EPIDEMIOLOGY AND MANAGEMENT OF BLACK ROT OF CAULIFLOWER IN PLAINS OF KERALA

By

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(2009-11-151)

THESIS

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DECLARATION

I, S. Lakshmi Prasanna (2009-11-151), hereby declare that this thesis entitled **"Epidemiology and management of black rot of in plains of Kerala"** is a bonafide record of research work done by me during the course of research and that the thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Epidemiology and management of black rot of cauliflower in plains of Kerala"** is a bonafide record of research work done independently by **Ms. S. Lakshmi Prasanna (2009-11-151)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

The cauliflower (*Brassica oleracea* var. *botrytis* subvar. *cauliflora*), a native of Northeast Mediterranean region belongs to the family Brassicaceae, is an important winter vegetable crop, widely grown in many parts of the world. Cauliflower is a good source of Vitamin C, Vitamin K and glucosinolates which are antioxidants. Antioxidants are beneficial in helping the body fight free radicals and in reducing inflammation. Cauliflower helps to increase the body's ability to detoxify itself; it boosts the body's antioxidant system, and helps to balance the levels of inflammation to anti-inflammation in the body. With these activities cauliflower helps to reduce the risk of several types of cancer.

India next to China, has larger area and production of cauliflower. About half of all cauliflower is raised in China and one fourth in India. The world's largest exporter of cauliflower was Spain which accounted for 36 per cent of world exports in 2003. The second largest exporter was France, accounting for 22 per cent of world exports (Hayley, 2011). There was no appreciable export of cauliflower from India. Report of FAO (2009) states India produced 6.532 million tonnes of cauliflower and broccoli which was cultivated in an area of 348900 ha. with a yield of 187214 Kg/ha. The major cauliflower producing states are West Bengal, Bihar, Orissa, Haryana, Gujarat, Jharkhand, Madhya Pradesh, Punjab, and Uttar Pradesh (National Horticulture Board, 2010).

With the development of new varieties suitable for tropical region, cauliflower cultivation is extended to non-traditional states like Andhra Pradesh, Tamil Nadu and Kerala. Cauliflower once confined to hill tracts of Idukki and Wynad districts of Kerala, is now cultivated widely in plains of Kerala as well (Pradeepkumar and George, 2009).

As in case of other crops, cauliflower is prone to many fungal, bacterial and viral diseases. Among these, black rot caused by *Xanthomonas campestris* pv. *campestris*, is one of the most destructive disease of cauliflower, affecting all the above ground parts throughout the growth stage. Any disease is a constraint in the production of a crop which affects the plant's ability to express its potentiality in yield. Black rot is a disease not only affects the yield in the field, but is also responsible for post harvest spoilage. The market acceptance of the infected curds is poor and hence a minor incidence on curd affects the farmer heavily.

The complexity of the disease – local infection, systemic infection, curd infection and dormant infection – makes the management of the disease quite difficult. Further, the disease is more aggressive in low areas where plants remain wet for longer periods and causes heavy losses during the periods of high temperature accompanied by high relative humidity. Losses often exceed 50 per cent due to rapid spread of disease.

In view of the potential crop losses and the economic significance of the disease, investigations were taken up with the following objectives:

1. Isolation and identification of the pathogen
2. Symptom expression
3. Progression of the disease and its severity in relation to the age of the plant
4. Assessment of severity
5. Screening of varieties against the disease and
6. Management of the disease

Review of literature

2. REVIEW OF LITERATURE

Black rot caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (commonly referred as *Xcc*) is one of the most damaging diseases of cauliflower and other crucifers which is world wide in occurrence. The disease was first reported from Kentucky (USA) in early 1880s (Harrison, 1891). Since then, black rot was recorded in many parts of the world from the USA to Russia (Jachewski, 1908; Smith, 1911). In Eastern Europe, *Xcc* causes root rot on winter rape as well as black rot of cabbages (Ivanuyk and Silvanovich, 1990). After 1980, the disease was found to occur widely in all parts of the world; Belgium (Achter *et al.*, 1977), Pakisthan (Akhtar, 1989), China (Shen and Chen, 1990), Brazil (Nakamura, 1990), Japan (Shiomi, 1992), Italy (Caponero and Iacobellis, 1994), Yugoslavia (Obradovic *et al.*, 1999), Colombia (Tamayo *et al.*, 2001), Turkey (Aksoy, 2007), Argentina (Romero *et al.*, 2008) and Nepal (Jensen *et al.*, 2010).

In India, black rot disease of crucifers was first reported by Patwardhan (1928) on cabbage. Cauliflower black rot was first reported by Patel *et al.* (1949) and subsequently it was reported from Himachal Pradesh (Rao and Srivastava, 1964), Varanasi in Uttar Pradesh (Lenka, *et al.*, 1977), Manipur (Gupta, 1991), Ranchi (Kumar and Kotur, 1991), Nainital (Harbola and Khulbe, 1994), Sikkim (Gupta and Choudhary, 1995), Maharashtra (Ingole *et al.*, 2008) and Karnataka (Varalakshmi *et al.*, 2009).

2.1 CROP LOSS

For many years the disease was considered to be of relatively minor importance to crucifer growers in the major production areas of USA and Western Europe. Outbreak of the disease was sporadic and limited. During the late 1960s,

early 1970s and 1990s the frequency and severity of the disease was increased. Approximately 70 per cent of several million transplants from one single seedbed were systemically infected in the USA in 1973 (Williams, 1980). In 1976, losses to the tune of $US 1 million were estimated (Kennedy and Alcorn, 1980). In Florida, two cabbage crops are commonly grown every year. If temperatures remain cool in the late winter and early spring, black rot does not become a problem, but if it is warm, serious outbreaks often occur (Schaad, 1988). In Illinois and Virginia, black rot is one of the most important diseases of cabbage and cauliflower (Lambe and Lacy, 1982; Eastburn, 1989).Cauliflower black rot losses may exceed 50 per cent due to the rapid spread of the disease (Anon, 1999). A crop loss upto 50 to 70 per cent in cauliflower has been recorded in India (Gupta and Thinol 2006). During 1989-1992, *Xcc* caused seed yield reductions in cauliflower in India (Shyam *et al.,* 1994). In Himachal Pradesh, curd rot of cauliflower has been a menace to the seed crop and is the cause of huge losses to farmers in India (Shyam *et al.,* 1994). Black rot appears annually in Manipur near the end of February and is severe (up to 50 per cent losses) in susceptible cultivars (Gupta, 1991). The widespread occurrence of black rot in Rajasthan with a high incidence of seed infection, can be the cause of severe losses (Sharma *et al.,* 1992). Losses to the tune of 100 per cent are not uncommon (Roberts *et al.,* 2007).

2.2 PATHOGEN

The genus *Xanthomonas* is classified under the family *Pseudomonadaceae* and the yellow-pigmented plant pathogen of this family has been unified in this genus (Schiegel, 1995). *Xanthomonas campestris* pv. *campestris* (*Xcc*) belongs to the genus causes diseases on at least 124 monocotyledonous and 268 dicotyledonous plant species including all major crop plants (Leyns *et al.,* 1984). Some of the problems found with the differentiation of *Xanthomonas* species are specifically related to their

high phenotypic similarity. Strains isolated from various crops were commonly placed in different pathovars of *X. campestris* on the basis of their host range and symptoms caused on the hosts from which they were first isolated and a range of experimental hosts (Dye and Lelliott, 1974). Vauterin *et al.* (1990, 1995) suggested an alternative taxonomy of *Xanthomonas* and placed the heterogeneous *X. campestris* pathovars into separate species according to the results of various tests including DNA-DNA hybridization. Although the designation of several newly proposed *Xanthomonas* species is opposed by results of some other tests including 16S rRNA gene and 16S-23S ITSR sequencing (Hauben *et al.*, 1997; Moore *et al.*, 1997; Goncalves and Rosato, 2002); Most strains of *X. campestris* from crucifers were clearly distinguished from other pathovars or species on the basis of fatty acid methyl ester (FAME) composition, soluble protein electrophoretic patterns, electrophoresis of total cell envelope proteins, DNA-DNA hybridization and rep-PCR (Minsavage and Schaad, 1983; Vauterin *et al.*, 1990; Rademaker *et al.*, 2000; Tsygankova *et al.*, 2001).

2.3 HOST RANGE

X. campestris is a pathogen of many cultivated cruciferous plants and weeds. Natural infection occur in more than 30 species of crucifers, of which majority belongs to the genus Brassica. Besides *Xcc*, several other pathovars of *X. campestris viz.*, pv. *aberranas*, pv. *armoraciae*, pv. *raphani* and some others were recognized within the species on the basis of variation in disease symptoms on host plants (Vauterin *et al.*, 1995). Rep-PCR analysis revealed an overlap in genetic and pathovar variation within *X. campestris* (Tsygankova *et al.*, 2001). In a few cases the difference between the pathovars was supported by serology and DNA analysis (Alvarez *et al.*, 1994; Rabenstein *et al.*, 1999) but environmental conditions and host variety can also alter disease symptoms (Ignatov *et al.*, 1999; Zhao *et al.*, 2000;

Vicente *et al.,* 2001). Alvarez *et al.* (1994) and Ignatov *et al.* (1998) found that *Xcc* isolates designated as serotype 1 were more common in areas with a warm climate, and bacteria of serotype 3 were more prevalent in cold regions.

Cauliflower and cabbage are the most readily affected hosts in the crucifers, although kale is equally susceptible. Broccoli and Brussels sprouts have intermediate resistance and radish is quite resistant, but not to all strains. Kohlrabi, Chinese cabbage, rutabaga (swede), turnip, collard, rape, jointed charlock (*Raphanus raphanistrum*) and mustard are also susceptible hosts (Sherf and MacNab, 1986).

2.4 DISSEMINATION OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS.*

The pathogen present in the seed serves as the main source of infection and it survives in soil and plant debris also (Schaad and White, 1974). Seeds are considered to be the most important source of inoculum of *Xcc* on cabbage and other cruciferous crops (Russel, 1898; Randhawa and Schaad, 1984; Schultz and Gabrielson, 1986; Walker, 1952). Seed infection usually varies from 0.25 to 80% (Shrestha *et al.,* 1977; Mariano *et al.,* 1985; Sharma *et al.,* 1992; Shiomi, 1992; Kobayashi *et al.,* 1994; Gaetan *et al.,* 1995).

Gupta and Choudhary, 1995 observed the pathogen in the seed coat, cotyledons and embryo of cauliflower, whereas in radish and rayosag it was noticed only in the cotyledon and embryo. Intra-embryonal infections have been reported in heavily infected rape and mustard seeds by Sharma *et al.,* 1992.

The bacterium spreads through rain and irrigation water (Roberts *et al.,* 1999; Krauthausen *et al.* 2006). It overwinters in the soil, especially in diseased plant material left in the field and in cruciferous weeds. It survives epiphytically in many

wild hosts, weeds and crop plants, which may be symptomless carriers of the bacterium. *Xcc* can also survive for 3 years or more in seeds. Primary infection usually occurs through infected seeds. In the absence of infected plant debris, symptomless infection of seeds and seedlings can be a major source of infection (Williams, 1980; Goto, 1992). Aerosol dispersal of inoculum from infected plants is an important source of black rot epidemics and a means of disease dispersal over short distances (Kuan *et al.,* 1986). Even 10 viable cells of the pathogen, when applied in droplets, can cause infection of cabbage plants. Infected plants usually do not show symptoms until the pathogen concentration reaches 100,000-1,000,000/40 cm^2 (Schultz and Gabrielson, 1986).

2.5 MORPHOLOGY, BIOLOGY AND PHYSIOLOGY OF *XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS*

Xanthomonas campestris is a gram-negative bacterium that belongs to the gamma-subdivision of proteobacteria (CaoJinRu *et al.,* 2008). The pathogen occurs singly or in pairs, motile with a single polar flagellum, strictly aerobic and rod shaped (0.4-0.7 x 0.7-1.8 μm) (Bradbury, 1970; 1984; Schaad *et al.,* 1998). It produces yellow, round, smooth, mucoid, convex, colonies on yeast dextrose chalk agar, catalase-positive, oxidase and urease-negative, hydrolyzed starch, gelatin and aesculin and did not produce nitrate or indole (Walter *et al.,* 1948; Guptha and Thinol, 2006; Romero *et al.,* 2008). The pigment is carotenoid. Non-pigmented variants have also been described by Bryan (1932).

Acid without gas is produced oxidatively from glucose, sucrose, fructose, galactose, arabinose, maltose and cellobiose and from mannitol, raffinose, and glycerol on prolonged incubation. Acid is not produced from inulin, dulcitol, *meso*-inositol, sorbitol or salicin. The pathogen produced lipase on Sierra's medium (Sierra,

1957), utilized malonate in Leifson's medium and citrate in Simmon's medium, produced H_2S. Optimum temperatures for growth of the bacterium are 80-86°F. However, it can grow from 40°-97°F (Kucharek, 2000).

Xcc produces both pectin methyl esterases and polygalacturonase in culture medium (Smith, 1958; Dye, 1960; Roth, 1961). Agglutination test was successfully used to differentiate *Xanthomonas* from other soft rot causing bacteria (Link and Taliaferro, 1928).

The bacterium produces a hetero-polysaccharide xanthan on culture plates which contributes to the mucoid character of the colonies (Morris, 1977). The presence of this viscous substance provides the basis for the use of viscosity tests in the preliminary identification of bacterial isolates (Pierce *et al.*, 1990). *Xcc* produces soft rot in potato slices and both pectin methyl-esterase and polygalacturonase in culture medium (CMI, 1965).

2.6 SYMPTOM EXPRESSION

Black rot symptoms can be observed in plants at any stage of growth. Symptoms induced by the pathogen are variable depending upon the host cultivar, age of plant and environment. The disease appears primarily on the above ground parts of the plant. Infected seeds usually do not show any sign of infection. However, seed discoloration or seed stain has been reported by Shiomi (1991). Severely affected rape and mustard seeds may appear shrivelled and discolored (Sharma *et al.*, 1992). The susceptibility of cauliflower to infection was greater when boron was deficient or excessive compared to plants grown at optimum levels of boron (Kumar and Kotur, 1991).

On leaf, symptoms appear first at the margins of leaves, indicating that the infection had occurred through the hydathodes (Lenka *et al.,* 1977). At this point tissue turns yellow, and the chlorosis progresses towards the center of the leaf, usually in a V-shape with the base of V towards the midrib. In chlorotic tissue the veins and veinlets become dark in color, latter the tissues turn brown, dehydrate and become brittle (Walker, 2004). The older lesions produced no bacterial exudation. When a vein is occasionally included in a spot, it may darken, but the infection was never observed to spread in it beyond the border of the lesion. During warm and humid periods in winter, severe leaf necrosis and vein rot symptoms were observed (Mirik *et al.,* 2008). Heavely infected leaves die prematurely (MuCulloch, 1929) and early defoliation observed (Walker, 2004). Young severely infected plants may be killed (Chadha and Kalloo, 1993).

Symptomless plants are common during the vegetative period until flowering where the pathogen remains dormant. Symptoms can be seen on plants after flowering is well advanced, by the time, the vascular system is usually invaded systematically (Cook *et al.,* 1952). Affected tissues become conspicuously black (Walker, 2004), followed by internal breakdown of the fleshy tissue (Chadha and Kalloo, 1993). Curds are no longer formed or the plants remain stunted (Knosel, 1960). Curds when invaded initially shows water soaked areas at the center followed by light brown discoloration of the affected tissue (Chakrabarty and Shyam, 1989; Guptha and Thinol, 2006).

Black rot bacteria may also enter plants through natural plant openings above and below the soil surface. Above-ground openings in the plant include hydathodes present at the edge of leaves, and stomata distributed over the lower and upper leaf surfaces. Another natural portal of entry is through root system. After plants are infected, marginal leaf lesions can be found within 8-12 days. After infection,

temperatures in the range of 68°-82°F are ideal for expression of symptoms. It may take up to 43 days for symptoms to appear on leaves after infection has occurred. Sometimes plants that are infected during the seedling stage may not develop symptoms until flowering time. The infected hosts are symptomless below 18°C (Kucharek, 2000; Walker, 1950).

Curd rot symptoms caused by the black rot organism are characterized by the appearance of yellowish-brown to black areas on the fringes of the bolt surface often accompanied with bacterial ooze in wet and humid weather. The rot spreads downwards towards the base of the branches forming dark-brown to black streaks. The affected branches may lodge down (Shyam *et al.*, 1994).

2.7 ASSESSMENT OF DISEASES

Assessment or measurement of disease is the basis of epidemiology, which is the study of disease at the level of populations of pathogens and hosts. It is also the basis of the study of the effects of disease on crop yield and of disease forecasting, which involves the prediction of the amount of disease that is likely to occur at some time in the future (Brown and Keane, 1997). Much of the published work on disease assessment and crop losses has been qualitative rather than quantitative. This has led to disease incidence being described in vague terms such as 'severe', 'debilitating', 'mild' or 'of little consequence'. Qualitative assessment of disease provides inaccurate and often misleading data. It does not enable comparisons to be made between the results of different workers or between results obtained from different seasons or locations.

Disease assessment involves the measurement and quantification of plant disease and therefore of fundamental importance in the study and analysis of plant

disease epidemics (Large, 1966). The need for accurate measurement methods has been elaborately stressed by Chester, 1950 and Large, 1966. Kranz (1988) stated that without quantification of disease no studies in epidemiology, no assessment of crop losses and no plant disease surveys and their application would be possible. One major difficulty associated with disease assessment is the rarity in nature of a 'one cause-one disease' situation. Under field conditions, plant growth and yield are influenced by many factors including nutrients, rainfall, insects, weeds and pathogens (Horsfall and Cowling, 1978).

For the first time, Horsfall and Barratt (1945) described a system of quantifying the plant diseases based on two principles: (a) that the human eye is a photocell that reads in logarithms and (b) that it sees diseased tissues below 50 per cent and healthy tissue above 50 per cent. This rule is put into practice by several workers in the quantification of the disease as the principles enhanced the accuracy and reproducibility.

2.8 INFLUENCE OF CLIMATIC FACTORS ON DISEASE INCIDENCE

Black rot infection may occur at any stage of plant growth generally initiated either by the germination of infected seed or by the entry of the pathogen through guttation droplets on the leaf margin, the bacteria are taken into the plant via the hydathodes (Robeson *et al.,* 1989). Out break of infection by *X. campestris* was reported in 1958 where the severity was enhanced due to the damp, warm weather and a mean temperature of 16.1^0C, where part of the cabbage crop was totally destroyed in the fielder plain Stuttgart (Knosel, 1958).

The disease was unknown in California owing to semi arid climate (Baker and Snyder, 1950). The development of black rot in cauliflower is dependent on the

weather; at high temperature the plant may be destroyed. A temperature of 20^0C for a few hours to several days coupled with high humidity suffices for the bacteria in the leaves. Heavy rain was found to be the main factor for transmission (Knosel, 1960). Under field conditions, the optimum temperature for disease development is about 26.5 to 30^0C, minimum being 5^0C and maximum 36^0C. Relative humidity above 85 per cent has been reported to be conductive for disease development, but it does not seem to be a very important factor for disease development as severe outbreaks of the disease have also been reported under hot and dry weather conditions (Gupta and Thinol, 2006).

Chakrabarty and Shyam (1989) studied correlation coefficients of weather parameters with disease incidence under field conditions. The study revealed that temperature, relative humidity and rainfall were positively correlated with the curd rot disease of cauliflower during the two seasons in Solan district of Himachal Pradesh. The pooled analysis showed that temperature had a profound influence on disease development.

According to Ingole *et al.* (2008) the per cent incidence and intensity of bacterial blight disease, caused by *X. axonopodis* pv. *malvacearum* were positively and significantly correlated with the maximum temperature (32.5^0C), sunshine temperature (18.1^0C), and morning and evening humidities.

The influences of abiotic stress on the development of bacterial curd rot of cauliflower caused by *Erwinia caratovara.* sub sp. *caratovora* (Shyam *et al.*, 2001) showed that temperature had significant positive correlation in two seasons with development of disease. Relative humidity had non significant and negative correlation. Rainfall exhibited non significant and positive correlation in one year and negative during second season.

The disease incidence was directly proportional to temperature between the extremes 19 ± 2^0C and 29.5 ± 0.5, when tomatoes were inoculated with *X. c.* pv. *vesicatoria* (Nakamura, 1990).

In an epidemiological study on Ascochyta blight of pea, Singh *et al.* (2005) observed that the average lesion area, apparent rate of infection and Area Under Disease Progression Curve (AUDPC) increased with increase in temperature. They observed significant effect of temperature, moisture duration and their interaction on disease development.

2.9 DISEASE IN RELATION TO AGE

Age of the plants plays an important role in the disease incidence and its severity in plants. The studies on development of bacterial wilt (*X. c. pv. graminis*) in relation to age of forage grasses by Wang and Sletten (1994) revealed that young plants were more susceptible to the pathogen than old ones.

Olanya *et al.* (1993) found that downy mildew of maize caused by *Pernosclerospora sorgi* showed that the disease incidence was negatively correlated with the age of the plant.

The disease incidence on corn plants inoculated with maize dwarf mosaic potyvirus was more in the young inoculated plants where as it was significantly lower in plants inoculated at later stages, suggesting that the resistance evolves after a certain developmental stage (Kovacs *et al.*, 1997).

Shaat (2003) reported that the incidence and severity of leaf spot disease caused by *Phoma medicagnis* in clover was found to be decreasing with the increasing in age of the plant.

There are also reports which state that with the increase in age of the plant the rate of disease increases. The disease incidence of *Psedocercospora psophocarpi* a leaf spot disease on winged bean showed a positive correlation with plant age (Gunasekera, 1990). Leaf spot disease of Indian mustered caused by *Alternaria brassicae* increased with the increase in age (Sinha, 1992). The effect of sheath blight caused by *Rhizoctonia solani* in rice was found to increase with increase in age of plant (Vanitha, 1996; Sarkar and Chowdhury, 2003).

2.10 CURD INFECTION AND ASSOCIATED ORGANISMS

Several micro organisms were found to infect the cauliflower curd. Some organisms are causing disease in the plants and are carried to the curds when the plants bolt and some are found to infect only the curds; a few are found in association with other pathogens. During advance stages of curd rotting several bacteria and fungi are associated with curd rot and each one enhances the rate of curd spoilage.

Chakrabarty *et al.* (1989) reported curd rot of cauliflower caused by *Fusarium equiseti*. The warm temperature and high relative humidity facilitated further invasion by *Erwinia caratovora* and *Fusarium lateritium* making it a complex rot. Nearly about 14 pathogenic fungal species and two bacterial pathogens were found to be associated with curd rot and categorized into four different types of rots.

Studies conducted by Shyam *et al.* (1994) revealed that curd rots were caused by *Erwinia caratovora, Sclerotinia sclerotiorum* and *Alternaria brassicae* together

with *Xanthomonas campestris* pv. *campestris* in cauliflower. Losses in seed crop ranged from 6 to 108 kg and 2 to 280 kg per ha by *Erwinia caratovora* and *Sclerotinia sclerotiorum* respectively.

Harbola and Khulbe (1994) reported that among 12 fungal pathogens tested for their association with curd rot, *Alternaria tenuissima, Aspergillus niger, Cordana musae* and *Fusarium moniliforme* were found to cause curd rot on cauliflower in Kumaun Himalaya.

Studies on diseased samples of cauliflower in Yugoslavia at different locations by Obradovic *et al.* (2000) showed that several pathogenic bacteria belonging to different genera *Erwinia, Pseudomonas and Xcc* were responsible for cauliflower head rot.

2.11 VARIETAL RESISTANCE

The development of crop varieties with disease resistance or tolerance to black rot has been the focus of many cole crop breeding programs worldwide, as it is the cheapest and ecologically sound disease management practice. In a screening test for resistance conducted by Taylor *et al.* (2002) among two hundred and seventy six accessions of *Brassica spp.* only 43 per cent showed resistance to 2, 3, 5 and 6 races of *Xcc.* Majority of accessions were susceptible.

Sharma *et al.* (2003) evaluated cauliflower germplasm for resistance to black rot and stalk rot. Among the 61 cauliflower genotypes evaluated 10 were moderately-resistant to stalk and black rot. None of the genotypes were resistant to both diseases. Two genotypes namely *Leamington* and *Late Enterprise* were found to be resistant to black rot.

Thakur *et al.* (2003) tested 25 cauliflower cultivars including their F_1, F_2 and backcross generations for their resistance to black rot disease caused by *Xcc*. The cultivars KN-81, BR-2, SN-445, ACC-641, KK-104 and RSK-1301 were resistant to black rot but PSB-1 and KJ-38 were highly susceptible to black rot. SN-445 and BR-2 carried the genes for black rot resistance which were quantitatively inherited and dominant in expression. Pusa Shubhra, an early-maturing variety was found to be field-resistant to black-rot and alternaria blight (Singh *et al.,* 1991).

Varalakshmi *et al.* (2009) reported that IIHR 73-56 and IIHR 250-4-1-11-28, were moderately resistant to black rot. Yunshan 2, a new cauliflower F_1 hybrid resistant to black rot (Zhang *et al.*, 2001).

2.12 MANAGEMENT OF BLACK ROT

2.12.1 Cultural Management

Several attempts were made by various workers to reduce losses due to the incidence of black rot of cauliflower. According to Agrios (1988), field sanitation is the first control measure, which should be taken against any bacterial pathogen. In an experiment done in Netherlands on survival and carry over of *X. campestris* pv. *campestris* in soil, it was found that good crop and soil management impeded survival of inoculum from one year to the next (Kocks *et al.,* 1998). Effectiveness of cultural measures like field sanitation, disinfecting harvesting implements, reducing water splash, culling diseased plants and removing alternate host are some of the control measures suggested by Nishijima and Fujiyama, (1985). Trkulja, (2006), suggested the use of healthy and disease-free seeds and seedlings, insect vector control, growing resistant cultivars, hybrids and crop rotation for at least three years (Popov, 1958).

Pre-drying seeds at 40^0C for 24 h followed by air treatment (75^0C) for 5-7 days is an effective method of disinfecting seeds infested by *Xcc* (Shiomi, 1992). According to Conroy (1960); Smith (1962); Shekhawat *et al.* (1982); Lambe and Lacy (1982), hot water treatment at 50^0C for 25-30 min. eliminated the bacteria effectively. Treating seed with low doses of hormetic ultraviolet light-C (UV-C) was found to eliminate *Xcc* (Brown *et al.,* 2001).

Susceptibility to black rot was more when boron was excess or deficient (Kumar and Kotur, 1991). Both Boron and lime application or lime combined with boric acid and ammonium molybdate reduced the incidence of curd rot caused by *X. campestris* (Kotur, 1992; 1998). Covering cauliflower nursery with nylon net, growing nursery under polycover, use of hessian cloth (shelter belt) in the field and removal of diseased foliage (Kashyap, 2010), mulching with *Eucalyptus hybrida* and *Thuja compacta* leaves and needles of *Casuarina equisetiifolia* reduced black rot incidence (Kumud and Shyam, 2003).

2.12.2 Chemical Control

Many attempts were made by various workers to manage the disease using antibiotics and chemicals. Seed, foliar and soil treatment and combination of these have been suggested by many workers. Achter *et al.* (1977) revealed that immersion of seeds for 30 min in a solution of chlortetracycline at 100 μ g/ml resulted in bacteria-free seeds with normal germination. Treatment with mercuric chloride (Bhat and Masoodi, 2000); 3% hydrogen peroxide for 30 min (Kim, 1986); 1 h soak in a 500 mg/ml solution of aureomycin (chlortetracycline), terramycin (oxytetracycline) or streptomycin (Humaydan *et al.,* 1980), is also effective for seed treatment. Seed treatment with streptomycin at 500 ppm for 1 h and 0.5% sodium hypochlorite for 30 min gave the best black rot control (Napoles *et al.,* 1991). Seed treatments with

antibiotic (plantomycin 0.1%, streptocycline 0.1% or aureofungin 0.1%) together with sodium hypochlorite solution (0.5%) effectively controlled *Xcc* (Kumar and Pandey, 1998). Kumud and Shyam (2003) reported good control of black rot with streptocycline 0.01% + mancozeb 0.25% for 3 h as seed treatment, streptocycline + mancozeb as foliar spray.

Bhat *et al.* (2000) reported that chlortetracycline is most effective as seed treatment, followed by the foliar spray of treatments chlortetracycline and oxytetracycline gave the best results. According to Lenka and Ram (1997) streptomycin was the most effective, giving 100 per cent disease control followed by oxytetracycline and chloramphenicol with 93.07 and 91.37 per cent disease control, respectively.

Beura *et al.* (2006) recommended seed treatment with streptocycline (100 ppm) for 15 min followed by seedling dip (100 ppm) for 15 min before planting and three sprays of streptocycline (200 ppm) at 10 days intervals starting from 15 days after planting for effective control of black rot. Treating the seeds with hot water at 52^0 C for 0.5 h, followed by dipping in streptocycline at 100 ppm and spraying the crop with streptocycline (50 ppm) at transplanting and again at curd and seed-pod formation was the best treatment for control of disease(Sharma, 1981).

2.12.3 Botanical Control

Tiwari *et al.* (2004) evaluated 925 extracts prepared from different plant parts for their activity against *X. campestris* pv. *campestris*. Seventy extracts, applied at 1 and 5 per cent possessed strong antibacterial activity and completely inhibited colony development of the pathogen. Twenty extracts completely inhibited the growth of the pathogen only at 5 per cent concentration.

Among aqueous extracts of 30 higher plants about eight plant species showed antibacterial activity *in vitro* against different pathovars of the phytopathogenic bacterium, *X. campestris* where *Prosopis juliflora, Oxalis corniculata and Lawsonia inerms* showed significant antibacterial activity (Satish *et al.,* 1999). Alcoholic extract of *Mikania glomerata* inhibited bacterial growth *in vitro* (at concentrations of 250, 500 and 1000 mg/l) and used for the preventive control of *Xcc* (Schultz *et al.,* 2006). Integrated management studies on black rot conducted by Bora and Bhattacharya (2000), revealed that plants treated with aqueous extracts of *Terminalia chebula* and *Sesbania aculeata* showed better control of the disease.

Extracts of turmeric and black pepper showed antibacterial and antifungal activity against pathogenic and spoilage microorganisms in food (Ram and Pranay, 2010). Experimental results of Kuhn *et al.* (2006) reported that turmeric extracts showed significant activities against *X. axonopodis* pv. *manihotis*. Gangopadhyaya (1998) found that inoculation with *Helminthosporium oryzae, Rhizoctonia solani, Sarocladium oryzae, Pyricularia grisea, Xanthomonas oryzae* pv. *oryzae* on seeds of rice treated with turmeric revealed that none of the turmeric treated plants developed disease symptoms. Dhanya and Mary (2006) recommended five sprays (at weekly intervals) of both turmeric powder and streptocycline gave 100 per cent control against bacterial blight of anthurium.

Garlic is effective against gram-positive, gram-negative, acid-fast bacteria and has antifungal, antiviral and antiprotozoal activities (Harris *et al.,* 2001). Garlic extracts (10, 20 and 30 g/100 ml) were toxic to *X. c.* pv. *vesicatoria* (Mangamma and Sreeramulu, 1991), inhibits the multiplication of *X. c.* pv. *citri in vitro* (Khan *et al.,* 2003). Curtis *et al.* (2004) showed the inhibitory activity of garlic against many plant pathogenic bacteria and fungi. Aqueous extract (10%) from leaves of zimmu (*Allium*

sativum L. × *Allium cepa* L.) were used against *X. c* pv. *malvacearum* (Satya *et al.*, 2007).

Kodam *et al.*, (1991) suggested the use of tea catechins in preventing bacterial plant diseases. Antibacterial activity of tea could be used to control vegetable bacterial diseases (Fukai *et al.*, 1991). About 20 plant extracts were screened against *Xcc* of which tea leaf extract *Camellia sinensis* was found to be effective against *Xcc* (Bharadwaj and Laura, 2009).

2.12.4 Biological Control

Biological control refers to the reduction of inoculum density or disease producing ability of pathogen or parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of environment, host or antagonists or by mass introduction of one or more antagonists (Baker and Cook, 1974).

Potential agents for biocontrol activity are rhizosphere competent fungi and bacteria which in addition to their antagonistic activity they are capable of inducing growth response by either controlling minor pathogens or producing growth stimulating factors (Weller *et al.*, 1988). Moreover, biocontrol agents being ecofriendly, is more attractive proposition for crop protection especially when the products are export oriented. In contrast to agrochemicals which gets leached off during incessant rains, biocontrol agents gets stabilized once an efficient strain that fits into the concerned ecological niche are introduced into the environment. Also, biocontrol agents fit well with organic farming, a proposition which is giving popularity in recent times.

Begum *et al.* (2001) worked on biological management of black rot by six saprophytic antagonists *in vitro*. *Pseudomonas fluorescens* and *Aspergillus terreus* were found to be best. But under field conditions disease severity was lowest when *P. fluorescens* was applied as seed treatment to *Xcc* charged seeds. Dzhalilov (1994) reported that Rizoplan (*Pseudomonas* sp. AP33) applied as a seed treatment followed two sprays in the field gave good control of black rot of cabbage.

Application of *B. subtilis* as seed treatment, seed+seedling treatment, seed+seedling treatment+soil drenching and seed treatment+soil drenching were equally effective in controlling *X. campestris* in cabbage (Bora and Bhattacharyya, 2000). Jalali and Parashar (1995) reported that sprays of the *Bacillus* str. (HSb-19) provided better control than streptocycline. Massomo *et al.* (2004) revealed that the incidence and severity of black rot in the foliage, stems and heads of the highly susceptible cultivar Copenhagen Market was significantly reduced, when antagonists were applied through the roots as compared to application through the seeds or foliage (cotyledons) which include *B. cereus*, *B. lentimorbus* and *B. pumilus*. Seed treatment with trichodermin (*Trichoderma harzianum*) and Bactofit (*B. subtilis*) gave the best control of black rot of cauliflower and cabbage (Papou and Grynko, 1994).

Chuaboon and Prathuangwong (2008) reported that the treatment of cauliflower seed with *Bacillus* sp. followed by four times foliar spray of *Pseudomonas fluorescens* at 14, 28, 32, and 46 days after planting reduced 82.08 per cent of disease incidence in cauliflower. The effectiveness of all the antagonists increased with increase in their concentration in the antagonist-pathogen mixture (Sanjay and Parashar, 2004). Dinesh *et al.* (2010) found that combination of *P. fluorescens* and *B. subtilis* is more effective for management of black rot disease.

The combined application of seed bacterization with *P. fluorescens* and two foliar spray with copper hydroxide has the highest potential for reduction of black rot disease (Chatnaparat *et al.*, 2008).

Materials and Methods

3. MATERIALS AND METHODS

The present studies is on the "Epidemiology and management of black rot of cauliflower in plains of Kerala" was carried out at Department of Plant Pathology, College of Horticulture, Vellanikkara and Agriculture Research Station, Mannuthy; during 2009-2011.

3.1 ISOLATION OF PATHOGEN

The pathogen causing black rot of cauliflower was isolated from naturally infected leaves and curds collected from the field, showing typical symptoms (Mirik *et al.*, 2008). Infected curds collected from the market were also used for isolation. The infected samples were washed thoroughly and the infected areas were cut into small bits, surface sterilized with 70% ethyl alcohol for a minute. These bits were then washed with three changes of sterile water and crushed with sterilized glass slide to get bacterial suspension. This suspension was streaked on Potato Sucrose Peptone Agar (PSPA) medium (Appendix I) to get single isolated colonies of the bacterium. Washed bits were also directly placed on PSPA medium. The plates were incubated for 48 h at room temperature. Characteristic single colonies were selected on the basis of its colour, fluidity and sliminess and purified by repeated streaking on PSPA medium so as to get single cell colonies. Then pure culture is maintained in slants as well as in sterile water under refrigerated condition.

Likewise, other organisms associated with black rot symptoms in leaves and curds were also isolated, purified and pure cultures were maintained for further studies. The fungal organisms which were obtained during isolation were purified by single fungal tip method.

3.2 PATHOGENICITY TEST

A thick suspension of 24 h old culture of the isolate was inoculated on 25 day old transplanted apparently healthy cauliflower plants by giving pin pricks along the leaf margins. The culture was smeared on the pin pricked area by means of cotton dipped in bacterial suspension. The inoculated plants were covered with polythene cover. Five plants were inoculated for this study.

A thick suspension of 24 h old culture of the isolate was inoculated on healthy curd by giving pin prick under net house conditions and on detached curds under lab conditions. The culture was smeared on the pin pricked surface of curd by means of cotton dipped in bacterial suspension. The curds were covered with polythene bags under net house and the detached curds were placed under bell jars padded with wet cotton.

The pathogen was re-isolated from artificially inoculated leaves and curds and was compared with original isolate for their typical morphological characters.

3.3 SYMPTOMATOLOGY

Symptoms produced by the pathogen under natural and artificial conditions were studied in detail.

3.4 CURD INFECTION AND ASSOCIATED MICRO ORGANISMS

Healthy detached curds were washed thoroughly with distilled sterile water and then surface sterilized with 70 % ethyl alcohol by dipping the whole curd for a minute followed by three successive washings with sterile distilled water. Then the

curds were inoculated with various isolates *viz.*, *Xanthomonas campestris* pv *campestris, Erwinia* sp., *Alternaria* sp., *Rhizopus* sp., and *Fusarium* sp., separately and also as a mixture. Symptoms produced by individual organisms were compared among themselves and with the symptoms produced in the combined inoculation.

3.5 CHARACTERIZATION AND IDENTIFICATION OF PATHOGEN

The cultural, morphological, physiological and biochemical characters of the pathogen were studied following the methods recommended in Laboratory Methods in Microbiology (Harringan and Mc Cane, 1996; Schaad and Stall, 1988), using 24-48 h old cultures.

3.5.1 Culture characters

The colony morphology was studied from 48 h old culture of the bacterium grown on PSPA medium. Colonies were observed for their colour, shape, size, elevation, margin and fluidity.

3.5.2 Pigment production

Production of water soluble and insoluble pigments by the isolates was studied by streaking on Yeast Glucose Agar (Appendix I) and King's B medium (Appendix I).

3.5.3 Morphology characters

3.5.3.1 Gram's reaction

For Gram's staining, 48 h old culture was used. Shape and grams reaction of bacteria was observed under oil immersion objective. KOH test was conducted to confirm the Gram's reaction.

3.5.3.2 Solubility in 3% KOH

A loopful of bacterial culture was placed on a clear glass slide. One drop of three per cent KOH solution was added, thoroughly mixed with the help of inoculation needle and moved up and down to know the solubility in KOH.

3.5.3.3 Endospore staining

A loopful of bacterial culture was taken and smeared on glass slide and fixed by heating, and then a few drops of 1.5 per cent amidoblack was added and allowed to stay for 70 sec. Then the slide was washed under gentle stream of running water, stained for 20 sec with 1 per cent carbol fuschin and washed thoroughly under tap water. Then the slide was blot dried and observed under microscope for endospore.

3.5.4 Physiological characters

3.5.4.1 Mode of utilization of glucose

To determine whether the bacterium utilized glucose only under aerobic condition or both under aerobic and anaerobic condition, one per cent glucose was

added to the prepared basal medium (Appendix I) and dispensed in tubes upto 4 cm. The medium was sterilized by tyndalization and inoculated in duplicate by stabbing with straight inoculation needle charged with bacterial growth. In one of the tubes the medium was sealed with 1 cm layer of sterilized liquid paraffin. The tubes were incubated at room temperature and observations on change in colour were taken at regular intervals up to 15 days.

3.5.4.2 Citrate utilization test

One day culture was streaked on the surface of Simmon's Citrate Agar (Appendix I) and observed for any colour change in the medium.

3.5.4.3 Starch hydrolysis

The ability of the bacterium to hydrolyze starch was tested using Nutrient Agar Medium (Appendix I) containg 0.2 per cent soluble starch. Test organism was spotted on Petri plates containing medium. The dishes were flooded with Lugol's iodine solution after 48 h of incubation and observed for the colour change.

3.5.4.4 Catalase test

Smear of one day old culture grown in PSPA medium was prepared on clear glass slide and covered with few drops of three per cent H_2O_2 and observed for the formation of effervescence.

3.5.4.5 Denitrification test

Bacterial culture was stab inoculated into the Vanden Mooter Succinate Medium (Appendix I) and sealed with three ml of one per cent molten agar and examined daily for production of gas under the seal.

3.5.4.6 Oxidase test

The 24 h old bacterial culture was spot inoculated on oxidase disc and the change in colour of the disc from white to purple or blue within 60 sec was observed.

3.5.4.7 Arginine dihydrolase reaction

The bacterial culture was stab inoculated into the semisolid medium of Thornley (1960) (Appendix I) and the tubes were incubated at room temperature for seven days and observed for colour change.

3.5.4.8 Production of hydrogen sulphide

The ability of bacterium to liberate hydrogen sulphide was tested using peptone water medium (Appendix I). Five ml of medium was dispensed in test tubes and autoclaved. Lead acetate paper stripes of 5 x 50 mm size were prepared by soaking them in super saturated solution of lead acetate. The stripes were dried, autoclaved and again dried. The tubes were inoculated in triplicates with bacterial isolates and lead acetate stripes were inserted aseptically by the side of the plug in the tube. The tubes were incubated at room temperature and observations were recorded at regular intervals up to 14 days for blackening of test strip.

3.5.4.9 Methyl Red Test

Five ml of methyl red broth medium (Appendix I) was dispensed in tubes and sterilized by steaming for 30 seconds for three successive days. Tubes were then inoculated with 48 h old culture of bacterial isolate. The tubes were incubated for seven days at room temperature. Few drops of 0.02 per cent methyl red in 50 per cent alcohol was added to culture tube and observed for colour change.

3.5.4.10 Gelatin liquefaction

Sterilized nutrient gelatin medium (Appendix I) was spot inoculated with 48 h old culture of bacterium. After incubation of seven days agar surface was flooded with 0.2 per cent $HgCl_2$ solution in dilute HCl and observed for the clear zone around the bacterial growth.

3.5.4.11 Production of Indole

Tryptophan broth medium (Appendix I) was used for this test. The medium was dispersed in tubes and autoclaved. Oxalic acid test stripes were used for detecting indole production. Filter paper stripes of size 5 x 50 mm were soaked in warm saturated solution of oxalic acid and cooled. When the stripes got covered with oxalic acid, they were dried at room temperature and used without sterilization. The tubes were incubated with bacterial isolate and oxalic acid stripes were inserted into the tube by the side of the plug, incubated and observed regularly for 14 days. Observed for change in colour of oxalic acid crystals on test strip to pink or red which indicates indole production.

3.5.4.12 Growth on 6 % NaCl

Peptone water with six per cent NaCl was used for the test. The medium was dispensed in tubes; autoclaved and inoculated with bacterium and incubated. The ability of bacterium to grow on medium was observed.

3.5.4.13 Lipolytic activity

Sierra's medium (Appendix I) was employed for this test. The medium was dispersed in 99 ml quantities in flasks, autoclaved and cooled to 45 ^{0}C. One ml of Tween 80 was added to the medium and thoroughly mixed. The medium was poured in sterile petridishes and the test bacterium was spot inoculated on the medium. The plates were incubated and observed at regular intervals for 15 days. Observed for opaque zone around the bacterial growth which indicates positive lipase production.

3.5.4.14 Utilization of Carbon sources

Basal medium for xanthomonads was supplemented with one per cent concentrated solution of carbon compound *viz,* dextrose, fructose, sucrose, glucose, maltose, lactose, mannose, cellobiose, arabinose, adinitol, inositol, glycerol, mannitol, dulcitol and sorbitol. 0.7 ml of five per cent alcoholic solution of bromocresol purple was added to get reddish violet colour. Medium was sterilized by tyndalisation and slants were inoculated with bacterium, incubated at room temperature. Observed for the change in colour of medium from reddish violet to yellow, which indicates the production of acid from carbon compounds.

3.5.4.15 Production of Ammonia

Peptone water was used for the test. The culture was inoculated in peptone water and incubated for 48 h. The accumulation of ammonia is detected by Nessler's reagent which gives brown to yellow precipitate with ammonia.

3.6. SUSCEPTIBILITY STUDIES ON CAULIFLOWER TO BLACK ROT PATHOGEN IN RELATION TO AGE

Apparently healthy plants were inoculated at regular intervals of 5 days from the 10^{th} to 60^{th} day after transplanting (DAT). Ten plants were inoculated at each interval and all the inoculated plants were observed individually for disease development. 30 days old seedling of variety Basant (transplanted on 30^{th} day) was used for this study. Inoculations were made on the 4-5 leaf, using sterile needle pin pricks were made along the leaf margin and 48 h old bacterial culture was smeared over the pin pricked area using cotton and sterile water. The plants were observed periodically for disease development.

3.7 REACTION OF VARIETIES OF CAULIFLOWER TO BLACK ROT UNDER NATURAL INFECTION

Cauliflower varieties/ lines grown at Agriculture Research Station, Mannuthy was observed for black rot disease progression under field conditions. The disease incidence was taken as actual area of infection on the leaves, recorded at 15 day interval for three times (at 10, 25 and 40 DAT) and unit area of infection was calculated by dividing the actual area of infection by the observed leaf area. As the multiple observations were taken on the disease incidence; Area Under Disease

Progression Curve (AUDPC) was calculated and expressed as A- value following the method described by Wilcoxon *et al.*, 1975.

$$\textbf{A-value} = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i-1})\mathrm{X}\, d$$

S_i – Disease severity at the end of the period

k- The number of successive evaluation of the disease

d- Interval between two evaluations

Based on these A – value obtained, the varieties were graded into four categories namely resistant, moderately resistant, moderately susceptible, and susceptible.

3.8 INFLUENCE OF CLIMATIC FACTORS ON THE INCIDENCE OF CAULIFLOWER BLACK ROT

Macro climatic conditions (recorded at College of Horticulture, Vellanikkara) were correlated with incidence of black rot of cauliflower at every five days interval. The disease incidence on a particular day was correlated with the climate which was prevailing just a day prior to the observation. To find out the exact day of climate which was having a bearing on the disease incidence, the observation was subjected to the correlation analysis by taking the climatical data that was prevailing two days prior to the observation. Similarly three, four, and then up to nine days prior to the observation were also subjected to correlation analysis. Average of two adjacent day's climates were worked out just prior to the day of observation 1and 2, 2 and 3, and then so on up to 8 and 9 day and these averages were also subjected to analysis. The same is followed for calculating three day average like average of 1,2 and 3 day

prior climate; 2,3 and 4 day prior climate and then up to 7,8 and 9 day prior climate in the similar manner and were subjected to analysis.

3.9 PREPARATION OF DISEASE ASSESSMENT SCALE

Natural disease incidence observed at 15 days interval on randomly selected 160 plants at 10, 25, and 40 DAT were used for developing a disease assessment scale for black rot of cauliflower. Correlation and linear regression analysis were done by taking yield as the dependent variable and the incidence of disease at 10, 25, and 40 DAT as independent variables. The original equation so obtained was compared with equations obtained from substitution of grades in the proposed assessment scale for the original disease incidence. The best equation was selected by finding the least varying equation from the original one by the substitution of disease incidence in the respective equation and calculating the yield.

3.10 DISEASE MANAGEMENT

The effectiveness of chemicals, botanicals and bioagents against black rot pathogen were tested under laboratory conditions. All these were evaluated by well method (Perez *et al.*, 1990). The efficacy was expressed as per cent inhibition using the following formulae (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Growth of pathogen in control (cm)

T = Growth of pathogen in treatment (cm)

3.10.1 *In vitro* evaluation of chemicals against black rot causing pathogen

For *in vitro* evaluation, 20 ml medium was transferred into sterilized Petri dishes. After solidification of medium, 0.1 ml of 48 h old bacterial suspension (one loop full in 10 ml water; from that 0.1 ml) was poured on it and was spread with spreader. Required quantity of chemicals were mixed with the sterile water to get desired concentrations and poured in to the well made in the center of the plate. Five replications were maintained for each concentration of the chemicals. The plate with sterile water in the well served as control. The inoculated Petri dishes were incubated at 28±2^{0}C. The diameter of the inhibition zone was recorded until its effect was lost. The per cent inhibition was calculated as per the method of Vincent (1927).

The chemicals selected and the concentrations used for *in vitro* evaluation are presented in Table 1.

Table: 1 Details of chemicals and their concentration used for *in vitro* evaluation

Sl.No.	Chemicals	Concentration
1	Streptocycline	100,200,250 ppm
2	Tetracycline	100,200,250 ppm
3	Copper oxychloride	0.15,0.2 %
4	Copper hydroxide	0.15,0.2 %
5	Copper oxychloride + Streptocycline	0.15 % + 100 ppm

3.10.2 *In vitro* evaluation of selected botanicals against pathogen

Four botanicals at two different concentrations were used for *in vitro* evaluation against the pathogen. The list and the concentrations are furnished in Table 2.

Table: 2 Botanicals used for *in vitro* evaluation against pathogen

Sl. No.	Botanicals	Concentration
1	Leaf extract of tea	5 and 10 per cent
2	Tea waste decoction	5 and 10 per cent
3	Garlic extract	5 and 10 per cent
4	Turmeric extract	5 and 10 per cent

Fifty gram leaves of tea, garlic and turmeric were taken separately, washed in sterile water, disinfected with 70 per cent ethyl alcohol and then exposed to U.V. light for one hour by keeping it upside down for every 15 min. The extract was prepared by macerating using sterilized pestle and mortar with 50 ml of sterile water under aseptic condition and filtered through clean, sterilized muslin cloth. In the case of tea spent waste, 50 g material is taken without washing and surface sterilized with alcohol, as the procedure may result in leaching of antibacterial agents. They were subjected to U.V. light treatment only and the extraction procedure followed. A bacterial lawn was prepared by adding 0.1 ml suspension on the mediated plates. A well was made at the centre using a cork borer and one ml of the suspension was poured into the well. Five replications were maintained for each treatment. The diameter of the inhibition zone was recorded until its effect was lost and the per cent inhibition was calculated using the formulae of Vincent (1927).

3.10.3 *In vitro* evaluation of bioagents against black rot causing pathogen

Two bioagents available at the Department of Plant Pathology *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Xcc.* A well was made at the centre using a cork borer and one ml (from one loop full in 10 ml water) of the bioagent suspension was poured into the well after preparing the bacterial lawn on the solidified medium. Five replications were maintained. The diameter of the

inhibition zone was recorded and the per cent inhibition was calculated using the formulae of Vincent (1927).

3.11 FIELD EVALUATION FOR THE EFFICACY OF SELECTED CHEMICALS, BOTANICALS AND BIOAGENTS AGAINST BLACK ROT PATHOGEN.

Based on the *in vitro* evaluation studies, three chemicals, three botanicals and a bioagent were selected for field studies regarding management of black rot. The experiment was carried out during Oct 2010-March 2011 at Agriculture Research Station, Mannuthy.

Details of field experiment were as follows:

Variety	: Basant
Design	: RBD
Treatments	: 8
Replications	: 3
Method of spraying	: Foliar spray at two times

Table: 3 Treatment selected for field experiment

Sl. No	Treatment	Chemicals	Concentration
1	T_1	Control	-
2	T_2	*Pseudomonas fluorescens*	1 %
3	T_3	Garlic extract	5 %
4	T_4	Garlic extract	10 %
5	T_5	Turmeric extract	10 %
6	T_6	Tetracycline	200 ppm
7	T_7	Tetracycline	250 ppm
8	T_8	Copper hydroxide	0.2 %

Foliar Spray

Selected chemicals, botanicals and bioagents were sprayed at two days interval; one at 20 and the other at 35 DAT. The actual area of black rot infection and the total area of the selected leaf were recorded before each spraying and at 50 and 60 DAT. The curd infection was noted at the time of harvest and the disease incidence was calculated.

3.12 STATISTICAL ANALYSIS

RBD, CRD, Correlation and regression and DMRT multiple comparisons among treatment means were done using statistical package SPSS.

Results

4. RESULTS

The results of the studies on 'Epidemiology and management of black rot of cauliflower in plains of Kerala' conducted at Department of Plant Pathology, College of Horticulture, Vellanikkara and Agriculture Research Station, Mannuthy during 2009-2011 is presented below.

4.1 ISOLATION OF PATHOGEN

The pathogen causing the black rot of cauliflower was isolated from the specimen showing typical symptoms of the disease on the leaf and on the curd on PSPA medium. The isolation yielded several organisms which are presented in the Table: 4

Gram positive bacteria were discarded as they were not the causative agent of black rot of cauliflower; they failed to produce infection on inoculation. Among the gram negative bacteria obtained from the curds, two produced yellow colonies and one white color colony. Among the yellow colored ones, the colony which showed more fluidity was taken for further studies. The white colony bacterium caused rotting in the curd was identified as *Erwinia* sp.

Table: 4 Microbes isolated from infected leaf and curd of cauliflower

Source	Organism	Description	Remarks
Leaf	Bacteria I	Rod shaped, gram positive, yellow colony	Failed to produce infection
	Bacteria II	Rod shaped, gram positive, yellow colony	Failed to produce infection
	Botryodiplodia sp.		Failed to produce infection
	Collectotrichum sp.		Failed to produce infection
Curd	Bacteria III	Rod shaped, gram negative	Take up infection
	Bacteria IV	Rod shaped, gram negative	Take up infection
	Bacteria V	Rod shaped, gram negative, white colony	Obtained from advanced infection. Take up infection on curd only. Identified as *Erwinia* sp.
	Alternaria sp.		Obtained from specimens showing advanced infection
	Fusarium sp.		Obtained from specimens showing advanced infection
	Rhizopus sp.		Obtained from specimens showing advanced infection

4.2 PATHOGENICITY OF THE ISOLATED ORGANISM

The pathogenicity of the isolated organism was established by inoculating on the apparently healthy leaves as well as on the curds of cauliflower. Inoculations resulted in typical production of symptoms which were attributed to black rot caused by *Xanthomonas campestris* pv. *campestris.*

4.2.1 On leaf

The symptoms of the disease on leaves appeared on the third day of inoculation (25 day old transplanted seedlings). Initially the symptom was seen as chlorotic flecks, developing into a chlorotic lesion, which enlarged in size and occupied larger area. The veins started to show discoloration. At this time, it seemed that the bacteria entering into the veins are moving towards the midrib. Within two to three days blockage occurred in the veins and the movement of nutrient and water supply was arrested, resulting in the formation of 'V' shaped lesion from the blocked point. The V shaped lesions were wider at the leaf margin and narrow towards the midrib. Later, it turned brown and black and finally into a necrotic lesion. The death of the tissues resulted in brittleness of leaf. Reisolation from the infected leaves resembled the original isolate.

4.2.2 On curd

Under net house conditions, on the inoculated curds it took three days to produce initial symptom. Symptoms started as small water soaked discoloration which enlarged rapidly. Within 5-6 days about $\frac{1}{4}^{th}$ of the curd was found to be infected and the infected portion appeared slimy in nature. As the disease progressed the tissue became soft and emitted foetid odour. When the inoculations were made on

detached curds in the lab which were provided with high relative humidity symptoms were produced within 36 h and the whole curd rottened and destroyed within 72 h. Reisolation from the curds confirmed the presence of *Xcc.*

4.3 STUDIES ON SYMPTOMATOLOGY

The symptoms which are produced due to black rot disease were studied in detail on the plants which were infected naturally under tropical field conditions as well as under artificial conditions.

4.3.1 Under natural conditions

4.3.1.1 On leaf

Different types of symptoms were produced by the pathogen under natural conditions *viz.,* chlorotic lesion, V shaped lesion, vein blackening, necrotic spot and vascular discoloration. Initially the infection started as small faint chlorotic flecks of less than 2 mm in diameter on the upper surface of the leaf near the leaf margin. This became increasingly yellow colored and these lesions expanded in all directions (Plate 1a). The affected leaf tissue became necrotic and brown. Appearance of V shaped lesion developed form a spot near the vein/midrib extending towards the margin. Affected leaves turned yellow and, veins and veinlets exhibited a discoloration turned brown and later became black.

The presence of bacteria in veins led to systemic infection. Microscopic examination proved the presence of bacteria in the veins. The systemically infected plant showed vascular discoloration which was conspicuously black, followed by internal breakdown of the fleshy tissues (Plate 1b).

Plate.1 Symptoms of black rot on cauliflower

1a. Marginal necrosis

1b. Vascular infection

1c. Curd infection

1d. Systemic infection-stunted growth

4.3.1.2 On curd

Initial symptom on the curd under natural conditions was seen as water soaked, slimy light brown lesion which progressed quickly to occupy an area of 1-2 cm in diameter. The spot turned dark brown and rotting setting in. Enlargement of infected area to nearly half of the curd could be seen within 3-4 days (Plate 1c). Secondary infections were also noticed. The infected curd emitted foetid odour. As the disease progressed the infected area became soft and pulpy.

4.3.2 Under artificial conditions

4.3.2.1 On leaf

On artificial inoculation, initial symptom of chlorotic flecks was visible on the third day. The spots enlarged in size and several adjacent spots coalesced together occupying a larger area. Development of V shaped lesion was seen from the fifth day onwards. All the inoculated plants showed the V shaped lesions and become systemically infected.

Once the systemic infection was established, plant growth was slow and it remains stunted (Plate 1d); the whole plant became chlorotic and lost turgidity. Slowly the leaves turned yellow and withered off. Within ten to fifteen days, the systemically infected plants were completely wilted.

4.3.2.2 On curd

Artificial inoculation on curds in the lab provided with congenial conditions resulted in slimy brown discoloration near the place of inoculation. Within 36 h the spot turned dark brown, progressed quickly and occupied more than half of the curd. The rotting of whole curd was noticed within three days.

4.4 CURD ROTTING AND ASSOCIATED MICRO ORGANISMS

Isolation from the severely infected curds yielded *Erwinia* sp., *Fusarium* sp., *Alternaria* sp., and *Rhizopus* sp., apart from *Xcc*. To know their role in the disease development in the curd, these isolates were inoculated on to the curd individually and also with a mixture of all along with *Xcc*.

All the isolates, when inoculated individually were able to produce infection and caused curd rot. All the isolates *Erwinia* sp., *Alternaria* sp., *Fusarium* sp. and *Rhizopus* sp. initiated the symptoms with in 36 h on individual inoculation. In all the inoculations, the curd was completely rottened and destroyed on the third day.

4.4.1 Symptoms of curd infection on inoculation with individual and mixture of organisms

Curds inoculated with *Erwinia* produced light brown colored water soaked, slimy spot at the place of inoculation on the next day of inoculation. The lesions turned soft, watery and black, and quickly spreading; the whole curd was found completely rottened within 72 h and emitted foul odour.

Curds inoculated with *Alternaria* produced dark brown colored lesions, near the place of inoculation after 36 h of inoculation. Later it became black followed by softening of the curd tissues, but the softening of the tissues were less compared to *Erwinia* infection. Sporulation was also noticed on the third day. The spread of the pathogen was slow and complete destruction of the curd was found on the fifth day.

Curds inoculated with *Fusarium* produced brown colored spot after 36 h of inoculation, followed by complete rotting of the curd with in 3 days. Mycelial growth was seen on the curd. The infected curd emitted foul odour.

Curds inoculated with *Rhizopus* produced discoloration after 36 h of inoculation at the place of inoculation later spreading of the spots were seen; mycelial growth covered almost half of the curd and then rotting followed (3 days).

It was found that most of the symptoms produced by all these pathogens including *Xcc* were found to be same, except that in case of fungal pathogens production of mycelial growth and sporulation was observed.

In case of inoculations with mixture of pathogens, all the symptoms *viz.*, discoloration on the curd, mycelial growth, rotting and softening were observed. The foetid smell also felt. The progression of the damage was very much faster compared to inoculations with single pathogens. Complete rotting and destruction of curd was observed within two days.

4.5 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

4.5.1 Cultural characters

The bacterium gave rise to yellow, circular, slimy, fluidal and convex colonies with entire margin on PSPA medium. A non water soluble yellow pigment on yeast glucose chalk agar medium was produced by the bacterium. The bacteria were found to be gram negative, short rods and produced no endospores (Table: 5).

The growth and change of blue colour of the Nutrient dextrose agar medium (containing 0.005% bromocresol purple) to yellow was observed only in case of tubes containing no liquid paraffin indicating the aerobic nature of the organism (Table: 5).

4.5.2 Physiological characters

The bacterium was found to utilize glucose oxidatively (aerobically) since the medium in the open tubes turns yellow from the top. It utilized citrate as a source of carbon which was evidenced by the change in colour of the medium. Hydrolyzed starch as indicated by colorless zone around the bacterial growth in contrast to the outer blue back ground of the medium. Positive to catalase reaction, arginine hydrolase as indicated by the change in colour of the medium to red liberated H_2S within 14 days which is evidenced by blackening of the lead acetate strip. Liquefied gelatin in the plates which were inoculated with the bacterium within a week. Produced lipases - observed by formation of opaque zone around the bacterial growth. The bacterium produced ammonia which is evidenced by the accumulation of yellow precipitate when Nessler's reagent was added to the peptone water inoculated with the bacterium after 48 h (Table: 5).

Table: 5 Cultural, morphological and biochemical characters of the pathogen

Sl. No.	Cultural, morphological & biochemical characters	Observation
I	Grams reaction	-ve
2	Margin	Entire
3	Surface	Small smooth
4	Configuration	Rod
5	Pigment production	
a	Water soluble	-ve
b	Non water soluble	+ve
6	Mode of utilization of glucose	
A	Aerobic	+ve
b	Anaerobic	-ve
7	Citrate utilization test	+ve
8	Starch utilization	+ve
9	Production of H_2S	+ve
10	MR test	-ve
11	Gelatin liquification	+ve
12	Production of indole	-ve
13	Nitrate reduction	-ve
14	Catalase test	+ve
15	Growth in 6 % NaCl	-ve
16	Lipolytic activity	+ve
17	Utilization of carbon compounds with acid production	
a	Glucose	+ve
b	Maltose	+ve
c	Lactose	+ve
d	Fructose	+ve
e	Dextrose	+ve

Sl. No.	Cultural, morphological & biochemical characters	Observation
f	Sucrose	+ve
g	Mannose	+ve
h	Arabinose	+ve
i	Sorbitol	-ve
j	Inositol	-ve
k	Cellobiose	-ve
l	Adinitol	-ve
m	Glycerol	-ve
n	Dulcitol	-ve
o	Mannitol	-ve
18	Ammonia production	+ve
19	Urease test	-ve
20	Arginine dihydroginase	-ve

+ve-Positive; -ve-Negative

Negative to methyl red test as evidenced by the absence of the development of distinct red colour in the culture tube when few drops of 0.002 per cent methyl red in 50 per cent alcohol was added. Nitrate was not produced as shown by the absence of gas production under the seal of molten agar in the test tube. The oxalic acid crystals on the strip did not turn pink or red which indicated that the bacterium did not produced indole. No growth of bacterium in 6 per cent NaCl was noticed (Table: 5).

Of the 15 carbon compounds tested, the bacterium produced acid from mannose, xylose, fructose, glucose, maltose, lactose, sucrose, dextrose and arabinose as indicated by the change of colour of the medium from reddish violet to yellow. There was no change in the colour of the medium from reddish violet to yellow in tubes containing sorbitol, inositol, cellobiose, adinitol, glycerol, dulcitol and mannitol (Table: 5).

4.6 PROGRESSION OF CAULIFLOWER TO BLACK ROT PATHOGEN IN RELATION TO AGE

The cauliflower variety Basant was transplanted on 30th day after sowing and 10 plants each were inoculated at 5 days interval starting from 10 Days After Transplanting (DAT) up to 60 DAT (total 11 intervals). The result obtained on the disease progression in relation to age of cauliflower is presented in Table 6, Fig: 1.

It was observed that all inoculated plants irrespective of age at which they were inoculated were infected locally, indicating the susceptibility of cauliflower in all stages of growth. The progression of the local lesion to the systemic infection was maximum in the case of plants when they were inoculated at 10 DAT. Then gradually, the development of local lesion to systemic infection decreased up to 40 DAT (which was the minimum). Then, the development of local infection to systemic

nature remained more or less same. The systemically infected plants developed from 10 to 50 DAT inoculations were mostly dead. During inoculations on 55 and 60 DAT plants, a small per cent of plants were systemically infected and they remained alive. All locally infected plants which were failed to produce systemic infection were found to be a live (Table: 6).

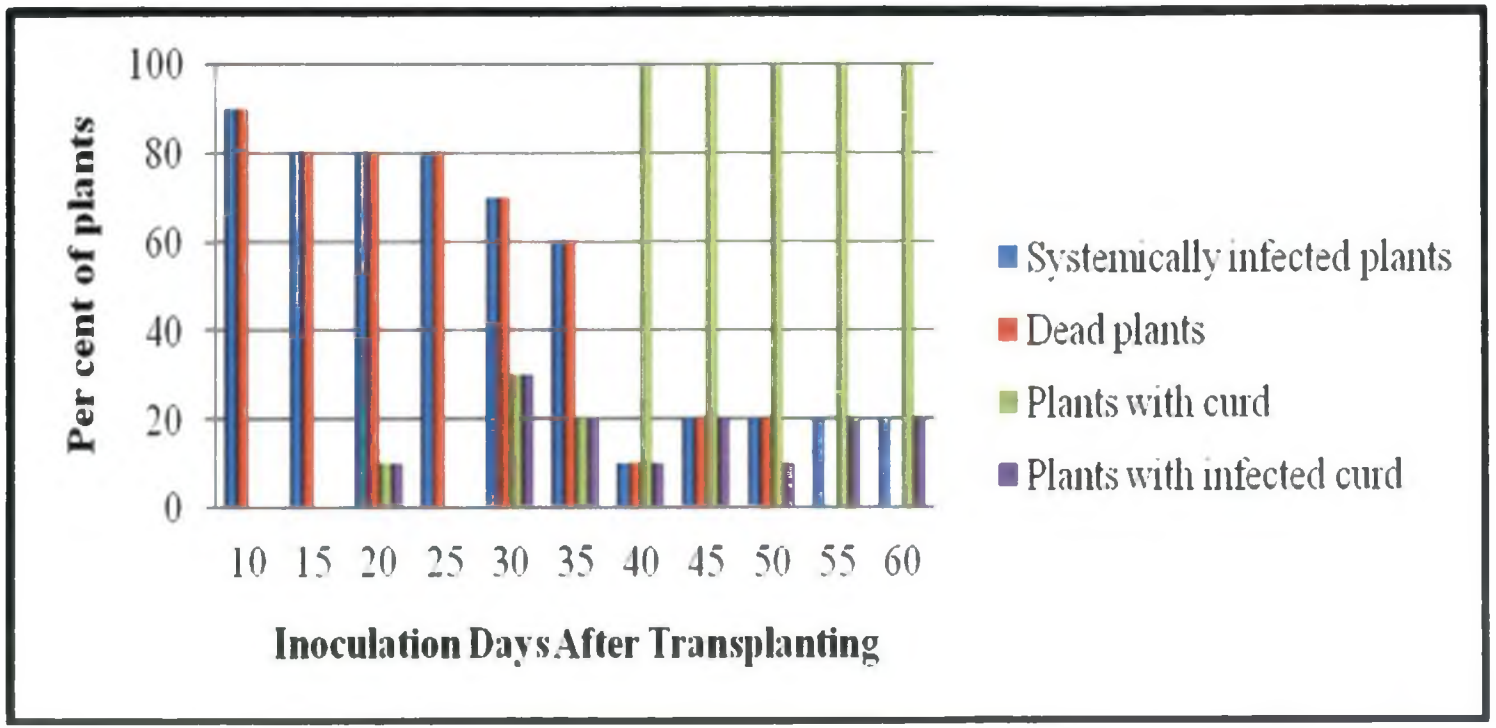
Up to 25 DAT inoculated plants the curd formation was nil or meager as the plants were either dead or failed to initiate curd and upto 35 DAT inoculations, the curds were invariably infected if curds were produced. There was an increase in the curd formation from the inoculation at 30 DAT plants to 60 DAT plants. Starting from artificial inoculation at 30 DAT more number of plants were alive and produced curd with infected or healthy curds (Table: 6). There was no escape for curds if the plants were systemically infected. Cent per cent of systemically infected plants produced infected curds if they developed curds.

Table: 6 Progression of black rot in relation to age of cauliflower

Sl no	Inoculation day (DAT)	Per cent					Average weight of healthy curd (g)	Average weight of infected curd (g)
		Locally infected	Systemically infected	Mortality	With curd	With infected curd		
1	10	100	90	90	#	@	@	*
2	15	100	80	80	@	@	@	*
3	20	100	80	80	10	10	Ñ	60
4	25	100	80	80	@	@	@	*
5	30	100	70	70	30	30	Ñ	85
6	35	100	60	60	20	20	Ñ	65
7	40	100	10	10	100	10	125	70
8	45	100	20	20	100	20	165	70
9	50	100	20	20	100	10	170	80
10	55	100	20	0	100	20	195	120
11	60	100	20	0	100	20	210	170

* No infected curd, @ No curd produced, # Curd initiation but no development, Ñ No healthy curds

Fig.1 Progression of black rot in relation to age of cauliflower



As the age progressed artificial inoculation on the leaves resulted in less number of curd infection. Only systemically infected plants produced curd infection when the plants were inoculated on the leaves.

4.6.1 Black rot symptom expression in relation to age of cauliflower

There was variation in black rot symptom expression in relation to the age at which they were infected. The time taken for the expression of symptom both local and systemic were less upto 20 DAT. Plants which were inoculated on 10^{th} and 15^{th} DAT, the local infection proceeded to systemic infection within 10-15 days and the disease progressed quickly and resulted in complete withering of leaf without production of bigger lesions or blackening of veins. Examinations of dead plants revealed the presence of bacteria in the stem indicating that they were systemically infected, demonstrating the increased vulnerability of young plants to systemic infection. When the older plants were inoculated, the time taken for expression of local lesion increased and all the plants were not infected systemically.

4.7 SCREENING OF VARIETIES TO BLACK ROT RESISTANT UNDER NATURAL INFECTION

Varieties/ lines grown at Agriculture Research Station, Mannuthy during 2009-2010 were scored for black rot leaf infection at 10, 25, 40 DAT and expressed as unit area infection. The multipoint disease scorings were made into A-value, which represents AUDPC (Area Under Disease Progression Curve) are presented in the Table 7.

Table: 7 Screening of varieties to black rot resistant under natural conditions

Sl.No	Variety/ line	Unit area infection			A- value
		10 DAT	25 DAT	40 DAT	
1	NS 121	0.07	0.00	0.00	0.53
2	NS 60	0.18	0.00	0.00	1.31
3	Greeshma	0.18	0.00	0.00	1.34
4	Pusa Meghna	0.20	0.00	0.00	1.5
5	Trisha	0.29	0.00	0.00	2.18
6	Megha	0.31	0.00	0.00	2.30
7	NS 60N	0.33	0.01	0.00	2.59
8	Ateseegra	2.66	0.00	0.10	2.75
9	Barkha	0.05	0.15	0.15	3.75
10	NS 131	0.22	0.10	0.09	3.79
11	2178	0.22	0.13	0.23	5.21
12	Pusa sarad	0.16	0.13	0.30	5.42
13	Himshot	0.18	0.17	0.25	5.75
14	NS 133	0.37	0.24	0	6.35
15	ACC 2235	0.34	0.06	0.80	9.41
16	Basant	0.37	0.42	0.49	12.81

These varieties/ lines were graded into different categories, between resistance and susceptibility.

Table: 8 Categorization of varieties of cauliflower to black rot

Sl. No	A-value	Categories	Varieties/ lines
1	0.0	Resistant	Nil
2	0.01-3.00	Moderately resistant	NS121, NS60, Greeshma, Pusa Meghna, Trisha, Megha, NS 60N, Ateseegra
3	3.01-5.00	Moderately susceptible	Barkha, NS131
4	Above 5.00	Susceptible	2178, Pusa Sarad, Himshot, NS 133, ACC 2235, Basant

Of the 16 genotypes screened, no variety was found to be resistant to black rot. NS 121, NS 60, Greeshma, Pusa Meghna, Trisha, Megha, NS 60N, Ateseegra were moderately resistant. NS 121 showed the least incidence and Basant was the most susceptible one (Table: 8).

4.8 INFLUENCE OF CLIMATIC FACTORS ON CAULIFLOWER BLACK ROT

The effect of macro climatic conditions on the incidence of black rot of cauliflower was conducted on a field. Observations were taken at five day interval from 10 to 60 DAT under natural conditions. Initially, the effect of series of one day climate *viz.,* maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, rain fall and sun shine hours were analyzed with black rot incidence occurring on every fifth day. The series of one day climate means, the climate just a day prior (one day prior climate) to the observation of the disease incidence, the climate two days prior (two day prior climate) to the disease observation and so on, up to nine days prior (nine day prior climate) to the observation. The relationship among the climatic factors and the disease incidence are presented in Table 9.

Table: 9 Relationship of one day weather parameters on the black rot incidence of cauliflower, r- values

Sl. No	Weather parameters (Independent variable)	One DPDI	Two DPDI	Three DPDI	Four DPDI	Five DPDI	Six DPDI	Seven DPDI	Eight DPDI	Nine DPDI
1	Maximum temperature	-0.252 NS	-0.23 NS	0.185 NS	0.425 NS	0.349 NS	0.066 NS	0.535 NS	0.349 NS	0.771*
2	Minimum temperature	-0.499 NS	-0.495 NS	0.298 NS	0.259 NS	-0.17 NS	-0.244 NS	-0.174 NS	-0.101 NS	0.046 NS
3	Maximum Relative Humidity	-0.09 NS	-0.047 NS	-0.323 NS	0.039 NS	0.418 NS	0.236 NS	-0.168 NS	-0.004 NS	0.048 NS
4	Minimum Relative Humidity	0.334 NS	-0.045 NS	-0.282 NS	-0.415 NS	-0.198 NS	0.312 NS	-0.365 NS	-0.645 NS	-0.174 NS
5	Rainfall	-0.003 NS	-0.003 NS	a	-0.553 NS	a	-0.553 NS	-0.097 NS	-0.479 NS	-0.132 NS
6	Sunshine Hours	-0.356 NS	-0.019 NS	0.49 NS	0.455 NS	0.342 NS	0.583 NS	0.451 NS	0.341 NS	0.443 *

* Significant at 0.05 level
NS Non significant
a. Cannot be computed because there was no rain on that particular day (0 mm rain fall)
DPDI- Day Prior to Disease Incidence

The analysis showed that climatic factors up to 8th day prior to the observations were not significantly affecting the disease incidence. There was a positive correlation between the maximum temperature and sunshine hours with the disease incidence on the climatic observation taken on ninth day prior to the observation, indicating a requirement of higher maximum temperature and sunshine hours for the disease development. As there was a significant correlation, the climatic data on ninth day prior to the disease observation was subjected to regression analysis. (Table: 10)

Table: 10 Extent of contribution of 9th day climatic parameters on the black rot incidence of cauliflower

Sl. No	Weather parameters (Independent variable)	β-value	Significance
1	Maximum temperature	-3.047	NS
2	Minimum temperature	11.928	NS
3	Maximum relative humidity	12.137	NS
4	Minimum relative humidity	-24.982	NS
5	Rainfall	-6.024	NS
6	Sunshine hours	-4.048	NS

NS - Non significant,
$R^2 = 0.081$

The regression analysis showed that there was no significant contribution of climate on the disease incidence even though the maximum temperature and sun shine hours were positively correlated. R^2 value obtained was 0.081 indicating that only 8.10 per cent of variation in the disease incidence would be predicted with the included variable on the ninth day prior climatic data observation, which is a very small value.

Normally, it is noted that, apart from single day climate, a period beyond a day may be responsible for disease development. With this idea, average of two adjacent day's weather was correlated with the disease incidence. Here also, a series of two day climatic mean such as, average of 1st and 2nd day prior to the observation, 2nd and 3rd day mean, 3rd and 4th day mean and up to 8th and 9th day mean be correlated with occurrence of the disease and the result is furnished in Table 11.

Table: 11 Relationship of two days mean of climatic parameters on the black rot incidence of cauliflower, r-values

Sl. No	Weather parameters (Independent variable)	Average of 1,2 DCPDI	Average of 2,3 DCPDI	Average of 3,4 DCPDI	Average of 4,5 DCPDI	Average of 5,6 DCPDI	Average of 6,7 DCPDI	Average of 7,8 DCPDI	Average of 8,9 DCPDI
1	Maximum temperature	-0.241 NS	-0.023 NS	0.317 NS	0.388 NS	0.212 NS	0.664 NS	0.444 NS	0.464 NS
2	Minimum temperature	-0.45 NS	-0.082 NS	0.279 NS	0.051 NS	-0.191 NS	-0.29 NS	-0.119 NS	0.00 NS
3	Maximum relative humidity	-0.057 NS	-0.182 NS	-0.2 NS	0.274 NS	0.323 NS	-0.283 NS	-0.079 NS	0.019 NS
4	Minimum relative humidity	0.136 NS	-0.167 NS	-0.348 NS	-0.307 NS	0.09 NS	.1.17 NS	-0.515 NS	-0.389 NS
5	Rainfall	-0.003 NS	-0.002 NS	-0.376 NS	-0.376 NS	-0.376 NS	0.277 NS	-0.338 NS	-0.324 NS
6	Sunshine hours	-0.194 NS	0.308 NS	0.465 NS	0.395 NS	0.457 NS	0.738 NS	0.389 NS	0.358 NS

* Significant at 0.05 level
NS-Non significant
DCPDI- Days Climate Prior to Disease Incidence

There was no significant correlation between the averages of two adjacent days climatic values with the disease incidence and hence the data were not taken for regression analysis and further, three adjacent days average of climatic factors were worked out and these data were analyzed for correlation with the disease incidence and the result obtained is presented in Table 12.

Three day mean of sun shine hours of 4, 5 and 6; 5, 6 and 7 and 6, 7 and 8 were found to be positively correlated with the disease incidence, like this three day mean maximum temperature of 7, 8 and 9 were also positively correlated. Hence the data were analyzed for regression and the result received is presented in Table: 13.

Table: 13 Extent of contribution of mean of three day climatic parameters on the black rot incidence of cauliflower

Sl. No	Weather parameters (Independent variable)	Mean of 4,5&6 DCPDI		Mean of 5,6&7 DCPDI		Mean of 6,7&8 DCPDI		Mean of 7,8&9 DCPDI	
		β-value	t- value	β-value	t- value	β-value	t- value	β-value	t- value
1	Max. temp.	-0.08	-0.19^{NS}	0.39	0.86^{NS}	-0.09	-0.19^{NS}	0.24	0.57^{NS}
2	Min. temp.	-0.08	-0.38^{NS}	-0.35	-1.65^{NS}	-0.13	-0.54^{NS}	0.05	0.20^{NS}
3	Max. RH	0.59	1.89^{NS}	0.23	0.58^{NS}	0.45	0.90^{NS}	0.29	0.6^{NS}
4	Min. RH	-0.04	-0.10^{NS}	0.57	1.08^{NS}	-0.02	-0.04^{NS}	-0.28	-0.50^{NS}
5	Rainfall	0.64	-0.56^{NS}	0.25	1.15^{NS}	-0.20	-0.84^{NS}	-0.24	-1.02^{NS}
6	Sunshine hours	-0.13	2.28^{NS}	0.74	2.56^{NS}	0.64	1.92^{NS}	0.17	0.42^{NS}
R^2		0.114		0.126		0.086		0.082	

Max. temp.: Maximum temperature;
Min. temp.: Minimum temperature;
RH: Relative Humidity;
NS-Non significant;
DCPDI- Days Climate Prior to Disease Incidence

The regression analysis showed there was no significance of t- value of any of the climatic parameters included in the study. R^2 value is also very less which can not be used in prediction models. The maximum R^2 value of 0.126 was obtained with the mean value of climatic factors for 6, 7 and 8 days. However, as of academic interest, equations were being made for the periods for which correlation was found to be significant. (Table: 14).

Table: 14 Prediction equations of climatic factors and the cauliflower black rot incidence for which significance was got in correlation

Sl. no	DCPDI	Prediction equation
1	9	y= -14.39-.412(Max)+1.376(Min)+.09(RH_1)-.192(RH_2)-1.557(RF)-.267(SH)
2	4,5,6	y =-0.189-0.006(Max)-0.007(Min) +0.005(RH_1) + 0(RH_2) -0.006(RF)+0.040(SH)
3	5,6,7	y=-0.813 +0.030(Max)-0.032(Min)+0.002(RH_1)+0.005(RH_2)+0.010(RF) + 0.042 (SH)
4	6,7,8	y =0.193-0.007(Max)-0.012(Min)+0.003(RH_1)+ 0(RH_2) -0.002(RF)+0.036(SH)
5	7,8,9	y = -0.079 +0.021(Max) +0.006(Min)+0.002(RH_1)-0.002(RH_2) -0.002(RF)+0.009(SH)

Where y- Disease incidence
Max- Maximum temperature
Min- Minimum temperature
RH_1- Maximum relative humidity
RH_2- Minimum relative humidity
SH- Sunshine Hours
DCPDI- Days Climate Prior to Disease Incidence

Table: 12 Relationship of mean of three day climatic parameters on the black rot incidence of cauliflower, r-values

Sl. No	Weather parameters (Independent variable)	Average of 1,2,3 DCPDI	Average of 2,3,4 DCPDI	Average of 3,4,5 DCPDI	Average of 4,5,6 DCPDI	Average of 5,6,7 DCPDI	Average of 6,7,8 DCPDI	Average of 7,8,9 DCPDI
1	Maximum temperature	-0.095 NS	0.152 NS	0.328 NS	0.287 NS	0.316 NS	0.315 NS	0.481*
2	Minimum temperature	-0.215 NS	0.023 NS	0.141 NS	-0.061 NS	-0.184 NS	-0.159 NS	-0.064 NS
3	Maximum relative humidity	-0.157 NS	-0.135 NS	0.034 NS	0.256 NS	0.149 NS	0.014 NS	-0.041 NS
4	Minimum relative humidity	-0.014 NS	-0.249 NS	-0.298 NS	-0.065 NS	-0.042 NS	-0.2 NS	-0.378 NS
5	Rainfall	-0.003 NS	-0.277 NS	-0.303 NS	-0.324 NS	-0.067 NS	-0.273 NS	-0.27 NS
6	Sunshine hours	0.167 NS	0.358 NS	0.425 NS	0.454*	0.453*	0.459*	0.389 NS

* Significant at 0.05 level
NS Non significant
DCPDI- Days Climate Prior to Disease Incidence

4.9 ASSESSMENT OF BLACK ROT DISEASE

Unit area of infection were calculated in 160 randomly selected and marked plants for black rot leaf infection at 10, 25, 40 DAT and the yield of each plants were also recorded at harvest. The data on disease at three points of time (independent variables) were subjected to correlation and partial regression analysis with the dependent variable yield.

Relationship of black rot incidence (unit area infection) on 10, 25 and 40 DAT on yield of cauliflower was worked out presented in Table: 15

Table: 15 Relationship of black rot incidence on yield of cauliflower

Sl.No	DAT	Original observation	
		r- value	Significance
1	10	0.023	NS
2	25	-0.037	NS
3	40	-0189	*

NS Non significant,* Significant at 0.05

The incidence of black rot on 10^{th} and 25^{th} DAT did not affect the yield significantly but, the incidence on 40^{th} DAT was negatively correlated, which was significant at 0.05 levels. The analysis gave the trend that the black rot incidence on 25^{th} and 40^{th} DAT was negatively correlated whereas the 10^{th} DAT incidence gave a positive trend. The data was further analyzed for partial regression and the regression analysis (given in Table: 16).

Table: 16 Extent of contribution of black rot incidence at 10, 25 and 40 DAT on the yield of cauliflower

Sl.No	DAT	Natural		
		B-value	t-value	Significance
1	10	0.780	2.353	NS
2	25	-0.235	-0.507	NS
3	40	-0.578	-1.895	NS

NS Non significant;
R^2= 0.079

y=389.061+3.371(10 DAT)-0.986(25 DAT)-2.391(40 DAT)

The analysis showed that, the independent variables were not significantly affecting the yield. The calculated R^2 value was 0.079; explaining that only 7.9 per cent of the variation of the yield would be contributed by the included variables. As it is well known that under non-epidemic seasons, the influence of disease on yield of lesser scale compared to the other yield contributing factors such as fertilizer application and irrigation. The data giving 7.9 per cent yield loss on a non-epidemic season is reasonably good to propose a black rot evaluation scale which is ultimately having a bearing on the yield. Since the data were collected to propose a black rot disease assessment scale, the disease incidence was collected as actual area of infection and then divided by leaf area to get the unit area of infection, which allow them to place the incidence under different proposed scales and to analyze the fitness to the real yield loss. Three set of scales were prepared – one representing the arithmetic scale, the second one based on the logarithmic scale and the third one a slightly modified logarithmic scales. The descriptions of these scales are given in the Table 17, 18 and 19.

Table: 17 Description of arithmetic scale (Scale I)

Grade	Description
0	No symptom – apparently healthy
1	0-25 per cent of leaf area infected (Chlorotic lesion +spot)
2	25-50 per cent of leaf area infected (Chlorotic lesion +spot)
3	50-75 per cent of leaf area infected (Chlorotic lesion +spot)
4	75-100 per cent of leaf area infected (Chlorotic lesion +spot)

Table: 18 Description of logarithmic scale (Scale II)

Grade	Description
0	No symptoms– apparently healthy
1	0-10 per cent of leaf area infected (Chlorotic lesion +spot)
2	10-25 per cent of leaf area infected (Chlorotic lesion +spot)
3	25-50 per cent of leaf area infected (Chlorotic lesion +spot)
4	50-75 per cent of leaf area infected (Chlorotic lesion +spot)
5	75-90 per cent of leaf area infected (Chlorotic lesion +spot)
6	More than 90 per cent of leaf area infected (Chlorotic lesion +spot)

Table: 19 Description of modified logarithmic scale (Scale III)

Grade	Description
0	No symptom– apparently healthy
1	0-5 per cent of leaf area infected (Chlorotic lesion +spot)
2	5-10 per cent of leaf area infected (Chlorotic lesion +spot)
3	10-25 per cent of leaf area infected (Chlorotic lesion +spot)
4	25-45 per cent of leaf area infected (Chlorotic lesion +spot)
5	45-65 per cent of leaf area infected (Chlorotic lesion +spot)
6	65-80 per cent of leaf area infected (Chlorotic lesion +spot)
7	80-85 per cent of leaf area infected (Chlorotic lesion +spot)
8	85-90 per cent of leaf area infected (Chlorotic lesion +spot)
9	More than 90 per cent of leaf area infected (Chlorotic lesion +spot)

The prepared grades in the scales were substituted individually to all the unit area of infection in all the observations on 160 plants and so three sets of data were obtained. These data were subjected to correlation and regression analysis individually and compared with original one (Table 20).

Table: 20 Comparison of relationship among the original data and proposed scales of disease incidence at different time of observation with the yield.

Sl. No	DAT	Original		Scale I		Scale II		Scale III	
		r- value	Sig	r- value	Sig	r- value	Sig	r- value	Sig
1	10	0.023	NS	0.099	NS	-0.300	NS	0.021	NS
2	25	-0.037	NS	-0.004	NS	-0.121	NS	-0.091	NS
3	40	-0.189	*	0.004	NS	-0.180	*	-0.089	NS

NS Non significant,* Significant at 0.05, Sig-Significance

All the three substituted grades in the proposed scales I an II were found to be not significantly correlated with the yield but, the proposed scale I behaved as that of the original one and the substituted grade at 40 DAT was negatively correlated at probability at 0.05 levels with the yield which was same as that of the original data. Even though the scale III was not significantly correlated the trend of the observations on 10^{th}, 25^{th} and 40^{th} DAT were same as the original one.

The data was analyzed for regression and all the proposed scales were compared with the original one (Table: 21).

Table: 21 Extent of contribution of black rot incidence at different interval of time on the yield in original and proposed scales

Sl.No	DAT	Original		Scale 1		Scale 2		Scale 3	
		B-value	t-value	B-value	t-value	B-value	t-value	B-value	t-value
1	10	0.78	2.35^{NS}	0.19	1.68^{NS}	0.32	1.94^{NS}	0.21	1.29^{NS}
2	25	-0.24	-0.51^{NS}	-0.19	-1.41^{NS}	-0.23	-1.74^{NS}	-0.07	-0.41^{NS}
3	40	-0.58	-1.89^{NS}	0.06	0.53^{NS}	-0.2	-1.36^{NS}	-0.19	-1.21^{NS}
R^2		0.079		0.026		0.045		0.044	

NS Non significant

The regression analysis showed that the t values of all the components of the proposed scales were non significant. The R^2 of the original data was 0.079 indicating the contribution of 7.90 per cent by the included variables in the yield, whereas it was as low as 2.60 per cent in scale I. Scale II and III came were very close with 4.50 and 4.44 per cent influence on the yield. With the available regression analysis data, four equations were formulated. (Table: 22)

Table: 22 Prediction equations based on the regression analysis of original and formulated scales on the yield of cauliflower

Eq. No	Scale	Regression equations
1	Original observation	y=389.061+3.371(10 DAT)-0.986(25 DAT)-2.391(40 DAT)
2	Scale I	y=289.664+ 59.042(10DAT)-33.493(25 DAT)+7.304(40 DAT)
3	Scale II	y=396.018+24.488(10 DAT)-21.205(25 DAT)-19.663(40 DAT)
4	Scale III	y=365.631+12.558 (10 DAT)-5.191 (25 DAT)-13.416(40DAT)

y- Disease Incidence;

10, 25, 40 DAT- Disease incidence on 10^{th}, 25^{th}, 40^{th} Days After Transplant

In order to find a best suited scale among the proposed scales, the variables were substituted in the equations with respective values (actual observation in the case of Equation 1, respective grades in the case of Equation 2,3 and 4) to find the variation and closeness among the equations. On comparison with the original one, it was found that equation 3 (Scale II) was found to be closely related to the original value as it showed 95 per cent similarity to original where as Equation 4 (Scale III) and Equation 2 showed only 90 and 77.5 per cent similarity respectively.

4.10 MANAGEMENT OF BLACK ROT OF CAULIFLOWER

Initially *in vitro* evaluations were made to study the efficacy of chemicals botanicals and bioagents to identify the suitable ones to be carried to the field for further studies.

4.10.1 *In vitro* evaluation of chemicals

Two chemicals copper hydroxide and copper oxychloride at two different concentrations 0.15 and 0.20 per cent, two antibiotics: tetracycline and streptocycline at three concentrations 100, 200 and 250 ppm and a combination of chemical and antibiotic: copper oxychloride (0.1 per cent) + streptocycline 100 ppm were tested *in vitro* against *Xcc* and calculated for per cent zone of inhibition. The results were presented in the Table 23.

Table: 23 *In vitro* inhibitory effects of plant protection chemicals against *Xanthomonas campestris* pv. *campestris*

Sl. No.	Treatments	Concentration	Per cent inhibition *				
			1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
1	Copper oxychloride	0.15 %	23.67 ^{e}	20.00 ^{d}	17.56 ^{d}	17.22 ^{cd}	14.78 ^{a}
		0.2 %	32.89 ^{de}	30.56 ^{c}	29.44 ^{bcd}	29.44 ^{bcd}	26.11 ^{a}
2	Copper hydroxide	0.15 %	25.33 ^{de}	22.00 ^{cd}	21.22 ^{cd}	21.11 ^{bcd}	20.11 ^{a}
		0.2 %	29.56 ^{de}	28.44 ^{cd}	28.11 ^{cde}	27.33 ^{bcd}	26.67 ^{a}
3	Streptocycline	250 ppm	56.22 ^{b}	50.67 ^{b}	47.67 ^{ab}	41.78 ^{ab}	36.44 ^{a}
		200 ppm	46.00 ^{c}	42.78 ^{b}	41.11 ^{abcd}	39.00 ^{abc}	38.33 ^{a}
		100 ppm	34.22 ^{d}	26.78 ^{cd}	20.89 ^{cd}	14.56 ^{d}	13.89 ^{a}
4	Tetracycline	250 ppm	70.67 ^{a}	66.44 ^{a}	43.11 ^{abcd}	41.11 ^{ab}	17.00 ^{a}
		200 ppm	61.78 ^{ab}	60.11 ^{a}	57.00 ^{a}	55.56 ^{a}	34.78 ^{a}
		100 ppm	54.78 ^{bc}	51.11 ^{b}	46.84 ^{abc}	40.89 ^{ab}	26.44 ^{a}
5	Copper oxychloride + Streptocycline	0.1 % + 100 ppm	32.11 ^{de}	25.67 ^{cd}	23.67 ^{bcd}	22.78 ^{bcd}	22.11 ^{a}

* Mean of three replications DAI - Days After Inoculation

In each figure followed by same letter do not differ significantly

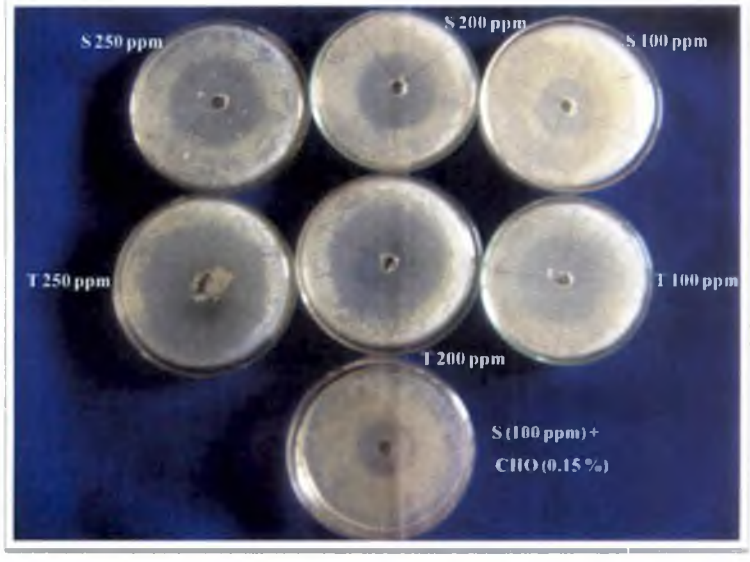


Plate.2a *In vitro* inhibitory effect of antibiotics

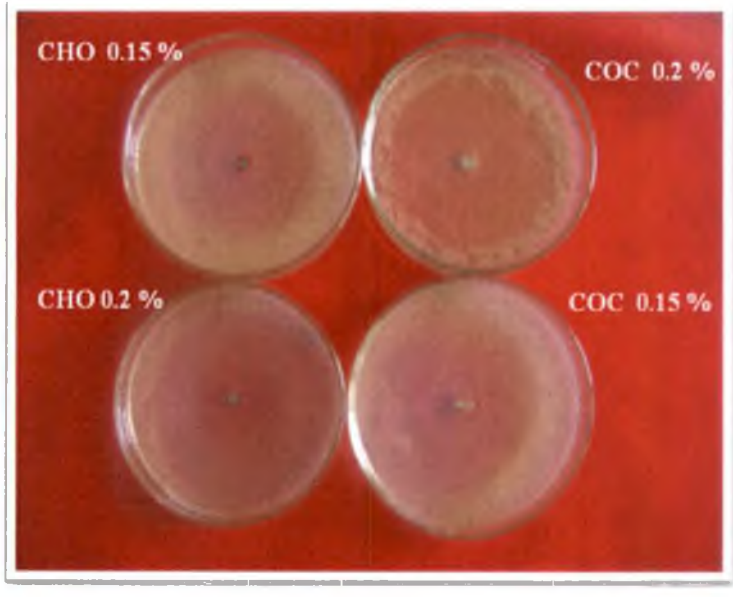


Plate.2b *In vitro* inhibitory effect of chemicals

S-Streptocycline; T-Tetracycline;

CHO- Copper hydroxide; COC-Copper oxychloride

Fig.2 *In vitro* inhibitory effect of antibiotics against *Xanthomonas campestris* pv. *campestris*

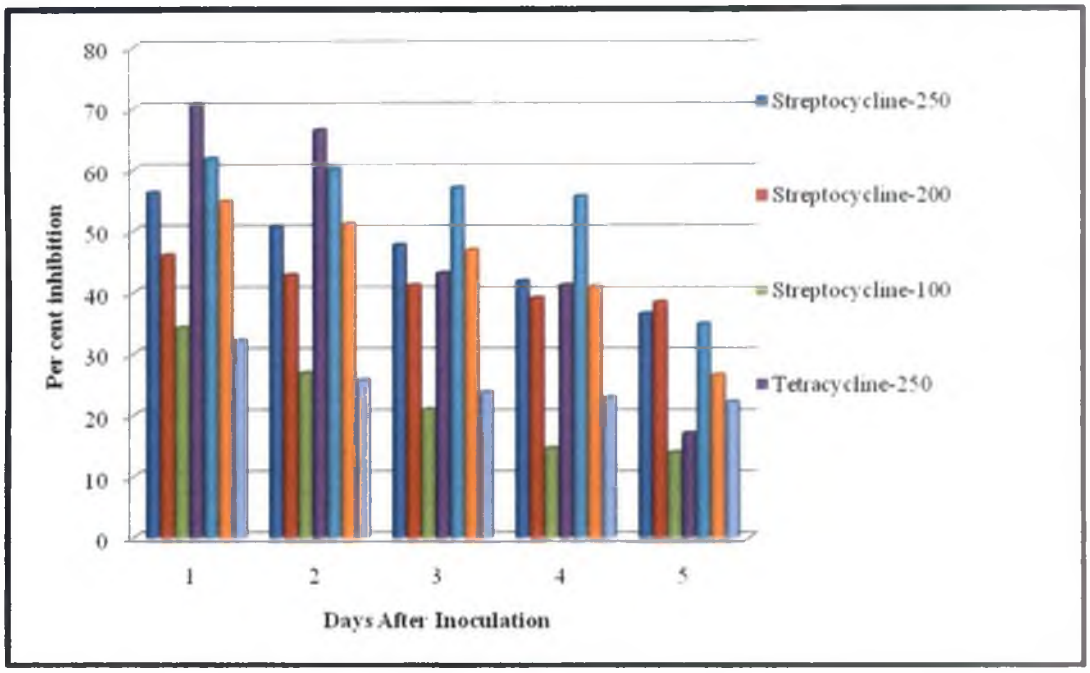
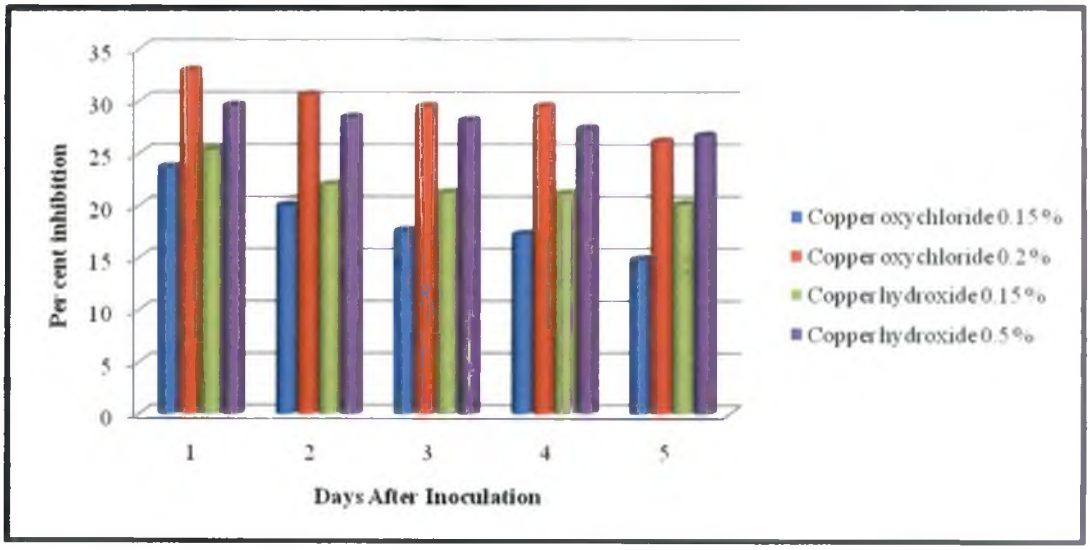


Fig.3 *In vitro* inhibitory effect of chemicals against *Xanthomonas campestris* pv. *campestris*



The results showed that all the antibiotics tested irrespective of the concentrations were more effective in inhibiting the *Xcc* compared to other chemicals and its combination with antibiotic. Maximum inhibition of 70.67 per cent was noticed with tetracycline 250ppm, followed by tetracycline 200ppm (61.78 per cent), streptocycline 250ppm (56.22 per cent), tetracycline 100ppm (54.78 per cent), streptocycline 200ppm (46.00 per cent) and 100ppm (34.22 per cent) (Plate 2a, Fig. 2).. Among the chemicals, copper hydroxide 0.20 per cent recorded 29.56 per cent inhibition. Copper oxychloride 0.15 per cent had shown least inhibition among the chemicals and antibiotics tested (Plate 2b, Fig. 3).

4.10.2 *In vitro* evaluation of botanicals

Turmeric extract, garlic extract, tea leaf extract and tea spent waste decoction at 5 and 10 per cent concentrations were tested against *Xcc*. The effects of these botanicals against *Xcc* were presented in the Table 24 (Fig. 4).

Table: 24 *In vitro* inhibitory effects of botanicals against *Xanthomonas campestris* pv. *campestris*

Sl. No.	Treatments	Concentration (%)	Per cent inhibition *				
			1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
1	Turmeric extract	5	22.56 [b]	38.11 [a]	16.22 [a]	15.56 [a]	14.78 [a]
		10	30.33 [b]	51.67 [a]	24 [a]	23.67 [a]	23.67 [a]
2	Garlic extract	5	54.56 [ab]	45.11 [a]	37.00 [a]	18.89 [a]	18.11 [a]
		10	67.22 [a]	54.00 [a]	49.22 [a]	44.00 [a]	41.78 [a]
3	Tea leaf extract	5	0	0	0	0	0
		10	0	0	0	0	0
4	Tea decoction	5	0	0	0	0	0
		10	20.67 [b]	17.56 [a]	17.22 [a]	12.56 [a]	12.22 [a]

* Mean of three replications; In each figure followed by same letter do not differ significantly

DAI - Days After Inoculation

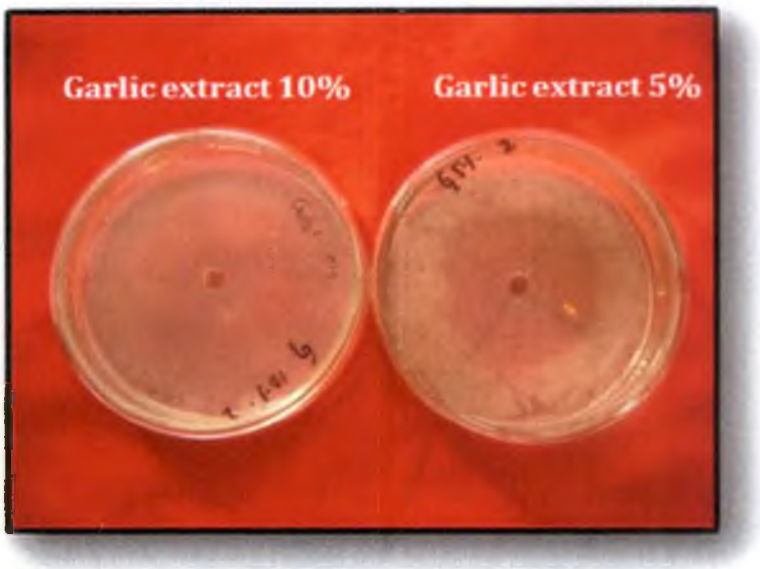


Plate 3a. ***In vitro*** **inhibitory effect of garlic extract**

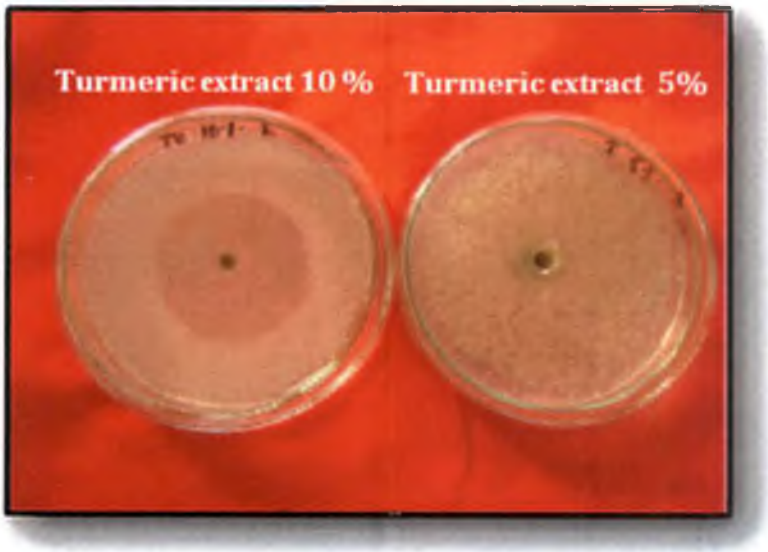
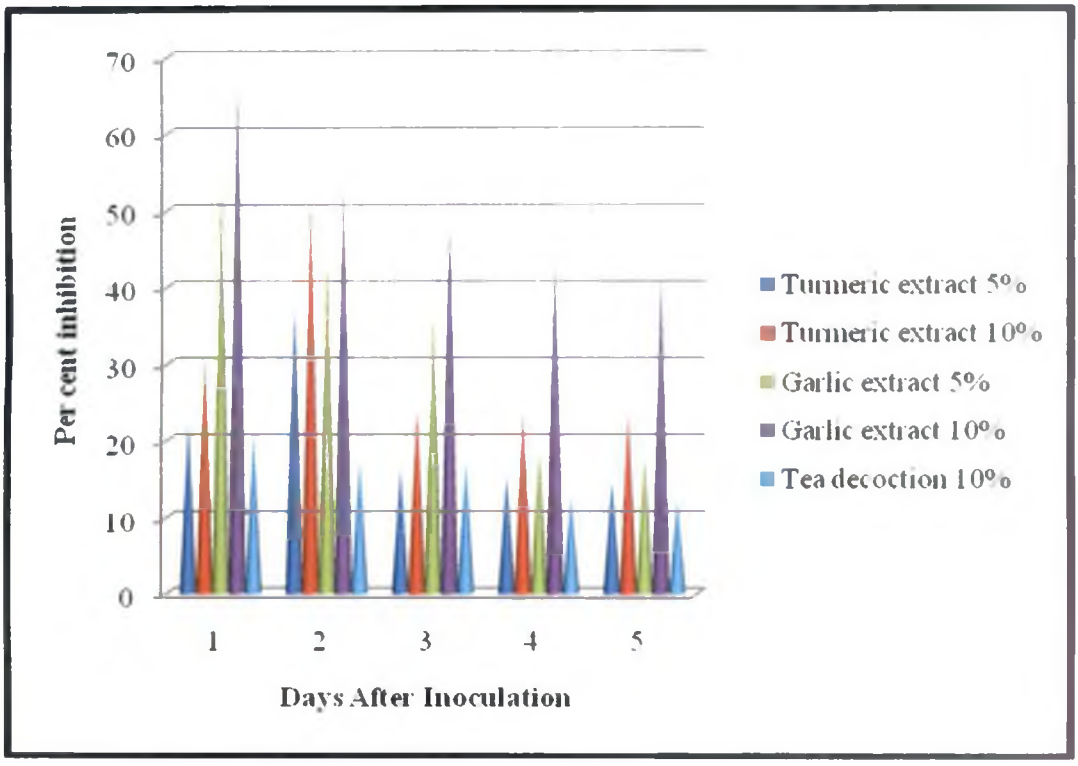


Plate 3b. ***In vitro*** **inhibitory effect of turmeric extracts**

Fig.4 *In vitro* inhibitory effect of botanicals against *Xanthomonas campestris* pv. *campestris*



Garlic extract 10 per cent was found to be the best botanical with an inhibition of 67.22 per cent, followed by lesser concentration of garlic at 5 per cent level (54.56 per cent) (Plate.3a). Turmeric extract 10 per cent (30.33 per cent) and turmeric extract 5 per cent (22.56 per cent) (Plate.3b) were found to be on par and minimum was recorded by tea decoction. Tea leaf extract and tea waste decoction 5 per cent were found to have no effect on the black rot pathogen.

4.10.3 *In vitro* evaluation of bioagents

Two bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *Xcc* and the effects of these bioagents on *Xcc* were presented in Table 25.

Table: 25 *In vitro* inhibitory effects of bioagents against *Xanthomonas campestris* pv. *campestris*

Sl. No.	Treatments	Per cent of inhibition *				
		1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
1	*P. fluorescens*	31.11	27.89	26.11	24.44	11.44
2	*B. subtilis*	70.00	67.33	64.44	61.22	52.22

* Mean of three replications

DAI-Days After Inoculation

It was observed that *B. subtilis* was found to be the best among the two bioagents which recorded an inhibition of 70.00 per cent.

4.11 FIELD EVALUATION FOR THE EFFICACY OF SELECTED CHEMICALS, BOTANICALS AND BIOAGENTS AGAINST BLACK ROT PATHOGEN

Based on the *in vitro* studies three chemicals (including antibiotics), three botanicals and a bioagent were selected for field studies.

Among the chemicals tetracycline 250 ppm, tetracycline 200 ppm (antibiotics) and copper hydroxide 0.2 per cent were selected. Though third best was recorded by streptocycline 250ppm, copper hydroxide 0.2 per cent was selected with an intention to include at least one chemical.

Among botanicals garlic 10 per cent, 5 per cent and turmeric 10 per cent were selected for field studies which were found to be the best in *in vitro* evaluation studies.

Though *B. subtilis* recorded maximum inhibition of *Xcc* in *in vitro,* due to non availability of bulk culture or commercial formulation in the market it was decided to include *P. fluorescence* which was commercially available.

The observations on the effect of different treatments on the incidence of black rot on the leaf were recorded at different intervals (20, 35, 50 and 60 DAT) and curd infection was taken at the time of harvest are presented in Table 26.

Table: 26 Effect of various treatments on the incidence of black rot of cauliflower

Treatment no.	Treatment	Leaf (Unit area infection)				A-value	Curd (per cent incidence)	Average curd yield (g)
		20 DAT	35 DAT	50 DAT	60 DAT		At harvest	
T1	Control	0.02^{c}	0.70^{d}	0.75^{d}	0.77^{e}	23.95^{d}	90.0^{e}	76.80^{e}
T2	*P. fluorescens*	0.02^{c}	0.36^{b}	0.37^{b}	$0.40b^{c}$	12.18^{b}	29.88^{b}	295.50^{b}
T3	Garlic 5%	0.01^{a}	0.46^{c}	0.48^{c}	0.45^{cd}	15.29^{c}	41.68^{c}	256.80^{c}
T4	Garlic 10%	0.01^{ab}	0.29^{a}	0.30^{a}	0.31^{a}	9.76^{a}	20.55^{a}	331.20^{a}
T5	Turmeric 10 %	0.01^{ab}	0.50^{c}	0.49^{c}	0.49^{d}	16.24^{c}	65.60^{d}	216.00^{d}
T6	Tetracycline 200 ppm	0.01^{ab}	0.38^{b}	0.40^{b}	0.42^{bc}	12.88^{b}	35.25^{b}	255.00^{c}
T7	Tetracycline 250 ppm	0.01^{b}	0.35^{b}	0.37^{b}	0.38^{b}	11.81^{b}	24.45^{b}	310.80^{b}
T8	Copper hydroxide 0.2%	0.01^{ab}	0.39^{b}	0.40^{b}	$0.41b^{c}$	12.97^{b}	30.9^{b}	285.00

In each figure followed by same letter do not differ significantly

The observations on 20 DAT, on the first spraying day, served as base value. Since multipoint observations (observations were recorded as unit area of disease incidence) were made, for easy interpretation, A-value (Area Under Disease Progression Curve) was calculated.

The result showed that garlic extract 10 per cent was found to be the best with least incidence of black rot at all observation intervals and the Area Under Disease Progression Curve also the least, followed by tetracycline 250 ppm and *P. fluorescens* application. The untreated control recorded the maximum disease incidence, followed

by turmeric extract 10 per cent application. But there observed no significant difference among the treatments Tetracycline 250, 200 ppm, *P. fluorescens,* copper hydroxide 0.2%, garlic extract 5 per cent and turmeric extract 10 per cent are found to be on par.

Garlic extract 10 per cent sprayed plots recorded the least curd infection, which is followed by tetracycline 250 ppm sprayed plots. The unsprayed control plots recorded the maximum curd infection.

Maximum yield was obtained in the plots treated with garlic extract 10 per cent where minimum disease incidence on curds was recorded, followed by tetracycline 250 ppm and *P. fluorescens*. The lowest yield was recorded in the control plots where maximum damage was recorded.

Discussion

5. DISCUSSION

Cauliflower is an important vegetable among cruciferous, grown in temperate regions where climate is cool, but with development of tropical varieties it is now being cultivated in plains under tropical conditions. In recent years, cauliflower cultivation in the plains of Kerala is increasing with more farmers adopting the crop for higher remuneration (Pradeepkumar and George, 2009). Although the black rot of crucifers including cauliflower was known to be prevalent from 1880s, their role in causing appreciable yield loss was not fully experienced till 1960s, from there on the frequency and severity of the disease increased. Several scientists all over the world are earnestly attempting to study the disease and to combat but it is still remaining as a crux in cauliflower cultivation. Black rot caused by *Xanthomonas campestris* pv. *campestris* is one of the major constraints in cauliflower production. The present study is not a result of complete investigation on the subject but to have an insight into some aspect of the disease under conditions of tropical plains of Kerala.

For better understanding of the disease and its correct diagnosis, it is essential to have a clear cut idea about symptoms and the way in which they are expressed. Therefore, the symptom expression of the disease was studied under natural and artificial conditions. Naturally infected plants showed variations in symptoms. The initial symptoms were produced in the form of small chlorotic flecks on leaf margin. They increased in size light yellow colored expanded in all the directions and V-shaped symptom with the base V towards the midrib appeared. Latter the tissues turned to brown, dehydrated and leaves became brittle and began to shed. These symptoms have been described by many workers (McCulloch, 1929; Knosel, 1959; Walker, 2004; Mirik *et al.*, 2008 and Watt *et al.*, 2009). Sherf and MacNab (1986) described the progression of symptom as the production of numerous brown specks resembling papery leaf spots leading to entire leaves turning yellow or wilt and

finally drop to the ground. Occasionally, affected plants have a long bare stalk with a tuft of leaves at the top. Symptomless plants are common during the vegetative period until flowering (Cook *et al.,* 1952). Low temperature seems to suppress the symptom expression by *Xcc*. Symptoms developed and progressed very quickly at high temperatures. But at low temperatures, marginal lesions occur at low temperatures (16°C), symptom were expressed as chlorotic spotting ('pale mottle') or marginal lesions only which are very difficult to diagnose (Stall *et al.,* 1993). Presence of downy mildew caused by *Peronospora parasitica* also mask the *Xcc* symptom development in cabbage (Walker, 1941).

Symptoms were also present on the curd initially as water soaked lesions then light brown colour discoloration appears at the center which later changed to black colour. Similar results were also reported by Cook *et al.* (1952); Chakrabarty and Shyam (1989); Guptha and Thinol (2006) and then followed by secondary infection. Symptoms of the disease are not normally expressed in cabbage plants growing at temperatures below 18-20^{0}C (Schultz and Gabrielson, 1986). Djalilov *et al.* (1989) reported that even minor, visually undetectable development of black rot may considerably increase damage to plants by soft rot caused by *Erwinia carotovora*, *Pseudomonas* sp. and other opportunistic pathogens.

As the disease is systemic in nature, infection of the vascular system was invariably noticed. Discoloration of the vascular bundles in the main stem was seen followed by internal breakdown of the fleshy tissues. Young plants were killed when they were infected with the pathogen at early stages. Similar symptoms have been reported earlier by Chadha and Kalloo (1993); Walker (2004) and Guptha and Thinol (2006) with slight variations.

The masking of the systemic infection was not observed during this study; may be because, the climate during this period at the plains of Kerala is normally above 30^0C with the relative humidity ranging around 70-75 per cent.

The bacterium causing black rot of cauliflower *Xanthomonas campestris* pv. *campestris* was isolated from diseased leaves and curds with typical symptoms. The colonies were yellow colored mucoid, slimy, glistening, convex and round colonies were obtained. Similar type of colonies were isolated by Bradbury (1984); Schaad and Stall, (1988); Guptha and Thinol (2006) and Romero *et al.* (2008)

The yellow colonies obtained on isolation were identified as *Xanthomonas campestris* pv. *campestris* on the basis of colony and morphological characters, staining reaction, physiological and biochemical characteristics as per the methods described by Bradbury, (1984); Harringan and Mc Cane (1996) and Schaad *et al.* (1988).

The bacterial cells were small straight rods (0.4-0.7 x 0.7-1.8 μm long) gram negative, aerobic, motile by single polar flagellum, produces no endospore. Colonies are mucoid, convex and shiny on YDC and NGA. Non water soluble pigments were produced on YDCA medium. They were weak producers of acids from carbohydrates. The different physiological and biochemical characteristics such as utilization of glucose, maltose, fructose, dextrose, sucrose, mannose and arabinose and did not grow on media containing sorbitol, inositol, cellobiose, adinitol, glycerol, dulcitol and mannitol. The isolate hydrolyzed starch, liquefied gelatin, produced hydrogen sulphide, positive to catalase reaction, did not produce indole and negative to urease and methyl red reaction, did not reduce nitrate to nitrite, growth at 6 per cent NaCl was absent. Thus, based on cultural, morphological and biochemical

characters coupled with pathogenicity, the bacterium causing black rot of cauliflower is tentatively identified as *Xanthomonas campestris* pv. *campestris*.

Starr *et al.* (1977) described the *Xcc* as aerobic, gram-negative, 0.7-3.0 x 0.4-0.5 µm rods, occurring singly or in pairs, motile with a single polar flagellum. On solid medium containing a suitable carbohydrate, colonies are yellow, convex, shiny and of a slimy mucoid consistency.

Five isolates obtained from infected curds were tested for their ability to produce infection in curds. It was found that all the organisms namely *Erwinia* sp., *Alternaria* sp., *Fusarium* sp., *Rhizopus* sp., together with *Xcc* were found to cause curd rot individually and similar results were reported by Chakrabarty *et al.* (1989); Harbola and Khulbe (1994); Shyam *et al.* (1994); Obradovic *et al.* (2000) and Tamayo *et al.* (2001).

It seems that presence of *Xcc* on the curd predisposes to infection by other organisms. Complete rotting of the curd was observed at much faster rate when the mixture of inoculum was inoculated along with *Xcc* compared to the individual inoculations. Djalilov *et al.* (1989) reported the presence of black rot pathogen resulted in considerable increase in damage to plants by soft rot caused by *Erwinia carotovora*, *Pseudomonas* sp. and other opportunistic pathogens.

Plant tissues often show different levels of resistance to pathogens depending on their age. Some of the many types of age-related resistance have similarity to known plant defense systems, including preformed defenses, race-specific gene-for-gene resistance, systemic acquired resistance and induced systemic resistance (Panter and Jones, 2002). Chauhan *et al.* (2000) reported the cauliflower plants up to 20 days

old were more prone to attack by *Rhizoctonia solani* causing stem rot than 35 day old plants. In 50-day-old plants susceptibility to the disease decreased drastically.

All the inoculated plants, in this study, irrespective of age at which they were inoculated were infected locally, indicating the susceptibility of cauliflower in all stages of growth. But the disease development was faster and progression to systemic nature was more when the plants were infected at a young age.

Systemic infection was severe and most destructive in young inoculated plants. The local infection proceeded to systemic infection within 10-15 days and the disease progressed quickly and resulted in complete withering of leaf without production of bigger lesions or blackening of veins. Microscopic examinations of dead plants revealed the presence of bacteria in the stem indicating that they were systemically infected, demonstrating the increased susceptibility of young plants to systemic infection. This is because young plants do not have developed a hardened defensive system and are highly susceptible to the disease. Their leaves being very soft less thick with less waxy coating the pathogen smeared can easily enter in to the veins and cause sudden collapsing of plants.

The plant utilizes most of its energy for its growth during the young stages where rate of respiration will be more. The movement of water in the xylem will be more rapid and so there is every possibility of bacteria to cause systemic infection. The bacteria block the fluid conducting tissue which leads to wilting and finally death of plant.

When the older plants were inoculated, only a few plants were systemically infected and they remained alive. Inoculations at this stage may have little effect on the death of the plants this is because the movement of bacteria was restricted by the

well developed defense system of old plants. These findings were in tune with the work done on different crops by Wang and Sletten (1994); Rathore (2000) and Shaat (2003).

All the systemically infected plants produced infected curds, if they were alive and produced the curds. This may be due to easy entering of bacteria in to the curds through conducting tissues. Young infected plants which showed systemic infection died before the initiation of curd. This showed that the young plants were easily consumed by the pathogen while the older plants can combat the disease for some period of time without fully succumbing.

The three parameters - the host, the pathogen and the environment - must be present and interact appropriately for occurrence of plant disease. The climate – temperature (maximum and minimum), moisture (rainfall and RH) and duration of sun light – can impact all three legs of the plant disease triangle in various ways.

In the present study different climatic factors *viz.*, maximum and minimum temperature, maximum and minimum relative humidity, rain fall and sunshine hours occurring on a particular day, the climatical average of two adjacent days and climatical average of three adjacent days were analyzed for their correlation with the disease incidence separately. From the results it was found that maximum temperature and sunshine hours were found to be positively correlated with the disease incidence coming on 9^{th} day prior to disease incidence, only maximum average temperature on 7, 8 and 9^{th} day prior to disease incidence and only average sunshine hours on 4, 5 and 6; 5, 6 and 7; and 6, 7 and 8. This means that there was increase in disease incidence with increase in temperature and sunshine hours, which are interdependent. Similar reports were produced by many workers on different

crops against different diseases (Chakrabarty and Shyam, 1989; Nakamura, 1990; Shyam *et al.*, 2001 and Ingole *et al.*, 2008).

The disease incidence was found to be more during warm temperature and high humidity periods where maximum losses were observed as per the reports of Knosel (1960) and Achter *et al.* (1977). As these studies were carried during the months of December-march where sufficient humidity and even dew in morning hours was observed, high temperature together with relative humidity resulted in increased disease incidence which is on line with that of the reports.

Based on the significance obtained on a particular day or average of day's climatical data prior to the disease incidence, regression analysis was worked out and prediction equations (regression equations) were made. Similar kind of equations were made by Chakrabarty and Shyam (1989) by pooling the climatical data of two seasons calculated for two years, where as here it was calculated for only one season because of time limitation. So the R^2 values obtained in this correlation analysis were found to be very less 0.081 (maximum obtained 9^{th} day climate, prior to disease incidence observation) indicating that only 8.10 per cent variation in the diseases incidence would be predicted, which can not be used in prediction models. But on academic interests prediction equations were made for the periods for which correlation was found to be significant.

Studies on the effect of climatical factors on the incidence requires comparatively large volume of observations to have a meaningful interpretation as most of the factors influencing the disease incidence are interdependent and produce the same type of interaction with a minor change in one or two parameters such as rainfall, cloudiness and duration of sunshine on prevailing temperature. The observations need to be taken for at least 3-4 years to get a concrete prediction model.

Sources of resistant to black rot in cauliflower were reported by many workers but most of them are not commercially grown by Indian farmers due to poor curd quality. MGS 2-3, Pusa Kea and S-445 were resistant to black rot shown to be governed by dominant polygenes but curd quality of these lines was not acceptable (Singh, *et al.,* 1987). Based on qualitative and quantitative parameters of the cauliflower and the disease reaction ranked by AUPDC. Pandey *et al.* (2003) reported that the varieties Kunwari-18, Phool Gobhi Kunwari, Kataki-7 and BT-10-2 were moderately resistance to black rot. Even though the cultivation of cauliflower is being done widely, the seeds produced by commercial firms suitable for tropical plains are used. There is no report of disease reaction of cauliflower varieties/ line to black rot from Kerala.

Sixteen varieties/lines were screened for their reaction to *Xcc* under natural conditions. The multipoint disease scorings were made into A-value, which represents AUPDC. Based on this A-value these were grouped into resistant, moderately resistant, moderately susceptible and susceptible. The results showed that of this sixteen varieties, no variety was found to be resistant. NS 121, NS 60, Greeshma, Pusa Meghna, Trisha, Megha, NS 60N and Ateseegra were found to be moderately resistant, Barkha and NS 131 were moderately susceptible and 2178, Pusa Sarad, Himshot, NS 133, ACC 2235 and Basant were susceptible. Basant was found to be the most susceptible variety as it recorded the maximum A- value. Disease screening on different crops caused by different pathogens was done by calculating the A-value by many researchers (Wilcoxon *et al.*, 1975; Jeger and Viljanen, 2000; Zambenedetti *et al.,* 2007) and the method gives the slow diseasing varieties a distinct advantage over the sudden epidemic prone varieties.

It is usually not sufficient to determine whether a disease is present or absent. The critical information required is the amount of disease that is present. Disease

often has to exceed a certain threshold before it reduces the yield of a crop. Small amounts have little effect on yield and the disease may not be worth controlling (Brown and Keane, 1997). This correlation with the threshold level make the standard scoring necessarily to have a bearing on the yield of the crop, hence in this study an attempt was made to correlate with the yield.

Much of the published works on disease assessment and crop losses has been qualitative rather than quantitative. One major difficulty associated with disease assessment is the rarity in nature of a 'one cause-one disease' situation. Under field conditions, plant growth and yield are influenced by many factors including nutrients, rainfall, insects, weeds and pathogens. It is difficult, therefore, to determine the relative importance of different factors in limiting yield. There are often complex interactions between the constraints on yield. Measurement of disease severity is more difficult and error-prone than measurement of disease incidence (James, 1974).

Determining disease severity often requires estimating the proportion of the total photosynthetic area of the crop to that of diseased 'proportion' which is often called 'leaf area affected' (Zadoks, 1985). In this study, the measurement of original disease in the plants were precise and taken as unit of area of infection which is the proportion of actual area infected to the total area of that leaf.

Disease severity assessment relies on visual judgments which tend to be deceptive and to vary greatly from person to person. The human eye tends to detect grades of disease severity in logarithmic steps (5%, 10%, 20%, 40%, 80%) rather than in the uniform, arithmetic steps (5%, 10%, 15%, 20%, 25% etc.) by which we like to express quantities. Accordingly, in this study we included three disease measurement scales – one following arithmetic, second one logarithmic and the third a slightly modified logarithmic to test the best key for disease measurement.

The analysis of the original data showed that the black rot incidence on 25th and 40th DAT was negatively correlated, whereas the 10th DAT incidence gave a positive trend on the yield. On substitution of proposed grades for the original values, the scale II is best near to the original which is based on logarithmic perception with a variation of only 5 per cent from the original one. There is a need for verification and validation of this proposed scale before being put into use.

Ultimate aim of any studies on plant disease is to have a management system thereby the loss occurring to the farmers can be minimized. Nowadays, due to the awareness on pollution and non target effects of plant protection chemicals, a lot of emphasis is being given to non chemical means of plant disease management including biological agents and botanicals.

Control measures have been recommended to minimize the threat of black rot in cauliflower, whenever it crossed threshold level. Since no chemicals, botanicals or bioagents were tested scientifically against *Xcc* for plains of Kerala, *in vitro* studies were conducted to find out the suitable chemicals/ botanicals / bioagents to be carried over to the field studies. Final selection was made in such a way that each category was represented by at least one treatment. Finally two treatments having antibiotics, one chemical, three botanicals and a bioagent were selected for the field studies. In the case of bioagents, availability to the farmers was considered in fixing the field treatment.

Of the two chemicals copper hydroxide and copper oxychloride tested *in vitro* under two different concentrations 0.15 per cent and 0.2 per cent (copper compounds), copper hydroxide 0.2 per cent was found to be the effective one. Similar results have been reported by Fukaya, *et al.* (1988). Copper hydroxide 0.2, 0.15 per

cent was found to be on par with copper oxychloride 0.2 per cent. The best among the chemical copper hydroxide 0.2 per cent was taken for field studies.

Among the two antibiotics (streptocycline and tetracycline) tested with three different concentrations 100, 200 and 250 ppm and a combination of antibiotic plus chemical (copper oxychloride 0.15ppm + streptocycline 100ppm), tetracycline 250 ppm was found to be the best among the chemicals which showed maximum inhibition of growth of pathogen *in vitro,* followed by tetracycline 200 ppm. Similar reports have been reported by many workers (Thirumalachar, *et al.,* 1956; Bhat, 2000; Santos *et al.,* 2008).

Among all the botanicals tested *viz.*, garlic, turmeric, tea leaf extract and tea waste at a concentration of 5 and 10 per cent, garlic irrespective of concentration had shown the better performance under *in vitro* conditions. Garlic has a wide spectrum of actions; antibacterial, antiviral, antifungal and antiprotozoal. Allicin, a major component of garlic was found to have antibacterial property (Harris *et al.,* 2001). Effect of garlic against many bacterial pathogens was reported by many workers Mangamma and Sreeramulu (1991); Khan *et al.* (2003); Curtis *et al.* (2004). Though tea decoction recorded minimum inhibition among all the botanicals it was found to be on par with turmeric 10 and 5 per cent.

Of the two bioagents, *Bacillus subtilis* showed the maximum inhibition on growth of pathogen. This is in conformity with the results reported by Papou and Grynko (1994); Jalali and Parashar (1995); Bora and Bhattacharyya (2000) and Massomo *et al.* (2004). Due to the non availability of commercial formulation of *B. subtilis* and culture in bulk quantity, *Pseudomonas fluorescens* was selected for field studies.

The result showed that garlic 10 per cent was the best of all the treatments which showed less area under disease progression curve (A-value), followed by tetracycline 250 ppm which was on par with *P. fluorescens,* tetracycline 200ppm and copperhydroxide 0.2 per cent. Maximum yield was recorded in garlic 10 per cent treated plot followed by tetracycline 250 ppm and *P. fluorescens* treated plot. There are several reports confirming the efficacy of tetracycline in the control of *Xcc* (Sharma, 1981; Kishun, 1984; Shah *et al.,* 1985; Napoles *et al.,* 1991; Lenka and Ram, 1997).

Summary

6. SUMMARY

One of the major constraint in cauliflower production is black rot incited by *Xanthomonas campestris* pv. *campestris*. It may cause losses of more than 50 per cent and some times even 100 per cent. Considering the serious nature of this disease the present investigation was undertaken to study the epidemiology and management of black rot of cauliflower in plains of Kerala.

The black rot pathogen was isolated from the infected leaves and curds showing typical symptoms from the samples collected from the field and also from the market on Potato Sucrose Peptone Agar medium. The colonies showing typical characters of *Xanthomonas* with the best virulence one were purified by repeated streaking on PSPA medium. The bacteria produced slimy yellow colonies and were aerobic gram negative rods. It produced acid oxidatively from sucrose, glucose, maltose, lactose, dextrose, fructose, mannose and arabinose where no acid was produced from dulcitol, inositol, adinitiol and sorbitol. It produced H_2S, liquefied gelatin and hydrolyzed starch. It utilized citrate, produced lipase and ammonia whereas it showed negative reaction for methyl red and urease test. Based on morphological, physiological and biochemical characters coupled with pathogenicity the pathogen was identified as *Xanthomonas campestris* pv. *campestris*.

Symptoms of disease both under natural and artificial conditions were studied on leaf and also on curd. The typical symptoms such as chlorotic flecks at the margins of leaf, V shaped lesions, drying of lesion and vein blackening were observed on leaves. On infected curd, discoloration, rotting and foul smell were noticed. In severe cases, pathogen become systemic and the symptom exhibited were vascular discoloration, cracking of vascular tissue and rotting. Systemic infection in

young plants resulted in death of the plants. All the general symptoms of naturally infected plants could be reproduced on artificial inoculation.

Susceptibility of plant to black rot in relation to age was studied by inoculating plants with an interval of 5 days. Plants of all stages irrespective of age were found to be susceptible to black rot and resulted in local lesions. The progression of local lesion to systemic infection was found to be more when the plants were inoculated at young stage. Infections at early stages of the crop resulted in the mortality of the plants. Up to 25 DAT inoculated plants the curd formation was nil or meager as the plants were either dead or failed to initiate curd. All the systemically infected plants produced infected curds if they develop curds.

Cauliflower varieties/lines were subjected to black rot scoring. Multipoint observation on disease was taken and AUPDC was calculated and expressed as A-value. Based on this A-values all these varieties/lines were graded into four categories, resistant (0), moderately resistant (0.01-3.0), moderately susceptible (3.01-5.0) and susceptible (above 5.0). No variety was found to be resistant to black rot, NS 121, NS 60, Greeshma, Pusa Meghna, Thrisha, Megha, NS 60N and Ateseegra were categorized as moderately resistant. Barkha and NS 131 were grouped under moderately susceptible category. Cauliflower line 2178, Pusa Sarad, Himshot, NS 133, ACC 2235 and Basant were the susceptible varieties/ lines. Off all this 16 varieties Basant was found to be the most susceptible variety as it recorded the maximum A-value (12.81).

Influence of climatic factors *viz.*, maximum and minimum temperature, maximum and minimum humidity, rainfall and sunshine hours on disease incidence were calculated by correlating the disease incidence with these climatic parameters starting from the day prior to the disease incidence upto the ninth day, average

climate of two and three days. Results showed that sunshine hours and maximum temperature on ninth day, three day mean of sun shine hours of 4,5 and 6; 5,6 and 7 and 6,7 and 8 were found to be positively correlated with the disease incidence, like this three day mean maximum temperature of 7,8 and 9 were also positively correlated. Regression analysis was worked out for these correlated values and regression equations were made.

A black rot measurement scale was proposed which is having a bearing on the yield. The scale was logarithmic in nature and having only 95 per cent similarity with the original loss value. Multipoint observations were used for this study.

In vitro evaluations were made to identify chemicals, botanicals and bioagents which are having inhibitory effect on the black rot pathogen. Of all the chemicals tested, copper hydroxide 0.2 per cent was found to be the best and among the antibiotics, tetracycline 250 ppm was found to be the best. Among different botanicals, garlic extract 10 per cent was the best. Among the two antibiotics tested *Bacillus subtilis* was found to be best compared to *Pseudomonas fluorescens.*

A field experiment was conducted to evaluate the results obtained *in vitro.* For these best performed three chemicals (antibiotics), three botanicals and a bioagent were selected. Tetracycline 250, 200 ppm and copper hydroxide 0.2 per cent; garlic extract 10, 5 per cent and turmeric extract 10 per cent; *Pseudomonas fluorescens* were selected because of the non availability of *Bacillus subtilis* in bulk.. Garlic extract 10 per cent was found to be the best treatment followed by tetracycline 250 ppm and *Pseudomonas fluorescens.* The garlic 10 per cent treated plot gave the highest yield.

Statistical models and prediction equations need to be based on huge volume of data which minimizes the error in the relationship arising out of several known and unknown interacting factors. Climatic factors and yield contributing factors are too large and their interactions are very high. The period of study of this thesis was only single season and the data were insufficient to make perfect models. Normally 4 to 5 season data are required to make a meaningful model. Further all models have to be tested, verified and validated at different levels. Hence these types of epidemiological studies have to be continued for a minimum period of five years.

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Annuexure

APPENDIX

Media composition

(Ingredients per liter)

1. Potato sucrose peptone agar medium (PSPA) (pH 7.0)

KH_2PO_4	:	0.2 g
Na_2HPO_4	:	0.5 g
$Ca(NO_3)_2$	:	0.5 g
$FeSO_4$	:	0.5 g
KCl	:	0.05 g
Peptone	:	2.0 g
Sucrose	:	20.0 g
Potato	:	300.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

2. Basal medium (ph 7.0)

Peptone	:	1.0 g
$NH_4H_2PO_4$	:	1.0 g
KCl	:	0.2 g
$MgSO_4$	:	0.2 g
Bromothymole blue	:	0.03 g
Agar	:	3.0 g
Distilled water	:	1000 ml

3. Methyl red broth (pH 7.0)

Peptone	:	5.0 g
Glucose	:	5.0 g
KH_2PO_4	:	0.2 g
Distilled water	:	1000 ml

4. Yeast Glucose Chalk Agar media (YGCA) (Ph 7.0)

Yeast extract	:	10.0 g
Glucose	:	10.0 g
Chalk ($CaCO_3$)	:	20.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

5. Kings 'B media (pH 7.2)

Peptone	:	20.0 g
Glycerol	:	10.0 g
K_2HPO_4	:	1.5 g
$MgSO_4$ 7 H_2O	:	0.5 g
Agar	:	20.0 g
Distilled water	:	1000 ml

6. Peptone water (pH 7.0)

Peptone	:	10.0 g
NaCl	:	5.0 g
Distilled water	:	1000 ml

7. Nutrient Agar media (NA) (pH 7.2)

Peptone	:	20.0 g
Beef extract	:	1.0 g
NaCl	:	5.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

8. Nutrient Gelatin media (pH 7.2)

Peptone	:	10.0 g
Beef extract	:	5.0 g
Gelatin	:	4.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

9. Sierra's media (pH 7.0)

Peptone	:	10.0 g
NaCl	:	5.0 g
$CaCl_2$ H2O	:	0.1 g
Agar	:	20.0 g
Distilled water	:	1000 ml

10. Simmon's Citrate Agar (pH 7.0)

$NH_4H_2PO_4$	:	1.0 g
KH_2PO_4	:	1.0 g
NaCl	:	5.0 g
Sodium citrate	:	2.o g

$MgSO_4$	:	0.2 g
Agar	:	15.0 g
Bromothymol	:	0.08 g

11. Thronley's media (pH 7.0)

Peptone	:	1.0 g
NaCl	:	5.0 g
K_2HPO_4	:	0.3 g
Agar	:	3.0 g
Phenol red	:	0.01 g
L arginine	:	1.0 g
Distilled water	:	1000 ml

12. Vanden Mooter Succinate Medium (pH 6-7.0)

K_2HPO_4	:	0.5 g
KH_2PO_4	:	0.5 g
$K_2SO_4.7H_2O$	:	o.2 g
Sodium Succinate	:	2.0 g
KNO_3	:	3.0 g
Yeast extract	:	5.0 g
Agar	:	3.0 g

13. Tryptophan Broth (pH 7.0)

Tryptophan	:	10.0 g
NaCl	:	5.0 g
Water	:	1000 ml

Abstract

EPIDEMIOLOGY AND MANAGEMENT OF BLACK ROT OF CAULIFLOWER IN PLAINS OF KERALA

By

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ABSTRACT OF THE THESIS

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Master of Science in Agriculture

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ABSTRACT

The present study "Epidemiology and management of black rot of cauliflower in plains of Kerala" was taken up at Department of Plant Pathology, College of Horticulture, Vellanikkara and Agriculture Research Station, Mannuthy during 2009-2011 with an aim to study the epidemic factors influencing the incidence, development and severity of black rot caused by *Xanthomonas campestris* pv. *campestris* in plains of Kerala and to conduct field studies on its management.

The pathogen was isolated from the leaves and curds of cauliflower showing typical symptoms of the disease, on PSPA medium. The colonies of the isolate were yellow in colour, circular, slimy and smooth convex with entire margin. Studies on morphological, cultural and biochemical characters confirmed the bacteria as *Xcc*.

Various types of *viz.*, chlorotic lesion, V shaped lesions, vein blackening; necrosis and vascular discoloration were produced by the pathogen. The symptoms were initially localized and later became systemic and stunting of plants was noticed due to systemic infection.

Progression and severity of the disease was studied by inoculating the plants at five days interval starting from 10 days after transplant (DAT) up to 60 DAT. Cauliflower plants of all age group were found to be susceptible to this disease. But, young plants were succumbing to death due to infection. As the age increased there was gradual decrease in the systemic infection. Plant mortality also decreased with the increase in age at which they got infected.

Sixteen cauliflower varieties were screened for black rot disease under field conditions. The incidence, calculated as A- value (AUDPC), varied from 0.53 to 12.81. These varieties were grouped in to four categories based on the scale; 0-

resistant, 0-3 moderately resistant, 3-5 moderately susceptible and >5 susceptible. Out of sixteen varieties, none was found to be resistant, eight were found to be moderately resistant, two were moderately susceptible and remaining six were susceptible.

An attempt was made to formulate a score card for this disease for easy observation and which has a bearing on the yield. The actual area of infection in the leaf at 10, 25 and 40 days old plants were correlated with yield and a equation y=389.061+3.371(10 DAT)-0.986(25 DAT)-2.391(40 DAT) was obtained. Three system of score card were formulated and their relationships with the yield were re-correlated statistically and three additional equations were arrived. The equation two was comparable with the original equation and that score card can be better adopted as it showed 95 per cent similarity to the original, where scale 1 and 3 showed 77.5 and 90.1 per cent similarity respectively.

An attempt was made to determine the role of weather parameters on incidence of this disease. The weather factors such as maximum temperature and sunshine hours were positively correlated prevailing to the observation on 9^{th} day and average of 7, 8 and 9 days. Similarly average of 4, 5, 6; 5, 6, 7 and 6, 7, 8 days were positively correlated with only sunshine hours. A partial multiple regression equation is also derived for predicting the disease incidence. Such correlation studies have to be conducted for at least five years consecutively to arrive at a better prediction model.

Initial *in vitro* evaluations were done to identify the chemicals, botanicals and bioagents to be carried to the field. Field trail showed that garlic extract 10 per cent, tetracycline 250 ppm and *Pseudomonas fluorescens* were best treatments against *Xcc.*