ANTHRACNOSE DISEASE OF VEGETABLE COWPEA

[*Vigna unguiculata* subsp. sesquipedalis (L.) Verdcourt]

By

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THESIS

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To my
Parents and Teachers

DECLARATION

I hereby declare that the thesis entitled '**Anthracnose disease of vegetable cowpea (*Vigna unguiculata* subsp. sesquipedalis [L.] Verdcourt.)**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title of any other university or society.

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We, the undersigned members of the Advisory Committee of **Mr.M.Praveen Kumar,** a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Pathology**, agree that the thesis entitled '**Anthracnose disease of vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* [L.] Verdcourt.)**' may be submitted by Mr.M.Praveen Kumar in partial fulfilment of the requirement for the degree.

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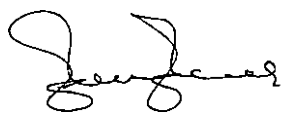

PRAVEENKUMAR, M.

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ABBREVIATIONS

PDA	- Potato dextrose agar
PCA	- Potato carrot agar
DAS	- Days after sowing
kg	- Kilogram
g	- Gram
l	- Litre
ml	- Millilitre
m	- Meter
cm	- Centimeter
mm	- Millimeter
µm	- Micrometer
h	- Hour
°C	- Celsius
RH	- Relative humidity
mha	- Million hectare
mt	- Metric tonne

Introduction

INTRODUCTION

In India cowpea occupies a lion share of total area under legumes. It is getting popularity day by day due to large scale cultivation and use. It is grown over an area of 0.73 m ha with an annual production of 0.72 mt. In Kerala also pole and bush type cowpea is grown in large scale which is used as a major vegetable/pulse crop.

Major factor which seriously impede the pulse production is the problem of pests and diseases. Among the various diseases, anthracnose is the most important and serious disease of legumes. Recently, anthracnose disease has become one of the constraints in the cultivation of cowpea in Kerala taking a heavy toll of the crop. An yield loss up to 50 per cent has been reported from certain areas of Kerala due to this disease (KAU, 1996). This disease not only reducesthe quantity but also the quality by causing discolouration and shrivelling of the seeds.

In India anthracnose of cowpea caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. was first reported in 1910. Although different aspects of cowpea anthracnose were studied elsewhere, informations on this disease are scanty from India. Moreover this disease has not received any attention in Kerala so far. With this view, the present study was undertaken to unveil the details of certain aspects of the disease such as etiology, host resistance, influence of weather conditions, assessment of crop loss due to disease and the management of disease. Continuous/repeated use of the same fungicide for the same pathogen, results in the development of fungicide resistant strains of the pathogen besides polluting the environment. As an alternative to chemical fungicides, use of biocontrol agents and plant products are gaining importance in recent years. In the present study, efficacy of certain plant products and biocontrol agent (*Trichoderma viride*) was assessed in the management of anthracnose disease of cowpea. Details of the findings of the study are presented here with which are expected to add to our knowledge of the disease.

REVIEW OF LITERATURE

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. is principally a stem disease of cowpea which is wide spread throughout Africa, Asia, South America, Bangladesh, Bhutan, Brazil, Cuba, India, Malaysia, Nigeria, Tanzania and Zambia (Allen, 1983). The relative importance of cowpea anthracnose varies with ecological zones. In Nigeria, this stem anthracnose is a devastating disease under humid forest conditions. In India, cowpea anthracnose caused by *C. lindemuthianum* has been first reported from Maharashtra in 1966 (Rao, 1966). In Kerala, cowpea anthracnose has become a serious problem in recent years especially in May-June sown crop and an yield loss up to 50 per cent has been reported (KAU, 1996).

2.1 Symptomatology

Onesirosan and Barker (1971) and Williams (1975) studied the symptomatology of anthracnose disease of cowpea. They observed that, the symptoms appeared as individual lenticular to circular, brown to tannish pink, sunken stem lesions with dark red margins. Later on these lesions, developed into large, spreading, dark lesions which girdled stem, branches and petioles. Coalscence of lesions led to chlorosis and death of the leaves. Brown sunken lesions developed on the pods also.

Emechebe and McDonald (1979), Emechebe (1981), Oladiran and Oso (1983) described a brownblotch disease of cowpea caused by *Colletotrichum capsici* and *Colletotrichum truncatum*. The disease developed as purplish or reddish brown blotches on petioles, leaf veins, stems, peduncles and pods without the formation of definite lesions. Girdling resulted in stem collapse. Necrosis of flowering axes caused floral abortion and/or distortion and shrivelling of immature pods. On pods sporulation appeared as alternating black and brown bands, is the

diognostic of the disease and the anthracnose severity was greater on older pods. It also caused dark brown to purplish seed discolouration.

Singh (1985) described a stem and pod anthracnose disease caused by *C. truncatum* on cowpea. The anthracnose on the stem was seen as elongated cankers which girdled the stem causing death of the parts above the point of infection. The entire plant was killed when the cankers developed near the base of the stem. Vascular browning was also seen in the stem. Cankers were dark red in colour. On leaves the cankers appeared first along the veins on the lower surface. Pod blight was first visible as small reddish streaks or blotches. Later the spots became pale brown to greyish in colour and were thickly covered with black fruiting bodies of the fungus.

2.2 Etiology

Anthracnose disease of cowpea caused by *C. lindemuthianum* is a common and important disease. The pathogen belongs to Melanconiales of class Coelomycetes under Deuteromycotina. Its perfect stage is *Glomerella lindemuthianum* (Sacc. and Magn.) in family Polystigmataceae, order Sphaeriales, class Pyrenomycetes of Ascomycotina.

Anthracnose disease, caused by *C. lindemuthianum*, is one of the most serious diseases of common bean and has been intensively studied in North America and Europe. (Hubbeling, 1957; Fouilloux, 1979; Allen, 1983; Alam and Rudolph, 1988).

Onesirosan and Barker (1971) and Williams (1975) suggested that, cowpea isolates of *C. lindemuthianum* were pathogenically distinct from bean isolates. Differences in fungal morphology had also been found between isolates of *C. lindemuthianum* from cowpea and bean. Manifestation of cowpea anthracnose

as a stem disease also differsfrom bean anthracnose where the pod is commonly affected.

Onesirosan and Sagay (1975) pointed out that, the pathogen *C. lindemuthianum*, incitant of stem anthracnose of cowpea, survived the dry season on diseased stem tissues either left on the soil surface or ploughed under.

Allen (1983) reported two species, *C. capsici* and *C. truncatum* causing brown blotch of cowpea and were considered a major threat to cowpea production in African savannas.

Although *C. capsici* was generally regarded as an unspecialized pathogen, no biotypes apparently developed high frequency on cowpea (IITA, 1983). *Colletotrichum dematium* has also been recorded on cowpea in India and Malaysia.

Anthracnose disease of cowpea caused by *C. lindemuthianum* was reported for the first time in Islamabad by Qureshi *et al.* (1985).

Singh and Shukla (1988) reported a leaf blight disease of *Vigna mungo* caused by *C. truncatum*. Pathogenicity of the fungus was confirmed by artificial inoculation of the pathogen into the plant.

Outbreak of a new anthracnose of mung and mash was recorded in Punjab by Bains *et al.* (1989). Red/brown lesions were observed on leaves, stems, branches and pods of mung and mash. The causal organism was isolated and identified as *C. truncatum* and the pathogen was found to be seed borne.

Thakur (1992) observed that *C. dematium* and *C. lindemuthianum*, the pathogens causing anthracnose in mungbean survived on crop residues till the next season. Some weeds were suspected to be the collateral hosts of the pathogen.

Adebitan (1994) showed that a temperature of 15-30°C was suitable for *C. truncatum* to grow on PDA, 28°C being optimum for growth and 25°C for sporulation. Eventhough the growth of fungus was more in complete darkness, sporulation was severely affected.

The infection process of a hemibiotrophic *Colletotrichum* species causing anthracnose disease in cowpea was studied by light microscopy and electron microscopy. During the biotrophic phase, the fungus produced unusual, large, multilobed, multiseptate infection vesicles with elongated neck region. The necrotrophic phase was characterized by rapid development of invasive secondary hyphae (Dada *et al.*, 1994).

Gil *et al.* (1994) studied the morphological and cellular characteristics of *C. lindemuthianum* and *Colletotrichum gloeosporioides* of cowpea using cytochemical techniques, lectins, monoclonal antibody, labelling and DNA sequence analysis.

From the studies conducted by Rahman *et al.* (1994) it has been observed that temperature has a significant role in the mycelial growth and acervuli development of *C. dematium* on culture medium. The fungus grew faster at 30°C with high acervuli formation on PDA + bean extract medium.

2.3 Studies on mycoflora associated with cowpea seeds

Kanapathipillai (1982) isolated many fungi from two seed lots of dolichos bean and cowpea, but *Colletotrichum* sp. and *Macrophomina phaseolina* were found only in cowpea.

Gil *et al.* (1983) observed various species of *Aspergillus* on 40 species of leguminous seeds.

Barros *et al.* (1985) isolated *Fusarium* sp., *Phomopsis* sp., *M. phaseolina*, *Botryodiplodia theobromae*, *Diplodia* sp. and *Phoma* sp. while studying the seed mycoflora of 34 cultivars of cowpea.

Sharma *et al.* (1988) observed *Fusarium moniliforme*, *Penicillium* sp., *Fusarium oxysporum*, *Aspergillus niger* and *C. gloeosporioides* as the dominant mycoflora in cowpea seeds.

Ambika (1991) isolated 20 species of fungi from cowpea and the maximum inhibition in germination of the seeds was found to be caused by *A. flavus*, *Chaetomium brasiliense*, *F. oxysporum* and *Rhizopus* sp.

2.4 Seed borne infection

Seed transmission of anthracnose disease caused by various species of Colletotrichum in different legumes have been reported by many workers.

Ellis *et al.* (1976) observed that *C. gloeosporioides*, causing anthracnose disease of *Stylosanthes* species is seed borne in nature and seed transmission is believed to be responsible for the rapid spread of the disease.

Suryanarayana (1978) stated that in cowpea seeds infection by *M. phaseolina* and *C. lindemuthianum* caused failure of germination and seedling blight.

Emechebe and McDonald (1979) reported that *C. capsici* and *C. truncatum* causing brown blotch of cowpea was seed borne in nature causing pre- and post-emergence damping-off of seedlings. Cowpea pods with brown blotch yielded 100 per cent seed infection in case of *C. capsici*, but only 0-7 per cent for *C. truncatum*.

Seed to plant transmission was 38 per cent and 3 per cent respectively for *C. capsici* and *C. truncatum*.

Prasanna and Ramaprasanna (1980) studied the seed borne aspects of anthracnose of cowpea caused by *C. lindemuthianum* and found that, out of 50 seed samples examined, 18 were infected and the infection was higher in white seeded than in dark seeded varieties. TVX 201 and TVX 30 were proved highly resistant to the disease.

Hepperly *et al.* (1983) observed 70 per cent seed infection in case of soybean anthracnose in seed samples having 30 per cent brown discolouration.

Tu (1983) observed that *C. lindemuthianum* causing anthracnose of beans can survive on seed for two to five years under low moisture condition and the degree of seed transmission is highly correlated with pod infection.

Prasanna (1985) measured the seed infection of cowpea anthracnose pathogen (*C. lindemuthianum*) as 30 to 90 per cent, the fungus being located in the seed coat, cotyledons and embryo. Seed infection is proved to be the primary source of disease spread.

2.5 Host resistance

The use of resistant varieties is a simple, effective and economical means of controlling plant diseases. Many workers have studied the host resistance against *Colletotrichum* sp. in cowpea and other legumes. But the informations on this aspect from India are meagre and scanty.

Williams (1974) exposed several hundred varieties of *Vigna sinensis* to *C. lindemuthianum* by detached petiole inoculation tests and reported that the susceptibility of petiole tissue was a valuable indication of varietal susceptibility.

Williams (1975) observed that 61 ICDN lines of cowpea were immune or highly resistant to *C. lindemuthianum*.

Williams (1977) also screened 5000 cowpea lines against important bacterial and fungal diseases including *C. lindemuthianum* under field condition and the results indicated that 16 cowpea lines had multiple resistance to fungal and bacterial diseases.

In the studies on combined resistance to bacterial blight, scab, leaf spot and brown blotch, one line VITA-4 was found to have resistance to all four pathogens (Allen *et al.*, 1983).

Oladiran and Oso (1983) observed that the severity of brown blotch of cowpea caused by *C. truncatum* and *C. capsici* increased with age of the pods except VITA-1 which didn't show any disease symptoms. The varieties Kano 1696 and IA12 339-1 were found to be moderately resistant to the disease.

Sohi and Rawal (1983) screened 141 cowpea varieties against *C. lindemuthianum* and 21 varieties were found to be resistant to this pathogen.

Dhiman *et al.* (1989) evaluated Sel-263, Pusa Dofasli, Pusa Barasati, L-1552, brown seeded and red seeded cowpeas for multiple resistance to bacterial blight, cowpea mosaic como virus and anthracnose under field condition. Among the varieties tested, only Sel-263 showed multiple resistance to all four diseases.

Out of the 27 cultivars of *Vigna radiata* screened for resistance to *C. dematium* and *C. lindemuthianum*, none had shown resistance in 1985, but Pusa 109 was highly resistant and ML-353 resistant during 1986. Some cultivars showing susceptible or moderately resistant reactions in 1985 had different reactions in the following year (Thakur and Khare, 1989).

Twusami *et al.* (1989) evaluated 62 IITA and local cowpea lines under natural infection for fungal diseases and found out that 30 lines were apparently resistant to multiple diseases, 19 were moderately susceptible and 13 susceptible to more than one disease. When seedlings of 44 lines showing resistance to multiple diseases, were inoculated with *C. truncatum*, 16 were resistant, 15 moderately resistant and 13 susceptible.

In a varietal trial conducted by Wang and Li (1990), it was observed that out of 805 *Phaseolus vulgaris* varieties, 10 were resistant to *C. lindemuthianum*.

Thakur and Khare (1991) noticed that incidence of anthracnose of *Vigna radiata* was higher in cv. J 45 (44.3%) than in P5 16 (18.4%) and Pusa Baisakhi (14.01%), but did not vary significantly among plant populations of different density.

Adebitan *et al.* (1992) screened 12 cowpea cultivars for reaction to infection by *C. lindemuthianum* and *C. truncatum*. Cowpea cultivars IT82E-60, IT81D-1137 and Vita-7 were susceptible to *C. lindemuthianum* whereas TVX 3236, IT 81D-994 and IT 81D-975 were resistant. Cultivars IT 82E--60, IT 82D-699 and Ife brown were susceptible to *C. truncatum* whereas TVX 3236, Vita-7 and IT 81D-1137 were resistant.

2.6 Effect of toxin on disease development

Many fungi which cause leaf spot diseases are also reported to produce toxins. Production of toxins of *Colletotrichum* spp. has also studied by various investigators. Lin (1948) reported the production of a toxic metabolite by *G. cingulata*.

Goodman (1960) reported that the toxin produced by *Colletotrichum fuscum* caused spotting of tomato foliage and this toxin affects plants, which were not attacked by the pathogen.

Production of toxin by *C. gloeosporioides* causing citrus die-back was reported by Sharma and Sharma (1969). Production of toxin *in vitro* took place in Richards solution after 22 days growth.

Janardhanan and Hussain (1970) studied the role of toxic metabolites produced by *G. cingulata* causing blight of jasmine. This toxic metabolite induced rapid wilting and necrosis of jasmine cuttings, symptoms similar to those caused by the fungus.

Narain and Das (1970) reported the production of toxin by *C. capsici* causing anthracnose of chillies. The toxic metabolites were assayed on seed germinability, seedlings, leaves and fruit tissues of chillies and all the test samples exhibited severe toxic injury.

Nair and Ramakrishnan (1973) conducted detailed studies on the toxin produced by *C. capsici* causing leaf spot disease of turmeric. In treatment with exo- and endo toxin solutions, visible alterations in the inoculated area of turmeric leaves were noticed within four hours of inoculation.

Karunakaran (1981) studied the toxin production of three isolates of *C. gloeosporioides* from clove, nutmeg and cinnamon and found that Richard's medium is the best for maximum toxin production.

Varma (1991) studied the effect of exo and endo toxins of *C. gloeosporioides* causing leafspot of *Plumbago indica* and observed that endotoxic metabolites produced symptoms much earlier than that produced by exotoxic metabolites. Richard's medium was found best for toxin production and maximum toxic activity was observed at 20th day of incubation at room temperature.

2.7 Disease management

Efforts to control the diseases caused by *Colletotrichum* species have been made since the early phase of plant protection as this fungus causes severe damage on cultivated crops. The effect of plant protection chemicals, botanicals and bioagents in the management of disease has practical importance and scientific interest. Many workers have conducted extensive studies in these aspects.

2.7.1 Effect of chemicals

Oladiran (1980) tried combined application of fungicide and insecticide for the control of cowpea diseases and the study revealed that fungicide + insecticide treatments together gave the highest yields and the applications of benomyl, difolatan or brestan with gammalin were found to be most effective. He sprayed four fungicides alone or each in combination with the insecticide lindane (gammalin) against stem and pod anthracnose of beans caused by *C. lindemuthianum*. Best control and highest yield were obtained in plots treated with benomyl + gammalin and no phytotoxicity could be observed in plants treated with insecticide and fungicide mixtures.

Barros *et al.* (1981c) observed good control of *C. lindemuthianum* when seeds of cowpea cultivar Alagoano were treated with captafol (100 ppm) and benomyl and thiabendazole each at 500 ppm.

Sindhan and Bose (1981a) tested certain fungicides against *C. lindemuthianum* causing anthracnose of French beans. Benlate followed by bavistin, ziram, vitavax, ferbam and lime sulphur were effective in reducing disease incidence and in increasing the seed yield when applied as foliar sprays. With seed treatment, benlate followed by bavistin, vitavax, ziram and agrosan GN were found effective in reducing the infection. Benlate, bavistin, vitavax and ziram were found effective against the disease as seen dresser as well as foliar sprays. Benlate, bavistin, vitavax and calixin persisted within the plant upto 15-20 days where as all other fungicides lost their effectiveness within 5-10 days after foliar sprays and seed treatment.

Studies on the efficacy of various fungicides for the control of anthracnose of cowpea caused by *C. lindemuthianum* was carried out by Sohi and Rawal (1984). They found that benomyl and bavistin performed best and reduced yield losses from 42.9 per cent to 4.9 per cent and 3.2 per cent respectively.

Interactions between fungicides, insecticides and spraying regimes were tried for controlling many diseases of cowpea. Two sprays of benomyl (1.5 kg ai/ha) + monocrotophos (0.75 kg ai/ha) at 35 and 49 days after planting gave the best control of brown blotch of cowpea caused by *C. truncatum* and infestation of pod borer. The mixtures were not phytotoxic and gave the highest grain yield in two consecutive years (Oladiran and Oso, 1985).

The efficacy of different fungicides on the severity of mung bean anthracnose was evaluated by Thakur and Khare (1989b). The best control of *C. dematium and C. lidemuthianum* on *Vigna radiata,* and highest yields were obtained by foliar sprays of carbendazim (0.1%) and triforine (0.15%).

In another field experiment conducted by Oladiran (1990) it was seen that benomyl @ 1.0 kg ai/ha in combination with either monocrotophos or permethrin @ 0.08 kg ai/ha as foliar spray gave the best control of brown blotch and pod borer infestation of cowpea. The fungicides and insecticides mixtures also gave significantly higher grain yield than when fungicide and insecticide were applied singly.

Rajkumar and Mukhopadhyay (1990) observed that seed treatment with thiram + carbendazim followed by three sprays of carbendazim gave minimum anthrocnose incidence and maximum yield in urd bean. Seed treatment and three sprays of mancozeb were also found to increase yield and reduce disease incidence. They have also noted that carbendazim eradicated the internal seed borne pathogen and thiram eradicated the external seed borne pathogen.

Ravi and Anilkumar (1990) studied about the resistance development in *C. truncatum* to carbendazim and thiophanate fungicides. They found that the resistance obtained was highly stable and the pathogen showed increased sensitivity to copper oxychloride, mancozeb, carboxin and triademefon.

Bharadwaj and Thakur (1991) suggested the application of 0.25 per cent mancozeb, 0.1 per cent carbendazim and 0.25 per cent captafol at 45, 60 and 75 days after sowing respectively for the best control of leaf spot and blight diseases of *Vigna mungo* caused by *Cercospora cruenta, Colletotrichum truncatum* and *Ascochyta phaseolorum.*

Alabi and Emechebe (1992) applied fungicides as seed treatment and foliar spray to control cowpea brown blotch induced by *C. capsici*. Benlate and Delsene M (carbendazim + maneb) were the most effective and the soak method giving the best control of seed borne infection.

Rahman *et al.* (1994) conducted *in vitro* and *in vivo* evaluation of fungicides for controlling anthracnose of country bean and found that tilt 25 EC (propiconazole) and knowin 50 WP (carbendazim) were highly effective.

2.7.2 Effect of botanicals

The scope of regular use of fungicide is limited due to the cost and adverse environmental hazards, besides development of resistance to pathogen. During recent years, use of plant secondary metabolites for the control of fungi is gaining importance. The presence of naturally occurring substance in plants with fungicidal properties has been recognized and tested against a wide range of fungi infecting various crops by many workers. However a search on literature revealed no information about the antifungal effect of plant extracts against *C. lindemuthianum.*

Singh and Dwivedi (1990) tested fungicidal properties of neem and blue gum against *Sclerotium rolfsii* and found that neem oil was the most effective on the volatile and non volatile fractions tested against *S. rolfsii.*

Upadhyaya and Gupta (1990) studied the effect of some medicinal plant extracts on the growth of *Curvularia lunata* and obtained inhibitory effect with ethanol extracts of garlic followed by those of *Ocimum sanctum* and *Datura alba* and aqueous extracts were less effective.

Sundrival (1990) pointed out the inhibition of spore germination and germ tube growth of *Alternaria solani* with the flower extracts of *Acacia arabica, Cassia fistula, Lantana camara* L., *Rhododendron arboreum* and *Thevetia peruviana*. Conidial germination remained completely inhibited by flower extracts from *L. camara.*

Mohan and Ramakrishnan (1991) observed that the extracts of *Allium sativum, L. camara, Azadirachta indica* were highly inhibitory to spore germination and mycelial growth of the pathogen *Exserohilum turcicum* causing leaf blight of sorghum.

Tewari and Nayak (1991) studied the activity of four plant extracts on three fungal pathogens of rice and reported that leaf extracts from betel vine and *Ocimum sanctum* could control *Pyricularia oryzae, Cochliobolus miyabeanus* and *Rhizoctonia solani.*

Patil *et al.* (1992) observed that water extracts of *O. sanctum* inhibited spore germination of *Rhizopus arrhizus* and *B. theobromae* under *in vitro* condition, which checked mycelial growth, reduced protein content and production of pectinolytic and cellulolytic enzymes.

Bhat and Sivaprakasm (1993) observed that cold water extracts of *Polyalthia longifolia* gave maximum inhibition of brinjal damping off pathogen, *Pythium aphanidermatum.* Among the hot water extracts tested, *Parthenium hysterophorus* L. gave maximum inhibition of the pathogen.

Jacob and Sivaprakasam (1993) observed that seed treatment of brinjal seeds with eucalyptus leaf extract as seed soak treatment for 30 minutes prior to sowing was effective in protecting against *Pythium aphanidermatum.*

Evaluation of some plant extracts for antifungal properties were investigated by Kazhmi *et al.* (1993) and observed that hexane extracts of *A. indica* seed, turmeric and *Valeriana officinalis* rhizomes and seed oil of mustard and *Anethum graveolens* were inhibitory to *Alternaria alternata, Aspergillus flavus, A. fumigatus* and *A. asentii,* the fungi community causing spoilage of stored grain.

Meena and Mariappan (1993) showed that leaf extracts of *Azadirachta indica, Mentha arvensis, Aegle marmelos, Catharanthus roseus, Lantana camara, Pongamia pinnata, Vitex negundo* and *Nerium odorum* and flower extracts of *C. roseus* inhibited mycelial growth and spore germination of the seed borne mycoflora of sorghum such as *Alternaria tenuis, Aspergillus flavus, C. lunata, F. moniliforme* and *Rhizopus stolonifer.* The extracts of neem, *Croseus* and *L. camara* were more effective than the other plant extracts tested.

Leaf extract of vilvam (10%) and prosopis (10%) were effective in inhibiting the mycelial growth of *Alternaria tenuis* inciting fruit rot disease of chilli under *in vitro* condition. Spraying vilvam leaf extract on the plant was also effective in reducing the disease intensity followed by that of prosopis, besides giving increased yield (Muthulakshmi and Seetharaman, 1993).

Ramanathan and Sivaprakasam (1993) found that extracts of *Parthenium hysterophorus, Crinum asiaticum* and *Carica papaya* reduced the incidence of pre and post emergence damping off of chilli.

Senthilnathan and Narasimhan (1993) pointed out that leaf extract of vilvam and prosopis were also effective in inhibiting the spore germination and mycelial growth of *A. tenuissima,* causing leaf blight of onion.

Mahapatra and Tewari (1994) reported that ethanol and essential oil extracts of *O. sanctum* inhibited growth and multiplication of *Aspergillus niger* and *A. flavus* and increased seed germination of groundnuts.

Sobti *et al.* (1995) found that the extracts of *Polyalthia longifolia* was effective in controlling *A. niger, M. phaseolina* and *A. flavus*, pathogen of groundnut.

Anandaraj and Leela (1996) observed complete inhibition of mycelial growth, sporangia and zoospore production and zoospore germination of *Phytophthora capsici* causing foot rot of black pepper with *Chromolena odorata* extract at 2.0 per cent concentration.

Simon (1996) reported that leaf extract of *O. sanctum* (10%) was found to be effective in controlling the downy mildew of bittergourd caused by *Pseudoperonospora cubensis* among the four leaf extracts tested under *in vitro, in vivo* and field conditions.

Asha *et al.* (1997) studied fungitoxic properties of ethanol extracts of 10 plant species against *Alternaria brassicola, C. capsici, Fusarium oxysporum, R. solani* and *Sclerotinia sclerotiorum* under *in vitro* condition and leaf extracts of *A. indica, Datura stramonium, O. sanctum* and *P. longifolia* were more fungitoxic than others against all the test fungi.

Kurucheve and Padmavathi (1997) assayed five plant products for fungitoxicity against *P. aphanidermatum* causing damping off of chillies and found that extracts of *Allium sativum* bulbs (10%) recorded the minimum mycelial growth followed by *Lawsonia inermis*.

2.7.3 Effect of antagonists

It is beyond the scope of present studies to go through the extensive literature about the antagonists tested against various pathogens. Leaving aside the vast literature on the antagonist *Trichoderma viride*, its effective use against some of the pathogens causing vegetable diseases may be mentioned for the sure of pertinence.

Weindling (1938) showed that *T. viride*, a common saprophytic fungus, was able to parasitize the mycelia of other fungi. Later he showed that the lethal action of *T. viride* was due to the secretion of an antibiotic substance which he called 'gliotoxin'. Brian and McGowan (1945), isolated another antibiotic substance from *T. viride* which they named 'viridin'.

Wright (1956) found that gliotoxin producing strains of *T. viride* were more effective than the viridin producing strain in controlling seedling diseases caused by *Pythium* sp.

T. viride reduced severity of symptoms due to *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae* and *R. solani* making early flowering and harvesting in tomato (Sporteli *et al.*, 1983).

Krishnamoorthy and Bhaskaran (1990) obtained good control of *Pythium indicum* causing damping off of tomato by soil inoculation with *T. viride*, *T. harzianum* and *Laetisaria arvalis*.

In vitro studies conducted by Marchetti *et al.* (1992) revealed that *R. solani*, *Pythium ultimum* and *Chalara elegans* were strongly inhibited by the antagonistic activity of *T. viride*, *T. harzianum* and *T. pseudokoningii*.

Manomohandas and Sivaprakasam (1993) showed that treating chilli seeds with the spore suspension of *T. hamatum*, *T. harzianum* and *T. reesei* and *T. viride* was found to be more effective than the application of antagnosits to the soil, in enhancing the germination and vigour of the seedlings and reducing the incidence of pre-emergence damping off.

Ramanathan and Sivaprakasam (1993) observed that seed treatment with the antagonists *T. viride* and *T. hamatum* was found to be as effective as TMTD and captan in protecting chilli seeds from damping off caused by *P. aphanidermatum*.

Bankole and Adebanjo (1996) studied the biocontrol of brown blotch of cowpea caused by *C. truncatum* with *T. viride*. Seed dip and soil drenching of this biocontrol agent was found to be very effective. Foliar application also significantly reduced brown blotch incidence in the field.

Ushamalini *et al.* (1997) observed that seed treatment of cowpea with *T. viride* and *T. harzianum* not only increased germination percentage but also reduced charcoal rot incidence significantly.

2.8 Seasonal occurrence

Mordue (1971a)reported that temperature and humidity are important factors in symptom expression of bean anthracnose caused by *C. lindemuthianum*. Infection is favoured by warm wet condition with a temperature of 15-26°C and humidity greater than 92 per cent.

Sindhan and Bose (1981b)found that anthracnose of beans caused by *C. lindemuthianum* in the hills of north India appeared in the second or third week of June and reached its maximum intensity in August-September. He also pointed

out that a relative humidity of 92 per cent and above is essential for infection, the optimum being close to 100 per cent. Infection was found to be higher at 17°C and disease intensity increased with the age of the plant and the best sowing time of French bean was between mid April to mid May for maximising the yield and minimising the disease incidence.

Siddique *et al.* (1983) observed that stem and pod infections of soyabean anthracnose occurred during warm (20-25°C) moist weather. Anthracnose of maturing plants caused serious losses during humid periods.

Thakur and Khare (1991) observed that spores of *C. lindemuthianum* and *C. dematium* were trapped in crops of *Vigna radiata* in highest numbers at a temperature range of 26-29°C, RH 91 to 96 per cent, rainfall 0-21.6 mm and wind velocity of 6-10 km hr^{-1}. The highest count was obtained at the end of July. A diurnal cycle was observed, with a peak in spores trapped during the morning 07.00-12.00 hr. Increase in lesion size was greatest at 100 per cent RH, 27°C and with extended exposure to light (72 h).

Alabi and Emechebe (1992) observed that growth and sporulation of cowpea brown blotch pathogen were greatly influenced by temperature. Cowpea brown blotch is unlikely to develop rapidly in the field when the temperature exceeds 35°C or falls below 20°C for several days. Air temperature during the growth season is conducive to spread the disease, with minimum and maximum mean values of 21.5°C and 31.3°C respectively in July-October.

Thakur and Khare (1992) reported that *V. radiata* is severly affected by *C. dematium* and *C. lindemuthianum* during monsoon season. Disease intensity was positively correlated with RH and rainfall and negatively with maximum and minimum temperature. Disease scores were highest at 90-100 per cent RH and

26-29°C with low rainfall (2.5-53.5 mm). Early sowings in June were most vulnerable than late sowings in July.

In vitro and *in vivo* studies conducted by Thakur and Khare (1993) revealed that a temperature of 25°C and RH 90-100 per cent were most favourable for maximum sporulation and germination of *C. lindemuthianum* and *C. dematium* on *V. radiata*.

Carma *et al.* (1994) reported that the incidence and spread of anthracnose of bean was same when bean was grown as monocrop and intercrop with maize and disease spread was severe when the row spacing of 0.5 m was adopted.

2.9 Crop loss assessment

The effect of *C. lindemuthianum* on the growth of *Vigna sesquipedalis* was studied by Wong and Thrower (1978) and observed significant reduction in the dry weight of the plant due to infection.

The extent of yield losses resulting from anthracnose was determined in three bean cultivars by Sohi and Rawal (1984). There were highly significant differences in disease level and yield between the sprayed and unsprayed plots. Highly significant yield losses of 86 per cent occurred in the highly susceptible cultivars and 27 per cent in the moderately susceptible cultivar. There was an estimated net gain of approximately US $ 1010.40 ha^{-1} resulting from controlling anthraconose by benomyl and US $ 652.70 ha^{-1} by growing an anthracnose resistant cultivar.

Dada (1990) assessed crop loss due to anthracnose disease in some cultivars of cowpea caused by *C. lindemuthianum*. Disease progress was assessed in five cowpea cultivars differing in susceptibility to *C. lindemuthianum* by six

weekly measurements of the length of necrotic lesions on stems and peduncles as well as by a visual symptom assessment key. A better correlation was found between visual scores and lengths of peduncle lesions than between visual scores and lengths of stem lesions. An antifungal compound was detected in resistant but not in susceptible ones.

Materials and Methods

MATERIALS AND METHODS

Investigations on various aspects of anthracnose disease of cowpea were conducted in the vegetable plot of Department of Olericulture, College of Horticulture, Vellanikkara. The soil of the experimental plot was laterite type. The major experiments were conducted during October to December 1997 except the studies on seasonal occurrence which was conducted during June-August (SW monsoon period), September-November (NE monsoon period) and January to March 1998 (Summer). The variety "Pusa Komal" was used for various experiments unless otherwise mentioned. "Pusa Komal" is a bush type short duration (65-70 days) variety, highly susceptible to anthracnose disease.

3.1 Collection, isolation and identification of the pathogen associated with anthracnose disease

Cowpea plants infected with anthracnose disease were collected during September 1997 from four locations viz., Vellanikkara (Thrissur district), Angamali (Ernakulam district), Kanakkari (Kottayam district) and Vellayani (Trivandrum district). The collected specimens of anthracnose disease from different locations were brought to the laboratory, washed under tap water and dried with blotting paper after separating into stem, leaves, petioles and pods. Isolation of the pathogen was done using standard technique. Various parts of the plant such as, stem, leaf lamina, leaf vein, petioles and pods were used for the isolation. The infected plant parts were cut into small bits of 5 mm size and then surface sterilized with 0.1 per cent mercuric chloride for 15 seconds and washed with three changes of sterile water. The bits were then transferred aseptically to sterile petridishes containing potato dextrose agar medium and also in sterile petridishes lined with sterilized moistened blotting paper. All petridishes were incubated at room temperature (25±2°C) and examined daily for the growth of the pathogen for three days. Fungi

developed on PDA were isolated, purified by hyphal tip method and identified (Riker and Riker, 1936).

The host tissues kept in the moist chamber were observed under microscope after 48 hours of incubation to identify the pathogen.

As the preliminary study indicated slow growth and less sporulation of the fungus in PDA medium, pure cultures of different isolates were maintained in potato carrot agar medium for various other experiments. Vellanikkara isolate was used for host resistance, bioassay and toxin studies.

3.2 Pathogenicity of the fungi associated with anthracnose disease

Pathogenicity of the fungi associated with anthracnose disease was studied in pot culture by artificial inoculation under *in vitro* and *in vivo* condition.

3.2.1 *In vitro* condition

A total number of ten seeds were sown in each plastic pot. When the seeds germinated, five healthy seedlings were retained in each and excess seedlings were thinned out. The seedlings were sprayed 20 days after sowing, with 10 ml of spore suspensions of different isolates having a concentration of 10^6 spores/ml by using an atomizer. Inoculated plants were covered with bell jar to provide proper humidity and kept in laboratory condition. Three pots were kept for each location. Inoculated plants were observed daily for the disease appearance and the symptoms produced were noted. Seedlings inoculated with sterile water served as control. Pathogen was reisolated from the infected plants and then compared with the original culture.

3.2.2 *In vivo* condition

Cowpea seeds were sown in earthen pots of size 20 cm diameter @ 15/pot. Three pots were maintained for each isolate and kept under natural condition in iron framed cages covered with muslin cloth. After germination, ten plants were retained in each pot. The seedlings were inoculated 20 days after sowing, with spore suspensions of different isolates, as mentioned under *in vitro* condition.

3.3 Morphological characters of the pathogen causing anthracnose disease

The identification of the fungi associated with anthracnose disease was done based on their morphological characters. Different media such as potato dextrose agar, potato carrot agar and neopeptone-glucose agar (Appendix I) were used for studying the morphological characters of the fungi. Neopeptone-glucose agar is a selective medium for the cultivation and sporulation of *Colletotrichum lindemuthianum* (Mathur *et al.*, 1950).

3.4 Symptomatology

Symptoms produced by the pathogen on different parts of the plants under natural condition and on artificial infection were observed.

3.5 Studies on mycoflora associated with the cowpea seeds

3.5.1 Blotter method

The germination percentage and mycoflora associated with cowpea seeds collected from healthy and diseased plants were studied by blotter method (ISTA, 1966).

Twenty cowpea seeds, each of healthy and diseased seeds, were placed in large sterile petridishes lined with sterilized moistened filter papers with three replications. These petridishes were incubated at room temperature (28 ± 2°C). Observations on the germination was taken daily for a period of seven days. Ungerminated seeds were taken out and placed on PDA medium in sterilized petridishes and incubated at room temperature and examined daily for the growth of the fungi. Fungi developed were isolated, purified by hyphal tip method and identified. Similarly seeds treated with carbendazim @ 1.0 g/kg seed were also kept to find out the per cent inhibition in germination due to mycoflora.

3.5.2 Agar plate method

Five seeds were placed in each of the five sterilized petridishes containing PDA medium. Seeds of healthy and diseased plants were kept separately. These dishes were incubated at room temperature and examined daily for the growth of the fungi. The fungi developed were isolated as mentioned above.

3.5.3 Pot culture method

Fifty numbers of treated (carbendazim @ 1.0 g/kg seed) and untreated healthy and diseased seeds were sown separately in big plastic trays of size 40x30cm. Observations on the germination was taken daily for 7 days and ungerminated seeds were removed, surface sterilized and transferred aseptically to PDA medium as mentioned in 3.5.1.

3.6 Studies on the seed borne nature of the pathogen

3.6.1 Agar plate method

Seeds collected from diseased and healthy plants were surface sterilized with 0.1 per cent mercuric chloride for 15 sec. and passed through three changes of sterile water and then plated on PCA medium.

Similarly diseased and healthy seeds were soaked separately in sterile distilled water for 4 h. Seed coat, embryo and cotyledons were separated out, surface sterilized and transferred aseptically to sterilized petridishes containing PCA medium. Three petridishes were kept for each sample. Fungi developed were isolated, purified and identified.

3.6.2 Pot culture method

Hundred seeds of each diseased and healthy plants were sown separately in earthen pots of size 20 cm diameter @ 10 seeds/pot. These pots were kept in iron framed cages covered with muslin cloth to prevent the natural infection and the seedlings were observed periodically for the symptom appearance.

3.7 Production of toxin and disease development

An experiment was conducted under laboratory condition to find out the effect of toxin on disease development.

Ten seeds were sown in each plastic pot. Seedlings were thinned out retaining five healthy seedlings in each pot. The pathogen was cultured in selective liquid medium. Thirty ml of the medium was taken in each 250 ml flask and sterilized by autoclaving. All flasks were inoculated with uniform mycelial discs of

5 mm diameter obtained from the actively growing seven day old culture of the fungus. These flasks were incubated at room temperature for 20 days, as the production of toxin was found to be maximum at this period (Varma,1991). After the desired period of incubation, medium containing the fungal mycelium was filtered through Whatman No.1 filter paper to get the exotoxin. Then the mycelial mat was homogenised with five volumes of water and centrifuged at 1000 rpm for 15 minutes. Pellets were discarded and the supernatent was taken, containing the endotoxin. Seedlings were sprayed with endotoxin and exotoxin separately. Three replications were maintained for both toxins. Seedlings were covered with bell jar to maintain the humidity and observed daily for the development of symptoms. Seedlings sprayed with sterile distilled water served as control.

3.8 Evaluation of cowpea genotypes for resistance against anthracnose disease

Different genotypes of cowpea were collected from the Department of Olericulture, KHDP and NBPGR, Vellanikkara. These genotypes were screened for their resistance to anthracnose disease during October 1997 after categorised into bush type, semierect and pole type. Experiment was laid out in RBD with two replications. The following varieties/lines were used for the experiment.

Bush type:

VS-4, VS-12, VS 14, VS-18, VS-28, VS-29, VS-29-1, VS-81, VS-389, RCV-7, Cowpea-263, Selection-16, Pusa Komal, Kanakamony.

Semi erect type:

VS-15-1, VS-15-9-1, VS-19-2, VS-35-1, VS-36-1, VS- 36-2, VS-37, VS-51, VS-82, VS-84, CWP-16.

Pole type:

VS-2, VS-5, VS-6, VS-11, VS-19-1, VS-21, VS-34, VS-48, VS-53, VS-58, VS-87, VS-88, VS-91, VS-94, IC-97857, IC-97860, IC-97862, IC-97784-A, IC-97784-B, NIC-13655, NIC-13659, NIC-13755, Call No.V90/062-A, Call no.V90/062-B, Malika.

3.8.1 Preparation of field

Land was ploughed thoroughly, weeds and stubbles were removed. Channels of 1.5 m long, 30 cm broad and 15 cm deep were taken. Two seeds were sown per hole at a spacing of 40 cm between rows and 15 cm between plants. Crop received the respective cultural and manurial practices as recommended by Package of Practices, KAU (1996). Thinning was done at two leaf stage thereby retaining one plant per hole. There were 18 plants for each variety (9 plants/channel). Pole type and semi spreading genotypes were trailed on cut tree branches. Observations on disease incidence and disease severity were taken at 10 days interval after the first appearance of the disease. Twelve plants were selected from each variety for scoring the disease after tagging the plants serially. Yield data were also recorded. Disease severity was assessed using 0-5 scale as mentioned below (Dada, 1990).

0 - No infection

0.5 - Hypersensitive spots on main stem only

1 - Trace of infection - Small anthracnose lesions on main stem and petioles of lower leaves only

2 - Slight infection - Lesions on stem, petioles and branches

3 - Moderate infection - Advanced anthracnose lesions on stem, petioles, branches, veins on the abaxial surface of leaves

4 - Severe - Advanced anthracnose lesions on stem, petioles, branches, leaves, veins and peduncles

5 - Very severe - Advanced lesions on stem, petioles, branches, leaf veins spreading lesions on peduncles and pods.

Based on the percentage of plant area infected, disease severity/intensity was calculated using the following formula (Wheeler, 1969).

$$\text{Per cent disease severity} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of plants taken for observation}} \times \frac{100}{\text{Max. disease category}}$$

Per cent disease incidence was calculated by using the following formula

$$\text{Per cent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

Based on the per cent disease severity, the genotypes were grouped into 5 categories as adopted by Rajkumar *et al.*,(1995).

Disease severity (%)	Category
0	Immune
1-10	Highly resistant
10.1-25	Moderately resistant
25.1-50	Moderately susceptible
Above 50	Highly susceptible

3.8.2 Screening of the resistant genotypes by artificial inoculation of the pathogen

The genotypes found to be resistant were sown again in the field as mentioned in 3.7.1. Inoculum was prepared in sterilized water having concentration of 10^6 spores/ml. Artificial inoculation was done twice, one at seedling stage and

second at flowering stage. Plants were thoroughly sprayed with spore suspension using a plastic sprayer, in the evening. Quantity of the spore suspension required varied with the size of the plants. Infected plant debris of the previous crop were also retained in the field, to provide sufficient inoculum for the infection.

Similarly, seeds of resistant genotypes were sown in plastic pots and artificial inoculation was done as mentioned above. Inoculated plants were covered with moistened polythene bags to provide humidity and kept in the laboratory for disease appearance.

3.9 *Management of anthracnose disease of cowpea*

Effectiveness of the selected plant protection chemicals, botanicals and the antagonist *Trichoderma viride* (Perr. ex. Fr.) were tested against anthracnose pathogen under *in vitro* and field conditions.

3.9.1 Details of the plant protection chemicals used

For the study, contact and systemic fungicides were used as given below:

Sl.No.	Common/generic name	Trade name	Concentration used
1	Chlorothalonil	Kavach 75 WP	0.2%
2	Copper oxychloride	Blue copper 50 W	0.3%
3	Mancozeb	Indofil M-45 75 WP	0.2%
4	Captan	Captaf 50 WDP	0.2%
5	Carbendazim	Bavistin 50 WP	0.1%
6	Tridemorph	Calixin 75 EC	0.1%
7	Hexaconazole	Contaf 5 EC	0.1%
8	Triforine	Saprol EG	0.1%
9	Triademefon	Bayleton 25 WP	0.1%

3.9.1.1 Details of the plant extracts used in the experiment

Aqueous plant extracts were prepared by macerating the plant material using pestle and mortar in a ratio of 1:1 (W/V). The extract was filtered through muslin cloth and from this extract, the required concentration (10%) of the various plant extracts were prepared.

The following plants were used for the study.

Sl. No.	Botanical name	Common name	Malayalam name	Family
1	*Allium sativum* L.	Garlic	Veluthulli	Alliaceae
2	*Leucas aspera* (Willd.) Link	Leucas	Thumba	Labiatae
3	*Lantana camera* L.	Lantana	Poochedi	Verbenaceae
4	*Ocimum sanctum* Linn. Mant	Ocimum	Thulasi	Labiatae

3.9.1.2 Preparation of antagonist suspension

A talc based formulation of *T. viride* obtained from TNAU Coimbatore, was used for the experiment. For the seed treatment, it was used @ 4g/kg seed and for foliar spraying aqueous spore suspension of the fungus was prepared having concentration of 10^6 spores/ml.

3.9.1.3 Details of the treatments used in the various disease management studies are as follows.

Treatments	Chemical/botanical/ antagonist	Concentrations used
T_1	Chlorothalonil	0.2%
T_2	Copper oxychloride	0.3%
T_3	Mancozeb	0.2%
T_4	Captan	0.2%
T_5	Carbendazim	0.1%
T_6	Tridemorph	0.1%
T_7	Hexaconazole	0.1%
T_8	Triforine	0.1%
T_9	Triademefon	0.1%
T_{10}	Garlic bulb extract	10%
T_{11}	Leucas leaf extract	10%
T_{12}	Lantana leaf extract	10%
T_{13}	Ocimum leaf extract	10%
T_{14}	*Trichoderma viride*	10^6 spores/ml
T_{15}	Control (No treatment)	

3.9.2 *In vitro* evaluation of chemicals, botanicals and antagonist against anthracnose pathogen

In bioassay studies, selective medium and potato carrot agar medium were used as both were found supporting the growth of the pathogen. In the case of chemicals, recommended doses of various fungicides were used, whereas in the case of botanicals, the concentration was 10 per cent.

3.9.2.1 *In vitro* evaluation of contact fungicides

Efficacy of different contact fungicides were studied by the poisoned food technique (Zentmyer, 1955). The fungicides tested were chlorothalonil (0.2%), copper oxychloride (0.3%), mancozeb (0.2%) and captan (0.2%).

Sixty ml of each media was taken in 250 ml conical flask and sterilized at 1.05 kg/cm^2 pressure for 20 minutes. The chemicals were mixed with the media in suitable proportion to get the desired concentrations and poured into sterilized petridishes @ 20 ml per plate. Mycelial discs of 5 mm diameter were cut from actively growing seven day old culture of the fungus and placed in the centre of each petridish containing poisoned medium. Three replications were maintained for each fungicide in different media. Checks were maintained, without the addition of the fungicide. Mean radial growth of the fungal colony was recorded when the growth in the control plates fully covered the medium.

The per cent inhibition of growth was calculated by the formula suggested by Vincent (1927)

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

C = radial growth of the fungus in control

T = radial growth of the fungus in treatment

3.9.2.2 *In vitro* evaluation of botanicals

Twenty five gram leaves of Ocimum, Lantana, Leucas and garlic bulb were taken separately, disinfested with 70 per cent ethyl alcohol and then washed thrice with sterile distilled water. The extract was prepared by macerating in sterilized pestle and mortar with 25 ml of sterile distilled water under aseptic condition. The extract was filtered through sterilized Whatman No.1 filter paper. Two ml of the standard extract was added to 20 ml cool and melted medium and poured into sterile petridishes. Mycelial discs of 5 mm diameter were cut from actively growing culture of the fungus and placed in the centre of medium. Each treatment was replicated thrice along with the control. Mean radial growth of the fungal colony was recorded when the growth in the control plates were fully covered the medium. The per cent inhibition of growth was calculated as mentioned in 3.9.2.1.

3.9.2.3 *In vitro* evaluation of fungal antagonist *Trichoderma viride*

The antagonistic property of *T. viride* against anthracnose pathogen was determined by dual culture method (Johnson and Curl, 1972). Twenty ml of each medium was transferred into sterilized petridishes. After solidification of the medium, mycelial discs of 5 mm diameter was cut from actively growing culture of the fungal antagonist *T. viride* and placed in the centre of one half of the petridish. Mycelial disc of 5 mm diameter of actively growing test fungus from another plate was similarly transferred and placed at the centre of other half of the same petridish.

The growth measurements were taken daily for nine days. The type of antagonism exhibited was recorded. Three replications were maintained for each media. The pathogen and antagonist grown in monocultures served as control.

3.9.2.4 *In vitro* evaluation of systemic fungicides

Required concentrations (0.1%) of the fungicides were prepared in sterile water. Roots of the seedlings were washed free of soil and then washed with sterile water. They were dipped in the fungicidal solution taken in test tubes for 2 h. The test tubes were covered with black paper to prevent the roots from turning green. Seedlings were taken out from the fungicidal solution and stem portion was disinfested with 70 per cent ethyl alcohol and then washed twice with sterile distilled water. Spore suspension of the fungus was prepared from seven day old culture with sterile distilled water (10^6 spores/ml). Twenty ml of the cool and melted medium was poured into sterile petridishes and allowed to solidify, 0.1 ml of the spore suspension was poured on the medium and spread with a sterilized glass spreader. Mid stem portion of the seedlings were taken in bits of size 5 mm and placed at the centre of the seeded medium. Seedlings dipped in sterile water served as control. Three replications were kept for each treatment for different media. Plates were incubated at room temperature and inhibition zone of the fungal growth around the bit was measured.

The per cent inhibition of growth was calculated by using the formula

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

C = radial growth of the fungus in control

T = radial growth of the fungus in treatment

3.9.3 Evaluation of fungicides/botanicals and bioagent under field condition

To find out the efficacy of fungicides, plant extracts and antagonist, in reducing the severity of the disease, a field trial was conducted during October-December, 1997.

3.9.3.1 Details of the field experiment were as follows:

Variety	- Pusa Komal
Design	- RBD
Replication	- 2
Treatments	- 15

Field was prepared thoroughly and channels were taken as mentioned in 3.7.1. There were 18 plants for each treatment. Crop received the respective cultural and manurial practice as recommended by the package of practices, KAU (1996). Treatment consisted of seed treatment alone, seed treatment + foliar application and foliar application alone. For the seed treatment, 150 seeds were soaked in 100 ml solutions each of the fungicides, botanicals and antagonist having desired concentrations, for 2 h. Seeds dipped in sterile water served as control. Details of the treatments are given in 3.9.1.3. Spraying was given at 15 days interval after sowing i.e., during the vegetative and flowering stage of the plant. In each treatment, 12 plants were selected and tagged for taking the observations. Observations were recorded at 10 days interval after the last spraying. Disease incidence and severity were scored as mentioned in 3.8.1. Yield data was recorded and the cost:benefit ratio was worked out.

3.10 Seasonal influence on the incidence of anthracnose disease

In order to find out the seasonal effect on the incidence of anthracnose disease, a field experiment was conducted during three seasons of the year i.e., SW monsoon (June-August), NE monsoon (September-November) and summer periods (January-March). Seeds were sown in raised beds of size 2 m x 1 m at a spacing of 50 cm between rows and 20 cm between plants. Disease incidence and disease severity were scored using the standard score chart. Yield and meteorological data were also recorded in all seasons.

3.11 Assessment of crop loss in cowpea due to anthracnose disease

To assess the crop loss due to anthracnose disease, a field experiment was conducted on susceptible variety, Pusa Komal, during October to December 1997. The experiment was conducted in RBD with six replications and two treatments. There were 18 plants per plot. The crop was raised as mentioned in 3.8.1. In the first treatment disease was kept at minimum by seed treatment with carbendazim @ 1 g/kg seed and the plants were sprayed with same fungicide (0.1%) at fortnightly interval which was found to be one of the effective systemic fungicide in *in vitro* experiment and the second one, without any chemical treatment. For quantifying the amount of infection in each plot, the per cent disease incidence and disease severity of 12 plants were recorded at 60 days after sowing. Replication wise, yield data were taken and the cost:benefit ratio was worked out. From percent disease incidence and percent disease severity (intensity) values, coefficient of disease index (CODEX) was then calculated as suggested by Datar and Mayee (1981).

$$\text{CODEX} = \frac{\text{Per cent disease incidence} \times \text{Per cent disease intensity}}{100}$$

For recording yield loss due to disease, yield of every plot was taken separately and yield loss was calculated by the following formula

$$\text{Per cent yield loss} = \frac{\text{Yield in treatment} - \text{Yield in control}}{\text{Yield in treatment}} \times 100$$

Yield in treatment = Yield of plots treated with carbendazim

Yield in control = Yield of plots without fungicidal treatment

3.11.1 Extent of plant damage due to anthracnose disease

To study the extent of plant damage due to anthracnose disease, 10 plants were selected and tagged in each plots of treated and untreated, diseased and healthy (completely disease free) plants. Height, total number of leaves, vines, pods, extent of infection in all these parts were recorded at 60 days after sowing.

3.12 Statistical analysis

Data related to different experiment was statistically analysed as described by Panse and Sukhatme (1978).

RESULTS

Investigations on various aspects of anthracnose disease of cowpea such as, symptomatology, etiology, seed borne nature of the pathogen, host resistance, management of disease, influence of environmental condition on disease development and crop loss due to anthracnose disease were carried out and the results are presented.

4.1 Collection, isolation and identification of the pathogen associated with anthracnose disease

Cowpea plants infected with anthracnose disease were collected from four locations viz., Vellanikkara, Angamali, Kanakkari and Vellayani. Fungal growth was observed on PDA medium in all infected plant parts used for isolation except in leaf lamina showing black dots (mildew) symptom. In all infected plant parts obtained from Vellanikkara, Angamali and Vellayani, the pathogen associated with the disease was found to be *Colletotrichum lindemuthianum* (Sacc and Magn) Br and Cav. Whereas the infected plant parts collected from Kanakkari areas showed the association of two pathogenic fungi, *C. lindemuthianum* and *Colletotrichum capsici* (Syd.) Butler and Bisby, causing different types of symptoms. These fungi were identified based on the morphological characters mentioned later in this chapter.

In host tissues kept in blotter method, numerous acervuli with conidiophores and conidia were observed and the fungi was same as that obtained on PDA medium.

4.2 Pathogenicity of the fungi associated with anthracnose disease

Pathogenicity of different isolates of the pathogen was proved under *in vitro* and *in vivo* conditions. In both conditions typical symptoms of anthracnose disease were observed on stem, petioles and leaf veins of the inoculated seedlings except mildew symptom.

In both conditions, symptoms were first observed on 3-4 days of inoculation. The pathogen was isolated from the infected portion and compared with the original culture. The pathogenicity test thus indicated that, *C. lindemuthianum* and *C. capsici* are the pathogens causing anthracnose disease of cowpea and *C. lindemuthianum* was found to the most prevalent pathogen in Kerala.

4.3 Morphological characters of the pathogen causing anthracnose disease

Different isolates of the pathogen associated with anthracnose disease were identified based on the morphological characters (Mordue, 1971a & b). Studies on colony characters and spore characters revealed that all isolates were *Colletotrichum lindemuthianum* (Sacc & Magn.) Br & Cav. except one of the isolate obtained from Kanakkari area. The common morphological characters of *C. lindemuthianum* in three different media, potato dextrose agar medium, potato carrot agar medium and neopeptone glucose agar medium, are presented below:

Potato dextrose agar medium

Slow growth (9 cm diameter growth in 12 days at room temperature), colonies at first grey, then rapidly turned darker with compact aerial mycelium and reverse of the colony almost black. No pink pigmentation, setae present, no

appressoria and chlamydospores, dark, hard spherical sclerotia were abundant in old cultures [Plate I(k)] and less sporulation (5.5 x 10^2 spores/ml).

Potato carrot agar medium

Fast growth (9 cm diameter growth in 9 days at room temperature), colonies at first whitish grey with compact fluffy aerial mycelium, dark pink pigmentation, then rapidly turned darker with reverse of the colony black, setae present, numerous appressoria which were dark brown, ovate to obclavate ranging from 10.68-17.8 µm x 7.12-17.8 µm in size [Plate I(i)], intercalary chlamydospores were abundant in old cultures [Plate I(j)], sclerotia absent and high sporulation (39 x 10^4 spores/ml).

Neopeptone glucose agar medium (selective medium)

Colonies very fast growing (9 cm diameter growth in 7 days at room temperature), sparse whitish mycelium, light pink pigmentation, no black discolouration either on or reverse side of the colonies. Setae abundant in old culture. Appressoria and intercalary chlamydospores absent. Very few sclerotia like structures observed and high sporulation (35 x 10^4 spores/ml).

Conidia hyaline, cylindrical with both ends obtuse, aseptate, uninucleate measuring 14.2-17.75 µm x 3.56 µm [Plate I (a to d)]. In the host tissues, setae were dark brown, septate, swollen at the base, slightly tapered to the rounded apex, 3.56 µm wide and 46.2-99.68 µm long [Plate I(f) and Fig.1(a)].

One of the isolate obtained from Kanakkari region was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby, based on the morphological characters, which are mentioned below (Mordue, 1971b).

On PDA at room temperature, colonies were dense, first whitish grey, rapidly turning grey, reverse of the colony black, abundant acervuli with dark setae. Setae dark brown, septate, rigid, swollen at the base, slightly tapered to the paler acute apex measuring 60.52-153.08 µm in length and 3.56-7.12 µm wide [Plate I(g & h)].

Conidia hyaline, falcate with acute apex and narrow truncate base, aseptate, uninucleate measuring 17.8-24.92 µm x 3.56 µ in size, formed from unicellular, hyaline,phialidic conidiophores [Plate :I(e)].

4.4 Symptomatology of anthracnose disease of cowpea under natural condition

Anthracnose disease of cowpea is principally a stem disease. Eventhough all parts of the plant above ground are affected, the manifestation of the disease is mainly seen on the stem. Symptoms of the anthracnose disease observed at different locations were almost same with slight variations. The common symptoms observed are mentioned below:

Vellanikkara (Thrissur district)

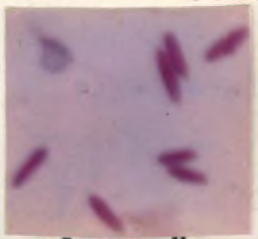
The symptoms of the disease were found to be varied with the wet and dry conditions.

In wet conditions, i.e., during S.W. monsoon period, the symptoms first appeared as reddish brown streaks on stem. The streaks later coalesced and spread all over the stem resulting in wilting of seedlings at early stage [PlateII(a)]. As the age advanced, plants showed rotting of the top portion of the stem, which resulted in the toppling of the foliage. Similar type of symptoms as developed on the main stem was noticed on petioles. On leaves, reddish brown streaks appeared on the veins of

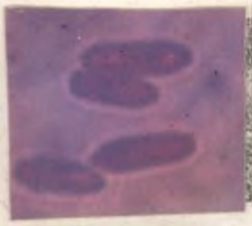
Plate I MORPHOLOGICAL CHARACTERS

CONIDIA

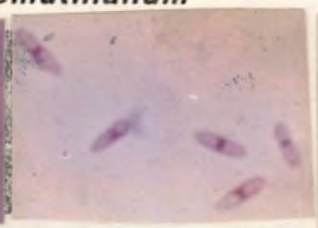
Colletotrichum lindemuthianum *Colletotrichum capsici*



a Angamali (10X10x)

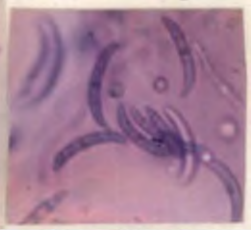


b. Kanakkari (10 X40x)



c. Vellanikkara (10 X10x)

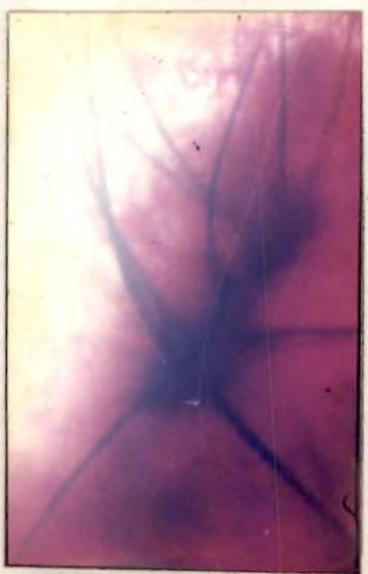
d.Vellayani (10 X10x)



e.Kanakkari (10 X10x)

ACERVULI

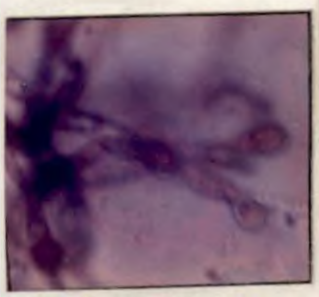
f.*C. lindemuthianum* on host tissue (10X20x)



g. *C. capsici* on host tissue (10X20x)

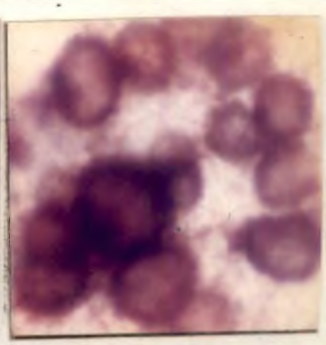
h. *C. capsici* in culture (10X20x)

Colletorichum lindemuthianum



i.Appressoria (10X20x)

j. Chlamydospores (10X40x)



k. Sclerotia-like bodies (10X40x)

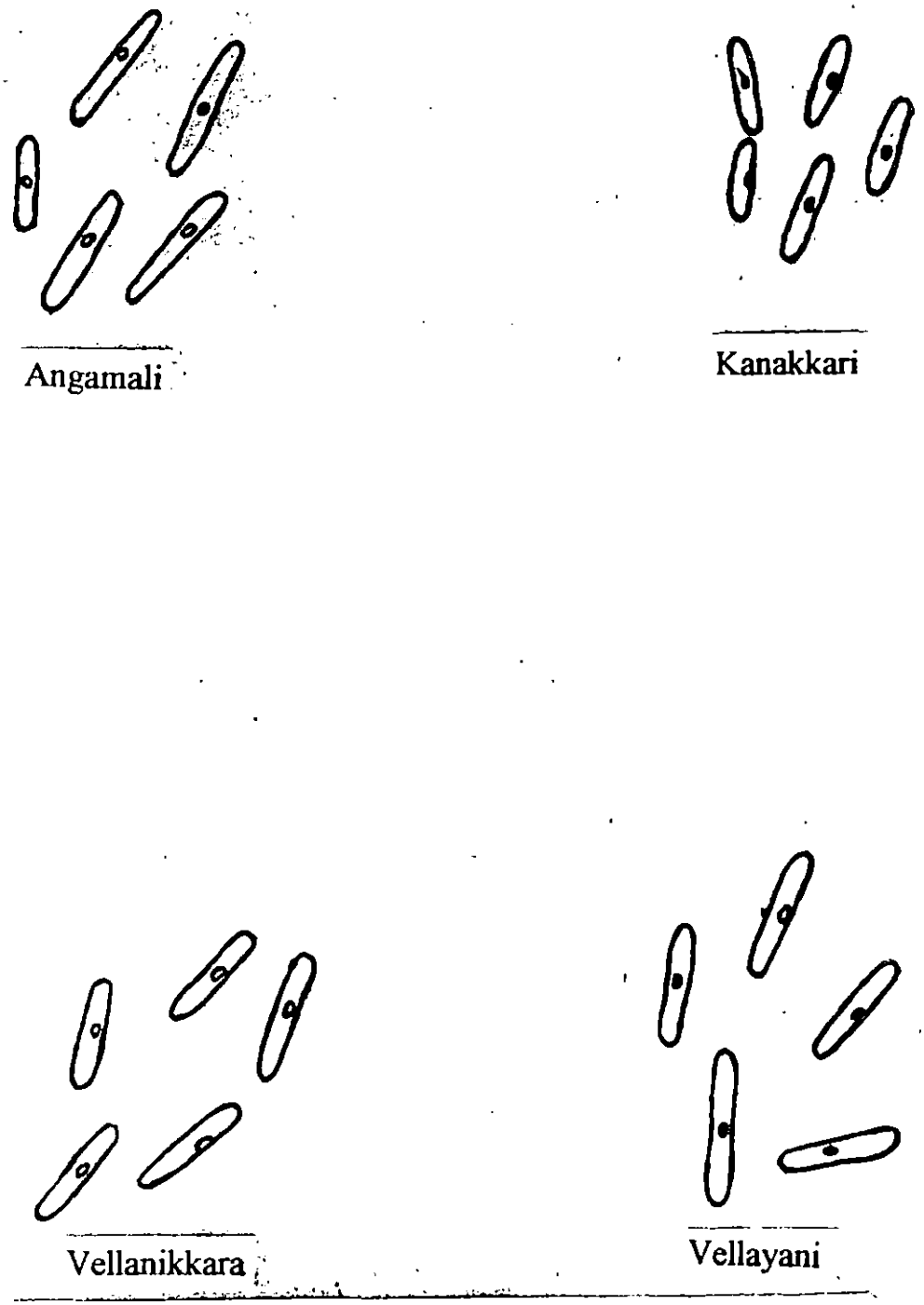


Fig.1(a) Spores of *Colletotrichum lindemuthianum* from different locations

lower surface, later extended to upper surface also. In few plants, numerous minute black dots (mildew appearance) were observed on leaf lamina. Finally the leaves became chlorotic and defoliated. Reddish brown streaks were developed on pods and resulted in rotting of the pods. Plant as a whole looked chlorotic and stunted [Plate II(b)]. Disease spread very rapidly to the neighbouring plants and caused heavy losses.

In dry conditions (other seasons), anthracnose on the stem was seen as individual lenticular to oval light brown lesions with dark reddish brown margins. These lesions coalesced and extended all over the stem and vines leading to drying up of the stem and vines. Black fruiting bodies of the fungus were observed on the dried parts [Plate II(c & d)].

On leaves reddish brown streaks appeared along the veins on the lower surface, then spread to veinlets and later, on to the upper surface [Plate II(e)]. The affected leaves became chlorotic and defoliated. In rare cases, mildew symptom was observed on the leaf lamina.

Reddish brown streaks appeared on petioles which later turned black showing mildew appearance as seen on clothes.

On pods reddish brown streaks/lesions developed, which subsequently became greyish in colour and were covered with black fruiting bodies of the fungus. Seeds were shrivelled and discoloured [Plate II(f)].

Angamali (Ernakulam district)

The symptoms observed were similar as described above. In addition to the anthracnose symptoms, basel swelling, splitting and rotting of the stem were

also observed in few plants [Plate II(h)]. But on artificial inoculation with *C. lindemuthianum*, no basal swelling or splitting or rotting of the stem was noticed.

Kanakkari (Kottayam district)

In Kanakkari areas of Kottayam district, two types of symptoms were observed on infected plants. In one type, reddish brown lesions developed on the stem. These individual lesions coalesced to form large sunken lesions and covered the whole stem causing drying up of the vines. On leaves, reddish brown streaks were very prominent on veins and veinlets. Mildew symptoms were also observed in mild forms on leaf lamina.

In the second type, symptoms on the stem were observed as small individual black sunken lesions which later enlarged and spread all over the stem and vines showing typical mildew or vine blackening symptom. On leaves, the streaks were black and were very prominent on the veins and veinlets. Prominent mildew symptoms were also observed on the leaf lamina [Plate II(g)].

Vellayani (Thiruvananthapuram district)

In this area, the disease symptoms were the same as that observed at Vellanikkara.

Symptomatology on artificial inoculation

On artificial inoculation, same type of symptoms as described above were observed in the laboratory as well as in the field conditions with the exceptions of the basal swelling symptoms, which was noticed only in Angamali area and the black mildew symptom, observed in few plants.

PlateII SYMPTOMATOLOGY

a. Seeding Wilt

b. Toppling of the foliage

c. Plant showing anthracnose symptom

SYMPTOMS ON VARIOUS PARTS

d. Stem

e. Leaves

f. Pods

g. Symptoms of *C. capsici*

h. Basal swelling and anthracnose symptoms observed at Angamali

4.5 Studies on mycoflora associated with cowpea seeds

Mycoflora associated with cowpea seeds were collected from healthy and diseased plants and were studied by blotter, agar plate and pot culture methods. Germination percentage of seeds and per cent inhibition of germination due to mycoflora were recorded and presented in Table 1. Both saprophytic and pathogenic fungi were found associated with both healthy and diseased seeds. As compared to the healthy, association of mycoflora was more in diseased seeds A number of fungi were observed on diseased seeds in agar plate as compared to that of the blotter and pot culture methods. In all the three methods, *C. lindemuthianum*, the causal agent of anthracnose disease, was noticed on diseased seeds in addition to *Fusarium* sp. *Choenephora cucurbitarum* and *Rhizoctonia bataticola*, the pathogens of different cowpea diseases were also observed on diseased seeds.

With regard to the germination percentage of seeds, 90 per cent germination was noted in untreated healthy seeds. However, carbendazim treated seeds gave 100 per cent germination and the inhibition of germination due to mycoflora was only 10 per cent in blotter method. Whereas in case of diseased seeds, treated seeds gave 90 per cent germination, while the untreated seeds showed only 60 per cent germination and the percent inhibition of germination was also upto a maximum of 30 per cent.

In pot culture methods, germination percentage was the same in treated seeds, while it was slightly more in untreated seeds as compared to blotter method. The per cent inhibition of germination due to mycoflora was also less when compared to blotter method and it was 6.7 per cent and 21.7 per cent for healthy and diseased seeds respectively.

Table 1. Germination percentage and mycoflora associated with cowpea seeds

Category	*Mean germination percentage						Fungi associated		
	Blotter method			Pot culture method			Blotter method	Agarplate method	Pot culture method
	Treated with carben-dazim	Untreated	Per cent inhibition of germination due to mycoflora	Treated with carben-dazim	Untreated	Per cent inhibition of germination due to mycoflora			
Diseased seeds	90	60	30	90	68.30	21.70	*Aspergillus flavus* *Fusarium* sp. *Colletortichum linde-muthianum* *Asochyta* sp. *Alternaria* sp.	*C. lindemuthi-anum* *Coanephora cucurbitarum* *Rhizoctonia bataticola* *A. flavus* *Fusarium* sp. *Penicillium* sp. *Curvularia lunata*	*C. lindemuthi-anum* *Fusarium* sp.
Healthy seeds	100	90	10	100	93.30	6.70	*A. flavus* *Fusarium* sp. *Alternaria* sp.	*Aspergillus niger* *Aspergillus fumigatus*	*Fusarium* sp.

*Means of 3 replications

4.6 Studies on the seed borne nature of the pathogen

Seed borne nature of the pathogen was studied by agar plate and pot culture method.

4.6.1 Agar plate method

Seed coat, cotyledons and embryos of the diseased and healthy seeds kept on PCA, showed fungal growth on the medium which indicated that *C. lindemuthianum* the pathogen associated with anthracnose disease is externally as well as internally seed borne.

4.6.2 Pot culture method

In pot culture method, healthy seeds recorded 92 per cent germination whereas only 68 per cent germination was noticed in diseased seeds. Out of 68 per cent seeds germinated, 20 to 42.85 per cent with an average of 30.88 per cent seedling infection was noticed, indicating the seed borne nature of the pathogen (Table 2).

4.7 Effect of toxin on disease development

In order to find out the effect of toxin on disease development, the seedlings were sprayed with the endotoxin and exotoxin of the pathogen. Seedling sprayed with either of the toxins did not produce any symptom indicating that the toxin of *C. lindemuthianum* has no role in causing anthracnose disease of cowpea.

Table 2. Percentage of seed infection due to anthracnose disease

Category	No. of seeds sown	No. of seeds germinated	Percentage germination	No.of seedlings infected	Percentage of infection
Healthy seeds	100	92	92	No infection	Nil
Diseased seeds					
Ist pot	10	7	70	2	28.57
2nd pot	10	5	50	1	20.00
3rd pot	10	6	60	2	33.30
4th pot	10	10	100	3	30.00
5th pot	10	7	70	2	28.57
6th pot	10	6	60	2	33.33
7th pot	10	6	60	2	33.33
8th pot	10	6	60	2	33.33
9th pot	10	7	70	3	42.85
10th pot	10	8	80	2	25.00
	100	68	68	21	30.88

4.8.1 Evaluation of cowpea genotypes for resistance against anthracnose disease

Fifty cowpea genotypes belonging to bush, semi erect and pole types were screened against anthracnose disease under natural condition. The results of the experiment are furnished in Table 3 and 4. From the data given in Table 3 it was found that the genotypes differed significantly for disease resistance and yield. Among the 50 genotypes tested, Kanakamony (bush type) was completely free of disease. Seven genotypes viz. VS-28, VS-389, VS-14, VS-29, VS-12, VS-81 of bush type and CWP-16 of semi erect were found to be highly resistant to the disease in which the disease severity ranged from 1.67-10 per cent. In all these varieties disease incidence was also comparatively low and ranged from 3.85-15 per cent. Among the resistant types, VS-28 recorded lowest disease severity (1.67%) and disease incidence (3.85%). Genotypes VS-28, VS-29, VS-389 showed late infection and were free of disease upto 60 days after sowing (Table 4). None of the pole type was found to be resistant to the pathogen.

Among the genotypes evaluated, twelve showed moderately resistant reaction of 12-25 per cent and most of them were pole types. In VS-34 disease incidence and disease severity were low and were 16.67 per cent and 15 per cent respectively, whereas VS-94 showed only 12.5 per cent disease severity, but the disease incidence was 50 per cent.

In moderately susceptible 16 genotypes, much difference could not be noticed in disease incidence and disease severity values. Malika, a popular pole type variety showed 32 per cent infection.

Popular bush types, Pusa Komal was highly susceptible to the disease and showed 100 per cent disease incidence and disease severity and cowpea selection-16 also recorded 98.63 per cent disease severity and 73.87 per cent disease

Table 3. Evaluation of cowpea genotypes for resistance against anthracnose disease

Sl. No.	Genotypes	Disease incidence (%)	Disease severity (%)	Category	Yield t ha^{-1}
1	2	3	4	5	6
Bush type					
1	VS-4	11.55	20.00	MR	2.61
2	VS-12	15.00	10.00	HR	4.53
3	VS-14	5.00	5.00	HR	4.33
4	VS-18	50.00	71.67	HS	1.92
5	VS-28	3.85	1.67	HR	3.08
6	VS-29	12.94	5.00	HR	5.00
7	VS-29-1	66.67	75.00	HS	1.47
8	VS-81	8.33	10.00	HR	2.58
9	VS-389	4.17	3.67	HR	3.25
10	RCV-7	54.17	53.34	HS	2.31
11	Cowpea-263	45.46	48.34	MS	3.47
12	Selection-16	73.87	98.35	HS	2.61
13	Pusa Komal	100.00	100.00	HS	4.89
14	Kanakamony	0	0	I	6.28
Semi erect					
15	VS-15-1	54.17	38.34	MS	4.72
16	VS-15-3-1	45.84	56.67	HS	4.75
17	VS-19-2	37.50	33.34	MS	5.67
18	VS-35-1	20.84	21.67	MR	6.83
19	VS-36-1	41.67	36.67	MS	4.95
20	VS-36-2	30.56	31.67	MS	1.89
21	VS-37	37.50	33.34	MS	4.61
22	VS-51	37.50	30.00	MS	2.50
23	VS-82	25.00	26.67	MS	1.06
24	VS-84	31.67	38.34	MS	1.47
25	CWP 16	8.34	5.00	HR	2.86

Contd.

Table 3. Continued

1	2	3	4	5	6
Pole type					
26	VS-2	83.34	40.00	MS	2.50
27	VS-5	75.00	55.00	HS	9.17
28	VS-6	75.00	47.50	MS	2.50
29	VS-11	30.00	18.00	MR	1.40
30	VS-19-1	58.34	28.34	MS	1.33
31	VS-21	22.50	13.00	MR	1.22
32	VS-34	16.62	15.00	MR	0.78
33	VS-48	33.34	13.34	MR	1.39
34	VS-53	45.00	25.00	MR	1.68
35	VS-58	63.34	22.67	MR	1.67
36	VS-87	30.00	12.00	MR	1.83
37	VS-88	28.34	15.34	MR	4.08
38	VS-91	41.67	23.34	MR	1.58
39	VS-94	50.00	12.50	MR	1.00
40	IC-97857	100.00	80.00	HS	1.67
41	IC-97860	32.50	37.50	MS	3.89
42	IC-97862	50.00	32.00	MS	1.20
43	IC-97784-A	100.00	60.00	HS	1.67
44	IC-97784-B	75.00	60.00	HS	3.89
45	NIC-13655	90.00	60.00	HS	2.78
46	NIC-13659	50.00	30.00	MS	6.11
47	NIC-13755	100.00	55.00	HS	1.11
48	Call No.V90/062-A	100.00	60.00	HS	1.61
49	Call No.V90/062-B	100.00	70.00	HS	5.56
50	Malika	58.57	32.67	MS	1.92

I - Immune
HR - Highly resistant
MR - Moderately resistant
MS - Moderately susceptible
HS - Highly susceptible

incidence. Most of the semi erect and pole type genotypes were found more susceptible to the disease.

With regard to yield, certain genotypes gave good yield of 4 to 9.2 t ha^{-1}. Kanakamony, immune type recorded an yield of 6.28 t ha^{-1}. Highest yield (9.17 t ha^{-1}) was recorded in pole type VS-5. Semi erect VS-35-1, a moderately resistant genotype also recorded 6.83 t ha^{-1}.

From the Table 4, it was observed that 22 per cent of the genotypes were infected 40 DAS, while 64 per cent showed infection only on 50 DAS. However, genotypes VS-28, VS-29, VS-389 showed late infection and were free of disease upto 60 DAS.

4.8.2 Screening of the resistant genotypes by artificial inoculation of the pathogen

The genotypes found resistant in field experiment were artificially inoculated with pathogen under natural and laboratory conditions. In both conditions, variety Kanakamony did not show any symptom on artificial inoculation, indicating that this variety is immune to anthracnose disease. Seven resistant genotypes VS-28, VS-389, VS-14, VS-29, VS-12, VS-81 and CWP-16 also showed highly resistant reaction in which disease severity ranged from 3.8-10 per cent on artificial infection.

4.9 *In vitro* evaluation of chemicals/botanicals and antagonist against anthracnose pathogen

The inhibitory effect of different contact fungicides, botanicals and antagonist on the growth of *C. lindemuthianum* was studied by poisoned food

Table 4. Reaction of cowpea genotypes to anthracnose disease at different stages of the crop

Sl. No.	Genotypes	40 DAS		50 DAS		60 DAS	
		DI(%)	DS(%)	DI(%)	DS(%)	DI(%)	DS(%)
1	2	3	4	5	6	7	8
Bush type							
1	VS-4	0	0	0	0	11.55	20.00
2	VS-12	0	0	0	0	15.00	10.00
3	VS-14	0	0	0	0	5.00	5.00
4	VS-18	3.85	1.67	19.23	25.00	50.00	71.67
5	VS-28	0	0	0	0	3.85	1.67
6	VS-29	0	0	0	0	12.94	5.00
7	VS-29-1	50.00	30.00	66.67	46.67	66.67	75.00
8	VS-81	0	0	0	0	8.33	10.00
9	VS-389	0	0	0	0	4.17	3.67
10	RCV-7	20.84	10.67	50.00	30.00	54.17	53.34
11	Cowpea-263	20.84	16.67	40.91	26.67	45.46	48.34
12	Selection-16	8.34	3.34	51.89	38.33	73.87	98.35
13	Pusa Komal	20.84	11.67	73.08	86.67	100.00	100.00
14	Kanakamony	0	0	0	0	0	0
Semi erect							
15	VS-15-1	0	0	20.84	10.00	54.17	38.34
16	VS-15-3-1	4.15	1.67	16.67	10.00	45.84	56.67
17	VS-19-2	4.15	1.67	12.50	6.67	37.50	33.34
18	VS-35-1	0	0	12.50	5.00	20.84	21.67
19	VS-36-1	0	0	16.67	6.67	41.67	36.67
20	VS-36-2	0	0	21.56	5.00	30.56	31.67
21	VS-37	0	0	4.17	3.34	37.50	33.34
22	VS-51	0	0	12.50	5.00	37.50	30.00
23	VS-82	5.00	1.67	13.34	6.67	25.00	26.67
24	VS-84	16.67	6.67	20.83	15.00	31.67	38.34
25	CWP-16	0	0	4.15	1.65	8.34	5.00

Contd.

Table 4. Continued

1	2	3	4	5	6	7	8
Pole type							
26	VS-2	0	0	50.00	10.00	83.34	40.00
27	VS-5	0	0	50.00	10.00	75.00	55.00
28	VS-6	0	0	75.00	32.50	75.00	47.50
29	VS-11	25.00	5.00	30.00	6.00	30.00	18.00
30	VS19-1	0	0	0	0	58.34	28.34
31	VS-21	0	0	0	0	22.50	13.00
32	VS-34	0	0	0	0	16.67	15.00
33	VS-48	0	0	0	0	33.34	13.34
34	VS-53	0	0	0	0	45.00	25.00
35	VS-58	0	0	0	0	63.34	26.67
36	VS-87	0	0	0	0	30.00	12.00
37	VS-88	0	0	0	0	28.34	15.34
38	VS-91	0	0	0	0	41.67	23.34
39	VS-94	0	0	37.50	7.50	50.00	12.50
40	IC-97857	0	0	90.00	30.00	100.00	80.00
41	IC-97860	0	0	20.00	10.00	32.50	37.50
42	IC-97862	0	0	30.00	12.00	50.00	32.00
43	IC-97784-A	0	0	30.00	28.00	100.00	60.00
44	IC-97784-B	0	0	75.00	15.00	75.00	60.00
45	NIC-13655	0	0	30.00	20.00	90.00	60.00
46	NIC-13659	0	0	50.00	10.00	50.00	30.00
47	NIC-13755	0	0	50.00	10.00	100.00	55.00
48	Call No.V90/062-A	0	0	90.00	30.00	100.00	60.00
49	Call No.V90/062-B	0	0	50.00	30.00	100.00	70.00
50	Malika	0	0	30.00	6.00	58.57	32.67

DAS - Days after sowing
DI - Disease incidence
DS - Disease severity

technique in PCA and selective medium. The results of the experiment are given in Table 5 to 7.

From the data presented in Table 5, it was observed that in selective medium the full growth of the fungus in control plates was obtained on 7th day after inoculation. There was significant difference among the treatments. Maximum inhibition of the pathogen was observed in mancozeb (92.73%). Copper oxychloride was found to be least effective giving only 67.65 per cent inhibition and the inhibitory effect was found to be reducing from 4th day of inoculation. All other treatments were found equally effective, giving 84.4-88.9 per cent inhibition of pathogen over control.

In PCA medium (Table 6), the fungus required 9 days for the full growth in control plates. Leucas leaf extract (10%) gave the maximum inhibition of 92.7 per cent and the lowest inhibitory effect was noticed in Lantana leaf extract (74.40%) and mancozeb (74.43%) treatments. In case of chemicals, a drastic decrease in inhibitory effect was noticed between 6th and 9th day of inoculation.

On comparison of selective medium and PCA, it was observed from Table 7 & Fig. 10 that, the effect of treatments varied with different media. In selective medium maximum inhibition of the pathogen was obtained with mancozeb (92.73%) whereas in PCA, mancozeb gave the lowest inhibition (74.43%) in which Leucas leaf extract (10%) was found to be most effective (92.7%).

As regard to per cent inhibition of pathogen in different treatments, selective medium was found to be better than PCA as they recorded 84.42 to 94.03 per cent inhibition except in copper oxychloride treatment. In both media there was significant difference among the treatments and both chemicals and botanicals were equally effective against the pathogen *C. lindemuthianum*.

Table 5. *In vitro* evaluation of contact fungicides/botanicals against *C. lindemuthianum* in selective medium

Treatment	Days after inoculation - [*Mean colony diamter (cm)]				Per cent inhibition over control
	2nd	4th	6th	8th	
Chlorothalonil (0.2%)	0.50^{d}	0.70^{dc}	0.75^{e}	0.80^{f}	$88.90(0.10)^{b}$
Copper oxychloride (0.3%)	0.50^{d}	1.00^{bc}	2.00^{b}	2.50^{b}	$67.65(0.75)^{c}$
Mancozeb (0.2%)	0.50^{d}	0.50^{e}	0.50^{e}	0.65^{g}	$92.73(1.19)^{a}$
Captan (0.2%)	0.65^{c}	0.70^{dc}	0.75^{e}	0.80^{f}	$88.90(1.10)^{b}$
Garlic bulb extract (10%)	0.50^{d}	0.70^{dc}	0.70^{e}	0.70^{g}	$86.70(1.05)^{c}$
Leucas leaf extract (10%)	0.80^{b}	1.20^{b}	1.20^{c}	1.20^{d}	$86.70(1.05)^{c}$
Lantana leaf extract (10%)	0.70^{bc}	1.10^{bc}	1.10^{d}	1.10^{e}	$88.90(1.10)^{b}$
Ocimum leaf extract(10%)	0.50^{d}	0.80^{cd}	1.00^{d}	1.35^{c}	$84.40(1.01)^{d}$
Control	1.40^{a}	4.00^{a}	7.00^{a}	9.00^{a}	

Figures in parentheses are transformed values

* Mean of 3 replications

In each column figures followed by same letter do not differ significantly according to DMRT

Table 6. *In vitro* evaluation of contact fungicides/botanicals against *C. linderouthianum* in Potato Carrot Agar medium

Treatment	Days after inoculation - [*Mean colony diamter (cm)]					Per cent inhibition over control
	2nd	4th	6th	8th	9th	
Chlorothalonil (0.2%)	1.00^{c}	1.35^{c}	1.35^{d}	1.45^{e}	1.70^{e}	81.10(0.95)^{d}
Copper oxychloride (0.3%)	0.50^{e}	0.70^{e}	0.92^{e}	1.30^{f}	2.00^{c}	77.70(0.89)^{f}
Mancozeb (0.2%)	0.60^{d}	0.65^{e}	0.65^{e}	1.70^{d}	2.30^{b}	74.43(0.84)^{g}
Captan (0.2%)	0.65^{d}	1.20^{d}	1.25^{d}	1.45^{e}	1.60^{f}	82.17(0.96)^{c}
Garlic bulb extract (10%)	0.50^{e}	0.60^{ef}	0.80^{e}	1.05^{g}	1.10^{g}	87.73(1.07)^{b}
Leucas leaf extract (10%)	0.50^{e}	0.50^{f}	0.60^{e}	0.60^{h}	0.64^{h}	92.70(1.19)^{a}
Lantana leaf extract (10%)	1.10^{b}	2.30^{b}	2.30^{b}	2.30^{b}	2.30^{b}	74.40(0.84)^{g}
Ocimum leaf extract(10%)	0.50^{e}	1.35^{c}	1.80^{c}	1.80^{c}	1.80^{d}	80.00(0.93)^{c}
Control	1.45^{a}	4.00^{a}	5.50^{a}	7.80^{a}	9.00^{a}	

Figures in parentheses are transformed values

* Mean of 3 replications

In each column figures followed by same letter do not differ significantly according to DMRT

Table 7. Comparison of selective medium and PCA medium for *in vitro* evaluation of contact fungicides and botanicals

Treatments	Selective medium		Potato Carrot Agar medium	
	Mean colony diameter (cm)	Per cent inhibition over control	Mean colony diameter (cm)	Per cent inhibition over control
Chlorothalonil (0.2%)	0.80	88.90	1.70	81.10
Copper oxychloride (0.3%)	2.50	67.65	2.00	77.70
Mancozeb (0.2%)	0.65	92.73	2.30	74.43
Captan (0.2%)	0.80	88.90	1.60	82.17
Garlic bulb extract (10%)	0.70	86.70	1.10	87.73
Leucas leaf extract (10%)	1.20	86.70	0 65	92.70
Lantana leaf extract (10%)	1.00	88.90	2.30	74.40
Ocimum leaf extract (10%)	1.35	84.40	1.80	80.00
Control	9.00		9.00	

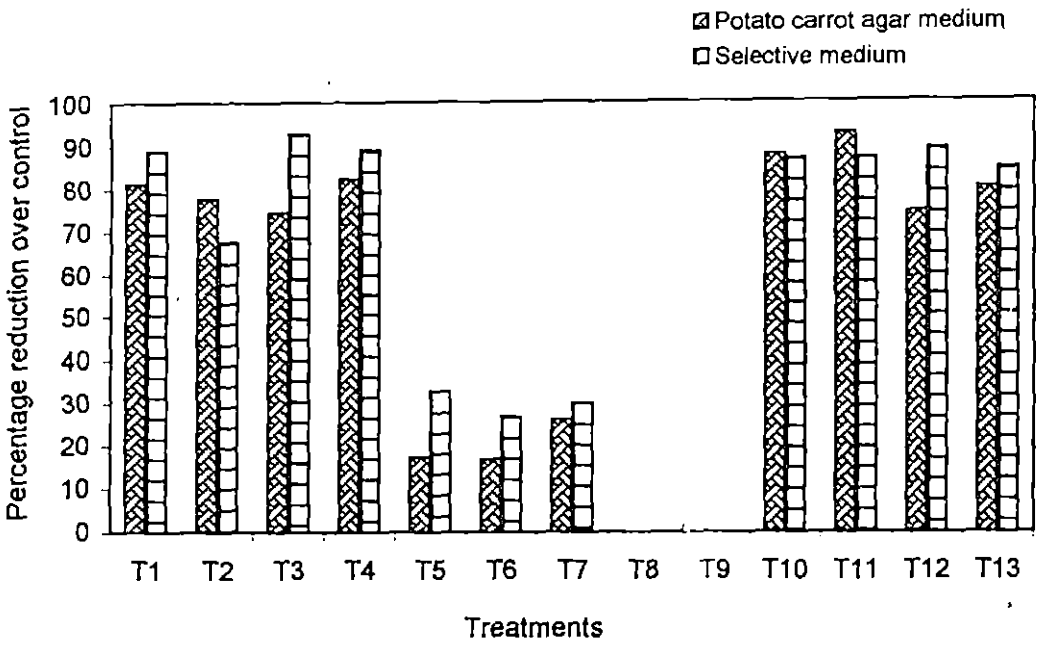


Fig.1(b). Comparison of selective medium and potato carrot agar medium for *in vitro*

4.9.1 *In vitro* evaluation of fungal antagonist *Trichoderma viride*

The antagonistic activity of *T. viride* against *C. lindemuthianum* in selective medium and PCA was assessed and the results are presented in Table 8.

In selective medium, initial growth rate of the test organism and antagonist was same in both mono and dual culture. But the growth of antagonist was faster as compared to the pathogen as evidenced by 4.5 cm against 1.7 cm on 2nd day after inoculation. On the 4th day after inoculation the antagonist started over growing pathogen and the complete over growth of the antagonist was observed on the 6th day. The growth of the test organism was arrested from 4th day onwards.

In monoculture also the full growth of antagonist was observed on 6th day whereas the pathogen took 7 days to complete the growth.

In PCA medium also the initial growth rate of the test organism and antagonist was same in both mono and dual culture two days after inoculation. From the 5th day onwards, no further growth of the pathogen was noticed and the antagonist started overgrowing on 7th day after inoculation and gave full coverage on the 8th day.

In monoculture, antagonist took only 7 days to complete the growth and the full growth of test organism was observed on 9th day after inoculation.

4.9.2 *In vitro* evaluation of systemic fungicides

The fungitoxic effect of different systemic fungicides (0.1%) on *C. lindemuthianum* was studied under laboratory condition by inhibition zone technique. The data of the experiment are presented in Table 9. From the table, it

Table 8. *In vitro* evaluation of *T. viride* against *C. lindemuthianum* (diameter in cm)

Type of medium	Days after inoculation								
	1	2	3	4	5	6	7	8	9
Selective medium									
D-A	1.00	4.50	5.90	6.40	8.60	9.00			
D-T	0.50	1.70	2.30	3.00	3.00	3.00			
M-A	1.20	4.70	5.40	6.50	7.70	9.00			
M-T	0.60	1.80	2.70	3.00	6.00	7.40	9.00		
Potato carrot agar medium									
D-A	1.00	2.00	3.40	4.90	6.80	7.10	8.30	9.00	
D-T	0.50	1.50	1.70	1.82	1.85	1.85	1.85	1.85	
M-A	1.20	2.20	3.10	3.90	5.00	7.50	8.10	9.00	
M-T	0.50	1.50	4.00	5.00	5.50	7.80	8.20	9.90	9.00

D-A - Dual culture - Antagonist
D-T - Dual culture - Test organism
M-A - Monoculture - Antagonist
M-T - Monoculture - Test organism

Table 9. *In vitro* evaluation of systemic fungicides by inhibition zone technique in selective medium and PCA medium

Treatments	Selective medium		PCA medium	
	Diameter of inhibition zone (cm)	Per cent inhibition over control	Diamter of inhibition zone (cm)	Per cent inhibition over control
Carbendazim (0.1%)	2.95	32.70	1.55	17.20
Tridemorph (0.1%)	2.40	26.70	1.50	16.70
Hexaconazole (0.1%)	2.70	30.00	2.35	26.10
Triforine (0.1%)	0	0	0	0
Triademefon (0.1%)	0	0	0	0
Control	0	0	0	0

was observed that, out of the five systemic fungicides tested, only three chemicals viz. carbendazim, tridemorph and hexaconazole showed inhibitory effect against the pathogen in both media. The maximum per cent inhibition was observed in selective medium in which carbendazim was found to be most effective (32.7%) followed by hexaconazole (30%).

In PCA, hexaconazole gave the maximum inhibition of 26.1 per cent followed by carbendazim (17.2%). Triforine and triademefon did not show any inhibitory effect on the pathogen, in both the media.

4.9.3 Evaluation of fungicides/botanicals and bioagent under field condition

Effect of different treatments on disease severity, incidence and on yield was studied under field condition. Observations were recorded 10 to 40 days after last spraying to find out the effect as well as the persistance of different treatments and the data are presented in Table 10 to 18.

From the Table 10 and 11, it is found that all treatments were highly effective in reducing the severity and incidence of the disease when recorded 10 days after the last spraying (40 DAS). However, mild infection was noticed in the case of foliar application alone with treatments T_{10} and T_{14}, in which disease severity was only 1.82 per cent and 3.64 per cent and disease incidence 4.55 per cent and 18.18 per cent respectively. Whereas the control plots recorded 45.61 per cent severity and 43.18 per cent incidence. Anthracnose infection was not noticed in any of the treatments upto 30 DAS except in control, in which 25 per cent severity and 27.82 per cent incidence were observed.

Data presented in Table 12 and 13 indicated that, all treatments were significantly superior over control in reducing the severity and incidence of the

Table 10. Effect of different treatments on severity of anthracnose disease at 10 days after last spraying

Treat-ments	Seed treatment		Foliar application		Seed treatment + foliar application		Mean	
	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control
T_1	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_2	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_3	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_4	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_5	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_6	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_7	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_8	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_9	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_{10}	0.00	100.00	1.82	96.01	0.00	100.00	0.61	98.67
T_{11}	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_{12}	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_{13}	0.00	100.00	0.00	100.00	0.00	100.00	0	100.00
T_{14}	0.00	100.00	3.64	92.02	0.00	100.00	1.21	97.34
T_{15}	45.61		45.61		45.61		45.61	

Table 11. Effect of different treatments on incidence of anthracnose disease at 10 days after last spraying

Treatments	Per cent disease incidence			
	Seed treatment	Foliar application	Seed treatment + Foliar application	Mean
T_1	0	0	0	0
T_2	0	0	0	0
T_3	0	0	0	0
T_4	0	0	0	0
T_5	0	0	0	0
T_6	0	0	0	0
T_7	0	0	0	0
T_8	0	0	0	0
T_9	0	0	0	0
T_{10}	0	4.55	0	1.5
T_{11}	0	0	0	0
T_{12}	0	0	0	0
T_{13}	0	0	0	0
T_{14}	0	18.18	0	6.1
T_{15}	43.18	43.18	43.18	43.18

disease upto 20 days of last spraying. Treatments T_1 and T_6 also gave 100 per cent control in all the three methods of application. However these two treatments were on par with other treatments as they could give 80.28-100 per cent control over check. Lowest disease reduction (80.28%) was noticed in T_{14}, in case of foliar application alone, but the same treatment gave good control (96.8-100%) when the seeds were treated with antagonist. However in case of disease incidence, all treatments were in one homogenous group (Table 13). As far as the methods of application are concerned, all methods were equally effective as evidenced by complete inhibition in all the treatments except T_2, T_{12} and T_{13} in case of seed treatment; T_1, T_6, T_8, T_9 in foliar application alone and the treatments T_1 to T_6 in case of seed treatment + foliar application.

All treatments were comparatively better in checking the severity and disease incidence even after 30 days of last spraying i.e., 60 DAS (Table 14 and 15). However gradual decrease in the persistance was observed especially in case of botanicals and antagonist when applied as foliar spraying. From the data, it is also seen that all the treatments differed significantly in their effect from control. All treatments except T_1 formed one homogenous group in case of disease severity and incidence. The plants which received cholorothalonil showed better protection of anthracnose disease by reducing disease severity (4.37%) and incidence (10.53%) irrespective of the methods of application. Regarding the method of application seed treatment and seed treatment + foliar application were superior as compared to foliar application alone as they yielded 80-100 per cent reduction of disease.

As the days progressed, the persistance of the treatments were found to be decreasing after 40 days of spraying as indicated by the increase in the disease severity and incidence (Table 16 and 17). From the data, it is evident that all treatments were superior to control in checking the anthracnose disease, but no significant difference could be noticed among the different treatments. T_1, T_3 and T_4

Table 12. Effect of different treatments on severity of anthracnose disease at 20 days after last spraying

Treatment	Seed treatment		Foliar application		Seed treatment + Foliar application		Mean	
	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control
T_1	0.00(0.15)	100.00	0.00(0.15)	100.00	0.00(0.15)	100.00	0.00(0.15)^{c}	100.00
T_2	6.67(0.26)	88.90	5.30(0.23)	91.17	0.00(0.15)	100.00	3.99(0.21)^{bc}	93.36
T_3	0.00(0.15)	100.00	4.17(0.22)	93.10	0.00(0.15)	100.00	1.39(0.17)^{bc}	97.70
T_4	0.00(0.15)	100.00	2.50(0.19)	95.83	0.00(0.15)	100.00	0.83(0.16)^{bc}	98.61
T_5	0.00(0.15)	100.00	3.33(0.20)	94.45	0.00(0.15)	100.00	1.11(0.16)^{bc}	98.15
T_6	0.00(0.15)	100.00	0.00(0.15)	100.00	0.00(0.15)	100.00	0.00(0.15)^{c}	100.00
T_7	0.00(0.15)	100.00	3.34(0.20)	94.44	1.12(0.15)	98.14	1.48(0.17)^{bc}	99.52
T_8	0.00(0.15)	100.00	0.00(0.15)	100.00	0.91(0.14)	98.50	0.30(0.14)^{bc}	99.50
T_9	0.00(0.15)	100.00	0.00(0.15)	100.00	1.67(0.16)	97.21	0.56(0.15)^{bc}	99.07
T_{10}	0.00(0.15)	100.00	10.00(0.30)	83.33	4.28(0.22)	92.80	4.76(0.22)^{bc}	92.04
T_{11}	0.00(0.15)	100.00	6.22(0.25)	89.63	1.00(0.14)	98.33	2.41(0.18)^{bc}	95.99
T_{12}	1.67(0.16)	97.23	5.85(0.25)	90.25	2.51(0.19)	95.81	3.34(0.20)^{bc}	94.43
T_{13}	5.00(0.23)	91.67	3.34(0.20)	94.44	4.18(0.21)	93.02	4.17(0.21)^{bc}	93.04
T_{14}	0.00(0.15)	100.00	11.83(0.33)	80.30	1.91(0.14)	96.82	4.58(0.20)^{bc}	92.37
T_{15}	60.00(0.89)		59.99(0.89)		59.97(0.89)		59.99(0.89)^{a}	
Mean	6.25(0.23)^{ab}		7.72(0.26)^{a}		5.17(0.21)^{b}			

Figures in parenthesis are transformed values

Figures followed by same letter do not differ significantly according to DMRT

Table 13. Effect of different treatments on incidence of anthracnose disease at 20 days after last spraying

Treatments	Per cent disease incidence			
	Seed treatment	Foliar application	Seed treatment + Foliar application	Mean
T_1	0(0.15)	0(0.15)	0(0.15)	0(0.15) [b]
T_2	9.09(0.29)	17.43(0.43)	0(0.15)	8.38(0.29) [b]
T_3	0(0.15)	16.67(0.38)	0(0.15)	5.55(0.22) [b]
T_4	0(0.15)	12.50(0.33)	0(0.15)	4.17(0.71) [b]
T_5	0(0.15)	14.29(0.35)	0(0.15)	4.76(0.22) [b]
T_6	0(0.15)	0(0.15)	0(0.15)	0(0.15) [b]
T_7	0(0.15)	16.67(0.38)	5.55(0.24)	7.41(0.25) [b]
T_8	0(0.15)	0(0.15)	4.55(0.23)	1.52(0.17) [b]
T_9	0(0.15)	0(0.15)	6.25(0.25)	2.08(0.18) [b]
T_{10}	0(0.15)	27.28(0.49)	14.29(0.35)	13.58(0.33) [b]
T_{11}	0(0.15)	22.73(0.47)	5.00(0.23)	9.24(0.29) [b]
T_{12}	5.55(0.24)	29.17(0.51)	12.50(0.33)	15.74(0.36) [b]
T_{13}	12.50(0.33)	10.00(0.30)	16.67(0.38)	13.06(0.35) [b]
T_{14}	0(0.15)	27.73(0.44)	9.55(0.31)	10.76(0.30) [b]
T_{15}	59.43(0.89)	59.43(0.89)	59.43(0.89)	59.43(0.89) [a]
Mean	6.25(0.23) [b]	16.59(0.37) [a]	8.92(0.28) [ab]	

Figures in parentheses are transformed values
Figures followed by same letter do not differ significantly according to DMRT

Table 14. Effect of different treatments on severity of anthracnose disease at 30 days after last spraying.

Treatmnts ments	Seed treatment		Foliar application		Seed treatment + Foliar application		Mean	
	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control
T_1	3.34(0.20)	96.54[a]	8.34(0.30)	91.37[a]	1.43(0.17)	98.52[a]	4.37(0.22)[d]	95.48[a]
T_2	16.67(0.41)	82.76[ab]	21.22(0.18)	78.04[ab]	2.00(0.18)	97.93[a]	13.30(0.33)[bcd]	86.24[ab]
T_3	5.00(0.23)	94.82[a]	13.34(0.33)	86.20[a]	1.67(0.17)	98.27[a]	6.67(0.25)[cd]	93.10[a]
T_4	10.00(0.30)	89.66[a]	10.84(0.30)	88.79[a]	1.67(0.17)	98.27[a]	7.50(0.29)[cd]	92.24[a]
T_5	6.67(0.25)	93.10[a]	11.33(0.32)	88.28[a]	9.29(0.30)	90.39[a]	9.10(0.30)[cd]	90.60[ab]
T_6	1.67(0.17)	98.27[a]	20.00(0.63)	79.31[ab]	12.23(0.32)	87.35[a]	11.30(0.32)[bcd]	88.31[ab]
T_7	3.34(0.20)	96.54[a]	18.57(0.45)	80.79[ab]	12.78(0.32)	97.12[a]	11.56(0.32)[bcd]	91.48[ab]
T_8	0.00(0.15)	100.00[a]	10.00(0.30)	89.66[a]	7.12(0.29)	-92.63[a]	5.71(0.23)[cd]	94.10[a]
T_9	6.67(0.25)	93.10[a]	8.57(0.30)	91.13[a]	7.84(0.29)	91.88[a]	7.33(0.29)[cd]	92.04[a]
T_{10}	11.67(0.32)	87.93[a]	21.37(0.63)	77.89[ab]	18.86(0.45)	80.49[ab]	17.30(0.44)[bcd]	82.13[ab]
T_{11}	18.34(0.45)	81.03[ab]	23.94(0.63)	75.24[abc]	11.00(0.32)	88.62[a]	17.76(0.45)[bcd]	81.63[ab]
T_{12}	15.00(0.38)	84.48[ab]	15.84(0.38)	83.61[a]	7.50(0.29)	92.24[a]	12.78(0.32)[bcd]	86.78
T_{13}	19.17(0.45)	80.17[a]	20.00(0.63)	79.31[ab]	12.50(0.32)	87.07[a]	17.22(0.34)[bcd]	82.18
T_{14}	6.34(0.25)	93.44[a]	22.43(0.63)	76.80[ab]	12.37(0.32)	87.20[a]	13.71(0.34)[bcd]	85.81
T_{15}	96.67(1.40)		96.67(1.40)		96.67(1.40)[a]		96.67(1.40)[a]	
Mean	16.52(0.38)[b]		23.63(0.63)[a]		14.22(0.35)[b]			

Figures in parentheses are transformed values

Figures followed by same letter do not differ significantly according to DMRT

Table 15. Effect of different treatments on incidence of anthracnose disease at 30 days after last spraying

Treatments	Per cent disease incidence			
	Seed treatment	Foliar application	Seed treatment + Foliar application	Mean
T_1	9.09(0.30)	17.50(0.42)	5.00(0.23)	10.53(0.31) [c]
T_2	30.95(0.60)	31.22(0.61)	10.00(0.30)	24.06(0.51) [bc]
T_3	11.11(0.32)	20.51(0.47)	5.56(0.23)	12.39(0.32) [bc]
T_4	18.18(0.43)	26.39(0.54)	4.55(0.23)	16.37(0.41) [bc]
T_5	17.43(0.42)	33.12(0.62)	32.83(0.61)	27.79(0.55) [bc]
T_6	12.50(0.38)	44.44(0.69)	38.89(0.69)	31.93(0.62) [bc]
T_7	7.15(0.29)	38.10(0.69)	21.67(0.47)	22.31(0.50) [bc]
T_8	0.00(0.15)	18.34(0.43)	24.43(0.51)	14.26(0.35) [bc]
T_9	17.43(0.42)	21.43(0.47)	20.00(0.47)	19.62(0.46) [bc]
T_{10}	22.22(0.480	57.20(0.74)	29.29(0.58)	53.07(0.73) [b]
T_{11}	29.17(0.57)	36.37(0.65)	23.34(0.50)	29.63(0.58) [bc]
T_{12}	22.22(0.48)	25.00(0.52)	12.50(0.33)	19.91(0.46) [bc]
T_{13}	26.67(0.54)	29.29(0.58)	16.67(0.41)	24.21(0.51) [bc]
T_{14}	19.55(0.46)	46.47(0.70)	33.64(0.61)	33.22(0.61) [bc]
T_{15}	100.00(1.43)	100.00(1.43)	100.00(14.3)	100.00(1.43) [a]
Mean	26.01(0.54) [b]	36.59(0.65) [a]	25.23(0.54) [b]	

Figures in parentheses are transformed values
Figures followed by same letter do not differ significantly according to DMRT

recorded lowest disease severity of 11.40 per cent, 12.23 per cent and 12.23 per cent respectively with a maximum control of 88.61 per cent and 87.77 per cent. Maximum disease severity (30.51%) was observed in T_{11}. It is also observed that effect of botanicals were more reduced as compared to chemicals on 40th day after last spraying. Regarding the disease incidence, lowest incidence (17.75%) was noticed in T_4 and all other treatments were in one homogenous group (Table 17).

In a clear justification of results already observed in different intervals, it is revealed that T_1 (chlorothalonil, 0.2%) was best followed by T_4 (captan 0.2%) and T_3 (mancozeb, 0.2%) in reducing anthracnose disease.

From the data presented in Table 16, it is noted that all methods of application were effective in case of contact fungicides (T_1 to T_4), however, seed treatment + foliar application gave maximum disease control especially in T_2 as compared to either seed treatment or foliar application alone. While in case of systemic fungicides, seed treatment and seed treatment + foliar application were most effective than foliar application alone. It is also found that seed treatment alone gave good control of disease and the same trend was observed in case of antagonist treatment (T_{14}) also. In case of botanicals, any notable difference could be observed among different methods of applications, however, seed treatment + foliar spray was more effective. None of the extracts showed adverse effect on seed germination and growth with seed treatment.

Comparison on the effect of fungicides and botanicals on *C. lindemuthianum* under *in vitro* and field conditions are given in Fig.2. From the figure it was observed that, as compared to *in vitro* evaluation, per cent reduction over control was high under field condition when recorded 10 days after last spray. Both contact fungicides and botanicals were equally effective whereas among the

Table 16. Effect of different treatments on severity of anthracnose disease at 40 days after last spraying.

Treatmnts ments	Seed treatment		Foliar application		Seed treatment + Foliar application		Mean	
	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control	Per cent disease severity	Per cent decrease over control
T_1	15.00(0.39)	85.00^{a}	13.34(0.36)	86.67^{a}	5.84(0.24)	94.17^{a}	11.40(0.33)^{d}	88.61^{a}
T_2	30.95(0.59)	69.05^{ab}	29.10(0.57)	70.91^{ab}	5.68(0.23)	94.34^{a}	21.94(0.46)^{bcd}	78.10^{ab}
T_3	10.00(0.30)	90.00^{a}	20.86(0.46)	79.16^{a}	5.84(0.24)	94.17^{a}	12.23(0.34)^{cd}	87.78^{a}
T_4	15.00(0.39)	85.00^{a}	18.36(0.43)	81.67^{a}	3.33(0.20)	96.67^{a}	12.23(0.34)^{cd}	87.78^{a}
T_5	15.00(0.39)	85.00^{a}	16.30(0.40)	83.70^{a}	17.19(0.42)	82.83^{a}	16.16(0.41)^{cd}	83.84^{ab}
T_6	3.34(0.20)	96.67^{a}	30.00(0.56)	70.00^{ab}	21.10(0.47)	78.89^{a}	18.14(0.42)^{bcd}	81.85^{ab}
T_7	5.00(0.23)	95.00^{a}	27.88(0.55)	72.15^{ab}	22.78(0.49)	77.23^{a}	18.55(0.43)^{bcd}	81.46^{ab}
T_8	6.67(0.25)	93.34^{a}	19.17(0.45)	80.84^{a}	20.29(0.46)	79.70^{a}	15.38(0.39)^{cd}	84.62^{a}
T_9	5.00(0.23)	95.00^{a}	36.86(0.65)	67.12^{a}	20.03(0.46)	80.00^{a}	20.63(0.47)^{cd}	80.75^{a}
T_{10}	23.34(0.50)	76.67^{a}	35.36(0.63)	64.62^{ab}	28.16(0.55)	71.86^{ab}	28.95(0.56)^{bcd}	71.10^{ab}
T_{11}	33.34(0.62)	66.67^{ab}	36.86(0.65)	80.00^{abc}	21.35(0.47)	78.67^{a}	30.51(0.58)^{bcd}	69.50^{ab}
T_{12}	23.33(0.50)	76.67^{ab}	20.01(0.41)	80.00^{a}	12.50(0.33)	87.50^{a}	18.62(0.42)^{bcd}	81.39^{ab}
T_{13}	27.50(0.55)	72.50^{ab}	31.66(0.60)	68.33^{ab}	28.37(0.56)	71.67^{ab}	29.18(0.57)^{bc}	70.83^{ab}
T_{14}	16.67(0.42)	83.33^{a}	32.88(0.60)	67.12^{abc}	25.85(0.53)	74.18^{ab}	25.13(0.52)^{bcd}	74.86^{ab}
T_{15}	100.00(1.43)		100.00		100.00(1.43)		100.00(1.43)	
Mean	26.01(0.61)^{b}	79.29^{a}	33.37(0.78)^{a}	71.43^{b}	22.55(0.51)^{b}			

Figures in parentheses are transformed values
Figures followed by same letter do not differ significantly according to DMRT

Table 17. Effect of different treatments on incidence of anthracnose disease at 40 days after last spraying

Treatments	Per cent disease incidence			
	Seed treatment	Foliar application	Seed treatment + Foliar application	Mean
T_1	20.63(0.47)	23.76(0.49)	19.30(0.44)	21.23(0.47) ^{bc}
T_2	30.95(0.59)	52.11(0.94)	15.58(0.40)	36.21(0.64) ^{bc}
T_3	16.67(0.38)	28.83(0.53)	19.85(0.45)	21.78(0.46) ^{bc}
T_4	18.18(0.44)	30.54(0.56)	4.54(0.23)	17.75(0.41) ^{c}
T_5	17.43(0.43)	49.34(0.78)	38.39(0.66)	35.05(0.62) ^{bc}
T_6	12.50(0.33)	47.91(0.76)	38.88(0.67)	33.10(0.59) ^{bc}
T_7	7.15(0.27)	42.29(0.71)	26.66(0.54)	25.37(0.51) ^{bc}
T_8	15.48(0.40)	35.87(0.64)	50.59(0.79)	33.98(0.61) ^{bc}
T_9	17.48(0.43)	21.44(0.43)	24.31(0.50)	21.41(0.47) ^{bc}
T_{10}	27.78(0.55)	61.33(0.90)	41.46(0.70)	43.52(0.72) ^{b}
T_{11}	41.67(0.70)	45.45(0.74)	32.53(0.60)	39.88(0.68) ^{bc}
T_{12}	22.22(0.48)	24.98(0.47)	21.65(0.47)	22.95(0.47) ^{bc}
T_{13}	29.17(0.57)	34.30(0.63)	37.50(0.66)	33.66(0.62) ^{bc}
T_{14}	34.55(0.62)	57.54(0.87)	38.18(0.67)	43.43(0.72) ^{b}
T_{15}	100.00(1.43)	100.00(1.43)	100.00(1.43)	100.00(1.43) ^{a}
Mean	28.18(0.55) ^{b}	44.38(0.73) ^{a}	33.96(0.61) ^{b}	

Figures in parentheses are transformed values
Figures followed by same letter do not differ significantly according to DMRT

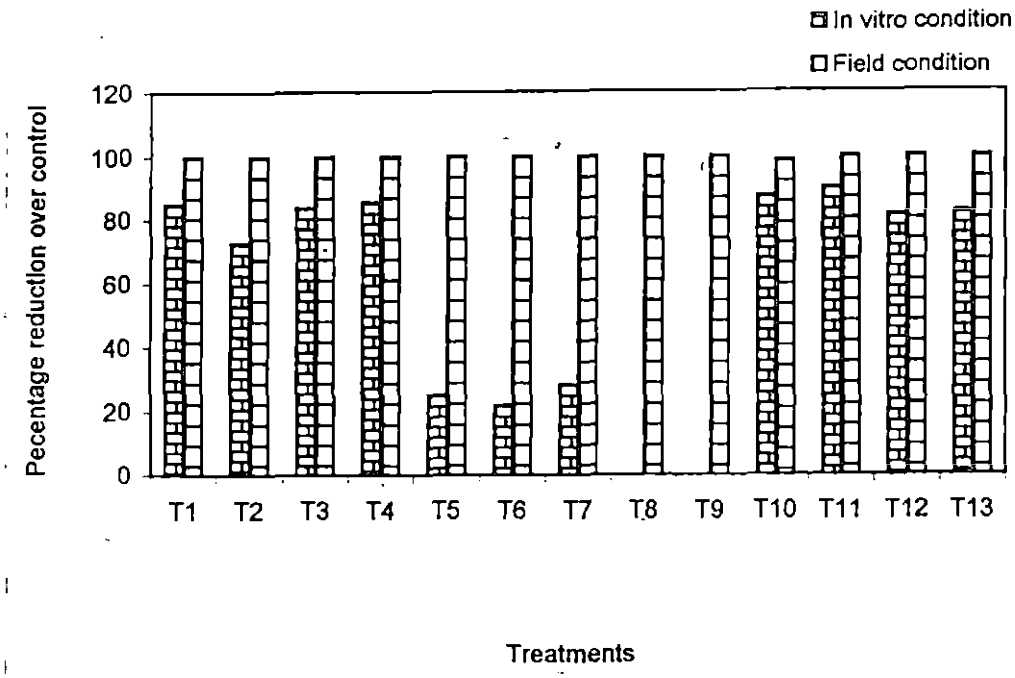


Fig.2. Comparison on the effect of different treatments under *in vitro* and field conditions

systemic fungicides triforine and triademefon which showed no inhibition under laboratory conditions gave 100 per cent control under field condition.

Data on yield presented in Table 18 showed that highest mean yield (1.85 kg plot^{-1}) was recorded from plots treated with mancozeb (T_3) which gave 740.45 per cent increase in yield over control. This was statistically superior to other treatments and control, but was on par with chlorothalonil (T_1) treatment (1.17 kg plot^{-1}). Lowest yield (0.61 kg plot^{-1}) was recorded in Lantana leaf extract (T_{12}) treatment which gave only 240.45 per cent increase in yield over control.

With respect to methods of applications, mean yield obtained was maximum in the case of seed treatment + foliar application (1.01 kg plot^{-1}) which was closely followed by seed treatment alone (0.98 kg plot^{-1}) (Table 18).

Since all the cultural operations except plant protection measures were common in all treatments, only the additional expenditure incurred is taken into account while calculating C:B ratio. The highest C:B ratio was noticed in seed treatments, in which, mancozeb treatment recorded the highest C:B ratio of 1:9.8. In case of foliar application, Ocimum leaf extract recorded the maximum C:B ratio of 1:2.2 followed by mancozeb (1:1.9). In seed treatment + foliar application, the highest C:B ratio (1:2) was observed in mancozeb and garlic treatments. When all methods are taken into account, the highest C:B ratio (1:4.57) was observed in mancozeb treatment.

4.10 Effect of seasonal influence on anthracnose disease of cowpea

In order to study the seasonal influence on anthracnose disease, three crops were taken during South-West monsoon period, North-East monsoon period and summer. Observations on disease incidence and disease severity are presented

Table 18. Effect of different treatments on yield of cowpea

Tteatments	Seed treatment			Foliar application			Seed treatment + Foliar application			Mean		
	Yield (kg)	C:B ratio	Per cent increase over control	Yield (kg)	C:B ratio	Per cent increase over control	Yield (kg)	C:B ratio	Percent increase over control	Yield (kg)	CB ratio	Percent increase over control
T_1	1.17(1.08)	1:1.8	565.71	1.04(1.01)	1:1.6	475.00	1.30(1.14)	1:1.5	619.44	1.17(1.07)^{ab}	1:1.63	554.49
T_2	1.23(1.11)	1:4.1	602.86	0.56(0.75)	1:1.7	211.11	1.08(1.04)	1:1.7	499.44	0.96(0.96)^{bc}	1:2.17	437.10
T_3	3.09(1.60)	1:9.8	1662.86	1.08(1.03)	1:1.9	500.00	1.39(1.18)	1:2.0	672.22	1.85(1.27)^{a}	1:4.57	940.45
T_4	1.02(0.99)	1:3.3	480.00	0.75(0.86)	1:1.2	313.89	1.17(1.04)	1:1.8	550.00	0.98(0.97)^{bc}	1:2.1	448.88
T_5	0.94(0.95)	1:2.8	437.14	1.28(1.12)	1:2.2	608.33	1.09(1.04)	1:1.6	505.56	1.10(1.04)^{abc}	1:2.2	519.10
T_6	0.95(0.95)	1:2.2	440.00	1.10(1.04)	1:1.7	511.11	1.45(1.20)	1:1.6	702.78	0.78(0.84)^{bcd}	1:1.83	336.52
T_7	1.14(1.03)	1:3.7	548.57	0.82(0.90)	1:1.3	352.78	1.15(1.07)	1:1.7	596.11	1.03(1.00)^{abc}	1:2.23	479.78
T_8	0.73(0.85)	1:2.1	317.14	0.89(0.93)	1:1.4	394.44	0.67(0.82)	1:0.8	271.67	0.763(0.87)^{bcd}	1:1.43	328.65
T_9	1.15(0.35)	1:3.6	557.14	0.98(0.98)	1:1.1	441.67	0.89(0.94)	1:1	394.44	1.005(0.99)^{abc}	1:1.9	464.60
T_{10}	0.75(0.86)	1:2.8	328.57	0.47(0.69)	1:1.8	161.11	1.18(1.08)	1:2	555.56	0.80(0.88)^{bcd}	1:1.87	349.44
T_{11}	0.75(0.86)	1:2.2	320.00	0.81(0.89)	1:1.7	347.22	0.98(0.98)	1:1.6	441.67	0.84(0.91)^{bcd}	1:1.83	370.79
T_{12}	0.87(0.93)	1:2.8	394.29	0.48(0.66)	1:0.8	166.67	0.47(0.68)	1:0.6	162 78	0.61(0.76)^{cde}	1:1.4	240.79
T_{13}	1.16(1.08)	1:4	560.00	0.98(0.98)	1:2.2	441.67	1.03(1.02)	1:1.8	473.89	1.05(1.03)^{abc}	1:1.7	492.13
T_{14}	1.18(1.09)	1:3.9	574.29	0.67(0.82)	1:1.2	272.22	0.89(0.94)	1:1.3	391.67	0.91(0.95)^{bcd}	1:2.67	412.36
T_{15}	0.18(0.41)			0.18(0.41)			0.18(0.41)			0.18(0.67)^{e}		
Mean	0.98(0.92)			0.76(0.85)			1.01(0.98)				1:2.13	

Figures in parentheses are transformed values

Figures followed by same letter do not differ significantly according to DMRT

in Tables 19, 21, 23 and Fig.3 and 4. Climatic factors like maximum and minimum temperature, relative humidity and rainfall during the cropping period were recorded to correlate the climatic factors on disease development and its severity (Tables 20, 22 and 24).

4.10.1 First crop (June to August - SW monsoon period)

The crop was sown on 17-6-97 and the crop duration was only 45 days due to early appearance and severity of the disease. During this period the disease appeared within 25 days after sowing. The main symptom during this period was rotting of the top portion of the stem and toppling of the foliage in addition to the anthracnose symptoms on stem. It is seen that on 25th DAS, disease incidence was 21.17 per cent and severity was 16.11 per cent (Table 19). But 35 DAS disease increased drastically showing 81.94 per cent disease incidence and 74.44 per cent severity. The infection has reached the maximum level at the end of the crop i.e. 45 DAS which recorded 95.27 per cent incidence and a high severity of 87.78 per cent. As the pod infection was severe due to rotting of the pods, yield was reduced drastically giving an average of 0.39 t ha^{-1}. Moreover the crop lasted only for 45 days due to early appearance and severity of disease which also caused reduction in the yield. During this period maximum temperature ranged from 26.6°C-31.3°C and minimum from 21.8°C-23.4°C and the relative humidity ranged from 85-95 per cent. Total number of rainy days during this period was 43 and the total rainfall ranged from 99.2 mm-492.2 mm (Table 20).

4.10.2 Second crop (September to November - NE monsoon period)

The crop was sown on 15-9-1997 and the duration of the crop was 63 days. In this case the disease appeared only at the later stage of the crop (52 DAS). So the scoring was done at five days interval. It has been observed that on 52 DAS

Table 19. Effect of seasonal influence on anthracnose disease of cowpea during June-August (SW monsoon period)

No.	25 DAS		35 DAS		45 DAS		Yield (t ha^{-1})
	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	
1	33.33	6.67	83.33	70.00	100.00	93.33	0.00
2	16.67	3.33	66.67	63.33	100.00	96.67	1.33
3	50.00	36.67	83.33	80.00	83.33	83.33	0.33
4	16.67	3.33	96.67	60.00	96.67	66.67	0.00
5	0.00	0.00	100.00	96.67	100.00	96.67	0.70
6	66.67	56.67	100.00	83.33	100.00	96.67	0.00
7	33.33	6.67	83.33	66.67	100.00	96.67	0.00
8	16.67	13.33	66.67	63.33	100.00	96.67	1.30
9	66.67	40.00	83.33	80.00	83.33	83.33	0.33
10	0.00	0.00	96.67	50.00	96.67	60.00	0.00
11	0.00	0.00	100.00	96.67	100.00	100.00	0.70
12	50.00	26.67	83.33	83.33	83.33	83.33	0.00
Mean	29.17	16.11	81.94	74.44	95.27	87.78	0.39

DAS - Days after sowing
DI - Disease incidence
DS - Disease severity

Table 20. Weather data at weekly intervals during 17-6-97 to 1-8-97

Period		Temperature °C		Relative humidity(%)	Rain (mm)	No. of rainy days
		Minimum	Maximum			
June						
	3rd week	23.2	31.3	85	99.2	4
	4th week	21.8	26.6	95	492.2	7
July						
	1st week	22.1	27.7	88	239.5	5
	2nd week	22.3	28.8	93	192.9	7
	3rd week	22.8	29.8	86	207.0	6
	4th week	22.7	29.0	91.5	252.1	6
	5th week	23.3	29.1	86.5	143.6	5
August						
	1st day	23.4	30.2	86.5	37.0	1

disease incidence was 6.95 per cent and severity was 4.45 per cent (Table 21). But after an interval of 5 days (57 DAS) disease incidence and severity reached upto 63.19 per cent and 24.31 per cent respectively. No drastic increase of disease was noticed when the scoring was done at 62 DAS which recorded 68.8 per cent incidence and the maximum severity was only 30.83 per cent. Since the disease appeared at the fag end of the crop, yield was not much affected as observed during June - August months and an average yield of 2.01 t ha^{-1} was obtained. During this period maximum temperature ranged from 29.5°C - 33.3°C and minimum temperature from 22.7°C - 24.7°C and the relative humidity ranged from 70-86.5 per cent. Total number of rainy days during this period was 27 and the total rainfall ranged from 13.2-76.1 mm (Table 22).

4.10.3 Third crop (January to March - summer period)

Summer crop was sown on 16-1-98 and the crop lasted upto 27-3-98 (70 days duration). Infection appeared only in very late stage of the crop and the disease incidence and severity were also comparatively low during this period (Table 23). A mild infection was noticed on 56 DAS, with a disease incidence of 5.56 per cent and severity 5 per cent. Even after ten days of symptom appearance no noticeable increase could be observed in case of incidence and disease severity, the incidence and severity had reached only to 6.94 per cent and 6.95 per cent respectively. The maximum incidence and severity recorded during this season were 9.72 per cent and 10.83 per cent only. As the disease appeared during the later stage of the crop, an average yield of 2.24 t ha^{-1} was obtained. During this period the maximum temperature ranged from 33.4°C - 37.5°C and minimum from 22.1°C - 24.3°C. Relative humidity ranged from 64-73.5 per cent. Total rainfall was only 11 mm (Table 24).

Table 21. Effect of seasonal influence on anthracnose disease of cowpea during September-November (NE monsoon period)

No.	52 DAS		57 DAS		66 DAS		Yield ($t\ ha^{-1}$)
	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	
1	0.00	0.00	50.00	20.00	100.00	40.00	2.73
2	33.33	26.67	100.00	53.33	100.00	56.56	2.40
3	0.00	0.00	33.33	16.67	50.00	23.33	3.27
4	16.67	10.00	100.00	36.67	100.00	46.67	2.20
5	16.67	6.67	66.67	26.67	66.67	36.67	1.67
6	0.00	0.00	33.33	13.33	33.33	16.67	1.00
7	0.00	0.00	25.00	25.00	75.00	30.00	2.93
8	16.67	10.00	83.33	40.00	83.33	40.00	1.67
9	0.00	0.00	66.67	13.33	66.67	20.00	1.93
10	0.00	0.00	83.33	33.33	83.33	40.00	1.73
11	0.00	0.00	50.00	10.00	50.00	13.33	1.53
12	0.00	0.00	16.67	3.33	16.67	6.67	1.10
Mean	6.95	4.45	63.19	24.31	68.80	30.83	2.01

DAS - Days after sowing
DI - Disease incidence
DS - Disease severity

Table 22. Weather data at weekly intervals during 15-9-97 to 17-11-97

Period	Temperature °C		Relative humidity(%)	Rain (mm)	No. of rainy days
	Minimum	Maximum			
September					
3rd week	22.7	29.5	86.5	76.1	6
4th week	24.3	30.8	81.5	26.1	3
October					
1st week	24.7	31.7	78.5	13.2	1
2nd week	23.1	33.3	72.5	84.3	2
3rd week	23.6	32.4	74.0	53.1	5
4th week	23.5	31.7	78.0	28.9	2
5th week	23.1	31.7	78	45.5	3
November					
1st week	23.6	31.00	78	74.6	3
2nd week	22.8	32.3	77.5	30.4	2
16th day	23.2	31.3	70.00	0	-
17th day	23.3	32.3	76.5	0	-

Table 23. Effect of seasonal influence on anthracnose disease of cowpea during January-March (Summer)

No.	56 DAS		61 DAS		66 DAS		Yield (t ha^{-1})
	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	
1	0.00	0.00	0.00	0.00	0.00	0.00	2.33
2	0.00	0.00	0.00	0.00	0.00	0.00	1.93
3	0.00	0.00	0.00	0.00	33.33	20.00	2.40
4	0.00	0.00	0.00	0.00	0.00	0.00	2.27
5	0.00	0.00	0.00	0.00	0.00	0.00	2.20
6	0.00	0.00	0.00	0.00	0.00	0.00	2.13
7	33.33	20.00	33.33	36.67	33.33	36.67	2.10
8	0.00	0.00	0.00	0.00	0.00	0.00	2.47
9	0.00	0.00	0.00	0.00	0.00	0.00	2.20
10	16.67	20.00	16.67	20.00	16.67	26.67	2.33
11	0.00	0.00	0.00	0.00	0.00	0.00	2.40
12	16.67	20.00	33.33	26.67	33.33	26.67	2.10
Mean	5.56	5.00	6.94	6.95	9.72	9.17	2.24

DAS - Days after sowing
DI - Disease incidence
DS - Disease severity

Table 24. Weather data at weekly intervals during 16-1-98 to 27-3-98

Period		Temperature °C		Relative humidity(%)	Rain (mm)	No. of rainy days
		Minimum	Maximum			
January						
	3rd week	22.1	33.7	69.6	0	0
	4th week	24.3	34.2	65.0	0	0
	5th week	24.3	34.6	60.5	0	0
February						
	1st week	23.3	34.8	66.5	0	0
	2nd week	23.4	34.4	64.0	0	0
	3rd week	23.6	33.4	64.0	0	0
	4th week	24.3	35.3	73.5	0	0
March						
	1st week	23.6	35.9	69.5	0	0
	2nd week	23.8	35.5	64.5	0	0
	3rd week	23.7	37.5	64.0	0	0
	4th week	22.9	36.2	69.0	11	1

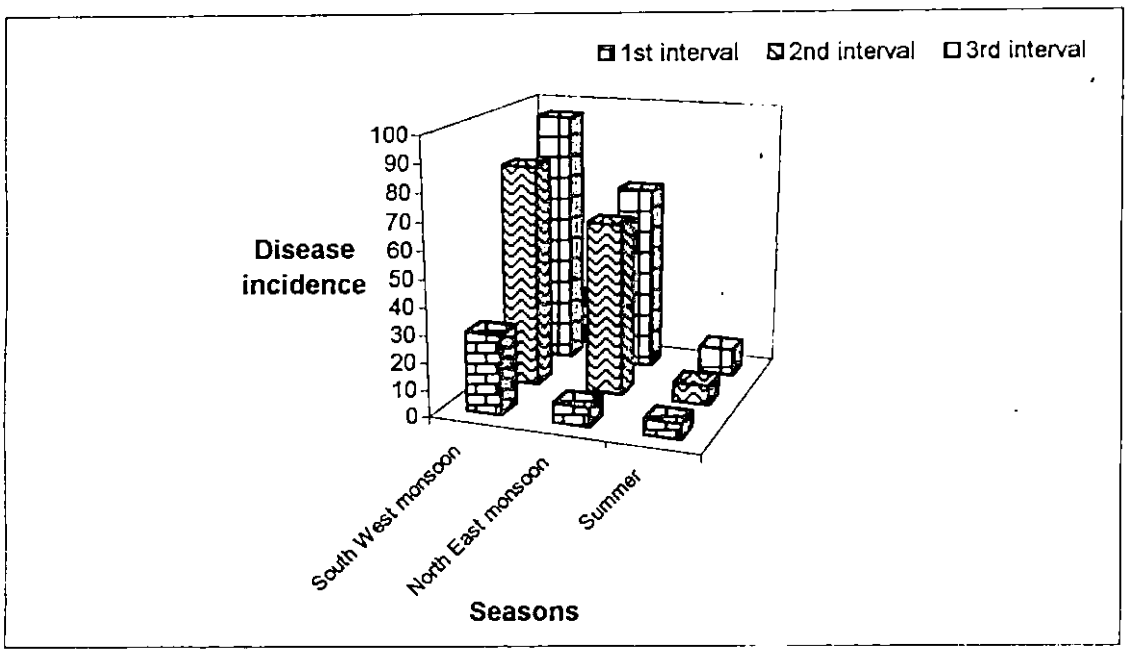


Fig. 3. Effect of different seasons on the incidence of cowpea anthracnose

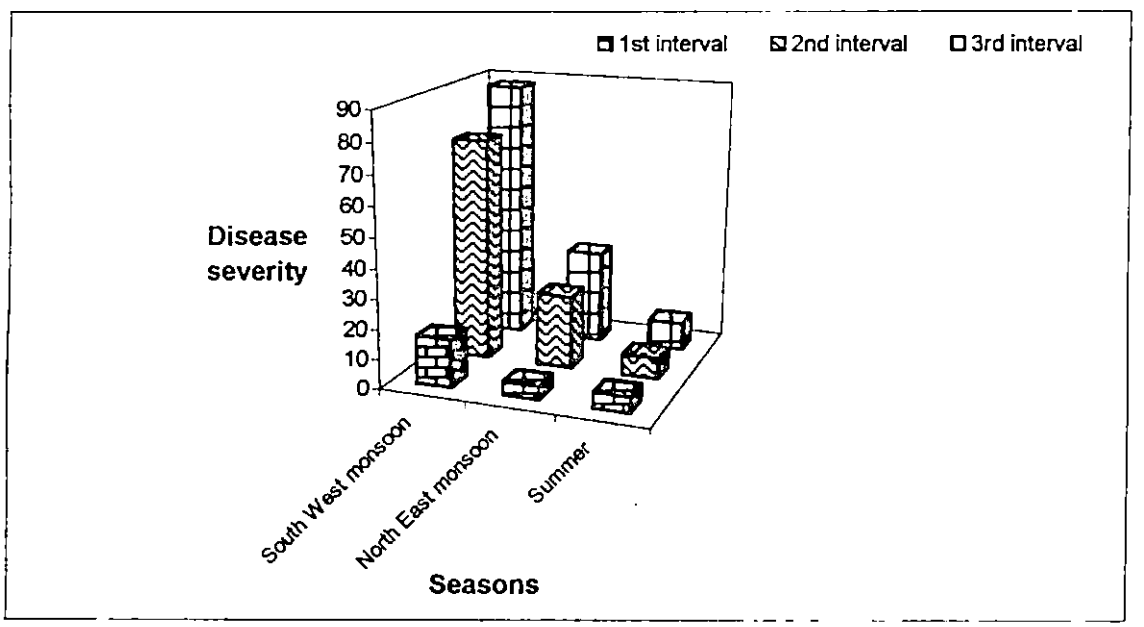


Fig. 4. Effect of different seasons on the severity of cowpea anthracnose

When three seasons were considered lowest disease incidence, severity and maximum yield were recorded during summer when the crop was raised in January-March. During this period disease appeared only at the later stage of harvesting.

4.11 Assessment of crop loss in cowpea due to anthracnose disease

To estimate the crop loss due to anthracnose disease a field experiment was conducted with two treatments, one with fungicidal application and the other without any treatment. Observations on disease incidence, severity, yield and cost benefit ratio are furnished in Table 25. The results showed significant difference between treated and untreated plots in case of disease incidence, severity and yield. The mean disease severity was found to be 14.96 per cent when treated with fungicide whereas it was 73.33 per cent in the control plot. In the case of disease incidence also, treated and control plots showed notable differences of 25.73 per cent and 75.56 per cent respectively.

It is also observed that coefficient of disease index decreased significantly as compared to the control plot and the highest CODEX of 55.41 was registered in the non treated while the lowest CODEX (3.85) was obtained with plots sprayed with Carbendazim.

In the case of yield also, plots sprayed with fungicide gave a higher yield of 776.93 whereas control plots recorded only 310.21 kg ha^{-1}. Similarly yield loss of 53.85 per cent was recorded as against the yield of 776.93 kg ha^{-1} in treated plot. On the whole, it was found that severe outbreak of disease (55.41) could cause a loss of 53.85 per cent in yield under natural condition. Yield loss decreases as the severity of the disease reduces.

Table 25. Assessment of crop loss in cowpea due to anthracnose disease

Replication	Disease severity (%)		Disease incidence (%)		Coefficient of disease index		Yield Kg ha-1		Yield loss (%)	C:B ratio
	Treated	Control	Treated	Control	Treated	Control	Treated	Control		
1	12.54	96.67	32.83	100.00	4.12	96.67	1020.45	553.75	45.73	1:14
2	17.19	100.00	38.39	100.00	6.60	100.00	1185.30	231.50	80.47	1:17
3	16.67	16.67	25.00	20.00	4.17	3.33	477.82	355.58	25.48	1:1
4	20.00	33.33	23.33	33.33	4.67	11.11	338.92	266.69	21.31	1:1
5	6.67	96.67	18.18	100.00	1.21	96.67	546.42	92.60	83.05	1:12
6	16.67	96.67	16.67	100.00	2.78	96.67	1092.68	361.14	6.95	1:15
Mean	14.96	73.33	25.73	75.56	3.85	55.41	776.93	310.21	58.85	1:13
t value (0.05)		(3.54)		(3.22)		(3.31)		(3.35)		

As the cultural operations were same in both treatments, only the expenditure incurred for fungicidal application is taken into account while calculating C:B ratio and it was observed that in the plot treated with fungicide gave an yield of 776.93 kg ha^{-1} as compared to control plot (310.21 kg ha^{-1}). The treated plot gave a cost : benefit ratio of 1:1.3.

4.11.1 Extent of plant damage due to anthracnose disease

The extent of plant damage due to anthracnose disease in treated and control plots are presented in Table 26 and Fig.5 & 6. There was a drastic reduction in the height of both untreated (46 cm) and carbendazim treated (57.17 cm) diseased plants as compared to untreated (55.75 cm) and treated (64 cm) healthy ones. However slight increase in height was observed in diseased treated plants as compared to healthy untreated ones. Same trend was observed in case of total number of leaflets also. Number of leaflets were more in carbendazim treated healthy and diseased plants. Moreover, number of leaflets were more in diseased plants (189.33) as compared to healthy treated ones (187.67), but no noticeable difference could be observed among the two. However, the number of leaflets were less in untreated diseased plants (111.00) as compared to healthy untreated ones (142.50). Regarding the disease incidence and severity on leaves, untreated plants recorded 21.62 per cent incidence and 20.67 per cent severity whereas treated ones showed only 9.90 per cent and 12 per cent incidence and severity respectively.

With regard to the stem infection, cent per cent incidence and severity was observed in untreated diseased plants whereas in treated ones, incidence and severity were 22.22 and 16.67 per cent respectively.

Table 26. Extent of plant damage due to anthracnose disease

Category of plants	Height* in cm	Leaves				Stem		Vines					Pods		
		Total No. of leaflets	No. of leaflets infected	Per cent disease incidence	Per cent disease severity	Per cent disease incidence	Per cent disease severity	Total No.of vines	No. of vines infected	Per cent disease incidence	Per cent disease severity	Total No.of pods	No. of pods infected	Per cent disease incidence	Per cent disease severity
Diseased untreated	46.00	111.00	24.00	21.62	20.67	100.00	100.00	23.00	14.67	63.78	56.67	10.00	0.17	1.70	6.67
Diseased treated	57.17	189.33	18.75	9.90	12.00	22.22	16.67	17.25	5.25	30.43	23.00	14.00	0	0	0
Healthy untreated	55.75	142.50	0	0	0	0	0	12.00	0	0	0	16.00	0	0	0
Healthy treated	64.00	187.67	0	0	0	0	0	19.00	0	0	0	20.67	0	0	0

*Average of 10 plants

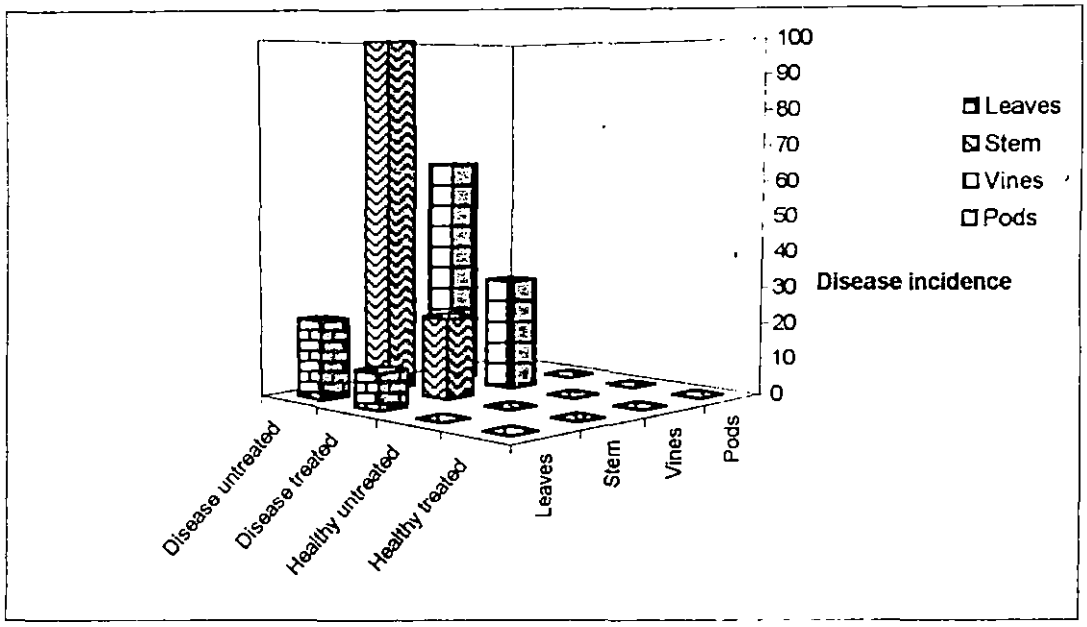


Fig.5. Extent of plant damage due to anthracnose disease

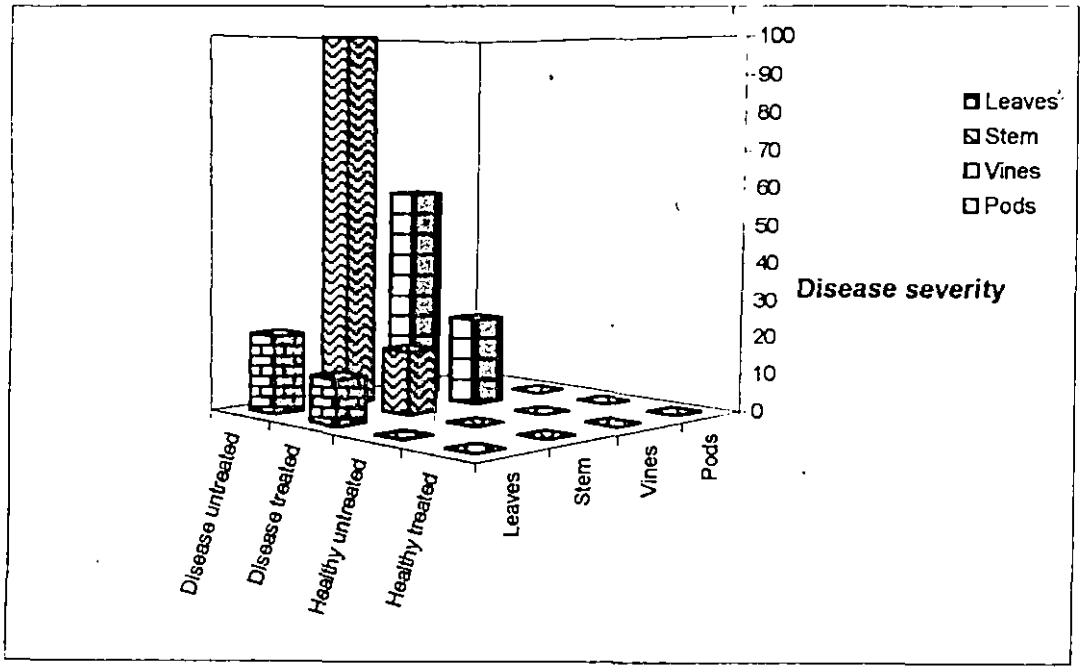


Fig.6. Extent of plant damage due to anthracnose disease

In case of infection on vines, 63.78 per cent incidence and 56.67 per cent severity were noticed in control plots whereas the per cent incidence and severity in treated plots were 30.43 and 23.00 respectively.

No pod infection was noticed in treated diseased plants whereas meagre incidence of 1.7 per cent and 6.67 per cent severity were observed in untreated diseased plants.

Discussion

DISCUSSION

Cowpea is an important pulse cum vegetable crop grown for food and fodder purpose both in tropics and subtropics. The crop is affected by a number of fungal, bacterial and viral diseases. Among the fungal diseases, anthracnose has become a serious problem in recent years in cowpea growing tracts of Kerala. This disease is found very severe in rainy season especially in May-June sown crop and 50 per cent crop loss has been reported from the pilot project areas of KHDP (KAU, 1996). Anthracnose disease of cowpea was first observed in India during 1966. But a perusal of literature revealed no information on anthracnose disease of cowpea in Kerala and the reports on Indian work are rather meagre and scanty. In view of the above facts, an investigation was carried out to study certain aspects of anthracnose disease of cowpea in Kerala particularly the etiology, host resistance, disease management and influence of climatic factors, which will add to our knowledge of the disease.

Eventhough the disease is a serious problem throughout Kerala, study has been limited to only four locations viz. Vellanikkara (Thrissur district), Angamali (Ernakulam district), Kanakkari (Kottayam district) and Vellayani (Trivandrum district). Isolation of the pathogen from various parts of the infected plants except leaf lamina collected from different locations showed the association of a fungus in both culture media and blotter method and its pathogenicity was proved by artificial inoculation under *in vitro* and *in vivo* conditions. Based on the morphological characters as described by Mordue (1971a) the fungus was identified as *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. In all infected plant parts obtained from Vellanikkara, Angamali, Kanakkari and Vellayani, the pathogen associated with the disease was found to be *C. lindemuthianum*. Onesirosan and Barker (1971), reported for the first time *C. lindemuthianum* as the cause of cowpea anthracnose. Rao (1966) also reported *C. lindemuthianum* as the

cause of anthracnose disease of cowpea from India. Thus the present findings on the etiology of disease are in conformity with the earlier reports. In addition to *C. lindemuthianum*, the infected plants collected from Kanakkari areas showed the association of *Colletotrichum capsici* (Syd.). Butler and Bisby which is identified on the basis of morpholigical characters which were identical with the descriptions of Mordue (1971b). The symptoms produced were slightly different from those produced by *C. lindemuthianum*. Allen (1983) reported *C. capsici* causing brown blotch of cowpea from African savannas. However, *C. lindemuthianum* was found associated with anthracnose in all four locations, indicating that this fungus may be the main pathogen responsible for anthracnose disease of cowpea in Kerala.

Studies on the morphological characters of the pathogen in different media like potato dextrose agar, potato carrot agar, neopeptone glucose agar showed variation in growth as well as in other colony characters. Growth of the fungus was slow in PDA, but it was fast in other two media. PDA showed less sporulation and no pink pigmentation, whereas in PCA and selective medium, pink pigmentation was observed due to high sporulation. Eventhough black discolouration was noticed on the reverse side of the colonies in PDA and PCA, no such pigmentation was observed in selective medium. Setae were present in all the three media, but were more in selective medium. Appressoria and intercalary chlamydospores were absent in PDA and selective medium, but it was abundant in PCA. Selerotia like bodies were observed abundant in PDA and very few in selective medium, but such type bodies were absent in PCA. However, some of these characters are similar to those observed by the earlier workers. Mathur *et al.* (1950) reported sparse and erratic sporulation of *C. lindemuthianum* on many natural and synthetic media, but observed excellent sporulation in neopeptone glucose agar selective medium. Mordue (1971a) reported slow growth and dark pigmentation in PDA medium as the main distinguishing character of *C. lindemuthianum* from *C. gloeosporioides*. The conidial characters of the fungus observed were similar in all the three media

and are in agreement with the description of Mordue (1971a) thereby confirming the findings of *C. lindemuthianum* as the main pathogen of anthracnose disease of cowpea.

Development of a disease in the plant is not a sudden effect. A chain of events are responsible for the causation of any disease. The symptoms and manifestation of injury to the plant is the last in this chain of events. Studies on the symptomatology are essential for the early detection of the disease. Hence, the symptomatology of anthracnose disease of cowpea collected from different locations were studied. In the present study, symptoms produced by *C. lindemuthianum* in different locations were almost same with slight variations. However, variation in the symptomatology was observed with weather conditions. In south west monsoon period plant showed rotting of the top portion and toppling of the foliage, in addition to anthracnose, which was not observed by any of the earlier workers. Whereas in other seasons, diseased plants showed typical anthracnose symptoms as described by earlier workers (Onesirosan and Barker, 1971). Other symptoms noticed were mildew appearance on the leaf lamina and the basal swelling, splitting and rotting of the stem, observed only in Ernakulam district. Symptoms observed on artificial inoculation were almost similar to those produced under natural condition. However the mildew symptoms and basal swelling could not be reproduced on artificial inoculation. It may be recalled that attempts to isolate the pathogen from the infected parts showing these symptoms also yielded negative results, as no fungal growth was noticed in case of mildew symptoms and *Fusarium* sp. was observed in case of basal swelling which indicated that, these two symptoms are not related to anthracnose disease of cowpea. A search through the relevant literatures did not give any information about the aforesaid symptoms. Because of the black lesions on the stem and mildew like appearance on petioles and leaf lamina, this disease is locally known as 'Karimbankedu' (vine blackening).

Several fungi found on the seeds of vegetables are known to cause considerable damage either directly to the seeds that carry them or to the crops that are raised from such contaminated seed stock. They may cause reduction or complete inhibition of germination or poor development of seedlings showing various kinds of disease symptoms. So the next attempt of the investigation was to find out the mycoflora responsible for poor germination of seeds. Mycoflora associated with cowpea seeds of healthy and diseased plants were studied by blotter, agar plate and pot culture methods. In all the three methods, *C. lindemuthianum*, the causal agent of anthracnose disease was noticed on diseased seeds in addition to *Fusarium* sp. *Choanephora cucurbitarum* and *Rhizoctonia bataticola*, the pathogens of pod rot and web blight of cowpea, were also observed on diseased seeds. As compared to healthy, association of mycoflora was more in diseased seeds. Ambika (1991) reported a number of pathogenic and saprophytic fungi associated with cowpea seeds, but no *Colletotrichum* species were observed. However, Sharma *et al.* (1988) observed *C. gloeosporioides* on cowpea seeds in addition to Fusarium, Penicillium and Aspergillus species.

In case of percentage of germination, both healthy and diseased seeds which were treated with carbendazim gave good germination as compared to untreated ones and it may be due to the growth promoting effect of the carbendazim fungicide. Nene and Thapliyal (1971) reported beneficial side effects of carbendazim like, stimulation of growth, flowering and yield of plants, besides disease control. With regard to the inhibition of germination, healthy seeds recorded only 10 per cent and 6.7 per cent in blotter and pot culture methods whereas diseased seeds showed 30 per cent and 21.7 per cent respectively which may be due to the association of more number of mycoflora on diseased seeds.

Studies on the seed borne nature of the pathogen by agar plate method showed the presence of the pathogen in the seed coat, cotyledons and embryos of

the diseased seeds indicating that *C. lindemuthianum* causing anthracnose disease of cowpea was externally as well as internally seed-borne and it is similar with the findings of Prasanna (1985). In the present study 20-42.85 per cent seedling infection was noticed, which also indicating the seed-borne nature of the pathogen. This result confirmed the earlier report of Prasanna (1985) who also observed 30-90 per cent seed infection in cowpea due to anthracnose disease.

The next aspect of investigation was to find out the effect of toxin on disease development which gave negative result indicating that, the toxin of *C. lindemuthianum* has no role in causing the anthracnose disease of cowpea. However, the toxin effect of *C. gloeosporioides* on disease development has been reported by Varma (1991).

Genetic response of the host to the pathogen is an innate plant factor determining success of infection. A genetically susceptible plant is infected readily and rapidly while a resistant plant poses obstacles in the way of penetration and establishment of pathogen. Moreover, use of resistant varieties for controlling plant disease is a cheap, simplest and effective method. Screening of large number of genotypes of a crop with considerable genetic diversity is a method for locating resistant types against disease which could be further utilized for the development of resistant varieties with desirable characters. With this idea, a number of genotypes of cowpea obtained from regional station of NBPGR, Vellanikkara, Department of Olericulture, College of Horticulture and KHDP were screened for host resistance against anthracnose disease under field condition during October 1997. The result showed that out of 50 genotypes evaluated, Kanakamony, a bush type cowpea variety was completely free of disease and seven genotypes viz. VS-28, VS-389, VS-14, VS-29, VS-12, VS-81 of bush types and CWP-16 of semi erect were found to be highly resistant to the disease in which the disease severity and incidence ranged only from 1.67-10 per cent and 3.85-15.0 per cent respectively. Twelve

genotypes showed moderately resistant reactions of 12-25 per cent and 16 genotypes were of moderately susceptible and 14 genotypes were found to be highly susceptible to the disease. Malika, a popular pole type variety of Kerala showed 32 per cent infection and the popular bush type variety Pusa Komal showed 100 per cent disease incidence and disease severity which is in accordance with the reports of Vander Plank (1968) who reported that the differential responses of genotypes in the case of disease resistance may be due to differential interaction of pathogens with the genotypes, they affected. Certain genotypes which were resistant at early stage of plant growth were found susceptible to disease at later stage. A variety may show resistant type of reaction when the conditions for infection are not conducive, the inoculum load is not sufficient, the races of the pathogen present are not pathogenic or due to the nutrient status of the soil in which the crops are cultivated (Yarwood, 1978; Khan, 1989). Most of the semi-erect and pole type genotypes were found more susceptible to the disease and none of the pole type was found resistant.

Varietal resistance of cowpea to *C. lindemuthianum* has also been studied by various workers (Williams, 1974; Prasanna and Ramaprasanna, 1980; Sohi and Rawal, 1983). A variety could be called as resistant only if it shows the resistant characters consistently under different sets of environmental conditions and under uniform inoculum pressure. The varieties which are found immune or highly resistant under natural conditions showed same type of reaction even on artificial inoculation under natural and laboratory conditions. Thus, the present study showed that the variety Kanakamony is immune and genotypes VS-28, VS-389, VS-14, VS-29, VS-12, VS-81 and CWP-16 are highly resistant to anthracnose disease. An intensive research is necessary to find out the facts and factors for the apparent physiological resistance manifested by Kanakomony which was beyond the scope of present investigation.

With regard to yield most of the genotypes recorded good yield as the disease appeared at the later stage. A highly susceptible pole type VS-5 recorded the highest yield of 9.17 t ha^{-1} and a moderately resistant semi-erect genotype VS-35-1 yielded 6.83 t ha^{-1}. However, Kanakamony, an immune bush type recorded an yield of 6.28 t ha^{-1}. Pole types are usually high yielders than bush type, because of the long poded character, long duration and more vegetative growth of the plant. Eventhough VS-5 is categorized as highly susceptible, it showed only 10 per cent disease severity on the 50th DAS and 55 per cent disease severity was recorded only on 60 DAS i.e. towards the fag end of the crop. In case of VS-35-1 also only 21.67 per cent disease severity recorded at 60 DAS. These may be the possible reasons for increase in yield in VS-5 and VS-35-1 as compared to the immune bush type one.

An appropriate approach for disease management will be integration of methods directed against the pathogen, in favour of the host and for modification of the environment. For this, the most effective way is the use of resistant varieties supplemented with cultural, chemical and biological methods. Present studies on varietal reaction to anthracnose revealed that most of the cowpea varieties especially yard long bean types which are mostly preferred in Kerala are susceptible to the disease. So the control of the disease by plant protection chemicals was found to be the another alternative method. Plant disease control aims at prevention or reduction in the incidence or severity of the disease. Among the various methods of plant disease control, use of chemicals offer comparatively more effectiveness and quick action in prevention or reduction of disease. As anthracnose of cowpea is found severe during rainy season, use of chemicals offer better control of the disease. Although the earlier reports revealed successful control of anthracnose by fungicidal application, the constant use of fungitoxicant chemicals had led to the occurrence of resistant races of pathogen, phytotoxicity and environmental pollution. Consequently, efforts are underway in finding alternatives to chemical fungicides. Studies conducted on the use of plant extracts and antagonists have opened a new

avenue for the control of plant diseases. Besides being safe and non phytotoxic, plant extracts and antagonists are known to be effective against various plant pathogens. So in the present investigation,an attempt was made to find out the effect of certain selected plant extracts and the antagonist *Trichoderma viride* to inhibit *C. lindemuthianum*, along with certain fungicides, which are reported to be effective against this pathogen. Contact fungicides such as chlorothalonil (0.2%), copper oxychloride (0.3%), mancozeb (0.2%), captan (0.2%), systemic fungicides like carbendazim, tridemorph, hexaconazole, triforine and triademefon each at 0.1 per cent concentration, botanicals such as garlic bulb extract, leaf extracts of Leucas, Lantana and Ocimum each at 10 per cent concentration and the antagonist *T. viride* were tested under *in vitro* and field conditions, to find out their effectiveness in reducing the anthracnose infection of cowpea caused by *C. lindemuthianum*.

In vitro evaluation of contact fungicides and botanicals showed that all treatments were found to be significantly superior to control and both chemicals and botanicals were equally effective against the pathogen *C. lindemuthianum* in both PCA and selective medium. However, the effect of treatments varied with different media. Mancozeb which showed maximum inhibition of the pathogen (92.73%) in selective medium gave the lowest inhibition (74.43%) in PCA. In PCA, Leucas leaf extract was found to be most effective (92.7%) while in selective medium, copper oxychloride was found to be least effective giving 67.65 per cent inhibition. It may be due to the synergistic reaction of mancozeb and inhibitory reaction of copper with other components of the selective medium. Pimental *et al.* (1971) reported effectiveness of dithane M-45 against *C. gloeosporioides* under *in vitro* condition. Singh *et al.* (1977) observed complete inhibition on the growth of *C. capsici* with captan, Bordeaux mixture, dithane Z-78 and blitox-50.

In vitro evaluation of *T. viride* against *C. lindemuthianum* in both media showed antagonistic effect by overgrowth on the pathogen. The effectiveness of

T. viride may be due to its fast rate of growth, thereby checking the growth of the pathogen. The antagonistic activity of *T. viride* on different pathogens of vegetables have been reported by many workers (Wright, 1956; Sporteli *et al.*, 1983; Krishnamoorthy and Bhaskaran, 1990; Ramanathan and Sivaprakasam, 1993 and Menon, 1996).

In bioassay of different systemic fungicides, only carbendazim, tridemorph and hexaconazole showed inhibitory effect against the pathogen in both media. Both carbendazim and hexaconazole were found effective in both media, however, carbendazim gave maximum per cent inhibition (32.7%) in selective medium whereas hexaconazole, gave maximum effect (26.1%) in PCA.

An optimal recomendation of a fungicide for control of anthracnose disease could be brought about only when applying under field conditions. The natural environment will definitely a limitating factor. A fungicide which is more effective even in such adverse conditions may be termed stable. With this view, effects of different fungicide as well as plant extracts and bioagent were tested under natural field conditions. All treatments were significantly superior to control in reducing the disease incidence and severity when recorded at different intervals from 10-40 days after last spray. All treatments were highly effective at 10 days after last spraying, as no infection could be noticed in any treatments except foliar application of garlic and antagonist which showed mild infection of 1.82 and 3.64 per cent respectively (Fig.7). At 20 days after last spray chlorothalonil and tridemorph gave 100 per cent control in all the three methods of application and lowest disease reduction was again noticed in case of antagonist and garlic foliar spray (Fig.8). All treatments were comparatively better in checking the disease infection even after 30 days of last spraying, of which chlorothalonil showed better protection by reducing incidence and disease severity irrespective of various methods of application. Gradual decrease of the persistance was observed especially

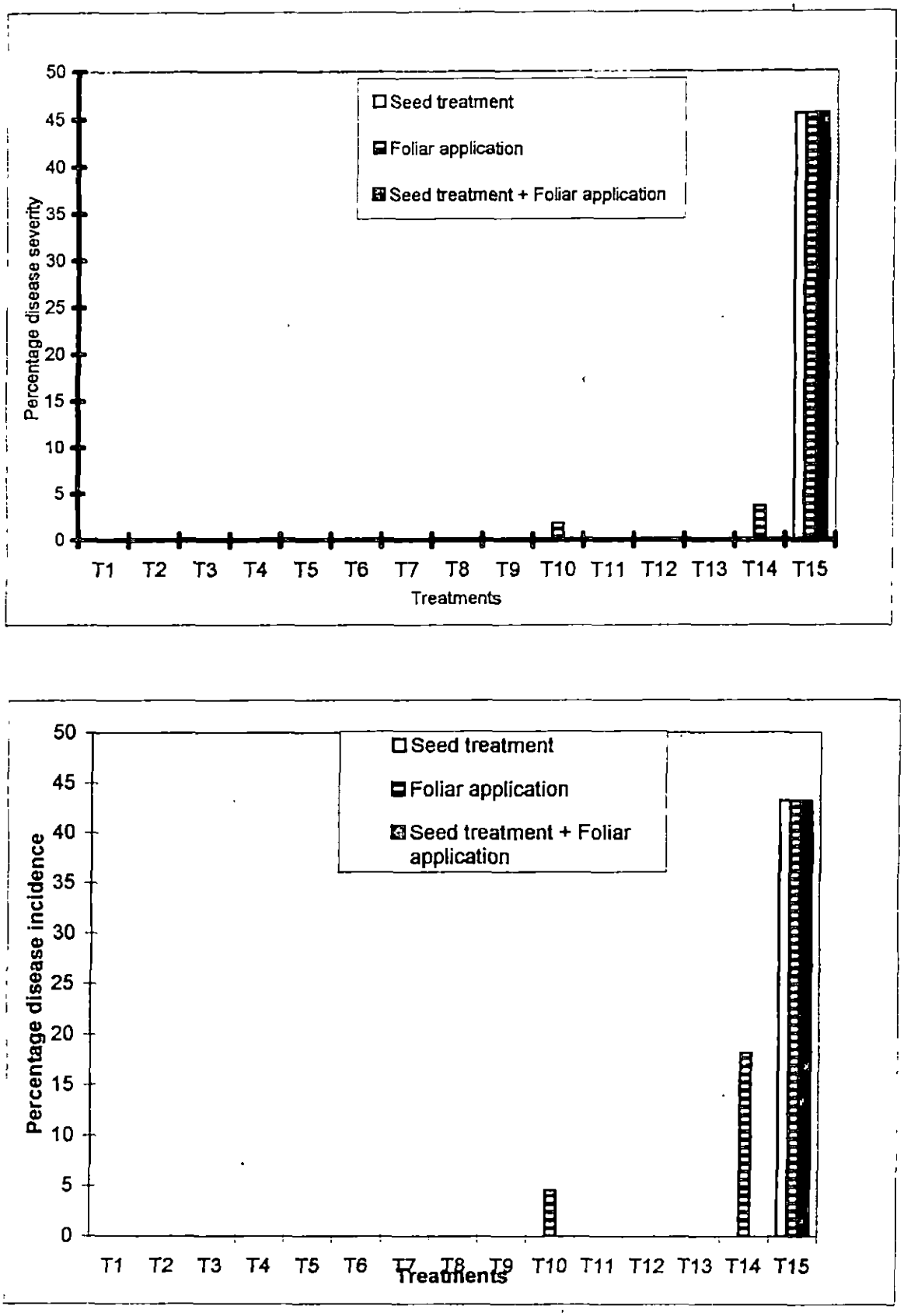
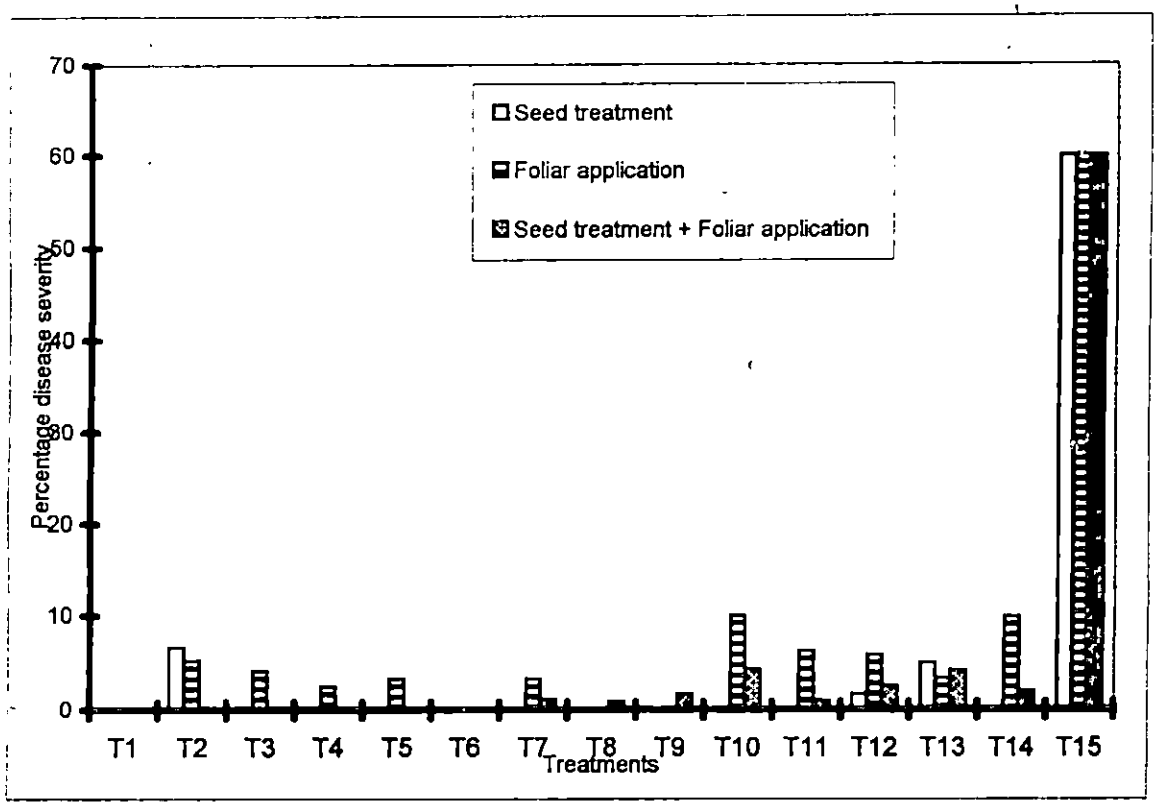


Fig.7 Effect of different treatments on the severity and incidence of anthracnose disease at 10 days after last spray



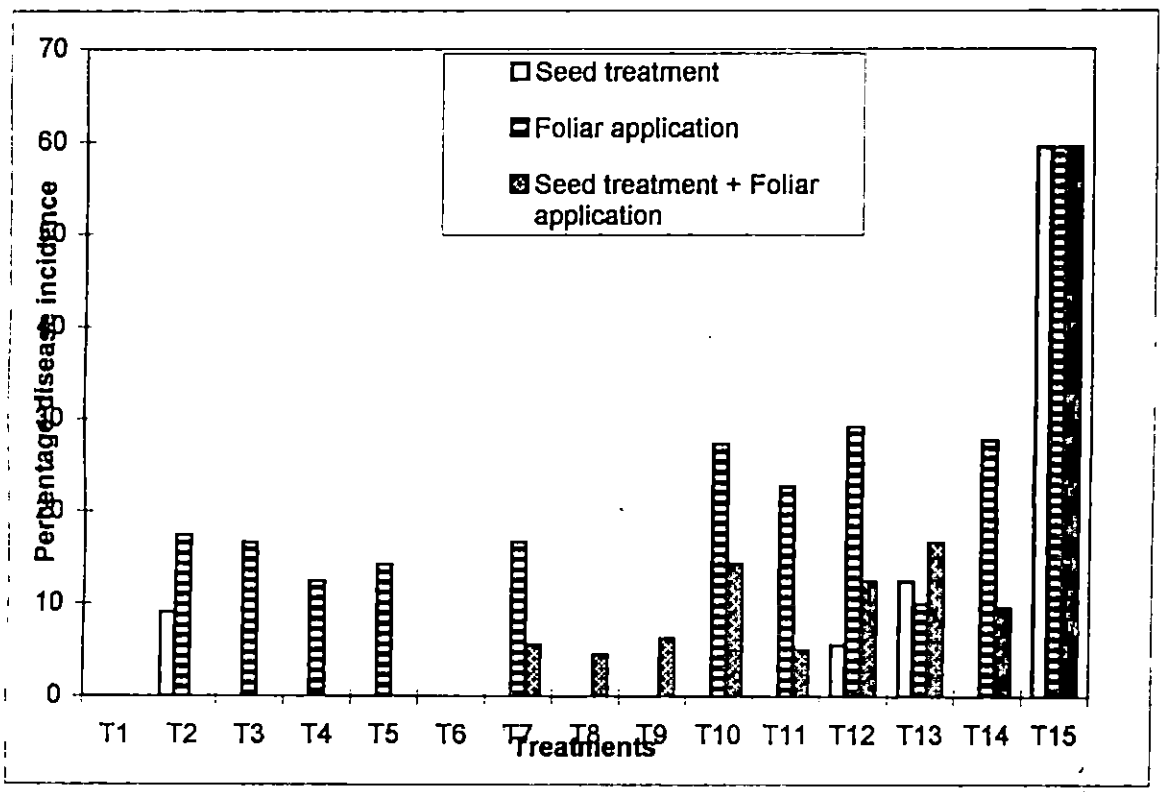
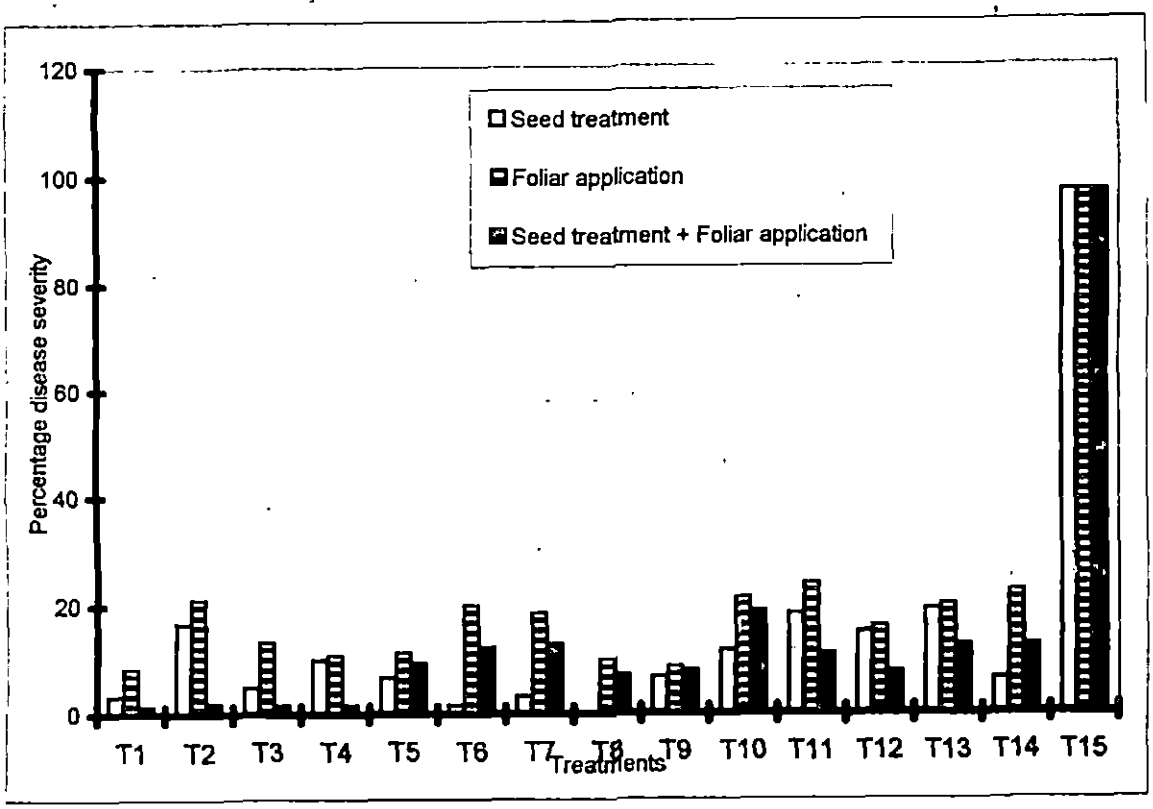


Fig.8 Effect of different treatments on the severity and incidence of anthracnose disease at 20 days after last spray

in case of botanicals and antagonist when applied as foliar spray (Fig.9). As the days progressed the persistance of the treatments were found to be decreasing as indicated by the increase in disease infection. Eventhough all treatments were superior to control, no significant difference could be noticed among other treatments. However chlorothalonil, captan and mancozeb showed lowest infection when recorded at 40 days after last spray (Fig.10).

Summing up the results already observed at different intervals revealed that all fungicides, botanicals and antagonist tested had inhibitory effect on *C. lindemuthianum*. However, fungicides were more effective than botanicals and *T. viride*, as they had long persistance. Among the various treatments chlorothalonil (0.2%) was best followed by captan (0.2%) and mancozeb (0.2%) in reducing anthracnose disease. Triademefon and triforine which showed no inhibitory effect on the pathogen under *in vitro* condition were however effective in controlling *C. lindemuthianum* in field condition. It may be due to the photoactivation of the compounds. Similar to the results obtained in the present studies, Ravi and Anilkumar (1990) and Thakur and Khare (1989b) reported the application of triademefon and triforine in reducing the disease incidence caused by *C. truncatum* in cowpea and *C. lindemuthianum* in *Vigna radiata*. Sohi and Rawal (1984) observed the effectiveness of carbendazim in controlling *C. lindemuthianum* in cowpea. Rajkumar and Mukhopadhyay (1990) found that seed treatment with thiram + carbendazim followed by three sprays of carbendazim gave minimum anthracnose incidence and maximum yield in bean. Seed treatment and three sprays of mancozeb was also found effective.

Skinner (1955) suggested that the presence of antibiotic constituents or some unknown substances contribute to the inhibitory activity of the plant extract. In the present study, active fractions of all plant extracts were comparable to each other showing fungicidal activity at par against the pathogen, *C. lindemuthianum*. Present



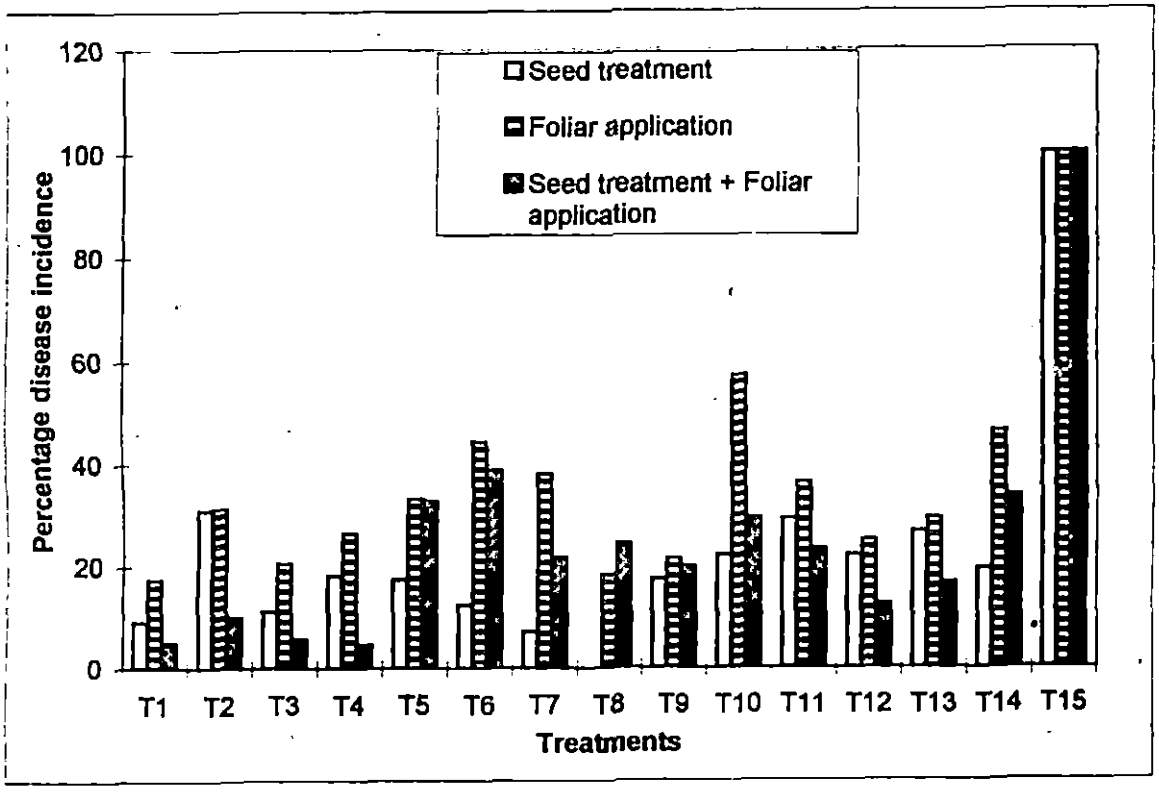
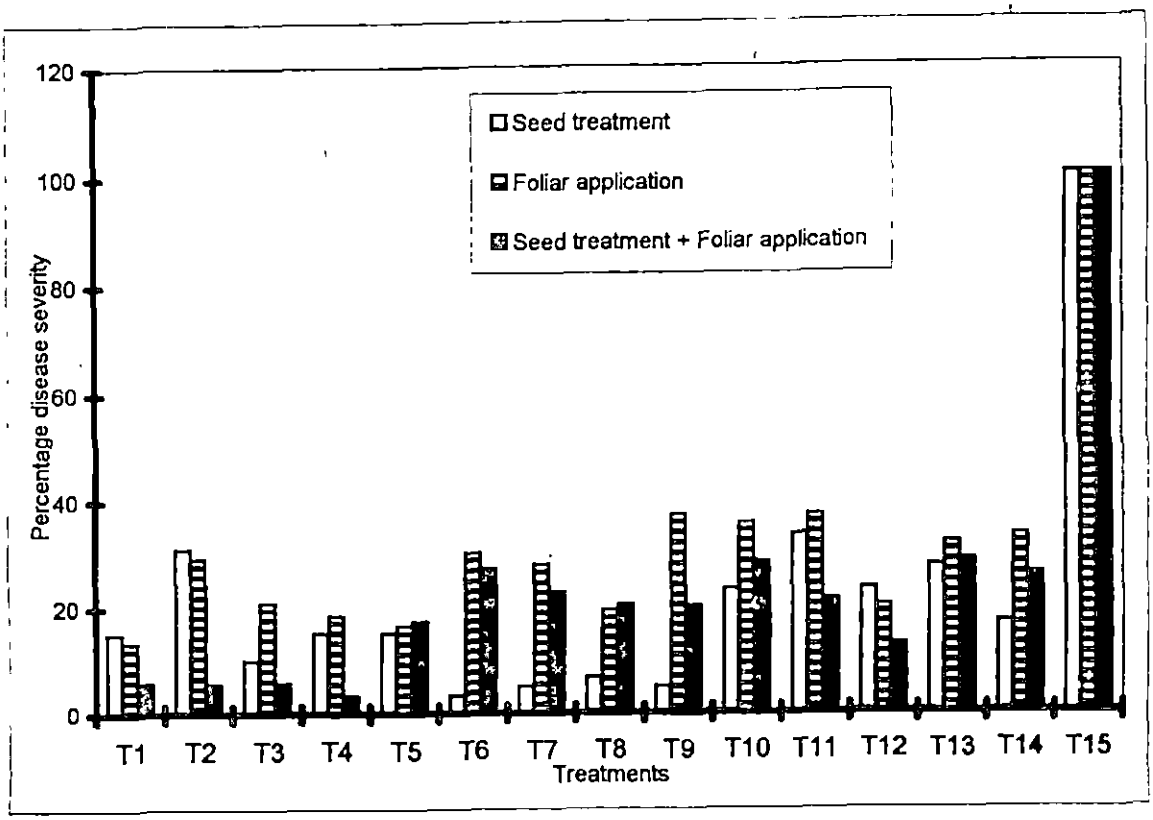


Fig.9 Effect of different treatments on the severity and incidence of anthracnose disease at 30 days after last spray



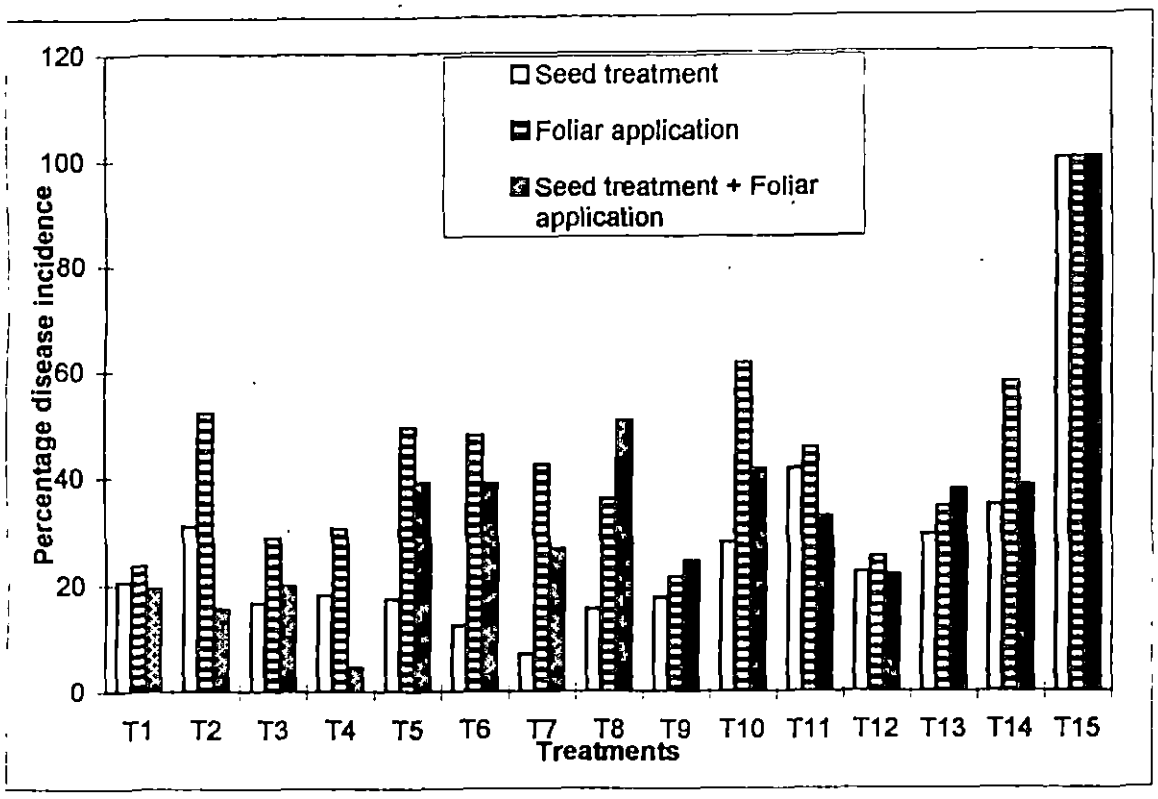


Fig.10 Effect of different treatments on the severity and incidence of anthracnose disease at 40 days after last spray

study also indicated that, plant products particularly Lantana, garlic and Ocimum offer a practicable and environmentally safer alternative to the use of fungicides and they can possibly be exploited for effective management of anthracnose of cowpea. The difference in the inhibitory effect of various plant products may be due to qualitative and quantitative differences in the antifungal principles present in them. Mohan and Ramakrishnan (1991) also observed the inhibitory effect of the extracts of *Allium sativum, Lantana camara* and *Azadirachta indica* on the pathogen *Exerohilum tauricum*. Simon (1996) observed the effect of *Ocimum sanctum* (10%) in controlling the downy mildew of bittergourd. Asha *et al.* (1997) also observed the fungitoxic property of ethanol extracts of *O. sanctum* on *C. capsici*. However, this is the first report on the antifungal properties of plant extracts against *C. lindemuthianum*.

With regard to the methods of application, eventhough all the three methods were effective in case of contact fungicides, seed treatment + foliar application was found to be most effective, while in case of systemic fungicides, seed treatment alone gave good control of disease. Maximum absorption of the active material is limited to a certain point. In this context, maximum active ingredient must have been absorbed in the seed treatment and the fungicide further applied on the foliage may not have absorbed. Sindhan and Bose (1981a) also tested certain fungicides by seed treatment, foliar spray and seed treatment + foliar spray and observed that benlate, bavistin, vitavax and ziram were effective in all three methods of application in reducing the anthracnose of French bean caused by *C. lindemuthianum*. Benlate, bavistin, vitavax and calixin persisted within the plant up to 15-20 days whereas, all other fungicides lost thier effectiveness within 5-10 days after foliar sprays and seed treatment. In case of antagonist also seed treatment was found to be effective. This is in line with the findings of Manomohandas and Sivaprakasam (1993) who reported that seed treatment of chilli with the spore suspensions of *Trichoderma* spp. was more effective than soil drenching in reducing

the incidence of pre emergence damping off. In case of botanicals also seed treatment + foliar application was more effective for the disease control. Thus in all cases seed treatment was found to be effective and essential which further confirmed the seed borne nature of the pathogen.

In reviewing the effect of application of fungicides and botanicals *in toto*, showed the drop in per cent inhibition over control, when the study is gradually shifted from the *in vitro* set up to field condition. Increase in inhibition percentage in natural condition may be due to the change from micro environment to the macro environment. Effect of fungicides may be due to the photoactivation of the compounds.

In case of botanicals, effect may be due to the activation and interconversions of the active principles of the extract due to the effect of sunlight. Lantana leaf extract which showed lowest inhibition under *in vitro* conditions showed maximum reduction under field condition when recorded at 40 days after last spray. By the action of sunlight, enzymes released help in the conversion of phenols to quinones which are more toxic than phenols against the pathogen. Leucas leaf extract which performed best in the *in vitro* studies however showed lowest control in field condition on 40^{th} day of last spray. The essential oils present in Leucas leaf extract must have slowly degraded due to the effect of sunlight and it is beyond the scope of the present studies to go deeper into this fascinating aspect.

Any treatment used in disease management will be effective when it reduces the infection and at the same time gives maximum yield. In the present study maximum yield was recorded from the plot sprayed with mancozeb followed by chlorothalonil and the lowest infection was also observed in these plots. This may be well explained from the fact that increase in yield is obtained corresponding to decrease in infection.

Maximum C:B ratio was obtained from mancozeb treated plots. Eventhough chlorothalonil gave good control was well as good yield, C:B ratio was comparatively less as compared to other treatments. There were discrepancies among the C:B ratio and the actual yield increase and disease control by various treatments. This is obviously due to the differences in the cost of fungicides.

Disease intensity in any crop is dependant upon the virulence of the pathogen, susceptibility of the host and prevailing environmental conditions. In cowpea, susceptibility to *C. lindemuthianum*, has been correlated with environmental factors by Williams (1975). In the present study to correlate the climatic factors on the incidence and severity of anthracnose disease, it was observed that the disease was severe in rainy season especially in June sown crop which recorded 95.27 per cent incidence and high severity of 87.78 per cent. Heavy crop loss was noticed due to the toppling of the foliage and rotting of the pods. Moreover the disease appeared early and duration of the crop was also less in this period. The higher incidence of anthracnose during rainy seasons related to its dependance on rain splash for the dispersal of spores. In addition, it may be due to the prevalence of favourable weather condition (22-28.9°C and 95% RH) for the disease development. In September sown crop, the disease appeared at later stage of the crop, i.e., 52 DAS. Eventhough 68.5 per cent incidence was noticed, disease severity was only 30.83 per cent and the yield was not much affected. The prevailing high temperature, 23.7-31.4°C and RH 98.2 per cent and less number of rainy days may not have much favoured the disease development. As compared to the September sown crop, disease incidence and severity was negligible in summer sown crop. The maximum incidence and severity recorded during this season were 9.72 per cent and 10.83 per cent only. Lowest incidence of disease during the season may be due to the prevalence of unfavourable weather conditions of high temperature (23.2-35.5°C), low RH (68.7%) and apparently no rainy days. Yield

was also not affected as the disease appeared at the later stage of the harvest. Thus the present study on seasonal influence on anthracnose revealed that, the environmental factors have a pivotal role on disease development and severity as reported by Colhoun (1973). Similar to the results obtained in the present studies, Mordue (1971a) reported that infection by *C. lindemuthianum* of bean anthracnose was favoured by warm wet conditions with a temperature of 15-26°C and humidity greater than 92 per cent. Sidhan and Bose (1981b) observed that anthracnose of French bean caused by *C. lindemuthianum* appeared in second or third week of June with a maximum damage from the beginning of August to middle of September in the hilly regions of Uttar Pradesh. He also observed that, best sowing time of French bean was summer for minimising the disease infection. Thakur and Khare (1993) observed high count of *C. lindemuthianum* on *Vigna radiata* at the end of July and a temperature range of 26-29°C, RH 91-96 per cent, rainfall 0-21.5 mm were found favouring the disease development. From the above findings, it can be concluded that best time for cowpea cultivation is during summer season (January-March) and southwest monsoon period (June-August), should be avoided in areas where anthracnose is a problem.

The next point for consideration was to find out the crop loss due to anthracnose disease. Crop loss was assessed by estimating the percentage of crop damage and loss in yield due to disease, in carbendazim (0.1%) treated and untreated plots. The study showed significant differences between treated and control plots in case of disease incidence, severity and yield. Mean disease severity was 73.33 per cent in control plots whereas treated ones showed only 14.96 per cent. In case of disease incidence also untreated plots showed a high incidence of 75.56 per cent whereas treated plots showed only 25.73 per cent. From the CODEX value and yield loss estimated, highest CODEX of 55.41 was registered in the non treated plot while the lowest CODEX 3.85 was obtained in carbendazim treated

plot. On the whole, it was found that severe out break of the disease could cause a loss of 53.85 per cent in yield under natural condition.

Sohi and Rawal (1984) determined the extent of yield losses due to anthracnose in three bean cultivars. They observed significant differences in disease levels and yield between the sprayed and unsprayed plots. An yield loss of 86 per cent occurred in the highly susceptible cultivars and 27 per cent in the moderately susceptible cultivar.

The last part of the discussion is on the extent of plant damage due to anthracnose disease. Leaves, stem, vines and pods of untreated diseased plant showed highest incidence and disease severity as compared to carbendazim treated ones. Drastic reduction in height of the diseased plants was also observed as compared to healthy ones in both carbendazim treated and untreated ones. However, treated diseased plants showed slight increase in height as compared to healthy untreated ones. Same trend was also observed in case of total number of leaflets. Increase in height and number of leaflets in treated healthy and diseased plants may be due to the stimulating effect of carbendazim. Nene and Taplial (1971) reported the growth promoting activity of carbendazim. The present study also confirmed the findings of Kumar (1996) who observed increase in height of cocoa seedlings with carbendazim treatment.

Summing up the discussion so far, it may be concluded that the present studies have enriched our knowledge in various aspects of anthracnose disease of cowpea in Kerala particularly the etiology, host resistance, disease management and influence of weather conditions on severity of the disease.

Summary

SUMMARY

Cowpea (*Vigna unguiculata* subsp. sesquipedalis [L.] Verdcourt) is one of the important vegetable crop cultivated throughout India. The damages caused by the attack of pests and diseases are the major constraints for the cowpea cultivation in India. Recently anthracnose disease has become a serious problem in cowpea growing tracts of Kerala. Hence the present investigations were carried out to study certain aspects of anthracnose disease with particular emphasis on etiology, host resistance, disease management and influence of climatic factors on disease development.

Isolation of the pathogen from various parts of the infected plants collected from four locations viz. Vellanikkara (Thrissur district), Angamali (Ernakulam district), Kanakkari (Kottayam district) and Vellayani (Trivandrum district) showed the association of a fungus and its pathogenicity was proved by artificial inoculation under *in vitro* and *in vivo* conditions. Based on the morphological characters the fungus was identified as *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. In addition to *C. lindemuthianum* another pathogen *C. capsici* (Syd.) Butler and Bisby was observed on the infected plants from Kanakkari areas. However the main pathogen responsible for anthracnose disease of cowpea in Kerala was identified as *C. lindemuthianum*, as the fungus was found associated with all the infected plants collected from different locations.

Morphological characters of the pathogen were studied in different media like potato dextrose agar, potato carrot agar and neopeptone glucose agar selective medium. Pathogen showed variation in growth as well as in other colony characters in these different media.

In the present study, symptoms produced by *C. lindemuthianum* in different locations were almost same with slight variations. Variations in the symptomatology was observed with weather conditions. In addition to the typical anthracnose symptoms of reddish brown lesions on the stem, toppling or breaking of the foliage was the another severe symptom observed in south west monsoon period. Symptoms produced on artificial inoculation were also similar to those produced under natural condition.

Mycoflora associated with cowpea seeds of healthy and diseased plants studied by blotter, agar plate and pot culture methods showed association of *C. lindemuthianum*, the causal agent of anthracnose disease in addition to various other fungi, on diseased seeds. It was also noted that the association of mycoflora was more in diseased seed when compared to healthy ones. Germination percentage of carbendazim treated healthy and diseased seeds was more as compared to untreated ones.

Studies on the seed borne nature of the pathogen showed that *C. lindemuthianum* causing anthracnose disease of cowpea is externally as well as internally seed borne. Effectiveness of seed treatment in disease management studies also confirmed the seed borne nature of the pathogen.

Exotoxin and Endotoxin of *C. lindemuthianum* were having no role in causing the anthracnose disease of cowpea.

Among the 50 genotypes screened, Kanakamony, a bush type cowpea variety was completely free of disease. Seven genotypes (VS-25, VS-389, VS-14, VS-29, VS-12, VS-81 and CWP-16) were highly resistant to the disease. Twelve showed moderately resistant reactions, 16 genotypes were moderately susceptible

and 14 genotypes were found to be highly susceptible to the disease. Some genotypes were high yielders with yield records of 6.28-9.17 t ha^{-1}.

In management of disease, all treatments were equally effective under *in vitro* and *in vivo* conditions. Chlorothalonil was found to be the best treatment followed by captan and mancozeb in field condition. Maximum yield was recorded in mancozeb followed by chlorothalonil treatment. Eventhough chlorothalonil gave good control as well as good yield, the C:B ratio was less compared to other treatments due to the high cost of the chemical. As far as disease control, yield and C:B ratio are concerned, mancozeb was found to be the best treatment. As the pathogen was seed borne, seed treatment was found to be essential and effective for reducing the disease incidence. Seed treatment alone gave good control especially in case of systemic fungicides and the antagonist *T. viride*. Additional two foliar sprayings at 15 and 30 DAS were also effective in case of contact fungicides and botanicals.

Summer season was found to be the best season for cowpea cultivation in Kerala in areas where anthracnose disease is a problem and south west monsoon crop should be avoided as the maximum infection and crop loss was observed during this period.

In crop loss assessment, significant difference was noticed between carbendazim treated and untreated plots for disease intensity and yield. Percentage of leaf, vine, stem and pod infection varied in treated and untreated plots. The highest CODEX of 55.41 caused loss of 53.85 per cent in yield under natural condition.

References

REFERENCES

Adebitan, S.A. 1994. Some factors influencing the growth and sporulation of *Colletotrichum truncatum*. *Int. J. Trop. Pl. Dis.* **12**(2):197-207

Adebitan, S.A., Ikotun, T., Dashiell, K.E. and Singh, S.R. 1992. *Pl. Dis.* **76**(10):1025-1028

Alabi, O. and Emechebe, A.M. 1992. Effect of temperature on growth and sporulation of cowpea brown blotch pathogen (*Colletotrichum capsici* (Syd.) Butler and Bisby). *Samaru J. agric. Res.* **9**:99-102

* Alam, M. and Rudolph, K. 1988. Recent occurrence and characterization of the races of bean anthracnose (*Colletotrichum lindemuthianum*) in West Germany. *Zeitschift Fur P Flanzenkrankheiten and Pflanzenzenschutz* **95**:406-413

Allen, D.J. 1983. The Pathology of Tropical Food Legumes: Disease Resistance in Crop Improvement. John Wiley & Sons, Chichester

Ambika, S. 1991. Bio-deterioration of important vegetable seeds due to Mycoflora II. M.Sc.(Ag.) thesis, Kerala Agricultural University, Vellanikkara, p.133

Anandaraj, M. and Leela, N.K. 1996. Toxic effect of some plant extracts on *Phytophthora capsici*, the foot rot pathogen of black pepper. *Indian Phytopath.* **49**(2):181-184

Asha, S., Sharma, O.P. and Jhamaria, S.L. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. *J. Mycol. Pl. Pathol.* **27**(1):29-31

Bains, S.S., Kaur, L., Dhaliwal, H.S. and Gill, A.S. 1989. Outbreak of a new anthracnose of mung and mash in Punjab. *Indian Bot. Rep.* **8**(2):164-165

Bankole, S.A. and Adebanjo, A. 1996. Biocontrol of brown blotch of cowpea caused by *Colletotrichum truncatum* with *Trichoderma viride*. *Crop Prot.* **15**(7):633-636

* Barros, S.T., Silva, I.P. and Menezes, M. 1981. Effect of fungicides on the control of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., artificially inoculated into seeds of the cowpea (*Vigna unguiculata* (L.) Walp.) cultivar Alagoano. *Fitopatolgia Brasileira*. **6**(3):465-468

* Barros, S.T., DE and Menezes, M., Fernandes, M.J. and Lira, N.P. 1985. Seed-borne fungi of 34 cowpea (*Vigna unguiculata*) cultivars in the state of Pernambuco Brazil. *Fitopatologia Brasileira* **10**:85-95

Bhardwaj, C.L. and Thakur, D.R. 1991. Economic management of black gram (*Phaseolus mungo*) disease with fungicides. *Indian J. Agric. Sci.* **61**(6):437-439

Bhat, N.M. and Sivaprakasam, K. 1993. Antifungal activity of some plant extracts. Crop diseases - Innovative techniques and management. (ed. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, p.335-340

Brian, P.W. and McGoman, J.C. 1945. Viridin: a highly fungistatic substance produced by *Trichoderma viride*. *Nature* **156**:144

* Carmo, M.G.F., Maffia, L.A., Haller, M.C.P. and Araujo, G.A. 1994. Progress and spread of anthracnose in beans grown as monoculture and intercrop with maize. *Fitopatologia Brasileira*. **19**(2):183-188

Colhoun, J. 1973. Effect of environmental factors on plant diseases. *Ann. Rev. Phytopath.* **11**:343-364

Dada, L.A.O. 1990. Assessment of anthracnose disease in some cultivars of cowpea (*Vigna unguiculata*) caused by *Colletotrichum lindemuthianum*. *J. Phytopath.* **130**(2):147-156

Dada, L.A.O., Connell, R.J., Nash, C., Pring, R.J., Lucas, J.A. and Bailey, J.A. 1994. Infection process and identity of the hemibiotrophic anthracnose fungus (*Colletotrichum destructivum*) from cowpea (*Vigna unguiculata*). *Mycol. Res.* **100**(9):1133-1141

Datar, V.V. and Mayee, C.D. 1981. Assessment of losses in tomato yields due to early blight. *Indian Phytopath.* **34**:191-195

Dhiman, J.S., Lal. T. and Singh, S. 1989. Reaction of cowpea cultivars to bacterial blight, anthracnose and cowpea mosaic diseases and their effects on economic performance. *Trop. Agric.* **66**(1):17-20

Ellis, M.A., Ferguson, J.E., Grof, B. and Sioclar, J.B. 1976. Transmission of *Colletotrichum gloeosprioides* and the effect of sulphuric acid scarification on internally borne fungi in seeds of *Stylosanthes* spp. *Pl. Dis. Rep.* **60**:353-361

Emechebe, A.M. 1981. Brown blotch of cowpea in northern Nigeria. *Samaru J. agric. Res.* **1**:20-26

Emechebe, A.M. and McDonald, D. 1979. Seed borne pathogenic fungi and bacteria of cowpea in northern Nigeria. PANS 25, 204-404

* Fouilloux, G. 1979. New races of bean anthracnose and consequences on our breeding programmes. In: Maraite, H. and Mayer, J.A. (eds.), Diseases of Tropical Food Crops. Universite Catholique de Louvai-La-Neuve, Belgium. pp.2151-253

* Gil, V.F., Bailey, J., O'connell, R. and Sheriff, C. 1994. Morphological and cellular characteristcs of colletotrichum isolates obtained from *Phaseolus vulgaris, Vigna radiata* and *Vigna unguiculata. Fitopatologia Colombiana.* **18**(1-2):42-52

Gil, L.S., Obi, J.U. and Husaini, S.W.H. 1983. Mycoflora of some Nigerian Leguminous seeds. *Legume Res.* **6**:27-33

Goodman, R.N. 1960. Colletotin a toxin produced by *Colletotrichum fuscum*. *Phytopathology* **50**:325-327

Hepperley, P.R., Mignucci, J.S., Sinclair, J.B. and Mendoza, J.B. 1983. Soybean anthracnose and its seed assay in Puerto Rico. *Seed Sci. Tecnol.* **11**:371-380

Hubbeling, N. 1957. New aspects of breeding for disease resistance in beans (*Phaseolus vulgaris* L.). *Euphytica* **6**:111-141

IITA. 1983. Annual report of International Institute of Tropical Agriculture, Ibdan, Nigeria

International Seed Testing Association. 1966. International rules for seed testing. *Proc. Int. Seed Test.Assoc.* **32**:107-115

Jacob, K.C. and Sivaprakasam, K. 1993. Evaluation of some plant extracts ad antagnosists for the control of pre-emergence damping off of brinjal (*solanum melongena* L.). Crop disease - innovative techniques and management. (ed. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, p.289-294

Janardhanan, K.K. and Hussain, A. 1970. Role of toxic metabolites and hydrolytic enzymes in jasmine blight disease. *Indian Phytopath.* **23**:541-545

Johnson, L.F. and Curl, E.A. 1972. *Methods of Research on the Ecology of Soil Borne Plant Pathogens*. Burgess Publishing Company, Minneapolis, p.247

Kanapathipillai, V.S. 1982. Seed mycoflora of hyacinth beans *Lablab niger* and long beans (*Vigna sesquipedalis*) and their pathogenic importance. *Trans. Brit. Mycol. Soc.* **78**:503-508

Karunakaran, P. 1981. Etiology and control of the disease of major tree spices (clove, nutmeg and cinnamon) in Kerala. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, pp.197

KAU. 1996. Final Research Report, on Farm Identification of Problems of Vegetable Grown in KHDP Pilot Project Area, Ernakulam, KAU, Vellanikkara

Kazhmi, A.R., Niaz, L. and Jilani, G. 1993. Evaluation of some plant extracts for antifungal properties. *Pak. J. Phytopath.* **5**:93-97

Khan, M.W. 1989. Powdery mildew of cucurbits - A three pathogen disease. *Int. J. Trop. Pl. Dis.* **7**:123

Krishnamoorthy, A.S. and Bhaskaran, R. 1990. Biological control of damping-off disease of tomato caused by *Pythium indicum* Balakrishnan. *J. biol. Control.* **4**(1):52-54

Kumar, A.K.M. 1996. Vascular streak dieback of cocoa and its management. M.Sc. thesis, Kerala Agricultural University, Vellanikkara, p.45

Kurucheve, V. and Padmavathi, R. 1997. Fungitoxicity of selected plant products against *Pythium aphanidermatum. Indian Phytopath.* **50**(4):529-535

Lin, K.H. 1948. Enzyme and toxic substance production by apple rotting fungi. *Lignan Sci. J.* **22**:139-142

Mahapatra, T.K. and Tewari, S.N. 1994. *Ocimum sanctum* L. leaf extract toxicity against collar rot (*Aspergillus niger*) and yellows root (*A. flavus*) diseases of groundnut. *Allelopath. J.* **1**(2):114-117

Manomohandas,T.P. and Sivaprakasam. 1993. Biological control of damping off disease in chilli nursery. Crop diseases - Innovative techniques and management. (ed. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, p.199-203

Marchetti, R., Nipoti, R., Ercole, N.D. and Guerzoni, M.E. 1992. Competition at atmospheric level of biocontrol mechanism in *Trichoderma* spp. *Petria*, 2(7):137-147

Mathur, R.S., Barnett, H.L. and Lilly, G.V. 1950. Sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopathology* **40**:104-113

Meena, S.S. and Mariappan, Y. 1993. Effect of plant products on seed borne mycoflora of sorghum. *Madras agric. J.* **80**(7):383-387

Menon, B. 1996. Etiology and management of damping-off of solanaceous vegetables. M.Sc. thesis, Kerala Agricultural University, Vellanikkara, p.

Mohan, S. and Ramakrishnan, G. 1991. Antifungal activity of various plant extracts/products on *Exerohilum turcicum* (Pass.) Lenonard. *Madras agric. J.* 78:57-59

Mordue, J.E.M. 1971a. *Colletotrichum lindemuthianum* Commonwealth Mycological Institute Description of Plant Pathogenic Fungi No.316, 2pp

Mordue, J.E.M. 1971b. *Colletotrichum capsici*. Commonwealth Mycological Institute. Description of Plant Pathogenic Fungi No.317, 2pp

Muthulakshmi, P. and Seetharaman, K. 1993. Use of plant extracts in the management of fruit rot disease of chilli caused by *Alternaria tenuis*. Crop diseases - Innovative techniques and management. (ed. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, p.295-302

Nair, M.C. and Ramakrishnan, K. 1973. Production of a toxic metabolite by *Colletotrichum capsici* (Syd.) Butter. and Bisby and its role in leaf spot disease of turmeric. *Curr.Sci.* **42**:362-363

Narain, A. and Das, D.C. 1970. Toxin production during pathogenesis of *Colletotrichum capsici* causing anthracnose of chillies. *Indian Phytopath.* **23**:484-490

Nenene, Y.L. and Thapliyal, P.N. 1971. Fungicides in plant disease control. Oxford and IBH Publishing Co., New Delhi, pp.278

Oladiran, A.O. 1980. Evaluation of fungicide and insecticide combinations in the control of cowpea diseases and on plant growth. *Trop. Grain Legume Bull.* **17-18**:13-15

Oladiran, A.O. 1990. The effect of fungicides and insecticides singly and in combination on the control of brown blotch, pod borer infestation and yields of *Vigna unguiculata* (L) Walp. *Trop. Pest Manage.* **36**(4):397-402

Oladiran, A.O. and Oso, B.A. 1983. Comparative susceptibility of some cowpea lines to brown blotch. *Trop. Grain Legume Bull.* **28**:10-17

Oladiran, A.O. and Oso, B.A. 1985. Interactions between fungicides, insecticides and spraying regimes in the control of fungal diseases, insect pests and yield of cowpea *Vigna unguiculata* (L.) Walp. *J. agric. Sci.* **105**(1):45-49

Onesirosan, P.T. and Barker, L.N. 1971. Stem anthracnose of cowpea in Nigeria. *Pl. Dis. Rep.* **55**:820-827

Onesirosan, P.T. and Sagay, S.O. 1975. Survival of two pathogens of cowpea over the dry season. *Pl. Dis. Rep.* **59**:1018-1020

Panse, V.G. and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*. 3rd edn. ICAR, New Delhi

Patil, R.K., Sharma, A. and Pathak, V.N. 1992. Inhibition of polyamine biosynthesis in *Botryodiplodia theobromae* Pat. And *Rhizopus arrhizus* Fisher by *Ocimum sanctum* leaf extract. *Indian J. Mycol. Pl. Pathol.* **22**(2):201-202

Pimental, C.P.V., Campacci, C.A. and Shirose, J. 1971. Preliminary *in vitro* testes with fungicides to inhibit *Glomerella cingulata. Biologica* **37**(4):79-83

Prasanna, K.P.R. 1985. Seed health testing of cowpea with special reference to anthracnose caused by *Colletotrichum lindemuthianum. Seed Sci. Technol.* **13**:821-827

Prasanna, K.P.R. and Ramaprasanna, K.P. 1980. Studies on seed borne aspects of anthracnose of cowpea caused by *Colletotrichum lindemuthianum* (Sac. & Magn.) Bri. & Car. in Karnataka. *Mysore J. agric. Sci.* **14**(2):273

Quereshi, S.H., Bashir, M. and Alam, S.S. 1985. Anthracnose of cowpea - a new disease record in Pakistan. *Trop. Grain Legume Bull.* **30**:26

Rahman, M.L., Akanda, A.M., Malek, M.A. and Khan, A.L. 1994. Some aspects of *Colletotrichum dematium* a pathogen of country bean anthracnose. *Bangladesh J. Pl. Pathol.* **10**(1-2):31-33

Rajkumar, B. and Mukhopadhyay, A.N. 1990. Chemical control of anthracnose of urd bean in field conditions. *Indian Phytopath.* **43**(1):102-105

Rajkumar, G., Kalloo and Pandey, P.K. 1995. Resistance in cowpea to *Pseudocercospora*. Paper presented in National Symposium on Recent Developments in Vegetable Improvement. 2-5 February at Raipur, p.23

Ramanathan, A. and Sivaprakasam, K. 1993. Effect of seed treatment with plant extracts, antagonists and fungicides against damping off disease of chilli. Crop diseases - Innovative techniques and management (ed. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, p.312-313

Rao, V.G. 1966. An acount of the market and storage disease of fruits and vegetables in Bombay, Maharashtra. *Mycopath. et Mycol. appl.* **28**:165-176

Ravi, K. and Anilkumar, T.B. 1990. Resistance development in *Colletotrichum truncatum* to carbendazim and thiophanate and growth and sporulation in resistant strains. *Indian J. Pl. Prot.* **18**(2):213-218

Riker, A.J. and Riker, R.S. 1936. Introduction to Research on Plant Disease. John Swift Co. St. Lows, Chicago, p.117

Senthilnathan, V. and Narasimhan, V. 1993. Effect of plant extracts/products on mycelial growth and spore germination of *Alternaria tenuissima* inciting the blight of onion and the nature of the antifungal components. Crop diseases - Innovative techniques and management. '(ed. Sivaprakasam, K. and Setharaman, K.). Kalyani Publishers, New Delhi, p.312-313

Sharma, M.C. and Sharma, B.C. 1969. Toxic metabolite production by *Colletotrichum gloeosporioides* causing citrus die-back in India. *Indian Phytopath.* **22**:67-74

Sharma, S., Raghunathan, A.N. and Shetty, H.S. 1988. Seed mycoflora of cowpea (*Vigna unguiculata* L. Walp) and their pathogenic importance. *Seed Sci. Technol.* **16**(2):541-548

Siddique, M.R., Mathur, S.B. and Neergaard, P. 1983. Longevity and pathogenicity of *Colletotrichum* species in seed stored at 5°C. *Seed Sci. Technol.* **11**:353-361

Simon, M. 1996. Varietal screening, host range and control of downy mildew of bittergourd (*Momordica chanrantia* L.) caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow. M.Sc. thesis, Kerala Agricultural University, Vellanikkara, p.85

Sindhan, G.S. and Bose, S.K. 1981a. Evaluation of fungicides against anthracnose of French bean caused by *Colletotrichum lindemuthianum*. *Indian Phytopath.* **34**:325-329

Sindhan, G.S. and Bose, S.K. 1981b. Epidemiology of anthracnose of French bean caused by *Colletotrichum lindemuthianum*. *Indian Phytopath.* **34**:484-487

Singh, R.S. 1985. Diseases of vegetable crops. Oxford and IBH Publishing Co. Pvt. Ltd. p.234

Singh, R.K. and Dwivedi, R.S. 1990. Fungicidal properties of neem and blue gum against *Sclerotium rolfsii* Sacc. a foot rot pathogen of barley. *Acta Botanica indiae.* **18**(2):260-262

Singh, R.P., Singh, P.N. and Singh, D.R. 1977. Note on fruit rot disease of chillies. *Indian J. Agrl. Res.* **11**(3):188-190

Singh, R.R. and Shukla, P. 1988. Collectotrichum leaf blight of black gram., *Farm Sci. J.* **3**(2):184-185

Skinner, F.A. 1955. Modern methods of plant analysis Vol.III. In: K.Peach and M.V.Tracey (Eds.), Springer-Verlog, Vierter-Band, pp.626-725

Sobti, A.K., Sharma, O.P. and Bhargava, A.K. 1995. A comparative study of fungicidal compounds and plant extracts against three pathogens of *Arachis hypogaea. Indian Phytopath* **48**(2):191-193

Sohi, H.S. and Rawal, R.D. 1983. Field resistance of cowpea varieties to anthracnose (*Colletotrichum lindemuthianum*) and stem blight (*Macrophomina phaseoli*) diseases. *Indian J. Mycol. Pl. Pathol.* **13**(1):58-60

Sohi, H.S. and Rawal, R.D. 1984. Studies on the efficacy of various fungicides for the control of anthracnose of cowpea caused by *Colletotrichum lindemuthianum. Pesticides* **18**(5):33-34

* Sporteli, M., Nipoti, P. and D'Ercole, N. 1983. Biological control trials against some fungus diseases of tomato grown under cover. *Informatore Fitopatologico.* **33**(6):35-38

Sundrival, R.C. 1991. Fungitoxic properties of flower extracts of some wild plants of Garhwal Himalaya. *Ad. Pl. Sci.* **4**(2):230-234

Suryanarayana, D. 1978. Seed Pathology. Vikas Publ. House Pvt. Ltd., New Delhi, pp.111

Tewari, S.N. and Nayak, M. 1991. Activity of four plant leaf extracts against three fungal pathogens of rice. *Trop. Agric.* **68**(4):373-375

Thakur, M.P. 1992. Survival of mung bean anthracnose. *Indian J. Pulses Res.* **5**(1):97-99

Thakur, M.P. and Khare, M.N. 1989a. Evaluation of mung bean varieties for resistance to anthracnose. *Indian J. Pl. Prot.* **17**(1):107-108

Thakur, M.P. and Khare, M.N. 1989b. Efficacy of different fungicides on the severity of mungbean anthracnose. *Indian J. Pulses Res.* **3**(1):94-96

Thakur, M.P. and Khare, M.N. 1991. Epidemiology and aerobiology of mung bean anthracnose. *Indian J. Mycol. Pl. Pathol.* **21**(3):233-240

Thakur, M.P. and Khare, M.N. 1992. Epidermiology of mung bean anthracnose. *Indian J. Pulses Res.* **5**(1):49-52

Thakur, M.P. and Khare, M.N. 1993. Factors affecting sporulation and conidial germination of two species of Colletotrichum from mung bean. *Indian J. Mycol. Pl. Pathol.* **23**(2):188-190

Tu, J.C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in Southern Ontario: Survival of the pathogen. *Pl. Dis.* **67**:402-404

Twumasi, J.K., Hossain, M.A. and Fenteng, P.K. 1989. Occurrence of some cowpea diseases and the evaluation of resistance of selected cowpea lines to brown blotch disease in the forest ecology of Ghana. *Trop. Grain Legume Bull.* **36**(7-9):4

Upadhyaya, M.L. and Gupta, R.C. 1990. Effect of extracts of some medicinal plants on the growth of *Curvularia lunata*. *Indian J. Mycol. Pl. Pathol.* **20**(2):144-145

Ushamalini, C., Rajappan, K. and Gangadharan, K. 1997. Management of charcoal rot of cowpea using biocontrol agents and plant products. *Indian Phytopath.* **50**(4):504-507

Vanderplank, J.E. 1968. Disease Resistance in Plants. Academic Press, New York, p.7-53

Varma, A.S. 1991. Fungal diseases of selected medicinal plants of Kerala. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, pp.176

Vincent, J.M. 1927. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*. **159**:850

Wang, X.M. and Li, Y.L. 1990. Evaluation of resistance to anthracnose in French bean varieties grown for seed. *Crop Genetic Res.* **3**:21-23

Weindling, R. 1938. Association effects of fungi. *Bot. Rev.* **14**:475-496

Wheeler, B.E.J. 1969. An introduction of Plant Disease. John Wiley & Sons Ltd., London, p.301

* Williams, R.J. 1974. Comparison of varietal reactions to cowpea anthrcnose in field plot and detached petiole inoculations. Proceedings of the American Phytopathological Society. p.87

Williams, R.J. 1975. An international testing programme for resistance to cowpea diseases. *Trop. Grain Legume Bull.* **2**:5-7

Williams, R.J. 1977. Identification of multiple disease resistance in cowpea. *Trop. Agric.* **54**(1):53-59

* Wong, P.Y.O. and Thrower, L.B. 1978. The effect of *Colletotrichum lindemuthianum* on the growth of *Vigna sesquipedalis*. *Phytopath-Z.* **93**(1):80-85

Wright, J.W. 1956. Biological control of soil-borne Pythium infections by seed inoculation. *Pl. Soil.* **8**:132-140

Yarwood, C.E. 1978. History and Taxonomy of Powdery Mildews. *The Powdery Mildew*. (Ed. D.M.Spencer). Academic Press, London, p.1-32

Zentmyer, G.A. 1955. A laboratory method for testing soil fungicides, with *Phytophthora cinnamomi* as test organism. *Phytopathology*. **45**:398-404

*Originals not seen

Appendix

APPENDIX

1. Potato dextrose agar medium

Peeled/sliced potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Water	:	1000 ml

2. Potato carrot agar medium

Sliced carrot	:	100 g
Peeled/sliced potato	:	100 g
Dextrose	:	20 g
Agar	:	20 g
Water	:	1000 ml

3. Neopeptone glucose agar selective medium

Neopeptone	:	2 g
Glucose	:	2.8 g
$MgSO_4.7H_2O$	:	1.23 g
KH_2PO_4	:	2.72 g
Agar	:	20 g
Water	:	1000 ml

ANTHRACNOSE DISEASE OF VEGETABLE COWPEA

[*Vigna unguiculata* subsp. sesquipedalis (L.) Verdcourt]

By

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ABSTRACT OF THE THESIS

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ABSTRACT

A study on various aspects of anthracnose disease of cowpea was conducted at College of Horticulture, Vellanikkara during 1997-98. Etiological studies revealed *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. as the main pathogen causing anthracnose disease in Kerala and the pathogen was found to be seed borne. Among the 50 genotypes tested, Kanakamony was found immune to the disease and seven genotypes were highly resistant to the disease. In disease management studies, all fungicides, botanicals and antagonist *Trichoderma viride* were equally effective under *in vitro* and field conditions. As far as disease control, yield and C:B ratio were concerned, mancozeb was found to be the best treatment. Summer season was found to be the best season for cowpea cultivation in areas where anthracnose is a problem. In crop loss assessment, significant difference was noticed between carbendazim treated and untreated plots in case of disease infection and yield, and yield loss of 53.85 per cent was recorded under natural condition due to this disease.