STUDIES ON CHARCOAL ROT OF SUNFLOWER CAUSED BY *RHIZOCTONIA BATATICOLA* (TAUB.) BUTLER

By

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CERTIFICATE - I

This is to certify that this dissertation entitled, "Studies on charcoal rot of sunflower caused by *Rhizoctonia bataticola* (Taub.) Butler", submitted for the degree of Ph.D. in the subject of Plant Pathology of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by Dalim Pathak under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

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DEAN, POSTGRADUATE STUDIES

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D E D I C A T E D
TO MY
ELDEST BROTHER
BHABENDRA PATHAK

✡ ✡ ✡ ✡ ✡ ✡ ✡ ✡ ✡ ✡

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CHAPTER - 1

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India. A native of Mexico and South Western U.S.A. and extensively grown in U.S.S.R., it was first introduced in India in 1969. Sunflower seeds contain 35 to 45 per cent high quality edible oil. The oil contains high quantity of linoleic acid (64%) which prevents increase of cholesterol level in human blood and thus helps in reducing heart diseases. Because of its short duration, photo and thermo insensitiveness the area under cultivation has been increasing in intensive agriculture in India (Das, 1997). During the last two decades, India has emerged as a major sunflower producing country in Asia. The area under sunflower cultivation is rising

gradually touching about 2.0 million hectares producing 1.8 million tonnes with a productivity of 900 kg/ha (Anon., 1996-97). In India, cultivation is mainly concentrated in the southern states of Maharashtra, Tamil Nadu, Karnataka and Andhra Pradesh. Now, with the development of sunflower hybrids, the farmers of other states are also cultivating sunflower (Seetharam, 1981).

In Haryana, sunflower has become an important oilseed crop and its area increased from nearly 18 thousand hectares in 1991 to about 50 thousand hectares in 1997 with a production of 75 thousand tonnes. In view of easy adaptability of the crop in diverse cropping system, the area is likely to increase further.

Sunflower is attacked by various fungal, bacterial and viral diseases (Anon., 1993). Charcoal rot of sunflower caused by *Rhizoctonia bataticola* (Taub.) Butler is a serious disease which causes significant loss in yield. The disease was first reported by Small from Sri Lanka in 1928. Kumar *et al.* (1994) reported reduction in 1000 seed weight and oil content by 20.16 and 36.88 per cent, respectively. In Haryana, the disease has been observed in almost all sunflower growing areas in varying degrees. However, in spite of the importance of the disease not much information is available on factors responsible for disease development and management through fungicides, biocontrol agents and conventional means.

Considering the prevalence and significance of the disease and importance of the crop, and lack of informations on various aspects, the

present investigation has been undertaken with the following objectives:

1. To study prevalence and severity of the disease in different sunflower growing areas in Haryana for disease incidence and losses.
2. To study effect of different edaphic and environmental factors responsible for disease development.
3. To identify genotypes resistant against the pathogen and fungicides, botanicals (plant extracts) and biocontrol agents for management of the disease.

CHAPTER - 2

REVIEW OF LITERATURE

Charcoal rot of sunflower caused by *Rhizoctonia bataticola* (Taub.) Butler [*Macrophomina phaseolina* (Tassi) Goid] was first reported by Small (1928) from Sri Lanka. Later on the disease was observed in many countries.

The pathogen causing the disease is seed and soil-borne in nature. The fungus survives as sclerotia in soil or in the form of sclerotia carried in crop residues. In seed, the organism harbours both externally and internally (Fakir *et al.*, 1976). The fungus belongs to Deuteromycotina, class Coelomycetes order Sphaeropsidales and family Sphaeropsidaceae. The hyphae is septate, 5 to 7μ thick, filliform, hyaline at first and later

becoming dark brown to black. The hyphae is branched and the branching is at a right angle, having a constriction at the point of origin and also a septum just after the constriction. The sclerotia are smooth, spherical to irregular in shape and dark brown to black in colour. The sclerotia are very minute. Pycnidia are globose, dark brown to greyish, 100 to 200 μ in diameter and contain single celled, hyaline, elliptical pycnidiospores (Kolte, 1985).

2.1 Occurrence and losses

After the first report of the disease by Small (1928) from Sri Lanka, many workers from different part of the world documented its occurrence and estimated loss due to this disease. Zelle (1937) reported that sunflower is infected by *R. bataticola* in Russia. There are reports of sunflower infection by *R. bataticola* from Argentina (Luciano and Davreux, 1967), Australia (Simmonds, 1965), Uruguay (Sackston, 1957), Hungary (Bekesi *et al.*, 1970), Romania (Hulea *et al.*, 1973), France (Acimovic, 1962), USA (Orellana, 1970), Egypt (El-Dahab *et al.*, 1980) and Spain (Jimenez-Diaz *et al.*, 1983).

In India, charcoal rot of sunflower has been reported by Kolte and Mukhopadhyay (1973). Gurha (1983) observed charcoal rot in some exotic cultivars of sunflower grown at the farm of IARI Regional Station, Kanpur. Chohan and Kaur (1976) reported the root rot of sunflower caused by *R. bataticola* from Punjab. Datta and Sharma (1976) observed root rot of sunflower caused by *R. bataticola* from West Bengal. From Karnataka, Sadashivaiah *et al.* (1986) reported this disease in sunflower. Pawar *et al.*

(1978) noticed the charcoal rot of sunflower as a destructive disease in Maharashtra. Sivaprakasam *et al.* (1975) observed the charcoal rot of sunflower in Tamil Nadu.

Sadashivaiah *et al.* (1986) found 15 per cent seed infection due to charcoal rot of sunflower. They also recorded 25.77 per cent less germination in infected seeds. Chohan and Kaur (1975) reported the seed infection of 4 to 49 per cent by *R. bataticola* in sunflower varieties EC-68413 and EC-68414. Jhamaria *et al.* (1975) found 8-10 per cent seed infection due to charcoal rot pathogen in sunflower. Fakir *et al.* (1976) reported seed borne infection on samples collected from Tintevanum, Nandigrama and Hyderabad which yielded 92.3, 37.5 and 7.0 per cent *Macrophomina phaseolina*, respectively. Tikhonov *et al.* (1976) from USSR found reduction in seed diameter and seed yield by 30 and 18-64 per cent, respectively. Bruniard and Luduena (1985) from Argentina reported negative correlation of growth cycle, oil content and seed yield per ha due to *M. phaseolina* infection. Pineda and Avila (1993) noticed 36.8 to 70.2 per cent yield loss due to *M. phaseolina* infection. Raut (1985) reported that charcoal rot caused reduction in height, girth of stem, root weight, stem weight and seed diameter to the extent of 13.77, 3.12, 36.44, 72.56 and 10.77 per cent, respectively.

Fakir *et al.* (1976) showed that in blotter test *M. phaseolina* often prevented germination, caused death of emerging radicle and discolouration of roots. El-Din *et al.* (1986) observed that fungal filtrate reduced seed germination. Kumar *et al.* (1994) reported reduction in 1000 seed weight and oil content by 20.16 and 36.88 per cent, respectively.

2.2 Symptomatology

According to Kolte and Mukhopadhyay (1973) early symptoms are not readily visible on infected plants, but affected plants become weak, mature early and when dry exhibit a black ashy discolouration of the stem. When the stem is split, black microsclerotia are found abundantly in the pith. On some of the plants pycnidia may be found. Flowers from affected plants do not attain full size and yield few seeds. Jimenez-Diaz *et al.* (1983) observed that symptoms become most conspicuous from late flowering to early ripening and include a dark brown black discolouration at the stem base, small distorted heads with a zone of aborted flowers and premature ripening. Datta and Sharma (1976) from West Bengal reported that the symptoms become apparent when the leaves appeared dull green in colour. The stem exhibited an ashy-black discolouration at the collar region and leaves started drying. The stem withered and ultimately turned black and when split profuse black sclerotia were noticed.

In some cases symptoms of the disease have been reported to become visible in the seedling stage (Acimovic, 1964).

2.3 Edaphic and Environmental factors for disease development

Kendrick (1933) observed that the bean seedlings grown in pots were infected more when the daytime mean soil temperature ranged from 95 to 113°F at one inch soil depth than where the daytime soil temperature at a depth of one inch ranged from 75 to 82°F. Edmunds (1964) studied the combined relation of plant maturity, temperature and soil moisture to charcoal rot development in sorghum. He observed that there was no

infection in plant supplied with 80 per cent or more available soil moisture. But at 25 per cent available soil moisture, plants that bloomed 14-28 days before being inoculated were killed within 5-7 days or 3-5 days after inoculation at soil temperatures of 35°C and 40°C, respectively, air temperature being 40°C in both the cases. Ghaffar and Erwin (1969) showed the effect of soil water stress on root rot of cotton caused by *Macrophomina phaseolina.* When plants at soil temperature of 20°C to 40°C were subjected to soil water stress and inoculated, the severity of disease was much greater in those plants subjected to water stress than in those plant provided with sufficient soil water.

Odvody and Dunkle (1979) observed that root infection of both fertile and male sterile sorghum by *M. phaseolina* occurred only after the onset of water stress condition. *M. phaseolina* on potato dextrose agar grew at high temperature (35°C) and low osmotic potentials.

Patel and Patel (1990) reported that charcoal rot caused by *M. phaseolina* increased with the progressive rise in environmental temperature and decrease in relative humidity. Pande *et al.* (1997) studied the effect of soil moisture stress in *M. phaseolina* infested soil on the development of charcoal rot in sorghum. They observed that lodging of sorghum plant was 3.18 per cent in no soil moisture stress condition as against 100 per cent in soil moisture stress condition. Due to soil moisture stress grain yield reduced from 20 to 33 per cent.

2.4 Screening for disease resistance

Host resistance offers one of the best means of controlling diseases,

hence attempts have been made to locate such resistance in different available germplasms/cultivars.

Orellana (1970) studied the response of sunflower genotypes to natural infection of *Macrophomina phaseolina.* He reported that early maturing varieties Krasnodarets and Armavirec were the most susceptible, whereas late maturing Lyng, Manchurian-26 and the moderately late T64001, Commander and NKH01 were the most resistant.

Mirza *et al.* (1982) evaluated that HS-90, Sorem-82, GH-20 and Cosmerama were highly susceptible whereas in Vniimk, NSH 33, Elliodaro and Golden Harvest there were less than 20 per cent plant killed.

Zazzerini *et al.* (1985) studied 10 cultivars for resistance to *R. bataticola* under conditions of natural infection in the field. The susceptibility of the cultivars varied from an average of 63 per cent of plant affected in Romsun HS52 to 92 per cent in Romsun HS301. El-Din *et al.* (1986) screened Giza-I and Miak as resistant to *M. phaseolina* and Hybrid 894 and Hybrid 8941 as susceptible. Lopes and Kimati (1987) showed that in glasshouse trials IDS-2, IDS-3, CMS-HA-290, Majak and Contisol were resistant under below temperature and RH stress condition. Mehdi and Mehdi (1988) observed that NK 212 and SF100 had the lowest mortality rates of 15.5 and 16.2 per cent, respectively. The hybrid Cargill 204 was the most susceptible with 48.2 per cent plant mortality. Gul *et al.* (1989) evaluated cultivars NK-212 and H-33 as resistant and CO-204 as susceptible. Early maturing varieties had less disease incidence than late maturing varieties. Ahmad *et al.* (1991) tested 6 hybrids under laboratory, field and greenhouse conditions and found that NSH-45 appeared to be most resistant in all conditions. Kumar and Kaushik (1994)

screened few sunflower cultivars against charcoal rot both in natural condition and artificial inoculation in screenhouse. They found that under natural conditions, sunflower hybrids MSFH-17, MSFH-31, LDMRSH-3 and U-5002 recorded less than 5 per cent incidence of charcoal rot in Kharif (August) sown crops, whereas MSFH-17, MSFH-31 and LDMRSH-3 were free from rot in spring (Jan.-Feb.) sown crops. After artificial inoculation the lowest disease incidence was recorded on LDMRSH-3 whereas maximum incidence (54%) was recorded in variety HS-1.

2.5 Biochemical basis of disease resistance

A number of substances have been implicated for providing resistance to the host against diseases. Further, plant diseases are often recognised by the characteristics morphological or cytological traits which are obviously the reflection of changes in the chemical and biochemical constituents in plant cell arising out of the interaction between host and the pathogen. It is, therefore, necessary to study the biochemical constituents responsible for host resistance and due to pathogenesis biochemical changes in the host plants.

Mahadevan (1970) reported some preformed inhibitory substances in the host providing protection from the pathogen. Quantities of such substances are said to be directly proportional to the degree of protection given to the host. Substances like phenols have also been attributed to provide resistance to host as reported by Thapliyal and Nene (1967). Other biochemical constituents like amino acids, pectin are also of great significance in determining the biochemical basis of disease resistance.

2.5.1 Total phenols

Phenolics have long been implicated as an active resistant factor in defence mechanism of plants against pathogens (Farkas and Kiraly,1962).

The importance of these compounds in host parasite interaction is that they act as hydrogen donors or acceptors in oxidation reduction reactions and their involvement in resistance by oxidation to quinones which are more toxic to microorganisms (Kotireddy and Prasad, 1974; Bajaj *et al.*, 1983). Anahosur *et al.* (1985) observed that phenols decreased in susceptible cultivars of sorghum compared to resistant ones due to charcoal rot [*Macrophomina phaseolina* (Tassi.) Goid]. Arora and Wagle (1985) reported that higher quantity of phenols were found in resistant genotypes as compared to the susceptible ones. Gupta *et al.* (1992) showed that total phenols was significantly higher both in healthy and diseased leaves of tolerant than in susceptible cultivars.

2.5.2 Free amino acids

The role of amino acids in host parasite system is significant in nitrogen metabolism of the pathogens (Wood, 1967). As a result of this, amount of amino acids in diseased plants either increased or decreased. Mogle and Mayee (1981) reported that free amino acids pool accompanying downy mildew infection decreased on moderately resistant and susceptible lines as compared to resistant lines of pearl millet. Decrease in free amino acids of banana leaves infected with fungal pathogen was reported by Rangaswami and Natarajan (1966). Sekhawat and Kothuri (1971) also observed the amino acids composition of healthy and downy mildewed plants of opium poppy (*Papaver somniferum*) and found that concentration of amino acids was either reduced in samples of diseased plants or it remain unchanged. Mitter *et al.* (1997) noticed that amount of sulphur containing amino acids, methionine and cystine was almost double in the resistant genotype compared to susceptible one.

2.5.3 Insoluble pectin

Pectin is a substance which is present in middle lamella of cells and responsible for keeping the plant straight. Pectin or pectic substances also make up a large portion of the primary cell wall, in which they form an amorphous gel filling the spaces between the cellulose microfibrills. Pectic substances are polysaccharides consisting mostly of chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and small side chains of galacturonan and some other five carbon sugars. The enzymes that degrade pectic susbtances are known as pectinases or pectolytic enzymes (Agrios, 1997). Pectin is responsible for resistance of plant against pathogen. Various pathogens produce different sets of pectinases and their isozymes which degrade the pectin.

2.5.4 Polyphenol oxidase

The polyphenol oxidase mainly catalyse the oxidation of phenolic substances through a polyphenol oxidase peroxidase - H_2O_2 system, whose reaction products are highly toxic to pathogens and are supposed to impart resistance to host (Tayal *et al.*, 1984). This enzyme is usually induced by external stimuli of infection or injury and affected tissue are reported to invariably exhibit an increase in the activities as well as number of isozymes in comparison to healthy tissue which has been associated with the resistance of plants (Farkas and Kiraly, 1962; Farkas and Stahmann, 1966). Velazhahan and Krishnaven (1994) observed higher activities of PPO in resistant cultivar of sunflower as compared to the susceptible cultivar infected by *Puccinia helianthi.*

2.6 Management of charcoal rot by fungicide

Macrophomina phaseolina inciting charcoal rot disease is mainly seed and/or soil borne in nature. Various workers have attempted to control the disease by seed treatment or soil application with chemicals.

Jhamaria *et al.* (1975) evaluated that Benlate (0.2%) was effective in reducing colony development of *Rhizoctonia* infected seeds when seeds were coated with the fungicide. Sivaprakasam *et al.* (1975) found that seed treatment with Benlate (0.3%) was most effective amongst 8 fungicides tested in inhibiting *M. phaseoli* in sunflower. Chohan and Kaur (1975) treated the seeds of sunflower with 8 fungicides and found that Bavistin (0.3%) and Benlate (0.3%) were effective in controlling preemergence and post emergence death of seedlings (seedling survival 89 to 92%). They also found that a soil drench of 0.3 per cent of Bavistin and Benlate only could successively eliminate soil borne pathogen (*R. bataticola*). Ilyas *et al.* (1976) observed the effect of soil fungicides on *Macrophomina phaseolina* sclerotium viability in soil and in soybean stem pieces. It was found that benomyl followed by thiophanate methyl were more effective in reducing sclerotium viability in soybean stem pieces. They also observed that at 200 µg concentration the time required to reach 50 per cent mortality of *M. phaseolina* in soil was less than 24 hours for benomyl 4 days for thiophanate methyl, 5 days for Thiram and 6 days for thiabendazole.

Pawar *et al.* (1978) treated the seeds of sunflower with different seed dressing fungicides to see their effect on charcoal rot of sunflower and observed that Benlate and Ceresan were more effective in reducing the mortality of plant and increased crop stand. Raut and Bhombe (1983) showed that Benlate treated seeds of sunflower gave only 1.5 per cent seed infection in comparison to 19.66 per cent in control. Suhag and Duhan (1983) observed that benomyl, carbendazim and thiophanate methyl at the rate of 0.1% gave good result in controlling gummy collar rot of muskmelon caused by *R. bataticola.* On seed treatment thiophanate methyl

reduced lesion size more (9.0 cm) followed by benomyl (9.5 cm) and carbendazim (10.5 cm). On soil drenching benomyl and carbendazim developed no lesion but thiophanate methyl developed 0.5 cm lesion. On seed treatment plus soil drenching thiophanate methyl did not develop lesion and carbendazim and benomyl developed lesions of 0.5 cm and 1.0 cm respectively. Gangopadhyay and Grover (1984) showed that seed treatment with carbendazim and soil drenching with carbendazim and thiophanate methyl gave good disease control of root rot of cowpea caused by mixed inocula of *R. solani, R. bataticola* and *Fusarium solani*. Suhag and Rana (1984) found that drenching of soil with carbendazim or quintozene followed by a second drench with captafol or thiram gave maximum protection against *Rhizoctonia solani* and *Pythium butleri* on inoculated seedlings of onions in screenhouse. Pandey and Srivastava (1990) showed that carbendazim, benomyl, chloroneb, carboxin and thiophanate methyl were most effective out of 10 fungicides when applied as soil drenching to control seedling disease of sugarbeet caused by *Rhizoctonia solani*. Patel and Patel (1990) studied with six fungicides *in vitro* to see their effect on growth of *R. bataticola* causing charcoal rot in sesamum. They found that carbendazim, benomyl and thiram were at par in inhibiting growth of the fungus though benomyl showed complete growth initiation at 0.05% concentration. Theradi Mani and Marimuthu (1994) proved that Bavistin was more effective in comparison to *Trichoderma harzianum* and *Trichoderma viride* in increasing survival of blackgram plants grown in *M. phaseolina* infested soil. Seed treatment with carbendazim (0.25%) showed reduction of charcoal rot incidence of Frenchbean (Bhardwaj, 1995). Sobti *et al.* (1996) made a comparative study of fungicidal compounds and plant extracts against *M. phaseolina*

in *Arachis hypogaea.* They found that carbendazim was more effective than plant extracts in mycelial inhibition and in reducing rotted seed and seedlings.

Ali and Pathak (1997) tested seven fungicides known to control *Rhizoctonia solani*, the cause of sheath blight of rice against *Trichoderma harzianum*, a well known biocontrol agent of *R. solani.* In the field, the lowest disease severity was recorded when the antagonist was combined with Contaf (hexaconazole) indicating that *T. harzianum* was not affected by hexaconazole. Sood and Kapoor (1997) reported that hexaconazole was effective in controlling neck blast of rice caused by *Magnaporthe grisea* in Himachal Pradesh. Tiwari (1997) reported the best result in hexaconazole (Contaf) out of six chemicals in controlling rice sheath blight caused by *Rhizoctonia solani.* Dillard and Cobb (1997) applied chlorothalonil at 7, 10 and 14 days intervals to tomatoes against black dot caused by *Colletotrichum coccodes* and found that recovery of pathogen from root segment at harvest was significantly reduced in 7 and 10 days interval treatments.

Zhilong and Xianming (1997) reported that anthracnose of *Allium chinense* caused by *Colletotrichum circinans* could be controlled by application of 75% chlorothalonil WP. Yong *et al.* (1998) studied the efficacy of chlorothalonil (Daconil) alone or in combination to inhibit growth of *Phytophthora.* Combination of chlorothalonil with mancozeb or Topsin-M was better than chlorothalonil alone in controlling *Phytophthora melonis.* Lopes *et al.* (1997) reported that chlorothalonil and combination of chlorothalonil and sulphur reduced early and late leafspots of groundnut in the field.

2.7 Management of charcoal rot by plant extracts

Plant extracts are known to have some antifungal, antibacterial and antiviral properties. For these properties, plant extracts are now-a-days being used by researchers for management of various plant diseases.

Sekhawat and Prasad (1971) tested the antifungal properties of some plant extracts on inhibition of spore germination of *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia penniseti*. Plant extracts of *Lawsonia alba*, *Datura stramonium* inhibited spore germination greatly. *Datura stramonium* was more inhibitory against all three pathogens. Misra and Dixit (1976) reported that crude leaf exract of *Clematis gouriana* Roxb. ex DC exhibited marked fungitoxicity against many test fungi. The active principle of the plant was 27.7 times more active than Blitox-50 and 55.4 times more active than Ziram agaisnt all test fungi. The active principle was found to be fungicidal, non-phytotoxic and non-systemic. Kumar and Sachan (199) showed that plant extracts of *Dioscorea sativa*, *Eucalyptus australiensis*, *Santalum album* caused effective inhibition while those of *Cassia fistula*, *Tagetes erecta* accelerated the germination of *Curvularia pallescens*. No germination occurred in *Dioscorea sativa* extract, while 100 per cent germination occurred in *Tagetes erecta* extract. Singh *et al.* (1980) studied the effect of aqueous extract and oil of Neem (*Azadirachta indica*) on four soil-borne pathogens, *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* which cause wilt in gram. Growth of the four pathogens in liquid medium was inhibited by extracts of leaf, trunk bark, fruit pulp and oil. Dwivedi and Dubey (1986) reported that volatile and non-volatile fractions of hydrodistillates of two medicinal plants, namely Neem (*A. indica*) and blue gum (*Eucalyptus globulens*) had deleterious effects on germination

of sclerotia of *M. phaseolina*. Volatile fractions were more effective than non-volatile fractions. In case of leaf distillate, blue gum was more effective. Neem oil was most inhibitory in sclerotial germination.

Misra and Tewari (1992) observed toxicity of *Polyatlhia longifolia* against five fungal pathogens of rice. They found that ethanolic extract besides being more effective on inhibiting mycelial growth, possessed broad spectrum fungitoxicity than essential oil. Bankole and Adebanjo (1995) studied the *in vitro* and *in vivo* efficacy of leaf extracts from five plants commonly used in traditional medicine in Nigeria in inhibiting growth of *Macrophomina phaseolina*. *Cymbopogon ciratus* reduced the colony diameter mostly followed by *Morinda lucida* and *Azadirachta indica*. *In vivo* test revealed that extract of *C. ciratus* inhibited growth of *M. phaseolina* more and gave more seedling emergence which was followed by *A. indica* and *M. lucida*. Bhore *et al.* (1995) investigated the efficacy of four natural plant extracts and two commercially available plant products against *Curvularia lunata* causing leaf spot of sunflower. The acetone extract of *Callistemon lanceolatus* and Indiara (1%) were found effective in inhibition of spore germination and minimising the leaf spot (60.45% and 65.74%, respectively). Kirkegaard *et al.* (1996) investigated the effects of volatile compounds released from the root, shoot and seed meal tissues of canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) on the mycelial growth of five soil borne pathogens of cereals. The roots and shoot tissues of both *Brassica* species were more suppressive at flowering than maturity and mustard tissues were generally more suppressive than canola. Rajappan *et al.* (1997) showed that dried leaf extract of *Ipomea* spp. effectively checked the growth of rice sheath rot pathogen (*Sarocladium oryzae*), while the growth of beneficial biocontrol

agents, viz., *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* was not affected in *in vitro* conditions. Srivastava and Lal (1997) studied the fungicidal properties in aqueous leaf extracts of *Calotropis procera*, *Azadirachta indica*, *Lantana camara* and *Ocimum basilicum* against *Curvularia tuberculata* and *Alternaria alternata* and found that all the extracts checked growth of the fungi in *in vitro* conditions and controlled fruit rot caused by these fungi from 64 to 85 per cent in *in vivo* conditions. Singh *et al.* (1984) reported that better control of powdery mildew of pea was observed with Neem extract in field trial.

2.8 Management of charcoal rot by biocontrol agents

The art and science of plant diseases control continues to more in the direction of biological control of plant pathogens. During the past several years some notable success of disease control was achieved through the introduction of antagonistic microorganisms.

Hadar *et al.* (1979) studied biological control of *Rhizoctonia solani* damping off with wheat bran culture of *Trichoderma harzianum*. They found that *T. harzianum* directly attacked the mycelium of *R. solani* when two fungi were grown together on a glucose plus mineral medium. In the greenhouse, *T. harzianum* applied in the form of wheat bran culture to *R. solani* infested soil, effectively controlled damping off of bean, tomato and egg plant seedlings. Parakhia and Vaishnav (1986) reported that the introduction of *Trichoderma harzianum* reduced the root rot disease of chickpea to an appreciable level in comparison to check. In seed treatment 18% plants were infected with *R. bataticola,* in soil drenching 28% and wheat husk bran culture 14% as compared to 70% in check. Pineda and Gonnella (1988) evaluated biocontrol agents against *Macrophomina phaseolina* in sesame. They found that two *Aspergillus* spp. and 2

Trichoderma spp. inhibited growth and sclerotia production by *M. phaseolina.*

Bedlan (1988) reported that hyphae of *Trichoderma viride* parasitized hyphae of *Rhizoctonia solani* both by encircling them and by penetrating and growing inside them. Use of *T. viride* as a biocontrol agent increased yield in field lettuce up to nearly 40%. Kehri and Chandra (1991) evaluated the efficacy of antagonist *Trichoderma viride* in controlling the pathogenic activity of *Macrophomina phaseolina*, responsible for the dry root rot of mung. The antagonist applied as seed coating reduced mortality due to *M. phaseolina* from 19 to 8 per cent in mung variety T-44 and from 19 to 10 per cent in variety Pusa Baisakhi in unsterilized soil under greenhouse conditions. The biocontrol efficacy of the antagonist showed an improvement in sterilized soil. The dry weight of shoots, grains and nodules showed an increase of 31.7, 16.6 and 100.0 per cent, respectively in T-44 and 27.0, 32 and 93.3 per cent, respectively in Pusa Baisakhi. Raguchander *et al.* (1993) showed that dry root rot in mungbean caused by *Macrophomina phaseolina* was reduced by the application of biocontrol agent *Trichoderma viride* isolates multiplied in organic substrates, such as coirpith, groundnut shell and press mud as row application in acid soil condition. Among the organic substrates, groundnut shell medium supported the production of maximum number of chlamydospores, better native *Rhizobium* nodulation and higher yield. Sclerotial number and root rot incidence were greatly reduced in groundnut shell as compared to coirpith and press mud. Sundar *et al.* (1995) studied the *in vitro* effect of five different species of *Trichoderma* on mycelial growth of root rot pathogen of castor, *Macrophomina phaseolina.* They observed that *T. viride* was the best in reducing mycelial growth up

to 84 per cent followed by *T. harzianum* upto 64 per cent. Selvarajan and Jeyarajan (1996) reported that *Trichoderma* spp. formed inhibition zones against chickpea root rot pathogens, *Fusarium solani* and *Macrophomina phaseolina.* In *F. solani* the sporulation was reduced and in *M. phaseolina* sclerotial size, germination and germ tube number were reduced. Sankar and Jeyarajan (1996) observed that *Trichoderma harzianum* and *Trichoderma viride* significantly reduced the sesamum root rot incidence to 10.1 and 12.8 per cent, respectively compared to 60 per cent incidence in control plots by seed treatment. Soil population of *Trichoderma* spp. also increased due to seed treatment. Seed treatment with *T. harzianum* significantly increased root length, shoot length, yield and oil content over control. Majumdar *et al.* (1996) reported that biocontrol agents, *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* exhibited antagonistic activity against *Macrophomina phaseolina*, the incitant of leaf blight of mothbean. Maximum growth inhibition of the pathogen was caused by *T. harzianum.*

Moody and Gindrat (1977) studied the potentiality of *Gliocladium virens* to control cucumber black root rot caused by *Phomopsis sclerotioides* and observed that *G. virens* reduced the disease significantly when applied to *P. sclerotioides* infested mineral soil. Pachenari and Dix (1980) found the increased β-1,3 glucanase activity and the formation of chitinase in cultures of *Botrytis allii* parasitized by *Gliocladium roseum.* Coagulation of cytoplasm and disintegration of hyphal walls occurred without physical contact between the hypae of the two species; coiling of *G. roseum* hyphae around those of the host was infrequent and penetration rare, indicating that intimate contact between hyphae is not an essential part of necrotrophic attack. Howell and Stipanovic (1983) isolated a

compound with antibiotic activity toward *Pythium ultimum* from potato dextrose broth shake cultures of *Gliocladium virens*. They have given its trivial name as gliovirin. Okhovvat and Karampour (1996) used *Trichoderma* spp. and *Gliocladium virens* to control chickpea root rot caused by *Fusarium solani* and observed that *Trichoderma* spp. were more effective in controlling the disease than *G. virens*. Prasad and Devaraj (1997) detected an endo-N-acetyl-galactosaminidase for the first time in the culture filtrate of *Gliocladium virens*. Sharma and Basandrai (1997) tried to see the effect of biocontrol agents, fungicides and plant extracts on sclerotial viability of *Sclerotinia sclerotiorum* and observed that all the treatments were effective in reducing sclerotial viability. Carbendazim, triadimefon, *Trichoderma harzianum* and *Azadirachta indica* were highly effective, but triadimenol, *Gliocladium virens*, *Lantana camara* were less effective. Chowdhury (1998) reported that when used as seed treatment and soil drenching *Trichoderma harzianum* reduced the infection of jute by *Macrophomina phaseolina* maximum. *T. viride* was slightly less effective than *T. harzianum*. *Gliocladium virens* was the least effective.

2.9 Effect of fungicides, plant extracts and biocontrol agents on phenol constituent of host plant

Different chemical substances, extracts of plants and biocontrol agents have ability to control plant diseases. They may directly act on the pathogen causing the disease or may change the physiology of the plant to defend the attack of the pathogen. Phenol is a chemical constituent of host plant governing resistance to plant and its content may be changed by application of the different substances used to control plant diseases.

Gautam *et al.* (1984) observed an increase of total phenolics in 10 and 20 days after drenching of triadimefon in soybean plants. Thomas

(1986) reported that carbendazim and thiophanate methyl application significantly increased the total phenol content in the leaves of groundnut and this increased phenol make the plants more resistant against disease. Sharma *et al.* (1990) reported that application of carbendazim as foliar spray and soil drenching increased total phenol content sharply in chilli plants.

Sindhan *et al.* (1991) reported that inoculation of *Bacillus subtilis*, *Aspergillus niger* and *Penicillium citrinum* significantly increased the level of total phenols, orthodihydroxy phenols, total sugars and reducing sugars in leaves of guar plants.

CHAPTER - 3

MATERIALS AND METHODS

3.1 Survey of disease in different parts of Haryana

In the present investigation, a survey for disease incidence was conducted in certain sunflower growing districts of Haryana viz., Karnal, Kurukshetra, Jind, Kaithal in the months of May-June in 1996. In each district, few villages were selected depending on availability of cultivation. In each village, five fields were selected and in each field five spots of 1 sq.m each were observed randomly. Diseased plants and total plants were counted in that square metre area and then per cent disease incidence was calculated. Other informations related to disease were also recorded as per availability.

3.2 Isolation, purification, identification and pathogenicity of the disease causing organism

3.2.1 Isolation

Diseased sunflower plants were collected from the experimental field of the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. Few pieces of bark of the plants from junction of diseased and healthy were cut into small bits and then treated in 0.1% mercuric chloride for one minute and were followed by repeated washing in sterile distilled water. The bits were transferred aseptically to potato dextrose agar (PDA) medium in Petriplates and kept in an incubator at 30±2°C for 7 days. White (pale white) mycelium of the pathogen started coming up around the bits within 2-3 days. The stock cultures were maintained at 4°C in refrigerator.

3.2.2 Purification

The fungus was purified by single hyphal tip method and maintained on PDA slants in refrigerator by subculturing at monthly interval.

3.2.3 Identification

The fungus was identified on the basis of its cultural and morphological characters i.e., size of colony, colour, growth pattern, size of sclerotia, etc. (Bernett and Hunter, 1972).

3.2.4 Pathogenicity test

For pathogenicity test, the pathogen was multiplied in maize-meal sand (MMS) medium. Maize-meal and sand were mixed in the ratio of 1:9 (w/w) and 250 g of it was taken in 500 ml conical flask and then moistened with required quantity of water. The flasks were plugged with non-

absorbent cotton and then sterilized twice in two consecutive days in autoclave at 20 lb pressure per square inch for 30 minutes each. Then the medium in flask was inoculated with a mycelial disc grown in PDA for 4 days at 30±2°C. The flasks were kept in incubator at 30±2°C for 15 days.

To prove the pathogenicity of the fungus *R. bataticola*, variety HS-1 of sunflower was employed. The pathogen multiplied in maize meal sand medium was mixed with sterilized soil at the rate of 5 per cent w/w and the soil was filled into earthen pots of 12 inches height. After 3 days the surface sterilized seeds of sunflower variety HS-1 were sown in pots. Twenty pots were maintained keeping 3 plants in each pot after thinning.

The isolation of the fungus was made from stem bits of the artificially diseased plants and the isolate was compared with the originally inoculated one and found similar which proved the fungus was pathogenic. The pathogenic isolate was employed in different experiments.

3.3 Effect of inoculum load on incidence of charcoal rot

The fungal culture in maize meal sand medium was mixed with sterilized soil collected from field at the rate of 1%, 3% and 5% w/w and filled into earthen pots of 12 inches height. After 3 days surface sterilized seeds of sunflower variety HS-1 were sown in pots, each pot with 5 seeds. Then six replications were maintained keeping 3 plants in each pot after thinning. Plants grown in uninoculated soil served as control. Plants were watered necessarily to keep required quantity of moisture in soil. Diseased plants were recorded starting from 25 days after sowing upto the date of harvest. Per cent disease incidence was calculated.

3.4 Sterilization of soil

For pathogenicity test, the soil was sterilized in autoclave at 20 lb pressure per square inch for 2 hours. This sterilized soil was exposed to air for 24 hours and then filled into earthen pots.

For other screenhouse experiments, the soil was sterilized by 1 per cent formaldehyde (40 per cent formaldehyde was diluted to 1 per cent with distilled water). The soil kept on concrete slab was drenched with 1 per cent formaldehyde and covered with polyethene sheets for 72 hours. The polyethene sheet was removed and the soil was exposed to air for two weeks for elimination of fumes. Such soil was then filled into earthen pots.

3.5 Sterilization of glasswares

Glasswares were sterilized by dry heat sterilization with the help of hot air oven kept at 160°C for two hours.

3.6 Effect of pathogen on seed germination, seedling root and shoot growth

To examine the effect of the pathogen on seed germination and seedling root and shoot growth, seeds of sunflower variety HS-1 were surface sterilized and rolled with fungus growth of 10 days old culture in Petri plates one hour before sowing. In the other case, the surface sterilized seeds were soaked in fungal metabolite (got from 10 days old culture in liquid medium) 12 hours before sowing. These treated seeds were then placed on the moist germinator papers. Fifty seeds were placed on each germinator paper. Then the germinator papers were kept in seed germinator at 25°C for 10 days (ISTA, 1966). Seeds rolled on medium (PDA) alone

and seeds soaked in liquid medium and incubated as above served as control. Four replications were maintained. The per cent germination, root and shoot length of seedlings were recorded.

3.7 Effect of edaphic and environmental factors on disease development

3.7.1 Effect of edaphic factors like soil moisture and soil temperature on disease development

For soil moisture and temperature, the experiment was conducted in microplots (1 m x 1m) with different levels of irrigation water.

The experiment was laid out in experimental field of Department of Plant Pathology in a randomized block design during spring (Rabi) season, 1998. The size of each plot was 1 m x 1 m. Application of fertilizers and other cultural practices were followed as per package of practices. Different levels of irrigation were given on the basis of cumulative pan evaporation (CPE = 5 cm). The treatments were as follows:

T_1 = 50% of CPE (5 cm) = 2.5 cm of water =25 litres of water/sqm

T_2 = 75% of CPE = 3.75 cm of water = 37.5 litres of water/sq.m.

T_3 = 100% of CPE = 5.0 cm of water = 50 litres of water/sq.m.

T_4 = 125% of CPE = 6.25 cm of water = 62.5 litres of water/sq.m

T_5 = 150% of CPE = 7.5 cm of water = 75 litres of water/sq.m

T_6 = Control (no water)

The plots were inoculated with the *Rhizoctonia bataticola* grown in MMS medium. Seeds of HS-1 variety of sunflower were sown at a space of 45 cm between rows and 30 cm between plants.

Cumulative pan evaporation data were collected from Department

of Agricultural Meteorology. When CPE was 5 cm the plots were irrigated according to different treatments. Irrigation treatments were given to the plots upto the time of harvesting of the crop.

Soil temperature was recorded in each plot everyday at the depth of 15 cm with the help of soil thermometer. Treatmentwise average temperature for the whole crop season was calculated.

Diseased plants were recorded throughout the crop season starting from 30 days after sowing and the last at the time of harvesting. Per cent disease incidence was calculated on cumulative basis.

Disease incidence was correlated with different irrigation levels representing varying moisture level and soil temperature.

3.7.2 Effect of environmental factors like air temperature, relative humidity (RH) and rainfall on disease development

In this experiment 4 plots, each with 4 m x 4 m size were sown using sunflower variety HS-1 during spring, 1998. The pathogen was inoculated in furrows. The plants were grown following the package of practices. Per cent disease incidence was recorded at 15 days interval, the first record was taken 33 days after sowing (after appearance of diseased plants in plots) and the last at one day before harvesting.

Air temperature (maximum), average relative humidity and rainfall data were collected from the Department of Agricultural Meteorology, CCS Haryana Agricultural University.

Per cent disease incidence was correlated with air temperature, relative humidity and rainfall.

3.8 Screening of germplasms to identify the source of resistance

3.8.1 Under natural conditions

A total of 50 germplasms including one susceptible variety HS-1 were grown in the field during spring season, 1996. Each entry was sown in two rows of 3 m length each. The inoculum grown in MMS medium (20 days old) was mixed in furrows. Plants were maintained at spacing of 45 x 30 cm following normal package of practices (Anon., 1989). Diseased plants were recorded for each entry throughout the crop season. Per cent disease incidence was calculated and grouped the entries into categories as per scale given below:

Per cent disease incidence	Category
0-5	Highly resistant (HR)
5.1-10	Resistant R)
10.1-20	Moderately resistant (MR)
20.1 -30	Moderately susceptible (MS)
30.1 - 50	Susceptible (S)
50.1 - 100	Highly susceptible (HS)

$$\text{Per cent disease incidence} = \frac{\text{No. of diseased plants in an area}}{\text{No.of total plants in that area}} \times 100$$

3.8.2 Under screenhouse conditions

A total of 39 germplasms selected after field screening were grown in screenhouse in spring, 1997 along with the susceptible variety HS-1. Plants were grown in earthen pots of 12 inches height filled up with

pathogen inoculated soil. The pathogen grown in MMS medium (20 days old) was mixed with field soil at the rate of 5 per cent w/w 3 days before sowing. Three plants in each pot for each entry were maintained and each entry was replicated four times. Diseased plants were recorded throughout the crop season and entries were grouped according to per cent disease incidence.

3.9 Biochemical basis of resistance in plants

Forty days old healthy plants from two varieties of each resistance group and from a single variety representing one resistance group viz., RHA-274 (HR); HRHA-7-2 (R), HRHA-6-2 (R); RHA-298 (MS), HRHA-1-4 (MS); RHA-347 (S), RHA-272 (S); RHA-857 (HS), HRHA-8 (HS) which were grown in pots in screenhouse were selected for this study. The uprooted plants were washed thoroughly with water and dried in hot air oven at 60°C. The dried samples were ground to fine powder with the help of mortar and pestle. The fine powdered samples were used for the analysis of total phenols, free amino acids, insoluble pectin. For assay of polyphenol oxidase fresh samples after thoroughly washed were used.

On the other hand, 40 days old infected plants from the same varieties grown in *Rhizoctonia bataticola* inoculated soil in pots were selected for the analysis of the above mentioned biochemical parameters.

3.9.1 Total phenols

Total phenols were determined according to Swain and Hillis (1959).

(a) Extraction: 0.5 g of dried and well ground sample was taken in 50 ml conical flask and 10 ml of methanol was added. The flasks were kept

overnight with intermittant shaking. The contents were filtered through Whatman Filter paper No. 42. The residue was washed with methanol two or three times and volume was made upto 50 ml with methanol. Two sets were maintained for each sample.

(b) Reagents

(i) Saturated sodium carbonate solution: 35 g of enhydrous Na_2CO_3 was dissolved in 100 ml of water at 70-80°C. The contents were cooled overnight and filtered.

(ii) 1N Folin Ciocalteau reagent

(iii) Standard tannic acid solution (1 mg/ml)

(c) Procedure

One ml of methanolic extract was taken in 50 ml volumetric flask to which 30 to 40 ml of distilled water was added followed by addition of 5 ml of saturated Na_2CO_3 solution and 2.5 ml of 1N Folin Ciocalteau reagent. The mixture was shaken thoroughly and volume was made to 50 ml and kept in dark for about half an hour. The absorbance was read at 725 nm against reagent blank. A standard curve was prepared with graded concentrations of tannic acid.

(d) Calculations

The results were expressed as g 100 g^{-1} dry weight.

3.9.2 Free amino acids

The procedure of Barnett and Naylor (1966) was followed for the estimation of amino acids.

(a) Extraction

0.5 g of dried and powdered sample was homogenised in 80% ethanol (v/v) and refluxed successively twice for 15 minutes on a steam bath and centrifuged. The supernatants were pooled together for free amino acids and volume was made to 20 ml. For each sample two sets were maintained.

(b) Reagents

(i) 0.01 M KCN: 0.163 g KCN was dissolved in 250 ml of 60% ethanol.

(ii) KCN acetone: 5 ml of 0.01 M KCN was diluted to 250 ml with acetone.

(iii) Acetone ninhydrin: 5% solution (w/v) of ninhydrin was prepared in acetone.

(iv) KCN acetone ninhydrin: 50 ml of acetone ninhydrin solution was mixed with 250 ml of KCN acetone solution.

(v) 0.2 M citrate buffer (pH 5.0): 21.008 g of citric acid was dissolved in 200 ml H_2O. Mixed with 200 ml of 2N NaOH and volume made to 500 ml.

(c) Procedure

A known volume (1 ml) of the ethanol extract was evaporated to dryness in a test tube. The residue was dissolved in 1 ml of distilled water and then added 1 ml of 0.2 M citrate buffer (pH 5.0) and 1 ml of KCN acetone ninhydrin solution. The mixture was heated for 20 minutes in a boiling water bath . After cooking this was diluted with 7 ml of distilled

water and the absorbance was read at 570 nm. Amino acids content of the sample was read from a standard curve prepared from glycine (100 µg/ml).

3.9.3 Insoluble pectin

Pectin was determined employing the method of Liu *et al.* (1993).

(a) Extraction

Pectic substances were extracted following the method of Moscoso *et al.* (1984). One gram of dried ground sample was transferred to 50 ml centrifuge tube. Two ml 95% ethyl alcohol and 40 ml distilled water were added. The mixture was stirred, allowed to stand for 10 minutes at room temperature and again stirred and centrifuged for 10 minutes at 1000 x g. The supernatant was decanted into 100 ml volumetric flask and the residue was extracted again with 40 ml distilled water. The second extract was added to the previous one and diluted to 100 ml with distilled water. The extract was filtered through Whatman No. 42 filter paper. Two sets were prepared for each sample.

(b) Reagents

(i) 95% ethyl alcohol

(ii) 2% NaCl

(iii) D-glucuronic acid

(iv) Concentrated sulphuric acid

(v) 2,5-dimethyl phenol (0.1% in glacial acetic acid)

(c) Procedure

To 0.25 ml of pectin extract, added 0.5 ml of 2% NaCl solution, followed by addition of 4.0 ml of cold concentrated sulphuric acid. The

contents of test tube were vortexed, heated in a temperature controlled water bath maintained at 70°C for 10 minutes and cooled under tap water for 10 minutes. The 0.1 ml of 2, 5-dimethyl phenol reagent was added and vortexed. After 15 minutes, absorbance was measured at 400 nm and 450 nm using spectronic 20. The absorbance difference between two wavelengths was used to calculate the glucuronic acid content from a standard curve prepared using different concentrations of glucuronic acid varying from 20-100 µg. The results are expressed as gram of glucuronic acid per 100 g of sample.

3.9.4 Polyphenol oxidase

(a) Enzyme extract for polyphenol oxidase

One gram of fresh sample was homogenized with 10 ml of cold 0.1M phosphate buffer (pH 7.0) containing 1 mM cysteine hydrochloride and 0.01% ascorbic acid in a previously chilled mortar using acid washed sand as an abrasive. The homogenate was strained through four layers of cheese cloth, the filtrate centrifuged at 10000 x g for 20 minutes in a refrigerated centrifuge at 4°C and the supernatant obtained was used for the enzyme assays.

(b) Reagents

(i) 0.1 M phosphate buffer (pH 7.0)

(ii) 0.05 M phosphate buffer (pH 6.6)

(iii) 1 mM cysteine hydrochloride

(iv) 0.01% ascorbic acid

(v) 1% catechol

(c) Assay of polyphenol oxidase

The polyphenol oxidase enzyme was assayed by the modified method of Taneja and Sachar (1974). The reaction mixture contained 2 ml of substrate (1% catechol), 0.2 ml of enzyme extract and 0.05 M phosphate buffer (pH 6.6) to make the total volume 4 ml. For each sample a separate control was prepared by taking boiled enzyme extract. Prior to mixing, all the ingredients were maintained at 37°C. The activity of polyphenol oxidase was expressed as change in absorbance at 430 nm/mg protein/ hour.

3.10 *In vitro* efficacy of fungicides, plant extracts and biocontrol agents against *R. bataticola*

3.10.1 *In vitro* efficacy of fungicides

To see the *in vitro* efficacy, two concentrations i.e., 0.1% and 0.2% of each of Benlate (benomyl), Topsin-M (thiophanate methyl), Kavach (chlorothalonil), Bavistin (carbendazim) and Contaf (hexaconazole) were tested by following Poisoned Food Technique (Nene and Thapliyal, 1979).

Stock solutions of double concentrations i.e., 0.2% and 0.4% for each fungicides were prepared by adding required quantity of sterile distilled water. In case of benomyl the fungicide powder was first dissolved in 20 ml acetone and then required concentration was made by adding sterile distilled water. Ten ml of each concentration was added to 10 ml of liquid PDA medium (double concentration becomes the required one) and then poured into Petriplates and allowed to solidify. Each Petriplate was then aseptically inoculated centrally with mycelial disc (3 mm diameter)

of *R. bataticola* taken from the periphery of 5 days old colony grown on PDA. The inoculated Petriplates were then incubated at 30±2°C and colony diameter was measured after 3 days. Three replications were maintained for each concentration. The Petriplates without fungicides served as control.

3.10.2 *In vitro* efficacy of plant extracts

3.10.2.1 Preparation of plant extracts (25%)

Leaves of the plants viz., Neem (*Azadirachta indica*), Ashok (*Polyalthia longifolia*), Mehendi (*Lawsonia inermis*) and Datura (*Datura stramonium*) were collected and 25 g leaves were washed 2-3 times in tap water and at last in distilled water. The leaves were mixed with 100 ml of sterile distilled water and then crushed in a mixie and squeezed the extract through two layers of muslin cloth. The extract was then filtered through What man no.1 filter paper. The filtrate was centrifuged at 5000 rpm for 30 minutes in refrigerated centrifuge. The supernatant was then passed through Millipore filter (0.22 µm pore size) for sterilization.

By this way the next concentration (50%) was also obtained.

3.10.2.2 Test of efficacy

Two concentrations, i.e., 25% and 50% of each of the plant extracts were tested against *R. bataticola in vitro* by Poisoned Food Technique (Nene and Thapliyal, 1979).

Ten ml of each concentration of plant extract was mixed aseptically with 10 ml of PDA thoroughly and poured into petriplates and then allowed to solidify. Mycelial disc (3 mm in diameter) taken from the periphery of

the 4 days old colony of the fungus *R. bactaticola* grown in PDA was inoculated centrally in Petriplates. The Petriplates were incubated at 30±2°C for 3 days and colony diameter was measured after 3 days. Three replications in each case were maintained. The Petriplates without the plant extract served as control.

3.10.3 Efficacy of biocontrol agents *in vitro*

Biocontrol agents, namely, *Trichoderma harzianum*, *Trichoderma viride* and *Gliocladium virens* were tested *in vitro* against *R. bataticola*. Mycelial discs (3 mm in diameter) of 4 days old both of the antagonist and the pathogen were placed in PDA in Petriplate at the opposite sides (dual plate technique) keeping at equal distance from centre of the Petriplate. The plates were incubated at 30±2°C for 3 days. The colony diameter of the pathogen was measured after 3 days. Three replications for each antagonist were maintained. The Petriplates inoculated only with the pathogen served as control.

3.11 Management of charcoal rot disease by fungicides, plant extracts and biocontrol agents with different modes of application

3.11.1 Management of charcoal rot disease by fungicides

3.11.1.1 Management by seed treatment with fungicides

Five fungicides viz., Benlate (benomyl), Topsin-M (thiophanate methyl), Kavach (Chlorothalonil), Bavistin (carbendazim), Contaf (hexaconazole) were evaluated for management of charcoal rot by seed treatment. The treatments were as follows:

Fungicides	Dose (g/kg)
Benlate (benomyl)	(i) 2.0
	(ii) 3.0
Topsin-M (thiophanate methyl)	(i) 2.0
	(ii) 3.0
Kavach (chlorothalonil)	(i) 2.0
	(ii) 3.0
Bavistin (carbendazim)	(i) 2.0
	(ii) 3.0
Contaf (hexaconazole)	(i) 2.0
	(ii) 3.0
Control	Not treated

Seeds of the sunflower variety HS-1 were surface sterilized with mercuric chloride for 2 minutes and washed with sterile distilled water and then treated with the chemical according to concentrations.

The treated seeds were then sown in earthen pots (10 seeds per pot) already filled with soil inoculated 3 days before sowing with *R. bataticola* grown on maize meal sand and medium at the rate of 5% w/w. For control, only surface sterilized seeds were sown. Four replications were maintained for each concentration. Three plants per pot were kept by thinning others at 15 days after sowing. Record of diseased plants was taken for the whole crop season starting from 25 days after sowing. Per cent incidence of disease was calculated. Per cent disease control was also calculated by the following formula:

$$\text{Per cent disease control} = \frac{\text{Per cent disease incidence in control} - \text{Per cent disease incidence in treatment}}{\text{Per cent incidence control}} \times 100$$

3.11.1.2 Management of charcoal rot by soil drenching with fungicides

Soil drenching with the above mentioned fungicides was done in pots in screenhouse to control the charcoal rot disease. Different treatments for soil drenching were as follows:

Fungicides	**Dose (g/l)**
Benlate (benomyl)	(i) 2.0
	(ii) 3.0
Topsin-M (thiophanate methyl)	(i) 2.0
	(ii) 3.0
Kavach (chlorothalonil)	(i) 2.0
	(ii) 3.0
Bavistin (carbendazim)	(i) 2.0
	(ii) 3.0
Contaf (hexaconazole)	(i) 2.0
	(ii) 3.0
Control	Not treated

The surface sterilized seeds of sunflower were sown in pots filled with soil inoculated 3 days before sowing with *R. bataticola* culture multiplied as earlier. Plants were thinned 15 days after sowing. Three plants per pot were kept and 4 replications of each concentration was maintained. Each pot was drenched with 500 ml of each concentration of

the above mentioned chemicals 20 days after sowing. The control was not drenched. Number of charcoal rot infected plants counted for the whole crop season starting from 25 days after sowing. Per cent disease incidence was calculated. Per cent disease control was also calculated.

3.11.1.3 Management of charcoal rot by seed treatment followed by soil drenching with fungicides

Combinations of seed treatment and soil drenching were tested in screenhouse with the above mentioned fungicides, against charcoal rot disease. The combinations were as follows:

Fungicides	**Dose (g/kg + g/l)**
Benlate (benomyl)	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Topsin-M (thiophanate methyl)	(i) 2.0 +2.0
	(ii) 3.0 + 3.0
Kavach (chlorothalonil)	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Bavistin (carbendazim)	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Contaf (hexaconazole)	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Control	Not treated

Surface sterilized seeds of sunflower variety HS-1 were treated first as in 3.8.2.1 for seed treatment. Seeds were sown and plants maintained as in 3.8.1.1 and 20 days after sowing, the pots were drenched with different concentrations as in 3.8.1.2. One control was maintained without

treatment. Diseased plants were recorded throughout the crop season starting from 25 days after sowing. Per cent disease incidence was calculated. Per cent disease control was also calculated.

3.11.2 Management of charcoal rot disease by plant extracts

3.11.2.1 Management by seed treatment with plant extracts

Plant extracts of different plants viz., Neem (*Azadirachta indica*), Ashok (*Polyalthia longifolia*), Mehendi (*Lawsonia inermis*) and Datura (*Datura stramonium*) were tested for their effectiveness to control charcoal rot disease by seed treatment (seed soaking). The treatments were as follows:

Plant extracts	**Dose (%)**
Neem	(i) 25%
	(ii) 50%
Ashok (*P. longifolia*)	(i) 25%
	(ii) 50%
Mehendi (*L. inermis*)	(i) 25%
	(ii) 50%
Datura (*D. stramonium*)	(i) 25%
	(ii) 50%
Control	Not treated

The surface sterilized seeds of sunflower variety HS-1 were soaked in different concentrations of the plant extracts as mentioned above for 2 hours (plant extracts were prepared as mentioned in 3.7.2.1 except centrifugation). Then the seeds were taken out and sown in pots filled up

with *R. bataticola* inoculated soil. Each concentration was replicated 4 times. A set of control was also maintained. In each pot 3 plants were kept after thinning. Record of diseased plants was taken throughout the crop season starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.11.2.2 Management of charcoal rot by soil drenching with plant extracts

By drenching the soil with different plant extracts (as mentioned above), it was tried to control the charcoal rot disease. The various treatments were as follows:

Plant extracts	**Dose (%)**
Neem (*A. indica*)	(i) 25%
	(ii) 50%
Ashok (*P. longifolia*)	(i) 25%
	(ii) 50%
Mehendi (*L. inermis*)	(i) 25%
	(ii) 50%
Datura (*D. stramonium*)	(i) 25%
	(ii) 50%
Control	Not treated

The surface sterilized seeds of sunflower were sown in pots filled up with soil inoculated with *R. bataticola* 3 days before. Four replications were maintained. Plants were thinned at 15 days after sowing and 3 plants per pot were maintained. Each pot was then drenched with 500 ml of each of

the concentrations of the plant extracts 20 days after sowing. The pots in control were not drenched. Diseased plants were recorded for the whole crop season starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.11.2.3 Management of charcoal rot by seed treatment followed by soil drenching with plant extracts

Both seed treatment and soil drenching methods were applied to control charcoal rot by plant extracts. The treatments were as follows:

Plant extracts	**Dose (% + %)**
Neem (*A. indica*)	(i) 25 + 25
	(ii) 50 + 50
Ashok (*P. longifolia*)	(i) 25 + 25
	(ii) 50 + 50
Mehendi (*L. inermis*)	(i) 25 + 25
	(ii) 50 + 50
Datura (*D. stramonium*)	(i) 25 + 25
	(ii) 50 + 50
Control	Not treated

Surface sterilized seeds of sunflower were treated with the plant extracts as in 3.8.2.1 and then sown in pots filled up with *R. bataticola* inoculated soil. Four replications were maintained. Three plants per pot were kept. The pots were then drenched with the plant extracts on 20 days after sowing as mentioned earlier in 3.8.2.2. One control set was also maintained. Diseased plants were recorded throughout the crop season

starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.11.3 Management of charcoal rot by biocontrol agents

3.11.3.1 Management by seed treatment with biocontrol agents

Biocontrol agents viz., *Trichoderma harzianum*, *Trichoderma viride* and *Gliocladium virens* were evaluated for controlling charcoal rot by seed treatment. The treatments were as follows:

Biocontrol agents	**Dose (g/kg)**
Trichoderma harzianum	(i) 2.0
	(ii) 3.0
Trichoderma viride	(i) 2.0
	(ii) 3.0
Gliocladium virens	(i) 2.0
	(ii) 3.0
Control	Not treated

The surface sterilized seeds of sunflower were treated with the above mentioned doses of the biocontrol agents grown on wheat bran-saw dust (4:1) medium. Then the coated seeds were sown in *R. bataticola* inoculated soil in pots. Four replication for each concentration were maintained. A set of control was also kept. Record of diseased plants was taken throughout the crop season starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.11.3.2 Management by soil drenching with biocontrol agents

Biocontrol agents viz., *T. harzianum*, *T. viride* and *G. virens* were

drenched in soil to control the charcoal rot disease. The treatments were as follows:

Biocontrol agents	**Dose (g/l)**
Trichoderma harzianum	(i) 2.0
	(ii) 3.0
Trichoderma viride	(i) 2.0
	(ii) 3.0
Gliocladium virens	(i) 2.0
	(ii) 3.0
Control	Not treated

The surface sterilized seeds of sunflower were sown in soil in pots inoculated with *R. bataticola* in four replications. In each pot 3 plants were kept after thinning. Then each pot was drenched with 500 ml of each concentration of biocontrol agents on 20 days after sowing. A set of control was also maintained. Diseased plants were counted for the whole crop season starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.11.3.3 Management by seed treatment followed by soil drenching with biocontrol agents

Combining effect of seed treatment and soil drenching with biocontrol agents was observed on the incidence of charcoal rot disease in screenhouse. The treatments were as follows:

Biocontrol agents	Dose (g/kg + g/l)
Trichoderma harzianum	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Trichoderma viride	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Gliocladium virens	(i) 2.0 + 2.0
	(ii) 3.0 + 3.0
Control	Not treated

Surface sterilized seeds of sunflower were treated with the biocontrol agents as in 3.11.3.1. Then the seeds were sown in *R. bataticola* inoculated soil in pots. Four replications were maintained. The pots were drenched on 20 days after sowing as in 3.11.2.2. A set of control was also maintained. Record of diseased plants was taken throughout the crop season starting from 25 days after sowing. Per cent disease incidence and per cent disease control were calculated.

3.12 Effect of fungicides, plant extracts and biocontrol agents on total phenols constituent of host plant

The experiment was conducted in screenhouse in pots filled up with uninoculated soil. Seed treatment, soil drenching and seed treatment followed by soil drenching were done with the fungicides, plant extracts and biocontrol agents as mentioned earlier in 3.11 only with the lower dose. Two replications in each case were maintained. A set of control was also kept.

Forty days old healthy plants were uprooted from each treatment and then washed thoroughly with water and dried in hot air oven at 60°C. The dried plants were then ground to fine powder with the help of mortar and pestle and these powdered samples were used to estimate total phenols.

Total phenols was estimated by using the method as mentioned earlier in 3.9.1.

CHAPTER - 4

EXPERIMENTAL RESULTS

4.1 Survey of charcoal rot disease in different parts of Haryana

Survey of the charcoal rot disease in different sunflower growing districts of Haryana viz., Karnal, Kurukshetra, Jind and Kaithal was undertaken during May-June in 1996. Data presented in Table 1 indicate that disease incidence was more in Karnal district amongst the districts surveyed. Different varieties showed different responses to the disease. The variety Jwalamukhi at Sangoha village in Karnal district scored maximum disease incidence (33.72%) and the same variety at Newal village in Karnal scored 33.17 per cent disease incidence. The lowest disease incidence (4.0%) was recorded in the variety Mahyco-8 at Vir

Table 1. Incidence of charcoal rot of sunflower in different parts of Haryana

Districts	Village	Variety	Date of sowing	Source of irrigation	Frequency of irrigation	Fertilizer used (kg/ha)	Soil type	Disease incidence (%)
Karnal	Ramba	Perry (DK-3848)	Ist week of Feb.	Shallow Tube Well (STW)	3-5	50 (DAP) 50 (Urea)	Silt loam	7.33
	Sangoha	Jwalamukhi	Ist week of Feb.	STW	3-4	50 (DAP) 50 (Urea)	Loamy sand	33.72
		1 KO-99	Ist week of March	STW	4-5	-do-	-do-	6.72
	Newal	Jwalamukhi	Ist week of Feb.	STW	6-7	50 (DAP) 75 (Urea)	Silt loam	33.17
		Mahyco-8	Ist week of March	STW	4-5	-do-	-do-	10.00
	Kalveri	Mahyco-8	Ist week of March	STW	5-6	50 (DAP) 50 (Urea)	Sandy loam	11.58
	Darrar	Mahyco-8	-do-	STW	4-5	50 (DAP) 50 (Urea)	Clay loam	10.22
	Taprena	Mahyco-8	2nd week of March	STW	4-5	50 (DAP)	-do-	10.06
Kurukshetra								
	Vir Mathana	Superzin	3rd week of Feb.	STW	3-4	100 (DAP)	Loamy sand	4.22
		Mahyco-8	Ist week of Feb.	STW	7-8	100 (DAP)	-do-	4.00
	Pipli	Mahyco-8	2nd week of Feb.	STW	5-6	50 (DAP)	Sandy	8.22
Jind	Asandh	Jwalamukhi	Ist week of March	STW	4-5	50 (DAP)	Sandy loam	16.50
Kaithal	Kaithal	Mahyco-8	-do-	STW	5-6	50 (DAP) 50 (Urea)	-do-	10.64

Mathana village in Kurukshetra district though elsewhere incidence ranged from 8.22 to 11.58 per cent. The variety Superzin though grown only at one location also showed less disease incidence (4.22%). Date of sowing, in general, did not affect disease incidence, however, delayed sowing of Mahyco-8 in Kurukshetra and adjoining areas resulted in higher disease incidence. Soil type, source of irrigation and other factors did not affect disease incidence.

4.2 Symptomatology

The first symptom of charcoal rot disease was noticed on 30 to 35 days old plant. The symptom first appeared at the collar region of the plant as dark brown to black lesion which later enlarged upward and downward and after a few days covered almost the whole plant. The infected plant showed wilting and became ashy black in colour. The roots were also found discoloured and dried. When the symptoms were found in later stage, the plants showed wilting and later on the whole plant became black in colour like charcoal. Those wilted plants produced small sclerotia in its pith. Some plants at later stage produced small dot like pycnidia on its stem surface (Fig. 1).

4.3 Isolation, purification and identification of the disease causing organism

The pathogen was isolated from stem bits of infected plants as described in materials and methods. The pathogen isolated on PDA medium was subsequently purified by single hyphal tip method. The cultural and morphological characteristics of the isolate was as under:

Fig. 1. Charcoal rot infected and healthy sunflower plants

On PDA medium, the growth is cottony, mycelium septate, 5 to 7 μ thick, filliform, hyaline at first and later becoming dark brown to black. The hyphae is branched and the branching is at a right angle, having a constriction at the point of origin and also a septum just after the constriction. The sclerotia are smooth, spherical to irregular in shape and dark brown to black in colour, 50-300 μ in diameter. On the basis of above characters the pathogen was identified as *Rhizoctonia bataticola* (Taub.) Butler.

4.4 Pathogenicity test

To prove the pathogenicity of the fungus *R. bataticola*, variety HS-1 of sunflower was employed. The pathogen multiplied in maize meal sand medium was mixed with sterilized soil at the rate of 5 per cent w/w and the soil was filled into earthen pots of 12 inches height. After 3 days the surface sterilized seeds of sunflower variety HS-1 were sown in pots. Twenty pots were maintained keeping 3 plants in each pot after thinning. At 30 days after sowing small brown to black coloured lesions at collar region of few plants were found which later enlarged through stem and roots. Koch's postulates were satisfied by reisolating the pathogen from these artificially infected plants which were similar to those inoculated earlier.

4.5 Effect of inoculum load on charcoal rot incidence

From the data presented in Table 2, it is observed that with the increase of inoculum level, the incidence of charcoal rot also increased being the highest at 5 per cent level (94.45%). The lowest disease incidence was found at 1 per cent inoculum level (11.11%).

Table 2. Effect of inoculum load on disease incidence

Inoculum load (%)	Per cent disease incidence	
1	11.1*	(11.8)
3	38.9	(35.9)
5	94.5	(84.1)
Control	0.0	(0.0)
CD (P=0.05)		18.4

* Each value is an average of six replications
Figures in the parentheses are angular transformed values

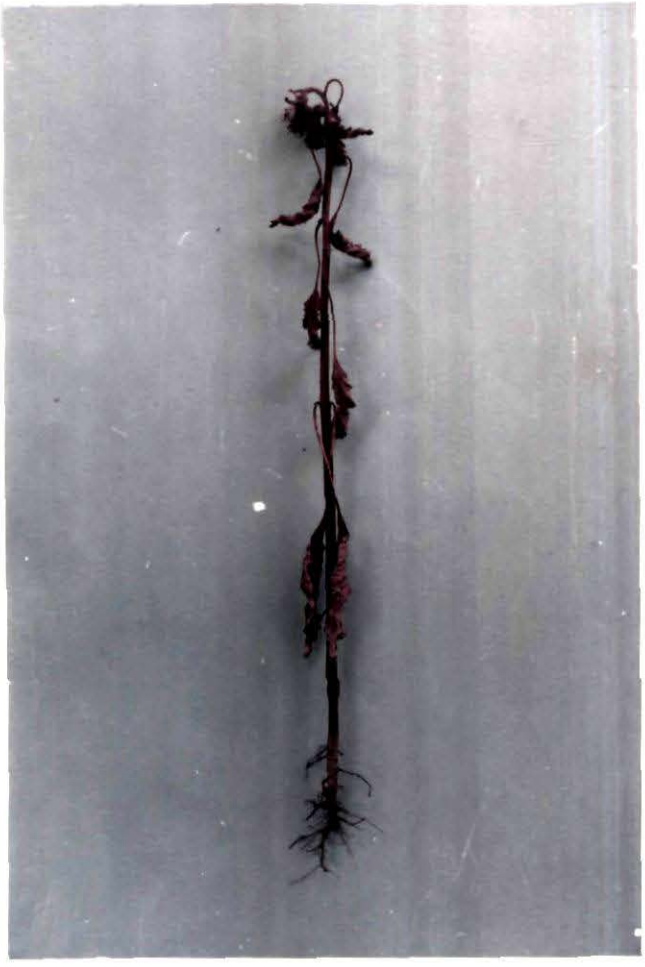


Fig. 2. Completely rotted sunflower plant showing charcoal rot symptom

4.6 Effect of pathogen on seed germination, seedling root and shoot growth

Seed treated with the fungus reduced the germination (49.5%) compared to control (80.5%). Root length and shoot length were also reduced significantly in germinator paper in contrast to control (Table 3).

Seeds treated with fungal metabolite also adversely affected the seed germination, seedling root and shoot growth. Seed germination was 43.5 per cent when treated with metabolite of the fungus as compared to 76.0 per cent in control (Table 4). Seedling root and shoot growth was also reduced to 5.40 cm and 4.85 cm, respectively as compared to 9.13 cm and 8.10 cm in control. A perusal of data of Tables 3 and 4 also reveal that fungal metabolite also reduced seed germination, seedling root and shoot growth more than the fungus itself.

4.7 Effect of edaphic and environmental factors on disease development

4.7.1 Effect of edaphic factors like soil moisture and soil temperature on disease development

Soil moisture as varying irrigation levels and soil temperature had influence on charcoal rot development. From the data presented in Table 5, it is evident that with the increase in levels of irrigation directed towards enhancing soil moisture there was corresponding decrease in per cent disease incidence. Likewise with the decrease in soil temperature there was decrease in per cent disease incidence i.e. with the increase of soil temperature, there was increase of the per cent disease incidence. The irrigation levels and soil temperature have been correlated with the

Table 3. Effect of *Rhizoctonia bataticola* on seed germination, seedling root and shoot growth in germinator paper

Treatments	Seed germination (%)[1]	Root length of seedling (cm)[1]	Shoot length of seedling (cm)[1]
R. bataticola	49.5* (44.7)	6.01	5.39
Control (untreated)	80.5 (63.9)	9.98	7.41
CD (P = 0.05)	4.1	0.42	0.90

[1] Record taken 10 days after sowing

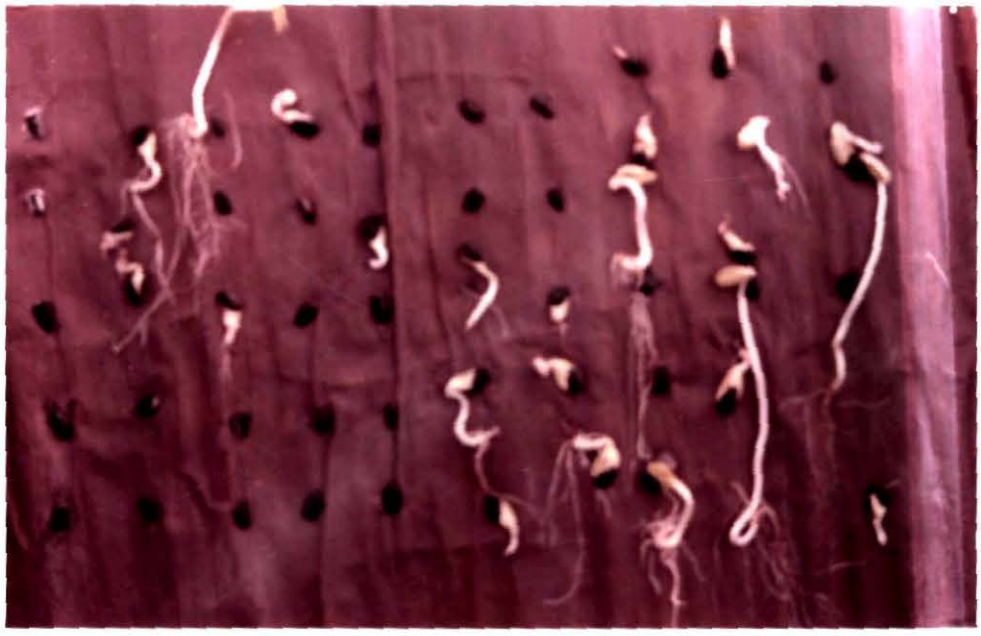
*Figures are average of four replications, each replication of 50 seeds. Figures in the parentheses are angular transformed values

Table 4. Effect of metabolite of *Rhizoctonia bataticola* on seed germination, seedling root and shoot growth on germinator paper

Treatments	Seed germination (%)[1]	Root length of seedling (cm)[1]	Shoot length of seedling (cm)[1]
Metabolite of *R. bataticola*	43.5* (41.3)	5.40	4.85
Control	76.0 (60.7)	9.13	8.10
CD (P = 0.05)	2.8	0.59	0.23

[1] Record taken 10 days after sowing

* Figures are average of four replications, each replication of 50 seeds. Figures in the parentheses are angular transformed values.



(a)

(b)

Fig. 3. Effect of fungal metabolite on seed germination, seedling root and shoot growth

(a) Effect of fungal metabolite
(b) Control

Table 5. Effect of irrigation levels and soil temperature on charcoal rot incidence

Irrigation levels (litres)	Soil temperature (°C)	Disease incidence (%)
0.0 (Control)	30.0	58.0
25.0	28.1	45.6
37.5	27.4	35.4
50.0	26.6	29.2
62.5	25.9	18.9
75.0	24.8	14.6

Regression equation and correlation of irrigation levels, soil temperature with charcoal rot incidence

Regression equation	R^2 (r)**
$Y = 412.946-1.402\ X_1 + 11.832X_2$	0.996 (0.998)**

** Significant at 1% level
Y = Disease incidence (%)
X_1 = Irrigation levels (litres)
X_2 = Soil temperature (°C)

[a] [b]

Fig. 4. **Effect of irrigation level and soil temperature on charcoal rot development**

[a] **Sunflower plants in irrigated plot**

[b] **Sunflower plants in control plot**

disease incidence and from R^2 value it is obvious that 99 per cent variation in disease development was due to cumulative effect of irrigation level and soil temperature.

4.7.2 Effect of environmental factors like atmospheric temperature, relative humidity and rainfall on disease development

An attempt was made to correlate disease incidence with prevailing environmental temperature, relative humidity and rainfall. As environmental temperature increased, per cent disease incidence also increased correspondingly (Table 6). With the decrease of relative humidity, the disease incidence increased. Rainfall had no linear relation with disease incidence. All the factors, i.e., environmental temperature, relative humidity and rainfall appeared to have direct impact on disease incidence. In this correlation, 97 per cent variation in charcoal rot incidence was due to combining effect of environmental temperature, relative humidity and rainfall.

4.8 Screening of germplasms to identify the source of resistance

4.8.1 Under natural conditions

A total of 50 germplasms which included some commonly grown varieties of sunflower were screened during spring season, 1996 at the experimental farm. Of the 50 germplasms tested, 3 viz., RHA-274, HRHA-7-2 and Acc.251 were found highly resistant, 3 such as HRHA-6-2, Acc. 350 and Acc.1445 resistant, 7 moderately resistant, 9 moderately susceptible, 17 susceptible and 11 as highly susceptible (Table 7).

Table 6. Effect of environmental temperature, relative humidity and rainfall on charcoal rot incidence

Temperature (°C)	Relative humidity (%)	Rainfall (mm)	Disease incidence (%)
22.7	69.0	1.1	13.3
32.5	51.3	0.1	21.5
37.0	39.5	0.0	26.3
38.5	40.3	0.1	31.2
42.0	30.3	0.2	41.0
44.3	28.0	0.1	51.0

Regression equation and correlation of environmental temperature, relative humidity, rainfall with charcoal rot incidence

Regression equation	R^2 (r)*
$Y = -144.62+0.3812\ X_1 + 0.7629X_2 + 17.86X_3$	0.9710 (0.9854)*

* Significant at 5% level

Y = Disease incidence (%)

X_1 = Air temperature (°C)

X_2 = Relative humidity (%)

X_3 = Rainfall (mm)

Table 7. Field screening of sunflower germplasms in pathogen inoculated soil in Rabi (Spring)

Category	Germplasms
Highly Resistant (HR)	RHA-274, HRHA-7-2, Acc. 251
Resistant (R)	HRHA-6-2, Acc. 350, Acc. 1445
Moderately Resistant (MR)	HRHA-10-3, Acc. 1141, Acc. 930, Acc.147, Acc. 912, RHA-298, EC-68415C*
Moderately susceptible (MS)	Acc. 1431, Acc. 251-3, Acc. 251-5, Acc. 917-4, HRHA-1-4, 83R6, RHA-272, Mahyco-8*, Superzin*
Susceptible (S)	RHA-297, RHA-265, Acc. 1391, Acc.300-4, Acc.300-7, Acc.251-2, Acc.275-1, RHA-347, RHA-296, RHA-273, RHA-271, HRHA-8, RHA-857, HRHA-9-1, RHA-586, Acc. 1881, Jwalamukhi*
Highly susceptible (HS)	Acc. 300-10, P28R, HRHA-9-2,RHA-856, Acc. 300, HRHA-4-1, Acc.7, Acc. 2010, Acc. 1351, Acc. 1888, HS-1*

*Grown on farmer's field.

4.8.2 Under artificial epiphytotics in screenhouse conditions

A total of 39 germplasms selected after field screening were grown in screenhouse in spring, 1997. Out of them, only RHA-274 was found highly resistant under heavy inoculum pressure while 4 viz., HRHA-7-2, Acc.251, HRHA-6-2 and Acc.1445 as resistant. None of the germplasms was found moderately resistant; 8 germplasms appeared moderately susceptible, 9 susceptible and 17 as highly susceptible (Table 8).

4.9 Biochemical basis of resistance in plants

With a view to find out biochemical basis of resistance, germplasms from different categories of resistance were analysed for various important biochemical components like total phenols, free amino acids, insoluble pectin and polyphenol oxidase and correlated with resistance. Attempt was also made to see the effect of infection on these constituents.

Forty days old healthy and infected plants from the varieties mentioned in 3.9 were taken for the analysis of the above mentioned biochemical components.

4.9.1 Total phenols

The data presented in Table 9 (Fig. 5) indicate that highly resistant cultivar, possessed the highest amount of total phenol (3.12 g 100 g^{-1}) while it was lowest in highly susceptible germplasm indicating thereby a direct correlation with resistance. As a result of infection phenol content decreased in all categories of germplasms irrespective of the degree of resistance they possessed.

Table 8. Screening of sunflower germplasms in screenhouse under artificial epiphytotic

Category	Germplasms
Highly Resistant (HR)	RHA-274
Resistant (R)	HRHA-7-2, Acc. 251, HRHA-6-2, Acc. 1445
Moderately Resistant (MR)	Nil
Moderately susceptible (MS)	RHA-298, HRHA-1-4, Acc.1141, Acc.930, Acc.147, Acc.350, HRHA-10-3, EC-68415C*
Susceptible (S)	83R6, RHA-296, RHA-347, RHA-273, RHA-272, RHA-271, Mahyco-8*, Superzin*, Acc.912
Highly susceptible (HS)	HRHA-9-1, RHA-857, RHA-586, HRHA-8, Acc. 1881, Jwalamukhi*, Acc.1431, Acc.251-3, Acc.251-5, Acc.917-4, RHA-297, RHA-265, Acc.1391, Acc.300-4, Acc.300-7, Acc. 251-2, Acc.275-1

*Grown on farmer's field.

Table 9. Total phenols (g $100g^{-1}$ sample) in different germplasms of sunflower

Germplasms	Total phenols (g $100\ g^{-1}$)	
	Healthy	Infected
RHA-274 (HR)	3.12*	2.80*
HRHA-7-2 (R)	2.94	2.57
HRHA-6-2 (R)	2.79	2.35
RHA-298 (MS)	1.78	1.26
HRHA-1-4 (MS)	2.12	1.55
RHA-347 (S)	1.72	1.10
RHA-272 (S)	1.69	1.05
RHA-857 (HS)	1.64	0.94
HRHA-8 (HS)	1.10	0.34
CD (P=0.05)	0.02	0.09

* Each value is an average of four estimations

HR = Highly resistant
R = Resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

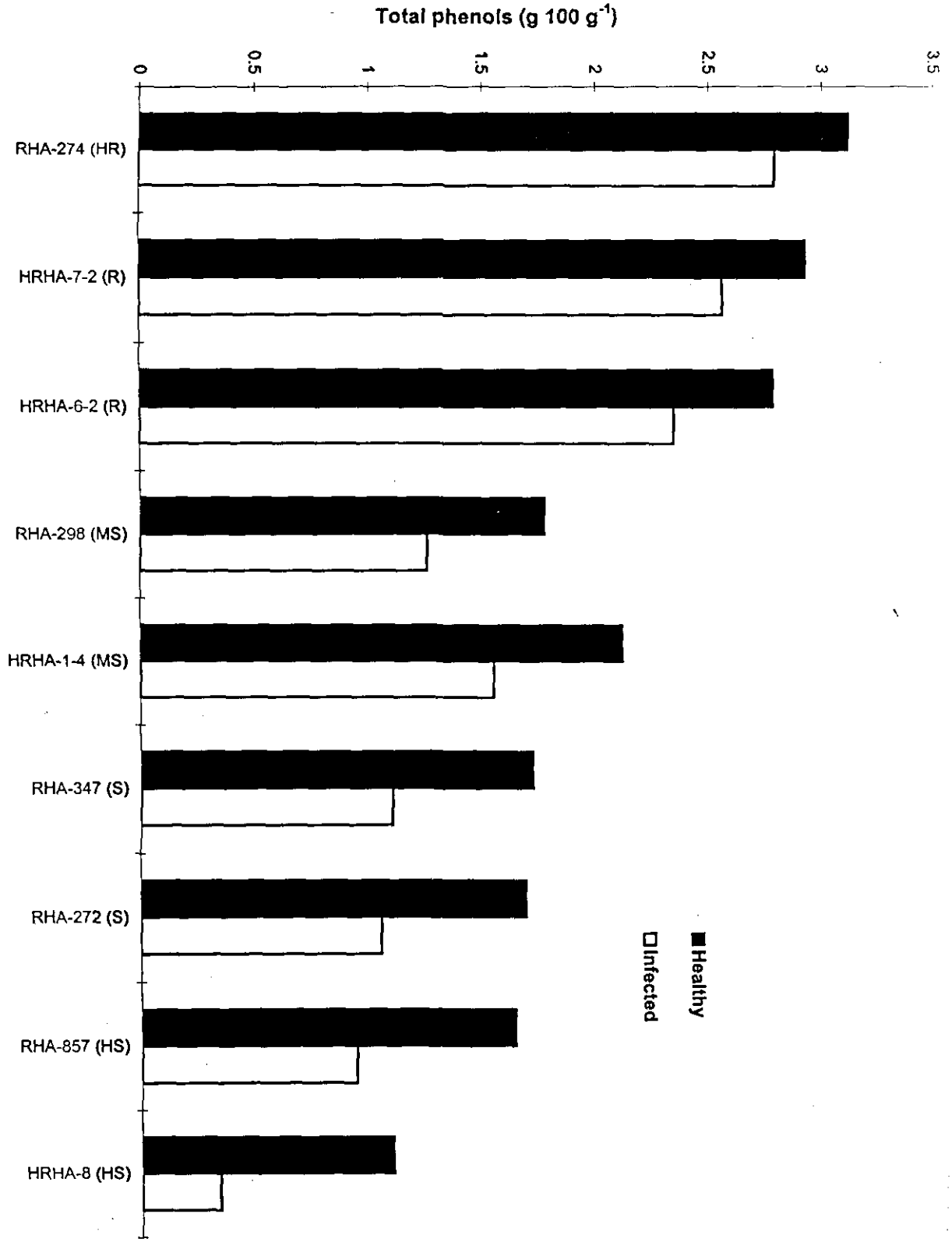


Fig.5. Total phenols in diffrent germplasms of sunflower

4.9.2 Free amino acids

The perusal of data presented in Table 10 (Fig. 6) shows that in healthy plants, the highly resistant cultivar RHA-274 scored maximum amino acids content (0.82 g 100 g^{-1} sample). The amino acids content showed gradual decline from highly resistant to highly susceptible cultivar (0.53 g 100 g^{-1}). The amino acid content showed reduction in all categories of germplasms after pathogenesis.

4.9.3 Insoluble pectin

Amount of insoluble pectin was found the highest in highly resistant cultivar (6.84 g 100 g^{-1} sample). The amount reduced with the susceptibility of the germplasms, i.e., the highest amount was found in the highly resistant cultivar and the lowest in highly susceptible cultivar (2.82 g 100 g^{-1} sample). With infections, the amount of insoluble pectin reduced in all the germplasms under study, i.e., infected plants from all the cultivars possessed less quantity of insoluble pectin than the healthy ones (Table 11; Fig. 7)

4.9.4 Polyphenol oxidase

Highest polyphenol oxidase activity was recorded in highly resistant cultivar RHA-274 amongst all the cultivars (Table 12; Fig. 8). Polyphenol oxidase activity reduced in the order of HR>R>MS>S>HS, being the lowest activity in the highly susceptible cultivar HRHA-8. In infected plants also same trend was found being the highest polyphenol oxidase activity in the highly resistant cultivar and the lowest in the highly susceptible cultivar. The polyphenol oxidase activity increased with infection in all

Table 10. Free amino acids (g 100 g^{-1} sample) in different germplasms of sunflower

Germplasms	Free amino acids (g 100 g^{-1})	
	Healthy	Infected
RHA-274 (HR)	0.82*	0.54*
HRHA-7-2 (R)	0.69	0.37
HRHA-6-2 (R)	0.67	0.34
RHA-298 (MS)	0.64	0.29
HRHA-1-4 (MS)	0.62	0.26
RHA-347 (S)	0.59	0.22
RHA-272 (S)	0.57	0.21
RHA-857 (HS)	0.54	0.18
HRHA-8 (HS)	0.53	0.17
CD (P=0.05)	0.01	0.01

* Each value is an average of four estimations

HR = Highly resistant
R = Resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

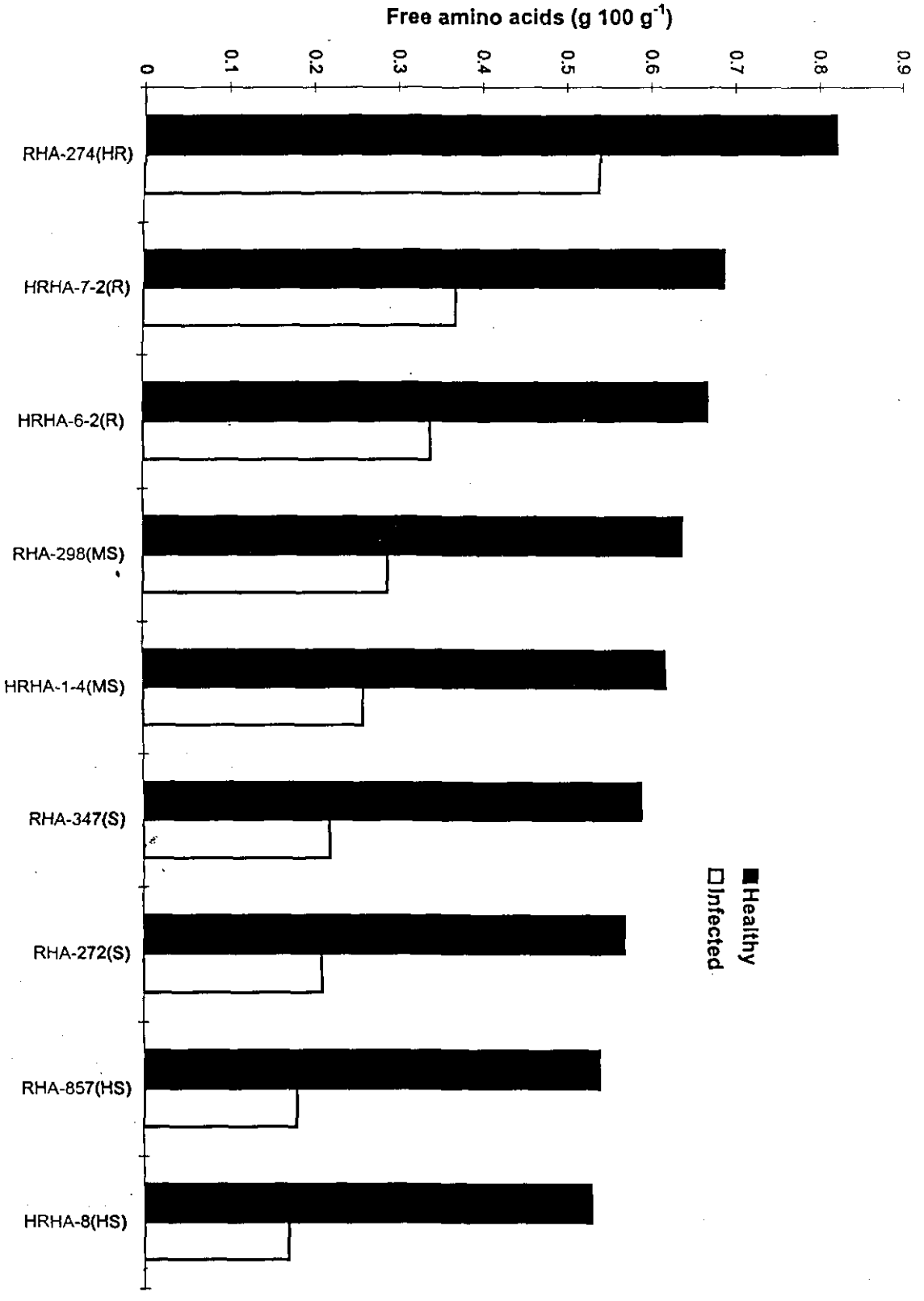


Fig. 6. Free amino acids in different germplasms of sunflower

Table 11. Insoluble pectin (g $100g^{-1}$ sample) in different germplasms of sunflower

Germplasms	Pectin (g 100 g^{-1})	
	Healthy	Infected
RHA-274 (HR)	6.84*	5.40*
HRHA-7-2 (R)	4.42	2.88
HRHA-6-2 (R)	4.26	2.74
RHA-298 (MS)	3.69	2.05
HRHA-1-4 (MS)	3.67	1.99
RHA-347 (S)	3.39	1.65
RHA-272 (S)	3.27	1.40
RHA-857 (HS)	3.11	1.26
HRHA-8 (HS)	2.82	0.89
CD (P=0.05)	0.02	0.29

* Each value is an average of four estimations

HR = Highly resistant
R = Resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

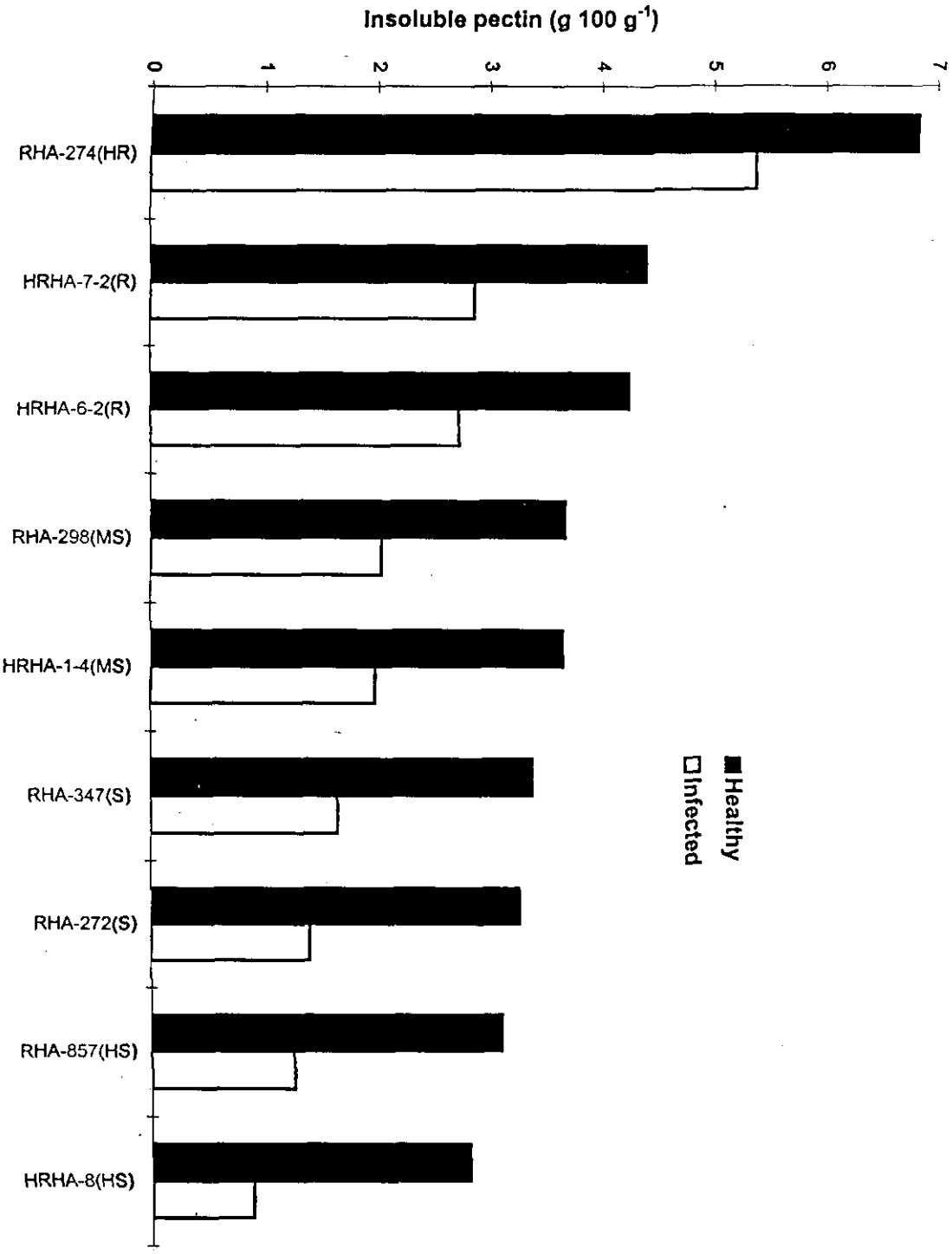


Fig. 7. Insoluble pectin in different germplasms of sunflower

Table 12. Polyphenol oxidase activity in different germplasms of sunflower

Germplasms	Polyphenol oxidase activity (ΔOD at 430 nm $h^{-1}mg^{-1}$ protein)	
	Healthy	Infected
RHA-274 (HR)	3.43*	3.98*
HRHA-7-2 (R)	2.21	2.75
HRHA-6-2 (R)	2.01	2.60
RHA-298 (MS)	1.71	2.11
HRHA-1-4 (MS)	1.68	1.99
RHA-347 (S)	1.41	1.68
RHA-272 (S)	1.31	1.60
RHA-857 (HS)	1.01	1.30
HRHA-8 (HS)	0.98	1.28
CD (P=0.05)	0.03	0.01

*Each value is an average of four estimations.

HR = Highy resistant
R = Resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

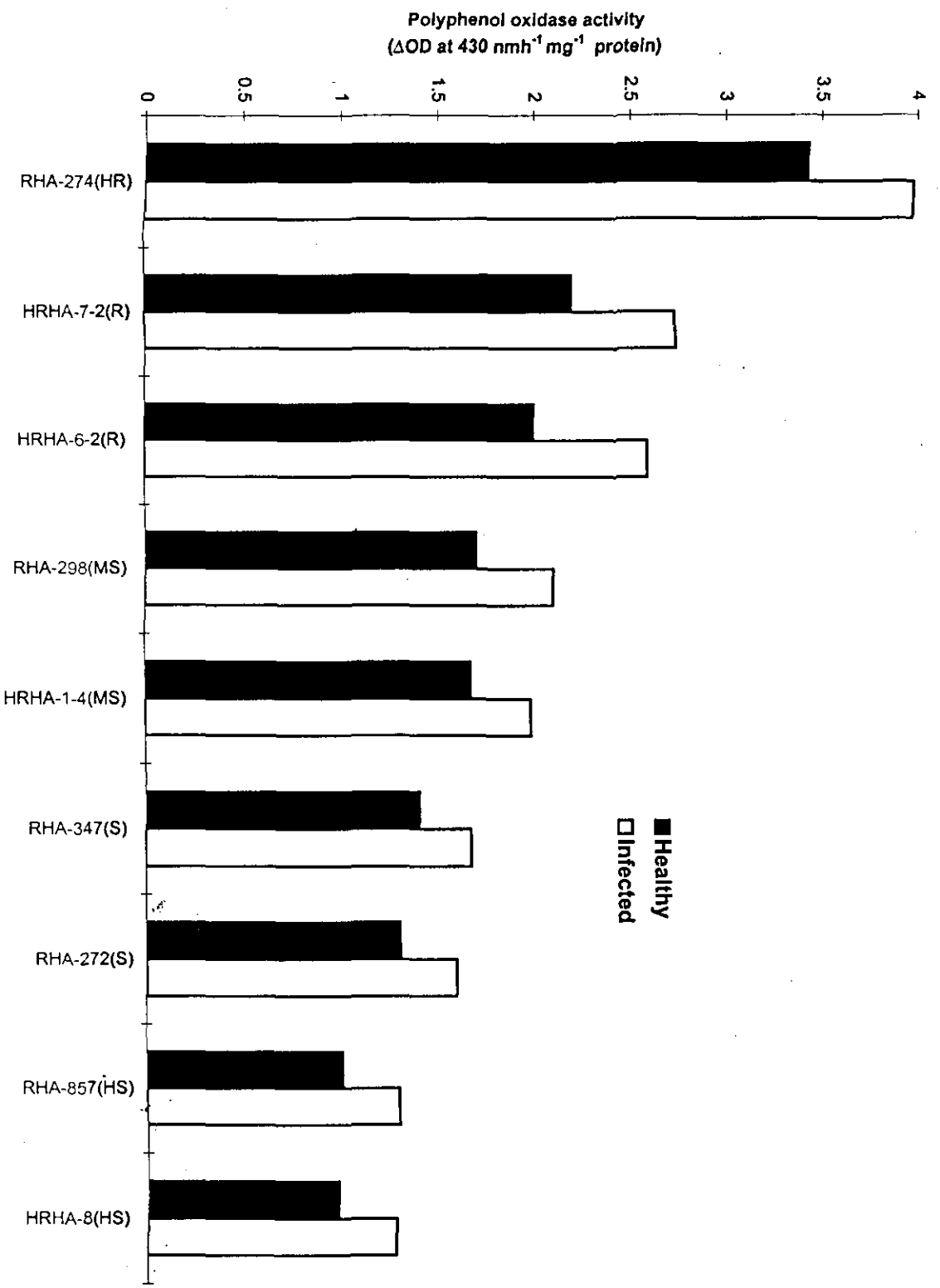


Fig. 8. Polyphenol oxidase activity in different germplasms of sunflower

categories of germplasms, maximum being in highly resistant RHA-274 and lowest in HRHA-8.

4.10 *In vitro* efficacy of fungicides, plant extracts and biocontrol agents against *Rhizoctonia bataticola*

4.10.1 *In vitro* efficacy of fungicides

From the data presented in Table 13, it is obvious that out of 5 fungicides tested, Benlate (benomyl) reduced the growth (colony diameter) of *R. bataticola* maximum at both the concentrations, i.e., 0.1 and 0.2 per cent though it was at par with Bavistin (carbendazim). Topsin-M followed Bavistin in reducing colony diameter. Chlorothalonil was the least effective fungicide. Of course all the fungicides significantly differed with control in their efficacy in reducing colony diameter of *R. bataticola*.

4.10.2 *In vitro* efficacy of plant extracts

Plant extracts of Neem (*Azadirachta indica*), Ashok (*Polyalthia longifolia*), Datura (*Datura stramonium*) and Mehendi (*Lawsonia inermis*) were tested against *Rhizoctonia bataticola* under *in vitro* conditions. All the extracts significantly reduced colony diameter of *R. bataticola*. However, at lower concentration Datura extract appeared best followed by Neem extract though at par with each other. Mehendi and Ashok were, however, least effective (Table 14). Conversely, at higher concentration Neem exhibited best potential in reducing colony diameter followed by Datura though at par with each other. Mehendi and Ashok exhibited similar trend of inhibition as at lower concentration.

Table 13. Effect of different fungicides on the growth of *Rhizoctonia bataticola in vitro*

Fungicides	Colony diameter (cm) at 30±2°C after 72 hrs		
	Concentrations		
	0.1%	0.2%	Mean
Benlate (benomyl)	0.93*	0.18	0.56
Topsin-M (thiophanate methyl)	3.00	1.50	2.25
Kavach (chlorothalonil)	4.82	2.43	3.63
Bavistin (carbendazim)	1.33	0.35	0.84
Contaf (hexaconazole)	3.35	2.08	2.72
Control	-	-	9.00
Mean	2.69	1.31	-

* Each value is mean of three replications

CD(P=0.05):
Fungicides (F) = 0.36
Concentrations (C) = 0.23
Fungicides x × Concentrations = 0.50

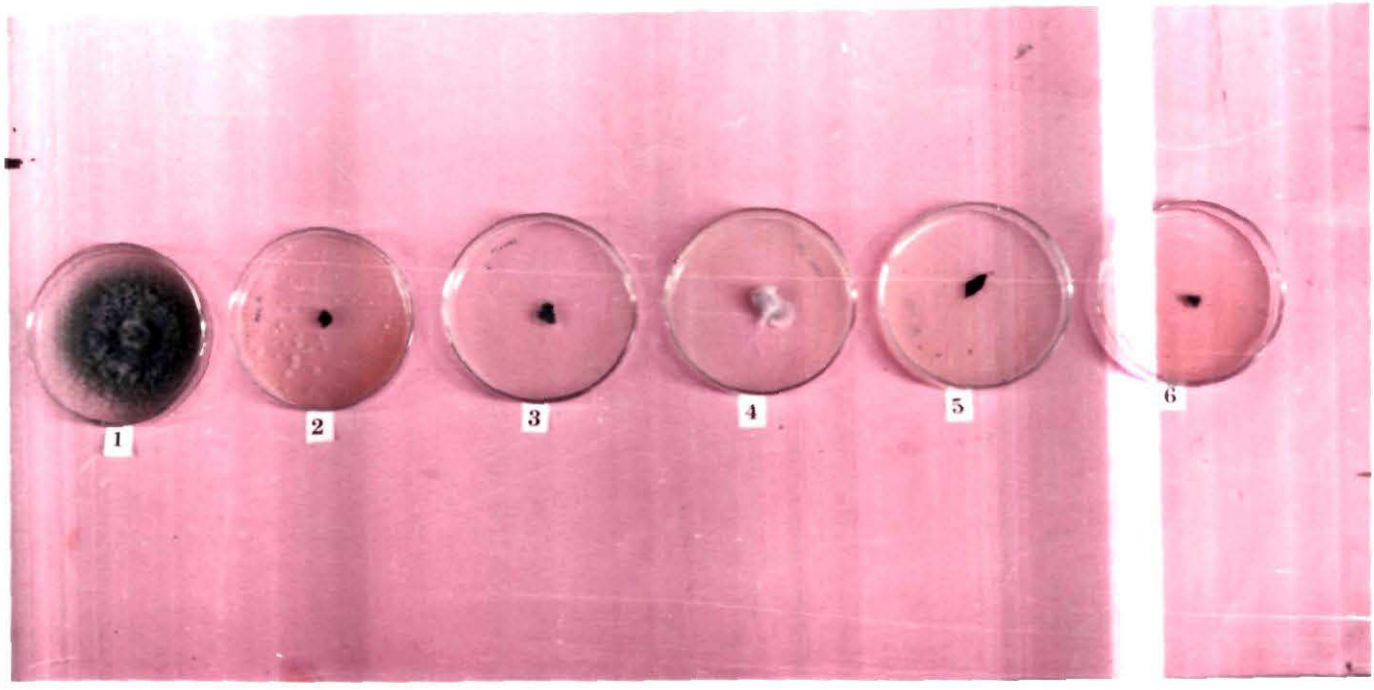


Fig. 9. **Effect of different fungicides on the growth of *Rhizoctonia bataticola in vitro***

1.	Control	3.	Topsin-M	5.	Bavistin
2.	Benlate	4.	Kavach	6.	Contaf

Table 14. Effect of different plant extracts on the growth of *Rhizoctonia bataticola in vitro*

Plant extracts	Colony diameter (cm)		
	Concentrations		
	25%	50%	Mean
Neem (*Azadirachta indica*)	2.02*	1.05	1.54
Ashok (*Polyalthia longifolia*)	6.77	5.73	6.25
Datura (*Datura stramonium*)	1.98	1.15	1.57
Mehendi (*Lawsonia inermis*)	6.18	4.35	5.27
Control	-	-	8.84
Mean	4.24	3.07	-

* Each value is mean of three replications

CD(P=0.05):
Plant extracts (PE) = 0.21
Concentrations (C) = 0.15
Plant extracts x Concentrations = 0.30

4.10.3 *In vitro* efficacy of biocontrol agents

Biocontrol agents viz., *Trichoderma harzianum*, *T. viride* and *Gliocladium virens* were tested for their efficacy *in vitro* against *Rhizoctonia bataticola*. Amongst the three species tried, maximum inhibition resulted due to *T. viride*, however, effectiveness of *T. viride* was statistically at par with *T. harzianum*. All the biocontrol agents reduced the colony diameter of *R. bataticola* significantly over control (Table 15).

4.11 Management of charcoal rot disease by fungicides, plant extracts and biocontrol agents with different modes of application

4.11.1 Management of charcoal rot disease by fungicides

4.11.1.1 Management of charcoal rot by seed treatment with fungicides

Five fungicides viz., Benlate (benomyl), Topsin-M (thiophanate methyl), Kavach (chlorothalonil), Bavistin (carbendazim) and Contaf (hexaconazole) were evaluated as seed treatment to control charcoal rot of sunflower in screenhouse during 1997 and 1998. From the data presented in Table 16, it is observed that Benlate recorded less incidence of disease in comparison to other fungicides. Though Benlate was at par with Bavistin, yet incidence of disease was less in Benlate. Benlate at 3 g/kg scored the least incidence of charcoal rot. In terms of disease control, seed treatment with Benlate at 3 g/kg seed appeared best with 83.3 per cent disease control followed by Bavistin (58.3%). All the fungicides except Kavach at 2 g/kg differed significantly with control in controlling the disease.

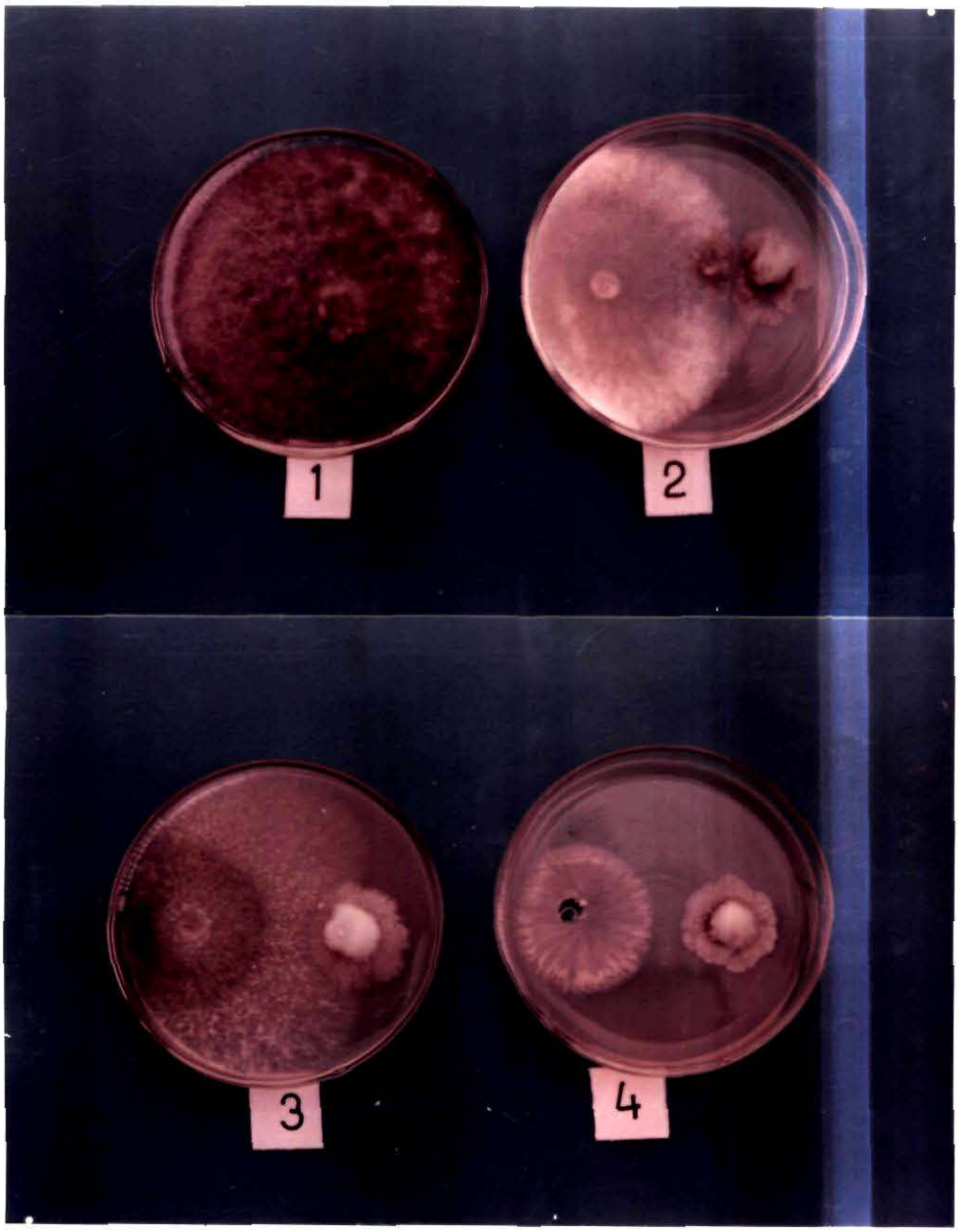


Fig. 10. **Effect of biocontrol agents on the growth of *Rhizoctonia bataticola in vitro***

1. **Control**
2. ***Trichoderma harzianum* treated**
3. ***Trichoderma viride* treated**
4. ***Gliocladium virens* treated**

Table 15. Antagonistic ability of biocontrol agents on *Rhizoctonia bataticola in vitro*

Biocontrol agents	Colony diameter (cm) of *R. bataticola* at 30±2°C after 72 hrs	% Inhibition
Trichoderma viride	2.50	72.2
Trichoderma harzianum	2.82	68.7
Gliocladium virens	5.13	43.0
Control	9.00	0.0
CD (P=0.05)	0.65	-

Table 16. Effect of seed treatment with fungicides on charcoal rot incidence

Fungicides	Dose (g/kg)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Benlate (benomyl)	2.0	33.3* (32.2)	41.7* (36.2)	66.7	58.3
	3.0	16.7 (17.6)	16.7 (17.6)	83.3	83.3
Topsin-M (thiophanate methyl)	2.0	66.7 (54.8)	58.4 (49.9)	33.3	41.6
	3.0	50.0 (45.0)	50.0 (45.0)	50.0	50.0
Kavach (chlorothalonil)	2.0	91.7 (81.2)	83.4 (72.4)	8.3	16.6
	3.0	75.0 (63.6)	75.0 (63.6)	25.0	25.0
Bavistin (carbendazim)	2.0	50.0 (45.0)	50.0 (45.0)	50.0	50.0
	3.0	41.7 (36.2)	41.7 (36.2)	58.3	58.3
Contaf (hexaconazole)	2.0	75.0 (63.6)	75.0 (63.6)	25.0	25.0
	3.0	66.7 (54.8)	58.4 (49.9)	33.3	41.6
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		22.1	23.8		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

4.11.1.2 Management of charcoal rot by soil drenching with fungicides

The five fungicides employed earlier for seed treatment were used as soil drenching to manage the charcoal rot disease. The findings have been furnished in Table 17. Though Benlate, Bavistin and Topsin-M were at par in their effectiveness to control charcoal rot, Benlate recorded less incidence at 3 g/l. Per cent disease control was also more in case of Benlate treated plants. Benlate was followed by Bavistin and Topsin-M. Kavach in both the years did not appear effective except 1998 at 3 g/l. Contaf on the contrary, appeared effective in both the years except at 2 g/l in 1997.

4.11.1.3 Management of charcoal rot by seed treatment followed by soil drenching with fungicides

Seed treatment and soil drenching application were done with the above mentioned fungicides to control the charcoal rot disease. The perusal of data presented in Table 18 reveals that all the treatments significantly controlled the disease over control. Benlate at 3 g/kg + 3 g/l recorded the lowest disease incidence though it was at par with Bavistin and Topsin-M. Benlate was followed by Bavistin and Topsin-M in lowering the disease incidence. Maximum control of disease was achieved by Benlate which was closely followed by Bavistin.

4.11.2 Management of charcoal rot disease by plant extracts

4.11.2.1 Management of charcoal rot by seed treatment with plant extracts

Plant extracts of Neem (*A. indica*), Ashok (*P. longifolia*), Mehendi (*L. inermis*) and Datura (*D. stramonium*) were tested as seed treatment for control of charcoal rot of sunflower. Per cent disease incidence and

Table 17. Effect of soil drenching with fungicides on charcoal rot incidence

Fungicides	Dose (g/l)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Benlate (benomyl)	2.0	41.7* (36.2)	41.7* (36.2)	58.3	58.3
	3.0	25.0 (30.0)	33.3 (35.2)	75.0	66.7
Topsin-M (thiophanate methyl)	2.0	66.7 (54.8)	66.7 (54.8)	33.3	33.3
	3.0	58.4 (49.9)	58.4 (49.9)	41.6	41.6
Kavach (chlorothalonil)	2.0	91.7 (81.2)	83.4 (72.4)	8.3	16.6
	3.0	83.4 (72.4)	75.0 (63.6)	16.6	25.0
Bavistin (carbendazim)	2.0	50.0 (45.0)	50.0 (45.0)	50.0	50.0
	3.0	41.7 (36.2)	41.7 (36.2)	58.3	58.3
Contaf (hexaconazole)	2.0	83.4 (72.4)	75.0 (63.6)	16.6	25.0
	3.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		29.6	21.3		

* Figures are average of four replications.

Figures in the parentheses are angular transformed values.

Table 18. Effect of seed treatment followed by soil drenching with fungicides on charcoal rot incidence

Fungicides	Dose (g/kg+g/l)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Benlate (benomyl)	2.0+2.0	25.0*(30.0)	33.3*(35.2)	75.0	66.7
	3.0+3.0	8.3 (8.8)	8.3 (8.8)	91.7	91.7
Topsin-M (thiophanate methyl)	2.0+2.0	50.0 (45.0)	50.0 (45.0)	50.0	50.0
	3.0+3.0	33.3 (35.2)	41.7 (36.2)	66.7	58.3
Kavach (chlorothalonil)	2.0+2.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
	3.0+3.0	58.4 (49.9)	58.4 (49.9)	41.6	41.6
Bavistin (carbendazim)	2.0+2.0	41.7 (36.2)	41.7 (36.2)	58.3	58.3
	3.0+3.0	25.0 (30.0)	16.7 (17.6)	75.0	83.3
Contaf (hexaconazole)	2.0+2.0	50.0 (45.0)	58.4 (49.9)	50.0	41.6
	3.0+3.0	41.7 (36.2)	50.0 (45.0)	58.3	50.0
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		25.7	20.9		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

Fig. 11. [1] Sunflower plants in control treatment

[2] Sunflower plants treated with Benlate by seed treatment + soil drenching

per cent disease control by these extracts have been presented in Table 19. The perusal of data reveals that though Neem and Datura were at par in their efficacy to reduce the disease, Neem gave better effectiveness. Ashok and Mehendi did not bring down disease significantly below from that of control except Mehendi at 50 per cent concentration in 1998.

4.11.2.2 Management of charcoal rot by soil drenching with plant extracts

Plant extracts as mentioned above were drenched in soil to see their effectivity to control charcoal rot. Neem and Datura showed their ability to reduce disease incidence, but Ashok at both concentrations and Mehendi at 25 per cent failed (Table 20). Though Neem and Datura were at par in their efficacy to control the disease, Neem was more effective.

4.11.2.3 Management of charcoal rot by seed treatment followed by soil drenching with plant extracts

Plant extracts as mentioned above were evaluated with their combining application as seed treatment and soil drenching to control charcoal rot. The data presented in Table 21 reveals that Neem extract and Datura extract were effective against charcoal rot. Ashok extract and Mehendi extract at lower dose appeared ineffective though Mehendi proved effective at higher concentration in both the years while Ashok only in 1998. Neem extract was more effective in controlling charcoal rot than Datura extract.

4.11.3 Management of charcoal rot by biocontrol agents

4.11.3.1 Management of charcoal rot by seed treatment with biocontrol agents

Biocontrol agents viz., *Trichoderma harzianum, T. viride* and

Table 19. Effect of seed treatment with plant extracts on charcoal rot incidence

Plant extracts	Dose (%)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Neem (*Azadirachta indica*)	25	75.0* (63.6)	75.0* (63.6)	25.0	25.0
	50	58.4 (49.9)	50.0 (45.0)	41.6	50.0
Ashok (*Polyalthia longifolia*)	25	100.0 (90.0)	91.7 (81.2)	0.0	8.3
	50	91.7 (81.2)	83.4 (72.4)	8.3	16.6
Mehendi (*Lawsonia inermis*)	25	100.0 (90.0)	83.4 (72.4)	0.0	16.6
	50	83.4 (72.4)	75.0 (63.6)	16.6	25.0
Datura (*Datura stramonium*)	25	83.4 (72.4)	75.0 (63.6)	16.6	25.0
	50	66.7 (54.8)	66.7 (54.8)	33.3	33.3
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		19.0	22.7		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

Table 20. Effect of soil drenching with plant extracts on charcoal rot incidence

Plant extracts	Dose (%)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Neem (*Azadirachta indica*)	25	75.0* (63.6)	75.0* (63.6)	25.0	25.0
	50	66.7 (54.8)	50.0 (45.0)	33.3	50.0
Ashok (*Polyalthia longifolia*)	25	100.0 (90.0)	100.0 (90.0)	0.0	0.0
	50	91.7 (81.2)	91.7 (81.2)	8.3	8.3
Mehendi (*Lawsonia inermis*)	25	100.0 (90.0)	83.4 (72.4)	0.0	16.6
	50	91.7 (81.2)	75.0 (63.6)	8.3	25.0
Datura (*Datura stramonium*)	25	83.4 (72.4)	75.0 (63.6)	16.6	25.0
	50	66.7 (54.8)	66.7 (54.8)	33.3	33.3
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		17.7	20.4		

* Figures are average of four replications.

Figures in the parentheses are angular transformed values.

Table 21. Effect of seed treatment followed by soil drenching with plant extracts on charcoal rot incidence

Plant extracts	Dose (%+%)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Neem (*Azadirachta indica*)	25+25	66.7* (54.8)	58.4* (49.9)	33.3	41.6
	50+50	41.7 (36.2)	41.7 (36.2)	58.3	58.3
Ashok (*Polyalthia longifolia*)	25+25	100.0 (90.0)	83.4 (72.4)	0.0	16.6
	50+50	83.4 (72.4)	75.0 (63.6)	16.6	25.0
Mehendi (*Lawsonia inermis*)	25+25	83.4 (72.4)	83.4 (72.4)	16.6	16.6
	50+50	66.7 (54.8)	58.4 (49.9)	33.3	41.6
Datura (*Datura stramonium*)	25+25	66.7 (54.8)	66.7 (54.8)	33.3	33.3
	50+50	50.0 (45.0)	41.7 (36.2)	50.0	58.3
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		19.5	25.0		

* Figures are average of four replications.

Figures in the parentheses are angular transformed values.

Fig. 12. [1] Sunflower plants in control treatment

[2] Sunflower plants treated with Neem extract by seed treatment + soil drenching

G. virens were tested by seed treatment to control charcoal rot in screenhouse. Per cent disease incidence and per cent disease control have been presented in Table 22. Amongst biocontrol agents *T. viride* appeared most effective in suppressing the disease though *T. viride* with *T. harzianum* and *T. harzianum* with *G. virens* were at par. However, in 1998, *T. viride* at 2 g/kg showed significant superiority over *T. harzianum* in controlling the disease. *G. virens* was effective only in 1998.

4.11.3.2 Management of charcoal rot by soil drenching with biocontrol agents

Soil was drenched with above mentioned biocontrol agents to see the efficacy of those biocontrol agents to control charcoal rot disease. The data presented in Table 23 reveals that *T. viride* was most effective in controlling the disease. *T. harzianum* has shown its effectiveness in reducing disease at par with *T. viride* but disease incidence for *T. harzianum* at 2 g/l dose was at par with control. *G. virens* was effective in controlling the disease only at 3 g/l in 1998.

4.11.3.3 Management of charcoal rot by seed treatment followed by soil drenching with biocontrol agents

Combining effect of seed treatment and soil drenching with biocontrol agents was evaluated for controlling charcoal rot of sunflower. The perusal of data presented in Table 24 reveals that all the biocontrol agents brought down disease significantly below from that of control. Though all the biocontrol agents were at par, *T. viride* recorded the least disease incidence. *T. viride* was followed by *T. harzianum* and then by *G. virens*.

Table 22. Effect of seed treatment with biocontrol agents on charcoal rot incidence

Biocontrol agents	Dose (g/kg)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Trichoderma harzianum	2.0	91.7* (81.2)	83.4* (72.4)	8.3	16.6
	3.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
Trichoderma viride	2.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
	3.0	58.4 (49.9)	58.4 (49.9)	41.6	41.6
Gliocladium virens	2.0	100.0 (90.0)	83.4 (72.4)	0.0	16.6
	3.0	83.4 (72.4)	66.7 (54.8)	16.6	33.3
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		21.1	16.9		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

Table 23. Effect of soil drenching with biocontrol agents on charcoal rot incidence

Biocontrol agents	Dose (g/l)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Trichoderma harzianum	2.0	91.7*(81.2)	83.4*(72.4)	8.3	16.6
	3.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
Trichoderma viride	2.0	75.0 (63.6)	66.7 (54.8)	25.0	33.3
	3.0	66.7 (54.8)	58.4 (49.9)	33.3	41.6
Gliocladium virens	2.0	91.7 (81.2)	83.4 (72.4)	8.3	16.6
	3.0	83.4 (72.4)	75.0 (63.6)	16.6	25.0
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		22.6	19.5		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

Table 24. Effect of seed treatment followed by soil drenching with biocontrol agents on charcoal rot incidence

Biocontrol agents	Dose (g/kg+g/l)	Per cent disease incidence		Per cent disease control	
		1997	1998	1997	1998
Trichoderma harzianum	2.0+2.0	75.0* (63.6)	75.0* (63.6)	25.0	25.0
	3.0+3.0	58.4 (49.9)	58.4 (49.9)*	41.6	41.6
Trichoderma viride	2.0+2.0	66.7 (54.8)	58.4 (49.9)	33.3	41.6
	3.0+3.0	50.0 (45.0)	41.7 (36.2)	50.0	58.3
Gliocladium virens	2.0+2.0	83.4 (72.4)	75.0 (63.6)	16.6	25.0
	3.0+3.0	66.7 (54.8)	58.4 (49.9)	33.3	41.6
Control	-	100.0 (90.0)	100.0 (90.0)		
CD (P=0.05)		17.1	22.1		

* Figures are average of four replications.
Figures in the parentheses are angular transformed values.

Fig. 13. [1] Sunflower plants in control treatment

[2] Sunflower plants treated with *Trichoderma viride* by seed treatment + soil drenching

4.12 Effect of fungicides, plant extracts and biocontrol agents on total phenols constituent of host plant

Studies were undertaken with a view to ascertain whether fungicides, biocontrol agents and plant extracts control the disease only by fungicidal property or by altering host physiology. Accordingly, plant treated with fungicides, plant extracts and biocontrol agents by different modes of application were analysed for phenol contents. The data presented in Table 25 (Fig. 14) reveals that in seed treatment, with different fungicides, plant extracts and biocontrol agents, Benlate treated plants contained the highest quantity of total phenols (1.81 g 100 g^{-1}). Benlate was followed by Bavistin ,Topsin-M, Contaf, Neem, Datura, *T. viride*. The phenol content in seed treatment with different substances was found in the order of Benlate > Bavistin > Topsin-M > Contaf > Neem > Datura > *T. viride* > Kavach > *T. harzianum* > Mehendi > *G. virens* = Ashok. Seed treated plants with Ashok extract contained the least quantity of total phenols which was at par with total phenols content in control. In soil drenching also, Benlate treated plants contained the highest amount of total phenols, but it was less than in seed treated plants. Different treatments followed the same trend as in seed treatment with regard to total phenols. Here, both Ashok and *G. virens* had no effect in enhancing phenol contents over that of control. In seed treatment + soil drenching also, Benlate treated plants contained the highest amount of total phenols. In containing total phenols in different treatments, the same trend was found. In comparison, seed treatment + soil drenching recorded higher quantity of total phenols than in seed treatment or soil drenching alone.

Table 25. Total phenols (g 100 g^{-1}) in sunflower plants treated with fungicides, plant extracts and biocontrol agents

Fungicides/ plant extracts/ biocontrol agents	Total phenols (g 100 g^{-1})		
	Seed treatment	Soil drenching	Seed treatment + soil drenching
Benlate (benomyl)	1.81*	1.75*	1.91*
Topsin-M (thiophanate methyl)	1.64	1.60	1.69
Kavach (chlorothalonil)	1.42	1.40	1.44
Bavistin (carbendazim)	1.70	1.63	1.75
Contaf (hexaconazole)	1.58	1.53	1.62
Neem (*Azadirachta indica*)	1.56	1.51	1.59
Ashok (*Polyalthia longifolia*)	1.13	1.13	1.15
Mehendi (*Lawsonia inermis*)	1.21	1.21	1.23
Datura (*Datura stramonium*)	1.48	1.45	1.57
Trichoderma harzianum	1.32	1.31	1.33
Trichoderma viride	1.44	1.43	1.49
Gliocladium virens	1.14	1.13	1.14
Control	1.12	1.12	1.12
CD (P=0.05)	0.01	0.01	0.01

*Each value is an average of four estimations.

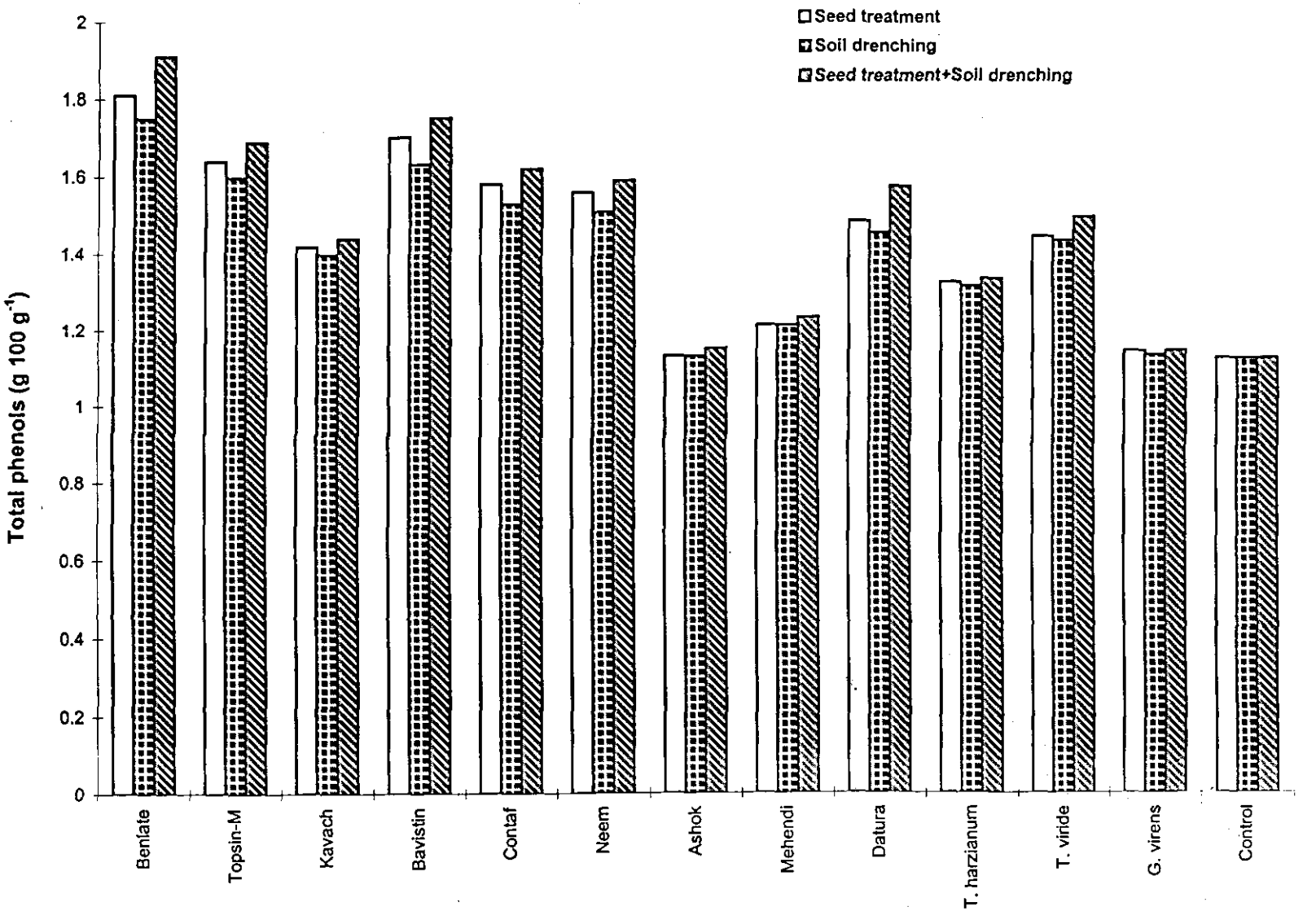


Fig. 14. Total phenols in sunflower plants treated with fungicides, plant extracts and biocontrol agents

CHAPTER - 5

DISCUSSION

Charcoal rot of sunflower caused by *Rhizoctonia bataticola* (Taub.) Butler is a serious disease which causes reduction in yield and oil content. Since its first appearance in India in 1973 (Kolte and Mukhopadhyay, 1973), the disease has become a serious problem in different sunflower growing states in India causing drastic reduction in yield as well as oil content. It, therefore, warrants a critical appraisal of prevalence in Haryana where sunflower has assumed significant importance in the cropping system and the existing control measures and other related aspects of the disease in the changing agricultural scenario. In this direction a proper survey of the disease prone areas for gathering informations on different factors responsible for disease development is of utmost importance

besides identification of resistant source, biochemical basis of disease resistance, edaphic and environmental factors responsible for disease development, induced resistance through different substances. Above all, how best the disease could be controlled with minimum use of fungicides coupled with plant extracts and biocontrol agents need to be observed critically. Results pertaining to above aspects of the study are discussed in subsequent paragraphs.

Survey of the disease in different parts of Haryana showed that in Sangoha village in Karnal the charcoal rot incidence was maximum (33.72%) in the variety Jwalamukhi while in the variety Mahyco-8 the incidence was minimum (4.0%) in Vir Mathana village in Kurukshetra district. Data showed that late sown crop of variety Mahyco-8 recorded high disease incidence. Gul *et al.* (1989) also found that early maturing varieties had less disease incidence than late maturing varieties. Varying incidence in different varieties surveyed obviously appeared due to differences in varietal resistance . Soil type did not appear to have any effect on incidence of charcoal rot in the present study though soil type is known to play an important role in disease prevalence of many soil borne diseases notably molya disease (*Heterodera avenae*) and flag smut of wheat (*Urocystis agropyrii*) flourishing in light soil.

Load of inoculum is known to play an important role on severity of diseases. Under present study too, increase in the inoculum load led to increase in disease incidence. At 5 per cent level of inoculum the disease incidence was recorded as 94.5 per cent. Baker (1971) showed increased

disease incidence with increased inoculum level of *Rhizoctonia bataticola* and thus the present results find support from the work of Baker (1971).

The pathogen, *R. bataticola* and its metabolite reduced seed germination, seedling root and shoot growth in germinator paper. Metabolite was more effective than the fungus (Table 3 and 4). Seed germination was reduced due to fungus by 38.5 per cent while 42.8 per cent by the fungal metabolite. Likewise, seedling root and shoot growth were also reduced more by the fungal metabolite than by the fungus itself. Fakir *et al.* (1976) showed that in blotter test *Macrophomina phaseolina* often prevented seed germination, caused death of emerging radicle and discolouration of roots. El-Din *et al.* (1986) observed that fungal filtrate reduced seed germination. Thus, the results obtained in the present investigation are in confirmity with those of these workers.

In present study, it was observed that increased irrigation level leading to consequential increase in moisture coupled with decreased soil temperature reduced the incidence of charcoal rot disease in the field. Conversely, decreased irrigation level and increased soil temperature enhanced disease incidence. Edmunds (1964) observed that there was no infection in sorghum plant with 80 per cent or more available soil moisture. But at 25 per cent available soil moisture, plants that bloomed 14-28 days before being inoculated were killed within 5-7 days or 3-5 days after inoculation at soil temperatures of 35 and 40°C, respectively. Pande *et al.* (1997) also observed that lodging of sorghum plant grown in *Macrophomina phaseolina* infested soil was 3.18 per cent in soil with no

moisture stress condition as against 100 per cent in soil moisture stress condition. Due to soil moisture stress grain yield was also reduced from 20 to 33 per cent.

Effect of environmental temperature, relative humidity and rainfall on charcoal rot incidence was studied. When temperature increased and relative humidity and rainfall decreased there was more disease development. Patel and Patel (1990) reported that charcoal rot in sesamum caused by *M. phaseolina* increased with the progressive rise in environmental temperature and fall in relative humidity.

With a view to find out resistant sources against charcoal rot a total of 50 germplasms including some cultivars grown on farmer's field were screened in the field in Rabi (spring) season, 1996. Out of them, 3 germplasms showed highly resistant reaction, 3 resistant, 7 moderately resistant, 9 moderately susceptible, 17 susceptible and 11 as highly susceptible. No information is available with regard to resistance of these germplasms tested from elsewhere. Orellana (1970) studied the response of sunflower genotypes to natural infection of *Macrophomina phaseolina* and reported that Krasnodarets and Armavirec were the most susceptible whereas Lyng, Manchurian-26, T 64001, Commander NKH01 were the most resistant. Zazzerini *et al.* (1985) also studied 10 cultivars for resistance to *Rhizoctonia bataticola* under conditions of natural infection in the field and reported that the susceptibility of the cultivars varied from an average of 63 per cent of plant affected in Romsun HS52 to 92 per cent in Romsun HS301.

A total of 39 germplasms which showed highly resistant to susceptible reaction against *R. bataticola* in field conditions were tested for their resistance under heavy inoculum pressure in screenhouse in spring, 1997. Out of 3 germplasms which were earlier found highly resistant only RHA-274 appeared highly resistant while other two were found resistant. Similarly, of the 3 earlier resistant germplasms only two viz., HRHA-6-2 and Acc.1445 continued to show resistant reaction while Acc.350 appeared moderately susceptible under heavy inoculum pressure. Likewise, other germplasms were grouped in moderately susceptible, susceptible and highly susceptible categories. Kumar and Kaushik (1994) screened some sunflower cultivars in field in natural infection in spring season and found that sunflower hybrids MSFH-17, MSFH-31 and LDMRSH-3 were free from rot while when tested under artificial inoculation in screenhouse conditions, the cultivars recorded disease incidence.

A number of biochemical constituents are known to offer resistance in the host against diseases. Therefore, germplasms with different degrees of resistance were analysed for their total phenols, free amino acids, insoluble pectin and polyphenol oxidase to find out correlation, if any, against charcoal rot disease.

Total phenols content was found maximum in the highly resistant cultivar and it decreased from highly resistant cultivar to highly susceptible cultivar, minimum being in the highly susceptible cultivar (Table 9; Fig. 5). After infection the total phenol content in each cultivar decreased

irrespective of the degree of resistance they possessed. Farkas and Kiraly (1962) implicated the phenolics as an active resistant factor in defence mechanism of plants against pathogens. In host parasite interaction phenolics act as hydrogen donors or acceptors in oxidation-reduction reactions and their involvement in resistance by oxidation to quinones which are more toxic to microorganisms (Kotireddy and Prasad, 1974; Bajaj *et al.*, 1983). Anahosur *et al.* (1985) observed that phenols decreased in susceptible cultivars of sorghum compared to resistant ones due to charcoal rot. Arora and Wagle (1985) reported that higher quantity of phenols were found in resistant genotypes as compared to the susceptible ones. Gupta *et al.* (1992) showed that total phenols was higher both in healthy and diseased leaves of tolerant than in susceptible cultivars.

Free amino acids were found highest in the highly resistant cultivar and it decreased in cultivars with increasing degree of susceptibility with lowest being in highly susceptible cultivar. Infection of plants by *R. bataticola* decreased the free amino acids content in each cultivar (Table 10, Fig. 6). Wood (1967) implicated the significant role of amino acids in host parasite system in nitrogen metabolism of the pathogen. As a result of this, amino acids in diseased plants either increased or decreased. Mogle and Mayee (1981) reported that free amino acids pool accompanying downy mildew infection decreased on moderately resistant and susceptible cultivars as compared to resistant ones of pearl millet. Sekhawat and Kothuri (1971) observed the amino acids composition of healthy and downy mildewed plants of opium poppy and found that concentration of

amino acids was either reduced in diseased plants or it remain unchanged. Mitter *et al.* (1997) noticed that amount of sulphur containing amino acids, methionine and cystine was almost double in resistant genotype compared to susceptible one.

Pectin is responsible for resistance of plants against pathogens. As such insoluble pectin was determined in different germplasms of sunflower. The highest amount of pectin was found in the highly resistant cultivar (Table 11, Fig. 7). It decreased accordingly with susceptibility of the germplasms. The minimum amount of pectin was recorded in the highly susceptible germplasm. In each germplasm pectin decreased due to infection. Various pathogens produce different pectinases and their isozymes which degrade the pectin (Agrios, 1997).

The activity of polyphenol oxidase in different germplasms of sunflower was determined (Table 12). Activity was maximum in highly resistant cultivar and it decreased accordingly with susceptibility of the germplasms being minimum in the highly susceptible one. Infected plants in each germplasm showed increase in polyphenol oxidase activity than the healthy plants. Polyphenol oxidase mainly catalyse the oxidation of phenolic substances through a polyphenol oxidase-peroxidase-H_2O_2 system, whose reaction products are highly toxic to pathogens and are supposed to impart resistance to host (Tayal *et al.*, 1984). This enzyme is usually induced by external stimuli of infection or injury and affected tissue are reported to invariably exhibit an increase in the activities as well as number of isozymes in comparison to healthy tissue which has

been associated with the resistance of the plants (Farkas and Kiraly, 1962; Farkas and Stahmann, 1966). Velazhahan and Krishnaven (1994) observed higher activities of polyphenol oxidase in resistant cultivar of sunflower as compared to susceptible cultivar infected by *Puccinia helianthi.*

In vitro efficacy of the fungicides viz., Benlate (benomyl), Topsin-M (thiophanate methyl), Kavach (chlorothalonil), Bavistin (carbendazim) and Contaf (hexaconazole) was evaluated against growth of *Rhizoctonia bataticola.* All the fungicides significantly reduced the growth (colony diameter) of *R. bataticola.* Benlate appeared most effective at both the concentrations in reducing the colony diameter though it was at par with Bavistin. Topsin-M followed Bavistin. Thus benzimidazoles showed their superiority over chlorothalonil and hexaconazole. Patel and Patel (1990) studied the effect of six fungicides *in vitro* on the growth of *Rhizoctonia bataticola* causing charcoal rot of sesamum and found that benomyl, carbendazim and thiram were at par in inhibiting growth of the fungus though benomyl showed complete growth inhibition at 0.05 per cent concentration. Sobti *et al.* (1996) made comparative study of fungicidal compounds (carbendazim and TMTD) and plant extracts against *Macrophomina phaseolina* in *Arachis hypogaea* and found that carbendazim was more effective than plant extracts in mycelial inhibition. Carbendazim was also superior to TMTD in reducing mycelial growth.

Different plant extracts tested against *R. bataticola* in *in vitro* conditions differed significantly in their efficacy with control. Datura extract at lower concentration was most effective against *R. bataticola*

though it was at par with Neem extract. However, at higher concentration, Neem extract was most effective though at par with Datura in reducing colony diameter of *R. bataticola*. Mehendi and Ashok were least effective (Table 14). Bankole and Adebanjo (1995) studied the *in vitro* efficacy of leaf extract of *Azadirachta indica* against *Macrophomina phaseolina* and observed the reduction of colony diameter by extract of *A. indica*. Dwivedi and Dubey (1986) reported that volatile and non-volatile fractions of hydrodistillates of two medicinal plants, viz., Neem (*A. indica*) and blue gum (*Eucalyptus globulens*) had deleterious effects on germination of sclerotia of *M. phaseolina*. Volatile fractions were more effective than non-volatile fractions. Srivastava and Lal (1997) studied the fungicidal properties in aqueous leaf extracts of *Azadirachta indica* and found the checking of growth of *Curvularia tuberculata* and *Alternaria alternata in vitro*. Sekhawat and Prasad (1971) tested the antifungal properties of some plant extracts on inhibition of spore germination of *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia penniseti*. Plant extracts of *Lawsonia alba*, *Datura stramonium* inhibited spore germination greatly. *Datura stramonium* was more inhibitory against all these pathogens. Misra and Tiwari (1992) studied toxicity of *Polyalthia longifolia* against five fungal pathogens of rice and found the inhibition of mycelial growth . Of course here, the toxicity of *Polyalthia longifolia* was not compared with the toxicity of *datura stramonium* or *Lawsonia inermis* or *Azadirachta indica*. The inhibition of mycelial growth of *R. bataticola* by the four plant extracts may be due to presence of different active principles (chemical substances) like Azadirachtin in case of *A. indica*.

Efficacy of biocontrol agents viz., *Trichoderma harzianum*, *T. viride* and *Gliocladium virens* were tested *in vitro* against *Rhizoctonia bataticola*. Amongst the three species tried, maximum inhibition of colony diameter resulted due to *T. viride* though it was at par with *T. harzianum*. *G. virens* was the least effective (Table 15). Hadar *et al.* (1979) studied biological control of *Rhizoctonia solani* and found that *T. harzianum* directly attacked the mycelium of *R. solani* when two fungi were grown together on a glucose plus mineral medium. Pineda and Gonnella (1988) evaluated biocontrol agents against *Macrophomina phaseolina* in sesame. They found that *Aspergillus* spp. and two *Trichoderma* spp. inhibited growth and sclerotia production by *M. phaseolina*. Bedlan (1988) reported that hyphae of *Trichoderma viride* parasitized hyphae of *Rhizoctonia solani* both by encircling them and by penetrating and growing inside them. Sundar *et al.* (1995) studied the *in vitro* effect of five different species of *Trichoderma* on mycelial growth of root rot pathogen of castor, *Macrophomina phaseolina*. They observed that *T. viride* was the best in reducing mycelial growth upto 84 per cent followed by *T. harzianum* upto 64 per cent. Sharma and Basandrai (1997) studied the effect of biocontrol agents, fungicides and plant extracts on sclerotial viability of *Sclerotinia sclerotiorum* and observed that all the treatments were effective in reducing sclerotial viability. However, carbendazim, triadimefon, *Trichoderma harzianum* and *Azadirachta indica* were highly effective, but triadimenol, *Gliocladium virens*, *Lantana camara* were less effective. Howell and Stipanovic (1983) isolated a compound with antibiotic activity toward

Pythium ultimum from potato dextrose broth shake cultures of *Gliocladium virens*. They have given its trivial name as gliovirin.

Five fungicides viz., Benlate (benomyl), Topsin-M (thiophanate methyl), Kavach (chlorothalonil), Bavistin (carbendazim) and Contaf (hexaconazole) were evaluated as seed dresser to control charcoal rot of sunflower in screenhouse during spring 1997 and 1998. Benlate recorded less disease incidence in comparison to other fungicides (Table 16). Bavistin, however, appeared at par with Benlate. The fungicide, Benlate at 3 g/kg scored the least incidence of charcoal rot. All the fungicides except Kavach at 2 g/kg were found to control the disease significantly. Jhamaria *et al.* (1975) also observed that Benlate (0.2%) was effective in reducing colony development of *Rhizoctonia* infected seeds when seeds were coated with the fungicide. Sivaprakasam *et al.* (1975) found that seed treatment with Benlate (0.3%) was most effective amongst 8 fungicides tested in inhibiting *Macrophomina phaseolina* in sunflower. Chohan and Kaur (1975) treated the seeds of sunflower with 8 fungicides and found that Bavistin (0.3%) and Benlate (0.3%) were effective in controlling pre-emergence and post-emergence death of seedlings. Raut and Bhombe (1983) showed that Benlate treated seeds of sunflower gave only 1.5 per cent seed infection in comparison to 19.66 per cent in control. Suhag and Duhan (1983) observed that benomyl, carbendazim and thiophanate methyl at the rate of 0.1 per cent gave good result in controlling gummy collar rot of muskmelon caused by *Rhizoctonia bataticola*. On seed treatment thiophanate methyl reduced lesion size more (9.0 cm)

followed by benomyl (9.5 cm) and carbendazim (10.5 cm). Sood and Kapoor (1997) reported that hexaconazole was effective in controlling neck blast of rice caused by *Magnaporthe grisea* in Himachal Pradesh. Dillard and Cobb (1997) applied chlorothalonil at 7, 10 and 14 days interval to tomatoes against black dot caused by *Colletotrichum coccodes* and found that recovery of pathogen from root segments at harvest was significantly reduced in 7 and 10 days interval treatments. Yong *et al.* (1998) found that combination of chlorothalonil with mancozeb or Topsin-M was better than chlorothalonil alone in controlling *Phytophthora melonis*.

The five fungicides employed earlier for seed treatment were used as soil drenching to manage charcoal rot disease. The findings furnished in Table 17 show that though Benlate, Bavistin and Topsin-M were at par in their effectiveness to control charcoal rot, Benlate recorded least disease incidence at 3 g/l. Kavach was effective only at 3 g/l in 1998. Contaf on the contrary, appeared effective in both the years except at 2 g/l in 1997. Ilyas *et al.* (1976) observed the effect of soil fungicides on *Macrophomina phaseolina* sclerotium viability in soil and in soybean stem pieces. They observed that at 200 µg concentration the time required to reach 50 per cent mortality of *M. phaseolina* in soil was less than 24 hours for benomyl, 4 days for thiophanate methyl, 5 days for thiram and 6 days for thiabendazole. Suhag and Duhan (1983) observed that on soil drenching, benomyl and carbendazim developed no lesion of gummy collar rot caused by *Rhizoctonia bataticola* but thiophanate methyl developed 0.5 cm lesion. Gongopadhyay and Grover (1984) showed that soil drenching with

carbendazim and thiophanate methyl gave good disease control of root rot of cowpea caused by mixed inocula of *Rhizoctonia solani*, *R. bataticola* and *Fusarium solani*. Suhag and Rana (1984) found that drenching of soil with carbendazim or quintozene followed by a second drench with captafol or thiram gave maximum protection against *Rhizoctonia solani* and *Pythium butleri* on inoculated seedlings of onions in screenhouse. Pandey and Srivastava (1990) showed that carbendazim, benomyl, chloroneb, carboxin and thiophanate methyl were most effective out of 10 fungicides when applied as soil drenching to control seedling disease of sugarbeet caused by *Rhizoctonia solani*. Tiwari (1997) reported the best result in hexaconazole (Contaf) out of six fungicides in controlling rice sheath blight caused by *Rhizoctonia solani*. Zhilong and Xianming (1997) reported that anthracnose of *Allium chinense* caused by *Colletotrichum circinans* could be controlled by application of 75 per cent chlorothalonil WP.

Seed treatment and soil drenching applications were done with the above mentioned fungicides to see their integrated effect on the control of charcoal rot disease of sunflower. The perusal of data presented in Table 18 reveals that all the treatments significantly controlled the disease. Benlate at 3 g/kg + 3 g/l recorded the lowest disease incidence though it was at par with Bavistin and Topsin-M. Suhag and Duhan (1983) observed that on seed treatment plus soil drenching thiophanate methyl did not develop lesion of gummy collar rot of muskmelon caused by *Rhizoctonia bataticola* but carbendazim and benomyl developed lesions of 0.5 cm and 1.0 cm, respectively. Lopes *et al.* (1997) reported that chlorothalonil and

combination of chlorothalonil and sulphur reduced early and late leaf spots of groundnut in the field.

Plant extracts of Neem (*A. indica*), Ashok (*P. longifolia*), Mehendi (*L. inermis*) and Datura (*D. stramonium*) were tested as seed treatment for control of charcoal rot of sunflower. The data presented in Table 19 reveals that though Neem and Datura were at par in their efficacy to reduce the disease Neem gave better effectiveness. Ashok and Mehendi did not bring down disease significantly below from that of control except Mehendi at 50 per cent concentration in 1998. Singh *et al.* (1980) studied the effect of aqueous extract and oil of Neem on four soil borne pathogens which cause wilt of gram and found growth inhibition in liquid medium by extracts of leaf, trunk bark, fruit pulp and oil. Bankole and Adebanjo (1995) studied *in vivo* efficacy of leaf extracts of five plant species and revealed that extract of *Cymbopogon ciratus* inhibited growth of *Macrophomina phaseolina* more and gave more seedling emergence which was followed by extract of *Azadirachta indica*. Srivastava and Lal (1997) studied the fungicidal properties of aqueous leaf extracts of *Calotropis procera*, *Azadirachta indica*, *Lantana camara* and *Ocimum basilicum* against *Curvularia tuberculata* and *Alternaria alternata in vivo* and found that fruit rot caused by these fungi was controlled from 64 to 85 per cent.

Soil drenching was done with the above mentioned plant extracts to control charcoal rot disease in screenhouse. The data presented in Table 20 shows that Neem and Datura extracts were effective in controlling the disease while Ashok at both concentrations and Mehendi at lower

concentration failed. Though Datura and Neem were at par yet Neem was more effective. Though Neem has been reported effective by other workers as seed treatment as mentioned in the preceding paragraph, no information is available with regard to its application as soil drench. Salama *et al.* (1988) reported that soil application of *Eucalyptus rostrata* reduced white rot of onion caused by *Sclerotium rolfsii*. Daya Ram (1998) observed that soil application of plant parts acts as systemic action, and also enhances microbial population which causes antagonistic, antibiosis, or directly inhibits the pathogens.

Seed treatment and soil drenching methods were integrated to control the charcoal rot by use of plant extracts as mentioned above. The data gathered from this experiment has been furnished in Table 21. Neem and Datura extracts were effective against charcoal rot. Ashok and Mehendi at lower concentration appeared ineffective though Mehendi at higher concentration proved effective in both the years, while Ashok only in 1998. Neem extract was more effective in controlling charcoal rot than Datura extract. Singh *et al.* (1984) reported that better control of powdery mildew of pea was observed with Neem extract in field trial.

Biocontrol agents viz., *Trichoderma harzianum*, *Trichoderma viride* and *Gliocladium virens* have been evaluated for their ability to control charcoal rot disease of sunflower in screenhouse by seed treatment. The data presented in Table 22 clears that *T. viride* appeared most effective in suppressing the disease though it was at par with *T. harzianum* only at 3 g/kg seed. In 1998, *T. viride* at 2 g/kg showed significant superiority over

T. harzianum in controlling the disease. *G. virens*, though was at par with *T. harzianum*, it was effective only in 1998. Hadar *et al.* (1979) reported that *Trichoderma harzianum* applied in the form of wheat bran culture to *Rhizoctonia solani* infested soil effectively controlled damping off of bean, tomato and egg plant seedlings in greenhouse. Parakhia and Vaishnav (1986) reported that the introduction of *Trichoderma harzianum* as seed treatment reduced the root rot disease of chickpea caused by *Rhizoctonia bataticola* upto 74.29 per cent. Kehri and Chandra (1991) observed that *Trichoderma viride* applied as seed coating reduced mortality of mung plants due to *Macrophomina phaseolina* from 19 to 8 per cent in the variety T-44 and 19 to 10 per cent in the variety Pusa Baisakhi. Sankar and Jeyarajan (1996) reported that *Trichoderma harzianum* and *T. viride* significantly reduced the sesamum root rot incidence to 10.1 and 12.8 per cent, respectively as compared to 60 per cent incidence in control plots by seed treatment. Okhovvat and Karampour (1996) used *Trichoderma* spp. and *Gliocladium virens* to control chickpea root rot caused by *Fusarium solani* and observed that *Trichoderma* spp. were more effective in controlling the disease than *G. virens*.

Soil drenching with the above mentioned biocontrol agents was done in screenhouse to manage the charcoal rot disease. The perusal of data presented in Table 23 reveals that *Trichoderma viride* was most effective in controlling the disease. Though *T. harzianum* was at par with *T. viride*, it recorded disease incidence which is at par with control at 2 g/l. *Gliocladium virens* was effective in controlling the disease only at 3 g/l in

1998. Parakhia and Vaishnav (1986) reported that soil drenching with *Trichoderma harzianum* controlled the root rot disease of chickpea by 60.0 per cent. Raguchander *et al.* (1993) showed that dry root rot in mungbean caused by *Macrophomina phaseolina* was reduced by row application of biocontrol agent, *Trichoderma viride* isolates multiplied in organic substrates. Vyas (1994) evinced that soil drenching with spore suspension of *Trichoderma* spp. reduced the incidence of dry root rot of soybean caused by *Rhizoctonia bataticola*. Efficacy of two species of *Trichoderma* in controlling the charcoal rot in the present study find support from other workers.

Biocontrol agents as mentioned above were applied as seed treatment followed by soil drenching to control charcoal rot disease of sunflower in screenhouse. The data presented in Table 24 reveals that all the biocontrol agents brought down the disease significantly below from that of control. Though all the biocontrol agents were at par, *T. viride* recorded the least disease incidence which was followed by *T. harzianum* and *G. virens* . Chowdhury (1998) reported that when used as seed treatment and soil drenching *Trichoderma harzianum* reduced infection of jute caused by *Macrophomina phaseolina* maximum. *T. viride* was slightly less effective than *T. harzianum* and *Gliocladium virens* was the least effective. Kulkarni (1994) reported that application of *Trichoderma harzianum*, *T. viride*, *Streptomyces* sp. and *Bacillus subtilis* to seed as well as soil was found to be effective in reducing the collar rot of groundnut caused by *Sclerotium rolfsii*.

Studies were undertaken to ascertain whether fungicides, plant extracts and biocontrol agents control the disease only by fungicidal properties or by altering host physiology or both. Accordingly, plants treated with fungicides, plant extracts and biocontrol agents by different modes of application were analysed for total phenols which undoubtedly has been implicated in conferring host resistance. The data presented in Table 25 (Fig. 14) reveals that in seed treatment with different fungicides, plant extracts and biocontrol agents did alter the host physiology by affecting the phenols. Benlate treated plants recorded maximum total phenols (1.81 g 100 g^{-1} sample). Total phenols in different treatments followed the order of Benlate > Bavistin > Topsin-M > Contaf > Neem > Datura > *T. viride* > Karach > *T. harzianum* > Mehendi > *G. virens* = Ashok. Seed treated plants with Ashok extracts possessed the lowest quantity of total phenols which was at par with total phenols content in control. In soil drenching also, Benlate treated plants scored the highest amount of total phenols (1.75 g 100 g^{-1} sample), but it was less than as in seed treated plants. Different treatments followed the same trend as in seed treatments with regard to total phenols content. Here, both Ashok and *G. virens* had no effect in enhancing total phenols over control. In seed treatment + soil drenching also, Benlate treated plants contained the highest total phenols (1.91 g 100 g^{-1} sample) and it was higher than total phenols in seed treatment or soil drenching. Different treatments followed the same trend as earlier in total phenols. In comparison, seed treatment + soil drenching recorded higher quantity of total phenols than in seed treatment or soil drenching alone.

From the foregoing discussion it may be concluded that fungicides, biocontrol agents and plant extracts do affect the host physiology in varying degrees and maximum when such agents were employed in integrated fashion as seed treatment and soil drench.

Gautam *et al.* (1984) observed an increase of total phenolics in 10 and 20 days after drenching of triadimefon in soybean plants. Thomas (1986) reported that carbendazim and thiophanate methyl application significantly increased the total phenols content in leaves of groundnut and this increased phenol make the plants more resistant against disease. Sharma *et al.* (1990) reported that application of carbendazim as foliar spray and soil drenching increased total phenols content sharply in chilli plants. Sindhan *et al.* (1991) reported that inoculation of *Bacillus subtilis*, *Aspergillus niger* and *Penicillium citrinum* significantly increased the level of total phenols, orthodihydroxy phenols, total sugars and reducing sugars in leaves of guar plants. However, no information is available with regard to plant extract in altering host metabolism and therefore the present finding appears to be new in this direction.

CHAPTER - 6

SUMMARY

Charcoal rot of sunflower caused by *Rhizoctonia bataticola* appeared widely prevalent in all sunflower growing areas of Haryana state. The survey revealed that its incidence was more in Karnal district amongst all the districts. Among varieties, Jwalamukhi suffered maximum (33.72%) in Sangoha village in Karnal. Disease incidence was the least (4.0%) in the variety Mahyco-8 in the village Pipli in Kurukshetra. Soil type, source of irrigation and other factors did not affect disease incidence. Date of sowing also, in general, did not have any influence on disease incidence, however, delay in sowing of Mahyco-8 in Kurukshetra and adjoining areas resulted in higher disease incidence.

Five per cent inoculum load caused maximum disease (94.5%). Both

fungus and fungal metabolite reduced seed germination, seedling root and shoot growth on germinator paper. Fungal metabolite was more effective than the fungus in reducing seed germination, root and shoot growth.

Increased irrigation level and decreased soil temperature decreased the disease incidence. Conversely, decreased irrigation level and increased soil temperature increased the disease incidence. Increasing environmental temperature, decreasing relative humidity and decreasing rainfall increased charcoal rot incidence.

With a view to find resistant source a total of 50 germplasms were screened in the field of which, 3 showed highly resistant reaction, 3 resistant, 7 moderately resistant, 9 moderately susceptible, 17 susceptible and 11 as highly susceptible. Thirty-nine germplasms selected after field screening were further screened under artificial epiphytotic conditions. Out of them only RHA-274 appeared highly resistant, 4 resistant, 8 moderately susceptible, 9 susceptible and 17 as highly susceptible.

Biochemical analysis of host and its correlation with resistance revealed that total phenols, free amino acids, insoluble pectin and polyphenol oxidase activity were maximum in the highly resistant germplasm RHA-274 and those reduced in decreasing order from highly resistant germplasm to highly susceptible germplasm being the minimum in the highly susceptible germplasm HRHA-8. After infection total phenols, free amino acids and insoluble pectin decreased irrespective of the germplasms. Polyphenol oxidase activity, of course, increased after infection in all the germplasms.

With a view to manage charcoal rot, efficacy of Benlate, Topsin-M, Kavach, Bavistin and Contaf was evaluated *in vitro* against *Rhizoctonia bataticola*. All the fungicides were found effective with being the Benlate being the best in reducing the colony diameter though at par with Bavistin followed by Topsin-M, Contaf and Kavach.

All the plant extracts viz., Neem (*Azadirachta indica*), Datura (*Datura stramonium*), Ashok (*Polyalthia longifolia*) and Mehendi (*Lawsonia inermis*) reduced growth of the fungus *in vitro*. Neem and Datura were more effective than others.

Amongst biocontrol agents (*Trichoderma harzianum, T. viride* and *Gliocladium virens*) *T. viride* was most effective in reducing colony diameter of the fungus, though it was at par with *T. harzianum.*

Effect of seed treatment, soil drenching and seed treatment followed by soil drenching with fungicides, plant extracts and biocontrol agents on charcoal rot incidence was observed in screenhouse. Amongst fungicides, Benlate proved most effective followed by Bavistin, Topsin-M, Contaf and Kavach. Seed treatment followed by soil drenching appeared more effective in comparison to seed treatment or soil drenching alone. Out of four plant extracts, Neem and Datura reduced the disease more in comparison to Ashok and Mehendi. Disease incidence was less in treatment with seed treatment followed by soil drenching than in other two methods. Biocontrol agents reduced the disease when used as seed treatment followed by soil drenching. *Trichoderma viride* reduced the disease maximum than *T. harzianum* and *Gliocladium virens*.

Biochemical analysis of the treated plants with fungicides, plant extracts and biocontrol agents with different modes of applications in relation to total phenols revealed that Benlate not only reduced the disease by its fungitoxic property but also induced resistance by increasing phenol content.

Seed treatment followed by soil drenching, comparatively enhanced phenol content more than plants with seed treatment or soil drenching alone. The quantity of total phenols decreased in different treatments in the order of Benlate > Bavistin > Topsin-M > Contaf > Neem > Datura > *T. viride* > Kavach > *T. harzianum* > Mehendi > *G. virens* = Ashok.

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ABSTRACT

a)	Title of Dissertation	:	Studies on charcoal rot of sunflower caused by *Rhizoctonia bataticola* (Taub.) Butler
b)	Full name of degree holder	:	Dalim Pathak
c)	Title of degree	:	Doctor of Philosophy in Plant Pathology
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e)	Degree awarding University/Institute	:	CCS Haryana Agricultural University Hisar-125 004 (Haryana), India
f)	Year of award of degree	:	1998
g)	Major subject	:	Plant Pathology
h)	Total number of pages in dissertation	:	108
i)	Number of words in the Abstract	:	Approximately 350

Charcoal rot of sunflower (*Helianthus annuus* L.) caused by *Rhizoctonia bataticola* has emerged as an important disease. Survey revealed that incidence of the disease was maximum in the variety Jwalamukhi in the village Sangoha in Karnal district and minimum in the variety Mahyco-8 in Pipli village in Kurukshetra district. Date of sowing, in general, did not have any influence on disease incidence, however, delayed sowing of Mahyco-8 in Kurukshetra district resulted in higher disease incidence. Soil type and source of irrigation too did not affect disease incidence.

Five per cent inoculum load caused maximum disease (94.5%). Both fungus and fungal metabolite reduced seed germination, seedling root and shoot growth on germinator paper. Fungal metabolite was more effective than the fungus in reducing seed germination, seedling root and shoot length. Decreased irrigation level and increased soil temperature increased the disease incidence. Increasing environmental temperature, decreasing relative humidity and decreasing rainfall increased charcoal rot incidence.

Out of 50 germplasms screened in the field in spring season, 3 showed highly resistant reaction, 3 resistant, 7 moderately resistant, 9 moderately

susceptible, 17 susceptible and 11 as highly susceptible. Thirty-nine germplasms selected after field screening were again screened under artificial epiphytotic conditions in spring season. Only RHA-274 appeared highly resistant while 4 resistant, 8 moderately resistant, 9 susceptible and 17 highly susceptible.

Biochemical analysis of host revealed that total phenols, free amino acids, insoluble pectin and polyphenol oxidase activity were maximum in the highly resistant germplasm RHA-274 and those reduced in decreasing order from highly resistant germplasm to highly susceptible germplasm being the minimum in the highly susceptible germplasm HRHA-8. After infection total phenols, free amino acids and insoluble pectin decreased irrespective of the germplasms. Polyphenol oxidase activity, however, increased after infection in all the germplasms.

Efficacy of Benlate, Topsin-M, Kavach, Bavistin and Contaf was evaluated against *Rhizoctonia bataticola in vitro*. Benlate was the best in reducing the colony diameter though it was at par with Bavistin followed by Topsin-M, Contaf and Kavach. Plant extracts of Neem (*Azadirachta indica*), Ashok (*Polyalthia longifolia*), Datura (*Datura stramonium*) and Mehendi (*Lawsonia inermis*) reduced growth of the fungus *in vitro*. Neem and Datura were more effective. Amongst biocontrol agents, *Trichoderma harzianum*, *T. viride* and *Gliocladium virens* tested *in vitro*, *T. viride* reduced the colony diameter of the fungus maximum though it was at par with *T. harzianum*.

Effect of seed treatment, soil drenching and seed treatment followed by soil drenching with fungicides, plant extracts and biocontrol agents on charcoal rot incidence was observed in screenhouse. Amongst fungicides tested under screenhouse Benlate proved most effective followed by Bavistin, Topsin-M, Contaf and Kavach. Seed treatment followed by soil drenching appeared more effective in comparison to seed treatment or soil drenching alone. Out of four plant extracts, Neem and Datura reduced the disease more in comparison to Ashok and Mehendi. Seed treatment followed by soil drenching reduced the disease maximum. Biocontrol agents reduced the disease more in seed treatment followed by soil drenching. *Trichoderma viride* reduced the disease maximum followed by *T. harzianum* and *Gliocladium virens*.

Biochemical analysis of the treated plants with fungicides, plant extracts and biocontrol agents with different modes of applications in relation to total phenols revealed that Benlate not only reduced the disease by its fungitoxic property but also induced resistance by increasing total phenols. Seed treatment followed by soil drenching comparatively enhanced phenols content more than seed treatment or soil drenching alone.

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