MASTERS' AND DOCTORAL SEMINAR REPORTS – 2014

Volume – II

Agrl. Meteorology
Agrl. Entomology
Pathology
Agrl. Microbiology

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Agrl. Meteorology

Applications of Remote Sensing in Agriculture

By

Aswany K S

(2014-11-174)

M.Sc.Agrl.Meteorology

Seminar report

Submitted in partial fulfilment for the requirement of the course

AGM 591, Seminar (0+1)

DEPARTMENT OF AGRL.METEOROLOGY

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CERTIFICATE

Certified that this seminar report entitle ' Applications of remote sensing in agriculture' for the course AGM.591 has been prepared by Aswany K S (2014-11-174) after going through the various references cited here in under my guidance , and she has not copied or borrowed from any of her fellow students.

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DECLARATION

I, Aswany K S (2014-11-174) hereby declare that this seminar report entitled 'Applications of remote sensing in agriculture' has been prepared by me, after going through the various references cited at the end of the report and has not borrowed from any of my fellow students.

Aswany

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Date : 08-01-2016

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2014-11-174

CERTIFICATE

Certified that the seminar report entitled 'Applications of remote sensing in agriculture' is a record of seminar represented by Ms. Aswany K S (2014-11-174) on 19th December, 2014 and is submitted for the partial requirements of the course AGM 591.

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1. Introduction

Remote sensing is the science and art of obtaining information about an object through the analysis of data acquired by a device that is not in contact with the object (Lillesand and Keifer, 1994). The term remote sensing was coined by Evelyn Pruitt in mid-1950 in the U. S. Office of Naval Research (O.N.R). We acquire much information about our surrounding through the senses of sight and hearing which do not require close contact between the sensing organs and the external objects. In another word, we are performing remote sensing all the time. The science of remote sensing in its broadest sense includes aerial, satellite, and spacecraft observations of the surfaces and atmospheres of the planets in our solar system, though the Earth is obviously the most frequent target of study. The term is normally limited to methods that detect and measure electromagnetic energy, including visible light that has interacted with surface materials and the atmosphere. Remote sensing of the Earth has many purposes, including making and updating planimetric maps, weather forecasting, and gathering military intelligence.

2. Remote sensing

Remote sensing is a tool to monitor earth's resources using space technology in addition to ground observations.. Our eyes are an excellent example of a remote sensing device. We are able to gather information about our surroundings by gauging the amount and nature of the reflectance of visible light energy from some external source (such as the sun or a light bulb) as it reflects off objects in our field of view. Without direct contact, some means of transferring information through space must be utilized. The capacity of remote sensing to identify and monitor land surfaces and environmental conditions has expanded greatly over the last few years and remotely sensed data will be an essential tool in natural resource management.

3. Elements of remote sensing

In much of remote sensing, the process involves an interaction between incident radiation and the targets of interest. This is exemplified by the use of imaging systems where different elements are involved. It also involves the sensing of emitted energy and the use of non-imaging sensors.

A Energy source or illumination

The first requirement for remote sensing is to have an energy source which illuminates or provides electromagnetic energy to the target of interest.

B Radiation and the atmosphere

As the energy travels from its source to the target, it will come in contact with and interact with the atmosphere it passes through. This interaction may take place a second time as the energy travels from the target to the sensor.

C Interaction with the target

Once the energy makes its way to the target through the atmosphere, it interacts with the target depending on the properties of both the target and the radiation.

D Recording of energy by the sensor

After the energy has been scattered by, or emitted from the target, we require a sensor (remote - not in contact with the target) to collect and record the electromagnetic radiation.

E Transmission, reception, and processing

The energy recorded by the sensor has to be transmitted, often in electronic form, to a receiving and processing station where the data are processed into an image (hardcopy and/or digital).

F Interpretation and analysis

The processed image is interpreted, visually and/or digitally or electronically, to extract information about the target which was illuminated.

G Application

The final element of the remote sensing process is achieved when we apply the information we have been able to extract from the imagery about the target in order to better understand it, reveal some new information, or assist in solving a particular problem.

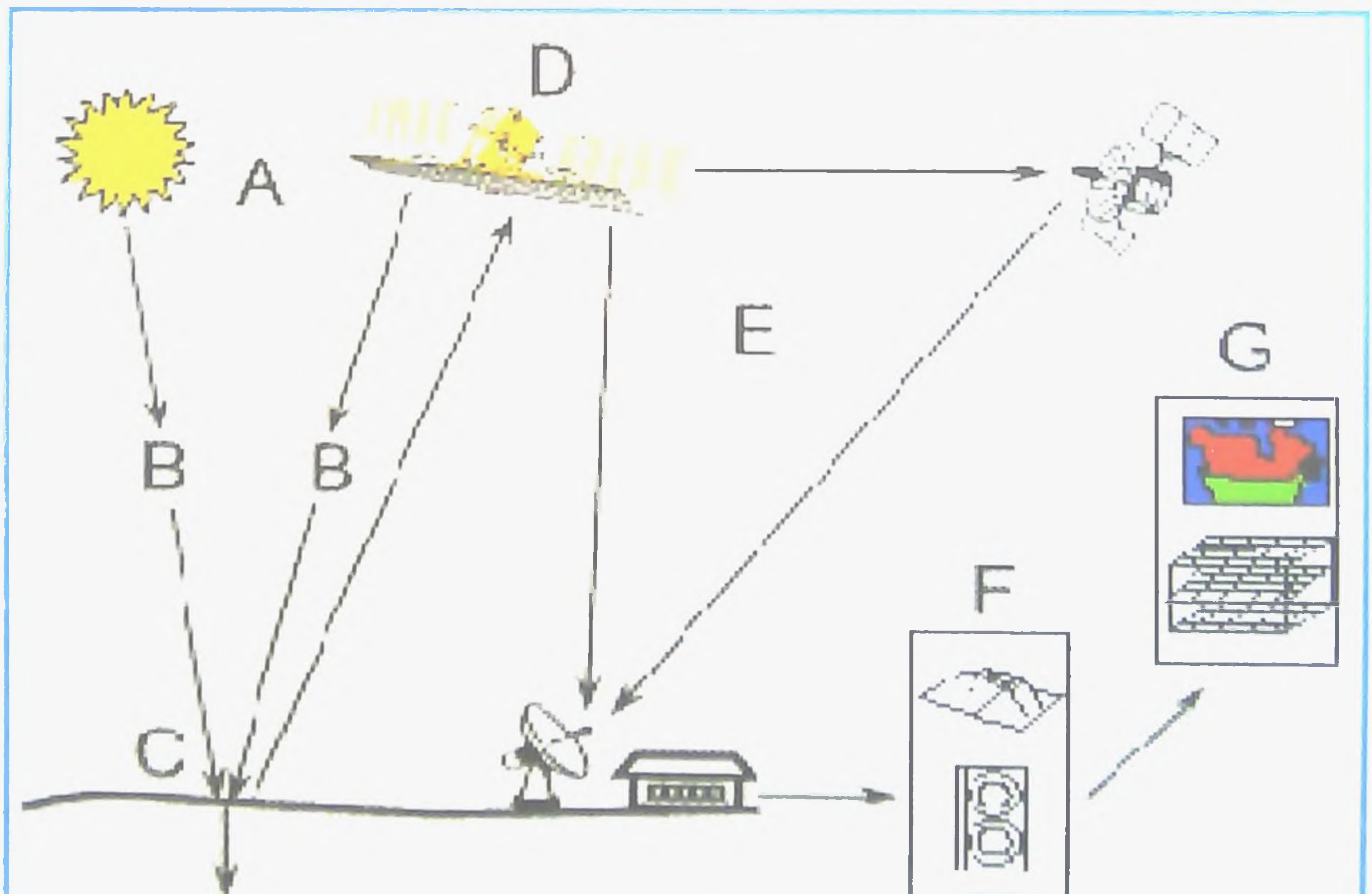


Plate 1. Elements of remote sensing

4. Electromagnetic Spectrum

The electromagnetic spectrum (EM) is the continuous range of electromagnetic radiation, extending from gamma rays (highest frequency & shortest wavelength) to radio waves (lowest frequency & longest wavelength) and including visible light. Remote sensing involves the measurement of energy in many parts of the electromagnetic (EM) spectrum. The major regions of interest in satellite sensing are visible light, reflected and emitted infrared, and the microwave regions. The measurement of this radiation takes place in what are known as spectral bands. The design of satellite sensors is based on the absorption characteristics of Earth surface materials across all the measurable parts in the EM spectrum.

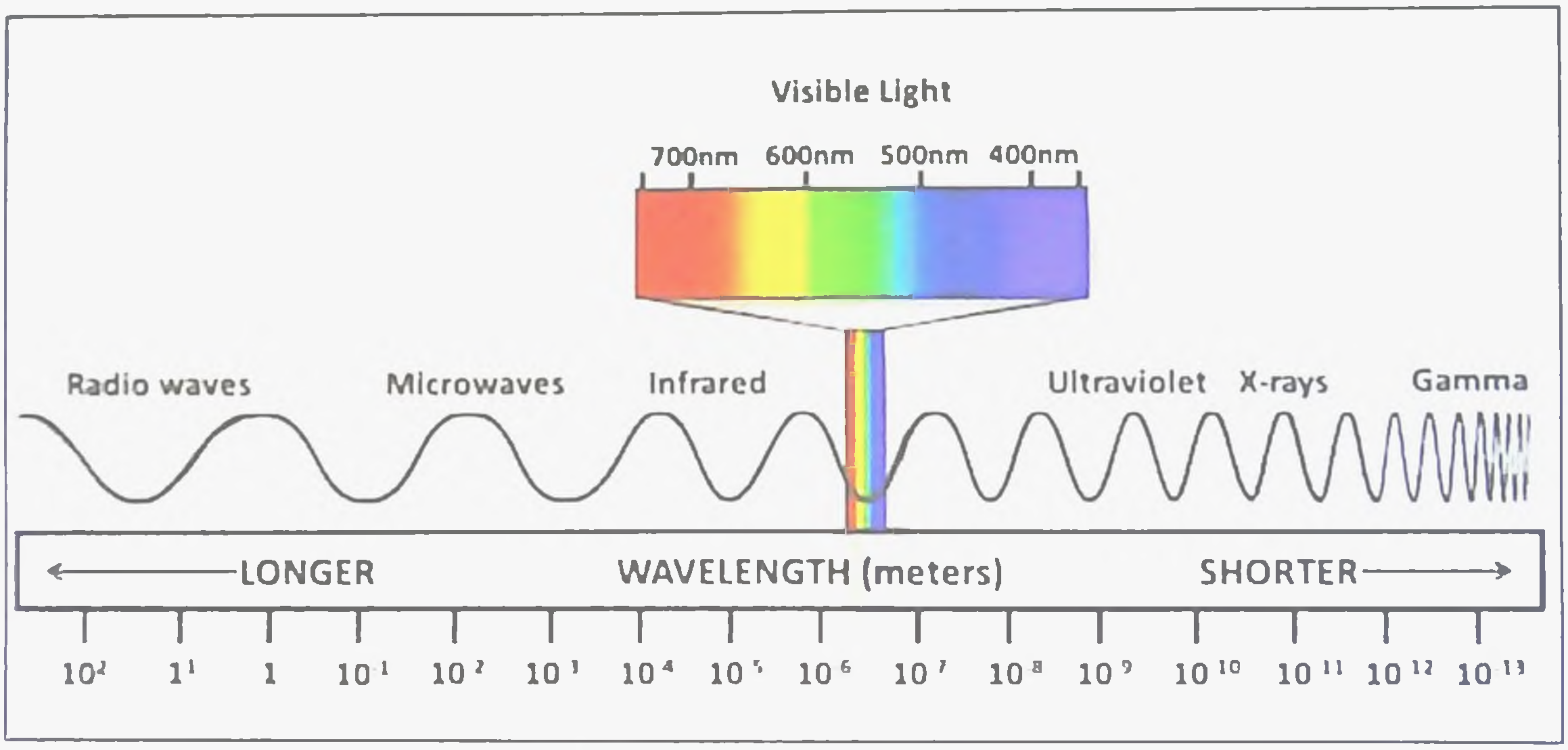


Plate 2. The electromagnetic spectrum

5. Interaction processes in remote sensing

Remote sensors measure electromagnetic (EM) radiation that has interacted with the earth's surface. Interactions with matter can change the direction, intensity, wavelength content, and polarization of EM radiation. The nature of these changes is dependent on the chemical and physical structure of the material exposed to the EM radiation. Changes in EM radiation resulting from its interactions with the Earth's surface therefore provide major clues to the characteristics of the surface materials. As sunlight initially enters the atmosphere, it encounters gas molecules, suspended dust particles, and aerosols. These materials tend to scatter a portion of the incoming radiation in all directions, with shorter wavelengths experiencing the strongest effect. Most of the remaining light is transmitted to the surface; some atmospheric gases are very effective at absorbing particular wavelengths.

As this modified solar radiation reaches the ground, it may encounter soil, rock surfaces, vegetation, or other materials that absorb a portion of the radiation. The amount of energy absorbed varies in wavelength for each material in a characteristic way, creating a sort of spectral signature. Most of the radiation not absorbed is diffusely reflected (scattered) back up into the atmosphere, some of it in the direction of the satellite.

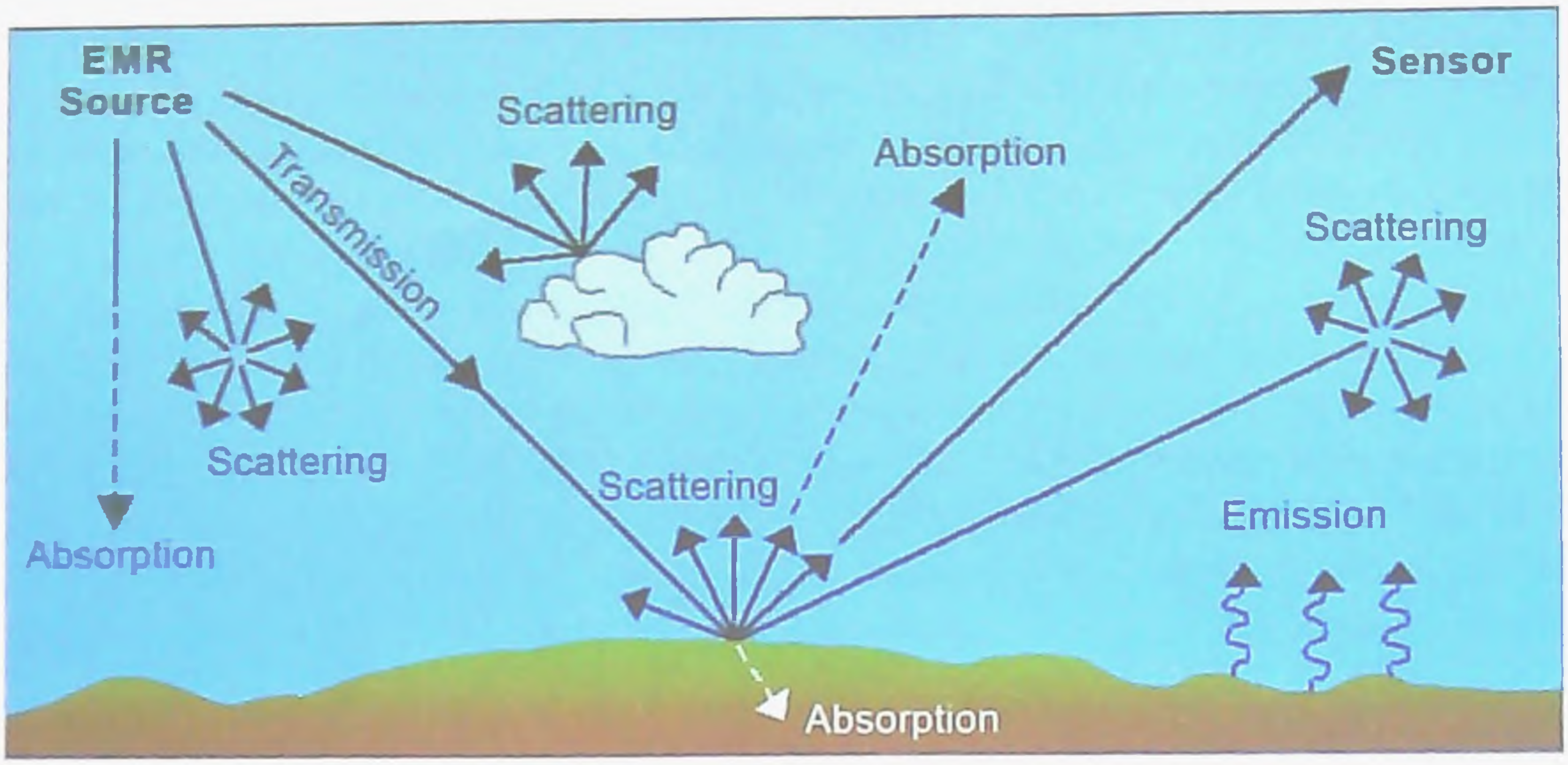


Plate 3. EMR interactions in the atmosphere and at the earth's surface

6. Types of remote sensing

The sun provides a very convenient source of energy for remote sensing. Remote sensing systems which measure energy that is naturally available are called passive sensors. Passive sensors can only be used to detect energy when the naturally occurring energy is available. For all reflected energy, this can only take place during the time when the sun is illuminating the Earth. There is no reflected energy available from the sun at night. Energy that is naturally emitted (such as thermal infrared) can be detected day or night, as long as the amount of energy is large enough to be recorded. Active sensors, on the other hand, provide their own energy source for illumination. The sensor emits radiation which is directed toward the target to be investigated. The radiation reflected from that target is detected and measured by the sensor. Advantages for active sensors include the ability to obtain measurements anytime, regardless of the time of day or season. Active sensors can be used for examining wavelengths that are not sufficiently provided by the sun, such as microwaves, or to better control the way a target is illuminated.

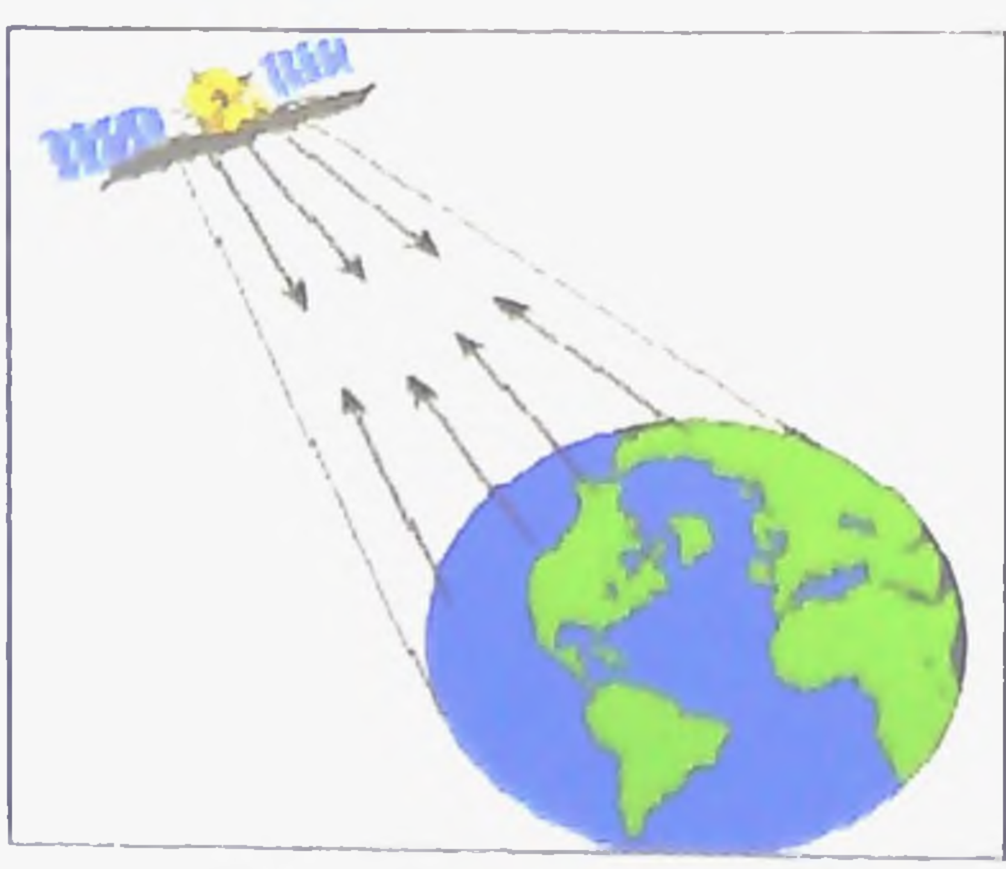


Plate 4. Active remote sensing

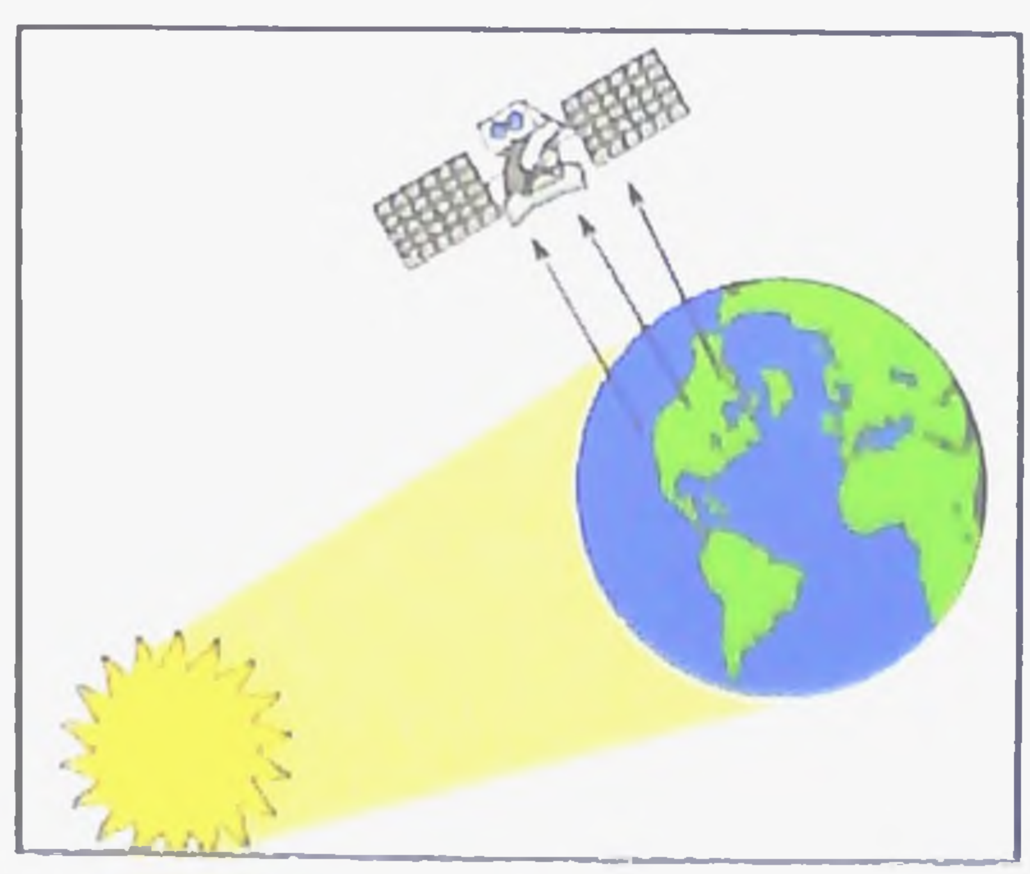


Plate 5. Passive remote sensing

7. Platform

Platforms are the vehicles or carriers for remote sensors. Platforms for remote sensors may be situated on the ground, on an aircraft or balloon, or on a spacecraft or satellite outside of the Earth's atmosphere. Ground-based sensors are often used to record detailed information about the surface which is compared with information collected from aircraft or satellite sensors. e.g. Sensors may be placed on a ladder, scaffolding, tall building, cherry-picker, crane, etc. Aerial platforms are primarily stable wing aircraft, although helicopters are occasionally used. Aircraft are often used to collect very detailed images and facilitate the collection of data over virtually any portion of the Earth's surface at any time. In space, remote sensing is sometimes conducted from the space shuttle or, more commonly, from satellites. Satellites are objects which revolve around another object. Because of their orbits, satellites permit repetitive coverage of the Earth's surface on a continuing basis.

Ground based platform

Space based platforms

Air based platform

Plate 6. Types of platforms

8. Growth indices

Table 1. Growth indices

Parameter	**Equation**	**Reference**
Normalized Difference Vegetation Index(NDVI)	(NIR-Red)/ (NIR+Red)	Rouse *et al* (1974)
Water Band Index(WBI)	900/970nm	Pefluelas *et al* (1997)
Water Moisture Index (WMI)	1600/820nm	Hunt and Rock (1989)
Photosynthesis Index	(531-570)/(531+570)nm	Gamon *et al* (1990)
Chlorophyll Based Difference Index (CI)	(850-710)/(850-680)nm	Datt (1999)

8.1 Normalized Difference Vegetation Index

The Normalized Difference Vegetation Index (NDVI) is an index of plant greenness or photosynthetic activity, and is one of the most commonly used vegetation indices. Vegetation indices are based on the observations that different surfaces reflect different types of light differently. NDVI is calculated on a per-pixel basis as the normalized difference between the red and near infrared bands from an image using the formula.

NDVI= (NIR-RED) / (NIR+ RED)

Where NIR is the near infrared band value for a cell and RED is the red band value for the cell. NDVI can be calculated for any image that has a near infrared band. The value ranges from -1.0 to 1.0 for each pixel in an image; helps identify areas of varying levels of plant biomass. Very low values of NDVI (0.1 and below corresponds to barren areas of rock, sand. Moderate values represent shrub and grassland (0.2 to 0.3), while high values indicate temperate and tropical rainforests (0.6-0.8).

9. Spectral signature

Radiation emitted as a function of wavelength is called spectral signature. The spectral signatures produced by wavelength-dependent absorption provide the key to discriminating different materials in images of reflected solar energy. The property used to quantify these spectral signatures is called spectral reflectance. The spectral reflectance of different materials can be measured in the laboratory or in the field, providing reference data that can be used to interpret images.

9.1 Spectral signature of earth's surface

The spectral reflectance curve of healthy green vegetation has a significant minimum of reflectance in the visible portion of the electromagnetic spectrum resulting from the pigments in the plant leaves. Reflectance increases dramatically in the NIR. Stressed vegetation can also be detected because stressed vegetation has a significantly lower reflectance in the infrared.

The spectral reflectance curve of bare soil is considerably less variable. The reflectance curve is affected by moisture content, soil texture, surface roughness, presence of iron oxide and organic matter.

The water curve is characterized by a high absorption at near infrared wavelength range and beyond. Because of this absorption property, water bodies as well as features containing water can easily be detected, located and delineated with remote sensing data. Turbid water has a higher reflectance in the visible region than clear water.

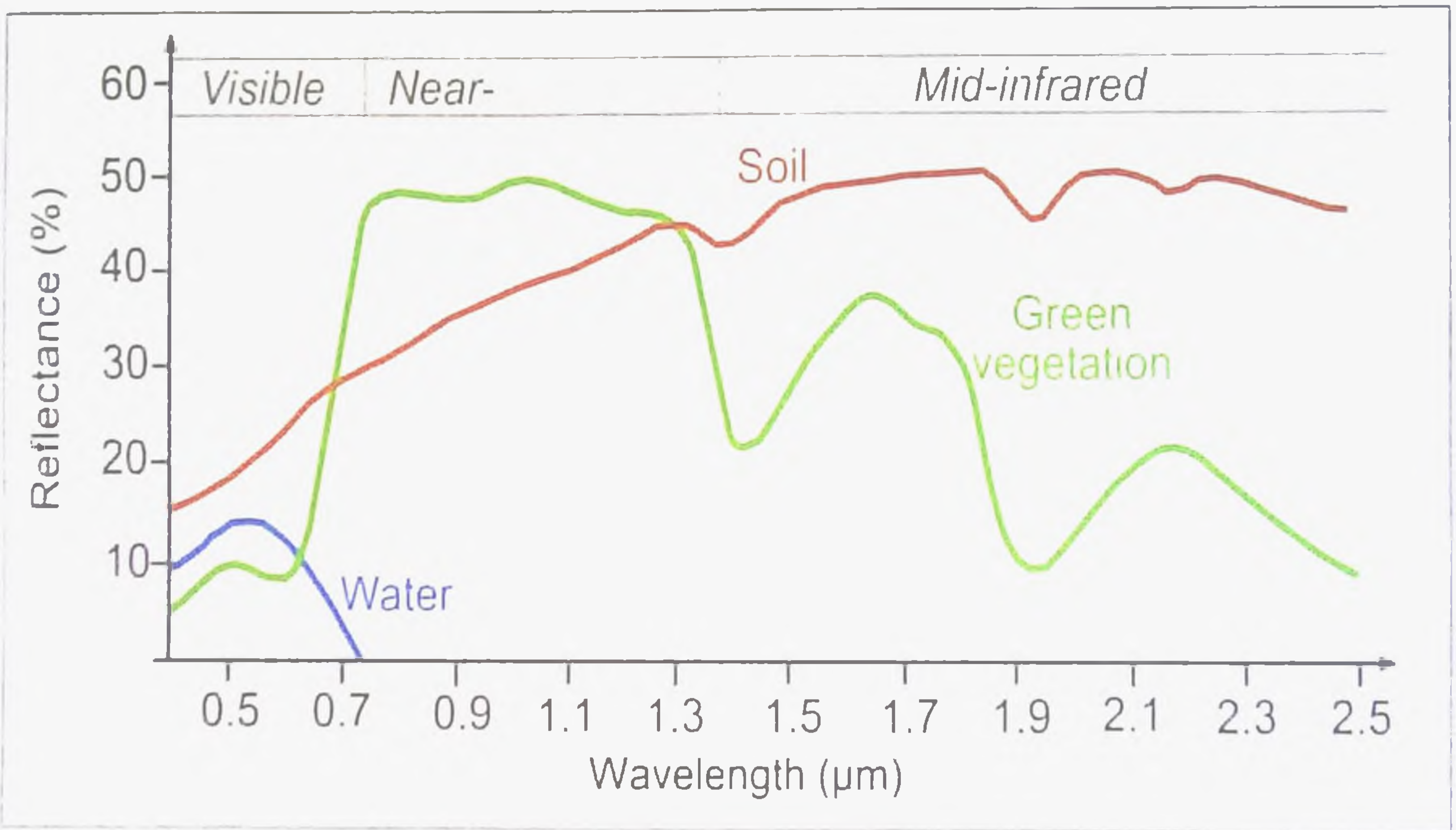


Plate 7. Spectral signature of earth's surface

9.2 Spectral signature of healthy leaf

Plants absorb red and blue light, less absorbs the green weakly absorbs the NIR and strongly absorbs FIR wave bands. Under the upper epidermis there are primary two layers of cells. The top one is the palisade parenchyma and consists of elongated cells, tightly arranged in vertical manner. In this layer contains most of the chlorophyll, carotenoids, anthocyanins which are responsible for the absorption of light. Because of those pigments, most of the visible electromagnetic energy is absorbed, especially in the blue and red region. Absorption in the green region is slightly weaker, which is why vegetation appears green to our eyes. As a result, very light energy escapes the palisade parenchyma and is reflected back towards the sky. NIR energy is not affected by these pigments and almost completely penetrates the palisade parenchyma, when reaches the spongy parenchyma, the presence of air spaces causes the reflection of the NIR energy in various directions. Remote sensors record the reflected energy in the visible and NIR regions of the spectrum.

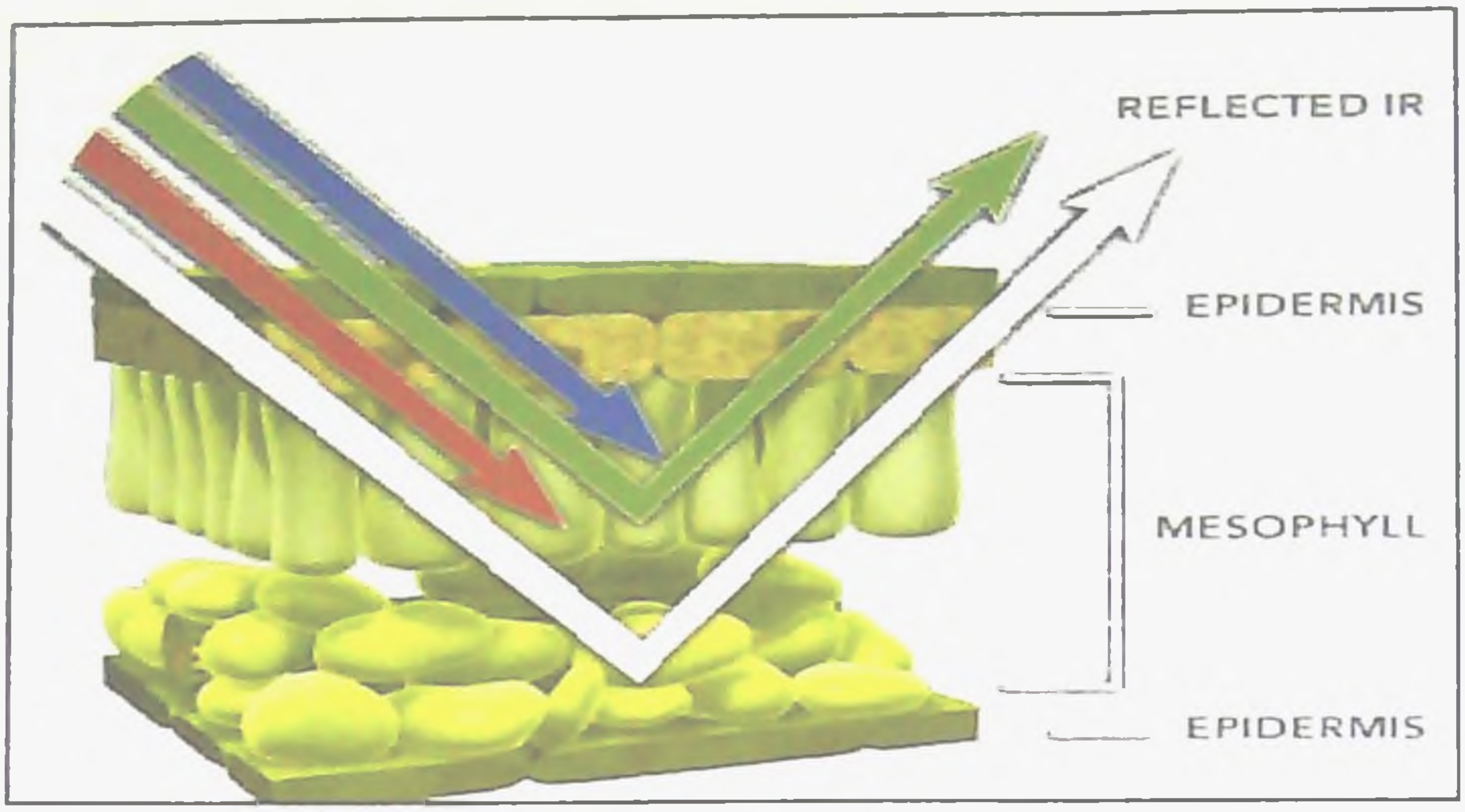


Plate8. Spectral signature of healthy leaf

10. Types of resolution

Resolution is the capability of the sensors to observe the smallest object clearly with distinct boundaries. There are four types of resolution namely spatial, spectral, temporal and radiometric resolution. The data collected by each satellite sensor can be described in terms of spatial, spectral and temporal resolution.

10.1 Spatial resolution

The spatial resolution specifies the pixel size of satellite images covering the earth surface. It is the measure of how closely lines can be resolved in an image. It depends on properties of the system creating the image, not just the pixel resolution in pixels per inch (ppi). In remote sensing, spatial resolution is typically limited by diffraction, observation, imperfect focus and atmospheric distortion.

10.2 Spectral resolution

It is the ability to resolve spectral features and bands into their separate components. The spectral resolution of a remote sensing system can be described as its ability to distinguish different parts of the range of measured wavelength.

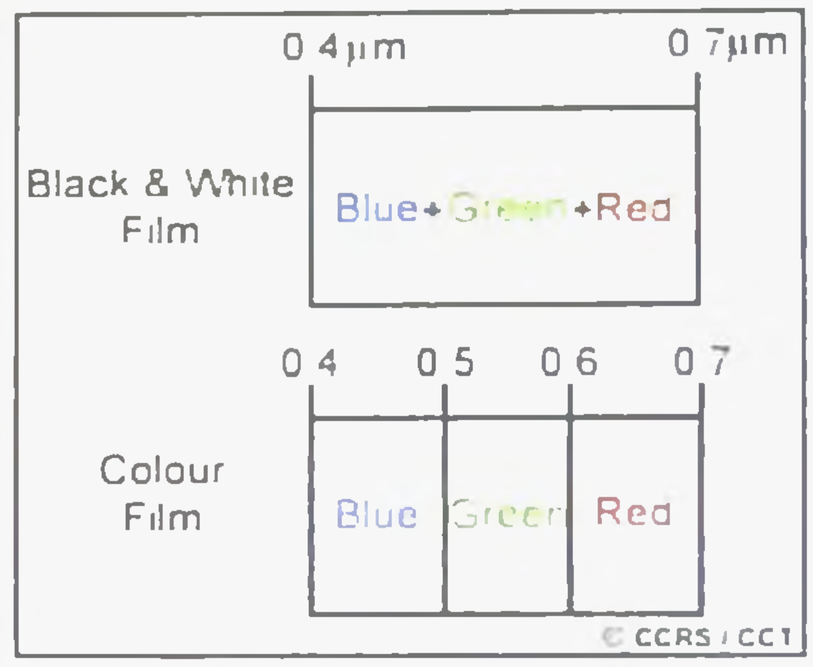
10.3 Temporal resolution

It is the precision of a measurement with respect to time. The absolute temporal resolution of a remote sensing system to image the exact same area at the same viewing angle a second time is equal to the revisit period of the satellite. The actual temporal resolution of a sensor depends on a variety of factors, including the satellite/ sensor capabilities, the swath overlap and latitude.

10.4 Radiometric resolution

Determines how finely a system can represent or distinguish differences of intensity. Usually it is expressed as a number of levels or a number of bits. In practice the effective radiometric resolution is typically limited by the noise level, rather than by the number of bits of representation.

Spatial resolution



Spectral resolution

Temporal resolution

Radiometric resolution

Plate 9. Types of resolution

11. Indian remote sensing satellites

Satellite remote sensing involves gathering information about the earth's surface using satellite orbiting around the earth. Data collected by the satellites are then transmitted to ground stations where images of earth's surface are reconstituted to obtain the required information. From the Indian remote sensing satellites, data is available in a variety of spatial resolution.KALPANA-1 is the first meteorological satellite built by ISRO. INSAT -3A is multipurpose satellite for communication, broadcasting and meteorological services along with KALPANA-1. CARTOSAT series, RESOURCESAT-2, TES (Technology Experiment Satellite) and IMS (Low cost Microsatellite Imaging Mission) are earth observation satellites. MEGHA-TROPIQUE is a meteorological satellite which give informations about water cycle in the tropical atmosphere. OCEANSAT-2 gathers data for oceographic, coastel and atmospheric applications.

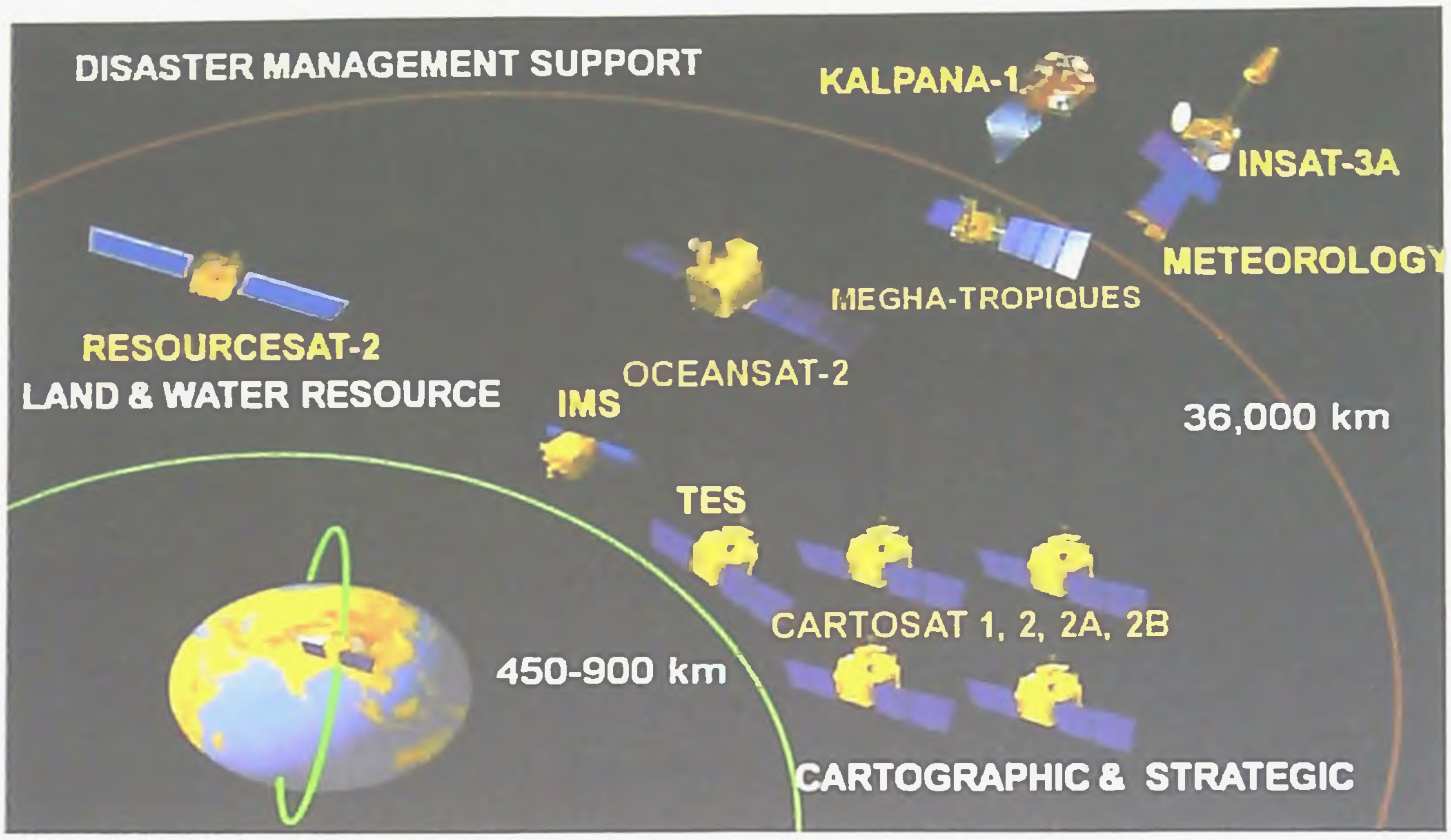


Plate 10 .Indian remote sensing satellites

12. Global Positioning System (GPS)

Space based navigation system that provides location and time information in all weather conditions anywhere on earth. Satellite based GPS provide information about the latitude, longitude and height information. GPS receiver monitors multiple satellites and solves equations to determine the exact position of receiver and its deviation from true time.

13. Geographic Information System (GIS)

Geographic Information System is any system designed to capture, store, manipulate, analyze, manage and present all types of spatial or geographic data.GIS is a computer based tool for mapping and analyzing feature events on earth surface. It integrates common database operations, such query and statistical analysis with maps. GIS can show different kinds of data on one map such as street, building and vegetation. This enables people to more easily see, analyse and understand patterns and relationships.

13.1 Software requirements in GIS

The software part of GIS includes the programs and the user interface for driving the hardware. Software includes not just core GIS modules but also various data drawing, manipulation, statistical, analysis, drawing, visualization, integration, imaging, database and other software required for the capture, display and analysis of data.

13.1.1. ERDAS IMAGINE

It is a raster based software package designed specifically to extract information from imagery.

13.1.2. ARCGIS

It is a geographic information system for working with maps and geographic information. It is used for creating maps, compiling geographic data.

14. Applications of remote sensing in agriculture

Agriculture plays a dominant role in economics of both developed and undeveloped countries. Remote sensing systems have the capability of providing regular, synoptic, multi-temporal and multi-spectral coverage of the country.

14.1 Remote sensing applications in vegetation

Remote sensing is used in crop identification, crop condition assessment, crop monitoring, damage assessment, crop area estimation, crop growth measurement, yield prediction. In order to identify a particular crop we need to familiar with its growth cycle and also know how the crops reflect NIR at each of their various growth stages. Crop condition assessment in the early growing stage is essential for crop monitoring and crop yield prediction. A normalized difference vegetation index based method is employed to evaluate crop condition.

14.1.1 Damage assessment

A plant becomes stressed when any biotic or abiotic factor adversely affects growth and development. Remote sensing is a means of detecting and assessing changes in plant canopies. Changes in spectral signature due to deficiencies of nutrients and damages by pathogens, pest and diseases, environmental factors, reduced amount of photosynthetic pigments causes an increase in red and blue reflectance and often affects the yellow region. Detection of crop stress is one of the major applications of hyper spectral remote sensing in agriculture.

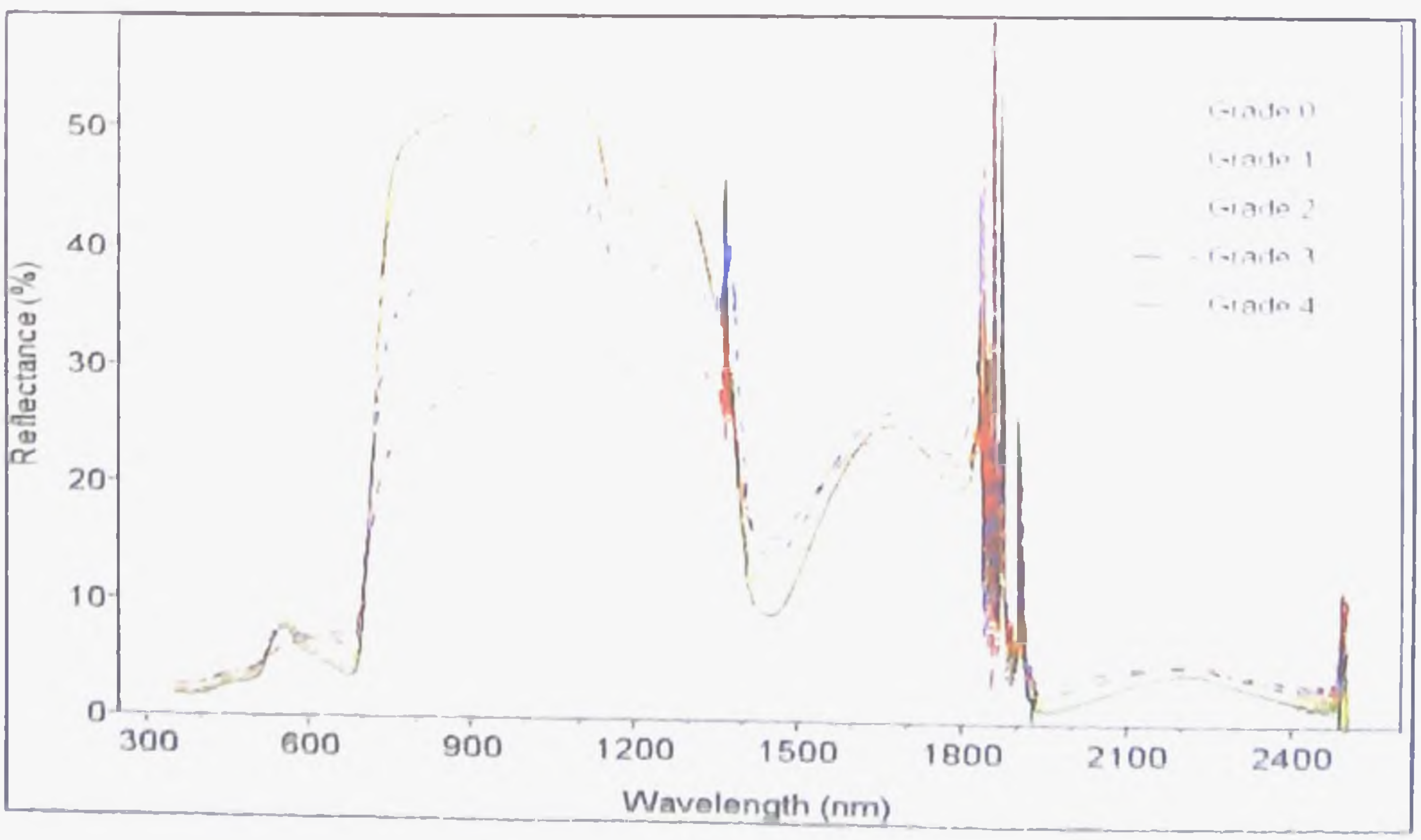


Plate 11. Reflectance spectra of cotton plant

This graph shows the mean reflectance spectra of plants with different levels of leaf hopper infestation. The sampled plants were graded into five levels of infestation starting from Grade 0 (healthy plant), and Grade 1(low infestation) to Grade 4 (severe damage). Reflectance spectra between healthy and leaf hopper infested plants showed significant decrease in blue and red region and increased reflectance spectra in NIR region (Prabhakar *et al.*, 2011). Even though the plants show reflectance in different wavelength region, the severity of leaf hopper infestation is clearly distinguished in NIR region.

14.2 Remote sensing applications in land use and land cover

Land suitability analysis can help to formulate the strategies for improvement in agricultural productivity. GIS based multi- criterion decision making using IRS P6 LISS-IV data set was used to analyze land suitability for agriculture in hilly zone (Zolekar and Vijay, 2015). For this study they selected spatial information regarding 12 criterions like slope, LULC, depth, texture, moisture, organic carbon, maximum water holding capacity, pH, N,P,K, soil erosion. For obtaining data regarding organic carbon, maximum water holding capacity, pH, N, P, K soil samples were collected from the selected sites and analyzed in laboratory to detect physical and chemical properties of soil. GIS software ARC 10 and ERDAS 9.2 were used for processing the remotely sensed data and field maps. The experts opinion and correlation analysis were used to decide the ranks of influencing criterion where as pair wise comparison matrix is used to determine the weights (Plate .12).

All thematic layers were integrated with each other in GIS according to their rank using weighed overlay analysis and land suitability for agriculture have been extracted (Plate.13). About 17% of area is classified in the class "highly suitable", 29% (12,372 ha) in "moderately suitable", 16% (6514 ha) in "marginally suitable", 38% (15,798 ha) in "not suitable for agriculture (Table 2).

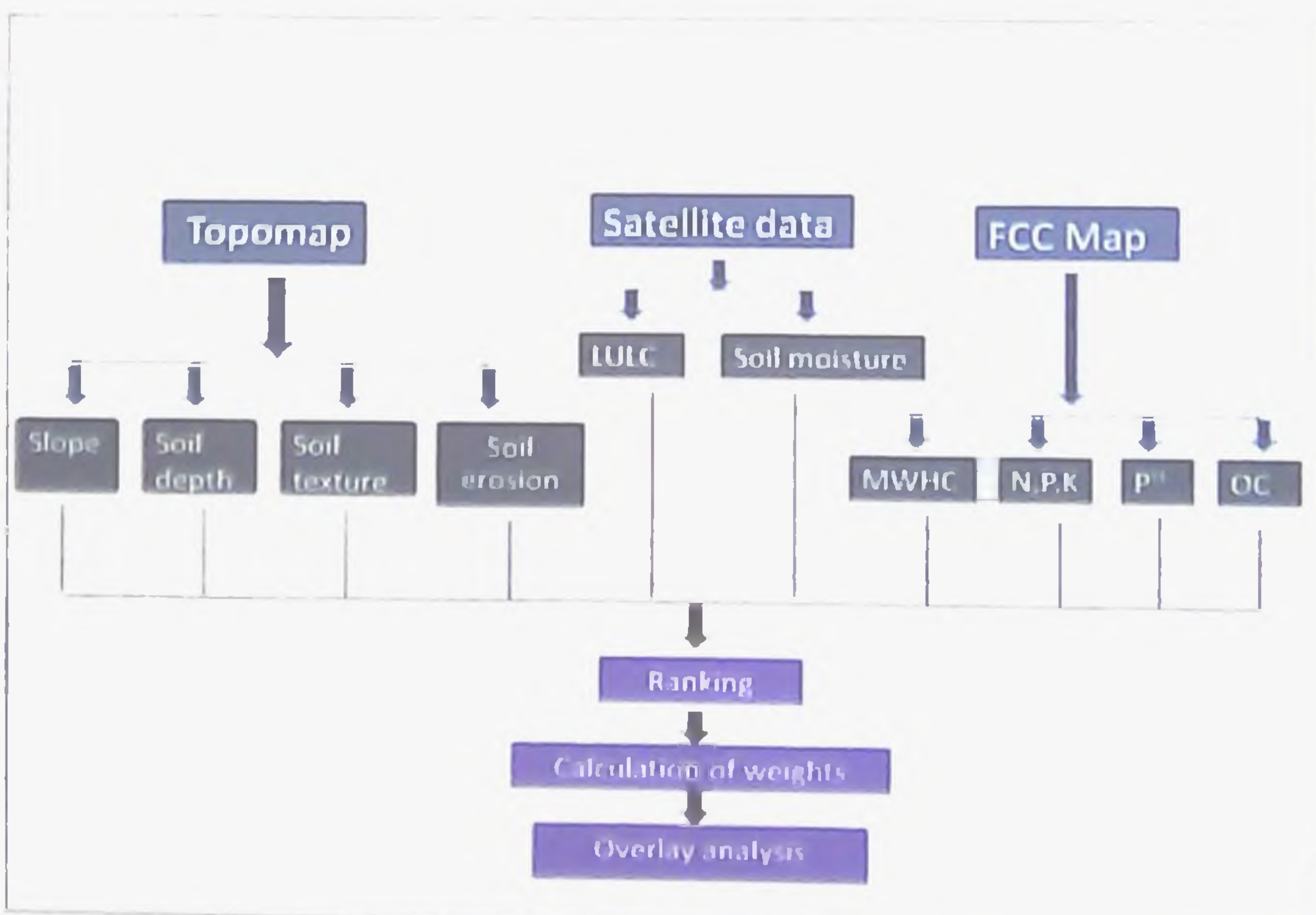


Plate 12. Schematic preparation of image processing and land suitability analysis

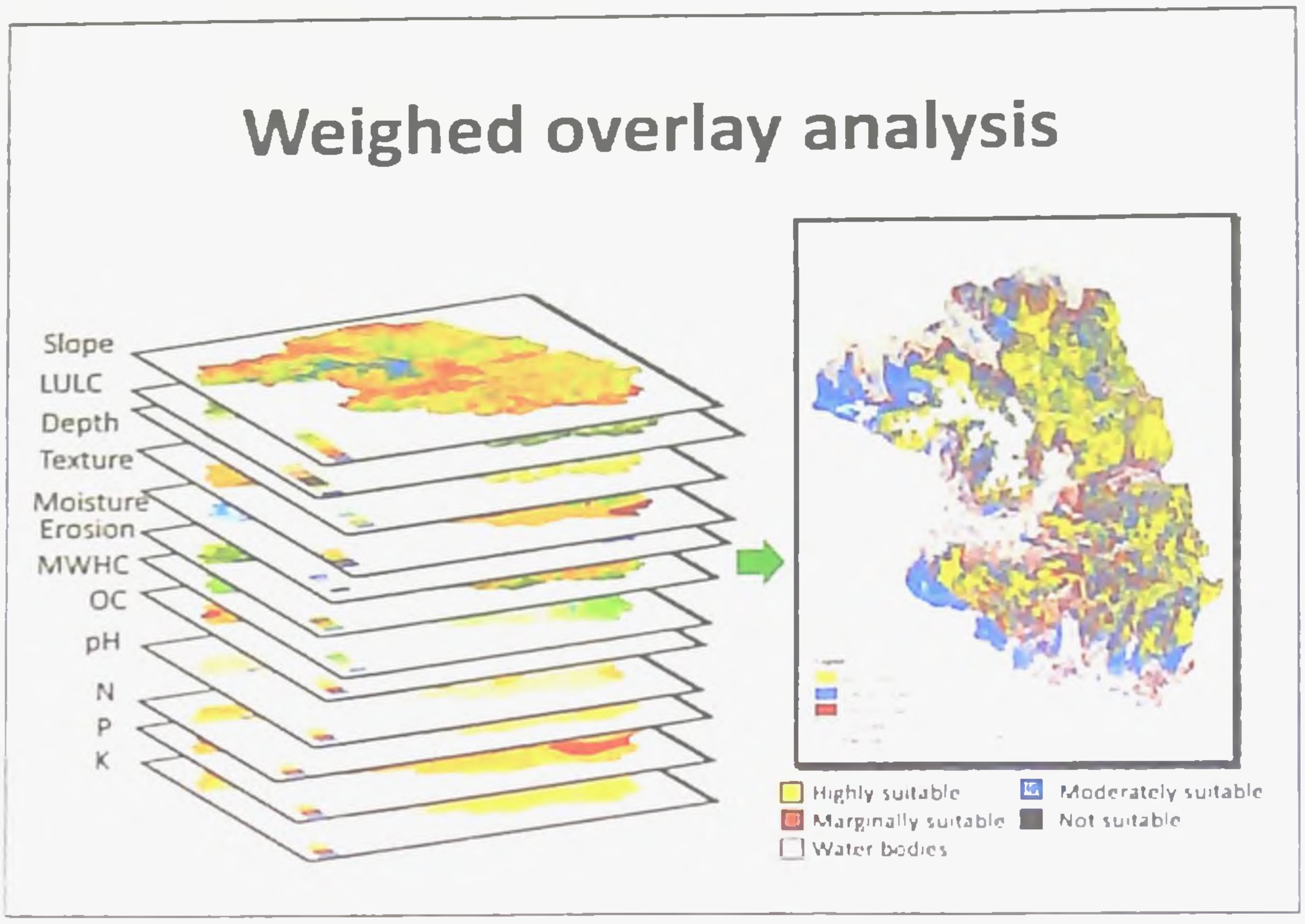


Plate 13. Weighed overlay analysis

Table .2. Land suitability classes

Category	Area		Suitability level
	Ha	%	
Highly suitable	7326	17	Agriculture
Moderately suitable	12,372	29	Arable farming
Marginally suitable	6514	16	Terrace cultivation
Not suitable	15,798	38	Not for agriculture

14.3 Remote sensing application in impact assessment tropical cyclone

Tropical cyclone is a rapidly rotating storm system characterized by a low pressure centre, strong winds and a spiral arrangement of thunderstorms that produce rain. Remote sensing data from United States Geological Survey (USGS) website was downloaded. The USGS satellite LANDSAT 8 of multi spectral scanner was selected for this study for the better identification of settlement, vegetation and water bodies (Vivek and Kumar, 2015) (Plate 18). Images of 4th October 2014, 20th October 2014 and 7th December 2014 were obtained from LANDSAT 8 (Table3). Classification map has been prepared for showing the pre-event and post event condition of that area (Plate 19). The changes will be identified by calculating the area in km^2 (Table 4).

The results showed, two week after cyclone passing over the region showed an increase in sparse vegetation and decrease in dense vegetation. This is due to trees uprooting. During the cyclone trees are mostly affected. The water level is also increased due to the heavy rainfall. Rise in the area of settlement is due to debris or raw materials from the buildings (Table 4).

Table 3. Details of satellite data

Image	Source	Details
LANDSAT 8 (4^{th} Oct.2014)	United States Geological Survey (USGS)	Spatial resolution: 30m
LANDSAT 8 (20^{th} Oct.2014)		
LANDSAT 8 (7^{th} Dec.2014)		

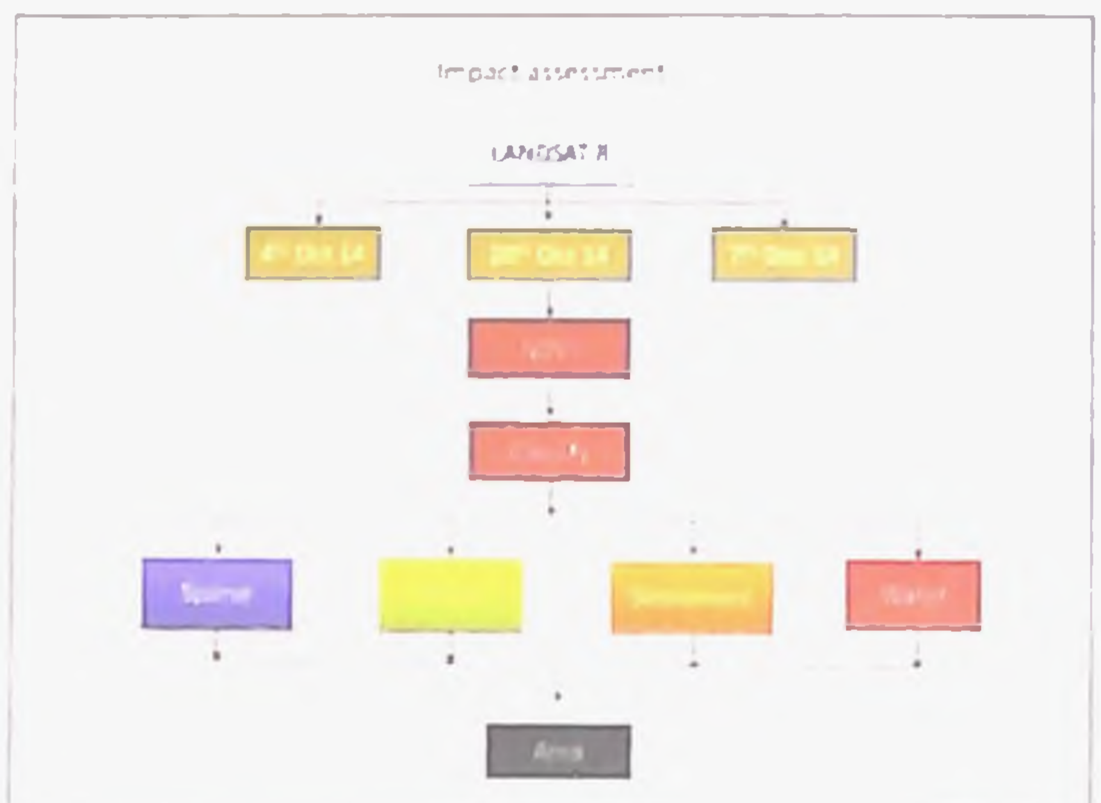


Plate 14. Flow chart of methodology

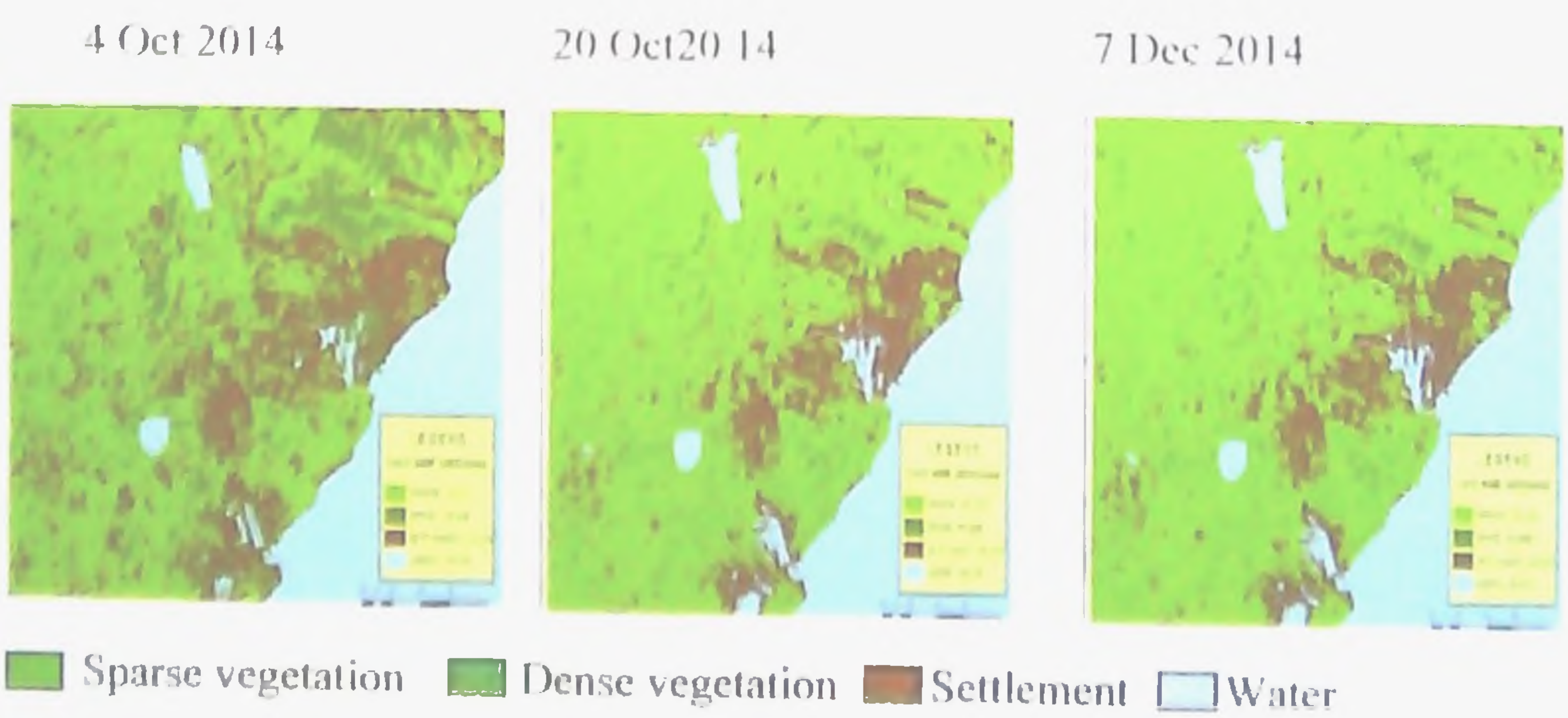


Plate 15. Classification map

Table 4. Impact assessment

Class name	Area km^2		
	4th Oct. 2014	20th Oct.2014	7th Dec. 2014
Sparse vegetation	275.01	362.31	385.54
Dense vegetation	126.43	41.83	39.59
Settlement	132.22	147.39	90.35
Water	174.27	182.29	180.58

14.3.2. Drought assessment

The occurrence of drought is mainly a climatic phenomenon which cannot be eliminated. A study was conducted by Dutta *et al.*, 2013 for the assessment of agricultural drought in Rajasthan using remote sensing derived vegetation condition index (VCI). In this study NOAA-AVHRR NDVI data were used for monitoring agricultural drought through NDVI based VCI. Plate.16 illustrates the vegetation condition index for different fortnights of kharif crops for the year 2002 and 2003. It was found that severe drought condition prevailed during kharif season of the year 2002 over a large area of Rajasthan.

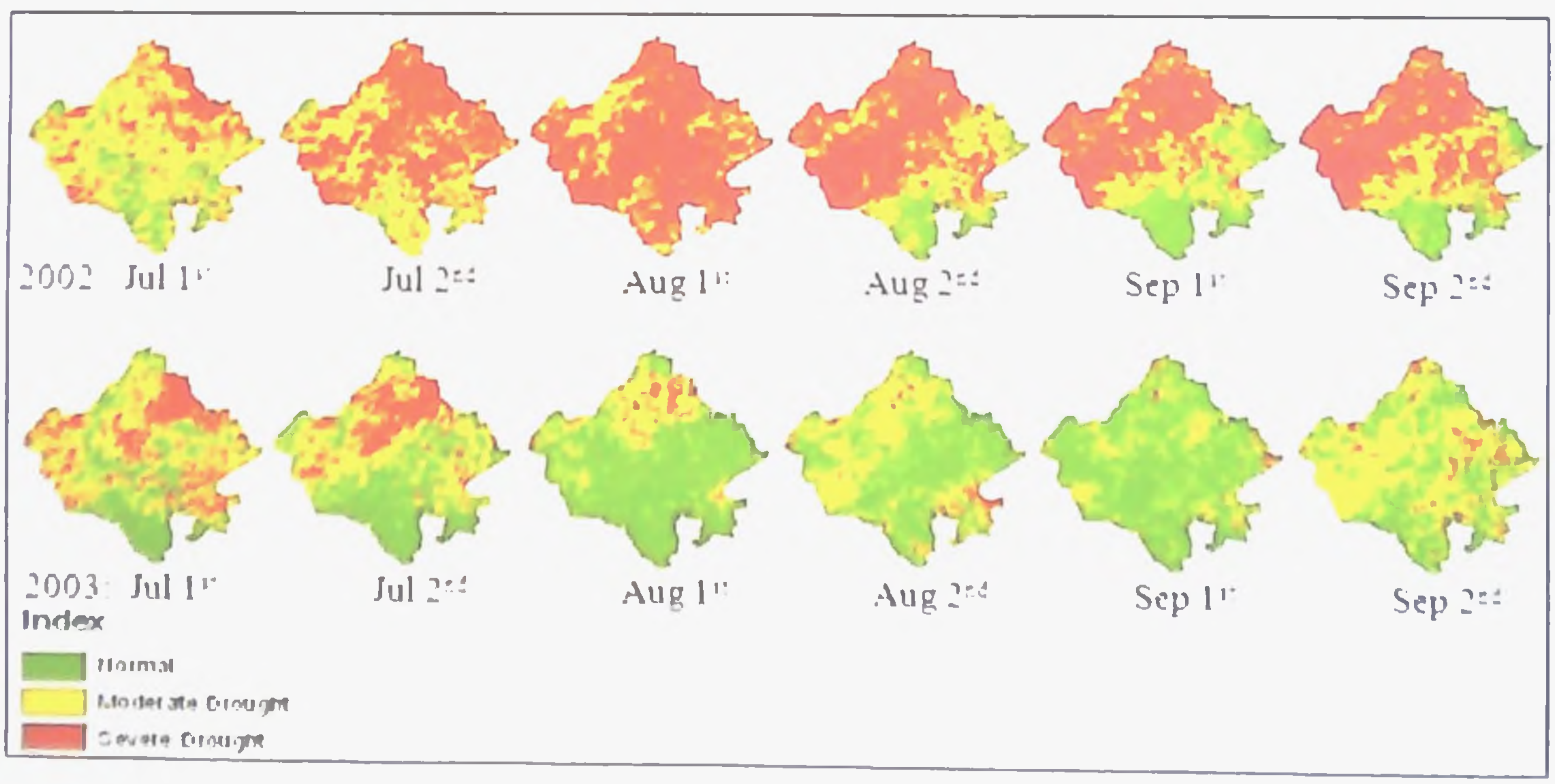


Plate 16. Spatio- temporal pattern of vegetation condition index

Conclusion

Remote sensing is the measurement of object properties on Earth's surface using data acquired from aircraft and satellites. It attempts to measure something at a distance, rather than in situ, and, for this research's purposes, displays those measurements over a two-dimensional spatial grid, i.e. images. Remote-sensing systems, particularly those deployed on satellites, provide a repetitive and consistent view of Earth facilitating the ability to monitor the earth system and the effects of human activities on Earth. Satellite remote sensing techniques are being operationally used to provide intra seasonal information on the spatial distribution of crops at different levels. Analysis of satellite data for crops along with the information on other natural resources provides valuable information towards sustainable agriculture. When utilizing satellite images to assess most types of land cover change, primarily those involving change in vegetation coverage, variations in climate must be considered. For better control and accuracy in these analyses, comparing images acquired during the same month or season is advisable.

Discussion

1. How remote sensing is used for detecting plant diseases?

The presence of diseases or insects feeding on plants or canopy surface causes changes in pigment, chemical concentrations, cell structure, nutrient, water uptake and gas exchange. These changes result in differences in color and temperature of the canopy, and affect canopy reflectance characteristics, which can be detectable by remote sensing.

2. How remote sensing is used for detecting earthquakes?

Earthquakes are hard to predict. But remote sensing could improve forecasts using Synthetic Aperture Radar. This technique combines two or more sequential radar images to measure ground motion between them very accurately on the scale of a few centimetres or even millimetres.

3. What is agricultural drought?

When there is insufficient soil moisture to meet the needs of a particular crop at a particular time.

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Meteorology

AGM 591: Master's Seminar

Name: Aswany K. S.

Admission No: 2014-11-174

Major advisor: Dr. B. Ajithkumar

Venue: Seminar Hall

Date: 19.12.2015

Time: 10.00 a.m.

Applications of remote sensing in agriculture

Abstract

Remote sensing is the science of acquiring information about the earth's surface without actually being in contact with it. This is done by sensing and recording reflected or emitted energy and processing, analyzing, and applying that information. In remote sensing, the process involves interaction between incident radiation and the targets of interest.

Identification of crops and discrimination from other crops is a primary step for studies about crop condition, monitoring of crop growth and crop disease analysis. Each crop has its own unique architecture, growing period enables its discrimination from other crops through remote sensing data (Goswami *et al.*, 2012). Information on crop area is the backbone of agricultural statistical system. Reliable and timely information on crop area can be obtained by remote sensing. This information is of great importance to planners and policy makers for taking important decisions with respect to procurement, storage, public distribution, export, and other related issues.

Detection of crop stress is one of the applications of hyperspectral remote sensing in agriculture (Prabhakar *et al.*, 2011). Use of remote sensing technique for detection of crop stress due to pests and diseases is based on the assumption that stresses induced by them interfere with photosynthesis and physical structure of the plant and affect absorption of light energy and thus alter the reflectance spectrum of plants.

Land is a stable part of the earth's surface. Land suitability analysis can help to formulate the strategies for improvement in agricultural productivity. Geographic Information System (GIS) based multi-criterion decision making approach using IRS P6 LISS-IV satellite dataset was used to analyze land suitability for agriculture in hilly zones (Zolekar and Bhagat, 2015).

The natural calamities such as drought and floods, cyclones and landslides *etc.* can effectively be monitored using the remote sensing data. Drought monitoring through satellite based information has been popularly accepted in recent years for its low cost, synoptic view, repetition of data acquisition and reliability (Dutta *et al.*, 2015). Remote sensing and GIS are essential components of the environmental impact assessment process. Remote sensing

methods and ground based spectral measurements can greatly simplify the monitoring of crop development and making decision to optimize inputs on agricultural production and reduce its harmful effects on environment.

References:

Dutta, D., Kundu, A., Patel, N. R., Saha, S. K., and Siddiqui, A. R. 2015. Assessment of agricultural drought in Rajasthan using remote sensing derived vegetation condition index and standardized precipitation index. *Egyptian J. Remote sensing Space Sci.*18: 53-63.

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Microclimate modification to enhance crop production

By

Sushna K.

(2014-11-230)

M. Sc. Agricultural Meteorology

Seminar report

Submitted in partial fulfilment of requirement of the course Agm. 591 Seminar (0+1)

DEPARTMENT OF AGRICULTURAL METEOROLOGY

COLLEGE OF HORTICULTURE

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VELLANIKKARA- 680656

THRISSUR, KERALA, INDIA

CERTIFICATE

Certified that the seminar report entitled **'Microclimate modification to enhance crop production'** for the course Agm.591 has been prepared by Sushna K (2014-11-230), after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

Vellanikkara
08-01-2016

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DECLARATION

I, Sushna K (2014-11-230), hereby declare that the seminar report entitled **'Microclimate modification to enhance crop production'**, has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Sushna K

Vellanikkara

Date: 08.01.2016

Sushna K

2014-11-230

CERTIFICATE

Certified that the seminar report entitled **'Microclimate modification to enhance crop production'**, is a record of seminar presented by Sushna K (2014-11-230) on 11th December, 2015 and is submitted for partial requirement of the requirement of the course Agm.591.

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1.Introduction

Now days scientists are curious about the impact of climate change in different areas. Agriculture is very sensitive to climate change. Higher temperature eventually reduces the yields of desirable crops while encouraging the weeds and pest proliferation. Changes in the precipitation pattern will cause short run production failure and which leads to a long run production decline. Despite the technological advances such as genetically modified organisms, improved varieties, irrigation systems etc. weather is still a key factor for agriculture production. The effect of climate on agriculture relates its variabilities in local climate rather than a global climate patterns. So the control or modification of the climate is only possible at the microclimate level.

2. Climate

Climate is a long term regime of weather variable such as rainfall, humidity, temperature, wind etc. Based on the horizontal, vertical and time scale basis the climate can be categorized into maroclimate, mesoclimate, microclimate (Rao, 2003).

Table 1. Scales of climate

Type of climate	Horizontal scale (km)	Vertical scale (km)	Time scale
Macroclimate	500 – 5000	10	4 to 16 days
Mesoclimate	1 – 100	1 – 10	1 – 10 hrs
Microclimate	< 100 m	200 m	6 – 12 min

Macroclimate is the study of physical process of atmosphere for a larger area as compared to the others and that of mesoclimate which lies between macro and microclimate. Microclimate is the study of atmospheric process over a small area as compared to the other

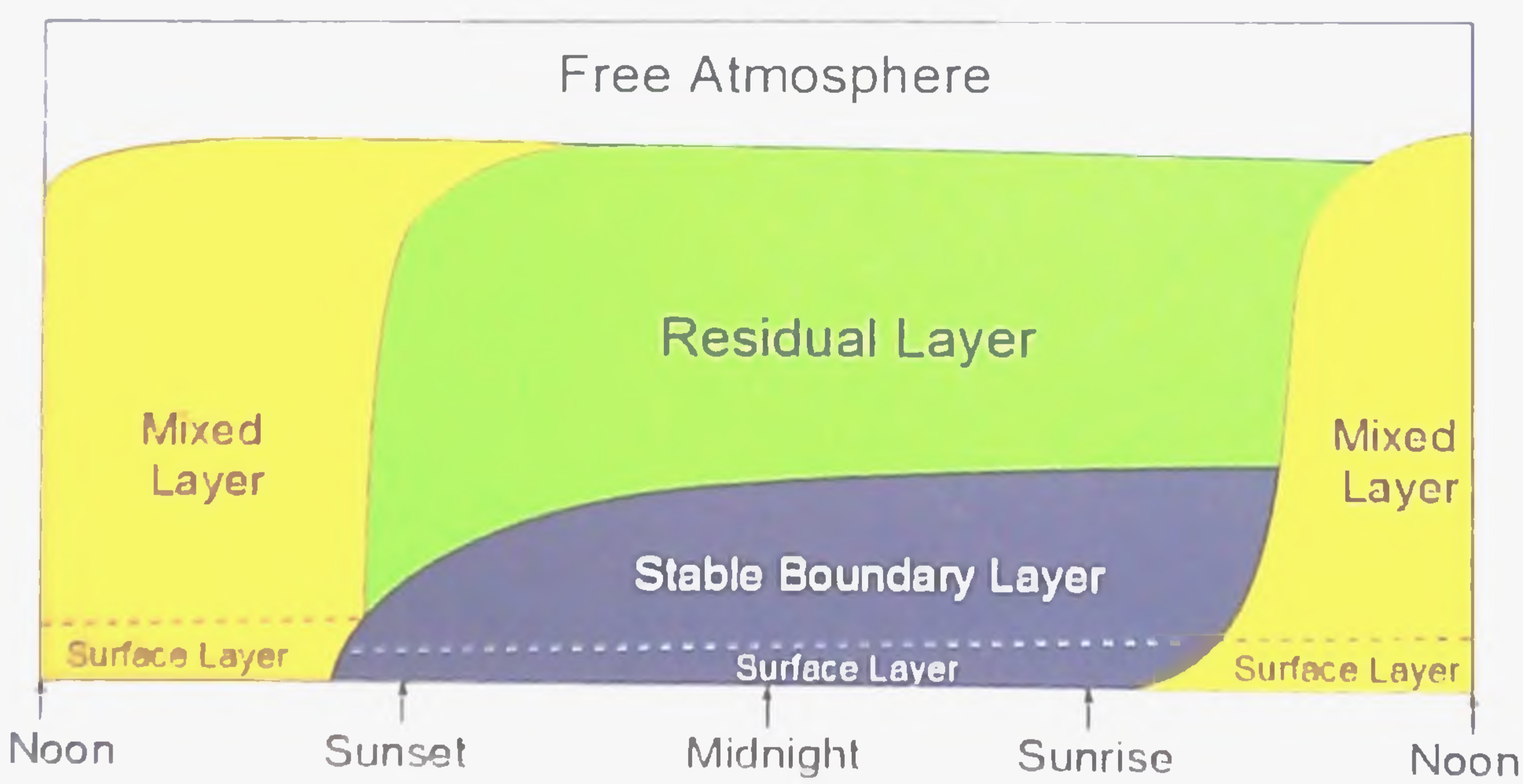
3. Microclimate

Microclimate is the atmospheric phenomena and physical processes taking place over limited region of the surface of the earth in the lowest layer of the atmosphere (Mavi, 1996).

4. Structure of microclimate

Troposphere is regarded as the lowest layer of atmosphere and which is nearest to the earth surface and has more or less uniform decrease of temperature with increase in height. It has got two layers the upper free atmosphere and the lower, planetary boundary layer. Within the planetary boundary layer the microclimate develops. Planetary boundary layer is also known as the atmospheric boundary layer, the structure of planetary boundary layer varies with the seasons, weather conditions and time of a day. Based on the time of a day the structure of atmosphere varies (Pic. 1). The surface layer is the lowest layer of planetary boundary layer and by the beginning of the day the short wave radiation will heats the soil surface and it will leads to the convective air motion this convective air motion will encourages the development of turbulent mixing. Turbulent mixing is the mixing of warmer and cooler air. The process called turbulent mixing will creates the mixed layer and which persist up to the sunset. After the sunset this mixed layer will collapses and which forms the stable boundary layer and which is capped by a residual layer where the residue of the convective air motion will be seen.

Plate 1. Structure of planetary boundary layer



5. Interactions in microclimate

The interactive process in microclimate varies in day and night. Beginning at the sunrise the short wave radiation will heats the soil and plant surfaces and which will leads to the movement of heat from the surface to the atmosphere which is known as the sensible heat flux. The sensible heat flux will creates turbulence in the atmosphere and which leads to a unstable condition in the atmosphere. The turbulence will encourages the evaporative loss from the surfaces and also it will promote the exchange of CO_2 .

According to Wright, 1967 after the sunset the situation will quickly reverses than that of day time. The soil and plant surfaces will radiate energy towards the atmosphere and so surfaces will become cooler. So the there is temperature gradient develops which is exactly opposite that of the day time and is known as the temperature inversion. The absence of turbulent mixing will reduces the evaporative loss and also reduces the exchange of CO_2 between the plants and atmosphere and it increase the O_2 transport associated with the respiration.

6. Transport processes in microclimate

The energy transport is primarily concerned with radiation and heat transport. Heat is transported by mainly three mechanisms that are conduction, convection and radiation.

Conduction is the transport of heat through the material from a region of higher temperature to region of lower temperature, and that of convection is the transfer of heat through the movement of particle. Radiation is the direct transfer of heat without the aid of any material medium.

Mass and momentum transport is associated with the transfer of CO_2, water vapor and other gases. Mass is transported by diffusion and momentum is transported by shearing stress which is produced due to the presence of wind in the atmosphere.

7. Importance of microclimate

The microclimate is the lowest layer of atmosphere and many of its characteristic property defines the presence of life on earth. The microclimate is the region where the turbulence is present and which helps the exchange of different gases and also the transfer of energy, radiation and transport of water vapor.

Microclimate is the region where the water or hydrological cycle present. This is the important cycle for living beings.

8. Modification of microclimate

The microclimate modification can be achieved by three ways by controlling the heat load, controlling the water balance and controlling the turbulence or wind velocity (Lowry, 2000).

8.1. Control of heat load

According to Perry, 2011 controlling the heat load means the heat energy which is required to add to a system for maintain the temperature at an acceptable range. This can be achieved by different ways such as,

a) Heat evasion
b) Heat trapping
c) Retardation of sensible and latent heat flux
d) Protection from frost damage

8.1.1. Heat evasion

In many areas in tropic and subtropics, heat load on some of the plants is above the tolerance limit. In such cases it is desirable to evade the thermal energy in order to achieve good results. Shading of plants is a common method of evading solar radiation. A number of shade structures are used and these are opaque. The shade can be from wood or fibre. Its beneficial effects are that it keeps the temperature low and retards evapotranspiration. The material acts as a thermostat and can be applied or removed as required.

A study was conducted by Louis in 2000 in Kerala Agricultural University, where the effect of shade levels on growth and yield of three different varieties have been compared. Different shade levels such as 25%, 50%, 75% and a control was maintained. The variation on leaf temperature was also compared (Table 2.) as compared to the control the leaf temperature was reduced

Table 2. Effect of shade levels on leaf temperature of turmeric varieties

Treatments	Leaf temperature (°C)			Mean
	Kanthi	Alleppey	Shobha	(°C)
S_0	38.67	40.29	38.70	39.22
S_1	30.67	30.98	31.01	30.88
S_2	33.22	32.65	29.94	31.93
S_3	30.61	31.52	33.89	32.01

The yield from all the three varieties also have been analyzed, the yield have been increased by using the shade levels and a higher yield was obtained for the turmeric varieties which have been planted under 25% shade levels.

Table 3. Effect of shade levels on yield of turmeric varieties

Shade levels	Yield ($t\ ha^{-1}$)			Mean ($t\ ha^{-1}$)
	Kanthi	Alleppey	Shobha	
S_0	26.22	25.64	20.39	24.08
S_1	31.46	31.18	27.38	30.00
S_2	26.84	28.95	20.75	25.51
S_3	26.66	26.62	20.06	24.45

8.1.2. Heat trapping

It is opposite of heat evasion, and which is extremely beneficial in temperate climates where the growing period is comparatively short. Heat trapping can be achieved by taking into account the angle of solar radiation relative to plants. By proper placement of the crop canopy, the flow of solar radiation and temperature can be increased. Heat trapping is accomplished by planting the trees on steep sunny slopes and erecting alternate low stone walls. These stone walls reflect the light back towards the lower portions and shaded sides of trees. Additionally, the thermal capacity of the wall material will increases the local source of heat by night. This type of heat trapping is practiced in cool temperate areas where the summer temperatures are not high.

Experiments have been proved that heat can also be trapped by employing an intermittent space covering practice. The soil between plant rows is covered with plastic sheets. These sheets reflect the light to the lower section of the canopy. An additional benefit of this device is that it directs rainfall towards the plants and reduces evaporative loss from the soil.

Another method of heat trapping is cultivation of plants under poly house. In day time it allows the penetration of short wave radiation towards the poly house and it restricts the flow of long wave radiation in the night time. Both the effect will leads to the warmed condition inside the poly house.

Plate 2. Heat trapping

Adjustment of plant canopy

Intermittent space covering by white plastic sheet

In Kerala Agricultural University a work was conducted on tomato, in open field and poly house. In that different weather variables like air temperature, relative humidity, and light intensity were compared in poly house and open field.

The weather variables such as relative humidity (%) and light intensity (lux) have been less inside the poly house condition and that of air temperature (^{0}C) have been higher inside the poly house as compared to the open field.

Figure 1. Air temperature in open field and poly house

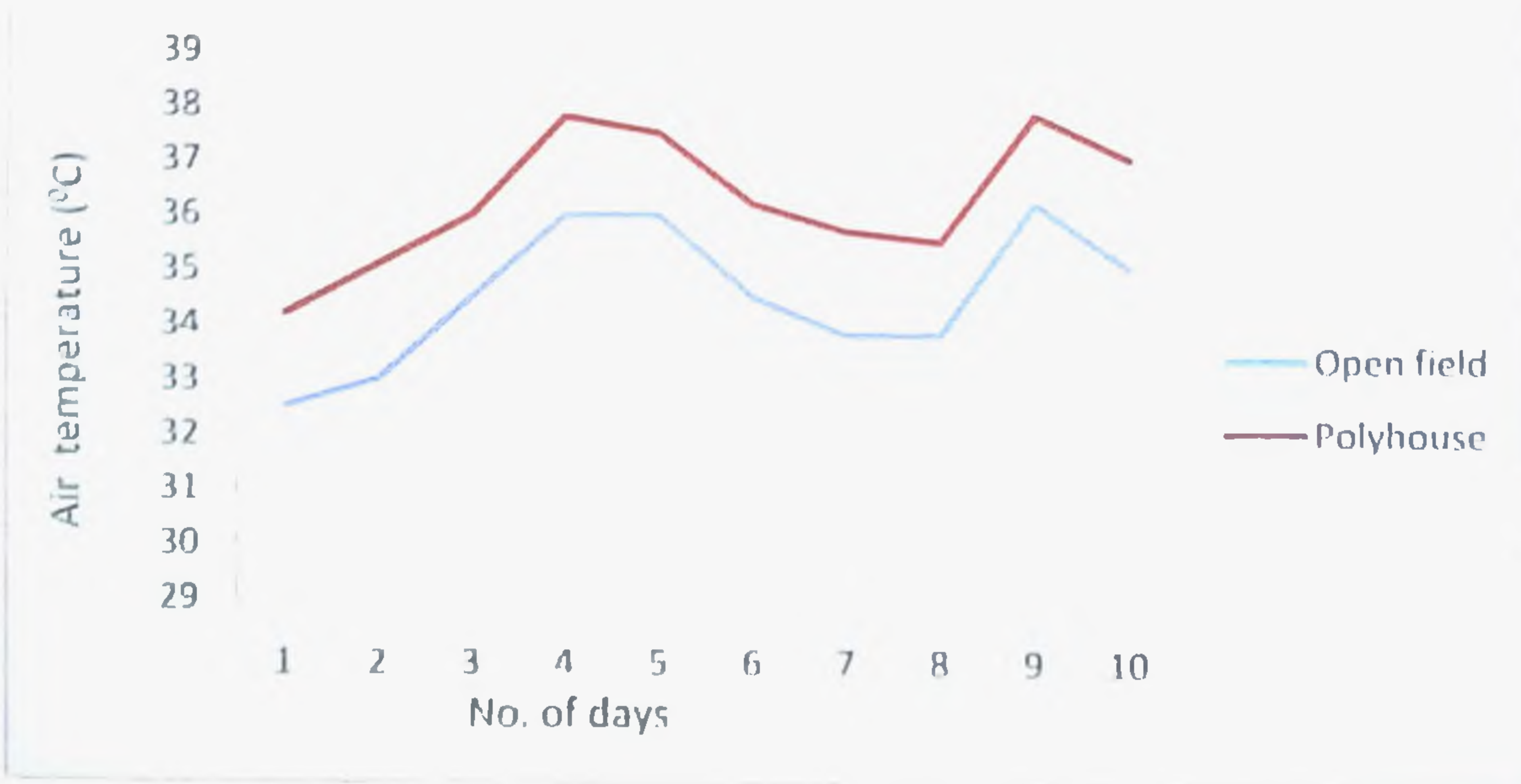


Figure 2. Relative humidity in open field and poly house

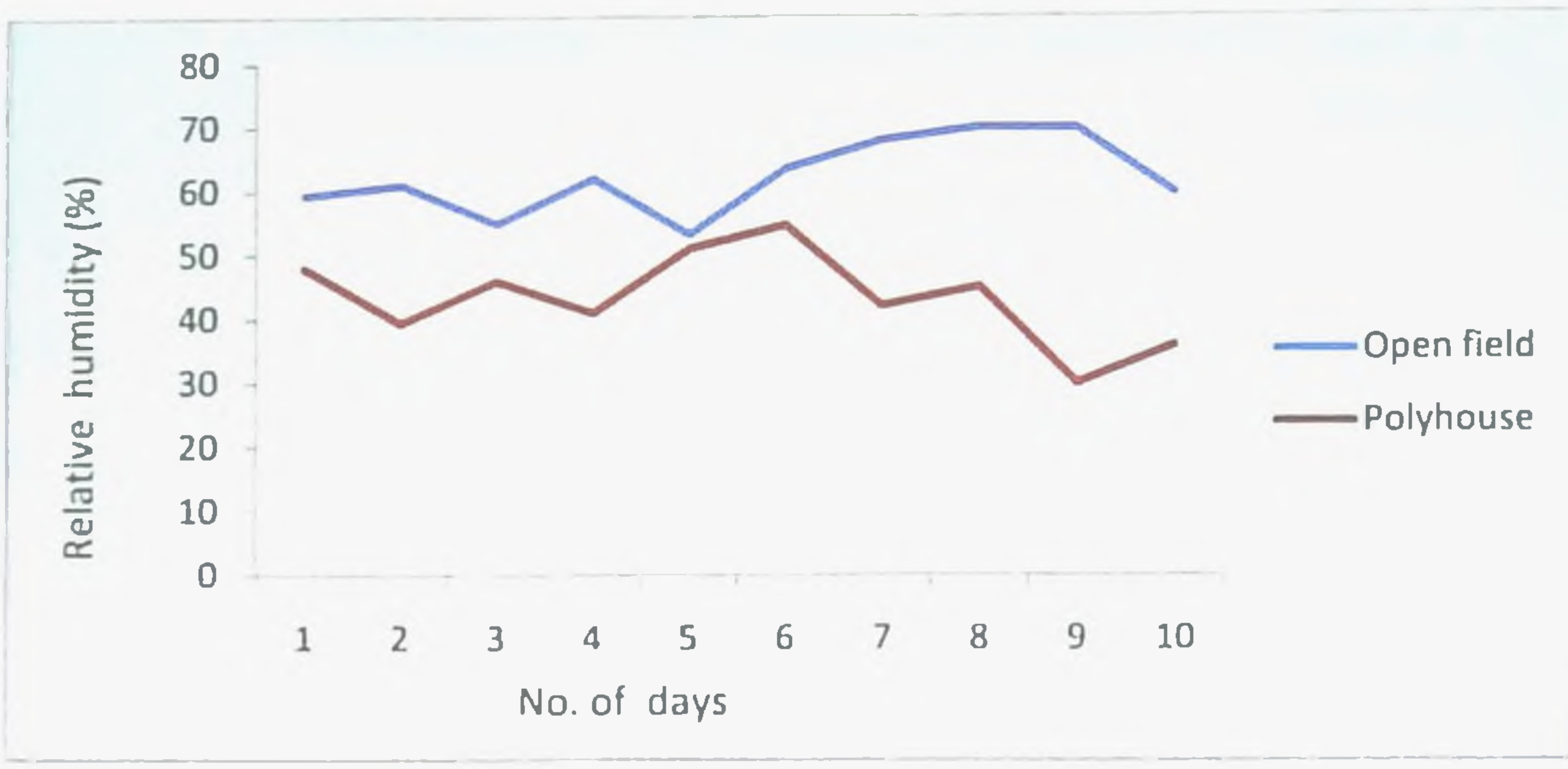
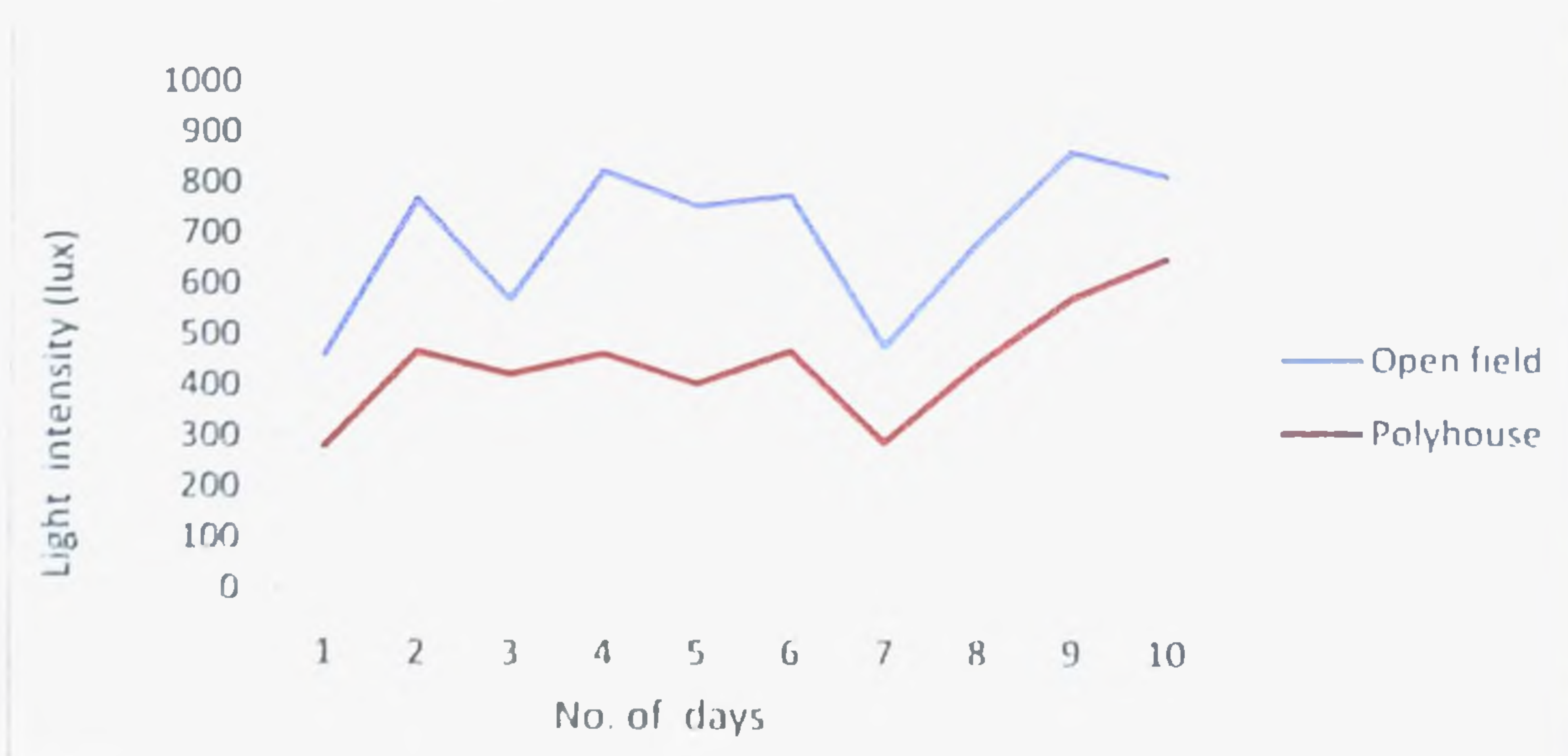


Figure 3. Light intensity in open field and poly house



Due to the microclimate modification the abiotic and biotic stresses have been noticed less inside the poly house, and that of open field the mosaic incidence and spotted wilt incidence is high.

Table 4. Effect of stress on tomato by growing environments

Variety	Cracking (%)	Mosaic incidence (%)	Spotted wilt incidence (%)
Anagha (O)	1.63	26.19	36.93
Anagha (P)	0	17.19	12.51
LE 643 (O)	7.88	32.14	13.10
LE 643 (P)	3.52	28.57	12.51

8.1.3. Retardation of soil sensible and latent heat flux

Heat sensitive crops can be covered with plastic enclosures during the night. These enclosures retard the loss of heat from the surroundings of the crop. This is an effective measure against cold damage. This method however, cannot be used for larger plants on a major scale.

Mulching and ploughing are the effective methods to retard flux from soil and to save crops from excessive cooling. Mulching and introduce a sort of barrier which reduces the radiation from the soil and tends to decrease transpiration. These mulches used can be of different types. These can be blanket of particles spread over the soil.

Plate 4. Types of mulches

Organic mulch

Inorganic mulch

A study was conducted in College of Horticulture, KAU in 2015 by Faras Bin Muhammed, he used different types of mulches in okra field. The soil temperature was taken from nine and eleven weeks after sowing. The soil temperature was increased by using mulching and higher soil temperature was obtained in the field where the plastic mulches have been used (Table 5).

Due to the increased soil temperature the weed seed cannot burst and it cannot germinate, so by mulching the weed can be controlled efficiently. In Table 6, the weed control efficiency is compared in all mulched field, the weed control efficiency have been increased in all cases and a higher efficiency to manage weeds can be obtained with the field were the black polythene sheet was used.

Efficient weed management will increases the yield of crops. The microclimate modification created by using different mulches will control the weeds and increases the yield of okra (Table 7).

Table 5. Effect of soil temperature on okra

Mulching material	Soil temperature at 10 cm depth (^0C)	
	9 WAS	11 WAS
Mango leaves	38.70	28.96
Coconut fronds	38.56	28.83
Fresh weeds	37.50	29.13
Paddy straw	39.00	28.83
Newspaper (2 layers)	39.50	28.80
Black polythene sheet	40.50	29.00
Control	38.40	28.76

Table 6. Effect of mulching on weed control efficiency

Mulching material	Weed control efficiency (%)		
	30 DAS	60 DAS	90 DAS
Mango leaves	90.77	43.96	34.43
Coconut fronds	49.32	31.23	28.74
Fresh weeds	54.67	37.53	20.58
Paddy straw	79.50	43.91	28.71
Newspaper (2 layers)	94.21	55.98	53.40
Black polythene sheet	95.21	96.27	91.67
Control	0.00	0.00	0.00

Table 7. Effect of mulching on okra yield

Mulching material	Total fruit yield ($t\ ha^{-1}$)
Mango leaves	10.06
Coconut fronds	4.55
Fresh weeds	4.22
Paddy straw	5.01
Newspaper (2 layers)	9.37
Black polythene sheet	15.63
Control	1.05

8.1.4. Protection against frost damage

At the onset of frost, when temperature decreases below the freezing, the aqueous solution which fills the intercellular spaces may turn solid. Prevention of frost and protection of plants from damaging frost can be achieved through both, direct and indirect methods (Jones, 2013).

Indirect methods include choice of sites, resistant cultivars and the use of crop growth regulators and chemicals, to alter flower bud formation during the frost occurring season. Direct methods include screens, heaters, sprinklers and sanding.

8.1.4.1. Indirect methods

It includes the choice sites which means on before planning a crop or an orchard, the site should be selected depending on the climatic conditions prevailing in that location, its slope and soil characteristics. If the temperature prevailing in a particular location is not suitable to the crop to be grown, another location should be chosen.

Depending on the prevailing climatic conditions, cultivars of any appropriate crop should be chosen. For frost prone climates, cold resistant varieties are the obvious choice.

Use of growth regulators and chemicals is done mainly with the purpose of delaying or advancing the most frost susceptible growth stages of crops to avoid frost damage. Ethephon is used to improve the winter bud hardening and also it delays the flowering by 4 to 7 days.

8.1.4.2. Direct methods

It includes the use of screens, heaters, over head sprinkler irrigation and sanding.

8.1.4.2.1. Screens

Screens made of any material with low transmissivity of long wave radiation can be used to prevent radiation frost. Chemicals have also been used to form smoke cover or fog cover over crop surfaces. Enclosing house orchards in low cost metal screen and sprinkling water on them was found to increase the temperature by several degrees. Under ideal condition orchard temperature may be raised by 1 or $2^{0}C$. Cetylalcohol was used to stabilize fog and resulted in an increase of 1 to $1.5^{0}C$ (Holeman *et al.*, 2012).

Plate 5. Screens for frost protection

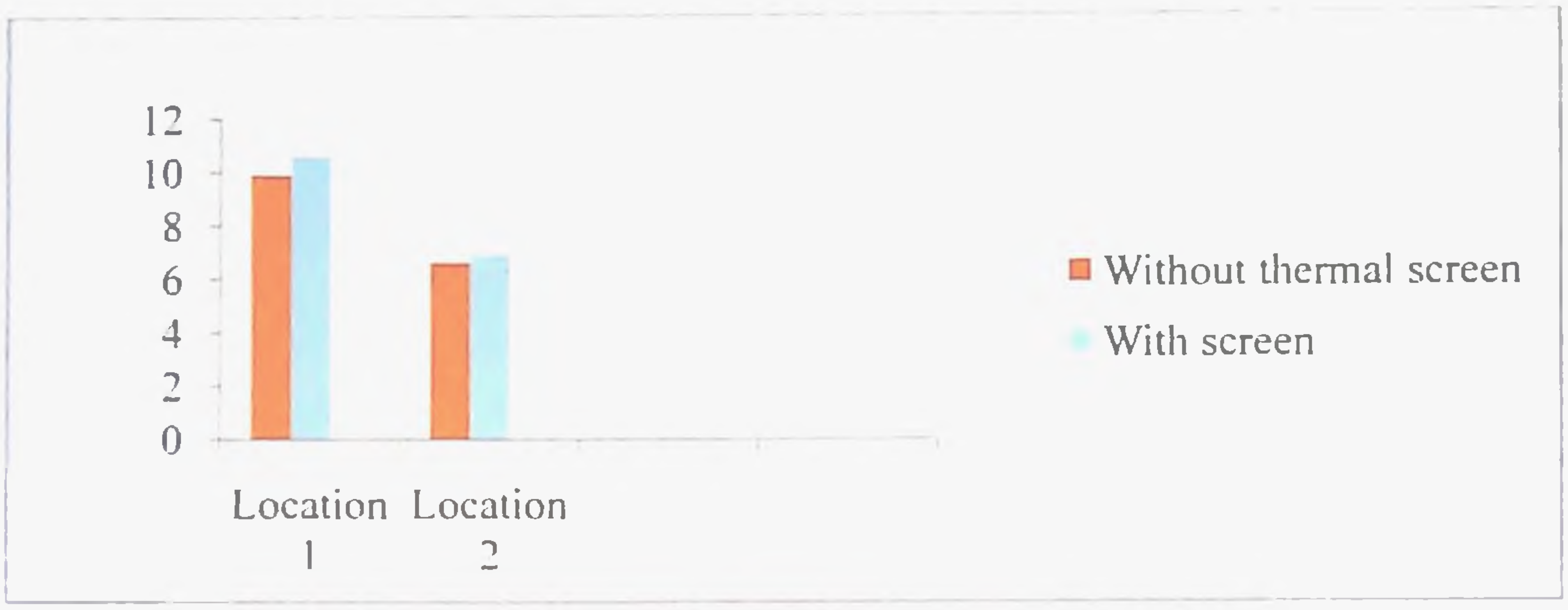
Gil and coworkers conducted an experiment on by using thermal screens in tomato fields, the weather elements such as air temperature and relative humidity is compared in both conditions. The day time and night time temperature have been increased and relative humidity decreased which will reduce the frost damage.

Table 8. Effect of thermal screens on air temperature and relative humidity in okra field

Location	Treatment	Temperature (^{0}C)		Relative humidity (%)	
		Day	Night	Day	Night
Location 1	Without thermal screens	17.5	10.5	69.3	91.6
	With thermal screens	17.7	12.0	67.6	89.9
Location 2	Without thermal screens	19.1	11.2	68.3	93.0
	With thermal screens	19.4	12.3	67.5	91.9

The modified climate provided protection against frost damage and which was lead to increased tomato yield in both the locality where the screens have been used.

Figure 4. Effect of thermal screens on yield of tomato



8.1.4.2.2. Heaters

Heaters are most effective on nights with a strong temperature inversion. Heaters increase the temperature by radiating energy to plants and soil. They also produce smoke which constitutes a moderate screens against radiation loss from the ground. Heaters fueled with the petroleum based products can be used to protect orchards. A heating system which is capable of producing 3.5 to 5.5 million British Thermal Unit (BTU) per acre per hour if the burning is in periphery and that of 2 to 3 million BTU per acre per hour if it is in between the rows. This would provide protection by raising temperatures from 3 to 5^0 C. This type of heating requires 35 to 40 heaters per acre. Around the periphery more heaters are required because the ascending plumes of hot air allow an inflow of cold air. The high costs coupled with fuel shortages, air and noise pollution regulations and labour problems, are increasing the interest in other methods of frost protection to substitute for heaters (Jones, 2013).

Plate 6. Heaters for frost protection

Heaters in between the rows

Heaters in periphery

8.1.4.2.3. Over head sprinkler irrigation

Sprinkling the canopy with water provides protection from cold by the release of latent heat of fusion. when water turns from liquid to ice. As long as the mixture of ice and water is maintained. the transfer of energy to the plant is efficient.

If too little water is sprinkled. the damage is worse than if no sprinkling was undertaken. When ice forms. energy is released but when evaporation is occurs energy is required. In fact as much as seven and half times more energy is needed to evaporate one gram of water. as is released by freezing. The net result will be a lowering of temperature. The temperature of the wetted plant under these conditions. may reach the wet bulb temperature which is generally lower air temperature. Thus the leaves must be sprinkled atleast once a minute for successful protection. It is necessary to make sure that distribution of water is excellent. the irrigation system is reliable. the sprinklers rotate and eject water at each minute. and that enough water can be applied to provide protection. The major disadvantage of this method is that it is difficult to regulate the system (Jiang *et al.*, 2014).

8.1.4.2.4. Sanding

Adding sand in small quantities to the soil every few years reduce frost damage. A sandy surface warms easily and cools slowly. by radiation. Sanding can raise the temperatures of clay soil by several degrees (Jones, 2013).

8.2. Control of water balance

Below a certain level of water supply crop production is not possible. Relatively small increases in moisture supply may produce a marked increase in crop yield. By Brun moisture regimes in the soil can be improved in two ways:

a) By increasing the amount of water stored in the root zone
b) By reducing the losses due to evapotranspiration

8.2.1. Water storage in crop root zone

The amount of precipitation taken in by the soil depends on run off and infiltration. By reducing run off and increasing infiltration the amount water stored in the soil can be increased.

8.2.1.1. Run off control

Most soil conservation methods such as strip cropping. contour ploughing. terracing. etc. aim at reducing run off. Various studies on moisture conservation of India. on shallow loam soil receiving 400mm rainfall annually. showed that bunding is increased soil moisture content by 50 to 100 per cent. The protection provided by vegetation is also usually a major factor in run off control. Plants intercept part of the rainfall and reduce the velocity of rain drops. They also slow down the movement of water on soil surface.

8.2.1.2. Increasing infiltration

The rate of infiltration of water into soil depends on the soil structure and texture, soil cover, and on the duration and intensity of rainfall. Mulches of straw or crop residues by breaking the impact of the raindrops, markedly improve infiltration.

The effect of tillage methods on crop growth and yield are to a large degree attributable to an increased soil moisture reservoir. This is achieved by creating soil conditions that favour root growth and penetration, and improved infiltration and conservation of water.

The study by Ajith in COH in 2000, paddy waste taken as water conserving material and which is applies in field of water melon at different depths. The Mean moisture Content (MMC) of soil was analyzed before and after the irrigation.

Table 9. Effect of paddy waste on MMC of soil before and after irrigation

Treatments	MMC (%) at different soil depth					
	0-15 cm		15-30 cm		30-60 cm	
	BI	AI	BI	AI	BI	AI
M_0	7.85	16.36	12.23	18.39	13.22	19.6
M_1	12.38	19.01	14.3	20.24	15.66	22.4
M_2	11.28	17.36	13.39	19.44	14.97	21.5
M_3	11.15	16.86	13.7	18.65	15.75	21.35

The moisture content in soil was increased after the irrigation which means the paddy waste as moisture conserving material is improved the soil moisture and ultimately increases the water holding capacity.

8.2.2.Modification of evapotranspiration

8.2.2.1.Reducing evaporation from soil

Evaporation from the soil can be minimized in number of ways

1. By decreasing turbulent transfer of water vapour to the atmosphere, by windbreaks, mulches etc
2. By decreasing capillary continuity of the surface layer

3. By decreasing capillary flow and the moisture holding capacity of the surface soil layers
4. By chemical treatments to control evaporation. It has been found that hexadecanol, a long chain alcohol, mixed with one quarter inch of the soil reduced evaporation by 40 per cent. This material which is resistant to microbial activity remains effective for more than a year.

8.2.2.2.Modification of transpiration

Three types of chemicals are being tried to reduce transpiration from plant canopies and evaporation from water bodies (Rosenberg, 1984).

1. The substances which form films on leaves or water bodies include long chain alcohols such as hexadeconol which form mono molecular layers. These are usually used on open water bodies and are unsatisfactory in reducing the transpiration from leaves. Low viscosity silicone was tested and found to reduce the water use by leaves without any detrimental effects on growth.
2. Stomata closing materials include common herbicides like attrazine. These act like a pump affecting the turgur of the stomata guards. The anti transpirant found to be most successful under field conditions is glycoryl half ester of decenyl succcini acid. A 12 per cent reduction in water loss was applied to the underside of leaves of broad leaf trees.
3. The third type of chemicals used to reduce evapotranspiration are called reflectants. It has been estimated that by doubling the albedo of the plants, transpiration can be reduced by 15 per cent. So far the best reflectant tried is kaolinite, which when applied in a mixture of plant gum and surfactant to a soya bean crop at the rate of 196kg per hectare produced satisfactory results

8.3.Control of turbulence or wind velocity

Wind is an important weather element and both its direction and velocity are significant. The influence of wind is both local and regional. It influences the configuration and distribution of plants in region. It influences plant life, both mechanically and physiologically. The influence is more pronounced on plants on flat lands near the seacoast, and on the higher slopes of mountains. The wind affects plants directly by increasing transpiration and the intake of carbon dioxide, and by causing several types of mechanical damage. Less significant effects are numerous including the generation of cold and heat waves, the movement of clouds and fogs, and the changing of water, light and temperature conditions (Venkataraman and Krishnan, 1992).

Under normal conditions, wind increases transpiration. However, this increases is only up to a certain point, beyond which either it becomes constant or begins to fall. With increasing wind velocity, there is a greater increase in cuticular transpiration than stomatal transpiration. Wind increases turbulence in the atmosphere, thus raising the supply of carbon dioxide to the plants and resulting in greater photosynthetic rates. However, the increase in photosynthesis is again up to a certain wind speed, beyond which its rate becomes constant.

When the wind is hot, it accelerates the desiccation of the plants by replacing humid air by dry air in the intercellular spaces. When a hot dry wind blows during the period when the cells are expanding and maturing, dwarfing of plants result. This is because the cells cannot attain full turgidity in the absence of optimal hydration, thus remaining at subnormal sizes. When the developing shoots come under the influence of strong and wind pressure from a fixed direction, the normal form and position of the shoots are permanently deformed. Another sever injury to plants caused by strong wind is lodging. The injury is most common in crop such as maize, wheat and sugarcane. Strong wind breaks the twigs and shed fruit from plants. Further, crops and trees with shallow roots are often uprooted.

Crops grown on sandy soils in areas where strong wind prevails are because of abrasion. When the plant cover is not thick, strong wind removes the dry soil, exposing their roots and then killing them.

8.3.1.Shelter belts

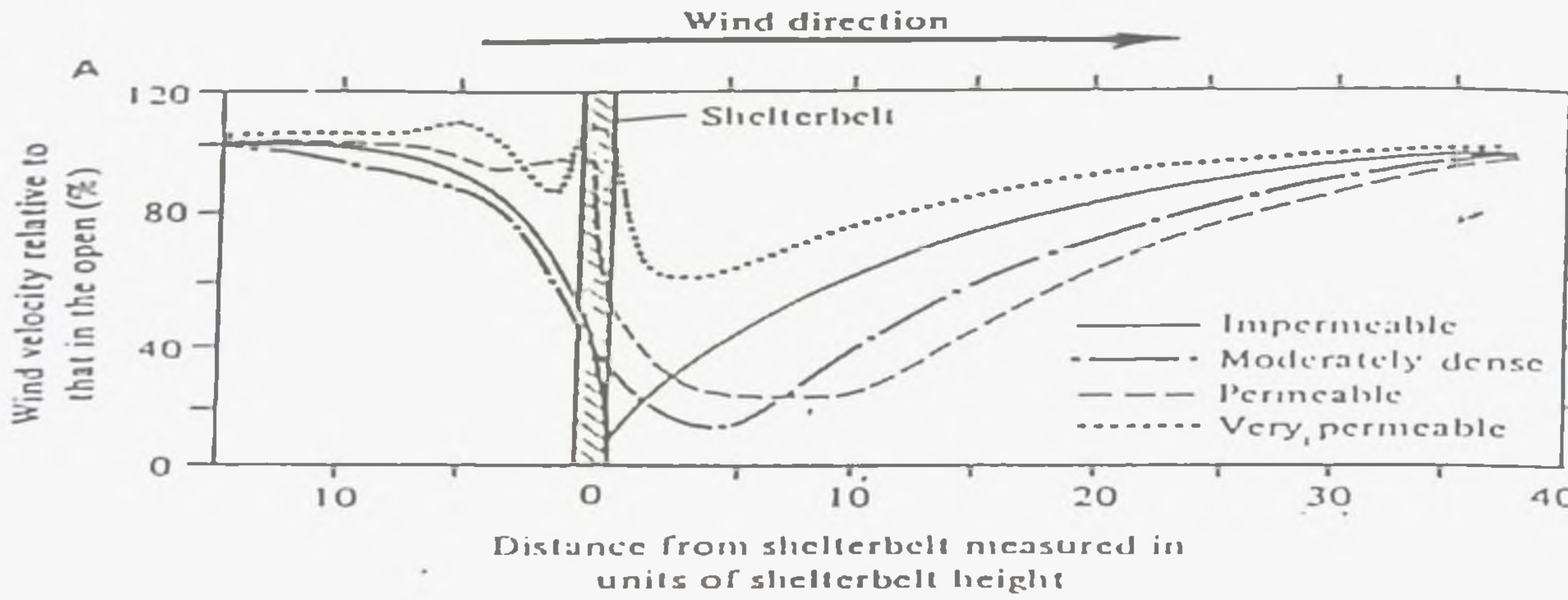
The use of shelter belts has been one of the oldest methods employed by man for modifying the climate. Properly oriented and designed shelter belts are very effective in stabilizing agriculture in regions where strong winds causes mechanical damage and impose severe moisture stress on growing crops. In cold climates wind breaks save plants from freezing and mechanical damage caused by cold winds. Windbreaks save the loose soil from erosion; aid in the uniform distribution of snow cover; and increase the supply of moisture to the soil in spring.

Various tall crops such as sorghum, corn, sunflower, wheat and oats, are being successfully used as temporary wind barriers to protect crops like sugar beet, soya bean, groundnut and tomato. Crop yields have been positively affected by these wind barriers in dry continental climates.

Wind speed reduction is based on permeability, height, density and shape of the shelterbelts.

8.3.2. Wind speed reduction based on shelter belt permeability

Plate 7. Wind speed reduction based on shelter belt permeability

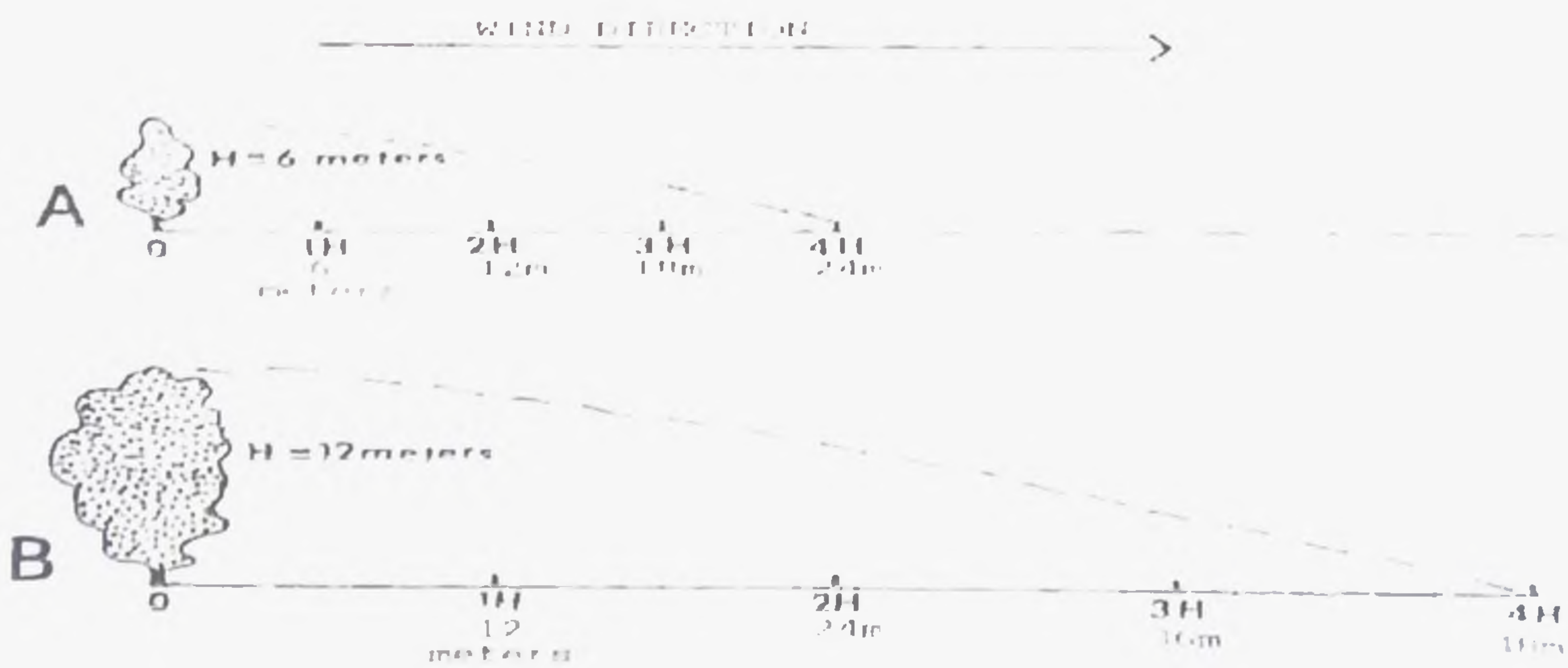


The optimal permeability of the shelter belt depends on the purpose for which the shelter belt is erected. For equal distribution of snow shelter belts should be more porous, than for controlling wind erosion. Numerous research studies can be cited to show that the optimum protection to crop plant is provided by a barrier which has permeability of 40 - 50 per cent.

8.3.3. Wind speed reduction is based on shelter belt height

The area protected by a shelter belt increases proportionally with the increase in the height of the barrier. Hence higher trees planted in the belt, result in a greater area protected.

Plate 8. Wind speed reduction based on shelter belt height



8.3.4. Effect of shelter belt on air temperature and relative humidity

The work was conducted by Rosenberg and coworkers in Punjab in the winter season in order to protect the maize from the heavy wind and also for to reduce the cold damage and the shelter belts used were sugar beets.

The air temperature has been increased due to the use of shelter belts and also the relative humidity was decreased so it will provide protection from the cold damage.

Table 10. Effect of shelter belt on air temperature and relative humidity

Date	Air temperature (^0C)		Relative humidity (%)	
	Open	With shelterbelt	Open	With shelterbelt
Aug. 14	19.5	19.6	54	60
18	24.6	28.1	66	69
25	23.1	24.6	73	74
Sep. 1	20.2	22.6	86	88
3	27.2	25.7	57	65
5	18.4	18.5	59	64

So the modification of the climate by shelter belts also given optimum condition to the plants. It reduces the damage due to heavy wind. Shelter belts have been used to provide protection from the cold damage.

9. Advantages and limitations of microclimate modification

Microclimate modification will provides suitable growing condition for the production of crops. Different methods are adopted in microclimate modification and the ultimate aims of all these methods are to protect the crops from different stresses and so it provides suitable growing conditions

Some of the methods of microclimate modification will provides protection from the weeds, pest and damage because it is for enhanced crop production, so the modified climate will restrict the biotic stresses

Another important advantage is it will provides the off season crops in entire year regardless of the climatic conditions

There are some limitations also for the microclimate modification the first one is it requires proper management in case of poly house the air temperature should be controlled properly. too much warming may negatively affect the crops. In case of micro sprinkler irrigation plenty of water should be ensured. Mulching material should properly select according to the soil.

Other limitation is the cost. many of the methods are costly. The initial cost of poly house is very high and that of the heaters. the operational cost is very high because it requires petroleum based products to fuel the heaters. The initial and the operational cost are very high for the micro sprinkler irrigation.

10. Summary

Microclimate modification is restricted only the modification in 100m of horizontal scale and 200m of vertical scale. There different methods of microclimate modification such as controlling the heat load, controlling the water balance and controlling the turbulence or wind velocity.

The control of heat load involves the heat evading, heat trapping, retardation of sensible and latent heat flux and prevention from frost damage and that of control of water balance includes increasing the water storage in root zone level and reduction of water loss through different ways. Use of shelter belts is the method to control the turbulence or wind velocity.

11.Conclusion

The future trends in agro meteorological research are focused to bring a breakthrough in the field of artificial modification of plant environment, to keep the optimum condition for the plant growth and yield. So great efforts are required to achieve this breakthrough because microclimate modification will serve as the basic tool to bring enhanced crop production in near future.

Discussion

1. Is there any specification for the installation of screens

   The screens can be placed according to the hemisphere. In case of India which is northern hemisphere so the shade effect is in southern side so the screens should be placed in southern side in order to achieve good radiation in the plant canopy.

2. Is this BTU is a standard unit. how it will convert to other unit

   BTU is a source unit not a standard unit. One BTU is equal to 1.05 kilo joule.

3. What is mean by heat waves

   Heat wave is a prolonged period of excessively hot weather. which may be accompanied by high humidity. especially in oceanic climate countries

4. How the ozone layer depletion will effect on the plants

   The changing climate will cause the depletion of ozone layer and this is causing much damage on plants animals too. Because the ozone is acts as a protective layer towards the earth and this will absorbs ultraviolet radiation also so the damage will definitely causing the transmission of UV radiation without the absorption so which will causing damages

5. What is the fuel used in the heaters

   Generally petroleum based fuels and natural gases also can be used as fuels in heaters.

6. How much percentage of modification in microclimate levels can be achieved through this methods

   A compete or 100 per cent modification of microclimate cannot be possible because there are some limitations like all the methods are depend on the prevailing weather conditions so we can modify about 60 to 70 per cent of the microclimate

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KERALA AGRICULTURAL UNIVERSITY

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Department of Agricultural Meteorology

AGM 591: Master's Seminar

Name: Sushna K. **Venue:** Seminar Hall

Admission No: 2014-11-230 **Date:** 11.12.2015

Major advisor: Dr. B. Ajithkumar **Time:** 10:00 a.m.

Microclimate modification to enhance crop production

Abstract

Climate change and agriculture are interrelated which takes place on a global scale. Climate change affects agriculture in a number of ways, through changes in average temperature, rainfall, climate extremes (e.g., heat waves), changes in atmospheric carbon dioxide, and ground-level ozone concentrations.

Climate change will probably increase the risk of food security globally. Despite the technological advances such as improved varieties, genetically modified organisms, and irrigation systems, weather is still a key factor in agricultural productivity. The effect of climate on agriculture is related to variation in local climate rather than in global climate patterns. In this context, modification of climatic variables is necessary, and which is possible only at the microclimate level.

Microclimate deals with the atmospheric phenomena and physical processes taking place over limited region of the surface of the earth in the lowest layer of the atmosphere (Mavi, 1996). The control or modification at the physical environment practiced can be grouped into three categories such as controlling the heat load, water balance and wind velocity or atmospheric turbulence (Lowry, 2000).

The control of heat balance is achieved through heat evasion, heat trapping, retarding the sensible and latent heat flux from the soil and protecting against frost damage (Perry, 2011). Protection of plants from damaging frost can be obtained through both direct and indirect methods (Jones, 2013). Indirect methods include choice of sites, resistant cultivars and the use of

growth regulators to alter flower and bud formation. Screens, heaters, overhead sprinkler irrigation, and sanding are direct methods adopted.

Improvement of water balance can be obtained by increasing the amount of water stored in the root zone and by reducing the losses due to evapotranspiration (Brun *et al.*, 1985). The amount of water stored in the soil can be increased by reducing the runoff and increasing the infiltration.

Wind is an important weather element and both its direction and velocity are significant. It influences plant life both mechanically and physiologically. The use of shelter belts has been one of the oldest methods employed for modifying the climate. In cold climates windbreaks save plants from freezing and mechanical damage caused by cold winds (Venkataraman and Krishnan, 1992).

The microclimate modification methods are serving as useful tools for increasing the crop production by providing the suitable environment for the crops. The major constraints of crop production like weeds, pests, and diseases can also be managed with this. Therefore, in the near future, the microclimatic modification may serve as the basic tool to manage crops.

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GENERAL CIRCULATION MODELS IN CLIMATE CHANGE PROJECTIONS

By

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(2014-11-235)

M.Sc. Agrl. Meteorology

Seminar report

Submitted in partial fulfillment of requirement of the course

AGM 591, Seminar (0+1)

DEPARTMENT OF AGRL. METEOROLOGY

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DECLARATION

I, K. Smitha (2014-11-235) hereby declare that the seminar entitled **"General circulation models in climate change projections"** has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

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Place: Vellanikkara

Date: 17-12-2015

CERTIFICATE

This is to certify that the seminar report entitled **"General circulation models in limate change projections"** has been solely prepared by **K.Smitha (2014-11-235)**, under ny guidance and has not been copied from seminar reports of any seniors, juniors or ellow students.

17/12/2015

Vellanikkara
17/12/2015

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CERTIFICATE

rtified that the seminar report entitled **"General circulation models in climate change jections"** is a record of the seminar presented by **K.Smitha (2014-11-235)** on 05-12-2015 and is mitted for partial fulfillment of requirement of the course AGM 591.

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1. Introduction

Widespread interest in understanding past, present, and future climate change and variability and the response and feedbacks of natural and managed ecosystems has motivated the development and application of models and techniques to provide climate data at relevant spatial and temporal scales. A wealth of observational data is available to support research over the historical record and geologic records provide indirect evidence of climate changes in the past. Quantitative estimates of paleo and future climate (including atmospheric circulation as well as surface-climate variables), however, must be obtained from climate models that account for the interactive changes in the global atmosphere and oceans that are driven by global boundary conditions, such as atmospheric trace-gas concentrations and aerosols, earth-sun geometry, sea ice, sea level, and continental ice sheets.

1.1. Climate model

In general terms, a climate model could be defined as a mathematical representation of the climate system based on physical, biological and chemical principles. As a consequence, climate models provide a solution which is discrete in space and time, meaning that the results obtained represent averages over regions, whose size depends on model resolution, and for specific times. For instance, some models provide only globally or zonally averaged values while others have a numerical grid whose spatial resolution could be less than 100 km. The time step could be between minutes and several years, depending on the process studied.

1.2 Hierarchy of climate models

Energy balance models (EBM)

As indicated by their name, energy balance models estimate the changes in the climate system from an analysis of the energy budget of the Earth. In their simplest form, they do not include any explicit spatial dimension, providing only globally averaged values for the computed variables. They are thus referred to as zero-dimensional EBMs. The basis for these EBMs was introduced by both Bitz (2001) and Sellers in (1969).

Intermediate complexity models

Intermediate complexity models include representation of the Earth's geography, i.e. they provide more than averages over the whole Earth or large boxes. Secondly, they include many more degrees of freedom than EBMs. As a consequence, the parameters of EMICs cannot easily be adjusted to reproduce the observed characteristics of the climate system, as can be done with some simpler models. The level of approximation involved in the development of the model varies widely between different EMICs. Some models use a very simple representation of the geography, with a zonally averaged representation of the atmosphere and ocean.

2. General circulation models (GCMs)

GCMs provide the most precise and complex description of the climate system. Currently, their grid resolution is typically of the order of 100 to 600 km. As a consequence, compared to EMICs (which have a grid resolution between 300 km and thousands of kilometers), they provide much more detailed information on a regional scale. A few years ago, GCMs only included a representation of the atmosphere, the land surface, sometimes the ocean circulation, and a very simplified version of the sea ice. Nowadays, GCMs take more and more components into account, and many new models now also include sophisticated models of the sea ice, the carbon cycle, ice sheet dynamics and even atmospheric chemistry.

GCMs essentially consist of extensive computer codes based on fundamental mathematical equations of motion, thermodynamics and radiative transfer. These equations govern the flow of the atmosphere and oceans, exchange of heat between the surface and the atmosphere, release of latent heat energy by condensation during cloud and precipitation formation, absorption of sunshine and emission of thermal radiation. The earth's atmosphere and oceans are represented by a three dimensional grid giving a three dimensional view of the global atmospheric and oceanic circulation. As climate research becomes increasingly relevant to industry and non-academic stakeholders, climate research output needs to become tailored to the end user

Types	Models	Institutes
Atmosphere General Circulation Model (AGCM)	HadSM3	Hadley centre
	HadAM3	Hadley centre
	HadAM3p	Hadley centre
Ocean General Circulation Model (OGCM)	ECHAM5-r1	Nederland's meteorological institute
	BCM	Sweden's meteorological and hydrological institute
	HadCM3Q3	Hadley centre
Atmosphere- Ocean General Circulation Model (AOGCM)	CM2.1	GFDL
	CM2.4	GFDL

Plate 1: Types of GCMs

3. Components of GCMs

1. Atmosphere

Gaseous part above the Earth's surface including traces amounts of other gaseous, liquid and solid substances. Weather, radiation balance, formation of clouds and precipitation, atmospheric flow, reservoir of natural and anthropogenic trace gases, transport of heat, water vapour, tracers, dust and aerosols.

2. Hydrosphere

All forms of water above and below the Earth's surface. This includes the whole ocean and the global water cycle after precipitation has reached the Earth's surface. Global distribution and changes of the inflow into the different ocean basins, transport of ocean water masses, transport of heat and tracers in the ocean, exchange of water vapour and other gases between ocean and atmosphere, most important reservoir of carbon with fast turnover.

3. **Cryosphere**

All forms of ice in the climate system, including inland ice masses, ice shelves, sea ice, glaciers and permafrost. Long-term water reserves, changes of the radiation balance of the Earth surface, influence on the salinity in critical regions of the ocean.

4. **Biosphere**

Organic cover of the land masses (vegetation, soil) and marine organisms. Determines the exchange of carbon between the different reservoirs, and hence the concentration of CO2 in the atmosphere, as well as the balances of many other gases, and therefore also the radiation budget. Influences the reflectivity of the surface, hence the radiation balance (e.g., tundra different from grassland), regulates the water vapour transfer soil-atmosphere, and via its roughness, the momentum exchange between the atmosphere and the ground.

A fifth component, which is particularly relevant for the assessment of future changes, is often treated as a distinct part of the climate system: the anthroposphere consisting of the processes which are caused or altered by humans. The most important ones are the emission of substances which alter the radiation balance, and land use change (deforestation, desertification, degradation and transformation into constructed areas). Most of the climate models treat processes and fluxes of the anthroposphere as an external forcing, i.e., the GCMs are run by prescribing atmospheric concentrations and emissions of CO_2. Prescribed are also dust and sulphate emissions from volcanoes: for the past based on documented data and paleoclimatic information of volcanic eruptions, for the future they may be based on the statistics of such events. A complete GCMs contains physical descriptions of all five components mentioned above and takes into consideration their coupling. It is part of the scientific work to select an appropriate model combination and complexity, so that robust results are produced for a specific science question. Each climate system component operates on a range of characteristic temporal and spatial scales. The knowledge of these scales is necessary for a correct formulation of GCMs.

3.1 Fundamental equations solved in GCMs

1. Conservation of energy (the first law of thermodynamics)

Input energy = increase in internal energy plus work done

2. Conservation of momentum (Newton's second law of motion)

Force = mass × acceleration

3. Conservation of mass

The sum of the gradients of the product of density and flow-speed in the three orthogonal directions is zero. This must be applied to air and moisture for the atmosphere and to water and salt for the oceans, but can also be applied to other atmospheric and oceanic 'tracers' such as cloud liquid water.

4. Ideal gas law

Pressure × volume is proportional to absolute temperature × density that describe the movement of energy, momentum and various tracers (e.g. water vapour in the atmosphere and salt in the oceans) and the conservation of mass is therefore required.

Generally the equations are solved to give the mass movement (i.e. wind field or ocean currents) at the next time step, but models must also include processes such as cloud and sea ice formation and heat, moisture and salt transport. The first step in obtaining a solution is to specify the atmospheric and oceanic conditions at a number of 'grid points', obtained by dividing the Earth's surface into a series of rectangles, so that a traditionally regular grid results. Conditions are specified at each grid point for the surface and several layers in the atmosphere and ocean. The resulting set of coupled non-linear equations is then solved at each grid point using numerical techniques. Various techniques are available, but all use a time step approach.

4. Structure of GCMs

- Construction of a GCMs is a task whose principles are easily understood but which in practice involves mastery of a multitude of technical details.
- For computational representation, the continuous fields of temperature, pressure, velocity, etc. in the atmosphere and ocean must be approximated by a finite number of discrete values. The most intuitive approach to this discretization is to divide the fluid up

into a number of grid cells and approximate the continuous field by the average value across the grid.

- Cell or the value at the center of the grid cell. This can approximately capture the behavior of motions at space scales much larger than the grid cell but obviously omits the infinite number of values that a continuous temperature field has at different points within the grid cell. For each of the discrete grid cells, there would be a single value of each variable (e.g. temperature). This would represent, for instance, the average value across the grid cell.
- Any feature of smaller scale than the grid cells cannot be explicitly represented in the model and must be parameterized.
- The vertical coordinate is essentially a pressure coordinate, but modified so it follows the large-scale topography.
- Grid spacing is not constant in the vertical. Typically the boundary layer has finer vertical resolution.
- The horizontal grid is in latitude and longitude. The zonal length of the grid cell (in kilometers).
- Each grid cell communicates with its neighbors. The arrows indicate transports (or fluxes) of mass, energy, and moisture into a particular grid cell.
- The fluxes are proportional to differences between the grid cells for each variable; The budgets of these fluxes are associated with the respective equation. For the velocity equations, neighboring grid cells affect each other via the pressure gradient force, since this depends on the difference of the pressure between a grid cell and its neighbors.
- By considering the balance of forces, fluxes etc. on a given box, the time rate of change of wind, temperature, etc. for a given grid cell can be computed. This is then used to calculate a new value for the wind, temperature, etc. one short time step (typically half an hour or less) later. This is then repeated for all the other boxes so the solution is available everywhere for the next time step.
- The time integration proceeds one time step at a time until the desired length of simulation (e.g. 100 years) is reached (about 2 million half-hour time steps).
- The equations are local in the horizontal in the sense that they only involve a grid cell and its immediate neighbors. However, since effects are passed from neighbor to neighbor,

the solutions eventually involve the entire grid. Thus as the simulation is carried forward in time, the solution involves the whole globe.

- All GCMs need observed values for part of their input, especially in order to specify the boundary conditions, and all require observational data with which to compare their results. Some variables, such as surface pressure, are available worldwide and pose only the problem of evaluating the accuracy of the observed dataset.Others, however, are sparse in either time or space. Knowledge of sea ice extent is largely dependent on satellite observations, so that there is only a short observational record and, although satellites offer information on extent and concentration of sea ice, there is little they can say about ice thickness. Thus it is difficult to compare such observations with any long-term average values obtained from models. As modellers include ever more sophisticated components of the climate system in their experiments, there is a growing need for information on other parameters for validation of models. One particular example is 'soil moisture'. The term could mean all the water in a soil column (which might, technically, include large reserves of groundwater not accessed by the biosphere) or might be limited to the amount of water accessible to the biosphere (possibly termed 'available soil water'). There is no consistent definition between different modelling groups and no validation set comparable to traditional observations of pressure and temperature. There is still much to be done in the field of model validation.

4.1 Parameterization

Subgrid scale phenomena such as thunderstorms, for example, have to be parameterized as it is not possible to deal with these explicitly. Other processes may be parameterized to reduce the amount of computation required. Certain processes may be omitted from the model if their contribution is negligible on the time scale of interest. For example, there is no need to consider the role of deep ocean circulation whilst modeling changes over time scales of years to decades. Some models may handle radiative transfers in great detail but neglect or parameterize horizontal energy transport. Other models may provide a 3-dimension representation but contain much less detailed radiative transfer information. Given their stage of development, and the limitations imposed by incomplete understanding of the climate system and computational constraints, climate models cannot yet be considered as predictive tools of future climate change. They can, however, offer a valuable window on the workings of the climate system, and of the processes that have influenced both past and present climate.

4.1.1 Clouds

Weather and climate model grid boxes have sides of between 5 kilometers (3.1 mi) and 300 kilometers (190 mi). A typical cumulus cloud has a scale of less than 1 kilometer (0.62 mi), and would require a grid even finer than this to be represented physically by the equations of fluid motion. Therefore, the processes that such clouds represent are parameterized, by processes of various sophistication. In the earliest models, if a column of air in a model grid box was unstable (i.e., the bottom warmer than the top) then it would be overturned, and the air in that vertical column mixed. More sophisticated schemes add enhancements, recognizing that only some portions of the box might convect and that entrainment and other processes occur. Weather models that have grid boxes with sides between 5 kilometers (3.1 mi) and 25 kilometers (16 mi) can explicitly represent convective clouds, although they still need to parameterize cloud microphysics.

4.1.2 Radiation

The amount of solar radiation reaching ground level in rugged terrain, or due to variable cloudiness, is parameterized as this process occurs on the molecular scale. This method of parameterization is also done for the surface flux of energy between the ocean and the atmosphere in order to determine realistic sea surface temperatures and type of sea ice found near the ocean's surface. Also, the grid size of the models is large when compared to the actual size and roughness of clouds and topography. Sun angle as well as the impact of multiple cloud layers is taken into account. Soil type, vegetation type, and soil moisture all determine how much radiation goes into warming and how much moisture is drawn up into the adjacent atmosphere. Thus, they are important to parameterize.

5. Downscaling

Downscaling is a method to convert the large-scale information that is created by coarse global climate models (GCMs) to higher resolution information for the region or location of interest. There are two types of downscaling: statistical and dynamical. Statistical downscaling finds statistical relationships between coarse climate model output and localized observations in order to produce a high resolution simulated output. This output is much closer to reality than GCMs, which cannot represent regional topographic features. Dynamical downscaling uses the same processes as GCMs, but at much finer scale. Dynamical downscaling models, called regional climate models (RCMs), are able to resolve physical processes of the general circulation of the atmosphere and surface processes, using the output from the GCM as the driving conditions in the finer scale RCM. Global climate models need to be downscaled in order to look at information on a local scale. Many models are available that are already downscaled.

5.1 Statistical downscaling

A second way of downscaling climate data is through the use of statistical regressions. There are a variety of such methods ranging from multiple regressions that link local variables to particular drivers in GCMs, to more complex methods using statistics designed for neural networks. The general strategy of these methods is to establish the relationship between large scale variables, such as the driving factors derived from GCMs, to local level climate conditions. Once these relationships have been developed for existing conditions, they can be used to predict what might happen under the different conditions indicated by GCMs.

5.2 Regional Climate Models (RCMs)

Simulating climate change at the regional and national levels is essential for policymaking. Only by assessing what the real impact will be on different countries will it be possible to justify difficult social and economic policies to avert a dangerous deterioration in the global climate. Furthermore, understanding processes on the regional scale is a crucial part of global research. Processes acting on local or regional scales, such as mountain ranges blocking air flow or dust clouds interacting with radiation will ultimately have impacts at the global level.

One technique used to overcome the coarse spatial resolution of coupled GCMs is that of nested modelling, depicted in the image. This involves the linking of models of different scales within a global model to provide increasingly detailed analysis of local conditions while using the general analysis of the global output as a driving force for the higher resolution model. Results for a particular region from a coupled GCM are used as initial and boundary conditions for the RCM, which operates at much higher resolution and often, with more detailed topography and physical parameterizations.

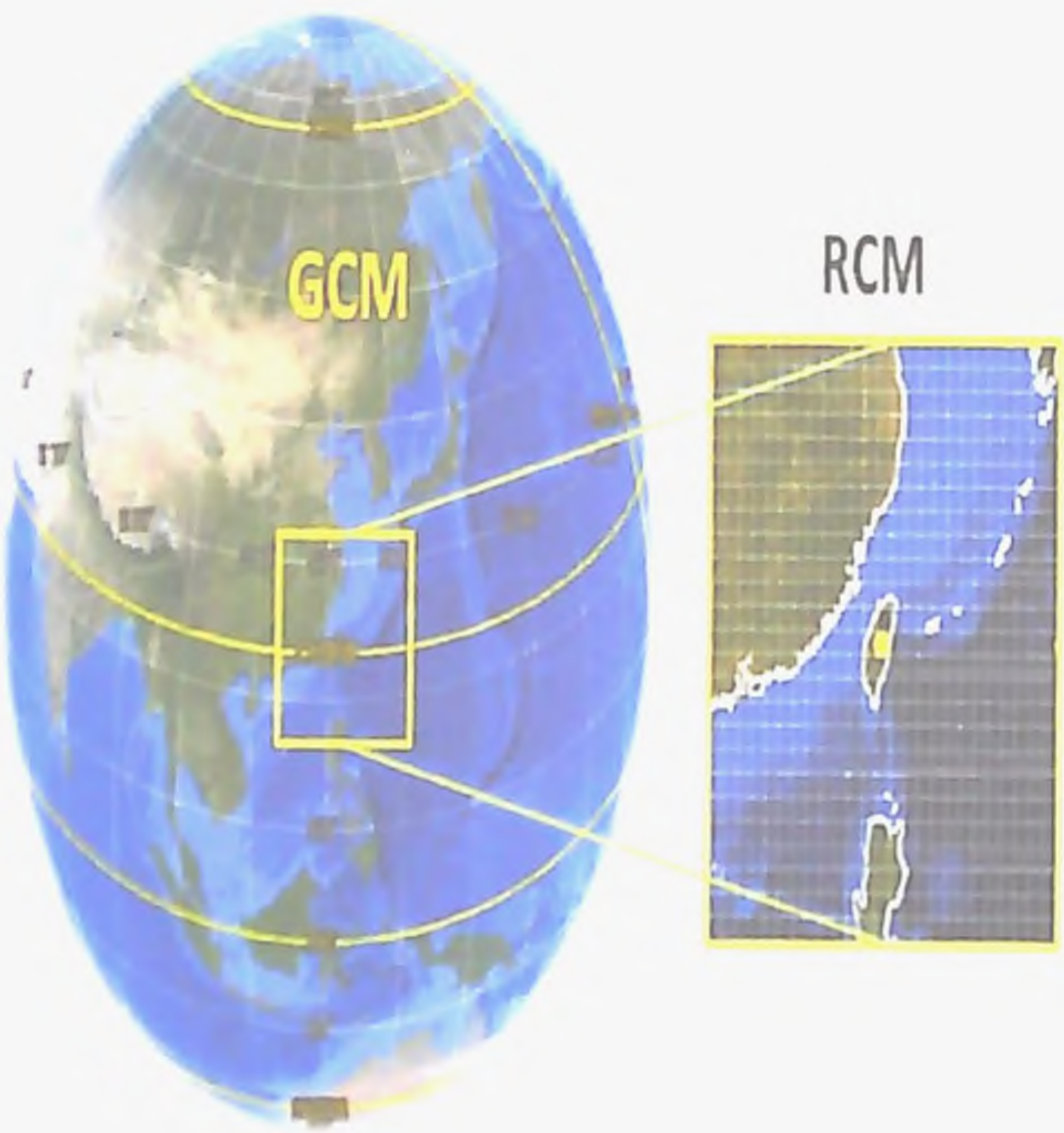

Plate 2 Regional climate model

6. Testing the GCMs

Testing the validity of GCMs Verification, validation, testing Despite very careful design, there is no guarantee that a computer model will be adequate for its intended use: some processes treated as negligible can turn out to be more important than initially thought; a parameterization may not be valid in the particular conditions of interest or may be incompatible with other hypotheses employed; the selection of parameters can be far from optimal; and so on. As a consequence, GCMs have to be tested to assess their quality and evaluate their performance. Although the principles remain the same, the tests performed with a model developed to analysing the development of the global carbon cycle over the last million years are clearly different from those for a model providing projections of future climate changes at the highest possible resolution.

6.1 Verification

A first step is to ensure that the numerical model solves the equations of the physical model adequately. This procedure, often referred to as verification only deals with the numerical resolution of the equations in the model, not with the agreement between the model and reality. It checks that no coding errors have been introduced into the program. The numerical methods used to solve the model equations must also be sufficiently accurate. Different methods are available to achieve this goal. A standard one is to compare the numerical solution with the analytical one for highly idealized test cases for which an exact solution is available. It is also possible to formally state that some parts of the code are correct, for instance, the one that solves large systems of n linear algebraic equations with n unknowns (which are often produced as part of the numerical resolution of the partial differential equations on the model grid).

6.2 Validation

The next step is the validation process, i.e. determining whether the model accurately represents reality. To do this, the model results have to be compared with observations obtained in the same conditions. In particular, this implies that the boundary conditions and forcings must be correctly specified to represent the observed situation. Validation must first be performed on the representation of individual physical processes, such as the formulation of the changes in the snow albedo in response to surface melting and temperature change. This is generally achieved for particular locations, during field campaigns specifically designed to study this process. They provide a much larger amount of very specific data than global data bases, allowing a detailed evaluation of the performance of the model on this topic. On a larger scale, the different components of the GCMs (atmosphere, ocean, sea ice, etc.) have to be tested independently, ensuring that the boundary conditions at the interface with the other components are well defined. Finally, the results of the whole coupled model have to be compared with observations. All those steps are necessary because bad surprises are always possible after the different elements are coupled together, due to non-linear interactions between the components. Some problems with the GCMs can also be masked by the formulation of the boundary conditions when components are run individually. However, having a coupled model providing reasonable results is not enough. In order to test whether the results occur for the correct reason, it is necessary to check that all the elements of the GCMs are doing a good job, and that the satisfactory overall behavior of the GCMs is not due to several errors in its various elements cancelling each other out.

7. Climate change projection

A climate projection is usually a statement about the likelihood that something will happen several decades to centuries in the future if certain influential conditions develop. In contrast to a prediction, a projection specifically allows for significant changes in the set of boundary conditions, such as an increase in greenhouse gases, which might influence the future climate. As a result, what emerge are conditional expectations (if this happens, then that is what is expected). For projections extending well out into the future, scenarios are developed of what could happen given various assumptions and judgments.

7.1 Intergovernmental Panel on Climate Change (IPCC)

IPCC is a scientific intergovernmental body under the auspices of the United Nations, set up at the request of member governments. It was first established in 1988 by two United Nations organizations, the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP), and later endorsed by the United Nations General Assembly through Resolution 43/53. Membership of the IPCC is open to all members of the

WMO and UNEP. The IPCC produces reports that support the United Nations Framework Convention on Climate Change (UNFCCC), which is the main international treaty on climate change. The ultimate objective of the UNFCCC is to "stabilize greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic [i.e., human-induced] interference with the climate system".IPCC reports cover "the scientific, technical and socio-economic information relevant to understanding the scientific basis of risk of human-induced climate change, its potential impacts and options for adaptation and mitigation.

The IPCC does not carry out its own original research, nor does it do the work of monitoring climate or related phenomena itself. The IPCC bases its assessment on the published literature, which includes peer-reviewed and non-peer-reviewed sources.

Thousands of scientists and other experts contribute (on a voluntary basis, without payment from the IPCC) to writing and reviewing reports, which are then reviewed by governments. IPCC reports contain a "Summary for Policymakers", which is subject to line-by-line approval by delegates from all participating governments. Typically this involves the governments of more than 120 countries.

7.2 Simulation of GCMs

Since the 1990 IPCC report, there has been a major international effort, known as the Atmospheric Model Intercomparison Project (Gates, 1992), to document the comparative performance of GCMs in simulating the contemporary climate. Thirty atmospheric GCMs were forced by observed sea surface temperatures and sea ice for the decade 1979-1988. Reports from 26 diagnostic subprojects were described at the First AMIP Scientific Conference (AMIP, 1995), covering a wide range of atmospheric features that included cyclone frequencies, tropical 30-60 day oscillation, large-scale southern hemisphere circulation, soil moisture, cloudiness, extreme events, and many others. Results have also been reported extensively in the open literature and in IPCC assessments.

Confidence in the ability of GCMs to estimate future climate changes comes from the fact that they are based on accepted physical laws such as conservation of mass, energy and momentum, as well as a wealth of observations for their more empirically-based components such as cloud reflectivities or infrared absorptive properties of greenhouse gases. Model simulations are also routinely compared against observations of the atmosphere, ocean and land surface. An example where the model surface temperatures are compared to observed global temperature changes over the 20th century. In this example, the climate models have been forced by known changes in natural factors (solar insolation and volcanic aerosols) and in human-caused factors (increasing greenhouse gas concentration and man-made aerosols). There is excellent agreement at the global scale between models and observations. Note how well the models simulate short-term cooling following large volcanic eruptions: this is because the models are 'told' when the eruption occurred and how much aerosol was deposited into the stratosphere. Irregular short-lived warmings are also apparent in the observations (e.g., early 1940s, and 1998), that lie within the model range but are well above the model average. These warmings occurred as a consequence of El Niño events in the equatorial Pacific, and are part of what is called 'internal variability' which cannot be specified in the way the computer simulations are set up. Thus, the climate models will develop El Niño events through the period of record, but they will not line up in time with their occurrence in the real world, any more than the simulated daily weather patterns will agree with observed weather sequences over long integrations.Global-mean surface temperature, relative to 1901-1950 average, from observations (black line) and from 58 simulations (orange lines) by 14 global climate models reported on in the IPCC Fourth Assessment. The climate models are driven by both natural and human-caused factors that influence climate. The average over all model runs is given by the red line, with the vertical grey bars signifying the dates of large volcanic eruptions.

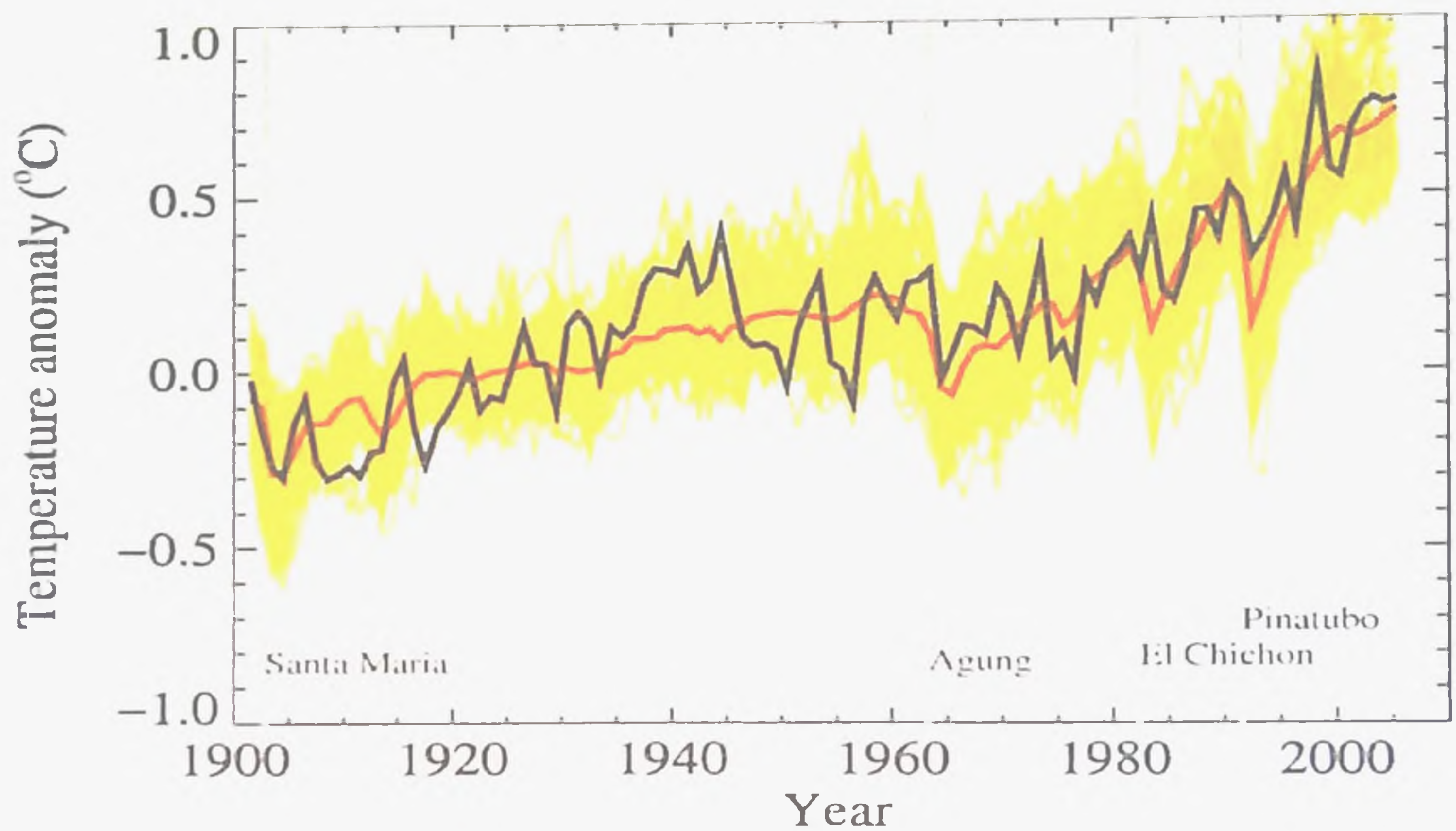

Plate 3: GCM simulations of global temperature

7.3 Emissions scenario

An emissions scenario, or Representative Concentration Pathway (RCP), is an estimate of the amount of change that might happen, generally measured by changes in the concentration of carbon or greenhouse gasses in the atmosphere. There could be a large-scale shift to clean energy in the next ten years or emissions could continue to rise. For this reason, models are usually run for at least three emissions scenarios: high, medium, and low. The "low" scenario projects future climate even if we stopped adding carbon to the atmosphere immediately. Because some changes take a decades or longer to affect the climate, we are still feeling the effects of actions from 50 years ago. The "high" scenario reflects what might happen if we do not make substantial emissions reductions.

7.3.1 RCP Primary Characteristics

RCP 8.5 was developed using the MESSAGE model and the IIASA, Austria. This RCP is characterized by increasing greenhouse gas emissions over time, representative of scenarios in the literature that lead to high greenhouse gas concentration levels (Riahi et al. 2007).

RCP6 was developed by the AIM modeling team at the National Institute for Environmental Studies (NIES) in Japan. It is a stabilization scenario in which total radiative forcing is stabilized shortly after 2100, without overshoot, by the application of a range of technologies and strategies for reducing greenhouse gas emissions (Fujino et al. 2006; Hijioka et al. 2008).

RCP 4.5 was developed by the GCAM modeling team at the Pacific Northwest National Laboratory's Joint Global Change Research Institute (JGCRI) in the United States. It is a stabilization scenario in which total radiative forcing is stabilized shortly after 2100, without overshooting the long-run radiative forcing target level (Clarke et al. 2007; Smith and Wigley 2006; Wise et al. 2009).

RCP2.6 was developed by the IMAGE modeling team of the PBL Netherlands Environmental Assessment Agency. The emission pathway is representative of scenarios in the literature that lead to very low greenhouse gas concentration levels. It is a" peak-and-decline" scenario; its radiative forcing level first reaches a value of around 3.1 W/m2 by mid-century, and returns to 2.6 W/m2 by 2100. In order to reach such radiative forcing levels, greenhouse gas emissions (and indirectly emissions of air pollutants) are reduced substantially, over time (Van Vuuren et al. 2007).

7.4 Criteria for selecting climate scenarios

Five criteria that should be met by climate scenarios if they are to be useful for impact researchers and policy makers are suggested:

- Consistency with global projections. They should be consistent with a broad range of global warming projections based on increased concentrations of greenhouse gases. This range is variously cited as 1.4°C to 5.8°C by 2100, or 1.5°C to 4.5°C for a doubling of atmospheric CO_2 concentration (otherwise known as the "equilibrium climate sensitivity").
- Physical plausibility. They should be physically plausible; that is, they should not violate the basic laws of physics. Hence, changes in one region should be physically consistent with those in another region and globally. In addition, the combination of changes in different variables (which are often correlated with each other) should be physically consistent.
- Applicability in impact assessments. They should describe changes in a sufficient number of variables on a spatial and temporal scale that allows for impact assessment. For example, impact models may require input data on variables such as precipitation, solar radiation, temperature, humidity and windspeed at spatial scales ranging from global to site and at temporal scales ranging from annual means to daily or hourly values.
- Representative. They should be representative of the potential range of future regional climate change. Only in this way can a realistic range of possible impacts be estimated.
- Accessibility. They should be straightforward to obtain, interpret and apply for impact assessment. Many impact assessment projects include a separate scenario development component which specifically aims to address this last point. The DDC and this guidance document are also designed to help meet this need.

Name	Concentration of co_2(ppm)
RCP2.6	>1370 in 2100
RCP4.5	850 in 2100
RCP6.0	650 in 2100
RCP8.5	490 before 2100 and then declines

Plate 4: Projection of carbon dioxide concentration

	2046-2065		2081-2100	
Scenario	Mean	Range	Mean	Range
RCP2 6	1.0	0.4-1.6	1.0	0.3-1.7
RCP4 5	1.4	0.9-2.0	1.8	1.1-2.6
RCP6 0	1.3	0.8-1.8	2.2	1.4-3.1
RCP8 5	2.0	1.4-2.6	3.7	2.6-4.8

Plate 5: Projection of surface temperature

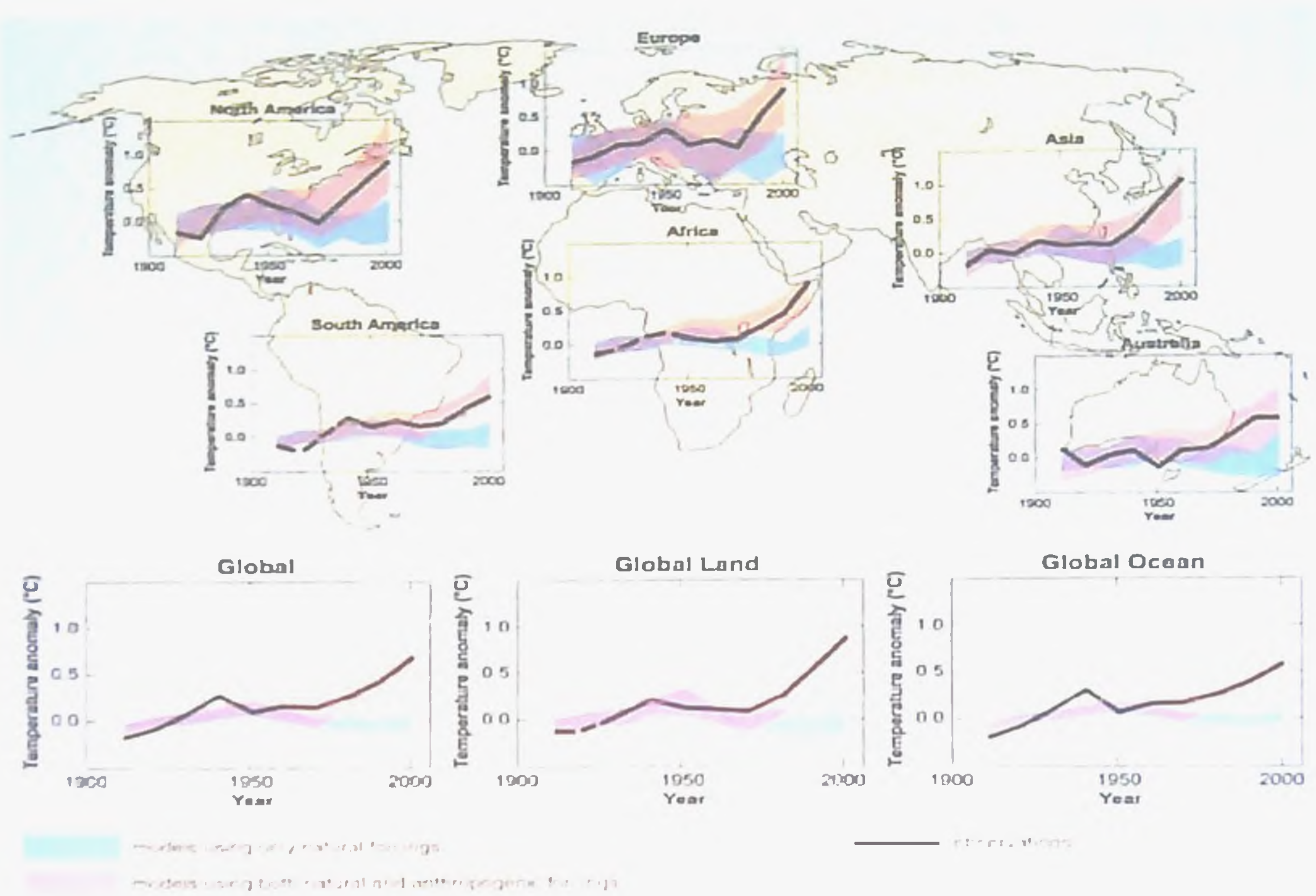

Plate 6: Observed and simulated surface temperature

Comparison of observed continental- and global-scale changes in surface temperature with results simulated by climate models using natural and anthropogenic forcings. Decadal averages of observations are shown for the period 1906 to 2005 (black line) plotted against the centre of the decade and relative to the corresponding average for 1901 to 1950. Lines are dashed where spatial coverage is less than 50%. Blue shaded bands show the 5% to 95% range for 19 simulations from 5 climate models using only the natural forcings due to solar activity and volcanoes. Red shaded bands show the 5% to 95% range for 58 simulations from 14 climate models using both natural and anthropogenic forcings.

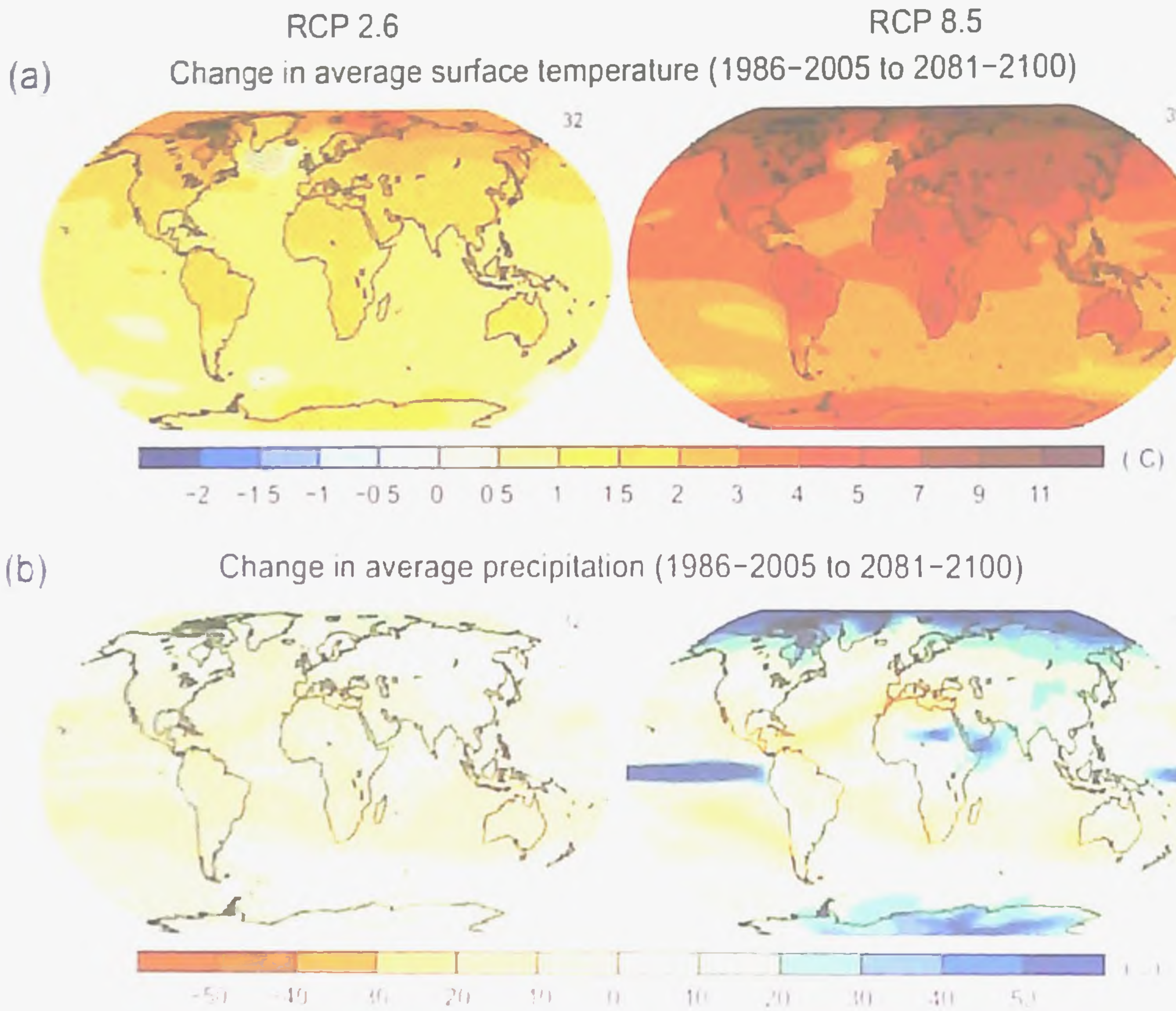

Plate 7 Projection of temperature and precipitation

Change in average surface temperature (a) and change in average precipitation (b) based on multi-model mean projections for 2081–2100 relative to 1986-2005 under the RCP2.6 (left) and RCP8.5 (right) scenarios. The number of models used to calculate the multi-model mean is indicated in the upper right corner of each panel. Stippling (i.e., dots) shows regions where the projected change is large compared to natural internal variability, and where at least 90% of models agree on the sign of change. Hatching (i.e., diagonal lines) shows regions where the projected change is less than one standard deviation of the natural internal variability.

8. Conclusion

GCMs are simplified descriptions of complex processes within the climate system. They are used for the quantitative testing of hypotheses regarding the mechanisms of climate change, as well as for the interpretation of instrumental data from paleo-data from various archives. GCMs are essential for the operational prediction of the economically important ENSO-phenomenon and other climate modes. A further important motivation for the development and application of GCMs remains the aim to assess future climate change. Research developing and using GCMs has become interdisciplinary and comprises domains of physics (thermodynamics, fluid dynamics, atmospheric physics, and oceanography), chemistry (organic, inorganic and surface chemistry, reaction kinetics, geochemistry, cycles of carbon, nitrogen, etc.) and biology (vegetation dynamics, ecology). Continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe, pervasive and irreversible impacts for people and ecosystems. Limiting climate change would require substantial and sustained reductions in greenhouse gas emissions which, together with adaptation, can limit climate change risks.

9. Discussion

1. What is the difference between projection and prediction? If both are same?

Projection and prediction are different. Prediction is the probabilistic statement that something will happen in the future based on what is known today where Projection is the probabilistic statement that it is possible that something will happen in the future if certain conditions develop.

2. Whether any GCMs are used in India?

Yes, GCMs are used in India mainly IMD (India Meteorological Department) uses GCMs to project climate change.

3. In RCM which equations are used?

Same equations which are used in GCMs, ie, Conservation of mass, conservation of momentum, conservation of energy and ideal gas law.

4. What is mean by vertical layer?

Layer of air above the ground is known as vertical layer.

5. What is the difference between emission and scenario?

Emission is the amount of pollutant matter released in the atmosphere from a specific pollutant source and in a specific time interval where scenario is the description of a possible future state of the world.

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11. Abstract

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Meteorology

AGM 591: Master's Seminar

Name: Smitha K. **Venue:** Seminar Hall

Admission No: 2014-11-235 **Date:** 05.12.2015

Major advisor: Dr. K. M. Sunil **Time:** 10:45 a.m.

General circulation models in climate change projections

Abstract

Changes in the atmospheric abundance of greenhouse gases and aerosols, in solar radiation and in land surface properties alter the energy balance of the climate system. There is considerable confidence that General Circulation Models (GCMs) provide credible quantitative estimates of future climate change, particularly at continental scales and above (Del Genio, 2003). GCMs are mathematical representations of the climate system, expressed as computer codes and run on powerful computers. Model fundamentals are based on established physical laws, such as ideal gas law, conservation of mass, energy and momentum, along with a wealth of observations. GCMs are routinely and extensively assessed by comparing their simulations with observations of the atmosphere, ocean, cryosphere and land surface.

GCMs depict the climate using a three dimensional grid over the globe, typically having a horizontal resolution of between 100 to 600 km and 20 to 30 vertical layers. Models are works based on their physical basis, and their skill in representing observed climate and past climate changes (Mizuta *et al.*, 2012). They have consistently provided a robust and unambiguous picture of significant climate warming in response to increasing greenhouse gases. The climate system includes a variety of physical processes, such as cloud processes, radiative processes and boundary-layer processes, which interact with each other on many temporal and spatial scales. Due to the limited resolutions of the models, many of these processes are not resolved adequately by the model grid and must therefore be parametrized (Soden and Held, 2006).

Most GCMs neither incorporate nor provide information on scales smaller than a few hundred kilometers. The effective size or scale of the ecosystem on which climatic impacts actually occur is usually much smaller than this. So there is a problem of estimating climate changes on a local scale from the essentially large-scale results of GCMs (Gates *et al.*, 1999). The prevailing approach for obtaining finer spatial resolution climate information is achieved by downscaling GCM output (Rajendran *et al.*, 2013). The two main approaches to downscaling climate information are dynamical and statistical. Dynamical downscaling requires running high-resolution climate models on a regional sub-domain, using observational data. Statistical downscaling is a two-step process consisting of the development of statistical relationships between local climate variables and large-scale predictors.

Limitations in computing power frequently result in the inability of models to resolve important climate processes. Low-resolution models fail to capture many important phenomena of regional and lesser scales, even though GCMs are credible tool which are currently used in more than a dozen centres around the world to enhance the understanding of climate and climate change and to support the activities of the Intergovernmental Panel on Climate Change (IPCC).

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Agrl. Entomology

Insect migration

By

Umamaheswary M. D.

(2014-11-124)

M. Sc. Agrl. Entomology

Seminar report

Submitted in partial fulfilment of requirement of the course

Ent. 591 Seminar (0+1)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

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DECLARATION

I, Umamaheswary M. D. (2014-11-124), hereby declare that the seminar report entitled **'Insect migration'**, has been completed by me independently after going through the references cited herein and I have not copied from any of the fellow students or previous seminar reports.

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2014-11-124

Vellanikkara
Date: 08/02/2016

CERTIFICATE

Certified that the seminar report entitled **'Insect migration'** for the course Ent.591 has been prepared by Umamaheswary M. D. (2014-11-124), after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

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1. Introduction

When animals move across the face of the Earth, sometimes making journeys of immense length, their behaviour is generally described as migratory. It is an adaptive behaviour of great significance that is observed cross the Animal Kingdom. Many birds, animals, mammals etc migrate. Like that several species of insects also display migratory behaviour like in case of monarch butterfly, locusts, dragonfly, whitefly etc. (Paintal, 2001)

Insect migration is an evolved adaptation and not a reaction to current adversity. In many cases migration is not immediately referable to prevailing adverse conditions, but commonly begins before these conditions are met. It forms an essential component of the life history and ecological niche of insects. Migration plays an important role in insect dominance (Dingle and Drake, 2007)

According to Kennedy (1961), "Insect migration is a behaviour which is persistent and straightened movement effected by the animal's own locomotory exertions or by its active embarkation on a vehicle. It depends on some temporary inhibition of station keeping responses but promotes their eventual disinhibition and recurrence". Persistent movement meant that dispersal was of long enough duration to carry the animal away from its original habitat to a new one and straightened-out meant that dispersal was in a general direction. Inhibition to station keeping cues, meant that insects were ignoring those signals provided by plant resources or congeners. During this time the insects are often responding to sky cues while ignoring others. After a period, they are disinhibited from responding to resource cues and again react to them.

Table 1 Orders under class insect showing migratory behaviour

Orders	Families	Examples
Orthoptera	Acrididae	*Dociostaurus maroccanus* (Moroccan locust), *Locusta migratoria* (Migratory locust), *Schistocerca gregaria* (Desert locust) , *Nomadacris septemfasciata* (Red locust)
Lepidoptera	Pieridae, Nymphalidae, Lycanidae, Sphingidae,Noctuidae	*Danaus plexippus* (Monarch butterfly) , *Vanessa cardui* (Painted lady butterfly), *Catopsilia florella* (Vagrant butterfly), *Agrotis infusa* (Bogong moth)
Odonata	Libellulidae, Aeshnidae	*Libellula depressa* (Broad bodied chaser), *Pantala flavescens* (Globe skimmer)
Coleoptera	Coccinellidae	*Hippodamia convergens* (Convergent lady beetle), *Adalia bipunctata* (Two spot ladybird)
Hymenoptera	Sphecidae, Tenthridinidae	*Sphex aegyptiacus* (Diger wasp) , *Athalia rosae* (Turnip sawfly)
Diptera	Syrphidae	*Eristalis sylvaticus* , *Syrphus lavendulae*

2. Why insects migrate?

Insects migrate from one place to another in search of resources like food, breeding place and also due to the overcrowding.

2.1 Food in temporary habitats

Insects migrate between temporary habitats such as ephemeral ponds, early successional habitat stages, or successively senescing host plants. Temporary habitat is ephemeral in nature that means habitats are highly variable through time. This impermanence is a function of season. Food will not be available throughout the time. Migration may be a favoured strategy to escape deteriorating habitats and to colonize new ones (Southwood, 1962). A number of species and populations of water striders (Hemiptera: Gerridae) occur over an array of habitats, from small, temporary ponds to large lakes and permanent streams to isolated permanent bogs. Species in the more temporary bodies of water have wings and undertake regular migrations to locate their aquatic habitats as they appear and disappear in the landscape (Harada *et al.*, 1997). In other cases, the insect's requirement may change at different life stages. So different habitats would be needed. The desert locust, *Schistocerca gregaria* (Forskal) different regions for feeding and for oviposition (Mathew and Mathew 2009).

Plate 1. Water striders, *Gerris* sp.

Plate 2. Desert locust, *Schistocerca gregaria*

2.2 Breeding

Insects migrate from one place to another for breeding purpose. New habitats will provide favourable conditions for breeding. Milk weed bug, *Oncopeltus fasciatus* (Dallas) is a wide ranging species found from Canada and South America. It reaches its northern areas of its range in between spring and early summer. The females settle on the patches of milkweed, they mate and lay their eggs close to the developing pods. These young bugs grow quickly and develop into breeding adults (Mathews and Mathews, 2009). Painted lady butterfly, *Vanessa cardui*, (Linnaeus) is a butterfly of world-wide distribution which, in the northern hemisphere, spreads each summer often to quite high latitudes, and retreats in the winter to dry sub-tropical areas. In the winter it breeds along the edges of the North African Desert. During the summer it regularly reaches the latitude of the British Isles, for breeding (Williams, 1957).

Plate 3. Milkweed Bug, *Oncopeltus fasciatus*

Plate 4. Painted lady, *Vanessa cardui*

2.3 Overcrowding

Insects also migrate due to overcrowding effect. Due to overcrowding there will be competition for food. It also lead to polymorphism in insects. In Brown plant hopper, *Nilaparvata lugens* (Stal) wing dimorphism occur due to overcrowding. So they will produce winged forms which will migrate to new host. Similarly in desert locust, *Schistocerca gregaria* overcrowding lead to phase polymorphism. Gregarious forms are produced which will migrate in swarms to new places.

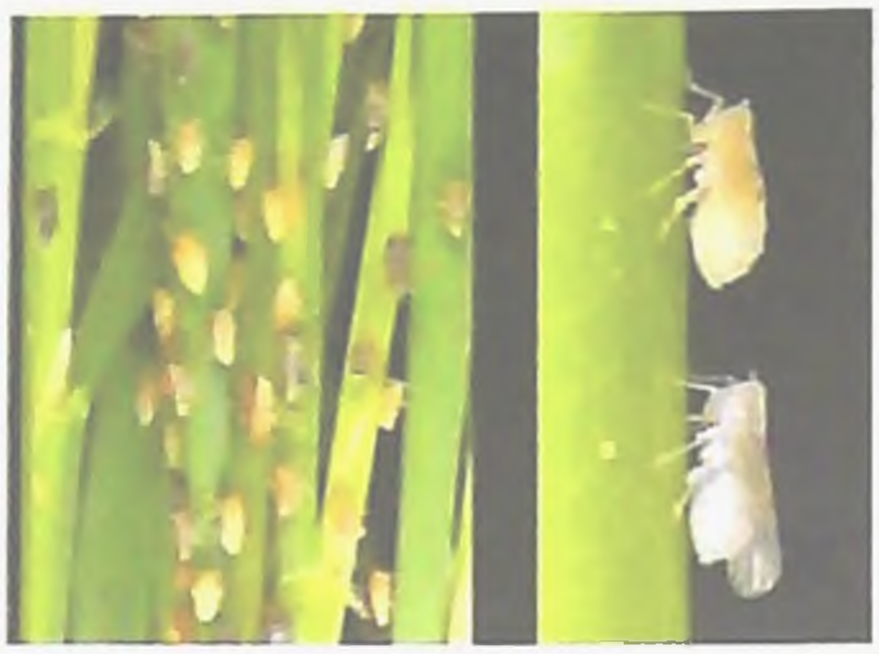

Plate 5. Brown plant hopper ***Nilaparvata lugens***

Plate 6. Desert locust, ***Schistocerca gregaria***

3. Types of migration

According to Taylor (1986), insect migration can be classified as dynamic migration and homeostatic migration based on frequency. Depending on the medium used migration can also be classified as aerial, pedestrian and waterborne (Reynolds *et al.*, 2014)

3.1 Based on frequency

3.1.1 Dynamic migration or One way movement

One way movement is a unidirectional movement. It carries insect from a location where they were produced to location where they breed and produce the next generation before dying (Dingle and Drake, 2007)

The Cotton strainer bug, *Dysdercus cingulatus* are tropical species which feed on the seeds of various shrubs and trees in the order Malvaceae. If newly emerged adult females find seeds still available, they feed and hystolyze their wing muscles and remain at natal site to produce another generation. If no seeds are available, they migrate to new hosts where feed and colonize. In case of host-alternating flying aphids, initially wingless young ones are produced through one or more generations on a primary host. Depending on aphid species, as females age, or as host plants senesce young ones are produced with wings. These winged offsprings then migrate to a new host plant and produce young there (Danthanarayana, 1986)

Plate 7. Cotton stainer bug,
Dysdercus cingulatus

Plate 8. Black bean aphid
Aphis fabae

3.1.2 Homeostatic migration or Return movement

Return movement are also called "round trip" migration. Round trip migration is a succession of oneway movement through a series of breeding areas. In this type of movement, insects return to their general breeding area from which they have originated (Dingle and Drake, 2007). The monarch butterfly, *Danaus plexippus* (Linnaeus) is considered as the classical example of migration. Formal studies on its migration was started by Urquhart. He was the first person who recorded the fall migration of monarch butterfly in 1961. He conducted the study by a tagging and recovery method. The monarch butterfly was tagged with a label and then released. He recruited several volunteers to observe this tagged monarch butterfly. He took almost 25 years to complete his research (Urquhart and Urquhart, 1977).

Population of monarch butterfly in North America undergo a dramatic migration in the spring and fall seasons of each year. In the fall, the monarch butterfly flies upto 2000 miles from Canada and the Northern United states to overwintering sites in southern California, Florida and Mexico. In the following spring they migrate back again to the north. The southward flight from the northern breeding areas in the fall occurs in large aggregations. The butterflies move south with the weather fronts, flying primarily in the middle of the day, accumulating in overnight roosts at night and during bad weather and replenishing fuel reserves by feeding on nectar. It is thought that most fall migrants are newly emerged individuals from the previous summer generation, most of whom are in adult reproductive diapause induced by short fall photoperiod and cool temperature. They therefore probably began migrating very soon after emergence prior to reproduction and remained in reproductive diapause throughout the cool winter in the Mexican mountains. In the spring, the day length and temperature increases and monarch come out of the diapause. There mating occurs and females migrate northward. The first generation leaving the overwintering site only migrates as far north as Texas and Oklahoma. The second, third and fourth generation return to their northern breeding locations in the United States and Canada in the spring (Dingle, 1977)

Plate 9. Monarch butterfly, *Danaus plexippus*

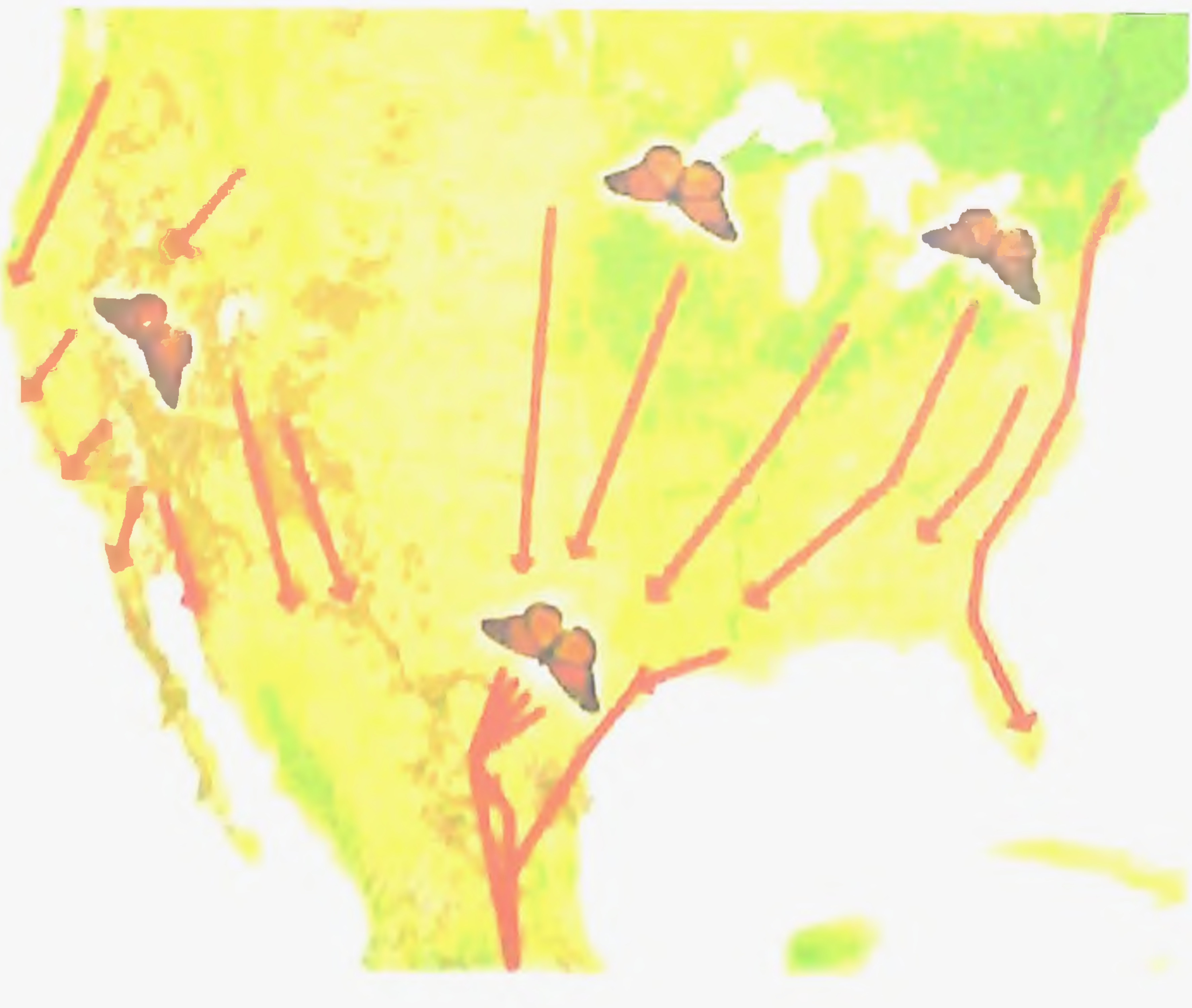

Plate 10. Migratory route of Monarch butterfly

Another example for round trip migration is Globe skimmer dragonfly, *Panatala flavescens* (Fabricius). This dragonfly take advantage of the moving weather systems and monsoon rains to complete the migration from Southern India to Maldives, then to East and Southern Africa and then likely back again to South India. The species involved breeds in temporary rainwater pools. So it is following the rains, taking sequential advantage of the monsoon rain of India, the short rains of East Africa, the summer rains of Southern Africa, the long rains of East Africa and then back to India for the next monsoon (May, 2012)

Plate 11. Globe skimmer, *Pantala flavescens*

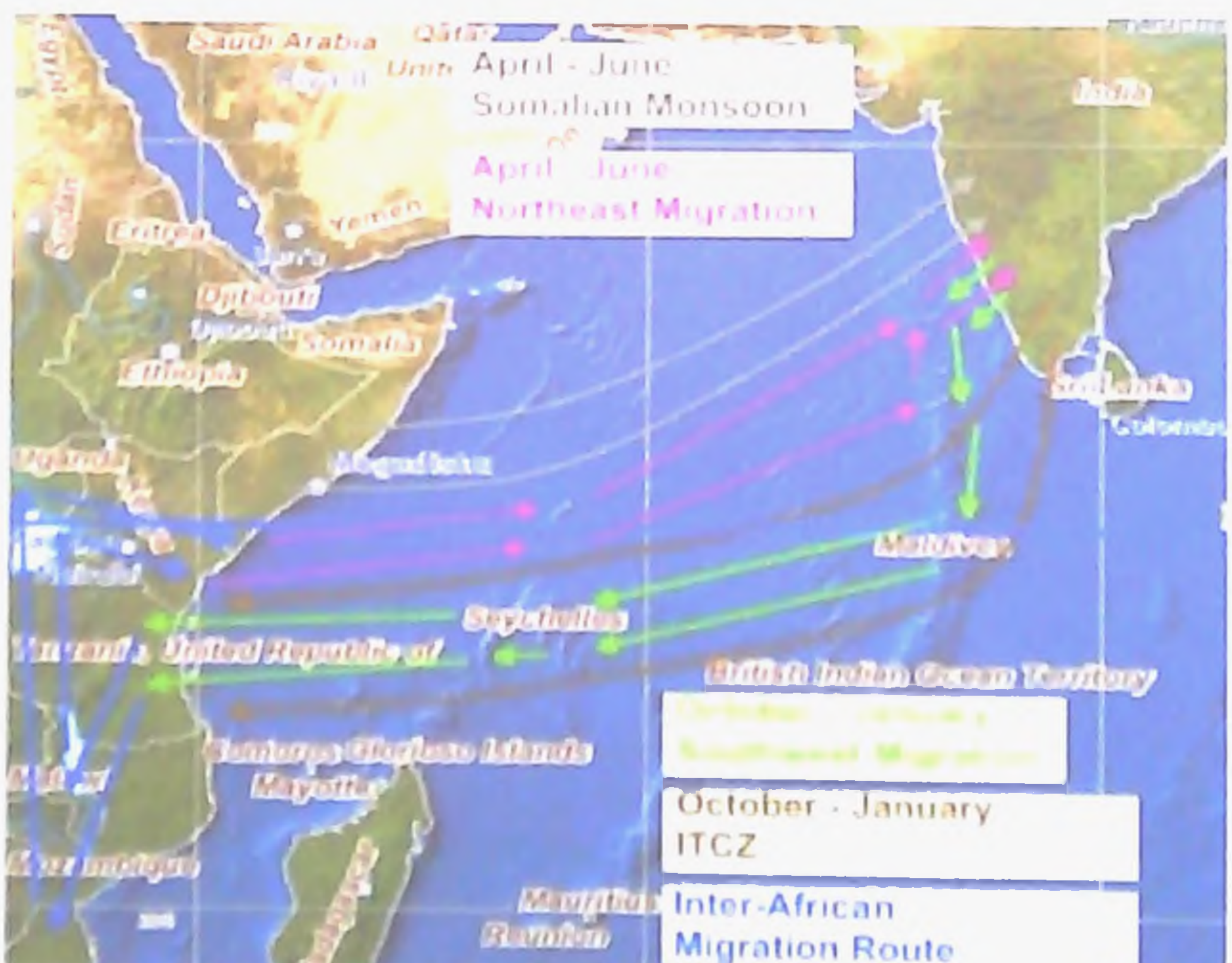

Plate 12. Migratory route of Globe skimmer

3.2 Based on medium used for migration

3.2.1 Aerial migration

Aerial migration is the migration on air currents. It is of two types, migration by ballooning (with the use of silk line) and migration without the use of silk line

3.2.1.1 Ballooning

Silk lines are used for migration. Ballooning is seen in young instar larvae of certain lepidopteran families like Cossidae, Geometridae, Lymantridae, Noctuidae etc. In some tree dwelling species the adult females of these families are flightless and ballooning is the only means of colonizing new hosts. In the case of winter moth, *Operophtera brumata* (Linnaeus) (Lepidoptera: Geometridae) the adult lay overwintering eggs on the top of the tree. The new emerged larvae spins silken threads. Then they migrate by ballooning. Ballooning is also seen in mites. In case of two-spotted spider mite, *Tetranychus urticae*, they congregate and these cluster of mites may produce a dense mass of silk at the apices of the host plant. These are then carried away by wind (Bell *et al.*, 2005)

Plate 13. Winter moth *Operophtera brumata*

Plate 14. Migration by ballooning in larva of winter moth

Plate 15. Two-spotted spider mite *Tetranychus urticae*

3.2.1.2 Migration without the use of silken lines

The insects launch themselves into the air which is then lifted of by the air currents. In Cochineal insect, *Dactylopius austrinus* (De Lotto) under the influence of positive phototaxis, female crawlers climbed to the uppermost parts of the Cactus host during the morning, which is then carried by the stronger winds. After the aerial movement female crawler becomes photonegative. Then the upward climbing behaviour is ceased and individuals undertake walks before finally settling (Moran *et al.*, 1982)

Plate 16. Cochineal insect
Dactylopius austrinus

3.2.2 Pedestrian migration

It is migration by walking and other types of limbed motions on land like running (cursorial movement), hopping or jumping (saltation). Walking type of migration is shown by Desert locust, *Schistocerca gregaria* (F). The nymphs of gregarious phase of the locust form large cohesive groups and march persistently in consistent direction. Estimate of the distances moved by bands of desert locust during the life span can total upto 30 km (Kennedy, 1956). In the case of *Hypogastrura socialis* (Uzel), it moves by jumping with anal sacs extended, allowing it to stick to the snow surface in a vertical stance. From this position it bends forward into the normal horizontal position, withdraws the anal sacs, and then rotates itself horizontally on the spot in order to select the direction of next jump.

Plate 17. Collembola, *Hypogastrura socialis*

Plate 18. Desert locust, *Schistocerca gregaria*

3.2.3 Waterborne migration

Larval stages of many insects dwell in rivers and streams use the water current to move from natal sites to colonize suitable microenvironments. First instar larvae of many chironomids show behavioural adaptation (phototaxis) which cause them to be temporarily planktonic in the surface of water and can be moved around by water current. There are two types of waterborne migration, they are rafting and floating type and sailing and skimming type (Davies, 1976).

Plate 19. Chironomid larvae

3.2.3.1 Rafting and floating type

In this type of migration insects are first carried by the wind, then it fall on sea or fresh water and are then drifted along the surface of water by wind. The arctic soil collembola, *Tetracanthella arctica* (Cassagnau), is carried by the wind and then fall into the water. It can remain alive in water for many days because of the presence of hydrophobic cuticle which allow the collembolan to obtain oxygen by diffusion through it (Coulson *et al.*, 2002).

Plate 20. Arctic soil collembola, *Tetracanthella arctica*

3.2.3.2 Sailing and skimming type

Insects on water surface may also be blown along the surface by the wind. This type of migration is seen in aster root aphid, *Pemphigus trehernei* (Foster). Primary host of the aster root aphid, is tidal aster. When erosion of marsh or overcrowding occur, the first instar larva become photopositive, crawl upto the soil surface and are then move by the incoming tide and thus reach new host along marsh edge. Floating on water surface for about 30 minutes make it photonegative resulting in movement into cracks containing aster root (Foster *et al.*, 1978).

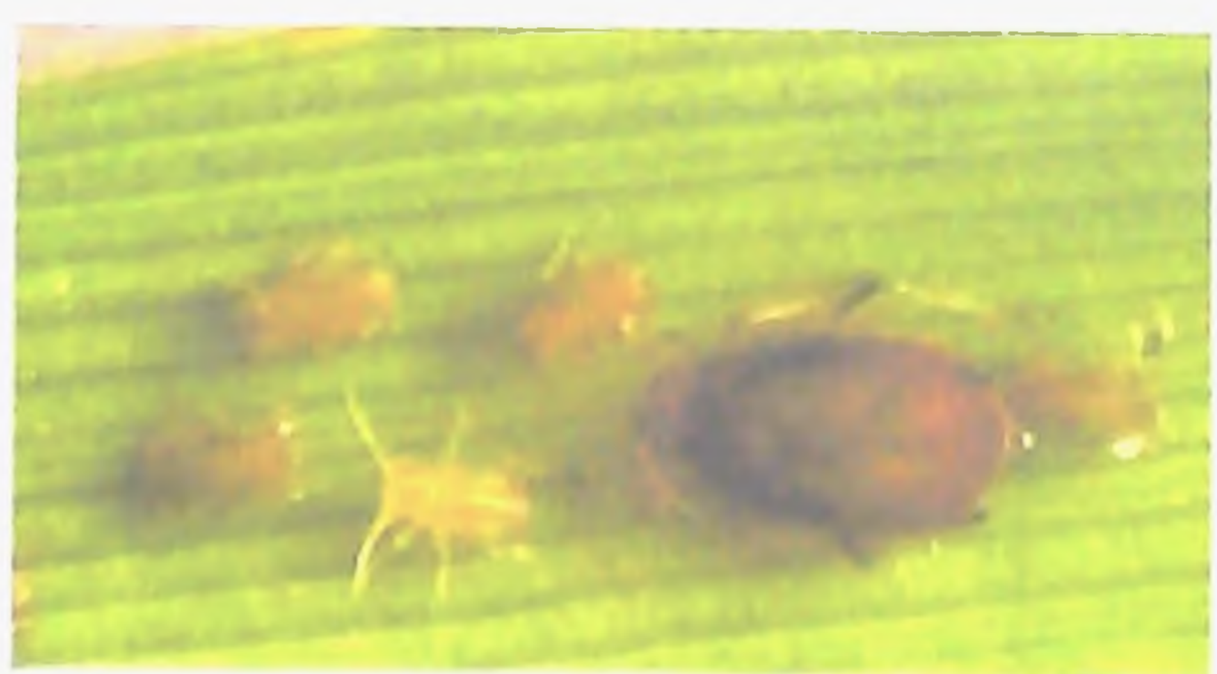

Plate 21. Aster root aphid, *Pemphigus trehernei*

4. Migratory syndrome

Several physiological and morphological traits that underlie migratory behaviour of insects are called migratory syndrome. The physiological traits include hormone titers, distribution of energy stores, flight propensity and age specific reproduction. Wing dimorphism and body size of the migrant are examples of morphological traits (Roff and Fairbarn, 2007).

4.1 Physiological traits

4.1.1 Hormone titers

Development in insects are controlled by age specific changes in hormone titers. There is a difference in hormonal profiles of migrants and non-migrants. Hormonal differences can produce long term irreversible effects such as the production of a winged and wingless morph. One particularly important hormonal pathway involved in migration is Juvenile Hormone (JH) pathway. During key developmental period, the titer of JH is regulated by Juvenile hormone Esterase (JHE). During this period the amount of JHE will be high which results in increased degradation of JH. Thus amount of JH will be less. This low level of JH promotes the development of wing and flight muscles and results in the formation of long winged morph (Zera, 2004).

4.1.2 Distribution of energy stores

Major metabolic fuels were carbohydrate which is stored as glycogen in muscle and trehalose in haemolymph. Fat is stored as triacylglycerol in adipose tissue and muscle. Migrants must synthesize and store flight fuel such as triglycerides. This is energetically demanding and may divert energy from early investment in reproduction (Zera and Denno, 1997). But non-migrants do not have to store this energy for flight and hence can utilize energy stores for reproduction.

4.1.3 Flight propensity

It is the capacity for flight. Individuals with fully developed wings are classified as migrants or long winged morphs. But flight propensity often varies in the long winged morph. It varies in the propensity to initiate a flight, mean duration of flight and propensity to terminate a flight in presence of particular cues such as host plants or habitat. Some individuals show little or no propensity to initiate a long distance flight, whereas other individuals may readily take flight and may require long duration flights before they are behaviorally and physiologically ready to settle down (Fairbairn and Desranleau, 1987).

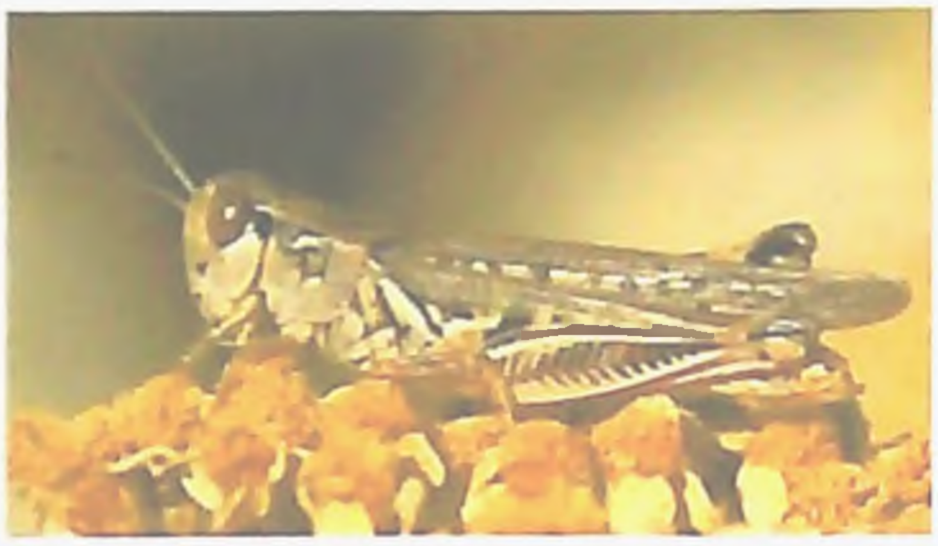

Plate 22. Red-legged grasshopper
Melanoplus sanguinippes

Plate 23. Codling moth,
Cydia pomonella

4.1.4 Age specific reproduction

Migration usually occurs in pre-reproductive period. The adult life of an insect is separated into migratory phase followed by the reproductive phase. This design is called "oogenesis flight syndrome". It is the pattern of delay in the onset of oogenesis, copulation and oviposition until the end or near the end of the migratory period (Johnson, 1969). The main mechanism behind this is change in hormone titers. During this period, the JH titers are low and physiological functions related to flight, flight muscle development and flight energy utilization are enhanced whereas reproductive functions such as oogenesis, copulation and oviposition usually are suppressed. Significance of oogenesis flight syndrome is that it reduces the competition for energy resources. If migration and reproduction are synchronised there will be competition for energy resources. Delay in reproductive functions thus reduce this competition. Beside this if females are carrying eggs it will cause aerodynamic constraints like reduction in flight capacity, flight duration etc. So oogenesis flight syndrome will help to reduce such constraints.

4.2 Morphological traits

4.2.1 Size of the migrant

Size also varies between migrants and non-migrants. Selection may favour larger size for migrants. This is to reduce the water loss or because larger size increases the energetic efficiency of flight (Roff, 1977).

4.2.2 Wing dimorphism

The most obvious morphological correlate of migratory capability is possession of fully developed wings with associated flight musculature. If the habitat is permanent and continuous, if there is a cost to the possession of flight machinery or if the flight is not used for foraging or mating, then short winged morphs are preferred. But if the habitat is temporary in nature then long winged morphs are preferred for migrating to new habitats. The main mechanism behind this is low level of Juvenile Hormone.

Plate 24. Long winged & short winged morphs of *Gryllus firmus*

In a study conducted by Zera and Herang in 1999, the functional response of JH esterase with wing polymorphism is represented. In this study they compared the JHE activity of last instar of short winged and long winged morphs of *Gryllus firmus* (Scudder). The results showed that the JHE activity of long winged *Gryllus firmus* (S) was maximum on day 5 of the last moult and it reduced drastically by day 7. The case was similar with short winged morph *Gryllus firmus* (S). But the JHE activity in long winged morph was as high as 80 percent compared to the quantity of JHE in short winged morph. This indicates that the increased content of JHE promotes long wings which in turn helps in migration

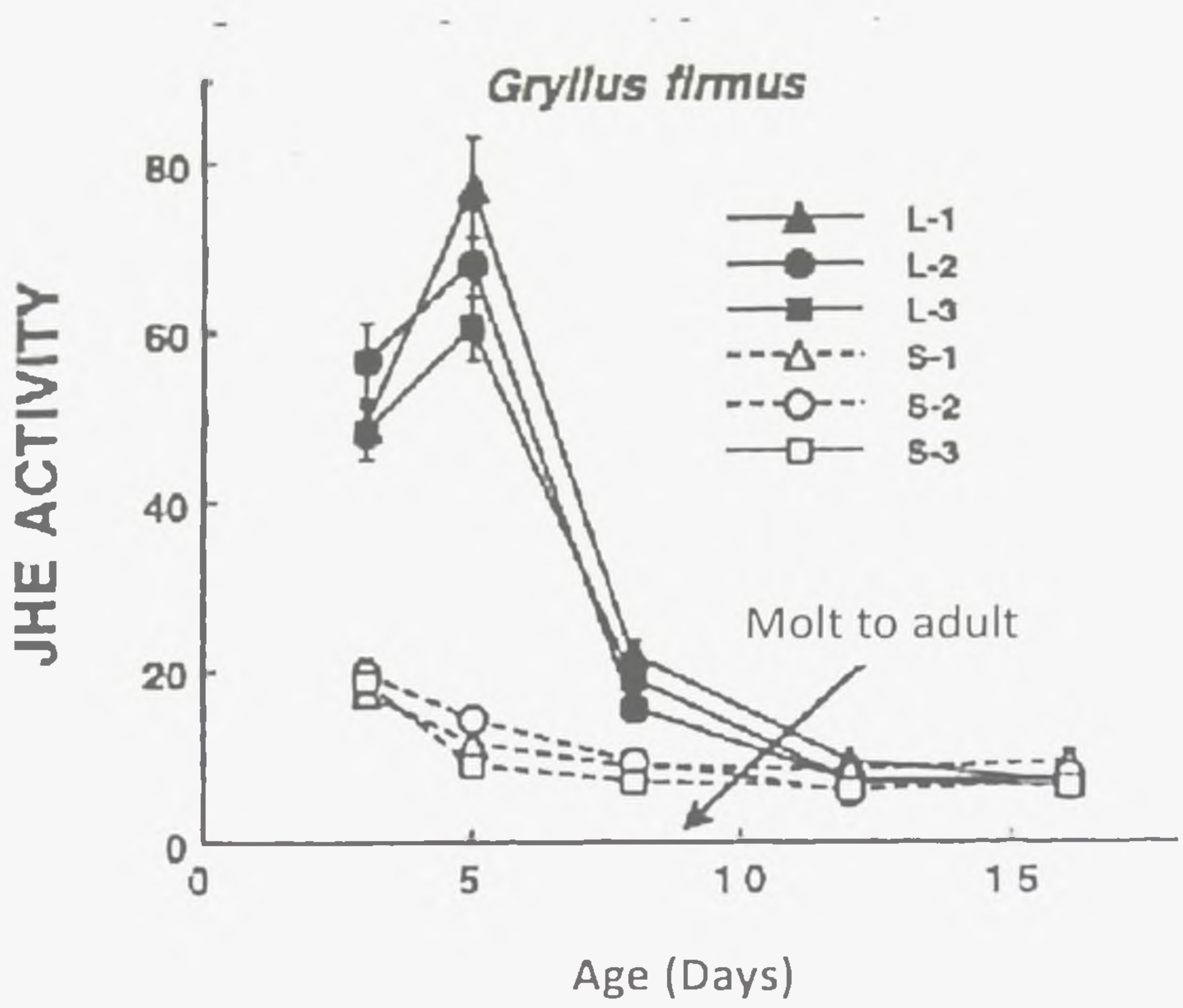

Fig 1. Functional relationship of Juvenile Hormone Esterase (JHE) with wing polymorphism in the cricket, *Gryllus firmus*

5. Methods used by insects for orientation and navigation

Insects are able to find their way during migration. They do so using the sensory adaptations and navigational capabilities. Different methods are used by insects for navigation like sun compass, earth's magnetic field and visual cues (Syrgley and Oliveira, 2001).

5.1 Sun compass

Insects use time compensated sun compass for orienting over long distances. Orientation is manifested by directing the body at a particular angle relative to the sun's position. They use polarized skylight associated with the position of the sun to detect the position of the sun even if it is hidden behind the clouds. It is a time compensation mechanism. Full compensation require innate or learned information about the position of the sun over the course of the day. Degree of compensation varies with latitude and time of the year. So insect might approximate the position of the sun with a time averaging function. For example a migrant might approximate the change in the sun's position as 15° per hour (Syrgley and Oliveira, 2001).

Plate 25. Statira Sulphur, *Aphrissa statira*

5.2 Earth's magnetic field

Magnetic field lines radiate between the North and South Poles of the earth. Many insects can sense and make use of magnetic fields for navigation purposes. The polarity, intensity and inclination angle of the Earth's field can provide a reliable sources of navigational information. These components can be used to either determine geographic position relative to destination or maintain a constant bearing in a particular direction (Akesson and Hedenstrom, 2007).

Plate 26. Earth's magnetic field

Plate 27. *Danaus plexippus*

5.3 Visual cues

Visual cues like land marks, shape of the coastline, topography of the land etc. are used for navigation by the migrant in short term as well as long term migration. Usually it is used for correcting its flight paths when exposed to a wind drift and also for positioning the distance from the earth. Pirpinto, *Ascia monuste* flew in a curvilinear track between islands off the Florida coast.

Plate 28. Pirpinto, *Ascia monuste*

6. Significance of insect migration

6.1 Pest management decision

6.1.1 Locust

Locust is an important migratory pest. According to Uvarov's phase theory there are two phases of locust like solitary and gregarious phase. Due to overcrowding effect solitary phase is transformed into gregarious phase. This gregarious phase is causing more damage to agricultural crops (Pener and Yerushalmi, 1997). The initial bands of locust are called outbreaks, when these bands join to form a large groups it is called upsurge and these upsurge when occur in a region from different breeding areas, plague will occur. Study of migratory locust can aid forecasting outbreak and thus help timely adoption of management measures.

Plate 29. Solitary and gregarious phase

Plate 30. Locust infestation in Madagascar

Table 2. Locust infestation occurred in India

Species	Area of distribution	Breeding grounds	Period of plague
Bombay locust, *Patanga succincta*	Gujarat, Madras and Bengal	Open areas of Western Ghats	1864-66, 1901-1908 and 1935-45
Migratory locust, *Locusta migratoria*	Madras Bangalore	Rajasthan, Gujarat	1898 – 1954

6.1.2 Whitefly

Whitefly is a polyphagous pest. It also act as a vector for viruses. The information on migratory behaviour of whitefly would aid growers in making decisions concerning the timing of crop planting and placement.

Plate 31. Whitefly, *Bemisia tabaci*

Byrne (1999) conducted field study to reveal that whitefly, *Bemisia tabaci* (Gennadius) exhibited migratory behaviour. He conducted both a lab and field study to determine the migratory behaviour of whitefly. In lab experiment he used a vertical flight chamber. It consisted of a large, open fronted box with a central opening in the roof. A small open box containing a sodium vapour light which provide sky cues is kept inside it. Mounted on the side of the chamber is a 550nm light source that stimulates a vegetative cues. Digital readings from the anemometer measure wind flow from above which is an indirect measure of insects rate of ascent. Insects ignoring the vegetative cues while flying towards the sky cues for an extended period were identified as being migratory. In this experiment approximately 5% of whiteflies flew towards the sky cues by ignoring the vegetative cues for more than 15 min. Also their movement was persistent and straightened out.

Similarly marked *B. tabaci* were released in the field and their distribution following dispersal across a 21 km² grid was noted. Their distribution was bimodal, one peak of alighting occurring at the field edge and another peak at a distance of 2.2 km. Those caught near the field were engaged in foraging while others are engaged in migratory flight by ignoring intervening crops.

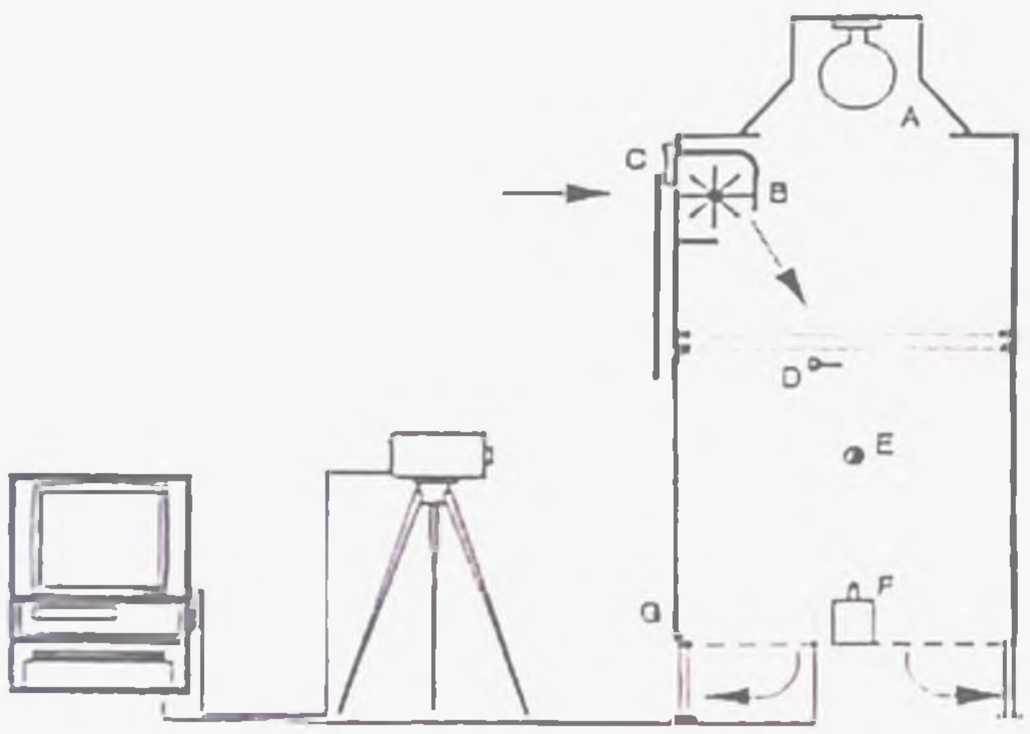

Fig2. Vertical flight chamber employed in examining flight behavior by *Bemisia tabaci*
A — mercury vapour lamp, B — air intake port, C — air intake fan, D — anemometer, E — 550 nm light, F — platform, G — door.

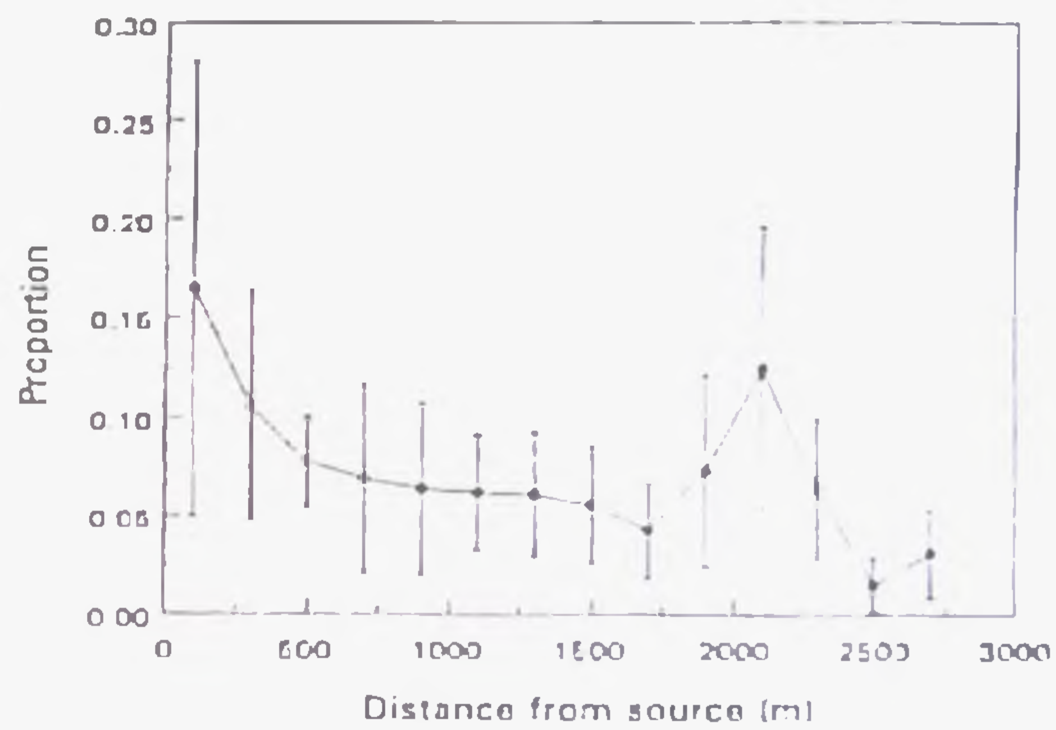

Fig 4. Proportion of *Bemisia tabaci* trapped in 200 m wide distance classes downwind

6.1.3 Pink boll worm

It is a major pest of cotton. In the late 1990s, Bt was introduced to control the pest. But resistance of this pest against Bt was of great concern. To prevent the development of resistance, it was suggested to plant a portion of the crop with refuge of non-Bt cotton crop so that pest could feed on plant that do not contain Bt toxin. Dispersal of this pest between these two crops increases chances that resistant adults mate with susceptible adults from refuges and dilute the alleles for resistance and prolong the pest population susceptibility (Byrne, 2008).

Plate 32. Larva of pink boll worm ***Pectinophora gossypiella***

Plate 33. Adult of pink boll worm ***Pectinophora gossypiella***

6.2 Beneficial insects

Some beneficial insects are being used to manage the pest. Many of the beneficial insects show migration. Understanding migratory behaviour also can help enhance the efficacy of natural enemies. For example Lacewings, *Chrysoperla sinica* (Tjeder) (Chen *et al.*, 1963) and Convergent lady beetle, *Hippodamia convergens* (Guerin-Meneville) (Williams, 1957) have reported undertaking long distance migration flights. In lacewings, because of the weak flight capability they have evolved certain morphological, physiological or behavioural adaptations to allow long distance flight. Study of different facets of *C. sinica* flight may aid its incorporation in Integrated Pest Management systems (Liu *et al.*, 2011). Convergent lady beetle show regular seasonal migrations between large summer breeding areas in coastal plains or lower valleys and winter quarters in the mountains, where there is a dormant period. For example in field when whitefly parasitoid *Eretmocerus eremicus* (Rose and Zolnrowich) are released, control of whitefly was less. It is because of the low relative dispersal distance of *E. eremicus* as compared with those of *Bemisia tabaci*. So study of migratory behaviour would help in selection of good parasitoid or combination of parasitoids.

Plate 34. Green lace wing ***Chrysoperla sinica***

Plate 35. ***Convergent lady beetle Hippodamia convergens***

Plate 36. ***Eretmocerus eremicus***

6.3 Ecotourism

Overwintering sites of migrating butterflies provide excellent ecotourism opportunities as in case of monarch butterfly. Several sanctuaries were established in the overwintering sites of monarch butterflies in Mexican countries.

Plate 37. El Rosario Monarch Butterfly Preserve (Central Mexico and Gulf Coast, Mexico)

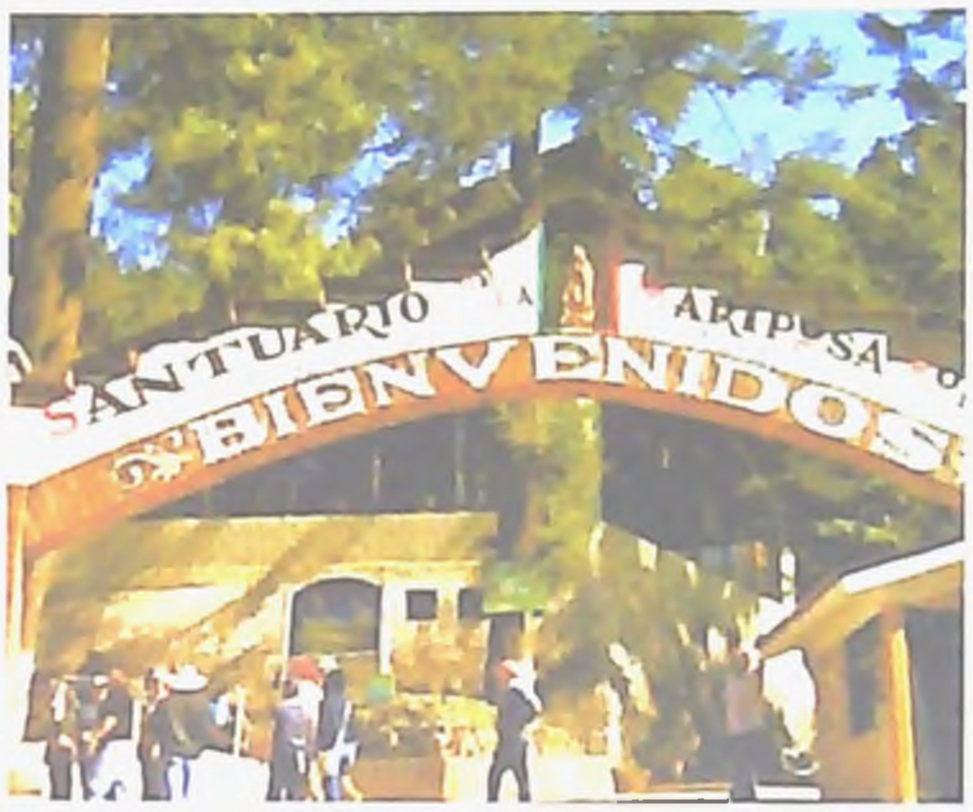

Plate 38. Monarch Butterfly Sanctuary in Michoacan, Mexico

Plate 39. Monarch butterflies in Overwintering sites

6.4 Edible insects

The migrating insects like locust can be used as a source of food. The red, yellow, spotted grey, and white locusts are edible. It is rich in protein, zinc and iron.

Plate 40. Fried locusts

Plate 41. Chocolate covered locust

7. Conclusion

Migration is an important natural phenomena. It is an adaptation evolved in insects to overcome the adverse conditions. The knowledge on insect migration would help in the development of simulation models that serves as an aid to growers in making pest management decisions. So detailed research is needed in this area. The overwintering sites of migratory butterflies provide excellent ecotourism opportunities. Migratory locusts can be utilized for edible purpose

Discussion

1. How many days the monarch butterfly will take to reach their overwintering sites in Mexico?

Monarch butterfly will take about 60 to 65 days to reach their overwintering sites in Mexico.

2. What is the life span of monarch butterfly?

Adult monarchs live only for 3 to 4 weeks. However, one of the mysteries of these insects is their capability to breed what has been termed by scientists as a 'Methuselah' generation. This is a very special generation of butterfly, born once a year near the end of the summer months. These butterflies can live up to 9 months and this generation will not breed in the north: instead the butterflies store up nectar from flowers and use this as fuel to enable them to migrate south for the winter.

3. Is there any research work going on in India?

Many Locust forecasting centres were established in India for the control of infestation of locust.

4. What is the difference between solitary and gregarious phase of locust?

Locusts are the swarming phase of certain species of short-horned grasshoppers in the family Acrididae. In the solitary phase, these are called grasshoppers which are innocuous, their numbers are low and they cause little economic threat to agriculture. In the gregarious phase, swarming occurs and cause extensive damage of agricultural crops. This behaviour is a response to overcrowding. Increased tactile stimulation of the hind leg causes an increase in levels of serotonin. There are also differences in morphology and development. The *gregaria* nymphs become darker with strongly contrasting yellow and black markings, they grow larger and have a longer developmental period while solitary phase are green in colour.

5. What is the importance of milkweed plant in monarch butterfly?

Monarch butterfly feed on milkweed which contains higher levels of cardiac glycosides. The monarch stores this cardenolides, or cardiac glycosides that it gets from the milkweed. This makes the butterfly unpalatable and thus protects it from predators.

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Entomology

ENT 591: MASTER'S SEMINAR

Name: Umamaheswary M. D.

Admission No.: 2014-11-124

Major Advisor: Dr. Maicykutty P. Mathew

Venue: Seminar hall

Date: 16-01-2016

Time: 10.00 a.m

Insect migration

Abstract

Migration is defined as the movement of animals in a direction for a certain distance which results in temporary or permanent change of habitat. It is an adaptive behaviour of great significance that is observed across the Animal Kingdom. Several species of insects display migratory behaviour like in case of monarch butterfly, locusts, dragonfly, whitefly *etc*. According to Kennedy (1961), "Insect migration is a behaviour which is persistent and straightened-out movement effected by the animal's own locomotory exertions on or by its active embarkation on a vehicle. It depends on some temporary inhibition of station keeping responses but promotes their eventual disinhibition and recurrence".

Insects migrate from one place to another in search of resources like food, breeding place and also due to overcrowding effect. Insect migration can be classified as dynamic migration and homeostatic migration based on frequency (Taylor, 1986). Depending on the medium used migration can also be classified as aerial, pedestrian and waterborne.

Several physiological, behavioural and morphological traits underlie the migratory behaviour of insects that are collectively referred to as migratory syndrome. The physiological traits include hormone titers, distribution of energy stores, flight propensity and age specific reproduction. Wing dimorphism and body size of the migrant are examples of morphological traits (Roff and Fairbairn, 2007).

Insects are able to find their way during migration by means of their sensory adaptations and navigational capabilities. Different methods are used by the insects for orientation and navigation includes solar navigation, use of earth's magnetic field, and visual cues (Srygley *et al*., 2001).

Phoresy: Hitch-hiking on insects

By

Chandini S.M.

(2014-11-150)

M. Sc. Agrl. Entomology

Seminar report

Submitted in partial fulfilment of requirement of the course

Ent. 591 Seminar (0+1)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA- 680656

THRISSUR, KERALA, INDIA

DECLARATION

I, Chandini S.M. (2014-11- 150), hereby declare that the seminar report entitled '**Phoresy : Hitch-hiking on insects**', has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Chandini S.M
2014-11-150

Vellanikkara
Date: 20.11.2015

CERTIFICATE

Certified that the seminar report entitled **'Phoresy : Hitch-hiking on insects'** for the course Ent.591 has been prepared by Manjushree G. (2014-11-214). after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

Dr. Susannamma Kurien

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95/10

CERTIFICATE

Certified that the seminar report entitled **'Phoresy : Hitch-hiking on insects'** is a record of seminar presented by Chandini S.M (2014-11- 10) on 11th December, 2015 and is submitted for partial requirement of the requirement of the course Ent.591.

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1. Introduction

Animal Kingdom is flourished with astounding number of symbiotic associations. Most of these associations are for getting food, shelter and transport. Phoresy is a special kind of commensal relationship in which one organism (phoretic or phoriont) attaches to another (phoront or carrier) for a limited time period to enhance dispersal of the phoront from the natal habitat to a new and potentially better habitat (Binche, 1994). The etymology of the word unites the perspective of both the phoriont (*phor* (Gr.) means "thief" and the phoront (*phoras* (Gr.) means "bearing" within the interaction.

Phoretic associations are strictly temporary and non- parasitic association. The phoriont is usually much smaller than its host and often several phorionts may attach on an individual phoront. The phoriont does not have an effective means of independent dispersal (e.g., wings or oars), whereas the phoront usually is quite mobile and larger in size than phoriont. Phoresy excludes any direct physiological benefit during transit. Phoront does not provide the phoriont with food while in transit nor does it contribute to the ontogeny or development of the phoriont during transit. Usually only one member of a complex life cycle of phoriont participates in the phoresy and the other members in the life cycle are not phoretic in nature. In such cases, the phoriont may come from any of the life stages (Mitchell, 1975).

Phoresy among arthropods has been recognized since at least the mid-seventies. First report of phoresy is on fly *Limosina sacra*, which is phoretic on dung beetle, *Scarabaeus damarensis* (Lesne, 1896). Both are inhabited in transient habitats. Among the arthopods, phoretic associations among insects are more prominent. Organisms with small size, limited mobility and inhabiting transient habitats are phoretically associated with insects for dispersal. Insects are the most popular phoronts due to their capacity for flight, diversity of their habitats and abundant population. Most of the insect orders have members that participate in phoresy; however, insects of order Diptera, Hymenoptera and Coleoptera form some of the most extensive phoretic associations with other insects, nematodes and mites (Wage, 1979).

2. Classification of phoresy

Phoresy can be classified based on ecology, phoront specificity and mode of attachment (Binche, 1994).

2.1 Ecology

Ecology is the study of interrelationships between living organisms with their environment. Environmental factors play important role in the existence of symbiotic associations. Based on the influence of environmental factors on phoretic associations, phoresy can be classified as either obligate or facultative.

2.1.1 Obligate phoresy

Phoriont totally depends on phoresy for completing their life cycle. The association is influenced by environmental factors like temperature, relative humidity, light *etc.* Usually mites associated with ephemeral habitats show obligate phoresy. Degradation of ephemeral habitat is influenced by environmental factors. Phorionts often have phoretic adaptations for enhancing phoresy. In obligate phorionts, phoretic migration is cyclic (recurrent) and regular. Mite *Poecilochirus* sp. is associated with American carrion beetle, *Necrophila americana* for their dispersal. Both these are inhabited on decaying human & animal carcasses (Gibbs and Edward, 2001).

Plate 1. *Poecilochirus* sp.

Plate 2. American carrion beetle, *Necrophila americana*

2.1.2 Facultative phoresy

Phoretic association is not compulsory for the phoriont to complete their lifecycle. The association is opportunistic in nature. Here association is not influenced by environmental factors. Phorionts randomly choose phoronts for their dispersal. Phoretic adpatations are absent for phorionts. Mite *Glyptholaspis* spp. hitches on dark flower beetle, *Euphoria sepulcralis* for its dispersal (Krantz, 1978).

Plate 3. ***Glyptholaspis*** **sp.**

Plate 4. Dark flower beetle, ***Euphoria sepulcralis***

2.2 Phoront specificity

The relationship between phoriont and phoront is termed as phoront specificity. Based on the phoront specificity of phoriont, phoresy can be classified into three types such as euryxenous, stenoxenous and oioxenous.

2.2.1 Euryxenous phoresy

In euryxenous phoresy, phoriont uses a wide range of phroronts for their dispersal. These associations are non-specific in nature. Mite *Histiogaster arborsignis* is phoretic on forty species of phoront for their dispersal. Longicorn beetle, *Chlorida festiva* and Southern pine beetle, *Dendroctonus frontalis* are common phoronts used by the mite (Lindquist, 1999).

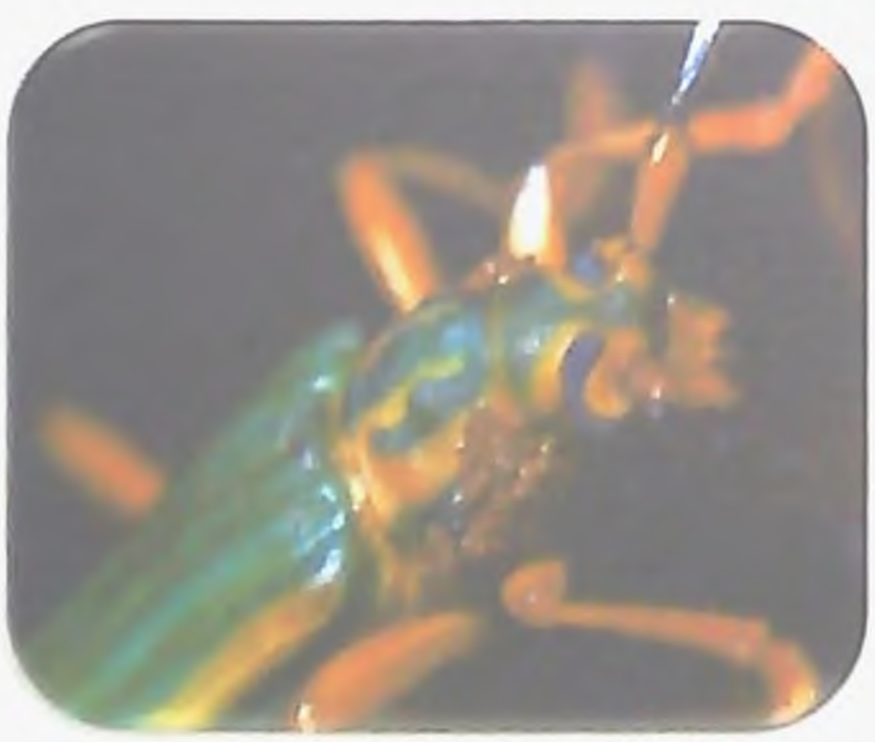

Plate 5. ***Histogaster arborsignis*** **on** ***Chlorida festiva***

Plate 6 ***Histogaster arborsignis*** **on** ***Dendroctonus frontalis***

2.2.2 Stenoxenous phoresy

Stenoxenous phoresy is specific in nature and phoriont prefer phoronts of same genus or family. Predatory mite *Hemisarcoptes* spp. prefers lady beetle *Chilocorus* spp as phoront. The mite prefers fourteen species of lady beetle of genus *Chilocorus* spp. for their dispersal (Gordon, 1985).

Plate 7. *Hemisarcoptes* spp.

Plate 8. *Chilocorus* spp

2.2.3 Oioxenous phoresy

Oioxenous phoresy is highly specific in nature. Phoriont constantly prefers a specific phoront for its dispersal. Mite *Macrocheles rettenmeyeri* attaches with army ant, *Eciton dulcium* for its dispersal. Mite attaches only to the leg membrane of the ant during phoresy (Ewing, 2001).

Plate 9. *Macrocheles rettenmeyeri*

Plate 10. *Eciton dulcium*

2.3 Mode of attachment

Phoriont can be attached both externally and internally with the phoront. Based on the mode of attachment phoresy can be classified into either endophoresy or ectophoresy.

2.3.1 Ectophoresy

In this type phoriont attaches externally to the phoront for its dispersal. Phorionts often attaches to the body wall of phoronts. Mite *Myialges anchora* attaches to the thoracic region of pigeon fly, *Pseudolynchia canariensis* during phoresy (Wallace, 1960).

2.3.2 Endophoresy

In endophoresy phoriont attaches internally to the phoront for its dispersal. Usually nematodes show this type of phoresy. Nematode *Diplogaster coprophila* attaches to the abdomen of fly *Sepsis fulgens* (Kethley and Johnson, 1975)

3. Stages in phoresy

Phoresy involves different stages such as searching of phoront, attachment to phoront and disembarkment (Morand and Binche, 1993).

3.1 Phoront seeking

Phoront seeking or searching of phoront is the first stage of phoresy. Phoriont identify its phoront using different cues like chemical, visual and auditory (Cheng, 1991).

Chemical cues are specific in nature and it represents a particular phoront. Broad mite, *Polyphagotarsonemus latus* exhibits a specific phoretic relationship with whitefly, *Bemisia tabaci*. Mite identify white fly by a chemical component triacontanyl triacontanoate present on the wax of white fly (Soroker *et al.*, 2003).

Plate 11. *Polyphagotarsonemus latus*

Plate 12. *Bemisia tabaci*

Egg parasitoids are often associated with phoresy for reaching their habitat. They use host insects as their phoront. Majority of parasitoid hosts are immature insects. One way of locating immatures is to hitch a ride on the adult and wait until it oviposits or returns to the nest. They find their phoront based on any specific chemical produced by them. Parasitoid *Mantibaria mantis* itself attaches to adult *Mantis religiosa*. If it attaches to male, it will transfer to the female during mating. When the female oviposit, it will parasitise freshly laid eggs (Morehead and Feener, 2000).

Table 1.Chemical cues used for phoront seeking

Phoriont	Phoront	Chemical cue	Reference
Trichogramma brassicae	*Pieris brassicae*	Benzyl cyanide	Anderson *et al.*, 2013
Telenomus calvus	*Podisus maculiventris*	2- hexenel	Aldrich *et al.*, 1989
Telenomus evanescens	*Pieris rapae*	Methyl salicylate	Arakaki, 1999
Apocephalus paraponerae	*Paraponera clavata*	4-methyl-3-heptanone	Reynolds *et al.*, 2014
Trissolcus basalis	*Murgantia histrionica*	2-decenal	Fric and Colazza, 2012
Trissolcus basalis	*Euschistus heros*	4-oxo-(E)-2-hexenal.	

Okumura and coworkers conducted an experiment on species specific attraction of nematode *Caenorhabditis japonica* towards borrower bug, *Parastrachia japonensis*. In this experiment, approximately thousand nematodes were inoculated on a filter paper in a 3 cm plastic petri dish, and then *C. japonica*-free bugs *Parastrachia japonensis*, *Erthesina fullo*, *Macroscytus japonensis* and *Armadillidium vulgare* were released in each dish. Twenty four hours after inoculation (at 25 C) the insects were dissected and their body parts were placed in water for 24 hours to release nematodes. The number of nematodes was assessed using a stereomicroscope. It was found that the body wall of *Parastrachia japonensis* contain maximum number of nematodes followed by *Erthesina fullo*, *Macroscytus japonensis* and *Armadillidium vulgare*. *Caenorhabditis japonica* appears to have a species-specific phoretic association with *Parastrachia japonensis* due to a chemical, 2- hexenel present on the body wall, whereas this chemical was absent in other three bugs

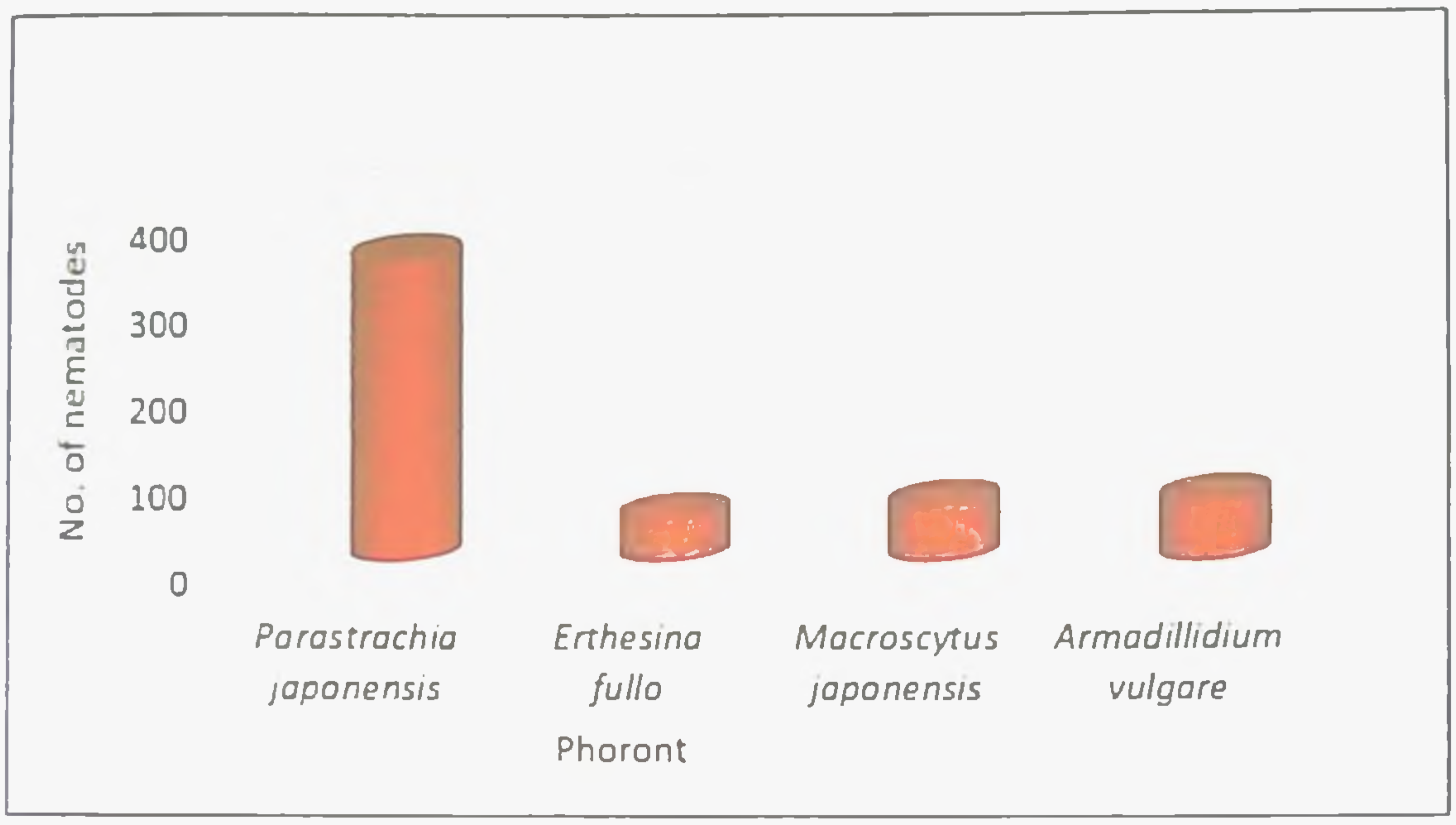

Figure 1.Species specific attraction of nematode, *Caenorhabditis japonica* on different bugs.

Ceroptera rufitarsis (Diptera: Sphaeroceridae) is a tiny fly phoretic on several roller dung beetles of the genus *Scarabaeus*. Niogret and Lumaret (2008) conducted an experiment on phoront seeking of fly *Ceroptera rufitarsis* towards dung beetle, *Scarabaeus sacer*. They released one hundred *C. rufitarsis* at one end of a 500 x 40 x 55 mm tunnel made of transparent polymethyl methacrylate. The fly was free to move within the tunnel. Later a live beetle without paint, live beetle with paint, live beetle fitted with magnetic bar and dead beetle were introduced at the opposite end of the tunnel. It was observed that about seventy five flies were found to be associated with live beetle without paint followed by live beetle with paint, live beetle fitted with magnetic bar and dead beetle. Reason for attraction of flies towards the live beetle without paint is its specific movement and metallic black colour. These act as specific visual cues for *C. rufitarsis* towards the beetles.

Plate 13. *Ceroptera rufitarsis*

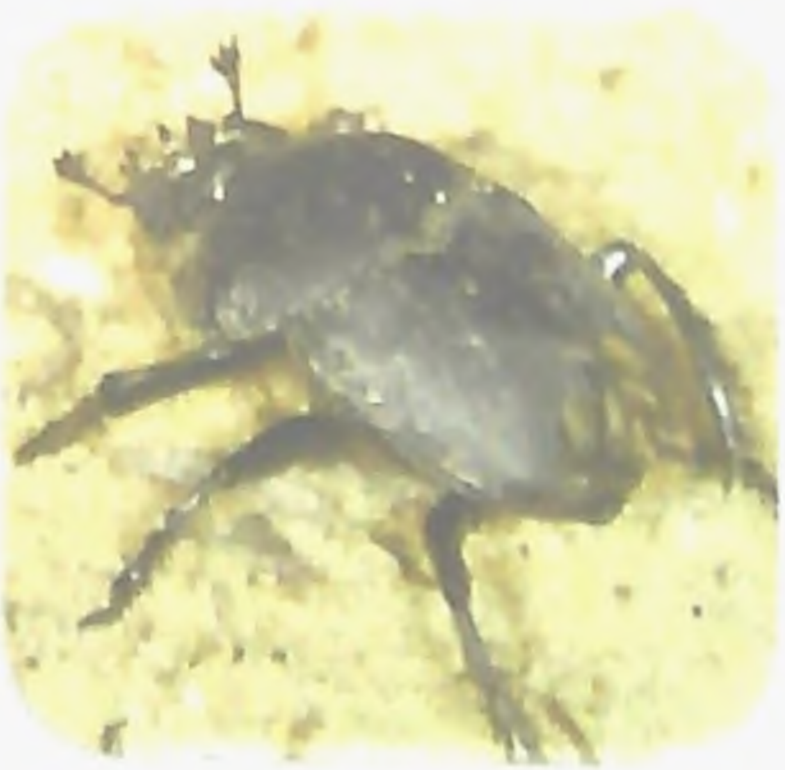

Plate 14. *Scarabaeus sacer*

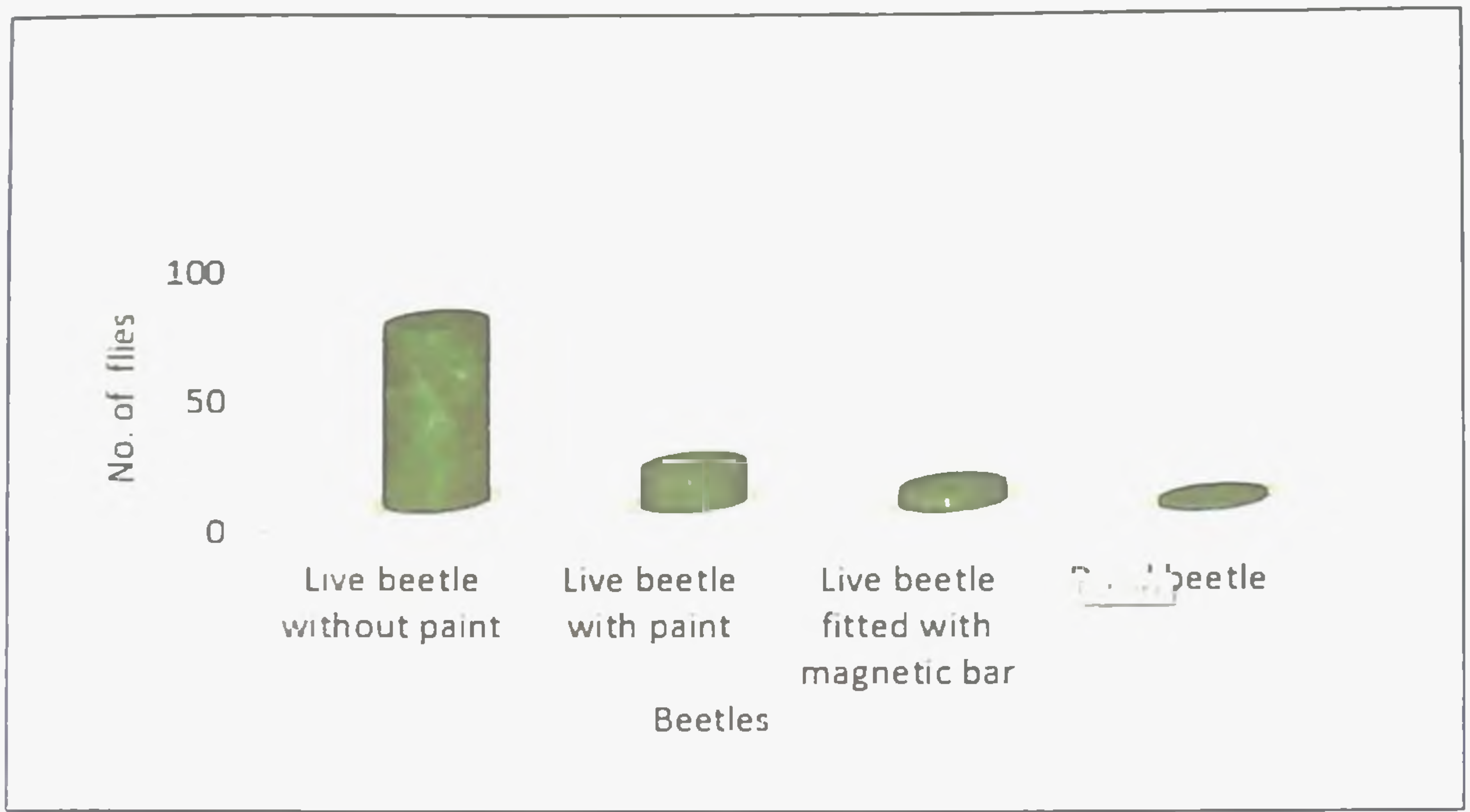

Figure 2. Attraction of *Ceroptera rufitarsis* towards dung beele, *Scarabaeussacer*

Like chemical and visual cues, auditory cues also play an important role in phoront seeking. Egg parasitoid *Telenomus calvus* identifies its phoront spined soldier bug, *Podisus maculiventris* by specific vibrations produced from hemelytra of the bug (Lumann *et al.*, 2009)

3.2 Attachment with phoront

After finding specific phoront, the phoriont attaches to the phoront. Certain phorionts have special structures like chelicera, sucker plate and anal pedicel which are used for attachment with phoront. Chelicerae are the mouth parts of mites. In the case of phoretic mite *Macrocheles muscaedomesticae*, chelicera is modified into toothed structure for the attachment with phoront stable fly, *Stomoxys calcitrans* (Axtell, 1964).

Plate 15. Chelicera of *Macrocheles muscaedomesticae*

Plate 16. *Macrocheles. muscaedomesticae* on stable fly

Deutonymphs of Uropodina (the third type) attach themselves to their phoront by means of anal pedicel. The secretion that builds the pedicel is produced by the gland that opens at the back of the alimentary canal. The secretion hardens when it comes in contact with air, and forms a stalk that integrates the deutronymph with an insect (Greenberg and Carpenter, 1999).

Plate 17. Anal pedicel of mite

Phoretic mites of family uropodidae and parasitidae exhibit special stage called hypopus stage. This stage is characterised with special structure called suckerplate. It is present at the caudal region of mites and aid in tight attachment with phoront (Hall, 2010).

Plate 18. Hypopus stage

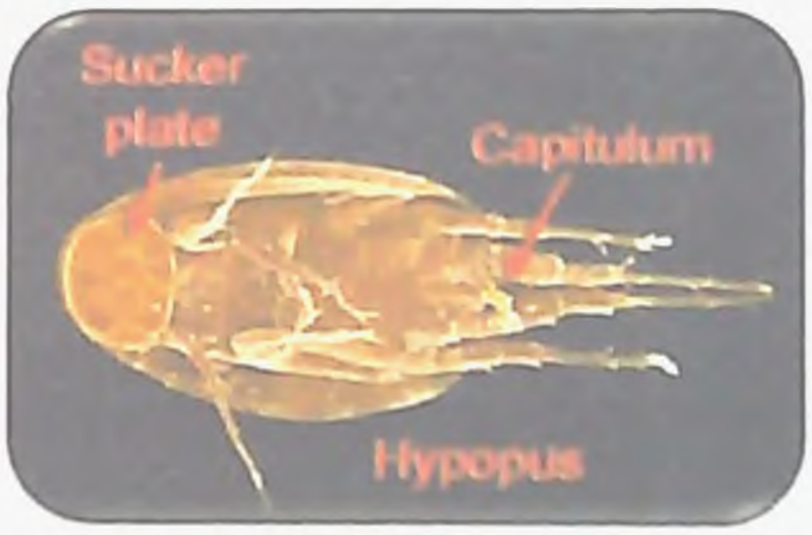

Plate 19. Suckerplate of uropodid mites

3.3 Disembarkment.

Disembarkment or detachment is the final stage of phoresy. In this stage phoriont detaches from its phoront when the suitable habitat is reached. Disembarkemnt is taken place according to the cues from the new and potential habitat. Nematode *Caenorhabditis japonica* is found to be phoretic on borrower bug, *Parastrachia japonensis*. They feed on nymphal cadaver of the bug. The detachment of nematode from the bug depends on the presence of nymhal cadaver (Kaya and Gaugler., 1991).

Plate 20. *Caenorhabditis japonica*

Plate 21. *Parastrachia japonensis*

4. Evolution of phoresy

Phoresy may begin with unrelated organisms moving independently among shared habitats. Relationship between a phoriont and a phoront can be established when some of the members of the population incidentally and randomly climb on board and are then delivered to a habitat suitable for population growth. If encounters are repeated and consistent, phorionts develop cues to the most productive of these associations. Successful relationships become even more specialized and eventually evolved into phoresy (Houck and Connor, 1991). A well-documented case of evolution of non – phoretic association into phoresy is in blister beetle. Blister beetles are largely known in the biological literature because of their importance for applied science (biological control of grasshoppers, pharmacology, veterinary and agricultural problems) as well as their distinctive biology (hypermetamorphic development, parasitoid larval habits, defensive attributes, and diverse courtship behavior) (Giulio *et al.*, 2014).

One of the most recent classifications of Meloidae is based on the evolution of phoretic first-instar larvae in the family. Family Meloidae form three subfamilies such as Meloinae, Eleticinae and Nemognathinae. Triungulins of subfamily Meloinae are comparatively gregarious and active than Eleticinae and Nemognathinae. In course of time, some triungulins of Meloinae evolved for phoresy and they are included under a new tribe called Meloini. These triungulins have morphological and physiological adaptations for enhancing phoresy. They have well developed sensory organs and legs. Presence of nine homologous spiniform and directed forward fronto-clypeal setae on medial anterior part of the head, ventrally denticulate mandibles, with teeth representing the apices of longitudinally serial, rounded indentations and parietalia with unique imbricate, squamiform microsculpture are highly specialized phoretic strategy of triungulins of Meloini. These morphological modifications enhances attachment with the abdominal intersegmental membranes of the phoront (Bologne and Pinto., 2001).

Plate 22. Triungulins of Nemognathinae

Plate no. 23 Triungulins of Meloini

Triungulins of blister beetle, *Meloe franciscanus* phoretic on digger bee *Habropoda pallida* They feed on pollen grains and developing egg, larvae and pupae of bee. The female beetle lay egg on soil. The emerged larvae will aggregate together on any vegetation near to them. They mimic the appearance of a female bee and produce a sex pheromone produced by females. Male bees confuse the larvae aggregate for a female and attempt to mate. This results in the larval aggregation attaching to the underside of the male. When the male finally finds a female and mates, the beetle larvae transfer themselves to the female and get transported back to the nest where they feed on the developing egg, larvae, pupae and pollen collected by the female for her offsprings (Leslie and Millar., 2006).

Plate 24. *Meloe franciscanus*

Plate 25. *Habropoda pallida.*

4.1 Phoresy to parasitism.

In some instances, phoretic association can become an intermediate step that grades into parasitism when the phoretic finds a way to get a meal as well as transport from the phoront.

Evolution of phoresy into parasitism is well explained in case of egg parasitoids. Egg parasitoid *Telenomes evanescense* phoretic on sixty five different phoronts. This species learn to hitch-hike specifically with mated female butterflies and after one successful transport leading to an oviposition into freshly laid eggs. So the phoretic parasitoid evolved into more specific association *i.e.* parasitism. They identify phoronts based on certain specific cues (Hulgens and Martins, 2010).

A well-documented case of a phoretic relationship becoming parasitic is that of the predatory mite *Hemisarcoptes* spp. and the coccinellid beetle *Chilocorus* spp. Both feed on diaspid scale *Aonidiella aurantii* and the association originated as a phoretic relationship. However, coccinellid beetles are reflex bleeders and *Hemisarcoptes* has become adapted to the reflexed alkaloid toxins in the haemolymph and use it for their molting. Because of feeding and completion of ontogenesis are part of the contribution of the phoront and this relationship has graded into parasitism. Other related members of the same mite family (Hemisarcoptidae), which use other phoretic hosts, remain phoretic. This is a good evidence that phoresy can be an end point and that it can also progress to other forms of symbiosis like parasitism (Gerson and Schenider, 1982).

5.Significance of phoresy

Phoresy is a simple symbiotic association aid in dispersal of organism into new habitats. There is immense scope for phoresy in agriculture and allied fields, provided the dispersed phoriont is a pest, natural enemy or decomposers.

5.1 Dispersal of pests

The coconut mite, *Aceria guerreronis* attacks young fruits of the coconut palm, *Cocos nucifera* (Andre *et al.*, 2011). Although the mites are small and populations can be extremely large. Their feeding can cause scarring and distortion of fruit, which may cause premature fruit drop. It is one of the most serious arthropod pests of coconut palm. Coconut mites probably disperse from one palm to another on air currents or by phoresy. Stingless bee *Trigona spinipes*, Italian honey bee *Apis mellifera*, Snout moth *Atheloca subrufella* and weevil *Parisoschoenus obesulus* are important phoronts used by coconut mite for its dispersal. When these phoronts move from an infested palm to healthy one it enhances dispersal of mites. It is important for understanding the process of colonization of coconut perianth mite *i.e.* essential for the improvement of control strategies of this serious coconut pest (Galvae *et al.*, 2012)

Plate 26. ***Aceria guerreronis***

Plate 27. ***Parisoschoenus obesulus***

Varroa mite *Varroa jacobsoni* is a highly destructive pest of Italian honey bee, *Apis mellifera*. It is a small mite that causes precocious reduction in foraging, increased drifting and high mortality of honey bees by parasite developing larvae and pupae in the bee colony. The varroa mite life-cycle can be divided into two stages, the phoretic stage and larval stage. In phoretic stage, mites are dispersed by adult bees (Tesfay, 2014).

Plate 28. ***Varroa jacobsonii***

Plate 29. ***Apis mellifera*** **with mite**

Dispersal and colonization of new areas by armored scale insects (Hemiptera: Diaspididae) is achieved by mobile first-instar nymphs, called crawlers. Crawlers are capable of actively wandering over short distances (generally < 1 m), their dispersal over longer distances is mediated through phoresy. Oleander scale, *Aspidiotus neri* is a serious pest of lemon dispersed by three insects such as

Argentine ant, *Musca domestica*, *Cryptolaemus montrozieri* and *Linepithe mahumile*. The crawlers use special structures to attach themselves to their phoront insects. They have four hairs on the end of each of their legs and these hairs end in a suction cup-like structure, reminiscent of the attachment structures possessed by phoretic mites (Castillo *et al.*, 2010).

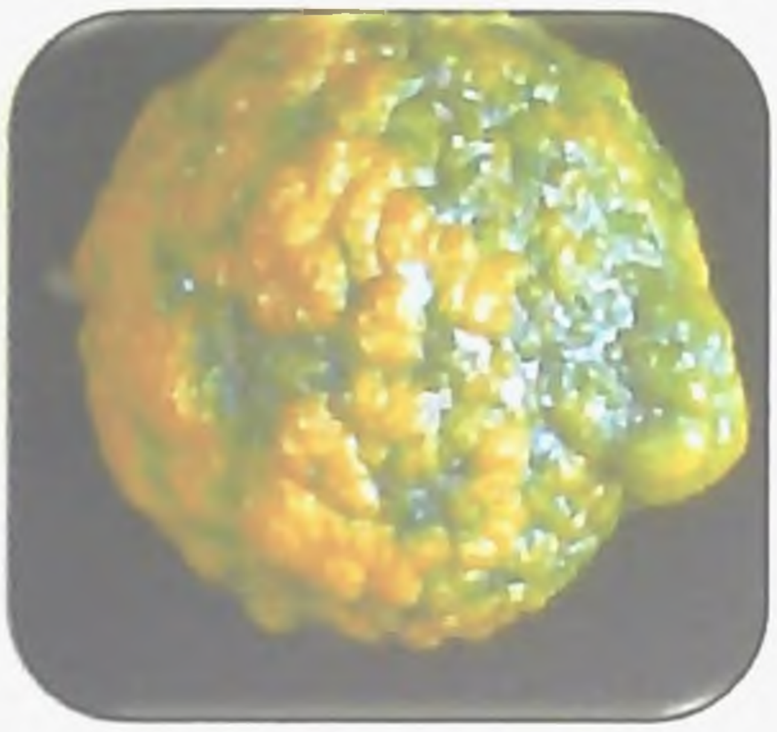

Plate 30. ***Aspidiotus neri*** **on lemon**

Plate 31. ***Linepithema humile***

5.2 Dispersal of natural enemies

Natural enemies that limit crop pests are key components of integrated pest management programs. Important natural enemies of insect and mite pests include predators and parasitoides. Phoresy plays an important role in the dispersal of natural enemies in the agro ecosystem. Predatory mite *Hemisarcoptes coccophagus* and *H. cooremani* are phoretic on three predatory beetle species *Chilocorus bipustulatus*, *C. cacti* and *C. infernalis*. Both mites and beetles are predacious on red scale, *Aonidiella aurantii*, which is a serious pest of citrus (Hill *et al.*, 1993).

The egg parasitoid *Trichogramma brassicae* is dispersed through phoresy. It is a parasitoid on cabbage white butterfly, *Pieris brassicae* This wasp identify the butterfly by a anti aphrodisiac pheromone benzyl cyanide.The pheromone is transferred by male butterflies to females during mating to enforce female monogamy. On detecting the anti-aphrodisiac, the tiny parasitic wasps ride on the mated female butterfly to reach the nest and then parasitize her freshly laid egg (Fatouros *et al.*, 2016).

Plate 33. ***Trichogramma brassicae***

Plate 33. ***Pieris brassicae***

5.3 Pollination

Bees, butterflies, wasps, flies, ants and beetles are important pollinators of flowers. Certain flower mites are phoretic on these pollinators and enhance pollination. Flower mite, *Parasitellus fucorum*are phoretic on buff-tailed bumblebee, *Bombus terrestris*.Both are pollinators of sunflower. Mite, *Proctolaelaps* spp. are commonly found on butterflies and moths. Mite *Xanthippe* sp. apparently feed on pollen and nectar in the inflorescences of date palm and is phoretic on nitulid beetle, *Stelidotaocto maculata* that pollinate the palm .

Plate 34. ***Parasitellus fucorum*** **on buff-tailed bumblebee,** ***Bombus terrestris***

5.4 Decomposition of carrion

Carrion is a highly attractive resource to a diverse array of arthropods. Compared with most other forms of detritus, carrion generates a very intense but brief hotspot of biological activity, including the rapid arrival and exploitation by arthropods. This activity results in the recycling of energy and nutrients through different organisms and trophic pathways, making it a critical part of ecosystem functioning. Two abundant groups of arthropod at carrion are beetles and mites (in addition to flies) Mites are a hyper-abundant component of the arthropod fauna at carcasses, where they may predate on fly larvae or nematodes, and scavenge on animal remains. Many mite species found at carrion are phoretic and they use flies and beetles for their dispersal (Perotti and Braig, 2009).

The role of phoretic mites in decomposition of human and animal carcasses are applied in forensic entomology. Forensic entomology is the study insects and other arthropod biology to solve criminal investigations. Insects arrive at a decomposing body in a particular order and then complete their life cycle. More than 212 phoretic mite species associated with carcases have been reported and among these, mites belonging to the order Mesostigmata form the dominant group, represented by 127 species. The composition of the phoretic mite assemblage on a carcass might provide valuable information about the conditions of and time elapsed since death. Sexton beetle, *Necrophorus* beetles often carry on their bodies the mite *Poecilochirus carabi*. These beetle are present on four week old dead bodies. If any chance the beetle is not present in the deadbody, the phoretic mite can act as the trace indicator of its carrier. Thus time of death can be determined through post mortem (Barton *et al.*, 2014).

Plate 35. ***Poecilochirus carabi***

Plate 36. Sexton beetle, ***Necrophorus vespilloides***

Table 2. Phoretic mites present on decaying carrion

Phoretic mite	Carrier insect	Reference
Ancistrocheles bregetovae	*Cochliomyia macellaria*	Norton, 1980
Glyptholaspis sp.	*Phaenicia eximia* *Cochliomyia macellaria*	OConnor, 2009
Macrocheles trogicolis	*Aphodius* spp *Onthophagus* spp.	Fain and Miessen 1997
Macrocheles penicilliger	*Trox scaber* *T. sabulosus* *T. tuberculatus* *T. sugayai*	Takaku and Yoshida, 2000
Parasitus coleoptratorum	*Onthophagus* spp.	Willson and Knollenberg, 2001
Poecilochirus sp.	*Necrodes surinamensis* *Necrophorus vespilloides*	Perotti and Braig, 2013

6. Conclusion

Phoresy is an important ecological phenomenon, which enhances the diversity and complexity of community interactions within and among habitats. Phoresy acts as connecting link for evolution of simple associations into higher and more specific associations like parasitism. It plays a key role in the dispersal of pests, natural enemies and other beneficial organisms into the agro ecosystem. So it has immense scope in integrated pest management. It is a prevalent form of commensalism among insects and one of the least understood potential ecological interactions. However, there is a need for more research works in this area for understanding the role of phoretic associations in dispersal of pest and beneficial organisms.

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8. Discussion

1. What is the relation between commensalism and phoresy?

Commensalism is a type of symbiotic relationship between two organisms where one organism benefits from the other without affecting it. Phoresy is a type commensal relation, where one organism associated with the other for their dispersal.

2. What is significance of phoresy in pest management ?

Pests like coconut mite, *Aceria guerreronis* and oleander scale, *Aspidiotus neri* are mainly dispersed through phoresy. By understanding the phoront insect aid in dispersal of pest, we can control the population of the pest through managing the phoronts.

Certain natural enemies are also dispersed through phoresy. Egg parasitoids like *Telenomus calvus, Trichogramma japonica, Telenomus evanescense* identifies its phoronts by sex pheromones. These sex pheromones can be used for attracting egg parasitoid. Thus we can control specific pests.

3. What is the reason behind phoretic association between *Ceroptera rufitarsis* and dung roller beetle ?

Ceroptera rufitarsis is a minute fly inhabited on cowdung pits. They are phoretic on dung beetle, *Scarabaeus sacer*. These beetles have a characteristic feature that, it will rolls down dung where ever they found. So these flies phoretic on the beetle i.e they will get both food as well as transport from the phoront.

4. What are different types of symbiotic associations?

Symbiosis is close and often long-term interaction between two different biological species. It is of four types such as parasitism, commensalism, ammensalism and mutualism. A parasitic relationship is one in which one member of the association benefits while the other is harmed. Commensalism is a relationship between two living organisms where one benefits and the other is not significantly harmed or helped. Amensalism is the type of relationship that exists where one species is inhibited and another one is unaffected. Mutualism is a relationship between individuals of same species where both individuals benefit.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Entomology

ENT 591: MASTER'S SEMINAR

Name: Chandini S.M | Venue: Seminar hall

Admission No: 2014-11-150 | Date: 11-12-2015

Time: 10.45 a.m.

Phoresy: Hitch-hiking on insects

Abstract

Phoresy is a special kind of relationship in which one organism (phoriont) attaches to another (phoront) for a limited time period to enhance dispersal from the natal habitat to a new one. Organisms with small size, limited mobility and inhabiting transient habitats such as mites, nematodes and small insects usually resort to phoresy for dispersal. Insects are the most popular phoronts due to their capacity for flight, abundant population and diversity of their habitats(Binche, 1994).

Phoresy can be classified either as obligate or facultative. Based on the specificity of phoront, phoresy is further classified as euryxenous, stenoxenous and oioxenous(Binche, 1994).

Phoresy involves different stages such as searching of phoront, attachment to phoront and disembarkment. The phoriont is guided by chemical, visual and auditory cues during each of these stages. Phorionts often have special structures like chelicera, anal pedicel and sucker plate for getting attached to the phoront. Disembarkment is the final stage where the phoriont detaches from its phoront upon receipt of appropriatestimuli from the new habitat (Morand and Benche, 1993).

Phoresy plays an important role in the dispersal of pests such as coconut perianth mite (*Aceriaguerreronis*), varroa mite (*Varroajacobsoni*) andoleander scale (*Aspidiotusnerii*) (Galvaet *al*., 2012). Similarly several natural enemies also reported to hitch- hike for dispersal. Fatouroset *al*., (2006) reported that the egg parasitoid, *Trichogrammajaponicum* was dispersed by cabbage butterfly, *Pierisbrassicae*. Similarly, Houck (1999) reported that the predatory mite, *Hemisarcoptersmalus* was dispersed by lady beetle, *Chilocorus*sp.

The role of phoretic mites in decomposition of human and animal carcasses is applied in forensic entomology. Phoretic mites can act as trace indicators of their phoronts and thus help in estimating the time of death (Barton *et al.*, 2014).

Phoresy appears to have evolved independently across Animal Kingdom. It is believed to have its origin in casual association between organisms with habitats. Many consider phoresy as an intermediate step in the evolution of higher association like parasitism, as in the case of mite *Hemisarcoptes* and the lady beetle *Chilocorus*(Bologna *et al.*, 2001).

Phoresy benefits the individual, but it can also enhance the diversity and complexity of community interactions within and among habitats. It has immense scope in pest management and applied science. However, there is a need for more research works in this area for understanding the role of phoretic associations in dispersal of pests and beneficial organisms.

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Killing for a living – predator prey interactions in insects

By

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(2014-11-150)

M. Sc. Agrl. Entomology

Seminar report

Submitted in partial fulfilment of requirement of the course

Ent. 591 Seminar (0+1)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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DECLARATION

I, Neenu Chandran (2014-11- 147), hereby declare that the seminar report entitled **'Killing for a living – predator prey interactions in insects'**, has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Neenu Chandran

2014-11-147

Vellanikkara

Date: 27.01.2016

CERTIFICATE

Certified that the seminar report entitled **'Killing for a living – predator prey interactions in insects'** for the course Ent.591 has been prepared by Neenu Chandran (2014-11-147), after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

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CERTIFICATE

Certified that the seminar report entitled '**Killing for a living - predator prey interactions in insects**' is a record of seminar presented by Neenu Chandran (2014-11-147) on 30th October, 2015 and is submitted for partial fulfilment of requirement of the course Ent.591.

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1. INTRODUCTION

The term predation is derived from the Latin word '*praederi*' which means 'to plunder' or 'to take by force'. Predation evolved independently several times in different group of organisms over millions of years and is considered to be the major driving force which have led to the evolution of vertebrates.

A predator is an animal that hunts and kills other animals for food and the prey is a term used to describe animals that are hunted and killed by predators. According to Coppel and Mertins (1977) predator is defined as "an animal which feeds upon other animals (prey) that are usually smaller and weaker than itself, frequently devouring them completely and rapidly". Dhaliwal and Arora (1996) defined predator as a free living organism throughout its life, it kills its prey, is usually larger than its prey and requires more than one prey to complete its development.

The interaction between a predator and its prey is the most powerful driving forces behind evolution. Predation is a strong selective pressure that drives prey organisms to find ways to avoid being eaten. Prey organisms that are difficult to find, catch or consume are the ones that will survive and reproduce. The result is that over evolutionary time, prey organisms have developed a stunning array of strategies to avoid being eaten.

About 25% of the insect species are predators or parasitoids. Nearly every order of insect contains predatory species. Some species are only predatory as immatures, others as adults, and still others are predatory throughout their life span. Some orders are entirely predatory like Odonata, Mantodea, Mecoptera and Neuroptera.

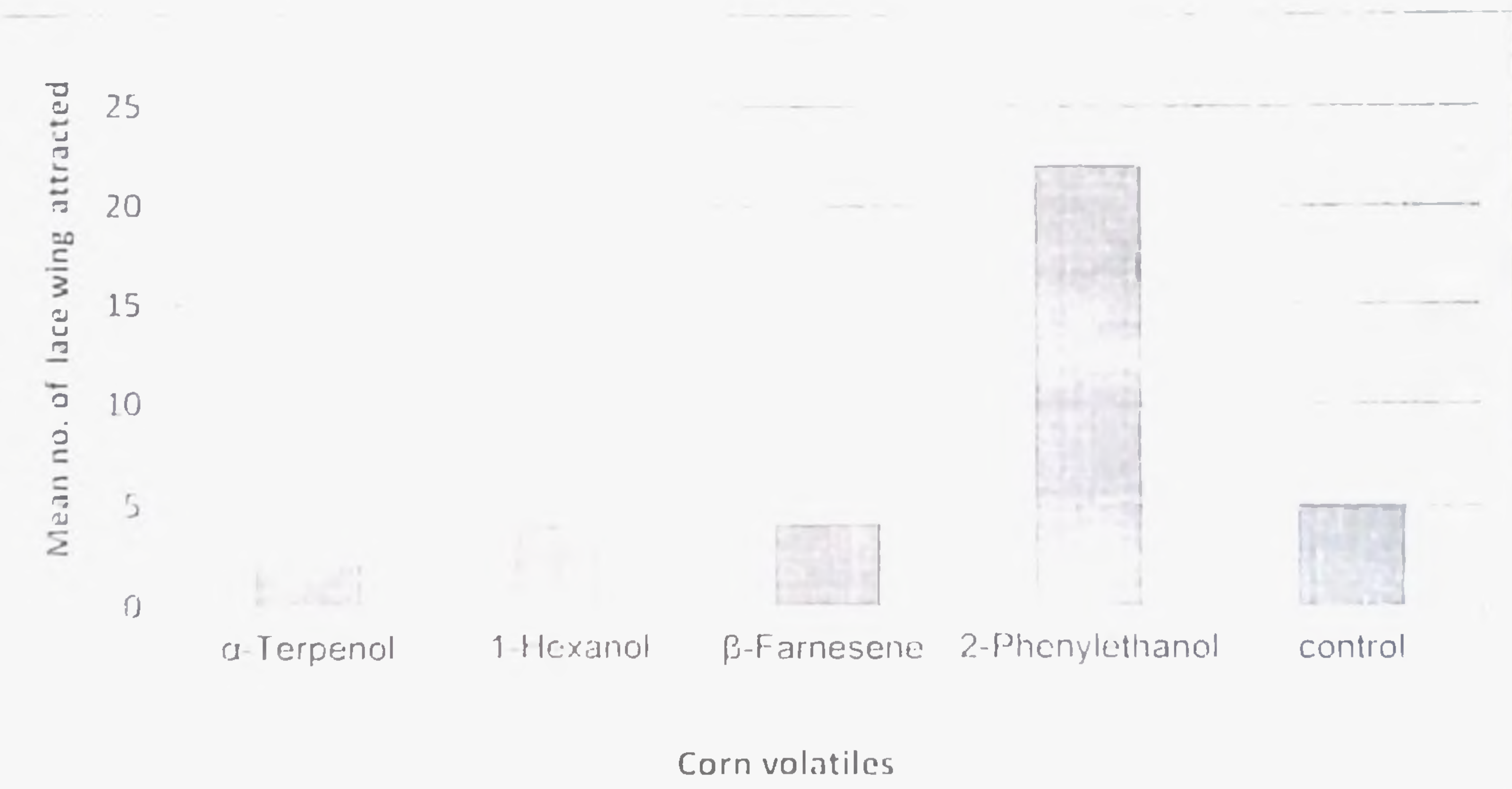
2. STEPS IN PREDATION

Predation involves four steps *i.e.* search, recognition, capture and handling.

2.1 Search: Predators search the environment for acceptable prey. Predator adaptations to improve foraging success include better visual acuity, development of a search image and limiting searches to prey-rich habitats. Search involves both habitat finding and prey finding.

2.1.1 Habitat finding: Predator is oriented towards prey rich habitats, mediated by long range cues like plant volatiles. For instance, an experiment was conducted by Zhu *et al.* (2005) in USA for eliciting the importance of corn volatiles in habitat finding by predatory green lace wing. They prepared traps using different corn volatiles such as α-terpenol, 1-hexanol, β-farnesene and 2-phenylethanol. Among these corn volatiles, 2-phenylethanol attracts more than 20 insects which indicate that there are specific cues for attraction of green lace wing to the corn ecosystem (figure 1).

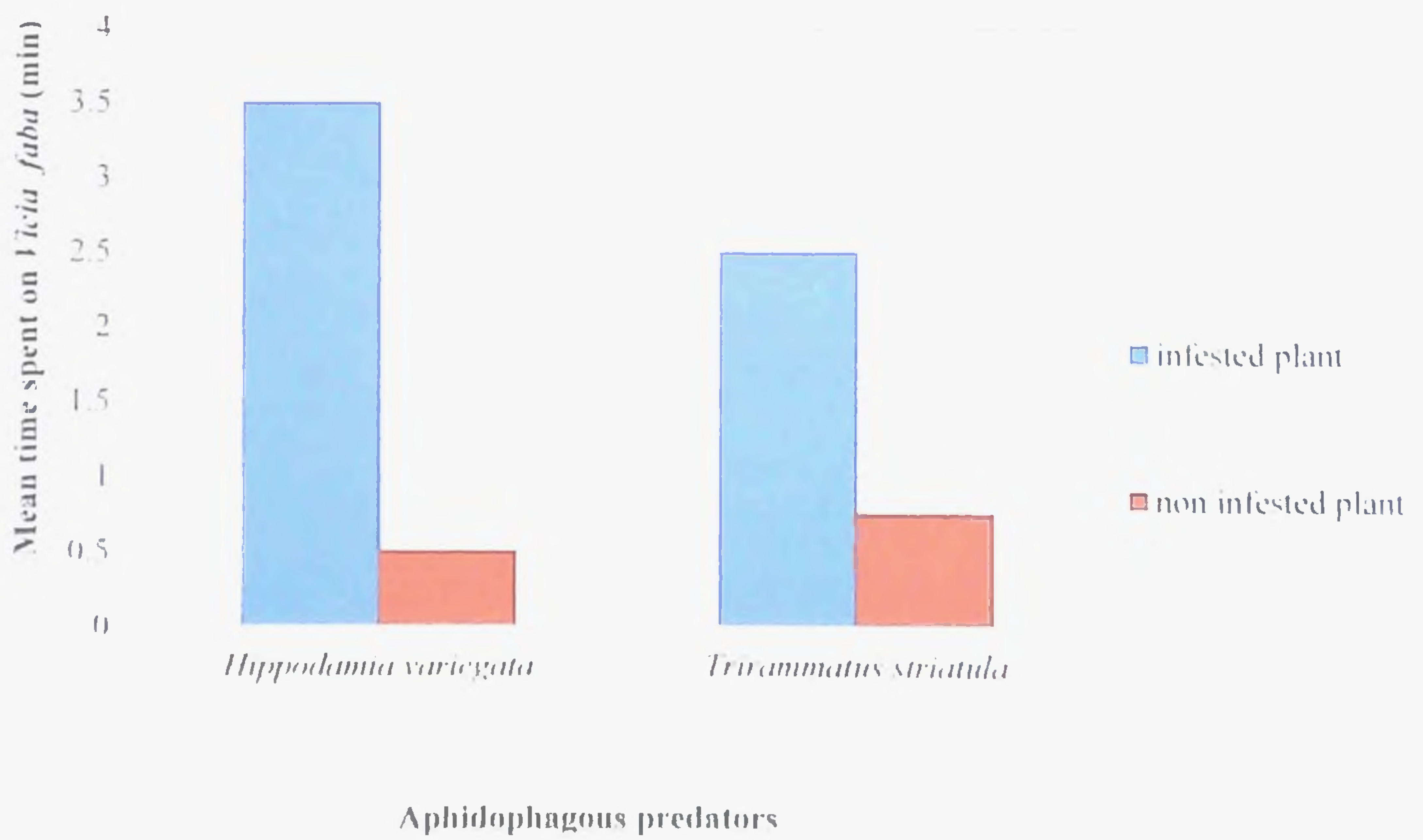
Figure1. Attraction of green lace wing to the plant volatile



2.1.2 Prey finding: It can be considered as process of actually finding suitable prey within the habitat. Prey finding is mediated by short range cues. It is often classified into two, i.e. random foraging and directional foraging. Random foraging is otherwise known as meat and eat strategy. Usually the immature stages of those predators show random foraging. Here the predators share similar habitat with their prey and capture the prey upon contact. Eg. Aphidophagous syrphid fly maggot. In directional foraging the predator orient to the prey from a distance with the help of visual, olfactory and acoustic cues. For example, the European bee wolf will be attracted towards any flying objects with similar size and shape of Italian bee. The carabid beetle utilises β-farnesene from aphid honey dew as an olfactory cue and certain ceratopogonid midges find their prey midges with the aid acoustic cue.

Figure 2 shows the importance of olfactory cues in prey searching by aphidophagous predators in Vicia faba. Olfactometries contrasting non-infested vs. infested plants with *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) were performed to study olfactory prey-searching in *Hippodamia variegate* (Coleoptera: Coccinellidae) and *Trirammatus striatula* (Fabricius) (Coleoptera: Carabidae). *H. variegata* and *T. striatula* were attracted to infested plants when contrasted with non-infested plants. These results corroborated the importance of semiochemicals produced by herbivory in the prey-searching behaviour of aphidophagous predators. In addition, presence of predators on the foliage may favour emission of aphid alarm pheromones, which could attract *H. variegata* and *T. striatula* (Tapia *et al.*, 2010).

Figure 2. Olfactory cues mediating prey searching behaviour in aphidophagous predators



2.2 Recognition: Predators quickly learn prey types and adapt to recognize prey and to avoid inedible species. Very specific visual, olfactory and gustatory stimuli involves in prey detection. The European bee wolf, *Philanthus triangulum* recognizes Italian bee, *Apis mellifera* by the presence of cuticular chemical (Z)-11-eicosen-1-ol. Whereas in case of predatory carabid beetle, *Anchomenus dorsalis* aphid honeydew sugar act as the stimuli (Merivee *et al.*, 2012). Figure 3 indicates the role of insect volatiles on prey recognition by *Philanthus triangulum*. Herzner *et al.* (2005) conducted an experiment using differently treated honey bee dummies and they recorded the attack rate by *Philanthus triangulum* in each category. The attack rate was around 45 per cent when the honey bee dummies are treated with natural honey bee extract. The honey bee dummies treated with a combination of hydrocarbon and (Z)-11-eicosen-1-ol shows 80 per cent attack rate whereas, literally no attack was observed when it was treated with hydrocarbon alone. This shows the importance of (Z)-11-eicosen-1-ol in prey recognition by *Philanthus triangulum*.

Figure 3. Role of insect volatiles on prey recognition by *Philanthus triangulum*

2.3 Capture: After detecting suitable prey organism, the predator must be able to capture it. Adaptations to improve capture efficiency include both morphological and behavioural modifications.

2.3.1 Morphological adaptations: The morphological adaptations are usually appendage modifications. In some predators the legs are modified for capturing and holding their prey. For example the raptorial forelegs in case of mantidfly and the basket forming legs in dragonflies. Also the mouthparts are variously modified in predatory insects. Eg. Horny proboscis in assassin bug, long sickle shaped mandibles of larval antlion, prehensile labial mask of dragonfly naiad.

2.3.2 Behavioural adaptations: Insect predators show a large number of behavioural adaptations for capturing the prey such as thanatosis, ambush predation and trapping

a. Thanatosis: The insect play dead. It is reported in the predatory Pselaphid beetle *Claviger testaceus* which prey upon the ant, *Lasius flavus*. The beetle shows feigning death infront of *ant* colony and chemically mimics insect cadaver. The worker ants carry this cadaver into the brood chamber. Once within the ant's colony the beetle become active and start feeding on egg, larva and pupa of ant.

b. Ambush predation: The predator remains in one place and strike at acceptable prey when they come within their range. Usually insect vision is limited to recognition of movement so that a non-moving predator is undetectable to a potential prey organism. Many of the ambush predators are cryptic, resembling things like bark, lichen, or leaves or some of them mimic an object that prey are attracted to like flowers. Eg. Orchid mantis concealed in the orchid flowers waiting for prey to pass by.

c. Trapping: Predators construct traps in which to capture prey. This could be considered as a form of ambush predation where the predator waits near the trap then pounces on the prey once it is ensnared in the trap. For example the snares of New Zealand Glowworm, *Arachnocampa luminosa*. The larva spins a nest out of silk on the ceiling of the cave and then hangs down as many as 70 threads of silk called snares from around the nest, each up to 30 or 40cm long and holding droplets of mucus. Larvae glow to attract prey into their threads, perhaps luring them into believing they are outdoors, for the roof of a cave covered with larva can look remarkably like a starry sky at night. Prey include midges, mayflies, caddisfly, mosquito, moth or even small snail or millipede. When prey is entangled in a snare, the larva pulls it up by ingesting the snare and starts feeding. Hydropsychid caddisfly larvae, an aquatic predator construct nets in which to trap prey and also the pit excavated by antlion grub is a highly efficient trap to capture prey.

2.4 Handling : Predators must handle prey by efficiently subduing them and detoxifying any defensive compounds. Adaptations promoting handling efficiency include improved foraging appendages to reduce the probability of injury and physiological specialization like secretion of toxins for rapid paralysis of the prey. The assassin bug species *Platymeris rhadamanthus* is capable of spitting venom up to 30 cm. The saliva of this insect, loaded with a mixture of at least six proteins including excess amounts of protease, Hyaluronidase, and Phospholipase causes intense local pain, Vasodilation, and edema in prey organism (Edwards, 1962). The wasps often paralyze their prey with a venomous sting which is the modified ovipositor.
Predators also improve foraging efficiency by learned avoidance, a behavior in which predators quickly learn to recognize poisonous or distasteful species by remembering adverse reactions from attempted predation events.

3. PREY DEFENCE

The prey defend its predator either by evasion or by active resistance to the predator attack. Prey defense or anti-predator adaptations are mechanisms developed through evolution that assist prey organisms in their constant struggle against predator. During evolution insects have developed an impressive set of mechanisms to defend themselves against natural enemies. This includes adaptations in their morphology, behaviour, physiology, and chemistry (Evans and Schmidt,1990). Predation pressure is one of the most important selective forces resulting in the evolution of escape behavior, crypsis, aposematism, chemical defense (Lima and Dill,1990) and possibly the most extreme defense like sacrificing a limb or other appendage to the predator i.e. autothysis and suicidal altruism.

3.1 Morphological adaptation: Many species of slug caterpillar (Limacodidae) have numerous protuberances and stinging spines along their dorsal surfaces. Species that possess these stinging spines suffer less predation than larvae that lack them. Another type of defensive structure called osmeterium, an eversible glandular process is present in papilionid caterpillar. They secrete repellents to avoid predation. Strong mandibles of termites, ants, wasps, and bees are also aid in anti predation.

3.2 Mechanical adaptations: Caddis fly larva evolved a special type of evasion mechanism by creating retreat from debris. They make shelter using debris over their body and it will act like a shell. This make most unwelcome to the predators.

3.3 Behavioural adaptations: Most widely adopted mechanisms of antipredation by insects are behavioual adaptations. This is mostly developed from the knowledge of previous predatory events through insect learning process.

3.3.1 Diematic behavior: The prey startles their predators by sudden display of eyespots or bright colours on the hidden body parts mostly on the hind wings. In case of owl butterfly, *Caligo* spp. when a potential predator approaches, they suddenly spread their hind wings which bear large eyespots which resembles the owl's eye.

3.3.2. Thanatosis: When a potential predator approaches certain prey will play dead. They release grip from the substrate, fall down and remain motionless. Eg. Pale Green Weevil, *Polydrusus impressifrons*

3.3.3. Protective colouration: Over the course of evolution insects develop certain adaptations to protect themselves from predation. The prey learned from previous predation event that certain insects are avoid risk of predation by merging with their background. This type of prey defense is termed as crypsis. It may either by camouflage or by masquerade. In camouflage the insect merges with their background like in case of sand dune grasshopper, katydid and geometer moth whereas in masquerade prey imitates inanimate objects like dry leaf as in case of katydid and camo moth and twig by stick insect.

3.3.4 Warning colouration: It is also called aposeimatism. Unpalatable or otherwise unprofitable prey species sometimes advertise their unsuitability to predators with bright colouration (Edmunds, 1974). The red colour in Milk weed bug, *Oncopeltus fasciatus*, orange colour in Velvet ant *Dasymutilla occidentalis* and yellow colour in Yellow jacket

Dolichovespula sp. Indicates the presence of toxins in them. The predator avoids such type of prey organisms based on their previous knowledge.

3.3.5. Mimicry: It is a false advertisement to the predators regarding the prey organism. The palatable insect mimics distasteful model so that they can escape from the risk of predation. A classical example for mimicry is the palatable viceroy butterfly, *Limenitis archippus* mimicking unpalatable monarch butterfly, *Danaus plexippus*

3.3.6. Autotomy: It is an active resistance mechanism where the self-amputation of a body part occurs, often as a reflexive action and along a predetermined breakage plane. It has been observed in a wide variety of taxa in defense against conspecifics or nonspecifics (Maginnis, 2006). Eg. Stick insects.

3.3.7. Phragmosis: Blocking of the nest entrance with any part of the body. In case of ant, *Cephalotes varians* nest ebtrance is blocked by the broad disc shaped head of soldier.

3.4. Chemical defense: The defensive chemicals where obtained by the insects either intrinsically or extrinsically. Many compounds are derived from the main food source of insect whereas other insects are able to synthesize their own toxins. Pasteels *et al.* (1983) have divided chemical defenses into different classes such as true poisons, repellents and other secretions

3.4.1 True poisons/class I compounds: interfere with specific physiological processes or act at certain sites which include butadienolides, cantharidin, cyanides, cardenolides, and alkaloids. Cardinolides present in milk weed plant is sequestered by milk weed bug, *Oncopeltus fasciatus* is used against its insect predators. Whereas some insects like bombardier beetle has specialized glands on the tip of its abdomen that allows it to direct a toxic spray towards predators. The spray is generated explosively through oxidation of hydroquinones with hydrogen peroxide outside its body chamber at the time of release and is sprayed at a temperature of 100 °C.

3.4.2 Repellents/Class II compounds: They irritate the chemical sensitivity of predators and stimulate scent and taste receptors so as to discourage feeding. They tend to have low molecular weight and are volatile and reactive, including acids, aldehydes , aromatic ketones, quinones, and terpenes. Ant attacks represent a large predatory pressure for many species of wasps, including the *Mischocyttarus Cerberus* (Vespidae). These wasps possess a gland located in the VI abdominal sternite (van de Vecht's gland) that is primarily responsible for making an ant repellent substance. Tufts of hair near the edge of the VI abdominal sternite store and apply the ant repellent, secreting the ant repellent through a rubbing behavior (Togni *et al.*, 2008).

3.4.3 Other secretions: Impairment of movement and sense organs is achieved through sticky, slimy, or entangling secretions that act mechanically rather than chemically. This last grouping of chemicals has both Class I and Class II properties. As with Class I and Class II compounds, these three categories are not mutually exclusive, as some chemicals can have multiple effects. For example, majority of termite soldiers secrete a rubber like and sticky chemical concoction that serves to entangle enemies, called a fontanellar gun and it is usually coupled with specialized mandibles. In nasute species of termites (subfamily: Nasutitermitinae), the mandibles have receded. This makes way for an elongated, syringic nasus capable of squirting liquid glue. When this substance is released from the frontal gland reservoir and dries, it becomes sticky and is capable of immobilizing attackers. It is highly effective against other arthropods, including spiders, ants, and centipedes.

3.5 Alarm pheromones: Many insects respond to the threat of predation by producing alarm signals that warn other individuals of the presence of danger. Alarm signaling benefits the fitness of the signaling individual itself, for the anti-predator or escape behaviors induced by the call reduce the probability of successful predation (Hogstedt, 1983; Sherman, 1985) or attract the predator away from the signaling individual (Charnov and Krebs, 1974). Alarm signals may be visual or auditory as well as chemical, alarm pheromones are common among insects. There are aggregative and dispersive response produced by the insects based on the proximity of predator. In case of social insects, they will produce an aggregative response when the predator approaches their nest and dispersive response when the predator is away from the nest. Whereas in non social insect like aphid alarm pheromone stimulate dispersal of colony. Table 1 shows different alarm pheromones present in various insects.

Table 1. Alarm pheromones in insects

Insect	Alarm pheromone	Reference
Aphid, *Myzus perciceae*	(E)-β-farnesene (Ebf)	Bowers *et al.*, 1972
Ant- *Hypoponera opacior* - *Lasius fuliginosus*	2,5-dimethyl-3-isopentylpyrazine	Duffield *et al.*, 1976
- *Atta* spp.	n-undecane (formic acid)	Stoeffler *et al.*, 2007
	4-methyl-3-heptanone	Hughes *et al.*, 2001
Honey bee, *Apis mellifera*	isopentyl acetate	Boch *et al.*, 1962

3.6 Suicidal altruism

An unusual type of predator deterrence is observed in the Malaysian exploding ant (*Componotus* sp.). Two oversized, poison-filled mandibular glands run the entire length of the ant's body. When combat takes a turn for the worse, the ant violently contracts its abdominal muscles to rupture its body and spray poison in all directions. These prevent predation and serve as a signal to other enemy ants to stop predation of the rest of the colony (Shorter & Rueppell, 2012).

4. PREDATOR-PLANT MUTUALISM

Highly evolved predator plant mutualisms are commonly seen in certain ants. The tropical ant, *Allomerus* spp. present in the Amazon rainforest have a stunning array of mechanisms to trap their prey. This is reported as a classical example for tritrophic interaction. The ant has a mutualistic relationship with *Hirtella physophora*, an Amazonian tree. The ants live inside leaf pockets in the tree. So that, they will get a safe place to live, and the plant secretes nectar in the leaf pockets for feeding the ants. The *Allomerus* ants are extremely aggressive insect predators, and help protect the tree from insect pests. These ants also have a friendly relationship with a fungus, in the order Chaetothyriales. Ants use this mould to construct traps. The ants trim away some of the hair-like projections on the plant, and then rearrange them as a structural base. Regurgitated mould acts as glue to hold these in place, and the mould will continue to grow once regurgitated, providing additional support. The ants then hollow out holes in the stem and tuck themselves in, just waiting for a prey organism.

5. OUTCOME OF PREDATOR-PREY INTERACTION

Predators and prey can influence one another's evolution. Traits that enhance a predator's ability to find and capture prey will be selected for in the predator, while traits that enhance the prey's ability to avoid being eaten will be selected for in the prey. The goals of these traits are not compatible, and it is the interaction of these selective pressures that influences the dynamics of the predator and prey populations. Predator prey interaction may be highly specific and intensive. Over the course of evolution it may lead to

(a) Coevolution

(b) Change in prey breadth

5.1 Coevolution: It is a change in one species (or group) in response to a change in closely associated species group. Payne *et al.* (1984) reported that Frontalin, an aggregative pheromone produced by female pine beetle, *Dendroctonus frontalis* act as a kairomone for its predator clerid beetle,*Thanasimus dubius*

There are similar reports by Nakamuta (1991) in the case of aphids. The alarm pheromone, β-Farnesene produced by the aphid act as an olfactory stimuli for coccinellid beetle, *Coccinella septumpunctata*

5.2 Change in prey breadth

Predator prey interaction often cause change in prey breadth. There are different classes of predator based on its host specificity prey breadth.

5.2.1 Monophagous: predator species that are highly restricted in their host range. Sometimes limited to one species of prey. The vedalia beetle, *Rodolia cardinalis* feeds only on cottony cushion scale, *Icerya purchasi*. Delucchi (1954) reported that *Scymnus impexus* is monophagy on balsam wooly aphid *Adelges piceae*

5.2.2 Oligophagous: predator species with a narrow host range confined to a genus or family. Usually the prey share similar life-history traits or exist in a common habitat with the predator. *Laricobius erichsonii* feeds on different species of bark aphids, *Adelges* spp & *Pineus* spp. certain aphidiophagous coccinellids like *Adalia tetraspilota* is also consider as oligophagous

5.2.3 Polyphagous: these types of predators are also called as general feeders and they are having a wider host range. Eg. praying mantis, dragonfly, ant

6. PREDATOR PREY DYNAMICS

Predator prey dynamics refers to change in the size of population of organisms through time. The predator prey interaction may play an important role in explaining population dynamics in many species. They are a type of antagonistic interactions, in which the population of one species (predators) has a negative effect on population of a second (prey), while the second has a positive effect on the first.

6.1 Predator responses

A real predator does not harvest at a fixed rate or with a constant effort. In nature, the rate of harvest by a predator population is determined by two attributes of the predator's response to changes in prey density: functional response and numerical response. Holling (1959) explained numerical and functional response of predators.

6.1.1 Numerical response: According to Holling, numerical response is the change in predator density as a function of change in prey density. There are two types of numerical responses demographic response and aggregational response. Demographic response consists of changes in the rates of predator reproduction or survival due to a changes in prey density whereas the aggregational response is change in predator population due to immigration into an area with increased prey population

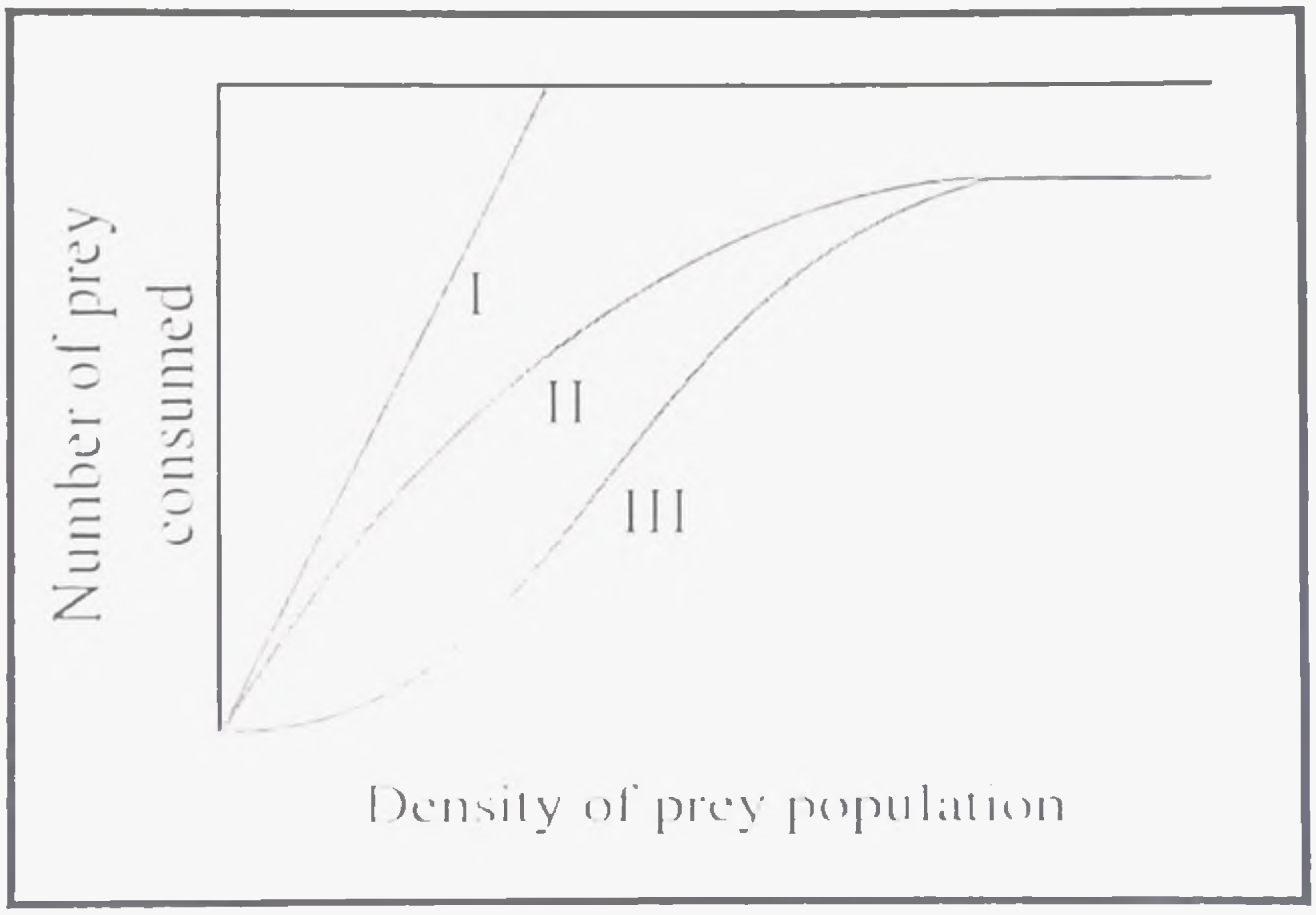
6.1.2 Functional response: Change in predator's rate of prey consumption with change in prey density is considered in functional response. It is again classified into Type I, type II and type III functional response. (figure 5)

6.1.2.1 Type I functional response: It is a linear increase in intake rate with food density, either for all food densities, or only for food densities up to a maximum, beyond which the intake rate is constant. It is found in passive predators.

6.1.2.2 Type II functional response: It is a decelerating intake rate, which follows from the assumption that the consumer is limited by its capacity to process food.

6.1.2.3 Type III functional response: This occurs in predators which increase their search activity with increasing prey density. Usually polyphagous predators follow type III response.

Figure 5. Graphical representation of functional responses



6.2 Factors influencing predator responses

As every biological system predator prey interaction is also dynamic in nature. The change in population of each component in this interacting system is interdependent. The response of predators to the changing population of prey can be influenced by several factors such as

a. Population size of the prey

b. Spatial distribution of prey

c. Obstructions in habitat

4.2.1 Population size of the prey

Most of the predators require a minimum number of preys to produce eggs and oviposit. A minimum number must be consumed by immature stages to provide energy for maintenance, searching, growth and development. This can be illustrated by several studies. Batool *et al.* (2014) confirmed the effect of prey population on biology of green lacewing, *Chrysoperla carnea*. They used *Sitotroga cerealella* eggs as feed for *C. carnea*. As the population size of the prey increases the pre-oviposition period of *C.carnea* decreases. This is because when the prey population increases the predator tends to reduce its life cycle to produce as many numbers of generations as possible before depreviating the food source. It is also observed that the number of eggs lay by the female increases with increase in food availability. This indicates the positive correlation between population size of prey organism to the functional response of the predator (table 2).

Table 2. Effect of prey population on biology of *Chrysoperla carnea*

Sitotroga cerealella (eggs/day)	Pre-oviposition period (days)	Eggs/female
20 eggs	9	7
30 eggs	9	8
60 eggs	6	15
70 eggs	5	25
80 eggs	1	12

Omkar and Srivastava (2003) demonstrated Hollings functional response with *Coccinella septumpunctata* (table 3). They observed that the consumption rate in *C. septumpunctata* was highest at the higher prey density. As the prey density increases from 50 to 800 the number of prey consumed by the predator increase whereas the per cent consumption shows a decreasing trend. When the prey population increases the the predator consumes more no. of preys but in a large population the fraction of prey consumed by the predator was too small or negligible. Prey handling time also declined as prey population increases. This is because the chances of encountering the prey were more in a large population compared to a smaller population.

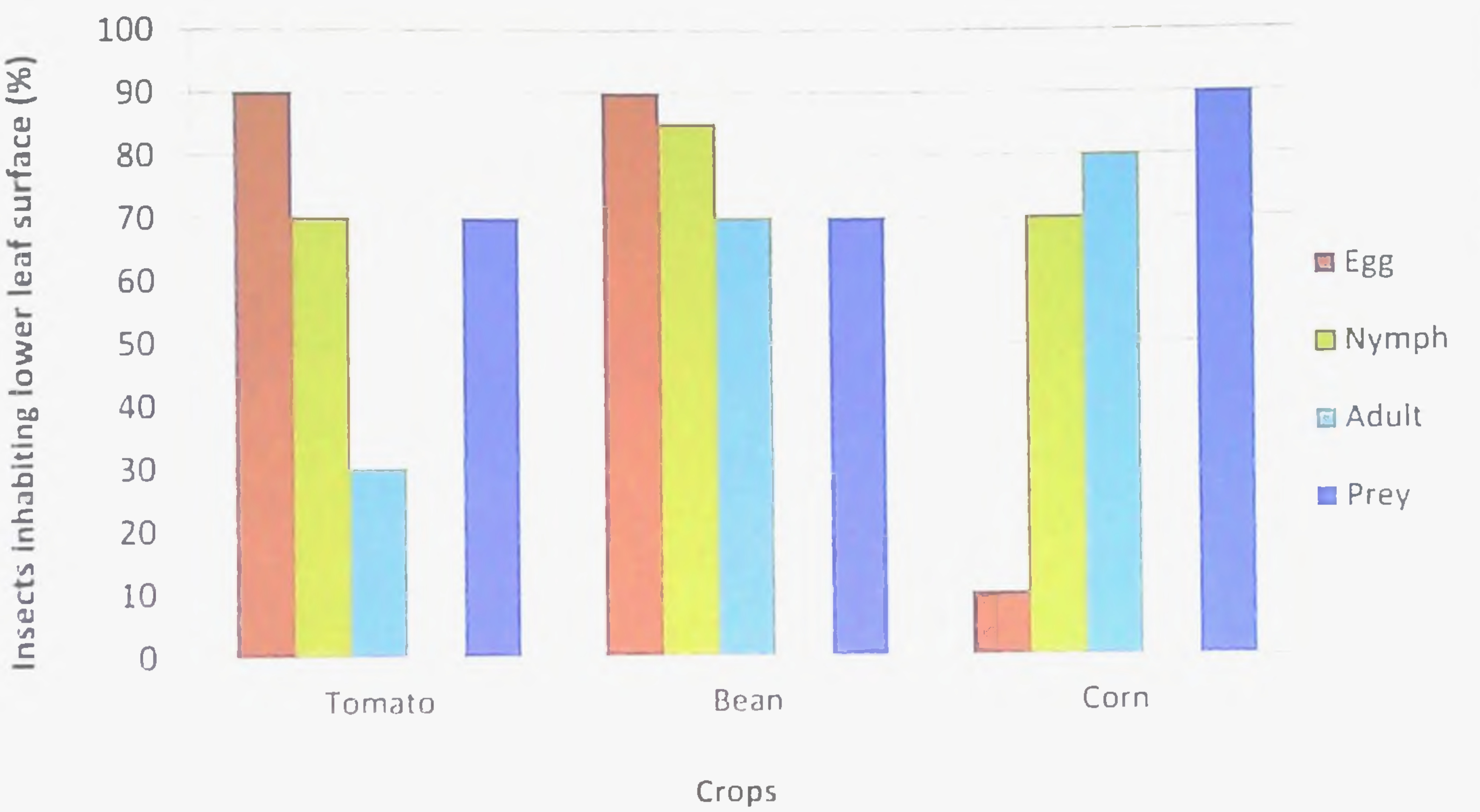
Table 3. Influence of prey density on prey consumption and handling time by *Coccinella septumpunctata*

Prey density	**Prey consumption**		**Handling time of prey (minutes)**
	Number	Per cent	
50	46	92.8	31.03
100	87	87.6	16.44
200	143	71.8	10.03
400	239	59.83	6.02
800	327	40.86	4.97

6.2.2 Spatial distribution of prey

The degree of predator-prey spatial overlap and predator searching efficiency differed greatly among plant species. A study was conducted by Coll *et al.* (1997) to study the prey searching efficiency of anthocorid bug, *Orius insidiosus* in varios crops like bean, tomato and corn (figure 6). During their study they observed that most life stages of *Orius insidiosus* inhabited the under leaf surface. Prey were more evenly distributed on both leaf surfaces in tomato and bean plants because proportionately fewer thrips inhabited the lower leaf surface of tomato and bean than of corn plants. Overall, the distributions of the prey and of the feeding stages of *O. insidiosus* (nymphs and adults) overlapped most on bean plants. On tomato and corn the degrees of overlap were lower. Only in bean did all life stages of *O. insidiosus* occur on the leaf surface where prey would be more abundant. In tomato and corn, the predator shifted its distribution during development. Most adult predators (about 80%) inhabited the underside of the leaves of bean and corn whereas most adults were recorded on the upper leaf surface in tomato (65%). In the absence of prey, the within leaf distribution of *O. insidiosus* eggs differed among plant species. It seems that the predator does not tend to deposit its eggs in places that are likely to harbour its preferred prey, thrips. In corn, *O. insidiosus* deposited almost 90% of its eggs on the upper surface of leaves likewise in bean and tomato, almost all predator eggs were deposited on the lower leaf surface where only about one third of the thrips occurred. Young predator nymphs were more abundant on the leaf surface where *O. insidiosus* eggs were laid. Probably, the high mobility of *O. insidiosus* nymphs favors oviposition in leaf structures that maximize egg survival even when only a few preys could be found nearby. Similarly in tomatoes, predator adults were more abundant on the upper leaf surface whereas their prey inhabited primarily the under leaf surface. It seems that the predator and its prey responded differently to plant leaf structures in different crops.

Figure 6. Effect of spatial distribution of prey on foraging behaviour of *Orius insidiosus*



6.2.3 Obstructions in habitat

Within a suitable habitat, predator faces several obstructions in the plant. The natural enemies often get affected by trichomes present on the plant surface. So they prefer glabrous plants over pubescent ones and the preference changes with the type of trichomes present in the plants. Elsey (1974) reported that *Chrysoperla carnea* were able to search for their prey at a much greater speed on the leaves of cotton than those of tobacco, because the movements of the larvae were seriously hampered by the glandular trichomes on tobacco. The trichomes present on cotton were not glandular and were sufficiently sparse that they did not impede the larvae.

7. IMPORTANCE OF PREDATORS IN AGRICULTURE

Biological control is one of the most important strategy in Integrated Pest Management where different groups of natural enemies and microorganisms are employed for the management of pest population. The widely used natural enemies against agricultural pests are predators and parasitoids. thre are mainly three approaches in biological control.

(a) introduction

(b) augmentation

(c) conservation

7.1 Introduction

There are many examples of successful classical biological control programs. One of the earliest successes was with the cottony cushion scale. Icerya purchasi a pest that was devastating the California citrus industry in the late 1800's. A predatory insect. the vedalia beetle. Rodolia cardinalis and a parasitoid fly were introduced from Australia. Within a few years the cottony cushion scale was completely controlled by these introduced natural enemies. Classical biological control is long lasting and inexpensive. Other than the initial costs of collection. importation, and rearing, little expense is incurred. When a natural enemy is successfully established it rarely requires additional input and it continues to kill the pest with no direct help from humans and at no cost.

7.2 Augmentation

This second type of biological control involves the supplemental release of natural enemies. Relatively few natural enemies may be released at a critical time of the season (inoculative release) or literally millions may be released (inundative release). Additionally, the cropping system may be modified to favor or augment the natural enemies. Lady beetles, lacewings etc. like predators are frequently released in large numbers (inundative release). The supplemental release of those predators is done through mass rearing in laboratory. *Chrysoperla carnea* is the important aphidophagpous predator that is mass reared and released in field.

7.3 Conservation

The conservation of natural enemies is probably the most important and readily available biological control practice available to growers. Natural enemies occur in all production systems, from the backyard garden to the commercial field. They are adapted to the local environment and to the target pest, and their conservation is generally simple and cost-effective. With relatively little effort the activity of these natural enemies can be observed. Lacewings, lady beetles and hover fly larvae are almost always present in aphid colonies. These natural controls are important and need to be conserved and considered when making pest management decisions. In many instances the importance of natural enemies has not been adequately studied or does not become apparent until insecticide use is stopped or reduced. Often the best we can do is to recognize that these factors are present and minimize negative impacts on them. If an insecticide is needed, every effort should be made to use a selective material in a selective manner.

8. CONCLUSION

Insect predators are important components of natural ecosystem. They play a major role in maintaining pest population. The act of predation involves several steps such as search, recognition, capture and handling. The predators exhibit several adaptations for successful predation such as behavioural and morphological adaptations. The prey also has evolved defensive mechanisms to avoid predation which includes morphological, behavioural, mechanical and chemical defense mechanisms. Successful regulation of population involves predator prey dynamics in an undisturbed ecosystem. There is great scope for the utilization of insect predators for pest management in agriculture. However, this requires more efforts at identification of potential predators, understanding their bioecology as well as interactions and, evaluation against crop pests.

9. DISCUSSION

1. Whether any toxins are responsible for the development of aposeimatic colouration in insects?

Ans. The aposeimatic colour development in insects is due to the presence of colour pigments but not by the toxins. The insect predator learns from their previous predatory event that these colourful insects are unpalatable or toxic. So they will avoid such type of insects and over the course of evolution the survived prey organisms are able to use this colour patterns as an antipredation mechanism.

2. What is meant by altruism?

Ans. Altruism means self sacrifice or selfless concern for the well-being of others. It is an animal behavior that benefits another at its own expense. It is most commonly seen in Malaysian exploding ant (*Componotus* spp.). When the predatory ants attacks their colony the soldiers of Malaysian exploding ants violently contracts its abdominal muscles to rupture its body and spray poison from the mandibular gland in all directions.

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

ENT 591: MASTER'S SEMINAR

Venue: Seminar Hall

Name: Neenu Chandran Date: 30.10.2015

Admission number: 2014-11-147 Time: 10.45 a.m.

Abstract

Killing for a living – predator prey interactions in insects

Predation is easily the most well known interaction of the animal world and involves the predator, an organism that kills, and the prey, which is killed by the predator. It is considered to be one of the most powerful driving forces behind evolution as it exerts a strong selective pressure on prey organisms to find ways to avoid being eaten and predators, ways to overcome prey defences.

About 25 per cent of all known insect species are predaceous in nature. Nearly every order of class Insecta contains predatory species. Some species are only predatory as immatures, others as adults, and still others are predatory throughout their life span. Some orders like the Odonata, Mantodea, Mecoptera and Neuroptera consists exclusively of predators.

The act of predation involves four distinct phases such as search, recognition, capture and handling, mediated by a range of cues that guide the predator. Such cues could be visual, olfactory or acoustic in nature. The European beewolf, *Philanthus triangulum* (Vespidae: Hymenoptera), for instance, are attracted to objects that are similar in size and shape to that of their prey, the Italian bees, *Apis mellifera*. Prey recognition, however, is mediated by (Z)-11-Eicosen-1-ol, a volatile component present in honey bee cuticle (Herzner *et al.*, 2005). Insect predators have evolved several morphological and behavioural adaptations that serve them admirably in the different stages of predation. While morphological adaptations mainly include the modification of appendages such as legs and mouthparts, the behavioural adaptations include thanatosis, ambush predation and trapping.

Antipredator mechanisms that assist prey organisms in their constant struggle to avoid being eaten can also be equally varied and spectacular. The range of prey responses to threats from predators can vary from as the simple running away in cockroaches to suicidal altruism in case of Malaysian exploding ants (Shorter and Rueppell, 2012). Such responses by prey are aided by adaptations in their behaviour, morphology, physiology and chemistry (Evans and Schmidt, 1990).

The ability of a predator in regulating prey populations is often explained in terms of its functional and numerical responses. Functional response refers to the increased kill by a predator in response to an increase in prey population while numerical response refers to the increase in predator population corresponding to an increase in prey numbers (Holling, 1959). Both are influenced by several factors like the searching efficiency of the predator, population size as well as spatial distribution of the prey and obstructions in the habitat. Omkar and Srivastava (2003) reported that *Coccinella septumpunctata* responded to an increase in prey density by an increase in the number of prey consumed as well as through reduced handling time.

Predators have been employed successfully in biological control of several important crop pests such as cottony cushion scale, *Icerya purchasi* and the coffee green scale, *Coccus viridis*. The commonly employed techniques involves introduction, augmentation and conservation. However, harnessing their potential call for greater efforts at identification of potential predators, understanding their bioecology as well as interactions and evaluation against crop pests.

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Sleeping through hardships - how insects overcome abiotic stress?

By

Manjushree, G.

(2014-11-214)

M. Sc. Agrl. Entomology

Seminar report

Submitted in partial fulfilment of requirement of the course

Ent. 591 Seminar (0+1)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

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VELLANIKKARA- 680656

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DECLARATION

I, Manjushree G. (2014-11-214), hereby declare that the seminar report entitled **'Sleeping through hardships - how insects overcome abiotic stress?'**, has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Manjushree G.

Manjushree G.

2014-11-214

Vellanikkara

Date: 29.10.2015

CERTIFICATE

Certified that the seminar report entitled **'Sleeping through hardships - how insects overcome abiotic stress?'** for the course Ent.591 has been prepared by Manjushree G. (2014-11-214), after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

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CERTIFICATE

Certified that the seminar report entitled **'Sleeping through hardships - how insects overcome abiotic?'**, is a record of seminar presented by Manjushree G. (2014-11-214) on 9th October, 2015 and is submitted for partial requirement of the requirement of the course Ent.591.

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1. Introduction

One of the reasons for dominance of insects is their ability to adapt quickly to adverse environmental conditions. Extremes of temperature, crowding, salinity *etc.*, often threatens survival of insect populations. One way of overcoming abiotic stress is by undergoing an inactive phase characterised by suppressed development till the environmental conditions favours. Such condition can be termed as dormancy. It can be defined as any state of suppressed development *i.e.*, ecologically or evolutionarily meaningful, usually accompanied by suppressed metabolism. It is further classified into quiescence, diapause and cryptobiosis (Kostal, 2006).

2. Quiescence

Quiescence is an arrest of development accompanied by suppression of metabolism which may occur at any stage of ontogenesis as a response to direct action of adverse factors (or a shortage of vital elements in the environment). It is determined by external limiting forces, and development is immediately resumed when forces are eliminated.

It is also a form of dormancy due to the direct response to external stress. Unlike other forms of dormancy, quiescence is not regulated by neuro-hormonal mechanism. It is considered as consecutive dormancy or exogenically-regulated dormancy.

Due to the scarcity of eco-physiological studies of non-diapause dormancy *i.e.*, quiescence whose role in life cycle regulation remains unclear and to the fact that most attention is now paid to diapauses (Belozerov, 2008).

3. Diapauses

Diapauses is defined as neuro-hormonally mediated, dynamic state of low activity that occurs during a genetically determined stage of metamorphosis, usually in response to environmental stimuli that precede unfavourable conditions (Tauber and Tauber, 1986).

3.1. Classification of diapause

Diapause is classified into different categories. According to destination it may be either a hibernation (winter diapauses) or an aestivation (summer diapause); according to the approached ontogenetic instar, it may be into egg (embryonic) or larval or nymphal or pupal or adult (reproductive) diapause; and according to the program may be into obligatory or facultative diapauses (Belozerov, 2008).

3.2. Stages of diapause

Diapause is divided into three main stages: pre-diapause, diapause and post-diapause stage. Each stage may further comprise some sub-phases, expression of which depends not only on genotype-driven physiological changes but is also influenced by environmental conditions (Kostal, 2006).

3.2.1. Prediapause stage

During pre-diapause stage, insects are induced to enter diapause as a response to specific environmental signals/conditions it receives during unfavourable conditions. Also certain preliminary preparations are carried out prior to arrest of direct development takes place. This helps in encountering the various problems during the resting stage. Further this stage is classified into two different phases.

3.2.1.1. Induction phase

Diapause is induced in advance of the advent of the environmental adversity. Diapause-inducing stimuli (or cues) are perceived during a fixed and specific sensitive period, which is genetically determined and it ranges from various periods within the parental generation through different stages of embryonal, larval and pupal development to the adult individual.

The inducing cues are signalling for the coming deterioration of environmental conditions and the term token stimuli is used in the literature to distinguish them from direct effects of other environmental factors on the rate of physiological processes. Also sensitivity to token stimuli may persist during further phases, where it takes on different roles, eg. in the diapauses maintenance or termination.

Other environmental factors usually modify, and sometimes even revert or overwhelm, the effect of token stimuli. The signalling nature of token stimuli is best understood in the case of photoperiod. In insects, receptors for photoperiodic signal are localized in various parts of brain or the compound eyes and the pathways by which this signal is transduced into a developmental programme have recently been investigated.

Other environmental factors such as temperature or oxygen also have acts as the token stimulus in those habitats where they seasonally change in a predictable and sufficiently slow manner and where photoperiodic or other token signals are less distinct or available (some tropical habitats, soil, caves, deeper layers in large water reservoirs, decaying wood).

The insects which undergoes facultative diapauses requires external cues *i.e.*, token stimuli for inducing diapauses. Where as in case of obligatory diapause, the initiation of developmental arrest needs no external cues because it represents a fixed component of the ontogenetic programme and is expressed regardless of the environmental conditions. Thus, the token stimuli are utilized to induce more widespread facultative diapause, where individuals can switch between two ontogenetic alternatives, i.e. direct development or diapause (Kostal, 2006).

3.2.1.2. Preparation phase

This phase is best documented in those organisms where the female parent exerts control over the developmental fate of her progeny. Two relatively well understood cases of maternally induced diapause: the egg diapause in the silkworm, *Bombyx mori* (Linnaeus) and the pupal diapause in the flesh fly, *Sarcophaga bullata* (Parker), clearly shows that diapause induction leads to specific alterations in gene transcription, neuro-endocrine milieu and metabolic pathways and that the individual is destined for later entry into a developmental arrest. The information about developmental destiny is "stored" during the preparation phase. Preparation phase is also characterized by different behavioural activities or physiological processes *viz.*, migration, location of suitable micro-habitats, aggregation, or the building-up of energy reserves before the final moult transition into the diapause stage (Denlinger, 2002).

Mitchell and Briegel (1989) reported that in the adults female mosquito of *Culex pipens* (Linnaeus) which are destined to enter, accumulation lipids and carbohydrates occurs twice as compared to equivalently aged non diapause female adults.

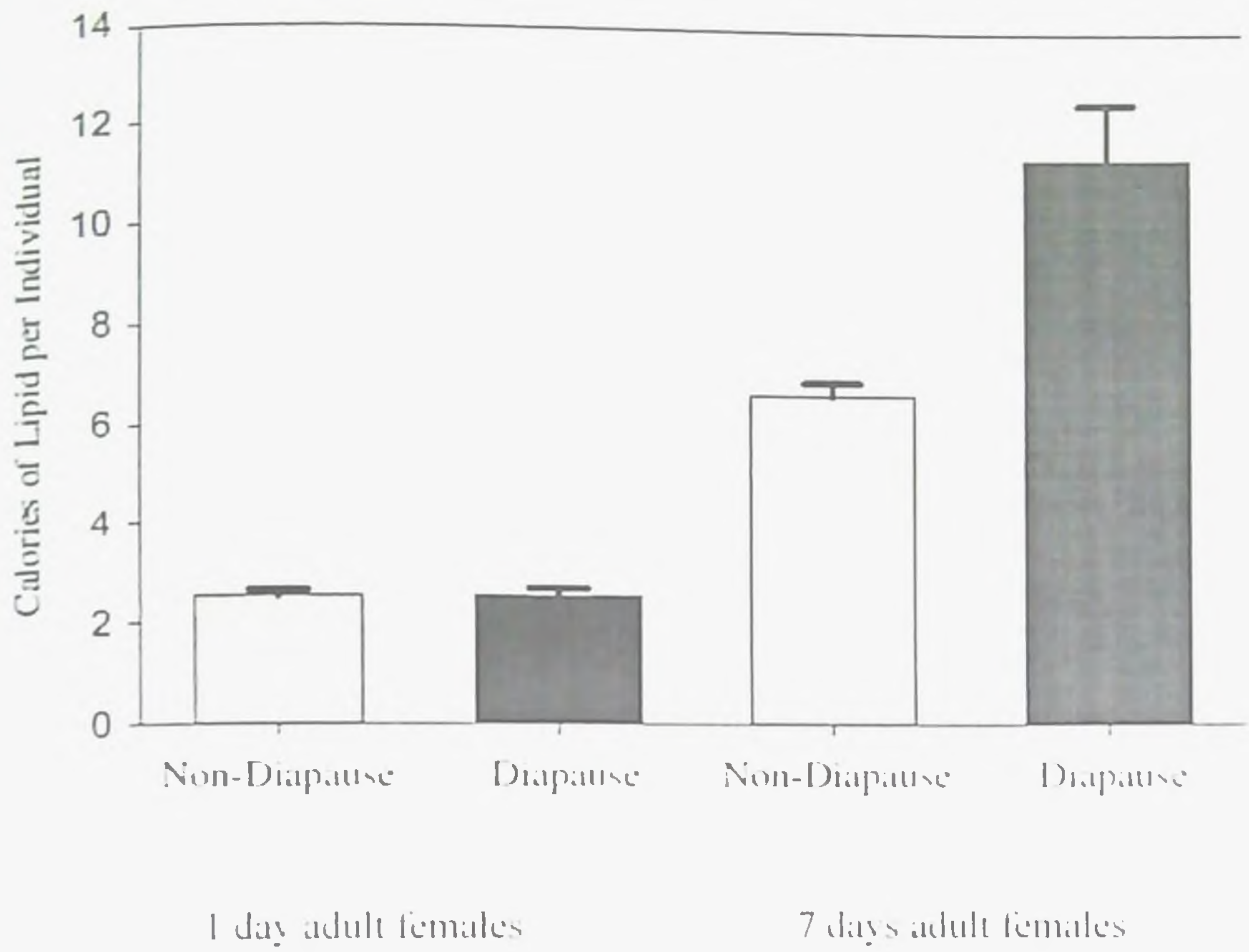

Figure 1. Increased accumulation of lipid content in diapausing adult females of mosquito, *Culex pipens* during diapause (Mitchell and Briegel,1989).

3.2.2. Diapause stage

During this phase, direct development (morphogenesis) is endogenously arrested and an alternative programme of physiological events proceeds, which is significantly modulated by changing environmental condition.

3.2.2.1. Initiation phase

This phase is an early part of diapause which can also be called by different names *viz.*, entry, onset, initiation, beginning, start, fixation, intensification. Considering morphological criteria, the initiation phase is found to begin when the ontogenetic stage is reached, at which direct development (morphogenesis) ceases.

During this phase either regulatory factors (unknown upstream factors- neuropetides, hormones), or competency of target tissues (hormonal receptors- members of signalling cascades- cell-cycle regulators *etc.*), or both found to be involved. Recently the roles of other factors *viz.*, heat shock proteins, have also received attention.

In addition to cessation of direct development, the initiation phase of diapause is also characterized by some other processes which allow it to be distinguished this phases from subsequent phases. The regulated decrease of metabolic rate probably represents the most general feature of the initiation phase. In many insects which diapause in mobile stages, a relatively slow decrease of metabolic rate is observed during the initiation phase. Although the developmental processes are blocked, high metabolic activity is required to support specific behavioural and physiological activities. It is also found, in some insects which are found in temperate region, stress tolerance mechanisms (typically cold-hardening) will become potentiated during the initiation phase and they are overtly expressed later, in response to a specific stimulus (cold). Stress tolerance commonly increases during diapause (Hayward *et al.*, 2005).

3.2.2.2. Maintenance phase

Despite the fact that environmental conditions after the initiation phase of diapause are usually still permissive for continuation of direct development, diapausing individuals remain locked in developmental arrest. During this phase, the metabolic rate is held relatively low and constant and the individuals maintain their diapause and remains in arrested state over the period of several weeks – months before the diapause is terminated.

Generally in the field, insects usually initiate winter diapause (hibernation-type) when it is still summer and maintain it during the warm summer/autumn. Similarly, insects with summer (aestivation-type) and tropical diapauses initiate and maintain their diapause before the adversity-period comes. The period of maintenance may extend to several years or even decades in some species and specific cases. Also the basic processes *viz.* energy-store depletion and somatic aging, likely contribute to gradual change of the physiological state during maintenance (Kostal, 2006).

3.2.2.3. Termination phase

Termination of diapause is spontaneous process. Token stimuli which plays a major role in inducing the insects to enter diapause is also found to important in terminating the diapause condition (Kostal, 2006).

3.2.3. Post-diapause stage

When environmental conditions which favours diapause termination differ from those favouring resumption of direct development insects are found to remains in exogenously locked in the state of post-diapause stage. At the end of post-diapause stage, when changes in limiting factors occurs it allows the insects to continue in direct development. Thus, post-diapause resumption of direct development is postponed to the vernal rise of temperatures in winter-diapausing insects and summer-diapausing insects must usually wait for the increase of humidity, or presence of liquid water.

In some cases, specific biotic factors, such as seasonal change of the biochemistry of the host plant or appearance of a food source signalled by allelochemicals, may stimulate the resumption of direct development (Kostal, 2006).

3.3. Environmental regulations of diapause

3.3.1. Photoperiod

Seasonal change in daylengths is a reliable indicator which is used in predicting upcoming periods of inclemency. It is accurate and can be used to effectively in detecting the advent of winter or other seasons which has to be avoided. The developmental period *i.e.* sensitive to photoperiod usually occurs far in advance of the actual diapause stage. Thus, diapause is not usually an immediate reaction to photoperiod but occurs in response to signals received at an earlier stage. Such early programming makes the insect to prepare themselves for diapause by sequestering food reserves and making other preparatory adjustments prior to the actual onset of the developmental arrest.

Many insects which overwinter in the temperate regions, short daylengths dictate the expression of diapause. And those that undergo a summer aestivation and reproduce in the autumn, long rather than short daylengths may be used to program diapause. Other species may respond to only a narrow range of daylengths for diapause induction, whereas daylengths both shorter and longer avert diapause. The stage sensitive to photoperiod varies among the insects (Denlinger, 2002).

Table 1. Photosensitive stage in insects

Insect	Sensitive stage	Diapausing stage
South-western corn borer *Diatraea grandiosella*	Early larval stage	Late larval stage
Flesh fly *Sarcophaga crassipalpis*	Early larval stage	Pupal stage
Colorado potato beetle *Leptinotarsa decemlineata*	Early adult	Late adult
Monarch butterfly *Danaus plexippus*	Early adult	Late adult
Silkworm, *Bombyx mori*	Late embryonic stage of female parent	Embryonic stage of offspring

3.3.2. Temperature

Temperature has generally been known to influence the photoperiodic control of summer diapause as well as winter diapause. In the both cases, high temperature acts in unison with long photoperiod, and low temperature with short photoperiod. This correlation is obviously due to the phase relationship between the annual cycles of photoperiod and temperature. Summer diapause thus shows again the reverse relation to temperature as compared with winter diapause. In many species, high temperature favours the induction of summer diapause and low temperature tends to prevent it (Masaki,1980).

Near the equator, where seasonal changes in daylengths are too subtle to be used as environmental cues, temperature replaces photoperiod as the primary environmental regulator of diapause. In case flesh fly, *Sarcophaga inzi* (Curran) which is living in East Africa low daytime temperature experienced during July and August, induces the flies to enter pupal diapause (Lees, 1955).

Jadhav *et al.* (2013) reported incidence of diapause in *Helicoverpa armigera* (Hubner) during winter and summer is governed by agroclimatic factors. It is found that multi-year data on the patterns of winter and summer diapause development and emergence correlated with the field activity of adult moth emergence is important in better understanding the role of local population dynamics in the ecology and genetics of *H. armigera*. However, one of the investigation revealed that shorter day length and cooler temperatures in November-

December and higher temperatures in March-April are influencing the pupae to enter diapause.

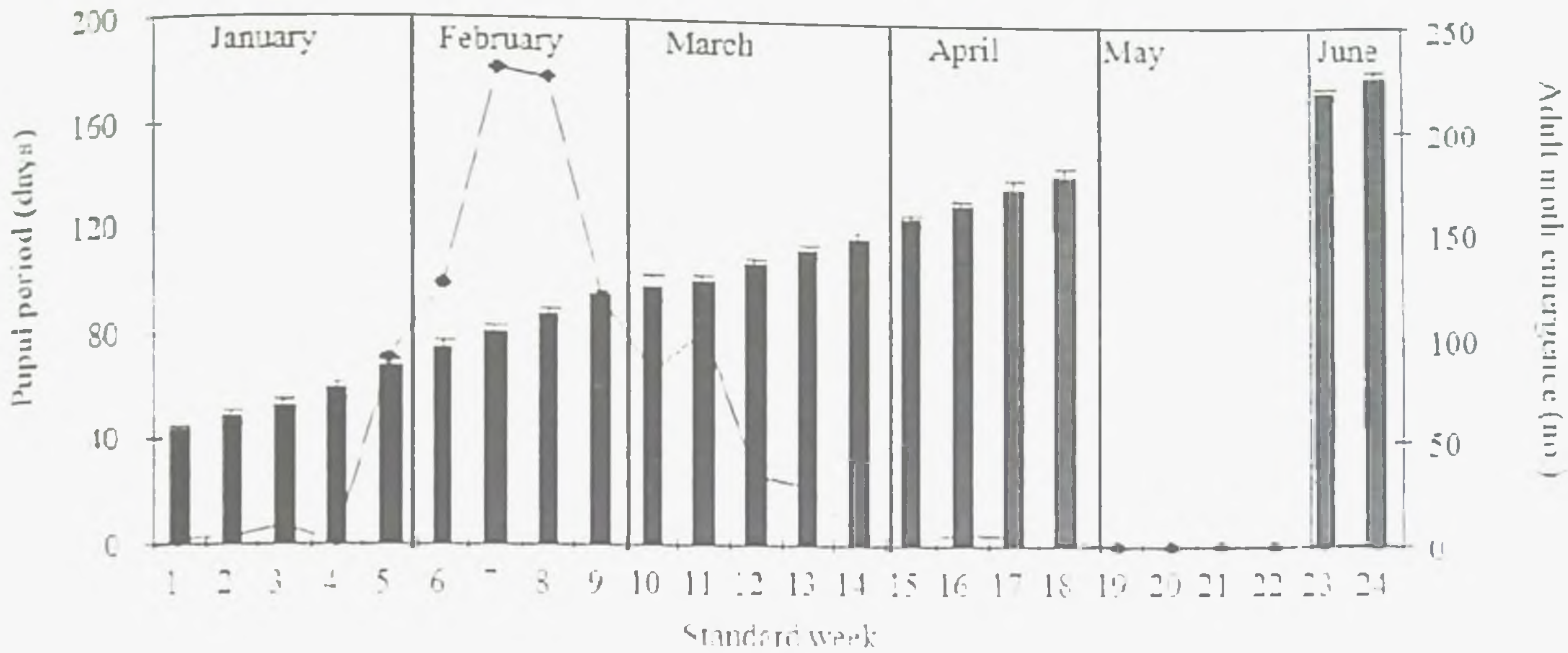

Figure 2. Pupal period and adult moth emergence from winter diapausing pupae of *H. armigera* in Andhra Pradesh, India (Jadhav *et al.*, 2013).

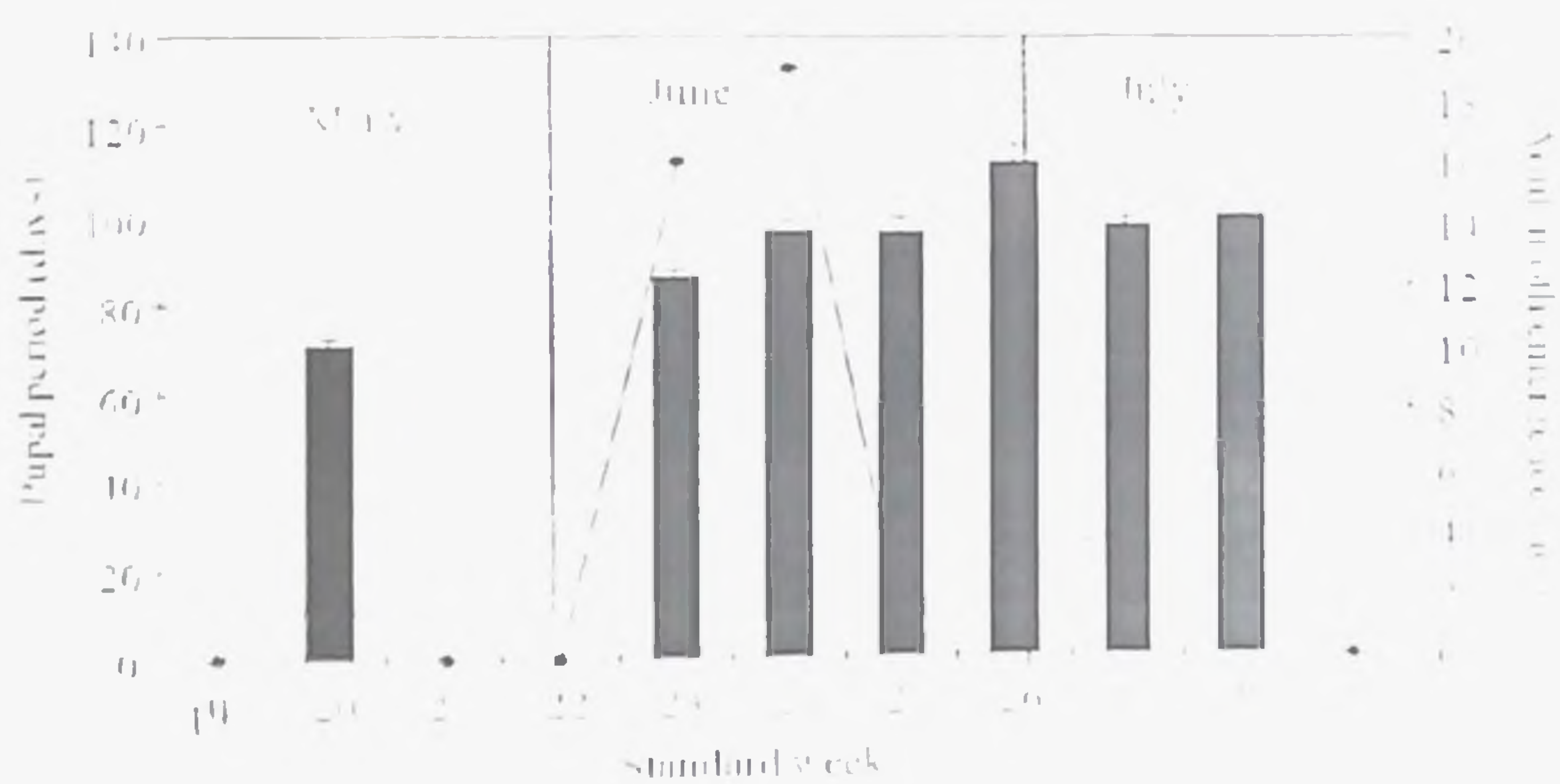

Figure 3. Pupal period and adult moth emergence from summer diapausing pupae of *H. armigera* in Andhra Pradesh, India (Jadhav *et al.*, 2013).

3.3.3. Moisture

It plays an important in post-diapause growth. Many orthopteran eggs, as well as hibernating larvae and adult insects, cannot begin their post-diapause growth until moisture has been taken up from their surroundings. In case of diapausing eggs of grasshopper.

Melanoplus differentialis (Thomas) and hessian fly *Mayetiola destructor* (Say) do not enter the post-diapause growth until they come in contact with moisture (Lees, 1955).

3.4. Behavirouràl adaptations during diapause

3.4.1. Site selection

During diapause insects retreat to protected sites like underground, inside stems, leaf bases of palm stems, in dry curled leaves, in tree holes, between root buttresses *etc*. The diapause site provides protection and buffering of the physical environment. The insect will further modify the site by lining the refugia with thick layers of silk or other waterproofing materials such as resin and wax. The site offers a microenvironment with humidity and temperature ranges which differ considerably from those of the environment at large. For example in diapausing female of *Culex Pipiens* during onset of diapause seeks a dark, humid, protected hibernaculum, a site buffered from the full brunt of winter (Denlinger and Armbruster 2013).

3.4.2. Migration

Diapausing insects are found to be migrating from feeding and breeding places to both aestivating and hibernating sites respectively. Long-distance flight has been known for noctuid moths. They aestivate at high altitudes and return to the breeding sites. This behaviour is found in different species in widely separated parts of the world: Southeastern Australia, North America, and Japan. Another noctuids in Europe and Mediterranean countries is also suspected to make aestivation flights, although the aestivating quarters have not been discovered. In Southeastern Australia, the adults of the bogong moth, *Agrotis infusa* (Boisdual) emerge in late spring to migrate from the plains to the mountains, where they aestivate, forming huge aggregations in rock crevices and caves. In any case, the microclimatic conditions at the aestivating site may decrease the metabolic rate and water loss, thereby favouring the survival of aestivating populations.

Similarly in case of monarch butterflies *Danaus plexippus* (Linnaeus) unlike most other insects in temperate climates cannot survive a long cold winter. The eastern North American monarch population is notable for its annual southward late-summer/autumn migration from the United States and southern Canada to Mexico. During the fall migration, monarchs cover thousands of miles, with a corresponding multi-generational return north. The western North American population of monarchs west of the

Rocky Mountains often migrates to sites in California but has been found in overwintering Mexican sites as well. Monarchs are the only butterflies to make such a long, two-way migration, flying up to 3000 miles in the fall to reach their winter destination (Masaki, 1980).

3.4.3. Aggregation

Formation of an aggregation with dispersal of individuals at the end of diapause is one of characteristic feature of diapausing insects. Distances traversed are unknown, and cues used to identify suitable aggregation sites and to provide group cohesion remain undefined.

Many of these species are likely to be distasteful and avoid predation by aggregating. In case of tropical beetles *Stenotarsus rotundus* (Arrow), the geckos and ants that live close to *beetles* assiduously avoid predating on them. Also the beetle aggregates up to eight layers deep. Such high densities significantly modify the insect's microenvironment by reducing evaporative water loss and lowering incident radiation. The aggregation is likely to serve another important function in case of hibernating lady beetle, *Hippodamia convergens* (Guerin) as soon as beetle terminate from diapause, they become reproductively active and thus the task of searching for a mate is eliminated (Masaki, 1980).

3.4.4. Colour polymorphism

Colour polymorphism is best documented in tropical species, where a link between diapause and seasonal patterns of colouration seen in lepidopterous larvae and adults. A light appearance caused by loss of pigmentation is one of the earliest and most conspicuous indicators of larval diapause in some noctuids and pyralid stem borers. Nondiapausing larvae remain dark and retain spots of cuticular pigmentation.

It is more prominent among certain adult butterflies and moths. The wet-season morph of the West African nymphalid, *Precis Octavia* (Cramer) is bright orange with black markings while the dry-season morph, which is in reproductive diapause, has a complex pattern of black markings with blue spots and very little orange. Dry-season morphs that occupy open habitats tend to be light coloured, inhabitants of shady, forested areas tend to be dark. In Costa Rica, Saturniid *Rothschildia lebeau* (Guerin) displays a range of colour morphs from light rust to dark chocolate. Adult moths do not diapause, but light morphs normally emerge from diapausing pupae that have bridged the dry season. As the rainy season progresses, more dark forms appear.

In all these cases, changes in colour pattern effectively track seasonal shifts in background colouration. In addition, light-coloured morphs should be able to reduce thermal stress by absorbing less solar radiation during a hot, dry season (Masaki, 1980).

3.4.5. Feeding habits

The species which diapause are capable of feeding and in species the nutrient intake may be absent, minimized, or distinctly different during diapause. For example, adult Colorado potato beetles, *Leptinotarsa decemlineata* (Say), do not feed during diapauses, they burrow into the soil and do not emerge until diapause is broken. In contrast, diapausing adults of the black blowfly, *Phormia regina* (Meigen), are active and will feed during diapause. However, they consume 80 per cent less than non-diapause adults. Similarly, diapausing adult females of the mosquito *Culex pipiens* actively feed on sugar, but they avoid taking blood meals (Hahn and Denlinger, 2007).

3.5. Physiological regulations during diapause

3.5.1. Water

Location of the refugia, formation of dense aggregations and specially constructed cells contribute immensely to the maintenance of a favourable humidity in the micro-environment. In addition to this, many tropical species have physiological or structural adaptations which render insects to resistant desiccation during diapause.

In case of diapausing larvae of *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) survive at low humidities and lose much less water than non diapausing larvae. Also the diapausing eggs of *Diabrotica virgifera* (LeConte) are more resistant to desiccation, they are protected by structural modifications of the chorion, the embryonic membrane, and encasements produced by the female accessory glands.

Apart from this, insects which diapause in different stages, resistance to desiccation is also found to be achieved by other two mechanisms like decreasing permeability of the cuticle by enhancement of the wax layer and decreasing evaporative water loss through the spiracles. Since oxygen demands are much lower during diapause, water loss is probably minimized by spiracular valves and setae that block escape of water vapour (Denlinger, 1986).

3.5.2. Storage proteins

Storage proteins are found to be synthesised before the onset of diapause and released into the hemolymph and remain in abundance throughout diapause. When diapause is terminated, they quickly disappear from the hemolymph. These storage proteins are first reported in Southwestern corn borer (*Diatraea grandiosella* Dyar). Later it is also noticed from larvae of several additional Lepidopterans including the codling moth (*Cydia pomonella* Linnaeus), pink bollworm (*Pectinophora gossypiella* Saunders), stem borer (*Busseola fusca* Fuller), spruce budworm (*Choristoneura fumiferana* Clemens) and the wax moth (*Galleria mellonella* Linnaeus), as well as adults of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) and the red fire bug (*Pyrrochoris apterus* Pallen).

These storage proteins are hexameric protein which is generally referred to as storage proteins. They are synthesised in fat body. Although these proteins do not appear to be utilized during diapause, they serve as an important amino acid source for the extensive tissue development that occurs immediately after the diapause termination. The proteins are also seen during other stages of development, and nondiapausing individuals. Thus, these proteins are not truly unique to diapause. The distinction is that they remain abundant in the hemolymph of diapausing insects for a long time because development has been arrested, where as in nondiapausing individuals the proteins are made and quickly utilized as the insect proceeds with development (Denlinger, 2002).

3.5.3. Hormonal control

Several key hormones also serve as regulators of diapause, but precisely which hormones are involved depends on the species and the developmental stage in which diapause occurs . In brief, embryonic diapauses are controlled in several ways. The best-known case is the regulation of early embryonic diapause in the silkmoth, *Bombyx mori*. In this species embryonic diapause is induced by the action of diapause hormone, a neuropeptide released from the suboesophageal ganglion of the female.

In larval diapause, responses of *Chilo* spp. are very similar to those of related temperate species. Juvenile hormone (JH) titer is high at initiation of diapause, drops to intermediate levels for the remainder of diapause, and then drops sharply at diapause termination. The elevated JH titer is essential for maintaining diapause and for dictating that the occasional moults occurring during diapause will be stationary (larva to larva) rather than progressive (larva to pupa). The drop in JH at the end of diapause signals release of the 20-hydroxyecdysone needed for pupation.

Regulation of pupal diapause results from a shutdown of the brain-prothoracic gland axis. In *Sarcophaga rotundus* appears to be the same in both tropical and temperate species. In both, diapause occurs when brain-prothoracic gland stop secreting 20-hydroxyecdysone. Thus diapause can be terminated readily with 20-hydroxyecdysone or other chemical agents that activate the brain or prothoracic gland.

Adult diapause takes place when JH is absent. A number of reproductive events in insects are regulated by JH, and blocking its production as occurs in diapause, results in the cessation of egg maturation, atrophy of accessory glands, degeneration of flight muscles, and a halt in mating activity. Activation of the corpus allata, the gland that secretes JH, terminates diapause: Flight muscles regenerate, mating activity ensues, and egg maturation begins.

A synergistic effect of JH in enhancing the efficacy of 20-hydroxyecdysone suggests that tropical species, like their temperate counterparts, utilize a JH pulse as part of the signal to initiate development. Adult diapause in *Stenotarsus rotundus* also fits the pattern of hormonal mediation observed in temperate species: JH deficiency during diapause prevents egg maturation and flight-muscle development.Application of exogenous JH promotes development of flight muscles and maturation of the gonads. Size of the corpora allata, the glandular source of JH is frequently correlated with synthetic activity (Denlinger, 2002).

3.5.4. Heat shock proteins

During diapauses HSPs either increase or decrease in amount or remain constant, with changes occurring in anticipation of rather than in response to stress. Diapause induced modifications in HSPs tend to last longer than those caused by stress and HSP production occurs without the general inhibition of protein manufacture seen in stressed cells. HSPs synthesis is differently regulated during diapause and within an insect individual HSPs may either increase or decrease in diapause versus non-diapause development. Additionally, in an insect undergoing diapause, one or more HSPs may rise in amount while others are reduced or do not change. The differential synthesis of HSPs and their responsibility for protein folding and storage as influenced by ATP, predict their role in protein maintenance and stress tolerance throughout diapauses (King and MacRae, 2015).

HSPS in insects undergoing diapause at different developmental stages

Table 2. HSPs in during embryonic diapause

Insect	Up-regulated HSPs	Down-regulated HSP
Silk worm. *Bombyx mori*	Hsp 20.8 A	-

Table 3. HSPs in diapausing larva

Insect	Up-regulated HSPs	Down-regulated HSPs
Corn stalk borer *Sesamia nonagrioides*	SnoHsp19.5	SnoHsp20.8
Bamboo borer *Omphisa fuscidentalis*	Hsp70	Hsp90
Asiatic rice borer *Chilo suppressalis*	Hsp90	-
Blowfly. *Lucilia sericata*	Hsp23, Hsp24 & Hsp 70	Hsp90

Table 4. HSPs in diapausing pupa

Insect	Up-regulated HSPs	Down-regulated HSPs
Flesh fly *Sarchophaga crassipalpis*	Hsp70, Hsp 60	Hsp90
Blue berry maggot *Rhagoletis mendax*	Hsp 70	Hsp90
Cotton bollworm *Helicoverpa armigera*	Hsp21.4	Hsp20.7 & Hsp90
Asian tiger mosquito *Aedes albopictus*	-	-

Table 5. HSPs in diapausing adult insect

Insect	Up-regulated HSPs	Down-regulated HSPs
Coloroda potato beetle *Leptinotarsa decemlineata*	Hsp70	Hsp23, Hsp26, Hsp90
Mosquito, *Culex pipiens*	-	Hsp70
Northern malt fly *Drosophila Montana*	Hsp20 and Hsp26	Hsp83

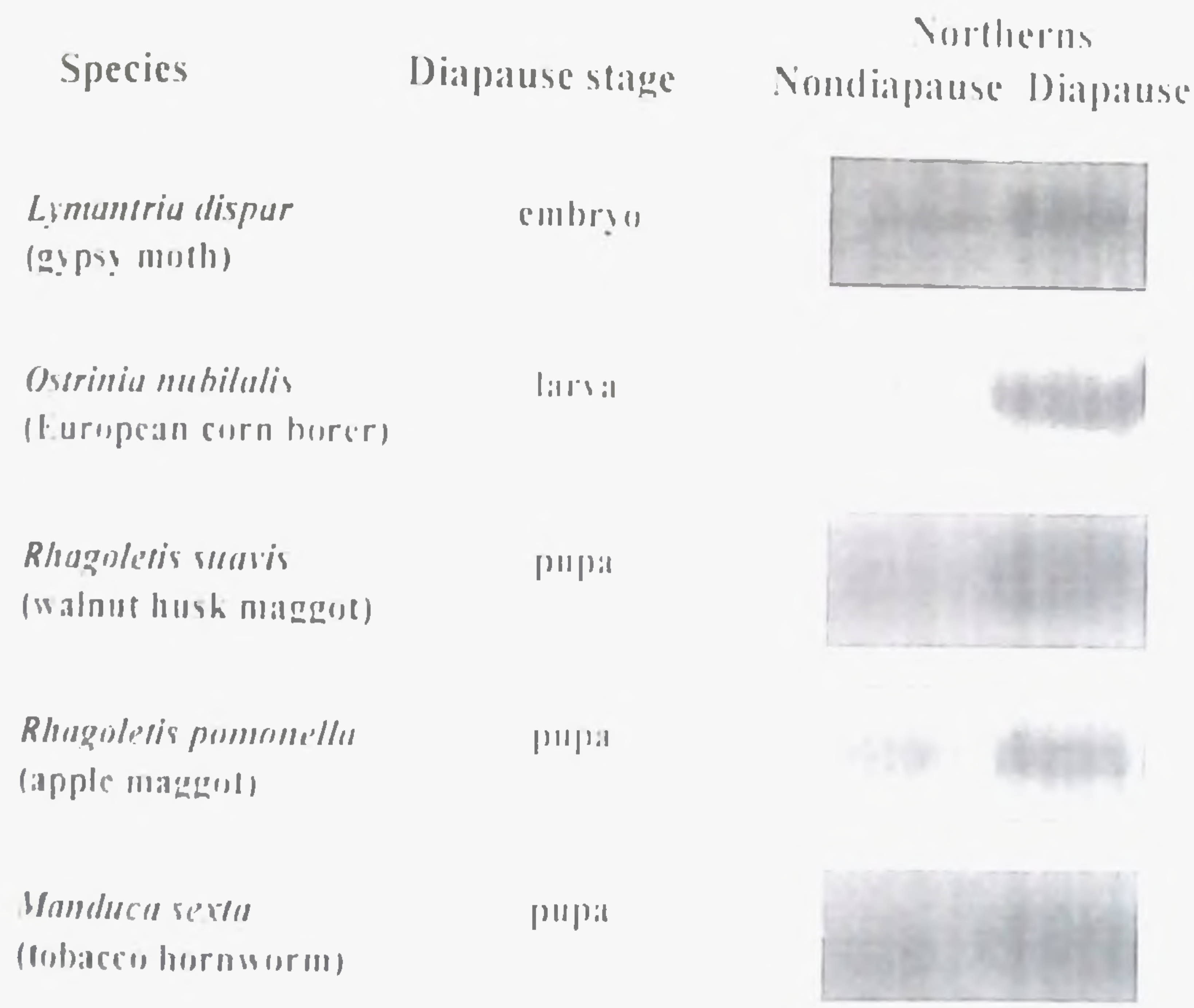

Figure 4. Up-regulation of Hsp70 during diapause in several insects at different diapausing stages (Rinehart *et al.*, 2004)

3.6. Practical significance of insect diapause

a. Early forecasting of the field emergence period and a good knowledge of the overwintering survival are the key feature for effective management of pest.

b. Spraying of diapause agonist in early larval stage helps in disrupting the entry into pupal diapause.

c. Inducing egg diapauses in natural enemies helps in long shipment or it can also used for long time storage.

d. Awakening the diapausing stage in insect during the non-crop season helps in effective management of pest.

e. By inducing diapause, blood feeding mosquitos are made into plant feeders. This helps in controlling of various populations which are vector for human diseases.

4. Cryptobiosis

Cryptobiosis is a state of an organism when it shows no visible signs of life and when its metabolic activity becomes hardly measurable or comes reversibly to a standstill.

It is found in many groups of invertebrates including insects (chironomids), tardigrades, nematodes, rotifers, collembolans and primitive crustaceans (brine shrimp, *Artemia salina* Linnaeus).

Cryptobiotic organisms can be divided into two types according to the developmental stage in which cryptobiosis occur: embryonic or post-embryonic. In the former, as in *Artemia salina* and collembola, female parents prepare the molecules necessary for cryptobiosis in their progenies (eggs) to some extent in response to external conditions. In contrast, in the latter case, as in chironomids, most tardigrades, nematodes and rotifers, parents do not affect induction of cryptobiosis in their progenies at all, and individuals themselves must be able to reversibly switch their physiology and biochemistry between development and cryptobiosis (Watanabe *et al.*, 2004).

4.1. Types of cryptobiosis

a. Cryobiosis (extremely low temperature)

b. Osmobiosis (extremely high level of solutes)

c. Anoxybiosis (lack of oxygen)

d. Anhydrobiosis (desiccation)

4.2. Regulation of cryptobiosis

The adaptive biochemical changes is found during cryptobiosis which involves the participation of large concentrations of polyhydroxy compounds, chiefly the disaccharides trehalose or sucrose. Stress heat shock proteins also reported to be involved, although the details are poorly understood and seem to be organism-specific (Clegg, 2001). And morphological changes also occurs where organism contracts itself and constructs the protective layer .

4.3. Cryptobiosis in chironomid larva

African chironomid larva, *Polypedilum vanderplanki* (Hint) is the most advanced and largest multicellular cryptobiotic invertebrate (length of larvae 6–7mm). This species breeds in small temporary pools formed in shallow hollows in unshaded rocks in Nigeria and Uganda. The cryptobiotic larvae show an extremely high thermal tolerance from −270 to 103°C and it can revive and grow after submersion in pure ethanol or glycerol. It can also survive in cryptobiotic condition for about 17 years. In the field, larvae usually live in pools by constructing soil nest tubes. Under normal condition larvae contains a small amount (3 per cent) of trehalose in their blood. During the cryptobiosis, larvae accumulate trehalose about 20 per cent of the dry body weight. Other sugars and polyols were not detected. The larvae make tubular shelters in the pools by incorporating detritus or soil with their saliva. The tubes of chironomids are found to have three functions. First, the tubes allow larvae to obtain oxygen and food efficiently, because the larvae produce water currents in their tubes by undulating their bodies, which serve to trap food in the water at the open mouth of the tubes, feces can also be washed out. Secondly, they have a protective role against predacious natural enemies. Because many species of chironomid larvae including *P. vanderplanki* are red in body colour due to hemoglobin in the hemolymph, without their tubes they would be easily visible to natural enemies. Thirdly, the tubes are to be protecting the chironomid larvae also from chemical toxicants (Kikawada *et al.*, 2005)

Kikawade *et al.* (2005) reported upon desiccation (DW evaporating at 0.22 ml day21 in a Petri dish with its glass top) larvae with a filter paper holding 0.44 ml distilled water started synthesizing trehalose 12 h after transfer to the desiccation box, and accumulated 38 mg trehalose/individual before complete dehydration.

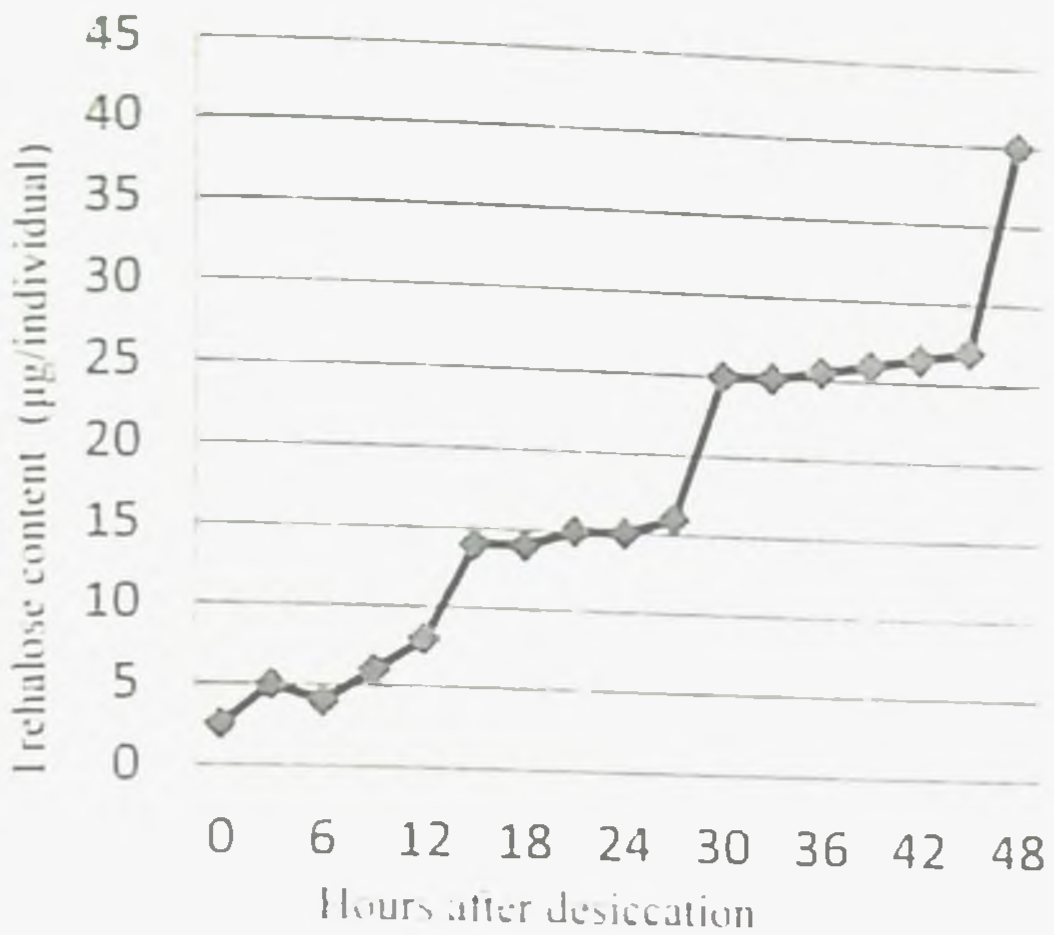

Figure 5. Changes of trehalose content in *P. vanderplanki* larvae during desiccation (Kikawada *et al.*, 2005)

Kikawada *et al.*, (2005) reported significant role of the larval tubular nests in inducing successful anhydrobiosis. Trehalose content and recovery in desiccated larvae with and without soil tubes were compared. Larvae in soil tubes were desiccated with 182 ml DW (total water volume is 200 ml) accumulated 38 mg trehalose individual before complete desiccation and 81.3% of them recovered after rehydration. When larvae without the soil tubes were desiccated with 200 ml DW, they accumulated trehalose at significantly lower level of 23 mg and only 43.3% of them recovered after rehydration. When total water volume in the dish was reduced to 100 ml in the soil tubes accumulated 37 mg of trehalose and 63.3% recovered after rehydration, whereas those without soil tubes synthesized trehalose at lower level (17.3 mg) and none of them recovered after rehydration.

Table 6. Recovery and trehalose content of desiccated larvae in with or without soil tubes

Water volume (µl)	Soil tube	n	Recovery (%)	n	Trehalose content (µl/individual)
200	+	32	81.3 a	6	38.8±2.1 A
200		30	43.3 b	6	23.3±6.3B
100	+	30	63.3 ab	5	37.0±6.2 A
100		35	0.0 C	6	17.3±4.3 B

5. Conclusion

Phenological studies pertaining to diapause and cryptobiosis are vital to both theoretical and applied biology. Detailed understanding of various factors involved in regulating dormant conditions and also behavioural and physiological changes occurring in insects during diapause and cryptobiosis helps in practical utility of pest management studies and such studies are now becoming widely recognized as essential for implementing modern pest management programmes.

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7. Discussion

1. What is corpora allata?

Ans: Corpora allata is a paired glandular bodies, usually present one on either side of oesophagus. It produces Juvenile hormone, whose principal function during direct development are in the metamorphosis and in the adult insects helps in development of flight muscles and reproductive organs.

2. What are changes occurring in membrane integrity during diapauses?

Ans: During diapauses, in order to resist desiccation under prolonged dry the cuticular permeability is reduced by deposition of large amount of lipid molecules.

3. What is token stimuli?

Ans : Token stimuli is a term given to the environmental factors which acts as signal for inducing diapause. This term is used to distinguish the direct effects of other environmental factors on the rate of physiological processes.

4. How insects will recognize the unfavourable conditions?

Ans: In insects, receptors for photoperiodic signal are localized in various parts of brain or the compound eyes, and these organs helps in recognizing the unfavourable conditions.

5. Is there any method to break diapause in eggs of silk worm in commercial seed production?

Ans: Yes, It is done by treating diapausing eggs with HCl of specific gravity of 1.075 with 15% concentration and heating to 46 C for about 4-7 minutes.

6. Why monarch butterfly migrates during diapauses?

Ans: The monarch butterfly cannot withstand freezing weather in the Northern and Cental continental climates in the winter. Also, the larval food plants do not grow in their winter overwintering sites. So the spring generation must fly back to north places where the plants are plentiful.

7. Weather there is any way to complete elimination of mosquito population?

Ans: No, the complete population cannot be eliminated. However, the population can be controlled by understanding the factors regulating the diapause in mosquito.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

ENT 591: MASTER'S SEMINAR

Venue: Seminar Hall

Name: Manjushree G.

Date: 09.10.2015

Admission number: 2014-11-214

Time: 10.00 a.m.

Sleeping through hardships - how insects overcome abiotic stress?

Abstract

One of the reasons for dominance of insects is their ability to adapt quickly to adverse environmental conditions. Extremes of temperature, crowding etc. often threatens survival of insect populations. One way of overcoming abiotic stress is by undergoing an inactive phase characterised by suppressed development till the environmental condition favours. Dormancy is a state of suppressed development which encompasses three different stages *viz.*, quiescence, diapause and cryptobiosis (Kostal, 2006).

Quiescence is a state of suspended development under unfavourable conditions and the insect resumes activity as soon as the conditions become favourable for development. Diapause is an induced dormancy which occurs through the effect of token stimuli signalling during unfavourable condition. Environmental factors *viz.*, photoperiod, temperature and moisture are involved in regulating diapause. Photoperiod acts as a reliable indicator for predicting upcoming period of inclemency. Temperature acts in unison with photoperiod and the moisture helps in promoting post-diapause growth. Behavioural adaptations like site selection, migration, aggregation and colour polymorphism are seen in insects which prepare to undergo diapause (Masaki, 1980).

During diapause, metabolic activities remain either low or constant. Under long dry season, the diapausing insects develop an efficient water conservation mechanism. In some other insects, hexameric proteins are synthesised which serve as an important source of amino acid for tissue development that occurs immediately following diapause termination. Hormonal mediation is also noticed in insects, which helps in the initiation, maintenance and termination of diapause. Heat shock proteins (HSPs) are also produced which help to prevent protein denaturation during stress period (King and MacRae, 2015).

Cryptobiosis is a unique phenomenon that occur in insect when subjected to extreme environment condition, where they show no visible sign of life and metabolic activity as in

African chironomid larva, *Polypedilum vanderplanki* Hint. Under extreme dry period, the larva contracts itself by forming soil tubes as a protective layer. Internally, trehalose content increases in the blood and acts as chemical chaperons to protect the cells (Watanabe *et al.*, 2002).

Phenological studies pertaining to diapause and cryptobiosis are vital to both theoretical and applied biology. Such studies are now becoming widely recognized as essential for implementing modern pest management programmes.

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Pathology

Antimicrobial peptides in plant disease management

By

Praveen N. M.

(2014-11-237)

M.Sc Plant Pathology

Seminar report

Submitted in partial fulfilment of requirement of the course

Pl. Path. 591 Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY
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Declaration

I, Praveen N. M. (2014-11-237), hereby declare that the seminar report entitled '**Antimicrobial peptides in plant disease management**', has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara
Date: 17-02-2016

Praveen N. M.
2014-11-237

Certificate

Certified that the seminar report entitled **Antimicrobial peptides in plant disease management'** for the course Pl. Path. 591 has been prepared by Praveen N. M. (2014-11-237), after going through various references cited herein under my guidance, and he has not copied or borrowed from any of his fellow students.

Vellanikkara
17-02-16

Dr. Reshmy Vijayaraghavan
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Certificate

Certified that the seminar report entitled **'Antimicrobial peptides in plant disease management'**, is a record of seminar presented by Praveen N. M. (2014-11-237) on 10.02.2016 and is submitted for partial requirement of the requirement of the course Pl. Path. 591.

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1. Introduction

Plant diseases have been a major concern to mankind all through the ages. There have been periodic outbreaks of certain diseases which caused large scale death and famine like Irish famine of 1845 in Ireland, devastating coffee rust in Srilanka and Panama wilt incidence in Australia made cultivation inconceivable. Such plant diseases caused by phytopathogenic microorganisms cause significant yield loss and it can also affect the quality and safety of agricultural products. For the past several decades, management of plant diseases has been increasingly dependent on the extensive use of toxic chemicals. However, continuous and widespread use of these chemicals has developed pathogenic strains that are resistant to certain fungicides and antibiotics. Moreover, public concerns about the potential side effects on human health and environment, has stimulated research to develop new antimicrobial agents that meet current health and safety standards. Since then antimicrobial peptides have been the object of attention in the past few years as candidates of plant protection.

1.1 Antimicrobial peptides

Peptides are short chains of amino acids joined together either by eupeptide or isopeptide bonds. They are distinguished from proteins purely on the basis of the number of residues present, conventionally understood to be less than 50, while those shorter than 20 residues tend to be labeled as oligopeptides. Antimicrobial peptides (AMPs) are portions of pathogen-related proteins which disrupt pathogen membranes, although this is not their only target, since they also act to inactivate ribosomes and thereby inhibit their synthesis of protein.

Majority of AMPs are encoded by genes, while others are products of secondary metabolism or synthesized by non-ribosomal peptide synthases (Giessen and Marahiel, 2012). Certain AMPs are generated after post-translational modifications, have cyclic structure or contain unusual amino acids (Laverty *et al.*, 2011). Sources of AMPs range from microorganisms to plants, vertebrates and invertebrates. Size of these compounds lie between 1-9 kDa and majority of the AMPs isolated are of cationic in nature due to the presence of basic amino acids like Lysine and Arginine. By their own nature, AMPs provide resistance to various plant pathogens including bacteria, fungi and viruses. Hence, they present innovative approaches for plant protection in agriculture.

2. History

Timeline of antimicrobial peptide advancement was by the discovery of first antibacterial peptide from wheat endosperm. purothionin which was first suggested by Alexander Fleming during 1921 as wheat flour containing lysozyme like peptide having antimicrobial properties. Later. Balls and collaborators during 1974 purified purothionin to α and β purothionin. Over the years. several additional peptides with antibacterial activity have been characterized, which having antimicrobial properties against many phytopathogenic organisms. Peptides like cecropin, was the first reported α helical AMP discovered by Boman *et al.* on 1981, also Zasloff *et al.* on 1987 isolated and characterized magainin from African clawed frog.

3. Classification

Antimicrobial peptides can be classified into several categories based on three dimensional structure, net charge and biological sources by which they are isolated. Based on 3-D structure there are five types of antimicrobial peptides like cyclic, helical, extended, looped or hairpin and α and β mixed peptides. Among these peptide classes cyclic, looped and α and β mixed peptides are having disulfide bonds which provide stability to the 3-D structure. Most of the AMPs are linear helical in structure and such 3-D folded structures are attained only when these compounds are interacting with a solid membrane thereby adopt a stable amphipathic conformation.

Secondly, these AMPs are classified based on net charge. AMPs isolated from different sources can either be cationic, anionic or neutral as far as net charge is concerned. The chart give below (Fig. 1.) is showing the similar result, out of the total AMPs isolated, more than thousand are cationic whereas eighty in number are neutral and approximately seventy five of them are anionic (Wang, 2010).

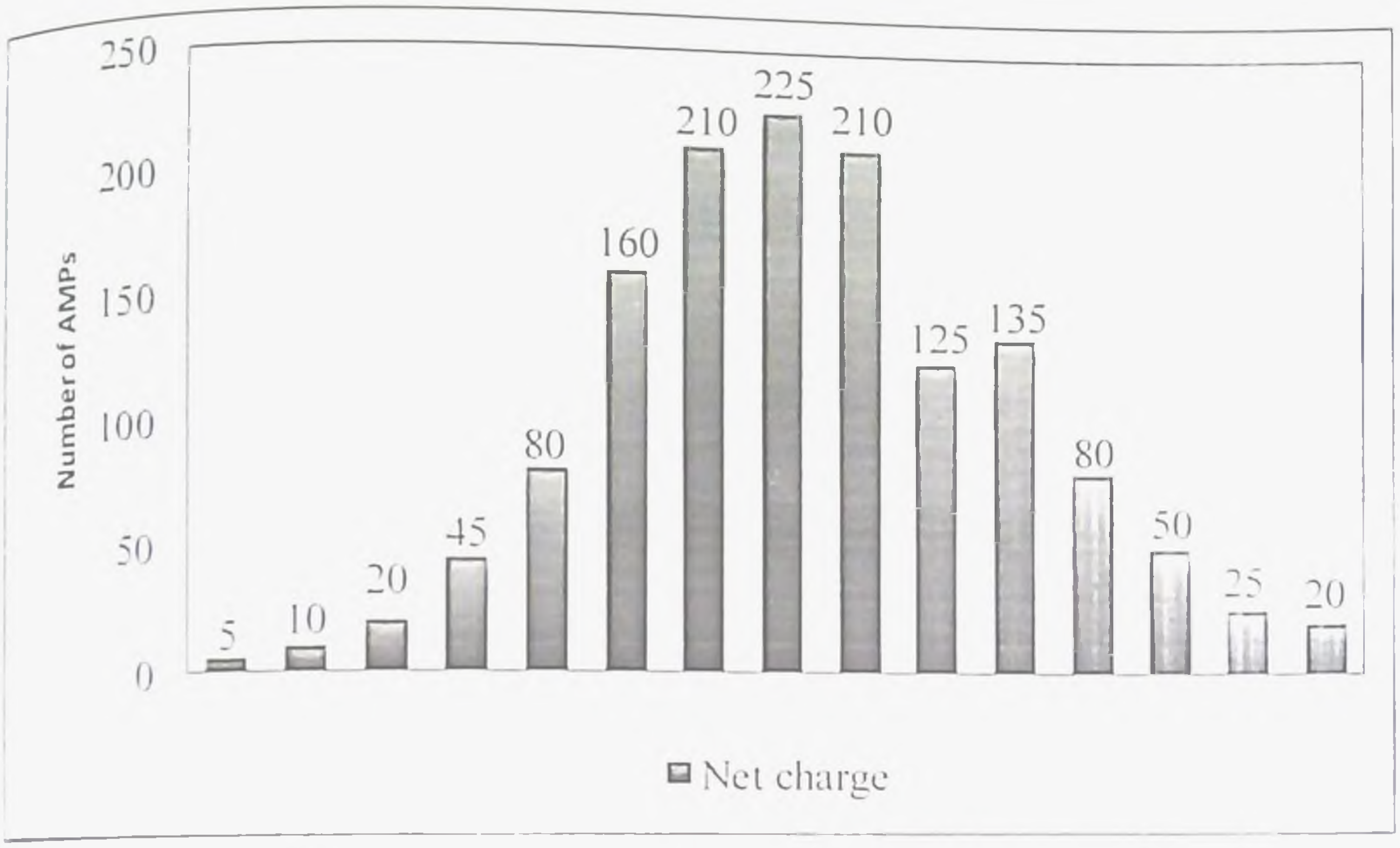


Fig. 1. Distribution of AMPs based on net charge

Third classification is based on the biological source in which they are isolated. Bacteriocins are AMPs isolated from bacteria. Similarly, there are Archaeal AMPs, Protozoan AMPs, Fungal AMPs, Plant AMPs, Animal AMPs and Human AMPs. About 73% of the AMPs isolated from animals, second comes plant as biological source, whereas least number of AMPs isolated from archaea, it is evident from the pie diagram (Fig. 2.).

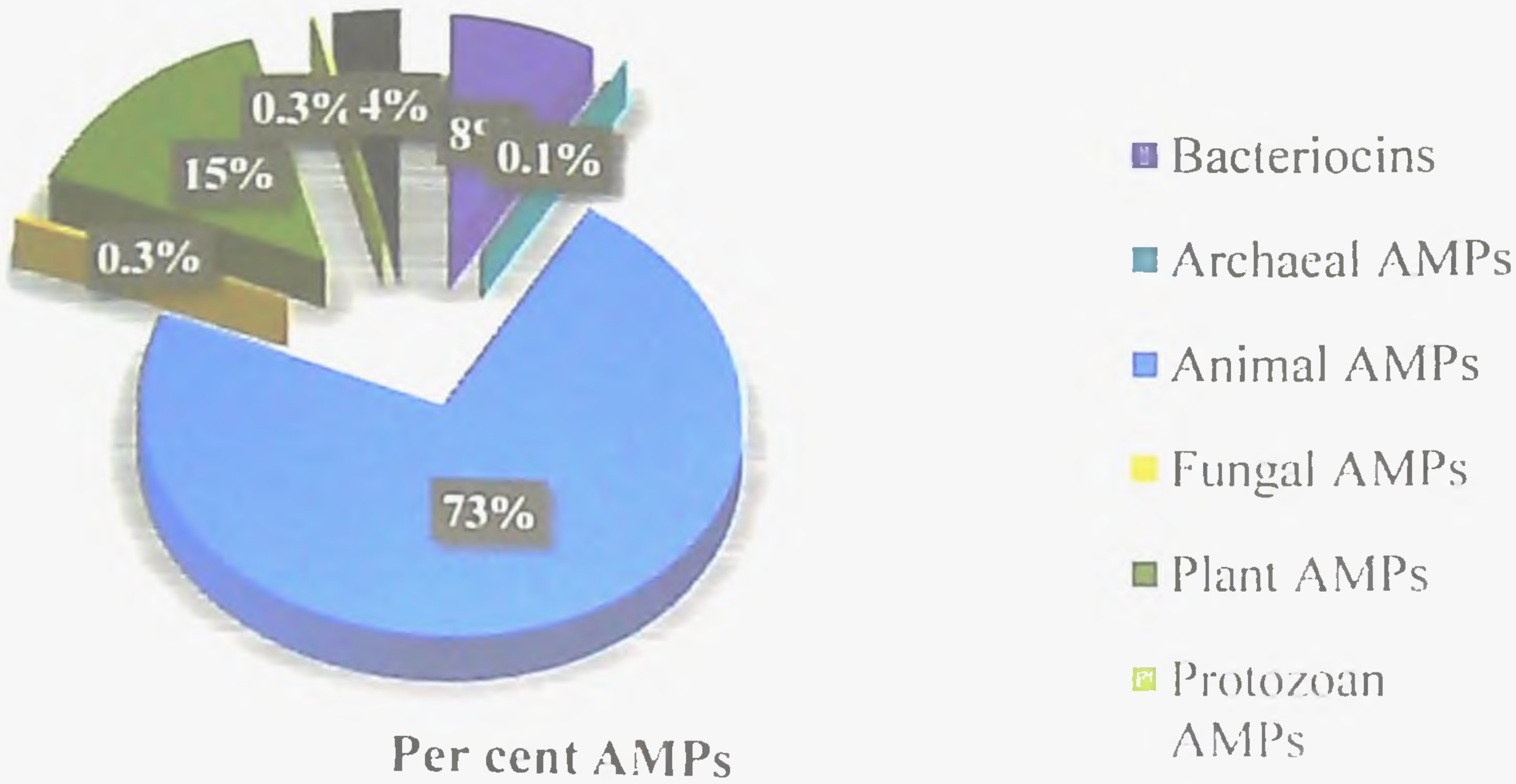


Fig. 2. Per cent AMPs isolated from different biological sources

3.1 Plant AMPs

Out of total AMPs isolated, 15% of them are from plants. A total of 271 plant AMPs have been recorded in current version of PhytAMP which is the database for plant AMPs. Different families of plants secreted AMPs are Amaranthaceae, Andropogoneae, Brassicaceae, Oryzeae, Santalaceae, Spermacoceae, Triticeae, Vicieae and Violaceae. Predominant genera of AMP producers are Viola (family Violaceae) and Arabidopsis (family Brassicaceae) as exhaustive studies have been carried out on these species. Classification of plant AMPs into different families has been proposed on the basis of primary structure (Castro and Fontes, 2005). Plant AMPs in the database are classified as cyclotides, defensins, Hevein-like, knottins, lipid-transfer proteins, snakins, thionins, vicilin-like (Fig. 3.). The majority of these AMPs possesses antifungal Also these possess insecticidal, anti-yeast and sodium channel blocker actions (fig. 4.). These findings may be useful in isolating and characterizing novel plant AMPs or designing novel peptides with higher potency against pathogens or with broad antimicrobial spectra. (51%), antibacterial (33%) and antiviral (10%) activities, as shown in pie diagram.

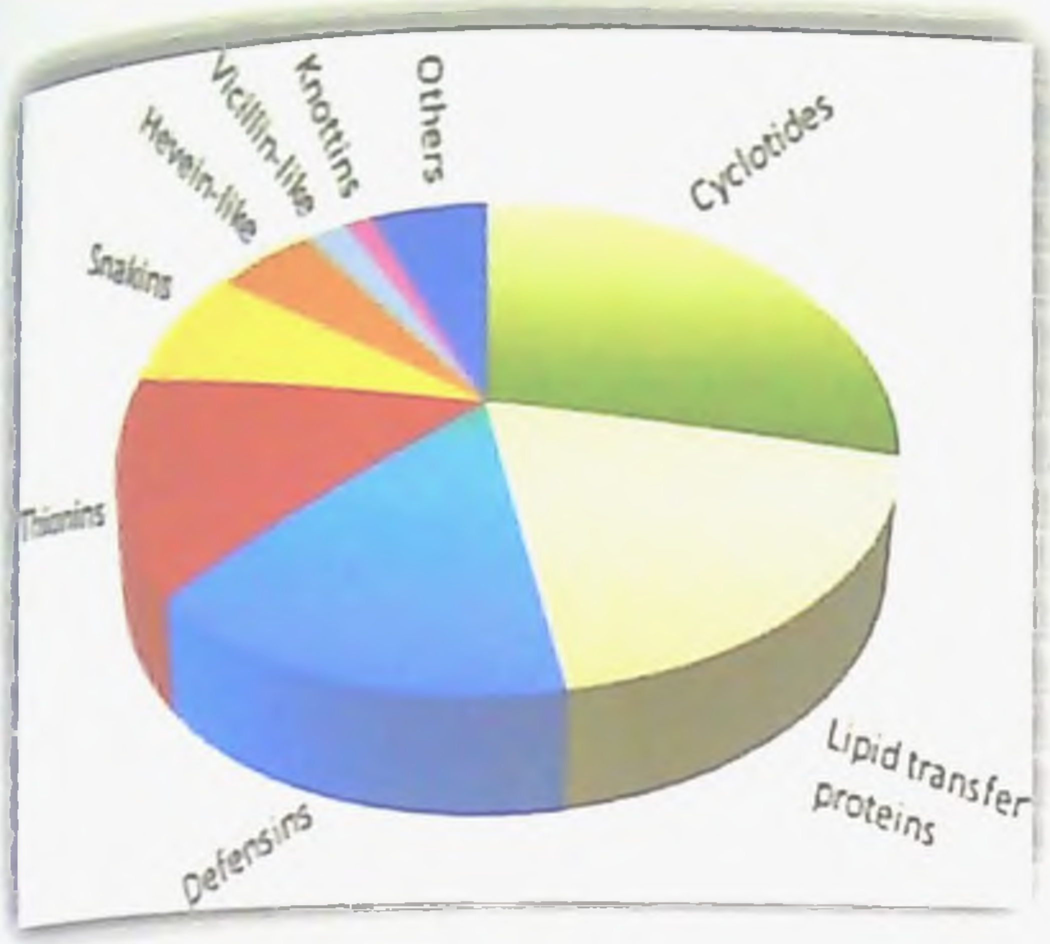


Fig. 3 Plant AMP families

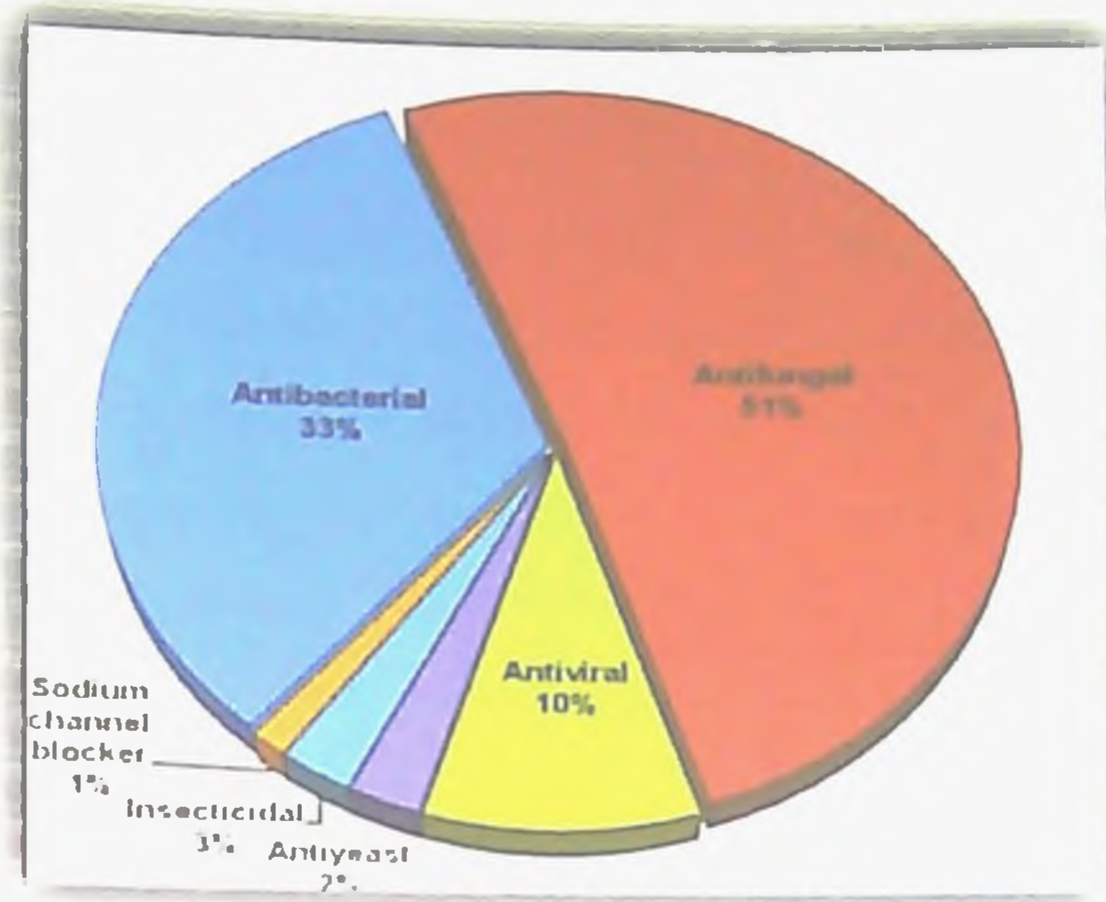


Fig. 4 Functions of plant AMPs

3.1.1 Structural features of plant AMPs

Structural features of AMPs relate with the mode of action by which they can act. Total amino acid number (residue number), net charge, number of disulfide bond and secondary structure are the major characteristic features determine the properties of AMPs. The table given below shows the characteristic features of families of plant AMPs (Table 1.).

h- helix, l- loop, s- sheet

Peptide family	Residue number	Charge	Disulfide bonds	Secondary structure
Thionins	45-47	+7 or +10	3 or 4	h, s
Defensins	45-54	+6	4	h, s
Hevein-like	43	-1	4	h, l, s
Knottins	30	+3	3	h, l, s
Cyclotides	28-37	-2 to +3	3	h, l, s
Snakins	63	+8, +9	6	h, l, s
Lipid transfer proteins	90-100	+8	4	h

Table 1. Structural features of plant AMPs

4. Role of AMPs in plant disease management

Antimicrobial peptide compounds are synthesized as part of innate defense mechanism of plants and animals at few concentrations. Thus use of such antimicrobial agents in disease management has immense scope to ward off infections caused by phytopathogens. One of the things that make AMPs attractive as antimicrobial compounds for plant disease management is the mechanism of action against the target microorganism. Mechanism by which these compounds are synthesized naturally as defense response is quite interesting. Such induction of compounds are initiated by plant-pathogenic interaction.

4.1 Innate defense mechanism of AMPs

Plant-pathogen interaction triggered by pathogen secreting enzymes that breakdown the plant cell wall and, once this defense is broken, plants distinguish a pathogen's presence through sensing pathogen-associated molecular patterns (PAMP) through transmembrane receptors. This enhances programmed cell death to the vicinity of pathogen action and such response is localized resistance mechanism which later leads to initiate systemic resistance, mediated by Systemic Acquired Resistance (SAR). SAR is facilitated by synthesis of Pathogenesis Related Proteins (PRPs), either by Salicylic acid (SA) or by simultaneous induction of Jasmonic acid (JA) and ethylene. Among the two pathways, JA and ethylene causes SAR to initiate against systemic management of phytopathogens (Dube, 2012).

An experiment conducted by Penninckx *et al.* in 1998 to prove influence of JA and ethylene in induction of AMPs. They used three cultivars of *Arabidopsis* sp., namely Colombian wild type (Col-0), Ethylene insensitive (Ein2-1) and Jasmonic acid insensitive (Col1-1) where Ein2-1 and Col1-1 are mutated which does not synthesis JA and ethylene respectively. Ethylene (50ppm), Paraquat (25μM), Methyl jasmonate (45 μM) and S drops leaf as control were applied on each cultivars. Their finding shows that only the cultivar, Col-0 can synthesis ethylene and JA could induce plant defensin whereas other cultivars lack either JA or ethylene could not induce. This proves that JA and ethylene have synergistic effect which act in parallel pathways for induction of plant defensin in *Arabidopsis* sp. (Fig 5.).

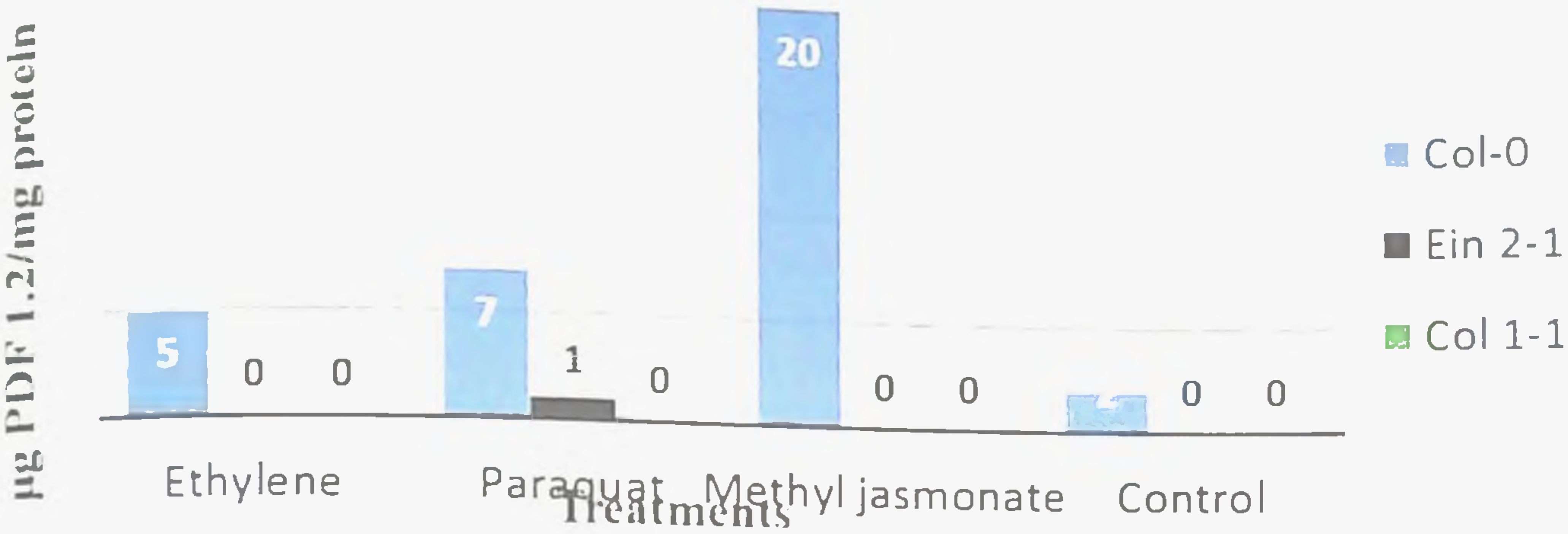


Fig. 5 Induction of plant defensisn in *Arabidopsis* sp.

4.2 Isolation of AMPs

Antimicrobial peptides synthesized in any organisms can be isolated and purified using usual protein extraction and purification procedures. First, the biological source is inoculated with a pathogen of our interest. It will trigger the synthesis of AMPs which will be antagonistic to the pathogen. And a control is also maintained as well. Then, usual protein extraction procedures is followed to for both treated sample and control. Compound which are synthesized due to pathogen inoculation is identified by comparing with running control sample also. SDS PAGE followed by HPLC and mass spectrometry can separate peptide fraction from other compounds.

4.3 Mode of action

AMPs typically have a broad spectrum of against pathogenic fungi, bacteria and viruses. Most eukaryotic peptides act on bacterial membranes or other generalized targets, in contrast to most antibiotics, which usually target specific proteins.

The mode of action of these peptides has been more clearly defined recently. AMPs are selective for prokaryotic membranes over eukaryotic membrane due to the predominantly negatively charged phospholipids in the outer leaflet of the prokaryotic membrane (Tossi *et al.*, 2000). Such preference is considered a regulatory function in target selectivity. Althought the overall charge of the peptide is important, it is known that other features play a role in potency and spectrum of the peptide. The size, sequence, structure (per cent helical content), overall hydrophobicity, amphipathicity, and width of the hydrophobic and hydrophilic regions of the peptide have a function in the efficiency of the peptide (Tossi *et al.*, 2000).

4.3.1 Antibacterial action

Lytic or pore forming action and non-lytic action are the two ways in which AMPs act against phytopathogenic bacteria.

4.3.1.1 Lytic or pore forming action

AMPs which are unstructured in aqueous solution when interact with a solid membrane as monomeric units. Later, unstructured monomeric peptides adopt a three-dimensional structure upon the interaction with the bacterial membrane and fold into amphiphilic molecules, with positively charged sides directly interacting with the anionic lipid headgroups of the bacterial membrane. The lipids are then displaced by the peptides, causing a thinning of the outer leaflet of the bacterial membrane leads to pore formation. Three pore forming models are barrel stave model, toroid pore model and carpet model. Polymeric complex peptide formed from monomeric peptides later transformed into barrel like structures and enter perpendicularly through the hydrophobic core. Toroid pore model is also similar to barrel stave but, the barrels formed is intercalated with the phospholipids of bacterial membrane. Third model, carpet model causes a phospholipid displacement that alters membrane fluidity and/or reduces the barrier properties of membrane. It also leads to membrane disruption and, further, to cell death. Due to the unfavourable energy observed after the membrane bilayer becomes curved, cell rupture and lysis will occur. All the three models later results in imbalance homeostasis of bacterial cell due to efflux of ions bring about death of bacterial cells.

Alan and Earle in 2001 compared three lytic peptides, MSI 99, Magainin II and Cecropin B against *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris* pv. *vesicatoria*, *Clavibacter michiganensis* pv. *michiganensis*, *Erwinia carotovora* subsp. *carotovora*, *Erwinia carotovora* subsp. *chrysanthemi* and *Ralstonia solanacearum*. The peptide Cecropin B has found inhibited two species of *Pseudomonas*, *Erwinia* and *Xanthomonas* sp. at lower concentration. Similarly, MSI 99 also inhibited both species of *Pseudomonas*, *Xanthomonas* sp. and *Clavibacter* sp. at same concentration. But, no peptide can resist growth of *Ralstonia solanacearum* even at highest concentration (Table 2.).

Bacterial pathogen	Peptide (µg/ml)					
	MSI-99		Magainin II		Cecropin B	
	MIC^R	MIC^L	MIC^R	MIC^L	MIC^R	MIC^L
Pseudomonas syringae pv. *tomato*	5	10	10	20	5	5
Pseudomonas syringae pv. *syringae*	5	10	5	20	5	5
Xanthomonas campestris pv. *vesicatoria*	5	5	5	10	5	5
Clavibacter michiganensis pv.	5	5	15	20	20	20
Erwinia carotovora subsp. *carotovora*	10	15	10	>30	5	5
Erwinia carotovora subsp. *chrysanthemi*	10	15	10	>30	5	5
Ralstonia solanacearum	>30	>30	>30	>30	>30	>30

Table 2. Sensitivity of bacterial plant pathogens to lytic peptides

4.3.1.2 Non-lytic action

Proline rich AMPs act without extensive membrane damage, mostly targeting Gram-negative bacteria (Casteels, 1989). They have been shown to penetrate into bacterial cells by translocating across the membrane, suggest a stereospecific mode of action involving interactions with a transport system followed by inhibition of specific intracellular target(s), rather than a non-specific disruption of membrane integrity. Apidaecins, pyrrhocoricins are the two major proline rich AMPs with antibacterial action. Casteels in 1989 tested purified forms of three apidaecins, Ia, Ib and 2 against *Agrobacterium tumefaciens, Erwinia amylovora,* var. *salicis, Pseudomonas syringae* pv. *tomato, Corynbacterium insidiosum.* He has found that, all the three purified forms were active against *Agrobacterium* sp. and *Erwinia* sp. at similar concentrations, whereas, the peptides could not resist *Cornybacterium insidiosum* (Table 3.). The table shows the examples of few antibacterial AMPs (Table 4.).

Bacterial strains	Minimal inhibitory concentration (µg/ml) of apidaecins		
	Ia	Ib	2
Agrobacterium tumefaciens	0.2	0.2	0.2
Erwinia amylovora var. *salicis*	0.02	0.02	0.02
Pseudomonas syringae pv. *tomato*	0.2	0.1	0.1
Corynebacterium insidiosum	50	50	100

Table 3. Effect of apidaecins against plant pathogens

Peptide	Source	Susceptible species	Reference
Purothionin (thionin)	Wheat	*Pseudomonas solanacearum*	Caleya *et al.*, 1972
		Xanthomonas phaseoli	
		Erwinia amylovora	
StSN2 (snakin)	Potato	*Clavibacter michiganensis* *Ralstonia solanacearum*	Lobo *et al.*, 2002
MsDef1 (defensin)	Alfalfa	*Erwinia caratovora*	Spelbrink *et al.*, 2004
PR-13 (thionin)	Tobacco	*Pseudomonas syringae* pv. *tomato*	Rayapuram *et al.*, 2008
Tachyplesin I	Horseshoe crab	*Erwinia caratovora*	Goyal and Mattoo, 2014
Attacin A	Ni moth	*Xanthomonas axonopodis* pv. *citri*	Goyal and Mattoo, 2014

Table 4. Examples of antibacterial AMPs

4.3.2 Antifungal action

Antifungal AMPs also utilize cationic and amphipathic property for membrane interaction and permeabilisation of fungal hyphae thus inhibiting fungal growth through altering homeostatic balance. Another mechanism is that, after entry of AMPs into fungal hyphae, it enhances production of Reactive Oxygen Species (ROS) which is deleterious to fungi. Few examples of antifungal AMPs are listed (Table 5.).

AMP	Source	Susceptible species	Reference
Ace-AMP1 (LTP)	Onion	*Fusarium oxysporum*	Cammue *et al.*, 1995
Rs-AFP1	Radish	*Botrytis cinerea*	De Lucca *et al.*, 1999
Mj-AMP2	Four O' clock	*Alternaria alternata*	Gao *et al.*, 2001
AFP	*Aspergillus*	*Botrytis cinerea*	Moreno *et al.*, 2003
Cecropin B	Silk moth	*Magnaporthe oryzae*	Goyal and Mattoo, 2014
Dermaseptin	Frog	*Phytophthora infestans*	Goyal and Mattoo, 2014
Cathelicidin	Human	*Fusarium oxysporum*	Goyal and Mattoo, 2014

Table 5. Examples of antifungal AMPs

VvAMP1, AMP isolated from *vitis vinifera* is inoculated against few pathogenic fungi like *Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Verticillium dahlia.* Experiment conducted by Beer in 2008 used two concentrations of VvAMP1, 6 µg ml^{-1} and 9 µg ml^{-1}. AMP at 9 µg ml^{-1} resist all the fungal pathogens successfully (Fig. 6.).

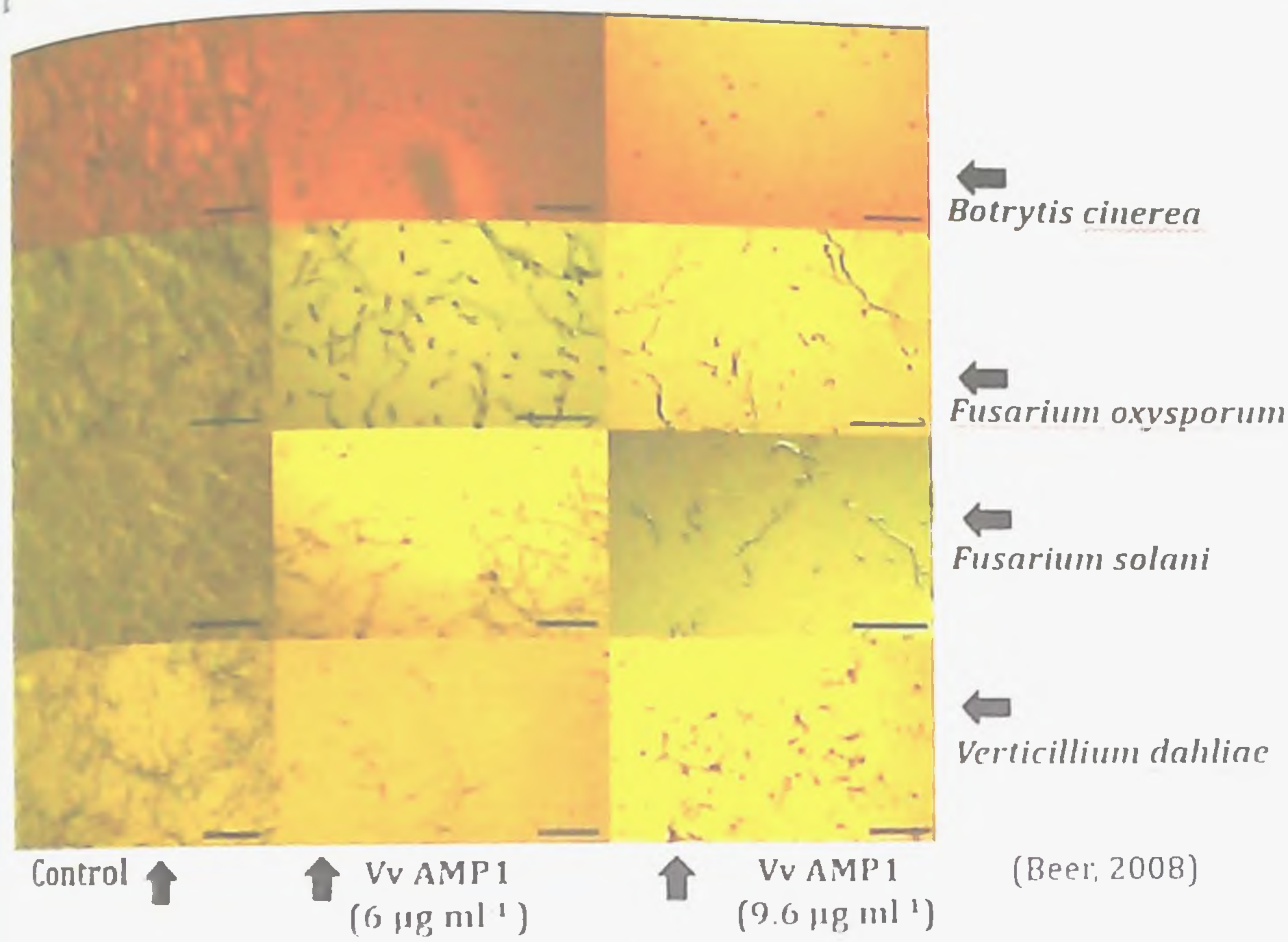
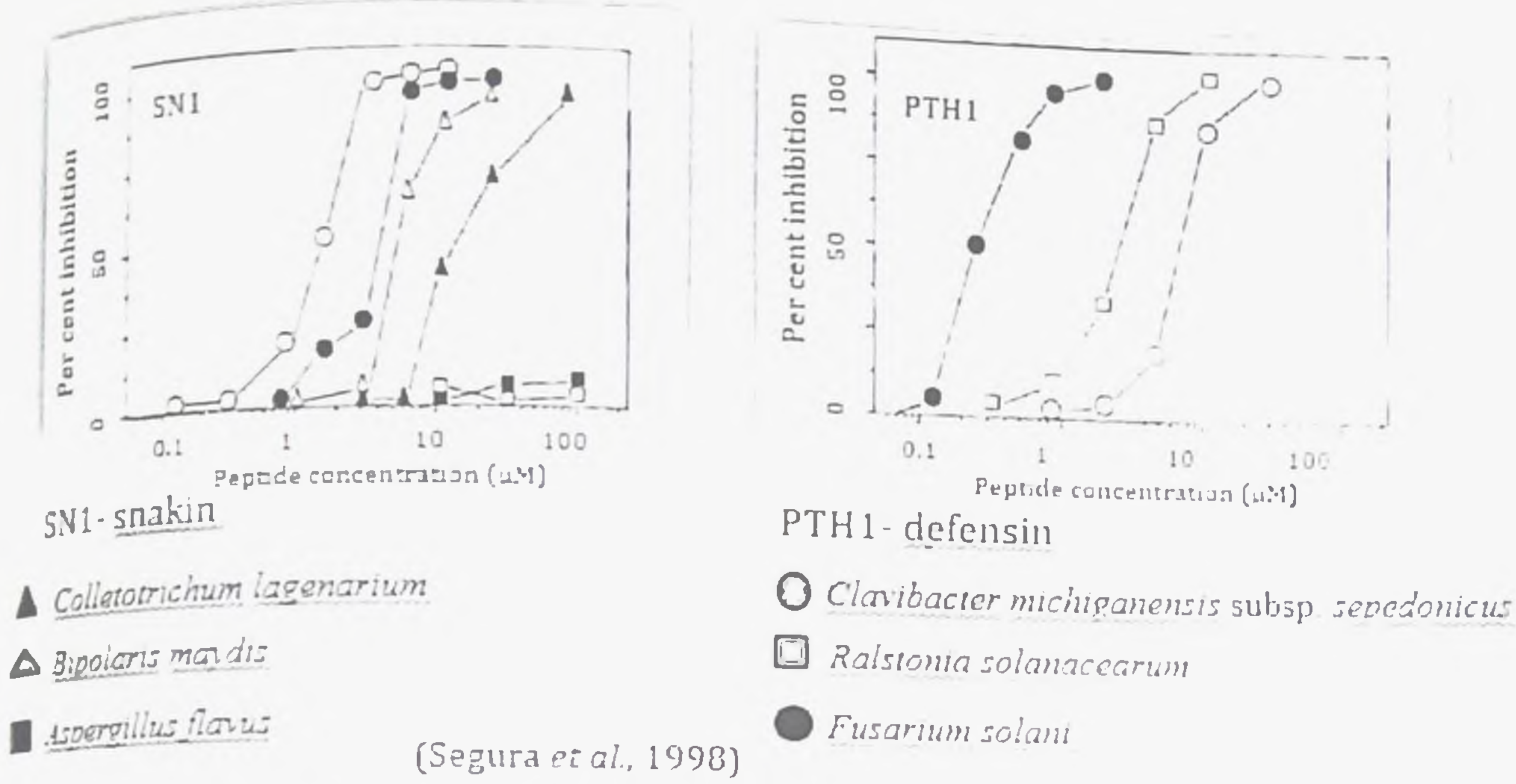


Fig 6. Inhibition of fungal plant pathogens by VvAMP1

Another experiment done by Segura *et al.*, 1998 examining effect of two peptides, snakin and defensin isolated from potato against both bacterial and fungal pathogens. Snakin (SN1) is tested against 6 pathogens and shown hundred per cent inhibition except *Ralstonia solanacearum* and *Aspergillus solani*. Defensin (PTH1) is tested against *Fusarium solani, Clavibacter michiganensis subsp. sepedonicus* and *Ralstonia solanacearum*. All the three pathogens were inhibited by PTH1 (Fig. 7.).



(Segura *et al.*, 1998)

Fig 7. Effect of snakin and defensin from potato against plant pathogens

Vasquez (2009) conducted a field study comparing the efficiency of AMP, Ib-AMP4 against two commercial agrochemicals, propiconazole and azoxystrobin on black sigatoka disease. 25, 50 and 75µg/ml concentrations of all the three chemicals were sprayed on the sigatoka affected banana crop. Table shows the result, at 75 µg/ml Ib-AMP4 and propiconazole found effective against the disease whereas azoxystrobin showed less efficiency at the same concentration (Table 6.)

Agrochemical	Concentration (µg/ml)	Per cent inhibition
Propiconazole	25	36.57
	50	96.72
	75	99.34
Azoxystrobin	25	13.92
	50	38.59
	75	62.91
Ib-AMP4	25	11.95
	50	46.53
	75	99.21

Table 6. Effect of AMP on black sigatoka disease

4.3.3 Antiviral action

AMPs isolated from several sources show antiviral property too. These compounds inhibit protein synthesis, either coat proteins or movement proteins which are essential for the systemic infection of viral diseases. Another mechanism by which viruses can be inhibited by blocking replication of viral particles. Certain AMPs like peptamine synthesized from *Pseudomonas chlororaphis* inhibit viral infection by stimulating Induced Systemic Resistance (ISR).

4.3.3.1 Mechanism of antiviral peptamine

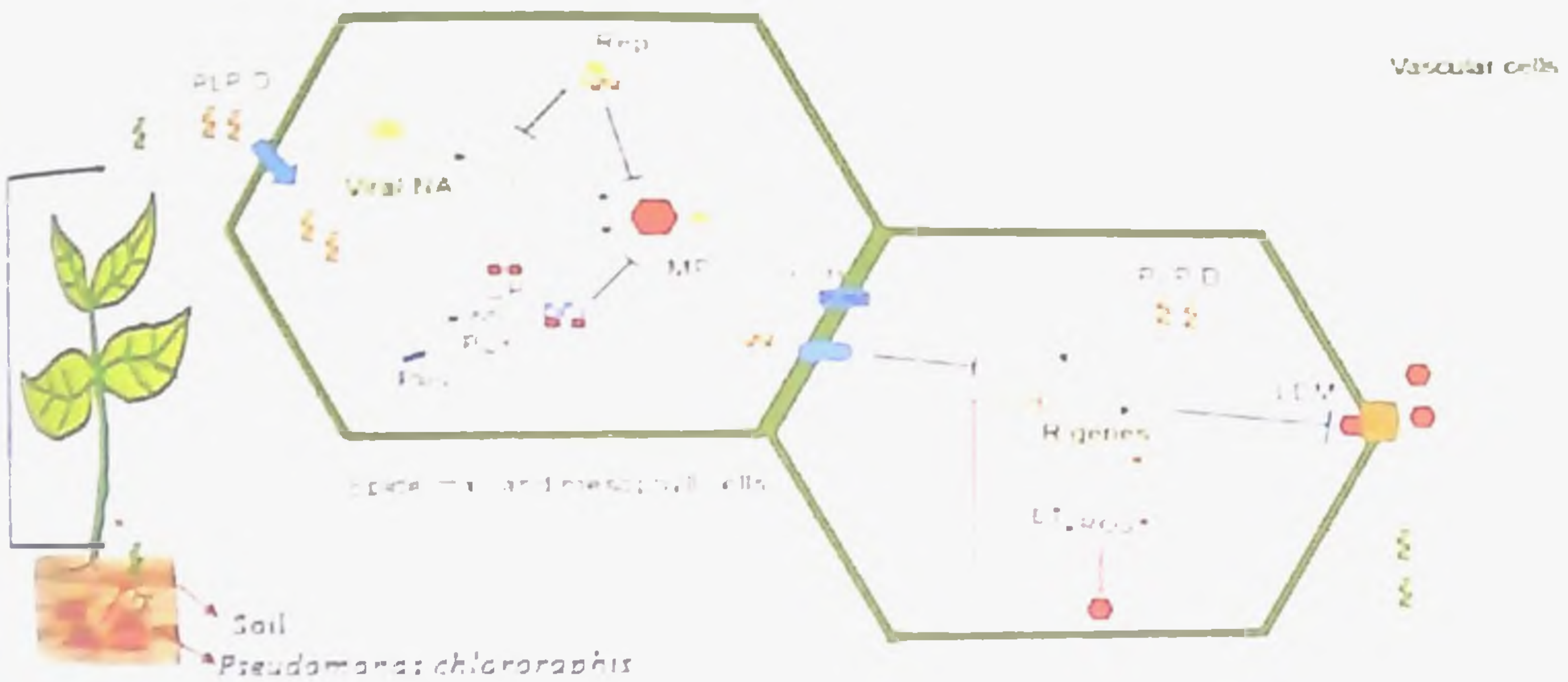


Fig. 8. Mechanism of antiviral peptamine

Plant peptide (PLP/D), Coat Protein (CP), Movement Protein (MP), Replicase (Rep)
Cell to cell movement (C-CM), Plasmodesmata (PLDM), Long Distal Movement (LDM)
Peptide aptamers (PLY), Nucleic Acid (NA), Salicylic acid (SA), Ethylene (ET),
Reactive Oxygen Species (ROS)

Peptamine, synthesized by non-pathogenic bacteria, *Pseudomonas chlororaphis* stimulate resistance genes which resist long distant movement of viral particles by inducing ethylene and Reactive Oxygen Species (ROS). Also these peptamine can be transformed into plant and thus the compound cause inhibiting cell to cell movement and long distant movement of viral particles. Effect of peptamine against Tobacco Mosaic Virus (TMV) is verified by Park *et al.* (2012) and got end result that at 1000 µg/ml resisted 95% infection (Fig. 9.).

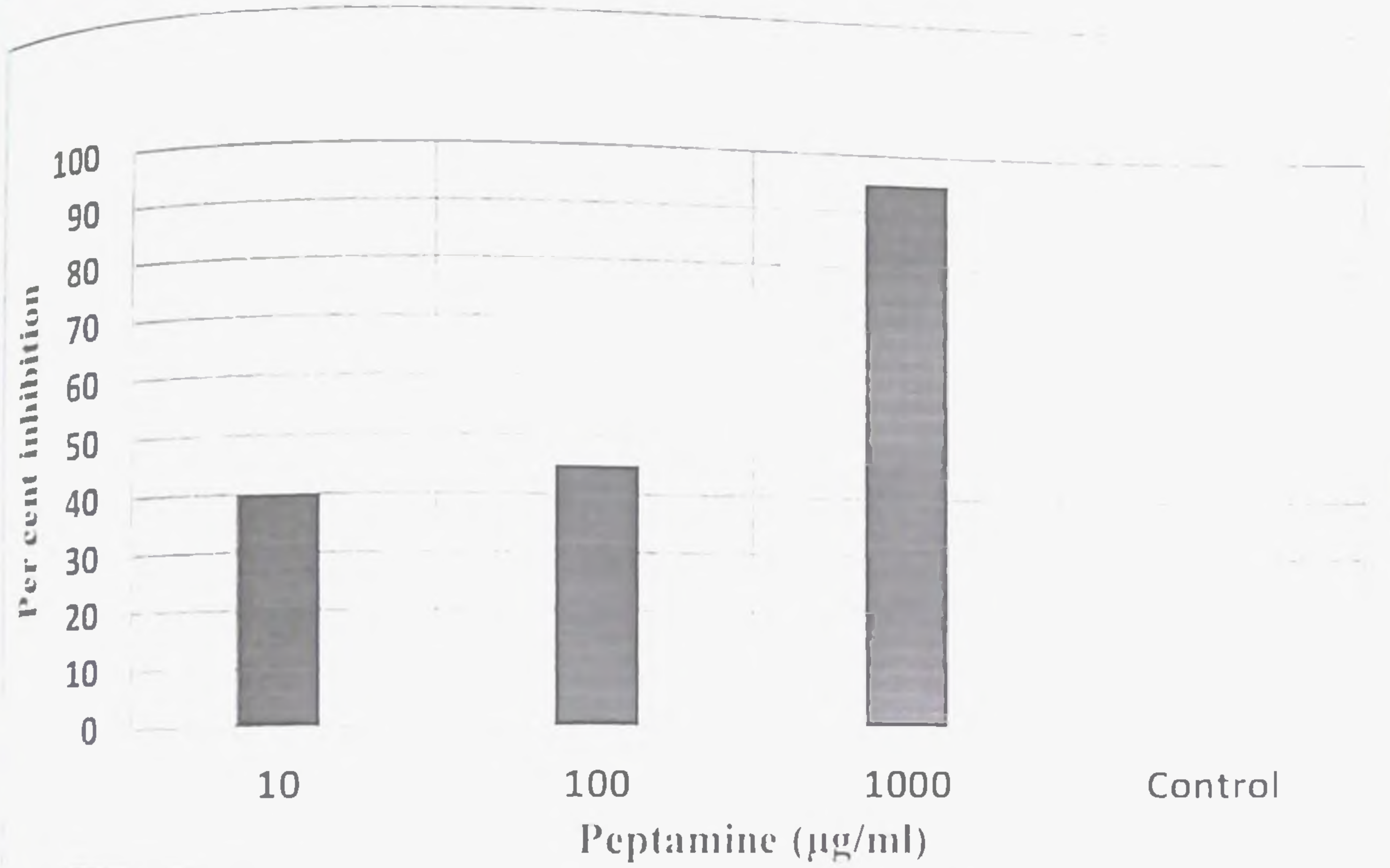


Fig 9. Effect of peptamine against TMV

Melittin is a natural AMP being isolated from honey bee is having antiviral property due to sequence homology on TMV infected crops (Culver *et al.* 1995) (Fig. 9.). Thus, Marcos in 1995 generated analogues of melittin and checked the efficiency against TMV. Analogues of melittin were formed, SubK7D, SubK7G, SubK7L and SubK7I by substituting amino acid lysine. SubK7I showed better result by causing lowest per cent necrotic lesion and this is cross infected with other viruses like ToMV, Ob, SHMV. Since ToMV showing highest homology with TMV, the melittin analogue exhibited highest resistance against it whereas SHMV is least homologous became impossible to manage (Fig. 11.).

Fig. 10 Homologous sequence of TMV coat protein and Melittin

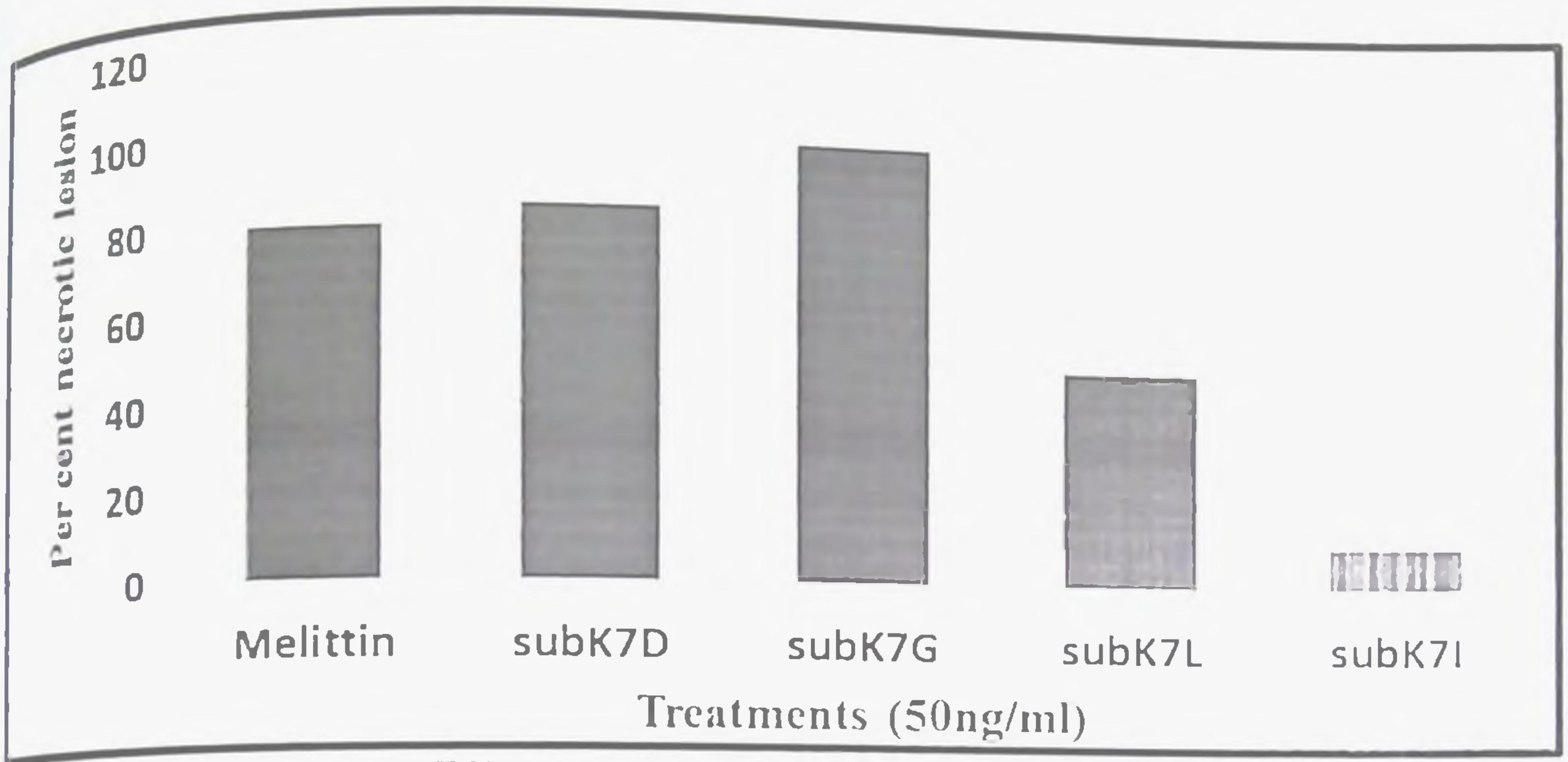
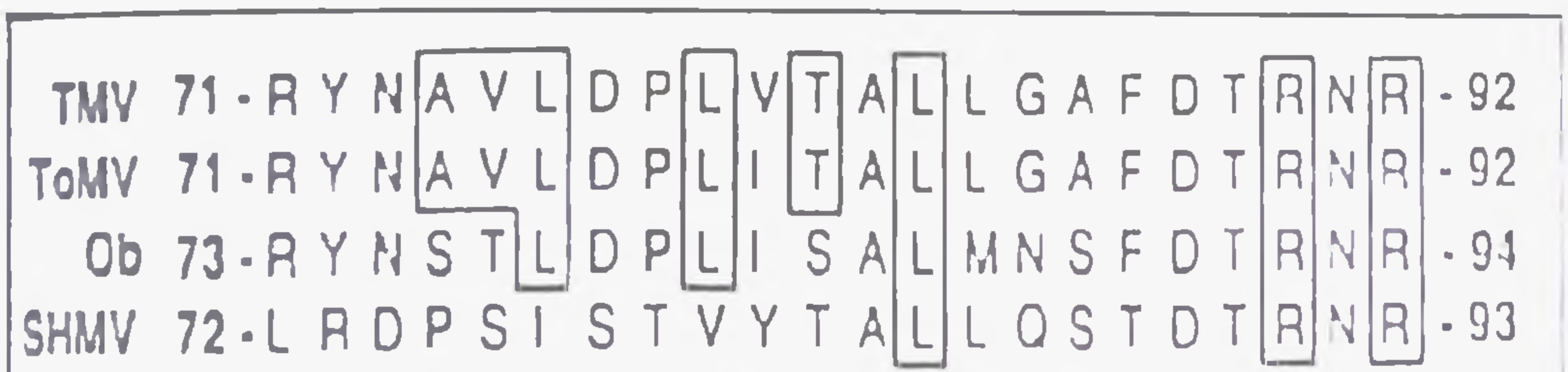


Fig 11. Effect of melittin analogues on TMV



Sequence homology of viruses

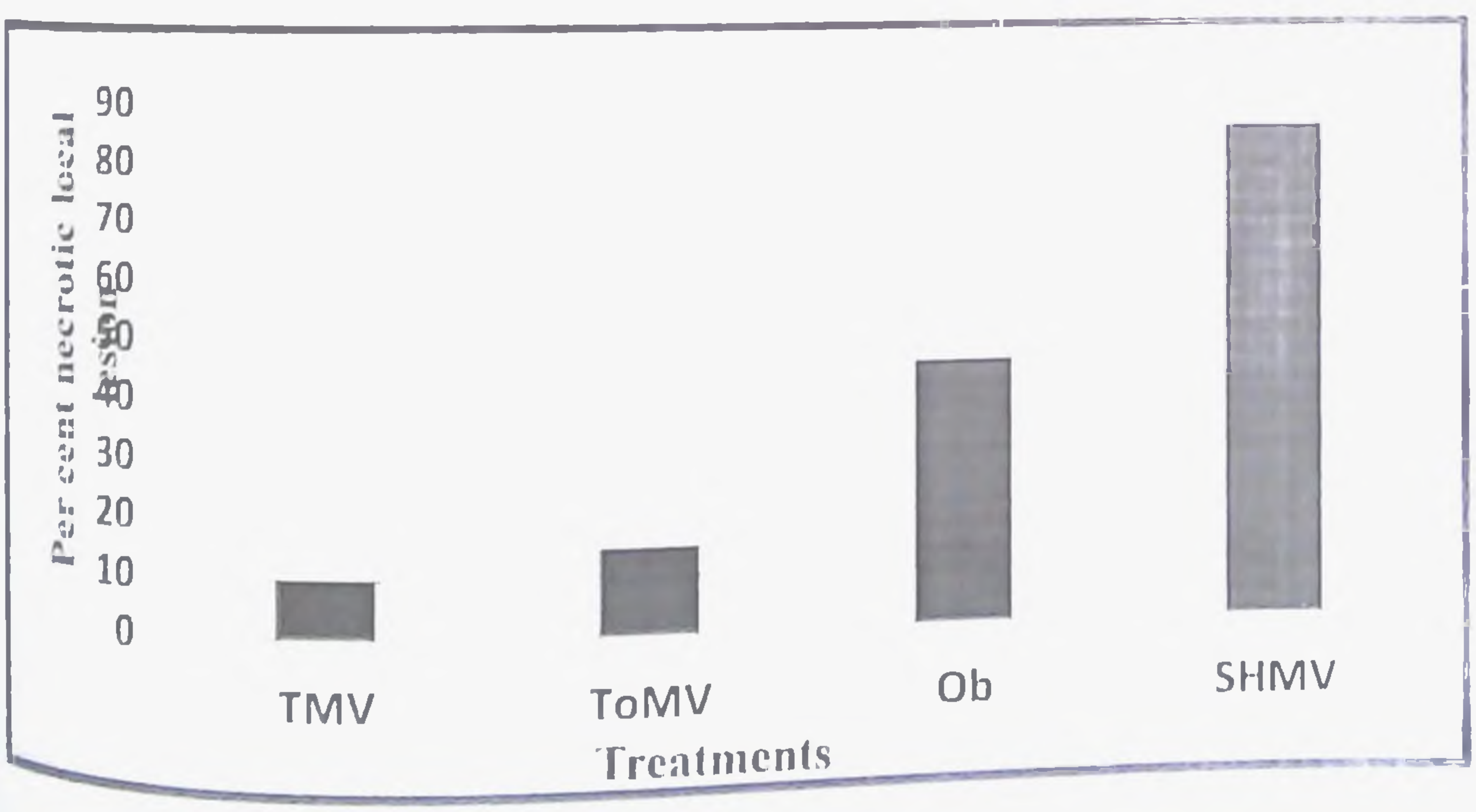


Fig 12. Effect of SubK7I on other viruses

Few examples for other antiviral AMPs are enlisted.

Peptides	Source	Susceptible virus	Mode of action	Reference
PAP	*Phytolacca americana*	Tobacco mosaic virus Cauliflower mosaic virus Cabbage mosaic virus	Inhibit protein synthesis	Chen *et al.*, 1991
Potide-G	*Solanum tuberosum*	Potato virus Y	Unknown	Tripathi *et al.*, 2006
Indolicidin	*Bos taurus*	Tobacco mosaic virus	Unknown	Bhargawa *et al.*, 2007

Table 7. Examples of antiviral AMPs

5. Synthetic AMPs

The purpose of designing synthetic peptides is to optimize their desired activity as gene products prior to clone them into a plants. Researches for shorter, more potent, non-toxic peptides have led to the discovery of synthetic peptides with broader and higher antimicrobial activity than their natural counterparts (Ali, 2000). Design of AMPs bioinformatics tools like Swiss model, I Tasser paves a new path in predicting and developing synthetic AMPs. Substitution, chain elongation or deletion of amino acids on natural conformation of AMPs, thus rectifying the defects in antimicrobial molecule make them highly effective than natural counterparts.

Ali (2000) examined the effect of four synthetic peptides, Pep 6, Pep 7, Pep 11, Pep 20 against *Phytophthora infestans*, *Alternaria solani*, *Erwinia caratovira* subsp. *caratovora*, *Erwinia caratovira* subsp. *atroseptica*. Experiment showed that synthetic AMPs, Pep 20 were effective against *Phytophthora infestans*, *Alternaria solani*, *Erwinia caratovira* subsp. *caratovora* whereas Pep 11 inhibited *Erwinia caratovira* subsp. *atroseptica* successfully (Table 8). Since the AMPs were non-natural these AMPs tested for phytotoxicity and proved less toxic (Table 9.).

Plant Pathogen	IC50 values (µM)			
	Pep 6	Pep 7	Pep11	Pep20
Phytophthora infestans	56.0	910.0	17.0	14.0
Alternaria solani	14.1	72.7	3.3	2.7
Erwinia caratovora sub sp. *caratovora*	>500	>500	6.3	5.2
Erwinia caratovora sub sp. *atroseptica*	>500	>500	2.2	2.6

Table 8. Inhibition of fungal and bacterial pathogens by synthetic peptides

Treatment (200µM)	Shoot length of potato (cm)	Fresh weight of potato plantlet (mg)
Pep 6	3.4±0.30	62.0±4.90
Pep 7	2.9±0.21	50.6±5.02
Pep 11	3.8±0.35	71.1±6.07
Pep 20	4.2±0.31	71.3±4.27
Kanamycin*	2.1±0.29	30.4±3.18
Control	4.5±0.21	71.8±2.62

Table 9. Testing phytotoxicity of synthetic AMPs

There are many synthetic AMPs being synthesized mimicking natural sources and exhibiting better performance against plant pathogens. Certain examples are enlisted (Table 10.)

Synthetic AMP	Natural source	Susceptible pathogens	Reference
D32R	Thionin	*Xanthomonas campestris* pv. *malvacearum*	Perello *et al.*, 2003
D4E1	Cecropin	*Thielaviopsis basicola*	Rajasekharan *et al.*, 2005
10 R	Indolicidin	Tobacco mosaic virus	Bhargava *et al.*, 2007
LL-37	Cathelicidin	*Xanthomonas campestris* pv. *vesicatoria*	Jung *et al.*, 2013
MsrA1	Magainin	*Alternaria brassicae*	Rustagi *et al.*, 2014

Table 10. Examples of synthetic AMPs and their sources

5.1 Transgenic approach

Novel approaches in plant genetic transformation and prompt development in recombinant DNA techniques have made possible to introduce selected genes into plants to confer disease resistant phenotypes (Owens, L. D. 1995). AMPs has received limited attention until recently in plant disease management through transgenics. Li et al. (2001) have reported disease resistance, to both a fungal and a bacterial pathogen, conferred by expression of a magainin analog, Myp30, in transgenic tobacco (*Nicotiana tabacum* var. Petit Havana).

Cecropins, a small family of naturally occurring lytic peptides found in *Hyalophora cecropia*, the giant silk moth. They exhibit a broad spectrum of antibacterial activity against both Gram negative and Gram positive bacteria. This observation incited scientists to propose the idea of using the genes cloned from insects to enhance bacterial disease resistance in plants. Certain examples of successfully transformed plants using cloned genes of AMPs has enlisted below, showing systemic resistance to susceptible pathogens.

Plants transformed	AMP	Susceptible pathogens	References
Rice	Cecropin A, B	*Magnaporthe grisea*	Coca *et al.*, 2004
Arabidopsis sp.	Rev 4	*Peronospora tabacina*	Ning *et al.*, 2006
Tobacco	Alf-AFP	*Verticillium dahliae*	Gao *et al.*, 2000
Banana	MSI-99	*Agrobacterium tumefaciens*	Vidal *et al.*, 2006
Brinjal	Dm-AMP1	*Botrytis cinerea*	Turrini *et al.*, 2004
Tomato	Mj-AMP1	*Alternaria solani*	Schaefer *et al.*, 2005

Table 11. List of transformed plants with AMPs expressing gene

Synthetic AMP	Natural source	Susceptible pathogens	Reference
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Table 11. List of transformed plants with AMPs expressing gene

6. Advantages and disadvantages

Action of AMPs are not site specific as they target cell membrane, transport systems, external proteins, outer surface lipids, nucleic acids and impairing plant pathogens. Antibacterial, antifungal and antiviral property of AMPs categorized them as broad spectrum compound which are regarded as future antibiotics or an alternative to conventional antibiotics. Even though these compounds are active at low concentration, biodegradable and low cytotoxic commercializing formulations as antibiotics are not much economical. Also there are few drawbacks by which many novel researches are pulled off, they are toxic effects of these compounds on beneficial microbes inside the plant species and degradation of AMPs by endogenous proteases in plants.

Certain bacteriocins like Gramicidin isolated from *Bacillus brevis*, Valinomycin from *Streptomyces fulvissimus* and Agrastatin are being commercialized as novel antibiotics against plant pathogens.

7. Conclusion

As in plant disease managemnt areas, AMPs could play strong roles as plant protection products. Successful use of AMPs has been achieved through the commercial development of biopesticides. However, exploitation of the great number of AMPs as active ingredients of pesticides has not been accomplished yet. The majority of AMPs with potential uses have been studied at the in vitro level, fewer compounds have been tested on plant pathosystems, and only a few are on the market. Development of compounds suitable for agricultural use as pesticide ingredients have several constraints mainly due to the intrinsic toxicity and low stability of some of the compounds, the requirement to develop suitable formulations, and the need for inexpensive products in plant protection. Therefore, future areas of interest consist of developing less toxic and more stable compounds.

as well as decreasing production costs by improving preparative synthesis and biotechnological procedures using microbial systems or transgenic crops as plant factories. Also, several transgenic plants expressing AMPs that confer different degrees of protection against diseases have been developed, commercial cultivars have not been marketed because of regulatory limitations and social concerns. Synthetic approaches to obtain AMPs are powerful tools to optimize molecules derived from natural compounds with improved activity against selected target pathogens, including decreased cytotoxicity and increased protease stability. Cost of production and commercializing AMPs as novel antibiotics are less reasonable, as a result major research works are focused on transgenic crop improvement. But such approaches have legal issues as obstacle. Thus commercializing them as novel biocides at low cost is the need of the hour.

8. Discussion

1. Whether AMPs are synthesized during stress conditions?

Yes, AMPs do synthesized during stress conditions also. Plants, microbes like bacteria, fungi produces AMPs as defense system in their tissues or cells under stress.

2. What is the difference between AMPs and antibiotics?

AMPs are regarded as future antibiotics. Many conventional antibiotics became ineffective due to existence of resistant strains of pathogen which was supposed to be destroyed. In this circumstances AMPs as novel source of antibiotics has its importance.

3. How does plant differentiate a pathogen and non-pathogen about to infect?

If a pathogen is coming in contact with a plant tissue, the receptor present in the plants recognize based on pathogen-associated molecular pattern (PAMP) which is the receptor of the pathogen.

4. Can we use AMPs as biocontrol agents?

Yes, AMPs are synthesized in many bacteria and fungi species, such species can be utilized as biocontrol agents. For example, cyclic lipopeptides from *Bacillus amyloliquefaciens* FZB42 has the ability to control *Fusarium oxysporum*. Mycosubtilin produced by Bacillus subtilis ATCC6633 were more effective in controlling Pythium damping-off on tomato.

5. Whether AMPs are synthesized as secondary metabolites?

AMPs synthesized by bacteria are by non-ribosomal synthesis as secondary metabolic process.

6. Is there any chances of using AMPs as prophylactic pesticide?

AMPs show systemic type of disease management as that of antibiotics and few other fungicides. These compounds are not advisable to apply as prophylactic since they are easily degradable and affected by endogenous protease critically. Thus applying it as prophylactic measures does not bring about management of plant diseases.

7. What is the status of commercial production of AMPs as pesticides in India?

Commercial production of AMPs as novel pesticides is not at its developing side. because of its cost of production. And producing transgenic crop that expressing finds more economical than manufacturing AMPs in large scale.

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Abstract

Phytopathogenic microbes cause significant yield loss and it can also affect the quality and safety of agricultural products. For the past several decades, management of plant diseases has been increasingly dependent on the extensive use of toxic chemicals. However, continuous and widespread use of these chemicals resulted in development of resistant strains of pathogens in addition to their adverse effect on the environment. Concern of these adverse effects resulted in the search for new antimicrobial agents and one such antimicrobial agent of promise is the antimicrobial peptides.

Antimicrobial peptides (AMPs) are the first line of defense in plants and animals against invasion of pathogenic organisms and they are short sequence peptides with generally fewer than 50 amino acid residues reported in living systems (Montesinos, 2007). About more than 900 AMPs have been reported from prokaryotes and eukaryotes. They are produced by ribosomal or non-ribosomal synthesis and expresses genes coding for the synthesis of these compounds conferring partial or total resistance to plant pathogens. In plants, innate defense mechanism is mediated by pathogenesis related proteins (PRPs) synthesis through hypersensitive response (HR) followed by salicylic acid independent pathway utilizing jasmonic acid and ethylene for the induction of AMPs (Dube, 2012). Such naturally synthesized AMPs can be isolated and purified by usual protein extraction procedures which are proved effective against many phytopathogens.

Mode of action of AMPs against fungal and bacterial pathogens depend upon the net positive and amphipathic nature of the compound. AMPs can lyse bacterial cells by pore forming mechanism or reduce bacterial growth by non-lytic methods. Antifungal action of AMPs depends on the membrane interaction of fungal hyphae and also by formation of reactive oxygen species (ROS) (Rahnamaeian, 2011). Antiviral peptides restrict viral replication and inhibit protein synthesis.

Design of AMPs using bioinformatic tools like Swiss model and I Tasser paves a new path in predicting and developing synthetic AMPs. Research on shorter, more potent, nontoxic peptides has led to the creation of synthetic peptides with broader and higher antimicrobial activity than their natural counterparts (Goyal and Mattoo, 2014). Another promising approach is by gene encoded AMPs which can be utilized for production of transgenic plants and this is likely to reduce the probability of a pathogen overcoming the resistance genes.

AMPs are biodegradable, less phytotoxic and active at lower concentration. However, these AMPs can be judiciously used for imparting resistance in host plants against pathogens. Indiscriminate use may lead to development of resistance in pathogens as reported in fungicides and antibiotics. Hence, future thrust should be relied on exploitation of a number of AMPs for plant disease management and to develop less toxic stable compounds using biotechnological tools employing microbial systems or transgenic crops.

SEMINAR REPORT

VOLATILE ORGANIC COMPOUNDS AS DIAGNOSTIC MARKERS FOR PLANT DISEASES

By

RESHMA RAJ. T.

(2014-11-157)

Submitted in partial fulfillment of requirement of the course

PL. PATH 591- Masters' Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY

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DECLARATION

I, Reshma Raj.T.(2014-11-157) hereby declare that the seminar entitled 'Volatile organic compounds as diagnostic markers for plant diseases'has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

Vellanikkara
28.10.2015

Reshma Raj. T.
(2014-11-157)

CERTIFICATE

This is to certify that the seminar report entitled 'Volatile organic compounds as diagnostic markers for plant diseases' has been solely prepared by Reshma Raj. T. (2014-11-157), under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

Vellanikkara

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CERTIFICATE

Certified that the seminar report entitled 'Volatile organic compounds as diagnostic markers for plant diseases' is a record of the seminar presented by Reshma Raj. T. (2014-11-157) on 17-10-2015 and is submitted for partial fulfillment of requirement of the course PL PATH 591.

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1. INTRODUCTION

Plants accumulate a diverse array of natural products, which are thought to be involved in their interactions with the environment. Many of these compounds have been referred to as secondary metabolites. These secondary metabolites are likely to be essential for successful competition (Theis and Lerdau, 2003). One per cent of these secondary metabolites are 'volatile organic compounds' (VOCs). VOCs are involved in a range of ecological functions, including indirect plant defense against insects, pollinator attraction, plant-plant communication and plant-pathogen interactions. More than 100,000 chemical products are known to be produced from 90 per cent of plants and at least 1,700 of these are known to be volatiles.

In fact, it has been estimated that the emission of VOCs by terrestrial plants accounts for the 36% of the whole photosynthates and in that more than 50 per cent of VOCs are released during plant-pathogen interaction (Kesselmeier and Staudt, 1999). These VOCs are released by plants at the time of recognition of the plant and the pathogen. Hence these can be utilized for early detection of diseases. At present techniques used for the detection of plant diseases are imaging and spectroscopic techniques, spore traps and molecular techniques. But these are unable to detect and identify a disease at very early stage. Moreover these are highly labour intensive and less accurate. Over the last decade protected cultivation has attracted some serious interest among people. Under such a concealed environment, VOCs emitted by diseased plants seems to be a novel approach to support early detection of plant diseases.

2. VOLATILE ORGANIC COMPOUNDS (VOCs)

Volatile organic compounds (VOCs) are defined as any organic compound with vapor pressure high enough under normal conditions to be vaporized into the atmosphere (Dicke and Loreto, 2010). VOCs are a complex mixture of carbon (carbon-hydrogen compounds) containing chemical species (excluding elemental carbon, carbon monoxide, and carbon dioxide), which are volatile at normal temperature and pressure. In precise terms, VOCs are those organic compounds whose vapor pressure range from 0.13 kPa to 101.3 kPa at 293K (Spinelli *et al.*, 2011).

3. DIVERSITY OF VOCs

VOCs are classified into two groups namely anthropogenic VOCs and biogenic VOCs (BVOCs). VOCs produced by vegetation and soil are called BVOCs. BVOCs are classified into microbial VOCs, fungal VOCs and plant emitted VOCs. Plant emitted VOCs accounts for two third of total VOC present in that atmosphere (Guenther, 1997). Plant emitted VOCS are produced during plant pathogen interaction, herbivore infestation, plant-plant communication, ripening of fruits and during abiotic stress.

4. CLASSIFICATION OF PLANT VOCs

Plant volatiles constitute for about 1per cent of plant secondary metabolites and are mainly represented by various classes like volatile plant hormones, green leaf volatiles, isoprene groups like monoterpenes, homoterpenes and sesquiterpenes (Plate 1) (Dudareva *et al.*, 2004). Plant VOCs emitted during plant pathogen interaction is represented in Table 1.

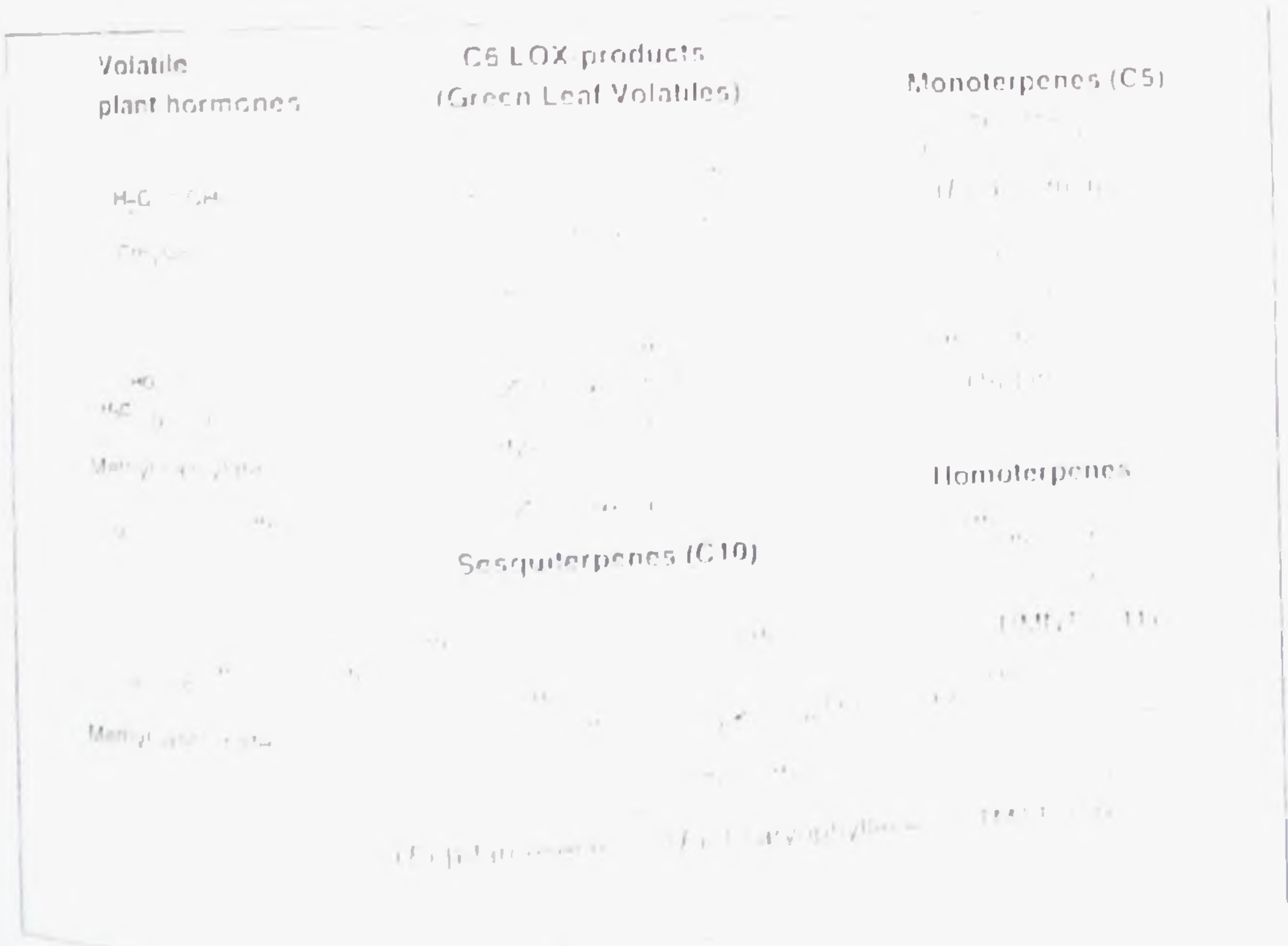


Plate 1- Classification of plant VOCs

Table 1- Plant VOCs emitted during plant- pathogen interaction

Classes of VOCs	Plant emitted VOCs	Pathogens
Alcohol	cis-3-hexen-1-ol	*Botrytis cinerea*
Monoterpenes	α-terpinene	*Oidium neolycopersici*
Sesquiterpenes	α-copaene	*Botrytis cinerea*
Homoterpenes	4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)	*Botrytis cinerea*
Phenolics	Methyl salicylate	Tobacco Mosaic Virus (TMV)

5. FACTORS AFFECTING PLANT VOCs EMISSION

Plants emit VOCs during abiotic stress like extreme temperature, lack or excess moisture, lack or excess light, during nutrient deficiency. Plant also emits VOCs during herbivore infestation, interplant communication, during ripening of fruits and during plant pathogen infection (Dicke and Loreto, 2010).

6. FUNCTIONS OF PLANT VOCS

a. Above ground

During herbivore infestation plant emits VOCs, which help in the attraction of natural enemies which feeds on the herbivores and reduce further attack. At the time of flowering, the VOCs emitted helps in the attraction of pollinators which results in fruiting. So VOCs increase the population of beneficial insects in the vicinity of plants.

b. Below ground

Soil borne pathogens attack the roots and eject exudates which emit VOCs from plants. This will attract beneficial microbes like rhizobia, PGPR in the rhizosphere of plants. This increases nutrient acquisition by plants.

6.1 PLANT VOCs AS COMMUNICATORS

Plant volatiles help the plant to communicate with the outside world and can carry both private and public messages and transmit information within a plant and potentially between plants. VOCs that are released from infected plants can serve as airborne signals and can be used as alarm cues. These airborne signals warn neighbouring plants about pathogen attack and elicit the activation of the defense mechanism thus allowing for a stronger response in future attacks (Kesselmeier and Staudt, 1999).

7. MECHANISM OF VOC EMISSION

Whenever a pathogen comes in contact with the host, elicitors are produced by the pathogen. Elicitors are chemical signals produced by the pathogen during their entry in to host which are recognized by specific receptor molecules in the plant. Host receptors interact with elicitors and receptors get activated in the plant cell. As a result nucleotide binding sites (NBS) in gene get activated and then protein binding to DNA alters gene expression leading to activation of defense response. Then lipoxygenases and phenoloxidases pathways get activated. This results in the release of phenolics, salicylic acid other transducers in the plant cell. These phloem mobile signal results in higher level of SA and methyl salicylate production and leads to activation of pathogenesis related proteins. This activates systemic acquired resistance (SAR) and further spread of pathogen is restricted. These volatile methyl salicylate esters induce SAR signals in other plants and increase the resistance to various diseases (Theis N and Lerdau M, 2003).

7.1 Signalling pathways

Two major defense signaling pathways have been recognized as important for plant biotic interactions, the jasmonic acid and the salicylic acid mediated pathways. These signaling pathways individually lead to defense responses, such as the production of a certain volatiles.

- Salicylic acid dependent defenses are activated more strongly in response to biotrophic pathogens such as Powdery mildew and Downy mildew.
- Jasmonic acid and ethylene-dependent defenses are activated to a higher extent in response to necrotrophic pathogens such as *Colletotrichum, Sclerotinia* and herbivorous insects.

8. TECHNIQUES TO MEASURE VOC EMISSION FROM PLANTS

There are three major steps in the measurement of plant VOCs. They are

(a) Collection of the plant-emitted VOCs

(b) Separation of the plant-emitted VOC blend

(c) Identification and quantification of the separated VOCs.

8.1 Collection of the plant-emitted VOCs

A fraction of the compounds emitted from the plants is collected. This sampling step is in general combined with the preconcentration of the VOCs present in the air to achieve the detection limits of commonly used analytical instruments.

Two methods are generally followed to preconcentrate the VOCs present in air.

8.1.1 Dynamic preconcentration of VOCs.

This method is referred to as dynamic because the air is actively pumped through a cartridge packed with a material that traps the compounds of interest.

8.1.2 Static preconcentration of VOCs.

In this case, a material is exposed to the air, in which the trapping of VOCs mainly depends on mass diffusion processes.

In both cases, the selection of the material is crucial in order to trap the VOCs of interest.

8.1.3 Selection of material for preconcentration

The commonly used materials are porous polymer tenax [poly-(2,6- diphenyl-*p*-phenylene oxide] (Plate 2) and carbon-based adsorbents, where the preconcentration depends on adsorption. For a few other materials, such as poly dimethyl siloxane (Plate 3), the preconcentration depends on absorption.

The criteria for the selection of material for preconcentration are

(a) Homogeneous and inert surface to avoid irreversible adsorption and catalytic effects during sampling and desorption

(b) Complete and fast adsorption or absorption of the VOCs of interest

(c) Low affinity with water

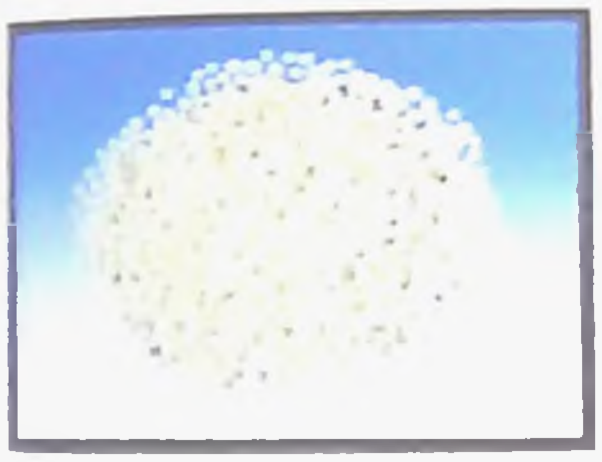


Plate 2- Porous polymer tenax

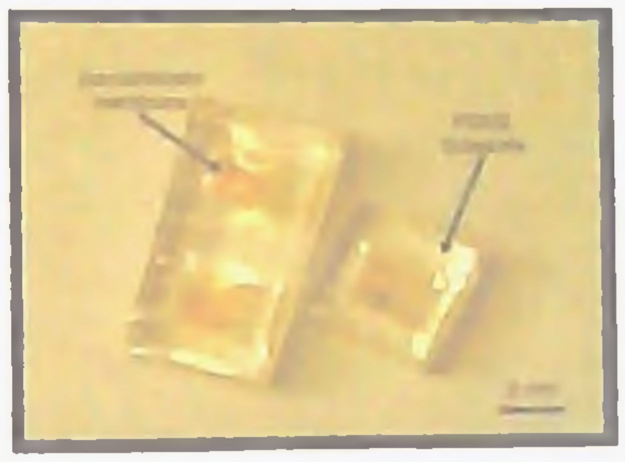


Plate 3- Poly dimethyl siloxane

8.2 Separation of the plant-emitted VOC blend

The VOC blend is often separated before identification and quantification of the individual compounds. The commonly used methods used for assessing the volatile metabolites released by diseased plants are by using gas chromatography (GC)-based techniques.

8.2.1 Gas chromatography (GC)

It is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium and the stationary phase is a layer of a polymer on an inert solid support inside a glass or metal column. The properties of this column should be selected with care because they have a large effect on the ability to separate plant-emitted volatiles.

An infection by *Phytophthora cactorum*, the fungus that causes crown rot diseases in strawberries, results in the release of *p*-ethylguaiacol and *p*-ethylphenol as characteristic VOCs from the infected portion of the strawberry. Profiling of such VOC could be used as a means to identify the type and nature of infection and can be used for disease diagnosis and confirmation. The volatile signature of plants could be analyzed using gas-chromatography (GC) technique to analyze the presence of the specific VOCs that is indicative of a particular disease (Jensen *et al.*, 2009).

8.3 Identification and quantification of the plant-emitted VOCs

After separation, a detector is used for the identification and quantification of the individual VOCs present in the sample. A key specification of any detector is its limit of detection (LOD) and limit of quantification (LOQ). LOD is generally defined as the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit. LOQ is considered if both detection and quantification of the concentration are required for the task of crop health monitoring (Jansen *et al.*, 2011). LODs are given in two different units: absolute amounts in nanograms (ng) or picograms (pg), or with respect to the concentration in air. LOQs are represented in nanograms per liter of air ($ng\,L^{-1}$) or picograms per liter of air ($pg\,L^{-1}$).

8.3.1 Types of detectors

The most popular detectors that are used for the identification and quantification of plant emitted VOCs are the flame ionization detector (FID) and the mass spectrometer (MS).

8.3.1.1 Flame Ionization Detector (FID)

This is a scientific instrument that measures the concentration of organic species in a gas stream. It is the most frequently used detector in gas chromatography. The operation of the FID is based on the detection of ions formed during combustion of organic compounds in a hydrogen flame. The generation of these ions is proportional to the concentration of organic species in the sample gas stream (Plate 4, 5). Flame ionization detectors are used very widely in gas chromatography because of a number of advantages.

- Cost: Flame ionization detectors are relatively inexpensive to acquire and operate.
- Low maintenance requirements: Apart from cleaning or replacing the FID jet, these detectors require no maintenance.
- Rugged construction. FIDs are relatively resistant to misuse.
- Linearity and detection ranges: FIDs can measure organic substance concentration at very low and very high levels, having a linear response of 10^6.

Plate 4- Flame Ionization Detector

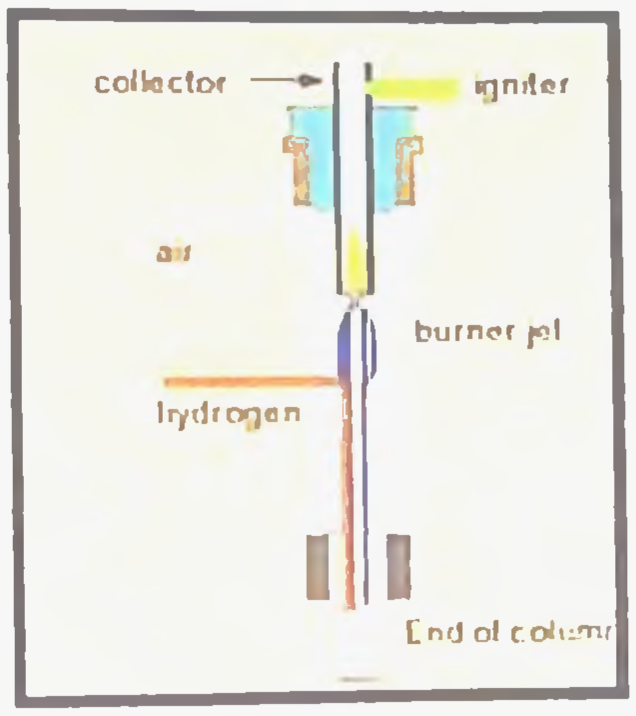


Plate 5- Parts of FID

The mechanism of MS is based on mass of charged molecule fraction. It is widely used for the detection of VOC's in wide dynamic concentration range.

8.3.1.3 Gas chromatography- mass spectrometry (GC–MS)

To enhance the performance of VOC separation and analysis, the gas chromatography is often combined with mass spectrometry (GC-MS). The GC-MS is commonly used technique for a qualitative as well as quantitative analysis of volatile metabolites released by plants. The main components of GC-MS are source, analyzer, ion detector and data analyzer (Plate 6). The GC-MS studies have been performed to evaluate the change in volatiles caused by bacterial or fungal infection.

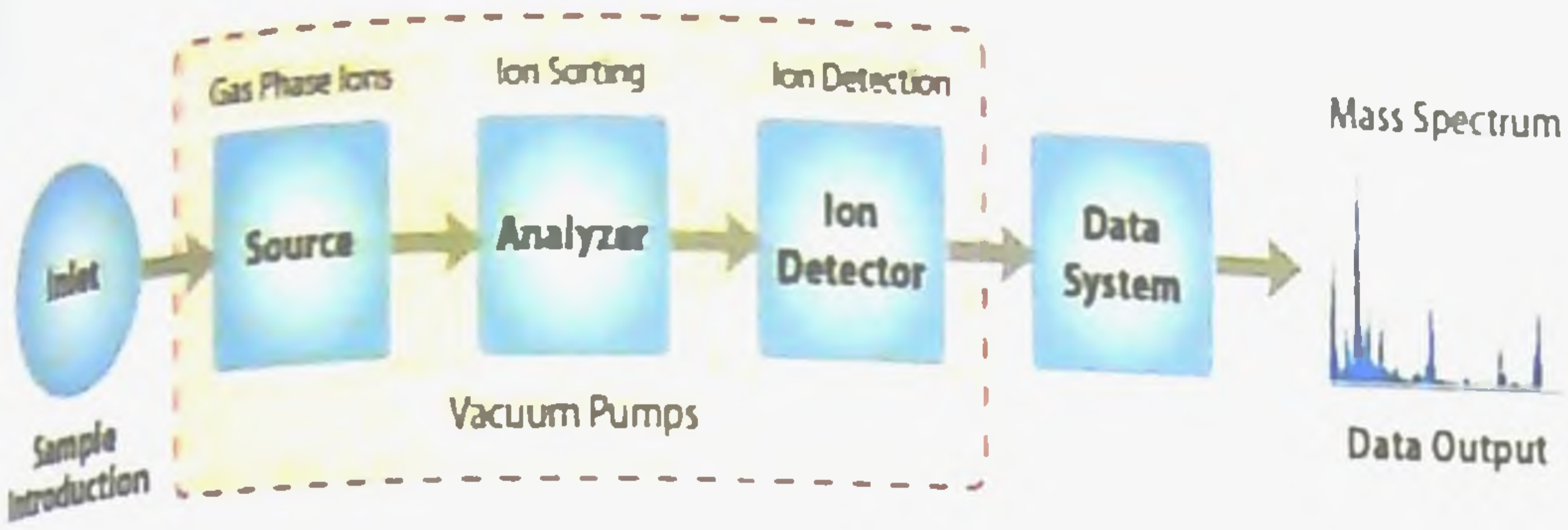


Plate 6-Components of GC-MS

Prithiviraj *et al.* (2004) assessed the variability in the volatiles released from onion bulbs infected with bacterial (*Erwinia carotovora* causing soft rot) and fungal species (*Fusarium oxysporum* and *Botrytis allii* causing basal and neck rots) using HAPSITE, commercial portable GC–MS instrument. The study indicated that 25 volatile compounds (among the 59 consistently detected compounds) released from onion can be used to identify the disease based on VOC profiling. Some of the examples of VOCs emitted by plants during pathogen infection is listed in Table 2.

Table 2-Plant VOCs emitted during pathogen attack

Crop	Pathogen	No. of VOCs	Reference
Potato	*Phytophthora infestans*, *Pythium ultimum* , *Botrytis cinerea*	32	(Lui *et al.*,2005)
Carrot	*Botrytis cinerea*, *Aspergillus niger*	39	(Vikram *et al.*, 2006)
Mango	*Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*	35	(Moalemiyan *et al.*, 2007)

9. HOW VOCS ACTS AS MARKERS IN PLANT DISEASE DETECTION?

Case study-An experiment was conducted by Laothawornkitkul *et al.*, 2010 regarding the use of VOCs as diagnostic markers for late blight disease in potato.

Volatiles from potato plants infected with *Phytophthora infestans* were monitored by *in situ* head-space sampling. The sampling was done in four periods *i.e.* 28–42, 52–66, 76–90, and 100–114 h after inoculation (HAI). The samples were analyzed by a gas chromatography–flame ionization detector (GC–FID) to assess the differences in volatile fingerprints between the infected-plant group and control groups. The samples were subsequently analyzed by gas chromatography–mass spectrometry to identify specific peaks observed by GC–FID. Spore germination, infection, symptom development and sporulation were also monitored to ascertain the disease developmental stage.

The first symptoms of infection were visible after two days. Three marker volatiles *i.e.* (E)-2-hexenal, 5-ethyl-2(5H)-furanone and benzene-ethanol were found in the third and fourth trapping periods (3–4 days after inoculation) when sporangiophores were already formed (Figure 1). The volatile metabolites from blighted plants could be applied for sensor development to detect the occurrence of the disease in the field as well as for investigation of volatile production in relation to plant responses to infection. Some of the examples of VOCs used as diagnostic markers for plant diseases are represented in Table 3.

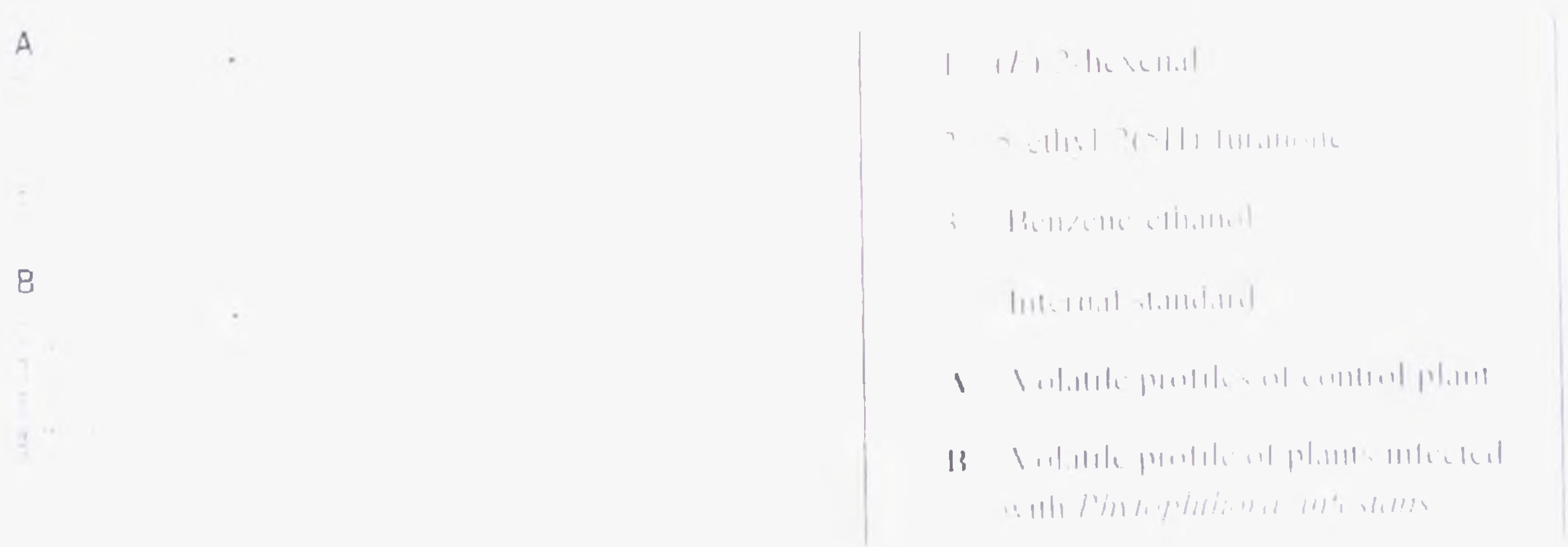


Figure 1. VOCs as diagnostic markers for late blight disease in potato

Table 3- VOCs as diagnostic markers for plant diseases

Crop	Pathogen	VOCs as diagnostic markers	Reference
Fungi-induced VOCs emission			
Groundnut	*Sclerotium rolfsi*	• Linalool	(Cardoza *et al*., 2002)
Tomato	*Botritis cinerea*	• α-terpinene	(Thelen *et al*., 2006)
Potato	*Phytophthora infestans*	• Benzene-ethanol • (E)-2-hexenal • 5-ethyl-2(5H)-furanone	(Laothawornkitkul *et al*., 2010)
Mustard	*Alternaria brassicae*	• 3-butenyl isothiocyanate	(Doughty *et al*. 1996)
Bacteria -induced VOC emission			
Apple	*Erwinia amylovora*	• 2,3-butendiole • Hexenal	(Spinelli *et al*., 2010)
Grapevine	*Agrobacterium vitis*	• Dimethyl disulfide	(Blasioli *et al*., 2010)
Tobacco	[illegible]	• m-cresol • Methyl benzoate(MeBA)	(Huang *et al*., 2003)
Virus-induced VOCs emission			
Tobacco	Tobacco Mosaic Virus	• Methyl salicylate • Isobutyl alcohol	(Shulaev *et al*., 1997)

10. ADVANCED TECHNIQUES FOR PLANT VOC ANALYSIS

In order to enhance the efficiency of disease detection using volatile profiling, more sensitive instruments are used which include biosensors and electronic noses.

10.1 Biosensor

A biosensor is a particular type of chemical sensor that uses the highly sensitive recognition properties of VOCs. Since its inception, biosensors were predicted to play a significant analytical role in agriculture. The basic principles of a biosensor are sensitivity, reliability and stability (Trasco and Chaniotakis., 2009).

10.1.1 Elements of Biosensor and its uses

- Bioreceptor - It is the VOC that is to be analyzed
- Transducer - Commonly used transducers for the detection of VOCs are nanoparticle, nanotube, nanowire
- Detector - Used for the amplification of signals

10.1.2 Nanoparticle based biosensor

For improving detection of VOCs released by diseased plants, nanoparticle based biosensor are commonly used. Gold nanoparticles are the widely used nanomaterial due to its high electronegativity and electronic conductivity for electron transfer.

The application of gold nanoparticle (AuNP) modified electrode has been reported for the electrochemical detection of methyl salicylate, a key plant volatile organic compound released by plants during infections. In addition to gold nanoparticles, semi conductive metal oxide nanoparticles have also been reported for VOC detection due to its advantages such as low cost, suitability for electron conduction signal and the ease at which to obtain desired size and shape.

Fang *et al.* (2014) studied the sensitivity of two different metal oxides to the VOCs emitted from diseased plants. Metal oxide nanoparticles such as SnO_2 and TiO_2 were used for the detection of VOCs such as *p*-ethylguaiacol produced from crown rot infected strawberry (Figure 2). They represented the limit of detection in nanomolar concentration range.

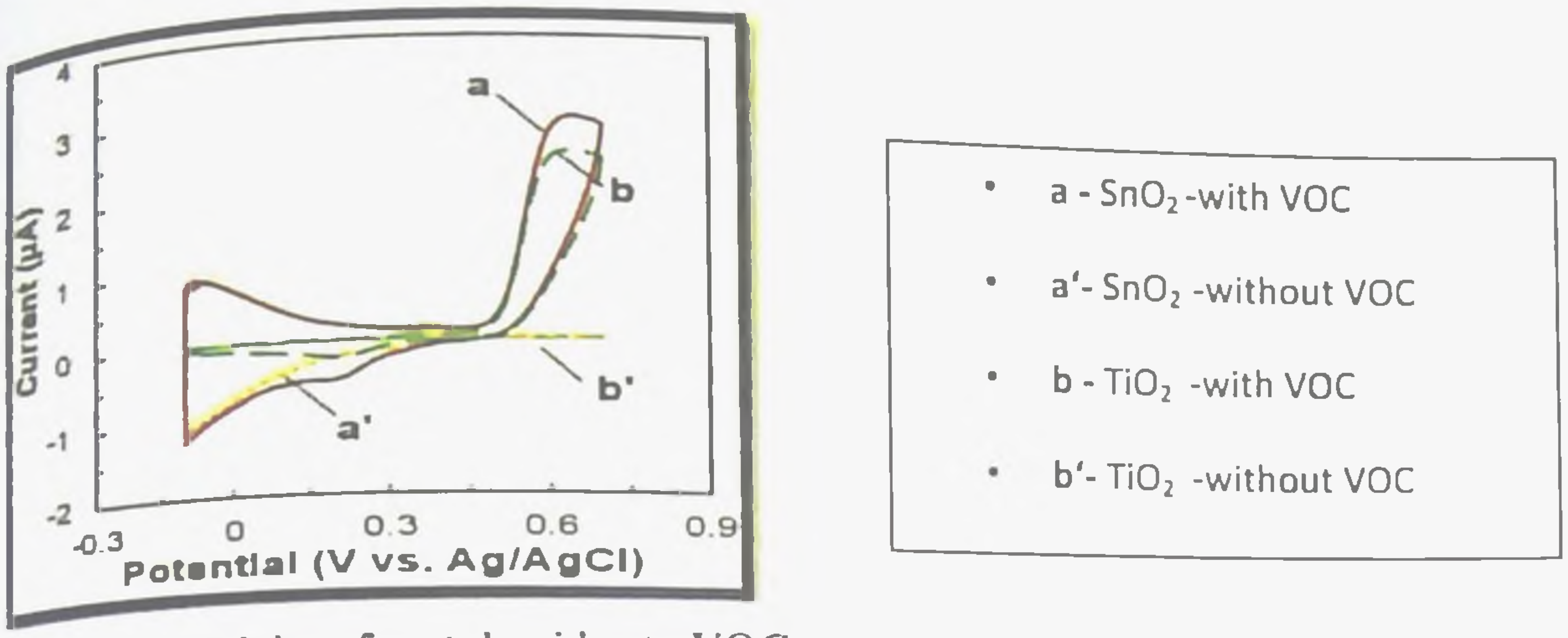


Figure 2- Sensitivity of metal oxides to VOCs

Cyclic voltammetry responses of (a and a') SnO_2 and (b and b') TiO_2 (a and b) with and (a' and b') without the presence of 0.17 mM *p*-ethylguaiacol in strawberry infected with *Phytophthora cactorum* causing crown rot disease (Fang *et al.*, 2014). Figure 2 shows the increased sensitivity of metal oxide SnO_2 to the VOC *p*-ethylguaiacol and this show that SnO_2 is the best metal oxide that can be used for the analysis of VOC than TiO_2

10.1.3 Enzymatic electrochemical biosensor

The use of enzyme as bio-recognition element can provide highly selective detection of the target VOC due to the high specificity of enzymes towards the VOC. An enzyme specific for the VOC of interest is immobilized on the nanomaterial modified-electrode. The amperometric detection is based on the bio-electrocatalytic reaction between the target VOC and electrode, which results in an electrical signal (current) that can be used for quantitative detection of the VOC (Fang *et al.*, 2014).

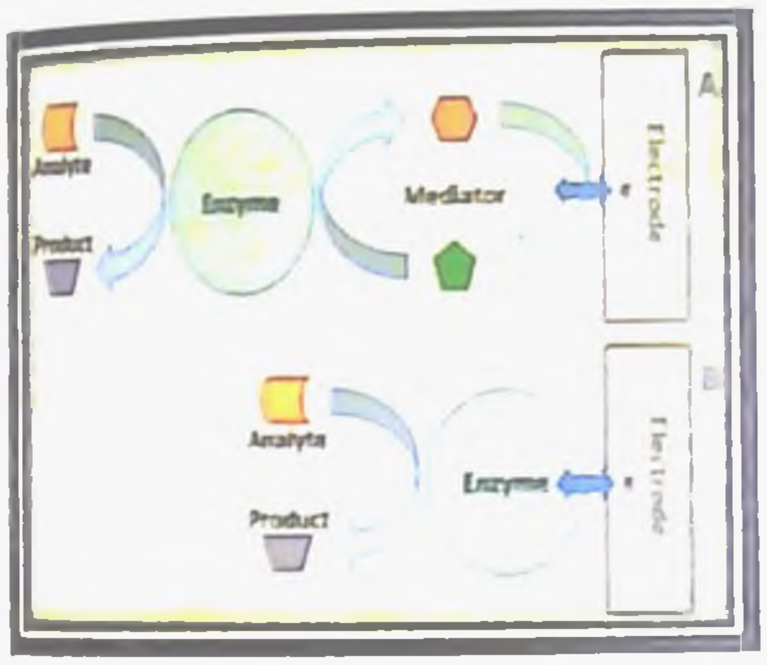
The amperometric signal can be obtained through either direct or mediated electron transfer based electrochemical reactions. Because of its high specificity, the enzymatic electrochemical biosensors have been successfully commercialized. Enzymes specific for particular VOC is listed in Table 4.

Table 4- Detection of VOCs using enzymatic biosensors

Volatiles emitted from tomato plant	Enzyme	Pathogen
cis-3-hexen-1-ol	Alcohol dehydrogenase	*Botrytis cinerea*
Methyl salicylate	Salicylic acid methyl transferase	Tobacco Mosaic Virus

10.1.3.1 Types of enzymatic nanoparticle based biosensor

There are mainly two types of enzymatic nanoparticle based biosensor. They are



Protein mediated enzymatic biosensor-For analyzing VOCs in liquid state

Direct enzymatic biosensor-For analyzing VOC in gaseous state

Plate 7-Types of biosensors

10.2 Electronic nose (E-nose)

An electronic nose system consists of a series of gas sensors that are sensitive to a range of organic compounds (Sankaran *et al.*, 2010). As each sensor has specific sensitivities, the sensitivities of a series of sensors could be used to discriminate different compounds present in the atmosphere.

Moreover E-noses are sensor array systems that can mimic human olfaction (Plate 8). The application of electronic nose systems for identifying plant diseases is relatively a new domain. E-nose instruments are good at addressing the chemical integrity of a sample, which is to determine whether the sample is the same as or different from a certain standard. Early and rapid detection of disease is the most important characteristics of E-noses. The main drawback of E-nose systems is that the LOD of most of these systems is in the μg L^{-1} range (Markom *et al.*, 2009).

Sensor used in E-noses are metal–oxide–semiconductor (MOSFET) devices which is a transistor used for amplifying or switching electronic signals. This works on the principle that molecules entering the sensor area will be charged either positively or negatively, which should have a direct effect on the electric field inside the MOSFET. Thus, introducing each additional charged particle will directly affect the transistor in a unique way, producing a change in the MOSFET signal that can then be interpreted by pattern recognition computer systems. So each detectable molecule will have its own unique signal for a computer system to interpret.

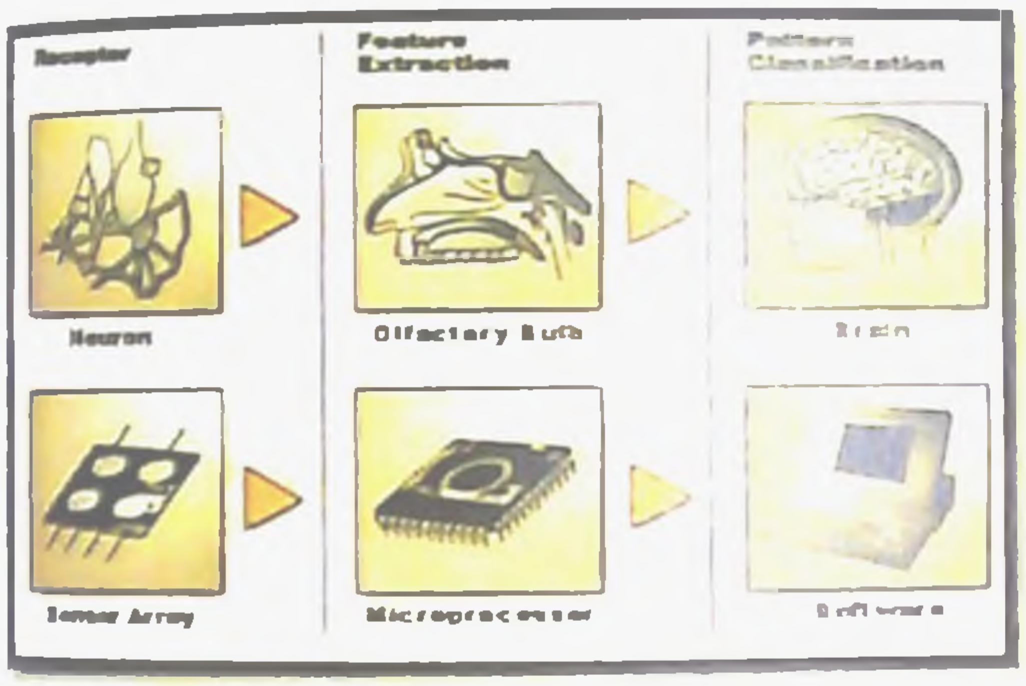


Plate 8- Parts of E-nose

Plate 9- Cyranose® 320

10.2.1 Types of E-nose

The two main types of electronic noses are Cyranose® 320 and PEN2. Cyranose® 320 is an array of 32 conducting polymer-based sensors to detect postharvest fungal disease in blueberries in a controlled environment whereas PEN2, an E-nose developed in Germany, consists of an array of 10 metal oxide-based sensors for measuring the profile of VOC's released from wheat damaged by insects.

Li *et al.* (2009) conducted an experiment for the detection 0f VOCs using E-nose system (Table 5). He used a Cyranose® 320 to detect postharvest fungal disease in blueberries in a controlled environment. Blueberries were first disinfected with ethanol to eliminate any naturally present fungal spores and bacteria. Once the blueberries were rinsed with distilled water to remove residual ethanol, they were inoculated with spore suspensions of three fungal species: *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Alternaria* sp. that cause gray mold, anthracnose, and *Alternaria* fruit rot in postharvest blueberries, respectively. The berries were placed in a 500ml bottle and headspace gases were tested using Cyranose® 320. GC-MS analysis was also performed to identify specific compounds that could be related to fungal diseases.

Table 5- Detection of volatiles using e-nose system

VOC concentration (ng)	Control	*Alternaria* sp.	*Botrytis cinerea*	*Colletotrichum gloeosporioides*
Styrene	0.01458	0.01569	0.01694	0.02551
1-Methyl-2-(1-methylethyl)-benzene	0.00493	0.01428	0.01646	0.02966
Eucalyptol	0.00404	0.03115	0.03897	0.01791
Undecane		0.01671	0.02460	
Thujopsene		0.03178		0.02135

Principal component analysis plots indicated a clear delineation between the control (fresh berries) and berries with fungal infections. The berries with *C. gloeosporioides* could be distinctively differentiated from the other groups, though there was some overlap in the VOC profiles of the berries infected with *B. cinerea* and *Alternaria* sp. GC-MS data indicated that styrene, 1-methyl-2-(1-methylethyl) benzene, eucalyptol, undecane, and thujopsene contributed to the classification of the four groups (three diseases and one healthy). This research work demonstrates that the total volatile blend is constant for specific plant and disease and the potential for applying VOC profiling-based technique for non-destructive detection of plant diseases (Li *et al.*, 2009).

10.3 Application of VOC profiling in protected cultivation

A novel approach to support the inspection of polyhouse crops is based on the measurement of volatile organic compounds emitted by unhealthy plants. This approach has attracted some serious interest over the last decade. Nowadays polyhouses fitted with e-noses are constructed which helps in rapid detection of diseases as early as 2-3 days (Lang *et al.*, 2014).

11. MERITS OF VOC PROFILING OVER OTHER TECHNIQUES

The merits of using VOC profiling are:

- Early detection of disease
- Application in hi-tech agriculture and in polyhouses
- Detection of post harvest diseases
- Non-destructive method of plant disease detection
- Used as plant vaccines

11.1 VOC's as plant vaccines

Song and Ryu in 2013 conducted an experiment to demonstrate the reduction of bacterial disease in cucumber by the treatment with different VOCs. Effectiveness of volatile organic compound (VOC) mediated induced resistance against the bacterial angular leaf spot pathogen, *Pseudomonas syringae* pv. *lachrymans*, in the open field is studied. There was an induction of systemic resistance by 10 nM 3-pentanol and 10 nM 2-butanone against

Pseudomonas syringae pv. Lachrymans (Figure 3). The severity of symptoms was scored from 0 to 5 as follows: 0 - no symptoms: 1- yellowish color; 2 - chlorosis only; 3 - partial necrosis and chlorosis; 4 - necrosis of the inoculated area and expanded chlorosis and 5 - complete necrosis of the inoculated area.

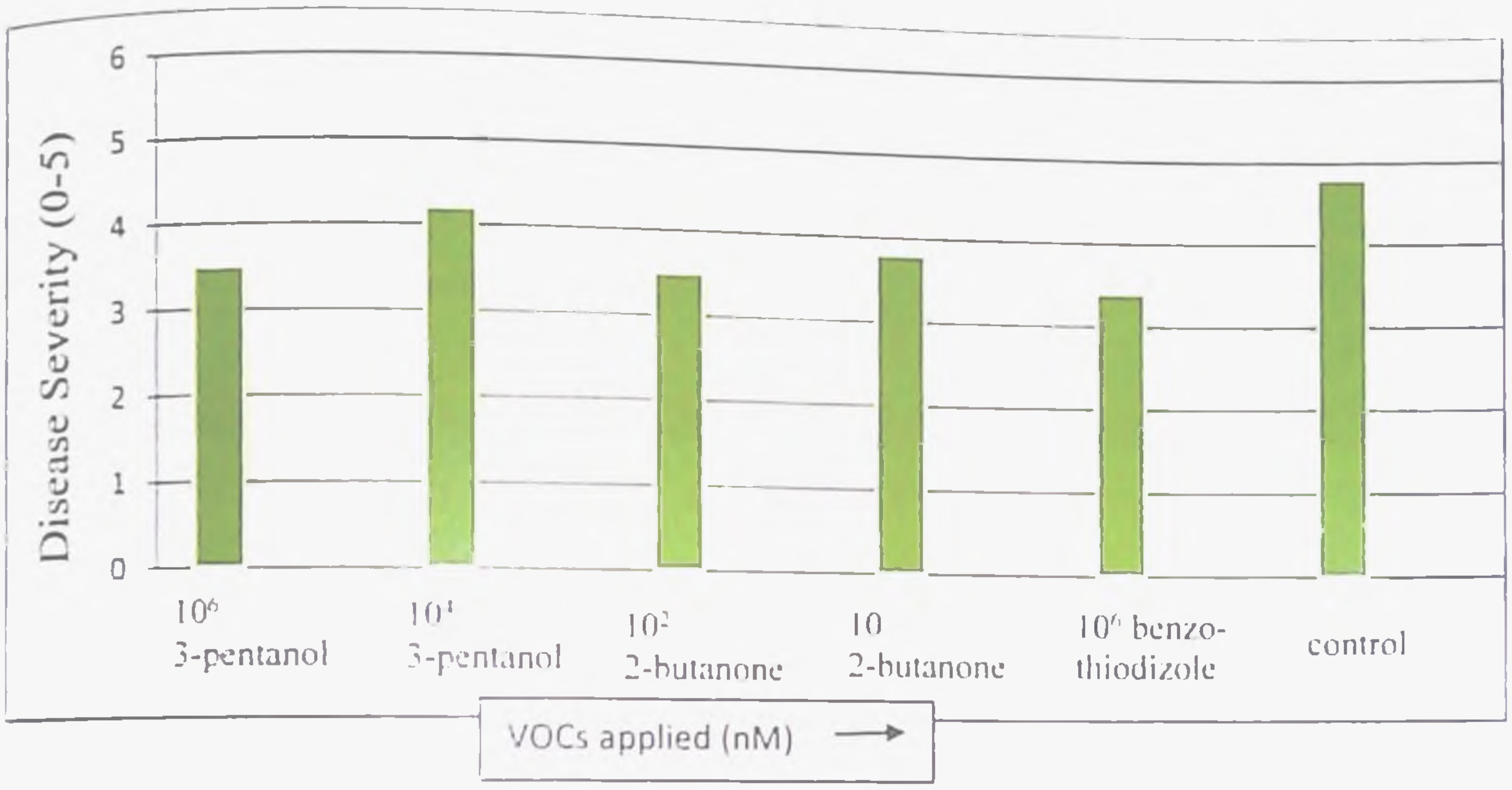


Figure 3- Supression of cucumber bacterial angular leaf spot pathogen, *Pseudomonas syringae* pv. *lachrymans* using VOCs

Disease severity of cucumber treated with 3-pentanol and2-butanone was assessed 7 days after infection with *P. syringae* pv. *lachrymans*. Water and 1mM benzothiadizole (BTH) were used as negative and positive controls respectively. From the experiment it was known that VOCs like 3-pentanol and 2-butanone has same effect in the disease suppression as that of benzothiodizole (BTH), a systemic resistance inducer

Since application of VOCs helps in the suppression of diseases in plants, this method can be explored for the production of engineered crops.

12. CHALLENGES IN VOC ANALYSIS

- Automation of the technique in field conditions which is costly
- Specificity of plant VOCs
- Loss processes of plant VOCs

12.1 Specificity of plant VOCs

Eventhough volatile profiling provides a scope for early detection of plant diseases, there are controversies regarding the specificity of VOC for a particular plant and disease. Jansen *et al.* (2009) through their experiments proved that plant VOCs emitted during disease infection are unspecific. They have shown that when different plant species were challenged with a similar infection, there was the emission of same VOC. For example, TMV infection in tobacco as well as in tomato induced an increase in the emission of methyl salicylate. They have also shown that when same plant species is infected with different pathogens, there was emission of same VOC. For example, infection on tomato plant by *Botritis cinerea* and *Oidium neolycopersici* emits the same VOC α-pinene.

However, Li *et al.*, (2009) conducted experiments which proved the specificity of VOCs emitted during plant-pathogen interaction. They have shown that eventhough a certain plant species may emit similar VOCs upon induction by different diseases and different plant species may emit the same VOCs after being challenged with a similar disease, the total VOC blend emitted is specific for a particular plant and the pathogen (Table 5).

12.2 Loss processes of plant VOCs

Due to the low concentration and high vapour pressure of plant emitted VOCs, there are chances for the escape of these VOCs to the atmosphere. Some of the reasons for these losses are

1. The first loss process for plant emitted VOCs is the removal of these VOCs by air transport. Air transport may be natural *via* wind or mechanical *via* fans in a polyhouses.

2. The second loss process is the degradation of VOCs due to gas phase reactions. In the atmosphere, the major degradation processes for plant VOCs are reactions with hydroxyl radicals (OH), nitrate radicals (NO_3), and ozone (O_3), leading to a number of breakdown products.

3. The third process to be taken into account as an important loss process is the solution of VOCs in water bodies such as raindrops or condensate.

4. The fourth process for losses of VOCs is uptake by the plant itself. These losses can occur by adsorption on the cuticle and uptake through the stomata .Uptake of VOCs through stomata requires a lower concentration of the compounds in the stomatal cavity than in the surrounding air. This concentration difference is important because gases move along the concentration gradient between the inside and the outside of the leaf. The stomatal cavity is covered by water. Therefore, VOC's that can be dissolved in this water and there after metabolized in plant tissues can maintain a continuous uptake potential (Jansen *et al.*, 2011).

13. FUTURE DIRECTIONS

VOC profiling is still an emerging field of analysis. Instrumentation and equipment are still being developed and refined and further advances in sampling allow greater coverage of VOCs to be collected. More sensitive instruments with lower detection limits and faster scan rates detect ultra-low abundance compounds, and suitable data analysis methods for data interpretation are all essential for further development of VOC profiling. Eventhough more studies are needed to prove the specificity of VOCs, there is enough scope for using these techniques for plant disease detection in open field with the help of more sensitive instruments. Real time monitoring of plant diseases may be possible by integrating volatile profiling equipment with an automated agricultural vehicle. VOC profiling has a great scope in early disease detection and thus made its application of VOC as diagnostic markers in the detection of diseases in open field and in polyhouses.

14. CONCLUSION

Diseased plants emit different types and amounts of volatiles. It will be a challenge to identify the disease based on VOC emission only. But, plant VOCs can be used to characterize the disease. In addition, instruments are available that meet the required technical specifications to detect these VOCs in an agricultural setting. Detection of VOCs is based on highly sensitive instruments including GC-FIDs, GC-MS, biosensors and e-noses. Among them E-noses pave way for early and rapid detection of diseases by analyzing the VOCs. Besides this, VOCs will generate a novel way for plant vaccination. Some of the challenges in these techniques are optimization of the technique for a specific plant and disease and automation of the technique for continuous monitoring of plant diseases field conditions.

15. DISCUSSION

Q1. How VOCs act as communicators in neighbouring plants?

Whenever a pathogen comes in contact with a plant, VOCs will be emitted from the infected plants and the neighbouring healthy plants have a capacity to sense the VOCs emitted from the diseased plant. Then a defense mechanism is activated in the neighbouring healthy plants protecting them from future attack by the same pathogen.

Q2. What is the scope for VOC profiling in India?

As VOC analysis is now widely used in protected cultivation, currently it has less scope in India. But in coming years these techniques can be used in open field condition by minimizing the cost and increasing the sensitivity of the equipment.

Q3. Which are the agencies undertaking the study on VOCs in India?

At present biosensors are being used in India for the detection of human diseases and scientists in GP Pant University, Uttar Pradesh are trying to develop the same for the detection of plant diseases.

Q4. What are the advantages of VOC profiling over molecular techniques?

1. Real time monitoring of disease at the time of recognition of the plant and the pathogen.

2. Even if a single plant is infected, VOC profiling helps in the detection of disease in very early stage.

3. Specificity in analysis.

Q5. How transducers detect VOCs in biosensors?

The main transducers used in biosensors are gold nanoparticles which is having high electronegativity and electro conductivity. Gold nanoparticles have piezoelectric effect and when a pressure is exerted by the VOCs on transducer, the pressure is converted into electrical signals which will be amplified and detected using a detector.

Q6. What types of enzymes are used for detection of VOCs in enzymatic electrochemical biosensor?

For analysis of VOC comming in the class alcohols (hexan-1-ol), alcohol dehydrogenase enzymes are used

Q7. Can we use VOCs for engineering new crops?

Yes. VOCs help in the suppression of diseases and this helps in the development of new disease resistant plants. This is a novel concept yet to be explored

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Plant Pathology

PL PATH 591: Master's seminar

Name: Reshma Raj. T

Admission number: 2014-11-157

Venue: Seminar Hall

Date: 17.10.2015

Time: 10.00 a.m.

Volatile organic compounds as diagnostic markers for plant diseases

Abstract

Volatile organic compounds (VOCs) are defined as any organic compound with vapour pressure high enough under normal conditions to be vapourized into the atmosphere (Dicke and Loreto, 2010). The emission of VOCs from plants accounts for about two third of the total VOCs present in the atmosphere. Various physico-chemical and biological factors affect the VOC release from the plants. Plants have been shown to change the VOC emission, when infectious plant diseases affect them (Sankaran *et al.*, 2010). These changes may be informative enough for the detection of plant disease at a very early stage *i.e.*, at the time of recognition between the plant and the pathogen. In addition, VOCs can induce systemic resistance to a particular disease.

Generally the study of plant VOC emission involves collection and separation of the plant emitted VOC blend, followed by identification and quantification of the separated VOCs. Approaches to detect plant diseases by analyzing VOCs are based on highly sensitive instruments including GC-FID, GC-MS, biosensors and Electronic noses (Jansen *et al.*, 2011). Laothawornkitkul *et al.* (2010) evaluated the prospective of VOCs as a diagnostic marker of late blight of potato using GC-MS. They reported that the compounds emitted by the late blight infected plants, such as (E)-2-hexenal, 5 ethyl-2(5H)-furanone and benzene ethanol could be used as a diagnostic marker to identify late blight of potato. They also found that these compounds could induce resistance to late blight in neighbouring plants.

Song and Rye (2013) conducted an experiment to demonstrate the effectiveness of VOCs as plant vaccines against the bacterial angular leaf spot pathogen, *Pseudomonas syringae* pv. *lachrymans*, in the open field. There was an induction of systemic resistance by the VOCs, 10^{0}nM 3-pentanol and 10^{-2}nM 2-butanone against the bacterial disease, similar to that of 10^{6}nM benzothiadizole (BTH), a systemic resistance inducer which was used as control.

Biosensors and E-noses are the latest and highly advanced instruments used to detect VOCs at very early stage of plant disease. Li *et al.* (2010) used E-noses and found that infection by post-harvest fungal pathogens in blueberries can be detected by the change in volatile profile. At present, potential of VOC based techniques for early detection of plant disease has been demonstrated only in protected cultivation. Eventhough more studies are needed to prove the specificity of VOCs, there is enough scope for using these techniques for plant disease detection in open field with the help of more sensitive instruments. Real time monitoring of plant diseases may be possible by integrating volatile profiling equipment with an automated agricultural vehicle

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Indigenous technical knowledge in plant disease management

By

Priyanka B.

(2014-11-219)

M. Sc. Plant Pathology

Seminar report

Submitted in partial fulfilment of requirement of the course

Pl. Path. 591 Seminar (0+1)

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

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CERTIFICATE

Certified that the seminar report entitled **'Indigenous technical knowledge in plant disease management'** is a record of seminar presented by Priyanka B. (2014-11-219) on 22nd January, 2016 and is submitted for partial fulfillment of the course Pl. Path. 591.

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DECLARATION

I, Priyanka B. (2014-11-219), hereby declare that the seminar report entitled **'Indigenous technical knowledge in plant disease management'**, has been completed by me independently after going through the references cited herein and I have not copied from any of the fellow students or previous seminar reports.

Priyanka B.

2014-11-219

Vellanikkara

Date: 01.03.2016

CERTIFICATE

Certified that the seminar report entitled **'Indigenous technical knowledge in plant disease management'** for the course Pl.Path. 591 has been prepared by Priyanka B. (2014-11-219), after going through various references cited herein under my guidance, and she has not copied or borrowed from any of her fellow students.

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CONTENTS

1. Introduction

Indigenous Technical Knowledge is the local knowledge – knowledge that is unique to a given culture or society. It contrasts with the international knowledge system generated by universities, research institutions and private firms. It is the basis for local–level decision making in agriculture, health care, food preparation, education natural resource management and a host of other activities in rural communities (Warren 1991). The advent of the concept of sustainable agriculture in late eighties in Indian agricultural scenario has evoked interest on indigenous technical knowledge (ITK) that has the element of use of natural products to solve the problems pertaining to agriculture and allied activities. Indian farmers, over centuries have learnt to grow food and to survive in difficult environments, where rich tradition of ITK has been interwoven with the agricultural practices followed by them. The enhancement of the quality of life of the Indians who in great majority live in and depend on agricultural production systems would be impossible by keeping this rich tradition of ITK aside.

2. Indigenous technical knowledge

Indigenous technical knowledge is defined as the actual knowledge of given population that reflects the experiences based on tradition and includes more recent experiences with modern technologies (Haverkort, 1995).

ITK embraces people's knowledge of tool and techniques the assessment, acquisition, transformation and utilization of resources, which are specific to a particular location.

3. Diversity of ITK

3.1. Agriculture

Indigenous agricultural practices (IAPs) are an unwritten body of knowledge. There is no systematic record to describe what they are, what they do and how they do what they do, how they can be changed, their operations, their boundaries and their applications.

3.2. Ethnobotany

Ethnolog - study of culture and botany - study of plants is the scientific study of the relationships that exist between peoples and plants.

Ethnobotanists aim to document, describe and explain complex relationships between cultures and (uses of) plants, focusing primarily on how plants are used, managed and perceived across human societies. This includes use for food, clothing, currency, ritual, medicine, dye, construction, cosmetics and a lot more.

3.3. Ethno- ecology medicine

Ethno- ecology medicine is the interdisciplinary study of dynamic relationships among peoples, biota, and environments with relation to medicinal properties. Ethnoecological studies are based on a multidisciplinary perspective that draws on the insights from the natural and behavioural sciences at multiple levels- from the views of villagers in developing nations to those of policy-makers in industrial nations.

3.4. Meteorology

Meteorology is the interdisciplinary scientific study of the atmosphere. Studies in the field stretch back millennia, though significant progress in meteorology did not occur until the 18th century.

The beginnings of meteorology can be traced back to ancient India, as the Upanishads contain serious discussion about the processes of cloud formation and rain and the seasonal cycles caused by the movement of earth around the sun. Varāhamihira's classical work *Brihatsamhita*, written about 500 AD, provides clear evidence that a deep knowledge of atmospheric processes existed even in those times.

3.5. Social science and humanities

Social science is concerned with society and the relationships among individuals within a society. The main social sciences include economics, political science, human geography, demography and sociology.

Humanities are more frequently contrasted with natural, physical and sometimes social sciences as well as professional training. The humanities include ancient and modern languages, literature, philosophy, international relations and musicology.

4. Nature of ITK

1. ITK is dynamic in nature and may include experimentation in the integration of new plant or tree species into existing farming systems or a traditional healer is test of new plant medicines.

2. ITK does not mean that the knowledge is old or non technical in nature, but tradition based, the way in which that knowledge is created, preserved and disseminated.

3. ITK is social in nature and often considered to be the property of the entire community and not belonging to any single individual within the community.

4. It is transmitted through specific cultural to traditional information exchange mechanism. For example, it is maintained and transmitted orally through elders or specialists (breeders, healers etc.) and often to only a selected few people within a community.

5. Difference between traditional knowledge system and western scientific system

Sl. No.	Traditional knowledge system	Western scientific system
1	All parts of the natural world are regarded as animate, all life forms as interdependent	Human life is generally regarded as superior, with a moral right to control other life forms
2	Knowledge is transmitted largely through oral media	Knowledge is transmitted largely through the written word
3	Knowledge is developed and acquired through observation and practical experience	Knowledge is generally learned in a situation, which is remote from its applied context
4	Knowledge is holistic, intuitive, qualitative and practical	Knowledge is essentially reductionist, quantitative, analytical and theoretical.
5	Knowledge is generated by resource users in a diachronic (long term) time scale	Knowledge is generated largely by specialist researchers on a synchronic (short term) time scale

6	The nature and status of particular knowledge is influenced by socio cultural factors such as spiritual beliefs, and is communally held	The nature and status of particular knowledge is influenced on peer review, and is held by individual specialists
7	Explanations behind perceived phenomena are often spiritually based on subjective	Explanation behind perceived phenomena are essentially rational and objective
8	Knowledge is used to make suitable decisions under variable conditions	Knowledge is used to put forward hypothesis and to verify underlying laws and constants

6. Sources of ITK

There are sources of ITK hidden in our village, communities and countryside. The main sources are

1. Farmers or tribes
2. Community leaders
3. Elder persons
4. Folklore, song and poetry
5. Ancient records
6. NGOs
7. Extension agencies
8. Published materials of different languages

7. Methods of ITK collection

There is no fixed method for collection of ITK. It depends on type of ITK, situation, people, social system, cultural values and other aspects.

7.1. Interaction with community leaders or elders: Contacting with the leaders or elder persons of village or a family, we can get information locally practised from that particular locality.

7.2. Rapid Rural Appraisal: An approach used by non-governmental organizations (NGOs) and other agencies involved in international development. The approach aims to incorporate the knowledge and opinions of rural people in the planning and management of development projects and programmes.

7.3. Case study: A case study is a "published report about a person, group, or situation that has been studied over time." If the case study is about a group, it describes the behaviour of the group as a whole, not behaviour of each individual in the group.

7.4. Participatory video: A form of participatory media in which a group or community creates their own film. The idea behind this is that making a video is easy and accessible, and is a great way of bringing people together to explore issues, voice concerns or simply to be creative and tell stories. It is therefore primarily about process, though high quality and accessible films (products) can be created using these methods if that is a desired outcome.

7.5. History: Through histories we can seek many traditional practices practiced by farmers in earlier days

7.6. Interview method: An interview is a conversation between two or more people where questions are asked by the interviewer to elicit facts or statements from the interviewee.[1] Interviews are a standard part of qualitative research.

7.7. Participant observation: Participant observation is one type of data collection method typically done in the qualitative research paradigm.

7.8. Brain storming: Brainstorming is a group creativity technique by which efforts are made to find a conclusion for a specific problem by gathering a list of ideas spontaneously contributed by its members.

7.9. Group discussion: Group Discussion! Is a methodology or in a simple language you may call it an interview process or a group activity.

7.10. Field observations: Field observations are a method where you observe people in 'real' locations and situations, such as workplaces, homes, etc. They can be particularly helpful if the causes of 'wasteful' energy behaviour are not clear to you.

7.11. Surveys: Method for collecting quantitative information about items in a population

7.12. SWOT Analysis: A structured planning method used to evaluate the strengths, weaknesses, opportunities and threats involved in a project . A SWOT analysis can be carried out for a product, place or person.

ITK in plant disease management

8. History

In the past when Indians were gaining knowledge on the prediction of rainfall, management of agriculture, farm operations, harvesting, and storage, nothing was known about plant protection. The only methods to protect the crop were prayers and mantras. It was believed that the crop is protected if the mantra was written with red lac-dye and tied to the crop. But it cannot be said that the people of that time were unaware of insects and other pests and their damage. Some of the pests (in Sanskrit) affecting crops were gandhi, Shankhi, Pandarmundi, dhuli, and shringari. It is certain that gandhi (offensive odour) is what is called today the gandhi bug (Leptocorisa varicornis F.); shankhi must be a snail (Pila sp.); and pandarmundi means white head which is the typical symptom of the attack of rice stem borer (Tryporyza incertulus Walker). It is certain that they knew the rice stem borer and its symptom of attack. Dhuli means powder and it is possible that this word must have been used for powdery mildew of wheat and barley. The word "shringari" in Sanskrit indicates something adorned with red colour and it is possible that the term was used for rust diseases.

It is significant that people at that time considered that plants and human beings have similar physiology. Therefore, they divided the diseases of plants into two categories like internal and external. The internal diseases were those which were caused by "vata" (gradual defoliation, lower and fruit drop, generally yellowing of leaves and fruits), "pitta" (leaf yellowing; premature drop, decay of flowers and fruits), and "kapha"(Fruit-bearing delayed and fruits are tasteless and ripen prematurely; oozing without wounds) and external diseases were those which were caused by insects, birds, and weather. These categories can be attributed today to fungi, bacteria, viruses, and nematodes as internal diseases and insects, non-insect pests, frost, water logging, and drought as external diseases.

9. Materials recommended in ancient days to control diseases

9.1 Botanical showing anti fungal property

Sl. No.	Materials	Author/ period
1	Root of vasika (*Justicia adhatoda*)	Varahamihira (505 - 587 AD)
2	Branches and leaves of atimuktaka (*Hiptage benghalensis*)	Varahamihira (505 - 587 AD)
3	White mustard (*Brassica alba*)	Surapala (1000 AD)

9.2 Botanicals showing anti bacterial property

Sl. No.	Materials	Author/period
1	Bidanga (*Embelia ribes*)	Surapala(1000AD), Someshwara Deva(1126 AD)
2	Mahua (*Madhuca* spp.)	Surapala (1000 AD)
3	Bhilata (*Semecarpus anarcardium*)	Surapala (1000 AD)

9.3 Materials and practices that need our early attention

9.3.1. Milk and milk products :

Milk and ghee have been used for centuries. Even buttermilk was found useful. About 40% of total aminoacids in milk are glutamate, leucine, and proline. Milk is reported to contain plant growth promoters. A recent report claimed that milk sprays induced systemically acquired resistance in chilli against leaf curl, a viral disease. Milk (10% aqueous suspension) also has been effectively used for controlling powdery mildews. Besides, milk has excellent sticker-spreader properties. The aminoacid proline has been found to systemically induce resistance in plants. It stimulates production of antimicrobial phenolics. High amounts of endogenous proline increase contents of cytokinin and auxins. Besides milk, proline is present in the connective tissues of animals including fish.

9.3.2. Application of cow dung :

Use of cow dung for dressing seeds, plastering cut ends of vegetatively propagating units such as sugarcane setts, dressing wounds, sprinkling diluted suspension on plants, and applying to soil has been indicated since the time of Kautilya (300 BC). Indian farmers continue to use cow dung in various ways. The metabolic fraction comprises substances originating in the body such as residues of the bile and other digestive juices, epithelial cells from the alimentary tract, and the bacterial residues. In short, fecal residues comprise undigested fiber, debris from sloughed-off intestinal epithelium, some excreted products derived from bile (eg. pigments), intestinal bacteria, and mucus. There are more than 60 species of bacteria and over 100 species of protozoa encountered in the rumen of a cow. A majority of the bacteria are cellulose, hemicellulose, and pectin fermenters. Enterohepatic circulation involving bile salts, a small part is lost through bacterial degradation in the feces as dyslysin which is the slimy material.

9.3.3. Liquid manure (kunapajala)

Preparation of *kunapajala*, or liquid manure, involves boiling the flesh, fat and marrow of animals such as deer, pigs, fish, sheep or goats in water, placing the boiled matter in an earthen pot and adding milk; the powders of sesame oil cake; black gram boiled in honey, a decoction of pulses, ghee and hot water. There is no fixed proportion for the ingredients. The pot should be put in a warm place for two weeks. The resultant fermented liquid is manure called *kunapajal*. With plant-based composts, there is always a danger of passing on dormant pathogens to fields. There should be no such danger with the application of *kunapajala*. Also, animal wastes are likely to have microflora that might provide better biocontrol of plant pathogens and diseases than plant-based composts.

10. Application of ITK practices in plant disease management

10.1. Control of bacterial leaf blight by spraying cow dung slurry

Disease and causal organism: Bacterial leaf blight (*Xanthomonas oryzae* pv. oryzae)

Location of use of ITK: Koipuram, Ezumattoor of Pathanmathitta district, Kerala.

Cow dung (20 kg) is mixed with 250 l water thoroughly and kept for 3-4 hours till the coarse materials settled down. The solution on top is filtered and sprayed on paddy leaf for control of BLB.

Scientific reason: Bactericidal action of cow dung helps to reduce the population of the bacteria. Cow dung also acts as media for biocontrol agents

Cow dung slurry

Bacterial leaf blight
(*Xanthomonas oryzae* pv. *oryzae*)

(ICAR, 2003)

10.2. Control of fungal diseases in paddy fields by markati (*Baccaurea ramiflora*)

Diseases and casual organism: Sheath blight (*Rhizoctonia solani*), false smut (*Ustilaginoidea virens*), black kernel (*Curvularia lunata*)

Location of use of ITK: Mishing tribe of East Siang district, Arunachal Pradesh

After transplanting, spreading leaves of markati (*Baccurea ramiflora*) in the fields.

Scientific reason: Chemical like ramifloside. sapidolide and picrotoximaesin which is present in plant parts of markati acts as anti fungal property

Markati
(*Baccaurea ramiflora*)

Sheath blight
(*Rhizoctonia solani*)

False smut
(*Ustilaginoidea virens*)

(ICAR, 2003)

10.3. Use of kavalusaraka(*Careya arborea*) bark to control blast disease of paddy

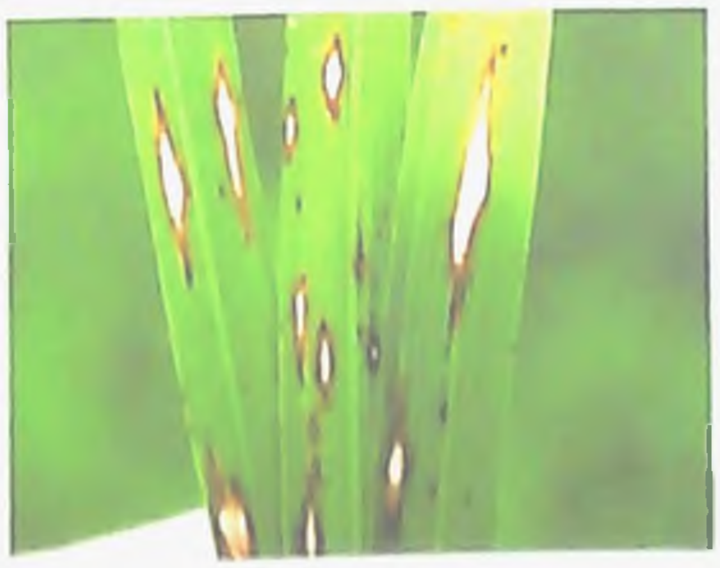
Disease and casual organism: Blast disease (*Pyricularia oryzae*)

Location of use of ITK: Shimoga district, Karnataka

2-3 kg bark of kavalusaraka (*Careya arborea*) is crused in water. About 500 ml of extract is mixed with 15 liters of water and sprayed on affected crop. Three sprays each, after 12 days interval are required to control blast in paddy

Scientific reason: Methanol extract of Careya arborea (MECA) stem and bark having antimicrobial activities which inhibit Gram positive and Gram negative bacteria and some fungal species

Kavalusaraka
(*Careya arborea*)



Blast disease
(*Pyricularia oryzae*)

(ICAR, 2003)

10.4. Control of downy mildew in cumbu(*Pennisetum thyphoides*)

Disease and causal organism: downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.)

Location of ITK used: Periyakulam, Tamil nadu

Pongemia decoction is prepared from leaves and 200g cooked rice in 10 litres of water. This solution is sprayed on the plants affected by downy mildew.

Scientific reason: Anti-inflammatory, anti-plasmodial, antinonciceptive, anti-hyperglycamic, anti-lipidperoxidative, anti-diarrhoeal, anti-ulcer, antihyperammonic and antioxidant activity

Pongemia
(*Pongemia pinnata*)

Cooked rice
in water

Downy mildew
(*Sclerospora graminicola*)

(ICAR, 2003)

10.5. Use of bark of mukul (*Commiphora mukul*) to control leaf curl in chilli

Disease and causal organism: chilli leaf curl virus

Location of ITK used: Baria district, Dahod, Gujarat

The bark of mukul and its gum are mixed with maize flour and sugar. It is burnt, smoke of this mixture is helps in controlling chilli leaf curl

Scientific reason: Antibacterial, Antiviral property present in leaf, bark and stem

Mukul
(Commiphora mukul)

Chilli leaf curl

(ICAR, 2003)

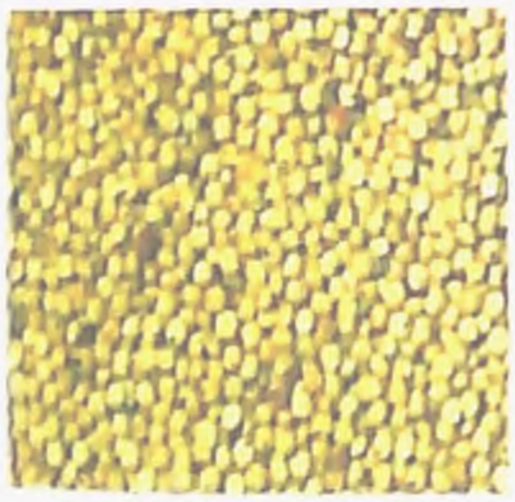
10.6. Control of head smut of sorghum by using cow urine

Disease and causal organism : Head smut of sorghum (*Sphacelotheca* reiliana)

Location of ITK used: Bijapur district, Karnataka

Soak the sorghum seeds in cow urine for half-an-hour and sun drying them before sowing to control head smut and to induce drought tolerance.

Scientific reason: Antimicrobial, antifungal and antibacterial property



Sorghum seeds

Cow urine

Head smut of sorghum
(*Sphacelotheca* reiliana)

(ICAR, 2003)

10.7. Control of banana diseases from different ITK practices.

Location of ITK used: Kerala

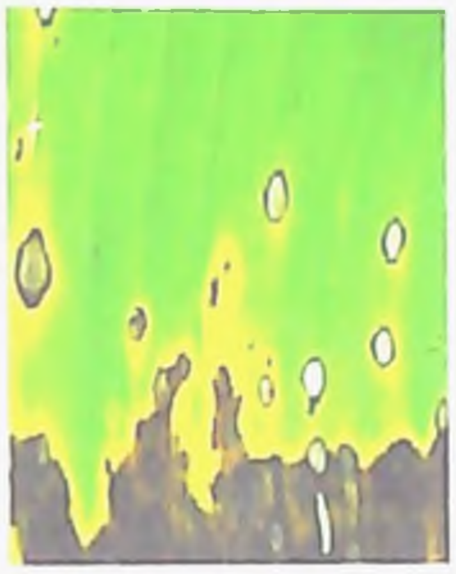
10.7.1. Control of leaf spot disease of banana by tobacco

Disease and causal organism: Leaf spot disease (*Mycosphaerella musicola)*

Diluted tobacco leaf extract is sprayed on banana crop to control leaf spot diseases.

Scientific reason: Tobacco has antifungal property which causes lysis of the germ tubes and/or growth inhibition

Tobacco
(Nicotiana tabacum)



Leaf spot disease
(Mycosphaerella musicola)

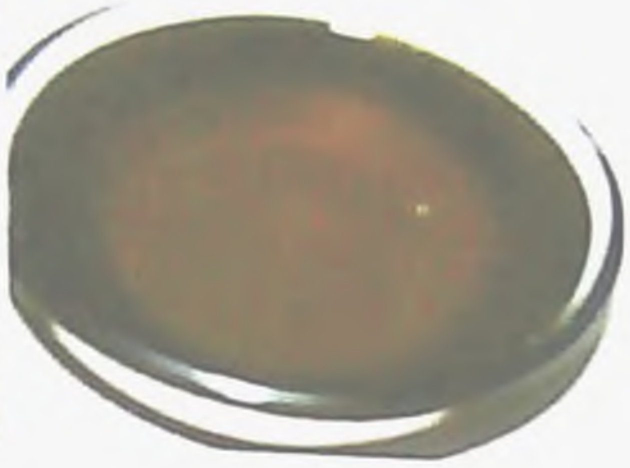
10.7.2. Control of rhizome rot by neem oil in banana

Disease and causal organism: Rhizome rot (*Eriwinia carotovora* subsp. *Carotovora*

Banana suckers are immersed for a while in 1 lit. of neem oil dissolved in 100 lit. of water before planting in order to prevent rhizome rot

Scientific reason: Antifungal, antibacterial, antiviral, antioxidant properties. Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms

Banana suckers



Neem oil

Rhizome rot
(*Eriwinia carotovora)*

(KAU, 2009)

10.8. Controlling powdery mildew of orange using *Aloe vera*

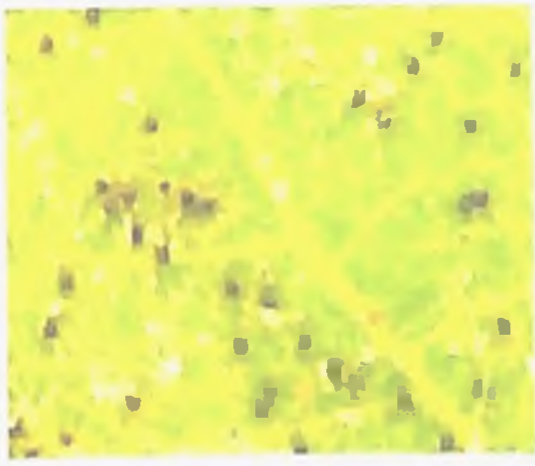
Disease and causal organism: Powdery mildew (*Oidium citri and O. Tingitaninum).*

Location of ITK used: Thane, Maharastra

Greenish *Aloe vera* plants are cut into small pieces and spread to a radius of 2 feet around the tree during flowering to control powdery mildew.

Scientific reason: *Aloe vera* contains 6 antiseptic agents like lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses.

Aloe vera



Powdery mildew
(*Oidium citri)*

(ICAR, 2003)


Banana suckers


Neem oil


Rhizome rot
(*Eriwinia carotovora)*

(KAU, 2009)

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Aloe vera


Powdery mildew
(*Oidium citri)*

(ICAR, 2003)

Some of ITK practices practiced in Kerala

Sl. No.	Crop	Diseases	ITK practices
1	Banana	Sigatoka leaf spot (*Mycosphaerella* sp.)	Power oil (Mineral oil) 1 % emulsion is effective in controlling the disease.
2	Rice	Blast	Apply rice hull ash @ 100 g / m2 which will help to reduce the incidence of blast in the Nursery
3	Amaranths	All diseases	One kg of fresh cowdung is put in 10 litres of water and the clear solution after filtering the supernatant liquid is sprayed at regular intervals
4	Bindhi	Yellow mosaic virus	Spraying neem oil-garlic mixture (2%)

(KAU, 2009)

10.9 Cultural methods

There are many agronomical practices practiced to manage the plant diseases they are

- Roguing
- Burning
- Pruning
- Weeding
- Flooding
- Trap crop
- Border crop

11. Case studies

Measurement of plant diseases

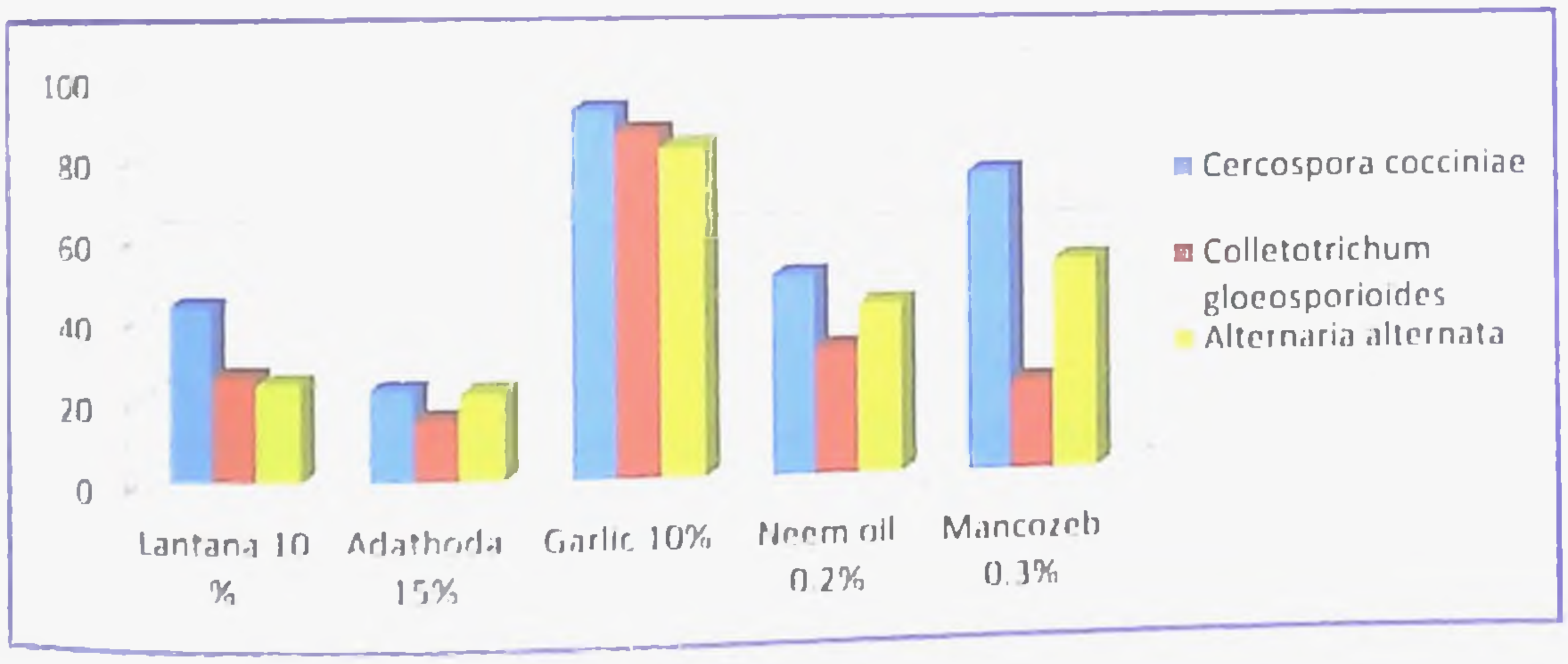
Percent Disease Incidence (PDI): It is the percentage of diseased plants or parts in the sample or population of plants. It can be the proportion or percentage of diseased leaves in a plant, diseased stalks or a tiller or diseased seedlings in a field.

Disease incidence: No. of infected plants x100 /Total no. of plant assessed

Percent Disease severity (PDS): Disease severity is the percentage of relevant host tissues or organ covered by symptom or lesion or damaged by the disease. Severity results from the number and size of the lesions.

Disease severity or Infection index = Sum of all disease rating x 100 / Total no. of rating x maximum disease grade

11.1. Effect of indigenous botanicals in management of leaf spot of gourd (*Coccinia grandis* (L.) Voigt)



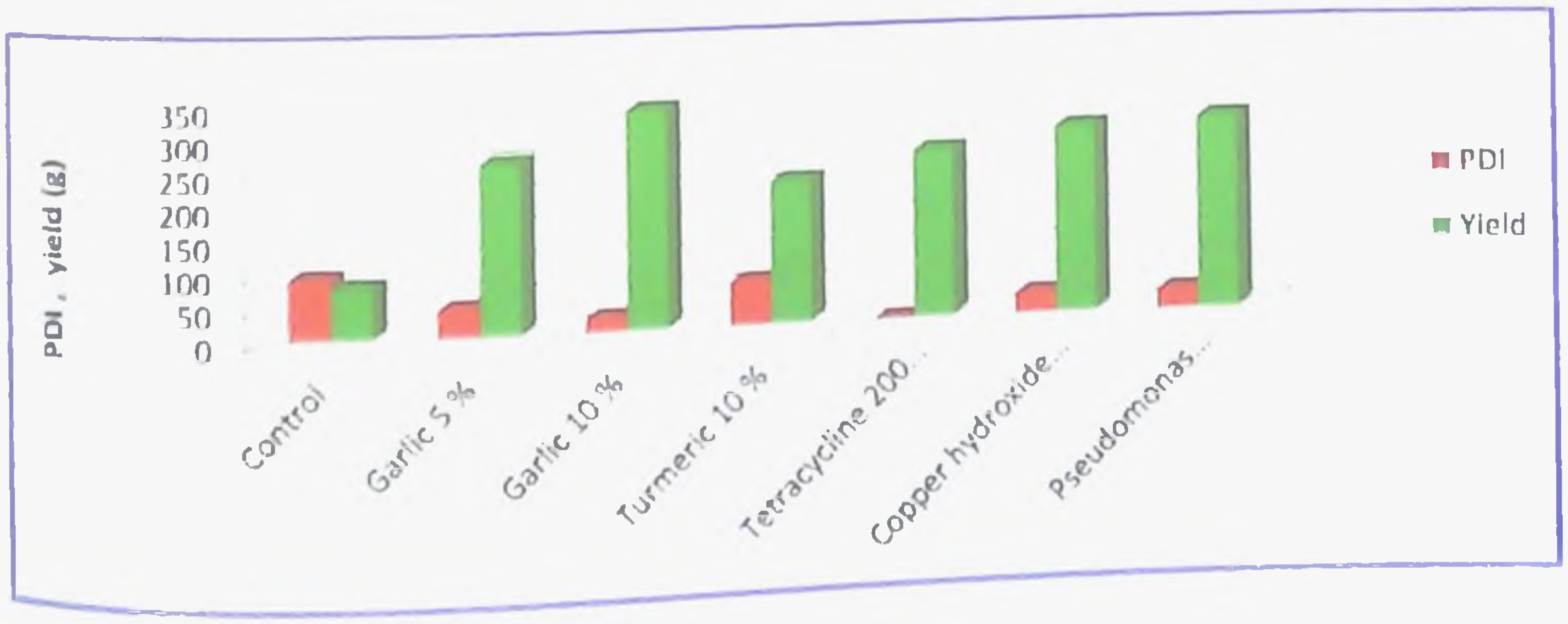
Davis(2003) conducted a study in the Department of Plant Pathology, College of Horticulture, Vellanikkara on management of leaf spot of ivy gourd caused by *Cercospora cocciniae, Colletotrichum gloeosporioides and Alternaria alternata*. The treatment involving adathoda and lantana 10 % were found to be more effective to control this disease when compared to Mancozeb 0.3%.

11.2. Effect of different treatment on disease incidence and disease severity of *Bitter gourd distortion mosaic virus* (BGDMV),

Treatment no.	Treatments	Per cent disease incidence	Per cent disease severity
T_1	Neem oil – garlic emulsion 2 %	87.50^{bcd}	60.46^{cd}
T_2	Neem seed kernel extract 5 %	88.88^{bcd}	56.90^{cd}
T_3	*Thespesia populnea* leaf extract 10 %	90.27^{bcd}	52.15^{cd}
T_4	Infected plant extract 10-3 dilution	93.04^{bcd}	53.11^{bc}
T_5	Coconut toddy 1: 3 dilution	92.49^{bcd}	50.61^{bc}
T_6	Coconut vinegar 1 %	85.54^{abc}	42.13^{ab}
T_7	Imidacloprid 0.025 %	76.38^{a}	37.84^{a}
T_8	Control	97.21^{d}	71.00^{d}

Zacharia (2006) reported that the botanical coconut vinegar 1% and neem oil garlic emulsion 2% were found to be effective with low PDI of 85.54 per cent and 87.50 per cent over the control with PDI of 97.21 per cent. Similarly PDS of 42.13 per cent was recorded in coconut vinegar 1% which indicates its efficiency to manage the BGDMV.

11.3. Effect of ITK treatments on the disease incidence of black rot of cauliflower



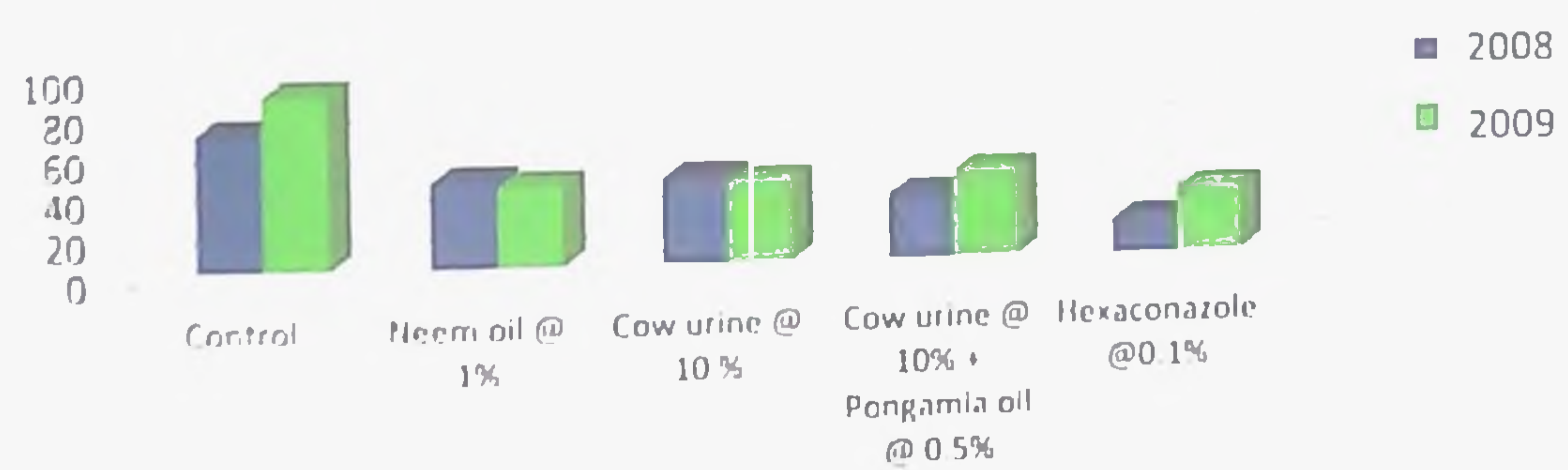
Prasanna (2009) conducted a study by application of various botanicals to control black rot of cauliflower and it was reported that the botanicals garlic 5%, garlic 10% and turmeric 10% has significant effect with lower PDI and higher yield over the control.

11.4. Effect of indigenous treatments in management of *Tobacco mosaic virus* (TMV)

Treatments	PDI	Cured leaf yield (kg/ha)
Control	66.8	1058
Panchagavya @ 5%	39.7	1117
Neem leaf extract 1500 ppm	31.7	1188
Bougainvillea leaf extract @ 5%	40.5	1247
Butter milk @5%	53.1	1269
Vermiwash @ 10%	42.9	1078

Hundekar *et al.*, 2010 studied management of Tobacco Mosaic Virus (TMV) management by indigenous treatment and observed PDI and yield that neem leaf extract 1500 ppm showing less PDI. Butter milk @ 5% and bougainvillea leaf extract @ 5% showed high yield.

11.5. Effect of ITK treatments on rust incidence in soybean



Jahagirdar, 2010 conducted study on management of soybean rust by ITK treatment in two different years and reported cow urine @ 10% + pongemia oil @0.5% sowing effective control disease in 2008 and 2009.

12. Why we document ITK?

Girach (2007) explained the reasons for documentation of ITK. They are

1. Economic, social and political factors are gradually uprooting many such untapped resources from their native habitats resulting in loss and erosion of very rich indigenous knowledge.

2. Rapid pace of acculturation / urbanization has tremendous influence on the lives of indigenous communities.

3. Modernization has resulted into loss of their peculiar culture and heritage.

4. The knowledge survives through word of mouth particularly among the old generation. Documentation of their vital knowledge on different subjects is necessary before the old generation passes away.

5. Documentation has great practical utility in almost every activity of human life such as health, animal health, livestock management, food, agriculture, timber, dye, religious ceremonies, shelter etc.

6. It provides useful clue for planning projects for conservation of biological diversity, sustainable uses of natural resources, indigenous health practices etc.

7. The data is the intellectual property of the informant (individual or community).
Benefit sharing should be there when data will be used for raising any benefit.

13. Attempts made to protect ITK

Globally and nationally there are many steps taken to document, protect and disseminate the traditional knowledge exiting in particular country or locality. Internationally

13.1. Traditional Knowledge Digital Library(TKDL)

TKDL is an Indian digital knowledge repository of the traditional knowledge, especially about medicinal plants and formulations used in Indian systems of medicine. Set up in 2001, as a collaboration between the Council of Scientific and Industrial Research (CSIR) and the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (Dept. of AYUSH), Ministry of Health & Family Welfare, Government of

India, the objective of the library is to protect the ancient and traditional knowledge of the country from exploitation through biopiracy and unethical patents, by documenting it electronically and classifying it as per international patent classification systems. Apart from that, the non-patent database serves to foster modern research based on traditional knowledge, as it simplifies access to this vast knowledge of remedies or practices.

13.2. World Intellectual Property Organization (WIPO)

In 1998, World Intellectual Property Organization (WIPO) began a new set of activities designed to explore the Intellectual Property (IP) aspects of the protection of ITK. The main objective of these activities was to identify and explore the IP needs and expectations of the holders of TK in order to promote the contribution of the IP system to their social, cultural and economic development. .WIPO Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore towards the development of an international legal instrument or instruments for the effective protection of traditional cultural expressions and traditional knowledge, and to address the intellectual property aspects of access to and benefit-sharing in genetic resources.

13.3. Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS)

TRIPS is an international agreement administered by the World Trade Organization (WTO) that sets down minimum standards for many forms of intellectual property (IP) regulation as applied to nationals of other WTO Members. It was negotiated at the end of the Uruguay Round of the General Agreement on Tariffs and Trade (GATT) in 1994. In 4th WTO Ministerial Meeting in Doha, 2001, one of the key issues raised that there is need of amendments in the Trade Related Aspects of Intellectual Property Rights (TRAIP) Agreement, so that the members shall require to provide that an application for a patent relating to biological materials or to Traditional Knowledge.

In India national level acts protect traditional knowledge. They are

13.4. Geographical indications Act, 1999

Geographical indications are closely related to functions assigned to trademarks and are well established in unfair competition law. Even if GIs have no property holder perse, they nevertheless count towards an intellectual property right, because their benefit stream is the geographical area in relation to the producer of a product. In addition to IPRs and competition law. GIs are subject to consumer laws, as they embody the preference a consumer may express for locally produced goods. The benefit stream (value) of TK is encapsulated in the intellect of the human mind, while the benefit stream of a Geographical indication is a particular product originating in a particular geographical region.

It has been suggested that Geographical indications Act will be helpful in protecting the traditional knowledge of the indigenous people. The development of plant varieties would not be in the picture unless these indigenous people were there. The important linkage between geographical indications and developing countries interest, corresponds to the protection of traditional knowledge. Probably the only existing category of intellectual property rights that may directly applied to the protection of traditional knowledge is that of geographical indications.

13.5. Protection of Plant Variety and Farmers Right Act, 2001 (PPVFR Act)

It is an Act of the Parliament of India enacted to provide for the establishment of an effective system for protection of plant varieties, the rights of farmers and plant breeders, and to encourage the development and cultivation of new varieties of plants. This act received the assent of the President of India on the October 30, 2001.

The provisions on the right to send specify that farmers are entitled to save, use, sow, re-sow, exchange, share and sell farm produce, including seeds of varieties protected by plant breeder's right. They are, however, not allowed to sell seeds of protected varieties as branded packages. All the same, this stands as the most liberal legislation to date in this sphere, allowing farmer's all the customary rights they previously enjoyed

13.6. Biological Diversity Act, 2002

It is an Act of the Parliament of India for preservation of biological diversity in India, and provides mechanism for equitable sharing of benefits arising out use of traditional biological resources and knowledge. The vision of NBA is the conservation and sustainable use of India's rich biodiversity and associated knowledge with people participation, ensuring the process of benefit sharing for well being of present and future generations. The mission of NBA is to ensure effective implementation of Biological Diversity Act, 2002 and the Biological Diversity Rules 2004 for conservation of biodiversity, sustainable use of its components and fair and equitable sharing of benefits arising out of utilization of genetic resources.

14. Advantages of ITK

It has minimum risk factor

It relies heavily on genetic and physical diversity

It exploits optimum utility of local resources

It is environmental healthy

It takes a holistic worldwide

It is readily available and easily understandable

It fits into local farming system and is adaptable to meet multipurpose need

It is based on cultural values of community

It is arrested by evidences from trust worthy sources

It encourages transparency and accountability

15. Limitations of ITK

Lack of replicability

Uneven distribution across individuals, communities and regions

Break down into faces of crises or external interventions must be proper attention

It is limited to the local pool of techniques, materials and genetic resources

Variation in capacity to generate implement and transfer from individual to individual

No scope for manipulation of social, political and economic structure within which they occur

16. Summary

Indigenous Technical Knowledge (ITK) is traditional based where transferred generation to generation by specific a community. ITK is diversified in different fields with unique nature. Indigenous practices like application of botanicals, animal products and some of cultural methods plays important role in management of plant diseases. Documentation of ITK serve as a ready references for the agricultural scientists for further study to determine their scientific rationality and effectiveness Attempts are made to protect ITK nationally and internationally.

17. Conclusion

Indian agriculture has rich traditional values existing and carried out till today. Documentation, inventory and validation of indigenous traditional practices should consider. Awareness among the farmers about traditional knowledge and locally available materials which can be effectively used in plant disease management should be done through extension agencies and media. ITK practices should be majorly recommended in Integrated Disease Management.

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18. Discussion

1. *How do organic farming and ITK differ?*

ITK is basics for organic farming, ITK may or may not be organic. In ITK, farmers use locally available materials along with lime, Bordeaux, mixture etc., where chemicals is not recommended in organic farming.

2. *How intercropping practices reduces plant diseases?*

Intercropping is the c ultivation of two or more crops simultaneously on the same field. The rationale behind intercropping is that the different crops planted are unlikely to share disease causing pathogens and to conserve the soil

Eg. Ground nut intercropping with bajra was found to be the best intercrop in reducing the Peanut bud necrosis disease incidence followed by sorghum

(Sunkad *et al*., 2015)

3. *What is difference between sea salt and common salt?*

The most notable differences between sea salt and table salt are in their taste, texture and processing. Sea salt is produced through evaporation of ocean water or water from saltwater lakes, usually with little processing. The minerals add flavour and colour to sea salt, which also comes in a variety of coarseness levels. Table salt is typically mined from underground salt deposits. Table salt is more heavily processed to eliminate minerals and usually contains an additive to prevent clumping. Most table salt also has added iodine, an essential nutrient that helps maintain a healthy thyroid.

4. *Which ITK is practiced worldwide?*

5.

Bordeaux mixture is widely used ITK.

In the 19th century, several outbreaks of vine diseases occurred among the *Vitis vinifera* vines of the classical European wine regions. These outbreaks were caused by pests to which these vines lacked resistance, carried on vines brought to Europe as botanical specimens of American origin. These pests included not only the Great French Wine Blight caused by the aphid *Phylloxera vastatrix*, but also mildew and other diseases caused by fungi.

After the downy mildew had struck, botany professor Pierre-Marie-Alexis Millardet of the University of Bordeaux studied the disease in vineyards of the Bordeaux region. Millardet then noted that vines closest to the roads did not show mildew, while all other vines were affected. After inquiries, he found out those vines had been sprayed with a mixture of $CuSO_4$ and lime to deter passersby from eating the grapes, since this treatment was both visible and bitter-tasting. This led Millardet to conduct trials with this treatment. The trials primarily took place in the vineyards of Château Dauzac, where he was assisted by Ernest David, Dauzac's technical director. Millardet published his findings in 1885, and recommended the mixture to combat downy mildew. In France, the use of Bordeaux mixture has also been known as the Millardet-David treatment. Now this is widely used in all countries in the world.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE

Department of Plant Pathology

PATH 591: Master's seminar

Name: Priyanka B.

Admission No.: 2014-11-219

Major advisor: Dr. S. Beena

Venue: Seminar hall

Date: 22-01-2016

Time: 10:40 am

Indigenous technical knowledge in plant disease management

Abstract

India is a vast country with rich biodiversity and treasure of traditional knowledge. Over the years, farmers and sages developed innumerable effective practices to grow crops and raise animals in agro-ecological regions of the subcontinent. Indigenous Technical Knowledge (ITK) is the actual knowledge of a given population that reflects the experiences based on tradition and includes more recent experiences with modern technologies (Haverkort, 1995).

ITK is diversified in different fields like agriculture, ethno-botany, meteorology, ethno-ecology medicine, social sciences and humanities. ITK is dynamic, traditional knowledge based, descriptive and analytical. ITK can be collected by different methods based on the situations, social systems and cultural values. In ancient history, people were aware of agricultural management, harvesting, storage and animal husbandry, but unaware about crop protection. Later, people observed external damages and also believed in internal diseases (vata, pitta and kafa).

In Vrikshayurveda, botanicals such as vasika (*Justicia adhatoda*), atimuktaka (*Hiptage benghalensis*), bidanga (*Embelia ribes*), Mahua (*Madhuca* spp.) *etc.* were mentioned as effective for plant disease management. Some of the animal products *viz.* cow dung and urine, milk, honey, animal fat, liquid manure *etc.* were also mentioned in other ancient literature. Application of cow dung slurry and cow urine in paddy fields, sea salt in coconut basins, asafoetida and turmeric for wilted plants are being practiced by the farmers.

Scientists established the specificity, concentration and methods of application of these traditional practices with modern scientific tools.

Research works carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara recorded the effectiveness of various indigenous methods for the management of plant diseases. Davis (2003) showed the effectiveness of adathoda (*Justicia adhatoda*) against leaf spot of ivy gourd. Zacharia (2006) reported that one per cent coconut vinegar was found to be effective against bitter gourd distortion mosaic. Effectiveness of garlic extract in reducing black rot of cauliflower was also reported (Prasanna, 2009).

Documentation of ITK is essential for conserving traditional practices in its original form. This is internationally done through Traditional Knowledge Digital Library (TKDL), World Intellectual Property Organization (WIPO) and nationally by Geographical Indications (GI) Act, Plant Varieties & Farmer's Rights (PV& FR) Act and Biological Diversity Act.

Even though application of ITK in agriculture has many advantages, there are some limitations like lack of replicability, uneven distribution, adaptation by society *etc.* In order to maintain a healthy environment, present generation need to be aware of the traditional practices. Thus, ITK should be a major component in integrated plant disease management.

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Agrl. Microbiology

Microbial herbicides

By

Janish Rose Jacob

(2014-11-184)

M.Sc. Agricultural Microbiology

Seminar report submitted in partial fulfilment of requirement of the course

Micro. 591: Master's Seminar (0+1)

DEPT. OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF HORTICULTURE

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DECLARATION

I, Janish Rose Jacob (2014-11-184), hereby declare that the seminar report entitled 'Microbial Herbicides' has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara

Date: 23/01/2016

Janish Rose Jacob

2014-11-184

CERTIFICATE

This is to certify that the seminar report entitled **'Microbial Herbicides'** has been solely prepared by Janish Rose Jacob (2014-11-184), under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

Vellanikkara

22-01-2016

Major Advisor,

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CERTIFICATE

Certified that the seminar report entitled 'Microbial herbicides' is a record of seminar presented by Janish Rose Jacob (2014-11-184) on 19th December, 2015 and is submitted for partial requirement of the course Micro. 591.

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1. Introduction

Weeds are a problem in both crop production and turf grass system, associated with decline in crop yield and quality; as an aesthetic nuisance and as a source of allergenic pollen (Gadermaier *et al.*, 2014). Since the post-world war II introduction of the first selective herbicides, 2,4-D and MCPA (2-methyl-4-chlorophenoxyacetic acid), such products have significantly changed the management techniques that are employed by farmers and other managers of anthropogenic ecosystems (Mithila *et al.*, 2011). The primary benefit offered by selective herbicides is the ability to control certain weed species without harming crops, based on physiological differences between species. This ability has enabled significant yield increases in many crops, and continues to be an important aspect of agroecosystem management (Mithila *et al.*, 2011). Currently, there are about 25 known herbicide target sites at the molecular level, e.g., disruption of EPSP (5-enolpyruvylshikimate-3-phosphate synthase) required for branched amino acid synthesis by glyphosate (Sammons and Gaines, 2014) or interference of auxin pathways by 2,4-D (Grossmann, 2010). Despite this variety, in many cases a limited number of herbicide mechanisms have been continuously employed by operators based on the low cost or ease of use associated with those products (Beckie, 2011). This practice has in many cases created artificial selection pressure on weed populations, causing the widespread emergence of herbicide-resistant weeds (Darmency, 2013). As of June, 2015, resistant-weed populations have been reported in association with 22 of the 25 known herbicide targets.

It is apparent that as new herbicides are developed, weeds will continue to evolve in response to whatever selective pressure that is applied. For this reason, the continuous development of novel weed control methods is essential to the ongoing maintenance of agricultural yields. These developments are needed both to control weed populations that are resistant to currently available modes of action, as well as to diversify weed control platforms in order to delay the emergence of new resistant traits. Additionally, increasing public concern with the negative effects of pesticide residues, particularly in residential areas (e.g., turfgrass), has led to increasing demand for alternative methods of controlling weeds and other pests (Bailey *et al.*, 2011).

1.1 Biological control of weeds

Biological control as a general term refers to the introduction of organisms into an ecosystem with the intention of controlling one or more undesirable species (Bailey *et al.*,

- ✓ They should be stable in storage
- ✓ They should be genetically stable *viz*. the ability of the organism to maintain its genotype over population
- ✓ They should be effective under field conditions
- ✓ They should be tolerant to variations in temperature
- ✓ They should be compatible with other chemicals

5. Types of microbial herbicides

Microbial herbicides can be divided into microbial preparation herbicide and microbe-derived herbicide by virtue of the effective components from the pathogen itself or its phytotoxin.

5.1 Microbial- derived herbicides

Microbial-derived herbicides, especially microorganism secondary metabolites, are a new kind of microbial herbicide to control weeds, which are always phytotoxins. Microorganism can produce a lot of metabolites whose characteristics are diversity in structure and biological activity, and easily degraded. These bioactive components invade into the host plant, cause pathogenicity, destroy their structure and lead them to produce necrotic lesions or chlorotic halo (Li *et al.*, 2003). Now, biologists and agriculturalists are paying more attention to natural products that have herbicidal activity, for these natural phytotoxins have a specific target and new different chemical structures which are difficult to synthesize by common pesticide synthesis method. They are less poisonous to most of mammalian systems, easily degraded and so far result in no biological disaster compared to chemical herbicides (Charudattan, 1991). These phytotoxins are very different in chemical structure and size. Some of them are polypeptides, terpenes, macrocylic and bakelite (Strobel *et al.*, 1991). They are also different in their host plant speciality. They either apply to a single species or to one kind of plant. Phytotoxins used for microbial herbicides can be divided into three types: bacterial, fungal, actinomycete derived product.

5.1.1 Phytotoxins from fungi

Junko Ohra and Kenji Morita (1995) found that phytotoxin, ferricrocin from *Colletotrichum gloeosporioides* have weed control activity. The experiment of controlling 7 different kinds of weeds indicated that Jointvetch (*Aeschynomene virginica*) was extremely damaged. Pigweed (*Amaranthus retroflexus*) and Florida beggar weed (*Desmodium tortuosum*) were severely burned and would not grow out of the damage, and Johnson grass (*Sorghum halepense*) was stunted but not killed by its extract. Phytotoxins from other fungi have weed control activity such as AAL (*Alternaria alternata*)-toxin, cornexistin and tentoxin.

AAL-toxin and its analog in structure can suppress cearmide synthetase and result in sphingol accumulation that makes membrane break. Cornexitin is metabolin inhibitor and action mechanism of this is similar to aminoacetic salt. It inhibits one isoenzyme of asparagines aminotransferases, but once acid from tricarboxylic acid cycle such as aspartic acid and glutamic acid is added, the activity of toxin will disappear. Tentoxin have two different action mechanisms under different conditions. One is interrupting the formation of chloroplast by blocking synthesis of coding nucleocytoplasmic protein and the other is energy tranferase inhibitor of ATPase's coupling factor for controlling photophosphorylation (Duke *et al.* 1996).

5.1.2 Phytotoxins from bacteria

Most of the bacteria with an ability to produce toxins are Gram-postive such as Streptomyces, Corynebacterium and very few are gram negative like Pseudomonas (Kremer *et al.*, 1990). *Pseudomonas syringe* pv. *phaseolicola* is a bacterial plant pathogen which causes halo blight disease and localized death on common bean (*Phaseolus vulgaris* L.) and kudzu (*Pueraria lobatan*), whose toxin was called phaseolotoxin. Once it infects plant root, it will spread to shoot terminus, then causes stunting, chlorosis and even causes foliage necrotic lesions (Zidack and Backman, 1996). Gurusiddaiah and Gealy (1994) have purified the phytotoxin from D7, which was inhibitory to downy brome (*Bromus tectorum*) and was a complex consisting of at least two polypeptides, a chromophore, fatty acid esters, and a lipopolysaccharide matrix. Further purification of this compound resulted in near complete loss of phytotoxin because purification procedures may damage the phytotoxin (Gurusiddaiah and Gealy, 1994). For further exploring possible mechanism of D7, Patrick *et al.* (1993) evaluated effects of a crude preparation of D7 on various physical process in roots of downy brome seedlings. It was found that cell division, respiration, and synthesis of protein, RNA, and DNA were not inhibited or only slightly inhibited, whilst disruption of lipid synthesis and membrane integrity were significant, which might account for inhibition of root elongation. Tranel repeated this experiment with the further purified toxin and found the same result (Patrick *et al.*, 1993). Other phytotoxins from pseudomonas have different physiological mechanisms. Phaseolotoxin, a tripeptide from *Pseudomonas syringe* pv. *phaseolicola*, inhibits arginine synthesis by competing with carbamoyl phosphate for the binding site on ornithine carbamoyl transferase (Daly, 1981). Tabtoxin from *P. syringe* pv. *tabaci* can be hydrolyzed to tabtoxinine-b-lactam, which inhibits glutamine synthetase. Syringomycin, a peptide based phytotoxin from *P. syringae* pv. *syringae*, was originally believed to cause cell membranes hydrolysis. Further studies showed that this toxin could specifically increase K^{+}

efflux and H^+-ATPase activity and was revealed to have a possible role in Ca^{2+} transport (Patrick *et al.*, 1993).

5.1.3 Phytotoxins from actinomycetes

Herbicidines and herbimycins are higher-plant toxins both produced by *Streptomyces saganonensis*. The former is used to control grassy weeds in paddy field as selective herbicides, the latter controls monocotyledonous and dicotyledonous weeds (Stephen and Lydon, 1987). Anismycins from Streptomyces is a kind of growth inhibitor for annual grassy weeds such as barnyardness and common crab grass and broad-leaved weeds. Its mechanism is to destroy synthesis of plant chlorophyll (Yufen Zhang, 1987). Bialaphos is a metabolite of *Streptomyces viridochromogenes* and is widely used to control annual or perennial grassy weeds and broad-leaved weeds. The mechanism here is to be metabolized to phosphinothricin in plant, and then phosphinothricin inhibits glutamine synthesis. Anismycein can make small seedlings of barnyardness and common crabgrass die above 50 ppm, and inhibit radicle growth under 12.5 ppm. Its synthetase may accumulate ammonia, and control photosynthetic phosphorylation causing plant death (Liu, 1999). Carbocyclic coformycein and Hydantocidin is produced from *Streptomyces hygrosoopies*, which can decrease synthetase of adenylosuccinate by increasing content of ATP and hold back synthesis of protein (Pilimoor, 1998). In addition, phthoxazolin, hytantocidin and homoalanosin from Streptomyces can control several weeds (Shen,1995).

5.2 Microbial preparation herbicide

Microbial preparation herbicide is defined as microorganism that can control weeds. Most research reports relate to fungal plant pathogens, for bacterial pathogens with weed control activity are scarce. The earliest reported mycoherbicide is a culture suspension of *Colletotrichum gloeosporioides* f. sp. *cuscutae* for controlling dodders with the name "Lubao No. 1", which was studied by Shandong Academy of Agriculture Science in China in the 1960s. *Pseudomonas fluorescens* strain D7, *P. fluorescens* strain BRG 100 and *Xanthomonas campestris* are bacterial agents used as microbial herbicides. *C. gloeosporioides* f.sp. *aeschynomene*, *Fusarium pallidoroseum* and *Phoma macrostoma* are some of the fungal agents used as microbial herbicides. Tobacco Mild Green Mosaic Tobamovirus (TMGMV) is the only viral agent used.

5.3 Evaluation of bioherbicidal potential of fungal pathogens against water hyacinth (*Eichhornia crassipes*)

Water hyacinth is one of the most invasive aquatic weeds of Kerala. In a study by Praveena and Naseema (2004), a survey was conducted in the waterways of four southern districts of Kerala to document the fungal pathogens of water hyacinth. Out of the 21 fungi recorded, 17 were pathogenic. Among these, *Myrothecium advena* Sacc. is a new report on water hyacinth. The extent of damage produced by the pathogenic fungi on water hyacinth (Table 1) ranged from 16.67 (*Curvularia lunata* and sterile fungus) to 61.11% (*M. advena*). Only *M. advena* and *Fusarium pallidoroseum* (Cooke) Sacc. caused more than 50% infection of the weed and hold promise as biocontrol agents of water hyacinth.

Table 1. Extent of damage caused by pathogenic fungi on water hyacinth

Pathogenic fungi	Intensity of infection (%)	Pathogenic fungi	Intensity of infection (%)
Alternaria eichhorniae	44.44	*F. pallidoroseum* isolate 1	45.09
Colletotrichum gloeosporioides isolate 1	35.24	*F. pallidoroseum* isolate 2	43.14
C. gloeosporioides isolate 2	46.21	*F. pallidoroseum* isolate 3	53.44
Curvularia lunata	16.67	*Helminthosporium* sp.	27.77
Fusarium equiseti	42.11	*Myrothecium advena*	61.11
F. moniliforme isolate 1	41.72	*Pestalotia* sp.	22.22
F. moniliforme isolate 2	41.21	*Rhizoctonia solani*	31.24
Sterile fungus	16.67		

6. Mode of action

The mechanism(s) behind the suppressive activity of a given biocontrol agent is in many cases only partially understood. Generally, bacteria, fungi and actinomycetes produces metabolites which will interfere in metabolic pathways of weeds and thus supresses their growth. For example, *Pseudomonas* spp. Producing Hydrogen cyanide (HCN) and *Phoma macrostoma producing* macrocidins. Future research into the mechanisms underlying these effects will be important to achieve consistent efficacy with biocontrol agents, as well as to evaluate potential impacts on human and ecosystem health. This in turn will be of value to gaining regulatory approval. Additionally, understanding bioherbicidal mechanisms may generate novel herbicides to overcome current resistance traits (Boyetchko *et al*., 2009), and will likely also be of peripheral value to the field of plant pathology.

7. Mass production

Commercialization of bioherbicides requires economically feasible methods of production of the bioherbicide agents (Stowell, 1991). In the case of fungi, the preferred method of industrial production is by means of liquid fermentation, but many fungi do not produce spores under submerged conditions. In this case, a biphasic production system, wherein a fungus is first cultured in liquid shake cultures, followed by slow drying in a shallow layer (Chandramohan and Charudattan, 1998) or over solid support (Stowell, 1991), have been shown to be practical. Use of natural substrates like grains, weed seeds, and other plant tissues (W[illegible] *et al*., 1999) may offer an economical and facile low-technology method of spore production. Basidiomycetes such as *Chondrostereum purpureum* and *Cylindrobasidium laeve* can be produced as infective mycelia in liquid culture or on sterilized wood blocks (Morris *et al*., 1999). Amsellem *et al*. (1999) have developed a method to produce stable mycelial inocula of *Fusarium arthrosporioides* and *F. oxysporum* that could be stored without loss of infectivity for more than 9 months. Adoption of the solid-substrate production methods used in the mushroom-spawn industry is another commercially feasible option.

7.1 Evaluation of culture medium for microbial herbicide (*Bipolaris euphorbiae*)

Moraes *et al* (2014) evaluated different culture media for the microbial herbicide. *Bipolaris euphorbiae*. The liquid medium made with sugar cane molasses stood out from the others because it provided great sporulation (23×10^4 conidia ml^{-1} of medium), conidial viability (99.7%), and formation of mycelial fungal biomass (1.26 g 100 ml^{-1} of medium). On solid media conidial production was markedly higher than in liquid media, especially the medium composed by a blend of sorghum grain (40%) and soybean hulls (60%) where the fungus produced 2.3 x 10^7 conidia g^{-1} of medium. The cultivation of *B. euphorbiae* in biphasic system not promoted a significant increase in the production of conidia. The solid media were more effective for the mass production of fungus and mixtures of grains and derivatives were effective for increasing conidia production.

Table 2. Conidia production and viability of *Bipolaris euphorbiae* in liquid media made from waste and agro-industrial by-products used at different

Liquid media	Conidia production ($\times 10^4$/ml)	Viability (%)
Sugar cane stillage	5.8	97.4
Cheesy whey	1.5	89.1
Cassava press water	[illegible]	99.2
Yeast cream	15	99.1
Sugar cane molasses	[illegible]	99.7

Observations after 10d of cultivation at 25 ± 0.5 °C in a 12h photoperiod

Table 3. Conidial production and viability of *Bipolaris euphorbiae* in solid media prepared with mixtures of substrates at various concentrations

Solid media	Mixture and concentration	Conidia production of *Bipolaris euphorbiae* ($\times 10^{7}$/g medium)	Viability (%)
Sorghum grains (SG) + Soya bean hulls (SH)	100 % SG + 0 % SH	1.37	99.9
	80 % SG + 20 % SH	1.15	99.9
	60 % SG + 40 % SH	1.15	100
	40 % SG + 60 %SH	2.3	99.8
	20 % SG + 80 % SH	1.3	99.9
	0 % SG + 100 % SH	1.4	99.9

Observations after 10 days of cultivation at 25 ± 0.5 °C in a 12h photoperiod

Table 4. Conidial production and viability of *Bipolaris euphorbiae* after biphasic cultivation for 10 days

Biphasic combination	Conidia production of *Bipolaris euphorbiae* (×10 g solid medium)	Viability (%)
Sugar cane molasses (8%)/ SG + SH (40:60 %)	0.7	99.9
Sugar cane molasses (8%)/ SG + cracked wheat (80:20 %)	0.8	99.7
Sugar cane molasses (8%)/ SH + cracked wheat (40:60 %)	1.0	99.9
Yeast cream (70%)/ SG +SH (40:60%)	1.3	99.9

Yeast cream (70 %)/ SG +cracked wheat (80:20%)	1.0	99.7
Yeast cream (70 %)/SH +cracked wheat (40:60 %)	1.4	99.9

Observations after 10 days of cultivation at 25 ± 0.5 °C in a 12h photoperiod

8. Formulations

As stated by Greaves *et al.* (1998), innovations in formulation technology are vital if we are to succeed with the next generation of bioherbicides. Our types of materials/formulations

have received much attention: various kinds of emulsions, organosilicone surfactants such as Silwet L-77, hydrophilic polymers, and alginate-, starch-, cellulose-, or gluten-based encapsulation systems. Each of these types has its specific advantage as well as disadvantage. Emulsions can be constituted to predispose weeds to bioherbicide agents and thereby improve the efficacy and consistency of weed control. Some surfactants, such as Silwet L-77, can facilitate direct entry of small cells (bacterial cells and small spores) into the weeds' tissues. Hydrophilic polymers, as a broad group, include numerous types of natural and synthetic polymers with different levels of water-holding qualities. Encapsulation methods offer possibilities to apply bioherbicides as dry material, to soil, water, and aerial plant surfaces. On the negative side, formulations composed of expensive materials or those that require technical sophistication are bound to increase the cost of bioherbicide products. Moreover, some materials used in these formulations may not be acceptable from a toxicological perspective. Furthermore, the formulation type will also affect the choice of application tools and methods; for example certain emulsions cannot be applied by conventional sprayers used by farmers.

Table 5. List of microbial herbicidal formulations available.

Pathogen	**Target host**	**Formulation/ carrier**	**Reference**
Alternaria destruens	*Cuscuta* sp.	Wettable powder and granules	Simmons, 1998
Chondrsterium purpureum strain HQ1	Regrowth of deciduous trees	Paste containing myecelia	Setliff, 2002

Alternaria eichhorniae	*Eichhornia crassipes*	Oil emulsion	Shabana, 2005
Colletotrichum gloeosporioides	*Senna obtusifolia*	Invert emulsion	Boyette, 2006
Fusarium oxysporum f. sp. *orthoceras*	*Orobanche cumana*	Pesta granules	Muller-stover and Sauerborn, 2007

9. Methods of application

Collego and DeVine are applied with conventional application tools and methods: with land-based or aerial sprayers . Some attempts have been made to apply foliar pathogens through soil or on the surface of soil, as broadcasted pellets or in-row applications (Pitelli *et al.*, 1994), but these methods generally have not been put into practical use. Thus, the preferred method of application is clearly by post-emergent spraying with conventional sprayers. Likewise, most evaluations in the laboratory have used aerosol sprayers, hand-pumped sprayers, and pressurized sprayers propelled by CO^2 or compressed air. The field applicators produce a wide range of droplet sizes and usually are operated at application volumes of less than 250 l ha to 500 l ha.

10. Application rate

Rate of application of microbial herbicides varies with the microbial agent used.rate of application of some of the microbial herbicides is listed in table No. 6.

Table 6. Application rate of some of the microbial herbicides

Microbial agent/ toxin	**Trade name**	**Weed host**	**Rate of application**	**Reference**
Phosphoinothricin	Rubout	Grasses and broadleaved weeds	1 kg/ ha	Ahmad and Malloch, 1995
TMGMV	Solvinix	Tropical soda apple	400 mg/ha	Charudattan and Hiebert, 2007
Phoma macrostoma	Not specified	Dendelion	32 g/ m^2	Bailey *et al.*, 2013

11. Commercial microbial herbicides

Some of the commercially available microbial herbicides are given in table No. 7

Table 7. List of commercially available microbial herbicides.

Pathogen	Weed host	Trade name
Colletotrichum gloeosporioides f. sp. *aeschynomene*	*Aeschynomene virginican*	Collego
C. gloeosporioides	*Hekea sericea*	Hakatak
Chondrostereum purpureum	*Prunus serotina*	Biochon
Colletotrichum gloeosporioides f. sp. *aeschynomene*	*Aeschynomene virginican*	Collego
C. gloeosporioides	*Hekea sericea*	Hakatak
C. gloeosporioides f. sp. *cuscutae*	*Cuscuta* sp.	LuBao
Cylindrobasidium laeve	*Acacia* spp.	Stumpout
Colletotrichum coccodes	*Abutilon theophrasti*	Velgo

12. Manufacturers

Majority of the microbial herbicides are manufactured in USA and Canada.Some of the manufacturers of microbial herbicides are listed below

- A. S. Joshy & Company, Mumbai, India
- Upjohn Company, USA
- Abbott Laboratories, USA
- Gold Biotechnology, USA
- Encore Technology group, USA
- Marrone Bio Innovations, USA

13. Advantages

13.1 Host specificity

Microbial herbicides are specific to the host plant.For this reason we can apply them without harming the main crop.

13.1.1. Host specificity of *Phoma macrostoma* 94- 44B

Bailey *et al* (2011) evaluated *Phoma macrostoma* 94-44B against economically important agricultural, horticultural and ornamental species, as well as target and nontarget weeds. *P. macrostoma* was pathogenic to its host plants like dandelion and dutch clover compared to pink periwinkle, bell pepper, water melon Kentucky blue grass, and gladiolus. Commercial applications for weed management in turfgrass, agriculture, horticulture and forestry seem probable, while domestically management of weeds in lawns, transplanted ornamental and annual flowering species may provide alternative markets.

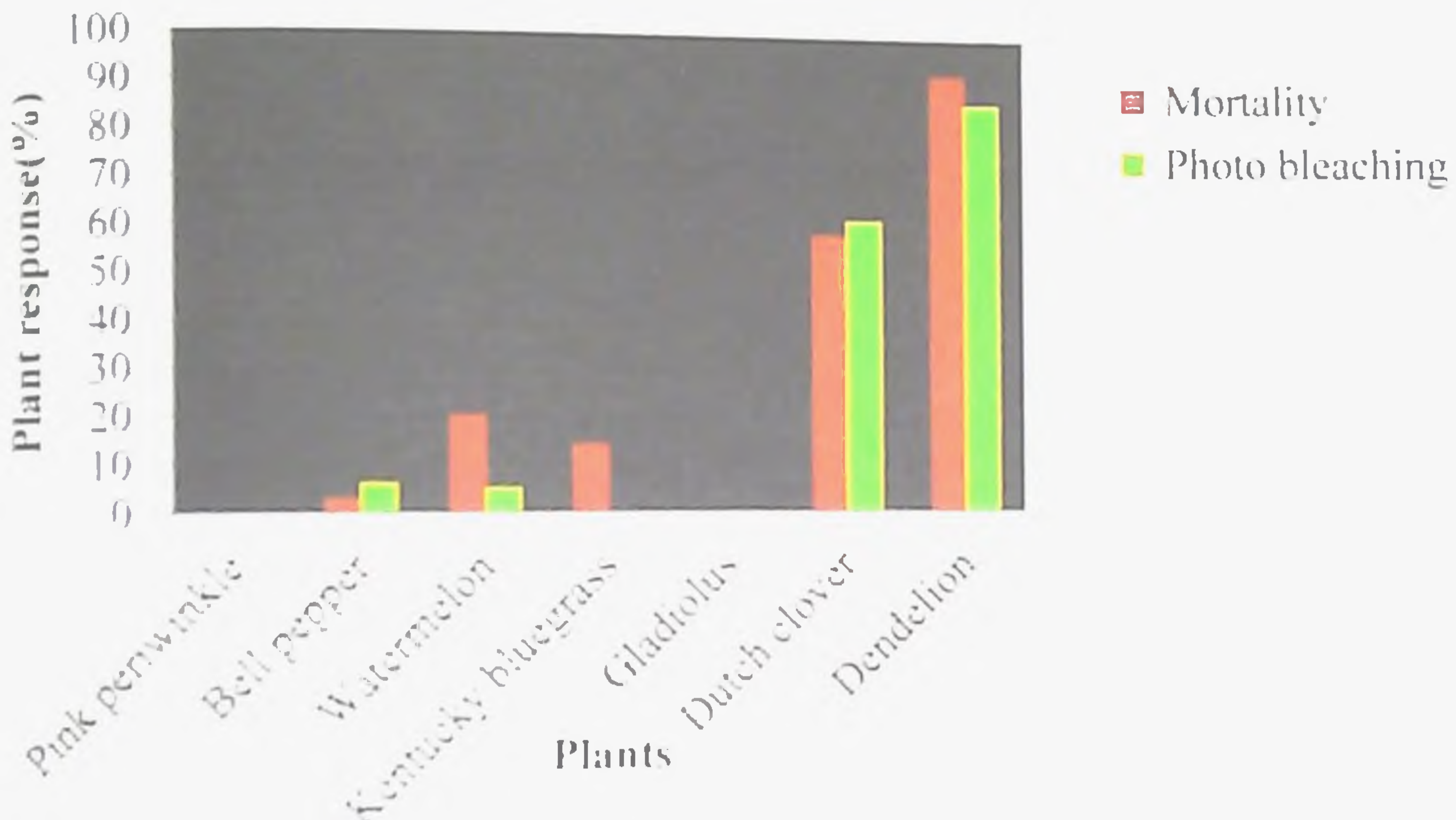

Fig 1. Photo-bleaching (PB), mortality (MT) and resistant or susceptible assessments of ornamental crops, vegetables and weeds to inoculation with *Phoma macrostoma*

13.2 Environmentally safe

Microbial herbicides are safe to the user as well as to the environment. They neither pose any residue problems nor contamination to the water bodies.

13.2.1 Environmental safety studies of *Phoma macrostoma* 94- 44B

Several isolates of the fungus *Phoma macrostoma* demonstrated bioherbicidal activity against dandelion seedlings when applied to soil. Using microbiological and molecular genetic techniques, the ability of these isolates to disperse and persist in soil were determined by Zhou *et al* (2004). PCR primers highly specific to the biocontrol isolates of *P. macrostoma*, were used to detect the isolates at rates of application between 4 and 1000 g/m^2. The biocontrol fungus appeared to have limited mobility in the soil since it was not often detected away from the area where it was placed. It persisted in the soil at detectable levels for up to 4 months, but then its presence declined with time. One year post application, *P. macrostoma* was either not present or significantly reduced in both soil and plant samples depending on the year of sampling. The results suggested that the isolates of *P. macrostoma* used for biological weed control would have minimal environmental impact due to its ubiquitous nature, limited mobility, and weak persistence over seasons.

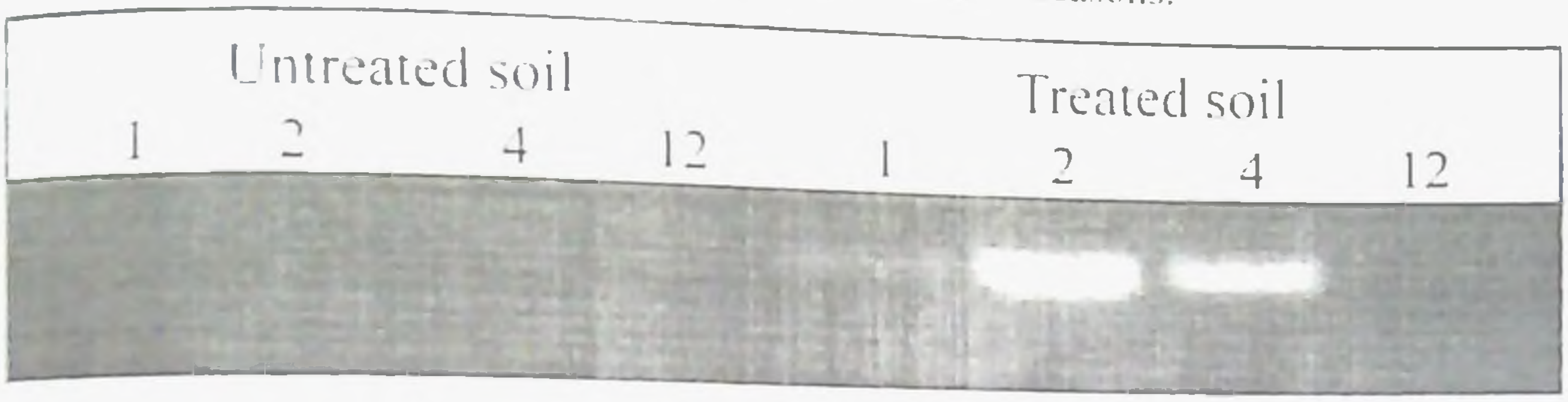

Fig 2. Persistence of *P. macrostoma* 85-24B in field soil after 1, 2, 4, and 12 months after application of inoculum at rates of 0 or 1000 g/m ^{2} (untreated or treated, respectively).

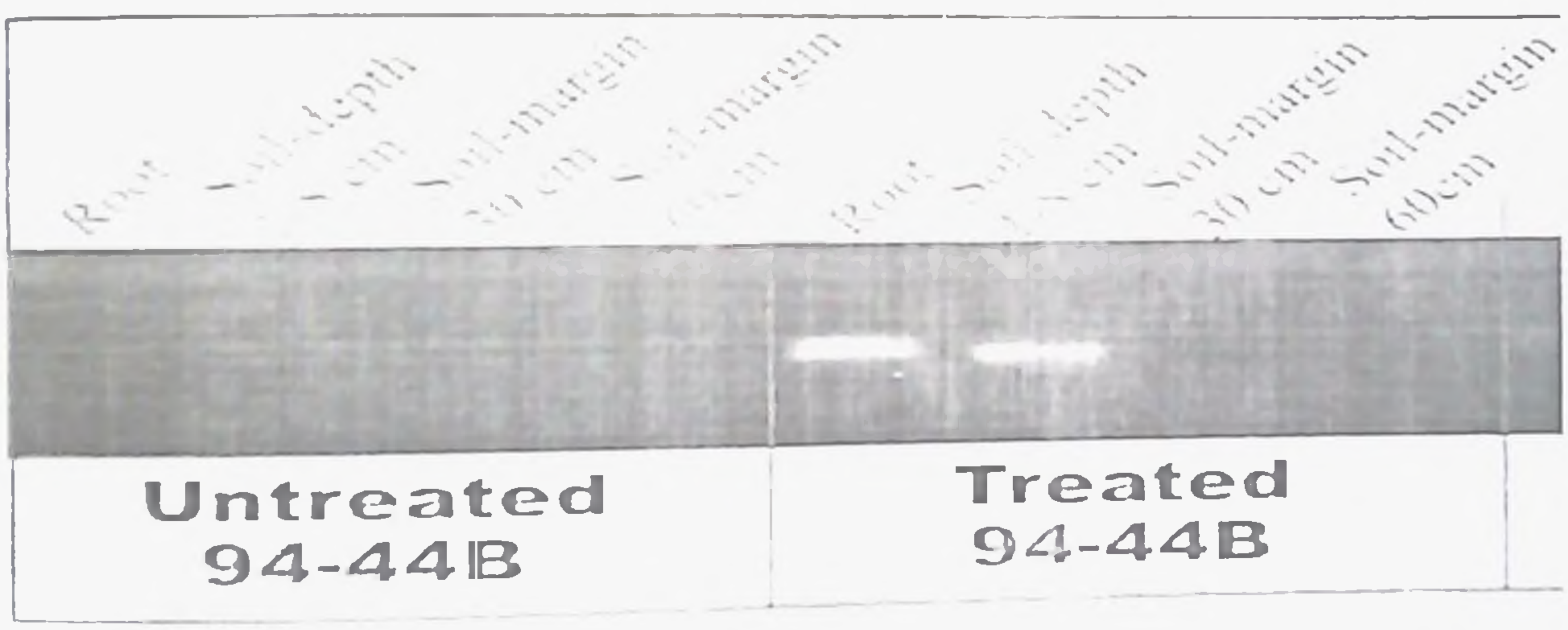

Fig 3. PCR detection of the dispersal of three *P. macrostoma* isolate 94-44B applied at either 0 or 500 g/m ^{2} (untreated or treated, respectively) under field conditions using DNA samples extracted from plant roots, soil from within plots at a depth of 1–8 cm, and soil from outside the plots at a distance of 30 and 60 cm.

13.3 Multiple mode of action

Microbes infects plants by more than one mode of action so that if one mechanism fails the other works successfully thus development of resistance by the weed plants will be less.

13.3.1 Multiple mode of action of *Phoma macrostoma* 94- 44B

Isolates of the fungus *Phoma macrostoma* cause intense photobleaching and mortality of Canada thistle (*Cirsium arvense*), dandelion (*Taraxacum officinale*) and other broadleaved weeds when applied to the soil. In this study, Bailey *et al* (2011) found out that these symptoms are caused by the production of macrocidins which have been extracted from cultured mycelium. The fungus entered the hosts at sites proximal to root hairs where wounding of the cells was most likely, and growing intercellularly towards the root core. In dandelion, the mycelium proliferated around the vascular trachea disrupting the competence of neighboring cells. *P. macrostoma* boasts potential as a bioherbicide to control susceptible broadleaved weeds through more than one mode of action.

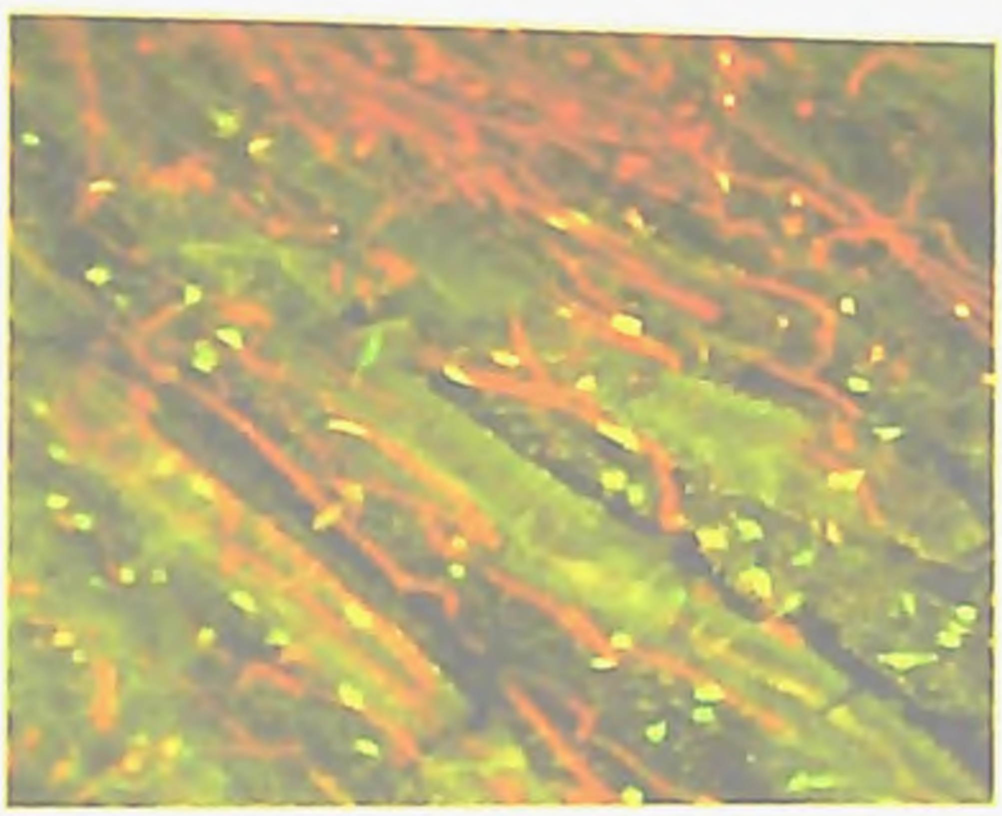

Fig 4. Root colonization of dandelion (green) following inoculation with *Phoma macrostoma* 94-44B (red), dandelion vascular tract (green).

14. Disadvantages

14.1. Host specificity

Most of the pathogens are specific to their host plants. In our cropping system several weeds will be present. So inorder to control each one of them, it is not economic to apply different microbial herbicides. In this aspect, host specificity is a disadvantage.

14.2. Regional specificity

The existing commercial bioherbicides are all used on a relatively small scale (a maximum of few thousand hectares) and on a regional (one or two states or provinces) or local (a few countries) basis. Hence, the market value of bioherbicides is relatively modest compared to chemical herbicides, said to be not more than $200,000 to $500,000 per biocontrol agent per year (anonymous industry sources). It is hoped that some weeds of worldwide importance, such as *Amaranthus* spp., *Cyperus* spp., *Echinochloa crusgalli*, *Portulaca oleracea*, weedy grasses, and others may provide for larger returns and hence generate sufficient economic incentives for development of commercial bioherbicide products (Charudattan, 2001).

14.3. Variable effects of temperature and dew period

One commonly reported disadvantage is the need for continuous moisture availability during the period in which the biocontrol agent infects the plant (Boyette and Hoagland, 2015). It was reported that dew periods of more than 12 hours are commonly necessary for bioherbicide candidates to successfully infect their hosts. The interplay of temperature and humidity also has a significant effect on the success or failure of infection by many pathogens (Ghosheh,2005) and may alter the efficacy of biological control agents (Casella *et al.*,2010). Cold air can retain less total moisture than warm air and thus the relative humidity is more commonly elevated at lower temperatures. Elevated humidity is generally beneficial to successful bioherbicide colonization because it decreases evaporation rates, thus increasing the duration of leaf wetness following inoculant application (Casella *et al.*, 2010).

14.4. Effect of fertilizer-pesticide interaction on infectiousness of weed control agent

It is possible that interactions with fertilizers and pesticides could affect the infectiousness of a candidate biological weed control agent (Boyetchko, 1997). For example, an investigation of the ability of *P. macrostoma* to control dandelions in turf found that co-application with a high rate of nitrogen fertilizer improved its efficacy, whereas co-application with phosphorus had no effect, and potassium sulphate decreased efficacy (Bailey *et al.*, 2013).

14.5. Lack of suitable production methods

Significant challenges were encountered in maintaining product consistency while scaling up production volumes (Boyetchko *et al.*, 2007). In the case of Sarritor (S. minor strain IMI344141) the commercial failure has been attributed to challenges with increasing production volume and product consistency, as well as in consistent efficacy of the product due to the narrow range of environmental conditions in which successful infection will occur (Watson and Bailey, 2013).

14.6. Fertilizers + bioherbicide, *Phoma macrostoma*, to control dandelions

Phoma macrostoma is registered as a bioherbicide in North America to control broadleaved weeds species in turfgrass. A study was conducted by Bailey *et al* (2013) to examine the effect of nitrogen, phosphorus, potassium, lime, and commercial fertilizers with or without applications of the bioherbicide on the reduction of dandelion under greenhouse and field conditions. The bioherbicide provided 70–100% reduction of dandelion. The addition of nitrogen with the bioherbicide, in the form of urea (45-0-0), Scotts Turf Builder Pro (32-0-4 plus 2% Fe), and Scotts Lawn Pro (26-0-3, with no iron), significantly reduced dandelion more than in soil that was not amended with fertilizers in the greenhouse and field locations. Bioherbicide efficacy on dandelion was 10–20% better with these fertilizer treatments. Phosphate (0-46-0), potassium sulphate (0-0-42), and lime had either no effect or did not reduce dandelions under greenhouse conditions. This study showed that *P. macrostoma* retained bioherbicide efficacy on dandelion in conjunction with typical fertility practices and the combination of the bioherbicide with nitrogen fertilizers improved bioherbicide efficacy, especially in low nitrogen soils.

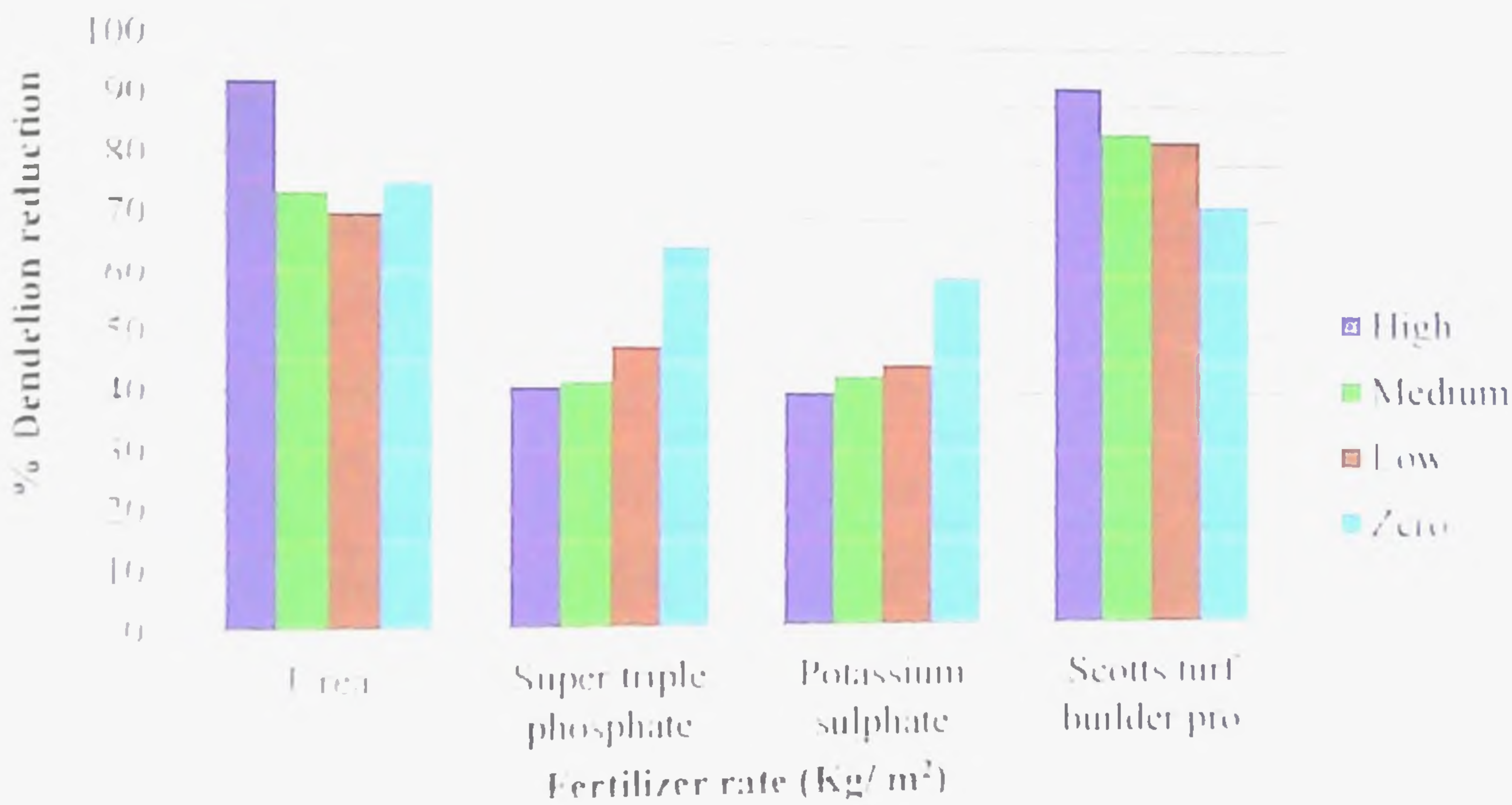

Fig 5. Effect of fertilizer types and levels applied with 4 g/m2 of the bioherbicide Phoma macrostoma on the percent dandelion reduction at three weeks

15. Novel approaches

Many approaches are being tested to improve the spectrum of weeds controlled or to increase the level of control while improving consistency. The first is the attempt to use a mixture of three pathogens to control several weeds. This approach, a 'multiple-pathogen strategy', has promise for further development (Chandramohan and Charudattan, 2003). Other approaches includes the use of chemical herbicides along with microbial herbicides, genetic manipulation of microbes and also the application of metagenomics

15.1 Multiple-Pathogen System

A novel system using four host-specific fungal plant pathogens applied in a single, post emergent spray to control pigweed, sickle pod, and showy crotalaria was tested under greenhouse conditions by Chandramohan and Charudattan (2003). The four pathogens were *Phomopsis amaranthicola* (a pathogen of pigweed species), *Alternaria cassiae* (a pathogen of sicklepod and showy crotalaria), *Colletotrichum dematium* f. sp. *crotalariae* and *Fusarium udum* f. sp. *crotalariae* (pathogens of showy crotalaria). Spore suspensions of each pathogen alone (10^6 spores ml^{-1}) or a mixture of the four pathogens (1:1:1:1, v/v, $2.5/10^5$ spores ml^{-1} of each pathogen, total 10^6 spores ml^{-1}) were tested on four- to six-leaf stage seedlings of the three weed species grown together in pots. One week after inoculation (WAI), all sicklepod and showy crotalaria seedlings were killed, and all pigweed seedlings were killed by 6 WAI when inoculated with their respective pathogen(s) alone or a mixture of the pathogens. None of the weeds inoculated with the root-infecting pathogen *F. udum* developed wilt disease by the time the experiment was completed (6WAI). The results demonstrate the feasibility to control three weeds simultaneously with different fungi without loss of efficacy or alterations in host specificity of each fungus in the given mixture. Scanning electron microscopy showed visual differences in the appearance or germination and further development of conidia of each pathogen on its respective host leaf surface compared to nonhost leaf surfaces, whether the pathogen was applied alone or in a mixture with the other pathogens studied. Application of several host specific fungal pathogens in a bioherbicide mixture as a multi-component bioherbicide system may be advantageous for further development of simultaneous, broad-spectrum weed control.

Fig 6. Scanning electron micrographs of developmental patterns of individual pathogens applied in a pathogen mixture to leaf surfaces.

Row 1: *Alternaria cassiae*. White arrows indicate healthy appressoria and black arrow indicates a lysed appressorium. (A) Tissue maceration on sicklepod (host); (B) formation of appressoria on showy crotalaria (alternative host); (C) lysis of an appressorium on smooth pigweed (nonhost).

Row 2: *Colletotrichum dematium* f.sp. *crotalariae*. White arrow indicates a healthy appressorium and black arrows indicate lysed appressoria. (A) Formation of a normal appressorium on showy crotalaria (host); (B) lysis of an appressorium on sicklepod (nonhost); (C) lysis of an appressorium on smooth pigweed (nonhost).

Row 3: *Phomopsis amaranthicola* . White arrows indicate initiation of spore germination. (A) Spore germination on smooth pigweed (host) leaf; (B) ungerminated, collapsed spores on showy crotalaria (nonhost); (C) absence of spores on sicklepod (nonhost).

Row 4: *Fusarium udum* f. sp. *crotalariae*. White arrows indicate spore germination and germtube formation. (A) Spore germination on showy crotalaria (host); (B) absence of

15.2 Microbial herbicides + chemical herbicides

Synthetic herbicides have been used as tools to synergize microbial herbicides for improved efficacy or management of hard - to- control weeds. Herbicides may weeken weeds and impair their defence systems, thus making weeds more vulnerable to mycoherbicide infection. The fungus *pyricularia setariae* Nisikido is a mycoherbicide for control of green fox tail (*Setaria viridis*). However, its efficacy suffers from insufficient disease severity on young leaves . as a result . green fox tail treated with *P. setaria* alone often recovered from initial injuries due to continuous growth of young leaves. After extensive assessment based on Colby's standard, several herbicides were found synergestic with the fungus. especially some of the graminicides at reduced rates that boosted the virulence of the fungus substanitially.(Peng and Byer, 2005). In this case study, Peng and Wolf (2011) have used a combination of sethoxydym and the fungus *Pyricularia setaria*. Sethoxydym inhibits cell division and is particularly toxic to actively growing young tissues. so a combination of these killed green fox tail completely. This study highlights the potential of synergy in broadening weed targets of mycoherbicides.

Fig 7. Effect of *Pyricularia setaria* on green fox tail in green house conditions. From left to right: untreated control, herbicide alone, fungus alone, and fungus plus herbicide

15.3 Genetic manipulation of microbes

Brooker *et al* (1996) conducted an experiment in which *Colletotrichum gloeosporioides* f. sp. *aeschynomene* was transformed with a gene (bar) for resistance to bialaphos (a natural herbicide) and evaluated for pathogenicity and virulence. A *C.g.aeschynomene* transformant (48-5b) containing the bar gene was stable and resistant to bialaphos up to a concentration of 100 mg/ml. Plants were stem-inoculated for pathogenicity tests and foliar-sprayed for virulence tests with the wild-type or the 48-5b isolate at rates of 0 to 2.5×10^{10} spores/m^2 with bialaphos at 0 and 0.56 kg a.i./ha. The host range of *C.g.aeschynomene* was not altered by the transformation. Disease severity on northern jointvetch (*Aeschynomene virginica*) was similar for treatments with the wild-type and transformant isolates without bialaphos, with 90% or greater disease severity occurring at 2.5 $\times 10^7$ spores/m^2. There was no significant difference in disease severity on northern jointvetch between treatments of the transformed isolate with or without bialaphos. At 2.5 × 10^6 spores/m^2, however, the coapplication of the transformant with bialaphos resulted in a higher level of disease severity than that of the wild-type isolate without bialaphos. Disease severity on northern jointvetch from the treatment of wild-type isolate with bialaphos was significantly less than that of the transformant with bialaphos at spore concentrations of 2.5 × 10^6 to 2.5 × 10^5 spores/m^2. On Indian jointvetch (*Aeschynomene indica*), the coapplication of bialaphos and *C.g.aeschynomene* resulted in a significant synergistic effect (increase in disease severity) with the transformant and an antagonistic effect (decrease in disease severity) with the wild-type. The results demonstrate that at recommended rates of *C.g.aeschynomene* for the control of northern jointvetch, there is no benefit from coapplying the fungus with subleathal levels of bialaphos. However, at spore concentrations lower than that recommended, significantly greater disease development can occur when sublethal levels of bialaphos are applied with bialaphos-resistant isolate. Furthermore, the coapplication of such a resistant isolate with sublethal levels of bialaphos can significantly extend the control range of this fungus toward Indian jointvetch. This suggests that improvements in the virulence and control range of a mycoherbicide may be realized if it were altered to endogenously produce a non-selective phytotoxin such as bialaphos.

Table 8. Disease Development on Northern Jointvetch (*A. virginica*) 35 Days 35 Days after Treatment with Wild-Type (WT) and bar Transformed (T) *C. g. aeschynomene* Isolates with (1) and without (2) Bialaphos at 0.56 kg a.i./ha

Isolates with or without bialaphos (0.56 kg a./ha)	Disease severity (%) on Indian jointvetch (*Aeschynomene indica*) Spores per m^2				
	2.5 × 10^6	2.5 × 10^7	2.5 × 10^8	2.5 × 10^9	2.5 × 10^{10}
Wild type without bialaphos	75	93	90	100	100
Wild type with bialaphos	15	51	82	100	100
Transformant without bialaphos	86	95	100	100	100
Transformant with bialaphos	91	98	100	100	100

15.4 Application of metagenomics

Metagenomics refers to the genomic assemblages of microorganisms isolated directly from their environment, without the need for prior culturing under laboratory conditions (Handelsman, 2004). Thus the term "culture independent" often accompanies descriptions of metagenomic techniques. Despite the high potential of biological weed control, few commercial products are available for use by producers and the general public (Hallett, 2005). A major obstacle is the extensive time involved with isolating microbial strains through an exhaustive screening process. Advances in molecular biology in the past decade have allowed researchers to discover novel plant microbial relationships relevant to weed control (Rector, 2008). The expanding field of metagenomics allows both microbial identity and function to be determined by extracting DNA and RNA directly from the environment, which circumvents the need to isolate and culture microorganisms (Handelsman, 2004). Direct isolation of DNA from the environment increases the diversity of microbial strains recovered. The massive diversity of bacterial and fungal genomes in soil provides a potentially vast pool of genes that code for the production of herbicidal compounds and herbicide resistance. As a consequence, two approaches to weed management can be developed using metagenomic techniques: (1) Isolation of novel herbicides produced by vector hosts expressing the biosynthesis genes, and (2) Identification of herbicide resistance genes in vector hosts exposed to high levels of herbicide.

DNA extraction kits are available for different environmental samples, including soil, water, plant, and stool. DNA extracted from environmental samples is commonly analyzed for phylogenetics or function. After DNA extraction, the sample can be sequenced following PCR, or cloned into a host organism for sequencing or functional analysis. Sequencing provides an analysis of the metagenomes in your environmental sample, whereas function-based cloning reveals the activity of a gene or gene cluster when expressed in a compatible host. Pioneering sequencing work required that environmental DNA fragments were ligated and transformed into *Escherichia coli* cells and then sequenced using Sanger based technology (Rondon *et al.* 2000). Next generation sequencing (NGS) technology, such as Roche 454, Illumina, or Life Technologies SOLiD allows for sequencing without the need for cloning DNA fragments into *E. coli* (Scholz *et al.* 2012). Functional-based screening allows for the discovery of compounds with new modes of action, structure, or genetic sequence. Metagenomic studies have been developed to examine plant pathogens with the goal of finding the genes involved with pathogenicity. The earliest studies in 2000 were on a virulence and xylanase deficient mutant of *Xanthomonas oryzae* pv. *oryzae* and wild-type

Xanthomonas campestris pv. *vesicatoria*. While the study on *X. oryzae* pv. *oryzae* focused on virulence genes (Ray *et al.* 2000), the other study focused on finding the genes conveying hypersensitivity responses in the tomato (*Lycopersicon esculentum*) (Astua-Monge *et al.* 2000). Additional studies have found and used avirulence genes in pathogens such as *Phytophthora infestans* (Whisson *et al.* 2001), *X. oryzae* pv. *pryzae* (Ochiai *et al.* 2001), and *Ustilago hordei* (Linning *et al.* 2004). Other researchers have sought genes encoding secretion proteins important to toxin release (Bell *et al.* 2002). Plant pathogen metagenomic studies have focused on specific, targeted analyses with isolated cultures. A similar, targeted approach with noxious weed species could prove beneficial for herbicide discovery.

16. Conclusion

The wealth of genetic potential of microorganisms on this earth is boundless. There have been many investigations of potential products for weed management. Some have been successful at suppressing weeds in the field and a select few are marketed products that now reduce weed infestations. Further studies are needed to continue to search for additional tools to combat weeds. Increasing our understanding of plant-microbe interactions will assist in this effort. Biocontrol agents need to be specific, competitive and well matched with the weed of interest. The search for biocontrol agents from the environment entails not only finding microorganisms that inhibit a weed, but that are specific for the weed or related plant species and have an economically viable market. Hostrange testing and non-target species testing are needed early in the process. In addition, the development of formulations and delivery systems is necessary to prolong the shelf-life and efficacy of the biocontrol agents in a variety of environments. Biocontrol should not be considered a stand alone option, but may be best if integrated with other methods of control, especially with those that are ecologically sound. Biocontrol agents to reduce or complement chemical herbicides expand options in weed management and tend toward the use of ecologically based systems. They add additional tools in the arsenal of weed management efforts. There is a wealth of genetic potential in the soil and the environment to be explored, screened and tested for weed suppression.

17. Discussion

1. Why solid media more effective for the mass production of fungus?

Ans: The enhanced surface area , lack of particle aggregation,equilibrium of nutrient content and appropriate moisture content provided by the mixture of grains and derrivatives promote fungus sporulation and to produce large amount of conidia.

2. What kind of weeds can be controlled by encapsulated forms?

Ans: It is used to control woody weed species like Parkinsonia found widely across North America.

3. Whether weeds acquire resistant to microbial pathogens?

Ans: No. weeds will never acquire resistance to pathogens.

4. What is meant by Pesta granules?

Ans: They are wheat based formullations.

5. Which microbial herbicides are commercially available in India?

Ans: Most of the commercial microbial herbicides are registered and available in America and Canada.Bialaphos is available in India.

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Department of Agricultural Microbiology

Micro 591: Master's Seminar

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Admission No : 2014-11-184
Major Advisor: Dr. D. Girija

Venue: Seminar Hall
Date: 19.12.2015
Time: 11. 30 am

Microbial herbicides

Abstract

Weeds prove to be a great menace in both crop production and turf grass systems as they are associated with decline in crop yield and quality. It is apparent that as new herbicides are developed, weeds will continue to evolve in response to whatever selective pressure that is applied. For this reason, the continuous development of novel weed control methods is essential for the ongoing maintenance of agricultural yield. Additionally, increasing public concern about the negative effect of pesticide residues has led to the increasing demand for alternative methods for controlling weeds and other pests. Bio-herbicides, especially microbial herbicides, are highlyeffective for weed control and are eco-friendly as well.

Microbial herbicides are living organisms or their products that are useful in suppressing weed populations (Olkowski,1995). The first studied microbial herbicide in China was a culture suspension of *Colletotrichum gloeosporioides* f. sp. *cuscutae* with the name "Luhao no. 1" to control dodder (*Cuscutae australis*) in the 1960's (Gao,1992). Microbial herbicides can be divided into microbial preparation herbicide and microbial-derived herbicide Microbial preparation herbicide is defined as microorganism that can control the weeds. Microbial derived herbicides are secondary metabolites produced by microorganisms which are phytotoxic to weeds (Li *et al.*, 2003).

Most of the research publications available now relate to fungal plant pathogens alone, as bacterial pathogens with weed control activity are scarce. A study conducted at Kerala Agricultural University by Praveena and Naseema (2003) revealed the herbicidal potential of *Fusarium pallidoroseum* isolate 1 and *Myrothecium advena* against water hyacinth. Some of the commercially registered microbial herbicides areCollego®, BioMal®, Stumpout®, Chontrol^{TM}, SolviNix^{TM}, Sarritor *etc.* (Charudattan and Dinoor, 2000).

Despite the promise shown by many microbial herbicides, only a few have achieved long-term commercial success. The possible reasons are narrow spectrum of activity, regional specificity, lack of suitable production methods and altered efficacy due to fluctuations in temperature and humidity (Auld and Morin,1995).

Inorder to increase the efficacy of microbial herbicides, several novel techniques *viz.*, multiple-pathogen system, combined use of microbial herbicides and chemical herbicides,genetic manipulation of microbes and application of metagenomics have been experimented. Metagenomic studies helps to examine plant pathogens with the goal of finding the genes involved with pathogenicity.(Kao- Kniffin *et al.*, 2013)

With intensive research in this field, there is ample scope for the development of new weed control strategies that can be employed to delay herbicide resistance, produce food in accordance with consumer concerns and reduce the environmental impact of modern agriculture and ecosystem management.

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Biofilmed microbial inoculants: A novel technique for survival under adverse environmental conditions

By

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(2014-11-156)

M.Sc. Agricultural Microbiology

Seminar report submitted in partial fulfilment of requirement of the course

MICRO 591: Master's Seminar (0+1)

DEPT. OF AGRICULTURAL MICROBIOLOGY

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DECLARATION

I, Manju Mohan E. (2014-11-156), hereby declare that the seminar report entitled 'Biofilmed microbial inoculants: A novel technique for survival under adverse environmental conditions' has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara

Date: 26-01-2016

Manju Mohan E

2014-11-156

CERTIFICATE

This is to certify that the seminar report entitled '**Biofilmed microbial inoculants: A novel technique for survival under adverse environmental conditions**' has been solely prepared by Manju Mohan E. (2014-11-156), under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

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1. Introduction

Most of our Kerala soils are deteriorated. The indiscriminate use of agrochemicals such as pesticides, herbicides, synthetic fertilizers have resulted in soil organic carbon depletion, physical and biological degradation of soil health and quality. It has become a constraint for improving crop yields and productivity. Considering the consequences of imbalanced use of chemical fertilizers, deployment of microbial inoculants as biofertilizers in crop improvement is a promising alternative. But, the poor survival and establishment of single strain biofertilizers in the field conditions often limits their widespread utilization in Integrated Nutrient Management strategies. So, the utmost importance has to be given to increase their survivability in the change in the environmental conditions. A research in Sri Lanka has found a solution for the survivability problems of single strain biofertilizers through biofilms. Development of biofilmed microbial inoculants of agriculturally important microorganisms (enclosed in a mucilaginous matrix) to overcome the survivability problems can be a new generation biofertilizers.

2. Biofilm

A Biofilm is a complex aggregation of microorganisms characterized by the excretion of a protective adhesive matrix. The cells living in a biofilm are embedded in this polymeric substance, which permits them to adhere and colonize the surfaces of different materials (Flemming, 2002). A biofilm consists of two components: microbial cells and Extracellular Polymeric Substances (EPS). The EPS are biopolymers produced by the microbial cells involved in the biofilm production. It provides structure and stability to the microbial cells. It will also act as a protective covering for the living microbial cells involved in the biofilm formation from the adverse environmental conditions such as extreme pH, high temperature, UV radiation, osmolarity etc.

In soil, mainly there are three types of biofilm: bacterial, fungal and fungal-bacterial biofilms. A bacterial biofilm is formed by the colonization of bacteria on root surface or any abiotic surface such as soil particles. Fungal biofilm is formed by the colonization of fungal mycelium on any abiotic surface such as soil particles or on any root surface. The fungal-bacterial biofilm is formed by the colonization of bacteria on any fungal mycelia. Here, the fungal mycelium will act as the biotic surface for the growth of bacteria.

3. Biofilm formation

Formation of biofilm occurs through different steps (Swarnalakshmi *et al.*, 2013).

3. 1. Surface conditioning

Organic molecules are absorbed on an inert surface and form a conditioning layer. The adsorbed organic molecules serve as a nutrient source for the microorganisms.

3. 2. Adhesion

The primary colonizers are attached with conditioned surface through electrostatic attraction and physical forces. Adherence of partner organisms to the surface is mediated through adsorption, desorption and irreversible adsorption.

3. 3. Slime formation

Microorganisms in biofilm excrete extracellular polymers (glycocalyx) that hold the biofilm together and cement it to the surface.

3. 4. Secondary colonizers

Other types of microbial cells attach to the extracellular polymers. The secondary colonizers metabolize wastes from the primary colonizers.

3. 5. Functional biofilm

The mature, functional biofilm is a complex, metabolically cooperative consortium comprising different species living in a customized microniche. Individual organisms have less function compared with communities which survive under multiple niches.

4. Applications of biofilm

The positive behavior of biofilm can be effectively utilized in different areas such as crop improvement, waste water treatment and bioremediation. Biofilmed microbial inoculants can be effectively utilized as a best solution for the problems due to the single strain biofertilizers. In waste water treatment, biofilm can be used as a biological filter and the treated water will be free of organic matter and other waste materials. Bioremediation is an emerging technology for clean-up of environmental pollutants using microorganisms. The potential of biofilm in the process of bioremediation have identified recently.

5. Importance of biofilm in crop improvement

Since the biofilm protects the microorganisms present in the biofilm formation, it increases the survivability of beneficial microflora in the soil under the adverse environmental conditions. The extracellular polymeric substances produced by the microorganism cements the soil particles together, enhance the soil aggregation and maintains a good soil structure (Amellal *et al.*, 1998); thereby help plants to avoid water stress.

6. Applications of biofilm in crop improvement

Biofilm in crop improvement can be applied as biofilm based biofertilizers, biofilm based biocontrol agents and biofilm based plant growth promoting rhizobacteria.

6. 1. Biofilm based biofertilizers

For the successful development of biofilm based biofertilizers, many studies have been conducted. To know the effect of *Bradyrhizobium–Penicillium* spp. based biofilm on the nutrient availability in the soil was studied by Jayasinghearachchi and Seneviratne (2005).

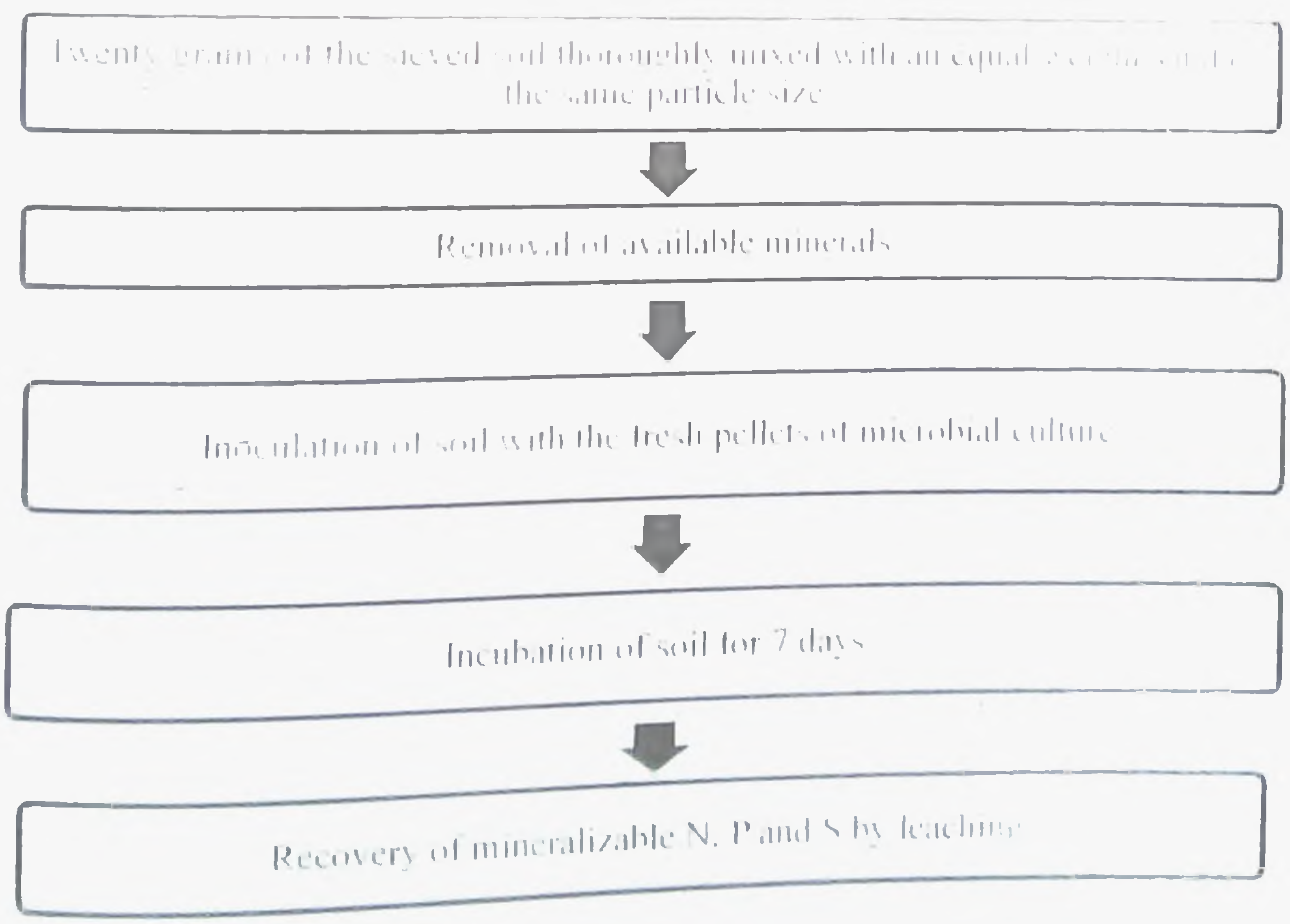


Figure 1: Procedure of experiment

The experiment was done with the method described earlier by Stanford and Smith (1972) with some modifications. To incubate the soil fifty milliliters leaching tubes were used. Twenty grams of the sieved soil (<2 mm) was thoroughly mixed with an equal weight of washed, oven-dried and sieved sand of the same particle size. Then the mixture was moistened using a fine spray of distilled water to prevent particle size segregation during transfer to the leaching tubes. The soil mixture was retained in the leaching tube by means of a glass wool pad fixed at the neck of the tube. A thin glass wool pad (about 5 mm) was placed over the soil in the tube to avoid dispersion of the soil when solution was poured into the tube. Minerals, initially available in the soil were removed by leaching with 200 mL of distilled water in 10 mL aliquots, by applying suction. Excess water was removed under vacuum. Then, 0.2 g of fresh pellet of each microbial culture variant dispersed in 1 mL of autoclaved distilled water was introduced to the soil in each tube. Six leaching tubes were maintained for each treatment. Then the tubes were incubated in the dark at 30 °C for 1 month. After the incubation, mineralizable N, P and S were recovered by leaching with 100 mL of 0.01 M $CaCl_2$ and leachates were analyzed for NH_4^+, NO_3^-, PO_4^{3-} and SO_4^{2-} by colorimetric and turbidimetric methods using a spectrophotometer (Anderson and Ingram, 1993).

Table 1: Mineral nitrogen, phosphorus and sulphur concentrations in treated and untreated soils after incubation

Treatments	NH_4^+ (µg g^{-1} soil)	NO_3^- (µg g^{-1} soil)	PO_4^{3-} (µg g^{-1} soil)	SO_4^{2-} (µg g^{-1} soil)
Control soil	9.20	31.19	1.047	5.05
Bradyrhizobium sp. alone	31.45	12.89	0.623	6.96
Penicillium spp. alone	25.17	23.01	0.666	6.10
Bradyrhizobium sp. + *Penicillium* spp. (biofilm)	36.35	103.29	9.18	4.19
MSD (0.05)	2.039	2.443	0.242	0.732
CV (%)	1.80	2.93	1.93	7.90

Availabilities of NH_4^+, NO_3^- and PO_4^{3-} in the soil were significantly higher with the *Bradyrhizobium* sp.-*Penicillium* spp. based biofilm than with the other microbial culture variants at the end of the incubation (Table. 1). However, the biofilm lowered soil SO_4^{2-} concentration compared to other treatments. The *Bradyrhizobium* sp. alone increased NH_4^+, NO_3^- and SO_4^{2-} concentrations whereas it lowered PO_4^{3-} concentration compared to the control soil. The *Penicillium* spp. alone enhanced the availabilities of $NH_4^+$and SO_4^{2-}in the soil, but it decreased $NO_3^-$and PO_4^{3-}concentrations.

To know the effect of *Rhizobium* based biofilm (Fungal-*Rhizobium* biofilm) in the nitrogen fixation of non-leguminous plants a study was conducted in Sri Lanka. The root system of wheat was inoculated with both *Rhizobium* monoculture and a fungal-*Rhizobium* biofilm.

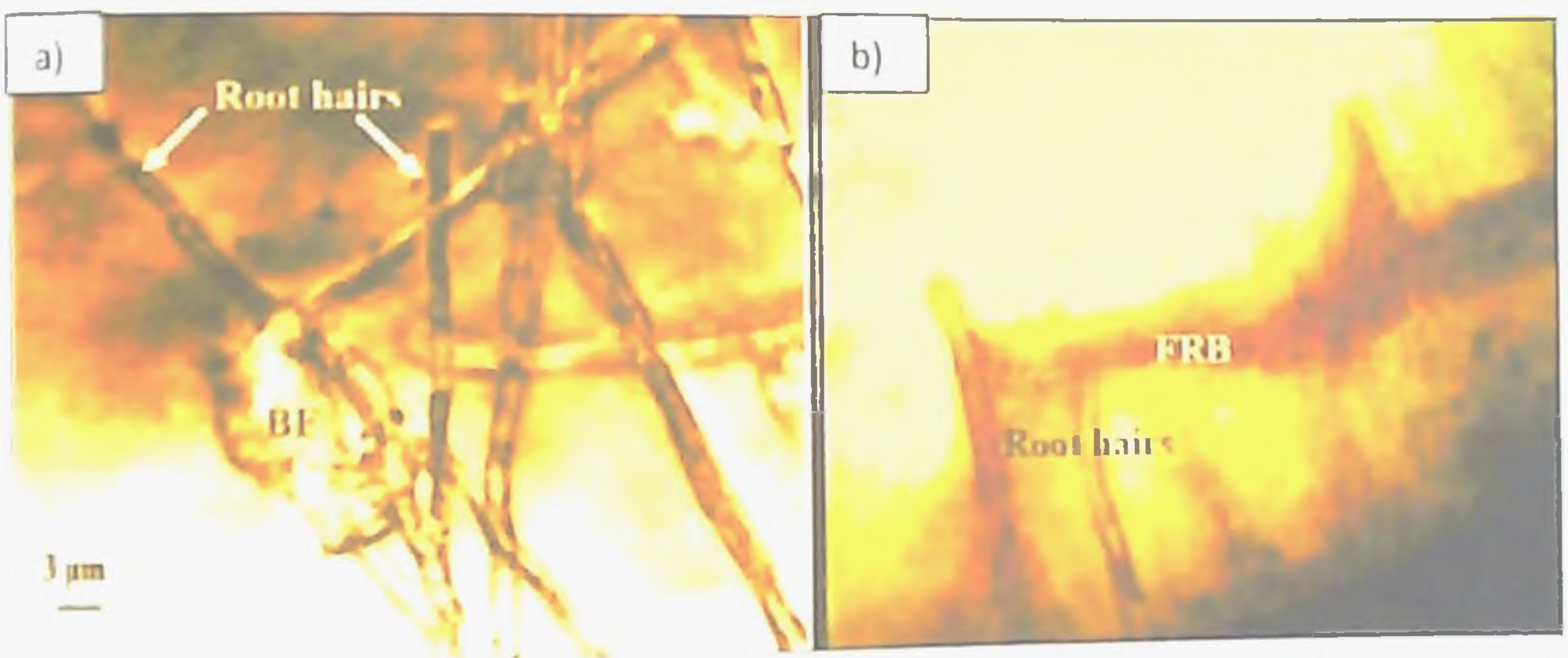


Plate 1: Rhizobial biofilm formation on non-leguminous plants: a) Rhizobial biofilms (BF) developed on root hairs of wheat inoculated with rhizobial monocultures; b) After inoculation of Fungal- *Rhizobium* Biofilms (FRB)

The root system of wheat which was inoculated with the Fungal-*Rhizobium* biofilm maintained a higher cell density of rhizobia as compared to monoculture of *Rhizobium* The Fungal-*Rhizobium* biofilm acted as nodule like structure or 'pseudonodules' for nitrogen fixation in non-leguminous plants (Jayasinghearachchi and Seneviratne, 2004).

Herath *et al.* (2015) studied the fungal bacterial biofilm under *in vitro* conditions by using *Acremonium* sp. as fungus and nitrogen fixers such as *Rhizobium*, *Bradyrhizhobium*, *Azotobacter* and *Acetobacter* as bacteria. During this study, the different maturity stages of biofilm formation were observed under microscope (Plate 2). Simultaneously, nitrogenase activity of the developed fungal-bacterial biofilm was measured and found to increase with the maturation stage of fungal-bacterial biofilm (Figure 2).

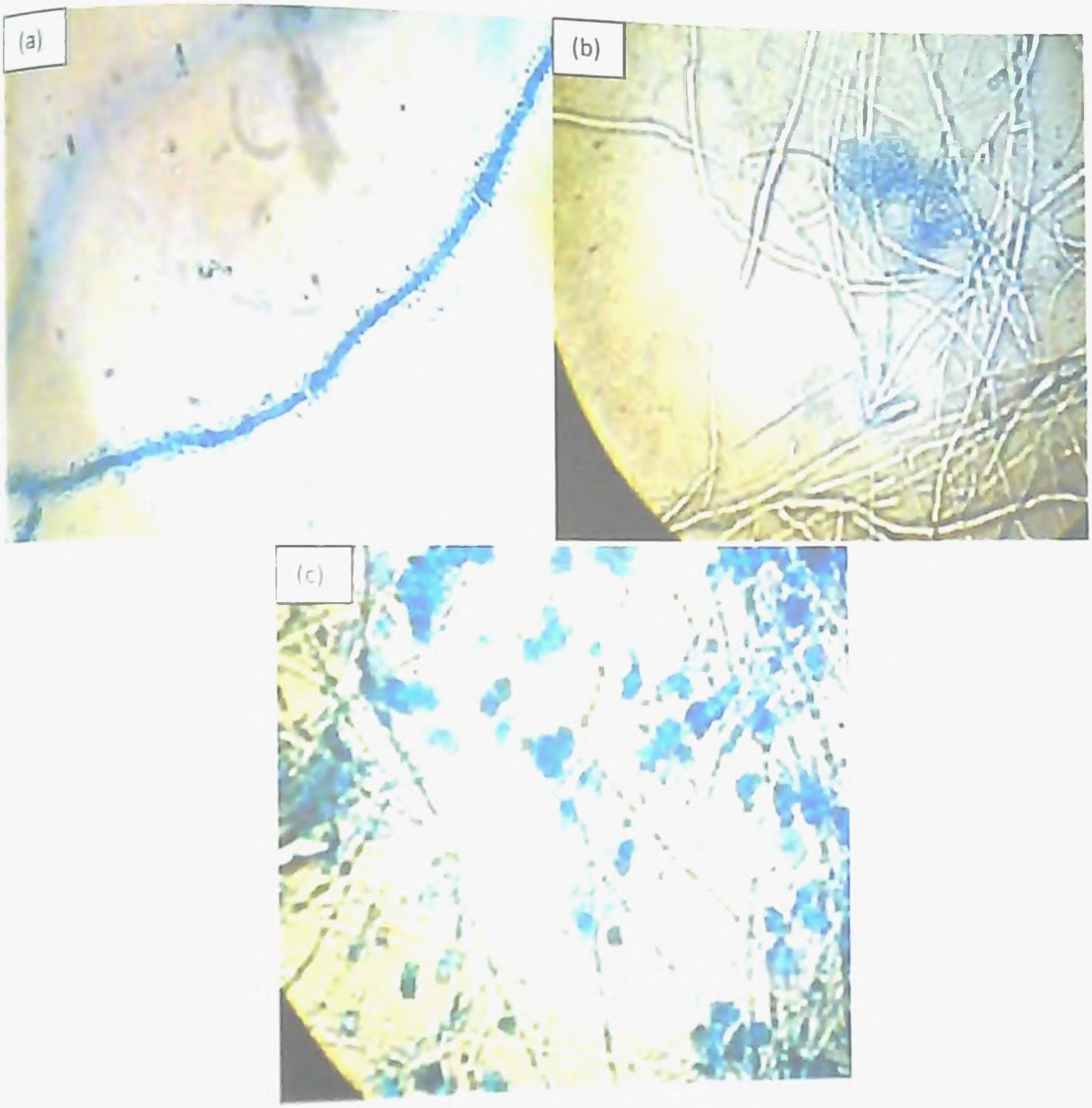
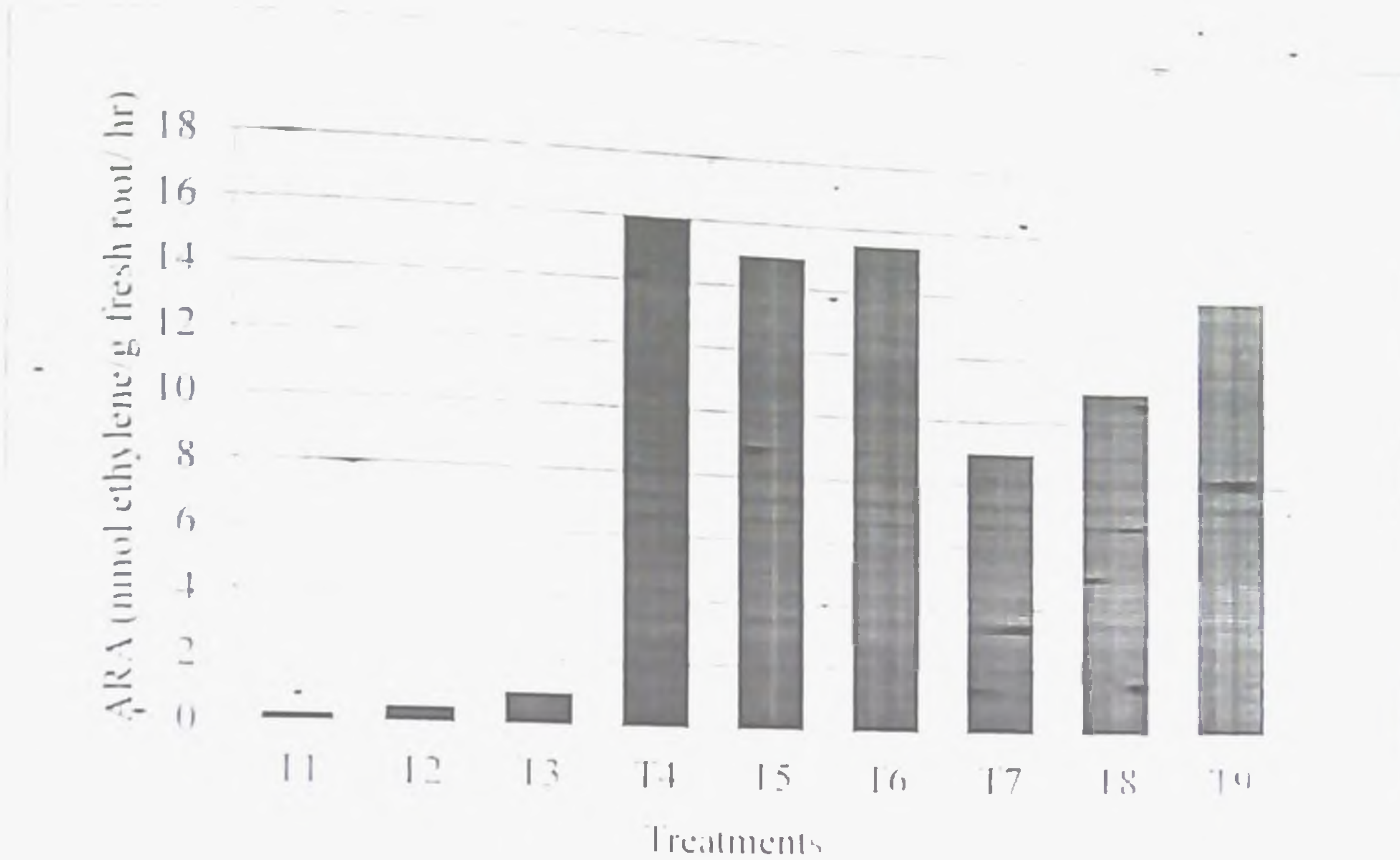


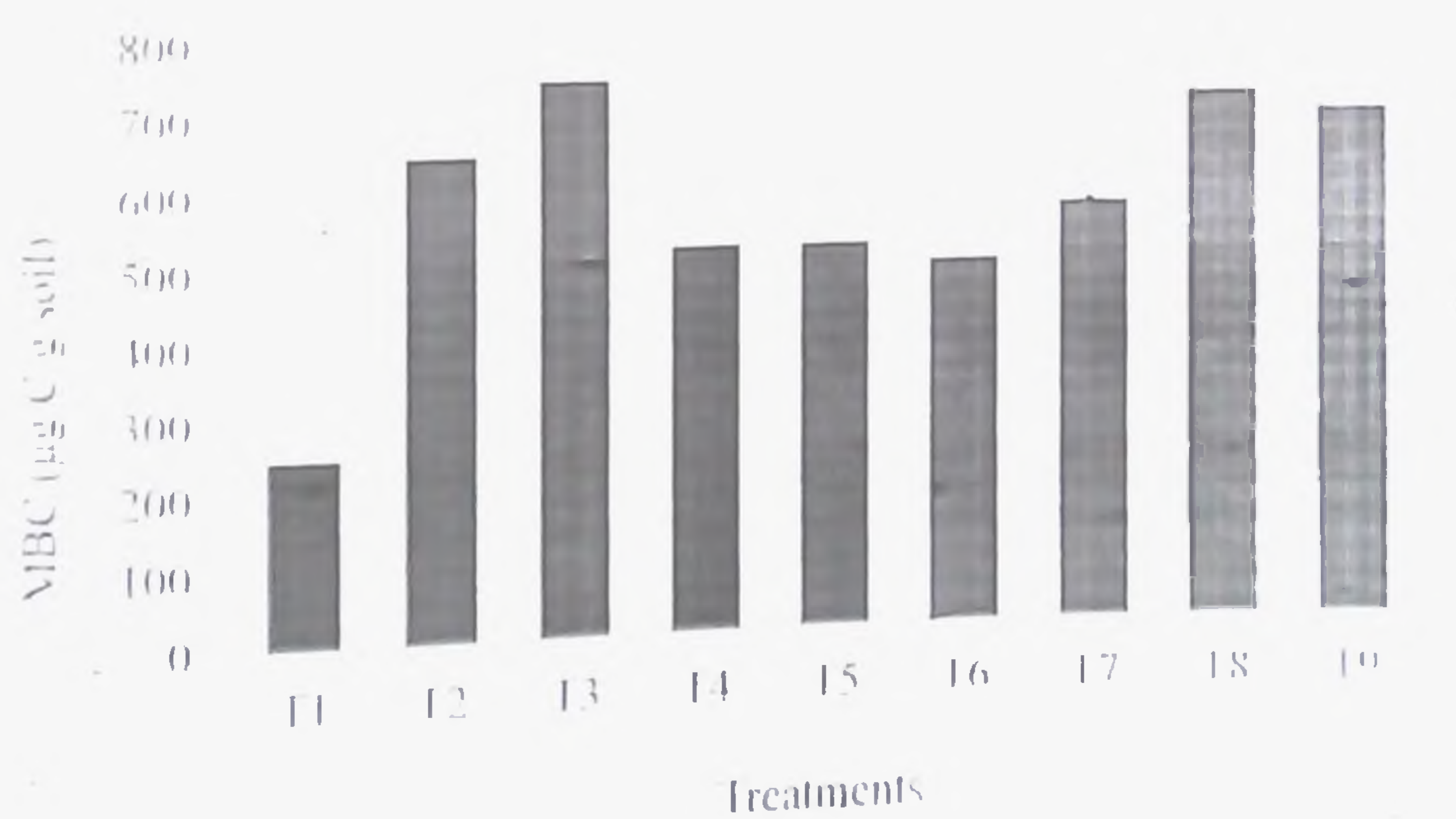
Plate 2: *Acremonium* sp. mycelial colonization by N_2-fixing bacteria forming fungal-bacterial biofilms, when developed under *in vitro* conditions. (a) Initial colonization of bacteria of the fungal mycelium, (b) a cell cluster formed by multiplication of the colonized bacteria, and (c) matured cell clusters on the mycelium. Darkness is due to cotton blue stain absorbed by exopolysaccharides produced by the biofilms.

Figure 2. Change in nitrogenase activity during fungal mycelial colonization of bacteria and biofilm formation at three stages of maturation, described in Plate 2.

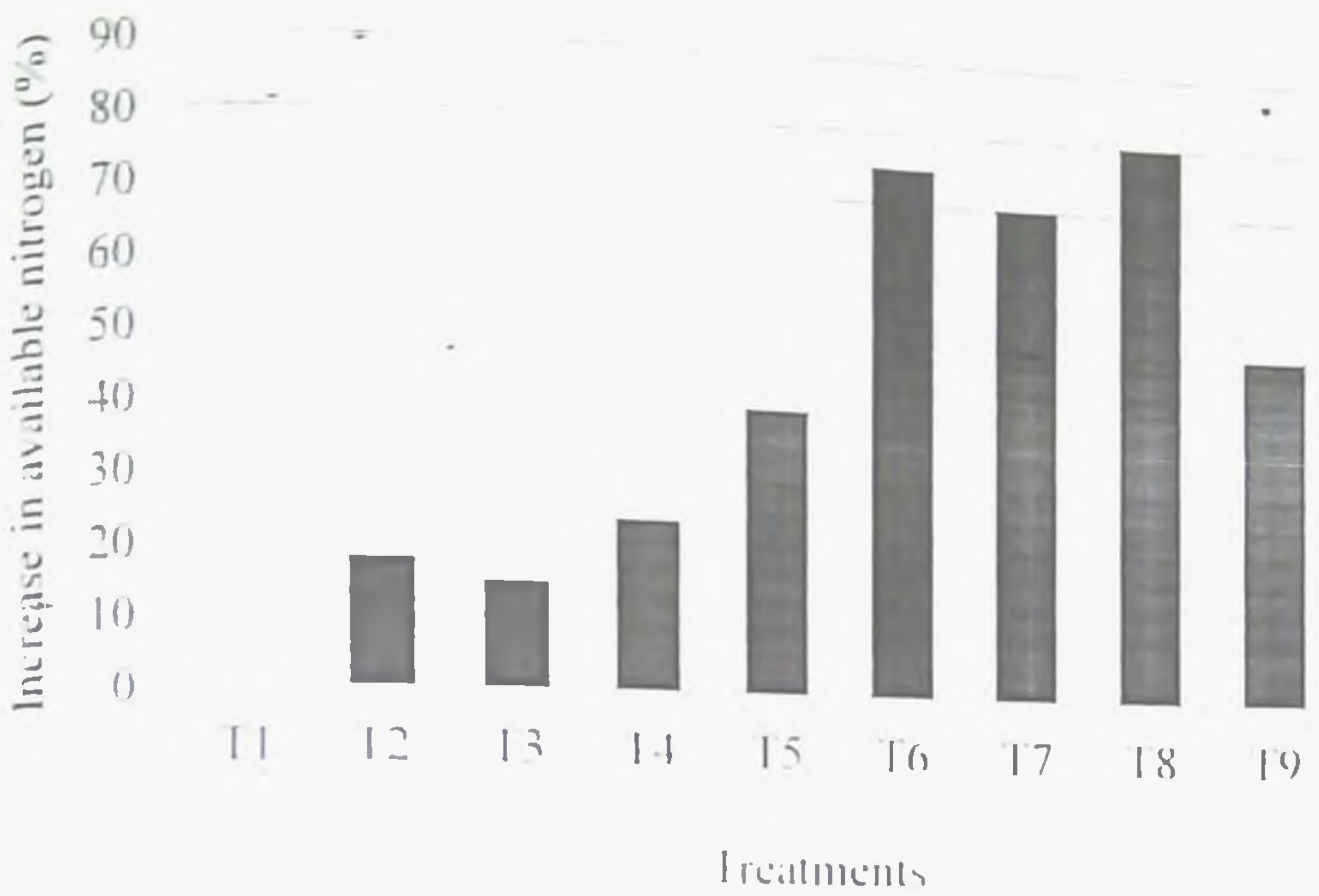
A study was conducted by Prasanna *et al.* (2014), to evaluate the potential of the most promising *Trichoderma* and cyanobacteria based biofilms and selected cyanobacteria as biofertilizer options in leguminous crops in terms of plant growth promotion and enhancing soil fertility. The treatments were T₁ control; T₂ *T. viride–B. subtilis* biofilm; T₃ *T. viride–P. fluorescens* biofilm; T₄ *T. viride–A. chroococcum* biofilm; T₅ *T. viride–Bradyrhizobium* biofilm; T₆ *Calothrix* sp.; T₇ *Anabaena* [illegible] T₈ [illegible] *Bradyrhizobium* biofilm and T₉ *Anabaena–A. chroococcum* biofilm. All treatments were taken in triplicate (which is generally the routine in all experiments) [illegible] were coated with [illegible] formulation such that the cfu per seed was 10^8–10 [illegible] (bacteria) and 10 [illegible] (*Trichoderma*) using carboxy methyl cellulose (1% CMC) as the sticking agent and air dried in shade for 1 h before sowing. Only carrier without any inoculum (T₁) was used as the control. Sowing was done on 2 August 2012. The experiment was undertaken up to the harvest stage (135 days), and midterm sampling was done after 40 days.



3. a. Acetylene reduction activity (ARA) as an index of nitrogen fixation of mungbean plants at mid crop stage



3. b. Microbial biomass carbon of soil samples (MBC) at the midstage of mung bean crop



3. c. Per cent increase in available N in the inoculated treatments (over control)

Figure 3: Effect of biofilmed microbial inoculants on mung bean.

The nitrogenase activity, measured as ARA (ARA of the root nodules), ranged from 15.67 to 0.03 nmoles of ethylene g^{-1} fresh wt. roots h^{-1} (Figure 3.a). The highest values were recorded in T[illegible] followed by T[illegible] and T[illegible]. The microbial biom[illegible] [illegible]ent was statistically at par in T[illegible] and T[illegible] (Figure 3.b). In terms of available N, T[illegible] and T[illegible] recorded 80% increase over control, followed by 72% and 67.5% increase in T[illegible] and T[illegible] treatments (Figure 3.c).

6. 2. **Biofilm based biocontrol agents**

Biofilmed microbial inoculants can be an effective tool for biological control of diseases. In order to evaluate the biocontrol potential of the different biofilms and individual biofilm partners on collar rot of cotton plants, a pot experiment was set up by Triveni *et al.* (2015) under the controlled conditions in the National Phytotron Facility, IARI, New Delhi.

The pots (14 inches diameter) were filled with 12 kg of soil with sandy clay loam texture; pH 7.5; organic C 0.42%; available N 156.8 kg ha^{-1}; available P 18.8 kg ha^{-1}; available K 616 kg ha^{-1}. The soil was pre-inoculated with 15 g of *Macrophomina phaseolina* colonised sorghum seeds (containing 5 × 104 microsclerotia kg $soil^{-1}$). The recommended chemical control treatments were the application of Vitavax

(DhanukaAgritech Ltd: contains Carboxin 75% wettable powder /WP) at a rate of 3 g kg^{-1} of seed. as soil drench. *Trichoderma* formulation available commercially (1%. Indore Biotech Inputs and Research Pvt. Ltd. Indore. India) at a rate of 4 g WP kg^{-1} of seed was applied as the recommended biological control agent. The rates of application of fertilizers were 120:40:40 NPK kg ha^{-1} in all pots except. in the treatments involving biofilms. inclusive of *A. chroococcum* or *Anabaena torulosa.*

In these pots. only 75% of the recommended N application (i.e. 90 N kg ha^{-1}) was given along with full dose of P and K fertilizers. as *A. chroococcum* and *Anabaena torulosa* are nitrogen fixers. The other treatments were all given 100% dose of all fertilizers. Sufficient humidity conditions required for efficient disease establishment and development of fungal mycelia were maintained.

The pots were inoculated. The treatments were T_1 :*M. phaseolina*. T_2 :*T. viride*. T_3 :*P. fluorescens*. T_4 :*B. subtilis*. T_5 :*A. chroococcum*. T_6:*T. viride* + *P. fluorescens* dual. T_7: *T. viride* + *B. subtilis* dual. T_8: *T. viride* + *A. chroococcum* dual. T_9: *T. viride*-*P. fluorescens* biofilm. T_{10} :*T. viride*- *B. subtilis* biofilm. T_{11}:*T. viride* - *A. chroococcum* biofilm. T_{12}:*Anabaena* sp. - *B. subtilis* biofilm. T_{13}:*Anabaena* sp. - *T. viride* biofilm. T_{14}: Chemical control. T_{15} and T_{16} served as biological control (*Trichoderma* formulation) and absolute control (no inoculation with *Macrophomina* or bacterial cyanobacterial biofilms). respectively. The cotton variety used is Pusa 86.

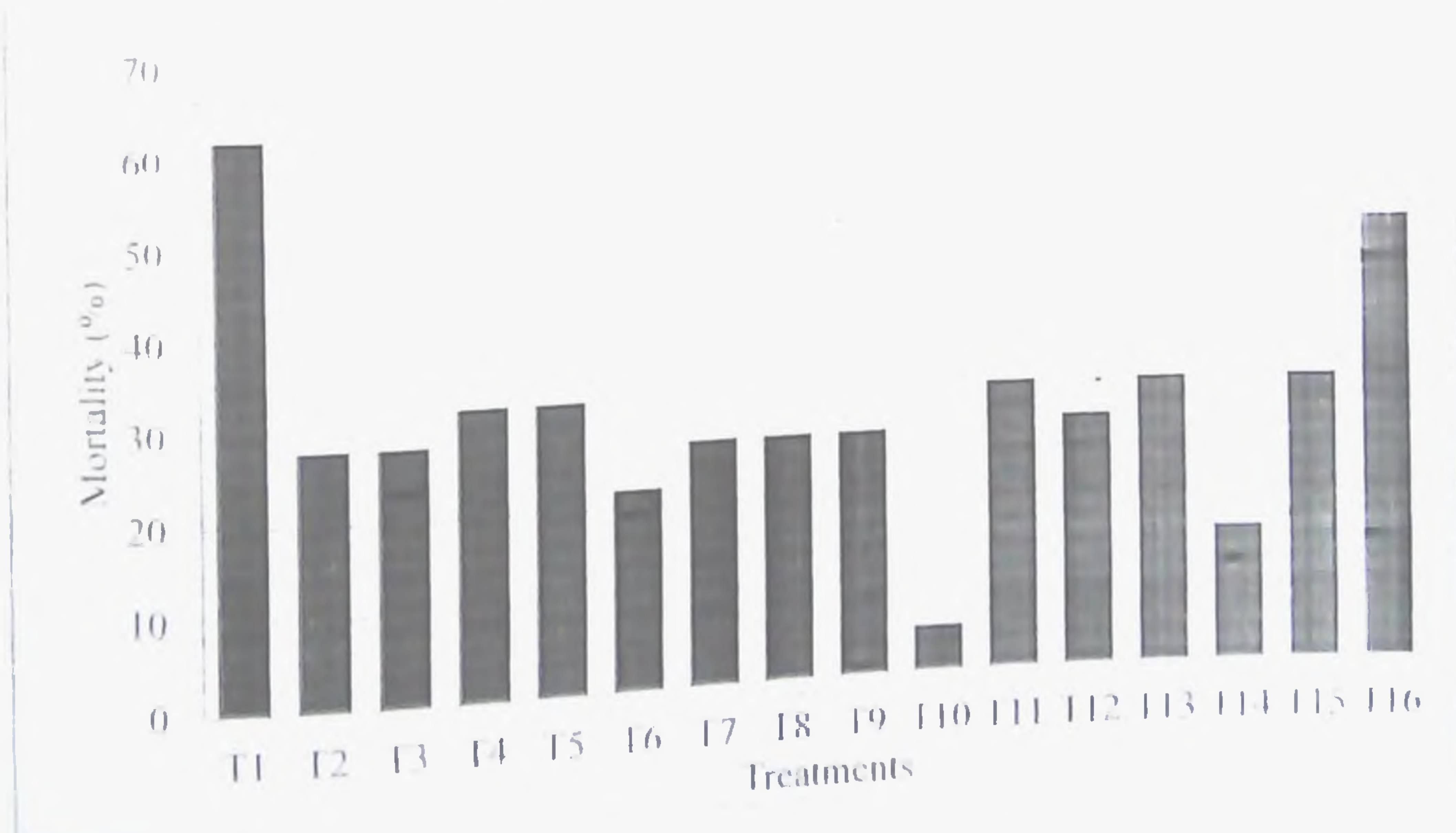


Figure 4: Effect of biofilmed biofertilizers on mortality of cotton seedlings

In order to ascertain the biocontrol ability of the biofilms, mortality was taken as an index (Figure 4). The lowest mortality (5.67%) was observed in *T. viride–B. subtilis* biofilm, followed by the chemical control treatment, which showed 16.67% mortality. The highest mortality was observed in the treatment involving only *Macrophomina phaseolina* (61.17%) treated pots, followed by absolute control (50%).

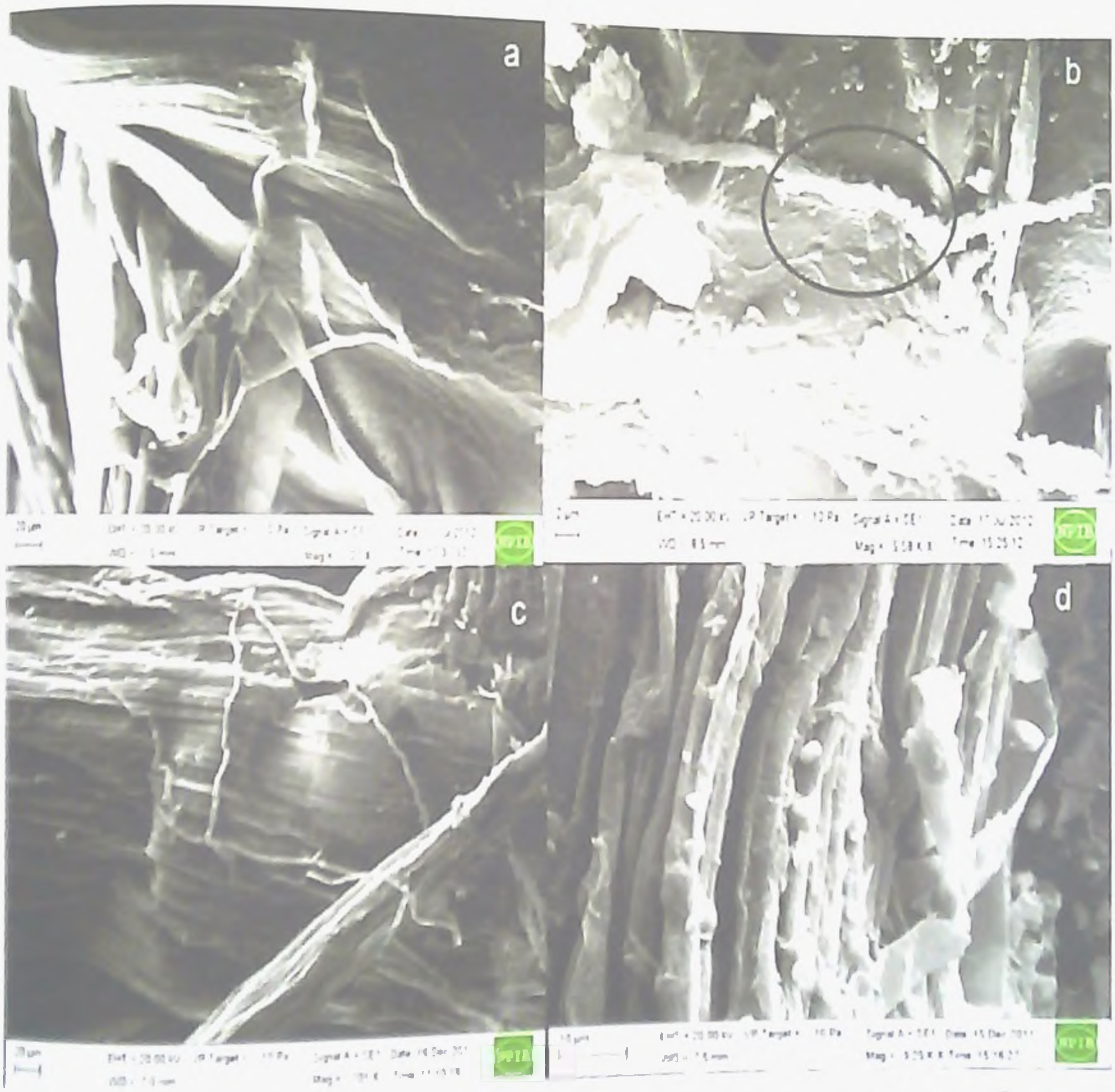


Plate 3: Scanning electron micrographs of cotton plants showing colonisation of roots mycelia/filaments and mat-like biofilms (left–right). (a) Healthy root tissues; (b) Roots colonised by *Trichoderma-Bacillus* biofilms; (c) *Anabaena-Trichoderma* biofilms entangled with root tissues; (d) Cyanobacterial filaments with attached bacteria (*Anabaena-Bacillus* biofilms) covering the root tissues. Black circle indicates biofilm-like growth on the roots.

SEM of root samples revealed colonisation of the inoculated organisms (Plate 3), resembling a network like growth comprising fungal cyanobacterial filaments along with bacterial colonies.

To increase the endophytic colonization of *Pseudomonas fluorescens* in tomato, a study has been conducted by Jayasinghearachchi and Seneviratne (2006).

Table 2: Endophytic populations of *Pseudomonas fluorescens* in leaf, shoot and root tissues of 21 days old tomato plants treated with *P. fluorescens-Pleurotus ostreatus* biofilmed inoculum and an inoculum of *P. fluorescens* alone.

Microbial treatment		Population of *P.fluorescens* Log10 (CFU/g fresh weight)
Pseudomonas fluorescens alone	Leaf	5.01
	Shoot	3.25
	Root	5.37
P. fluorescens - Pleurotus ostreatus biofilm	Leaf	5.87
	Shoot	3.77
	Root	6.45
F value		110
MSD (0.05)		0.30
CV (%)		[illegible]

***p < 0.001, MSD: Minimum Significant Difference, CV: Coefficient of Variation

Significantly high endophytic populations of *P. fluorescens* was recorded in the leaf, shoot and root when the plants were grown with biofilm inoculum compared to the plants grown with a planktonic inoculum (Table 2). A significantly higher root endophytic population of *P. fluorescens* was recorded as compared to that of leaf and shoot (p < 0.001). Shoot showed the least bacterial colonization. No negative effects on plant growth were observed due to the inoculation of *Pleurotus ostreatus*.

6. 3. Biofilm based plant growth promoting rhizobacteria

Biofilm based microbial inoculants has an important role in the enhancement of plant growth promotion activities. To know the role of biofilm based microbial inoculants in the plant growth promotion, several studies have been conducted.

Hettiarachchi *et al.* (2014), studied the effectiveness of biofilmed microbial inoculants on enhancement of root growth of rubber seedlings.

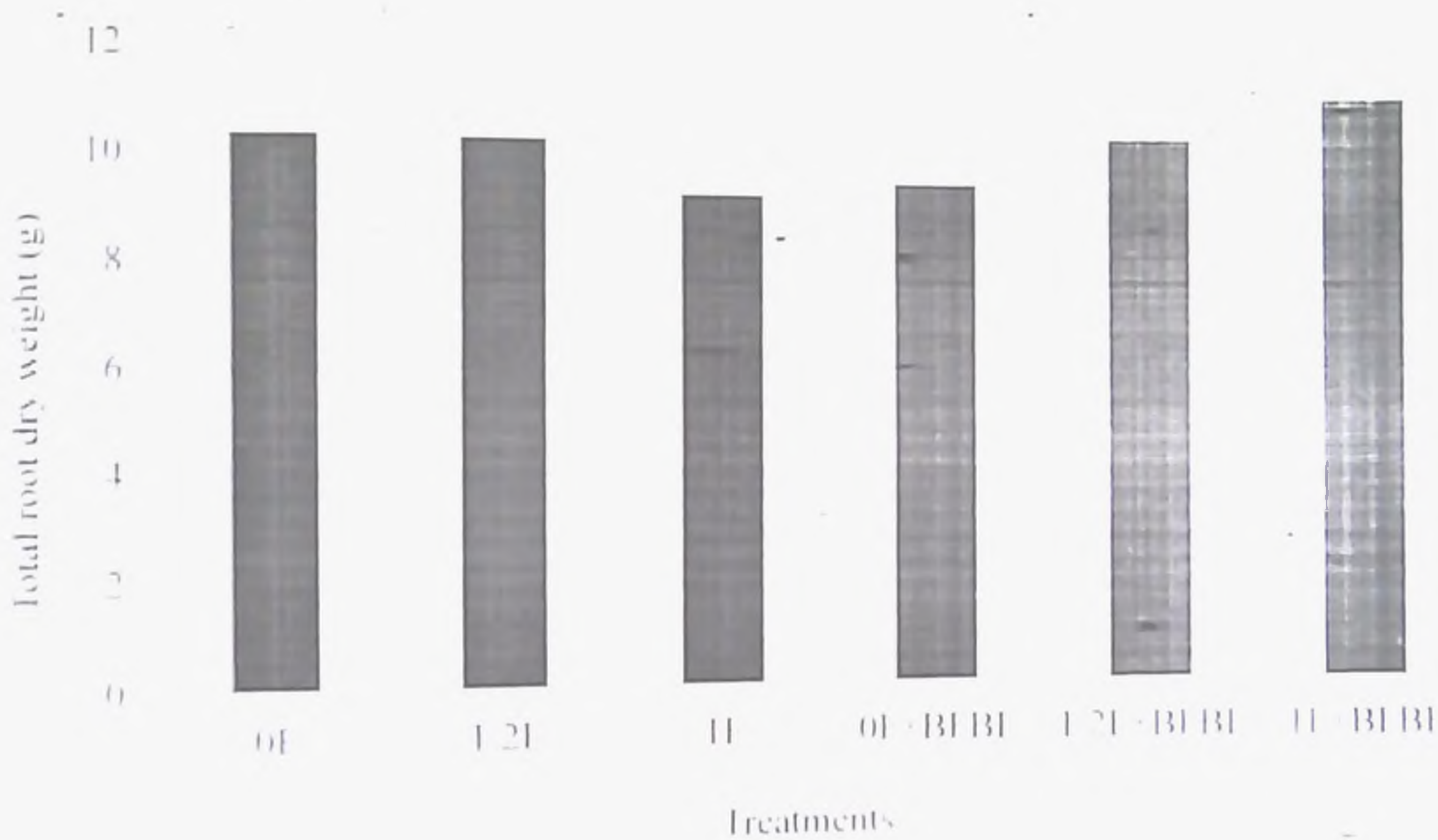


Figure 5: Effect of different fertilizer applications on total root dry matter of rubber plants

Total root dry matter production of the plants was measured at the end of the experiment is given in Figure 5. Considering zero (0F), half (½F) and full (1F) recommended inorganic fertilizer only application treatments (T_1, T_2, T_3 respectively) fertilizer application was inversely correlated with root growth. Biofilm biofertilizers only treatment (0F+BFBF-T4) gave slightly higher root growth than the recommended fertilizer level (T_3). Combine use of BFBFs with inorganic fertilizer from half (T_5) to full (T_6) of the recommended fertilizer level treatments greatly influenced root growth than their non BFBFs application treatments (T_2 and T_3). The treatments having combined use of half recommended inorganic fertilizer with BFBFs (T_5) and full recommended inorganic fertilizer with BFBFs (T_6) gave significantly higher root growth compared to full recommended inorganic fertilizer treatment (T_3).

To evaluate the efficiency of *Trichoderma* based biofilms, seed germination assay was done (Triveni *et al*., 2013). Cotton seeds were surface sterilized by immersing in 70 % ethanol for 30 s, followed by 0.1 % mercuric chloride for 3 min and then rinsing several times with sterile distilled water. These seeds were air-dried under laminar flow. The percent germination of the seeds was tested by keeping the surface-sterilized seeds on 1 % water agar for 48–96 h with different cultures/mixtures/biofilms. The treatments used were T_1: *Trichoderma viride*, T_2: *Bacillus subtilis*, T_3: *Azotobacter chroococcum*, T_4: *Pseudomonas fluorescens*, T_5: *B. subtilis* + *T. viride*, T_6: *A. chroococcum* + *T. viride*, T_7: *P. fluorescens* + *T. viride*, T_8: *T. viride*- *B. subtilis biofilm*, T_9: *T. viride*- *A. chroococcum biofilm*, T_{10}: *T. viride*- *P. fluorescens biofilm*, T_{11}: Control with *Macrophomina phaseolina* and T_{12}: Control.

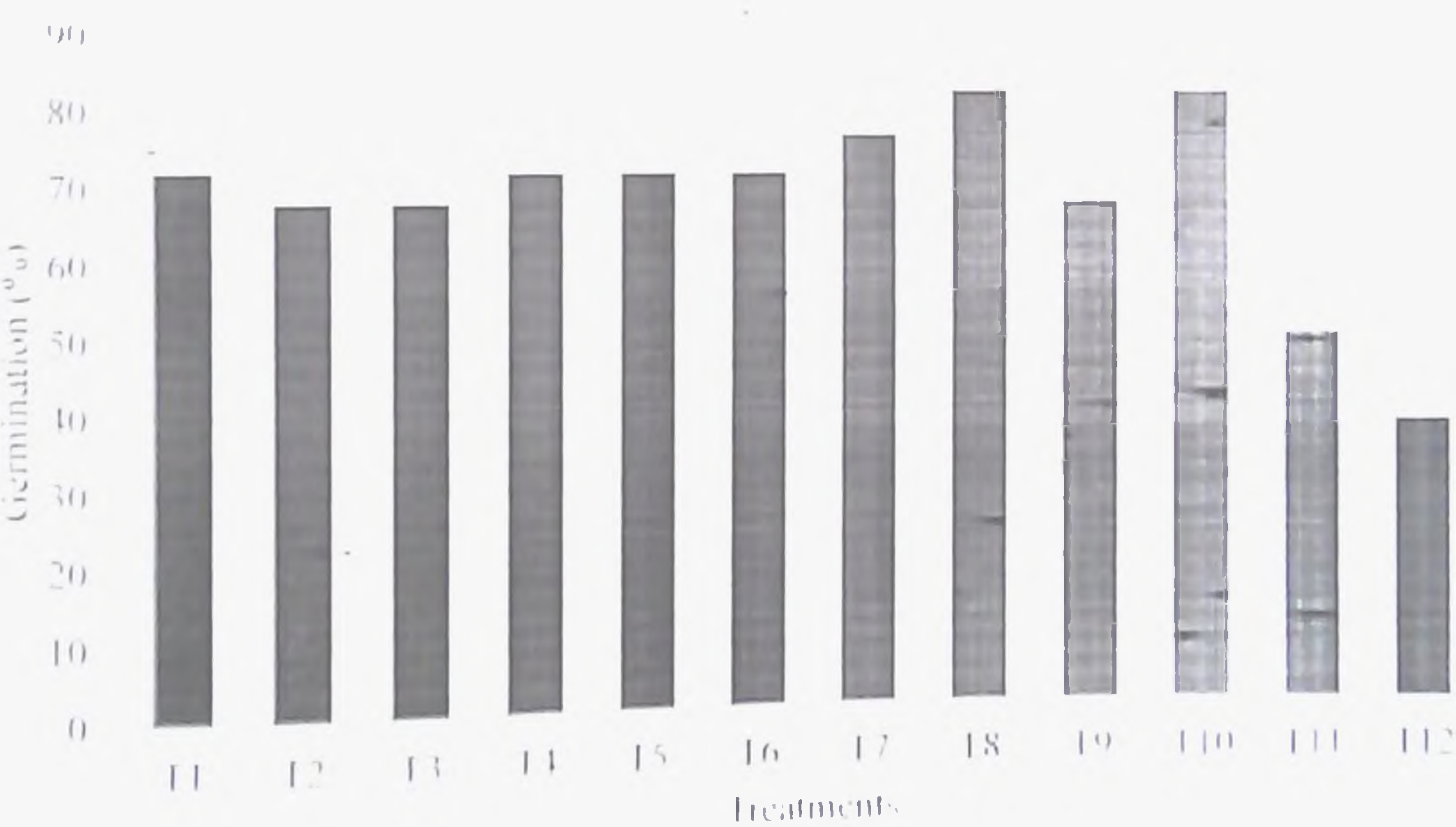


Figure 6: Percent germination of cotton seeds, as influenced by biofilms and their partners.

The highest germination percent was showed by the inoculation of *T. viride*+ *B. subtilis* biofilm and *T. viride*+ *P. fluorescens* biofilm. The germination percentage was lowest in the control treatment.

The IAA production ability of these above treatments was also measured (Hartmann *et al.*, 1983). It is found that all the cultures used were IAA producers, with values ranging from 0.013 to 0.082 μg/ml (Figure 7), and biofilms showed higher IAA production compared with the single cultures. The highest values of IAA production were observed with the *P. fluorescens*- *T. viride* dual culture and biofilms of *T. viride*- *P. fluorescens* and *T. viride*- *B. subtilis* (0.082 μg/ ml, 0.072 μg/ml and 0.074 μg/ml respectively).

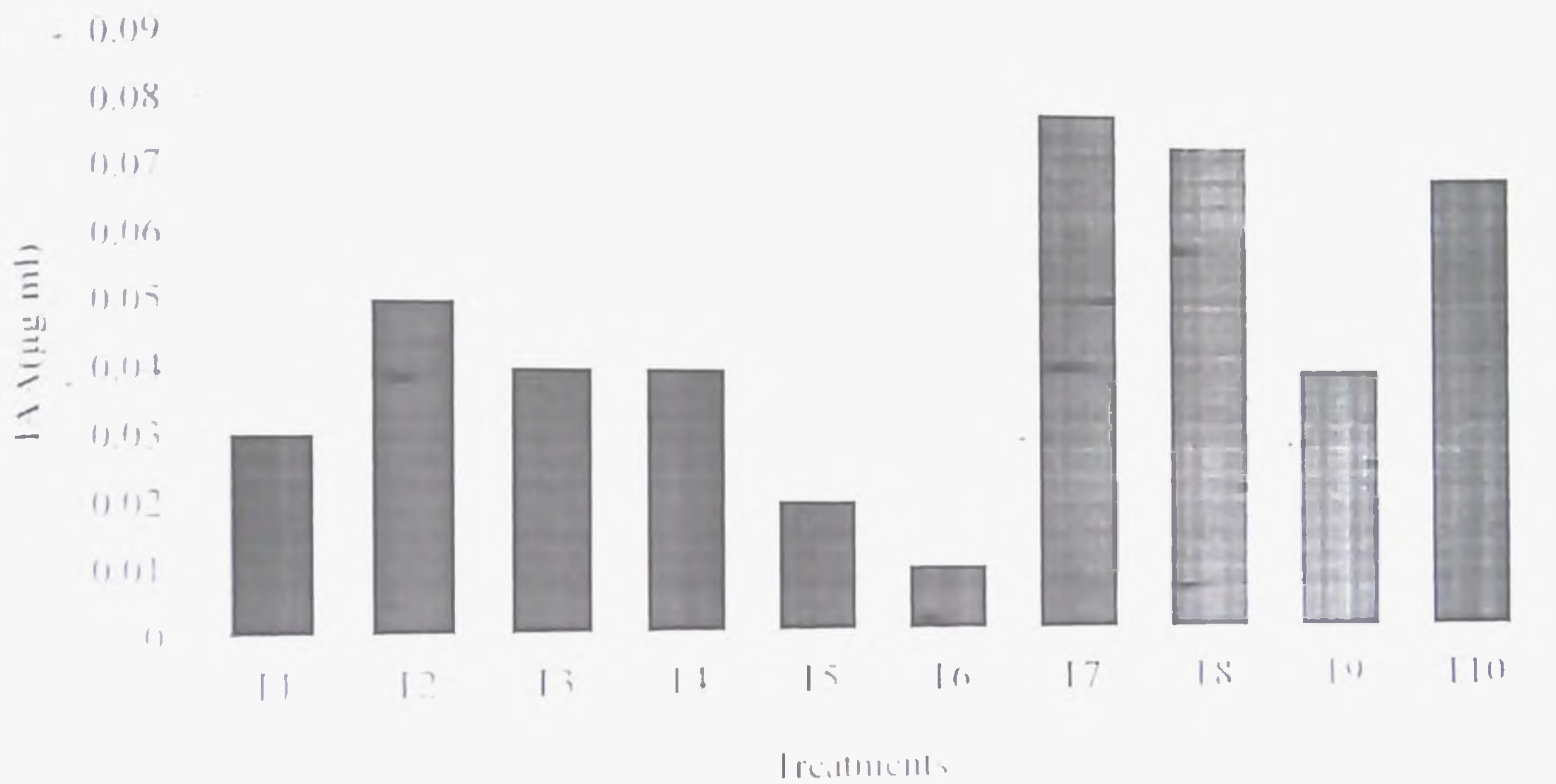
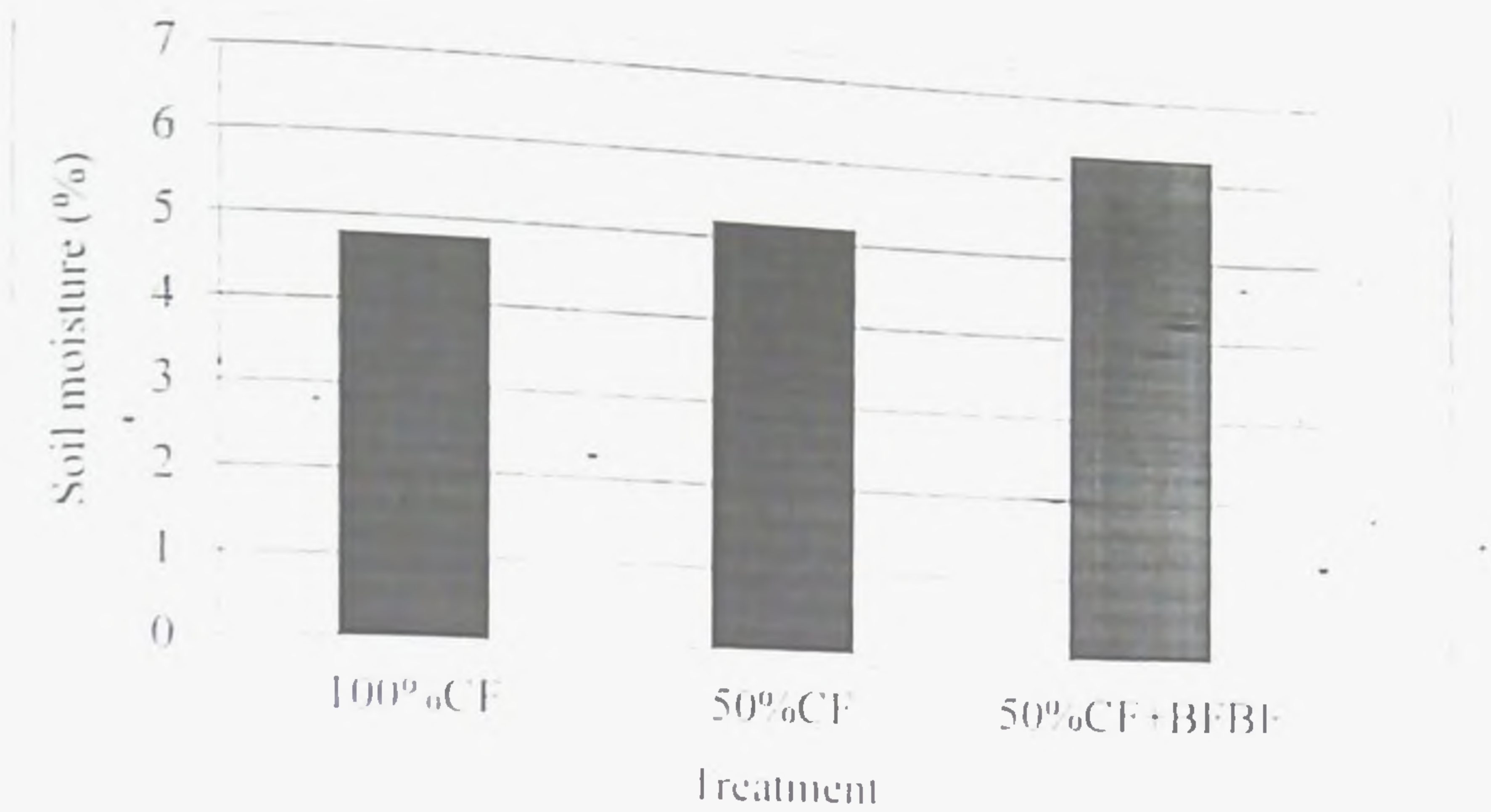


Figure 7: IAA production by the bacteria, individually, in dual cultures and as biofilms. Treatment numbers denote T1: *T. viride*; T2: *B. subtilis*; T3: *A. chroococcum*; T4: *P. fluorescens*; T5: *B. subtilis* + *T. viride*; T6: *A. chroococcum* + *T. viride*; T7: *P. fluorescens* + *T. viride*; T8: *T. viride* - *B. subtilis* biofilm; T9: *T. viride*- *A. chroococcum* biofilm; T10: *T. viride*- *P. fluorescens* biofilm.

7. Restoration of deteriorated soils

An experiment was done in Sri Lanka to know the role of biofilmed microbial inoculants in the restoration of deteriorated soils (Seneviratne *et al.*, 2011). The study was conducted in a tea plantation. The selected field was under the application of fertilizers and agrochemicals for the past 50-55 years. The treatments used were 100% of recommended chemical fertilizer (100%CF), 50% of recommended chemical fertilizers (50%CF) and 50% chemical fertilizers and biofilmed biofertilizers (50%CF+BFBF).

(a)



(b)

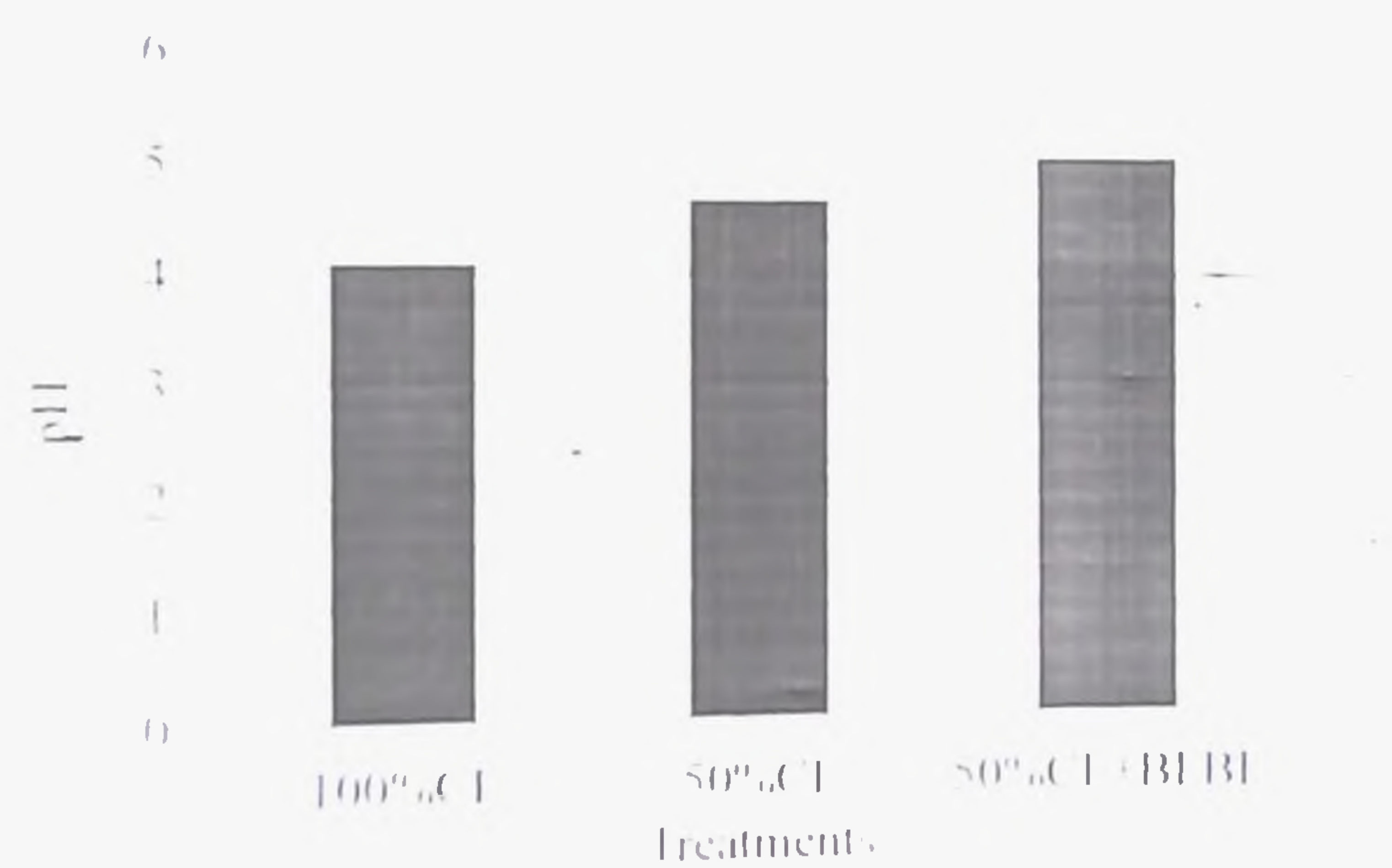
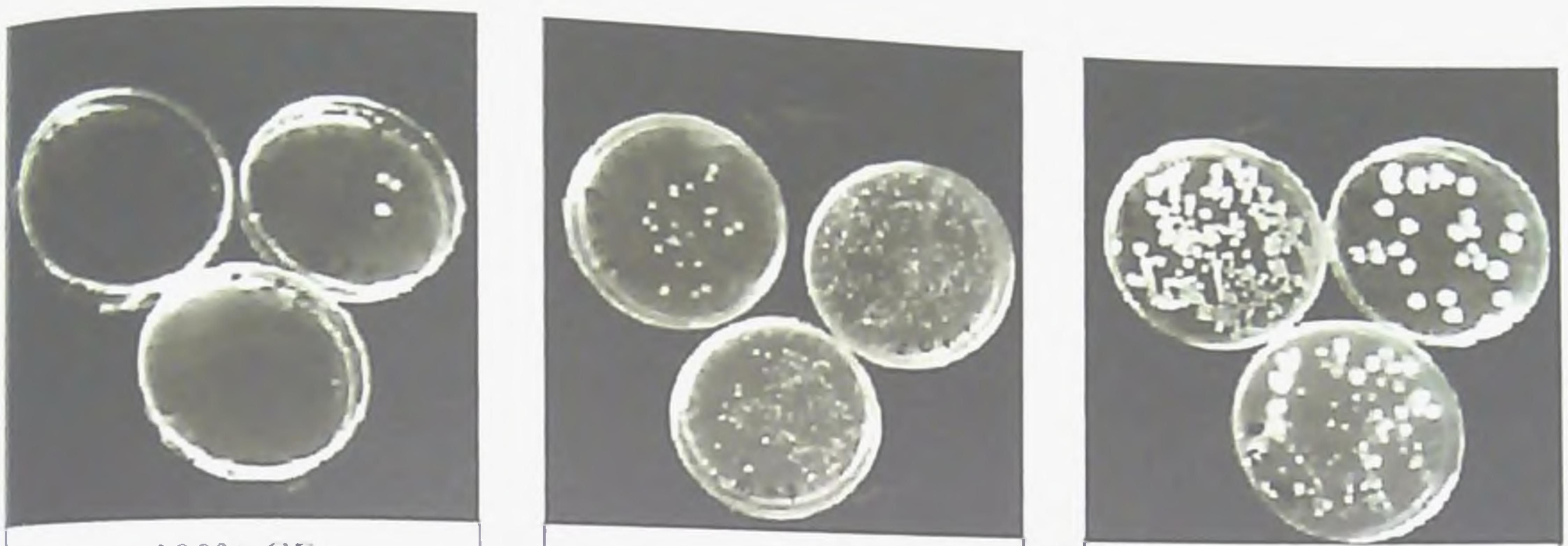


Figure 8. Effect of biofilmed biofertilizers (BFBFs) applied under nursery and field conditions of tea cultivation in Sri Lanka

The application of biofilmed biofertilizers along with the 50% of recommended chemical fertilizers increases the soil moisture content as compared to the 100% chemical fertilizer application. It also increases and maintains the pH level of the soil from a very strongly acidic condition (Figure 8).

They also isolated the total bacterial from the each treatment (Plate 1). The soils from each treatment is serially diluted and plated on nutrient agar. After [illegible] days, the observation was taken. 50% reduction in the application of recommended chemical fertilizer itself increases the population in a considerable level. The highest population of bacteria was recorded in the combined use of 50% chemical fertilizer and biofilmed biofertilizers.



100% CF | 50% CF | 50% CF+BFBF

Plate 4: Total soil bacterial colonies isolated on nutrient agar, from soils treated with 100% of recommended chemical fertilisers (CF), 50% of CF or 50% of CF together with biofilmed biofertilizers (BFBFs) in a tea nursery were plated on petri dishes and observed on day 7. Persistence of the applied BFBFs in the soil was evident from larger colonies developed from large colony forming units or biofilms of the 50% CF and BFBFs treatment.

8. Mass production

Mass production of biofilmed microbial inoculants involved the separate culturing of partner organisms such as bacteria and fungi (Figure 9). Fungi are generally cultured in potato dextrose broth media. The culturing of bacteria can be done in any specific media (eg: for *Pseudomonas fluorescens*, King's B media, for *Azotobacter*, Jensens' media etc.). The fungal culture has to be incubated at 30°C in a static position for one week and bacterial culture at a temperature of 30°C in a shaker incubator for 48 hours. After 16 days of biofilm formation, the biofilms produced by the fungus and bacteria has to be extracted and washed thoroughly with distilled water. Then the fungal and bacterial cultures have to be centrifuged and vortexed separately, to get a uniform suspension. The next important step is the co-culturing of bacteria and fungi to produce fungal bacterial biofilms. The vortexed uniform suspensions of bacteria and fungi have to be cultured together in a suitable media (eg: The most suitable media for the growth of *Trichoderma*-based biofilms with *B. subtilis* and *P. fluorescens* were found to be Pikovskaya broth + 1% $CaCO_3$, while for *A. chroococcum* the highest values were produced in Jensen's medium (Triveni *et al.*, 2012)). After 3 weeks of incubation the culture can be mixed with the carrier material.

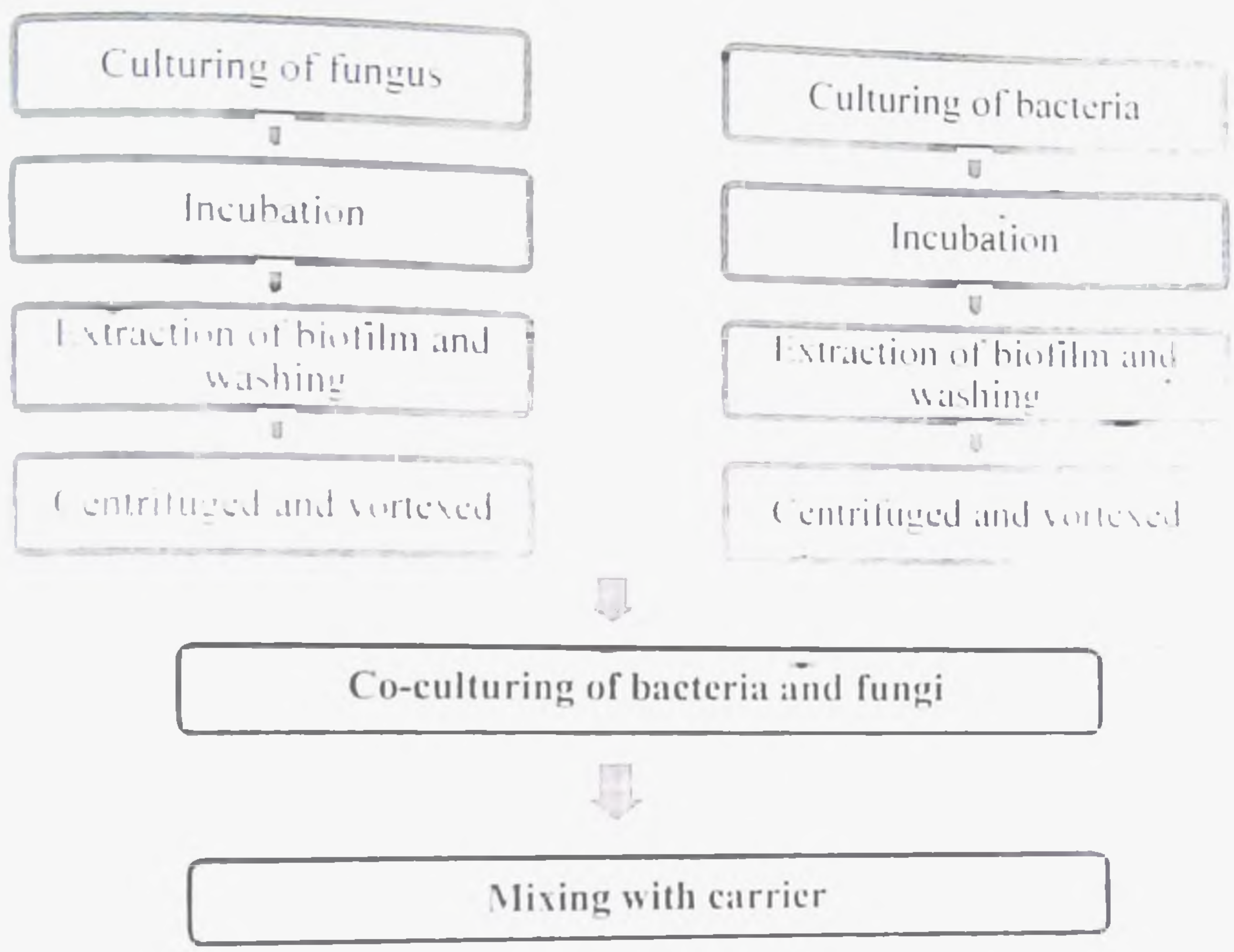


Figure 9: Mass production of biofilmed microbial inoculants

9. Commercial formulations

The initial studies on the biofilmed biofertilizers were carried out by [illegible] scientists from Institute of Fundamental Studies, Sri Lanka and Tea Research Institute of Sri Lanka. They conducted field experiment in tea plantation with the application of biofilmed biofertilizers. The only commercial formulation available is "Biofilm Liquid biofertilizers" produced by Lanka Biofertilizers (Pvt.) Ltd. They developed Biofilm- T, Biofilm- M, Biofilm- R and Biofilm- Veg for tea, maize, rice and vegetables respectively. They are prepared using the native isolates of the beneficial microbes from Sri Lanka. So they can be applied to the Sri Lankan soils only. There is no commercial formulation available in India because there are no field studies have been done in India.

10. Advantages

- The EPS produced by the microorganisms helps their survival under stress conditions such as high temperature, extreme pH, UV radiation etc.
- The microbial cells in the biofilm are highly protected from the action of toxic substances such as antibiotics.
- The fungal-rhizobium biofilm can be effectively utilized in N_2 fixation of non-legumes
- Enhances plant growth by the production of plant growth hormones.
- Plants withstand environmental stress such as moisture stress. The EPS can trap water molecules within their structure with the help of hydrogen bonding.

11. Disadvantages

- The biofilm is very destructive in their nature. About 65% of Infectious diseases are caused by biofilm.
- The microorganisms will become drug resistant and more resistant to antibiotics due to the biofilm formation.
- It causes blockage of irrigation pipes especially in laterals.

12. Conclusion

Biofilmed microbial inoculants using agriculturally useful microorganisms enhances the better persistence of microbes under adverse environmental conditions. Application of biofilm have to be extended to other areas of crop improvement such as microbial herbicides, biopesticides etc. The soil application of FRBs as biofilmed inocula appears to be important if soil fertility is to be sustained in nutrient depleted lands and survival of rhizobia is to be improved in the soil in the absence of their hosts. However, applications of this biotechnology are scarce because it is still understudied. Selection of combinations of microbes for highest efficiency, simultaneous biofertilizing and biocontrolling activities is a key in future research in this technology. Thus, more research should be done under laboratory and field conditions in order to optimise biofilmed inocula for various crops.

13. Discussion

1. What are the methods used for removing biofilm from irrigation pipes?

Ans: Normal chorine treatment can't remove biofilm. It is resistant to chlorine. Continuous ozone treatment and chlorine dioxide treatment can remove biofilm from irrigation pipes.

2. Why it is not commercially available in India?

Ans: Since there are no extensive studies conducted in India on a pilot scale, there are no biofilmed biofertilizers are commercially available.

3. How we will ensure the biofilm formation on root in field condition?

Ans: Biofilm formation can be observed through scanning electron microscope.

4. Field application of liquid biofertilizers?

Ans: Spraying in the root zone with a gap of one week of the application of half of the recommended chemical fertilizers.

5. "Biocapsule" is a recent technology in biofertilizers technology. How it differs from biofilmed biofertilizers?

Ans: Biocapsules increases only the shelt lite of biofertilizers up to 1 year as compared to conventional biofertilizers, but not in the field conditions. But, the biofilmed biofertilizers increases the survivability of the biofertilizers in adverse field conditions such as high temperature, extreme pH etc.

6. Can the microbes form biofilm naturally in soil? If yes, then what is the need of biofilmed biofertilizers?

Ans: Microbes form biofilm naturally in soil. So the aim of biofilmed biofertilizers, is to increase the cell population by helping them to multiply and to produce biofilm in laboratory condition. Then it is applied to the soil, so that the biofilm will protect the microorganisms.

7. Where the studies related to biofilm in agricultural field is initiated?

Ans: Prof. Gamini Seneviratne of Institute of Fundamental Studies (IFS), Sri Lanka initiated the research on biofilmed biofertilizers in 2003. In 2005, IFS together with Tea Research Institute (TRI) commenced field trial of biofilmed biofertilizers for tea plantation.

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Microbiology

Micro 591 Master's Seminar

Name: Manju Mohan E.

Admission No.: 2014-11-156

Major advisor: Dr. K. Surendra Gopal

Venue: Seminar hall

Date: 07-01-2016

Time: 10.45 am

Biofilmed microbial inoculants: A novel technique for survival under adverse environmental conditions

Abstract

Biofilm is a community of microbial cells enclosed in a mucilaginous matrix called Extracellular Polymeric Substances (EPS). It helps in the survival of microorganisms under adverse environmental conditions such as high temperature, extreme pH, UV radiation, *etc*. Biofilm also helps to maintain good soil structure. Hence, these benefits of biofilm can be effectively utilized in crop improvement.

Several studies have been conducted to develop biofilm based biofertilizers to enhance the efficiency and survivability of biofertilizer strains. According to Jayasinghearachchi and Seneviratne (2004), a fungal-*Rhizobium* biofilm can act as nodule like structures or "pseudonodules" by maintaining higher cell density of rhizobia on the root hairs of non legumes. A recent study showed that the nitrogenase activity of nitrogen fixers increased along with the maturity of biofilm formed (Herath *et al.*, 2015). The results on the efficacy of *Trichoderma* and *Anabaena* based biofilms for leguminous crops showed that, biofilm improved the acetylene reduction activity, microbial biomass carbon and percentage nitrogen availability in mung bean (Prasanna *et al.*, 2014). *Trichoderma* based bacterial biofilm significantly reduced the collar rot in cotton as compared to the monocultures (Triveni *et al.*, 2015).

The total root dry weight of rubber was highest with the application of biofilmed biofertilizer along with the chemical fertilizer, than the chemical fertilizer alone (Hettiarachchi *et al.*, 2014). Application of *Pseudomonas fluorescens* - *Pleurotus oestreatus* biofilm increased the endophytic colonization of *P. fluorescens* in tomato as compared to the application of *P. fluorescens* alone. Application of biofilm based biofertilizers along with 50 percent chemical fertilizers maintained the soil pH and also

increased the soil moisture content (Seneviratne *et al.*, 2011).

Prasanna *et al.* (2014), developed a method for mass production of carrier based biofilmed microbial inoculants. However, such inoculants are not available commercially.

Biofilmed microbial inoculants have better persistence in soil under harsh environmental conditions and hence improves crop growth and yield, besides maintaining soil fertility. The synergy in the multiple mode of action combined with increased survivability of biofertilizers can provide enhanced nutrients and biological control. It will also result in long term sustainable crop production, besides widespread adoption of biofertilizers.

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VERMIFILTRATION – A LOW COST SUSTAINABLE TECHNOLOGY FOR WASTEWATER TREATMENT

By

R. SRI VITHYA

(2014-11-229)

Seminar report

Presented on 29/01/2016

Submitted in partial fulfillment of requirement of the course

MICRO 591- Masters' Seminar (0+1)

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

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THRISSUR, KERALA

DECLARATION

I, R. Sri Vithya (2014-11-229) hereby declare that the seminar entitled **"Vermifiltration – a low cost sustainable technology for wastewater treatment"** has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

Sri vithya . R

Place: Vellanikkara
Date: 10-02-2016

R. Sri vithya
(2014-11-229)

CERTIFICATE

This is to certify that the seminar report entitled **"Vermifiltration – a low cost sustainable technology for wastewater treatment"** has been solely prepared by **R. Sri Vithya (2014-11-229)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

Place: Vellanikkara

Date: 10-02-2015

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INTRODUCTION

Wastewater treatment is the removal of contaminants from any form of wastewater and it includes physical, chemical and biological processes so that the water can be re-used (Pons, *et al.*, 2004). The most suitable wastewater treatment technique to be applied must meet the recommended microbiological and chemical quality guidelines at low cost, minimal operational and maintenance requirements (Trivedy, ., 2007; Nhapi, 2009).

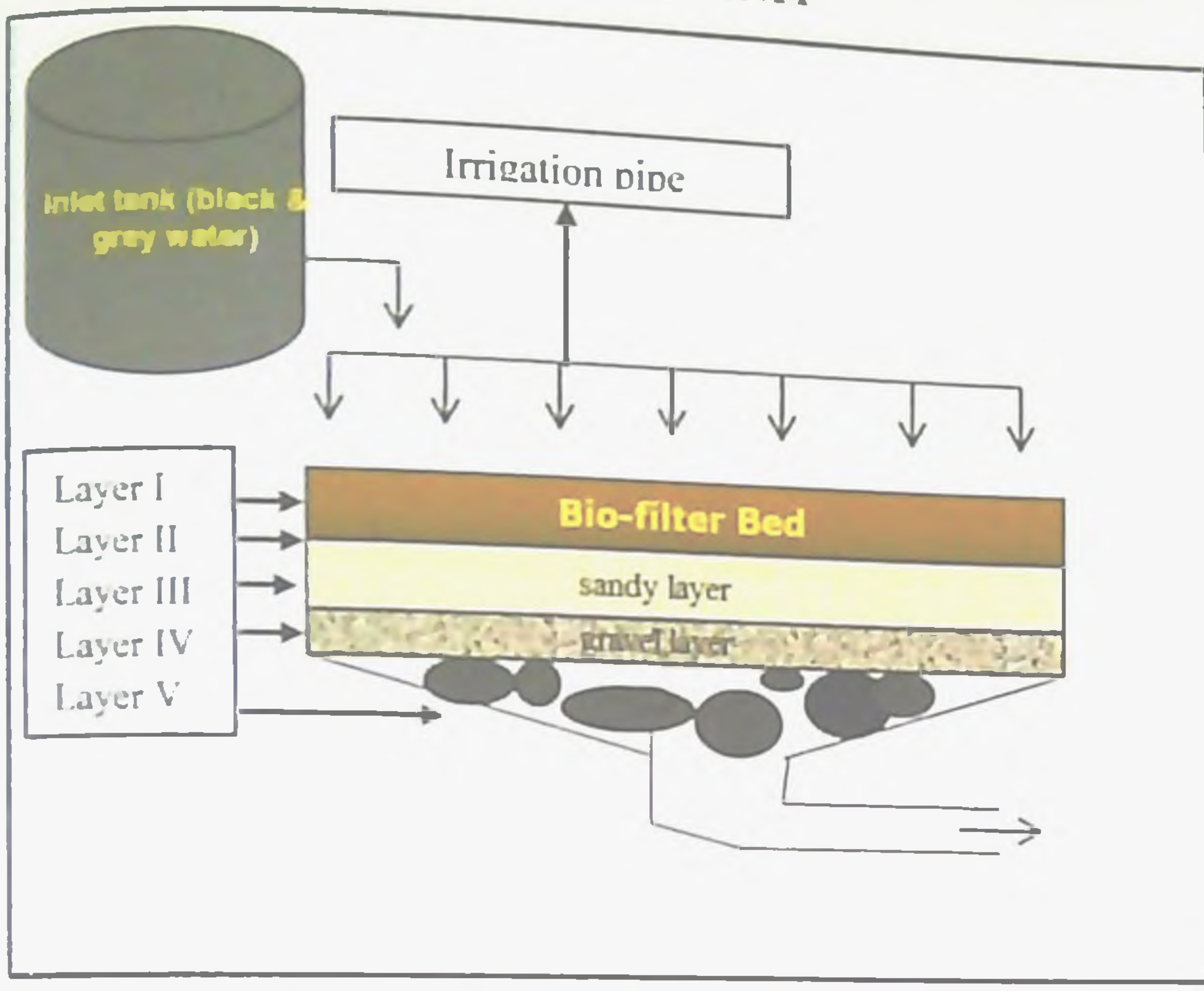
Vermifiltration technology which uses epigeic earthworms has increasingly became an eco-friendly wastewater treatment technique (Azuar and Ibrahim, 2012). The idea was first advocated by Jose Toha from Chile in the year 1992(Bouche and Qiu, 198; Aguilera, 2003; Li, *et al.*, 2008). It is a treatment combination of conventional filtration process with vermi composting technology. It is a low cost, odourless and non-labour intensive method of wastewater treatment (Malek, *et al.*, 2012). The resulting vermi-filtered water is clean and disinfected enough to be reused for farms irrigation, in parks and gardens (Ghatnekar, *et al.*, 2010).

During vermifiltration, there is no sludge formation in the reactor which requires additional expenditure on landfill disposal, instead a vermicompost which is a bio-fertilizer is formed (MPDB, 2006). Vermifiltration also removes heavy metals, solid and liquid organic waste in the wastewater through the action of earthworms (Xing, *et al.*, 2010). Various earthworm species have been used in vermifiltration of municipal wastewater (Sinha, *et al* 2010).

The earthworms body work as a bio-filter and earthworms have been found to reduce biological oxygen demand (BOD5), chemical oxygen demand (COD), total dissolved solids (TDS), total dissolved and suspended solids (TDSS) and turbidity from wastewater (Malek, *et al.*, 2012; Xing, *et al.*, 2010).

Sewage wastewater carries hazardous chemicals and very high loads of organic matter which must be treated before its disposal. Treatment of this wastewater for sustainable development with environmental protection can be achieved by the use of earthworm's species which promises to provide cheaper solutions to social, economic, environmental and health. Vermifiltration of wastewater can be used for irrigation purposes (Sinha, *et al.*, 2010).

STRUCTURE OF VERMIFILTRATION UNIT



Layer I

Soil bed + Earthworms

Layer II

Net wire mesh

Layer III

Sand + 5 mm aggregate

Layer IV

10 to 16 mm aggregate

Layer V

20 mm aggregate

(Lakshmi, et al., 2014)

FUNCTION OF VERMIFILTRATION UNIT

Vermifiltration unit consists of wastewater collecting tank, vermifiltration bed and treated water collecting tank. Wastewater will be collected in the wastewater collecting (PVC) drum. These drum are kept on an elevated platform just near the vermifilter bed. The PVC drum has tap at the bottom to which a sprinkler system is attached. The treated water collecting tank will be attached at the bottom of the vermifilter bed.

In the top most layer of the filter system, earthworm bed material is placed in which earthworms are released. The bed materials consists of pure garden soil. The sprinkler system is used for trickling the wastewater through uniform distribution on the soil surface. Wastewater from the PVC drums flows through the irrigation pipes by gravity.

The wastewater is percolated down through various layers in the vermifiltration bed passing through the soil layer inhabited by earthworms, sandy layer, and gravel layer. The treated water will be collected at the bottom in a treated water collecting tank. Earthworms plays a major role in the vermifiltration unit. Optimum number of earthworms added in the vermifiltration bed is 8000-10000/ m^2

PRINCIPLES OF VERMIFILTRATION UNIT

Vermifiltration which reduces the physico-chemical parameters such as Biological Oxygen Demand (BOD), Chemical oxygen demand (COD), total dissolved and suspended solids (TDSS), and turbidity.

Biological Oxygen Demand

BOD refers to the amount of dissolved oxygen requires to degrade the organic contaminants by aerobic bacteria. In vermifiltration the BOD reduces through microbial and enzymatic action which are present in the earthworms gut and intestine.

Chemical Oxygen Demand

Chemical decomposition of organic and inorganic contaminants can be increased in the vermifiltration through enzymes which are present in the earthworms gut.

Total Dissolved and Suspended Solids

Organic and inorganic solids which are either in the form of dissolved or suspended solids can be reduced through the solids eaten by earthworm and expelled as vermicasts.

Turbidity

Turbidity is cloudiness of the wastewater. This can be reduced through adsorption of suspended solids by earthworms and geological material which are present in the filter bed. For removing the turbidity, geological material plays a major role.

ROLE OF EARTHWORMS IN THE VERMIFILTRATION UNIT

Earthworms are versatile waste eaters and decomposers. It promotes the growth of beneficial decomposing bacteria in the wastewater and acts as an aerator, grinder, crusher, chemical degrader and a biological stimulator (Sinha *et al.*, 2008).

The wastewater will be sprinkled on the top layer of the vermifiltration bed, the dissolved solids will be trapped by the solid particles and then it will be processed by the earthworms and fed to the soil microbes immobilized through the soil bed. Then, the earthworms body also adsorb the dissolved and suspended solids and stabilized through complex degradation process (Garkal, *et al.*, 2015). Stabilization will occur in the soil layer

Earthworms increases the hydraulic conductivity and natural aeration of the organic particles by granulating the organic particles into small particles (Ghatnekar, *et al.*, 2010). In addition, the earthworms grind the silt and sand particles, increasing the total specific surface area which enhances the ability to adsorb the organic and inorganic particles from the wastewater (Sinha, *et al.*, 2007). Increment of soil processes and soil aeration by the earthworms enables the soil stabilization and filtration system to become efficient. The processes of microbial stimulation, biodegradation as well as the enzymatic degradation of waste solids by earthworms simultaneously work in the vermifiltration system (Sinha, *et al.*, 2007).

UNIQUE FEATURES OF EARTHWORM IN THE VERMIFILTRATION UNIT

Earthworm host millions of decomposing microorganisms in their gut and intestine. Edward and Fletcher. (1988) showed that the number of bacteria and actinomycetes contained in the ingested material increased up to 1,000 fold while passing through the gut. A population of earthworms numbering about 15,000 will in turn foster a microbial population of billions of millions.

Singleton *et al.*, (2003) also studied the bacterial flora associated with the intestine and vermicasts of the earthworms and found species like *Pseudomonas, Mucor, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes,* and *Acidobacterium* which has potential to degrade several categories of organics. *Acaligenes* can even degrade PCBs and *Mucor* dieldrin.

ECOLOGICAL ADAPTATION FOR SURVIVAL IN HARSH ENVIRONMENT

Earthworms are burrowing animals and form tunnels by literally eating their way through the soil. The distribution of earthworms in soil depends on factors like soil moisture, availability of organic matter and pH of the soil. They occur in diverse habitats specially those which are dark and moist. Earthworms are generally absent or rare in soil with a very coarse texture and high clay content or soil with pH <4. Earthworms are also tolerant to moderate salt salinity in soil, but some species like the tiger worms (*Eisenia fetida*) has been found to be highly salt tolerant (concentration of 15 g/kg of soil and survival ranges from 80–100%). This means that *E. fetida* can tolerate soils nearly half as salty as seawater.

Earthworms can also tolerate toxic chemicals in environment. After the Seveso chemical plant explosion (1976) in Italy, when a vast area was contaminated with extremely toxic chemical like TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), several fauna perished except for some species of the earthworms that survived. Earthworms which ingested TCDD contaminated soils were shown to bioaccumulate dioxin in their tissues and concentrate it on average 14.5 fold (Satchell, 1983). *E. fetida* also survived 1.5% crude oil containing several toxic organic pollutants (OECD, 2000).

Earthworms can also tolerate high concentrations of heavy metals in the environment. Some species have been found to bioaccumulate up to 7600 mg of lead (Pb)/gm of the dry weight of their tissues (Ireland, 1983). They can tolerate a temperature between 5 and 29°C. A temperature of 20–25°C and a moisture of 60–75% are optimum for good worm function (Hand, 1988). Generally earthworms can also tolerate extensive water loss by dehydration (Roots, 1956).

Earthworms also graze on the surplus harmful and ineffective microbes in the wastewater selectively, prevent choking of the medium and maintain a culture of effective biodegrader microbes to function.

Their activity is significantly slowed down in winter, but heat can kill them instantly. It seems worms are not very sensitive to offensive smell as they love to live and feed on cattle dung and even sewage sludge. However, offensive smell can persist only for a short while in any environment where worms are active. They arrest all odour problems by killing anaerobes and pathogens that create foul odour through the celeomic fluid which has anti-bacterial properties and arrest the formation of all microbes that cause rotting (Pierre *et al.*, 1982).

Some bacteria and fungi fostered by the worms also produce 'antibiotics' which kills the pathogenic organisms in the waste biomass making the medium virtually sterile and odourless. Fungus *Penicillium* spp. producing antibiotics 'penicillin' has been reported from the intestine of earthworms. The removal of pathogens, fecal coliforms (*E. coli*), *Salmonella* spp., enteric viruses, and helminth ova from sewage and sludge appear to be much more rapid when they are processed by *E. fetida*. Of all, *E. coli* and *Salmonella* were greatly reduced (Bajsa *et al.*, 2003).

ENORMOUS POWER OF REPRODUCTION AND RAPID RATE OF MULTIPLICATION

Earthworms are bisexual animals and multiply very rapidly. After copulation each worm ejects lemon-shaped 'cocoon' where sperms enter to fertilize the eggs. Up to 3 cocoons/ worm / week are produced. From each cocoon, about 10–12 tiny worms emerge. It indicate that they double their number at least every 60 – 70 days.

In the optimal conditions of moisture, temperature, and feeding materials, earthworms can multiply by 28, i.e. 256 worms every 6 months from a single individual. Each of the 256 worms multiplies in the same proportion to produce a huge biomass of worms in a short time. The total life cycle of the worm is about 220 days. They produce 300–400 young ones within this life period (Hand, 1988). A mature adult can attain reproductive capability within 8–12 weeks of hatching from the cocoon. Red worms takes only 4–6 weeks to become sexually mature (ARREPT, 2005). Earthworms continue to grow throughout their life

Earthworm species used

- Tiger Worm (*Eisenia fetida*).
- Red Tiger Worm (*E. andrei*).
- Indian Blue Worm (*Perionyx excavates*).
- African Night Crawler (*Eudrilus euginae*).

- Red Worm (*Lumbricus rubellus*) are best suited for vermitreatment of variety of solid and liquid organic wastes under all climatic conditions (Graff, 1981).
- *Eisenia fetida* and *E. andrei* are closely related. An army of the above five species combined together works meticulously.
- The *E. fetida*, *P. excavatus*, and *E. euginae* are voracious waste eaters and biodegraders.

CRITICAL FACTORS AFFECTING VERMIFILTRATION PROCESS

- Hydraulic Retention Time (HRT)
- Hydraulic Loading Rate (HLR)
- Selection of filter media
- Earthworm biomass

Hydraulic retention time (HRT)

Hydraulic retention time is the time taken by the wastewater to flow through the soil profile (vermifilter bed) in which earthworms inhabits. It is very essential for the wastewater to remain in the vermifiltration system and be in contact with the worms for certain period of time. HRT depends on the flow rate of wastewater to the vermifiltration unit, volume of soil profile, and quality of soil used.

HRT is very critical, because this is the actual time spent by earthworms with wastewater to retrieve organic matter from it as food. During this, earthworms carry out the physical and biochemical process to remove nutrients, ultimately reducing BOD, COD, and the TDSS. The longer the wastewater remains in the system in contact with earthworms, the greater will be the efficiency of vermiprocessing and retention of nutrients. Hence, the flow of wastewater in the system is an important consideration as it determines the retention of suspended organic matter and solids (along with the chemicals adsorbed to sediment particles).

Maximum HRT can results from 'slower rate of wastewater discharge' on the soil profile (vermifilter bed) and hence slower percolation into the bed. Increasing the volume of soil profile can also increase the HRT. The number of live adult worms, functioning per unit area in the vermifilter bed can also influence HRT.

HRT of vermifiltration system can be calculated as: $HRT = (\rho \times Vs)/Q$

HRT = Theoretical hydraulic retention time (hours); Vs = volume of the soil profile (vermifilter bed), through which the wastewater flows and which have live earthworms (cum). q = porosity of the entire medium (gravel, sand, and soil) through which wastewater flows. Qwastewater = Flow rate of wastewater through the vermifilter bed (cum/h).

Hydraulic loading rate (HLR)

The volume and amount of wastewater that a given vermifiltration system (measured in area and depth of the soil medium in the vermifilter bed in which the earthworms live) can reasonably treat in a given time is the hydraulic loading rate of the vermifilter system. HLR can thus be defined as the volume of wastewater applied, per unit area of soil profile (vermifilter bed) per unit time. It critically depends upon the number of live adult earthworms functioning per unit area in the vermifilter bed. The size and health of the worms is also critical for determining the HLR.

HLR of vermi-filtration system can be calculated as

$$HLR = V / (A \cdot t)$$

V - volumetric flow rate of wastewater (cum). A - Area of soil profile exposed (sq.m). t - time taken by the wastewater to flow through the soil profile (h). High hydraulic loading rate leads to reduced hydraulic retention time (HRT) in soil and could reduce the treatment efficiency. Hydraulic loading rates will vary from soil to soil.

Selection of filter media

Filter media plays an extraordinary role in the vermifiltration, since changes of the exotic environment play a crucial role on the structure and function of the earthworm's body wall, which is closely related with earthworm's activity and respiratory metabolism (Xing *et al.*, 2011).

Soil in some quantity needs to be employed because soil is the most suitable substrate for nitrifying bacterial communities in this reactor, and that earthworm activities had no significant effect on the community structure of nitrobacteria at different soil depths (Wang *et al.*, 2011).

Coal cinder-convertor slag mix as a medium was particularly identified for its ability to reduce phosphorus levels in the effluent stream (Wang *et al.*, 2010). A mix of black cotton soil and gravel has been found to work very well for domestic wastewater (Kharwade and Khedikar, 2011). Similarly, quartz sand and ceramsite studied for domestic wastewater treatment showed positive impacts on the vermifilter performance (Xing *et al.*, 2010).

Earthworm biomass

The earthworm biomass increased slowly in vermifiltration bed. The increase in weight of earthworm biomass during the vermifiltration period varied between 7.4% and 20.7%. Maximum growth of earthworms was at hydraulic loading rate 2.5 $m^3m^{-2}d^{-1}$ during which earthworms increased to 326. For optimal function of the unit, it needs 8000 numbers of earthworms per square meter of the worm bed and in quality as 10 kg per m^3 of soil (Komarowski, 2001).

APPLICATION OF VERMIFILTRATION TECHNOLOGY

Vermifiltration had wide application on different wastewater sources such as,

- Domestic wastewater (Lakshmi, *et al.*, 2014)
- Sewage wastewater (Manyuchi *et al.*, 2013)
- Dairy wastewater (Telang and Patel, 2013)
- Industrial wastewater (Das *et al.*, 2015)

Domestic wastewater treatment by vermifiltration

a. Collection of Earthworms

Indian blue worm.

Initial density of 75g was selected for the experiment.

b. Preparation of bed material for Earthworms

In the top most layer of the filter system, bed material placed in which Earthworms were released. The bed materials consists of pure garden soil, saw dust and cow dung. Soil and sawdust were mixed at a volume ratio of 3:1. Sawdust was added as a bulking agent because it has been shown to improve soil permeability and enhance earthworm growth and survival. Cow dung was added to provide nutrients of earthworms during acclimatization period of the experiment.

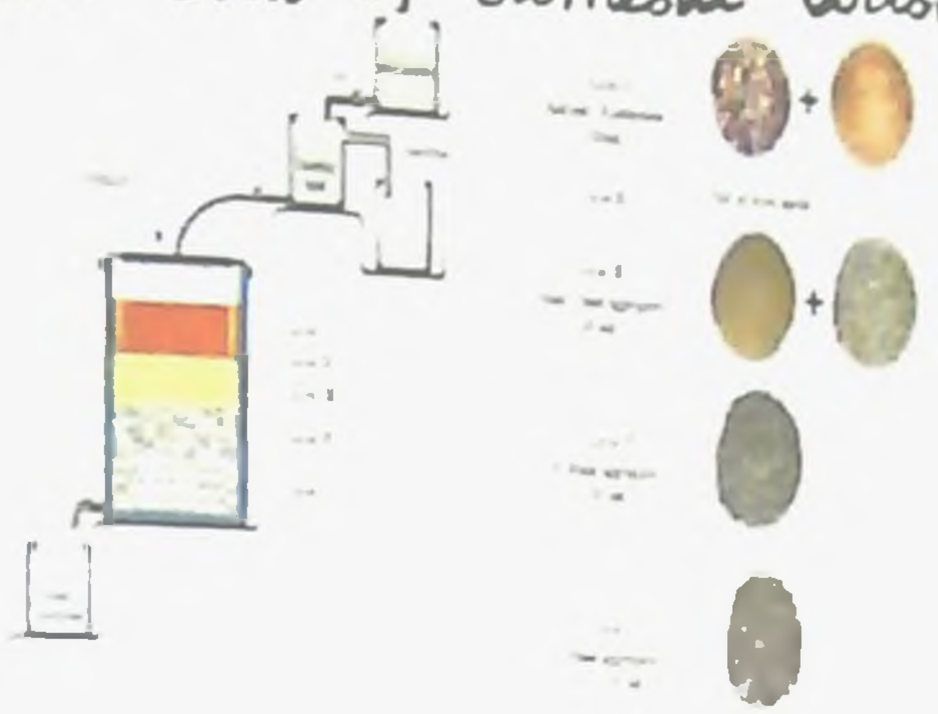
c. Design of vermifilter system - Bench Scale Reactor

A laboratory model of vermifilter system was designed in which arrangement has been made to supply the wastewater from top as well as collect the treated wastewater from the bottom of the system. The wastewater was fed by gravity flow and with the help of sprinkler.

The body of the reactor was made of PVC drum. The designed model has been treat 40 liters of wastewater per day. Size of the unit was 40×40 m^2 of area. The depth of 40 cm has been divided into 4 parts in which gravel, sand and soil bed for earthworm were placed from bottom layer to top.

The topmost layer of about 10 cm consists of soil bed in which the earthworms were released. The worms were given around one week settling time in the soil bed to acclimatize in the new environment.

Plate 2 : Experimental unit of domestic wastewater treatment



d. Operation of the reactor

Around 45 liters of wastewater was kept in PVC drum. These drums were kept on an elevated platform just near the vermifilter unit. The PVC drums had tap at the bottom to which a sprinkler system was attached. The sprinkler system consisted of simple 16 mm polypropylene pipe with holes for trickling water that allowed uniform distribution of wastewater on the soil surface.

Wastewater from the drums flowed through the irrigation pipes by gravity. The wastewater percolated down through various layers in the vermifilter bed passing through the soil layer inhabited by earthworms, the sandy layer, and the gravels. Next day this treated wastewater from both systems were collected and analyzed for BOD, COD, pH, turbidity and the TSS.

To start up the non-vermifilter, seeding was adopted using less polluted wastewater to develop microbial film layer on the filter bed, this will help to degrade the organic matter content. The seeding was done for duration of 7 days before starting actual wastewater feeding. After seeding, the actual procedure was repeated as same as vermifilter operation.

e. Results of the experiment

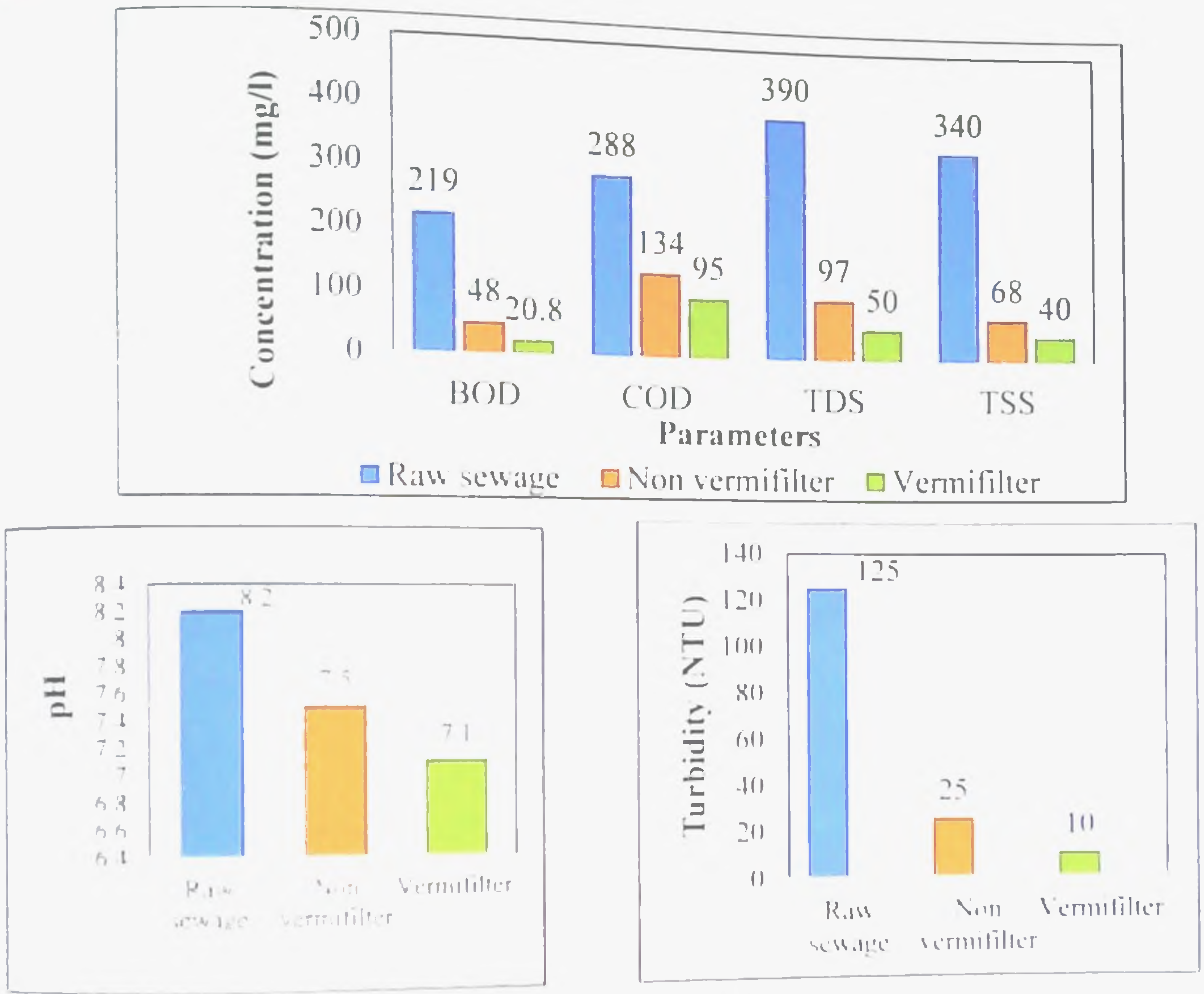
Variation in pH value of treated wastewater

Results indicated that the pH value of raw wastewater is almost neutralized by the earthworms in the vermifilter unit. The pH value of treated wastewater without earthworms also improved but it was not consistent throughout the experiment.

Turbidity removal

Results indicated that the average reduction in turbidity by earthworms was over 96%

while in the control unit was also significantly high (80%). It appears that the filter media of the unit plays very important role in turbidity removal by adsorption of suspended particles on the surface of the soil, sand and the gravels. Turbidity of treated wastewater is affected by HLR.



ig 1 : Effect of vermifiltration on BOD, COD, TDSS, pH and turbidity in domestic wastewater treatment

Removal of Total Suspended Solids

Results showed that the earthworms could significantly remove the suspended solids from the wastewater by over 88%, which in the control unit where geological and microbial system works together was over 60% only.

Removal of Total Dissolved Solids

Total suspended solids (TSS) and total dissolved solids (TDS) showed drastic reduction during bio filtration (control) and vermifiltration process. The total reduction in TDS content was about 95% in vermifiltration unit and that was significantly higher than total removal in control unit (75%).

Removal of Biochemical oxygen demand (BOD)

BOD is also an important indicator of organic load of wastewater. The BOD load in effluents from control unit and vermifiltration unit was significantly lower than initial levels, but vermi-biofiltration showed more removal efficiency than control unit. Results show that the earthworms can remove BOD loads by over 92%. BOD removal in the control unit is just around 78%.

Removal of Chemical Oxygen Demand (COD)

Results showed that the average COD removed from the wastewater by earthworms is over 65% while that without earthworms is just over 52%. COD removal by earthworms is not as significant as the BOD. as but at least much higher than the microbial system. Again, the enzymes in the gut of earthworms help in the degradation of several of those chemicals which otherwise cannot be decomposed by microbes.

From the experimental data it was found that vermifilter is more efficient than non-vermifilter in efficiency of removal of BOD, COD as well as solids. Results of vermifilter technology are most cost effective, odour free for treatment with efficiency, economy and potential decentralization. When compared to the acceptable value for BOD in treated wastewater is 1-15mg/l, COD is 40-70mg/l and pH is 7.0. The values obtained from the experiment are well within the limits. it shows vermifiltration system has good performance in treatment of waste water.

SEWAGE WASTEWATER TREATMENT

a. Type of earthworm used

500 *Eisenia fetida* earthworms were used.

b. Vermifiltration Bed

The vermifiltration bed constituted of a layer of the fibrous plastic filter to cover the base of the bed. The bottom most layers were made up of aggregates of sizes 7.0-8.0 cm and filled up to the depth of 0.2 m. On top, was a layer of aggregates 3.0-5.0 cm and filled up to another depth of 0.2 m. Another 10cm layer of small gravel mixed with sand was introduced with a depth of about 0.25 cm. The top most layer was then made up of 15cm of garden soil and was the one in which worms were introduced into. The worms were given one week settling time in the soil bed to acclimatize in the new environment.

As the earthworms played the critical role in wastewater purification, their number and population density increased. The untreated sewage water was evenly distributed in the vermifiltration bed.

The hydraulic retention time (HRT) in the vermifilter bed was kept uniformly for 2 hours. All experiments for both the vermifilter and the control bio-filter were replicated 3 times.

c. Results of this experiment

Change in pH value of treated sewage

The raw wastewater's pH value (6.45) of the raw sewage water was neutralized by the earthworms in the vermifilter bed to a pH of around 7. However, pH value of treated sewage wastewater without earthworms also improved to 6.6 but not as high as those in the vermifilter. Furthermore, this pH is ideal for optimum earthworm activity.

Removal of BOD

The earthworms in the vermifilter removed BOD loads by about 98% whilst the control bio-filter bed indicated a BOD removal of around 75%. These are still acceptable for water to use in irrigation purposes.

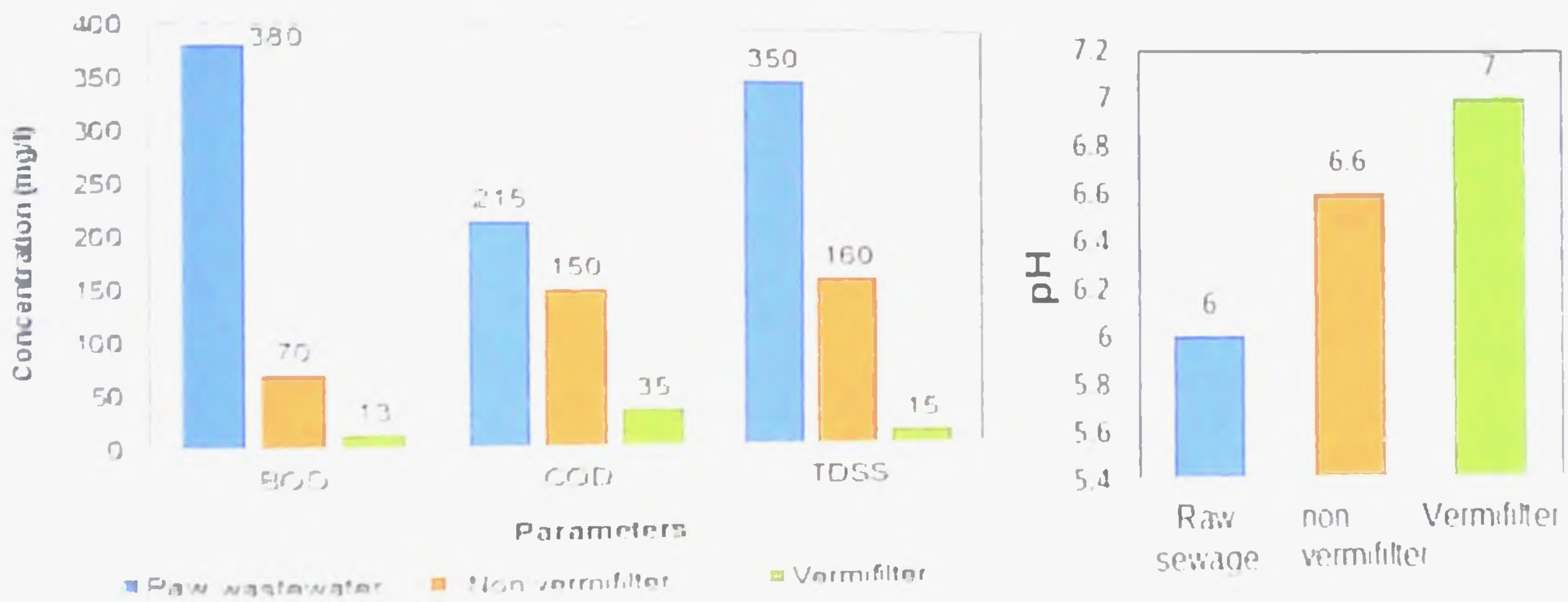


Fig 2 : Effect of vermifiltration in BOD, COD, TDSS, pH and turbidity in sewage wastewater

Removal of TDSS

The earthworms in the vermifilter significantly removed the TDSS from the sewage water by about 95% while the control bio-filter bed indicated a 60% decrease. The TDSS values (14-15 mg/L) remaining after vermifiltration of the sewage water were also acceptable for use of the treated sewage water for irrigation purposes.

Removal of turbidity

There was a reduction in the sewage water turbidity by earthworms in the vermifilter by over 98% while the sewage water in the control bio-filter bed also showed a significant decrease of about 95%. The successful reduction of BOD_5, COD and TDSS has a direct link to turbidity reduction as well such that the water can be successfully used for irrigational purposes.

Vermifiltration technology is a recommended solution for sewage wastewater treatment in developing countries. Vermifiltration of sewage wastewater results in treated water which can be used for irrigation purposes and a biofertilizer,

Sewage wastewater treatment by consortia vermifiltration

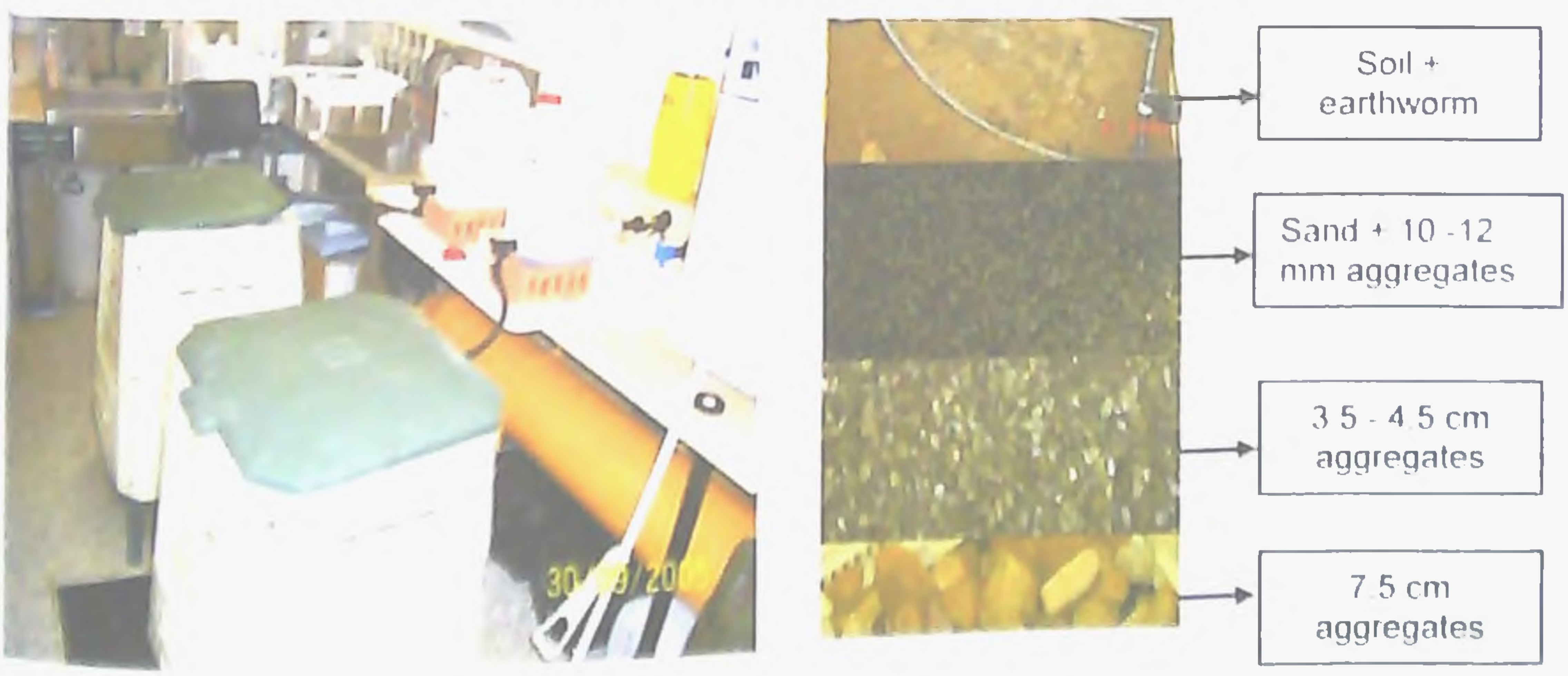
a. Earthworm collection

E. fetida, P. excavatus, and *E. euginae* are voracious waste eaters and biodegraders. Mixture of these three species started with 500 worms in 0.025 cum of soil bed used in this experiment.

b. Formation of vermifilter bed

The bottom most layer is made of gravel aggregates of size 7.5 cm and it fills up to the depth of 25 cm. Above this lies the aggregates of 3.5–4.5 cm sizes filling up to another 25 cm. On the top of this is the 20 cm layer of aggregates of 10–12 mm sizes mixed with sand. The topmost layer of about 10 cm consists of soil in which the earthworms were released. The worms were given around one week settling time in the soil bed to acclimatize in the new environment.

Plate 3: Experimental unit of sewage wastewater treatment



c. Result of the experiment

Improvement in pH value of treated sewage

Results indicate that the pH value of raw sewage is almost neutralized by the earthworms in the vermifilter kit. pH value of treated sewage without earthworms also improved but it was not consistent in all experiments.

Removal of BOD

Results show that the earthworms can remove BOD loads by over 98% or nearly complete at hydraulic retention time of 1–2 h (Chaudhary, 2006). BOD removal in the control kit was just around 77%.

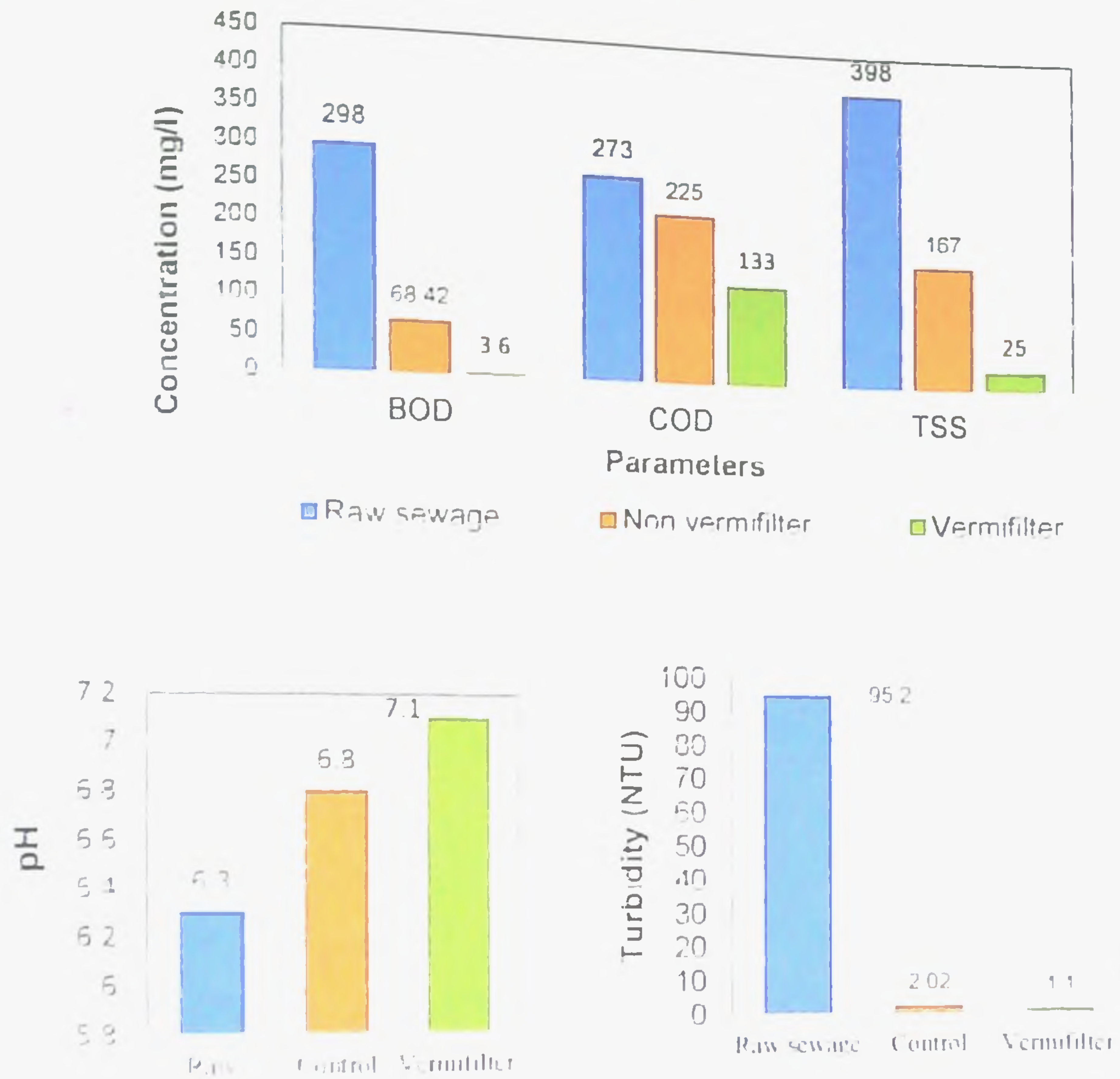


Fig 2: Effect of consortia vermifiltration on BOD, COD, TDSS, pH and turbidity in sewage wastewater treatment

Removal of COD

Results show that the average COD removed from the sewage by earthworms was over 45% while that without earthworms was just over 18% (Chaudhary, 2006)

Removal of TSS

Results shows that the earthworms can significantly remove the suspended solids from the sewage by over 90%, which in the control kit where geological and microbial system works together was over 58% only (Chaudhary, 2006)

Turbidity removal

Results indicate that the average reduction in turbidity by earthworms is over 98% while that without earthworms in the control kit was also significantly high and over 97% (Chaudhary, 2006).

Any wastewater from the households and commercial organizations can be successfully treated by the earthworms and the technology can also be designed to suit a particular wastewater (UNSW ROU, 2002).

DAIRY WASTEWATER

a. Earthworm collection

200 *Eisenia fetida* earthworms were used based on 5000-10000 worms/m^2.

b. Preparation of vermifilter bed

Vermifilter bed made up of different layers of gravel, sand and garden soil with earthworms. The bottom most layer was made of 4-5 cm of aggregate at a depth of 40 mm, above this layer 2-3 cm of aggregate at a depth of 30 mm. Another layer aggregate 1-2 cm size mixed with sand was introduced. The top most layer consists of garden soil with worms at a depth of 120 mm.

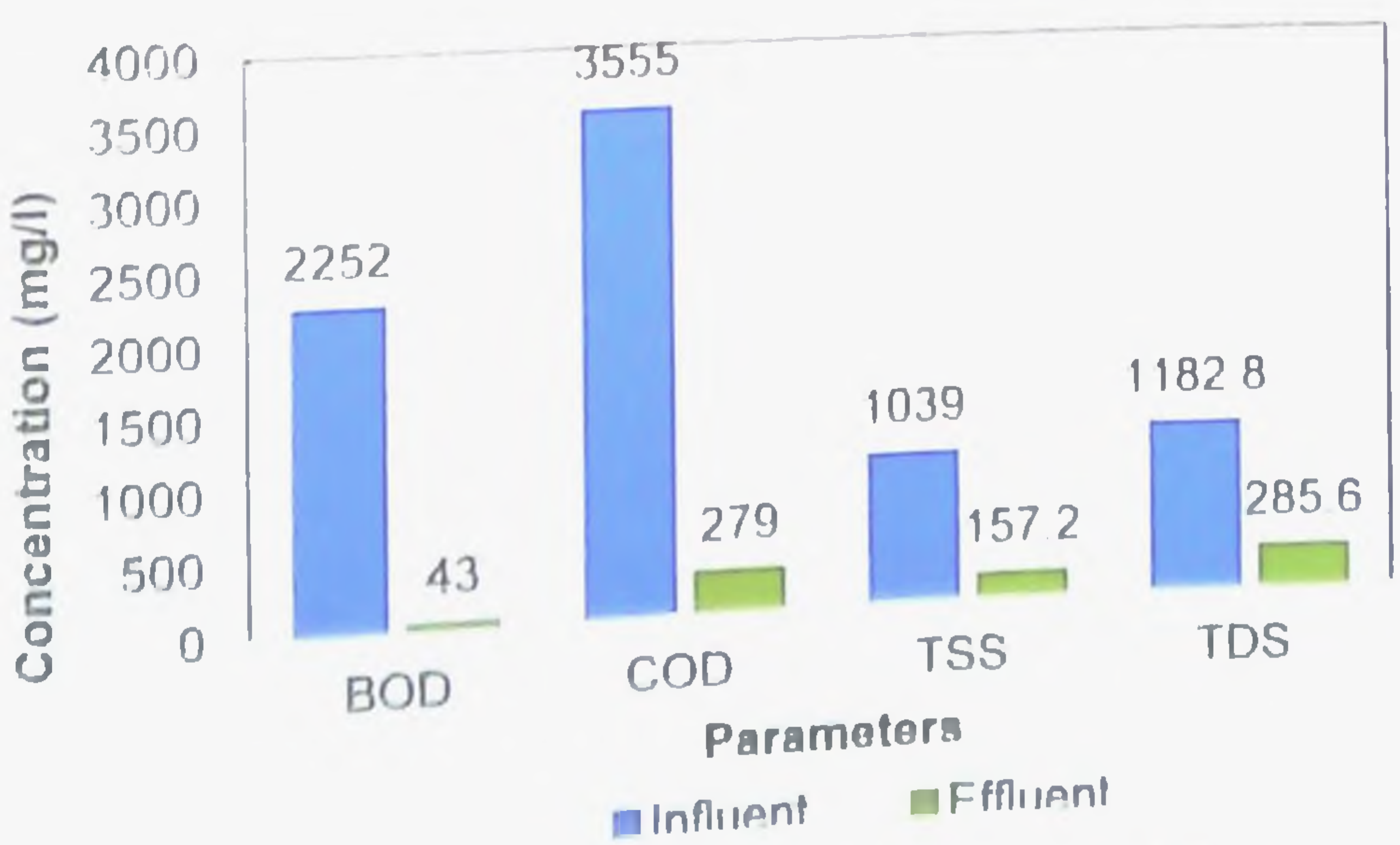
c. Result of this experiment

Variation in pH

No considerable variation was observed between vermi treated wastewater and untreated dairy wastewater. The graph shows an increase in pH of effluent but within limit.

BOD and COD Removal

Results indicate that the overall efficiency of BOD and COD of the treated dairy wastewater from the vermifilter were found to be always greater than 90 % and 85%. The BOD values remaining after vermibiofiltration of the dairy wastewater was acceptable for use of irrigation purpose.



(Manyuchi, M.M. et. al, 2012

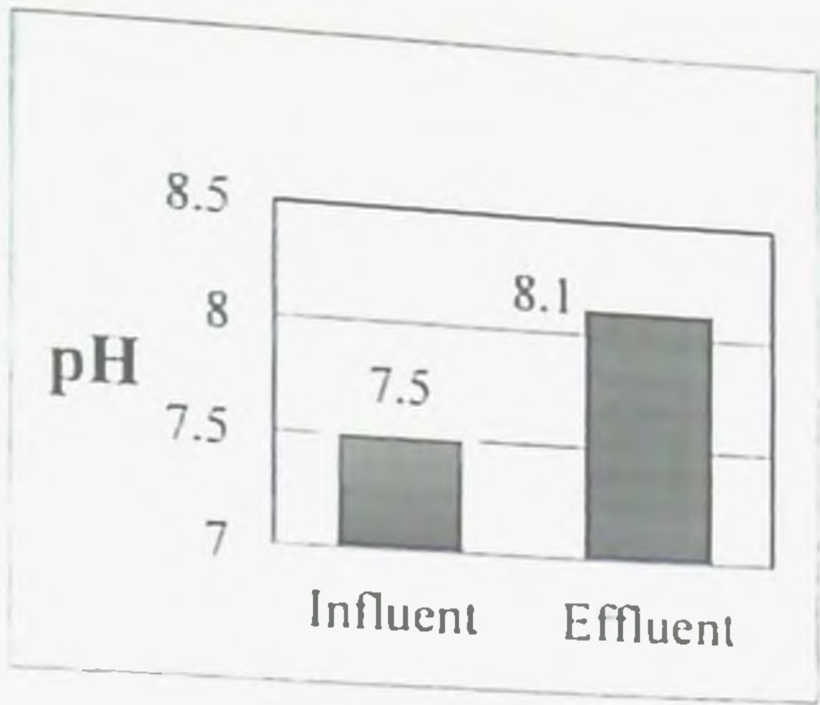


Fig4: **Effect of vermifiltration in BOD, COD, TDSS, pH and turbidity in dairy wastewater**

Removal of TSS and TDS

Earthworms in the vermifilter significantly removed the TDS from the dairy wastewater by about 80-85%. The TSS from the dairy wastewater by about 90-95%. Earthworms consume the solid biomass and clear the path for efficient process conditions and resulted into high removal efficiency of TSS and TDS from wastewater.

Results showed that vermifilter achieves good performance; the results were better than conventional wastewater treatment. The vermifilter treatment was cost effective, 60 to 70% of cost reduction is possible, odour free with the good efficiency of removal of parameter. The BOD, COD, TSS, TDS and Oil &Grease were reduced by 97.95%, 91.64%, 84.27%, 76.39%, and 84.13% respectively.

Integrated microbial-vermifiltration technique for ayurvedic industrial effluents

a. Collection of earthworm species

Eisenia fetida earthworms were collected from the composting units of Kerala Agricultural University (KAU), Mannuthy. Liquid effluents were collected from Ayurveda Industry.

b. Earthworm bed materials

Bedding material consisting of straw, cow dung, vegetable scraps were used after sterilization in the upper most layer of vermicompost.

c. Isolation of enzyme producing strains

Protease and lipase producing strains were isolated from the effluent by standard plating techniques. A protease (*Bacillus* sp.) and lipase (*Bacillus* sp.) producing bacteria were selected.

d. Pre-treatment of the effluent

Experiments were conducted on a lab scale. Each conical flasks were inoculated with protease producing strain (*Bacilllus* sp), lipase producing strain (*Bacilllus* sp), and consortium (Mixture of protease and lipase producing strains) with 1 ml of overnight cultures. Effect of pre-treatment of effluent with consortium and selected strains on reduction of BOD and COD was studied.

e. Vermifiltration of raw effluent

The reservoir was filled with 1l of raw effluent without pre-treatment, and was uniformly distributed at a flow rate of 9.3 l/hr to the surface of the vermifilter set up. The entire system was retained for 2hrs. After 2 hrs the treated water was collected at the bottom outlet. The entire set up was allowed to remain to convert the bedding material into humified vermi compost. The treated water collected at the bottom outlet was checked for its BOD, COD, oil and grease content.

f. Vermifiltration of pre-treated effluent ayurveda wastewater

2L of raw effluent was pre-treated with individual enzyme producing strains and consortium. The reservoir was filled with 1L of pre-treated effluent, and was uniformly distributed at a flow rate of 9.3 L/hr to the surface of the vermifilter set up. The entire system was retained for 2hrs. After 2 hrs the treated water was collected at the bottom of the outlet and checked for its BOD, COD, Oil and grease content.

g. Result

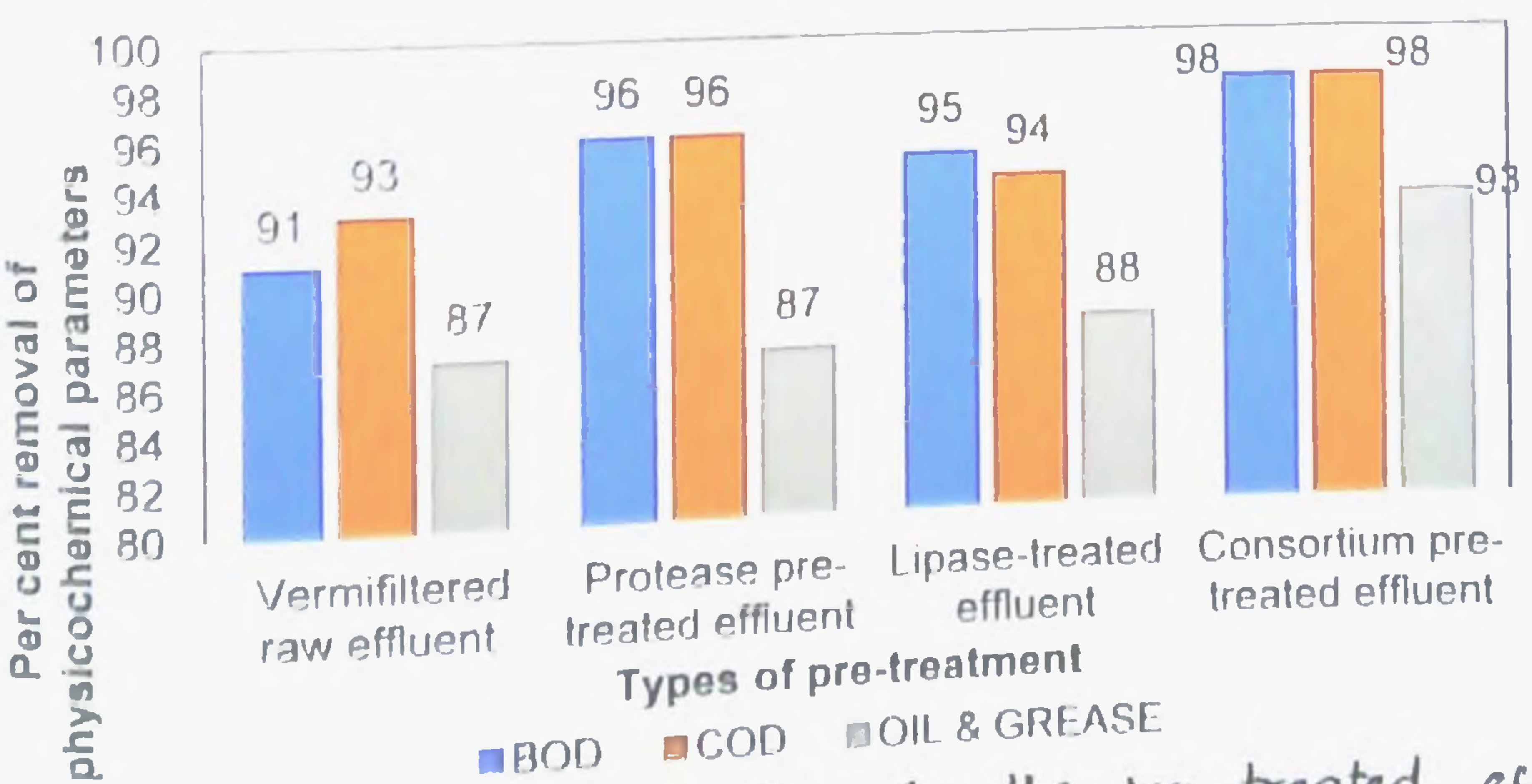
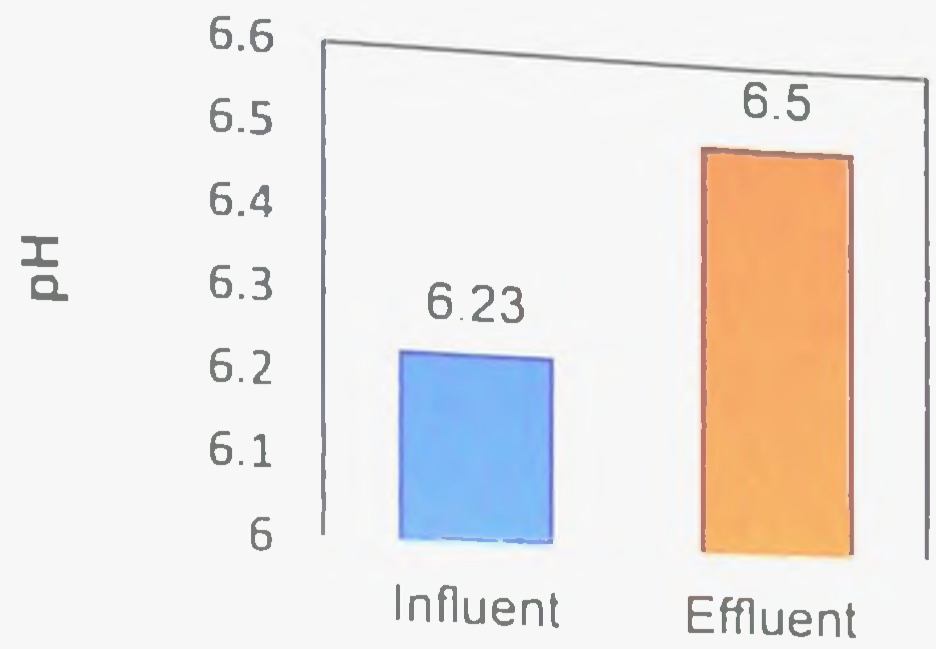


Fig 5: Performance of vermifiltration in the pre-treated effluent

Pre-treatment of the effluent

Ayurvedic effluent was inoculated with protease producing strain (*Bacillus* sp), lipase producing strain (*Bacillus* sp), and consortium (mixture of protease and lipase producing strains) incubated at 37°C. Reduction of BOD and COD by microbial pre-treatment. Protease and lipase action helps in degradation of proteins and lipids present in the effluent and make it readily available for consumption by microbes and earthworms.



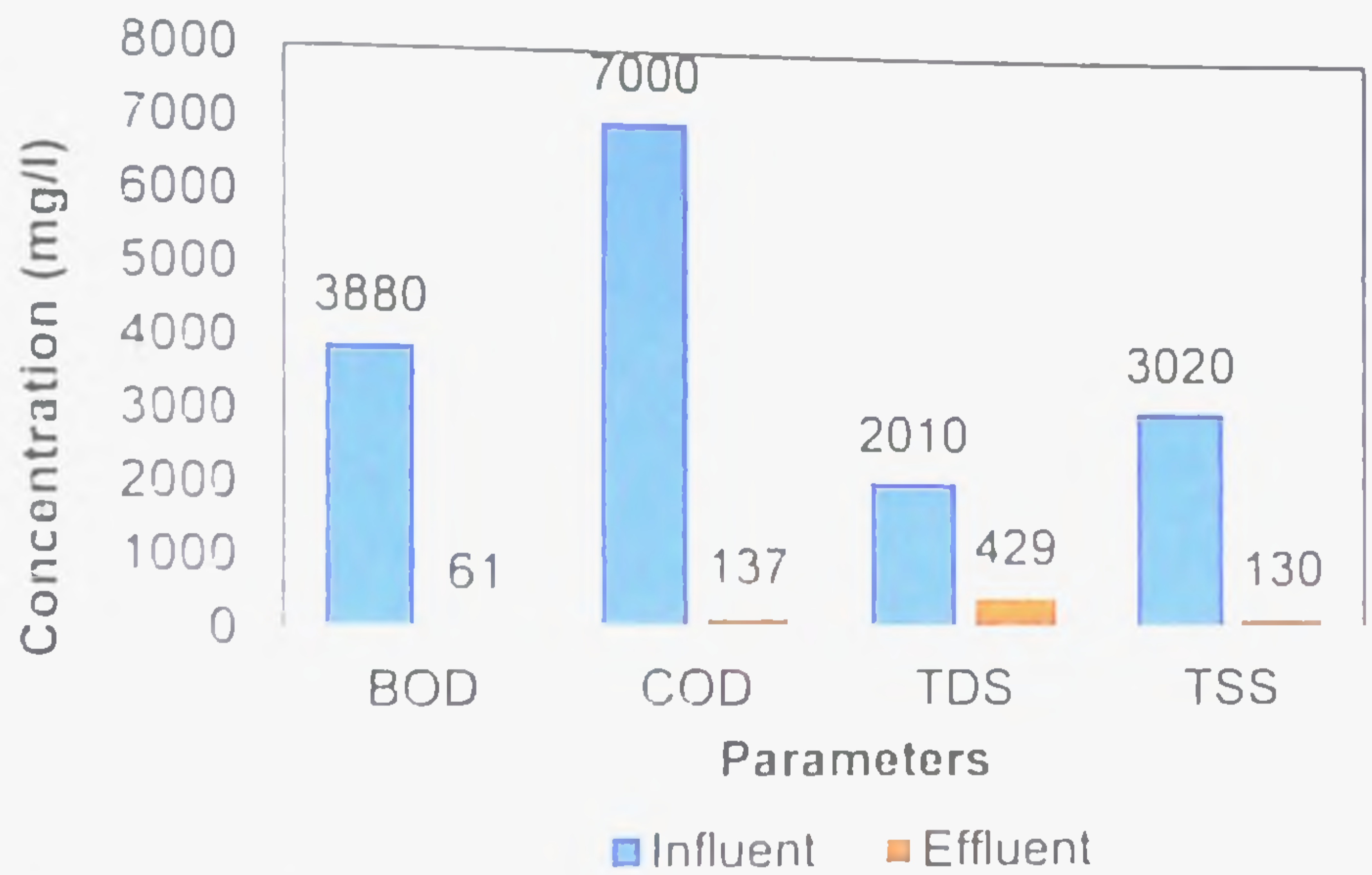


Fig 6: Effect of Integrated microbial - vermifiltration on ayurveda wastewater treatment

Variation in pH value of treated wastewater

Results indicate that the pH value of both raw effluent and treated effluent have an average value of 6.2.

Removal of Total Suspended Solids

Results showed that the microbes and earthworms can significantly remove the suspended solids from the wastewater by over 95%.

Removal of Total Dissolved Solid

Total suspended solids (TSS) and Total Dissolved Solids (TDS) showed drastic reduction during integrated microbial-vermifiltration process. The total reduction in TDS content was about 75% in vermifiltration unit with pre-treatment. Results thus clearly suggested the capability of earthworms to remove solid fractions of wastewater during integrated microbial -vermifiltration processes.

Removal of Biochemical Oxygen Demand (BOD)

The BOD load in effluents vermifiltration unit with pretreatment was significantly lower than initial levels and integrated microbial-vermifiltration showed more removal efficiency. Results show that the earthworms can remove BOD load by over 98.43%.

Removal of Chemical Oxygen Demand (COD)

Results showed that the average COD removed from the wastewater by vermifiltration with pretreatment was over 98.03% indicating degradation of several chemicals by enzymes in the gut of earthworms which is not usually degraded by microbial pre-treatment.

Integrated microbial- Vermifiltration technique for the treatment of ayurveda liquid effluent was developed. It is a decentralized and cost effective method which can be applied to treat both domestic and industrial waste water treatment. A huge reduction in various effluent parameters like BOD, COD, TSS, TDS, Oil & grease, was observed. Presence of heavy metals in ayurvedic effluent was almost zero. Treated effluent well suited the irrigational water quality criteria.

GLOBAL STATUS OF VERMIFILTRATION

Due to its simplicity and cost-effectiveness, vermifiltration of both municipal and industrial wastewater is destined to become a global movement. In Chile, over 100 sewage treatment plants of different sizes, going from individual houses to plants for 12,000 persons and bigger plants for industries are already working. It has been introduced on commercial scale in Mexico and Venezuela (Soto and Toha, 1998).

India and Brazil are also introducing the technology on commercial scale. It is being considered in US, Australia, China, Russia, Vietnam, Cambodia and Zimbabwe too for decentralized treatment of wastewater. In India the TRANSCHEM Agritech Ltd., has also commercialized the technology and installed a vermifiltration plant in Bhavanagar, Gujarat for treatment of 400 KL sewage every day in about 400 sq. m of land transformed into bio-filter (Sinha *et al.*, 2008).

ADVANTAGES OF VERMIFILTRATION TECHNOLOGY

- **Vermi-filtration of wastewater is low energy & efficient system**

  Activated Sludge Process, Trickling Filters and Rotating Biological Contactors which are highly energy intensive, costly to install and operate. In vermifilteration the capital and operating costs are less.

- **No sludge formation**

The sludge is a 'biohazard' and requires safe landfill disposal at high cost. The greatest advantage of vermifiltration system is that there is no formation of 'sewage sludge' (Huges *et. al.*, 2005). The worms decompose the organics in the wastewater and also devour the solids. They feed readily upon the sludge components, rapidly convert them into vermicompost..

- **No foul odour**

Earthworms arrest rotting and decay of all putrescible matters in the wastewater and the sludge.

- **Disinfected and detoxified water fit for non-potable uses**

Vermifiltered water is free of pathogens and toxic chemicals (heavy metals & endocrine disrupting chemicals) and suitable for 'reuse' as water for non-potable purposes.

- **Worms remove endocrine disrupting chemicals from sewage**

Earthworms have also been reported to bio-accumulate 'endocrine disrupting chemicals' (EDCs) from sewage which otherwise is not removed by our conventional sewage treatment plants.

- **Treated water fit for park irrigation**

The vermifiltered water also becomes 'highly nutritive' rich in NKP and other nutrients as the worms release them into water during the process. The water is very suitable for irrigation in parks and golf courses.

DISADVANTAGES OF VERMIFILTRATION TECHNOLOGY

- **Cleaning of the tank**

Because it was a permanent set up for treating wastewater so cleaning of the vermifiltration bed is very critical.

- **Require more tanks for treatment**

Some of the wastewater contain higher amounts of pollution so treating that wastewater it requires more than one tank or bed.

- **Temperature variation**

While degradation of organic and inorganic compounds some heat will be generate this will affect the earthworms activity.

Conclusion

Vermifiltration is a low cost, eco-friendly technology which is the most suitable one for treating wastewater both developed and developing countries.

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Question and answer

1. Which is the largest earthworm species and the type of earthworm species used for wastewater treatment?

   In sirumalai, Dindugal district of Tamilnadu where the largest earthworm species were found. The choosen earthworm for vermifiltration process should be an epigeic in nature.

2. Cost of earthworm?

   Cost of earthworm will be around Rs. 1200/kg

3. Whether pH can affect the earthworm activity?

   No, pH cannot affect the earthworms activity. Because, earthworms are present in the pH 4 to 8.5

4. What does endocrine disrupting chemicals mean?

   This the toxic chemicals which are present in the sewage wastewater which cannot be removed through conventional treatment. Because this chemicals will present in our usage water means it will affect the endocrine gland functions.

5. For what purpose the coal cinder and convertor slag mix will be used for P contaminated wastewater?

   Because, the excellent phosphorus removal efficiency of converter slag-coal cinder filters can be attributed to the excellent phosphorus adsorption from the wastewater.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Microbiology

MICRO 591: MASTER'S SEMINAR

Name: R. Sri Vithya

Admission number: 2014-11-229

Major advisor: Dr. K. Surendra Gopal

Venue: Seminar Hall

Date: 29.01.2016

Time: 11.30 am

Vermifiltration – a low cost sustainable technology for wastewater treatment

Abstract

Wastewater treatment is the biggest problem in many developing countries, due to high establishment and running cost of a wastewater treatment units. Majority of urban areas in developing countries dispose the wastewater without any treatment. So, they need to treat wastewater at a low cost without any negative impact on the environment.

Vermifiltration is a low cost and eco-friendly technology over conventional systems with immense potential for decentralization in rural areas (Taylor *et al.*, 2003). It was first proposed by Jose Toha in 1992 (Bouche and Qiu, 1998). The term vermifiltration indicates utilization of earthworms in the wastewater treatment. Its body works as a bio-filter and enhances the microbial action through increasing the microbial population. It hosts millions of decomposer aerobic bacteria, enzymes and hormones in their guts and intestine (Sinha *et al.*, 2008).

Vermifiltration bed consists of multilayers namely soil, sandy and gravel layer arranged one below the other. The gravel layers will act as trickling filter of the conventional system. The earthworms will be introduced in the top layer of the vermifilter bed. The earthworms are introduced in the bed at the rate of 8,000-10,000/m² area of the bed. The Indian blue worms are used in large scale units and for the small scale, tiger worms are used (Pathania *et al.*, 2007).

Earthworm can reduce the organic and inorganic contaminants, pathogens and toxic chemicals through ingestion, biodegradation and also adsorption through their body walls. It will intensify the stabilization through soil layer by granulating the clay particles and grinds the silt and sand particles which enhance the surface area of the vermifiltration bed. The Hydraulic Loading Rate, Hydraulic Retention Time and filter media will affect the vermifiltration unit by reducing the earthworm activity.