SEED-BORNE *Colletotrichum capsici* (Syd.) Butler and Bisby AND IT'S MANAGEMENT

Thesis submitted in part fulfilment of the requirement for the degree of ***Master of Science (AGRICULTURE)*** *in* ***Plant Pathology*** *to the Tamil Nadu Agricultural University, Coimbatore.*

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CERTIFICATE

This is to certify that the thesis entitled **"SEED-BORNE *Colletotrichum capsici* (Syd.) Butler and Bisby AND IT'S MANAGEMENT"** submitted in part fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in Plant Pathology to the Tamil Nadu Agricultural University, Coimbatore is a record of *bona fide* research work carried out by **Mr. R. RAJAVEL (ID.No.98-614-006)** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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Abstract

ABSTRACT

SEED-BORNE *Colletotrichum capsici* (Syd.) Butler and Bisby AND IT'S MANAGEMENT

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DEGREE : **Master of Science (Agriculture) in Plant Pathology**

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Chilli is an important spice cum vegetable crop and popular among the Indians because of it's food and medicinal values. The surplus production was exported to over 90 countries. The demand was growing and was 90,000 tonnes by 2000 and 1.13 lakh tonnes by 2005 in addition to 9.45 lakh tonnes of dry chilli (1996-97). There is an obstacle mostly by pests and diseases to achieve the demand. The enormous loss was accounted by fruit rot of chilli (*Colletotrichum capsici*) which is seed-borne in nature and can survive for four years on the seeds.

A survey was conducted to find out the status of fruit rot incidence and seed-borne nature of *C. capsici* in different chilli growing parts of Tamil Nadu.

The disease incidence on fruit and seed-borne nature have been found to be 45.30 PDI and 33.3 per cent, respectively.

Different techniques were tried to standardize the detection of *C. capsici* from chilli seeds. Chilli seeds soaked in 3 per cent calcium chloride have recorded maximum of 23.33 per cent seed infection over other methods. The light periods and pH have influenced the seed infection count and the maximum count was achieved at pH 7.5 and exposing the seeds to 12 h (Phillips TLF 40W/34) + 12 h NUV (T1 40W/08) light periods. In SDS-PAGE analysis, the disappearance of 29 kDa protein was observed due to *C.capsici* seed infection. This can be used as an indicator for detection of *C. capsici* in seeds.

Morphological variation in respect to conidia and setae was observed between the isolates. Seeds infected with *C. capsici* had direct effect on seed morphology and seed germination, which results in poor seedling vigour.

Increased levels of peroxidase, polyphenol oxidase and total phenol content were observed. The increasing activity was directly proportionate to the level of seed infection. The reduced levels of protein and sugars (reducing, non-reducing and total sugar) were also observed and the reduction is proportionate to the level of seed infection.

In order to manage the *C. capsici* causing fruit rot *in vitro* evaluation of antagonists, plant products and fungicides were studied. Among the fungicides tried carbendazim (0.1 per cent) and mancozeb (0.3 per cent) were highly effective in inhibiting the mycelial growth (86.54 per cent). The strains PF1 (*Pseudomonas fluorescens*) and Uv10 (*Trichoderma viride*) were also equally effective in

inhibiting the mycelial growth. Among the plant extracts tried, *Catharanthus roseus* had recorded higher reduction in mycelial growth (44.15 per cent) over other plant extracts.

All the treatments were found to inhibit the *C. capsici* spore germination. However, 10 per cent extracts of *Prosopis juliflora* had inhibited the spore germination by 100 per cent. The treatments had showed their effectiveness in checking the fruit rot/anthracnose incidence in the field that is conducted at two different locations in the same season. Spraying of 4 per cent talc based *Pseudomonas fluorescens* (PF1) reduced the incidence of fruit rot to the tune of 72.80 per cent and this was followed by spraying (10 per cent) leaf extracts of *Prosopis juliflora* (59.90 per cent). The use of biocontrol agents or plant extracts proved superior in checking fruit rot incidence at field condition, when compared to fungicides, carbendazim (42.40) and mancozeb (56.80).

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CHAPTER I

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most important spice crops and it forms an indispensable adjunct in every house in the tropical world. It is specially liked for its pungency, spicy taste besides the appealing colour it adds to the food. It is very popular and occupies major area under cultivation because of it's preference in house hold (Siripurapu, 1993).

Chilli cultivation in India occupies an area of 9.565 lakh ha with an annual production of 9.45 lakh tonnes (1996-97), of which 93.94 per cent is consumed in India and remaining 6-7 per cent is exported to the third world countries. The major chilli producing states are Andhra Pradesh, Karnataka, Tamil Nadu, Madhya Pradesh, West Bengal, Uttar Pradesh and Rajasthan.

In Tamil Nadu, it is cultivated in an area of 66,107 ha with an annual production of 39,663 tonnes (1994-95). The major contribution is from Ramanathapuram District, the other chilli growing areas are Trichy, Tirunelveli, Tuticorin, Madurai, Villupuram, Salem, Tenkasi and Coimbatore.

The fresh fruits of chilli contain insignificant amount of vitamin B, C, E and provitamin A. The pungent principle in chillies has got so many effects on our body such as anti-inflammatory, anti-obesity and desensitization against different chemical analgesic effect. `Capsaicin', the pungent principle has the ability to lower the blood cholesterol and hence could be used as an anti-obesity agent (Menon, 1995). The recently isolated "vitamin P" from chilli has the ability to prevent the heart attack by dialating the blood vessels.

It's importance in household and medicine has been exploited for long, but it is being affected by many diseases mainly due to deleterious seed-borne diseases (Durairaj, 1972). Chilli has been affected by more than 51 pathogens consisting of fungi, bacteria and viruses. There are 26 fungi reportedly present in chilli seeds (Basak *et al.*, 1996). Among the seed-borne pathogens, the fruit rot / anthracnose is a deleterious one causing considerable damage to the crop.

Basak *et al.* (1996) reported the *C. capsici* seed- infection in chillies to an extent of 58.39 per cent in Bangladesh. This disease leads to a yield loss of 12-30 per cent in Assam (Chowdhury, 1957), 10-35 per cent in Haryana (Bansal and Grover, 1969) and 10-30 per cent in Punjab (Rai and Chohan, 1966). The yield loss in South India has been reported to be 30 per cent (Durairaj, 1972).

In view of the value of chilli and it's products, severity of the disease and the economic losses it causes, a study was undertaken with the following objectives.

1. Survey for *C. capsici* infected chilli seeds.
2. Standardization of techniques to detect *C. capsici* from chilli seeds.
3. To study the effect of *C. capsici* seed infection on seeds and seedling quality.
4. Assessment of field incidence of dieback in relation to level of *C. capsici* seed infection.
5. Production of disease free chilli seeds using IPM strategies.
6. Detection of *C. capsici* in chilli seeds on the basis of molecular techniques.

Plate 2.

CHILLI HEALTHY AND INFECTED FRUITS

HEALTHY FRUITS

INFECTED FRUITS

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

2.1. Causal organism

Fruit rot and dieback of chilli (*Capsicum annuum* L.) is one of the deleterious diseases gaining importance in recent years. The pathogen causing fruit rot is not clear as the responsible organisms are several, they are *Colletotrichum, Gloeosporium, Alternaria, Helminthosporium, Fusarium, Curvularia, Diplodia* and *Phoma*. The fruit rot of red pepper was first reported in America (Halstead, 1890). In India, Sydow (1913) first recorded it in Madras Presidency and he reported the causal organism as *Vermicularia capsici* Syd. (Syn. *Colletotrichum capsici* (Syd.) Butler and Bisby).

2.2. Prevalence and occurrence

Chowdhury *et al.* (1957) reported the presence of fruit rot incidence in Assam and observed 12-32 per cent incidence. Rai and Chohan (1966) reported that *C. capsici* was severe on chilli fruits in Punjab and it over wintered in plant debris in field and caused 10-30 per cent reduction in yield.

Grover and Bansal (1968) observed the incidence of *Colletotrichum piperatum* and *C. capsici* while surveying for chilli diseases in Punjab and Haryana. They reported the per cent incidence for each pathogen and noticed severe damage when the area was irrigated frequently.

Bansal and Grover (1969) reported that the fruit rot of chilli caused 10-35 per cent yield loss in Haryana. The disease was serious in Orissa, exclusively in the area where intensive cultivation was followed (Panigrahi and Narain, 1971).

Subramanian *et al.* (1971) reported that the disease first appeared when the crop was in flowering stage. In severe cases, it infects the fruits and branches causing fruit rot and die back respectively.

Siddiqui *et al.* (1977) reported that the incidence of *C. capsici* on chilli seed was the primary source for symptom development in the field. They also performed different interesting management practices and recommended difolatan (0.25 Per cent).

Thind and Jhooty (1985) reported the prevalence of *C. capsici* in chilli and found to be quite serious. The extent of damage ranged from 66-84 per cent in Punjab.

2.3. Susceptible stages of fruits

There were several thoughts about the stages of infection. Several workers reported that only red fruit of chilli was vulnerable to pathogen attack. However, Higgins (1926) reported that *Gloeosporium piperatum* attacked fruits in all stages of development.

Natarajan (1973) showed that *C. gloeosporioides* produced not only in red fruit, but when inoculated on young green fruits with injury it caused rotting. which was supported by Adikaram *et al.* (1982).

Prakasam (1983) reported the susceptible stages of fruits. He observed most susceptibility in twenty-five days old chilli fruits and recorded negative results when inoculated on five days old green fruits.

Basak *et al.* (1994) studied the prevalence and relative incidence of fruit rot causing fungi on capsicum fruit. The infection was also observed on different stages of

fruits and found higher incidence in fully ripened fruits. Roy *et al.* (1997) observed garden that yielded poor quality fruits and analyzed for the cause and found that the causative agent was *C. capsici*. The yield loss ranged from 3-15 per cent and observed more severity during ripening.

2.4. Growth, sporulation and symptom development

High disease incidence and development was recorded when the plants were exposed to 28°C and 90.00 per cent relative humidity (Chowdhury *et al.*, 1957).

Misra and Mahmood (1960) observed the growth and sporulation of *C. capsici* in different sources of sugars and nitrogen. They found increased growth and sporulation with increased levels of sugar and nitrogen and found maximum growth and sporulation at 1: 0.25 C/N ratio.

Misra and Dutta (1963) observed the differences in morphological character, carbon nitrogen requirement, growth rate, sporulation and virulence of two isolates collected from two different location of Orissa and found that the variation was due to strain.

The growth and sporulation of *C. capsici* were correlated with temperature (30°C), relative humidity (85 per cent) and pH (Misra and Mahmood, 1960). The sporulation was heavier at pH 5 with alternate light and darkness.

Louis *et al.* (1988) studied the influence of extracellular conidial matrix on germinability of *C. capsici* and found increased germinability with decreased spore density. The spore failed to germinate when the spore density was high and also age of culture.

Ouyang *et al.* (1993) studied the effect of toxin on symptom development. The partially purified toxin would infect the capsicum when subjected to the temperature 25°C and pH 6.7 and found less radial growth of *C. capsici*.

The existence of variation among the *C. capsici* isolates was correlated with nitrogen content and symptom development. Highly virulent isolates had high content of nitrogen and would develop symptom within 15 days after inoculation, whereas it was 30 days in less virulent isolates (Kolte *et al.*, 1994).

Palarpawar and Ghurde (1997) studied the effect of different nitrogen sources on growth and sporulation of *C. capsici* and *C. curcumae*. Increased growth and sporulation was observed when media was supplemented with peptone and the reverse was the case when supplemented with ammonium oxalate, sodium nitrate and urea in *C. capsici,* whereas in *Colletotrichum curcumae*, the sporulation was abundant in media containing potassium nitrate, ammonium carbonate and urea.

Hong Jeumkya and Hwang Byungkook (1998) studied the influence of inoculum density, wetness duration, plant age and inoculation method and cultivar resistance on infection of pepper. The symptom development was positively correlated with inoculum density and leaf wetness.

2.6. Seed-borne nature of pathogen

Acervuli could clearly be distinguished and noticed on seed coats. The strands of hyphae were found ramifying into various portions of internal seed tissues (Ridgway, 1912).

Several workers have reported the seed-borne nature of anthracnose. *C. capsici* was known to be seed-borne in Georgia (Anon., 1933). Smith and Crossan (1958) found that *C. piperatum* and *C. capsici* were capable of surviving in/on the seeds of *Capsicum frutescens* for atleast nine months.

Rai and Chohan (1966) reported twenty-nine per cent mortality in chilli seedlings when it was infected with *C. capsici*. The seeds obtained from rotten chilli fruits carried the pathogen both internally and externally (Grover and Bansal, 1968).

Rout and Rathi (1972) reported the seed-borne nature of several fungi in chilli seeds. They also observed the presence of *C. capsici* on seed coat (external).

Menon and Nik (1988) reported the seed-borne nature of *C. capsici* and observed the morphological character and acervuli distribution under microscopic examination.

Padaganur and Naik (1991) observed the external nature of *C. capsici* in the chilli seed lot. The damage caused by the pathogen to seeds ranged from 1.25 to 75.5 per cent.

Patil *et al.* (1993) observed the seed-borne nature of *C. capsici* and correlated the fruit rot incidence with fruit characters. Less incidence was noticed in fruits having thick pericarp.

2.6.1. Seed and seedling quality

Seeds collected from chilli fruits were subjected to blotter technique and the observation showed that *C. capsici*, *Phytophthora nicotianae* var. *nicotianae* and *X. campestris* pv. *vesicatoria* were present (Jindal *et al.*,1994) They performed the germination study and recorded 17.3 per cent germination in infected seeds compared to

healthy seeds (71.00 per cent). They also reported the decreased root and shoot length in infected seed sample.

Kamlesh Mathur (1995) studied the effect of culture filtrate on seed germination and found poor germination. The marked inhibition and the poor germination (17.3 per cent) was due to the presence of *C. capsici* on seed (Jindal *et al.*, 1994).

Basak *et al.* (1996) observed the seed-borne nature of several fungi in chilli seeds and seed infection by *C. capsici* was 58.39 per cent.

2.7. Biochemical changes

Azad (1991) reported the presence of chemical constituent in three different varieties infected with *C. capsici*. The increased level of sugars (reducing sugar, non-reducing sugars and total sugars) was reported while the reverse case was observed in sulphur, ascorbic acid and capsaicin content.

Jeyalakshmi *et al.* (1999) reported the change in biochemical constituents during infection and reported low level of sugars in infected fruits. The result obtained was confirmatory with the report of Madhukar and Reddy (1991) and Subbaraja (1981).

2.8. Disease management

Fruit rot and dieback diseases incited by *C. capsici* are disastrous and cause severe yield losses. With a view to control the disease, scientists have tried fungicides (Chauhan and Duhan, 1977), plant derivatives (Shivpuri *et al.*, 1997) and antagonistic organisms (Jeyalakshmi and Seetharaman, 1998). In recent years, antagonistic organisms and plant products gained importance because of increased resistance to fungicide application.

2.8.1. Plant products / their components that are effective against plant pathogenic fungi

Plant synthesizes its own food and several biochemical components by utilizing the organic and inorganic substances available in nature for their own food as well as for their self defense requirement. The chemical substances produced and stored in plants were well demonstrated for their antifungal, antibacterial and antiviral activities. The plant products possessing anti-fungal activity and their use were well furnished below.

Charya *et al.* (1979) stated that the extracts of different parts of 40 plants, when tested against *Drechslera rostrata* and *Curvularia lunata*, those of *Lawsonia inermis, Punica granatum, Prosopis juliflora* roots and rose flower completely inhibited the germination of conidia.

Gupta *et al.* (1981) stated that the phytonicides of onion, garlic, *Azadirachta indica*, *Ocimum basillicum* and *Leucas* spp., were inhibitory to the conidial germination of *Colletotrichum graminicola* and *C. capsici.*

Rathee *et al.* (1982) reported that the essential oil of *Nigella sativa* L., had strong antimicrobial activity against *Pythium vexans*, *R. solani* and *C. capsici* and the active ingredient involved was carvine.

Kalpana Dixit *et al.* (1986) screened seedling extracts of 40 taxa belonging to ten families. Extracts of *Abrus precatorius, Carum copticum*, cabbage and radish gave 100 per cent inhibition of spore germination of *Botrytis* sp. and *Colletotrichum gloeosporioides.*

Babu and Reddy (1986) reported that the extracts of *Eucalyptus globulus*, *Punica granatum*, *Lawsonia inermis* and *Datura stramonium* were effective in checking fruit rot of lemon caused by *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. Pre inoculation treatments were more effective than post inoculation.

Kaanna *et al.* (1989) stated that the fresh carrot leaves were hydrodistilled and the essential oil (0.07 per cent yield) was analyzed. Anti-fungal activity was tested against *C. capsici* and *Sclerotium rolfsii*. The oil was found to inhibit the growth of *C. capsici* and *S. rolfsii* by 36 per cent and 80 per cent, respectively.

Khanna *et al.* (1991) screened 10 essential oils and oil from *Blumea lociniata* was effective against *C. capsici* and tobacco mosaic tobamovirus. While oil from *Ocimum grassimum* was the most effective against the plant pathogenic fungi *C. capsici* and *Sclerotium rolfsii* infecting *Piper betle*.

Rajeswari and Mariappan (1992) stated that the cold water, hot water and ethanol extracts of *Prosopis juliflora*, *Adhatoda vesica* and *Vitex negundo* were more inhibitory for the radial growth and biomass production of *Pyricularia oryzae*. Bandara *et al.* (1992) studied the effect of root extracts of *Eupatorium ripaium* against *C. gloeosporioides* and it was comparable to that of benlate.

Johri *et al.* (1992) studied the fungicidal activity of coumarins of plant origin and evaluated against *C. capsici* and found the effect to be on par with Blitox (COC) and streptocycline.

Johri *et al.* (1994) studied the effect of saponins extracted from *Mimusaps elengi* against *C. capsici*. Encouraging results were obtained under field conditions.

Renuka Devi (1995) reported that the extract of *Prosopis juliflora* (10 per cent) was effective against *C. capsici* and obtained reduced incidence of fruit rot (50 per cent) and die back (61 per cent) over control.

Shivpuri *et al.* (1997) tested the fungitoxic properties of 10 plant species, which included *Allium cepa, A. sativum, Ocimum sanctum, Polyalthia longifolia, Tagetes erecta, Catharanthus roseus* and *Withania somnifera* and found that *D. stramonium, O. sanctum* and *C. roseus* were most effective.

Jeyalakshmi and Seetharaman (1998) studied the effect of plant products and antagonists against *C. capsici* in chilli and recorded positive results when treated with palmarosa oil, *Ocimum sanctum* leaf extract and neem oil.

2.8.2. Effect of antagonistic organism on *Colletotrichum capsici*

Jeyalakshmi *et al.* (1998) conducted an experiment with *Trichoderma* spp., *Pseudomonas fluorescens, Bacillus subtilis* and *S. cerevisiae* for the management of *C. capsici* and reported the maximum reduction of mycelial growth in the plate treated with *S. cerevisiae, T. viride* and *B. subtilis*. They also performed the same study under field condition and attained positive results.

2.8.3. Effect of fungicides on *Colletotrichum capsici*

2.8.3.1. Fungicides reported to eliminate fungi in seeds and its efficacy against *Colletotrichum capsici*

Mali and Joi (1985) reported that difolatan (Captafol), thiram and carboxin were most effective against colony growth and sporulation of *Alternaria alternata, C. capsici, Curvularia calavata* and *Macrophomina phaseolina.*

Kumar and Mahmood (1986) observed enhanced germination of chilli seeds when treated with fungicides. Best germination was achieved with thiram, followed by difolatan, brassicol, captan and carbendazim. The best control was achieved with aureofungin, thiram, captan, carbendazim, and difolatan.

Perane and Joi (1988) reported best control of *C. capsici* with seed treatment (Thiram). They observed the seed infection and seedling infection under field condition and reported that the seed-borne nature of *C. capsici* was not responsible for symptom developed under field condition, they found that the source was from infected debris, which were buried with dieback symptom.

Kore and Apes (1989) tested nine fungicides against *C. capsici* in PDA plates at 50-3000 ppm. Growth on Blitox (COC), Dithane Z.78 (zineb), Dithane M-45 (mancozeb) and captafol at 50 ppm, was enhanced but inhibition occurred at higher concentration. Bavistin (carbendazim) and thiophanate did not permit good growth of *C. capsici* at 50 ppm.

Chilli seeds with natural infection were treated with benomyl, mancozeb, carboxin and fungal infection was recorded using the standard blotter method (Mridha and Chowdhury, 1990). Infection by *Colletotrichum capsici* was reduced by all the treatments.

Azad (1992) studied the efficacy of six antibiotics (agrimycin, actidione, aureofungin, streptomycin, grisovin and tetracycline) and four fungicides (Dithane @.78, Calixin, Bavistin and Vitavax) on *C. capsici*. They found less contamination on seeds when treated with antibiotics (except aureofungin) and noticed

10 per cent incidence in carbendazim treated seeds. Increased germination was observed in all the cases.

2.8.3.2. Fungicides against *C. capsici* under field condition

Raju and Rao (1985) reported that dithane M-45 (mancozeb) applied to control *C. capsici* on capsicum was compatible with different insecticides. Effective control of fruit rot and three insect pests were observed by six rounds of combined application of mancozeb + monocrotophos at 15 days interval.

Eleven fungicides were tested against chilli fruit rot caused by *C. capsici* and *A. solani*. However, "Foltaf" (captafol) 0.2 per cent gave best control followed by fytolan (COC) 0.25 per cent and carbendazim 0.1 per cent (Jeyasekar *et al.*, 1987).

The most effective and economical management of *C. capsici* and *A. alternata* on chilli under Punjab condition was achieved with spraying of 'difolatan', 'Dithane M-45' at 0.2 per cent (Thind and Jhooty, 1987).

Eswaramurthy *et al.* (1988) studied the efficacy of ten fungicides against *C. capsici* (chilli) and observed less incidence with 'Foltaf' 0.2 per cent, Fytolan' (coc) at 0.25 per cent. They also observed less incidence in 'carbendazim' at 0.1 per cent treated plot.

Mishra (1988) recommended application of fungicides for the control of *C. capsici* in view of getting high C:B ratio, and came with high cost benefit ratio when the field was treated with carbendazim and Dithane M-45 at regular intervals (4 time).

In field trials during 1982 and 1983, among the nine treatments tested, best control was obtained in sprays of carbendazim, followed by benomyl and captafol (Das and Mohanty, 1988).

Malraja and Narayanaswamy (1988) reported that *Capsicum annuum* seedlings were artificially inoculated with *C. capsici* and sprayed three times with one/two per cent 'Dithane Z.78' (zineb), 'Dithane M-45' (mancozeb), 'Fytolan' (coc) and 'bavistin' (carbendazim) at 105, 120 and 135 DAT. They found that mancozeb was the most effective treatment followed by zineb, copper oxy chloride and carbendazim.

Perane and Joi (1988) studied efficacy of fungicides in seed treatment and field spray. They obtained best result, when the study was performed with combination of seed treatment and fungicidal spray. Fruit rot was least after seed treatment with thiram and spraying with mancozeb.

Abbaiah and Reddy (1989) studied the effect of different fungicidal mixtures against fruit rot of *Capsicum annuum* and found that single fungicide at recommended rates was often better than the synergistic combination. Combination of COC + zineb, zineb + carbendazim and tridemorf + zineb at recommended field concentration was determined by *in vitro* studies.

Sinha (1990) studied the effect of seven commonly available fungicides for the control of *C. capsici*. Increased C:B ratio was obtained with Dithane M-45 and Blitox 50.

Biswas (1992) reported that, in field trials of six fungicides against *C. capsici* on chilli, the best result was given by bavistin (carbendazim) at 0.1 per cent applied once in the nursery bed and again at one month and two months after transplanting.

Acharya and Das (1995) recommended various chemicals for the control of *C. capsici* in *Piper betle*. The prescribed fungicides were 'bitertanol' 0.05 per cent, 'mancozeb' 0.2 per cent and "ziram" 0.1 per cent. High C:B ratio was obtained when the interval of spray was 20 days.

Renuka Devi (1995) studied the effect of fungicides against *C. capsici* and increased yield / decreased incidences were obtained with carbendazim and probenphos.

Kumawat (1997) reported the effect of eight fungicides on anthracnose of chilli, the maximum control was recorded in the plots treated with thiram followed by mancozeb.

Materials and methods

CHAPTER III

MATERIALS AND METHODS

3. Survey for the occurrence of *Colletotrichum capsici* infected seeds and disease situation in Tamil Nadu

The chilli seed samples employed in present studies were collected from 30 different locations of chilli growing areas in Tamil Nadu.

3.1. Disease incidence

The fruit rot incidence of chilli was observed and recorded from 10 hot spots of Tamil Nadu. The fruits in the field were categorized based on the scale given by Bansal and Grover (1969).

Sl. No.	Category	Grade
1.	No disease	0
2.	1-5% Fruit area infected	1
3.	6-25% Fruit area infected	2
4.	26-50% Fruit area infected	3
5.	51-100% Fruit area infected	4

Per cent disease index (PDI) was calculated (Wheeler, 1969) as mentioned below.

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Number of fruits assessed}} \times \frac{100}{\text{Maximum disease category}}$$

3.2. Preparation of media

The cultural and growth characters of *C. capsici* were studied in potato dextrose agar (PDA) Riker and Riker (1936).

Potato (peeled)	-	250 g
Dextrose	-	20 g
Agar agar	-	20 g
Distilled water	-	1000 ml
pH	-	6.0 - 6.5

Potato dextrose agar was prepared as per the procedure (Riker and Riker, 1936) and sterilized at 1.1 kg/cm^2 for 15 min.

3.3. Isolation and maintenance of culture

C. capsici was isolated from infected chilli seeds collected from Ulagankathan. Seeds collected from infected fruits were surface sterilized with 0.1 per cent sodium hypochlorite solution for three minutes and washed with three changes of sterile distilled water. Three seeds were placed in Petri dishes containing PDA medium and incubated at 25-28°C for 7 days. The culture was purified by single spore isolation and maintained in PDA slants.

3.3.1. Identification of the fungus

The fungal culture was identified based on the morphological characters such as type of acervulus, conidia and setae and this was compared with the description given by Butler (1918)

3.3.2. Morphological studies

Morphological characters like conidia and setae were observed from two different isolates through microscope. Fifteen observations were taken of width and length of spore with the help of stage and ocular micrometer.

Stage division = **x**
Ocular division = **y**

$$z = \frac{x}{y}$$

where z = calibrated value (μ)

3.4. Standardization of detection techniques

Seed-borne associations of fungus on chilli seeds were common. Hence, the standardization of techniques is vital for identification of different species of *Colletotrichum.*

3.4.1. Standard blotter methods

Plastic Petri plates of 9 cm diameter were placed with three layers of Whatman No.1 moist filter paper. Twenty-five seeds were placed on the filter paper at equi distance and incubated under NUV at 20 ± 2°C for 7 days. The plates were then examined for the presence of *C. capsici* seed infection on eighth day. Seed samples from different places were subjected to this method. One hundred seeds formed one replication and three replications were maintained in each sample.

3.4.2. Deep freeze blotter method

The seeds were plated as in standard blotter method and incubated for 24 hours at room temperature and they next 24 h in deep freezer (-10°C). Subsequently, the plates were kept under controlled condition as in standard blotter method for five days (Limonard, 1966; 1968). Finally, the seeds were examined for the presence of *C. capsici* on eighth day of incubation.

3.4.3. 2, 4-D method

The blotters were dipped in 0.1 per cent solution of 2,4-dichlorophenoxy acetic acid in distilled water. Twenty-five seeds were placed on blotter and were incubated under NUV at 20 ± 2°C for 7 days and the seeds were examined for the presence of *C. capsici* seed infection on eighth day after incubation.

3.4.4. Influence of calcium chloride on *C. capsici* seed infection count

Chilli seeds were soaked in calcium chloride solution of 2 per cent, 3 per cent and 5 per cent concentration (Renukeswarappa and Shethna, 1985)

individually for 24 h and plated on Petri plate containing moist blotter paper. It was kept for incubation under 12 h NUV and 12 h dark at 20 ±2°C for 7 days and observations were made on eighth day for the presence of *C. capsici* seed infection.

3.4.5. Effect of blotter pH on infection count

The blotters were dipped in different pH levels of water *viz.*, 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 and the seeds were plated, incubated and observed as in standard blotter technique.

3.4.6. Effect of light on *C. capsici* seed infection count

Near ultra violet (NUV) light source was provided by Philips T_1 40W/08 black tube light and artificial day light (ADL) by Philips TLF 40W/34 white cool fluorescent tubes (Leach, 1967; Valluvaparidasan, 1994). The seeds were plated as in standard blotter methods and were incubated at 20°C + 12 h NUV light + 12 h darkness, 20°C + 24 h artificial day light, 20°C + 24 h darkness and 20°C + 12 h ADL + 12 h darkness. The observations were made on eighth day after incubation.

3.5. Seedling symptom test

This test was based on the distinguishing symptoms produced by seed-borne fungi on growing seedling under controlled conditions.

3.5.1. Germination test (ISTA, 1999)

Germination test was performed by paper towel method. Final count on normal seedlings was recorded on 14^{th} day and per cent seed germination was computed.

3.5.2. Drymatter production (g/seedling)

The seedlings used for growth measurement were dried in a hot air oven at 80°C for 24 h and cooled in a desiccator. The dry matter production was

calculated with five seedlings and the average was calculated. The results were expressed as g/seedling.

3.5.3. Vigour index

The vigour index was computed by adopting the procedure of Abdul-Baki and Anderson (1970).

Vigour index = Germination percentage x Total seedling length (cm)

3.5.4. Effect of *C. capsici* seed infection on fruit and seed weight of chilli

The infected and apparently healthy fruits of chilli were collected at random and the weight of ten fruits was calculated and expressed in g. The seeds from both healthy and infected were extracted separately and 100 seed weight was worked out and expressed in gram.

3.6.1. Seed quality analysis

The change in biochemical constituents and enzyme activities due to seed infection was estimated in six different seed samples with varying degrees of *C. capsici* seed infection. The changes in constituents were recorded and expressed as per the method adopted.

3.6.2. Estimation of Total Sugars (Hedge and Hofreiter, 1962)

Total sugars from chilli seeds (100 mg) were extracted with warm 80 per cent ethanol twice (5 ml each). This was centrifuged for 10 min and 0.1 ml of supernatant was poured into the test tube and allowed in a water bath for evaporation of ethanol. The residue was dissolved in 1 ml of water and 4 ml of anthrone reagent was added and kept in a water bath for 8 min. After rapid cooling, the absorbance was measured at 630 nm and the results were expressed in percentage.

3.6.3. Estimation of Reducing Sugars (Somogyi, 1952)

The residue was obtained as in total sugar and was dissolved in 2 ml of water. Then it was added with 1 ml of alkaline copper tartarate and kept in water bath for 10 min. Test tubes were cooled and added with 1 ml of arsenomolybdate, 6 ml of distilled water. The absorbance was measured at 620 nm and the results are expressed in percentage.

3.6.4. Estimation of non-reducing sugars

The difference between total sugars and reducing sugars yield the non-reducing sugars.

3.6.5. Estimation of Total Phenol (Malik and Singh, 1980)

Ethanolic extract (3.6.2) was centrifuged and the supernatant was collected and made upto 25 ml. The residue was mixed with 6 ml of water, 0.5 ml of Folin ciocalteau reagent and kept undisturbed for 10 min and then two ml of 20 per cent sodium carbonate solution was added and the absorbance was measured at 660 nm. The reagent blank was maintained with distilled water and the results are expressed in μg/g of sample.

3.6.6. Estimation of protein content (Ali-Khan and Young, 1973)

The powdered chilli seeds of 100 mg were taken in 50-ml polyethylene screw cap bottle with 25 ml of 1N NaOH. The mixture was placed in a shaker for 30 min to disperse the protein. Ten ml of this suspension was poured into a separate test tube and used as a blank. The remaining solution was added with 0.25 ml of 10 per cent $CuSO_4$ solution and kept overnight. After the prescribed period, the solution was centrifuged for 10 min. The absorbance was recorded in collected supernatant solution at 620 nm.

Protein percentage = 378 + 61.6 x OD value

3.6.7. Estimation of peroxidase (Hartee, 1955)

Seed samples were homogenized at 4°C in 10 mM sodium phosphate buffer (pH 7.0) using pre-chilled pestle and mortar. The homogenate was centrifuged at 15000 g for 10 min at 4°C and the supernatant was immediately used for enzyme assay. Peroxidase activities were assayed using guaiacol as a hydrogen donor.

One hundred ml of enzyme extract was added with 1.5 ml phosphate buffer (pH 7.0) and 1.5 ml of guaiacol. Before reading the absorbance, the hydrogen peroxidase (250 µl) was added and the changes in absorbent were recorded at 420 nm. Reference cuvette was performed simultaneously with 100 µl of distilled water. The activity was expressed as the change in absorbance at 420-nm min^{-1} g^{-1} from tissue.

3.6.8. Estimation of Polyphenol oxidase (Mayer *et al.*, 1965)

Two hundred ml of enzyme extract was used for estimation. 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) was added to the enzyme extract along with 200 µl of 0.1 M catechol. The activity was expressed as the change in absorbance at 495-nm min^{-1} g^{-1} from tissue.

3.6.9. Sodium dodecyl sulphate polyacrylamide gel electrophoresis analysis of seed protein (SDS-PAGE)

Chilli seeds were ground in a chilled pestle and mortar with extraction buffer (0.1-M phosphate buffer) pH 7.0 at 1:10 ratio. Crude extracts were obtained by centrifuging these at 15000 g at 4°C for 15 min and the extracts were stored at -20°C until further use.

Crude extracts equivalent to 100 µg of protein were precipitated using 40 per cent TCA (ice cold) for 45 min at 4°C. Then the pellet was dissolved in 2 x sample buffer (30 µl) containing 1.51 per cent Tris, 20 per cent glycerol, 4 per cent SDS, 10 per cent 2-mercaptoethanol and 0.002 per cent bromophenol blue prior to loading them on to polyacrylamide gel.

SDS-PAGE was performed by the method described by Laemmli (1970) using a separating gel of 15 per cent acrylamide with an overlay stacking gel of 4 per cent. Samples were heated at 90°C for 3 min and then cooled on ice. They were then loaded onto polyacrylamide gels. Gels were run using Sigma Aldrich dual mini gel apparatus at 20-milli amps constant current per gel and electrophoresis was carried out till the dye front had migrated to the bottom of the gel.

3.7. *In vitro* evaluation of plant products

3.7.1. Plant species tested for antifungal activity against *C. capsici*

Extracts from six plant species were collected for evaluating their antifungal activities. Among them, four plant species that were effective have been selected for testing their effectiveness under field condition.

3.7.2. Preparation of plant extracts

Fresh plant leaves were used for extraction (for garlic, cloves are selected). The selected parts were washed with sterile distilled water. They were ground with sterile distilled water at 1:1 (w/v) using pestle and mortar and filtered through cheesecloth. This formed the standard plant extract solution (100 per cent).

3.7.3. Screening of plant products/extracts

Sl.No.	Scientific name	Common name	Family
1	*Aegle marmelos* L. Corr	Bael	Rutaceae
2	*Allium sativum* L.	Garlic	Alliaceae
3	*Catharanthus roseus* (L.)	Periwinkle	Apocynaceae
4	*Eucalyptus* sp. Sieb	Eucalyptus	Myrtaceae
5	*Lawsonia inermis* (L)	Henna	Lythraceae
6	*Prosopis juliflora* (Sw.) DC	Seemaikaruvel/mesquite	Mimosaceae

3.7.4. *In vitro* evaluation of plant extracts on the radial growth of *C. capsici*

Twenty ml of PDA was poured into sterilized Petri plate and allowed for solidification. Four wells at four corners were made with sterilized (8-mm) cork borer and filled with two ml of plant extracts. Seven days old *C. capsici* culture disc was taken from periphery and placed in the middle of Petri plate and appropriate control was maintained. Three replications were maintained in each treatment. The plates were incubated at room temperature (25 ±1°C) and the diameter of the colonies were measured on 7^{th} day and expressed in cm.

3.8. *In vitro* evaluation of fungicides

3.8.1. Fungicides tested for antifungal activity against *C. capsici*

Three fungicides were selected and tested for their effectiveness against *C. capsici*. The fungicides tested were carbendazim, mancozeb and chlorothalonil.

3.8.2. *In vitro* evaluation of fungitoxic effect of fungicides on radial growth of *C. capsici* (Schmitz, 1930)

Potato dextrose agar medium was amended with 100, 200, 300 mg / 100 ml of fungicides (carbendazim, mancozeb and chlorothalonil) and poured separately in a Petri dish and allowed for solidification. The seven days old *C. capsici* fungal disc (8-mm) was placed in the middle of the Petri plates and appropriate control was maintained along with three replication. The plates were incubated at room temperature (25 ±1°C) and the diameter of colonies were recorded on 7^{th} day and expressed in cm. The per cent inhibition of growth was calculated by using the formula given below by Vincent (1927).

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

Where,

I = Inhibition

C = Rate of growth in control

T = Rate of growth in treatment

3.9. *In vitro* evaluation of biocontrol agents

3.9.1. Sources of antagonists

Native chilli rhizosphere strains were isolated from sample obtained from Ulagankathan and Kolathur of Tamil Nadu. One gram of rhizosphere soils were collected and transferred to a 200 ml conical flask containing 100 ml of sterile water. After thorough shaking, different dilutions were prepared and one ml each of 10^{-5} and 10^{-6} dilutions were poured into the sterile Petri dishes. Later, King's B medium (King *et al.*, 1954) was poured, rotated in clock and anticlock wise direction. The fluorescent colonies were observed after 24 h incubation. The bacterial strains were isolated and characterized (Stainer *et al.*, 1966). In addition to the isolated strains, the strain PF_1, Bacillus *subtilis* and *T.viride* strains obtained from TNAU were included.

3.9.2. Preparation of talc based formulation of Bacterial antagonist

The talc-based formulation of fluorescent pseudomonads was prepared by following the method (Vidhyasekaran and Muthamilan, 1995). A loopful of bacterium was inoculated into the KB broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (25 ±2°C). The bacterial population in the broth was 9 x 10^8 cfu/ml after 48 h growth. One kg of sterilized talc powder (105°C for 12 h) was taken in a metal tray and pH was adjusted to neutral by adding $CaCO_3$ (15 g/kg). Ten g of carboxy methyl cellulose (CMC) was added to one kg of talc powder before mixing. Two hundred ml of 48h grown inoculum was mixed with 1/2 kg of talc powder under aseptic conditions. After shade drying for over night, it was packed in polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was 2.5 to 3 x 10^8 cfu/g.

The talc-based formulation of *B. subtilis* was prepared as in 3.9.2. by using nutrient agar broth.

3.9.3. *In vitro* evaluation of fungitoxic effect of biocontrol agent on the radial growth of *C. capsici*

The strains of fluorescent pseudomonads and *B. subtilis* were tested for their antagonistic effect against *C. capsici*. Mycelial discs of 7 day old culture of *C. capsici* were placed on Petri plate containing PDA and were allowed to grow for two days. Then, the bacterial antagonist was streaked in such a way that equally quarters the plate and incubated for 5 days. Three replications were maintained along with control. The colony growth was measured on seventh day and the results were expressed in cm. The per cent inhibition was calculated as in 3.8.2.

3.9.4. In vitro evaluation of fungitoxic effect of *T. viride* (strains) on the radial growth of *C. capsici*

The strains of *T. viride* were tested for their antagonistic effect against C. capsici. Mycelial discs of 7 day old culture of *C. capsici* were placed on Petri plate containing PDA along with *T. viride* strains and incubated for 7 days. Three replications were maintained along with control. The colony growth was measured on seventh day and the results were expressed in cm. The per cent inhibition was calculated as in 3.8.2.

3.10. Spore germination studies

3.10.1. Effect of plant extracts on germination of *C. capsici* spores

The host leaf extract was placed on cavity slide and allowed to air dry. A drop of spore suspension($4x10^3$-conidia/cubic ml) was poured and covered with coverslip. Then, the slides were kept in moist chamber and incubated. The spore germination was observed from 6^{th} hour on-wards.

3.10.2. Effect of Bacterial antagonists on *C. capsici* spore germination

Culture filtrates of strains of fluorescent pseudomonads and *B. subtilis* were prepared and a drop of cell free culture filtrate was placed on cavity slide and allowed for air-drying. A drop of spore suspension (4×10^3 conidia / cubic ml) was dropped in

the cavity and covered with coverslip. Three replications were maintained along with control. The germination of conidia was viewed through compound microscope from 6^{th} hr onwards.

3.10.3. Effect of fungicides on germination of *C. capsici* spores

The different concentration of fungicides was prepared and spore germination study was conducted as in 3.10.2.

3.11.1. Field experiment

Two-field experiments were conducted simultaneously at two locations (one at Puduvelamangalam near Kolathur, Salem district and another at Masthigoundanpathi near Madukkarai, Coimbatore District during June 1999.

The experiment was conducted in randomized block design with a plot size of 4 x 2.5 m and 5 x 2 m in Puduvelamangalam and Masthigoundanpathi respectively. Three replication were maintained with control (Plain water).

3.11.2. Effect of biocontrol agent, botanicals and fungicides under field condition

Field experiment was conducted with eleven treatments and a control (plain water). The 4 per cent commercial formulation of biocontrol agent, viz., PF1, PFk, PFu and *B. subtilis*, 10 per cent plant extract viz., eucalyptus, henna, periwinkle and seemai-karuvel and the fungicides carbendazim (0.1 per cent), mancozeb (0.2 per cent) and chlorothalonil (0.2 per cent) were sprayed at 115, 130 and 145 days after transplanting. The fruit rot incidence was observed on 130 and 160 DAT. The per cent disease index was calculated by following the formula given by Bansal and Grover (1969).

Sl.No.	Category value	Grade
1	No disease	0
2	1-5 per cent fruit infected	1
3	6-25 per cent fruit infected	2
4	26-50 per cent fruit infected	3
5	51-100 per cent fruit infected	4

$$PDI = \frac{\text{Sum of individual rating}}{\text{Number of fruits assessed}} \times \frac{100}{\text{Maximum disease category}}$$

3.12. Statistical analysis

The data pertaining to the observations in the laboratory were analyzed in completely randomized design (Gomez and Gomez, 1984) and the field data were analyzed in Randomized block design. The significant effects of various treatments were compared using Duncan's Multiple Range Test (DMRT) and LSD.

Experimental Results

Plate 3.

SURVEY FOR COLLETOTRICHUM CAPSICI INFECTED SEEDS OF TAMIL NADU

HEALTHY SEED LOT

INFECTED SEED LOT

CHAPTER IV

EXPERIMENTAL RESULTS

4.1. Survey for the incidence of chilli anthracnose in Tamil Nadu

Survey was conducted to find out the status of chilli fruit rot caused by *Colletotrichum capsici* in ten chilli growing regions of Tamil Nadu and the per cent disease was recorded and presented (Table 1, Fig. 1) The intensity of fruit rot incidence varied between the regions and it ranged from 21.60 to 45.25 per cent. The higher incidence was recorded in Indili, (Villupuram District) and the lower incidence was in Attur (Salem District), where as the incidence of fruit rot in Coimbatore, Kolathur, Ulagankathan and Periyakulam were 28.9, 28.6, 29.1 and 29.5 per cent, respectively.

4.2. Survey for the occurrence of seed-borne *C. capsici* in chilli seeds of Tamil Nadu

Seed samples were collected from thirty different places of Tamil Nadu and used for the detection of *C. capsici* by blotter method. The per cent *C. capsici* seed infection was recorded and presented (Table 2, Fig. 1). It revealed that fifty per cent of the seed samples were found to be infected with *C. capsici*. Per cent seed infection was found to vary from 0.00-33.33 per cent. The higher (33.33 per cent) seed infection was recorded in local variety obtained from Indili (Villupuram District) and showed significant differences among the location. However, the seed sample collected from Kallakurichi (16.00 per cent), Kolathur (16.00 per cent) and Puduvelamangalam (13.33 per cent) were statistically on par with each other (Plate 3).

4.3. Standardization of different seed health methods for *C. capsici*

Seed health test was performed with seed samples showing different intensity of seed infection by *C. capsici* and the results were presented (Table 3, Fig. 2). In general irrespective of *C. capsici* seed infection, the blotters soaked in three per cent $CaCl_2$

Table 1. Survey for the incidence of chilli anthracnose in Tamil Nadu

Sl.No	Location	Per cent disease index *
1	Ariyalur	26.5
2	Attur	21.6
3	Coimbatore	28.5
4	Indili	45.3
5	Kolathur	28.6
6	Madukkarai	23.4
7	Palladam	31.3
8	Periyakulam	29.5
9	Tenkasi	22.6
10	Ulagankathan	29.1

* Mean of eight replications

Table 2. Survey for the occurrence of seed-borne *C.capsici* in chilli seeds of Tamil Nadu

Sl.No	Seed sample	Place of collection	*Occurrence of *C.capsici*(%)
1	Local	Ariyalur	0.00^{c} (4.05)
2	K2	Aruppukkottai	4.00^{c}(10.67)
3	K1	Bhavanisagar	0.00^{c} (4.05)
4	Local	Chinnasalem	1.33^{c} (6.55)
5	Co1	Coimbatore	1.33^{c} (6.55)
6	K2	Coimbatore	0.00^{c} (4.05)
7	Local	Coimbatore	1.33^{c} (6.55)
8	Pkm1	Coimbatore	0.00^{c} (4.05)
9	Local	Cuddalur	0.00^{c} (4.05)
10	Local	Cumbum	0.00^{c} (4.05)
11	Local	Indili	33.33^{a}(35.10)
12	Guntur Local	Kallakurichi	16.00^{b}(23.47)
13	Local	Karanur	0.00^{c} (4.05)
14	Local	Kolathur	13.33^{b}(21.09)
15	K1	Kovilpatti	2.67^{c} (9.04)
16	Local	Kudikadu	2.67^{c} (9.04)
17	K1	Madukkarai	2.67^{c} (9.04)
18	K1	Palladam	1.33^{c} (6.55)
19	Ceylon	Pallapatti	0.00^{c} (4.05)
20	Pkm1	Periyakulam	0.00^{c} (4.05)
21	Local	Pudukottai	0.00^{c} (4.05)
22	Local	Puduvelamangalam	16.00^{b}(23.47)
23	T1 Local	Tenkasi	0.00^{c} (4.05)
24	T2 Local	Tenkasi	0.00^{c} (4.05)
25	T3 Local	Tenkasi	2.67^{c} (9.04)
26	Local	Thirukanur	0.00^{c} (4.05)
27	Local	Thottium	0.00^{c} (4.05)
28	Pkm1	Vadugapatti	2.67^{c} (9.04)
29	Local	Vellur	1.33^{c} (6.55)
30	K2	Virudhunagar	0.00^{c} (4.05)
Mean			3.42 (8.42)

CD (P=0.05)=6.84

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

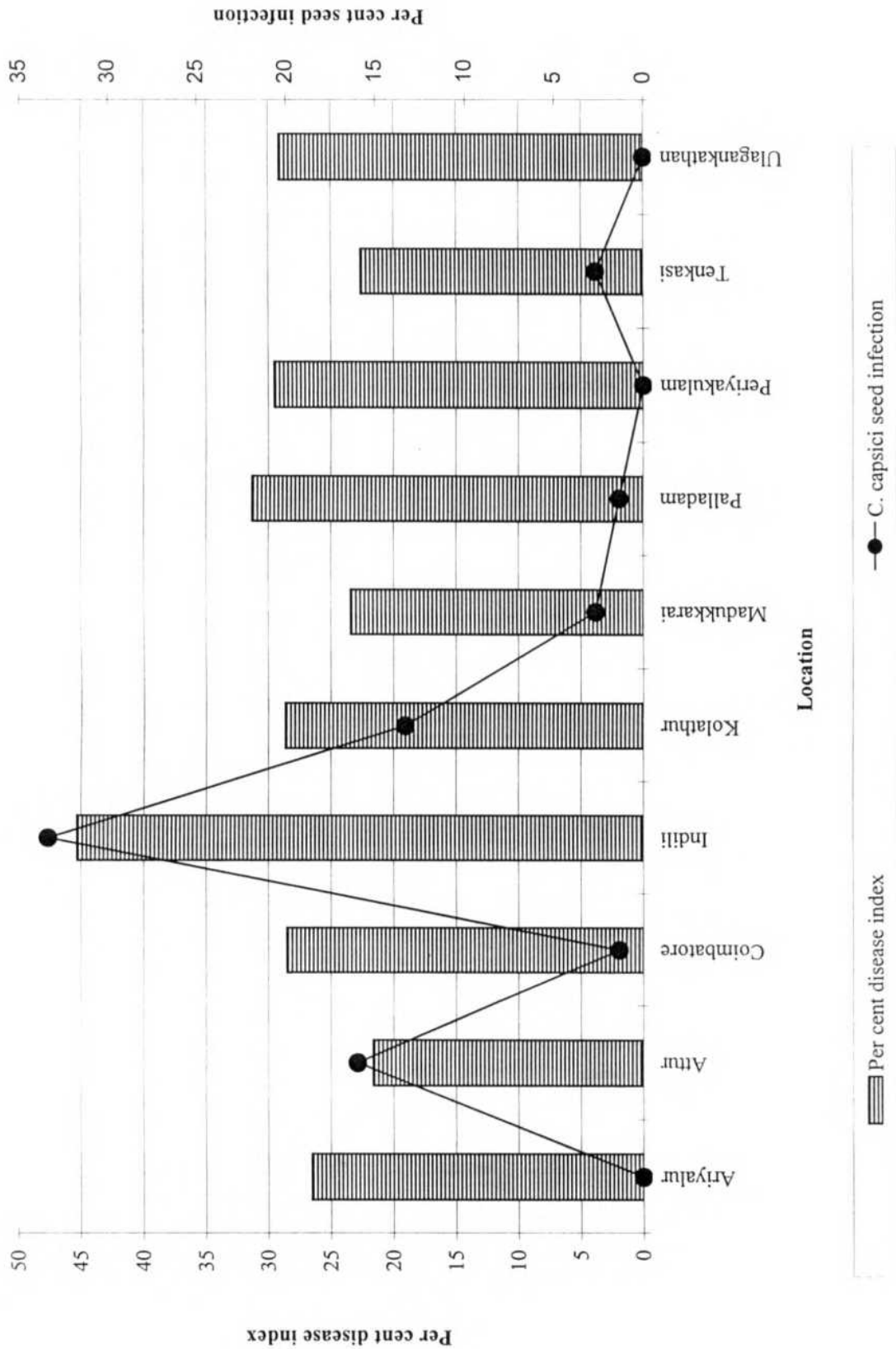


Fig.1. Survey for the *C. capsici* seed infection and incidence of chilli anthracnose in Tamil Nadu.

Table 3. Standardization of different seed health methods for *Colletotrichum capsici*

Sl.No	Seed samples	Place of collection	Standard blotter technique*	Deep freezing*	2,4-d*	Calcium chloride		
						2%*	3%*	5%*
1	Local	Kallakurichi	18.00^{b} (25.07)	22.00^{b} (27.76)	32.00^{b} (34.41)	28.00^{b} (31.89)	22.00^{bc} (27.76)	14.00^{bc} (21.92)
2	K1	Kolathur	10.00^{bc} (18.35)	4.00^{de} (10.24)	2.00^{cd} (7.80)	2.00^{de} (7.80)	10.00^{cde} (18.34)	14.00^{bc} (21.92)
3	Local	Puduvelamangalam	18.00^{ab} (25.07)	14.00^{bc} (21.92)	22.00^{b} (27.76)	38.00^{b} (38.05)	30.00^{b} (33.20)	22.00^{b} (27.95)
4	Local	Indili	32.00^{a} (34.28)	60.00^{a} (50.85)	70.00^{a} (56.80)	90.00^{a} (71.65)	94.00^{a} (76.02)	88.00a (70.00)
5	T3	Tenkasi	2.00^{d} (7.80)	2.00^{de} (7.80)	2.00^{cd} (7.80)	2.00^{de} (7.80)	8.00^{de} (15.90)	6.00^{cd} (13.98)
6	K1	Madukkarai	4.00^{cd} (11.54)	8.00^{cd} (16.43)	8.00^{c} (15.90)	0.00^{e} (4.05)	10.00^{cde} (17.56)	2.00^{d} (7.80)
7	K2	Aruppukottai	0.00^{d} (4.05)	2.00^{de} (7.80)	0.00^{d} (4.05)	6.00^{cd} (13.98)	12.00^{cde} (20.27)	4.00^{d} (10.24)
8	Pkm1	Periyakulam	2.00^{d} (7.80)	0.00^{e} (4.05)	8.00^{c} (15.90)	10.00^{c} (18.34)	18.00^{bcd} (25.07)	6.00^{cd} (13.98)
9	Pkm1	Vadugapatti	2.00^{d} (7.80)	2.00^{de} (7.80)	4.00^{cd} (10.24)	8.00^{cd} (15.90)	6.00^{e} (13.98)	0.00^{d} (4.05)
Mean			9.78 (15.75)	12.67 (17.18)	16.44 (20.07)	20.44 (23.27)	23.33 (27.57)	17.33 (21.32)

CD (P=0.05) = 9.22
Mean of two replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

Fig.2. Standardization of different seed health methods for *C. capsici.*

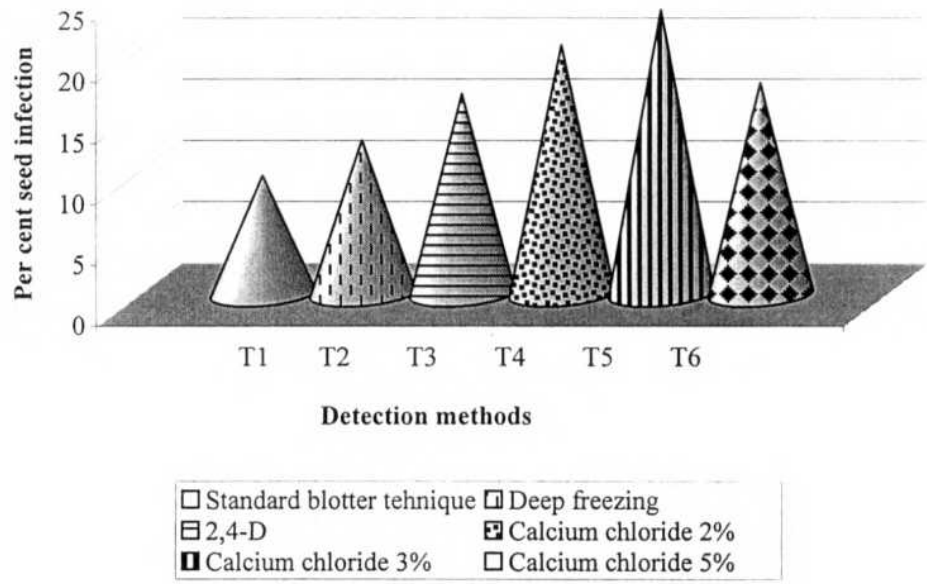


Fig.3. Effect of different light periods on of *C. capsici* infection count in chilli seeds.

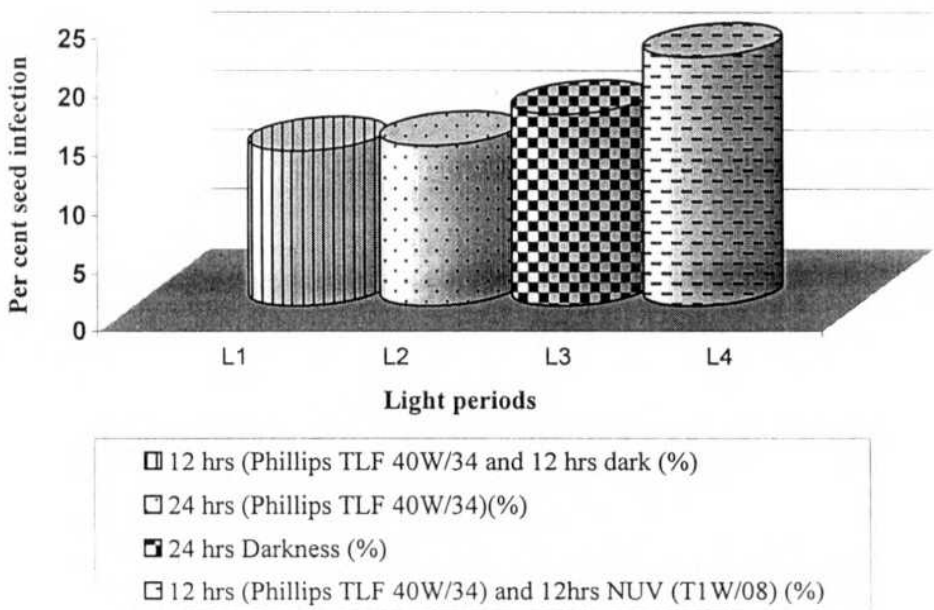


Plate 4.

recorded maximum seed infection when compared to other methods tried. The higher infection count of 94 per cent was recorded in local variety under three per cent $CaCl_2$ soaked blotter methods as against the standard blotter methods (Plate 4).

4.4. Effect of different light periods on *C. capsici* infection count in chilli seeds

The influence of different light sources and period of exposure on *C. capsici* infection count was recorded and depicted (Table 4, Fig. 3) The light period influenced the *C. capsici* infection count and was statistically different among the treatments and exposure period. The higher infection count of 70.00 per cent was recorded when plates were kept under 12 h (Phillips TLF 40W/34) + 12 h NUV (T_1 40W/08) in seed samples collected from Indili, Villupuram District. Which did Kallakurichi (42.00 per cent) and Puduvelamangalam (26.00 per cent) follow. Among the four light sources, the 12 h light, 12 h NUV showed higher seed infection count (21.33 per cent). However, the treatments were statistically on par with each other.

4.5. Effect of different pH level on *C. capsici* seed infection count on chilli seeds

The effect of six different pH levels (5.0, 6.0, 6.5, 7.0, 7.5 and 8.0) on *C. capsici* seed infection count was studied and presented (Table 5, Fig. 4). The per cent seed infection varied with pH levels of blotters. The variety, Indili (Local) showed higher (94.00 per cent) seed infection at pH 7.5 and it was followed by 82.00, 74.00, 88.00, 52.00 and 90.00 per cent at pH 5.0, 6.0, 6.5, 7.0 and 8.0, respectively. Among different pH levels tried, the pH 7.5 showed higher seed infection count (23.11 per cent) when compared to other levels tried. Differences in all the level were observed. However, they were statistically on par with each other. The lower incidence was recorded at neutral pH.

Table 4. Effect of different light periods on of *Colletotrichum capsici* infection count in chilli seeds (%)

Sl.No	Seed samples	Places of collection	Light periods			
			12 h (Philips TLF 40W/34)and 12 h dark*	24 h(Philips TLF 40W/34) *	24 h dark*	12h(Philips TLF 40W/34) and 12 h NUV(T_1 40W/08)*
1	Local	Kallakurichi	34.00a (35.66)	38.00b (38.05)	42.00b (40.39)	42.00b (40.36)
2	K1	Kolathur	10.00bc (18.35)	0.00d (4.05)	0.00e (4.05)	10.00d (18.35)
3	Local	Puduvelamangalam	14.00b (21.92)	10.00c (18.35)	14.00c (21.92)	26.00c (30.64)
4	Local	Indili	38.00a (38.50)	60.00a (50.79)	52.00a (46.15)	70.00a (56.80)
5	T3	Tenkasi	6.00bc (13.98)	2.00d (7.80)	14.00c (21.92)	10.00d (18.35)
6	K1	Madukkarai	10.00bc (18.35)	6.00c (13.98)	6.00d (13.98)	12.00d (20.00)
7	K2	Aruppukottai	0.00d (4.05)	0.00d (4.05)	10.00cd (18.35)	14.00d (21.92)
8	Pkm1	Periyakulam	6.00c (13.98)	8.00c (16.43)	10.00cd (18.35)	8.00d (15.90)
9	PKM1	Vadugapatti	0.00d (4.05)	0.00d (4.05)	0.00e (4.05)	1.00e (4.05)
Mean			13.11 (18.71)	13.78 (17.51)	16.44 (21.02)	21.33 (25.15)

CD (P=0.05)=5.62

*Mean of two replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

Table 5. Effect of different pH levels on *Colletotrichum capsici seed* infection in chilli seeds

Sl.No	Seed sample	Places of collection	pH levels*					
			5.0	6.0	6.5	7.0	7.5	8.0
1	Local	Kallakurichi	26.00^{b} (29.99)	26.00^{bc} (30.64)	30.00^{b} (33.20)	18.00^{b} (25.07)	38.00^{b} (38.05)	14.00^{c} (21.92)
2	K1	Kolathur	18.00^{b} (25.07)	18.00^{c} (25.07)	14.00^{c} (21.92)	10.00^{b} (18.35)	12.00^{c} (20.00)	8.00^{c} (15.90)
3	Local	Puduvelamangalam	22.00^{b} (27.95)	34.00^{b} (35.66)	42.00^{b} (40.39)	18.00^{b} (25.07)	40.00^{b} (39.15)	34.00^{b} (35.66)
4	Local	Indili	82.00^{a} (64.93)	74.00^{a} (59.36)	88.00^{a} (69.73)	52.00^{b} (46.18)	94.00^{a} (76.11)	90.00^{a} (71.65)
5	T3	Tenkasi	4.00^{c} (10.24)	0.00^{e} (4.05)	10.00^{c} (18.35)	2.00^{c} (7.80)	8.00^{c} (15.90)	0.00^{d} (4.05)
6	K1	Madukkarai	4.00^{c} (10.24)	2.00^{de} (7.80)	10.00^{c} (18.35)	2.00^{c} (7.80)	10.00^{c} (18.35)	2.00^{d} (7.80)
7	K2	Aruppukottai	0.00^{c} (4.05)	6.00^{d} (13.98)	2.00^{d} (7.80)	4.00^{c} (10.24)	6.00^{c} (12.16)	2.00^{d} (7.80)
8	Pkm1	Periyakulam	0.00^{c} (4.05)	0.00^{e} (4.05)	0.00^{d} (4.05)	0.00^{c} (4.05)	0.00^{d} (4.05)	0.00^{d} (4.05)
9	Pkm1	Vadugapatti	0.00^{c} (4.05)	0.00^{e} (4.05)	0.00^{d} (4.05)	0.00^{c} (4.05)	0.00^{d} (4.05)	0.00^{d} (4.05)
Mean			17.22 (20.21)	17.78 (20.52)	21.78 (24.20)	11.78 (16.51)	23.11 (25.30)	16.67 (19.21)

CD (P=0.05)=7.79

*Mean of two replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

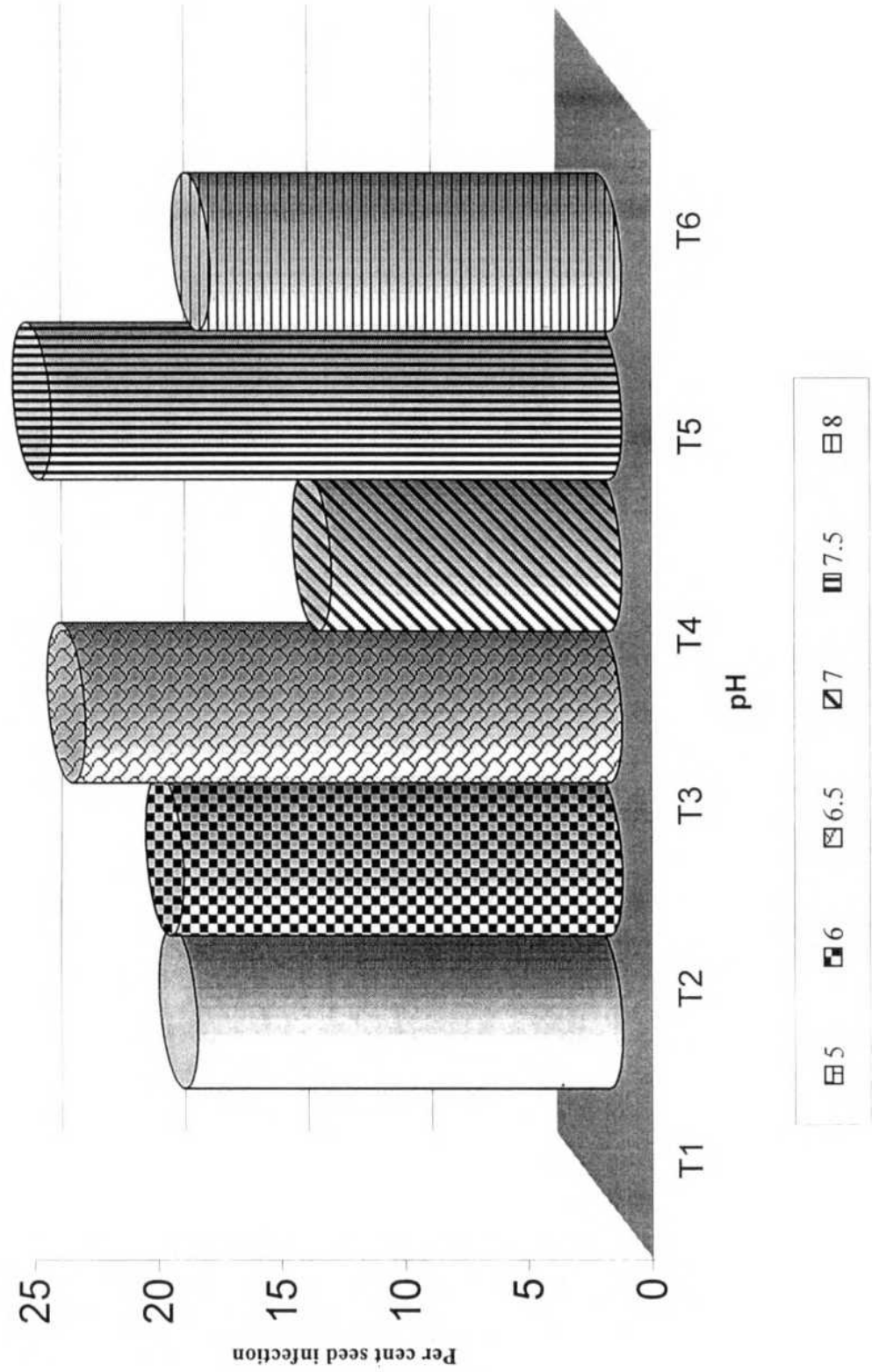


Fig.4.Effect of different pH level of *C.capsici* seed infection in chilli seeds.

Plate 5.

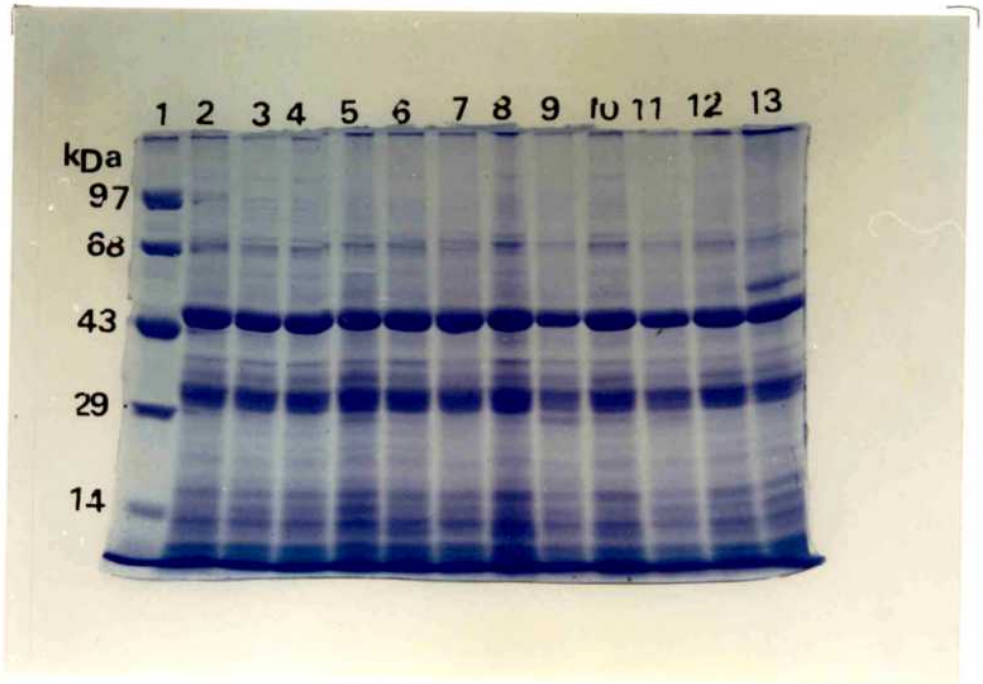


Plate 5a.

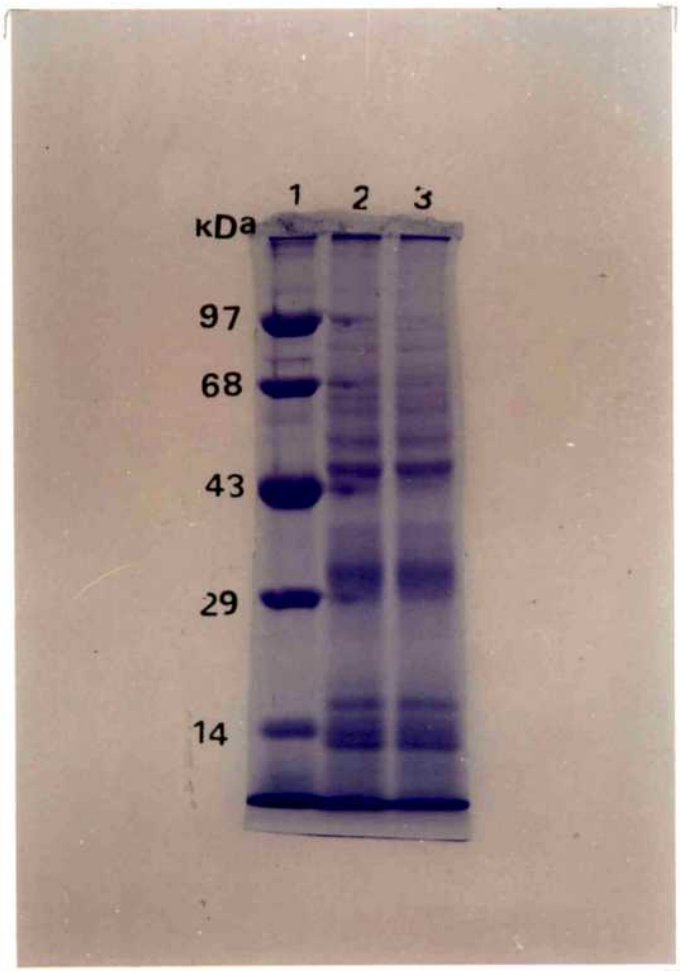


Plate 5b.

4.6.SDS-PAGE analysis for healthy and infected chilli seeds

SDS-PAGE analysis for crude extract of six varieties with healthy and infected seeds(*C. capsici*) showed variation in protein pattern with a molecular weight of 29 kDa disappeared in infected seeds of K1, Co 1and Sln 4-8 (Plate5). A new protein with a molecular weight of 50 kDa was induced as the result of *C. capsici* infection in UL (L).

4.7. Morphological features of two isolates of *C. capsici*

The morphology and cultural characters of two isolates of *C. capsici* were studied and presented (Table 6, Fig. 5). On 9th day after incubation, the isolate 1 (I_1) has grown 8.5 cm diameter where as it was 9.0 cm in diameter in Isolates 2 (I_2). The isolate I_2 started producing acervuli on 3rd day on wards where as it was on 4th day in I_1. The conidial and setae size was also found to be varied between the isolates. The conidial size of I_1 was 28.34 x 4.8 µ and was 28.34 x 4.8 µ in I_2 on 9th DAI. The setae size also showed similar result and was 102.78 x 4.8 µ in I_1 and 102.26 x 4.8 µ in I_2 on 9th DAI (Plate 6).

4.8. Morphological features of *C. capsici* isolated from chilli seeds

The conidia and setae measurement revealed the distinguishable differences in length and breadth (Table 7, Fig .5). The conidial size was found to vary from 20.48 x 4.8 µ - 30.78 x 4.8 µ and they were significantly different. Similar variation was also observed in setae size which ranged from 104.64 x 4.8 - 267.68 x 4.8 µ. The higher conidia size of 30.72 x 4.8 µ and setae size of 267.74 x 4.8 µ was recorded in the seed samples of Vadugapatti and Kallakurichi, respectively.

Plate 6.

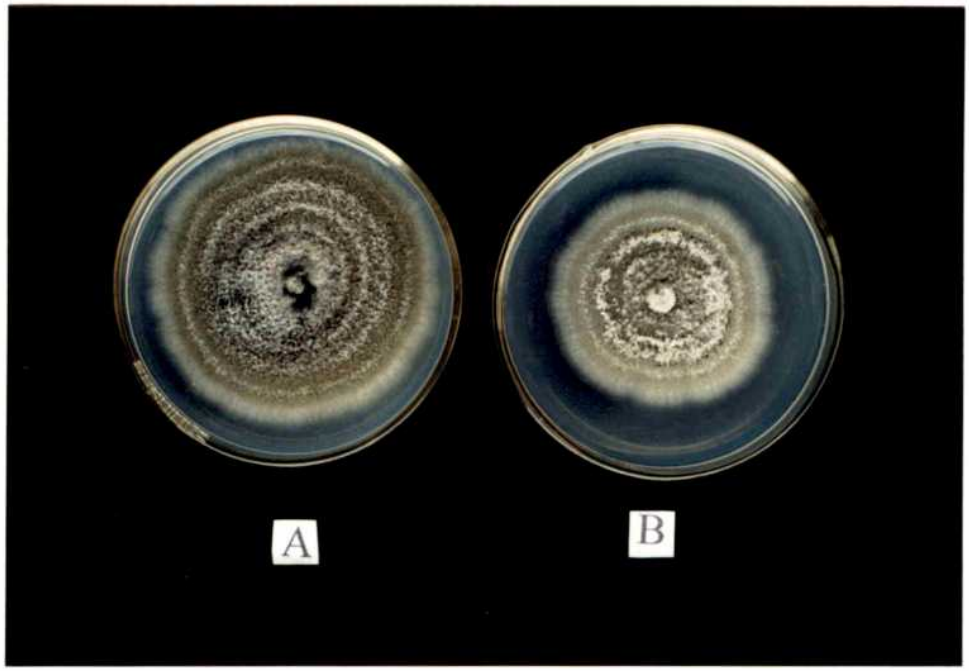


Plate7.

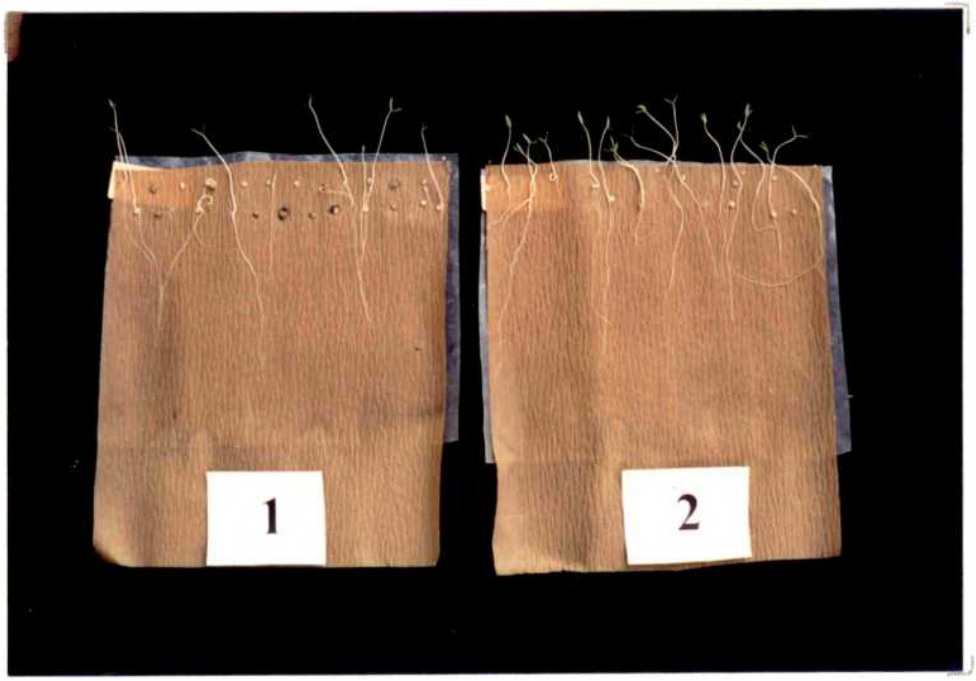


Table 6. Morphological features of two isolates of *Colletotrichum capsici*

Days after incupation	Colony growth(cm)		Conidia(μ)*				Setae(μ)*			
	Isolate1 (I_1)	Isolate2 (I_2)	Isolate1(I_1)		Isolate2(I_2)		Isolate1(I_1)		Isolate2(I_2)	
			length	breadth	length	breadth	length	breadth	length	breadth
1	2.0	1.5	–	–	–	–	–	–	–	–
3	3.0	4.0	–	–	–	–	–	–	–	–
5	6.0	7.0	28.22	4.8	27.25	4.8	100.32	4.8	100.75	4.8
7	7.5	9.0	28.32	4.8	28.27	4.8	100.81	4.8	102.24	4.8
9	8.5	9.0	28.34	4.8	28.27	4.8	102.72	4.8	102.26	4.8

*Mean of fifteen observations

Table 7. Morphological features of *Colletotrichum capsici* isolated from chilli seeds.

Sl.No	Seed sample	Places of collection	Conidia(μ)*		Setae(μ)*	
			Length	Breadth	Length	Breadth
1	K1	Kovilpatti	28.80^{bc}	4.8^{a}	244.80^{c}	4.8^{a}
2	Local	Vellur	29.70^{ab}	4.8^{a}	104.64^{k}	4.8^{a}
3	Co1	Coimbatore	26.50^{efg}	4.8^{a}	248.32^{b}	4.8^{a}
4	K2	Aruppukottai	25.60^{g}	4.8^{a}	247.68^{b}	4.8^{a}
5	Pkm1	Coimbatore	26.24^{fg}	4.8^{a}	131.84^{h}	4.8^{a}
6	Pkm1	Vadugapatti	30.72^{a}	4.8^{a}	241.90^{d}	4.8^{a}
7	Pkm1	Periyakulam	20.48^{h}	4.8^{a}	113.28^{ej}	4.8^{a}
8	Local	Puduvelamangalam	26.88^{dg}	4.8^{a}	215.68^{e}	4.8^{a}
9	Local	Kallakurichi	27.84^{cf}	4.8^{a}	267.74^{a}	4.8^{a}
10	T3	Tenkasi	26.88^{dg}	4.8^{a}	116.16^{i}	4.8^{a}
11	Local	Cuddalore	29.76^{ab}	4.8^{a}	145.60^{g}	4.8^{a}
12	Local	Indili	28.80^{bc}	4.8^{a}	130.88^{h}	4.8^{a}
13	K1	Palladam	28.48^{bcd}	4.8^{a}	215.36^{e}	4.8^{a}
14	Local	Pudukottai	28.16^{be}	4.8^{a}	179.52^{f}	4.8^{a}
Mean			27.49	4.80	185.92	4.80

CD (P=0.05)=1.618
*Mean of fifteen observations

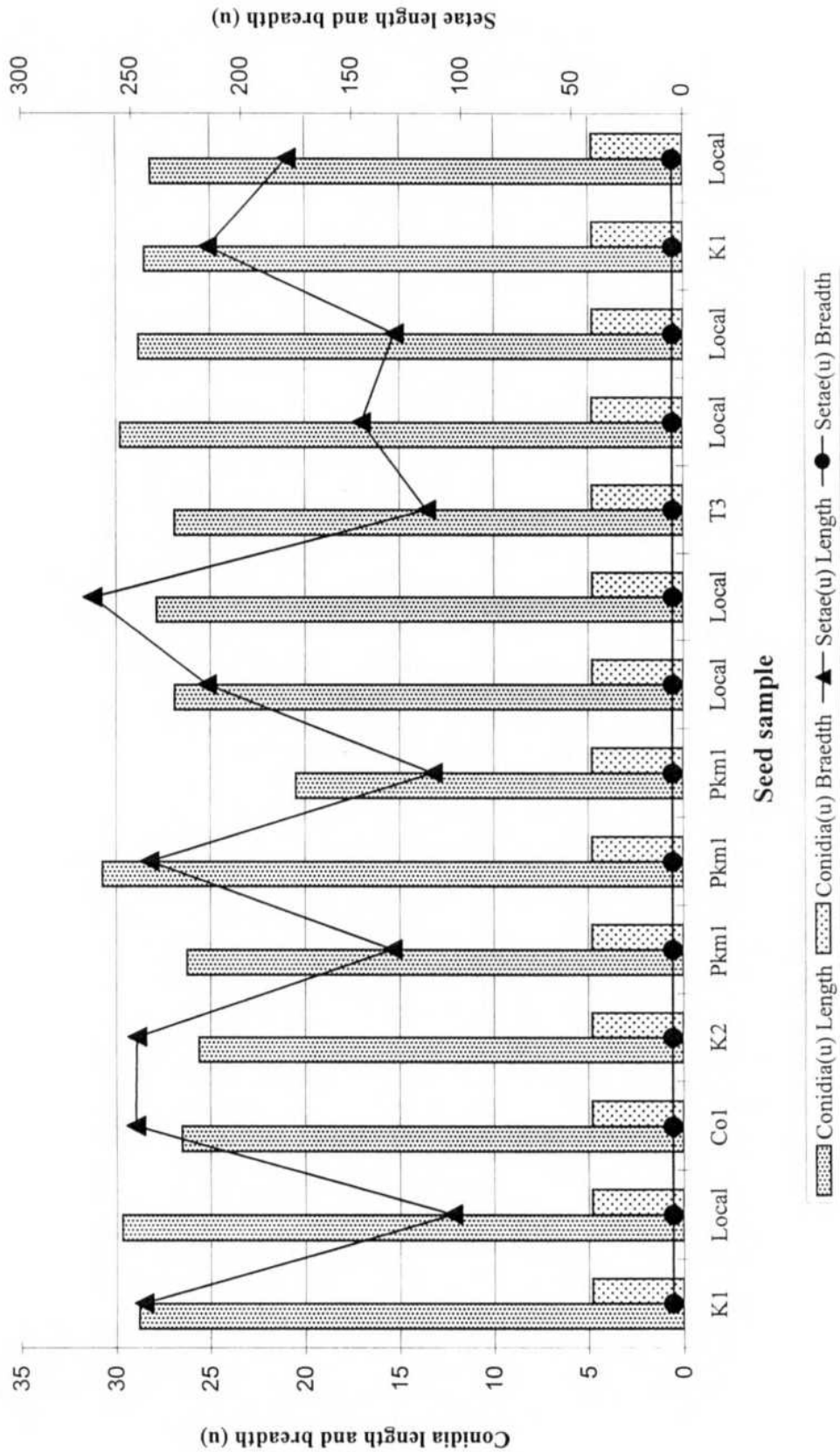


Fig.5. Morphological features of seed-borne *C. capsici* isolated from chilli seeds.

Table 8. Effect of *Colletotrichum capsici* seed infection on chilli seed germination

Sl.No	Seed sample	Places of collection	Germination (%)*	Dry matter production*	Vigour index*	Per cent seed infection
1	Local	Kallakurichi	6.00^{e} (13.98)	0.002^{a}	187.20^{c}	18.0
2	K1	Kolathur	20.00^{c} (26.56)	0.010^{a}	217.80^{c}	10.0
3	Local	Puduvelamangalam	40.00^{b} (39.21)	0.006^{a}	453.08^{b}	18.0
4	Local	Indili	6.00^{e} (13.98)	0.002^{a}	70.00^{c}	32.0
5	T3	Tenkasi	48.00^{a} (43.85)	0.008^{a}	713.32^{a}	2.0
6	K1	Madukkarai	46.00^{a} (42.69)	0.010^{a}	605.60^{a}	4.0
7	K2	Aruppukottai	38.00^{b} (38.05)	0.018^{a}	472.10^{b}	0.0
8	Pkm1	Periyakulam	10.00^{d} (18.35)	0.004^{a}	110.60^{c}	2.0
9	Pkm1	Vadugapatti	14.00^{c} (21.92)	0.008^{a}	139.19^{c}	2.0
Mean			25.33 (28.73)	0.0123	329.87	
		CD (P=0.05)	6.95	0.0487	195.77	

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

4.9. Effect of *C. capsici* seed infection on chilli seed germination

Effect of *C. capsici* seed infection on seed germination and seedling vigour was studied (Table 8, Fig. 6). The variation in vigour index was observed which ranged from 70.00 to 713.32. The lower vigour index and poor germination was recorded in local variety collected from Indili (Villupuram district). The highest germination percentage of 48 was recorded with highest vigour index in the samples collected from Tenkàsi. *C. capsici* seed infection had also influenced the dry matter production (DMP) and it ranged from 0.002-0.018. However, the dry matter production was not significantly different and was on par with others (Plate 7).

4.10. Effect of *C. capsici* infection on fruit and seed weight

C. capsici infected fruits were collected randomly from different locations and were used for assessing the effect of fruit rot infection on fruit weight, 100 seed weight and resultant seed infection and the results were presented (Table 9, Fig. 7). The results indicated that the fruit weight for infected chilli was significantly different and it decreased by 31.87 per cent over healthy fruit in K2. Similar effect was recorded in all the five varieties with different seed infection. The 100 seed weight was also found to be varied between healthy and infected seeds and the significant reduction was recorded in infected seeds. The per cent decrease in seed weight ranged from 5.26 to 35.39 per cent. The maximum reduction was recorded in K1 variety, which has 20.00 per cent *C. capsici* seed infection.

4.11. Effect of *C. capsici* seed infection on peroxidase and polyphenol oxidase activity

Peroxidase and polyphenol oxidase activities were studied in six different varieties with varying degree of seed infection (Table 10, Fig. 8). The peroxidase activity was found increased in infected seeds. The increased activity of 48.37 per cent was

Table 9.Effect of *Colletotrichum capsici* infection on fruit and seed weight

Sl.No	Seed sample	Places of collection	Per cent seed infection	Fruit wt(g)*		Per cent decrease over healthy	100 seed wt(g)*		Per cent decrease over healthy
				Healthy	Infected		Healthy	Infected	
1	K1	Madukkarai	20.0	5.08^{d}	4.15^{d}	18.87	0.50^{a}	0.33^{a}	35.39
2	K2	Aruppukottai	88.0	5.13^{d}	3.49^{d}	31.87	0.43^{a}	0.30^{a}	29.37
3	Co1	Coimbatore	6.0	8.30^{c}	5.98^{c}	27.98	0.55^{a}	0.44^{a}	19.45
4	Co3	Puduvelamangalam	50.0	7.44^{c}	6.26^{c}	15.81	0.48^{a}	0.36^{a}	25.10
5	Sln4-8	Coimbatore	12.0	10.36^{b}	7.26^{b}	29.92	0.38^{a}	0.36^{a}	5.26
6	UL(l)	Ulagankathan	74.0	12.37^{a}	9.87^{a}	20.20	0.66^{a}	0.50^{a}	24.17
Mean				8.15	6.17		0.50	0.38	

CD (P=0.05)=0.966

*Mean of two replications

In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

Fig.6. Effect of *C. capsici* seed infection on chilli seed germination and vigour.

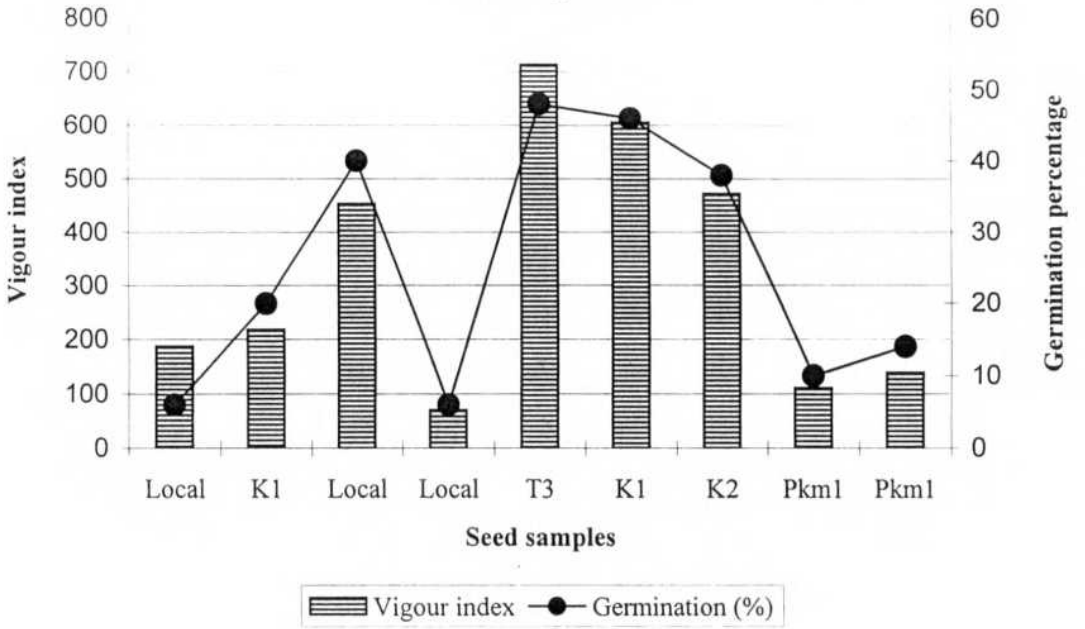


Fig.7. Effect of *C. capsici* infection on fruit and seed weight.

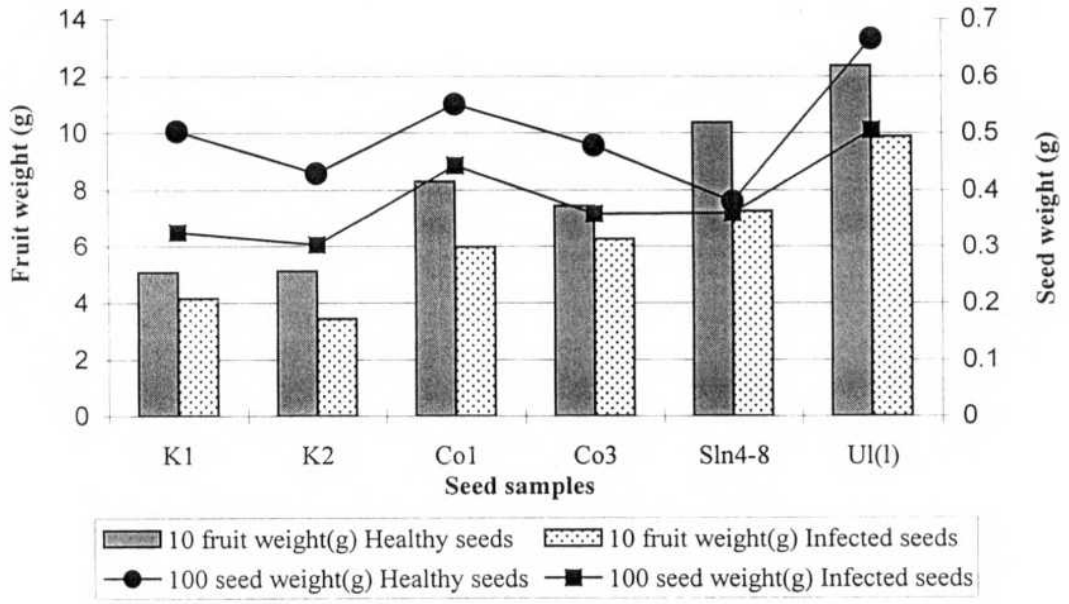


Table 10. Effect of *Colletotrichum capsici* seed infection on peroxidase and polyphenol oxidase activity in chilli seeds

Sl.No	Seed samples	Places of collection	Per cent seed infection	Peroxidase*		% increase over healthy	Polyphenol oxidase*		% increase Over healthy
				H	I		H	I	
1	K1	Madukkarai	20.0	0.42^{d}	0.56^{b}	25.13	0.011^{a}	0.041^{b}	73.17
2	K2	Aruppukottai	88.0	0.19^{de}	0.22^{d}	12.33	0.016^{a}	0.141^{a}	88.65
3	Co1	Coimbatore	6.0	0.29^{c}	0.38^{c}	24.54	0.034^{a}	0.079^{ab}	56.96
4	Co3	Puduvelamangalam	50.0	0.54^{a}	1.04^{a}	48.37	0.025^{a}	0.100^{ab}	75.00
5	Sln4-8	Coimbatore	12.0	0.16^{cd}	0.39^{c}	35.00	0.017^{a}	0.030^{ab}	42.28
6	UL(l)	Ulagankathan	74.0	0.23^{e}	0.41^{c}	43.20	0.013^{a}	0.043^{b}	69.76

CD (P=0.05)=0.075
*Mean of three replications
H - Healthy seeds
I -Infected seeds
In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

recorded in 50 per cent infected seed lot. The polyphenol oxidase activities were also recorded and were found increased in infected seeds and it ranged from 42.28 to 88.65 per cent. The higher activities of 0.016 were recorded in 88.00 per cent infected seed lots. The lower activities of 0.07 were recorded in 12 per cent infected seed lot. The increase in both peroxidase and polyphenol oxidase was directly proportionate to the percentage of seed infection.

4.12. Effect of *C. capsici* seed infection on protein content of chilli seeds

Protein content of both healthy and infected seeds of chillies (six varieties) were calculated and presented (Table 11, Fig. 9). The protein content of infected seeds was found to be decreased in the entire seed sample tested. The protein content of 88.00 per cent *C. capsici* infected K2 chilli seed was decreased to an extent of 66.21 per cent over healthy seeds, whereas variety Co1 (6.0 per cent seed infection) showed 20.11 per cent decrease in protein over healthy seeds. The results showed significant differences between the seed lots and indicated that the reduction in protein content was proportionate to the seed infection.

4.13. Effect of *C. capsici* seed infection on total phenol content of chilli seeds

Total phenol content of both healthy and infected chilli seeds were computed with *C. capsici* infected seeds and presented in (Table12, Fig.10). The total phenol content of infected seeds was found increased irrespective of the variety and seed infection level. The total phenol content in K1 and Sln 4-8 was statistically on par with each other. Similar was the case in Co 3 and UL (L).

The total phenol content of K2 variety infected with 88 per cent *C. capsici* seed infection was found to be 370.0 µg/g with an increased level of 48.64 per cent over healthy where as in Co 1 (6 per cent seed infection), it was 13.79 per cent over healthy.

Table 11. Effect of *Colletotrichum capsici* seed infection on protein content of chilli seeds

Sl.No	Seed sample	Places of collection	Per cent seed infection*	Protein content (%)		Per cent decrease over healthy
				H*	I*	
1	K1	Madukkarai	20.0	13.36^{b} (21.44)	6.83^{a} (15.15)	48.88
2	K2	Aruppukottai	88.0	15.48^{a} (23.16)	5.23^{b} (13.21)	66.21
3	Co1	Coimbatore	6.0	5.62^{d} (13.72)	4.49^{bc} (12.23)	20.11
4	Co3	Puduvelamangalam	50.0	9.42^{c} (17.87)	5.07^{bc} (13.02)	46.18
5	Sln4-8	Coimbatore	12.0	4.76^{d} (12.60)	4.12^{c} (11.70)	13.65
6	UL(l)	Ulagankathan	74.0	12.55^{b} (20.74)	8.04^{a} (16.47)	35.93
Mean				10.20 (18.25)	5.63 (13.63)	

CD (P=0.05)=1.33
*Mean of two replications
H-Healthy seeds
I-Infected seeds
Values in parentheses are arcsine transformed
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Fig.8. Effect of *C. capsici* seed infection on peroxidase and polyphenol oxidase activity.

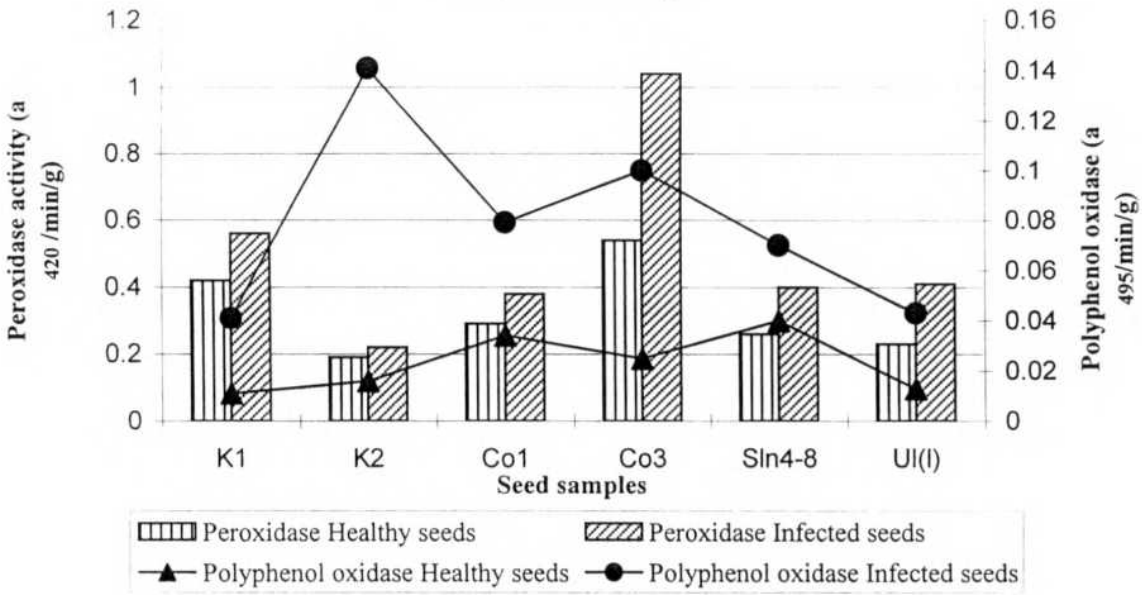


Fig.9. Effect of *C. capsici* seed infection on protein content of chilli seeds.

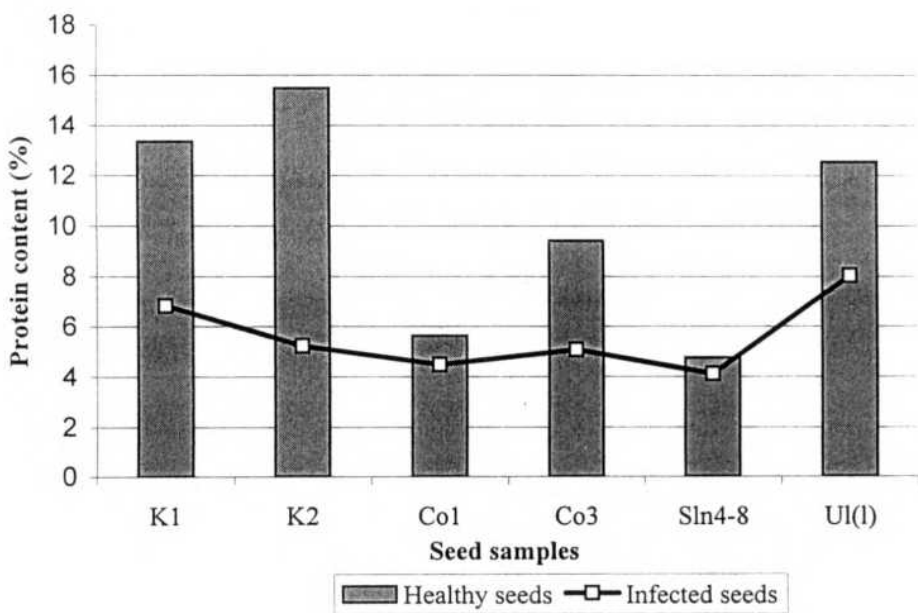


Table 12. Effect of *Colletotrichum capsici* seed infection on total phenol content of chilli seeds

Sl.No	Seed samples	Places of collection	Per cent seed infection	Total phenol (µg/g)*		Per cent decrease over healthy
				Healthy seed	Infected seed	
1	K1	Madukkarai	20.0	290.0^{b}	490.0^{ab}	40.82
2	K2	Aruppukottai	88.0	190.0^{c}	370.0^{d}	48.65
3	Co1	Coimbatore	6.0	500.0^{a}	580.0^{a}	13.79
4	Co3	Puduvelamangalam	50.0	230.0^{bc}	470.0^{bc}	51.12
5	Sln4-8	Coimbatore	12.0	320.0^{b}	390.0^{cd}	17.95
6	UL(l)	Ulagankathan	74.0	250.0^{bc}	510.0^{ab}	50.10
Mean				296.67	468.33	

CD (P=0.05)=91.15

*Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 13.Effect of *Colletotrichum capsici* seed infection on sugar content of chilli seeds

Sl.No	Seed samples	Places of collection	Per cent seed infection	Reducing sugar(%)*		Non reducing sugar(%)*		Total sugar(%)*		Per cent decrease over healthy
				H	I	H	I	H	I	
1	K1	Madukkarai	20.0	1.39^{b} (6.77)	1.21^{a} (6.31)	0.47^{c} (3.95)	0.41^{ab} (3.67)	1.86^{bc} (7.84)	1.62^{ab} (7.31)	12.90
2	K2	Aruppukottai	88.0	0.84^{d} (5.26)	0.45^{c} (3.87)	0.89^{a} (5.43)	0.48^{a} (3.99)	1.73^{c} (7.57)	0.94^{ad} (5.56)	45.66
3	Co1	Coimbatore	6.0	1.45^{b} (6.93)	1.25^{a} (6.43)	0.46^{c} (3.88)	0.45^{a} (3.89)	1.91^{b} (7.95)	1.71^{a} (7.52)	10.47
4	Co3	Puduvelamangalam	50.0	1.70^{a} (7.49)	1.24^{a} (6.39)	0.46^{c} (3.88)	0.27^{c} (3.00)	2.16^{a} (8.46)	1.51^{b} (7.07)	30.09
5	Sln4-8	Coimbatore	12.0	1.38^{b} (6.76)	1.23^{a} (6.37)	0.45^{c} (3.86)	0.43^{ab} (3.78)	1.84^{bc} (7.79)	1.66^{a} (7.41)	9.78
6	UL(l)	Ulagankathan	74.0	1.19^{c} (6.26)	0.92^{b} (5.52)	0.68^{b} (4.74)	0.37^{b} (3.48)	1.87^{bc} (7.87)	1.29^{c} (6.53)	31.10
Mean				1.33 (6.58)	1.05 (5.82)	0.57 (4.29)	0.41 (3.64)	1.90 (7.91)	1.46 (6.90)	

CD (P=0.05)=0.0312
*Mean of two replications
H - Healthy seeds
I - Infected seeds
Values in parentheses are arcsine transformed
In a column, means followed by a common letter are not significantly different at the 5%level by DMRT

Fig.10. Effect of *C. capsici* seed infection on total phenol chilli seeds.

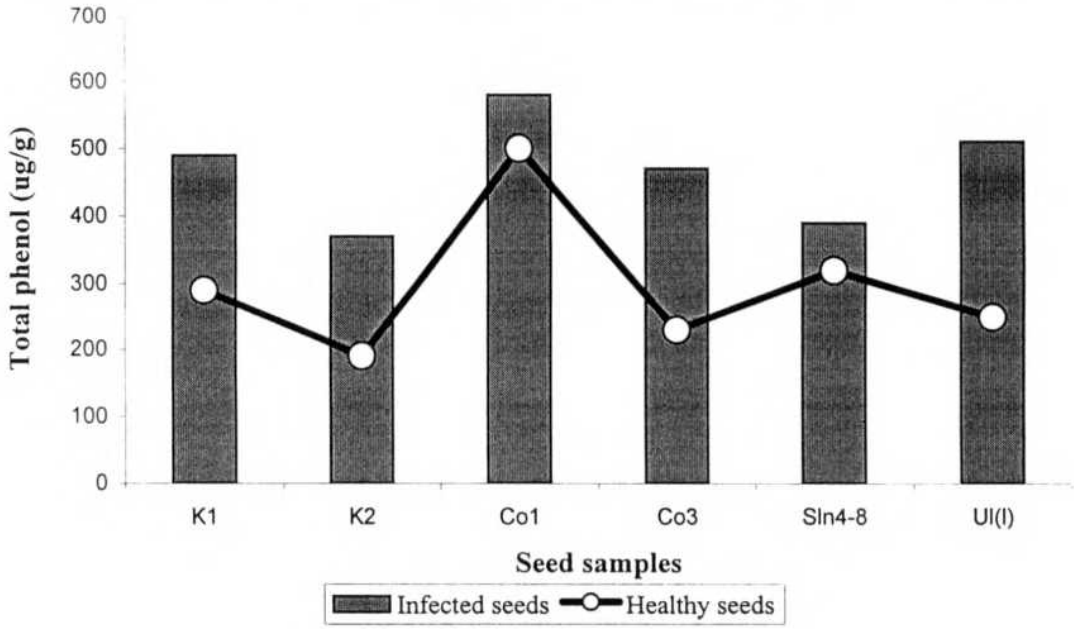


Fig.11. Effect *C. capsici* seed infection on sugar content of chilli seeds.

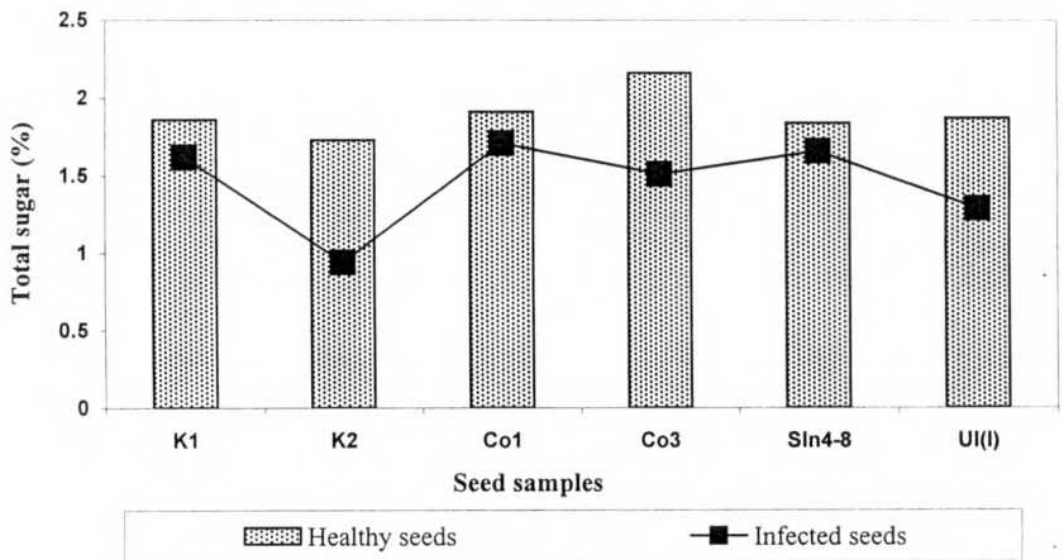


Plate 8.

Plate 8a.

Plate 8b.

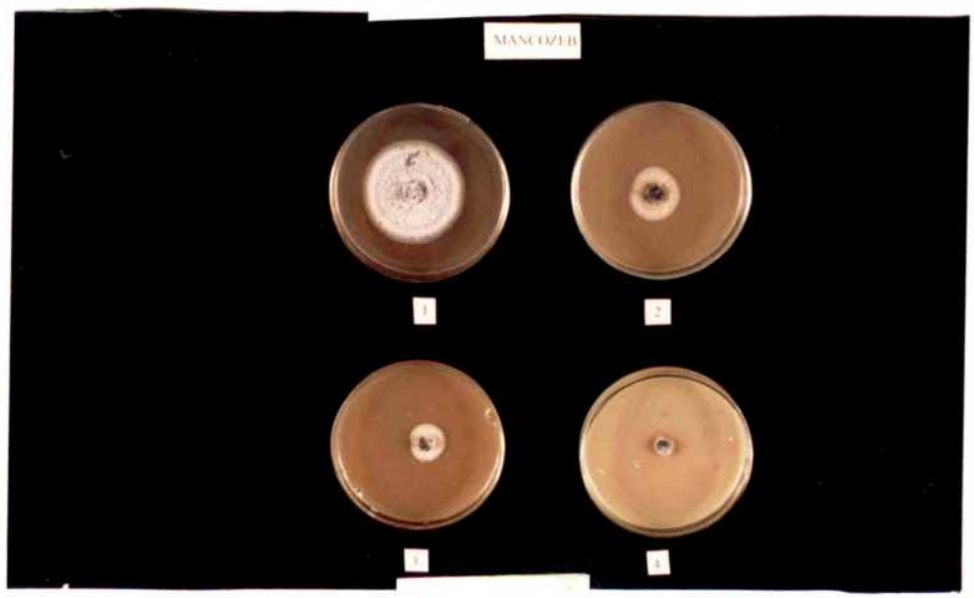


Plate 8C

4.14. Effect of *C. capsici* seed infection on sugar content of chilli seeds

Sugar content of both healthy and *C. capsici* infected seeds was estimated and presented (Table 13, Fig. 11). The sugar content of infected seeds was found to be decreased over healthy seeds. The decreased level of total sugar content ranged from 9.78 to 45.66 per cent. The higher decrease in total sugar content was recorded in the sample showing 88.00 per cent *C. capsici* seed infection and the lower level of 9.78 per cent was recorded in the sample showing 12.00 per cent seed infection. Similar trends were obtained in both reducing and non-reducing sugar content.

4.15. Screening of plant products, antagonistic organism and fungicides against *C. capsici*

4.15.1. Efficacy of fungicides against *C. capsici*

The efficacy of fungicides on mycelial growth of *C. capsici* was assessed and the results were presented (Table 14, Fig. 12). The reduced mycelial growth was recorded in all the treatments but their efficacy varied with fungicides and their concentrations (Plate 8) Among the fungicides tried, carbendazim (0.1 per cent) and mancozeb (0.3 per cent) exerted maximum reduction of mycelial growth which was followed by chlorothalonil (0.3 per cent). The reduction in mycelial growth in carbendazim, mancozeb and chlorothalonil was 86.54, 86.54 and 74.51 per cent over control, respectively.

4.15.2. Efficacy of bacterial biocontrol agents on *C. capsici* growth under *in vitro*

The efficacy of bacterial antagonists *viz.*, *Bacillus subtilis* and three strains of *P. fluorescens* were tested against *C. capsici* and the result obtained from the test indicated the bacterial biocontrol agents to inhibit the growth of *C. capsici* on solid medium. (Plate 9). Among the biocontrol agents, PFu and *B. subtilis* were found to inhibit the growth rate significantly to an extent of 47.70 and 44.98 per cent, respectively, over control. The reduced mycelial growth was also recorded in other strains. However,

Plate 9.

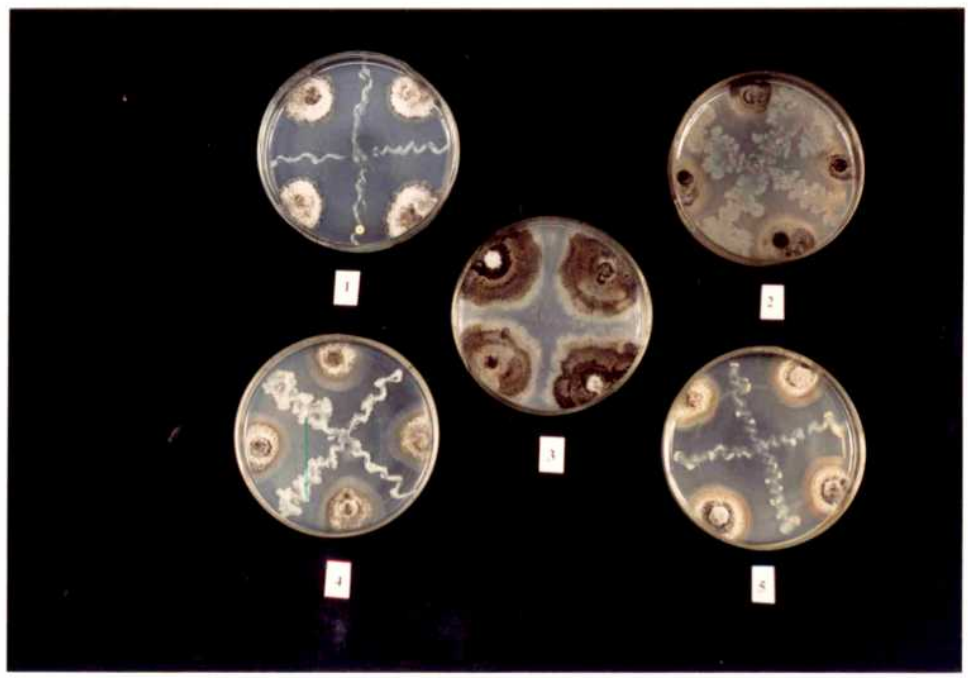


Table 14. Efficacy of fungicides on *C.capsici* growth under *in vitro* condition

Sl.No	Treatment	Concentration (%)	Colony growth (cm)*	Per cent decrease over control
1	Carbendazim	0.02	4.03^{e}	41.83
		0.05	3.20^{d}	53.84
		0.10	0.93^{a}	86.54
2	Chlorothalonil	0.10	3.07^{d}	55.76
		0.20	2.47^{c}	64.42
		0.30	1.77^{b}	74.51
3	Mancozeb	0.10	2.40^{c}	65.38
		0.20	1.50^{b}	78.36
		0.30	0.93^{a}	86.54
4	Control	–	6.93^{f}	–

CD (P=0.05)=0.33
*Means of three replication
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 15. Efficacy of bacterial antagonistic agents on *C.capsici* growth under *in vitro*

Sl.No	Treatments	Colony growth (cm)*	Per cent decrease over control
1	*Bacillus subtilis*	2.00[a]	44.95
2	PF1	2.43[c]	33.03
3	PFu	1.90[a]	47.70
4	PFk	2.20[b]	39.44
5	Control	3.63[d]	-

CD (P=0.05)=0.194
*Mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level of DMRT

Fig.12. Efficacy of fungicides on *C. capsici* growth under *in vitro* condition.

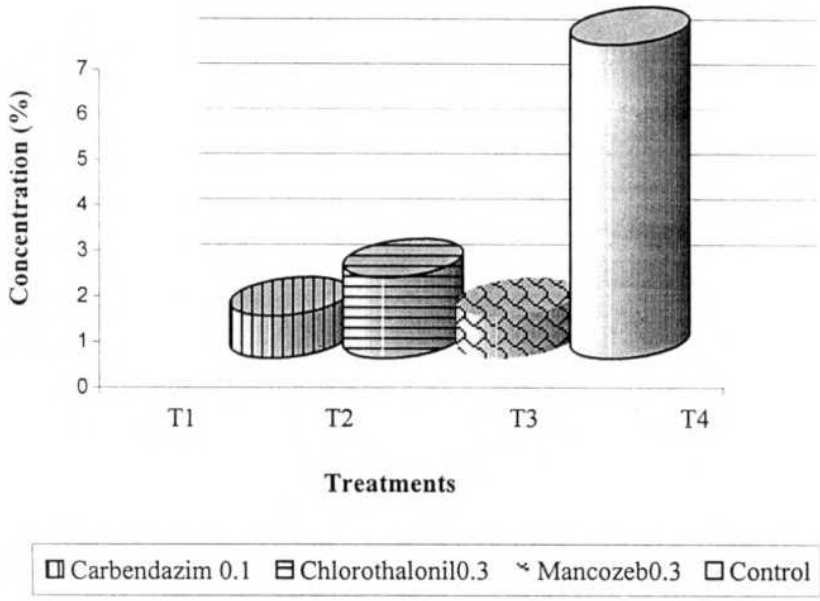


Fig.13. Effect of bacterial antagonistic organisms on *C. capsici* growth under *in vitro*.

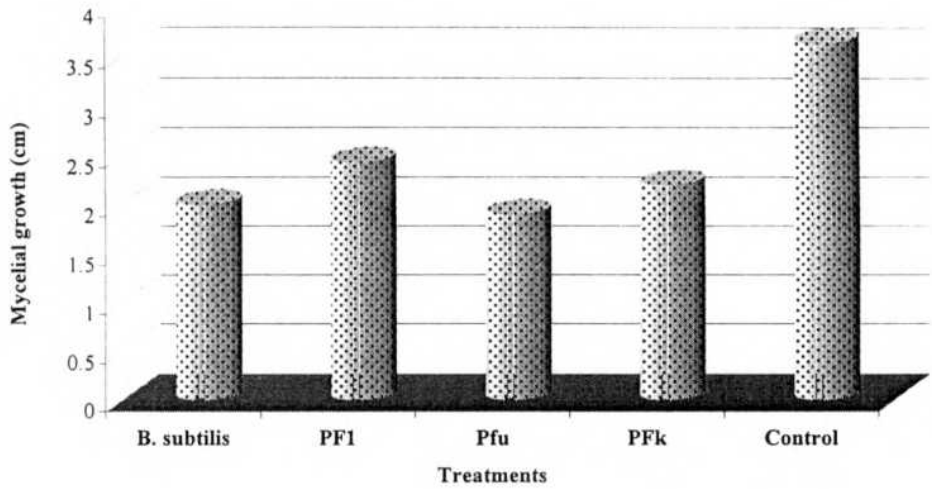


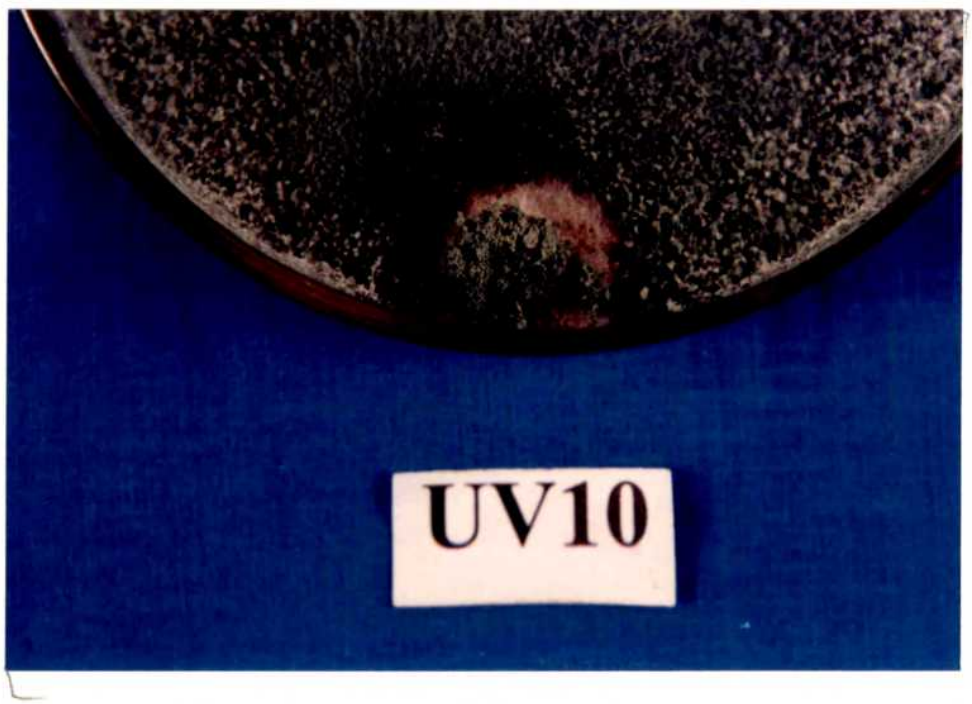
Table 16. Efficacy of fungal antagonists on *C.capsici* growth under *in vitro*

Sl.No	Fungal antagonists (strains of *Trichoderma viride)*	Colony growth (cm)*	Per cent decrease over control
1	Nat-TV	2.50[b]	62.27
2	Mg6	2.00[ab]	70.15
3	Mg3	2.50[b]	62.27
4	Uv10	1.50[a]	77.61
5	Mnt7	2.40[b]	64.18
6	Mnt5	2.40[b]	64.18
7	Control	6.70[c]	-

CD (P=0.05)=0.513
*Mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level of DMRT

Plate 10.

Plate 10a.



the reduction per cent was considerably low even after nine days after incubation (Table 15, Fig. 13).

4.15.3. Efficacy of fungal antagonistic against *C. capsici*

The inhibitory effect of six different strains of *Trichoderma viride* was studied against *C. capsici* by dual plate method and the results were presented (Table 16, Fig. 14). Among six strains of *T. viride* tried, the strain Uv 10 was found to inhibit the mycelial growth to an extent of 77.61 per cent over control. This was followed by Mg 6, MnT 7, MnT 5, Mg 3 and NAT-Tv and were equally effective as that of Uv10 in inhibiting the mycelial growth of *C. capsici* (Plate 10).

4.15.4. Efficacy of plant products on *C. capsici* growth under *in vitro*

The efficacy of plant extracts on the mycelial growth rate of *C. capsici* was tested and presented (Table 17, Fig. 15). All the plant species have been found effective in reducing the mycelial growth. Among the plant species tested, the *Catharanthus roseus* and *Prosopis juliflora* were found highly effective and inhibited the mycelial growth to an extent of 44.15 and 43.24 per cent, over control respectively. The other plant extracts also inhibited the growth. However, they were significantly different from C. *roseus* and *Prosopis juliflora* (Plate 11).

4.16. Effect of plant products, antagonistic organisms and fungicides against *C. capsici* spore germination

Effect of fungicides on spore germination revealed that mancozeb was highly effective in inhibiting spore germination to an extent of 87.51 per cent over control and the other fungicides also recorded appreciable result (Table 18, Fig. 16). Among the antagonistic organism PFk recorded higher reduction in percentage of spore germination (89.94 per cent) followed by PF1, PFu and *B. subtilis*. However, they were statistically different from each other. The plant products have also exerted appreciable results and

Plate 11.

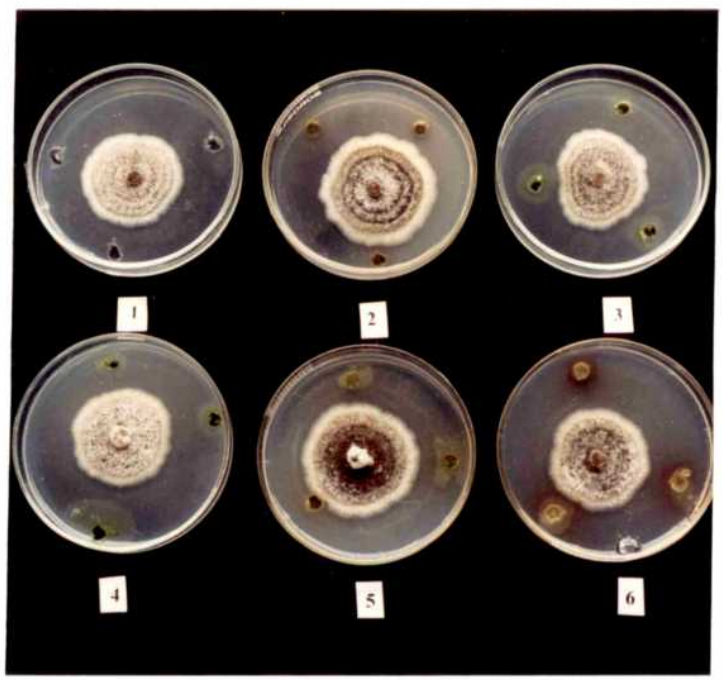


Table 17. Efficacy of plant products on *C.capsici* growth under *in vitro*

Sl.No	Plant species	Colony growth (cm)*	Per cent decrease over control
1	*Allium sativum*	4.83^{bc}	34.69
2	*Aegle marmelos*	5.17^{c}	30.18
3	*Prosopis juliflora*	4.20^{a}	43.24
4	*Eucalyptus* spp	4.43^{ab}	41.45
5	*Lawsonia inermis*	4.90^{bc}	33.78
6	*Catharanthus roseus*	4.13^{a}	44.15
7	Control	7.40^{d}	-

CD (P=0.05)=0.49
*Mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level of DMRT

Fig.14. Efficacy of fungal antagonists on *C. capsici* growth under *in vitro*.

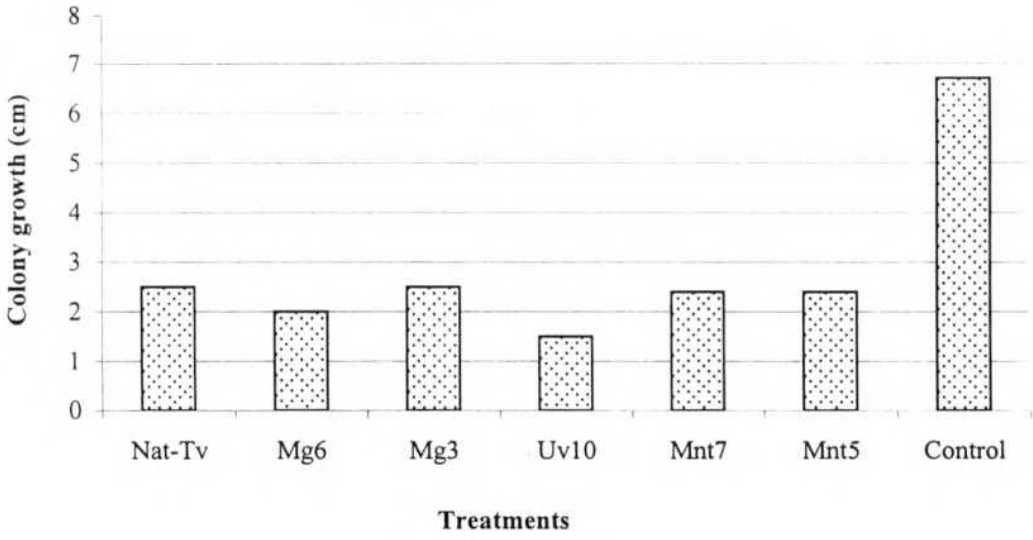


Fig.15. Efficacy of plant products on *C. capsici* growth under *in vitro*.

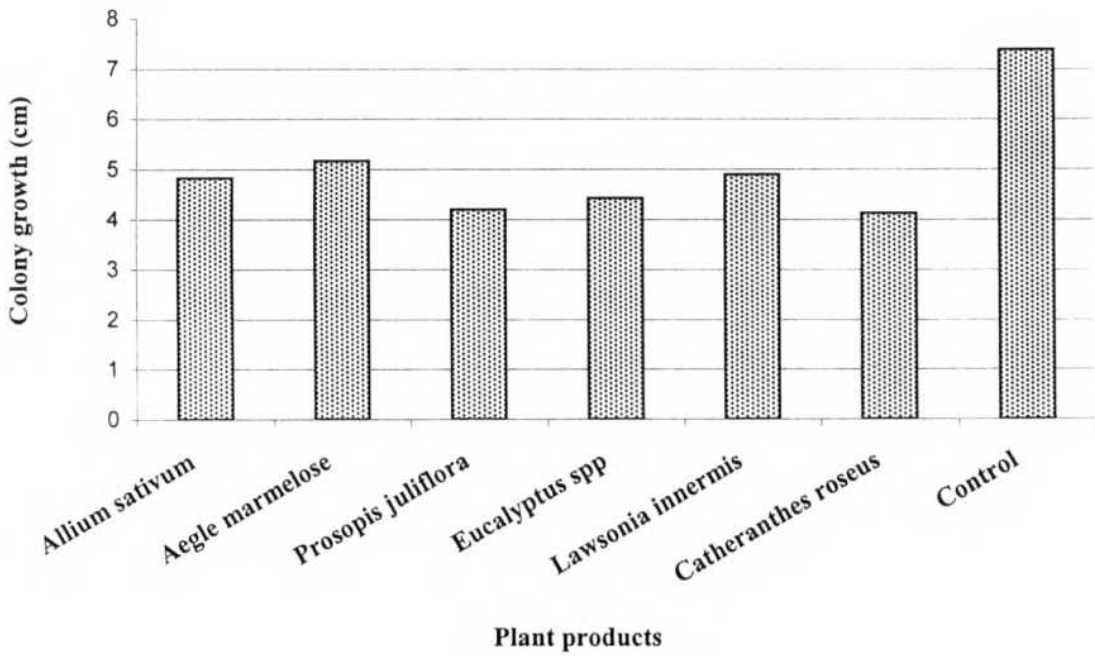


Table 18. Effect of plant products, antagonistic organisms and fungicides on *Colletotrichum capsici* spore germination

Sl.No	Treatments	Spore germination (%)*	Per cent decrease over control
	Fungicides		
1	Carbendazim	18.44^{def} (25.39)	80.24
2	Chlorothalonil	33.33^{g} (35.22)	64.43
3	Mancozeb	11.66^{bcd} (19.77)	87.51
	Antagonistic organisms		
4	*Bacillus subtilis*	21.66^{f} (27.71)	76.80
5	PF1	13.33^{be} (21.14)	85.72
6	PFu	13.33^{cf} (22.02)	85.72
7	PFk	9.39^{abc} (17.84)	89.94
	Plant species		
8	*Catharanthus roseus*	5.55^{ab} (15.49)	94.05
9	*Eucalyptus* spp	37.78^{g} (37.91)	59.52
10	*Lawsonia inermis*	20.48^{ef} (26.90)	78.06
11	*Prosopis juliflora*	0.00^{a} (12.92)	100.00
12	Control	93.33^{h} (73.40)	-
Mean		23.19 (27.98)	

CD (P=0.05) = 5.75

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Fig.16. Effect of plant products,antagonistic organisms and fungicides on spore germination of *C. capsici*.

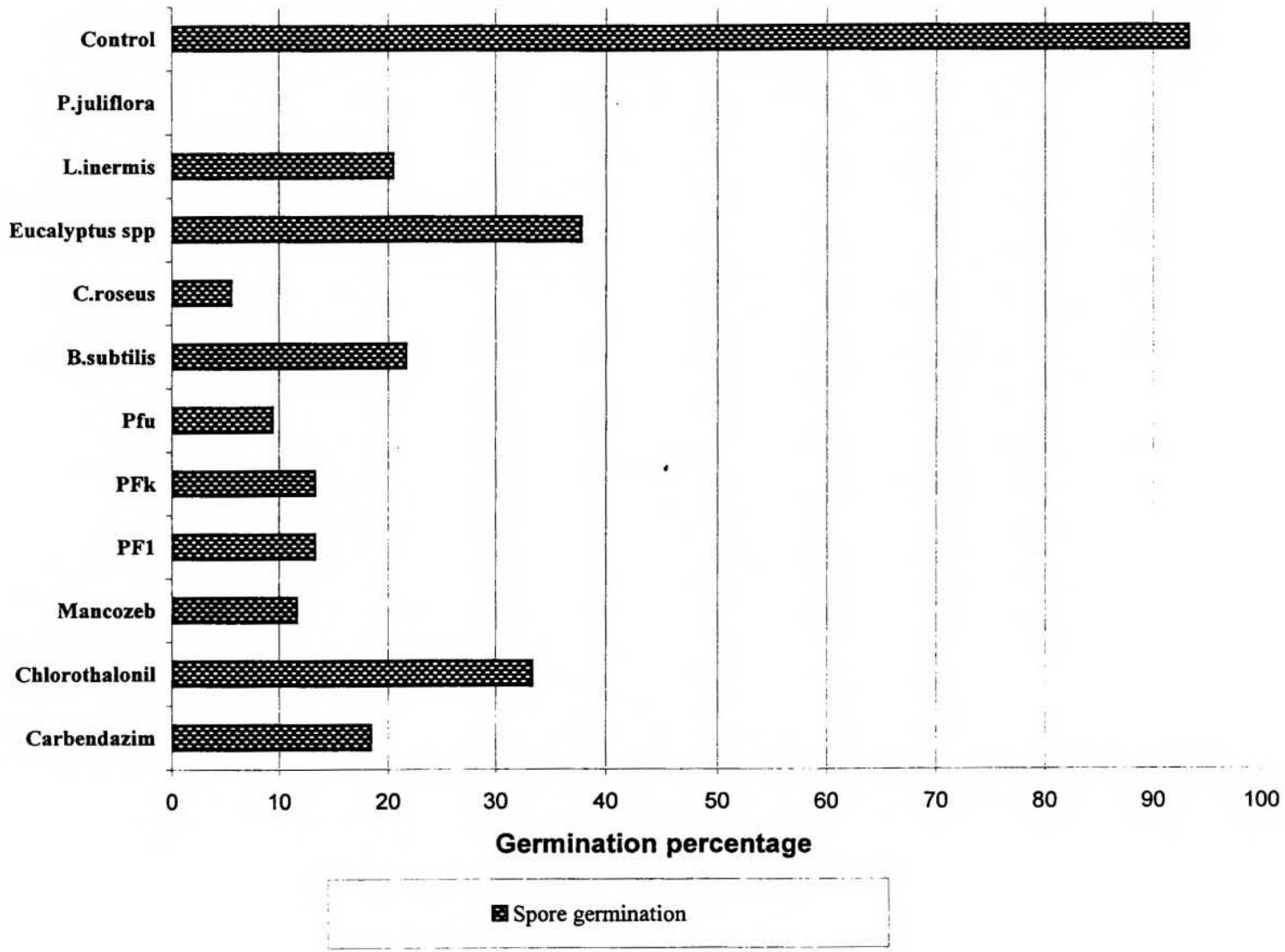


Plate 12.


TNAU
PLANT PATHOLOGY
DISEASE FREE SEED PRODUCTION STUDY IN CHILLI
CROP : CHILLI
SEASON : JUNE - JULY
TREATMENTS : 12
FUNGICIDES : 3
BIOCONTROL AGENTS : 4
PLANT EXTRACTS : 4
CONTROL : 1
VAR : K1
DATE : 12-07-01
LOCATION : KILLIKULAM
REPLICATION : 3
DESIGN : RBD
SOWINGS AT
STUDENT : R RAJAVEL
CHAIRMAN

the reduction in spore germination ranged from 59.42 to 100.00 per cent over control. The complete reduction in spore germination was recorded in *P. juliflora* followed by *C. roseus* (94.05 per cent). In general, the plant extract *P. juliflora* recorded higher reduction in spore germination. Which did *C. roseus*, PFk and mancozeb follow with a reduction in spore germination of 94.05, 89.94 and 87.51, respectively.

4.17. Performance of plant products, antagonistic organisms and fungicides against chilli fruit rot under field condition

The performance of plant extracts, antagonistic organisms, and fungicides were studied in two different locations of Tamil Nadu (Kolathur and Madukkarai) with same treatments. The strain PF1 (Plate 13) recorded higher per cent reduction in fruit rot incidence over control in both the trials and it was 72.80 and 75.75 per cent in Kolathur and Madukarai, respectively (Table 19and 20, Fig. 17).

Plant products and fungicides also caused the reduction in disease incidence over control. Significant difference in reducing the disease incidence was recorded among the treatments. In trial I, the *B. subtilis* had reduced the incidence to an extent of 64.00 per cent followed by *Prosopis juliflora* with the reduction of 59.90 per cent. The treatments like mancozeb, PFu, *Catharanthus roseus* and *Lawsonia inermis* were statistically on par with each other. However, significant differences were recorded among the treatments.

In trial II, the plant extract of *C. roseus* had shown appreciable result and the reduction in fruit rot incidence over control was 73.48 per cent, which was followed by *B. subtilis*, PFk and carbendazim, with a reduction of 65.15, 62.12 and 61.36 per cent over control, respectively. The treatments like, carbendazim, PFk, *B. subtilis* and *Prosopis juliflora* were statistically on par with each other. However, the reduction in disease incidence varied among the treatment tried (Plate 12)

Plate 13.

Plate 13a.

Plate 13b.

Table 19. Performance of plant extracts, antagonistic organisms and fungicides on chilli fruit rot under field condition (Kolathur) Trial (I)

Sl.No	Treatments	130 DAT		160DAT	
		Per cent disease index	Per cent reduction over control	Per cent disease index	Per cent reduction over control
	Fungicides				
1	Carbendazim	25.33^{e} (30.21)	34.45	24.00^{d} (29.31)	42.40
2	Chlorothalonil	25.00^{de} (29.99)	35.34	23.00^{cd} (28.50)	44.80
3	Mancozeb	24.00^{cde} (29.31)	37.93	18.00^{bcd} (25.09)	56.80
	Antagonistic organisms				
4	*Bacillus subtilis*	20.33^{bc} (26.73)	47.42	15.00^{ab} (22.74)	64.00
5	PF_1	13.33^{a} (21.33)	65.52	11.33^{a} (19.62)	72.80
6	PFu	19.33^{bcd} (25.97)	50.00	20.67^{bcd} (26.99)	50.40
7	PFk	23.67^{cde} (29.10)	38.80	22.67^{cd} (28.30)	45.60
	Plant species				
8	*Catharanthus roseus*	19.00^{bc} (25.72)	50.86	18.00^{bcd} (25.01)	56.80
9	*Eucalyptus* spp	24.33^{cde} (29.55)	37.07	23.67^{c} (28.87)	43.20
10	*Lawsonia inermis*	22.33^{be} (28.14)	42.24	19.33^{bcd} (26.05)	53.60
11	*Prosopis juliflora*	17.33^{ab} (24.57)	55.17	16.67^{abc} (24.08)	59.90
12	Control	38.67^{f} (38.44)	-	41.67^{f} (40.19)	-
Mean		22.72 (28.25)		21.14 (27.06)	

CD (P=0.05) = 3.692 CD (P=0.05) = 4.383

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5% lev by DMRT

Table 20. Performance of plant extract, antagonistic organism and fungicides on chilli fruit rot under field condition (Madukkarai) Trial II

Sl.No	Treatments	130 DAT		160DAT	
		Per cent disease index	Per cent reduction over control	Per cent disease index	Per cent reduction over control
	Fungicides				
1	Carbendazim	18.00^{ab} (25.10)	50.46	17.00^{abc} (24.31)	61.36
2	Chlorothalonil	25.00^{b} (29.84)	31.19	20.67^{c} (26.78)	53.03
3	Mancozeb	22.00^{d} (27.90)	39.45	18.33^{bc} (25.30)	58.33
	Antagonistic organisms				
4	*Bacillus subtilis*	19.67^{ab} (26.29)	45.87	15.33^{abc} (22.89)	65.15
5	PF1	12.33^{a} (20.46)	66.05	10.67^{a} (18.96)	75.75
6	PFu	19.00^{ab} (25.72)	47.70	18.00^{bc} (25.01)	59.09
7	PFk	22.00^{b} (27.94)	39.45	16.67^{abc} (24.05)	62.12
	Plant species				
8	*Catharanthus roseus*	17.33^{ab} (24.55)	52.30	11.67^{ab} (19.74)	73.48
9	*Eucalyptus* spp	23.00^{b} (28.51)	36.67	18.33^{bc} (25.34)	58.33
10	*Lawsonia inermis*	21.67^{b} (27.46)	40.36	18.00^{bc} (24.99)	59.09
11	*Prosopis juliflora*	16.33^{ab} (23.77)	55.05	13.33^{abc} (21.34)	69.70
12	Control	36.33^{c} (37.06)	-	44.00^{d} (41.54)	-
Mean		21.05 (27.05)		18.50 (25.03)	

CD (P=0.05)=5.90 CD (P=0.05)=5.07

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

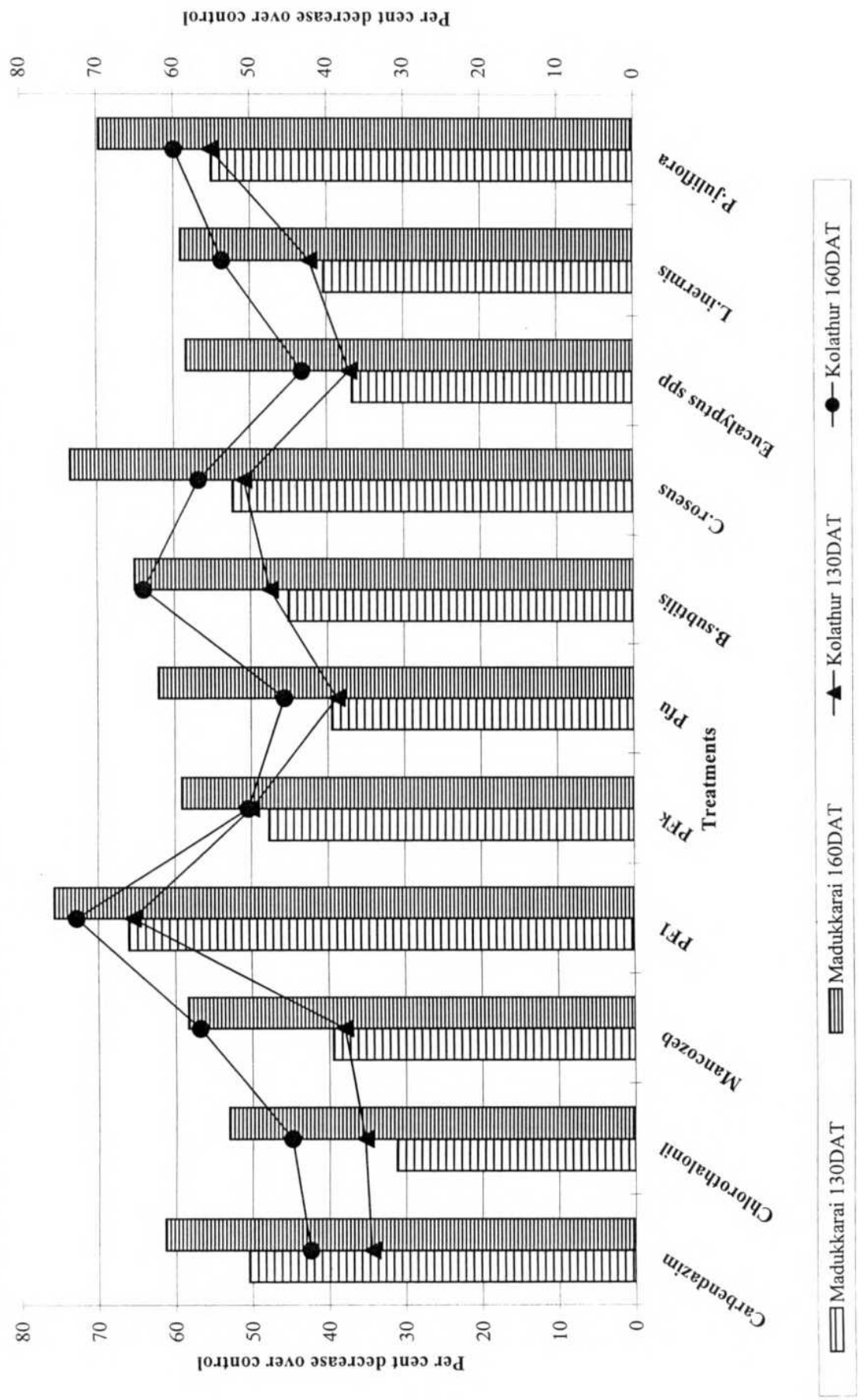


Fig.17. Performance of plant products,antagonistic organisms and fungicides on chilli fruit rot incidence.

Discussion

CHAPTER V
DISCUSSION

Chilli is an important spice cum vegetable crop being cultivated practically in all parts of India. Chilli cultivation is being affected by a number of diseases caused by fungi, bacteria and viruses. Among fungal diseases, the fruit rot and die back (*C. capsici*) is a serious threat in major chilli growing areas. None of the local and high yielding varieties are resistant to the disease. The management of disease under field condition is not economically viable and ecologically sound since the pathogen is seed-borne in nature (Anonymous *et al.*, 1933; Rout and Rathi, 1972; Rai and Chohan, 1966; Menon and Nik, 1988; Patil *et al.*, 1993; Kamlesh mathur *et al.*, 1995 and Basak *et al.*, 1996).

Owing to lack of comprehensive information about fruit rot of chillies in Tamil Nadu, a survey was conducted in 10 different locations of Tamil Nadu *viz.*, Ariyalur, Attur, Coimbatore, Indili, Kolathur, Madukkarai, Palladam, Periyakulam, Tenkasi and Ulagankathan. Higher incidence of fruit rot was observed (45.3 per cent) in Indili, Villupuram district. Similar results were obtained in the surveyed area of Punjab with 66-84 per cent *C. capsici* incidence. (Thind and Jhooty, 1985).

The extent of seed-borne nature of *C. capsici* was assessed in 30 different locations of Tamil Nadu and the occurrence ranged from 0.00 to 33.33 per cent. The maximum incidence was recorded in sample collected from Indili. Many workers found the seed-borne nature and incidence in several areas. Basak *et al.*(1996) reported 58.39 per cent seed infection with *C. capsici* in Bangladesh and Rout *et al.* (1972) reported 5.11 per cent seed infection of *C. capsici* in Orissa.

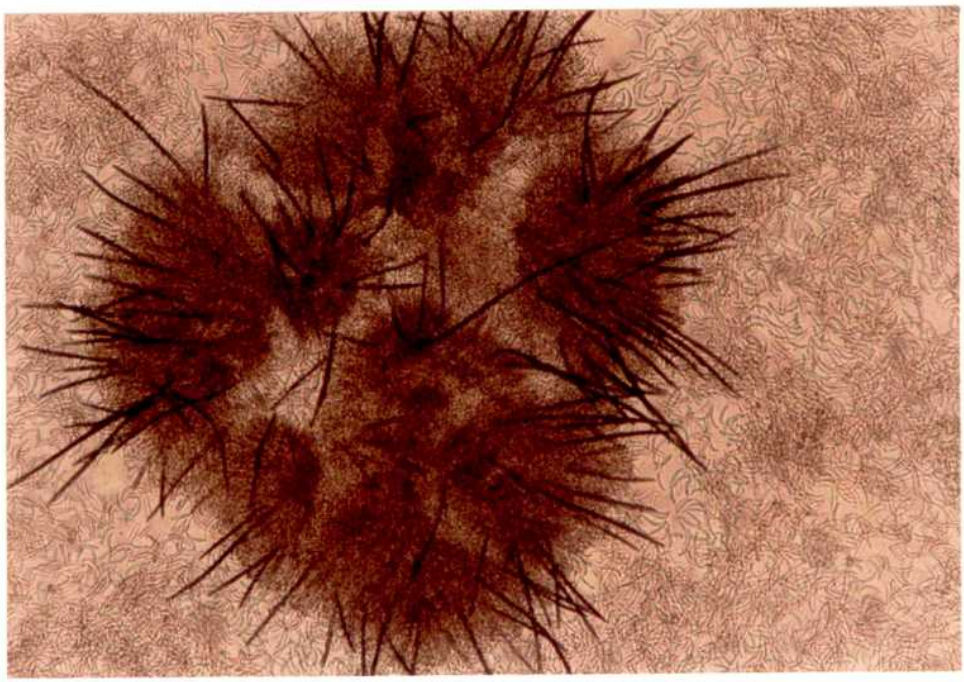
Pathogen free sound seeds are preferred for sowing to have desired germination, emergence and healthy population but the disease causing fruit rot is seed-borne and hence the seed health test was performed with four different methods. The maximum seed infection of pathogen was achieved in calcium chloride soaked seeds followed by 2, 4-D and deep freezing methods. Neergaard and Saad (1962) observed higher percentage of *Pyricularia oryzae* in 2, 4-D method. Shukla *et al.* (1990) also reported higher counts of *Phoma* sp. from soybean seeds. Limonard (1966; 1968) found maximum infection of *C. dematium* in *Capsicum* seed. Seed soaked in calcium chloride solution (5.00 per cent) yielded 50 per cent more *C. dematium* (Renukeswarappa and Shethna, 1983).

The influence of different light periods on seed infection of *C. capsici* revealed that 12 h (Phillips TLF 40W/34) + 12 h NUV (T1 40W/08) was exerted the maximum count (21.33 per cent). An NUV darkness cycle of 12/12 h has been widely employed for the detection of seed-borne fungi (*Drechslera oryzae*). Michail *et al.* (1977) recorded higher count of *R. solani* when employing the 12 h / 12 h light and darkness. The present investigation is in contrast with the earlier report, the higher count was in 12 h / 12 h light and NUV, which may be due to the influence of near ultraviolet on *C. capsici*.

The effect of pH level on *C. capsici* showed variation and the maximum seed count (*C. capsici*) was recorded at pH 7.5 (23.11 per cent). Singh *et al.* (1982) recorded the maximum growth of *A. padwickii* at pH 4.

SDS-PAGE analysis for crude extracts of chilli seed showed variation with the appearance of 50 kDa protein in infected seeds of chilli. There is also a disappearance of 29 kDa protein in K1, Co 1 and Sln 4-8. Here the protein pattern varied with variety and levels of *C. capsici* seed infection. Mahendran (1996) observed the induction of 23 kDa protein in IR 50 and Bhavani when inoculated with *H. oryzae*

Plate 14.



Morphological features of isolates of *C. capsici* revealed that there was a difference in length and breadth of conidia and setae. *C. capsici* has falcate conidia, thick stroma, conspicuous and abundant setae and densely aggregated acervuli (Plate 14).

While growing in culture media, the two isolates from chilli were different from each other. The acervuli are either scattered/arranged in concentric rings. This was in agreement with the early work of Dastur (1921). The measurement of conidia and setae were 28.34 x 4.8 μ, 102.72 x 4.8 μ in isolate I and 28.27 x 4.8 μ, 102.26 x 4.8 μ in isolate II, respectively. The wider variation was in agreement with the earlier work of Higgins (1930) and Leeling *et al.* (1944).

Higgins (1930) reported that the American fungus has the measurement of 48-96 x 3.5-6μ and 16.8-26.4 x 3-4.2 μ for setae and conidia, respectively.

The morphological features of *C. capsici* from different locations of Tamil Nadu varied in their conidia, setae, length and breadth and it ranged from 20.48-30.72 x 4.8 μ and 113.28 - 267.74 x 4.8 μ, respectively and this was in agreement with the earlier work.

Leeling *et al.* (1944) studied the cultural characters of *C. capsici* isolated from pepper, eggplant and tomato and found them in their cultural characters. In pepper, the conidia and setae size was 24.9 x 3.4 μ and 101 x 7.0 μ, respectively and it was 25.0 x 3.6 μ, 123 x 6.5 μ in the culture from tomato and in egg plant, it was 25.1 x 3.5 μ and 118 x 6.9 μ, respectively.

Pring *et al.* (1995) reported that the size of conidia and setae were significantly different. In the present investigation, the result obtained was in agreement with the earlier work of Leeling (1944) and Pring *et al.* (1995).

Effect of seed infection on germination of *C. capsici*

The seed germination percentage dropped to 6.00 per cent in the infected seed lot of chilli. This may be due to the influence of *C. capsici* on seed germination. The vigour index and dry matter production was also reduced in *C. capsici* infected seed lot. The poor germination was due to the decay of growing tip. The acervuli of *C. capsici* developed on the plumule of chilli seeds and it lead to decay of seeds. Similar study in chilli was performed by Jindal *et al* (1994) who recorded 17.3 per cent germination as against the ISTA recommendation. The present investigation confirmed the earlier report of Shekhawat and Chakravarti, (1976); Segehote and Jurangbhanic, (1984) and Bhardwaj *et al.* (1987).

The influence of *C. capsici* on fruit and seed weight revealed loss of 30 per cent in seed weight and fruit weight and it was heavier in highly infected seed lot.

Bio-chemical changes in *C. capsici* infected chilli seeds

The increased activity of peroxidase and polyphenol oxidase was observed in *C. capsici* infected seeds of chillies. The 40.00 per cent increase in peroxidase activity was due to 50.00 per cent *C. capsici* infection. The polyphenol oxidase activity was found to increase with increase in per cent seed infection.

The increased peroxidase activities were only the aftermath of necrosis. Farkas and Stahmann (1966) and Prasad (1986) reported that an increase in the PO, PPO activity was noticed in infected tobacco plants. The increased level might be toxic because

polyphenol oxidase and peroxidase oxidize phenols and produced oxidized phenols, which are more toxic.

In the present study, the total phenol content was found increased in infected seeds of chilli. The increased content of total phenolics in infected seeds might be either due to the triggered metabolism of their synthesis or by the transportation of phenolics from the neighbouring healthy tissue to infection site as a host defence against the pathogenic infection as reported by Madhukar and Reddy (1991). The result obtained in the present investigation was in conformity with the earlier report.

The result of estimated protein clearly indicated that the protein content was invariably low in infected seeds. The protein content was found to be reduced in infected chilli fruits than healthy fruits. This was in agreement with the earlier findings of Sujathabai (1992) and Jeyalakshmi *et al.*(1999).

The sugar content was lower in diseased seeds (Konger, 1974). In the present investigation, the reducing, non-reducing and total sugar was found to be reduced in infected seeds. This was in conformity with the earlier report of Subbaraja (1981). Jayalakhsmi *et al. (*1999) reported decreased level of sugars in infected fruits of chilli when compared to the healthy fruits.

The reduction in sugar content might be due to seed infection by *C. capsici*. The maximum percentage of sugar was recorded in susceptible variety and it tends to be reduced in infected susceptible variety. (Azad *et al.*, 1991) and the above all would support the present investigation.

Effect of fungicides, plant extracts and antagonistic organisms on *C. capsici* growth
Fungicides

Fungicides were tested for their efficacy in reducing the growth of *C. capsici*. In general, fungicides were found to be effective in checking the mycelial growth *in vitro*. Higher reduction was recorded at 0.1 per cent and 0.3 per cent in carbendazim and chlorothalonil, mancozeb, respectively. Kadu *et al.* (1977) stated that "Dithane M-45" could restrict the growth of the fungus completely. Narain and Panigrahi (1971) reported effectiveness of "ziram", "Dithane M-45" (0.25 per cent) and found complete inhibitory effect on *C. capsici*. Das and Mohanty (1988) reported that out of eight fungicides tried, "carbendazim" could restrict the growth of the fungus completely.

Plant products and antagonistic organism

Plant products were tested against the growth of *C. capsici* and found to reduce the growth. The plant extract of *Catharanthus roseus* has been found to be highly effective. The *P. juliflora* has also been effective. Renukadevi (1995) reported the effectiveness of *Prosopis juliflora* in checking the growth of *C. capsici*. This was in agreement with present investigation. This might be due to the presence of carbohydrate in plant species. The carbohydrates are the precursors of phenolics, which show antifungal, antibacterial and antiviral properties (Carrasco *et al.*, 1978).

The bacterial and fungal antagonists were effective in checking the mycelial growth *in vitro*. The strains PFu, PF1 and Uv10 of bacterial and fungal (*T. viride*) antagonists respectively, reduced the mycelial growth of *C. capsici*. This was in agreement with the earlier work.

A significant reduction in the number of germ tubes, conidia of *C. graminicola* in samples treated with the antagonists, *T. viride, T. harzianum, P. fluorescens* and

B. subtilis were observed earlier by Michereff *et al.*, 1993; Sas-Piotrowska and Dorszewski (1996) found that *T. viride* and *T. harzianum* significantly reduced the growth and sporulation of *C. capsici in vitro*.

The possible role of antagonistic action of the organisms, *viz.*, *T. viride*, *P. fluorescens* and *B. subtilis* on spore germination and mycelial growth of *C. lindemuthianum* as discussed by several workers. It is a well known fact that antagonism of *Trichoderma* spp is due to it's different mechanism of action *viz.*, competition, hyperparasitism and antibiosis (Malathi, 1996).

The volatile metabolites produced by *T. harzianum* were identified as alkyl pyrones, which were powerful inhibitors to several fungi *in vitro* (Claydon *et al.*, 1987). It is reported that several antibiotics like viridin, gliotoxin and tirchoderin were produced by *Trichoderma* sp. (Allen and Haenseler, 1935; Ayers and Adams, 1981 and Fravel, 1988).

Similarly several mechanism of action have been proposed to explain suppression of plant pathogens by fluorescent pseudomonads. The biosynthesis of antibiotic pyoluteorin (Howell and Stipanovic, 1980) was the important mechanism. *P. fluorescens* showed a distinct role in the spore germination and mycelial growth of *C. lindemuthianum*. (Muthuraj, 1998). However, it is well known that in a given biological system more than one mechanism may operate to suppress a pathogen and the relative importance of a particular mechanism may vary with physical or chemical conditions in the given situation (Weller *et al.*, 1988).

The strains of *B. subtilis* are known to produce a variety of antifungal polypeptides (Korzyski *et al.*, 1967). A proteinaceous antifungal antibiotic, bacillomycin

was isolated and purified from culture filtrate of *B. subtilis* (Esterhuizen, 1974) and was found to be more effective in inhibiting the growth of *Helminthosporium turcicum* (Esterhuizen and Merwe, 1977). It is clear that any of the mechanism of action discussed earlier may operate and suppress the growth of pathogen either by parasitism, lysis/antibiosis.

Effect of plant products, antagonistic organisms and fungicides on spore germination of *C. capsici*

Plant products have been reported to interfere/suppress the fungal spore. Singh *et al.* (1990) observed that "Ajoene", a compound derived from garlic (*A. sativum*), inhibited the spore germination of several fungi namely *Alternaria solani, Fusarium lini* and *Colletotrichum* sp.

Rajeswari (1992) reported that the extracts of *P. juliflora* and *A. vasica* reduced the spore germination of *P. oryzae.* Renukadevi (1995) reported that *C. capsici* spore germination was completely inhibited in *P. juliflora* extract. In the present study, the spore germination was completely reduced in extract of *C. roseus* and *P. juliflora.* This was in agreement with the earlier work of several scientists. The inhibitory effect might be due to the alkaloid on conidial germination. As the concentration increased the conidial germination got reduced..

The fungicides tested produced positive results, which might be due to the chemical compounds on germination. In the present investigation mancozeb and carbendazim were found to be highly effective. In support to this investigation, a naive report was recorded in Benlate, Brestan and Dithane M-45 on spore germination. The above mentioned fungicides were highly effective in inhibiting the spore germination of *C. capsici.* Mali and Joi (1985) reported that Difolatan, Thiram and Carboxin were the

most effective against colony growth and sporulation of *C. capsici.* Narain and Panigrahi (1971) recorded the minimum conidial germination of *C. capsici*, even at lower concentrations of ziram. They have also reported the effect of oxathin and plantvax compounds against the *C. capsici* conidial germination and the above results confirmed the present investigation. The spore germination was determined by the age of the isolate and density of conidia on slides, higher the age and density lower will be the spore germination (Anonymous, 1943). In the present study, *P. fluorescens* and *B. subtilis* showed distinct role in the prevention of spore germination.

Valluvaparidasan (1994) observed that the cell free culture filtrate of *Chaetomium globosum*, *B. subtilis* and *P. fluorescens* inhibited the spore germination of *H. oryzae.* Muthuraj (1998) reported the inhibition in spore germination of *C. lindemuthianum* when treated with the culture filtrates of *B. subtilis* and *Trichoderma* spp.

Vasudeva *et al (*1958) reported that *B. subtilis* produced an antibiotic namely bulbiformin which inhibited the spore germination of *Alternaria* sp. to an extent of 63 per cent. The above mentioned results were in agreement with the present investigation of *B. subtilis* and *P. fluorescens* on spore germination of *C. capsici.*

Field evaluation of plant products, antagonistic organisms and fungicides

The observation from the present study clearly indicated that all the treatments (Fungicides, antagonistic organisms and plant products) significantly reduced the incidence of fruit rot. Among the treatments PF1 was highly effective and recorded reduced fruit rot incidence (75.75 per cent). This was followed by leaf extract of *C. roseus* (73.48), *Prosopis juliflora* (69.70) and powder formulation of *B. subtilis* (65.15).

Similar observations were also recorded in trial I and the strain PF1 recorded reduced fruit rot incidence (11.33 PDI) which was followed by *B. subtilis, Prosopis juliflora*, mancozeb and *Catharanthus roseus*.

Among the plant products tried, *C. roseus* and *P. juliflora* had been highly effective in reducing the fruit rot incidence, which was in confirmation with the earlier work (Jeyalakshmi and Seetharaman, 1998; Renukadevi, 1995). They found that *P. juliflora* was most effective in reducing the fruit rot incidence under field conditions. Shivpuri (1997) tested plant products against *C. capsici* and found that leaf extract of *Ocimum sanctum, Polyalthia longifolia* and *Catharanthus roseus* were highly effective.

Antagonistic organisms

The observations of present study indicated that the strain PF1, *B. subtilis*, both with foliar spray controlled the incidence of fruit rot under field condition. This was in confirmation with the earlier work of Jeyalakshmi *et al.* (1998), who found that the foliar application of *Saccharomyces cerevisiae* and PF1 significantly reduced the fruit rot incidence.

Muthuraj (1998) reported the reduction in pod infection of *C. lindemuthianum* when treated with *P. fluorescens* and *T. viride*.

The increased action may be due to the induction of systemic resistance by *P. fluorescens,* which needs further investigation. The induction of systemic resistance due to treatment with *P. fluorescens* has already been reported by Dubeikaovsky *et al.* (1993) and Peterson and Pound (1960) indicating the increased efficacy of the antagonist in the disease management.

Fungicides on fruit rot incidence

The three fungicide treatments significantly reduced the fruit rot incidence. Lowest fruit rot incidence of 17 and 18 PDI was recorded in trial I and trial II with carbendazim and mancozeb, respectively. This was in confirmation with the earlier work of Kumawat (1995), who found that mancozeb was highly effective followed by thiophanate methyl, captafol, carbendazim and ziram.

Datar *et al.* (1990) reported the effectiveness of mancozeb @ 0.25 per cent followed by thiophanate methyl @ 0.05 per cent and carbendazim @ 0.05 per cent. Chauhan and Duhan (1977) however, found captafol and carbendazim to be most effective followed by mancozeb @ 0.2per cent which was in confirmation with the present investigation.

Raju and Rao (1985) reported the effective control of fruit rot and three insect pests by six rounds of combined application of "mancozeb + monocrotophos" at 15 days interval from November. Thind and Jhooty (1987) reported that spraying of "Difolatan" and Dithane M-45 at 0.2 per cent gave better management of fruit rot. However, Das and Mohanty (1988) came with carbendazim as the best and effective one against *C. capsici.*

Renukadevi (1995) reported that the fungicides "carbendazim" and "chlorothalonil" effectively reduced the incidence of fruit rot to the extent of 71.2 and 62.2 per cent respectively.

The results of the present investigations were in agreement with the earlier work done by several workers. However, the use of fungicides in the control of plant diseases gets reduced because of the growing concern about environment safety.

Summary

CHAPTER VI

SUMMARY

1. The maximum fruit rot incidence of 45.3 PDI was recorded at Indili in Villupuram District and the lower incidence of 21.60 PDI was recorded at Attur in Salem District of Tamil Nadu.

2. The occurrence of seed-borne nature of *C. capsici* was studied in 30 different locations of Tamil Nadu and more than 50 per cent of seed lot was found to be infected with *C. capsici*. The higher seed infection count was 33.33 per cent at Indili in Villupuram District.

3. The higher seed infection count was recorded in 3 per cent calcium chloride soaked seeds (23.33 per cent) when compared to standard blotter technique, deep freeze blotter and 2,4-D methods.

4. The influence of light period on seed infection count was studied and higher count (21.33 per cent) was noticed in 12 h (Phillips TLF 40W/34) + 12 h NUV (T_1 40W)/08).

5. The higher (*C. capsici)* seed infection count was recorded (23.11per cent) at pH 7.5. The other pH level also influenced the seed infection and came with significant differences.

6. The disappearance of 29 kDa protein was observed in SDS-PAGE analysis for *C. capsici* infected seed samples. Which was present in healthy seed samples.

7. Morphological features of two isolates revealed that there was a significant difference in size of conidia and setae and recorded the conidia and setae size of 28.34 x 4.8 μ and 102.72 x 4.8 μ, respectively in Isolate I and it was 28.27 x 4.8 μ gel 102.72 x 4.8 μ in Isolate II.

8. Measurement of conidia and setae observed on seeds of chilli varied with location of seed samples and the conidial, setae length and breadth varied from 20.48-30.72 x 4.8 μ and 113.28 - 267.74 x 4.8 μ, respectively.

9. The *C. capsici* seed infection had influence on germination, seedling vigour and dry matter production. The reduced seed germination percentage of 6.00 was recorded with vigour index of 187.20. The poor seed germination was due to the decay of growing tip.

10. The *C. capsici* seed infection directly influenced the fruit and seed weight with 31.87 and 29.37 per cent reduction in fruit and seed weight recorded in 88 per cent infected fruits.

11. The increased peroxidase and polyphenol oxidase activity was noticed with increased seed infection. The per cent increase of peroxidase (48.37 per cent) and polyphenol oxidase (75.00 per cent) was recorded in 50.00 per cent *C. capsici* infected seed lot.

12. The total phenol content tends to be increased in *C. capsici* infected chilli seed lot. The sample K2 collected from Aruppukottai recorded 88 per cent seed infection which in turn increase the total phenol content from 190.0, 370.0 μg.

13. Protein content in chilli seeds infected with *C. capsici* showed significant differences and recorded a 66.21 per cent reduction in 88.00 per cent infected seed lot and there was 13.65 per cent reduction in 12.00 per cent infected seed lot.

14. The reducing, non- reducing and total sugar content in healthy and infected seed sample revealed that there was a reduction in sugar content in infected seed samples. The reduction in total sugar content of 45.66 per cent was recorded in 88.00 per cent *C. capsici* infected seed sample.

15. Among the fungicides tested, the carbendazim (0.1 per cent) and mancozeb (0.3 per cent) were highly effective in reducing the mycelial growth (0.93 mm) *in vitro*.

16. The bacterial and fungal antagonists also inhibited the growth of *C. capsici in vitro*. Among the bacterial strain PF1 recorded the minimum growth, 1.9 mm was recorded in *T.viride* Strain Uv 10.

17. The leaf extracts were also effective in inhibiting the mycelial growth *in vitro*. The 44.15 per cent reduction over control was recorded in leaf extract of *Catharanthus roseus*.

18. *In vitro* spore germination of *C. capsici* was highly suppressed by fungicide mancozeb, biocontrol agent PFu and plant extracts of *Prosopis juliflora*. However, a maximum suppression of 100 per cent and 94.05 per cent was recorded in *Prosopis juliflora* and *C. roseus,* respectively.

19. The performance of plant products, antagonistic organism and fungicides were noticed in two different field trials. The three consecutive spray was performed irrespective of location. The highest reduction in fruit rot incidence was recorded in PF1 treated plots. The same results were exerted in two fields. However, all the treatment had it's own influence on the reduction of fruit rot incidence. The plant products like *Prosopis juliflora* and *C. roseus* have reduced the fruit rot incidence.

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