MANAGEMENT OF *PHOMOPSIS* BLIGHT AND FRUIT ROT OF BRINJAL (*SOLANUM MELONGENA* L.)

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(2009 – 11 – 152)

THESIS

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DEPARTMENT OF PLANT PATHOLOGY
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2011

DECLARATION

I hereby declare that this thesis entitled '**Management of *Phomopsis* blight and fruit rot of brinjal (*Solanum melongena* L.)**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Dedicated to

The Farming Community

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LIST OF ABBREVIATIONS

%	-	Per cent
°C	-	Degree celcius
µm	-	Micrometre
@	-	At the rate of
CD	-	Critical difference
cfu	-	Colony forming units
dia.	-	Diameter
et al.	-	And others
Fig.	-	Figure
g	-	Gram
h	-	hour
ha	-	Hectare
i.e.	-	That is
Kg	-	Kilogram
L	-	Litre
min.	-	Minute
ml	-	Millilitre
mm	-	Millimeter
ppm	-	Parts per million
rpm	-	Revolutions per minute
sp.	-	Species (singular)
spp.	-	Species (Plural)
t	-	Tonnes

Mts	-	Metric tonnes
viz.	-	Namely
w/v	-	Weight/volume

Introduction

1. INTRODUCTION

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. The name brinjal is popular in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties which are white and resemble in shape to chicken eggs. It is also called aubergine (French word) in Europe. Eggplant is a semitropical/tropical plant originating in Asia and India. Before it gained in culinary popularity eggplant was also termed "mala insana," which translates to "mad apple," owing to the belief that eating eggplant would lead to madness (Kemble et al., 1998). Brinjal is described as the 'King of vegetables' due to it's versatality in use in Indian food (Choudhary and Gaur, 2009). Brinjal is of much importance in the warm areas of Far East being grown extensively in India, Bangladesh, Pakistan, China and Philippines. Bitterness in eggplant due to the presence of glycoalkaloids in the Indian commercial cultivars varies from 0.37 mg/100 g fresh weight to 4.83 mg (Bajaj et al., 1981).

COMPOSITION AND USES OF BRINJAL

Eggplant has been a common vegetable in our diet since the ancient time. Its composition per 100 g of edible portion is given below (Chen and Li, 2009):

Calories	24.0	Sodium (mg)	3.0
Moisture content (%)	92.7	Copper (mg)	0.17
Carbohydrates (%)	4.0	Potassium (mg)	2.0
Protein (g)	1.4	Sulphur (mg)	44.0
Fat (g)	0.3	Chlorine (mg)	52.0
Fiber (g)	1.3	Vitamin A (I.U.)	124.0
Oxalic acid (mg)	18.0	Thiamine (mg)	0.04

Calcium (mg)	18.0	Riboflavin (mg)	0.11
Magnesium (mg)	16.0	B-carotene (ug)	0.74
Phosphorus (mg)	47.0	Vitamin C (mg)	12.0
Iron (mg)	0.9		

Brinjal is known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik, 1993). The global area under brinjal cultivation has been estimated as 1.85 million ha with total production of brinjal fruit of about 32 million Mts (FAO data, 1995). India accounts for about 8.7 million Mts with an area of 0.53 million ha under cultivation. In 2007-08, 34 million kg worth of Rs. 19 million was exported mainly to UK, Netherland, Saudi Arabia and Middle East countries (DGCIS, 2008). Brinjal occupies 928 ha in Kerala specifying 64 ha area in Thiruvananthapuram district (Farm Guide, 2011).

But the area under this crop is very low owing to many biotic and abiotic stresses. Among the biotic stresses, leaf blight and fruit rot caused by *Phomopsis vexans* is a major constraint. The disease occurs when spores are released from a fungal fruiting body (pycnidia) and dispersed by splashing rain, insects and contaminated equipment. Spores germinate rapidly when free moisture is present on leaves, stems or leaves. The fungus survives in crop debris, seeds and soil. *Phomopsis* blight and fruit rot can cause disease on eggplant leaves, stems, and fruit. and also causes seedling damping off. Leaf spots first appear as small (less than 0.4 inches) grey to brown lesions with light centers. Lesions often become numerous and cover large areas of leaves. Severely infected leaves torn become yellow and wither. Small black dots, the pycnidia are often apparent on older leaf, stem and fruit lesions. If a canker develops at the base of a stem, it can girdle and kill the stem. Fruit lesions are sunken, discolored and soft with a surrounding margin of black fruit bodies. If conditions become dry, infected fruit become shrivelled, dry and form black

mummies (Schwartz and Gent, 2007). *Phomopsis vexans* is a pycnidial fungus with an apparent sexual form in the genus *Diaporthe*, easily seedborne and producing large numbers of conidia. It causes disease in *Solanum melongena*, its only significant host, ranging from poor seed germination and damping off of seedlings to leaf and stem lesions and to fruit rot, both in the field and after harvest (Chalkley, 2010). Temperature of 25^0C, p^H 4.0 to 9.0 favoured growth and pycnidial formation. The pathogen was internally as well as externally seed borne and was transmitted to the seedlings. The inoculum on stem and fruits survived for 24 months while inoculum on leaves survived only for two months in field.

Being a vegetable crop, exclusive dependence of chemical methods of management of diseases is not always advisable (Campbell, 1989). This may lead to residue and persistence problems, risk of ground water pollution, death of non target beneficial flora and fauna and evolution of fungicide resistant pathogen population. In this context, the present investigation is carried out with the objective to develop a compatible, effective, economic and environmentally sound management practice for the control of this disease.

The main objectives of the investigation are:

- Study on the symptomatology of fruit rot of brinjal.
- Identification of the pathogen of the disease based on colony and conidial morphology.
- Growth studies of pathogen on different solid and liquid media, carbon sources, nitrogen sources and p^H.
- Isolation of antagonists of pathogen from phyllosphere and rhizosphere of healthy brinjal plants.
- *In vitro* screening of antagonists against the pathogen.
- *In vitro* screening of plant extracts against the pathogen.

- *In vitro* screening of oil cakes against the pathogen.
- To evolve a management practice for the blight and fruit rot of brinjal using biocontrol agents, plant extracts and oil cakes.

Review of Literature

2. REVIEW OF LITERATURE

Brinjal or Eggplant (*Solanum melongena* L.), an important vegetable crop belonging to the family Solanaceae, is grown for its fruits. Eggplant is the most important vegetable crop. Such a potential vegetable crop is known to suffer from many diseases and among them seedling diseases are very important. Seedling diseases are damping off and seedling blight caused by *Phomopsis vexans*, *Fusarium* sp, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici* etc. These diseases cause crop loss up to 30-50 per cent affecting eight million farm families involved in eggplant cultivation (Masuduzzaman et al., 2008). Phomopsis blight is an unsightly disease that not only harms eggplants but also makes them inedible and unmarketable. It is caused by a fungus *Phomopsis vexans*. This disease is equivalent to canker sores on human.

2.1. History, distribution and yield loss

Eggplant is probably a native of India and has been in cultivation for a long time. Vavilov (1928) considered its centre of origin as in the Indo-Burma region. It originated in India but has a secondary center of variation in China. In China, eggplant has been known for the last 1500 years (Chen and Li, 2009). Along with tomato and onion, brinjal is the second most important vegetable after potato in India. The market share for vegetables produced in India is as follows: 26.7 per cent potato, 8.6 per cent tomato, 8.5 per cent onion, 8.4 per cent brinjal, 7.3 per cent tapioca (cassava), 5.4 per cent cabbage, 4.8 per cent cauliflower, 3.4 per cent okra and 23.8 per cent others in the production share of vegetables in India in 2005 - 06. It is estimated that per capita consumption of vegetables in India is only about 190 gm per day which is far below the minimum dietary requirement of 280 gm per day per person (Rajya Sabha, 2006). Annually India consumes around 8-9 million Mts of brinjal which is produced on 5, 50,000 ha in different parts of

the country. After China, India is the second largest producer of vegetables in the world (Choudhary and Gaur, 2009). In India, Panwar et al. (1970) reported that the losses due to *Phomopsis* fruit rot ranged to the extent of 10-20 %. This pathogen causes over 50 per cent losses in production and productivity in various parts of the world (Akhtar et al., 2008).

Chalkley (2010) reported that the fungus has been widely distributed in areas of most continents, but only a few of those are in Europe and Africa. Thippeswamy et al. (2006) reported that among 145 brinjal seed samples collected from different agro-climatic regions of Karnataka, India during 2001-03, when analysed for mycoflora found that this crop is susceptible to *Phomopsis* blight (*Phomopsis vexans* [*Diaporthe vexans*]) and leaf spot (*Alternaria solani*) diseases. These are seed borne fungal diseases and reduce the yield up to 30-50 per cent. *Phomopsis vexans* and *Alternaria solani* caused 1-10 per cent spots in one month old and two month old seedlings and 20-30 per cent in three month old plants. In India fruit rot of brinjal caused by *Phomopsis vexans* was first reported in Gujarat by Harter in 1914. A description of *Phomopsis vexans (Diaporthe vexans),* its transmission, geographical distribution and host on *Solanum melongena* L. *was* given by Punithalingam and Holliday (1972). Kumar and Sugha (2004) reported 37 isolates of *Phomopsis vexans* infecting brinjal. Variability study of *Phomopsis vexans* causing blight in brinjal revealed significant and substantial differences in radial growth of the isolates on PDA (Akhtar and Chaube, 2006).

Seed transmission may explain the broad historical distribution of *Phomopsis vexans* but limitation of its host range to a non staple vegetable crop can allow for its avoidance and eradication by cultural methods. As a result, it does not appear often on lists of restricted pathogens, even though it may cause yield losses of more than 50 % (Chalkley, 2010). In Louisiana, USA in 1921, at least 50 per cent yield reduction was observed in eggplant crops due to infection in the field as reported by

Edgerton and Moreland (1921). Later, Martin (1930) in USA and Nolla (1929) in Puerto Rico also reported losses of 50 per cent or more due to Phomopsis blight in aubergines. In India, the yield losses due to fruit rot ranged from 10-20 per cent in Punjab and Delhi (Panwar et al., 1970). In an advanced stage of disease, seed quality is also adversely affected and infected seed becomes discoloured with poor germinability and reduced seed viability (Toole et al., 1941; Porter, 1943; Vishunavat and Kumar, 1993). Seed infection results in pre emergence and post emergence damping off of seedlings; approximately one third of the plants were lost at each stage as reported by Kaushel and Kumar (1995). *Phomopsis vexans* has been reported from many areas in the warmer parts of most continents but is unknown in Europe except in Romania (Smith et al., 1988) and known in only a few African countries. It is probably native to southern Asia the area of origin of the host *Solanum melongena* (Prance and Nesbitt, 2005) where it is also reported to infect some wild *Solanum* species (Datar and Ashtaputre, 1988). It is readily transmitted in and on the seed (Porter, 1943; Vishunavat and Kumar, 1993) of a crop that is only grown in limited areas may explain its lack of a continuous distribution in the tropics and subtropics. The fungus could be introduced to a region within a seed lot but then die out if its presence discouraged continuous local cultivation of eggplant (Chalkley, 2010).

Spegazzini (1881) described a fungus occurring on leaves of *Solanum melongena* in Italy as *Phyllosticta hortorum*. Halsted (1892) reported the same fungus on leaves and fruits of eggplants in New Jersey, USA, as *Phoma solani*. In 1905, Smith observing septate conidia in the USA proposed the name *Ascochyta hortorum* instead of *P. hortorum*. In Italy, Voglino (1907) studied a fungus on aubergine and agreed with Smith concluding that the fungus described by Spegazzini as *P. hortorum* was an *Ascochyta*. Cross inoculation tests and morphological studies indicated to Harter (1914) that *Phoma solani* and *Phyllosticta hortorum* were the same species. He also concluded that the genus to which the fungus belonged was

not *Phoma*, *Phyllosticta* or *Ascochyta* but *Phomopsis*. Unlike the previous workers, Harter observed and described the beta conidia (stylospores) as characteristic of the genus. He proposed the name *Phomopsis vexans* for the fungus and Spegazzini agreed that the American isolates were different from *Phyllosticta hortorum* (Harter, 1914). While the coelomycete on eggplant that produces both alpha and beta conidia is a true *Phomopsis* (Uecker, 1988), the species *Phoma hortorum* Speg. and *Ascochyta hortorum* (Speg.) recently has been synonymized with *Phoma exigua* Desm. var. *exigua*, a weak pathogen of many plants that may be found in older lesions caused by other fungi (Boerema et al., 2004). Gratz (1942) observed perithecia on two per cent potato dextrose agar in culture and assigned the name *Diaporthe vexans*. The current view is that *Diaporthe vexans* is the teleomorph of *Phomopsis vexans* (Cannon and Simmons, 2002). Nevertheless, although the known connections of some *Phomopsis* species are to sexual forms in the genus *Diaporthe,* the name *D. vexans* (Sacc. & Syd.) Gratz is illegitimate because Gratz did not provide a description in Latin of the new species (Punithalingam and Holliday, 1972). Species concepts in *Phomopsis* furthermore have often been based on host specificity but the phylogeny based on molecular data obtained so far indicates that either species have broader host ranges or significant changes 'jumps' between hosts have occurred in species evolution. Host debris and seed from infested fruit are primary sources of inoculum. Naturally infected seed germinates less well and more slowly (Punithalingam and Holliday, 1972)

2.2. Symptomatology

Phomopsis blight is caused by the fungus *Phomopsis vexans* and can infect above ground plant parts at all stages of development. Generally spots first appear on seedlings shortly after they emerge. Dark sunken lesions (cankers) form on the stem slightly above the soil line. Eventually these cankers encircle the stem resulting in the collapse and death of the plant. Leaf spots can occur at any time during the

season though older leaves are most susceptible. Spots are circular with a distinct narrow brown margin about one inch in dia. Lesions are typically grey to brown developing a light colored centre as they age. Black, pimple like structures develop in the centre of old lesions; these are pycnidia which are the fungal reproductive structures where spores are produced. These can easily be seen with a hand lens or magnifying glass. Diseased leaves may turn yellow and drop prematurely. Lesions can also form on stems and branches. Fruit spots are similar to those on leaves but are much larger leaving diseased fruit unmarketable. Symptoms first appear as pale sunken circular to oval areas on the fruit surface. These later turn brown and enlarge up to two to three inches in dia.; often two or more lesions merge to cover much of the fruit surface. Affected fruit become soft and watery at first. Decay may penetrate rapidly throughout the fruit causing a light brown discoloration of the flesh. Under dry conditions fruit shrivel and become mummified. Black pycnidia arranged in a concentric, target like pattern can usually be seen in the centre of fruit lesions (Kemble et al., 1998).

Fungi cause spots on leaves and fruits of eggplant (Schlub and Yudin, 2002) but the large dark *Phomopsis* pycnidia produced in the lesions are distinctive. *Phoma exigua*, which may colonize the lesions as well produces only small ellipsoid conidia some of which may be septate (Boerema et al., 2004). Fruit rot due to *Phomopsis* is more likely to begin at the top from infection of the calyx (Edgerton and Moreland, 1921). Chalkley (2010) reported the existence of several pycnidial fungi causing leaf spots on *Solanum melongena* L. resulting in difficulties with the identification of each one. Infection is easily visible in the field on close examination of leaves, stems and fruits; characteristic conidiomata appear as black pinhead sized structures which are often concentrically arranged on fruits. Infected fruits are soft and mushy or mummified and black.

The symptoms range from poor germination and seedling blight to fruit rot. Post emergence damping off of seedlings results from infection of the stem just above the soil surface. The symptoms on leaves are more prominent during the early stages of plant growth. The lesions first are small more or less circular and buff to olive later becoming cinnamon buff with an irregular blackish margin (Pawar and Patel, 1957). Irregular spots result from coalescence. After transplanting leaves coming in contact with the soil may become infected directly or develop leaf spot due to infection by conidia. Lesions on the petiole or the lower part of the midrib can result in death of the entire leaf. Affected leaves may drop prematurely and the blighted areas become covered with numerous black pycnidia. On stems and branches elongated blackish brown lesions are formed eventually containing pycnidia. The diseased plant bears smaller leaves and the axillary buds are often killed. When stem girdling occurs the shoot above the infected area wilts and dries up and the plant may be toppled by the wind (Edgerton and Moreland, 1921; Pawar and Patel, 1957). Pycnidia develop readily in lesions on young stems but rarely on older ones (Harter, 1914).On the fruits the symptoms appear first as minute sunken greyish spots with a brownish halo which later enlarge and coalesce producing concentric rings of yellow and brown zones. These spots increase in size and form large rotten areas on which conidiomata often develop concentrically covering most of the rotten fruit surface. Pycnidia on fruit are larger than those on stems and leaves (Harter, 1914). If the infection enters the fruits through the calyx the whole fruit may become mummified due to dry rot (Pawar and Patel, 1957). Rot may appear in fruit in transit after harvest.

Only economic host is eggplant or brinjal (*Solanum melongena*) and the disease is variously known as tip over, leaf blight or leaf spot, fruit rot, stem blight or canker and damping off. Leaf spots (up to 3 cm dia.) are conspicuous irregular in outline and may coalesce; lower leaves may be affected first. In stem lesions the cortex dries and cracks. Plants become stunted and girdling cankers cause death.

Fruit spots are pale, sunken, conspicuous and may affect the whole fruit; fruit may drop or remain attached, becoming mummified after a soft decay. Pycnidia are abundant (Punithalingam and Holliday, 1972).

2.3. Pathogenicity

Phomopsis vexans was predominantly isolated from the infected plant parts. During pathogenicity three distinct types of symptoms were produced on plant *i.e.* leaf spot (blight), fruit rot and stem blight. In seedling stage under artificial inoculation damping off and collar rots were produced. Inoculation on injured, on lower surface of leaf developed the disease more readily.

Lou et al. (2006) reported that a spore suspension of 10^6 spores/ml of *Phomopsis vexans* collected from PDA cultures was used to spray inoculate potted *Ilex crenata* Thunb. var. *convexa* plants that were kept for 48 h under a polyethylene sheet cover and grown at 22-25°C in a greenhouse which after 5 to 10 days when inoculated trees showed symptoms resembling those seen in nature. The same fungus used for inoculum was re isolated from the margins of necrotic tissues of inoculated plants but not from controls sprayed with sterile water. *Phomopsis vexans* has been previously recorded on eggplant (*Solanum melongena*) in China but this is the first report of its occurrence on *Ilex creanata* var. *convexa.*

Selfed seedlings of nine field tolerant lines along with two field susceptible lines were grown in plastic trays and inoculated with a spore concentration of $5x10^5$ spores/ml after 21 days and at seven days thereafter and proved Koch's postulate (Hazra et al., 2006). Mature leaves selected at random from the lower nodes of seedlings/mature plants were matched with standard area diagram and visually scored in a six-point scale. Disease severity (per centage of leaf area spotted by the disease) was estimated semiquantitatively by comparing the leaves with standard area diagram of *Phomopsis* leaf blight.

2.4. Disease assessment rating

After the onset of infection, disease severity (the area or proportion of plant tissue affected) was estimated semiquantitatively at 15 days intervals utilizing the standard area diagram. According to 1slam and pan (1993) the infection grades (fig. 1) used for visually estimated spot areas were:

0 = no infection.

1 = > 0 - ≤ 1.0.

2 = > 1 - ≤ 10.0.

3 = > 10.0 - ≤ 25.0.

4 = > 25.0 - ≤ 50.0.

5 = > 50.0.

2.5. The Pathogen, *Phomopsis vexans*.

Phomopsis blight in brinjal is caused by *Phomopsis vexans* (Sacc. & Syd.) Harter

Teleomorph: *Diaporthe vexans* Gratz

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

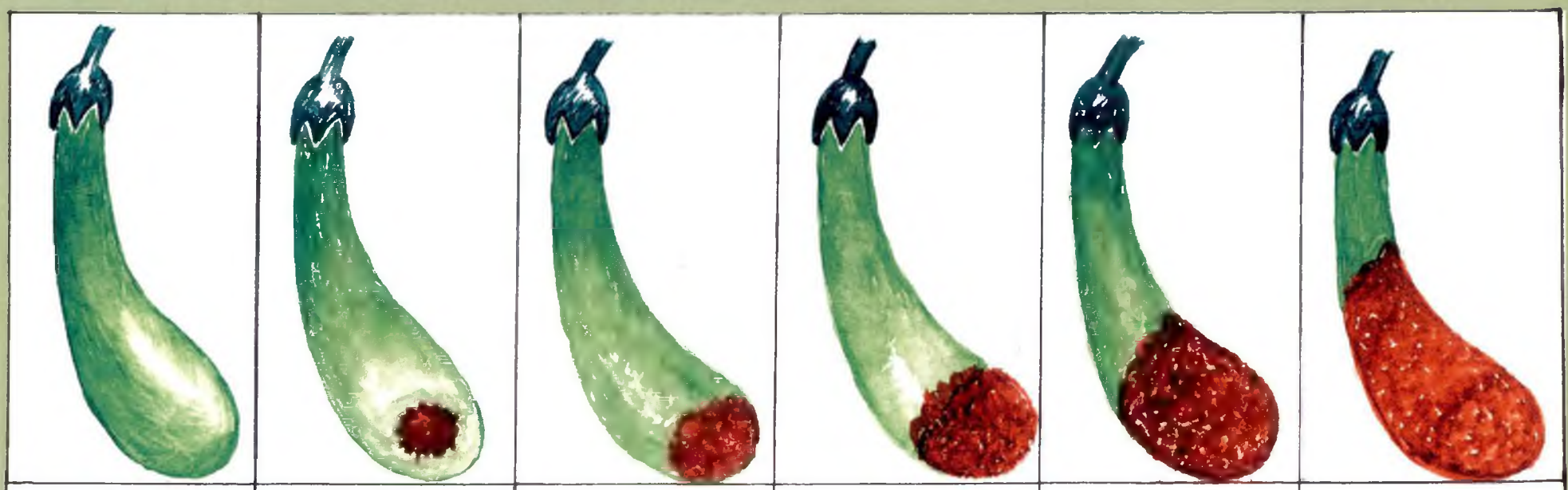
Subclass: Sordariomycetidae

Order: Diaporthales

Family: Diaporthaceae

Genus: *Phomopsis*

Fig : 1 DISEASE SCORE CHART OF BLIGHT & FRUIT ROT IN BRINJAL INCITED BY PHOMOPSIS VEXANS (ISLAM & PAN, 1993)



0	1	2	3	4	5
NO INFECTION	≤ 0 to 1 %	>1 to ≤10	>10 to ≤25	>25 to ≤50	>50

Species: *vexans*

Apparently *Phomopsis vexans* is the serious pathogen on *Solanum melongena*, however it has also been reported on multiple genera of Solanaceae as well as reports on *Acacia* sp. (Fabaceae), *Prunus sp.* (Rosaceae), and *Sorghum bicolor* (Poaceae). The *Phomopsis* spp. is found during all seasons and have been mostly found on woody host plants. Twigs up to one cm in dia. are the most common substrate. Species including *P. actinidiae, P. coneglanensis, P. juglandina, P. occulta, P. pulla, P. sarothamni* and *P. vepris* have only alpha type conidia and both types of conidia are found in *P. oncostoma, P. occulta, P. ramaelis* and *P. revellens*. Teleomorphs of some species were in some cases found on the same twigs as their anamorphs (*Phomopsis quercinum* and *Amphiporthe leiphaemia*; *Phomopsis revellens* and *Diaporthe eres; Phomopsis detrusa* and *Diaporthe detrusa*). Ali and Saikia (1993) described ten species of *Phomopsis*, three of which (*P. clerodendrumii, P. peperomiae* and *P. zingiberii*) recorded on *Clerodendrum splendidum, Peperomia* sp. and *Zingiber officinale* (ginger) respectively are new species. The following species have not been reported in India before: *P. corchoricola, P. phaseoli, P. tersa* and the *Phomopsis* state of *Diaporthe phaseolorum* var. *batatatis*. Additional features of *P. capsici, P. palmicola* and *P. vexans* recorded on *Capsicum annuum, Areca catechu* (arecanuts) and *Solanum melongena* (aubergines) respectively are also described by them.

2.6. Important species of *Phomopsis*

- *P. asparagi, P. asparagicola* and *P. javanica* : *Phomopsis* blight of asparagus
- *P. vexans* : *Phomopsis* blight of aubergine
- *P. cucurbitae* : Purple stem of cucurbits
- *P. sclerotioides* : *Phomopsis* black stem of cucurbits
- *P. viticola* : Cane and leaf spot of grapes
- *P. obscurans*: Leaf blight of strawberry

- *P. cocoina:* Leaf spot of coconut
- *P. psidii*: Stylar end rot of guava
- *P. vexans*: *P. elata*

2.7. Morphological and cultural characters of the pathogen

2.7.1. Morphological characters

2.7.1.1. Colony characters

Akhtar (2006) collected infected aubergine plants showing typical disease symptoms caused by *Phomopsis vexans* infection from Pantnagar (Uttaranchal) and several other locations including Kanpur, Lucknow, Bareilly and Mau (Uttar Pradesh), India and after routine isolation and purification of the pathogen single spore cultures were obtained and isolates from each location was designated as Pv 10, Pv 11, Pv 16, Pv 25 and Pv 36. The growth and type of colonies, diameter, zonations, production of biomass, pycnidial shape, location and distribution of all the five isolates grown on PDA were recorded and he repoted that three of the isolates exhibited aerial, one sub-aerial colonies and one isolate produced appressed colonies, two of the isolates produced spherical pycnidia while the remaining three isolates produced flask shaped pycnidia. Pv 11 and Pv 16 had scattered type of pycnidia distribution while Pv 36 exhibited pycnidia distribution at and around the point of inoculation. Pycnidia rarely developed in Pv 10 and Pv 25. He also observed that in majority of the isolates pycnidia were located on the colony surface and the morphological features of conidia also varied among the isolates.

On PDA, the fungus had white floccose mycelium and produced numerous black, globose to irregular pycnidia (up to 300 µm). Alpha conidia were one celled, hyaline and ellipsoidal (4.5-10.3 µm long × 1.8-2.1 µm wide); beta conidia were one celled, hyaline, filiform and straight or curved (16.8-27.5 µm long × 1.0 µm wide). A

pure culture was identified as *Phomopsis vexans* (Sacc. & Syd.) Harter based on morphology and host specificity. This is the second report of *Phomopsis vexans* on apricot since the disease was cited in Algeria by Bello and Sisterna (2000).

Islam and Pan (1990) collected a total of 16 isolates of *Phomopsis vexans* from diseased aubergines from various locations in India and compared their morphological characters, growth rate, sporulation and pathogenicity. They reported that isolates varied in colony dia. (69-94.33 mm), dry weight (436.66-642.66 mg) and relative abundance of pycnidia production. Variations were also observed in colour, texture, zonation and arrangement of pycnidia in the colonies. Pathogenicity also varied with isolates.

2.7.1.2. Morphology of Conidium

There are very few reports on conidial physiology of pathogen (Kumar, 1997). Numerous pycnidia were observed in the dead cortex of twigs and branches. A fungus isolated on PDA from symptomatic tissues had a white floccose mycelium and produced black, globose to irregular pycnidia up to 290 µm in dia. Alpha conidia were one celled, hyaline, and ellipsoidal (5.5-9.05 µm long x 1.9-2.3 µm wide); beta conidia were one celled, hyaline, filiform and straight or curved (19.9-28.2 µm long x 0.95-1.32 µm wide). The fungus was morphologically identical to *Phomopsis vexans* (Sacc. & Syd.) Harter (Punithalingam and Holliday, 1972).

Diaporthe vexans (Sacc. & Syd.) Gratz 1942:

Pycnidia subepidermal, erumpent, dark, thick-walled, flattened to globose, varying in size often 100-300 µm dia. with or without a beak; beak measuring 76 µm. Phialides hyaline simple or branched sometimes septate 10-16 µm long arising from the innermost layer of cells lining the cavity. Alpha conidia hyaline, aseptate, sub cylindrical, 5-8 x 2-3 µm. Beta conidia filiform, curved, hyaline, septate, 18-32 x

0.5-2.0 µm, non germinating. Hyphae hyaline, septate, 2.5-4 µm dia. (Singh, 1987). Perithecia in culture usually in clusters, 130-350 µm dia., beaked; beaks sinuous, carbonaceous, irregular 80-500 µm long. Asci clavate, sessile, 24-44 x 5-12 µm, eight-spored. Ascospores biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, one septate constricted at the septum, 9-12 x 3-4.5 µm (Gratz, 1942).

Kumar and Sugha (1999) described *Phomopsis vexans* (Perfect stage: *Diaporthe vexans*), as an incitant of leaf blight and fruit rot of brinjal is reported to produce alpha and beta conidia. Various studies revealed that formation of conidia in pycnidia of *P. vexans* is temperature dependent. At 10–16°C the pathogen produces beta conidia and at 25-28°C, the alpha conidia. These two forms of conidia get inter converted when subjected to a specific temperature. They proved from these studies that alpha and beta are two forms of the same conidium; *P. vexans* produced only one type of conidia in its pycnidia which are hyaline, one celled, sub cylindrical and 5-9 x 2-2.8 µm in size during summer months which gradually changed into the beta form. Inoculation of host plants with beta conidia caused intraveinal necrosis which progressed towards the leaf base and resulted in premature defoliation thus indicating their role in pathogenesis. Isolations from such leaves produced pycnidia with alpha conidia at 25°C and beta conidia at 16°C.

The morphological features of conidia varied among the isolates. The shape was either elliptical (Pv 11) or sub cylindrical. The number of guttulae in Pv 10 and Pv 36 was two, in Pv 16 was 2-3 and in Pv 11 and Pv 25 was one. The smallest alpha conidia was recorded in Pv 25 (6.9 x 1.8 µm) and the largest (12.0 x 3.0 µm) in Pv 10. On the other hand the extent of variation in stylospore morphology was relatively high. Sickle shaped stylospores were observed in one isolate and curved in two isolates. Stylospores that were straight and slightly curved at one end were recorded in Pv 10. Filiform stylospores were observed for Pv 16. The size of beta conidia ranged from 17.50 x 0.8 µm-27.5 x 1.25 µm. Minimum spore counts was recorded in

Pv 25 (6.33 x 10^4/ml) and the maximum in Pv 16 (44.00 x 10^4/ml). In the remaining isolates sporulation density ranged between 16.33 and 33.00 x10^4/ml. All the isolates took different durations for germination of alpha conidia. Pv 36 took minimum incubation period (40 h) while the longest incubation period (75 h) was recorded with Pv 11 and Pv 25. Maximum germination (72 %) was recorded with Pv 36 and minimum (52.67 %) with Pv 10. The largest germ tube (58.40 µm) was recorded with Pv 36 and smallest (15.60 µm) with Pv 10. In the remaining isolates, germ tube length ranged from 16.40-58.00 µm (Akhtar, 2006)

A variability study conducted by Akhtar and Chaube (2006) revealed significant and substantial differences in radial growth of the isolates of *P. vexans* causing blight of brinjal on PDA. Similarly, biomass production ranged between 20.0-353.0 mg. The morphological characters, notably the type of colony, colour, texture and zonation also differed significantly. The variation in stylospore morphology was relatively high. The germinability of pycnidiospore from different isolates differed significantly and it ranged from 31.0-72.0 %. Except for isolates Pv 36 where conidial germination was also bipolar in rest of the isolates the germination was unipolar. The germtube length and time taken for germination also differed significantly. The extent and magnitude of pycnidial development was variable. Pycnidia were black in some isolates while in some brown pycnidia developed. Pathogenicity revealed isolate Pv 36 as the most aggressive isolate while isolate Pv 11 as the least aggressive.

2.7.2. Cultural characters

2.7.2.1. Growth and sporulation of *Phomopsis vexans* in different solid and liquid media

Lou et al. (2004) compared the sporulation conditions of eight species of *Phomopsis* in pure culture and reported that the optimum media for *Phomopsis*

sporulation were alfalfa extract + Czapek's Dox medium and Oat Meal Agar and alfalfa stem + WA media. They also reported that the optimum range of temperature was 22°C-25°C. The optimum illumination time was 12 h each day (fluorescent light, 40 W, interval: 30 cm). The befitting p^H value was found as 5.6-6.8.

Five monoconidial isolates of *P. vexans* varied considerably in morphological and cultural level when grown on nine different culture media. The colony colour ranged from white to dull white to blackish white to yellowish white to greyish with circular to irregular shape of colony. Zonation in cultures of different isolates on different media varied from distinct to indistinct to absent. Colony growth rate ranged from slow to fast. Radial growth observed on culture media revealed that PDA as the best, while Richards' Agar as the least supporting medium.

The formation status of alpha and beta conidia of 23 isolates of *Phomopsis* (Sacc.) Bubak belonging to 18 species on different media were compared. The results showed that alfalfa extract + Czapek's Dox medium could impel most of isolates to produce alpha and beta conidia. The shapes and sizes of alpha and beta conidia on the medium were almost the same as those on the host plants. Therefore the original descriptions of *Phomopsis durionis, P. sterculicola, P. macadamii, P. lucumicola and P. tinea* which could not produce beta conidia on the hosts should be supplemented according to their morphological characteristics in pure culture (Lou et al., 2004).

PDA, Soil Extract and Oat Meal agar favoured growth while oat meal agar and host decoction agar were favourable for pycnidia production. *Phomopsis vexans* produces abundant conidiomata on 4–7 per cent Oat Meal agar medium at 30°C under light (Divinagracia, 1969). Pawar and Patel (1957) reported good production of pycnidia on agar made with an extract of the host.

2.7.2.2. Toxin production by the fungus

Pan et al. (1995) reported that *Phomopsis vexans* the causal organism of leaf blight and fruit rot of aubergine produced non-host specific phytotoxin(s) in culture medium. The toxin(s) produced showed a positive correlation with the virulence of the pathogen. The symptoms produced on groundnut leaves following dipping of seedlings in different concentration of toxin(s) preparation were not identical to the natural symptoms. The results suggested the presence of toxic principle(s) in the culture filtrate and its effect(s) to be non-host specific. The toxic principle(s) was thought to act as secondary determinant of disease.

2.7.3. Nutritional studies of *Phomopsis vexans.*

2.7.3.1. Effect of different carbon sources on growth and sporulation of *Phomopsis vexans.*

Akhtar et al. (2008) studied the effect of nutritional factors for the germinability of five isolates of *P. vexans* (Pv 10, Pv 11, Pv 16, Pv 25 and Pv 32), in different carbon sources such as glucose, sucrose and dextrose and different types of substrates *viz.*, leaf, fruit and stem, leachates of host plant and reported that two concentrations of three carbon sources stimulated germination of alpha conidia of *P. vexans*. Among the carbon sources, glucose was most suited and stimulated maximum germination in all the five isolates. Dextrose was found least conducive and effective as far as spore germination of the isolates of *P. vexans* was conducted. The germination in the isolate was increased by 15.19 and 34.18 per cent at 100 and 1000 ppm of glucose respectively. The isolate Pv16 was observed to be least sensitive with minimum change in germination per cent that is 4.39 and 17.96 per cent at 100 and 1000 ppm of glucose respectively and it was clear that isolate Pv 16 was least sensitive to all the carbon sources. They reported glucose as the best source of carbon.

Quan et al. (2007) reported that among carbon sources glucose and mannitose were useful types for the growth of *Phomopsis brevistylospora* causing *Phomopsis* rot on post harvest rockmelon.

Gurgel et al. (2002) reported that among the four carbon sources (starch, glucose, maltose and sucrose), starch, glucose or sucrose under continuous light conditions induced the best mycelial growth, pycnidia and spore production in the isolates of *Phomopsis anacardii* and *Phomopsis mangiferae* infecting cashew and mango.

Nine different fungal strains *viz.*, *Diaporthe perniciosa*, strain DHF, *Cytoeporina ludibunda*, strains CA_4, CC_2, and MK, *Phomopsis vexans, Fusarium* strains D and A at all concentrations of glucose (1.8 to 6 per cent), the rate of fungal growth fell with increasing acid while at all concentrations of acid the rate of growth fell with increasing glucose (Horne, 1931).

2.7.3.2. Effect of different nitrogen sources on growth and sporulation of *Phomopsis vexans.*

L-leucine was reported as the favourable nitrogen source for mycelium growth whereas glutamic acid could not be used by *Phomopsis brevistylospora* causing *Phomopsis* rot on postcharvest rockmelon (Quan et al., 2007).

Kumar and Sugha (2003) studied the effects of N, P and K on leaf blight and fruit rot caused by *Phomopsis vexans* on aubergine and evaluated different N fertilizer [calcium ammonium nitrate (CAN), ammonium sulfate (AS) and urea] at 100, 102.5 and 97.5 kg/ha and reported that N significantly increased disease incidence (DI) and yield. The average DI was higher with urea and CAN than with AS. Yield variation among the N fertilizers was not significant. *Phomopsis vexans* incidence was highest under 112.50 kg N/ha. Crop yield was not significantly

affected by the N rate. Thus Nitrogenous fertilizer at the recommended rate may increase crop yield without increasing *Diaporthe* incidence.

Gurgel et al. (2002) reported asparagine and potassium nitrate as the best among the four nitrogen sources (asparagine, arginine, ammonium sulfate and potassium nitrate) to induce the best mycelial growth, pycnidia and spore production of *Phomopsis anacardii* and *Phomopsis mangiferae* infecting cashew and mango respectively.

2.7.3.3. Effect of p^H on the growth and sporulation of *Phomopsis vexans*

Mycelium of *Phomopsis brevistylospora* causing the *Phomopsis* rot on post harvest rockmelon could grow at p^H 3-12 though the optimum p^H 4-8 was reported by Quan et al. (2007).

Phomopsis vexans produced macerating enzyme (ME), pectin methyl esterase [pectin esterase] (PME) and cellulase enzymes in the Richards' medium but not phospholipase enzyme and the activities were favoured at p^H 9.2 and 7.2 while the activity of Cx (cellulose enzyme) was slower and favoured at p^H 4.0 (Varma and Nema, 2005).

Islam and Pan (1991) reported that in culture filtrates of *P. vexans* the peak activity of cellulase (Cx) was at p^H 5.5 and of pectic enzyme at p^H 8.5 being the temperature optimum for both at 30°C. The alkaline p^H was optimum for lyase type pectic enzyme and the activities of both enzymes were highest nine days after fruits were inoculated.

2.8. Effect of environmental factors on the incidence of disease by *Phomopsis vexans.*

A number of workers have studied the factors affecting growth and sporulation of the fungus in culture.

Phomopsis fruit rot of brinjal (*Solanum melongena*) incited by *Phomopsis vexans* were found conducive at the temperature of 25°C, RH ≥ 90 per cent, fruits of younger age (5-10 day old), higher inoculum load (> 120 spores/ml) and inoculum of younger age (14 day old) for the development and progress of Phomopsis fruit rot (Sugha et al., 2002).

Kemble et al. (1998) reported that the fungus (*Phomopsis vexans*) can survive from season to season in plant debris in the soil as well as in or on seed for more than a year in fields where a diseased crop was grown and the disease is favoured by warm wet weather and is spread by splashing water.

Maximum incidence of fruit rot in brinjal occurred during the monsoon months of June to October when temperatures and relative humidity usually remain high was reported by Roy (1997).

2.9. Antagonistic Microorganisms for controlling *Phomopsis spp.*

Biological control of plant diseases involving the use of antagonistic microorganisms offers an excellent alternative to chemical control. A vast number of microorganisms present in rhizosphere have been considered as important in sustainable agriculture because of their biocontrol potentials and plant growth promotional activities (Jagadish, 2006).

Quan et al. (2007) reported that *Trichoderma* spp. were strong antagonists against *Phomopsis brevistylospora* causing rot on post harvest rockmelon.

Bhadrasree (2007) reported the use of biocontrol agents like *Trichoderma harzianum* and P_{22} culture of *Pseudomonas fluorescence* for the control of *Rhizoctonia solani* causing collar rot and web blight of cowpea.

The potential antagonistic property of different *Trichoderma harzianum* isolates (T2, T5, T8, T10 and 5 T3) was determined using dual culture plate method for *Macrophomina phaseolina, Rhizoctonia solani, Athelia rolfsii, Helminthosporium sativum* [*Cochliobolus sativus*], *Helminthosporium oryzae* [*Cochliobolus miyabeanus*], *Phytophthora parasitica* [*Phytophthora nicotianae*], *Fusarium culmorum* f.sp. *ciceri, Fusarium oxysporum* f.sp. *lycopersici* [*Fusarium oxysporum* f.sp. *lycopersici*], *Fusarium udum* [*Gibberella indica*], *Cephalosporium sacchari* [*Fusarium sacchari*] and *Colletotrichum capsici* inoculated 48 h earlier before planting the inoculum block of antagonist while *Aspergillus niger* was inoculated on the same day with *Trichoderma* isolates. *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum*, f.sp. *ciceri* and *Phytophthora parasitica* appeared to be more sensitive, hardly one isolate was promising against *Athelia rolfsii, Helminthosporium sativum* and *Fusarium oxysporum* f.sp. *lycopersici*. From the isolates of *Trichoderma*, isolates 5 T3 were selected. Four other isolates T2, T5, T8 and T10 were selected for their established higher antagonistic efficiency. *Colletotrichum capsici, Cephalosporium sacchari* and *Rhizoctonia solani* were almost sensitive to all the five isolates. *Macrophomina phaseolina* was generally sensitive to all test isolates. *Athelia rolfsii* and *Aspergillus niger* were not sensitive to any of the isolates. *Helminthosporium oryzae* was sensitive to T10 and 5 T3. On the other hand *Helminthosporium sativum* was less sensitive to T10, T8 and 5 T3, and more to T2 and T5. *Fusarium oxysporum* f.sp. *ciceri* showed mild susceptibility to all test isolates except 5 T3 to which it was a little more sensitive (Choudhary and Sen, 2006).

Srinivas et al. (2005) treated aubergine cultivars infected with *Phomopsis vexans* with biological control agents like *Pseudomonas fluorescens* and *Trichoderma harzianum* ($1x10^8$ cfu g/l) and talcum-based formulations of *Pseudomonas fluorescens* ($28x10^7$ cfu g/1) at 5 g/kg of seeds and *T. harzianum* ($19x10^7$ cfu g/1) at 10 g/kg of seeds and evaluated for reduction of *Phomopsis vexans*, seed germination, vigour index and field emergence and reported that pure culture of *Pseudomonas fluorescens* was more effective in reducing the *Phomopsis vexans* infection followed by *T. harzianum* compared with fungicide-treated (Bavistin [carbendazim], Dithane M-45 [mancozeb] and Captan) and untreated seeds. Formulation *of Pseudomonas fluorescens* was effective in reducing the *Phomopsis vexans* infection and increasing the seed germination, vigour index and field emergence followed by *T. harzianum* and fungicide treatments in comparison with control.

Among the twelve isolates of *Trichoderma* spp. (eight of *T. harzianum* and four of *T. viride*) screened against *Colletotrichum capsici, Sclerotinia sclerotiorum, Pythium aphanidermatum, Fusarium oxysporum* f.sp. *lycopersici, Alternaria brassicicola, A. alternata, Phomopsis vexans, Rhizoctonia bataticola* [*Macrophomina phaseolina*] and *Rhizoctonia solani* isolated from chilli, cauliflower, tomato and aubergine plants, *Trichoderma* treated phytotoxins exhibited mild symptom development compared to the control phytotoxins and the metabolites of *Trichoderma* isolates Th3, Th10, Th30, Th31 and Th32 were effective in reducing the disease symptoms on leaves and seedlings against phytotoxins even at a mixed inoculation ratio of 1 : 3 (Sharma and Dureja, 2004).

Jadeja (2003) reported that *Trichoderma koningii* exhibited the maximum antagonistic activity against *Phomopsis vexans* with 80.60 per cent growth inhibition among *T. longibrachiatum* (79.25 %), *T. viride* (78.88 %), *T. harzianum* (77.77 %), *T. hamatum* (74.81 %). Pycnidial formation was absent in all the treatments.

Unlike fungal antagonists, bacterial antagonists like *Bacillus* (5 strains) and *Pseudomonas fluorescens* were also found effective against *P. vexans* (Jadeja, 2003). He reported 70 per cent growth inhibition with *Bacillus* - D followed by *P. fluorescens* and *Bacillus* - A having 64.81 and 61.11 per cent growth inhibition in their part respectively. Pycnidial formation was absent in all the treatments.

A study conducted by Pan et al. (2001) evaluated different *Gliocladium virens* strains (11 Gv, 13 Gv, 15 Gv and 17 Gv) against *Fusarium oxysporum* f. sp. *udum* [*F. udum*], *Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii* [*Corticium rolfsii*] and reported that all strains were highly antagonistic against *F. oxysporum* f. sp. *udum, M. phaseolina* and *R. solani* but were less antagonistic to *S. rolfsii*. Only 15 Gv could antagonize *S. rolfsii*. Of the different *G. virens* strains tested 15 Gv was most effective against all pathogens. Dusting and dipping application with *Trichoderma harzianum* has been reported effective against *Phomopsis vexans* of brinjal (Rajkumar, 2000).

2.10. Plant extracts in management of diseases caused by *Phomopsis spp.*

Plant extracts might be a substantial alternative of chemical pesticides in controlling plant diseases. *Allamanda cathertica*, a recognised medicinal plant, its extract has been proved effective in controlling *Phomopsis vexans* (100 %) was reported by Rahman et al. (2009). Masuduzzaman et al. (2008) reported that compounds extracted from *Allamanda cathartica* using organic solvents prevented growth of *P. vexans* in culture at unspecified concentrations.

Dhakate et al. (2008) studied *in vitro* and *in vivo* studies of some plant extracts against *Phomopsis* blight that caused by *Phomopsis vexans* [*Diaporthe vexans*] using acetone extract at five per cent of datura (80.65 %), eucalyptus (53.60 %), aqueous extract of tulsi [*Ocimum tenuiflorum*] (53.27 %), acetone extract of neem [*Azadirachta indica*] (51.15 %) and neem aqueous extract (49.83 %) and

reported that neem aqueous extract and datura acetone extract at five per cent was effective in checking the disease incidence in brinjal.

Jadeja (2003) found that the 10 per cent rhizome/bulb/clove extracts of turmeric, ginger, mango ginger, onion and garlic inhibited the growth of *Phomopsis vexans* causing stem and branch blight in brinjal with maximum effectiveness of rhizome of turmeric (37.03 %) on mycelial growth and also yielded few scattered pycnidia; the same was found moderate in ginger extracts while low growth inhibition with good pycnidial formation was obtained in garlic clove extracts. He also studied the effect of eight different leaf extracts (10 %), *viz*, datura, congress grass, neem, lantana, ardusi, allamanda, tulsi and nafatia on growth inhibition and pycnidial formation of *Phomopsis vexans* and proved that leaf extracts of datura and congress grass inhibited 75.55 and 73.33 per cent of growth without a sign of pycnidial formation and that of neem and lantana were also effective as more than 50 per cent inhibition was recorded with no pycnidial formation in neem leaf extract and poor in lantana.

The influence of cashew leaf, fruit (apple) and shell extracts at 5, 15, 25 and 50 per cent (w/v) was tested on the growth of *Phytophthora palmivora, Alternaria solani, Fusarium oxysporum, Rhizoctonia bataticola, Sclerotium rolfsii, Pellicularia filamentosa, Macrophomina phaseolina* and *Phomopsis vexans* and was reported to have a tremendous effect in suppressing the growth of all the fungi especially *Phytophthora palmivora* in which an inhibition to the extent of 70 % (Joy et al., 2002). The products such as JMS stylet oil (paraffin oil), Serenade (*Bacillus subtilis*), Croplife (citrus and coconut extract) + Plantfood (foliar fertilizer), Armicarb (potassium bicarbonate), Elexa (chitosan), Milsana (giant knotweed [Fallopia sp.] extract), and AQ10 (*Ampelomyces quisqualis*) were tested for their efficacy in controlling powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*), black rot (*Guignardia bidwellii*), *Phomopsis* of grapes (*Phomopsis viticola*)

and *Botrytis* bunch rot (*Botrytis cinerea*) in grape and reported that JMS Stylet Oil, Armicarb, Serenade, AQ10, Elexa and Milsana provided moderate control of downy and powdery mildew; JMS Stylet Oil and Armicarb also reduced *Phomopsis* rachis infections, Armicarb gave adequate control of black rot control, Serenade and Croplife + Plantfood provided moderate to good control of *Botrytis* bunch rot and moderate control of downy mildew and *Phomopsis* leaf spot. The product Milsana was reported to provide moderate control of *Botrytis* bunch rot of grapes (Schilder et al., 2002).

Allamanda leaf extract showed miracle result in controlling plant diseases (Meah, 2002). So far it had been shown effective against *Phomopsis vexans* and *Sclerotium rolfsii* (Khan, 1999; Rumana, 2004). In the Republic of Georgia, extracts of garlic and celery were found effective as seed treatments for the control of *P. vexans* (Kuprashvili, 1996). Natural plant extracts (*Allamanda cathartica* and *Aegle marmelos)* and homeopathic drugs (thuja, teucrium) could prevent or reduce growth of the fungus (*Phomopsis vexans*) infecting eggplant *in vitro* and was an effective fungicide, though higher concentrations of active ingredient were required (Panda et al., 1996). Extracts from garlic and celery used for treatment of seeds of capsicum, aubergine, tomato, cabbage, carrot and onion infected by *Peronospora destructor, Phomopsis vexans, Fusarium oxysporum, Verticillium albo-atrum, Alternaria brassicae* and *A. radicina* showed that the plant extracts disinfected seeds and increased yields (Kuprashvili, 1996).

Panda et al. (1996) reported that among the homeopathic drugs (*Arsenicum album* [arsenic oxide], sulfur, Thuja, Teucrium and Baptisia) and leaf extracts (from *Polyalthia longifolia, Aegle marmelos, Azadirachta indica, Carthamus roseus, Ocimum sanctum* and *Allamanda cathartica*) used for the control of *Phomopsis* blight (caused by *Phomopsis vexans*) and fruit rot in brinjal, Thuja and Teucrium, each at two per cent concentration, were as effective as the fungicide bavistin

(carbendazim) in inhibiting the growth of *P. vexans*. However, leaf extracts of *A. cathartica* also had excellent potential as a fungicide.

Suhaila et al. (1996) reported that ethanolic extracts of *Piper betle* showed strong activity against all the pathogens tested (*Colletotrichum capsici, Fusarium pallidoroseum, Botryodiplodia theobromae, Alternaria alternata, Penicillium citrinum, Phomopsis caricae-papayae* and *Aspergillus niger*) with inhibition diameters significantly ($P < 0.01$) bigger than 2.5 mg ml-1 prochloraz or 10 mg ml^{-1} clotrimazole. Thirty four other plants (Kucing gala (unknown scientific name), Limau batik (unknown scientific name), *Bertholletia excelsa, Bixa orellana, Caesalpinia pulcherrima, Cerbera odollam* (fruits and leaves), *Colocasia gigantea, Curcuma domestica* [*C. longa*], *Curcuma mang[g]a, Derris el[l]iptica, Elephantopus scaber, Eleusine indica, Eugenia polyantha, Euphorbia hirta, Euphorbia tirucalli, Gardenia florida, Hedyotis auricularia, Hibiscus rosa-sinensis, Juniperus chinensis* (three varieties), *Lawsonia inermis, Lecythis ollaria, Mentha arvensis, Mimusops elengi, Ocimum sanctum, Phyllanthus niruri, Piper nigrum, Solanum nigrum* and *Tinospora tuberculata*) showed selective antifungal activity.

Leaf extracts of *Catharanthus roseus, Polyalthia longifolia, Azadirachta indica, Allamanda cathertica* and *Aegle marmelos* were effective for the control of *Phomopsis vexans* of brinjal. The allelopathic control of *P. vexans* by aqueous leaf extracts of five plants were tested and he reported that fungal growth was inhibited to a maximum by leaf extracts of *Allamanda cathertica* (93.75 %) followed by *Aegle marmelos* (85.38 %). Leaf extracts of *Catharanthus roseus, Polyalthia longifolia* and *Azadirachta indica* were equally effective but that of *Ocimum sanctum* was the least effective causing 52.23 per cent growth inhibition (Mohanty et al., 1995).

Arun Arya (1988) reported that 50 per cent crude leaf extract of *Eucalyptus occidentalis* was more effective than the extracts of *Aegle marmelos, Strychnos nux-*

vomica, Lawsonia inermis and *Ephedra foliate* at same concentration for the management of fruit rots of grapes and guava caused by *Phomopsis viticola* and *P. psidii.*

2.11. Effect of oil cakes in controlling the plant diseases.

Bhadrasree (2007) reported that gingelly oil cake and coconut oil cake were effective in managing the collar rot and web blight of cowpea caused by *R. solani.*

Ammendment of soil with individual applications of mustard oil cake, urea, triple super phosphate, muriate of potash, zinc sulphate ($ZnSO_4$) and calcium sulphate ($CaSO_4$) and their mixed application reduced the level of infection of anthracnose (caused by *Phomopsis* and *Macrophomina*) on immature guava fruits compared with applications of manures. Mixed applications of manures and fertilizers yielded the lowest fruit infection (Hossain et al., 1996).

2.12. Use of fungicides for controlling *Phomopsis spp.*

Beura et al. (2008) found carbendazim as the best control for *Phomopsis* and also maximized yield. In the laboratory, carbendazim completely inhibited culture growth of *Phomopsis vexans* (Mohanty et al., 1994). Sensitivity of spore germination to the fungicide is high though not as high as sensitivity to prochloraz (Kumar and Sugha, 2004). Akhtar (2007) evaluated the efficacy of carboxin, carbendazim and mancozeb at 50, 100, 250, 500 and 1000 ppm and reported complete growth inhibition and spore germination of *Phomopsis vexans* infecting aubergines. Fungicides such as mancozeb, carbendazim and captaf were found superior for the inhibition of seed borne pathogens like *Phomopsis vexans and Alternaria solani* and increased seed germination in brinjal (Thippeswamy et al., 2006).

Copper oxychloride (0.3 %), mancozeb (0.25 %), zineb (0.25 %), captan (0.25 %), thiophanate methyl (0.25 %), carbendazim (0.1 %) and tebuconazole (0.05 %) sprayed at 10 day intervals from disease initiation, were effective against *Phomopsis* blight with the greatest reduction in disease incidence using carbendazim (80.10 %) followed by tebuconazole (76.10 %) was reported by Manna et al. (2004).

Jadeja (2003) reported that carbendazim 0.05 per cent was effective in reducing disease incidence, dried branches and dried plants infected with *Phomopsis vexans* in brinjal. Increased yield of 19.4 q/ha was found with sprayings of 0.1 per cent carbendazim.

Singh and Agrawal (1999) reported the efficacy of mancozeb (0.3 %), carbendazim (0.1 %), mancozeb (0.3 %) + thiophanate methyl (0.1 %) and copper oxychloride (0.3 %) to control fruit rot (*Phomopsis vexans*) of aubergine with the sprayings carried out at the first appearance of symptoms and 15 days thereafter. All fungicides significantly reduced fruit rot and increased yield with carbendazim resulting in the lowest disease incidence (4.3 %) and highest yield (222.83 q/ha). Bavistin was the most effective fungicide for the control of aubergine rot caused by *Diaporthe vexans* (Meah et al., 1998).

Chemical seed treatment with captan, carbendazim, carboxin, metasulfovax, thiram and triadimenol was found to increase germination and to reduce the incidence of damping off of seedlings in artificially infested soil (Kaushal and Kumar, 1995).

Copper oxychloride, captafol, mancozeb, ziram, chlorothalonil and carbendazim were evaluated against *Phomopsis* leaf blight/fruit rot in aubergine caused by *P. vexans* and the results showed that three foliar sprayings with carbendazim (0.15 %) gave maximum protection against leaf blight and fruit rot (76.97 and 74.62 % respectively) and the highest net profit of Rs. 10,096.00/ha while

Mancozeb (0.3 %) provided adequate protection against the disease and gave a net profit of Rs. 9101.00/ha. Benefit/cost analysis indicated that mancozeb was superior to carbendazim showing benefit/cost ratio of 8.41 and 6.31 respectively (Das, 1995). The best control (53.8 %) of *P. vexans* on aubergines was given by topsin M - 70 [thiophanate methyl], applied at 0.1 per cent as a seed treatment and then as three foliar sprays at intervals of 20 days was reported by Pandey and Kumar (1993).

Five fungicides *viz.*, Blitox - 50 [copper oxychloride], Bavistin [carbendazim], Dithane M - 45 [mancozeb], Ridomil - MZ [mancozeb + metalaxyl] and Difolatan [captafol], were tested *in vitro* against *P. vexans*, the causal agent of blight and fruit rot in aubergine and the percentage inhibition of mycelial growth was investigated for three concentration (0.1, 0.2 and 0.3 %) of each fungicide and the results revealed that all the chemicals caused significant growth inhibition at all concentration with carbendazim causing 100 per cent inhibition (Mohanty et al. 1994).

Islam and Pan (1993) reported that field trial against *P. vexans* with carbendazim (0.1 %), tolclofos - methyl (0.5 %) and carboxin (0.25 %) provided nearly complete control of the disease, with three sprays at 15 day intervals at three fortnightly sprays of carbendazim, each at 700 litres/ha were the best in terms of increased yield (19.4 q/ha over the untreated control) and net profit (Rs 4645.15/ha) with a benefit/cost ratio of 3.95:1. John (1991) reported COC - 50 as the best fungicide for the control of blight and fruit rot of brinjal caused by *Phomopsis vexans*.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation entitled "Management of *Phomopsis* blight and fruit rot of brinjal (*Solanum melongena* L.)" was carried out at College of Agriculture, Vellayani during the period 2009-2011. Brinjal variety Swetha released by Kerala Agricultural University was used for the study. The materials used and the methods employed in the investigation are outlined below.

3.1. ISOLATION OF THE PATHOGEN

Brinjal fruits showing initial stages of infection were collected from different blocks of the College of Agriculture, Vellayani and used for the isolation of the organism. The fungal pathogen was isolated from diseased parts by routine mycological techniques (tissue plating) as described here-under:

The infected tissue (fruit) were cut into small bits (2-4 mm) with a margin of healthy tissue around it, surface sterilized with 0.1 per cent mercuric chloride solution for one min. and then rewashed in two to three changes of sterile distilled water. These bits were plated on solidified Potato Dextrose Agar (PDA) medium in sterilized petri dishes. The dishes were incubated at room temperature (28 ± 2°C). When the growth of the fungus was visible, mycelial bits were transferred aseptically to PDA slants. The slants were incubated at room temperature.

3.1.1. Single spore isolation

Culture obtained after isolation was purified by single spore isolation (Johnston and Booth, 1983). Ten ml of two per cent water agar was poured into sterile petri dishes and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from seven day old fungal culture. One ml of the prepared suspension was spread uniformly on agar plate. These plates were incubated at 28 ±

2°C for 12 h. The plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the dishes.

The growing hyphal tip portion of the isolate was cut with the help of a cork borer under aseptic conditions and using an inoculation needle it was carefully transferred to PDA slants and incubated at 28 ± 2°C. This culture was used for further studies.

3.1.2 PATHOGENICITY

Koch's postulates were proved for confirming the pathogenicity of the fungal culture. Disease free brinjal plants were raised in pots kept in green house. The fruits of different ages were artificially inoculated by placing seven day old culture bits of the isolated fungus on the pinpricked part of the healthy brinjal fruit. The culture bits were then covered with moist cotton wool and inoculated fruits were covered with polythene bags to ensure high humidity for disease development.

After incubation for five days the symptoms were expressed; the fungus was then reisolated from the fruit portions exhibiting typical symptoms of the disease and compared with purified original isolate. The morphological and cultural characters were studied.

3.2. SYMPTOMATOLOGY

Symptoms of disease caused by the culture was studied by observing the symptoms produced in the naturally infected brinjal fruits and also following the course of development of the disease under artificial inoculation.

3.3. MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE PATHOGEN

3.3.1. Morphological characters

3.3.1.1. Colony characters

Five mm discs taken from the fungal culture (of an actively growing seven day old sporulated culture) were inoculated separately at the centre of a 90 mm petri plate containing 15 ml solidified sterile PDA and incubated at room temperature. Colony colour and characters were recorded after seven days.

3.3.1.2. Conidial characters

The size of conidia of the culture isolate was measured with a standardized microscope using micrometer. The average size and shape of 100 conidia were taken. Based on the colony, conidial morphology and the pathogenicity tests, the fungus was identified.

3.3.2. Cultural characters

3.3.2.1. Growth and sporulation of *Phomopsis vexans* on different solid media

The following culture media were used to study the growth of the fungi.

1. Potato Dextrose Agar (PDA)
2. Czapek – Dox Agar (CDA)
3. Host Extract Dextrose Agar (HEDA)
4. Oat Meal Agar (OMA)

The compositions of the media used are given under Appendix I.

The media were prepared and sterilized by autoclaving at 15 pounds pressure for 20 min. which corresponds to a temperature of 121.6°C. Fifteen ml of each medium listed above was poured into 90 mm dia. petri plates. After solidification, five mm discs of *Phomopsis vexans* from an actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of the petri dish. Each set of experiment was replicated three times and the plates were incubated at room temperature. The measurement of the colony dia. was taken when the maximum growth was attained in any one of the media tested. Cultural characters such as colony dia., colony colour, type of margin and sporulation were also recorded.

The sporulation was categorized as follows.

Sl. No	Score	Category	Description (Conidia/microscopic field [100X]
1	++++	Excellent	> 150
2	+++	Good	101 – 150
3	++	Fair	51 – 100
4	+	Poor	1 – 50
5	-	No sporulation	0

3.3.2.2. Growth and sporulation of *Phomopsis vexans* in different liquid media

The composition and preparation of different liquid media used were the same as that of solid media except that agar was not added. Each media was prepared and dispersed at the rate of 30 ml into each of 100 ml conical flasks. These flasks were then sterilized at 15 pounds pressure for 20 min. The flasks were then inoculated

with mycelial discs of five mm dia. from the actively growing periphery of seven day old culture on PDA and incubated at room temperature (28 ± 2^0C) for 15 days. Each treatment was replicated three times. The mycelium was filtered out through Whatman No. 42 filter paper and the dry mycelial weight was determined after oven drying the mycelia at 50°C till constant weight was attained. Sporulation in each treatment was recorded.

3.3.2.3. Production of toxic metabolite by *Phomopsis vexans* in different liquid media

The following liquid media were tested to assess their comparative merits in supporting the production of toxic metabolites by *Phomopsis vexans.*

1. Potato Dextrose Broth (PDB)
2. Czapek's Dox Broth (CDB)
3. Host Extract Dextrose Broth (HEDB)
4. Oat Meal Broth (OMB)

Thirty ml of each medium was dispersed in 100 ml conical flasks and sterilized by autoclaving at 15 pounds pressure for 20 min. The medium was inoculated with five mm disc of seven day old growth of *Phomopsis vexans.* For each treatment three replications were maintained. After 15 days of incubation at room temperature the culture was filtered through Whatman No. 42 filter paper. The comparative toxic activity of each of the filtrate was studied by bioassaying the test solution on the host fruit as follows.

The toxic activity of each filtrate was measured by the intensity of symptoms produced on the host fruit. To detect the toxic activity, the test solution was applied on the detached fruits. Brinjal fruits of uniform maturity were collected and surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one min. followed by three washings with sterile distilled water. Culture filtrate (0.05 ml) was placed on the fruit

after giving pin pricks and without pinpricks. Control was kept by using sterilized water. The treated fruits were placed separately in sterilized polythene bags. A piece of moist cotton swab was placed inside the bag and mouth of the bag was tied. The bagged fruits were incubated at room temperature for 72 h. Each treatment was replicated three times. The lesion size produced on the fruit was measured and recorded. Development of lesions on the fruits were observed by measuring the average of the dia. of lesions in two directions perpendicular to each other.

Further studies on nutritional and physiological factors, *in vitro* and *in vivo* management were carried out using the obtained culture *Phomopsis vexans*.

3.3.3 Nutritional studies of *Phomopsis vexans*

3.3.3.1. Effect of different carbon sources

Growth and sporulation of *Phomopsis vexans* on different sources of carbon were studied in the best medium (Czapek-Dox broth medium) selected. The same quantity of carbon source as used in Czapek-Dox medium was substituted by other carbon sources *viz.*, dextrose, lactose, maltose and starch. Broth containing sucrose as carbon source served as control. Thirty ml of each of the above treated media was poured in 100 ml conical flasks plugged and sterilized. The broth containing respective carbon sources were aseptically inoculated with five mm mycelial discs of *Phomopsis vexans* obtained from actively growing periphery of seven day old culture and incubated at room temperature for a period of 15 days. After this the mycelium was filtered through Whatman No. 42 filter paper and the mycelia mat was oven dried till attaining constant weight in any one of the media tested.

3.3.3.2. Effect of different nitrogen sources on growth and sporulation of *Phomopsis vexans*

Both inorganic and organic forms of nitrogen were used for the study. The organic form of nitrogen used was urea and the inorganic forms were ammonium carbonate, ammonium chloride, ammonium nitrate and potassium nitrate. These were substituted for sodium nitrate in Czapek's Dox broth medium to give equivalent amount of nitrogen in each case. Each broth was prepared and dispersed at the rate of 30 ml into each of 100 ml conical flasks. These flasks were then sterilized at 15 pounds pressure for 20 min. The flasks were then inoculated with mycelial discs of five mm dia. from an actively growing periphery of seven day old culture on PDA and incubated at room temperature (28 ± 2^0C) for 15 days. Each treatment was replicated three times. The mycelium was filtered out through Whatman No. 42 filter paper and the dry mycelial weight was determined after oven drying the mycelia at 50°C till constant weight was attained. Sporulation in each treatment was recorded as described earlier.

3.3.4. Physiological factors affecting the growth of *Phomopsis vexans*

3.3.4.1. Effect of p^H on growth and sporulation of *Phomopsis vexans*

Phomopsis vexans was grown on Czapek-Dox broth at p^H ranges of 5, 6, 7, 8 and 9. The p^H levels were adjusted by adding one normal alkali (NaOH) or acid (HCl). Five mm mycelial disc from a seven day old actively growing culture was inoculated separately into conical flasks containing 30 ml medium at different p^H levels. Three replications were maintained for each p^H level. These flasks were incubated at room temperature for 15 days. The mycelium from each flask was filtered and dried in hot air oven till constant weight was obtained. The dry weight of each treatment was recorded.

3.4. ISOLATION OF ANTAGONISTIC ORGANISMS FROM PHYLLOSPHERE AND RHIZOSPHERE OF BRINJAL PLANTS

3.4.1. Isolation of antagonistic organisms from rhizosphere of brinjal plants.

Rhizospheric (soil) samples were collected from healthy brinjal plants from different blocks of College of Agriculture, Vellayani. The rhizospheric mycoflora was obtained by following the Serial Dilution Plate technique (Johnson and Curl, 1972). One gram soil was collected randomly dried, powdered, pooled, weighed and mixed thoroughly in 100 ml sterile distilled water/blank in 250 ml conical flasks to make 10^{-2} dilution. This aliquot was serially diluted aseptically to get 10^{-4} and 10^{-6} dilutions. One ml of aliquot from each of 10^{-4} and 10^{-6} dilution were transferred aseptically into petri dishes and plated with melted and cooled Martin's Rose Bengal Agar medium and Nutrient Agar medium respectively. Rotated the dishes for uniform distribution. Three replications were maintained. Petri dishes were then incubated at room temperature (28 ± 2^0C) for 24-48 h. The fungal as well as bacterial colonies developed were examined and transferred to PDA and nutrient agar slants respectively. The fungal cultures were purified by the single hyphal tip method (Parmeter et al., 1969) and the bacterial culture by streak plate method (Anon., 1957). The purified cultures were then stored in PDA and nutrient agar slants under refrigerated condition for identification and subsequent antagonism studies.

3.4.2. Isolation of antagonists from phyllosphere of brinjal plants

Serial Dilution Plate technique was followed for the isolation of mycoflora from the phyllosphere (fruit, stem, petal, calyx, flower bud, stamens and roots) of brinjal plants (Aneja, 2003). Disease free healthy brinjal samples collected from plants growing in different blocks of College of Agriculture, Vellayani were used for the isolation. Cut five discs each of six mm dia. from every collected leaf using cork borer. Transferred 50 discs to 100 ml water blank and ahaken for 20 min. in a rotary

shaker. Ten gram of the leaf bits were transferred to 100 ml of sterile distilled water in a 250 ml conical flask. Transferred 10 ml of the suspension to 90 ml sterile water blank using a sterile pipette to make a 10^{-1} dilution. The contents were shaken for 2-3 min. Transferred 10 ml of the suspension to another 90 ml sterile water blank using another sterile pipette to make a 10^{-2} dilution. Transferred one ml aliquots from 10^{-1} and 10^{-2} dilution to sterile petri plate and poured melted and cooled Martin's Rose Bengal Agar medium and Nutrient Agar medium respectively in these plates. Three replications were also maintained. The plates were then incubated at room temperature for seven days. Observed the Martin's Rose Bengal Agar plates after five to seven days and Nutrient Agar plates after two to three days of incubation for the appearance of fungi and bacteria. The fungal and bacterial colonies developed were examined and transferred to PDA and nutrient agar slants respectively. The fungal cultures were purified by the single hyphal tip method (Parmeter et al., 1969) and the bacterial culture by streak plate method (Anon., 1957). The purified cultures were then stored in PDA and nutrient agar slants under refrigerated condition for identification and subsequent antagonism studies.

3.5. *IN VITRO* MANAGEMENT OF *PHOMOPSIS VEXANS*

3.5.1. In *vitro* screening of fungal antagonists against *Phomopsis vexans*

The antagonistic effect of fungal isolates obtained from the phyllosphere and rhizosphere was tested on *Phomopsis vexans* by following dual culture technique by Morton and Stroube (1955).

Agar disc of five mm dia. was cut from the edge of a vigorously growing seven day old sporulated culture of *Phomopsis vexans* and this was placed in the media two cm away from the periphery of petri plates. On the opposite side, two cm away from the periphery, five mm sized one week old culture disc of antagonistic fungus cut from a vigorously growing culture was placed. Three replications were

maintained. The plates were incubated at room temperature for six days. Control was kept with five mm disc of *Phomopsis vexans* at the centre of PDA plate. Antagonist that exhibited highest percentage inhibition was selected.

Colony development was observed and assessment on interactions between the antagonist and the pathogen were made. Observations on the interaction and its classification were made using the method of Purkayastha and Bhattacharyya (1982) with five groups as follows:

A - Homogenous - Free intermingling between pairing organisms.

B - Overgrowth - Pathogen overgrown by the test fungus.

C - Cessation of growth at the line of contact of the cultures.

D - Aversion - A clear zone of inhibition was observed between the two organisms.

E - Overgrowth - Test fungus overgrown by pathogens.

Percentage inhibition of mycelial growth by each antagonist was calculated using the formula (Vincent, 1927).

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Percentage inhibition of mycelial growth of the pathogen.

C = Growth of the pathogen in the control plates (mm).

T = Growth of the pathogen in dual culture (mm)

3.5.1.1. Identification of fungal antagonist of *Phomopsis vexans*

The selected fungal antagonists were identified by the slide culture technique (Riddle, 1974). Plain agar was melted and poured into sterile petri dishes to a thickness of two mm and after solidification blocks of five mm^2 were cut out using a sterile needle. One such block was placed at the centre of a sterile microscopic slide. All the four corners of the agar block were inoculated with one antagonistic fungal spore. The block was covered with sterile cover slip. The slides were incubated separately in moist chamber for 48 h at room temperature. The cover slip was then gently lifted and was mounted with lactophenol cotton blue stain. The slides were observed under microscope. Based on their spore morphology they were identified at generic level.

3.5.2. In *vitro* screening of bacterial antagonist against *Phomopsis vexans*

Bacterial isolates obtained from the rhizosphere of brinjal plant were tested for their antagonistic effect on *Phomopsis vexans* by the dual culture technique by Morton and Stroube (1955). The bacterial isolate obtained was streaked two cm away from the periphery of the petri plate and opposite to that bacterial isolate a five mm disc of the seven day old culture of *Phomopsis vexans* was placed two cm away from the periphery of the petri plate. A triangle was marked on the bottom of another petri dish leaving two cm on each side from the centre of the dish and the mycelia discs of *Phomopsis vexans* was placed at the centre of the triangle, length of the streak being 4.5 cm. Three replications were maintained. Percentage inhibition of mycelial growth was calculated as described in section 3.5.1.

3.5.2.1. Identification of bacterial antagonist of *Phomopsis vexans.*

The morphological and colony characters of collected bacterial isolates were studied by growing them on Nutrient Agar and Kings' B medium. Gram staining,

Catalase production, production of fluorescent pigment on Kings' B medium and spore staining were conducted as per the procedure described in the Manual of microbiological methods by Society of American Bacteriologists (Anon., 1957).

3.5.3. Studies on the selected fungal antagonist *Trichoderma* isolate T2

3.5.3.1. Production of volatiles by *Trichoderma* isolate T2

The method adopted by Dennis and Webster (1971) was followed for studying the effect of volatiles on the suppression of *Phomopsis vexans*. The petri dishes containing PDA were inoculated with selected fungal antagonistic fungus by placing five mm disc cut from an actively growing culture and incubated at room temperature. After three days the lid of each plate was replaced by a petri plate bottom containing PDA inoculated with the pathogen. The two dishes were then sealed together with adhesive tape. Control was kept by keeping pathogen inoculated plate over the plate inoculated with the pathogen. Three replications were maintained in each case. After incubation the colony diameter of *Phomopsis vexans* was measured and compared with that of the control plates.

Percentage inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

$$I = \frac{(C - T) \times 100}{C}$$

Where, I = Percentage inhibition of mycelial growth of the pathogen.

C = Growth of the pathogen in the control plates (mm).

T = Growth of the pathogen in treatment plates (mm)

3.5.3.2. Production of non volatiles by *Trichoderma* isolate T2

The effect of non volatile substances produced by the selected antagonist is determined by following the methods of Dennis and Webster (1971). Twenty ml PDA was poured into petri plates. A single cellophane disc was placed aseptically over PDA in each petri plate. A mycelial disc of five mm cut from the margin of selected fungal antagonist was placed in the centre of the cellophane tape. The plates were incubated at room temperature for one day. Then the cellophane along with adhering fungus was carefully removed and in its place a five mm mycelial disc of the test pathogen (*Phomopsis vexans*) was placed. Instead of placing the fungal antagonist, controls were kept by keeping pathogen inoculated plate. Replications were maintained. After incubation the colony diameter of the test pathogen was measured and compared with that of the control plates. Percentage inhibition of mycelial growth was calculated as described in section 3.5.3.1.

3.5.3.4. Mass multiplication of fluorescent bacterial isolate or its formulation

Talc based formulation of fluorescent bacteria was prepared following the method by Vidhyasekaran and Muthamilan, 1995. For this the bacterium was grown in Kings' B broth and incubated for 48 h at room temperature. Hundred gram of talc was taken in polypropylene bags and about 0.5 g of CMC was added to this mixed, sealed and autoclaved. After autoclaving 40 ml of 48 h old culture was added, mixed thoroughly and stored at room temperature.

3.5.4. *In vitro* screening of plant extracts against *Phomopsis vexans*

Four plant extracts *viz., Lantana camara, Ocimum sanctum, Azadirachta indica,* and *Clerodendron odoratum* at two concentrations (20 and 30 %) were tested for their antimycotic behaviour against *Phomopsis vexans* following poisoned food technique (Nene and Thapliyal, 1982). Procedure followed for the preparation of plant extracts are detailed below.

Hundred gram fresh leaves from each plant was collected from field, washed well and ground with 100 ml distilled water. The macerate was filtered through four layered cheese cloth, again filtered through Whatman No. 42 filter paper and centrifuged at 5000 rpm for 10 min. The supernatant collected was filtered through Millipore membrane filter. Double strength PDA medium was supplemented aseptically with the different concentrations of each leaf extract. Fifteen ml sterilized medium was poured into each petri plate and inoculated with a five mm disc taken from the periphery of seven day old culture of *Phomopsis vexans* by placing it in the centre of the plate. The plates were incubated at room temperature (28 ± 2^0C). All the treatments were replicated three times. A control was also maintained where no leaf extract was added to PDA. When complete mycelial growth was observed in control plates observation was taken from the treatment plates. Percentage inhibition of mycelial growth was calculated as in section 3.5.3.1.

Plants used for preparation of leaf extracts

Sl. No.	Plant (Common name)	Scientific Name
1.	Lantana (Poochedi)	*Lantana camara* (Linn.)
2.	Holy basil (Tulsi)	*Ocimum sanctum* (Linn.)
3.	Neem (Vep)	*Azadirachta indica* (A. Juss)
4.	Peruvalam	*Clerodendron odoratum* (Linn.)

A lower concentration of the most effective leaf extract was tried further under *in vitro* condition.

3.5.5. *In vitro* screening of oil cakes against *Phomopsis vexans*

Four oil cakes were tested against *Phomopsis vexans* following poisoned food technique (Nene and Thapliyal, 1982). Each oil cake was used at 20 and 30 per cent concentration. The oil cakes tested were as follows:

Sl. No	Oil cake	Scientific Name of plants from which oil cake was obtained
1.	Coconut oil cake	*Cocos nucifera* (Linn.)
2.	Neem oil cake	*Azadirachta indica* (A. Juss)
3.	Gingelly oil cake	*Sesamum indicum* (Linn.)
4.	Groundnut oil cake	*Arachis hypogea* (Linn.)

Oil cakes were dried at room temperature (30^0C) for three days broken into small pieces and powdered thoroughly with pestle and mortar, sieved through a seive (2 mm porosity) and soaked in sterilized distilled water in the ratio 1 : 2 (w/v). After 24 h oil cake extract was obtained by squeezing the cake in water through four folds of muslin cloth. The extract was centrifuged (5000 rpm for 10 min) and supernatant was decanted into 100 ml flask. From this 20 and 30 ml was added to 80 and 70 ml prepared and sterilized Double strength PDA medium independently to the media to get the required concentrations. The media along with respective oil cake extract was melted, cooled and poured to petri dishes. After plating the media, a five mm dia. disc of actively growing seven day old culture of *Phomopsis vexans* was inoculated at the centre of the petri plate. Control plates were kept without adding oil cake extracts. Three replications were maintained for each treatment. The plates were then incubated at room temperature. When mycelial growth completely covered the

surface of the media in control plates, observations on the inhibition of mycelial growth due to the use of oil cakes were recorded. Percentage inhibition of mycelial growth was calculated as in section 3.5.3.1.

3.5.6. *In vitro* effect of carbendazim at various concentrations on the growth of *Phomopsis vexans*

Carbendazim at three different concentrations (0.025, 0.05 and 0.1 %) were tested for their antimycotic behavior against *Phomopsis vexans* using poisoned food technique (Nene and Thapliyal, 1982). Double strength PDA medium was prepared and sterilized. Carbendazim was then added independently to the media to get the required concentrations. After plating the poisoned media, a five mm culture disc of *Phomopsis vexans* was placed at the centre of the petri plate. The plates were incubated at room temperature. Control plates were kept without adding carbendazim. Three replications were maintained for each treatment. When mycelial growth completely covered the surface of the media in control plates, observations were recorded. Percentage inhibition of mycelial growth was calculated as in section 3.5.3.1.

3.6. *IN VIVO* MANAGEMENT OF FRUIT ROT OF BRINJAL CAUSED BY *PHOMOPSIS VEXANS*

3.6.1. Preparation of pathogen inoculum and foliar application

The procedure described by Laha and Venkataramanan (2001) was adopted with slight modification. Five mm culture disc of *Phomopsis vexans was* inoculated in 250 ml conical flasks containing 100 ml sterile water and incubated at room temperature. When the mycelial mat completely covered the liquid surface, it was thoroughly agitated and the liquid was filtered through a muslin cloth. Foliar spray of *Phomopsis vexans* containing 10^6 conidia per ml was given 20 days after fruit

formation. To ensure uniform infection of fruits by fruit rot, the plants were covered with polythene bags for two days to create a favourable microclimate for the successful infection by the pathogen.

3.6.2. In *vivo* management of fruit rot of brinjal

A pot culture experiment was laid out in Completely Randomized Design (CRD) with three replications and nine treatments using the brinjal variety Swetha which was found highly susceptible to fruit rot. The experiment was conducted during January-May 2011 at College of Agriculture, Vellayani. The manuring and all the cultural practices were done as per package of practice recommendations Crops (KAU, 2007).

The treatments were selected based on the maximum inhibition of growth of *Phomopsis vexans* obtained through *in vitro* management studies. The best antagonist, best plant extract and best oil cake and their combination were selected as treatments in this study. Talc based formulation of the selected antagonist was used for field spraying. The treatments were as follows.

T_1 - Seedling root dip in antagonist at the time of transplanting followed by foliar spray.

T_2 - Foliar spray with best plant extract.

T_3 - Soil amendment with best oil cake.

T_4 - Seedling root dip in antagonist at the time of transplanting stage followed by foliar spray with antagonist + Foliar spray with best plant extract.

T_5 - Soil amendment with best oil cake + Seedling root dip in antagonist at the time of transplanting stage followed by foliar spray with antagonist.

T_6 - Soil amendment with best oil cake + Foliar spray with best plant extract.

T_7 - Soil amendment with best oil cake + Seedling root dip in antagonist at the time of transplanting stage followed by foliar spray with antagonist + Foliar spray with best plant extract.

T_8 - Foliar spray with carbendazim 0.1 %.

T_9 - Control.

Seedlings were dipped in the talc based formulation of the selected antagonist for 20 min. The treatments T_1, T_4, T_5 and T_7 were treated with this as seedling dip at the time of transplanting. The selected antagonist, plant extract and the oil cake showing maximum antifungal activity against the pathogen under *in vitro* studies were used for *in vivo* studies. All the treatments were given seven days after artificial inoculation with the fruit rot pathogen. Carbendazim at 0.1 per cent was given as foliar spray two days after artificial inoculation of the fruit rot pathogen. The following observations were recorded during the course of the experiment.

3.6.3. Disease intensity

The intensity of the fruit rot was recorded before the treatment application *i.e.* two days after artificial inoculation of the pathogen after first treatment application (seven days after artificial inoculation of the pathogen), and after second treatment application (twelve days after artificial inoculation of the pathogen). Scoring of the disease was done using the 0 – 5 disease scale developed by Islam and Pan (1993). Score chart was described as in section 2.4.

Percentage disease index (PDI) of fruit rot was calculated using the formula developed by Mc Kinney (1923)

$$\frac{\text{Sum of individual ratings} \times 100}{\text{Total number of fruit assessed} \times \text{Maximum disease category}}$$

3.6.4. Effect of various treatments on biometric parameters of brinjal plant

After 60 days of transplanting, the treated plants were recorded for the following biometric observations:

3.6.4.1. Effect of various treatments on no. of leaves, no. of branches and height of brinjal plant.

3.6.4.1.1. No. of leaves

No. of leaves per plant was counted and recorded.

3.6.4.1.2. No. of branches

No. of branches per plant was observed and recorded.

3.6.4.1.3. Height of the plant

Length of each plant was measured and recorded.

3.6.4.2. Effect of various treatments on yield of brinjal.

3.6.4.2.1. Number of fruits per plant

No. of fruits per plant was observed and recorded.

3.6.4.2.2. Yield / plant

The yield of plants include the number of fruits per plant and its mean weight.

3.7. STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

Results

4. EXPERIMENTAL RESULTS

4.1. ISOLATION OF THE PATHOGEN

The pathogen causing fruit rot of brinjal was isolated from the naturally infected brinjal fruits. Fungal culture was obtained from the infected plants during March 2009 and it was designated as an isolate Pv. The culture was isolated following standard procedure on Potato Dextrose Agar (PDA) medium.

4.1.1. Single spore isolation

The fungal isolate Pv obtained was purified by single spore isolation procedure.

4.1.2. Proving the pathogenicity

Pathogenicity of the fungal culture (Pv) was proved following Koch's Postulate's on brinjal fruits, variety Swetha. Typical symptom of fruit rot (circular or elliptical sunken necrotic lesion) was reproduced within five days. The pathogen was reisolated and the pathogenicity was established.

4.1.3. Maintenance of the culture

The fungal isolate Pv culture was sub cultured frequently on PDA slants and was kept in refrigerator at 5 °C. Virulence of the culture was maintained by passing through the host once in three months.

4.2. SYMPTOMATOLOGY

4.2.1. Under natural conditions

Symptomatology of the isolate was studied. Symptoms first appeared as pale, sunken, circular to oval areas on the fruit surface. These later turned brown and enlarged up to 2 to 3 inches in dia; often two or more lesions merge to cover much of the fruit surface. Affected fruits became soft and watery at first; decay may penetrate rapidly throughout the fruit, causing a light brown discoloration of the flesh. Later black, pimple like structures the pycnidia developed in the centre of old lesions. As the infection progressed the spots were markedly delimited by a thick and sharp outline enclosing a lighter black or straw coloured area. When the diseased fruit was cut open, the lower surface of the skin was covered with minute, elevated, spherical, black, stromatic masses of the fungus. The characteristic conidiomata appear as black pinhead-sized structures, which are often concentrically arranged on fruits. Infected fruits were soft and mushy or mummified and black. Affected fruits get wrinkled and deformed. Finally the diseased fruits were shrivelled and dried. The infected fruits either remained on the plant or dropped off. The variety Swetha was prone to fruit rot infection with the culture (Pv). Symptoms occurred 15-20 days after fruit formation till harvest and extended upto post harvest stage (Plate 1 and 2).

4.2.2. Under artificial conditions

Symptoms started at the base of the fruits near the calyx region. On the fruits the symptoms appeared first as minute sunken greyish spots with a brownish halo which later enlarged and coalesced, produced concentric rings of yellow and brown zones. These spots increased in size and formed large rotten areas on which pycnidia often developed concentrically, covering most of the rotten fruit surface (Plate 3 and 4). After 8-9 days of artificial inoculation the whole fruit became mummified.

Symptoms under natural infection

Plate 1. Leaf infection.

Plate 2. fruit infection.

Symptoms under artificial infection.

Plate 3. Standing plant

Plate 4. Detached fruit

4.3. MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE PATHOGEN

4.3.1. Morphological characters

Morphology of the isolate Pv was studied on PDA medium.

4.3.1.1. Colony characters

On PDA, the culture appeared initially as white floccose mycelium which gradually turned to dark greyish white as it grew older. Diurnal zonations were prominent on the upper surface. Numerous black, globose to irregular pycnidia were also produced in a month old culture. A distinct olivaceous grey to black zone alternated with light creamish zone was observed on the reverse side of the colony (Plate 5).

4.3.1.2. Conidial characters

4.3.1.2.1. Conidial morphology of the isolate Pv.

On PDA the isolate grew as white floccose mycelium and produced black, globose to irregular pycnidia. The culture produced alpha conidia which were one celled, hyaline and ellipsoidal (2.5-12 µm long x 0.9-2.8 µm wide) and beta conidia which were one celled, hyaline, filiform and straight or curved (15.1-28.0 µm long x 0.30-2.3 µm wide) (Plate 6, Fig. 2).

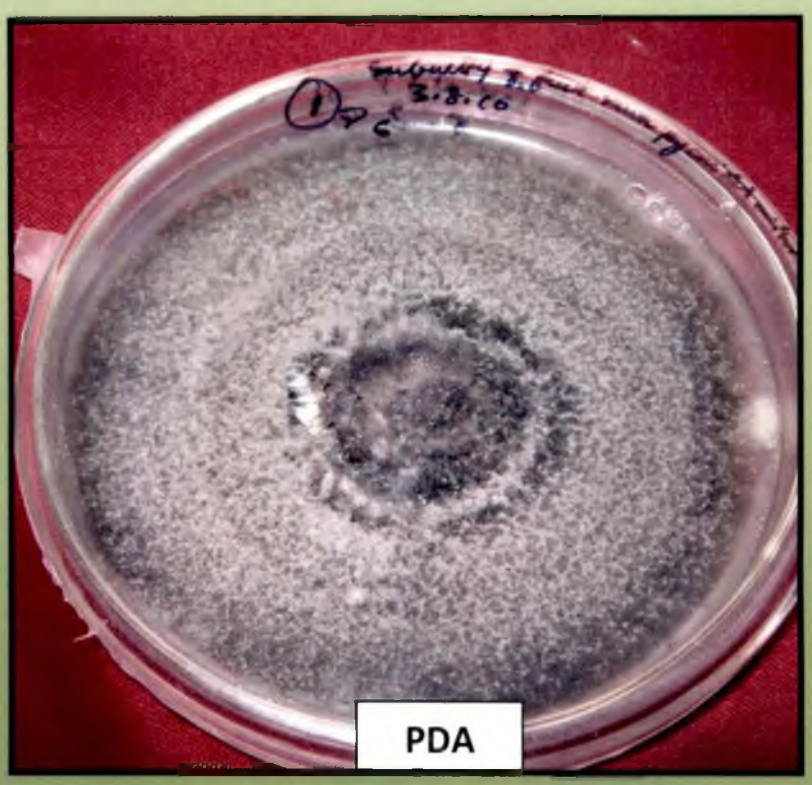
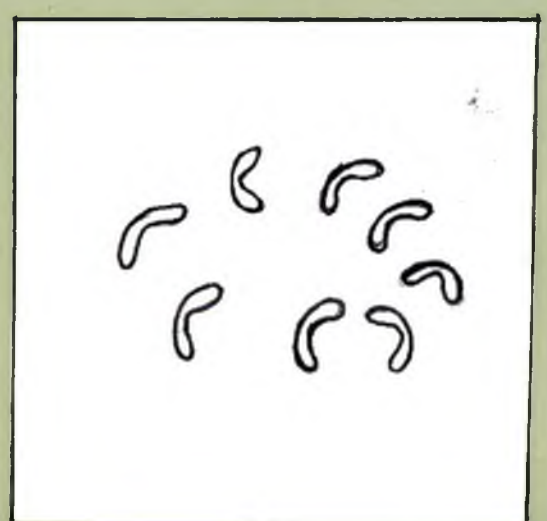


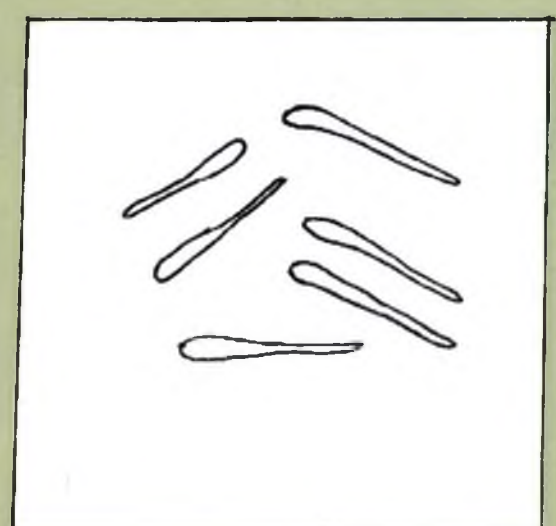
Plate 5. Growth of *Phomopsis vexans* on PDA.

Plate 6. Alpha and Beta conidia of *Phomopsis vexans*.

Fig : 2 DIAGRAM SHOWING ALPHA AND BETA CONIDIA OF PHOMOPSIS VEXANS CAUSING BLIGHT AND FRUIT ROT IN BRINJAL



(a) α CONIDIA



(b) β CONIDIA

Table 1. Colony and conidial morphology of the isolate Pv on PDA

Sl. No.	Characters	*P v*
1.	Type of growth	Felty
2.	Colony colour	Creamy white turned into greyish white
3.	Colony forms	Circular
4.	Type of margin	Entire
5.	Elevation	Slightly raised
6.	Spore mass colour	Grey
7.	Diurnal zonations	Prominent
8.	Mycelial dry weight (mg/30ml)	482.36-666.90 mg

Spore counts were recorded as 11.7 x 10^4/ml. From the colony and conidial morphology, the fungal isolate collected was identified as *Phomopsis vexans* (Pv).

4.3.2. Cultural characters

4.3.2.1. Growth and sporulation of *Phomopsis vexans* on different solid media.

The diversity in cultural and morphological characters of *Phomopsis vexans* was studied on four different solid media under laboratory conditions. The data are presented in Table 2.

Maximum radial growth was obtained on PDA medium (90.00 mm) within seven days and was significantly superior to all the other media tested. This was

Table 2. Growth and sporulation of *Phomopsis vexans* in different solid media

Sl. No.	1.	2.	3.	4.
Media	Potato Dextrose Agar	Czapek's Dox Agar	Host Extract Dextrose Agar	Oat Meal Agar
Growth Characters	Mycelium aerial, white at first then turned grey with entire but sometimes with wavy margin.	Mycelium aerial, white to greyish with entire margin.	Mycelium aerial, white to greyish with entire margin.	Mycelium aerial, white later turned to dark grey sunken with uneven margin
Sporulation	Very good sporulation.	Good sporulation.	Fair sporulation.	Fair sporulation.
Radial growth (mm)*	90.00	63.30	74.00	61.60
CD at 0.05 %		11.89		

* Mean of three replications.

followed by Host Extract Dextrose Agar (74.00 mm), Czapek's Dox Agar (63.30 mm) and Oat Meal Agar (61.60 mm). Minimum growth was observed on Oat Meal Agar. There was no significant difference in the growth of *Phomopsis vexans* in Host Extract Dextrose Agar, Czapek's Dox Agar and Oat Meal Agar media.

Sporulation was obtained in all the four media tested. Excellent sporulation of the fungus was recorded on PDA followed by Czapek's Dox Agar media. Sporulation was fair on Oat Meal Agar and Host Extract Dextrose Agar. With respect to mycelial colour, it varied from dull white to grey. The growth varied from slightly raised to slightly fluffy to sunken with smooth and entire or uneven margins. The growth of the fungus on PDA was circular, felty, greyish white with entire margin showing diurnal zonations. Mycelial growth on Host Extract agar was like sunken mat with circular entire margins, greyish, development of black pin head pycnidia in a month old culture at the centre of the colonies. On Oat Meal Agar, the fungus produced dark grey, sunken in centre and slightly raised on the margins, circular growth having smooth and uneven margins, produced white pin head pycnidia initially which later turned black. Mycelial character of *Phomopsis vexans* on Czapek's Dox Agar media and PDA media were similar (Plate 7, Fig. 3).

4.3.2.2 Growth and sporulation of *Phomopsis vexans* in different liquid media.

Dry mycelial weight and sporulation of *Phomopsis vexans* in four different liquid media after 15 days of growth was recorded and the data are presented in Table 3.

Among the various liquid media tested for the growth of the pathogen, Czapek's Dox broth (666.90 mg), Host Extract Dextrose broth (666.90 mg) and Potato Dextrose broth (665.40 mg) were on par and significantly superior over Oat Meal broth (479.03 mg) (Plate 8, Fig. 4).

Table 3. Growth of *Phomopsis vexans* in different liquid media after 15 days.

Sl. No.	Media	Growth characters	Dry mycelial weight * (mg)
1.	Potato Dextrose broth	Mycelium thick and greyish white, excellent sporulation. Filtrate was reddish brown.	665.40
2.	Czapek's Dox broth	Mycelium thick and greyish white. Filtrate was yellowish.	666.90
3.	Host Extract Dextrose broth	Mycelium thick and greyish white. Filtrate was brown.	666.90
4.	Oat Meal broth	Mycelium scanty and thin, white in colour. Filtrate was creamish brown.	479.03
CD at 0.05 %			56.99

* Mean of three replications.

Plate 7. Growth of *Phomopsis vexans* on different solid media.

Plate 8. Growth of *Phomopsis vexans* on different liquid media.

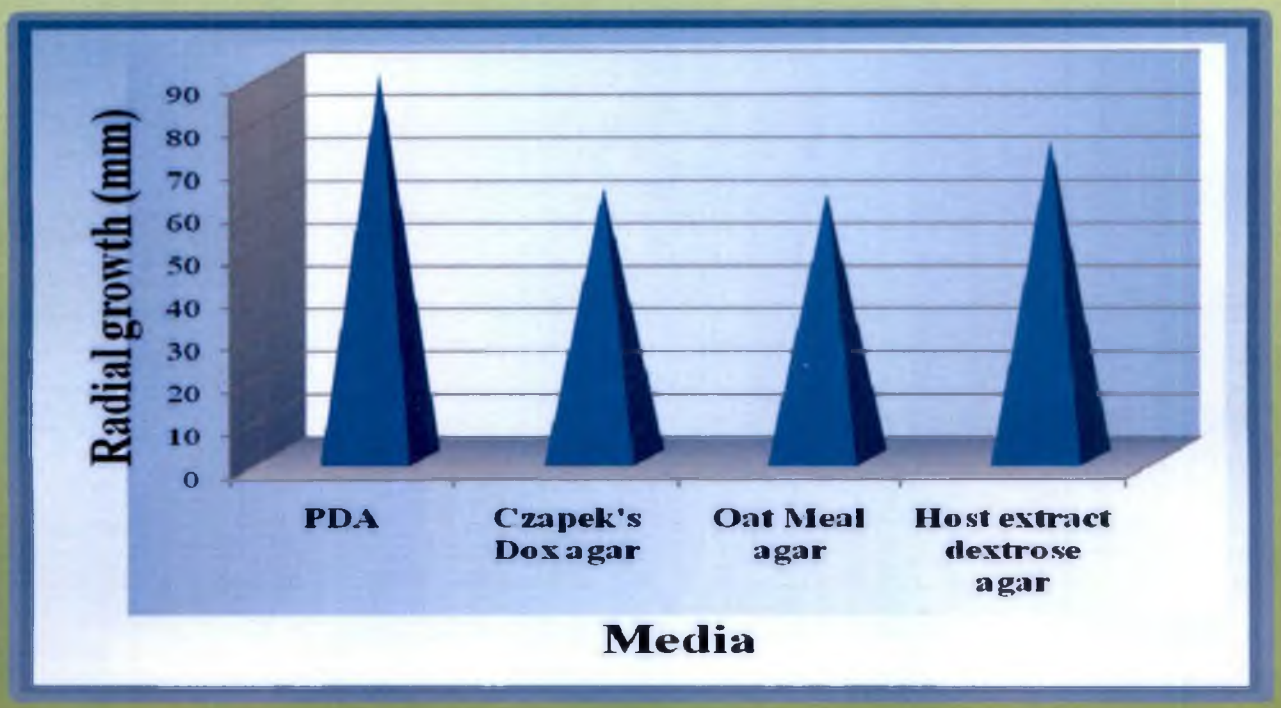


Fig. 3. Growth of *Phomopsis vexans* on different solid media

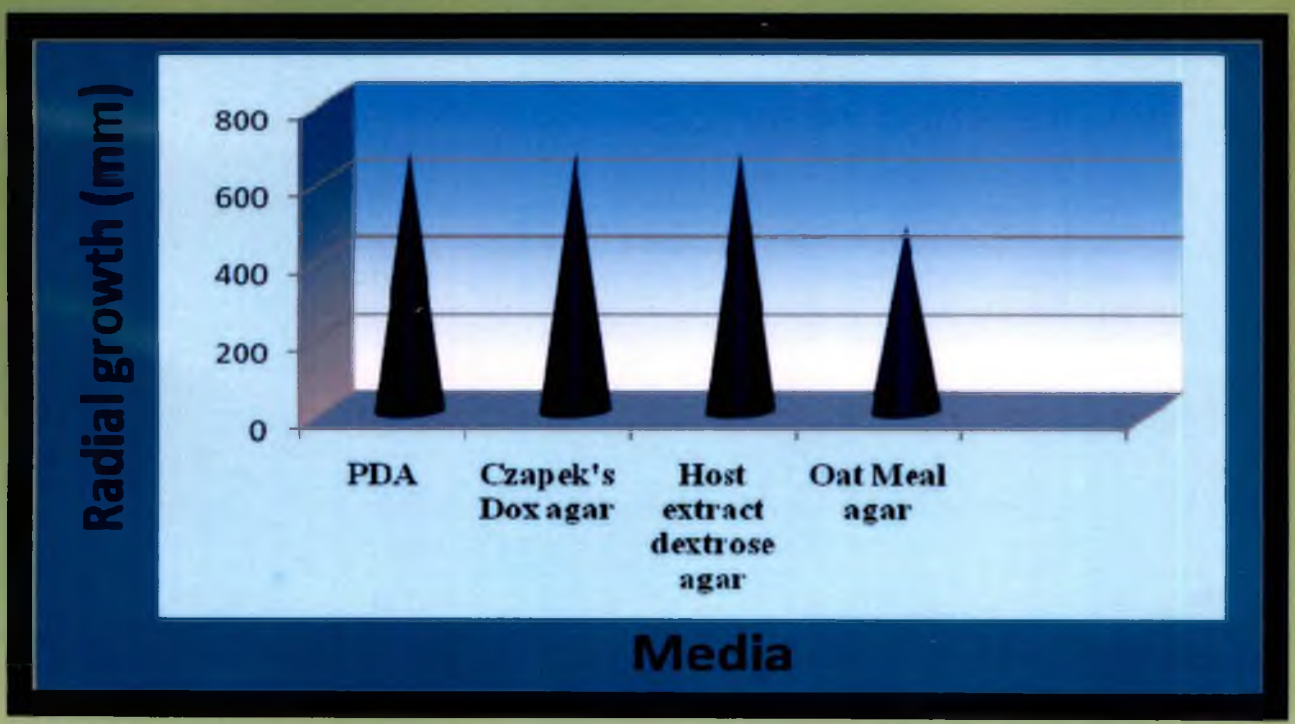


Fig. 4. Growth of *Phomopsis vexans* in different liquid media

4.3.2.3. Production of toxic metabolite by *Phomopsis vexans* (Pv) on different liquid media and its activity on brinjal fruits.

The toxic metabolite activity of the culture filtrate from different liquid media was studied by conducting bioassay on brinjal fruits.

The effect of the toxic metabolite produced by *Phomopsis vexans* on brinjal fruit (variety Swetha) are presented in Table 4. Size of lesions formed after three days of inoculation by the culture filtrate containing toxin of the fungus was maximum and significantly high in the case of Potato Dextrose broth. Next to Potato Dextrose broth significantly lower size lesions were produced by Host Extract Dextrose and Czapek's Dox culture filtrate. Minimum lesion size was observed in fruit inoculated with Oat Meal broth. Lesions were light brown and water soaked spreading an area of 2.03 cm when the culture filtrate from Potato Dextrose broth was artificially inoculated on the detached brinjal fruits. Colour of the lesions produced by toxin from different media were also slightly different. The lesions were observed as dark brown in the centre with outer margin light brown in Potato Dextrose and Czapek's Dox broth but in Host Extract Dextrose and Oat Meal broth, entire lesion was dark brown coloured (Plate 9, Fig.5).

4.3.3. Nutritional studies of *Phomopsis vexans*.

4.3.3.1. Effect of different carbon sources on growth of *Phomopsis vexans*.

The requirement and utilization of carbon sources by *Phomopsis vexans* was studied with five different carbon sources using Czapek's Dox media as the basal medium. Dry mycelial weight of *Phomopsis vexans* in different liquid media supplemented with different carbon sources after 15 days of inoculation were recorded and the data are presented in Table 5.

Table 4. Effect of toxic metabolite of culture filtrate of *Phomopsis vexans* on detached brinjal fruit (Swetha).

Sl.No.	Media	Lesion size (cm) * after 3 days.	Symptoms
1.	Potato Dextrose culture filtrate	2.03	Inner lesions were dark brown surrounded by light brown water soaked areas. Lesions appeared as circular which later extended in linear pattern.
2.	Czapek's Dox culture filtrate	0.77	Lesions appeared as dark brown in circular pattern.
3.	Host Extract Dextrose culture filtrate	1.35	Lesions appeared as dark brown surrounded by water soaked areas in linear pattern.
4.	Oat Meal broth	0.12	Inner portions of lesions were light brown surrounded by dark brown areas. Lesions were smaller in size.
5.	Control (Sterile water)	0.00	No lesions.
CD at 0.05 %		0.23	

* Mean of three replications

Table 5. Growth and sporulation of *Phomopsis vexans* in different carbon sources.

Sl. No.	Czapek's Dox media with Carbon source	Growth Characters	Mycelial dry weight (mg) * in Czapek's Dox medium
1.	Dextrose	White, scattered, round smooth, raised having uneven margins, thin mycelial growth.	376.66
2.	Lactose	White, thick, circular smooth and entire margins, concentric zonations present, fluffy mycelial growth.	653.33
3.	Maltose	Off white, thick fluffy mycelial growth with circular, smooth and entire margins.	626.66
4.	Starch	Yellowish white, circular, smooth and having entire margins.	543.33
6.	Control (Sucrose)	Loose textured, thin scattered, white sparse growth of mycelium.	436.66
CD at 0.05 %			143.39

* Mean of three replications

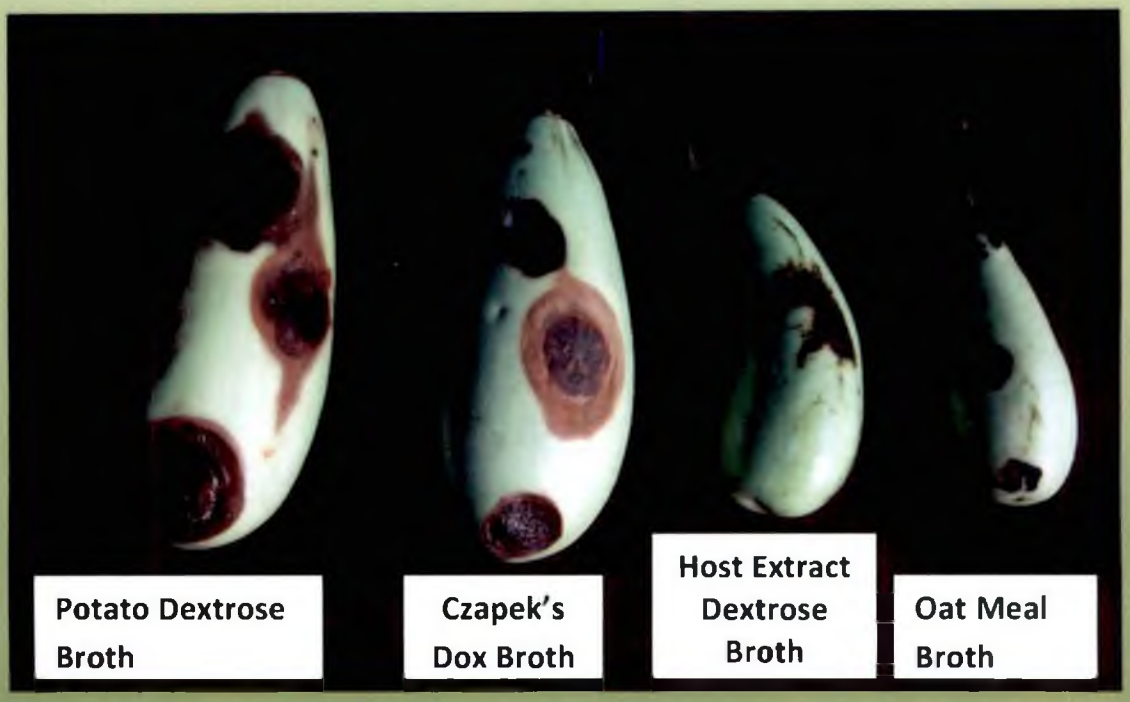


Plate 9. Symptoms produced by *Phomopsis vexans* culture filtrate on brinjal fruits.

Plate 10. Growth of *Phomopsis vexans* on Czapek's Dox Broth with different Carbon sources.

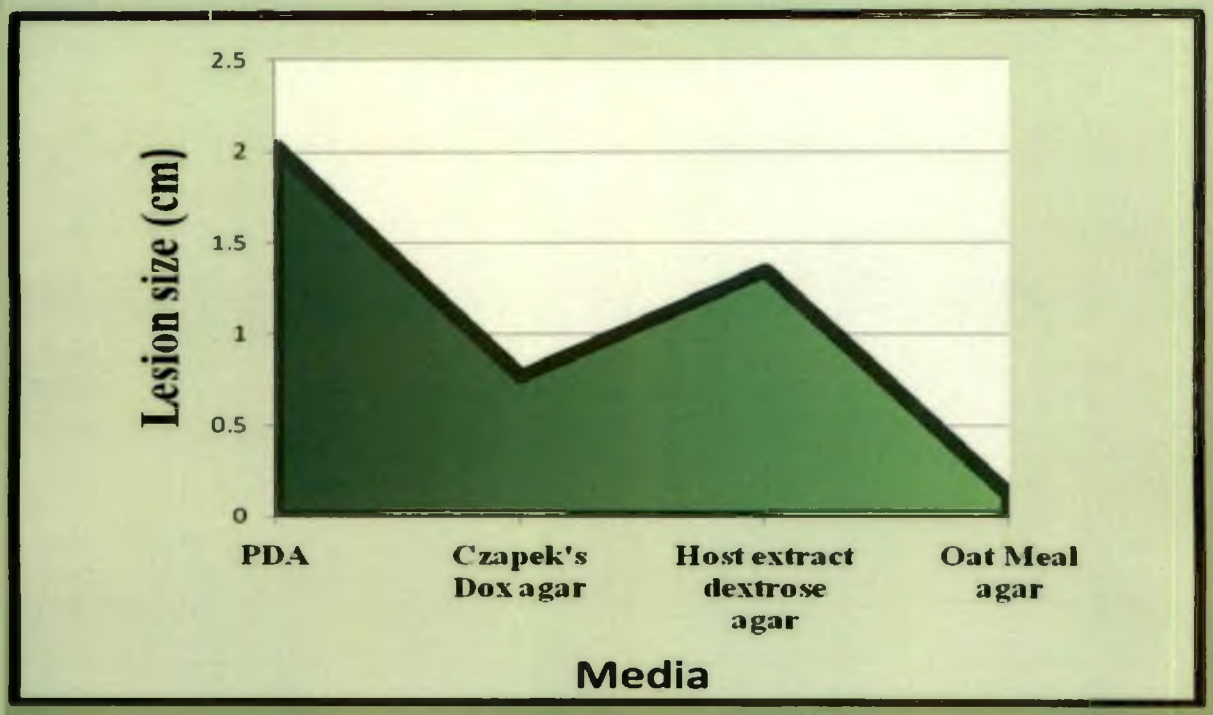


Fig. 5. Effect of toxic metabolite of culture filtrate of *Phomopsis vexans* on detached brinjal fruit (Swetha).

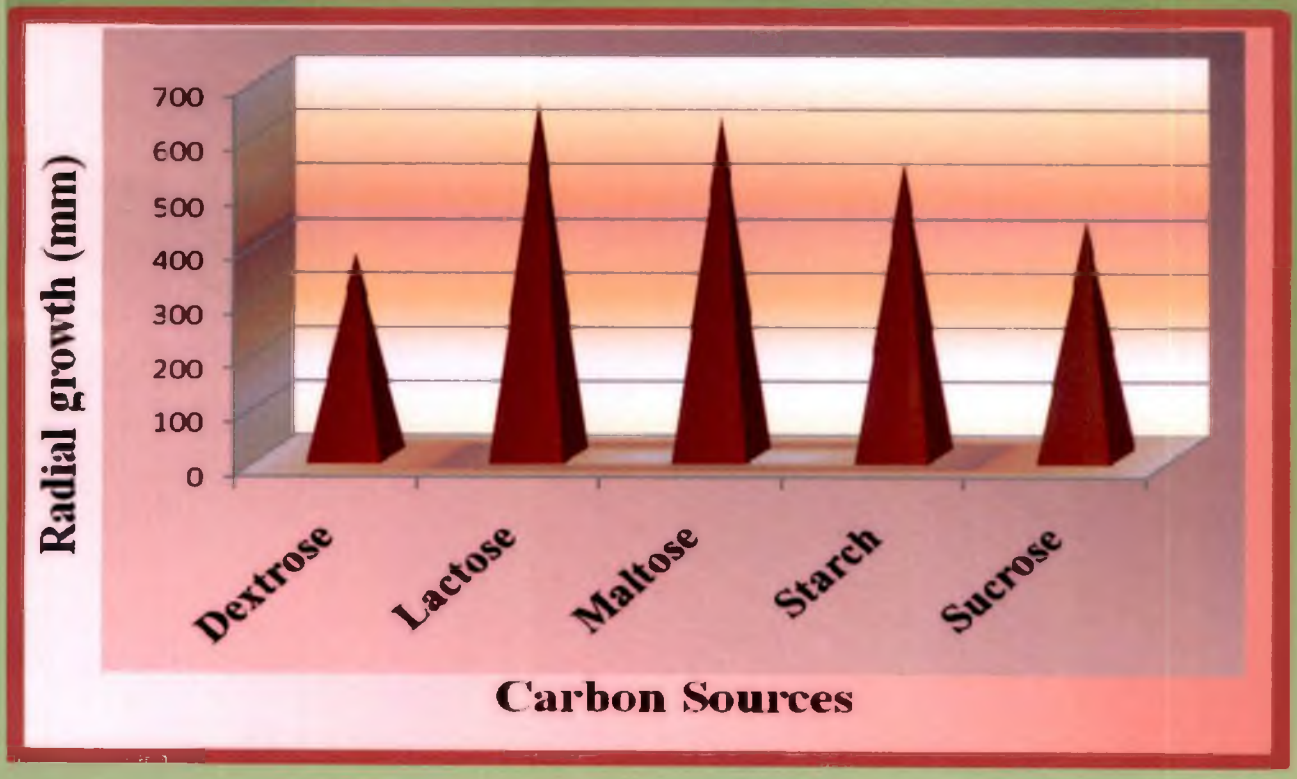


Fig. 6. Growth of *Phomopsis vexans* in Czapek's Dox broth with different Carbon sources

Among the five different carbon sources, lactose was the best one recording the maximum dry mycelial weight of 653.33 mg followed by maltose (626.66 mg) and starch (543.33 mg) and they recorded statistically significiant growth compared to dextrose. Dextrose showed minimum growth as compared to all other carbon sources including sucrose which was the carbon source in Czapek's Dox broth. There was no significant difference in the growth of pathogen in dextrose (376.66 mg) when compared with sucrose (436.66 mg). The mycelial growth appeared as off white to white and slightly fluffy to sunken. In dextrose media the growth was sparse and dirty white. Culture colour in different carbon sources varied slightly. In Czapek's Dox broth with lactose and sucrose as carbon source culture appeared white in colour but it was off white in maltose while starch medium showed slightly yellowish white (Plate 10, Fig. 6).

4.3.3.2. Effect of different nitrogen sources on growth of *Phomopsis vexans*

The requirement of nitrogen sources by *Phomopsis vexans* was studied using different nitrogen sources supplemented in Czapek's Dox broth. Dry mycelial weight of *Phomopsis vexans* in different nitrogen sources after 15 days of growth was recorded and the data are presented in Table 6.

Statistically, no significant differences in growth of *Phomopsis vexans* was recorded in Czapek's Dox media when it was supplemented with various nitrogen sources. Ammonium nitrate was found to be the best utilized nitrogen source other than sodium nitrate (626.60 mg) which was the main nitrogen constituent of Czapek's Dox broth though statistically there was no significant difference in dry weight of the mycelium among the four nitrogen sources, *viz.*, sodium nitrate, ammonium nitrate, ammonium chloride and potassium nitrate recording 626.60, 563.30, 556.60 and 543.30 mg dry weight respectively. All the sources recorded

Table 6. Growth and sporulation of *Phomopsis vexans* in different nitrogen sources.

Sl. No.	Czapek's Dox medium with Nitrogen Source	Growth characters	Mycelial dry weight (mg) * in Czapek's Dox medium
1.	Urea	No growth.	0.00
2.	Ammonium Carbonate	No growth.	0.00
3.	Ammonium Chloride	Off white and light grey in colour, smooth and thin mycelial mat with uneven margins.	556.60
4.	Ammonium nitrate	Dirty white, thick mat with smooth and entire margins.	563.30
5.	Potassium nitrate	White coloured, scattered, loose textured, smooth and thin mycelial mat with entire margins.	543.30
6.	Control (Sodium nitrate)	Off white to dirty white coloured, thick mycelial mat with smooth and entire margins.	626.60
CD at 0.05 %			115.76

* Mean of three replications.

significantly lower growth than control. No growth was observed in case of urea and ammonium carbonate.

Culture colour in different nitrogen sources varied. Mycelial colour was white, dirty white to off white in Czapek's Dox broth supplied with different nitrogen sources, off white in ammonium chloride and sodium nitrate, dirty white in ammonium nitrate and white in potassium nitrate (Plate 11, Fig. 7)

4.3.4. Physiological factors affecting the growth of *Phomopsis vexans.*

4.3.4.1. Effect of p^H on growth of *Phomopsis vexans.*

The fungus was grown in Czapek's Dox medium at different p^H levels and observations were recorded on dry mycelial weight. The results are presented in Table 7.

The fungus recorded maximum growth at p^H 8.0 (dry mycelial weight of 876.67 mg/30 ml Czapek's Dox broth). No significant difference in growth was recorded at p^H 6.0, 7.0, 8.0 and 9.0. At p^H 5.0, no growth was recorded indicating that this level is unfavourable for the growth of *Phomopsis vexans.* But at p^H 7.0 and 9.0, the fungus recorded 836.67 mg and 816.67 mg dry mycelial weight respectively and p^H 8.0 recorded as the optimum p^H for the growth since p^H levels above and below 8.0 recorded lower growth of the pathogen (Plate 12, Fig. 8).

4.4. ISOLATION OF ANTAGONISTS FROM PHYLLOSPHERE AND RHIZOSPHERE OF BRINJAL PLANTS

4.4.1. Isolation of antagonists from brinjal rhizosphere

From the micro organisms isolated from rhizosphere, five fungi and two bacteria were selected having antagonistic property against the pathogen *Phomopsis*

Table 7. Growth of *Phomopsis vexans* at different p^H.

Sl. No.	P^H	Mycelial dry weight (mg) * in Czapek's Dox medium
1.	5	0.00
2.	6	730.00
3.	7	836.67
4.	8	876.67
5.	9	816.67
	CD at 0.05 %	165.41

*Mean of three replications.

Plate 11. Growth of *Phomopsis vexans* on Czapek's Dox Broth with different Nitrogen sources.

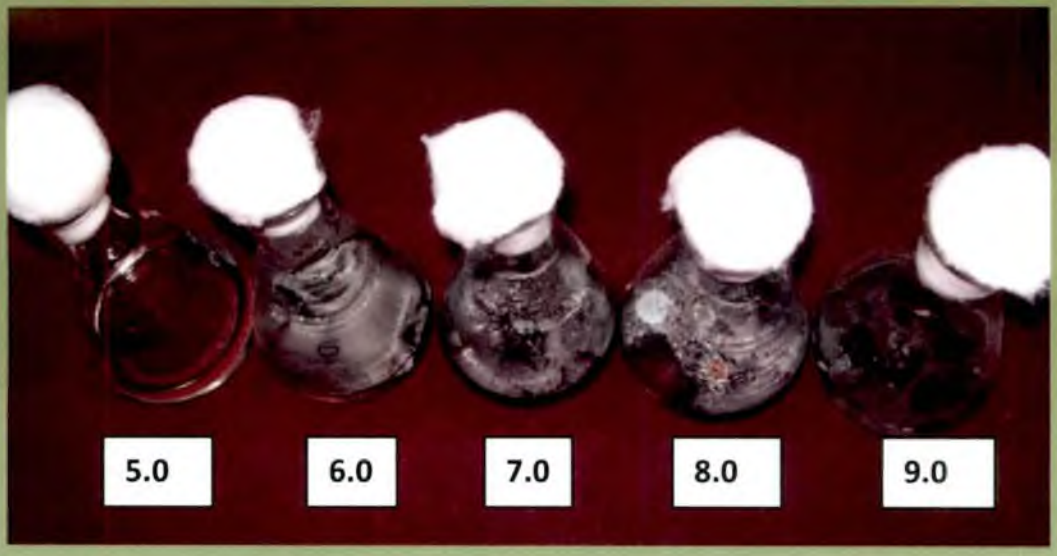


Plate 12. Growth of *Phomopsis vexans* on Czapek's Dox Broth at different p^H.

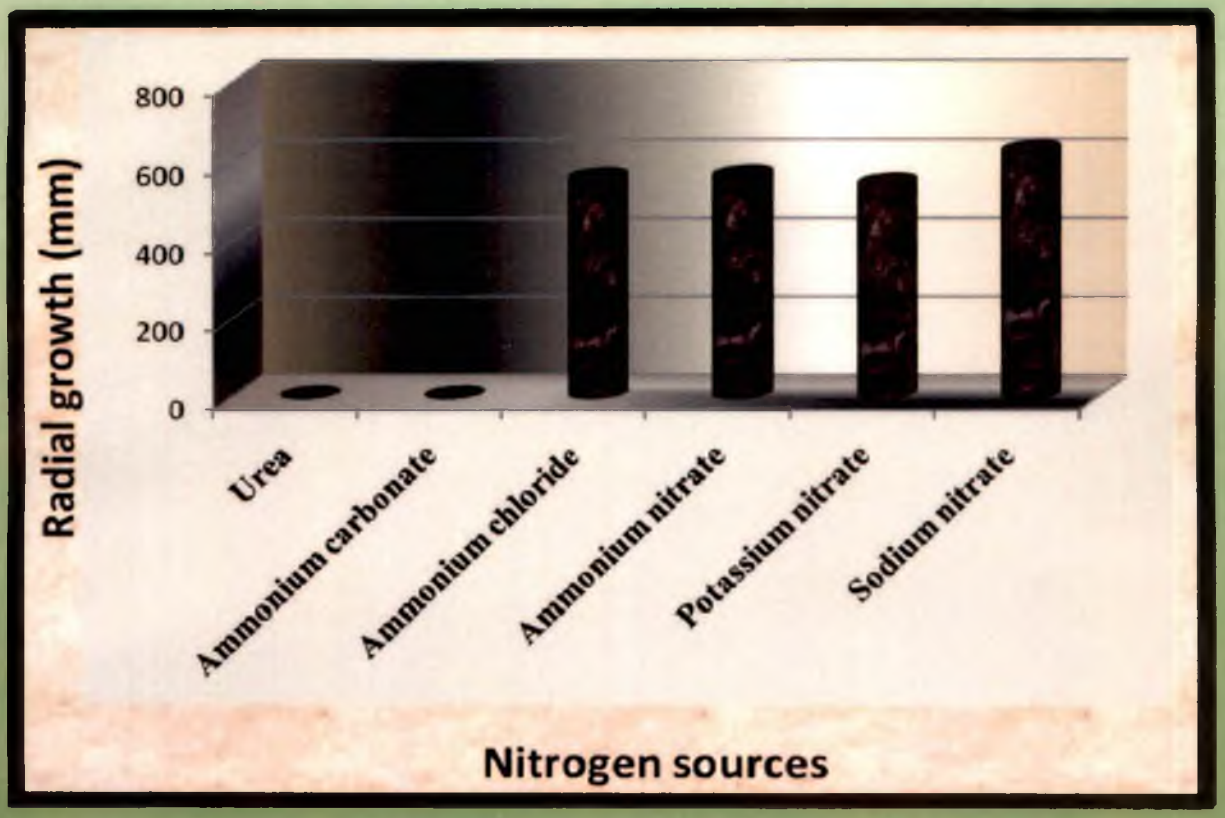


Fig. 7. Growth of *Phomopsis vexans* in Czapek's Dox broth with different Nitrogen sources.

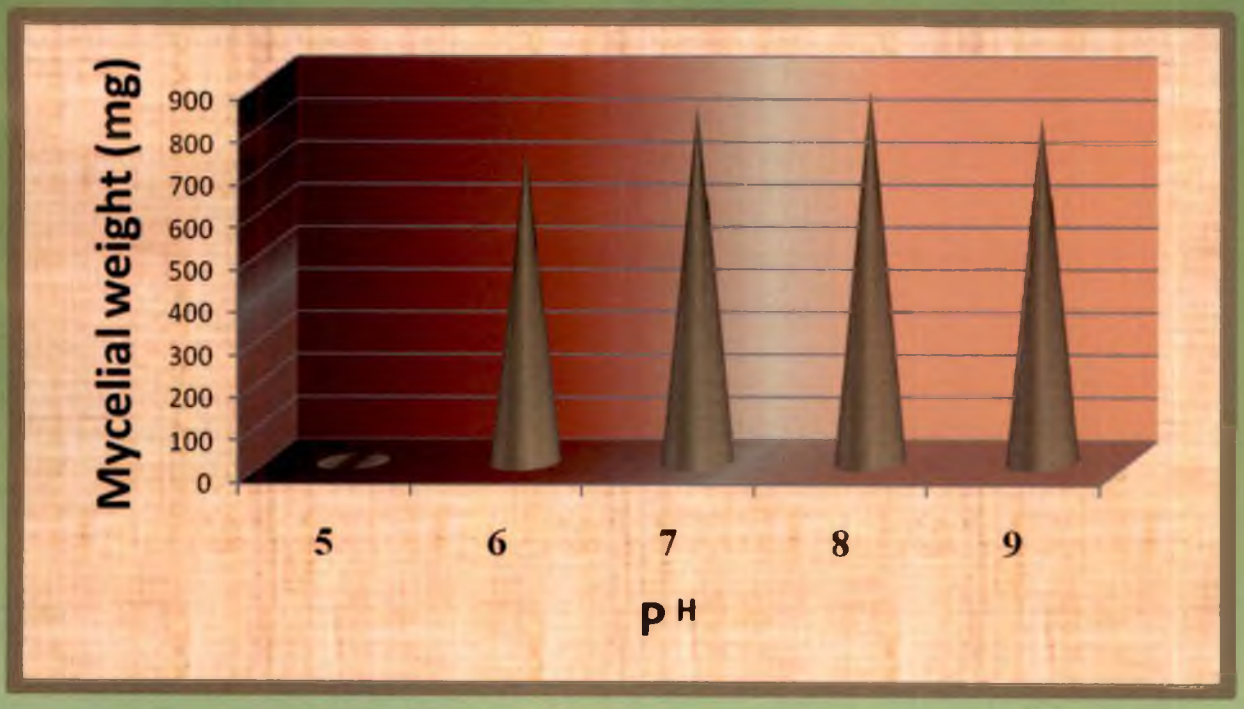


Fig. 8. Growth of *Phomopsis vexans* in Czapek's Dox broth at different P^H

vexans. The fungi were identified as *Trichoderma* sp. (T1 to T5). The bacteria selected were designated as bacterial isolate B1 and fluorescent bacterial isolate (FB - 1).

4.4.2. Isolation of antagonists from brinjal phyllosphere

From the brinjal phyllosphere one fungus and one bacterium was selected based on their inhibition percentage against the pathogen *Phomopsis vexans*. The fungus was identified as a species of *Aspergillus* (A1) and the bacterium as fluorescent bacterial isolate (FB - 2).

4.5. *IN VITRO* MANAGEMENT OF *PHOMOPSIS VEXANS.*

4.5.1. *In vitro* screening of fungal antagonists against *Phomopsis vexans.*

All the antagonists collected significantly suppressed the growth of *Phomopsis vexans* (Table 8). The *Trichoderma* isolate T2 was effective in inhibiting the growth of the pathogen (67.33 %). T2 had over grown and suppressed the growth of *Phomopsis vexans* within five days when they were placed in PDA media at five cm apart. Other *Trichoderma* isolates (T1, T3, T4 and T5) and *Aspergillus* sp (A1) also showed suppression of growth of the pathogen in six days of incubation. T2 was followed by T1 and T5 with 64.03 and 63.80 per cent growth inhibition of the pathogen. Among *Trichoderma* isolates T2, T1 and T5, T2 recorded maximum inhibition of *Phomopsis vexans*. But statistically there was no significant difference in antagonism between them. Among the five *Trichoderma* isolates T3 and T4 showed significantly lower antagonism than T2, T1 and T5 with 67.33, 64.03 and 63.80 per cent inhibition. *Aspergillus* sp. showed minimum antagonistic activity against *Phomopsis vexans* with 38.46 per cent inhibition which was significantly inferior to four of the *Trichoderma* isolates *i.e.,* T2, T1, T5 and T3. The least

Table 8. Percentage inhibition of *Phomopsis vexans* by fungal antagonists (*Trichoderma* and *Aspergillus* sp.) in dual culture.

Sl. No.	Microbial antagonists	Radial growth of *Phomopsis vexans* (cm) * in dual culture	% inhibition over control*	Type of interactions
1.	*T 1* (*Trichoderma* sp.)	3.23	64.03 (53.14)	B•
2.	*T2* (*Trichoderma* sp.)	2.93	67.33 (55.12)	B
3.	*T3* (*Trichoderma* sp.)	4.26	52.56 (46.45)	B
4.	*T4* (*Trichoderma* sp.)	5.13	42.7 (40.77)	B
5.	*T5* (*Trichoderma* sp.)	3.26	63.8 (52.99)	B
6.	*A1* (*Aspergillus* sp.)	5.53	38.46 (38.31)	B
7.	Control.	9.00	-	
CD at 0.05 %			3.23	

*Mean of three replications.

B• - Over growth - Pathogen overgrown by test fungus

(Figures given in parenthesis are transformed values).

effective *Trichoderma* isolate T4 and the *Aspergillus* sp. had no statistical difference in their antagonistic activity against *Phomopsis vexans* (Plate 13, Fig. 9).

4.5.1.1. Identification of fungal antagonist of *Phomopsis vexans*

Six different fungi obtained from the phyllosphere and rhizosphere of brinjal plants. They were identified based on the spore morphology, colony and cultural characters. The fungal antagonists selected were identified as the species of *Trichoderma* and *Aspergillus*.

4.5.2 *In vitro* screening of bacterial antagonist against *Phomopsis vexans.*

The efficacy of the bacterial isolates obtained from the rhizosphere and phyllosphere of brinjal plants in inhibiting the growth of *Phomopsis vexans* under *in vitro* condition was studied by dual culture technique. The bacterial isolates varied in their ability in inhibiting the pathogen.

All the bacterial isolates exhibited high antagonistic activity against *Phomopsis vexans.* Of these, fluorescent bacterial isolate FB - 2 exhibited 82.93 per cent inhibition of *Phomopsis vexans* (Table 9, Plate 14, Fig. 9).

The per cent growth inhibition by the bacterial and fungal isolates were compared. Among the fungal and bacterial antagonists tested, fluorescent bacterial isolate (FB - 2) which showed the maximum suppression of pathogen was selected for *in vivo* experiment.

4.5.2.1. Identification of selected bacterial antagonist of *Phomopsis vexans.*

The fluorescent bacterial isolate (FB - 2) was subjected to various tests as indicated under materials and methods. It was found as a gram –ve rod, catalase

Table 9. Percentage inhibition of *Phomopsis vexans* by bacterial antagonists in dual culture.

Sl. No.	Microbial antagonists	Radial growth of *Phomopsis vexans* (cm) * in dual culture	% inhibition over control *	Type of interactions
1.	B1	2.9	68.46	C•
2.	FB – 2	1.53	82.93	C
3.	Control.	9.00	-	

*Mean of three replications.

C• - Cessation of growth at the line of contact of the cultures.

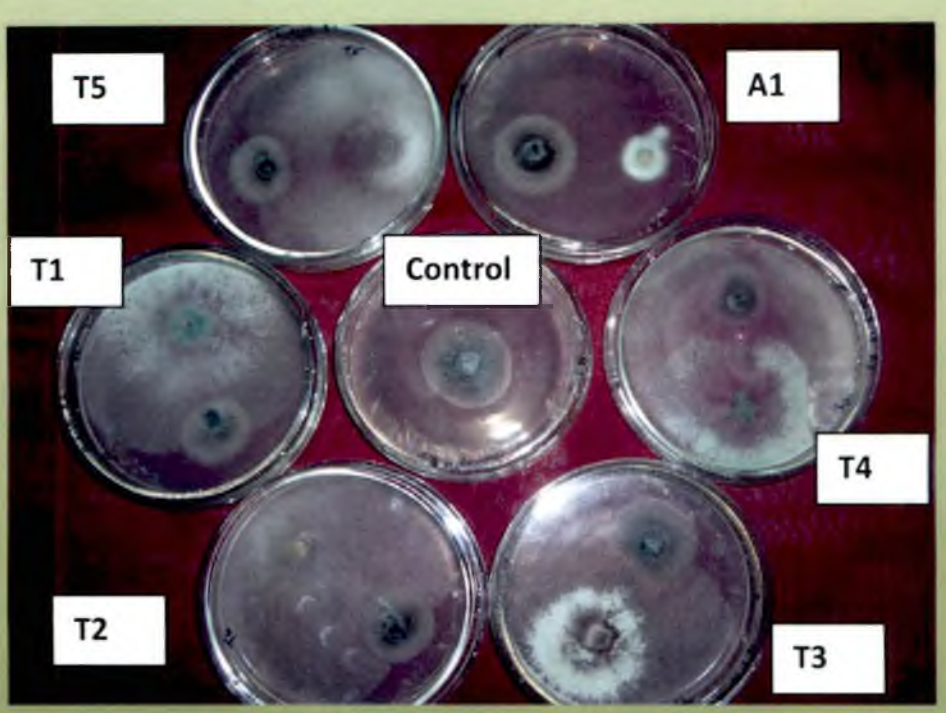
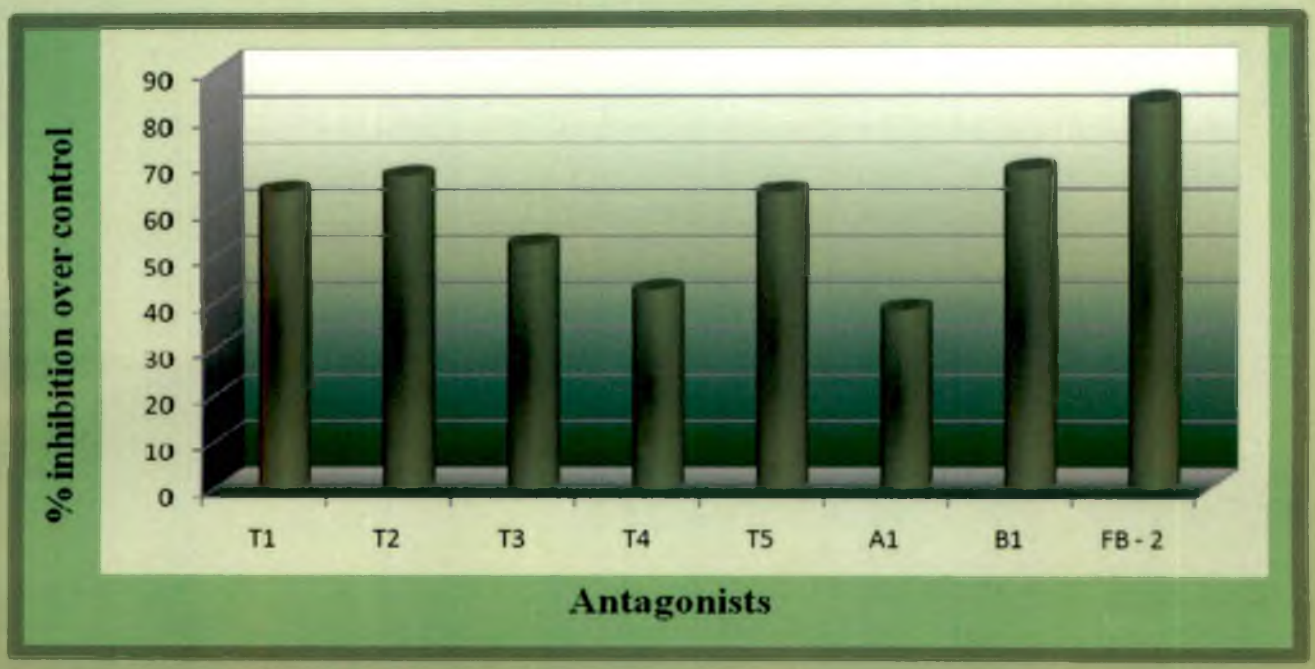


Plate 13. Inhibition of *Phomopsis vexans* by fungal antagonists.

Plate 14. Inhibition of *Phomopsis vexans* by fluorescent bacteria.



T1 to T5 - *Trichoderma* sp.

A1 - *Aspergillus* sp.

B1 – Bacterial isolate

FB - 2 – Fluorescent bacterial isolate

Fig. 9. *In vitro* inhibition of *Phomopsis vexans* by antagonists.

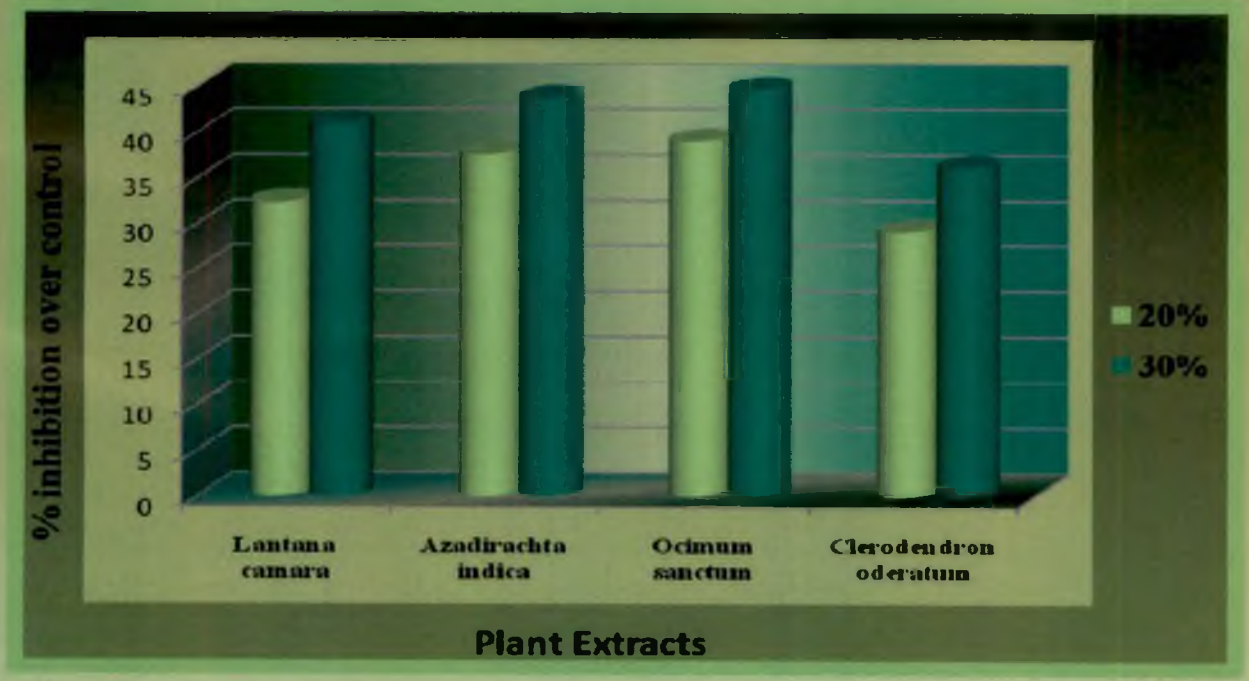


Fig. 10. Inhibition of *Phomopsis vexans* by different plant extracts

positive and produced raised, entire, slimy colonies on King's B medium with green fluorescence and identified as fluorescent bacteria.

4.5.3. Studies on the selected fungal antagonist T2.

Among the five fungal antagonists obtained, the isolate T2 which was identified as a species of *Trichoderma* showed maximum percentage inhibition against *Phomopsis vexans*.

4.5.3.1. Production of volatile compounds by the *Trichoderma* isolate T2.

Volatile compound released from the culture of *Trichoderma* sp. (T2) inhibited colony growth of *Phomopsis vexans*. The percentage inhibition of *Phomopsis vexans* by volatiles was 62.59 % (Table 10, Plate 15).

4.5.3.2. Production of non volatiles by the *Trichoderma* isolate T2.

The results showed that the fungal antagonist *Trichoderma* sp. (T2) inhibited the growth of *Phomopsis vexans* by the production of non volatile antifungal substances. The percentage inhibition of *Phomopsis vexans* when exposed to the volatiles was found to be less compared to that by non volatile compounds. The growth of *Phomopsis vexans* was inhibited by 54.44 per cent by the non volatiles produced by T2 (Table 10, Plate 16).

4.5.4. *In vitro* screening of plant extracts against *Phomopsis vexans*.

Four plant extracts *viz., Ocimum sanctum, Azadirachta indica, Lantana camara* and *Clerodendron oderatum* at two different concentrations (20 and 30 %) were evaluated in the laboratory for their efficacy against *Phomopsis vexans* through poisoned food technique (Table 11).

Table 10. Effect of volatiles and non volatile compounds of *Trichoderma* isolate T2 on growth of *Phomopsis vexans*

Treatments	Growth of the Pathogen (cm)*	% inhibition over control
Volatiles of *T2*	3.36	62.59
Non volatiles of *T2*	4.1	54.44
Control	9.0	-

*Mean of three replications

Table 11. Effect of various plant extracts on the growth of *Phomopsis vexans (in vitro)*

Sl. No	Plant Extracts	Growth of the Pathogen (cm) * at 20% concentration	% inhibition over control	Growth of the Pathogen (cm) * at 30% concentration	% inhibition over control	Mean	
						Growth of the Pathogen (cm)	% inhibition over control
1.	*Lantana camara*	4.73	32.37 (34.62)	4.13	40.94 (39.76)	4.43	36.65 (37.19)
2.	*Azadirachta indica*	4.36	37.61 (32.48)	3.93	43.80 (36.92)	4.14	40.70 (34.70)
3.	*Ocimum sanctum*	4.26	39.04 (38.65)	3.86	44.75 (41.96)	4.06	41.89 (40.30)
4.	*Clerodendron oderatum*	5.13	29.04 (37.81)	4.46	36.18 (41.41)	4.79	32.61 (39.61)
5.	Control	7.0		7.0			
CD at 0.05 % between concentrations						0.22	2.05
CD at 0.05 % between extracts						0.31	2.90

(Figures given in parenthesis are transformed values)

*Mean of three replications

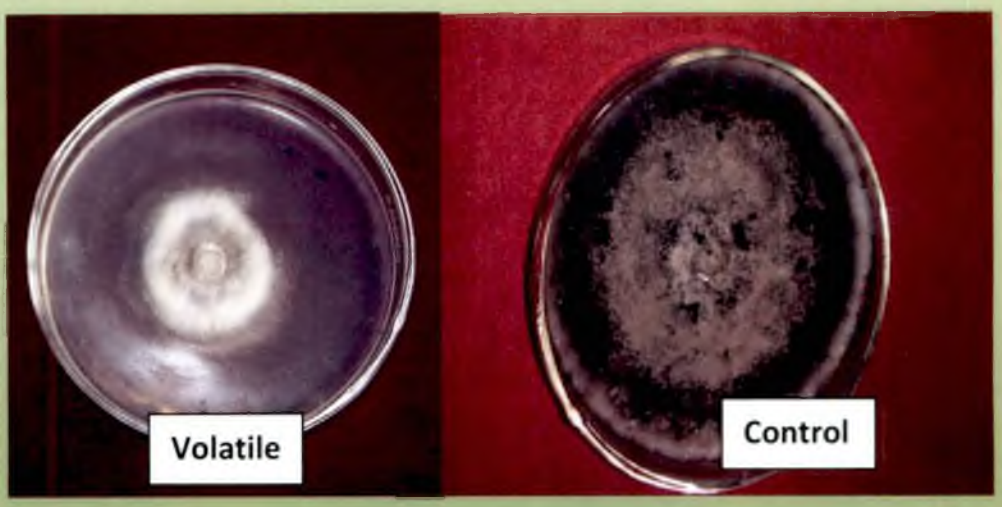


Plate 15. Inhibition of *Phomopsis vexans* by volatile compounds of *Trichoderma* sp. (T2).

Plate 16. Inhibition of *Phomopsis vexans* by non volatile compounds of *Trichoderma* sp. (T2).

All the plant extracts were highly effective against *Phomopsis vexans*. Among the four plant extracts evaluated, *Ocimum sanctum* was found to be the best in inhibiting the mycelial growth (41.89 %) of *Phomopsis vexans* and was significantly superior to *Clerodendron oderatum* (32.61 %). Though *Azadirachta indica* (40.70 %) and *Lantana camara* (36.65 %) recorded lower effect than *Ocimum sanctum*, statistically no significant difference was observed between them.

A similar trend in the inhibition of *Phomopsis vexans* was observed when all the plant extracts were evaluated at 20 and 30 per cent concentrations.

Among the four plant extracts evaluated at two concentrations (20 and 30 %), *Ocimum sanctum* at 30 per cent concentration was found to be superior in inhibiting the mycelial growth (44.75 %) of *Phomopsis vexans* and was significantly superior to all other plant extracts tested. Next best plant extract in inhibiting the growth of *Phomopsis vexans* was *Azadirachta indica* at 30 per cent concentration (43.80 %). There was significant difference in *Ocimum sanctum* at 30 per cent (44.75 %) and *Clerodendron oderatum* at 20 per cent concentration (29.09 %) in inhibiting the pathogen. It was also observed that *Ocimum sanctum* at 20 per cent (39.04 %) was on par with 30 per cent concentration of *Azadirachta indica* (43.80 %) and *Lantana camara* (32.37 %).

The higher concentrations of all the leaf extracts had shown significant superior effect than their lower concentrations in inhibiting the growth of *Phomopsis vexans*. The lower concentration (20 %) of *Ocimum sanctum* was selected for the *in vivo* experiment (Plates 17 to 20, Fig. 10).

4.5.5. *In vitro* screening of oil cakes against *Phomopsis vexans*

Four oil cakes *viz.*, coconut oil cake, neem oil cake, gingelly oil cake and groundnut oil cake were tested to check the suppression of radial growth of

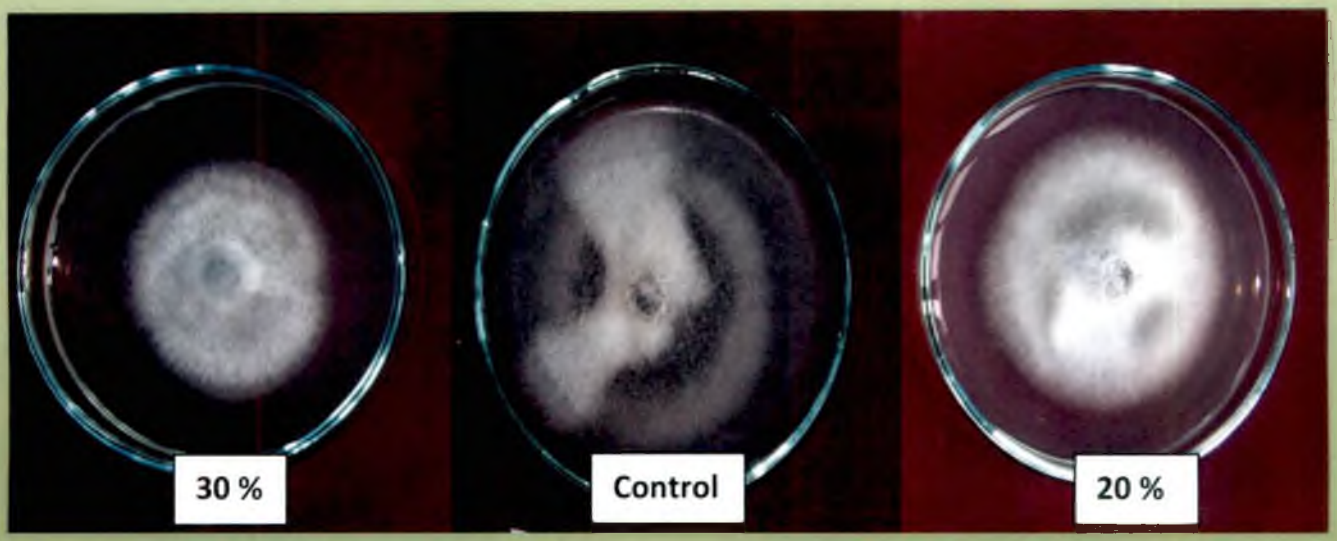


Plate 17. Inhibition of *Phomopsis vexans* by *Lantana camara*.

Plate 18. Inhibition of *Phomopsis vexans* by *Ocimum sanctum*.

Plate 19. Inhibition of *Phomopsis vexans* by *Azadirachta indica*.

Plate 20. Inhibition of *Phomopsis vexans* by *Clerodendron oderatum*.

Phomopsis vexans following poisoned food technique. Each oil cake was used at 20 and 30 per cent concentration (Table 12).

The four oil cakes tested were highly effective in inhibiting the growth of *Phomopsis vexans* when compared to control. Among the different oil cakes tested, groundnut oil cake gave the highest percentage growth inhibition of 60.46 and was significantly superior to all the other oil cakes tested. The effect of coconut oil cake, gingelly oil cake and neem oil cake were statistically on par with each other in inhibiting the pathogen.

All the oil cakes tested were highly effective in inhibiting the growth of *Phomopsis vexans*. Among all the four oil cakes evaluated at 20 and 30 per cent concentration, groundnut oil cake at 30 per cent concentration recorded 64.75 per cent inhibition. This was significantly superior over all the concentrations of oil cakes tested. However 20 per cent concentration of coconut oil cake (48.57 %) and gingelly oil cake (47.14 %) had lower effect in inhibiting the pathogen than 30 per cent concentration of all other oil cakes. Neem oil cake at 20 per cent concentration had least effect in checking the growth of *Phomopsis vexans* (38.09 %). Twenty per cent concentration of groundnut oil cake (56.18 %) had equal effect in inhibiting *Phomopsis vexans* compared to 30 per cent concentration of all other oil cakes except groundnut oil cake. The lower concentration of groundnut oil cake was selected for field trial (Plate 21 to 24, Fig. 11).

4.5.6. *In vitro* effect of carbendazim at various concentrations on the growth of *Phomopsis vexans*.

Among all the three concentrations of carbendazim evaluated (0.025 %, 0.05 % and 0.1 %), cent per cent growth inhibition of *Phomopsis vexans* was observed at the highest concentration (0.1 %). Hence 0.1 per cent concentration of carbendazim

Table 12. Effect of various oil cakes on the growth of *Phomopsis vexans (in vitro).*

Sl. No	Oil cakes	Growth of the Pathogen (cm) * at 20% concentration	% inhibition over control	Growth of the Pathogen (cm) * at 30% concentration	% inhibition over control	Mean	
						Growth of the Pathogen (cm)	% inhibition over control
1.	Coconut oil cake	3.60	48.57 (44.16)	3.26	56.66 (47.16)	3.43	52.61 (45.66)
2.	Gingelly oil cake	3.70	47.14 (43.34)	3.16	54.76 (47.72)	3.43	50.95 (45.53)
3.	Ground nut cake	3.10	56.18 (48.54)	2.46	64.75 (53.56)	2.78	60.46 (51.05)
4.	Neem cake	4.30	38.09 (38.00)	3.03	53.80 (48.83)	3.66	45.94 (43.41)
5.	Control	7.0		7.0			
CD at 0.05 % between concentrations					0.32		
CD at 0.05 % between extracts					0.45		

(Figures given in parenthesis are transformed values)
*Mean of three replications.

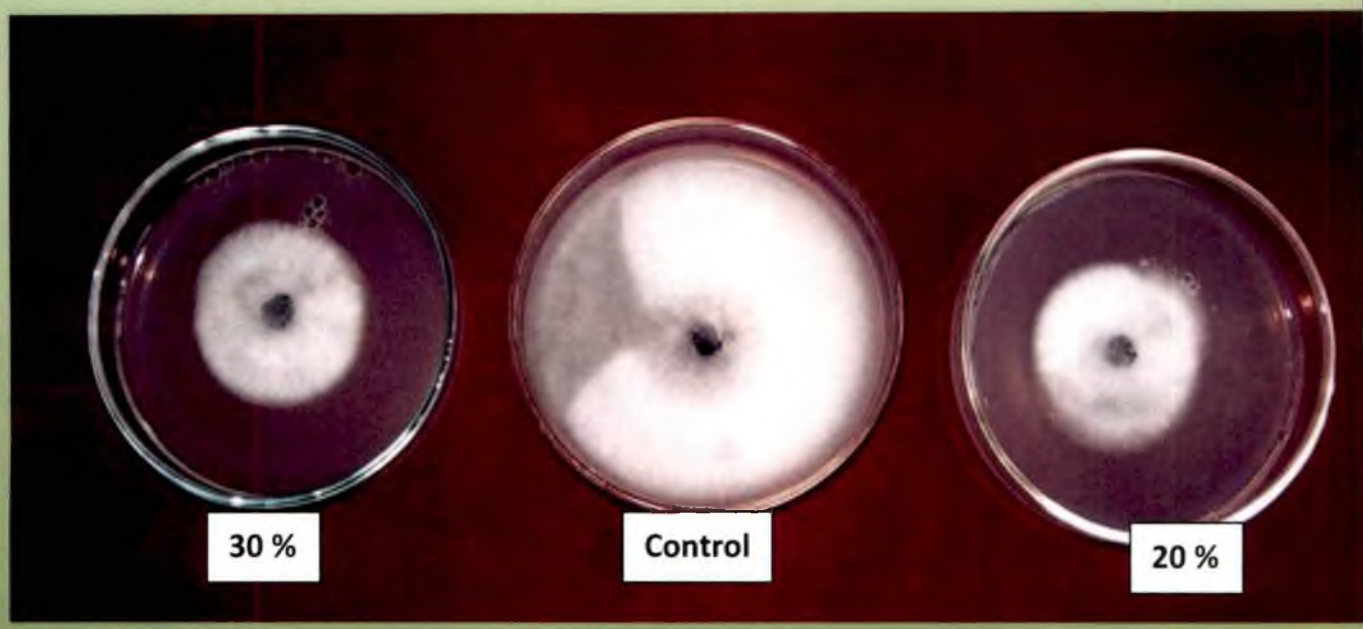


Plate 21. Inhibition of *Phomopsis vexans* by Coconut oil cake.

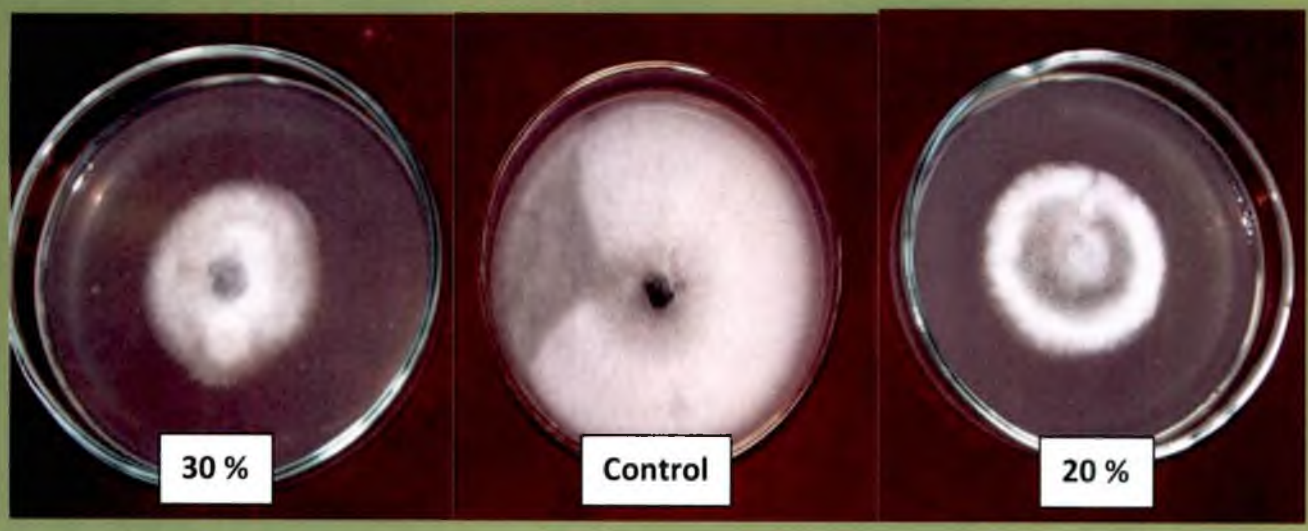


Plate 22. Inhibition of *Phomopsis vexans* by Gingelly oil cake.

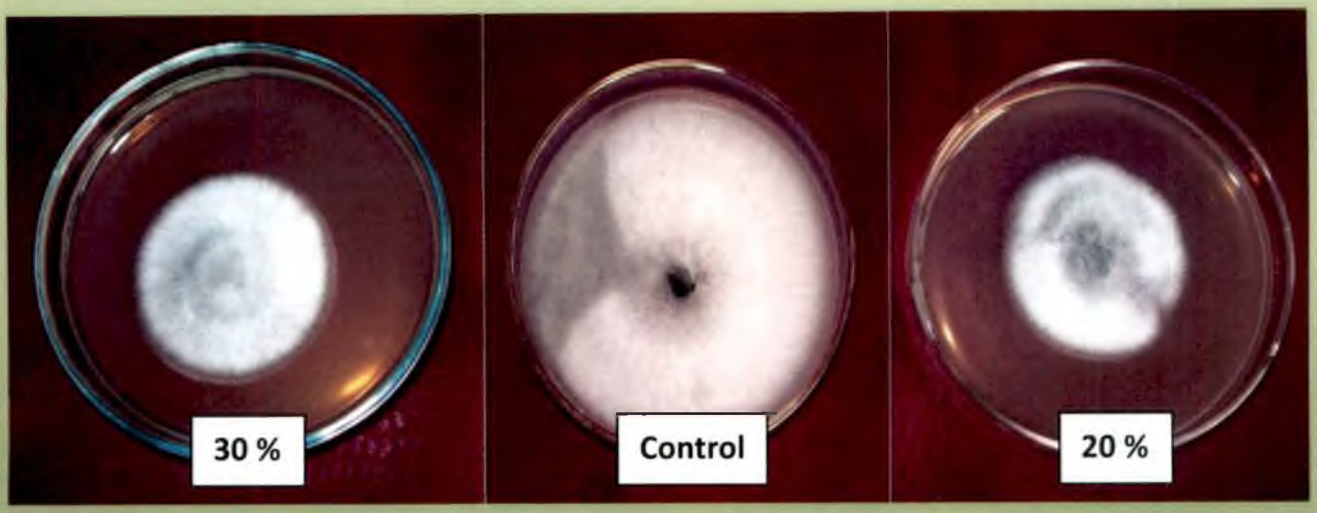


Plate 23. Inhibition of *Phomopsis vexans* by Neem oil cake.

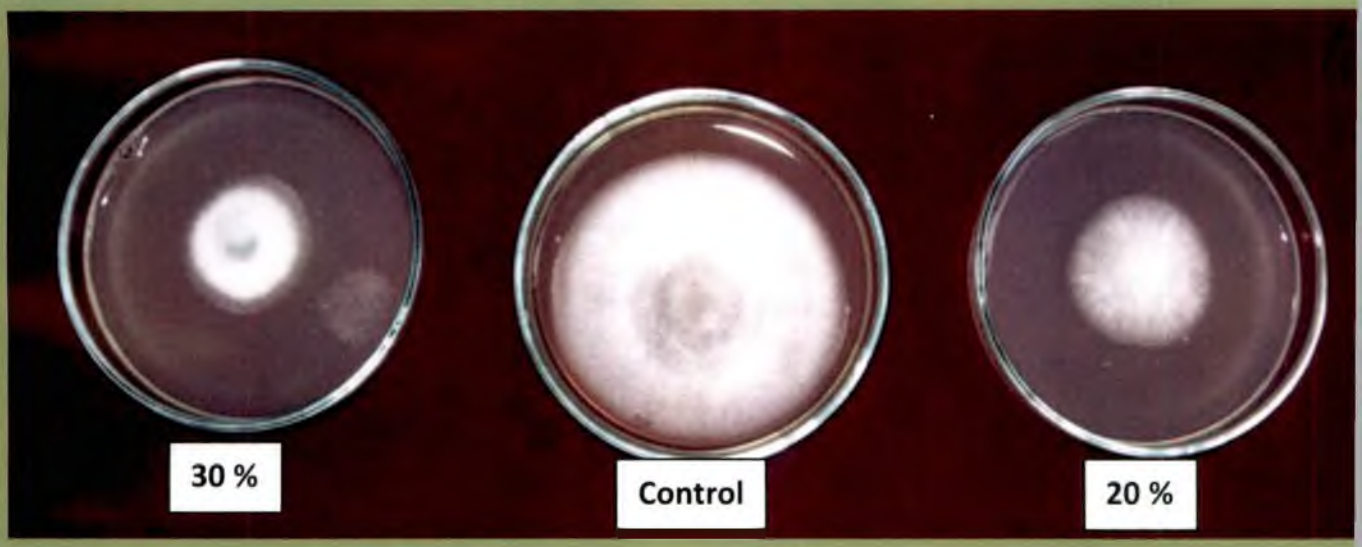
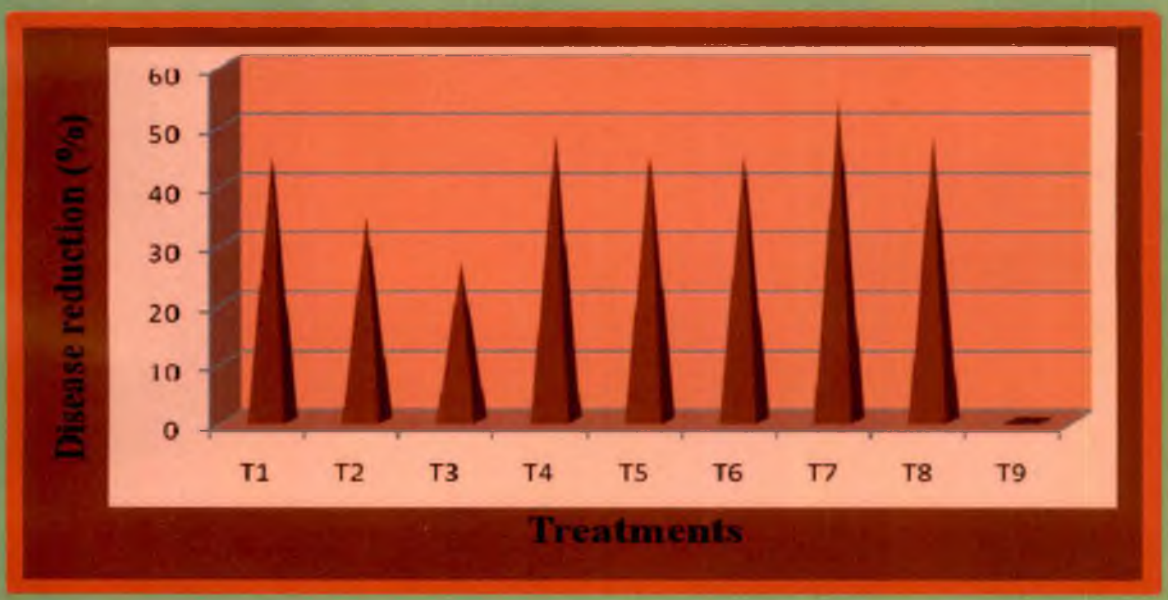


Plate 24. Inhibition of *Phomopsis vexans* by Groundnut oil cake.

Fig. 11. Inhibition of *Phomopsis vexans* by various oil cakes.



T1 - Talc based formulation of fluorescent bacteria

T2 - *Ocimum sanctum* leaf extract

T3 - Ground nut oil cake

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Carbendazim @ 0.1 %

T9 - Control.

Fig. 12. Effect of ecofriendly materials on disease reduction over control of fruit rot of brinjal.

(*i.e.* the concentration of carbendazim as per technical program) was used in the field trial (Table 13).

4.6. *IN VIVO* MANAGEMENT OF FRUIT ROT OF BRINJAL CAUSED BY *PHOMOPSIS VEXANS.*

4.6.1. Preparation of pathogen inoculum and foliar application

The brinjal plant were artificially inoculated by spraying with the conidial suspension (10^6 conidia/ml) of *Phomopsis vexans* on two weeks old fruit. Fruits were initially pin pricked and suitable microclimatic conditions were provided for disease development.

4.6.2. *In vivo* management of fruit rot of brinjal.

Based on the results of the *in vitro* management studies, the best antagonist (fluorescent bacteria 2 %), best plant extract (*Ocimum sanctum* 20 %) and the best oil cake (groundnut oil cake 200 g/pot), their combinations and carbendazim 0.1 per cent as fungicidal check were selected as treatments for the *in vivo* study. Fluorescent bacteria was used as talc based formulation for field application.

Table 13. Percentage inhibition of *Phomopsis vexans* by different concentrations of carbendazim *(in vitro)*.

Sl. No.	Bavistin Concentrations (%)	Growth of the Pathogen (cm)*	% inhibition over control
1.	0.025	2.86	68.14
2.	0.05	2.60	78.10
3.	0. 1	0.00	100.00
4.	Control	9.0	

*Mean of three replications.

The following were the nine treatments and their schedule of operation.

Treatments	**Particulars**	**Schedule of Application**
T_1	Talc based formulation of fluorescent bacteria (2 %)	Seedling root dip at the time of transplanting and foliar spray seven (Days after Inoculation) DAI
T_2	*Ocimum sanctum* leaf extract (20 %)	Foliar spray seven DAI
T_3	Groundnut oil cake (200 g / pot)	Soil amendment seven (Days before Transplanting) DBT
T_4	T_1 + T_2	Seedling root dip at the time of transplanting with fluorescent bacteria and foliar spray by the combination of fluorescent bacteria + *Ocimum sanctum*.
T_5	T_1 + T_3	Seedling root dip at the time of transplanting with fluorescent bacteria and foliar spray of fluorescent bacteria and soil amendment with groundnut oil cake.
T_6	T_2 + T_3	Foliar spray of *Ocimum sanctum* and soil amendment with groundnut oil cake.

Plate 25. POT CULTURE EXPERIMENT GENERAL VIEW

T_7	T_1 + T_2 + T_3	Seedling root dip at the time of transplanting with fluorescent bacteria and foliar spray by the combination of fluorescent bacteria + *Ocimum sanctum* leaf extract + soil amendment with groundnut oil cake.
T_8	Carbendazim 0.1 %	Foliar spray seven DAI.
T_9	Control	Water spray.

4.6.3. Disease intensity

Seven days after foliar spraying with the pathogen, all the inoculated fruits showed incidence of the disease in the form of water soaked lesions around the pin pricks. The percentage disease index (PDI) was calculated based on the damage caused by fruit rot pathogen on the brinjal fruits using the formula developed by Mc Kinney during 1923. Data on disease index before treatment application and after treatment application (seven days after artificial inoculation of the pathogen) are given in (Table 14).

All the eight treatments were effective in reducing the disease intensity of *Phomopsis* blight and fruit rot of brinjal. Among all the treatments applied T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) showed disease reduction of 54.22 per cent over control. This was statistically significant over all the other treatments. Treatment T_3 (groundnut oil cake) recording 26.81 per cent disease reduction had significant lower effect than all the other treatments. It was also observed that Per cent Disease Reduction obtained in between the maximum and minimum effective treatments, were statistically on par with each other (Plate 26, Fig. 12).

Table 14. Disease Index of fruit rot in brinjal before and after the treatment application.

Treatment		Disease index before treatment application	Disease index after treatment application	Disease reduction over control (%)
T_1	Fluorescent bacteria talc based formulation (2 %)	43.33 [6.59]	22.77 [4.68]	44.97 (41.78)
T_2	*Ocimum sanctum* leaf extract (20 %)	35.55 [6.04]	25.55 [5.05]	34.61 (35.55)
T_3	Ground nut oil cake (200 g /pot)	42.22 [6.51]	28.88 [5.37]	26.81 (30.47)
T_4	T1 + T2	37.77 [6.22]	20.00 [4.47]	48.67 (44.16)
T_5	T1 + T3	42.22 [6.57]	21.66 [4.65]	44.96 (42.03)
T_6	T2 + T3	43.33 [6.65]	21.66 [4.65]	45.1 (42.11)
T_7	T1 + T2 + T3	36.66 [6.09]	18.33 [4.27]	54.22 (47.40)
T_8	Carbendazim 0.1%	31.1 [5.66]	20.88 [4.55]	48.18 (43.93)
T_9	Absolute Control	35.55 [6.04]	40.55 [6.34]	-
CD at 0.05 %		[1.17]	[0.93]	14.00

[Figures given in parenthesis are square rot transformed values]
(Figures given in parenthesis are angular transformed values)
*Mean of three replications.

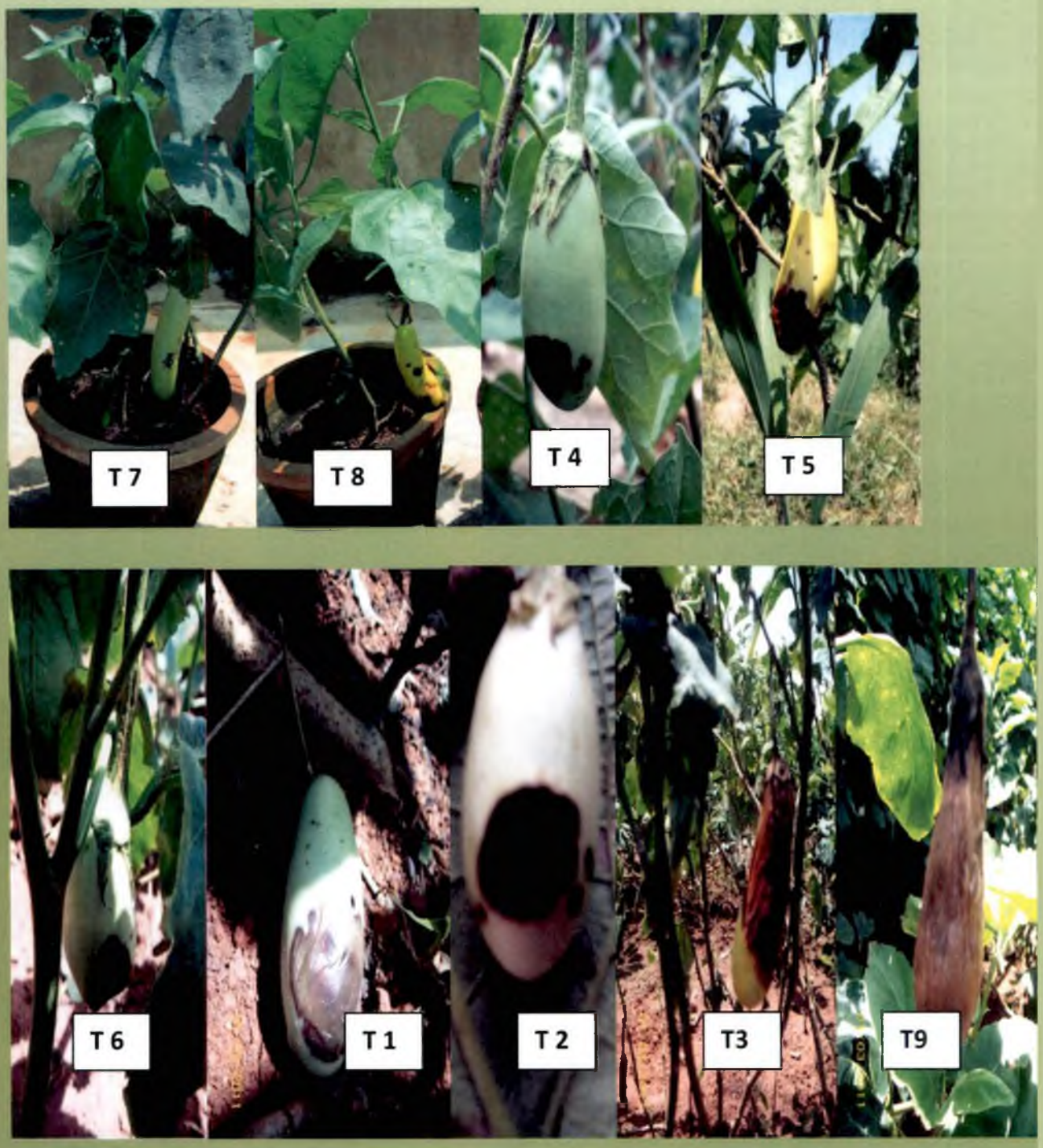


Plate 26. Effect of various treatments in the reduction of disease intensity of fruit rot in brinjal.

T1 - Talc based formulation of Fluorescent bacteria

T2 - *Ocimum sanctum* leaf extract

T3 - Ground nut oil cake

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Carbendazim @ 0.1 %

T9 - Control.

4.6.4. Effect of various treatments on biometric parameters of brinjal plant.

4.6.4.1. Effect of various treatments on no. of leaves, no. of branches and height of brinjal plant.

4.6.4.1.1. Effect of various treatments on no. of leaves.

The data on number of leaves per plant as influenced by different treatments are presented in Table 15. All treatments recorded increase in no. of leaves as compared to the control. Statistically, treatment T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) recorded significantly more number of leaves (177.66) per plant than all the other treatments (Fig. 13).

4.6.4.1.2. Effect of various treatments on no. of branches.

The data on total number of branches per plant as influenced by different treatments are given in Table 15 (Fig. 14). Statistically there was not much difference in the total number of branches due to different treatments. However the highest number of branches (8.00) was observed in treatment T_1 (Talc based formulation of fluorescent bacteria) and the lowest (5.33) in T_9 (control).

4.6.4.1.3. Effect of various treatments on height of the plant.

The data on height of the plant as influenced by different treatments are given in Table 15.

Application of the treatment T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) significantly increased the height of the plant compared to all the other treatments. However significantly lower plant height was recorded in T_8 (carbendazim 0.1 %) as 46.90 cm. Treatments

Table 15. Effect of various treatments on no. of leaves, branches and height of brinjal plant.

Treatments		No. of leaves*	No. of branches*	Height of plant* (cm)
T_1	Fluorescent bacteria talc based formulation (2 %)	136	8	60
T_2	*Ocimum sanctum* leaf extract (20 %)	104.66	6	59.33
T_3	Ground nut oil cake (200 g)	124	5.66	56.83
T_4	T1 + T2	114.33	7.33	65.5
T_5	T1 + T3	82.33	7.66	56.16
T_6	T2 + T3	103.33	6.66	63.33
T_7	T1 + T2 + T3	177.66	6.66	73.83
T_8	Carbendazim 0.1%	135	7.00	46.9
T_9	Control	78.33	5.33	60.26
CD at 0.05 %		19.57	1.71	6.32

*Mean of three replications

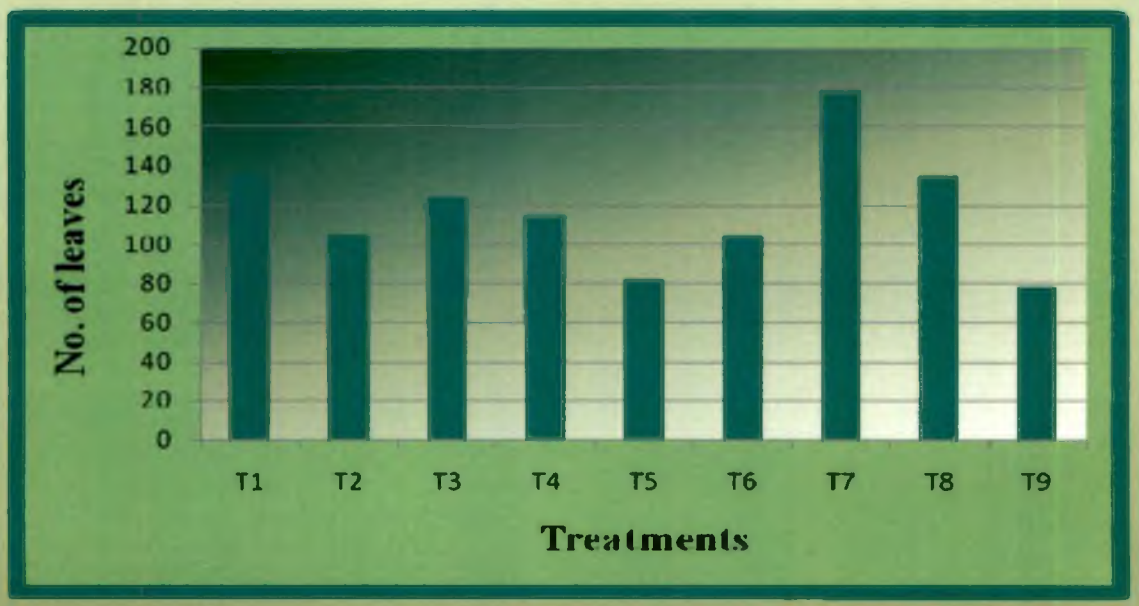


Fig. 13. Effect of ecofriendly materials on No. of leaves of brinjal.

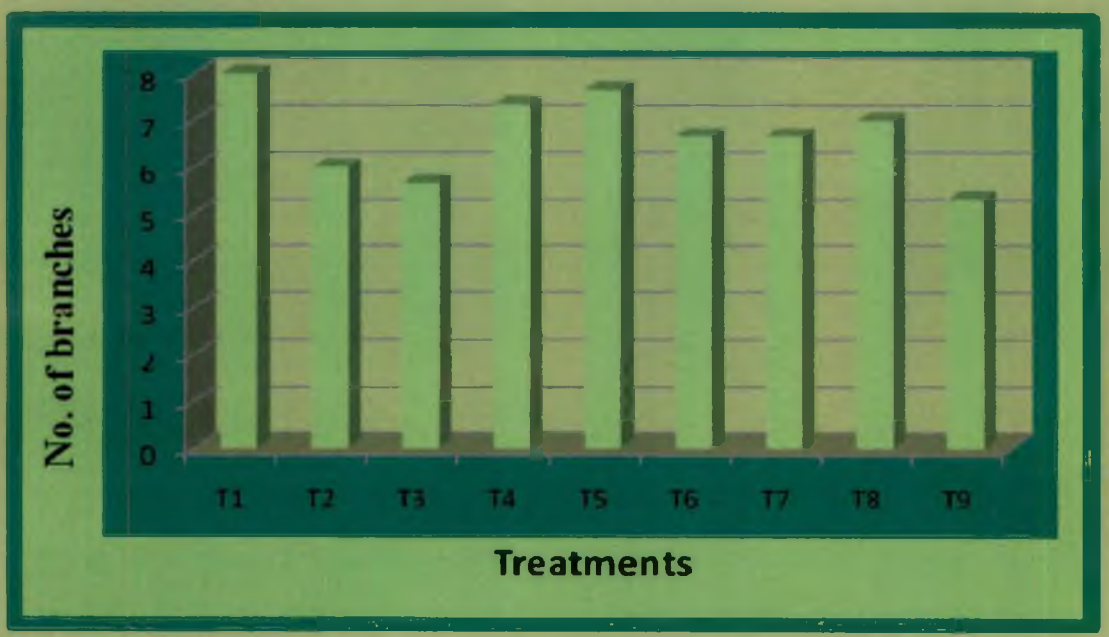


Fig. 14. Effect of ecofriendly materials on No. of branches of brinjal.

T1 - Talc based formulation of fluorescent bacteria

T2 - *Ocimum sanctum* leaf extract

T3 - Ground nut oil cake

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Carbendazim @ 0.1 %

T9 - Control.

T_1 (Talc based formulation of fluorescent bacteria), T_2 (*Ocimum sanctum* leaf extract), T_3 (groundnut oil cake), T_5 (Talc based formulation of fluorescent bacteria + groundnut oil cake) and T_8 (carbendazim 0.1 %) showing plant height of 60, 59.33, 56.83, 56.16 and 46.90 cm were lower than control (Fig. 15).

4.6.4.2. Effect of various treatments on yield of brinjal.

4.6.4.2.1. Number of fruits per plant

The data on number of fruits per plant as influenced by different treatments are presented in Table 16.

T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) recorded significantly higher number of fruits (12.33/plant) than all other treatments and was statistically on par with T_4 (fluorescent bacteria + *Ocimum sanctum* leaf extract) which recorded 10 fruits/plant. Treatments *viz.*, T_4 (fluorescent bacteria + *Ocimum sanctum* leaf extract) and T_1 (Talc based formulation of fluorescent bacteria) were statistically on par with each other. T_1 (Talc based formulation of fluorescent bacteria) and T_8 (carbendazim 0.1 %) were on par with each other in increasing the number of fruits. All the other treatments recorded lower number of fruits than control.

4.6.4.2.2. Yield per plant (kg)

The data on yield per plant are presented in Table 16. Different treatment applications significantly influenced yield per plant (Fig. 16).

The highest yield of 1.45 kg per plant was recorded in T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake), which was followed by T_8 (carbendazim 0.1 % as check) which was on par with T_1 (Talc based formulation of fluorescent bacteria) with an yield of 1.12 and

Table 16. Effect of various treatments on yield of brinjal plant.

Treatments		No. of fruits*	Weight (kg/plant)*
T_1	Fluorescent bacteria talc based formulation (2 %)	9.66	1.092
T_2	*Ocimum sanctum* leaf extract (20 %)	9	0.903
T_3	Ground nut oil cake (200 g)	8.66	0.853
T_4	T1 + T2	10	1.026
T_5	T1 + T3	9	0.928
T_6	T2 + T3	6.66	0.643
T_7	T1 + T2 + T3	12.33	1.45
T_8	Carbendazim 0.1%	9.66	1.122
T_9	Absolute Control	9.33	0.804
CD at 0.05 %		2.49	0.29

*Mean of three replications.

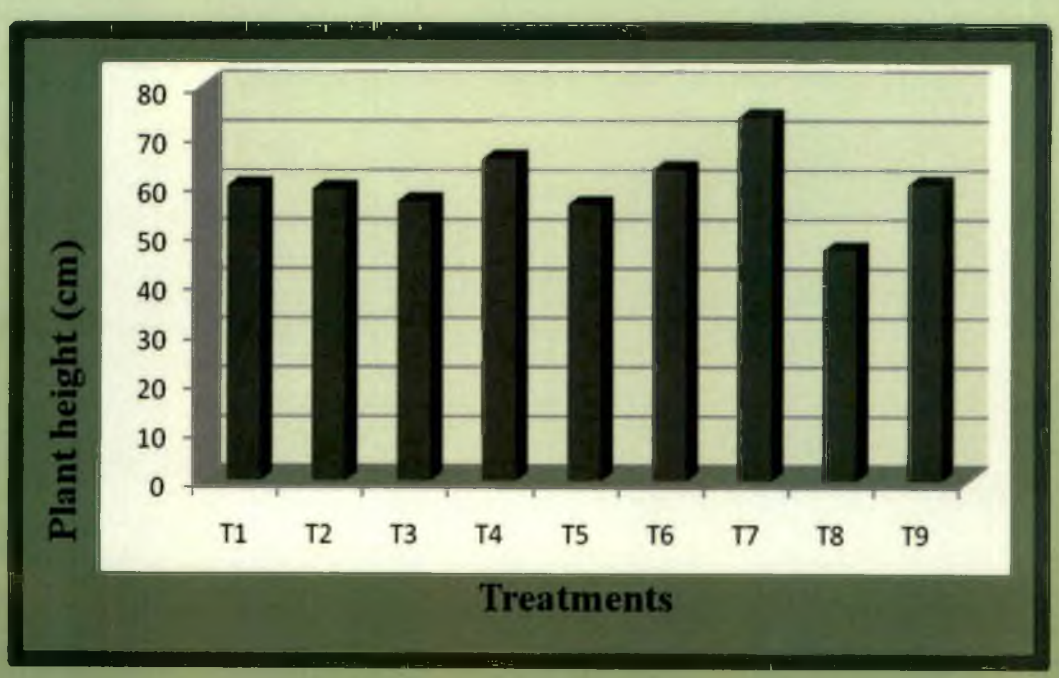


Fig. 15. Effect of ecofriendly materials on Height of brinjal plant (cm).

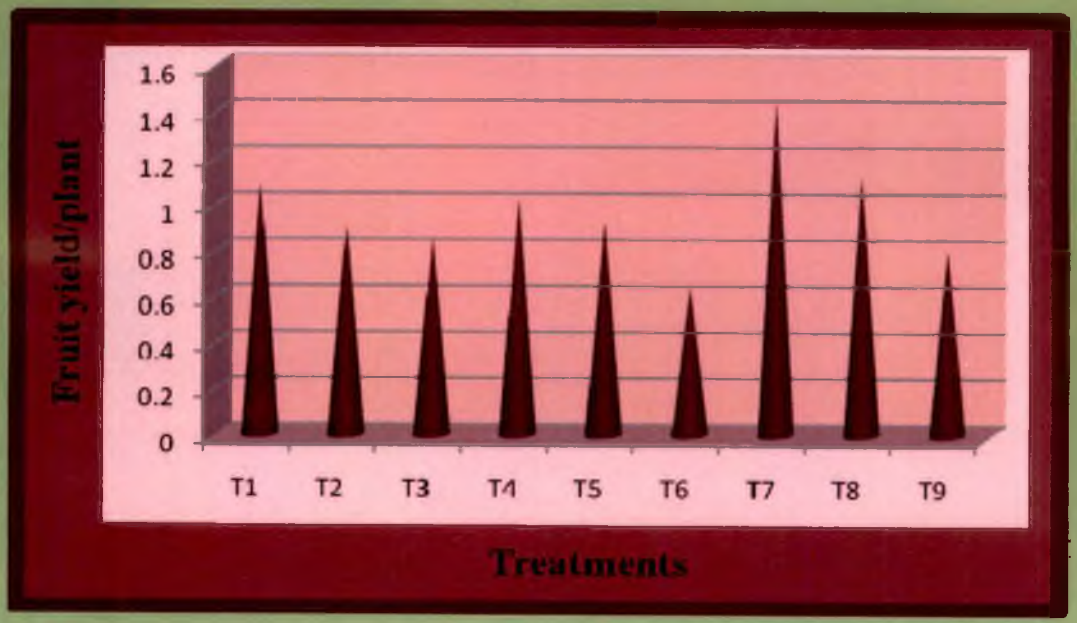


Fig. 16. Effect of ecofriendly materials on the brinjal fruit Yield/plant

T1 - Talc based formulation of fluorescent bacteria

T2 - *Ocimum sanctum* leaf extract

T3 - Ground nut oil cake

T4 - T1 + T2

T5 - T1 + T3

T6 - T2 + T3

T7 - T1 + T2 + T3

T8 - Carbendazim @ 0.1 %

T9 - Control.

1.09 kg per plant. While, the lowest yield of 0.64 kg per plant was recorded in treatment T_6 (*Ocimum sanctum* leaf extract + groundnut oil cake). Treatments T_1 (Talc based formulation of fluorescent bacteria) was on par with T_4 (fluorescent bacteria + *Ocimum sanctum* leaf extract). T_4 (fluorescent bacteria + *Ocimum sanctum* leaf extract) was on par with T_5 (talc based formulation of fluorescent bacteria + groundnut oil cake), T_2 (*Ocimum sanctum* leaf extract) and T_3 (groundnut oil cake) and these were on par with T_9 (control). However the treatment T_6 (*Ocimum sanctum* leaf extract + groundnut oil cake) recorded an yield of 0.64 kg/plant was lower than control.

The increase in yield obtained from T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) was 1.45 kg/plant and from T_8 (carbendazim) was 1.12 kg/plant when compared to control T_9 (control). About 50 per cent increase was obtained from T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) when compared to control.

Discussion

5. DISCUSSION

Brinjal (*Solanum melongena* L.) is an important vegetable crop which gives very high economic return to the growers (Islam and Pan, 1993). In recent years, the *Phomopsis* disease of brinjal caused by *Phomopsis vexans* (Sacc. & Syd.) Harter has become a major constraint in its successful cultivation. *Phomopsis* blight is an unsightly disease that not only harms eggplants but makes them inedible and unmarketable. This pathogen causes over 50 per cent loss in production and productivity in various parts of the world (Akhtar et al., 2008). In the present investigation, the etiology of the pathogen causing blight and fruit rot of brinjal, symptomatology of the disease, isolation of antagonists from the rhizosphere and phyllosphere of brinjal plants and their *in vitro* and *in vivo* screening along with various oil cakes and leaf extracts against the pathogen were carried out.

The symptoms of the disease particularly on the fruits were studied in detail and were found to be more or less identical to those reported by other investigators (Pawar and Patel, 1957). On tissue isolation, the pathogen was obtained from the infected brinjal fruits (variety Swetha). Fungal culture obtained from the infected plants during March 2009 was designated as isolate Pv. The isolate was isolated and brought into pure culture on PDA and its morphological and cultural characteristics were studied. The culture appeared initially as white floccose mycelium which gradually turned to dark greyish white as it grew older. Diurnal zonations were prominent on the upper surface. Numerous black, globose to irregular pycnidia were also produced in a month old culture. A distinct olivaceous grey to black zone alternated with light creamish zone was observed on the reverse side of the colony. This isolate Pv was identified as *Phomopsis vexans* which as the pathogen of blight and fruit rot of brinjal. The same organism was reported by earlier workers as the blight and fruit rot pathogen in brinjal. In India fruit rot of brinjal caused by

Phomopsis vexans was first reported in Gujarat by Harter (1914). The present finding was in tune with the findings of Akhtar (2006).

The present investigation showed that under natural condition, symptoms produced by *Phomopsis vexans* first appeared as pale, sunken, circular to oval areas on the fruit surface which later turned brown and enlarged up to 2 to 3 inches in dia.; often two or more lesions merged to cover much of the fruit surface. Affected fruit became soft and watery at first; decay may penetrate rapidly throughout the fruit, causing a light brown discoloration of the flesh. Later black, pimple like structures developed in the center of old lesions; these are the pycnidia. Similar observations were made by Schlub and Yudin (2002) who were of the opinion that *Phomopsis vexans* caused spots on leaves and fruits of eggplant but the large dark pycnidia produced in the lesions were distinctive. If the infection entered the fruits through the calyx, the whole fruit may became mummified due to dry rot (Pawar and Patel, 1957). Present investigation further showed that brinjal fruits (var. Swetha) were prone to fruit rot infection with *Phomopsis vexans* and symptoms occurred 15-20 days after fruit formation till harvest and extended upto post harvest stage. Similar observations were made by Sugha et al. (2002) and they reported that time taken for the appearance of disease symptoms was 5 to 30 days old fruits.

Under artificial conditions, symptoms of *Phomopsis vexans* appeared first as minute sunken greyish spots with a brownish halo which later enlarged and coalesced, produced concentric rings of yellow and brown zones. These spots increased in size and formed large rotten areas on which pycnidia often developed concentrically covering most of the rotten fruit surface. After 8-9 days of artificial inoculation the whole fruit became mummified. These symptoms were obtained when the brinjal fruits were sprayed with spore suspension along with placing of culture bit of the pathogen on the pin pricked area. Lou et al. (2006) reported that a spore suspension of 10^6 spores/ml of *Phomopsis vexans* collected from PDA culture

was used to spray inoculate potted *Ilex crenata* Thunb. var. *convexa* plants which after 5-10 days, showed symptoms.

Studies on the conidial physiology of pathogen were made during the present investigation. *Phomopsis vexans* isolated on PDA from symptomatic tissues produced two types of conidia, alpha conidia which were one celled, hyaline and ellipsoidal (2.5-12 µm long x 0.9-2.8 µm wide) and beta conidia which were one celled, hyaline, filiform and straight or curved (15.1-28.0 µm long x 0.30-2.3 µm wide). Similar findings were recorded in various studies (Gratz, 1942; Punithalingam and Holliday, 1972; Kumar and Sugha, 1999; Akhtar and Chaube, 2006). Alpha conidia were one celled, hyaline, and ellipsoidal (5.5-9.05 µm long x 1.9-2.3 µm wide); beta conidia were one celled, hyaline, filiform and straight or curved (19.9-28.2 µm long x 0.95-1.32 µm wide). The fungus was morphologically identical to *Phomopsis vexans* (Sacc. & Syd.) Harter (Punithalingam and Holliday, 1972). Alpha conidia were one celled, hyaline, and ellipsoidal (4.5-10.3 µm long × 1.8-2.1 µm wide), beta conidia were one celled, hyaline, filiform, and straight or curved (16.8-27.5 µm long × 1.0 µm wide) was reported by Bello and Sisterna (2000). Alpha conidia were hyaline, aseptate, sub cylindrical, 5-8 x 2-3 µm and beta conidia were filiform, curved, hyaline, septate, 18-32 x 0.5-2.0 µm, non germinating. Hyphae were hyaline, septate, 2.5-4 µm dia. (Singh, 1987).

The present investigations also revealed variations in the colony growth of *Phomopsis vexans* on different culture media. Maximum radial growth was obtained on PDA medium (90.00 mm) within seven days and was significantly superior to all the other media tested. This was followed by Host Extract Dextrose Agar (74.00 mm), Czapek's Dox Agar (63.30 mm) and Oat Meal Agar (61.60 mm). Minimum growth was observed on Oat Meal Agar. Statistically there was no significant difference in the growth of *Phomopsis vexans* in Host Extract Dextrose Agar, Czapek's Dox Agar and Oat Meal Agar media.

Sporulation was obtained in the four media tested. Excellent sporulation of the fungus was recorded on PDA followed by Czapek's Dox Agar media. Sporulation was fair on Oat Meal agar and Host Extract Dextrose Agar. With respect to mycelial colour, it varied from dull white to grey. The growth varied from slightly raised to slightly fluffy to sunken with smooth and entire or uneven margins. The growth of the fungus on PDA was circular, felty, greyish white with entire margin showing diurnal zonations. Mycelial growth on Host Extract Dextrose Agar was like sunken mat with circular entire margins grayish and developed black pin head pycnidia in a month old culture at the centre of the colonies. On Oat Meal Agar, the fungus produced dark grey, sunken in centre and slightly raised on the margins, circular growth having smooth and uneven margins, produced white pin head pycnidia initially which later turned black. Mycelial character of *Phomopsis vexans* on Czapek's Dox Agar media and PDA media were similar. These observations were in agreement with Lou et al. (2004), Divinagracia (1969), Pawar and Patel (1957).

Fungi in general utilized a wide range of nutrients as energy source. Among the various liquid media tested for the growth of the pathogen, Czapek's Dox broth (666.90 mg), Host Extract Dextrose broth (666.90 mg) and Potato Dextrose broth (665.40 mg) were statistically on par and significantly superior over Oat Meal broth (479.03 mg). The findings of the present investigation was exactly similar to the findings of Akhtar and Chaube (2006), Islam and Pan (1990).

The toxic metabolite activity of the culture filtrate from different liquid media was studied by conducting bioassay on brinjal fruit variety Swetha. Size of lesions formed after three days of inoculation by the culture filtrate containing toxin of the fungus was maximum and significantly high in the case of Potato Dextrose broth. Next to Potato Dextrose broth significantly lower size lesions were produced by Host Extract Dextrose and Czapek's Dox culture filtrate. Minimum lesion size was

observed in fruit inoculated with Oat Meal broth. Similar findings were made by Pan et al. (1995).

Further studies like nutritional and physiological factors, *in vitro* were conducted using *Phomopsis vexans* during the course of investigation. Carbon is the most important nutrient required by fungi and it is the essential structural component of the fungal cell. Five carbon sources were tested for the growth of *Phomopsis vexans* using Czapek's Dox media as the basal medium. There was significant difference in the growth of *Phomopsis vexans* in various carbon sources. Among the five different carbon sources, lactose was the best one recording the maximum dry mycelial weight of 653.33 mg followed by maltose (626.66 mg) and starch (543.33 mg) and they recorded statistically significiant growth compared to dextrose. Dextrose (D-Glucose) showed minimum growth as compared to all other carbon sources including sucrose which was the carbon source in Czapek's Dox broth. But glucose as the best source of carbon for the growth of *Phomopsis vexans* had been reported by Akhtar et al. (2008), Quan et al. (2007), Gurgel et al. (2002).

Nitrogen is an important component required for protein synthesis and other vital functions. Statistically no significant differences in growth of *Phomopsis vexans* was recorded in Czapek's Dox media when it was supplemented with various nitrogen sources. Ammonium nitrate was the best utilized nitrogen source other than sodium nitrate (626.60 mg) which was the main nitrogen constituent of Czapek's Dox broth though statistically there was no significant difference in dry weight of the mycelium among the four nitrogen sources, *viz.*, sodium nitrate, ammonium nitrate, ammonium chloride and potassium nitrate recording 626.60, 563.30, 556.60 and 543.30 mg dry weight respectively. Similar studies on growth of *Phomopsis* sp carried out by Gurgel et al. (2002). They reported asparagine and potassium nitrate as the best among the four nitrogen sources to induce the best mycelial growth, pycnidia and spore production of *Phomopsis anacardii* and *Phomopsis mangiferae*

infecting cashew and mango. L-leucine was reported as the favourable nitrogen source for mycelium growth, whereas glutamic acid could not be used by *Phomopsis brevistylospora* causing *Phomopsis* rot on post harvest rockmelon (Quan et al., 2007).

Hydrogen ion concentration is one of the most important factors influencing the growth of fungi. The p^H of the medium determines the rate and amount of growth and many other life processes. In this study the fungus recorded maximum growth at p^H 8.0 (dry mycelial weight of 876.67 mg/30 ml Czapek's Dox broth). The results are in confirmation with that of Quan et al. (2007).

Biological control of plant diseases involving the use of antagonistic microorganisms offers an excellent alternative to chemical control. A vast number of microorganisms present in rhizosphere have been considered as important in sustainable agriculture because of their biocontrol potentials and plant growth promotional activities (Jagadish, 2006). In the present study ecofriendly approach like use of biocontrol agents, plant extracts and oil cakes were evaluated for the management of fruit rot in brinjal.

During the present investigation, native fungi isolated from phyllosphere and rhizosphere of brinjal plants were tested for their antagonistic effect on the pathogen. Among the various antagonistic organisms obtained, six fungal antagonists (five *Trichoderma* sp and one *Aspergillus* sp.) and three bacterial isolates (B1, FB - 1 and FB – 2) were selected and they were further evaluated under laboratory conditions. Among all these fluorescent bacterial isolate (FB - 2) was found to show the maximum suppression of the pathogen. Srinivas et al. (2005) reported that pure culture of *Pseudomonas fluorescens* was more effective in reducing the *Phomopsis vexans* infection followed by *T. harzianum.* Among the various *Trichoderma* sp. tried against *Phomopsis vexans* in brinjal under *in vitro* conditions *Trichoderma*

oil cake (56.18 %) had equal effect in inhibiting *Phomopsis vexans* compared to 30 per cent concentration of all other oil cakes except groundnut oil cake.

Bhadrasree (2007) reported that gingelly oil cake and coconut oil cakes were effective in managing the collar rot and web blight of cowpea caused by *R. solani.* Amendment of soil with individual applications of mustard oil cake, urea, triple super phosphate, muriate of potash, ZnSO4 and CaSO4 and their mixed application reduced the level of infection of anthracnose (caused by *Phomopsis* and *Macrophomina*) on immature guava fruits compared with applications of manures (Hossain et al., 1996).

In the present investigation, among all the three concentrations of carbendazim evaluated (0.025 %, 0.05 % and 0.1 %), cent per cent growth inhibition of *Phomopsis vexans* was observed at the highest concentration (0.1 %). Similar results were reported by Beura et al. (2008), Akhtar (2007). John (1991) reported COC–50 as the best fungicide for the control of blight and fruit rot of brinjal caused by *Phomopsis vexans.*

Maximum inhibition of growth of *Phomopsis vexans* was obtained under *in vitro* evaluation by using combinations of talc based formulation of fluorescent bacteria (2 %) + *Ocimum sanctum* leaf extract (20 %) + groundnut oil cake (200 g).

Based on the results of the *in vitro* management studies the treatments were selected for *in vivo* study. The percentage disease index (PDI) was calculated based on the damage caused by fruit rot pathogen on the brinjal fruits using the formula developed by Mc Kinney during 1923. Disease index was calculated before and after treatment application. Among all the treatments applied T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) showed disease reduction of 54.22 per cent over control. This is statistically significant over all the other treatments. T_3 (groundnut oil cake) recorded 26.81 per cent disease

reduction had significant lower effect than all the other treatments. Manna et al. (2004) reported *Phomopsis* blight with the greatest reduction in disease incidence using carbendazim (80.10 %), followed by tebuconazole (76.10 %). Jadeja (2003) reported that carbendazim 0.05 per cent was effective in reducing disease incidence, dried branches and dried plants infected with *Phomopsis vexans* in brinjal. Increased yield of 19.4 q/ha was found in brinjal with sprayings of 0.1 per cent carbendazim. Bhadrasree (2007) reported that the use of biocontrol agents like *Trichoderma harzianum* and P_{22} culture of *Pseudomonas fluorescence* were effective for the control of *R. solani* causing collar rot and web blight of cowpea.

Srinivas et al. (2005) treated aubergine cultivars infected with *Phomopsis vexans* with biological control agents (*Pseudomonas fluorescens* and *Trichoderma harzianum)* at $1x10^8$ cfu g/l and talcum based formulations of *Pseudomonas fluorescens* ($28x10^7$ cfu g/l) at 5 g/kg of seeds and *T. harzianum* ($19x10^7$ cfu g/l) at 10 g/kg of seeds and recorded their effect on reduction of *Phomopsis vexans*, increased seed germination, vigour index and field emergence and reported that pure culture of *Pseudomonas fluorescens* was more effective in reducing the *Phomopsis vexans* infection, followed by *T. harzianum*, compared with fungicide treated (Bavistin, Dithane M - 45, Captan and untreated seeds). Dhakate et al. (2008) conducted *in vitro* and *in vivo* studies of some plant extract against *Phomopsis* blight caused by *Phomopsis vexans.*

Effect of application of ecofriendly materials on brinjal plant was also studied. All treatments recorded increase in no. of leaves as compared to the control. Statistically treatment T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) recorded significantly more number of leaves (177.66) per plant than all the other treatments. However the highest number of branches (8.00) was observed in treatment T_1 (Talc based formulation of fluorescent bacteria) and the lowest (5.33) in T_9 (control). Application of the

treatment T_7 significantly increased the height of the plant compared to all the other treatments. However significantly lower plant height was recorded in T_8 (carbendazim 0.1 %) as 46.90 cm. T_7 recorded significantly higher number of fruits (12.33/plant) than all other treatments and was statistically on par with T_4 (fluorescent bacteria + *Ocimum sanctum* leaf extract) which recorded 10 fruits/plant.

In the present study it was also observed that the highest yield of 1.45 kg per plant was recorded in T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake). While the lowest yield of 0.64 kg per plant was recorded in treatment T_6 (*Ocimum sanctum* leaf extract + groundnut oil cake). About 50 per cent increase was obtained from T_7 (Talc based formulation of fluorescent bacteria + *Ocimum sanctum* leaf extract + groundnut oil cake) when compared to control. Beura et al. (2008) found that carbendazim provided the best control of *Phomopsis* and also maximized yield. Extracts from garlic and celery used for treatment of seeds of capsicum, aubergine, tomato, cabbage, carrot and onion infected by *Peronospora destructor, Phomopsis vexans, Fusarium oxysporum, Verticillium albo-atrum, Alternaria brassicae* and *A. radicina* showed that the plant extracts disinfected seeds and increased yields (Kuprashvili, 1996). Panda et al. (1996) reported leaf extracts of *A. cathartica* had excellent potential as a fungicide. Fungicides such as mancozeb, carbendazim and captaf were found superior for the inhibition of seed borne pathogens like *Phomopsis vexans and Alternaria solani* and *increases* seed germination in brinjal (Thippeswamy et al., 2006). Copper oxychloride (0.3 %), mancozeb (0.25 %), zineb (0.25 %), captan (0.25 %), thiophanate methyl (0.25 %), carbendazim (0.1 %) and tebuconazole (0.05 %), sprayed at 10 days interval from disease initiation, were effective against *Phomopsis* blight with the greatest reduction in disease incidence when carbendazim (0.1 %) was used (80.10 %) followed by tebuconazole (76.10 %) (Manna et al., 2004). Singh and Agrawal (1999) reported that the fungicides *viz.*, mancozeb (0.3 %), carbendazim (0.1 %), mancozeb (0.3 %) + carbendazim (0.1 %), thiophanate methyl

(0.1 %), mancozeb (0.3 %) + thiophanate methyl (0.1 %) and copper oxychloride (0.3 %) significantly reduced fruit rot and increased yield in brinjal with carbendazim resulting in the lowest disease incidence (4.30 %) and highest yield (222.83 q/ha). Chemical seed treatment with captan, carbendazim, carboxin, metasulfovax, thiram and triadimenol was found to increase germination and to reduce the incidence of damping off of seedlings in artificially infested soil (Kaushal and Kumar, 1995).

From the results of the present investigation it was found that soil amendment with groundnut oil cake 200 g/pot, seedling root dip with talc based formulation of fluorescent bacteria (2 %) for 20 min. at the time of transplanting + foliar spray with *Ocimum sanctum* leaf extract (20 %) is effective for managing the fruit rot and leaf blight of brinjal incited by *Phomopsis vexans* under field conditions. Further field experiments should be done to confirm the results of the study. The results obtained in the present study could be further exploited to develop a non hazardous, pesticide free and pollution free organic plant protection measure and reach the vegetable growers.

Summary

6. SUMMARY

The *Phomopsis* leaf blight and fruit rot caused by *Phomopsis vexans* (Sacc. & Syd.) Harter, is a very destructive disease and considered to be the major constraint for limited production and productivity of brinjal. The chemical control measures though proven effective is costly and poses severe threat to human health and environment. Hence, the present investigation was carried out with the objective of evolving an ecofriendly management strategy for the disease using biocontrol agents, plant extracts and oil cakes.

The fruit rot pathogen was isolated from the brinjal plants grown in different blocks of COA, Vellayani and were used in the study. The study involved symptomatology of the disease under natural and artificial conditions. In both the situations symptoms appeared first as minute sunken greyish spots with a brownish halo which later enlarged and coalesced, produced concentric rings of yellow and brown zones. These spots increased in size and form large rotten areas on which pycnidia often develop concentrically covering most of the rotten fruit surface. During the present investigation, *Phomopsis vexans* was isolated and the pathogenicity of the disease was proved. Morphological and cultural characters of the pathogen was studied. On PDA *Phomopsis vexans* appeared initially as white floccose mycelium which turned to dark greyish white as it grew older from the fifth day. Diurnal zonations were prominent on the upper surface. Numerous black globose to irregular pycnidia were also produced in a month old culture. It produced two types of conidia, Alpha conidia which were one celled, hyaline and ellipsoidal and beta conidia were one celled, hyaline, filiform and straight or curved.

Growth of *Phomopsis vexans* was studied in different solid and liquid media, carbon sources, nitrogen sources and different levels of p^H (5 – 9). Best solid medium for the growth of *Phomopsis vexans* was found to be Potato Dextrose Agar whereas the best liquid medium was Czapek's Dox broth. It was found that the best

carbon source was lactose, best nitrogen source was ammonium nitrate and optimum p^H suitable for the growth of the pathogen was at 8.0.

Various fungal and bacterial antagonists were obtained from the rhizosphere and phyllosphere of healthy brinjal plants. The best antagonist, fluorescent bacterial isolate (FB – 2) showed 82.93 per cent inhibition of the pathogen under *in vitro* screening by dual culture technique.

From among the different leaf extracts *viz., Ocimum sanctum, Azadirachta indica, Lantana camara* and *Clerodendron oderatum* tried at two concentrations (20 and 30 %) for the inhibition study of the pathogen under *in vitro* conditions it was found that *Ocimum sanctum* was found to be the best (41.89 %). The lower concentration (20 %) of *Ocimum sanctum* was selected for the *in vivo* experiment.

Among various oil cakes *viz.,* coconut oil cake, neem oil cake, gingelly oil cake and groundnut oil cake tested under *in vitro* conditions against the pathogen, highest inhibition was obtained in groundnut oil cake at 20 per cent concentration.

Since variety Swetha being the most susceptible one among the KAU released varieties of brinjal to the *Phomopsis* blight and fruit rot disease, it was selected for *in vivo* management trial. A pot culture experiment in CRD with three replications and nine treatments *viz.*, the selected antagonist, fluorescent bacteria (2 %), plant extract *Ocimum sanctum* (20 %), groundnut oil cake (20 %) individually and their combinations along with standard fungicidal check carbendazim 0.1 per cent and unsprayed control was laid out at COA, Vellayani.

Among the nine treatments, soil amendment with groundnut oil cake (200 g/pot), seedling dip with talc based formulation of fluorescent bacteria (2 %), for 20 min. and foliar sprays with a combination of talc based formulation of fluorescent bacteria (2 %) + *Ocimum sanctum* leaf extract (20 %) was found best for the management of *Phomopsis* blight and fruit rot disease of brinjal.

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7. REFERENCES

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* Originals not seen

Appendices

APPENDIX – I

Composition of different media

Potato Dextrose Agar

Pealed and sliced potato	:	200 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Czapek's Dox Agar

Sucrose	:	30 g
$NaNO_3$	:	2 g
K_2HPO_4	:	1 g
$MgSO_4.7H_2O$	:	0.5 g
KCl	:	0.5 g
$FeSO_4$	:	0.01 g
Agar	:	20.00 g
Distilled water	:	1000 ml

Host Extract Dextrose Agar

Brinjal leaves	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1000 ml

King's medium B (KMB)

Agar	:	20.00 g
Peptone	:	20.00 g

K_2HPO_4	:	1.50 g
$MgSO_4.\ 7H_2O$	:	1.50 g
Glycerol	:	10.00 ml
Distilled water	:	1000 ml
pH	:	7.2

Martin's rose Bengal agar

Dextrose	:	10.00 g
Peptone	:	5.00 g
KH_2PO_4	:	1.00 g
$MgSO_4.\ 7H_2O$	:	0.50 g
Rose Bengal	:	33 mg/L
Streptomycin	:	30.00 mg
Agar	:	20.00 g
Distilled water	:	1000 ml

Nutrient Agar

Agar	:	20.00 g
Beef extract	:	3.00 g
Peptone	:	5.00 g
NaCl	:	8.00 g
Distilled water	:	1000 ml

Oat Meal Agar

Oat Meal	:	30 g
Agar	:	20 g
Distilled water	:	1000 ml

MANAGEMENT OF *PHOMOPSIS* BLIGHT AND FRUIT ROT OF BRINJAL (*SOLANUM MELONGENA* L.)

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ABSTRACT

MANAGEMENT OF *PHOMOPSIS* BLIGHT AND FRUIT ROT OF BRINJAL (*SOLANUM MELONGENA* L.)

Brinjal or Eggplant (*Solanum melongena* L.), an important vegetable crop belonging to the family Solanaceae is grown for its fruits. Due to it's versatality in use in Indian diet, brinjal is described as the 'King of vegetables' (Choudhary and Gaur, 2009). Such a potential crop is known to suffer from many diseases including damping off, seedling blight fruit rot etc caused by *Phomopsis vexans*, *Fusarium* sp, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici* etc. These diseases cause crop loss up to 30-50 % (Masuduzzaman et al., 2008).

In the organic era of cultivation of crops, use of chemical means of disease management is having least significance. In this context, an ecofriendly management strategy for the management of *Phomopsis* blight and fruit rot in brinjal was taken up.

Brinjal fruits showing the typical symptom of fruit rot were collected from different blocks of College of Agriculture, Vellayani, isolated and identified as *Phomopsis vexans*. Its' pathogenicity was proved following Kochs' Postulates.

Symptomatology studies of the disease under natural and artificial conditions were studied. Under both the situations symptoms appeared almost similar. The symptoms first appeared as pale, sunken, circular to oval areas on the fruit surface. Affected fruits became soft and watery at first; decay penetrated rapidly throughout the fruit, causing a light-brown discoloration of the flesh. Under dry conditions, fruits get shrivelled and became mummified. Black pycnidia arranged in a concentric pattern can generally be seen.

Cultural and morphological study of the pathogen were also done. Cultural and morphological characters of the pathogen was studied on PDA. Both types of conidia - Alpha and Beta conidia are produced by the pathogen. Four different media were tested, *viz.,* Potato Dextrose medium, Czapek's Dox medium, Host Extract Dextrose medium and Oat Meal medium. Best solid medium for the growth of *Phomopsis vexans* was PDA whereas the best liquid medium was Czapek's Dox broth. Growth of the pathogen was also tested in different p^H from 5.0 to 9.0 and optimum p^H for the growth of the pathogen was found as 8.0. Five different carbon sources were tested, viz., dextrose, lactose, maltose, starch and sucrose. Among these lactose was found as the best carbon source for the growth. The different nitrogen sources tried were ammonium carbonate, ammonium chloride, ammonium nitrate, potassium nitrate and sodium nitrate. Among them sodium nitrate was found to be the best.

During the isolation of antagonists from phyllosphere and rhizosphere of brinjal plants many organisms were obtained. Among them the fungal antagonists selected were *Trichoderma* sp. and *Aspergillus* sp. The bacterial isolates selected for the inhibition studies of the pathogen were B1 and fluorescent bacterial isolate (FB – 2). These were further evaluated under laboratory conditions and fluorescent bacterial isolate (FB - 2) was found to show the maximum suppression of the pathogen.

In vitro screening of bio control agents/plant extracts/oil cakes were also tested against the pathogen. The best antagonist obtained under *in vitro* screening by dual culture technique, was fluorescent bacterial isolate. Leaf extracts at 20 and 30 per cent concentrations from *Ocimum sanctum, Azadirachta indica, Lantana camara* and *Clerodendron oderatum* were tested against *Phomopsis vexans* under *in vitro* conditions by poisoned food technique and maximum inhibition of the pathogen was obtained in *Ocimum sanctum* at 20 and 30 per cent concentration. Among the oil

cakes *viz.,* coconut oil cake, gingelly oil cake, neem cake and groundnut oil cake tested against the pathogen, highest inhibition was obtained from ground nut oil cake.

The selected treatments from the *in vitro* tests were subjected to *in vivo* screening. For this a pot culture experiment in CRD with three replications and nine treatments were laid out in the College of Agriculture, Vellayani. The treatments used were fluorescent bacterial isolate (2 %), *Ocimum sanctum* leaf extract (20 %), ground nut oil cake (200 g/pot) individually and in combinations with standard fungicidal check carbendazim 0.1 per cent and inoculated control. The variety Swetha was used for the *in vivo* experiment.

Application of soil amendment with groundnut oil cake (200 g/pot), seedling root dip with talc based formulation of fluorescent bacterial isolate, FB - 2 (2 %) for 20 min. and foliar sprays with a combination of talc based formulation of fluorescent bacteria (2 %) + *Ocimum sanctum* leaf extract (20 %) was found to be the best for the management of *Phomopsis* blight and fruit rot disease of brinjal.