INTEGRATED MANAGEMENT OF ANTHRACNOSE OF COWPEA (*Vigna unguiculata* (L.) WALP.)

लोबिया (*विग्ना अंग्यूकुलाटा* (लि0) वाल्प0) के श्यामव्रण रोग का एकीकृत प्रबन्धन

THESIS

submitted in

Partial Fulfilment of the Requirements

For

the degree of

Doctor of Philosophy

in

Plant Pathology

2006

by

Santosh Kumar Singh

Id. No. CA- 5451/02

Department of Plant Pathology

C.S. AZAD UNIVERSITY OF AGRICULTURE & TECHNOLOGY, KANPUR- 208002 U.P., (INDIA)

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Dedicated to

My Venerable

Parents

Dr. P.N.Singh
M.Sc. (Ag.), Ph.D.
Associate Professor

DEPARTMENT OF PLANT PATHOLOGY,
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CERTIFICATE

*This is to certify that **Shri Santosh Kumar Singh,** Id. No. CA-5451/02 of College of Agriculture, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, a candidate for the degree of Doctor of Philosophy in Agriculture with major in Plant Pathology, has worked under my guidance and supervision during the course of the investigation and the present thesis entitled, **"Integrated Management of Anthracnose of cowpea (Vigna unguiculata (L.) Walp.)"** which is submitted is his genuine work.*

Date : August, 2006
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CERTIFICATE

*We, the undersigned, members of the Advisory Committee of Shri Santosh Kumar Singh, Id. No. CA-5451/02, a candidate for the degree of Doctor of Philosophy in Agriculture with major in Plant Pathology, agree that the thesis entitled, **"Integrated Management of Anthracnose of cowpea (Vigna unguiculata (L.) Walp.)"** may be submitted by him in partial fulfilment of the requirement for the degree.*

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Place: Kanpur
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Introduction

INTRODUCTION

Cowpea (*Vigna unguiculata* (L). Walp.) is one of the most ancient human food source and has probably been used as crop plant since Neolithic times (Summerfield *et al.*, 1974). One view is that cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago, at the same time as the introduction of sorghum and millet (Allen, 1983). Padulosi and Ng (1997) speculated that the Transvaal region of the Republic of South Africa was the centre of speciation of *V. unguiculata* due to the presence of most primitive wild varieties. Cowpea is now grown throughout the tropics and subtropics and has a wide variety of uses including hay, grazing grain, green manure and as a vegetable.

Cowpea has variable practices of cultivation. It is grown as solitary, mixed and as kitchen garden crop in Punjab, Uttar Pradesh, Bihar, West Bengal, Maharashtra, Karnataka, Haryana, Kerala, Delhi and Himachal Pradesh. It is grown mainly in *Kharif* season but in some areas farmers also grow during *Zaid* where irrigation facilities are available.

Cowpea has manifold uses, mostly it is used as vegetable but sometimes it is also consumed as pulse and boiled grain in the breakfast. Besides being a rich source of protein, minerals and vitamins for human beings, it is used as animal fodder also. It also enriches the soil through symbiotic nitrogen fixation from atmosphere by the root nodule bacteria (*Rhizobium* sp.) and has unique quality of preventing soil erosion by covering the soil surface. The vegetables as a whole occupy total production of 90 million tones (2000-2001) and the total beans productivity in India is 22 q/ha. But the exact data of area and production of cowpea are not available. However, it is grown in almost all the states of India including Uttar Pradesh. It is grown both in *Kharif* and *Zaid* seasons.

Cowpea belongs to the sub family - Fabaceae of Family-Leguminosae, in genus-*Vigna*. It is vigorously growing annual legume with a strong taproot, bearing numerous horizontally spreading laterals. Stem is prostrate and sinistore; more or less erect varieties also occur. Flowers shortly pediceled, white to pale-white in colour, borne on axillary recemes, 2 to 3 flowers to each peduncle, carrying 3 bracts at the small lobes, triangular and acute, standard whitish to violet with the wings violet and truncate at the base. Pods are 20 to 30 cm long, cylindrical and slightly curved with a thick obtuse beak, pendent and slightly constricted between the seeds.

Taking the world as a whole, beans or cowpea, French bean, Dolichoas bean, Cluster bean and Broad bean are cultivated in all the countries with an average production of different types of beans. Cowpea is specially liked due to its flavour, sweet taste in tender pods, colour and other characteristics. It has somewhat fleshier walled pods with less fibre in younger stages. It is used mainly as green pods but in dry form it is used as pulse. The foliage of this crop is also used for green manure, silage, hay and forage for animals.

The main use of cowpea is as a vegetable crop and as a legume, especially for small scale farmers in rural areas (Kay, 1979; Coetzee, 1995). It is very palatable, highly nutritious and relatively free of metabolites. The seeds also contain small amounts of β-carotene equivalents, thiamine, riboflavin, vitamin A, niacin, folic acid and ascorbic acid. The use of cowpea seeds as vegetable provides an inexpensive source of protein in the diet. The dried pulse may be cooked together with other vegetables to make a thick soup. The fresh, immature pods may be boiled as a vegetable. Fresh leaves and growing points are often picked and eaten in the same way as spinach (Coetzee, 1995, Quass, 1995). According to Kay (1979), cowpea is grown in many countries eg. as a dual purpose crop. The chemical composition of cowpea is given as below in Table 1.

Table 1: Chemical composition of Cowpea in percentage.

Composition	Seed	Hay	Leaves
Carbohydrate	56-66	-	8
Protein	22-24	18	4.7
Water	11	9.6	85
Crude fibre	5.9-7.3	23.3	2
Ash	3.4-3.9	11.3	-
Fat	1.3-1.5	2.6	0.3
Phosphorus	0.146	-	0.063
Calcium	0.104-0.076	-	0.256
Iron	0.005	-	0.005

(Kay, 1979; Tindall, 1983; Quass, 1995).

Inspite of the evolution of improved varieties and adoption of recommended package of practices, the average production of this crop is very low in India in comparison to many other countries of the world. Apart from the biotic stresses and other reasons, the main cause of its low production is attributed to onslaught of diseases of varied origins, which take away a heavy toll of the crop every year. Like other crops, cowpea is also susceptible to a wide range of pests and pathogens that attack the crop at all stages of growth. These include insects, bacteria, viruses and fungi. About 40 species of fungi are cowpea pathogens (Allen, 1983). However, fungal diseases namely wilt (*Fusarium tracheiphilium*); wilt and stem rot (*Sclerotium rolfsii*); stem rot (*Rhizoctonia* sp.); leaf spot (*Cercospora vignicola*); red stem canker (*Phytophthora vignae*) and damping off (*Corticium solani*) are the major limiting factors in successful cultivation of cowpea. If the ever burgeoning population of this country is to be steered out of malnutrition, suitable protection and production technology must be evolved to augment the production of this vegetable as well as pulse crop.

During the course of survey of cowpea around Kanpur, the important varieties of this crop were found moderately to highly infected with anthracnose disease caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. Except for fragmentary report of its occurrence in India, no other information is available so far on this important disease of cowpea.

Therefore, keeping in view the importance of crop and severity of the disease, it was thought necessary to carry out detailed study of the disease along with the pathogen with the following main objectives:

1. Survey and collection of diseased material from different locations.
2. Isolation, purification and pathogenicity test.
3. Morphological and cultural characters of the pathogen leading to its identification.
4. Survival and perpetuation of the pathogen.
5. Isolation of bio-agents and their screening against the causal pathogen *in vitro*.
6. Varietal screening against the pathogen for disease resistance.
7. To study the impact of the following on the disease severity:
    a) Date of sowing
    b) Soil pH
    c) Soil amendments
    d) Soil texture
    e) Different doses of P & K.
    f) Effect of bio-agents.

Review of Literature

REVIEW OF LITERATURE

Lobia (*Vigna unguiculata* (L.) Walp.) being an important vegetable and pulse crop has attracted the attention of plant pathologists all over the world whenever this crop is grown. A critical review of literature has revealed that lobia suffers from more than thirty pathogens of varied origins which lower the quality and quantity of the produce of this crop. Some of the common fungal diseases and their causal organisms reported on this crop are given below (Table 2).

Table 2: Major fungal diseases of "Cowpea" along with causal organism.

S. No.	Disease	Causal organism	Reported by
1.	Rust	*Uromyces appendiculatus*	Showden (1921)
2.	Leaf spot	*Cladosporium* sp.	Gardner (1925)
3.	Leaf spot	*Oidium* sp.	Brient & Martyan (1929)
4.	Wilt	*Fusarium tracheiphilium*	Kendrick (1929)
5.	Leaf spot	*Ascochyta pinodella*	Sparague (1929)
6.	Stem rot	*Rhizoctonia* sp.	Sen (1930)
7.	Leaf spot	*Cercospora vignicola*	Kawamura (1931)
8.	Stem Rot	*Glomerella vignicaulia*	Tehan (1937)
9.	Wilt	*Fusarium vasinfectum*	Thomas (1938)
10.	Damping-off	*Corticium solani*	Tavernetti (1942)
11.	Leaf spot	*Macrophomina phaseolina*	Weimer & Luttrell (1945)
12.	Leaf spot	*Rhizoctonia bataticola*	Vasudeva (1947)
13.	Red stem canker	*Phytophthora vignae*	Purss (1952)
14.	Anthracnose	*C. lindemuthianum*	

Amongst the fungal pathogens, *Colletotrichum lindemuthianum* appears to be quite severe which also cause various type of diseases on different hosts under suitable environmental conditions.

A critical review of literature revealed that there is only a report of the occurrence of anthracnose on cowpea and no information appears to be available particularly about severity of disease, perpetuation and spread of disease, resistant varieties and its management. There are number of aspects related to the present study on which the information is not available. So the work done in this direction on some other crops in relation to anthracnose caused by *Colletotrichum lindemuthianum* is being reviewed here to supplement the information.

Colletotrichum is one of the most important genus of plant pathogenic fungi worldwide, especially in sub-tropical and tropical regions. The genus causes economically significant diseases of cereals and grasses, legumes, vegetables and perennial crops including tree fruits. Above ground plant parts are affected at all stages of maturity from seedlings to mature plants and seed. Losses are caused by the effects of disease at different growth stages i.e. at establishment and during vegetative growth through loss of inflorescence and premature fruit fall through photosynthetic and other physiological disfunctions and through post harvest losses due to phenomenon of latent or quiescent infection (Bailey and Jegar, 1992).

The genus *Colletotrichum* was established by Corda (1931). The genus *Colletotrichum* belongs to Sub division-Deuteromycotina, Class- Coelomycetes, Order-Melanconiales and Family-Melanconiaceae (Ainsworth ,1971).

In the genus *Colletotrichum* formation of conidia and conidiophores takes place in acervuli. Acervuli are ennate, erumpent, discoid and elongate surrounded by long blade setae. Conidiophores aseptate usually unbranched and rarely branched producing conidia singly or in chain. conidiophores measures 20 x 8 μ in size. Conidia are exposed 16-42 x 4-28 μ in size.

The fungus *C. lindemuthianum* causing anthracnose disease of cowpea has globrous acervuli in pure culture. The growth is characteristically slow and the mycelial mat grayish black in colour.

The germinating spore of *C. lindemuthianum* typically forms a short germ tube that swells to form a spherical structure called an appressorium. The morphology and the role of appressoria have been reviewed by Emmett and Parberry (1975).

Colletotrichum lindemuthianum grew on different culture media like potato dextrose agar medium (PDA), Barnelt medium, bean seed agar, bean pod extract agar, bean leaf agar and pod agar (MVI). The cultures were kept at 24°C in the dark for 20 days. The best medium for growth and sporulation of fungus was MVI and PDA (Pria *et al.*, 1997).

Holdeman (1950) isolated a number of falcate spore of *Colletotrichum* on various hosts and divided into two types on the basis of cultural characters on Potato dextrose agar.

Symptomatology:

Bailey *et al.* (1990) reported that *Colletotrichum lindemuthianum* causing anthracnose of cowpea produced water soaked lesions in all tissues of cowpea seedlings. The hyphae formed in viable epidermal cells. The hyphae were large and highly branched. These infected epidermal cells died and soon afterwards the surroundings of uninfected cells were also died. When the initially infected epidermal cell become extensively colonized, thinner branched hyphae grow into and through the surrounding tissues producing lesions with large number of acervuli on the surface.

The symptoms of anthracnose disease were also reported by Paulo-Junior *et al.* (1994) on *Phaseolus vulgaris* and Rahaman *et al.* (1999) on Lablab.

Survival and perpetuation of the pathogen:

Rai and Chauhan (1966) stated that *C. capsici* survived in plant debris in the form of stromatic bodies for 10 months.

Onesirosan and Sagay (1975) reported that *C. lindemuthianum* incitant of stem anthracnose of cowpea, survived in the dry season at Ile-ife, Nigeria in diseased stem tissue either left on the soil surface or plowed under.

Prasanna and Rama Prasanna (1980) reported the seed borne aspects of anthracnose of cowpea caused by *C. lindemuthianum* (Sacc. & Magn.) Bri. & Cav. in Karnataka. In their study, out of 50 seed samples examined, 18 were found infected. These findings have been proved that the pathogen survives in seed also.

According to Prasanna (1985) the blotter method proved more suitable for detecting the pathogen (*C. lindemuthianum*) in the seed than the deep freezing blotter or agar plate methods. Infection was heavy (32-88% of the sample) in seeds with light coloured testas, compared with 2-19% in other seeds. Germination percentage decreased with an increase in seed infection, which caused seed rot and seedling mortality. The fungus was located in the seed coat, cotyledons and embryo. Transmission studies showed that infected seeds serve as a primary source of inoculum for spread of the disease.

Thakur (1992) reported the survival of *C. dematium* and *C. lindemuthianum* on crop debris of *Vigna radiata* until the following season.

C. lindemuthianum causing bean anthracnose could be detected up to 22 months from bean debris placed at 0, 10, and 20cm depth, using the host plant-assay. It was also recorded from naturally infested bean debris after exposure to winter conditions for 3 months. The inoculum concentration in over wintered bean debris was found sufficient to initiate disease on bean plants during the following growing season (Dillard and Cobb. 1993).

Ravi *et al.* (1999) evaluated various techniques for detecting the French bean anthracnose pathogen (*C. lindemuthianum*) in seeds and found that agar plate method was the best method and yielded maximum percentage of seed infection. The pathogen was present mostly in seed coats and cotyledons and rarely in embryonic axes.

Sharma *et al.* (2001) conducted field trails and reported that the bean anthracnose pathogen (*C. lindemuthianum*) can survive in seeds and act as main source of inoculum.

Qandah and Al-Momany (2003) reported that bean anthracnose pathogen (*C. lindemuthianum*) over winter in infected seeds which could be considered as the most important source of primary inoculum.

Isolation of bio-agents and their screening against the causal pathogen *in vitro*:

Trichoderma is easily isolated from any substrate using potato dextrose agar (PDA) medium by soil plate or dilution plate methods. Papavizas and Lumsden (1982) developed Trichoderma medium E (TME) for selective isolation from soil a specific medium named Trichoderma selective medium -TSM for quantitative isolation from soil. Elad and Chet (1983) and Elad *et al.* (1981) gave superior results than TME (Sana *et al.*, 1997). The TSM medium was further improved by adding Captan 50% 10mg/ litre (TSM) to avoid the contaminants like *Mucor*, *Rhizopus*, *Penicillium* especially *Fusarium* species (Askew and Lap, 1993). Kuling *et al.* (2000) isolated *Trichoderma* species from Himalyan soils using PDA and cellulose agar.

Two per cent malt extract agar (MA) and oatmeal agar (OA) were used to identify the species (Rafai, 1969; Biset, 1984).

The special nutrient agar, cornmeal dextrose agar (CMD) and potato dextrose agar were also used by Samules *et al.* (1998). The isolates were grown at 20°C, 25°C and 40°C for the study of morphological characters of different isolates.

Barros *et al.*(1995) observed that five *Trichoderma* species caused morphological changes in the hypha of *C. lindemuthianum* indicating an antagonistic reaction. The cellophane paper test showed that *T. harzianum* and *T. viride* produced a noticeable decrease in colony diameter of causal organism.

Trichoderma viride exhibited a high reduction in mycelial growth of *C. capsici*, causing disease in chilli (Jeyalakshmi *et al.*, 1998).

Varietal screening against the pathogen for disease resistance:

Williams (1974) compared the varietal reactions to cowpea anthracnose in field plot and detached petiole inoculations. Several hundred varieties of *Vigna sinensis* were exposed to *Colletotrichum lindemuthianum* in the field and those classified as immune, highly resistant or highly susceptible were subjected to detached petiole inoculation tests in the laboratory. Results indicated that the susceptibility of petiole tissue is a valuable indicator of varietal susceptibility.

Williams (1975) reported in Nigeria, 61 ICDN lines were found to highly resistant to *C. lindemuthianum* .

Sohi and Rawal (1983) found the field resistance of cowpea varieties to anthracnose (*C. lindemuthianum*) and stem blight (*Macrophomina phaseoli*) disease. Out of 141 varieties tested, 21 were resistant to *C. lindemuthianum* and *M. phaseoli*.

Adebitan *et al.* (1992) used three inoculation methods in screening cowpea genotypes for resistance to two *Colletotrichum* species. Twelve cowpea cultivars were screened for reaction to infection by *C. lindemuthianum* and *C. truncatum* using spraying seedling leaves with a spore suspension, stem injection with a spore suspension and wrapping wounded seedling stems with inoculum meal. The later method produced optimal conditions for infection and disease development.

Adebitan and Olufajo (1998) evaluated twelve cowpea varieties for yield, fodder production and disease resistance, during 1992-94 in the rainy season (July-October). Only IAR 7/180-4-5 showed multiple disease resistance to scab, anthracnose (*C. lindemuthianum*) and bacterial blight.

To study the impact of the following on the disease intensity:

Date of sowing:

Subramanyam (1979) observed that the sowing date of the crop has to be so adjusted that the best temperature and moisture conditions may prevail for the rapid growth of the host to escape the critical period of disease incidence. The disease incidence was high when the sowing of the crop was further delayed by August 27th but the decline in percentage of disease intensity was gradual.

Singh (1981) observed that early sowing of cowpea crop reduced the incidence of *Myrothecium roridum* to a great extent. Sinha *et al.* (1994) observed that early sowing of soybean susceptible variety (PK-262) reduced the incidence of disease caused by *Myrothecium roridum* in comparison to late sowing.

Alternation in date of sowing has great impact on the occurrence of the plant diseases. Thakur and Khare (1990) found that *C. dematium* and *C. lindemuthianum* were higher in early sowing (23rd June to 3rd July) than in late sowing (13th July). Disease progress was greater in the early planted crop and cv. J-45 was more severely affected than Pusa Baishakhi and K-851.

Singh (1999) reported that the rate of disease spread in a plant population and the severity of the disease were greatly influenced by the sowing time. The soybean crop sown on 25th June exhibited lesser intensity of *Myrothecium* leaf spot as compared to that sown on July 8th and 23rd.

Soil pH:

According to Chowdhary (1957) optimum pH for the growth of *Colletotrichum capsici* was between 5 to 6. *Colletotrichum leginarium* grew well at pH varying from 3.0 to 9.5. Maximum growth at 5.1 and beyond this a sharp fall in dry mycelia weight was observed under pH 6.3. However, the maximum sporulation was obtained at pH 5.6. No sporulation occurred at pH 2-3.

Chandrasekaran and Shanmugan (1984) observed that effect of soil pH on chickpea root rot and reported that infection was significantly higher at pH 6.0

followed by pH 5.0 and 7.0. Least infection was recorded at pH 9.0 followed by pH 8.0.

Soil amendments:

It has been observed that disease caused by *Sclerotinia sclerotiorum* and number of sclerotia per plant can be lowered down by the application of 10-40 tonnes of FYM/acre in cauliflower seed crop (Sandhu, 1992).

Singh *et al.* (2000) observed that soil amendments like pyrite and gypsum @ 2.0 tonnes/ha and mustard cake, castor cake, neem cake, paddy straw and wheat straw @ 20.0 tonnes/ha were more effective against sclerotinia stem rot of bishop weed. Maximum disease reduction was obtained by pyrite followed by neem cake, mustard cake and gypsum.

Soil texture:

Srivastava and Kamthan (2002) evaluated the effect of different soil types on chickpea wilt and observed that black soil supported highest wilt incidence (73.50%) where as it was 64.4, 59.9, 46.6 per cent in sandy loam, red soil, and clay soil respectively.

Ghasolia *et al.* (2004) found the highest disease incidence in loamy soil (29%) followed by clay (14.0%) and sandy loam (9%) soils, while lowest incidence (5.6%) was noted in the fields with sandy soil.

Different doses of P and K:

Most of mineral elements required for plant growth have been reported either to increase or to decrease the severity of some diseases. The effects of nitrogen (N), phosphorus (P) and potassium (K) on disease incidence have been most extensively reported, because of their limited availability in many soils in relation to their large quantity required for optimum plant growth (Huber, 1980).

Singh (1984) was of the opinion that good nutrition supports a luxuriant vegetative growth and provides conducive conditions for the foliar disease development.

Singh and Shukla (1985) reported that nitrogen significantly increased the severity of *Myrothecium* leaf spot disease in cowpea plants while phosphorus and potassium both, together or individually helped in minimizing the disease severity.

Adebitan (1996) reported in field trials during the rainy season 1991-93, the incidence of *C. lindemuthianum* was significant on the moderately resistant varieties like IT 822E-60 and IT 81D-1137 without phosphorus application. Anthracnose was most severe on varieties receiving no P and less severe with increasing P levels and was least with P_2O_5 at 80kg/ha.

Effect of bio-agents on disease severity:

Cowpea seeds infected with *C. truncatum* when treated with *T. viride* showed promising results against brown blotch disease. (Blankole and Adebanjo, 1996).

Ravi *et al.* (1999) tested six species of *Trichoderma* (*T. hamatum, T. harzianum, T. konigii, T. pseudokonigii, T. longibrachiatum* and *T. viride*), *Gliocladium virens, Bacillus subtilis* and *Pseudomonas fluorescens* for their antagonistic activity against seed borne *Colletotrichum lindemuthianum* in French bean. *T. viride* recorded the maximum inhibition of mycelial growth followed by *P.*

fluorescens and *T. harzianum* in a dual culture technique. Infected bean seeds are soaked in 10 per cent culture filtrate. treated with 0.4 per cent talk formulation of *T. viride* recorded minimum seed infection and maximum seed germination.

Kumar *et al.* (2000) studied about the control of seed borne fungal pathogens of pigeonpea and found that *Trichoderma viride* eliminated completely seed borne infection of *Alternaria alternata, Phyllosticta cajani, Rhizoctonia bataticola, R. solani, Curvularia lunata, Cladosporium cladosporiodes, Colletorichum* spp., *Trichothecium roseum* reduced the colony of *Aspergillus flavus, A. niger, Botrytis cineria, Fusarium moniliformae* and *F. semitectum* with significant increase in seed germination and emergence, root length, shoot length and fresh weight of seedling over *Chaitomium globosum* treated and untreated seeds (control).

Ravi *et al.* (2000) evaluated efficacy of botanicals in combination with the formulation of *Trichoderma viride* (0.4%) and *Pseudomonas fluorescens* along with fungicides as seed treatment and sprays against French bean anthracnose (*C. lindemuthianum*). They reported that seed treatments followed by foliar spray of different leaf extracts and bio-control agents were more effective than the seed treatment alone.

Gaikwad *et al.* (2002) evaluated various bio-control agents (*T. harzianum* and *T. viride* at 4g/kg seeds, *Pseudomonas fluorescens* and Vesicular Arbuscular Mycorrhiza at 10g/kg of seeds) and fungicides (Thiram and Carbendazim at 3g/kg of seeds) in a field experiments. They reported that seed treatment with bio-control agents and fungicides were highly effective against Charcoal rot of sorghum.

Adebanjo and Bankole (2004) evaluated the efficacy of some fungal and bacterial isolates obtained from cowpea phylloplane in inhibiting the *in vitro* and *in* *vivo* growth of *C. lindemuthianum*, causal agent of anthracnose of cowpea. Inhibition of growth of the pathogen with production of zone of inhibition was observed for *Aspergillus flavus*, *A. ochraceus*, *Penicillium ourantiogrisenum*, *Bacillus subtilis* BS21, *B. subtilis* BS22, and *B. subtilis* BS23. Inhibition of growth on contact was recorded for *A. niger* while *Trichoderma viride*- TH14 and *T. viride* – TH31 hyperparasitized the pathogen.

Material & Methods

MATERIAL AND METHODS

Survey and collection of diseased material:

Naturally infected leaves, stems and pods exhibiting different types of typical symptoms of anthracnose were collected at regular intervals during the crop season 2003 and 2004 from the Student's Instructional Farm, Legume Research Farm and Vegetable Research Farm of the C.S. Azad University of Agriculture & Technology, Kanpur. Diseased samples were also collected from farmer's field in the vicinity of Kanpur, Crop Research Station Deegh, Kanpur Dehat, Kannuaj, and Zonal Agricultural Research Station of the University at Bharari, (Jhansi).

Isolation and purification of the pathogen:

The diseased samples of the various cowpea cultivars showing different types of typical symptoms were thoroughly washed in tap water and separately cut into small pieces at about half a centimeter in size, showing half healthy and half diseased tissue with the help of sterilized blade. Those pieces were surface sterilized with aqueous mercuric chloride ($HgCl_2$) solution (1:1000) for 15-20 seconds, followed by 3-4 washings with distilled water. The excess of water was removed by drying the pieces in between the folds of sterilized blotting paper. The surface sterilized diseased pieces were then aseptically transferred on 2 per cent potato dextrose agar (PDA) medium in 90 mm Petri dishes. Three such pieces were placed at an equal distance in each Petri dish with the help of sterilized forcep aseptically and incubated at 24±2°C.

The Petri dishes used for isolation were already sterilized in hot air oven at 160°C for two hours and poured with 2 per cent potato dextrose agar medium. The medium was also sterilized before use in autoclave at the pressure 1.1kg/cm^2 for 20 minutes. After 48-72 hours of incubation, the growing mycelium from the margin of apparent colonies was sub-cultured on fresh PDA slants.

The cultures of the fungus thus obtained were purified by hyphal tip isolation and maintained on potato dextrose agar medium to keep the culture viable. Sub culturing was done at an interval of 15 days and preserved at low temperature (5 ± 1°C) in the refrigerator before use.

Pathogenicity test:

The pathogenicity of the isolated fungus, obtained from diseased plant parts, was tested and established according to Koch's postulate in pot culture at one month old plants of cowpea cultivar K-5269 that was found susceptible to the disease under natural conditions. For this study, the pots were filled with sterilized soil and five plants per pot were maintained.

The plants were inoculated by spore cum mycelial suspension prepared in sterile water, with the help of automizer. Both mechanically injured and uninjured plants used for inoculation purpose. The inoculated plants were kept in moist chamber for 48 hrs to provide sufficient humidity for infection. A separate set of plants sprayed with sterile water served as control.

After inoculation, the pots were shifted to glass house and watered periodically to maintain sufficient humidity for proper growth of the plants and disease development. These plants were examined periodically for recording the symptoms. Final data on disease were recorded 15 days after inoculation. The fungus was re-isolated from the artificially inoculated plants and compared with the original isolate of the pathogen.

Morphological and cultural characters of the pathogen leading to its identification:

The pathogen was found to produce the characteristic symptoms on different part of infected cowpea plants. It was identified on the basis of its morphological and cultural characters and pathogenic behaviour towards the host.

The morphological and cultural characters of the pathogen were studied on eight different natural, semi synthetic and synthetic media and their composition is given in Appendix-1.

(A) Preparation of media:

The media were prepared by the standard methods as described by Riker and Riker (1936). The different constituents of media were dissolved in distilled water separately and for its solidification 2 per cent agar was added. All the media were sterilized in autoclave at 1.1kg/cm^2 pressure for 20 minutes.

(B) Sterilization of Petri dishes and other glasswares:

Cleaned Corning or Borosil glasswares were used throughout the study. Unless otherwise mentioned all the Petri dishes were sterilized in hot air oven at 160°C for two hours.

(C) Pouring of media in Petri dishes :

Sterilized Petri dishes of 90 mm diameter used for pouring the medium. A set of three Petri dishes was maintained for each treatment and 20 ml sterilized media was aseptically poured in each Petri dish. The Petri dishes were handled aseptically in a sterilized inoculation chamber using a sprit lamp flame in the chamber. The medium in the dishes was allowed to solidify before inoculation.

Basal medium:

Potato dextrose agar medium (PDA) is well known medium to study morphological characters of most of the pathogens. Therefore, it was also used for studying the present fungus under study. The culture pieces of 5 mm size from 10-day-old culture were transferred to sterilized Petri dishes containing sterilized potato dextrose agar medium. The inoculated Petri dishes were incubated at 24 ± 2°C for 10 days.

Inoculum:

Ten-day-old culture of the pathogen grown on 2 per cent Potato dextrose agar medium was used as inoculum for all the studies. Petri dishes, containing equal quantity of agar medium, were inoculated with 5 mm culture disc cut with a sterilized cork borer from the actively growing region of the pathogen. Flasks containing liquid media, were similarly inoculated aseptically with 5 mm culture disc of the pathogen.

Determination of fungal growth in culture:

a) Qualitative growth:

To find out the redial growth rate of fungus, 90 mm diameter Petri dishes were poured equal amount (20 ml) of each medium. The radial growth of the colony was measured in two direction of right angle of each other and its average was taken as the diameter of the developing colony in mm.

The following morphological characters were studied:

(i) Mycelial characters:

Nature of growth, colour, branching pattern, septation in hyphae were recorded.

(ii) Acervulus characters:

The shape, size and colour of the acervuli were recorded.

(iii) Setae morphology:

For measurement and morphological observation of setae, pathogen was grown on a plate of PDA at 24 ± 2°C incubation temperature. Setae were harvested after 21-30 days from acervuli produced on the PDA plates. Setae were mounted in cotton blue in Lactophenol and measured at 40x with the aid of ocular and stage micrometer in compound microscope. Tests to characterize setae of the fungus on PDA were repeated twice. The number of setae measured per replication varied from 20-50 because setae production was not uniform.

(iv) Conidial morphology:

For measurements and morphological observations of conidia, the pathogen was grown on a plate of PDA at 24 ± 2°C incubation temperature. Conidia were harvested after 14 days from acervuli produced on the potato dextrose agar plate. Conidia were mounted in cotton blue in Lactophenol and measured in compound microscope.

b) Quantitative growth:

To determine the amount of mycelial mat produced by pathogen, 50 ml of each liquid medium was poured in 150 ml conical flask. After inoculation these flasks were incubated at 24 ± 2°C for 15 days after which the mycelial mat was filtered through Whatman's filter paper No.-42, dried in hot air oven at 60°C for 72 hrs, subsequently cooled in desicator and then finally weighed on electronic balance. The actual growth of fungal mat was obtained by deducting the weight of the filter paper from the final weight.

Survival and perpetuation of the pathogen:

To determine the survival of the pathogen through diseased plant debris, the following experiments were conducted both under laboratory and field conditions:

1. Under laboratory condition:

Diseased leaves and pods from naturally infected cowpea plants were collected in 2003 and kept in paper bags after drying between blotting papers and stored in the laboratory (15-25°C) till the next crop season. Monthly isolations were taken from material for one season to know the viability of the pathogen. Regular inoculation experiments were carried out to test the pathogenicity of the isolated fungus.

2. Under field condition:

Diseased plant debris, wrapped in tissue paper, were buried in the pot soil at the depth of 10cm and kept in open field condition (5-45°C). Regular isolations and pathogenicity tests were carried out at monthly intervals to find out the survival of the pathogen.

In order to study the role of diseased plant debris as a primary source of infection, two sets of experiments were conducted. In one set of pots, diseased plants debris, stored at room temperature (15-30°C) was mixed with sterilized soil and filled in earthen pots of 30cm diameter. In another set, the diseased plant debris, which was buried in soil and kept in open was thoroughly mixed with sterilized soil and filled in earthen pots. In all these pots, the surface sterilized healthy seeds were sown at the rate of 5 seeds per pot at equal distance. Data on seedling emergence and disease development were recorded. Plants raised from surface sterilized healthy seeds in sterilized soil in pots without mixing of any other plant debris served as control.

Role of seed:

Studies were carried out on the seed samples collected from the infected crop. The seed borne nature of the pathogen was tested by standard blotter method and agar plate method. A total number of 400 seeds were used for each method. Surface sterilized healthy seeds with 0.1 per cent mercuric chloride solution for 2-

3 minutes were also tested. The mercuric chloride treated seeds were thoroughly washed with sterilized water for 3-4 times to remove the traces of mercury from seeds. The Petri dishes containing seeds were incubated in incubation chamber for 10 days at 25 ± 2°C. After incubation the seeds were examined under stereoscopic binocular microscope for the presence of the fungus. Data on the occurrence of the pathogen on seeds were recorded.

Location of the pathogen in the seed:

To find out the location of the pathogen in seed, the naturally infected cowpea seeds after softening were broken apart with the help of sterilized forcep aseptically. The various parts of seed like seed coat, cotyledon and embryo were separated out and plated in Petri dishes containing 2 per cent potato dextrose agar medium. The Petri dishes were then incubated at 25 ± 2°C in an incubator. Observations on the growth of the pathogen were carried out regularly.

Role of seed in disease development:

For determining the role of seed as the primary source of infection in the next season crop, seeds were collected from the infected crop of cowpea during 2003. These seeds were stored in paper bags at room temperature (15-30°C) till the next sowing season. Healthy seeds were also collected which were artificially infected with the pathogen. For this purpose, fresh culture of the fungus was taken and mycelial cum spore suspension was prepared. The seeds were then heavily coated with this suspension and dried at room temperature in laboratory. These artificially infected seeds were stored in paper bags till the next crop season. These naturally and artificially infected seeds and surface sterilized healthy seeds were sown in autoclaved soil in pots during next crop season. Data on seedling emergence and number of infected seedlings were recorded.

Role of infested soil:

Experiments were carried out in two different sets for determining the role of infested soil as a primary source of infection. In one set, earthen pots of 30 cm diameter were filled with autoclaved soil. The soil was thoroughly mixed with the culture of the pathogen. The culture of the pathogen was raised on sand corn meal medium in 250 ml Erlenmayer flasks. The pots containing infested soil were kept at room temperature (15-30°C) till the next sowing season. In the next sowing season, five healthy surface sterilized seeds were sown in each pot. In the other set, the pots were filled with non-infested autoclaved soil and surface sterilized healthy seeds were sown in these pots. Data on seedling emergence and number of infected seedling, if any, were recorded.

Secondary spread of the disease:

Under natural conditions the disease occurs on the aerial plant parts. The typical spots produced by the pathogen on the leaves were suggestive of aerial infection. Abundant production of acervuli on the infected plants parts were frequently observed. Hence, the role of conidia (spores) in the aerial infection and secondary spread of the disease was examined during crop season.

The seeds obtained from completely healthy plants were surface sterilized before sowing. The autoclaved soil was filled in 30 cm diameter earthen pots and 5 seeds per pot were sown. These pots were kept in glass house to minimize secondary infection. After one month, these pots were transferred in the vicinity of naturally infected field. One set of pots was left exposed in open in close proximity of the crop in the field. The other set of spots was kept covered all around in a polythene tent, to prevent from aerial infection. Observations on disease development were taken after 10 days of placement.

Isolation of bio-agents and their screening against the causal pathogen *in vitro:*

Natural isolation of antagonists was made from the rhizosphere of cowpea plants. For this purpose, soil sampling was made from the rhizosphere of affected plants where disease was not noticed. They were taken to the laboratory separately in the poly bags. An homogenous mixture was prepared and out of the total soil, 10 g of sample was diluted with 90 ml of distilled water and make up the volume 10^{-7}. From this solution 10 ml each was added up to 10^{-7} concentration following standard serial dilution method. From this solution a drop was streaked over the Potato dextrose agar medium in the sterilized Petri dish or a drop was put and spread over where the cooled liquid medium was poured and mixed thoroughly by gentle shaking. Thus, prepared Petri dishes were kept for incubation for a week and whatever, colonies formed have to be evaluated their morphological characters. After confirmation the mycelium from colony as isolated and incubated to separate Petri plates containing media. After getting pure culture, the sub-culturing was followed at every 15 days for their maintenance. All the procedures were to be conducted in aseptic conditions under laminar flow chamber.

The bio-agents isolated from rhizosphere of cowpea plants were assayed in laboratory to find out their antagonistic activity following dual culture technique (Johnson and Curl, 1972). The antagonistic activity of micro-organisms was assayed on potato dextrose agar medium in sterilized 90 mm Petri dishes under aseptic conditions. Petri dishes were poured with 20 ml sterilized Potato dextrose agar medium and allowed to solidify. Five mm disc of antagonistic organism were placed at one corner and test fungus opposite to another corner. These discs were cut with the help of sterilized cork borer from 7-day-old culture and were placed in such a way so that they are approximately 6 cm away from each other. The plates were kept in an incubator at 24 ± 2°C for 7 days. Each treatment was replicated thrice. Data on antagonistic potential of different bio-agents were recorded.

Varietal screening against the pathogen for disease resistance:

Screening of cowpea varieties/cultures was carried out under natural conditions as well as under artificial conditions to find out the sources of resistance to anthracnose of cowpea caused by *Colletotrichum lindemuthianum*. Varieties/cultures were initially screened under natural conditions in the field during 2003 and 2004. The genotypes found free, resistant or moderately resistant under field conditions were subsequently tested under artificial conditions of inoculation in the glass house during 2005. For the study, 25 plants of each variety were raised and when the plants become 30 days old, they were inoculated with the spore-cum-mycelial suspension. The inoculation of plants was done in evening and inoculated plants were covered with polythene bags for 48 hrs to avoid unwanted infection and to provide favourable conditions. Reaction of different varieties/cultures was noted after 10 days of inoculation.

For categorizing cowpea varieties/cultures, 0-5 disease scale, was followed as suggested by James (1974) and modified by Singh and Srivastava (1987). The disease categories from 0-5 scales were established orbitrarily on the basis of percent leaf area affected as per the following key (Fig. 1)

Key for categorizing the reaction of cowpea varieties/cultures to anthracnose:

Numerical value	Reaction	Description
0	Immune	Leaf totally free from infection.
1	Resistant	Leaf area infected from 0.1-5.0 %
2	Moderately resistant	Leaf area infected from 5.1-10.0 %
3	Moderately susceptible	Leaf area infected from 10.1-25 %
4	Susceptible	Leaf area infected from 25.1-50 %
5	Highly susceptible	Leaf area infected more than 50 %

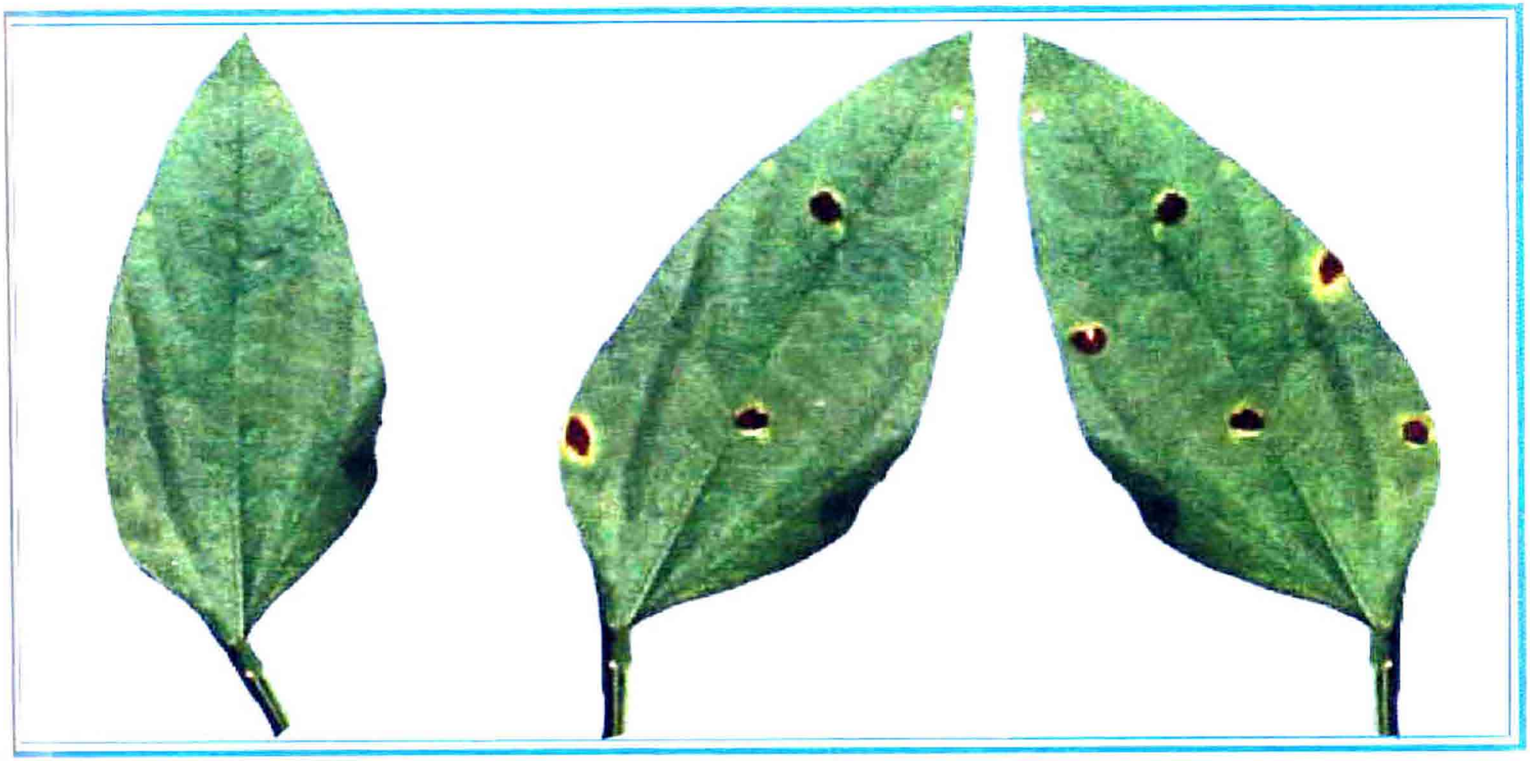
The disease index was calculated by adopting the formula of Chester (1950) and modified by Wheeler (1969) as given below:

$$\text{Per cent disease index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined}} \times \frac{100}{\text{Maximum disease category}}$$

To study the impact of the following on the disease intensity:

(a) Effect of date of sowing on disease intensity:

Experiments were conducted at Vegetable Research Farm, Kalyanpur, Kanpur of the University for two successive years i.e. 2003 and 2004. A susceptible variety of cowpea (K-5269) was sown in plots (3 x 2m) on different sowing dates with three replications which are as follows:



Immune Resistant Moderately Resistant

Moderately Susceptible Susceptible Highly Susceptible

Fig. 1: Gradation for varietal reaction

Second week of June. Last week of June.

Second week of July. Last of week of July

The data regarding disease intensity for different dates sown crops were recorded starting from the first appearance of the disease and subsequently at fortnightly intervals till the maximum disease intensity occurred. Per cent disease plants were assessed by calculating the disease index by using following formula:

$$\text{Per cent disease index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined}} \times \frac{100}{\text{Maximum disease category}}$$

(b) Effect of soil pH on disease intensity:

In order to fined out the impact of soil pH on disease intensity, experiments were conducted in glass house of the Department of Plant Pathology, C.S. Azad University of Agriculture & Technology, Kanpur during 2003 and 2004. Earthen pots of 30 cm diameter were taken and filled with sterilized soil, mixed with inoculum at the surface of sterilized soil. Different pH level viz., 5.0, 5.5, 6.0, 7.0, 7.5 and 8.0 were adjusted with $^{N}/_{10}$ NaOH and $^{N}/_{10}$ HCl before sowing of the healthy cowpea seeds. Each treatment was replicated thrice and suitable control was also maintained by using normal soil pH. Data on average disease intensity of both the years were recorded at the time of harvesting.

(c) Effect of soil amendments on disease intensity:

To see the impact of different soil amendments, experiments were conducted in the glass house of department during 2003 and 2004. Certain types of soil amendments like neem cake, mustard cake, castor cake, paddy straw and wheat straw @ 30 tonnes/ha and Pyrite and Gypsum @ 20 tonnes/ha were incorporated 15 days before sowing of healthy cowpea seeds. Prior to incorporation, the amount of soil amendments were calculated. Earthen pots of 30 cm diameter were taken for each treatment and filled with sterilized soil and each pot was incorporated with 5 g inoculum separately on the surface of soil. Healthy seeds were sown **in** pots and suitable control was also maintained. Each treatment was replicated thrice and irrigated from time to time. Observations on average disease intensity of both the years were recorded.

(d) Effect of different doses of P and K on disease intensity:

The effect of varying levels of phosphorus (P) and potassium (K) on disease incidence was studied during crop season of two consecutive year *viz*., 2003 and 2004. The nutrients were supplied in the form of Super phosphate (P) and Murate of potash (K) and their field doses were converted into plot doses on area basis. All the possible combinations were replicated thrice. The experiment was conducted in Randomized Block Design (Factorial arrangement) having plot size of 3 x 2 m. Different doses of P and K were applied as follows:

Doses of P and K:

$P_0 = 0$	$K_0 = 0$
$P_1 =$ 30kg/ ha	$K_1 =$ 20kg/ha.
$P_2 =$ 60kg/ha	$K_2 =$ 40kg/ha

Treatment combination:

P_0K_0	P_1K_0	P_2K_0
P_0K_1	P_1K_1	P_2K_1
P_0K_2	P_1K_2	P_2K_2

The susceptible variety of cowpea (K-5269) was sown and definite number of plants were maintained in each plot. Observations on the disease intensity were made from time to time in crop season and data of both the year were analysed statistically.

(e) Effect of different soil texture on disease intensity:

To see the impact of different soil types, experiments were conducted in the glass house of the Department during 2003 and 2004. Different types of soils i.e.–loam, sandy loam, silt loam and clay soils were collected from different growing regions of Uttar Pradesh. The earthen pots of 30 cm diameter were taken for each treatment and filled with different types of sterilized soils @ 5.0 kg per pot and each pot was incorporated with 5 g inoculum separately on the surface of soil. Healthy seeds were sown in the month of July. Each treatment was replicated thrice and irrigated from time to time as and when required. Observations on average disease intensity of both the years were recorded.

(f) Effect of different bio-agents on the disease intensity:

(i) Seed Dressers :

To see the impact of different bio-agents, experiments were conducted in the glass house of Department during 2003 and 2004. Earthen pots of 30 cm diameter were taken for each treatment and filled with sterilized soil and each pot was incorporated with 5 g inoculum separately. Before sowing, seeds were treated with the formulation of bio-agents @ 4 g/kg seed. Five seeds coated with bio-agents were sown in each pots and each treatment was replicated thrice. Observations on per cent infection were taken at the time of harvesting.

(ii) Soil applicants :

For evaluating the efficacy of bio-agents as soil applicants against the pathogen, experiments were conducted in the field during the crop season 2003 and 2004. A highly sick field with known history of anthracnose of cowpea was selected. The cowpea variety (K-5269) was sown in plots of 3 x 2 m size, with three replications. Untreated soil served as control. The observations on disease intensity were recorded at the time of harvesting.

Statistical analysis:

The data of the experiments conducted in the laboratory, pots and fields were subjected to statistical analysis. The data were transformed, whenever required. The critical difference was worked out at 5.0 per cent probability level to fined out the difference between the treatments (Panse and Sukhatme, 1967; Chandel, 1993).

Experimental Findings

EXPERIMENTAL FINDINGS

The present investigations were carried on the anthracnose of cowpea (*Vigna unguiculata* (L.) Walp.) caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. and Cav. During survey at different cowpea growing areas near Kanpur and its surrounding districts, it was observed that the cowpea crop was affected from the disease in varying degree of infection.

There is a meager report of the occurrence and it seems from the literature that not much information is available on the major aspects of the disease. Therefore, it was thought necessary to work out in detail about the symptomatology, disease intensity, identification of the pathogen, screening of varieties and suitable measures for disease management following the techniques described under "**Material and Methods**".

Symptoms of the disease:

Under natural conditions, the disease manifest itself mainly on the leaves but in severe conditions it also appears on stems, petioles and pods. In the beginning, the disease appears as minute spots on the leaf lets which later increase in size and turn light brown to dark brown in colour (Fig. 2)

The spots may be circular to irregular and scattered throughout the leaf lamina. Mostly the lesions remain separate but in advanced stages of disease development, they coalesce together to form large (up to 5-6 mm.), (Fig. 3) necrotic, circular to rectangular spots on leaves with shot holes (Fig. 4). Severe spotting may cause premature defoliation. In severe conditions, about 85 per cent area is covered with the necrotic spots on old and mature leaves. The symptoms appear on the petioles are small, reddish brown, enlarging 1 to 4 mm in diameter. On stem they appear as light grey, water soaked and irregular lesions, which later

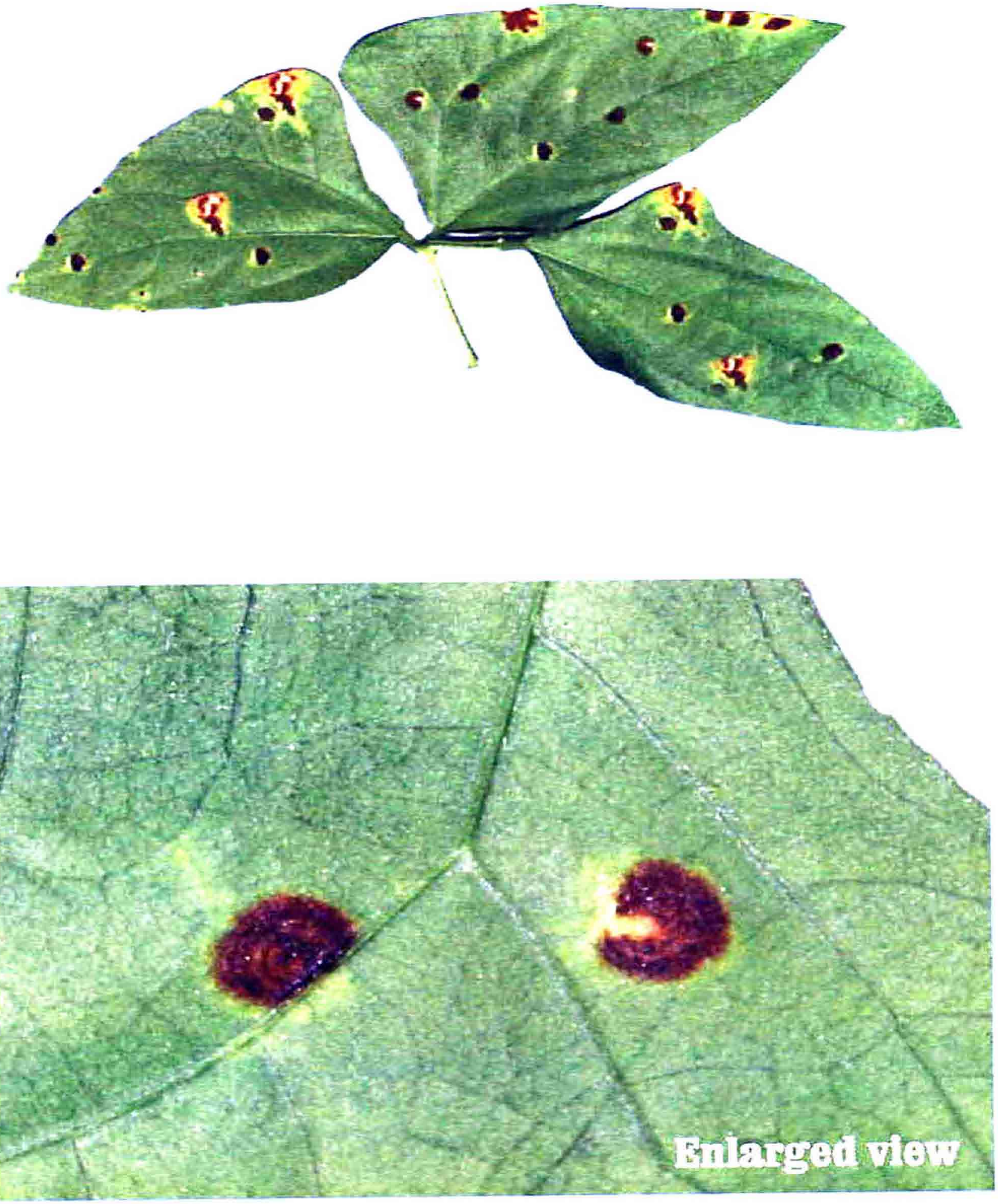


Fig. 2: Symptoms on leaves

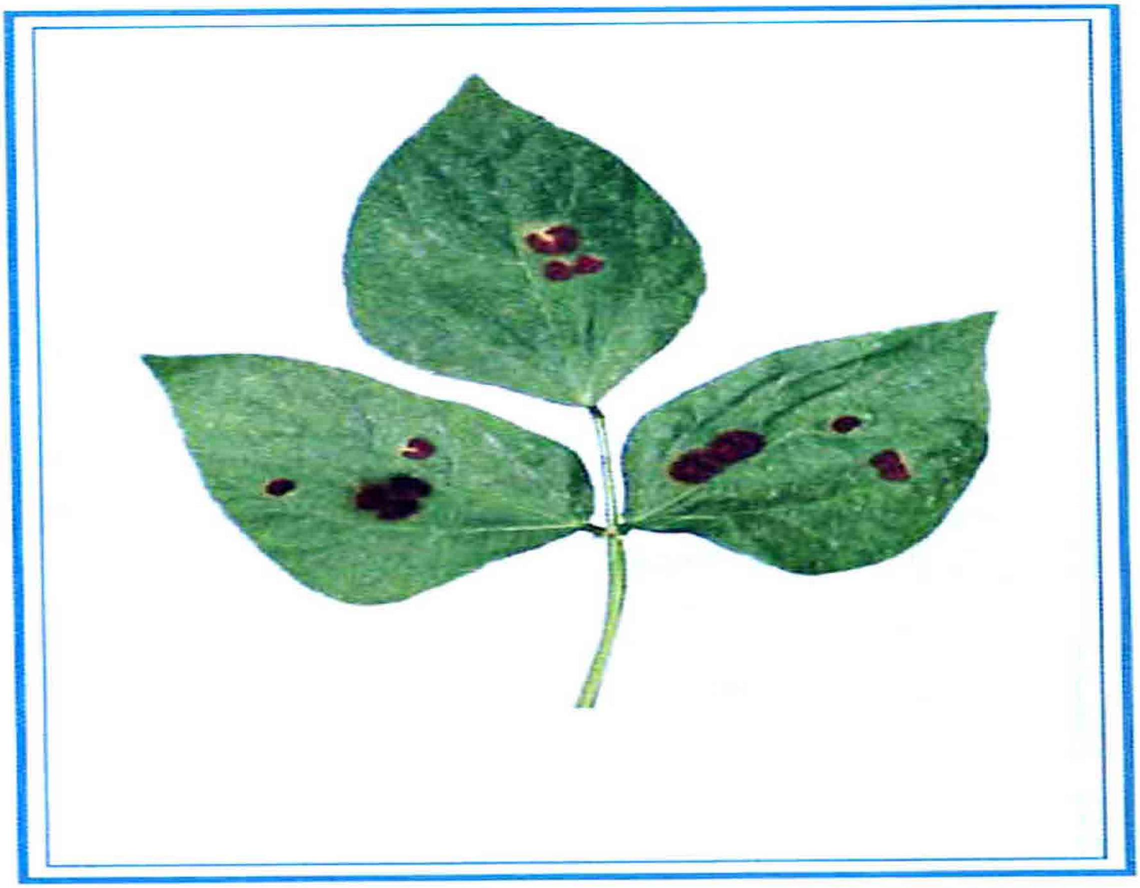


Fig. 3: Coalescence of spots

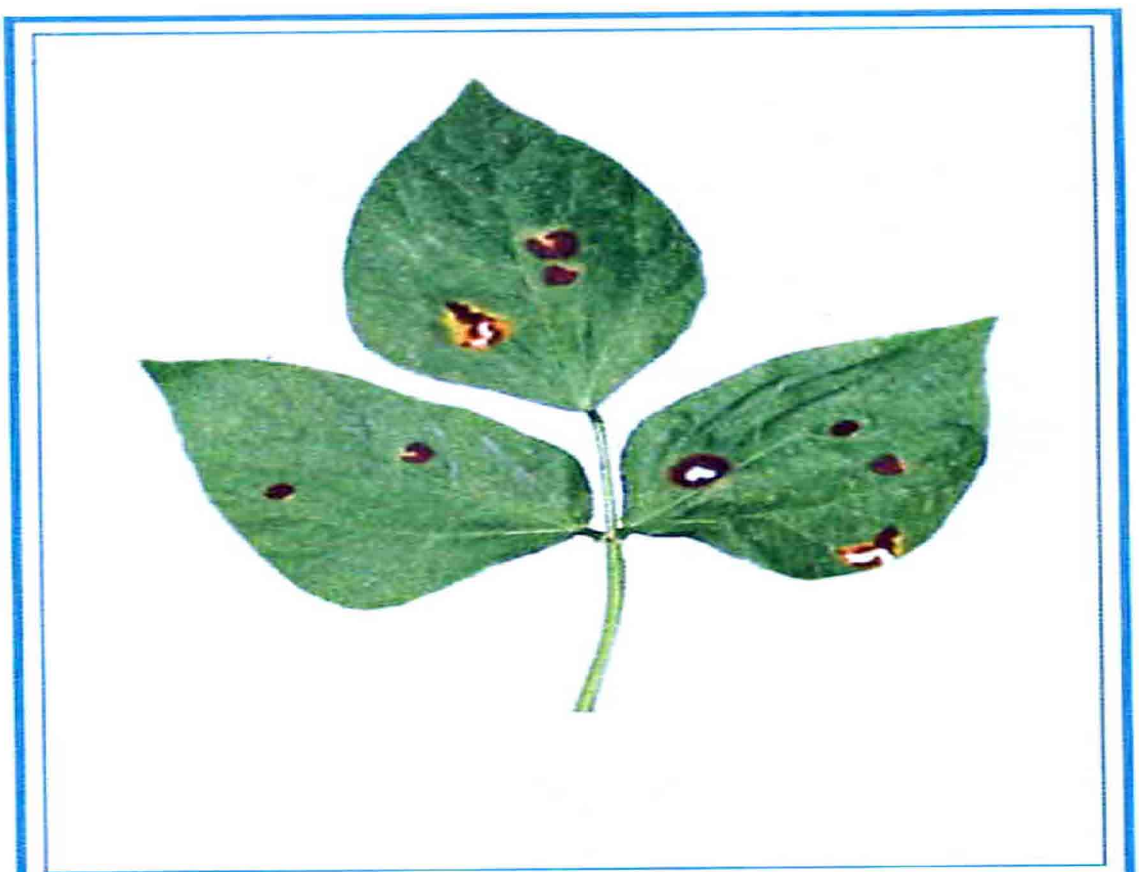


Fig. 4: Leaf spots with shot holes

on turn to rusty brown and enlarge up to 2-8 mm in length. On pods, the symptoms appear as small, reddish blotches, which later become light brown to grayish with numerous acervuli (Fig. 5).

Survey of the disease:

Cowpea crop grown at different places like Students Instructional Farm, Legume Research Farm and Vegetable Research Farm of the University, Crop Research Station-Deegh (Kanpur Dehat), Zonal Agricultural Research Station-Bharari (Jhansi) and Farmers field in Kanpur and Kannauj districts were regularly examined during the peak period of the disease development to assess the severity under natural conditions. Disease intensity was recorded as percentage leaf area affected and data thus obtained are prescribed in the Table -3.

Table- 3: Average disease intensity of anthracnose of cowpea at different locations.

District	Locality	Av. disease intensity (Per cent)
Kanpur	(i) Students Instructional Farm, C.S.A.U.A.&T. Kanpur.	43.80
	(ii) Legume Research Farm, C.S.A.U.A.&T. Kanpur.	41.00
	(iii) Vegetable Research Farm, C.S.A.U.A.&T. Kanpur.	32.00
	(iv) Farmers Field adjoining to Kanpur.	37.00
Kanpur Dehat	Crop Research Station, Deegh	39.70
Kannauj	Farmer's Field.	25.40
Jhansi	Zonal Agricultural Research Station, Bharari.	22.50

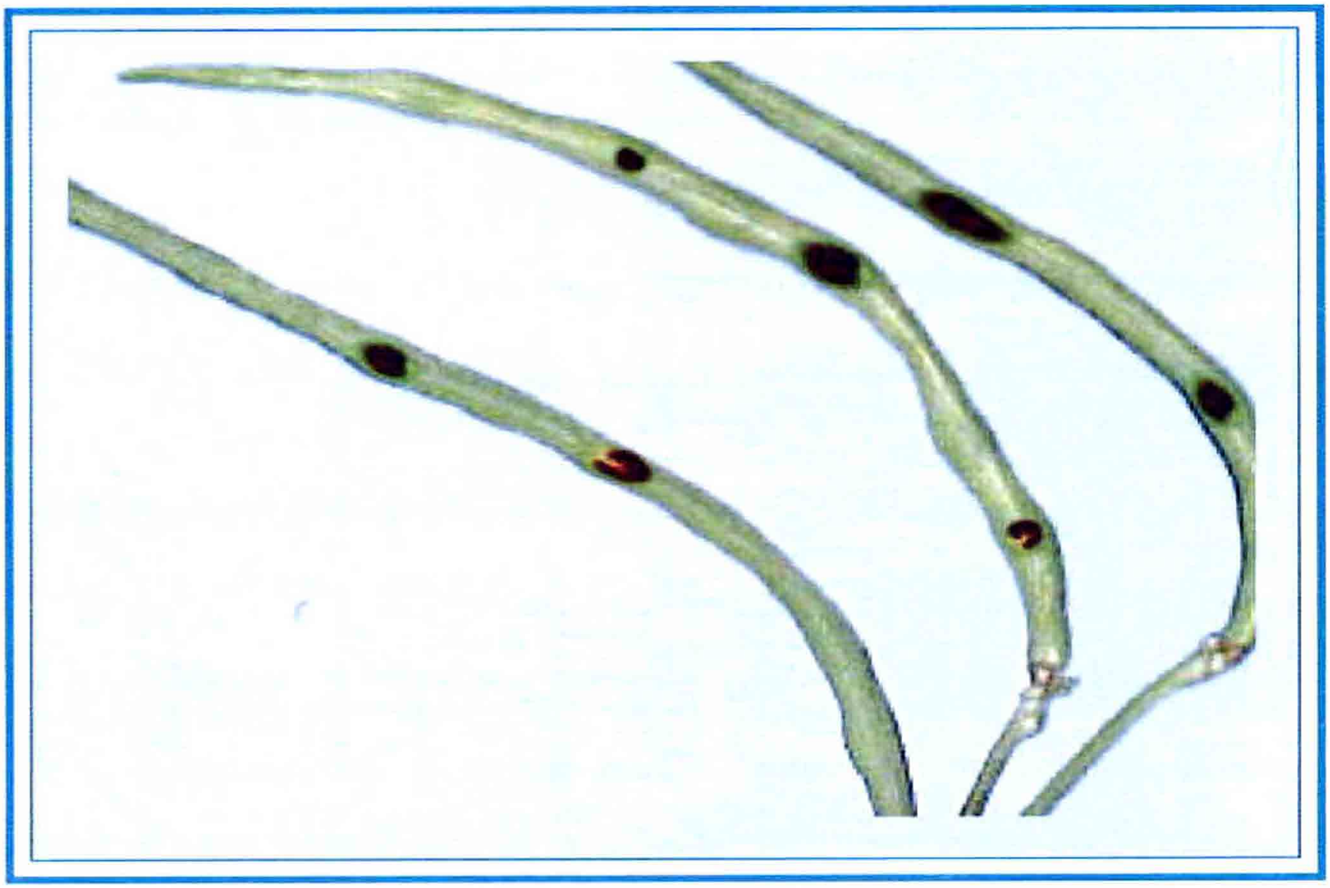


Fig. 5: Symptoms on pods

It is evident from Table 3 that disease intensity ranged from 22.50 to 43.80 per cent. Almost similar symptoms were absorbed on different varieties of cowpea. The maximum disease intensity was recorded at Student's Instructional Farm of the University, whereas, it was lowest in the Zonal Agriculture Research Station at Bharari (Jhansi). In the other fields, disease intensity varied from 25.40 to 41.00 per cent.

Isolation and Purification of the Pathogen:

Isolations were made from infected leaves, which were surface sterilized with 0.1 per cent mercuric chloride solution for 15-20 seconds, washed in sterilized water and these pieces were then transferred on 2 per cent potato dextrose agar medium as described under "**Material and Methods**". The fungus was later purified by subsequent transferring of fresh myceelium in culture tubes and kept in a low temperature (5 ± 1°C) for further studies.

Pathogenicity test:

One month old plants of cowpea raised in pots, under glasshouse conditions, were inoculated with isolated fungus. Forty plants for each treatment were maintained in which 20 plants were uninjured and 20 plants were injured mechanically. The plants were inoculated by spraying spore suspension, hyphal mass and spore masses and then covered with polythene bags. The plants sprayed with sterilized water served as control. Infection thus obtained in each case is given in Table-4.

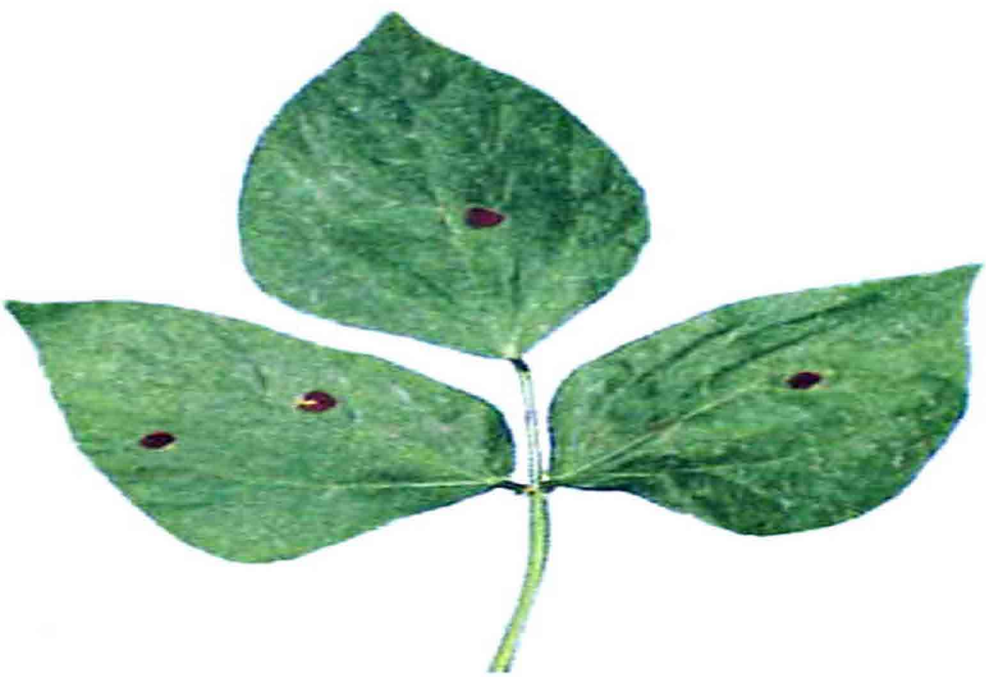
Table-4: Pathogenicity of fungus exhibited through different types of inoculation.

S. No.	Treatment	Uninjured			Injured		
		No. of plants inoculated	No. of plants showing infection	Per cent infection	No. of plants inoculated	No. of plants showing infection	Per cent Infection
1.	Spore suspension	20	10	50	20	14	70
2.	Hyphal suspension	20	13	65	20	18	90
3.	Hyphal mass	20	12	60	20	16	85
4.	Spore mass	20	11	55	20	15	75
5.	Control	20	-	Nil	20	-	Nil

The plants sprayed with spore and hyphal suspension produced numerous brown coloured spots on the leaves after 48 hours, which soon increased in size up to 3-5 mm and later these spots also produced fructifications while in the leaf areas covered by spores and hyphal masses, the entire portion of leaves become discoloured, infection spread rapidly and numerous fructifications made. Their appearance is related as time progresses (Fig. 6).

From the artificially produced leaf spots, the re-isolations were made, which resulted in production of the same fungus which was isolated previously from the naturally infected leaves. Thus, isolation, artificial inoculation and re-isolation proved Koch's postulate and in this way, the pathogenicity of the isolated fungus was established.

Healthy leaf



Inoculated leaf

Fig. 6: Pathogenicity test

Morphological characters of the pathogen:

Morphological characters are considered to be the basis for identifying the species in a particular group. With this view, different fungal structure were studied on potato dextrose agar medium, which are described as below:

A. Mycelium:

The mycelium is branched, septate when young, hyaline but with the age they become grayish black in colour (Fig. 7).

B. Acervulus:

Acervulus is mostly globrous measuring 150-250μm. The acervuli are dark brown to grayish black in colour (Fig. 8, 10).

C. Setae :

The setae are dark brown to black in colour, 1-3 septate, measuring 30-100 x 4-9 μm in size and varying in number from 2-15 in each acervulus (Fig. 9).

D. Conidiophore:

Conidiophores are hyaline, short, erect, unbranched and arranged in definite layers on the surface of stromatic tissues. They are packed together measuring 10.5-16.5 x 2.0-3.0 μm.

E. Conidia:

Conidia produced in pinkish masses are single celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed. They measure 11-20 x 2.5-5.5 μm and form appressoria on germination.

Fig. 7: Culture of pathogen

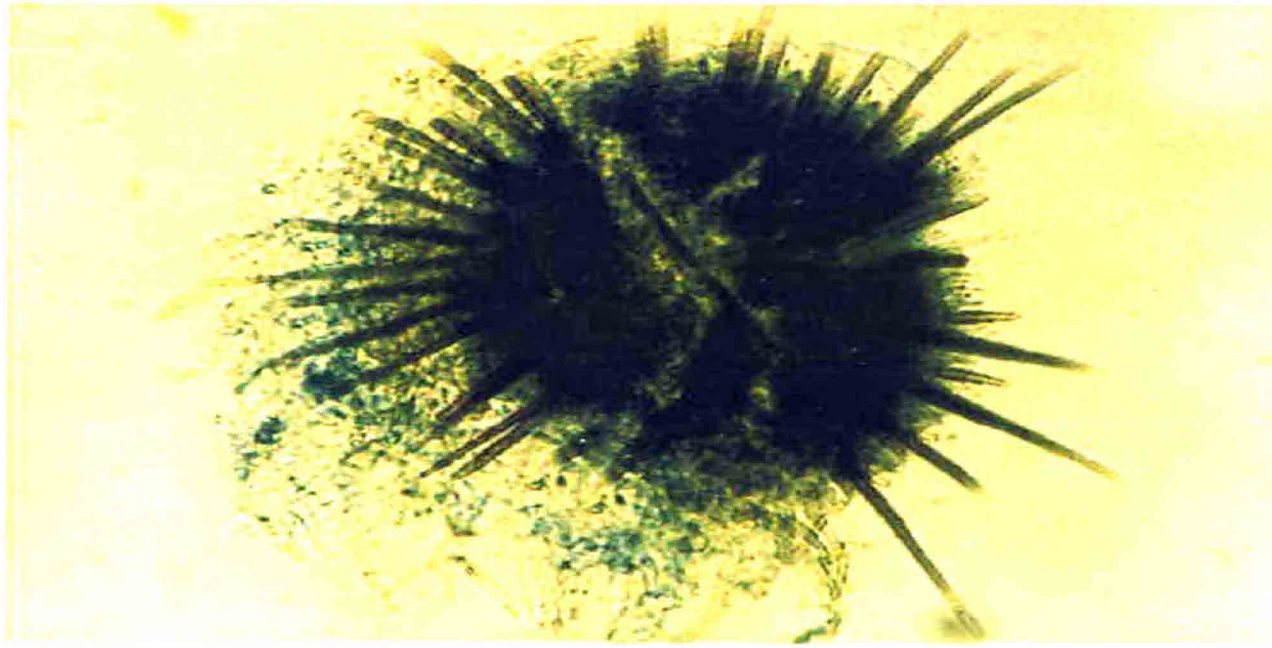


Fig. 8: Acervulus

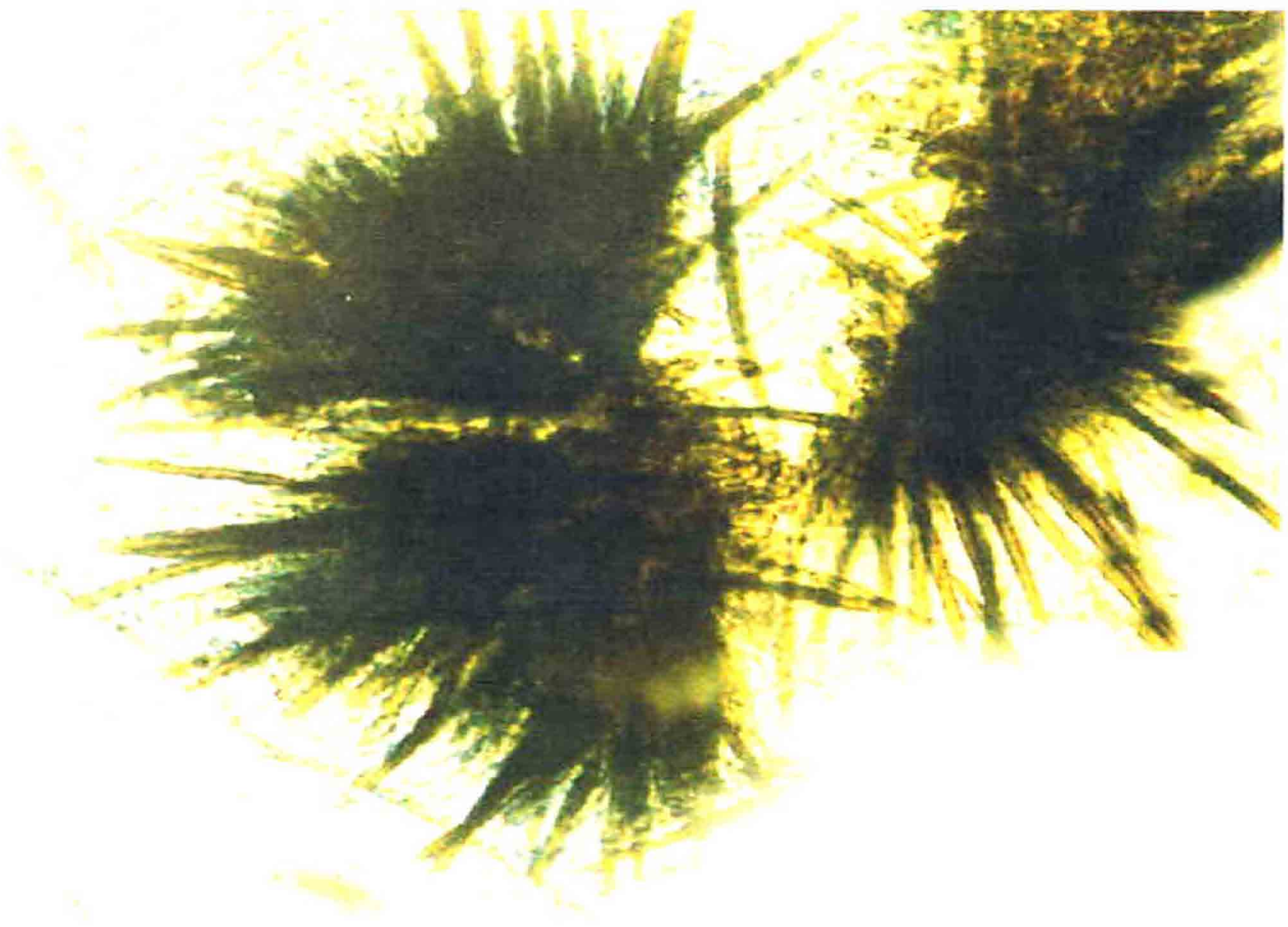


Fig. 9: Setae

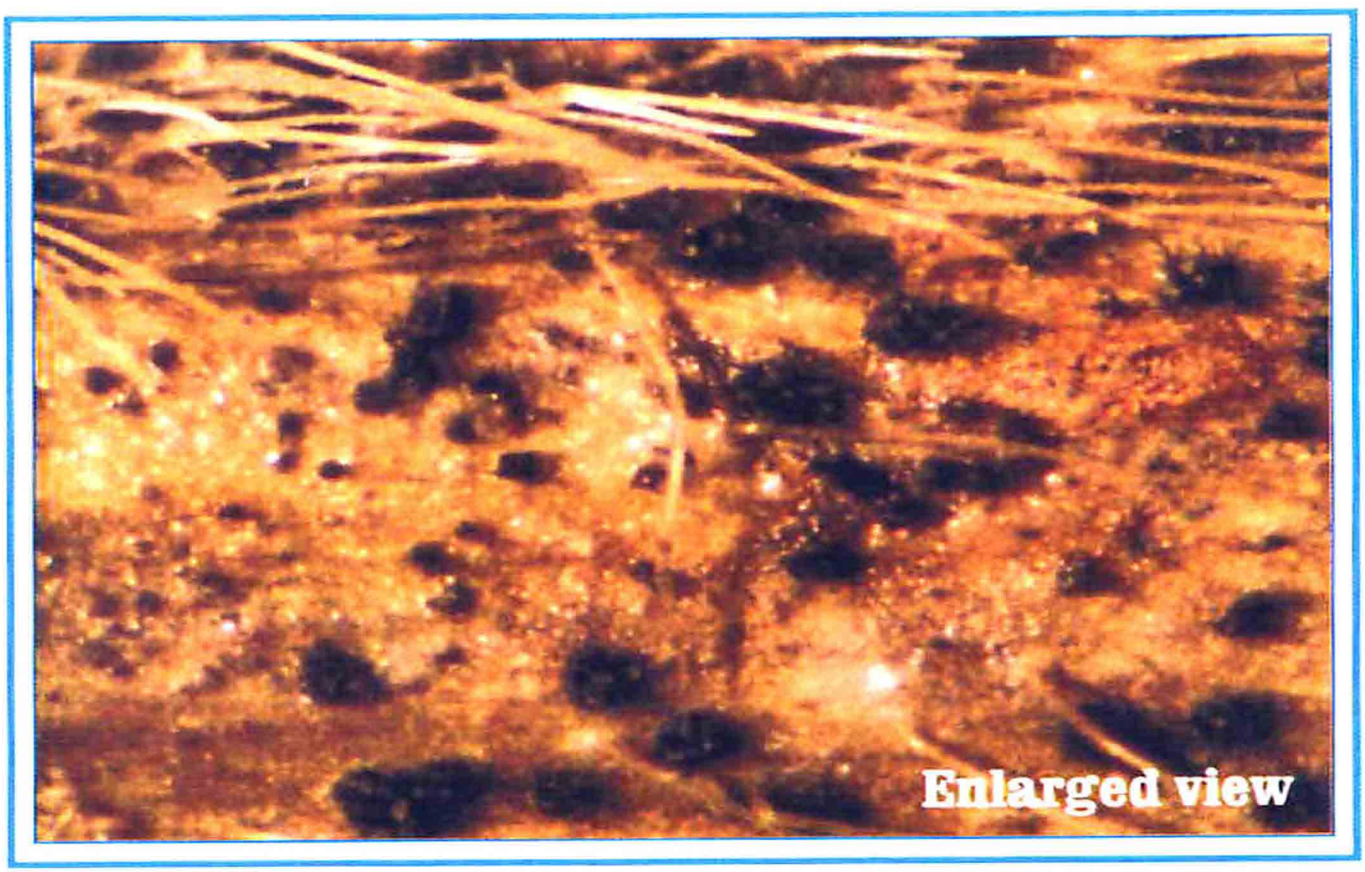


Fig. 10: Formation of acervuli on infected pods

Cultural characters of the pathogen:

The study was taken up to find out the minimum, optimum and maximum growth requirements of the pathogen in culture with regards to different media as per technique described under "**Material and Methods**".

Qualitative growth:

The pathogen was grown on eight different natural, synthetic and semi-synthetic solid media. The data on fungal growth were recorded 10 days after incubation at 24 ± 2°C. The results thus obtained are summarized in Table-5.

Table 5: Qualitative growth of the fungus and development of acervuli on different solid media.

S. No.	Solid media	Av. Diameter of fungal colony (mm)	Formation of acervuli
1.	Potato dextrose agar	82.34	++++
2.	Richard's agar	75.33	++++
3.	Czapek's (Dox) agar	71.34	+++
4.	Kirchoff's agar	70.44	+++
5.	Oatmeal agar	68.63	++
6.	Cornmeal agar	65.56	++
7.	Ashtana and Hawker's agar	58.38	++
8.	Host extract agar	53.67	+
	CD at 5% level	**4.89**	

Where,

++++ = Excellent

+++ = Good

++ = Fair

\+ = Poor

It is evident from the Table-5 and its Figure 11 that potato dextrose agar medium promoted best growth of the fungus and gave excellent acervuli formation followed by Richard's agar medium. Statistically they were different from each other. On rest of the media, growth of the pathogen varied from good to poor. However, the least growth was recorded on host extract medium with poor acervuli formation.

Quantitative growth :

The same media, which were taken for the measurement of radial growth in solid form, were employed in the liquid form for measuring the growth and formation of acervuli of the pathogen. The observations on the average fungal dry weight and formation of acervuli in different liquid media are summarized in Table- 6.

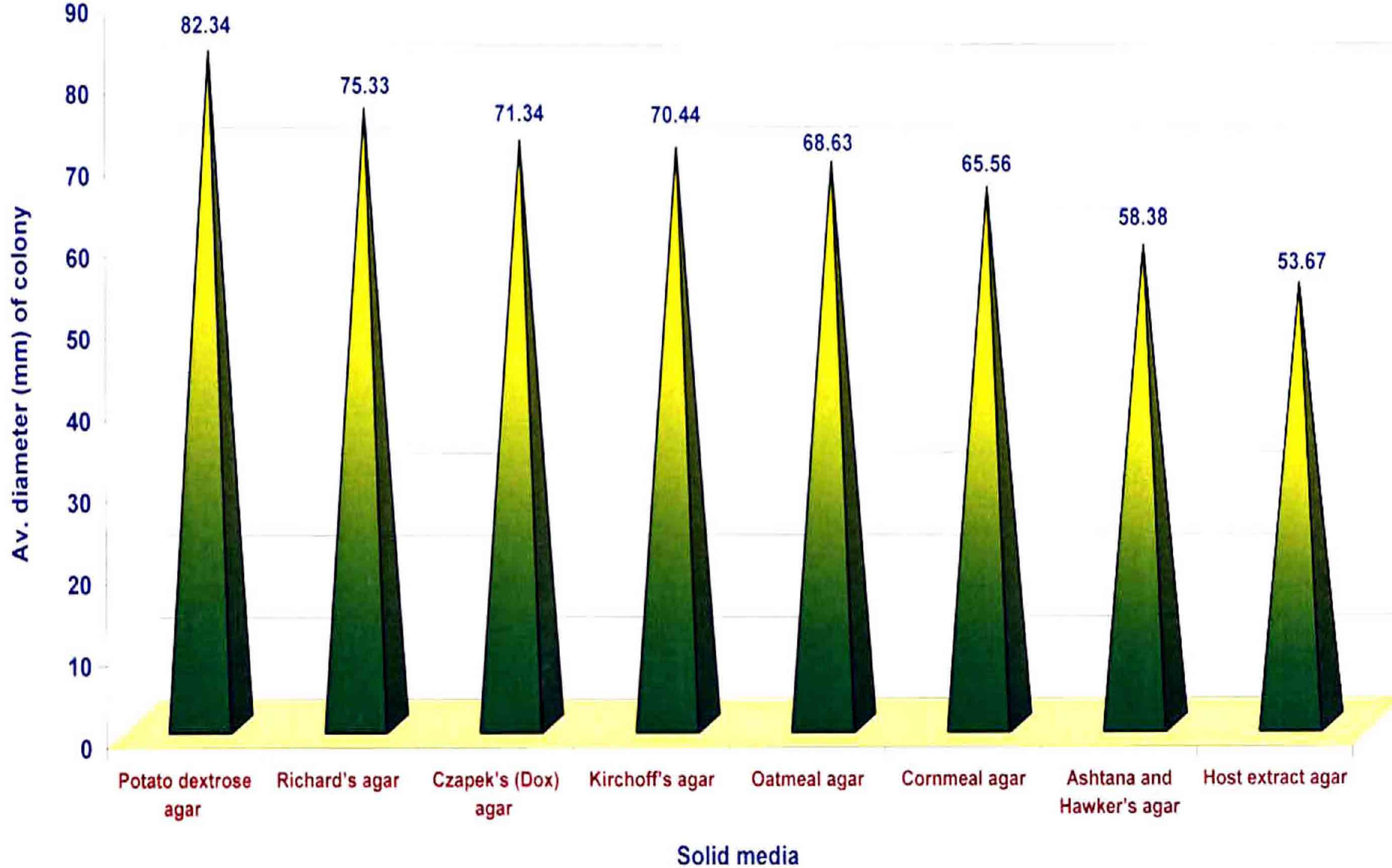


Fig.11 : Growth of the fungus and development of acervuli on different solid media

Table 6: Quantitative growth of fungus and formation of acervuli on different liquid media at 24±2°C.

S. No.	Media	Av. Mycelial dry weight (mg)	Formation of acervuli
1.	Potato dextrose	775	++++
2.	Richard's medium	761	++++
3.	Czapek's (Dox) medium	755	+++
4.	Kirchoff's medium	704	+++
5.	Oatmeal medium	684	++
6.	Corn meal medium	675	++
7.	Asthana & Hawker's medium	648	++
8.	Host extract medium	595	+
	CD at 5% level	**8.89**	

It is obvious from the Table 6 and its Figure 12 that potato dextrose medium supported the best growth of fungus and also gave excellent acervuli formation followed by Richard's medium. All the media differed significantly from each other except Richard's and Czapek's (Dox) media which were at par with each other. The host extract medium supported the average growth and poor sporulation of the fungus.

Mode of spread and perpetuation of the pathogen:

A. Survival of pathogen:

Knowledge of perpetuation of the pathogen as well as creation of artificial epiphytotics is a pre-requisite for devicing suitable control measures. In the present investigation the experiments were conducted to know the mode of perpetuation of the disease and its spread and the results thus obtained are being summarized here.

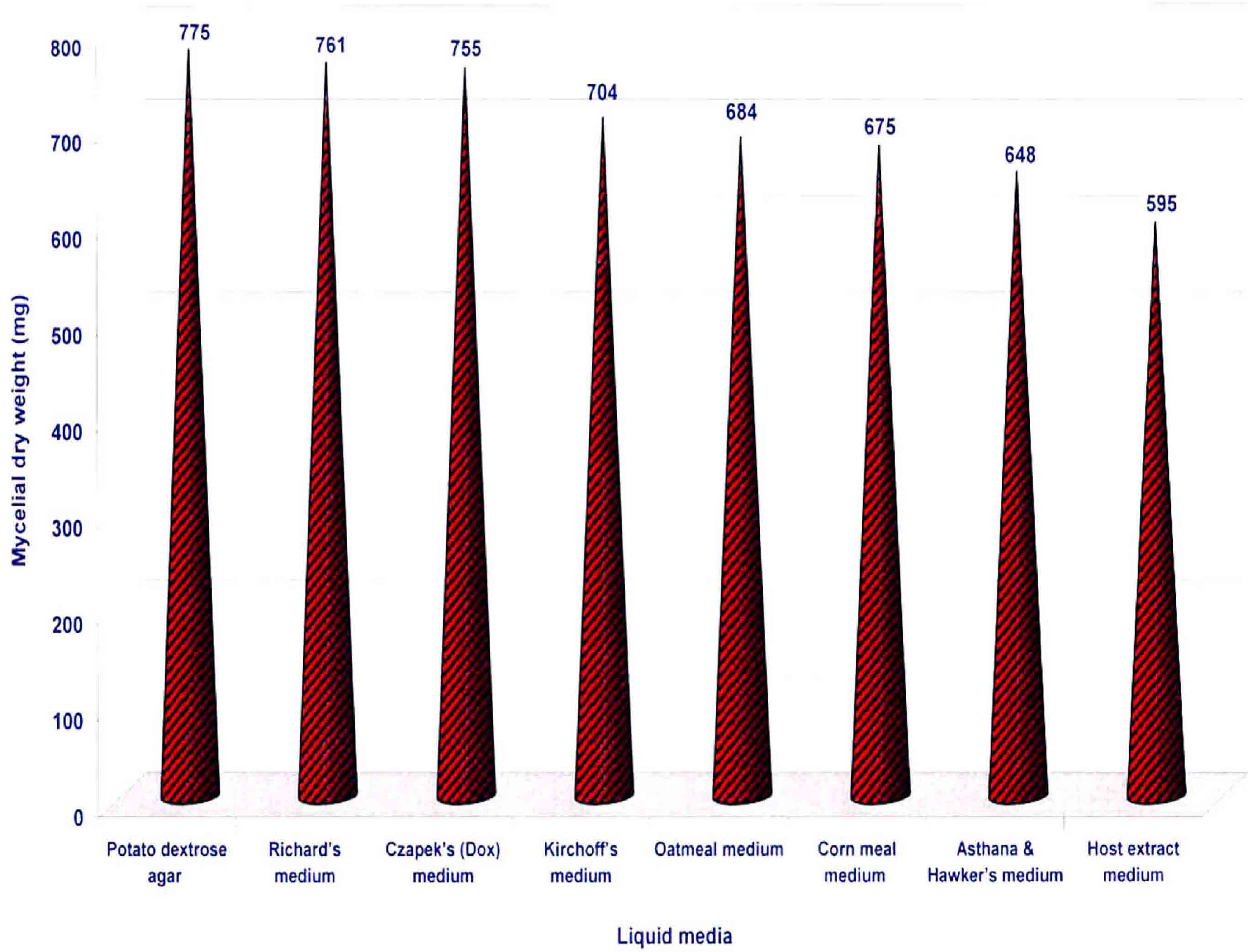


Fig. 12: Growth of fungus and formation of acervuli in different liquid media

(i) **Role of diseased plant debris:**

a) **Survival of the pathogen in plant debris:**

To find out the role of plant debris in the survival of the pathogen, naturally infected leaves and pods were collected during the crop season 2003. One part of the diseased specimen was kept in paper bags under laboratory conditions while other one part buried in pot soil kept in open (field conditions), in the month of August, 2003. Isolations were made from these specimens at monthly intervals to know the viability period of the pathogen. Every month ten pieces were used for isolation and the percentage of pieces yielding culture is summarized in Table-7.

Table-7: Survival of pathogen in infected plant debris under different storage conditions.

Storage period (month)	Month of Isolation	Laboratory conditions			Field Conditions		
		No. of pieces used for isolation	No. of pieces yielding culture	% of pieces yielding culture	No. of pieces used for isolation	No. of pieces yielding culture	% of pieces yielding culture
1	Sept.,2003	10	10	100	10	10	100
2	Oct., 2003	10	10	100	10	10	100
3	Nov., 2003	10	10	100	10	10	100
4	Dec., 2003	10	10	100	10	9	90
5	Jan., 2004	10	9	90	10	8	80
6	Feb., 2004	10	7	70	10	6	60
7	March, 2004	10	3	30	10	4	40
8	April., 2004	10	0	0	10	2	20
9	May, 2004	10	0	0	10	1	10
10	June, 2004	10	0	0	10	0	0
11	July, 2004	10	0	0	10	0	0

It was observed that the isolation made from plant debris, yielded the culture of *Colletotrichum lindemuthianum* upto 7 months when kept in laboratory conditions while upto 9 months when kept buried in pot soil.

It is evident from the results of Table-7 that up to first four (4) months of storage, the pathogen could be isolated from 100 per cent of pieces of plant debris kept in either conditions. But thereafter, the recovery of the culture from plant debris pieces declined sharply with the increase in storage period. The pathogen could not be isolated beyond seven months of storage from plant debris kept in laboratory conditions and after nine months of storage from plant debris kept under field conditions.

b) Role of plant debris in primary infection:

A pot culture experiment was carried out to find out the role of plant debris in the initiation of the disease, during the crop season 2003. The diseased plant debris stored in laboratory and field conditions were mixed with autoclaved soil in the pots separately. The autoclaved soil without plant debris served as control. The surface sterilized healthy seeds of cowpea (variety K-5269) were then sown in such pots. The observations on seedling emergence and percentage of infected seedlings were taken and summarized in Table-8.

Table 8: **Role of diseased plant debris as primary source of inoculum.**

S. No.	Treatment	No. of seeds sown	No. of seedling obtained	No. of seedling infected	% of infected seedlings
1.	Diseased plant debris kept in laboratory conditions + autoclaved soil + surface sterilized seed.	40	35	7	20.00
2.	Diseased plant debris kept in field conditions + autoclaved soil + surface sterilized seed	40	34	11	32.47
3.	Surface sterilized seed + autoclaved soil without plant debris (control)	40	37	0	0

It is obvious from Table 8 that the pathogen was able to survive in diseased plant debris and to cause primary infection in cowpea seedlings. The pathogen survive in diseased plant debris stored in laboratory conditions, caused infection to the tune of 20.00 per cent seedlings while it infected 32.47 per cent seedlings when surviving in plant debris stored in the field conditions. The seedlings raised in sterilized soil from healthy, surface sterilized seeds without mixing plant debris (control), remained healthy and did not show any infection.

(ii) Role of seed:

To study the role of seed in the perpetuation of the disease if any, isolations were made from the seeds collected from naturally infected cowpea plants, showing characteristic disease symptoms. Seeds from healthy plants were also collected for comparison, which served as control. The untreated and surface sterilized seeds were tested by using agar plate and standard blotter method for the presence of the pathogen as described under "**Material and Methods**". The observations on per cent seed showing infection are presented in Table-9a.

Table-9a: Survival of the pathogen in cowpea seeds.

S. No.	Treatment	No. of seed plated	No. of seeds showing infection	% of seed infected
1.	Potato dextrose Agar	400	90	22.50
2.	Standard blotter method	400	97	24.25
3.	Control	400	Nil	Nil

It is evident from the Table-9a that the pathogen was present in the seeds. *Colletotrichum lindemuthianum* was isolated by both the techniques employed in the experiment. Agar plate method showed that the pathogen was present in 22.50 per cent of plated seeds while 24.25 per cent seeds were found infected in standard blotter method. The pathogen could not be isolated from the seeds obtained from healthy plants. However, it was found associated with mercuric chloride treated seeds in lesser number of seeds, indicating that the pathogen was present in the internal tissues of the seed.

a. Location of pathogen in the seed:

In order to find out the location of the pathogen in the seed, naturally infected seeds were taken for the experiment. These seeds were then subjected to separation of seed coat, cotyledon and embryo aseptically. The seed parts were plated in Petri dishes containing 2 per cent potato dextrose agar medium separately. Such Petri dishes were examined regularly for the presence of the growth of the pathogen and data are summarized in Table -9b.

Table 9b: Location of the pathogen in the seed.

S. No.	Parts of seed	Presence or absence of the pathogen
1.	Seed coat	+
2.	Cotyledon	+
3.	Embryo	+

Where,

+ Denotes presence of the pathogen

- Denotes absence of the pathogen.

It is evident from the Table -9b that the pathogen could be isolated from the seed coat, cotyledon and embryo. Thus, it is clear from the results that the pathogen is also seed borne in nature (Fig. 13).

b. Role of seeds in disease development:

To study the role of seeds in the initiation of the disease, naturally and artificially infected seeds of cowpea collected in the previous year, were stored at room temperature (15-25°C) in the laboratory for sowing in the next season. Five seeds per pot (30 cm diameter) were sown in autoclaved soil. Sets of surface sterilized healthy seeds were also sown in autoclaved soil, which served as control. The data on percentage of infected seedlings were recorded and are being presented in Table -9c.

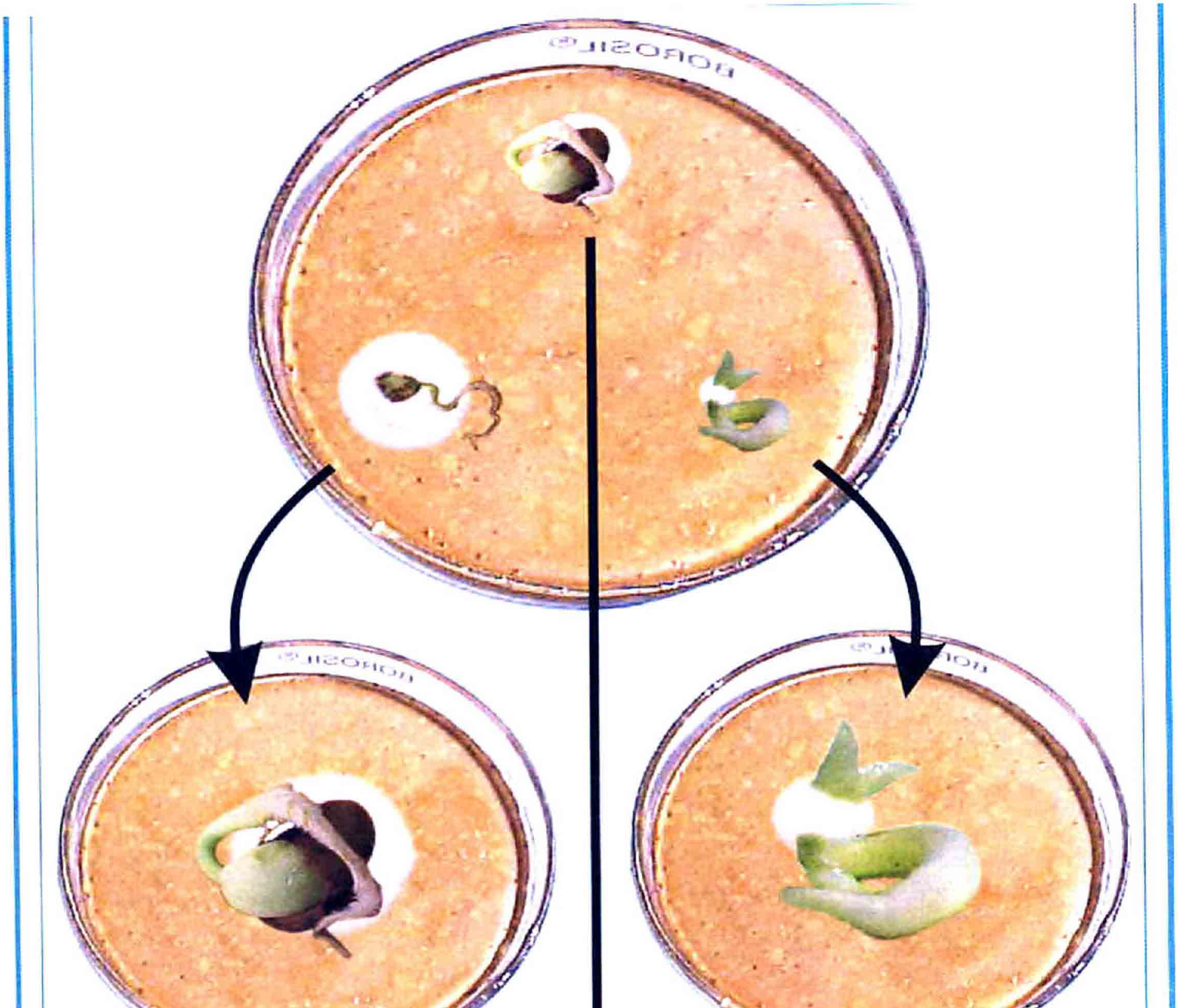


Fig. 13: Location of pathogen in the seed

Table 9c: **Role of infected seeds in disease development.**

Treatment	No. of seed sown	No. of seedlings obtained	No. of infected seedlings	% of infected seedlings
Naturally infected seeds	40	34	7	20.58
Artificially infected seeds	40	33	10	30.30
Surface sterilized healthy seeds (Control)	40	38	Nil	Nil

It is clear from the results presented in Table -9c that the disease was successfully reproduced from infected seeds sown in sterilized soil. Disease symptoms were observed on 20.58 per cent seedlings in case of naturally infected seeds while 30.30 per cent seedlings were found infected when raised from artificially infected seeds. On the other hand, plants raised from healthy surface sterilized seeds did not develop any symptom of the disease. This clearly indicates that the diseased seeds served as primary source of inoculum.

(iii) Role of infested soil:

Pure culture of the pathogen raised on sand cornmeal medium, having profuse sporulation was thoroughly mixed with autoclaved soil. The pots filled with this infested soil in the month of September were kept at room temperature (15-25°C) till the next season. The surface sterilized healthy seeds were sown in these pots. Pots filled with autoclaved non-infested soil and sown with surface sterilized seeds served as control. The data on seedlings were recorded and are presented in Table -10:

Table -10: Role of infested soil in the perpetuation of the disease.

Treatment	No. of seeds sown	No. of seedlings obtained	No. of seedlings infected	% of infected seedlings
Soil infested with fungal culture + surface sterilized seeds	40	37	7	18.91
Non infested autoclaved soil + surface sterilized seeds (control)	40	38	Nil	Nil

It is evident from the Table -10 that the plants raised from sterilized seeds in infested soil developed disease symptoms as produced by the pathogen. In this case 18.91 per cent seedlings were found to be infected. The plants grown in control from sterilized seeds in autoclaved non-infested soil did not show infection and remained healthy during the experiment. This clearly indicated that the infested soil served as the primary source of inoculum.

B. Secondary spread of the disease :

In order to determine the role of air borne inoculum in the secondary spread of the disease, cowpea plants were raised in pots from healthy, sterilized seeds sown in sterilized soil, under glass house conditions. Two sets of pots were placed in vicinity of infected cowpea field. Observations were made on the percentage of infected plants and the results are summarized in Table-11.

Table -11: Role of air borne inoculum in the secondary spread of the disease.

Treatment	No. of seeds sown	No. of seedlings obtained	No. of infected plants	% of infected plants
Sterilized seed+autoclaved soil exposed for aerial infection	40	36	26	72.22
Sterilized seed + autoclaved soil kept covered in polythene tent.	40	38	Nil	Nil

It is obvious from the results shown in Table -11 that the disease symptoms developed only on those sets of plants, which were exposed to aerial infection. In the experiment, the percentage of infected plants by air borne inoculum was 72.22. The other set of plants kept covered with polythene tent were free from any disease symptoms, during the experiment.

On the basis of results, it was concluded that the secondary spread of the disease was by no other means except the air borne spores (conidia), as there were no chance of disease development either through seed or soil since both were already sterilized at time of commencement of the experiment.

Isolation of bio-agents and their screening against the causal pathogen *in vitro*:

The following antagonists (bio-agents) were isolated from the rhizosphere of cowpea plants from different locations and identified on the basis of their morphological characters under microscope.

***Trichoderma viride* :**

Growth of *Trichoderma viride* is 8-9 cm within 4 days, dark bluish green, colony texture in floccose, mycelium is loose tufts in concentric rings. Conidiophores are irregularly branched, phialides not more than 2-3 in groups, often single, opposite, shape is often sigmoid or hooked. Conidia is globose to ovoid rarely ellipsoidal. Chlamydospores are common.

Trichoderma harzianum:

Growth rate is 7-9 cm within 3 days and colour is dark green, colony texture is floccose, compact mycelium. Flexuous regularly, highly branched conidiophores, whorls phialides which is ampuliform to lageniform in shape. Conidia, subglobose to ovoid, smooth and light green, chlamydospore is fairly abundant.

Trichoderma virens :

Growth rate 8.0 - 9.5 cm within 4 days, dark bluish green to dull blackish green in colour and structure is floccose, flat pustules, simple re-branched conidiophores. Phialides are in whorls of 2-3 occasionally single, pin shape and curved. Broadly ellipsoidal to ovoid, smooth conidia of green colour and chlamydospore is abundant.

Pseudomonas flourescens:

These are gram negative and flagellated rods. It produce a yellow green, water soluble pigment, which diffuses into the mycelium and is florescent under UV light.

***Bacillus subtilis*:**

The spore is oval, central relatively thin walled and its diameter is never greater than that of the vegetative cell. These form endospore which remain dormant for many years. These are gram positive.

Fungal and bacterial bio-agents isolated from rhizosphere of affected cowpea plants were tested to find out their efficacy against the pathogen *in vitro* by using dual culture technique as per procedure mentioned under "**Material and Methods**". Data recorded are presented in Table-12.

Table -12: Effect of bio-agents against the pathogen *in vitro*.

S. No.	Bio-agents	Average growth (mm)		% inhibition over control.
		Antagonist	Pathogen	
1.	*Trichoderma viride*	51.23	16.65	81.50
2.	*Trichoderma harzianum*	49.50	19.35	78.50
3.	*Trichoderma virens*	46.66	23.39	74.01
4.	*Pseudomonas flourescens*	39.40	31.44	65.07
5.	*Bacillus subtilis*	27.75	49.24	45.28
6.	Control	-	90.00	-
	CD at 5%			**4.62**

The results presented in Table -12 and its corresponding Fig (14) showed that the mycelial growth of *Colletotrichum lindemuthianum* was reduced to a great extent by extensive growth of bio-agents in dual culture technique. Maximum inhibition (81.50%) of growth of the pathogen was obtained with *Trichoderma viride* followed by *T. harzianum* and *Trichoderma virens*. *Pseudomonas flourescens* was comparatively less effective against the pathogen whereas *Bacillus subtilis* proved to be least effective as it inhibited only 45.28 per cent growth of the pathogen.

Screening of cowpea varieties / cultures for disease resistance:

Resistant varieties play an important role in controlling the disease and increasing the quality and quantity of produce without increasing the cost of cultivation, pollution and toxicity in the products and by products of crops. Therefore, this experiment was conducted for picking out the source of resistance against *Colletotrichum lindemuthianum* of cowpea.

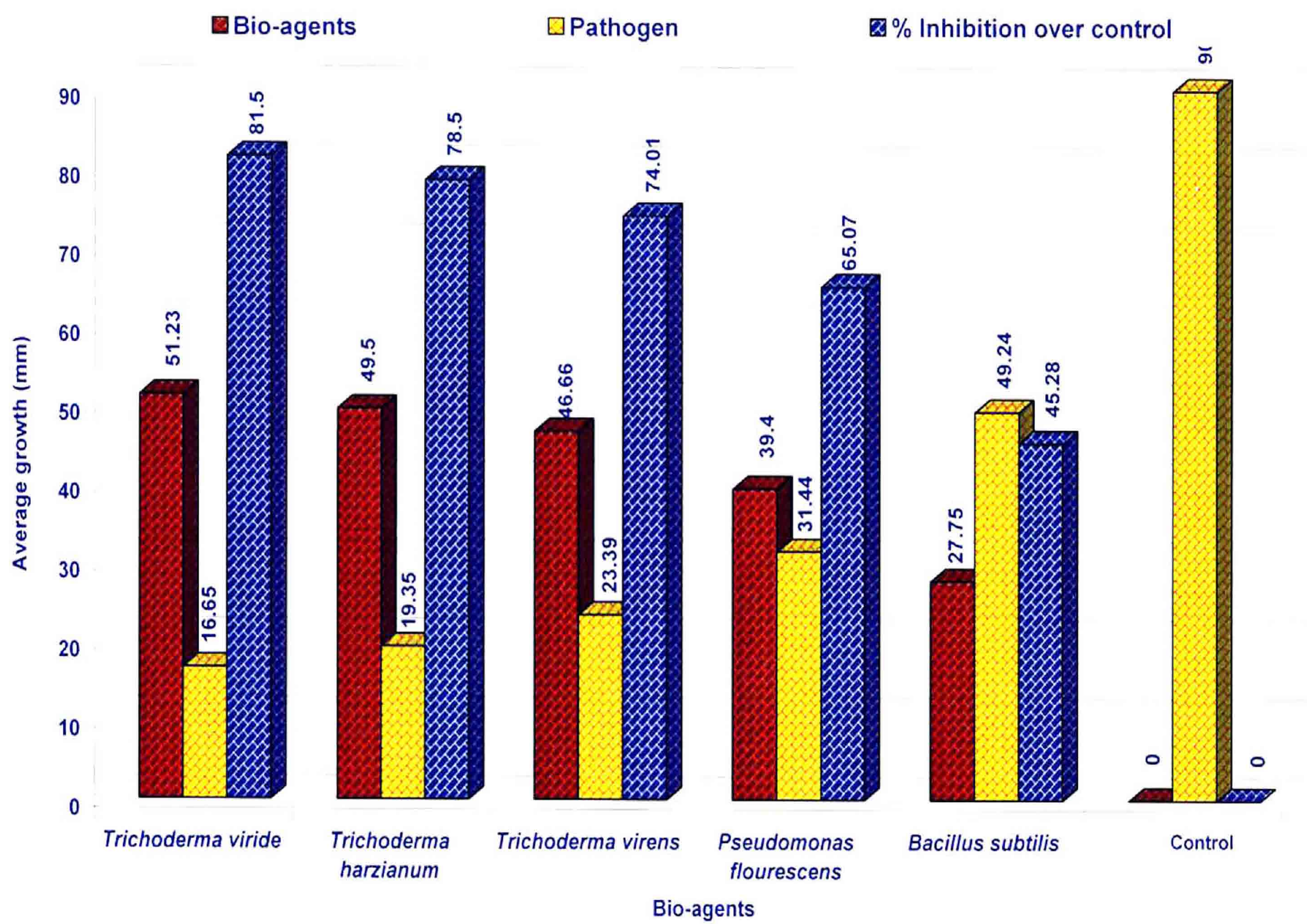


Fig. 14: Effect of bio-agents against the pathogen *in vitro*

A total number of 80 varieties/ cultures were screened under natu conditions at Vegetable Research Farm, Kalyanpur of the University during cr season, 2003 and 2004.

The varieties/ cultures were grouped in various categories resistance and susceptibility on the basis of leaf area affected and the results presented in Table -13.

Table 13: Reaction of cowpea varieties/cultures to *Colletotrich lindemuthianum* under natural conditions during 2003 and 200

Numerical value	Category	Name of varieties/ cultures
0	Immune	Nil
1	Resistant (01%-5.0% infection)	RCV-7, RCV-326, Sel-16, BCKV-2, CH 2, BCP-3, P-28, Type-2, M-33, JC-5, 1270, M-390.
2.	Moderately resistance (5.1%-10.0 % infection)	M-120, M-441, M-462, M-278, PCV- Pusa Rituraj, Cowpea-263, TVRCR-1, P 326 Pusa Komal, NP-1, VS-15, CPC-1, 10, Pusa Barsati, NDPC-13, C-2, C-13.
3.	Moderately susceptible (10.1%-25.0% infection)	C-263, C-152, M-44, M-312, M-481, 483, M-431, C-1, C-5, C-12, C-14, S-8 16, P-863 JC-5, JC-10, P-1270, P-8 Lobia Lal.
4.	Susceptible (25.1%-50.0% infection)	M-72, M-97, M-110, M-310, M-383, M- M-485, M-478, M-442, S-9, S-18, C-7 152, G-7, P-8203, P-8204.
5.	Highly susceptible (More then 50% infection)	S-14, C-6, G-5, BCKV-1, M-391, M- IG-16, IG-25, IG-5236, IG-8201, IG-8 P-696, K-5269.

It is evident from the Table -13 that out of 80 varieties/ cultures screened so far, 12 were proved resistant. 18 moderately resistant, 19 moderately susceptible, 16 susceptible and 15 highly susceptible. None of the variety/ culture was found consistently immune to the disease.

Thirty varieties/cultures showing resistant and moderately resistant reaction under natural conditions in two successive years (2003 and 2004) were further tested under artificial inoculation conditions in the crop season 2005. Plants of each variety/culture were raised from surface sterilized seeds in 30 cm earthen pots filled with autoclaved soil. The inoculation of plants and other procedures were the same as described under '**Material and Methods**'. The data on disease intensity were recorded at regular intervals and the results thus obtained are presented in Table-14.

Table-14: Reaction of cowpea varieties/cultures against *Colletotrichum lindemuthianum* under artificial inoculation conditions (2005).

Numerical value	Category	Name of varieties/ cultures
0	Immune	Nil
1	Resistant	RCV-7. RCV-326. Sel-16, BCKV-2, Pusa Rituraj, M-278, NP-1.
2	Moderately resistance	M-120. M-441, M-462, Cowpea-263, VS-15. P-1270, Pusa Komal, M-390, P-28, BCP-3
3	Moderately susceptible	Pusa Barsati, Type-2, TVRCR-1, NDPC-13, CPC-1, PCV-395, JC-10, CHCR-2.
4	Susceptible	C-2, C-13, JC-5, PCV-326 M-33.
5	Highly susceptible	Nil

A critical study of the results presented in Table-14 indicated that under artificial inoculation conditions, cowpea varieties/ cultures differed significantly in respect of their reaction against the pathogen. Out of 30 varieties/ cultures, only 7 varieties/ cultures proved resistant, 10 moderately resistant and the rest were proved moderately susceptible to susceptible.

Evaluation of the impact of date of sowing, soil pH, soil amendments, soil textures, different doses of P and K and bio-agents on disease intensity:

Different types of practices like different date of sowing, soil pH, soil amendments, soil textures, different doses of P and K and bio-agents were employed in the present study to access their impact on the management of anthracnose of cowpea.

(a) Effect of different date of sowing on the disease intensity:

Change in sowing date is a cultural measures for crop disease management. It reduces the period over which infection agents (propagules) meet the susceptible stage of the host. If the environmental factors for the pathogen are the same as for the host, disease incidence is likely to be more. In the mean time, it is also possible that the host can be grown at a much wider range of temperature and humidity, which may not be favourable for the pathogen. One of the methods to achieve goal is to alter the date of sowing so that the susceptible stage of plant growth does not coincide with the environment highly favourable for the pathogen. Early or delayed sowing of the crop enables the host to escape critical period. The results pertaining to the date of sowing and disease incidence recorded year wise are summarized in the Table -15.

Table -15: Effect of date of sowing on the disease intensity.

S. No.	Date of sowing	Average disease intensity (Per cent)	
		2003	2004
1.	Second week of June	37.92 (37.98)	38.62 (38.38)
2.	Last week of June	36.21 (36.99)	34.81 (36.15)
3.	Second week of July	29.51 (32.90)	30.67 (33.62)
4.	Last week of July	28.31 (32.13)	26.30 (30.82)
CD at 5%		**3.62**	**3.49**

The data presented in the Table -18 (Fig. 15) revealed that minimum disease intensity was 28.31 per cent in 2003 and 26.30 per cent in 2004 when crop was sown late in the season (Last week of July), whereas, maximum disease intensity 37.92 per cent and 38.62 per cent was observed when crop was sown early in both the crop season (2nd week of June). The disease intensity was also high when the crop was further delayed in sowing (Last week of June) in both the years.

Thus, finding showed the importance of sowing the crop when the best temperature and moisture conditions present for rapid growth of the host to escape critical period of disease incidence.

(b) Effect of different soil pH on disease intensity:

In order to find out the impact of different soil pH on disease intensity, experiments were conducted in the glass house of the Department during 2003 and 2004. The experiments were done according to the procedure described under '**Material and Methods**'. For this purpose, five seeds were sown in each pot

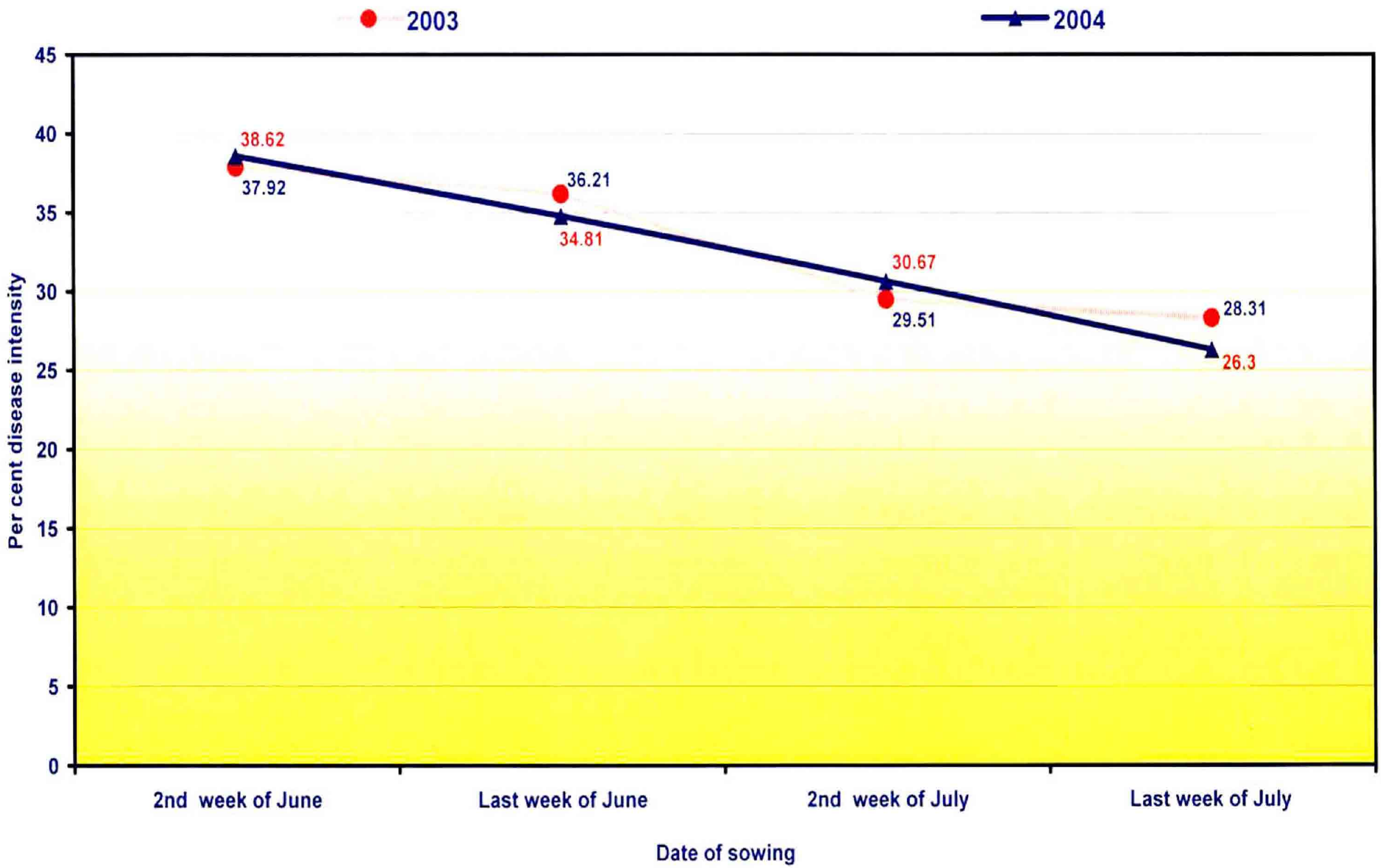


Fig.15: Effect of date of sowing on disease intensity

(30cm) and replicated thrice. The rate of disease intensity was recorded at the time of harvesting and results are summarized in Table -16.

Table- 16 : Effect of different soil pH on disease intensity.

S. No.	Soil pH	Average disease intensity (Per cent)		Average disease intensity (Per cent)
		2003	2004	
1.	5.0	23.34 (28.85)	24.58 (29.67)	23.96
2.	5.5	28.52 (32.27)	26.76 (31.12)	27.64
3.	6.0	34.82 (36.15)	35.18 (36.37)	35.00
4.	6.5	39.74 (39.07)	38.67 (38.45)	39.20
5.	7.0	32.74 (34.73)	32.96 (35.03)	32.85
6.	7.5	21.86 (29.93)	20.28 (26.74)	21.07
7.	8.0	22.39 (27.65)	23.01 (28.05)	22.70
CD at 5%		**2.28**	**2.47**	

The results presented in Table 16 (Fig. 16) revealed that the highest disease intensity was observed at pH 6.5 followed by pH 6.0 and pH 7.0. Least disease intensity was observed at pH 7.5 followed by pH 8.0, which were statistically at par with each other.

Thus, the finding showed the importance of soil pH in relation to disease development.

(c) Effect of different soil amendments on disease intensity:

To see the impact of different types of soil amendments, the experiments were conducted in the glass house of the Department during 2003 and 2004. The experiments were deployed as per procedure described under "**Material and**

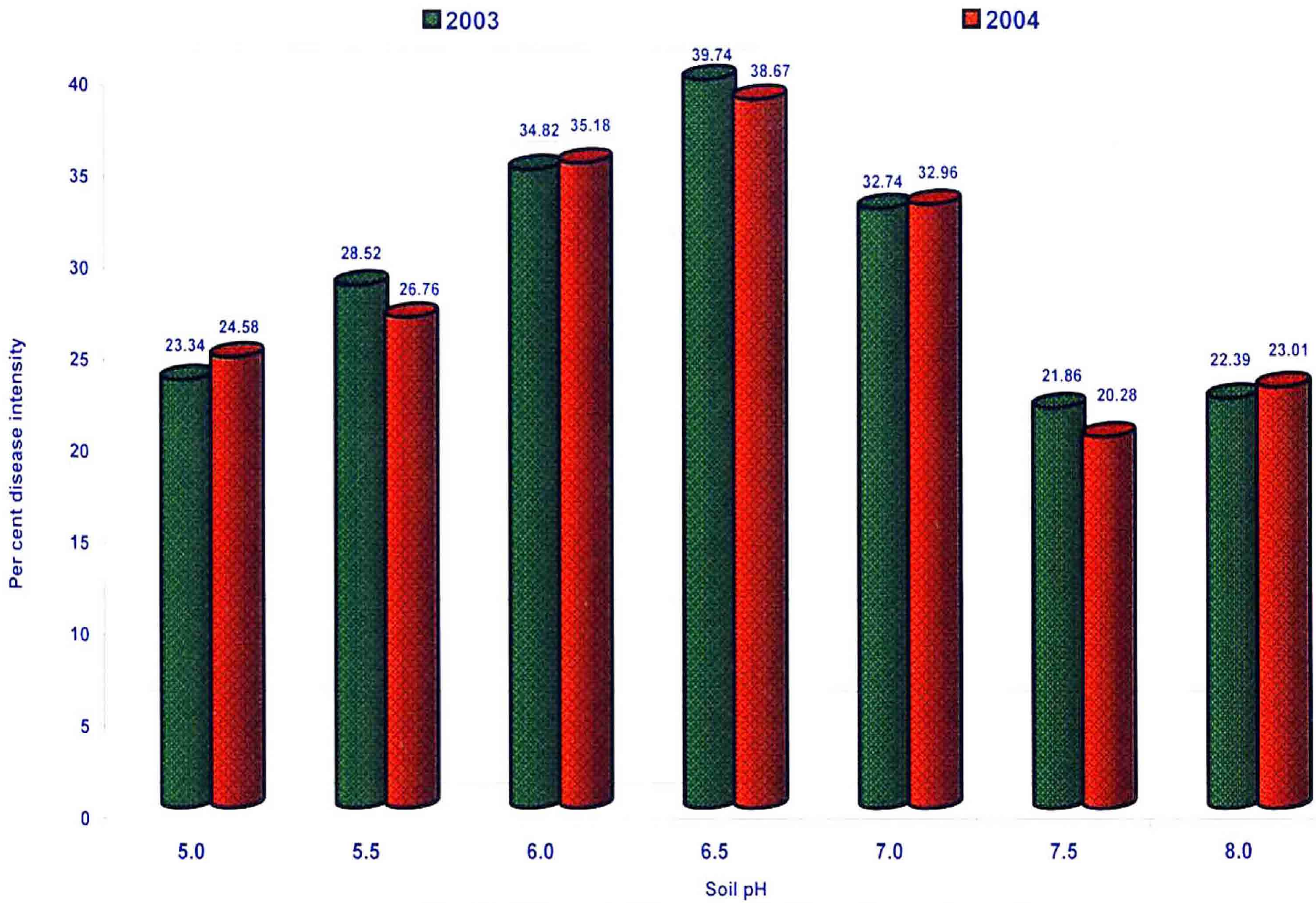


Fig.16: Effect of different soil pH on disease intensity

Methods". Each treatment was replicated thrice. Average disease intensity was recorded in both the years and results are summarized in Table – 17.

Table - 17: Effect of different soil amendments on disease intensity:

S. No.	Soil amendments	Average disease intensity (Per cent)		Average disease intensity (Per cent)
		2003	2004	
1.	Pyrite	13.46 (21.44)	14.58 (22.32)	14.02
2.	Neem cake	15.36 (23.04)	16.76 (24.11)	16.06
3.	Paddy straw	18.74 (25.65)	19.96 (26.53)	19.35
4.	Mustard cake	21.25 (27.74)	21.34 (27.51)	21.29
5.	Castor cake	23.14 (28.75)	24.38 (29.58)	23.76
6.	Gypsum	27.95 (31.91)	28.85 (32.48)	28.40
7.	Wheat straw	31.48 (34.12)	32.69 (34.87)	32.08
8.	Control	35.28 (36.41)	36.35 (37.06)	35.81
CD at 5%		**2.40**	**2.76**	

It is evident from Table-17 and its corresponding Fig. 17 that disease was minimized in varying degree by the use of different soil amendments as compared to control. However, disease intensity was reduced to a great extent with the addition of pyrite (20 tonnes/ha) as soil amendments followed by neem cake (30 tonnes/ha) in both the years and they were statistically at par with each other. Rest of the soil amendments were in descending order of merit as regards to disease intensity except wheat straw which was least effective and have maximum disease intensity.

Therefore, it is suggested that the soil amended with pyrite and neem cake would help in minimizing the disease as compared to others.

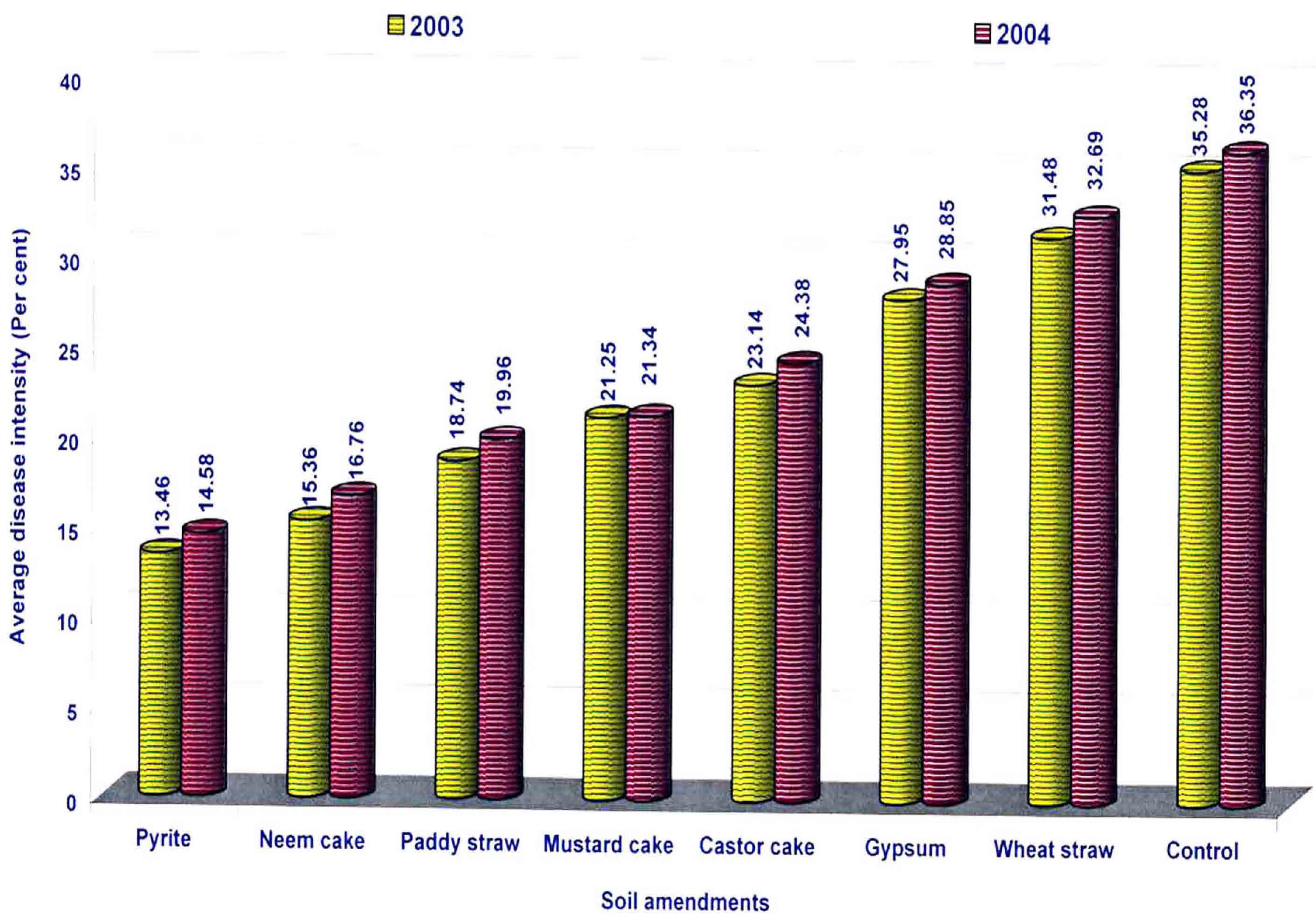


Fig.17: Effect of different soil amendments on disease intensity

d. Effect of different types of soil on disease intensity:

Anthracnose caused by *Colletotrichum lindemuthianum* is a major disease which hampers the cowpea production in the country. It has been observed that the pathogen have ability to survive in the soils and exhibits different reaction in different soils. Therefore, it was thought worthwhile to study the effect of different types of soil texture on the incidence of the anthracnose of cowpea.

To observe the impact of different types of soil on the disease intensity, the experiments were carried out during crop season 2003 and 2004 as per procedure described under "**Material and Methods**". For this purpose five seeds were sown in each pot (30cm) of each soil and replicated thrice. The rate of disease intensity was recorded and results are summarized in Table - 18.

Table – 18: Effect of different types of soil on disease intensity.

S. No.	Types of soil	Average disease intensity (Per cent)		Average disease intensity (Per cent)
		2003	2004	
1.	Clay soil	16.34 (23.81)	17.54 (24.61)	16.94
2.	Silt loam soil	19.28 (26.00)	20.38 (26.61)	19.83
3.	Sandy loam soil	23.44 (29.15)	24.96 (29.97)	24.35
4.	Loam soil	26.22 (30.79)	27.39 (31.54)	26.80
CD at 5%		**2.82**	**2.86**	

It is evident from the Table - 18 and its corresponding Fig. 18 that the minimum disease intensity was recorded in clay soil followed by silt loam soil in both the years and they were statistically at par. The maximum disease was recorded in loam soil.

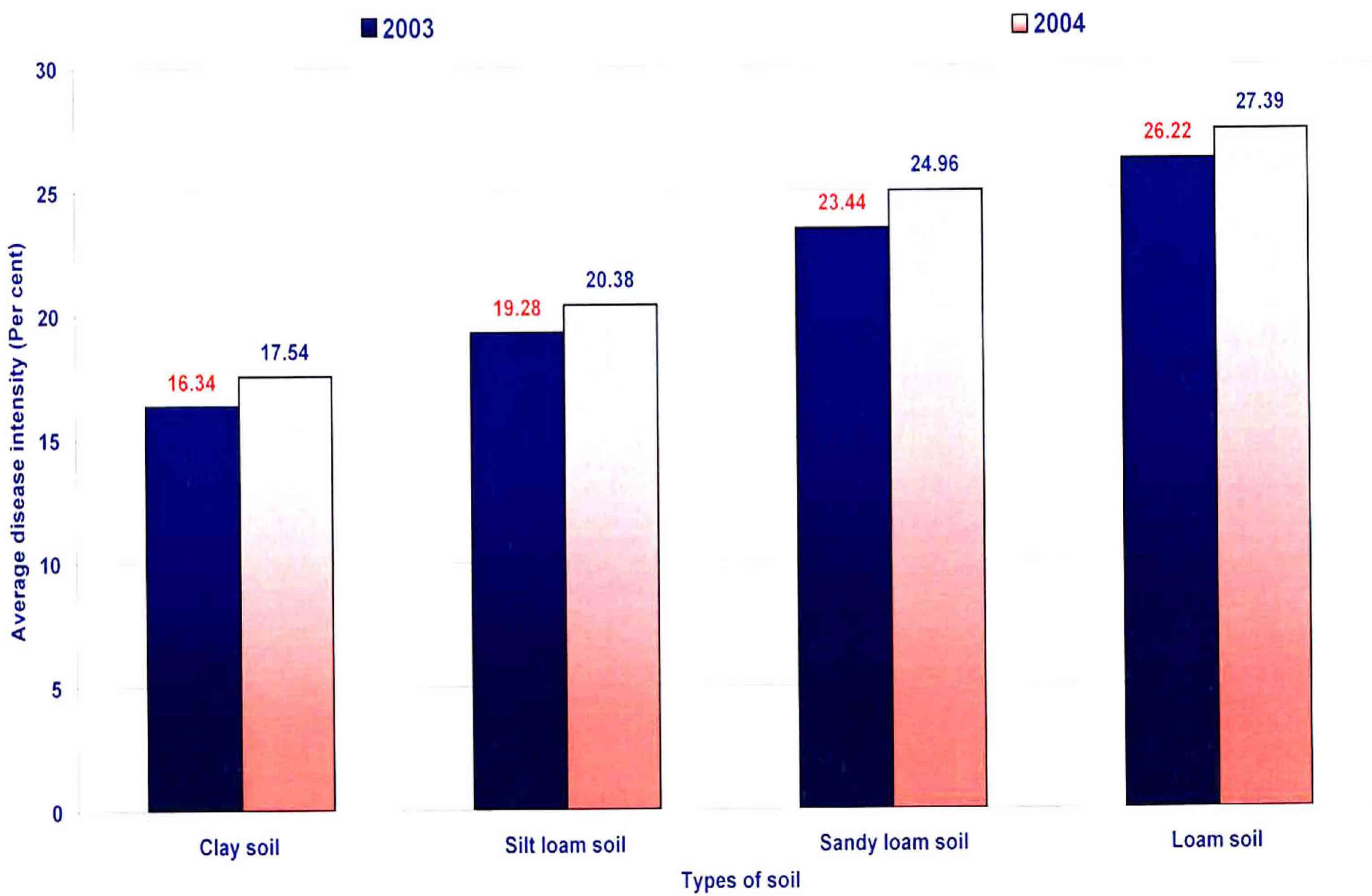


Fig.18 : Effect of different types of soil on disease intensity

e. Effect of different doses of phosphorus and potassium on intensity of the disease:

To determine the effect of phosphorus (P) and potassium (K) application on the severity of the disease an experiment was conducted at Vegetable Research Farm. Kalyanpur of the University in Factorial R.B.D. by using the method described in the "**Material and Methods**". Different combinations of P and K doses were applied in different plots. Disease severity in each treatment was recorded at the time of crop maturity and data thus recorded were analyzed statistically. The results are being summarized in the Table – 19.

Table - 19: Effect of phosphorus and potassium on disease intensity.

P/K	2003			Av.	2004			Av.
	K_0	K_{20}	K_{40}		K_0	K_{20}	K_{40}	
P_0	38.78 (38.5)	23.97 (29.2)	16.26 (23.7)	26.37 (30.5)	39.54 (38.9)	24.76 (29.7)	17.82 (24.9)	27.37 (31.2)
P_{30}	24.14 (29.4)	19.36 (26.1)	15.82 (23.4)	19.77 (26.3)	25.24 (30.1)	20.50 (26.9)	16.92 (24.2)	20.88 (27.1)
P_{60}	17.30 (24.57)	13.42 (21.48)	13.02 (21.06)	14.58 (22.37)	18.02 (25.11)	14.96 (22.75)	14.10 (21.92)	15.69 (23.2)
Average	26.74 (30.88)	18.92 (25.62)	15.03 (22.76)		27.60 (31.40)	20.07 (26.49)	16.28 (23.72)	

CD at 5% level of significance for the year **2003 = 1.75**

2004 = 1.35

The results given in Table – 19 Fig. 19 indicated that the disease intensity decreases with the increase in the doses of phosphorus and potassium. The lowest disease intensity was recorded when phosphorus was applied @ 60 kg/ha with the potassium @ 40 kg/ha. The disease intensity in the combination of $P_{60}K_{40}$ was 13.02 per cent and 14.10 per cent during the year 2003 and 2004, respectively.

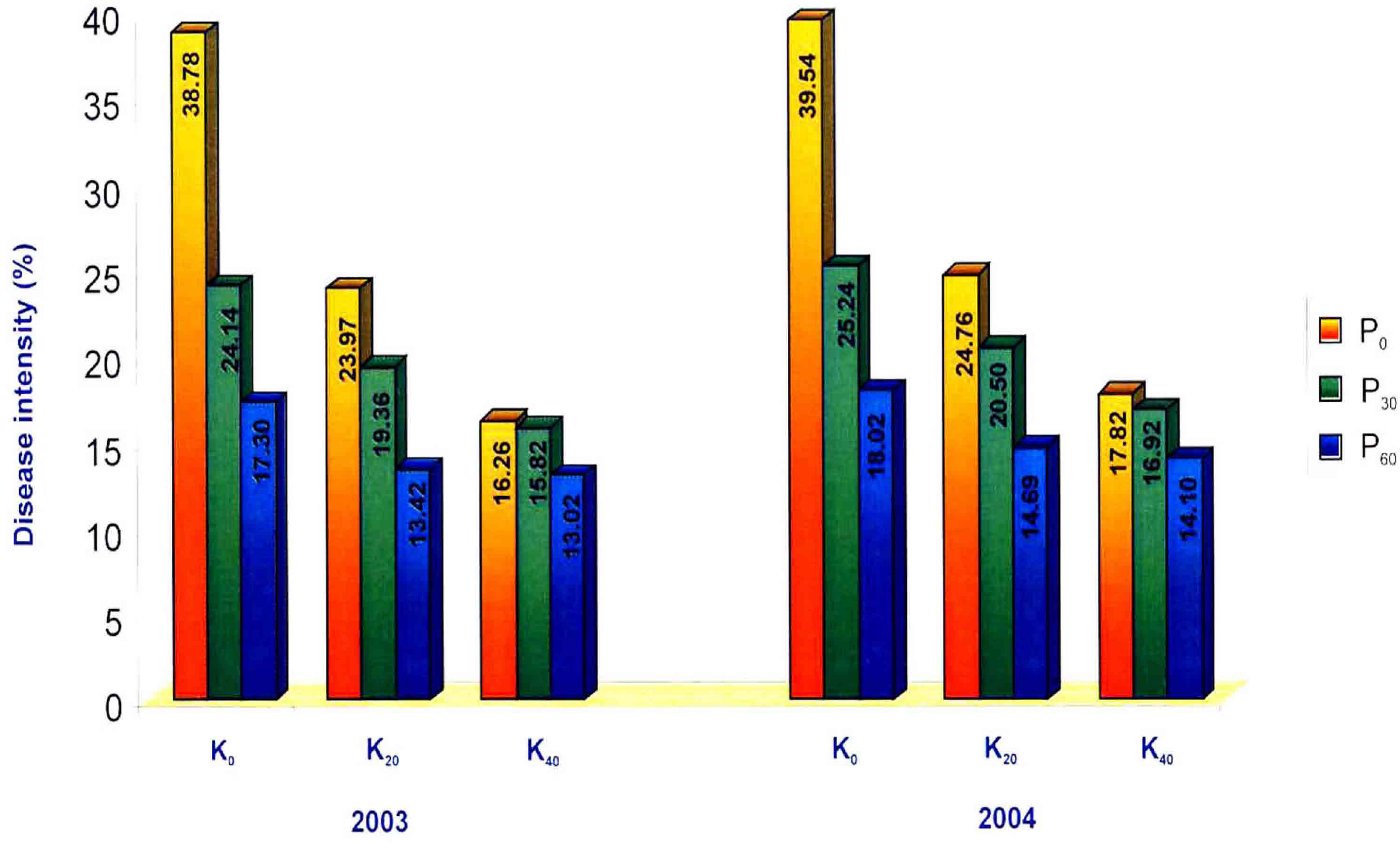


Fig. 19: Effect of Phosphorus and potassium on disease intensity

The highest disease intensity was recorded in treatment combination P_0K_0 which was 38.78 per cent and 39.54 per cent during 2003 and 2004, respectively followed by the disease intensity in treatment combinations P_{30} K_0, P_0K_{20} and P_{30} K_{20}, respectively in both the years.

f. Effect of different bio-agents on the disease incidence:

(i) Effect of bio-agents as seed dresser on the disease intensity:

Evaluation of bio-agents as seed dresser was made according to appropriate procedure described earlier under "**Material and Methods**". The results obtained are presented in Table -20.

Table - 20: Effect of bio-agents as seed dresser on the disease intensity.

S. No.	Antagonist	No. of plants per plot	Average no. of affected plants per plot	Av. Per cent (Disease intensity)
1.	*Trichoderma viride*	20	3	15.0 (22.59)
2.	*Trichoderma harzianum*	20	4	20.0 (26.52)
3.	*Trichoderma virens*	20	6	30.0 (33.21)
4.	*Pseudomonas flourescens*	20	8	40.0 (39.23)
5.	*Bacillus subtilis*	20	9	45.0 (42.13)
6.	Control	20	11	55.0 (47.87)
	CD at 5%			4.12

It is avid from the results presented in Table -20 (Fig. 20) that all the bio-agents were significantly superior over control. Minimum disease incidence (15%) was recorded in the trial where seeds were treated with *T. viride* followed by *T. harzianum* (20.0%) as regards to the management of the disease followed by *T. virens* (30.0%). The high disease intensity was noticed with *P. flourescens* (40%) and *Bacillus subtilis* (45.0%) which proved worst among all the bio-agents tested.

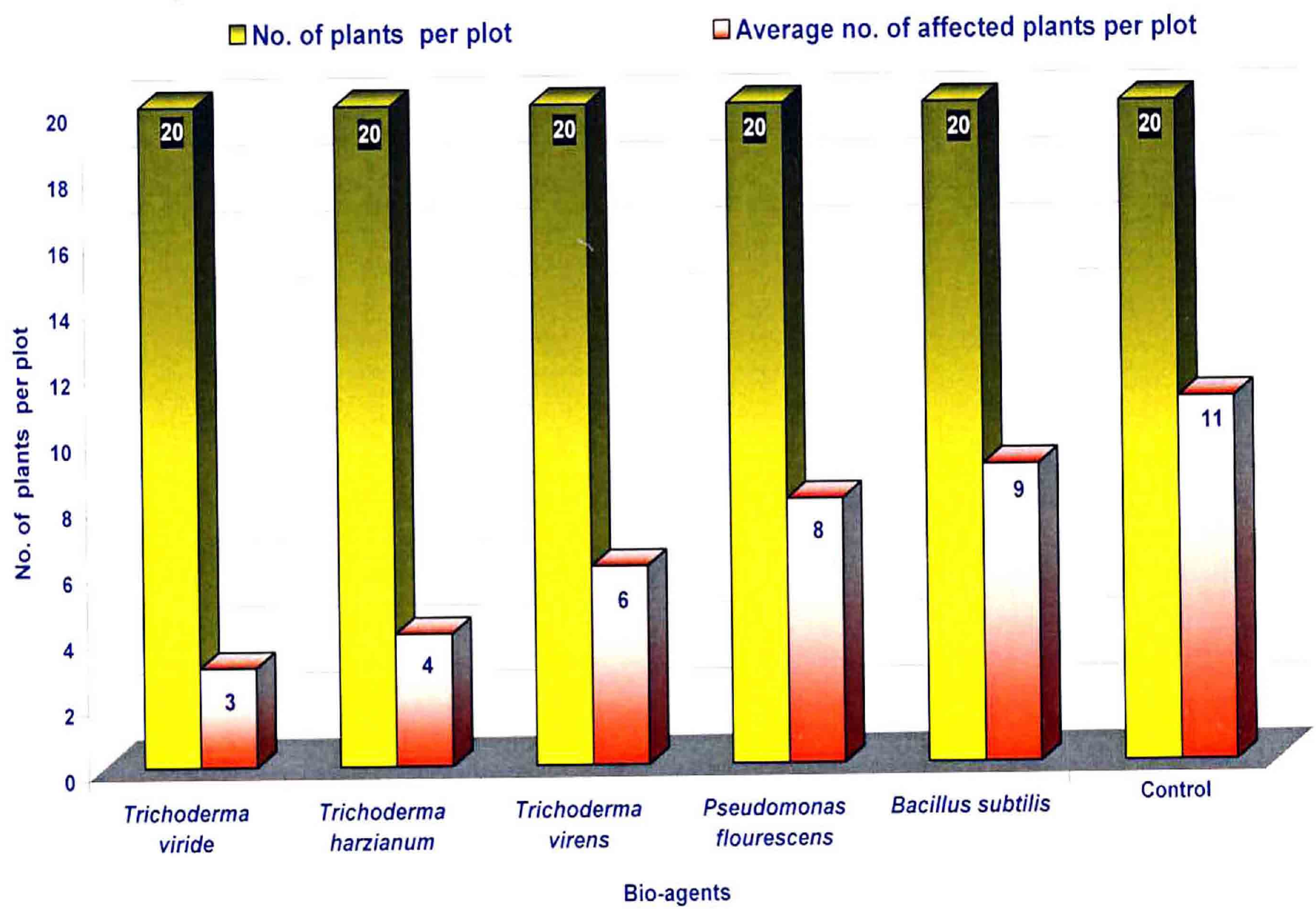


Fig. 20: Effect of bio-agents as seed dressers on the disease intensity

(ii) Effect of bio-agents as soil applicants on disease intensity:

To see the effect of bio-agents as soil applicants, experiments were conducted in plot size of 3 x 2 m. Each treatment was replicated thrice and irrigated periodically. The experiments were laid down as per procedure mentioned under "**Material and Methods**". Observations on disease intensity were recorded and data are presented in Table -21.

Table - 21: Effect of bio-agents as soil applicants on disease intensity:

S. No.	Bio-agents	Average disease intensity (per cent)	
		2003	**2004**
1.	*Trichoderma viride*	18.72 (26.59)	19.38 (26.07)
2.	*Trichoderma harzianum*	21.18 (27.40)	22.46 (28.26)
3.	*Trichoderma virens*	24.01 (29.28)	25.82 (30.53)
4.	*Pseudomonas flourescens*	28.20 (32.07)	29.56 (32.93)
5.	Control	36.92 (37.04)	39.24 (38.82)
	CD at 5%	**3.20**	**3.50**

It is evident from Table - 21 and its corresponding Fig. 21 that all the treatments were significantly superior in minimizing the incidence of anthracnose of cowpea over control. Minimum disease intensity (18.72 & 19.38 %) in both the years of the disease was recorded when soil was incorporated with *T. viride* formulation. However, the second best bio-agent was *T. harzianum* followed by *T. virens* as regards to the management of the disease. In case of bacterial bio-agents, formulation of *P. flourescens* proved least effective.

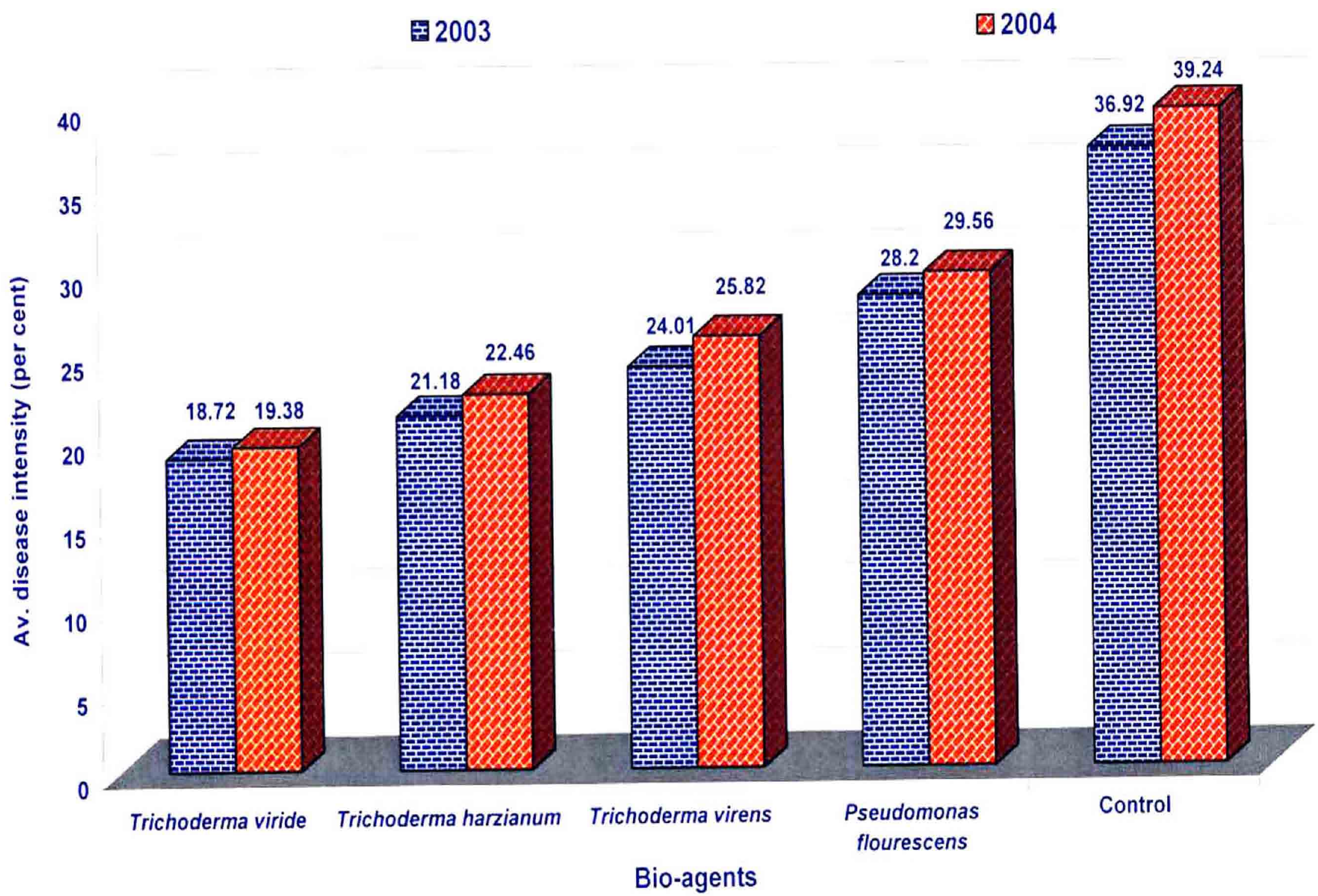


Fig. 21 : Effect of bio-agents as soil applicants on disease intensity

Discussion

DISCUSSION

Vegetable crops have remained as a mainstay of Indian economy for centuries. This is because of its high nutritive value and forming an integral part of daily diet of the predominantly vegetarian population in India. Over the last three decades of high yielding varieties era, the magnitude of vegetable production has not been proportionate to cereals and remained almost stagnant. As a result of ever increasing population of the country, the per capita availability of vegetables has been shown as sharp decline in recent past. Among the major factors limiting the production of cowpea, problem of diseases is the most important one.

Few diseases of this crop, which were of minor importance, now have assumed serious proportion and several new diseases have come into prominance. The anthracnose disease of cowpea is one of the such diseases. During the course of survey of cowpea diseases at Kanpur and adjoining areas, important varieties of cowpea were found moderately to heavily infected with anthracnose disease caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bric. & Cav.

Despite the introduction of high yielding varieties and improved cultural practices, production of cowpea per unit area is still low. Therefore, it is essential that due importance should be given to cowpea crop for stepping up its production.

As is evident from the available literature, there is only report of the occurrence of anthracnose of cowpea but no information is available regarding major aspects of the disease like perpetuation and spread of the pathogen, varietal screening and various methods of management with ultimate aim of finding out the best control measures.

Under natural conditions the disease manifests itself mainly on the leaves but in severe cases it may also appears as minute, circular to irregular spots scattered on leaf lamina. These spots are light brown to dark brown or reddish brown in colour. In the beginning, these lesions remain separate but later on, coalesce to form large necrotic, circular to rectangular spots on leaves with shot holes which may also cause defoliation. The lesions appear on petioles are small and reddish brown. On stem, the symptoms appear as light grey, water soaked and irregular lesions, which later becomes rusty brown and enlarge up to 2-8 mm in diameter. On pods the symptoms appear as small reddish brown blotches, which later become light brown to grayish and in advance stages, they may produce numerous fruiting bodies (acervuli). The symptoms appear on the cowpea plants are more-or-less similar to those as described by Baily *et al.* (1990); Rahman *et al.* (1999) on Lablab and Paula - Junior *et al.* (1994) on *Phaseolus vulgaris*.

Disease survey conducted in four district of Uttar Pradesh during the crop season of cowpea in 2003 and 2004, *Colletotrichum lindemuthianum* was found invariably associated with the cowpea plants of different stages. No previous information is available about the prevalence and severity of the disease under present conditions of cowpea crop husbandry and intensive cultivation. It is likely that the population of the fungus on the soil surface and plant residue has gradually built up higher and is making its presence felt with the occurrence of the anthracnose of cowpea.

During the course of present investigations, disease samples were collected by personal visit and some samples were obtained through plant pathologists and other scientists working at different research centers. According to present findings disease incidence at different places was quite high during crop season. This may be due to favourable environmental conditions or the pathogen may be more virulent or the interaction of both for the development of the disease.

During the investigation, disease samples were collected from fields and isolations were made from them. These isolations yielded the fungus, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. Pathogenicity tests were then carried out on cowpea plants by artificial inoculation to examine the pathogenic behaviour of the isolated fungus. In pathogenicity tests, it produced typical anthracnose symptoms on inoculated plants and thus proved the Koch's postulates.

After isolating the pathogen from diseased plants, its morphological characters were studied. Accordingly, the fungus produced branched, septate, hyaline mycelium in the beginning, which later becomes grayish black in colour. The acervulus is mostly glabrous, measuring 150-250 µm and dark brown to grayish black in colour. The setae are dark brown to black in colour, 1-3 septate measuring 30-100 x 4 -9 µm in size and 2-15 in each acervulus. Conidiophores are hyaline, short, erect, unbranched and arranged in definite layers on the surface of stromatic tissues. They are packed together, measuring 10.5 -16.5 x 2-3 µm. Conidia produced in pinkish masses are single celled, hyaline, oblong to cylindrical with rounded ends or with one end slightly pointed measuring about 11-20 x 2.5-5.5 µm. Thus, morphological characters of the fungus closely resemble with that of described by Corda (1931); Emmett and Parberry (1975).

Knowledge of cultural characters of a fungus is of great help in understanding its behaviour towards host under varying conditions. On the basis of composition, there are two general types of media, natural media that are composed of entirely natural products and synthetic media of known composition. In the preset study, Potato dextrose agar medium (PDA) was used as basal medium for cultural and morphological characters and it was found to be best one followed by Richard's medium for the vegetative growth and acervuli production. However, least growth of the pathogen was observed on host extract medium. On rest of the media, its growth varied from moderate to good. These findings are in agreement with the findings of Holdeman (1950) and Pria *et al.* (1997).

In general, the production of acervuli by the pathogen varied from excellent to poor on different media. The production of acervuli (sporulation) was good not only in those media, which supported good mycelial growth but also on Oatmeal and Kirchoff's media in which generally less mycelial growth occurred.

On the basis of different morphological and cultural characters, the fungus associated with the anthracnose of cowpea, is identified as *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav.

In the studies pertaining to the survival, primary infection and secondary spread, the pathogen was found to survive through diseased plant debris, seeds and infested soil. The pathogen could survive and remain viable for seven months under laboratory conditions and nine months in field conditions in infective stage in diseased plant debris, which served as primary source of inoculum.

The present results are more-or-less similar to the findings of Onesirosan and Sagay (1950), who reported that *C. lindemuthianum* survived in diseased stem tissues either left on the soil surface or plowed under. The present investigations are also in agreement with that of Thakur (1992) who also reported *Colletotrichum lindemuthianum* surviving to next season on mungbean crop residue in soil.

Besides these, the present investigations are also agree with findings of Dillard and Cobb (1993), who reported *C. lindemuthianum* on bean which over winters on plant debris lying on soil surface.

Seeds play an important role in survival and dissemination of many plant pathogens. In the present investigation seed borne nature of the pathogen was examined in seed samples obtained from affected plants showing characteristic disease symptoms. The pathogen was detected from naturally infected seeds by plating them on potato dextrose agar medium (PDA) and by standard blotter method. In case of PDA, 22.50 per cent seeds were found infected while 24.25 per cent seeds were observed infected in case of standard blotter method, whereas, the pathogen could not be detected from the control. Experiments were also conducted to determine the locations of the pathogen for its survival through seeds. In the present findings the pathogen was found to survive in seed coat, cotyledons and embryo. The role of infected seeds in the development of the disease in the next season sown crop was also established in pot culture experiment. Prasanna and Rama Prassanna (1980) reported *C. lindemuthianum* surviving in cowpea seeds. The present findings are also corroborated by the observations of Prassanna (1985); Ravi *et al.* (1995); Sharma *et al.* (2001) and Qandah and Al-Momany (2003).

The observations that the plants grown in pots containing either infested soil or diseased plant debris with sterilized soil, expressed disease symptoms and confirmed that infested soil and other plant debris harbour the pathogen and play an important role in development of disease in the next crop season.

Studies on the secondary spread of the pathogen revealed that secondary spread took place through the spore (conidia) formed by the pathogen on infected plant parts. Under natural conditions a large number of acervuli are formed on infected plant parts by the pathogen. The acervuli release number of conidia, which cause secondary infection when transported by air from lower leaf to upper leaf and from one plant to another plant.

In vitro study on biological control of *C. lindemuthianum* was carried out by using antagonists like *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma virens*, *Pseudomonas flourescens* and *Bacillus subtilis* by dual culture technique. All the bio-control agents inhibited mycelial growth of pathogen to a great extent. *Trichoderma viride* exhibited the maximum antagonistic activity causing an inhibition of 81.50 per cent in the growth of pathogen followed by *Trichoderma harzianum*, *Trichoderma virens* and *Pseudomonas flourescens* whereas, *Bacillus subtilis* showed the minimum antagonistic activity. Similar results are also reported by Barros *et al.* (1995) and Jeyalakshmi *et al.* (1998).

Breeding for disease resistance is an important and the first line measure to control a disease. This measure is not only simple, practical and effective but also environmentally safe and sustainable, which saves time and energy and also lowers the cost of production. Out of 80 varieties/cultures of cowpea were screened, 30 varieties/cultures remained resistant to moderately resistant against the disease under natural conditions for two consecutive years 2003 and 2004. When these 30 varieties/ cultures were further subjected to screening under artificial inoculation only 7 varieties/cultures *viz.*, RCV-7, RCV-326, Sel-16, BCKV-2, Pusa Rituraj, M-278 and NP-1 were found to be resistant and 10 varieties/ cultures *viz.*, M-120, M-441, M-462, Cowpea-263, VS-15, P-1270, Pusa Komal, M-390, P-28 and BCP-3 were proved moderately resistant whereas, the remaining varieties/ cultures were graded under moderately susceptible to susceptible. The present findings emphasized the need for further evaluation of more number of genotypes against the disease so as to get genotypes with high degree of resistance in combination to high yield potential. The present results also supported the findings of Sohi and Rawal (1983), Adebitan *et al.* (1992) and Adebitan and Olufajo (1998).

Sowing date is a cultural measure for crop disease management. It reduced the period over which infection agents (propagules) meet the susceptible stage of the host. If the environment is favourable for the pathogen, the same as for host, disease incidence will likely to be high, but it is also possible that the host can be grown at a much wider range of temperature and humidity which may not be favourable for the pathogen. This dissimilarity in favourable environment for host and pathogen can be explained for disease management. One of the methods to achieve the reasonable management of the disease is to alter the date of sowing so that the susceptible stage of plant growth does not coincide with the environment highly favourable for the pathogen.

In order to know the effect of different date of sowing of cowpea on the disease intensity, four different dates of sowing were taken for observing the disease occurrence. The maximum disease intensity was observed when crop was sown early in season (2nd week of June) and it gradually decreased with the increase in sowing dates. The disease intensity was lowest when crop was sown late in season (last week of July). Thus, the finding confirmed that the crop should be grown at that time when best temperature and moisture conditions are present for rapid growth of the host to escape the critical period of disease incidence. These observations are also according to the findings of Thakur and Khare (1990) who reported that late sowing of cowpea reduced the disease incidence caused by *Colletotrichum lindemuthianum* in comparison to early sowing.

Studies were carried out to see the effect of different soil pH on the disease occurrence during crop season 2003 and 2004. It was observed that minimum disease intensity was observed at pH 7.5 followed by pH 8.0, which were statistically at par with each other. The maximum disease intensity was recorded at pH 6.5 followed by pH 6.0. Thus, it was clear from the experiment that high soil pH reduce the disease intensity. Choudhary (1957) and Chandrasekaran and Shanmugan (1984) also reported same type of results on different crops with different causal agents.

In order to see the impact of different soil amendments on the disease intensity, seven different soil amendments were used. Minimum disease intensity (13.46% and 14.58%) was observed when soil was amended with pyrite (20 t/ha) followed by neem cake (30 t/ha). Soil amended with wheat straw was found least effective in controlling the disease in both the years i.e. during 2003 and 2004. The present results also supported the findings of Sandhu (1992) and Singh *et al.* (2006) who worked on different crops.

In order to find out the effect of different types of soil (texture) on disease intensity, the pot culture experiments were carried out under natural conditions. It was observed that disease intensity was maximum (26.22% and 27.39 %) in loam soil, whereas, it was minimum (16.34% and 17.54 %) in clay soil in both the years followed by silt loam soil. These findings are in accordance with the results of various workers (Srivastava and Kamtham, 2002 and Ghasolia *et al.* 2004).

By enlarge, the nutrition of a plant determines the resistance or susceptibility to disease. Non availability of some nutrient elements to the plants may result in proneness to diseases, while extra availability of some other nutrients also increase disease susceptibility. The effect of phosphours (P) and potassium

(K) on disease intensity has been extremely worked out because of their limited availability in soil in comparison to their large quantity required for optimum plant growth. Present studies also deal with the effect of phosphorus and potassium application in cowpea crop on severity of anthracnose.

The results of study revealed that the disease intensity decreased with increase in doses of P and K. The lowest disease intensity was recorded when phosphorus was applied @ 60 kg/ha with potassium @ 40 kg/ha. The disease intensity in combination $P_{60}K_{40}$ was 13.02 per cent and 14.10 per cent during the year 2003 and 2004, respectively. The highest disease intensity was recorded in treatment P_0K_0 which was 38.78 per cent and 39.54 per cent during 2003 and 2004, respectively, followed by the disease intensity in treatment combinations like $P_{30}K_0$, P_0K_{20} and $P_{30}K_{20}$, respectively in both the years. These investigations showed that a balanced combination of phosphorus and potassium helped in minimizing the disease intensity. The present work is related with the work done by Adebitan (1996) who also reported that application of phosphorus decreased the disease.

Studies were conducted to find out the best bio-control agent as seed dresser in the pot culture. All the bio-agents were significantly superior in minimizing the disease over control. *Trichoderma viride* was more effective in minimizing the disease as it exhibited only 15 per cent disease incidence as compared to control (55 per cent). Next effective bio-agents were *T. harzianum*, *T. virens* and *Pseudomonas flourescens*. The least effective bio-agents was *Bacillus subtilis*. The present findings are similar with the results obtained by Gaikwad *et al.* (2002) and Ravi *et al.* (1999).

Studies were also conducted to find out the best bio-agents as soil applicant in field experiment. *Trichoderma viride*, *T. harzianum*, *T. virens* and *Pseudomonas flourescens* were significantly superior in reducing the disease . *Trichoderma viride* was most effective in reducing the disease as it showed only 18.72 per cent and 19.38 per cent disease in both the years, respectively when applied to the soil before sowing.

Present results are in accordance with the observations made by Ravi *et al.* (2000) who also reported that *T. viride* and *P. flourescens* as soil applicants for effective management of anthracnose of French bean. Adebanjo and Bankole (2004) obtained an effective control of *C. lindemuthianum* of cowpea through bio-agents like *Trichoderma viride* and *Aspergillus niger*.

Summary

SUMMARY

Cowpea [*Vigna unguiculata* (L.) Walp.] which is also known as 'lobia', is an important vegetable as well as pulse crop of Indian subcontinent. Endowed with several unique characteristics, cowpea finds an important place in the farming system adopted by small, medium and large scale farmers in our country. Its green seeds and tender pods are used as vegetable. Since it is a leguminous crop, it facilitates symbiotic nitrogen fixation in soil and restores soil fertility. Being a crop of such immense value, cowpea needs every endeavour to obtain its better yield per hectare. Among the major constraints for the low productivity of this crop, diseases of different origin are the main. It is in this context, the present investigations were carried out to find out effective disease management strategies for the management of anthracnose of cowpea.

During survey, it was found that most of the important varieties of cowpea were moderately to heavily infected with anthracnose caused by *Colletotrichum lindemuthianum* . The disease is characterized by the appearance of minute, circular to irregular spots, scattered on leaf lamina. These spots are light brown to dark brown or reddish brown in colour. Initially these lesions remain separate but later on coalesce to form large, necrotic, circular to rectangular spots on leaves with shot holes, which may also cause defoliation. Small reddish brown lesions also appear on petioles. Several light grey, water soaked, irregular lesions also appear on stem and become rusty brown in advanced stage. On pods the symptom appears as small, reddish brown blotches, which later become light brown to grayish colour and later on, numerous acervuli are also produced on it.

To know the prevalence of the disease. survey was conducted in four districts of U.P. It was found that crop suffered from the disease in varying degrees. The disease intensity was as high as 43.80 per cent in Student's Instructional Farm of the University during the crop season 2003 and 2004. In all the districts surveyed, the disease intensity was at its maximum during the months of July and September (2003 and 2004) when environmental conditions were favourable.

To Study the morphological characters of the pathogen, it was isolated on potato dextrose agar medium collected from four districts. It was observed that mycelium of isolated fungus was branched, septate, hyaline but later become grayish black in colour. Acervulus was globrous measuring 150-250 µm, dark brown to grayish black in colour. Setae were dark brown to black, 1-3 septate measuring 30-100 x 4-9 µm in size and varying in numbers. The conidiophores were hyaline, short, erect, unbranched and arranged in definite layers on the surface of stromatic tissues packed together measuring 10.5 – 16.5 x 2-3 µm. The conidia were single celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed and measuring about 11-20 x 2.5-5.5 µm.

The qualitative as well as quantitative growth of the pathogen was observed on eight different solid and liquid media being of natural, synthetic and semi synthetic in nature. The maximum radial growth of the fungus was obtained on potato dextrose agar medium followed by Richard's agar medium while the least growth was obtained on host extract agar medium. The acervulus formation was excellent on both the medium in which the vegetative growth was also good. Maximum vegetative growth of the fungus was harvested in Potato dextrose medium. It also supports excellent development of acervuli (sporulation).

In studies, pertaining to the survival of the pathogen, it was observed that the pathogen could survive through diseased plant debris for 7 months under laboratory conditions and for 9 months under field conditions and was able to cause infection in the next season grown crop. The pathogen could also survive in seed coat, cotyledon and embryo of infected seed and hence carry the disease to the next crop season. Like seeds and diseased plant debris, infested soil also play a key role in survival of the pathogen in the form of acervuli.

Secondary spread of the pathogen took place by means of air borne spores (conidia). The acervuli formed on infected plant parts release conidia in abundance and being minute in size, these conidia are propelled by air currents to new infection occurs.

Efficacy of bio-agents like *Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas flourescens* and *Bacillus subtilis* were tested under laboratory conditions against the pathogen. It was observed that all the bio-agents tested, inhibited the growth of test fungus. However, *T. viride* exhibited the maximum antagonistic activity followed by *T. harzianum*, *T. virens*, *Pseudomonas flourescens* whereas least effective bio-agent was *Bacillus subtilis*.

Screening of 80 varieties/ cultures of cowpea was carried out under natural conditions in two consecutive years i.e. 2003 and 2004. The observation on disease intensity revealed that none of the varieties/ cultures was found immune to anthracnose for the two consecutive years. Out of 80 varieties cultures screened so far, 30 varieties / cultures were found resistant to moderately resistant. In the third year (2005), these 30 varieties/ cultures were inoculated artificially. The varieties/ cultures found consistently resistant to the disease were RCV-7, RCV-326, Sel.-

16, BCKV-2, Pusa Rituraj, M-278 and NP-1. These varieties/ cultures may be further used in breeding programme against the present disease.

In the present time, cultural practices are the safest and cheapest tool of management of the diseases where there is restriction in the use of chemicals as they are injurious to human health and hazardous to environment viz., alternation in date of sowing, soil pH, soil amendments, soil texture and different doses of phosphorus and potassium were evaluated to see their effect on disease intensity.

Different dates of sowing of the crop had a great impact on disease occurrence. Minimum disease intensity was observed when the crop was sown in last week of July whereas it was increased when the crop was sown early.

Six different soil pH were tested, in which maximum disease intensity was observed at soil pH 6.5 followed by 6.0 and minimum disease intensity was observed at soil pH 7.5 and pH 8. High pH was not favourable for growth of cowpea.

Management of the disease by soil amendments revealed that minimum disease incidence (13.46% and 14.58%) was recorded when soil was amended with pyrite (20 tonnes/ha) in both the years.

Out of different soil types (textures) tested, disease intensity varied from 16.94 to 26.80 per cent in both the years. However, it was minimum (16.34% and 17.54%) in clay soil and maximum (26.22% and 27.39%) in loam soil in both the years.

The effect of different doses of phosphorus and potassium on disease intensity revealed that highest disease intensity (38.78% & 39.54%) was recorded in treatment P_0K_0 where phosphorus and potassium were not applied. However, application of increasing doses of both phosphorus and potassium resulted in decreasing incidence of the disease. The disease intensity was found the lowest (13.02% for 2003 and 14.10% for 2004) when 60Kg of phosphorus was applied in combination with 40kg of potassium per hectare.

Studies on the efficacy of bio-agents against *Colletotrichum lindemuthianum* as seed dresser and soil applicants showed that *Trichoderma viride* was most effective in both the experiments.

ABSTRACT

Cowpea is used as vegetable as well as pulse crop in Indian subcontinent. Among the major constraints responsible for low productivity, diseases are the main and Anthracnose is one of them. During survey, it was found that most of the cowpea varicties/cultures were infected with the disease, under study. Disease appears as minute, circular to irregular spots on leaf lamina brown in colour, which later spread on petioles, twigs and pods. Maximum disease intensity was 43.80% at Students Instructional Farm in both the years in the month of July to September. Mycelium of the fungus was branched, septate, hyline which later becomes grayish black in colour. Acervuli globours, 150-250μ m in size; setae 1-3 septate, 30-100 x4 –9 μm in size. Conidiophore arranged in defnite layers and 10.5-16.5 x 2-3 μm in size and conidia are single celled, hyline with rounded ends and 11-20 x 2.5- 5.5 μm in size. Maximum vegetative growth of the fungus was observed both in solid and liquid Potato dextrose medium. Pathgoen could survive through diseased plant debris for 7-month under laboratory conditions and 9-month in field conditions. The disease is internally as well as externally seed borne. Secondary spread of pathogen took place by means of conidia. Among the bio-agents tested against fungus, *T. viride* exhibited maximum antagonistic effect cowpea varieties/cultures. Like RCV-7, RCV-326, Sel-16, BCKV-2, Pusa Rituraj, M-278 & NP-7 were resistant against the disease. In late sown crop (Last week of July) the disease, intensity was low in comparison to early sown second week of June crop. Maximum disease intensity was observed in acidic soil at pH 6.5 while disease intensity was low in alkaline soil at pH 7.5 and 8.0. Minimum disease intensity was recorded when pyrite was applied @ 20 tonnes/ ha. In clay soil the disease intensity was low (16.34 and 17.54) while in loam soil it was found maximum (26.22 and 27.39). Application of higher dose of phosphorus and potash reduced the disease incidence. The formulation of *Trichoderma viride* was found best as seed dresser as well as soil applicants.

सारांश

भारतीय उपमहाद्वीप में लोबिया का प्रयोग सब्जी के साथ–साथ दाल के रूप में भी होता है। इसकी उत्पादकता के प्रभावित करने वाले प्रमुख कारक रोग हैं जिनमे श्यामव्रण रोग प्रमुख स्थान रखता है। सर्वेक्षण के दौरान यह पाया गया कि लोबिया की सभी प्रमुख प्रजातियां इस बीमारी से ग्रसित हैं। शुरूआत में रोग के लक्षण छोटे, गोल या अनियमित आकार के भूरे धब्बे के रूप में पत्ती पर दिखाई पड़ते हैं जो बाद में पर्णकंचुक, टहनियों एवं फलियों पर भी फैल जाते हैं। सर्वाधिक रोग सघनता 43.80 प्रतिशत जुलाई से सितम्बर माह में विद्यार्थी प्रक्षेत्र पर पाई गई। इस रोग का कारक *कोलेटोट्राइकम लिण्डेमुथियेनम* की कवक तन्तु, शाखायुक्त, विभाजित एवं सफेद थे, जो बाद में काले रंग के हो गये। एसरवुलाई गोल तथा 150–250μm आकार के, जिसमें 1 से 3 शूक (सीटी) उपस्थित थीं, जिनका आकार 30–100 x 4–9μm था। कोनिडियोफोर एक निश्चित परिधि में व्यवस्थित थे और उनका आकार 10.5–16.5 x 2–3μm था तथा कोनिडिया एक रंगीन तथा गोल शिराओं वाली व 11–20 x 2.5–5.5 μm लम्बाई की थी। सर्वाधिक गुणात्मक वृद्धि इस कवक की पोटैटो ड्रेक्सट्रोज अगर माध्यम पर पाई गयी और सर्वाधिक मात्रात्मक वृद्धि भी पोटैटो डेक्स्ट्रोज माध्यम पर ही पाई गयी। कृत्रिम परिस्थिति में रोग जनक 7 माह तक फसल अवशेष पर जीवित रहता है जबकि प्राकृतिक परिस्थितियों में यह 9 माह तक जीवित रहता है। यह रोग अन्तः एवं बाह्रय बीज जनित है। द्वितीयक फैलाव कोनिडिया के द्वारा होता है। सभी जैव कारकों में *ट्राइकोडर्मा विरिडी* में प्रतिरोधक क्षमता सर्वाधिक पाई गई। RCV-7, RCV-326, Sel-16, BCKV-2, Pusa Rituraj, M-278 & N-1 प्रजातियां इस रोग के प्रति प्रतिरोधी पायी गई। देर से बोई गई फसल में जल्दी बोई गई फसल की अपेक्षा रोग सघनता कम थी। सर्वाधिक रोग सघनता अम्लीय भूमि में जिसका पी0एच0 मान 6.5 था, पाई गई जबकि रोग सघनता क्षारीय भूमि में जिसका पी0एच0 7.5 व 8.0 था, कम पाई गई/ जब पायराइट का प्रयोग 20 टन/हे0 की दर से किया गया तो रोग सघनता कम थी। चिकनी भूमि में रोग सघनता कम थी जबकि दोमट भूमि में सबसे अधिक थी। उच्च मात्रा में फास्फोरस व पोटाश का प्रयोग करने पर रोग में बहुत कमी आई। *ट्राइकोडरमा विरिडी* बीज शोधन एवं भूमि उपचार हेतु सर्वाधिक उपयुक्त पाया गया।

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Appendices

ANOVA TABLE OF DIFFERENT EXPERIMENTS

Appendix 1: Qualitative growth

Source of variation	D.F.	T.S.S	M.S.S	F. Value
T	7	1741.081	248.725	31.09
Error (A)	16	128.000	8.000	
Total	23	1869.082	81.264	

Appendix 2: Quantitative Growth

Source of variation	D.F.	T.S.S	M.S.S	F. Value
T	7	80987.625	11569.660	428.51
Error (A)	16	432.000	27.000	
Total	23	81419.625	3539.983	

Appendix 3: Bio-agents *in vitro*:

Source of variation	D.F.	T.S.S	M.S.S	F. Value
T	5	7902.355	1580.471	86.35
Error (A)	12	183.038	15.253	
Total	17	8133.042	478.414	

Appendix 4: Date of sowing -2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	3.784	1.892	0.57
T	3	76.379	25.459	7.71
Error (A)	6	19.804	3.300	
Total	11	99.969	9.088	

Appendix 5: Date of sowing -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	4.147	2.073	0.68
T	3	95.456	31.818	10.40
Error (A)	6	18.359	3.059	
Total	11	117.963	10.723	

Appendix 6: Soil pH -2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	5.155	2.577	1.57
T	6	288.849	48.141	29.24
Error (A)	12	19.756	1.646	
Total	20	313.761	15.688	

Appendix 7: Soil pH -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	2.736	1.368	0.71
T	6	299.020	49.836	25.83
Error (A)	12	23.148	1.929	
Total	20	324.904	16.245	

Appendix 8: Soil amendments - 2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	3.137	1.568	0.83
T	7	590.648	84.378	44.90
Error (A)	14	26.311	1.879	
Total	23	620.097	26.960	

Appendix 9: Soil amendments -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	3.640	1.820	0.73
T	7	564.297	80.613	32.32
Error (A)	14	34.915	2.493	
Total	23	602.852	26.210	

Appendix 10: Soil type -2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	6.647	3.323	1.66
T	3	88.076	29.358	14.65
Error (A)	6	12.025	2.004	
Total	11	106.749	9.704	

Appendix 11: Soil type -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	4.308	2.154	1.05
T	3	81.938	27.312	13.25
Error (A)	6	12.364	2.060	
Total	11	98.612	8.964	

Appendix 12: Phosphorus and potassium -2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	3.442	1.721	0.95
P	2	298.575	149.287	82.35
K	2	301.619	150.809	83.19
PK	4	106.410	26.602	14.68
Error (B)	16	29.004	1.812	
Total	26	739.051	28.425	

Appendix 13: Phosphorus and potassium -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	5.843	2.921	0.98
P	2	286.237	143.118	48.01
K	2	272.284	136.142	45.67
PK	4	98.010	24.502	8.22
Error (B)	16	47.697	2.981	
Total	26	710.073	27.310	

Appendix 14: Bio-agents as seed dresser

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	7.569055	3.784528	0.74
T	5	1389.366482	277.873296	54.00
Error (A)	10	51.455189	5.145519	
Total	17	1448.390747	85.199456	

Appendix 15: Bio-agents as soil applicant -2003

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	7.461	3.730	1.29
T	4	255.758	63.939	22.10
Error (A)	8	23.149	2.893	
Total	14	286.369	20.454	

Appendix 16: Bio-agents as soil applicant -2004

Source of variation	D.F.	T.S.S	M.S.S	F. Value
Replicates	2	4.243	2.121	1.15
T	4	288.484	72.121	39.04
Error (A)	8	14.780	1.847	
Total	14	307.508	21.964	