CHAPTER II

2. Literature Review

Little information is available on effect of seed-borne *Macrophomina phaseolina* in mungbean and blackgram including physical and biological seed treatment. However, some informations are available in relation to other crops on these aspects. The existing literatures with respect to present investigation are reviewed below:

2.1. Fungi associated with mungbean and blackgram seeds

Mungbean and blackgram seeds are subjected to attack by a number of fungi. According to Bilgrami *et al.*, (1976) a large number of fungi associated with the seed of mungbean and blackgram when they were assayed by blotter method. In mungbean seeds, *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Alternaria alternata*, *Fusarium semitectum*, *Curvularia lunata*, and *Helminthosporium hawaiiense* were detected. Likewise, in blackgram seeds, all these fungi found to be associated including *Cladosporium* sp.

The contamination of infected soil with the pod has been detected as one of the main cause of seed infection with many fungi including *M. phaseolina*. Ellis *et al.*, (1976) studied the effect of pod contact with soil on fungal infection of dry bean seeds. They reported 64 to 92 percent fungi were detected in the pods, which contacted with the soil and 3 to 30 percent in pods, which did not contact the soil. Among the recovered fungi *M. phaseolina*, *Fusarium spp.*, *Rhizoctonia solani*, *Phomopsis sp.*, and *Alternaria spp.* were prominent. Moreover, Fuhlbohm *et al.*, (1997) found that semi-lodged portion of mungbean plants produced 20 percent *M. phaseolina* infected seeds while non-lodged portion of that crop contained only 2 percent infected seeds. They unveiled as the cause of high infection in semi lodged

portion was due to the direct contact of pods with the soil and soil splash on the pod.

2.2. Pathogenicity of *Macrophomina phaseolina*

Although very few pertinent information are available regarding the pathogenicity of *M. phaseolina* in mungbean and blackgram but its pathogenic effect has been investigated in several other crops. Gangopadhyay *et al.*, (1970) tested the pathogenicity of *M. phaseolina* in soybean. They inoculated the soybean plants at the age of 3 weeks with infected toothpicks by *M. phaseolina*. All the plants began to wilt about 1 month after inoculation. Many of the seeds from inoculated plants were blemished by presence of black spots with reduction in seed weight whereas seeds from non-inoculated plants did not show such blemishness or loss in weight.

Nath *et al.*, (1970) studied the pathogenicity of *M. phaseolina* in mungbean seeds by soil inoculation method. When they sowed the mungbean seeds in the *M. phaseolina* infested soil, the germination of seed was completely checked. After 15 days, when the seeds were dug up, all of them observed to be germinated but rotten. Incase of transplanting healthy seedlings in infected soil, none of them could survive and were killed within 5-7 days of transplanting.

In kidney bean, pathogenicity of *M. phaseolina* was observed by soil inoculation method (Watanabe, 1972). The result showed about 30.0 percent diseased seedlings including abnormal plants. The pathogen was also re-isolated from the infected plants.

Pathogenicity test has been done in potato tuber and sunflower seeds by Fakir *et al.*, (1976). They found *M. phaseolina* to be highly pathogenic in potato tuber causing charcoal rot symptom. In sunflower, damping-off of germinating seeds and linear necrotic lesions on stems of older plants were observed. Eventually, pycnidia and sclerotia were developed on the infected

parts of the inoculated plants. The pathogen was isolated from infected seeds and stems of the inoculated plants.

Thirumalachar *et al.*, (1977) reported the pathogenicity of *M. phaseolina* following pre-emergence and post-emergence damping-ff method in the seed of sesame, sunflower, okra, chili, and horsegram. In the inoculated samples, 30 to 60 percent damping-off seedlings were noticed. Among them, sesame, sunflower, and okra gave 45 to 60 percent infection while chili and horsegram showed 30 to 40 percent infection.

Ellis *et al.*, (1979) observed that *M. phaseolina* was found to be highly pathogenic to soybean seed. They inoculated the seed with the fungi following plated on PDA and planted in sterile soil. In both case it was noticed that the fungi reduced the germination significantly.

Scholefield and Griffin (1979) studied the seed-borne nature of *M. phaseolina* in mungbean. They dipped the sprouts of mungbean (48 hours old) in a suspension of sclerotial and mycelial fragments of *M. phaseolina* prepared from an eight-day-old culture on PDA. Then the inoculated sprouts were kept to a humid chamber. Within 24-48 hours, blackish-brown lesions were developed on the radicles of the beans in the inoculated sprouts. From the lesions, *M. phaseolina* was re-isolated.

The role of *M. phaseolina* was investigated by Reuveni *et al.*, (1983) in melon by artificial seed infestation with sclerotial inoculum. The effect on emergence of seedlings was found to be drastic and severe pre-emergence damping-off while the control treatment showed 100 percent emergence. The fungus was recovered from the roots and all upper parts of the plants.

Nayak and Behera (1994) reported the pathogenicity of *M. phaseolina* in blackgram seed. They found that *M. phaseolina* caused loss in germination. They also observed that rotting was mostly in radicle than

plumule. Rotting of radicle was noticed from 2nd to 4th day and incase of plumule it occurred at 5th day.

However, *M. phaseolina* causes infection not only in the seeds and seedlings but also in the mature plants. Investigations on pathogenicity have been done on the mature plants of alfalfa and white clover (Pratt *et al.*, 1998). They inoculated by toothpicks infested with isolate of *M. phaseolina* in stolons of white clover and stems of alfalfa. After inoculation, a brown-black progressive necrosis with expanding lesions was appeared. In taproot and crown of alfalfa, *M. phaseolina* caused dark discoloration in bands or streaks above and below inoculation points and death of cortical tissues, lateral roots and stems. Sclerotia formed in all tissues of both species. All isolates of *M. phaseolina* were re-isolated from margins of necrosis in all types of inoculated tissues and re-grown in pure culture. These results fulfill Koch's postulates for *M. phaseolina* as a pathogen of mature plants of white clover and alfalfa.

2.3. Location of *M. phaseolina* in mungbean and blackgram seed

As the location of the fungus within the seed plays an important role during seed germination, so it is in need to detect the site of *M. phaseolina* in mungbean and blackgram seeds. However, the literatures show that the site of *M. phaseolina* differs depending on the crop and even on variety. Gangopadhyay *et al.*, (1970) studied on the site of *M. phaseolina* in soybean seed. They observed the presence of *M. phaseolina* in three parts of seed viz. seed coat, cotyledon and embryo in both naturally and artificially infected seeds. In both cases, the presence of *M. phaseolina* was detected only in the seed coat. The existence of this fungus was found neither in the embryo nor radicle.

From the study of Chaudhary and Pal (1982) in sannhemp (*Crotalaria juncea*) seeds, maximum *M. phaseolina* was evolved from seed coat,

although the other parts of seed like cotyledon and embryo also showed presence of *M. phaseolina* to a lesser extent.

In sunflower seed, the location of *M. phaseolina* was detected by Raut (1983). The various components of seed were tested by blotter method. *M. phaseolina* was detected in the pericarp of all infected seeds, in the endosperm 57 to 89 percent incase of presoaked and 54 to 71 percent in the dry dissected seeds, and in embryos 5 to 25 percent seeds. The fungus formed both sclerotia and pycnidia on the pericarp and endosperm.

According to Sharada and Shetty (1987) the incidence of *M. phaseolina* in blackgram was found to be higher in seed coat by 61 to 41 percent followed by 34 to 22 percent in radicle, 30 to 19 percent in cotyledon, and 21 to 13 percent in plumule. They also reported that the surface sterilization of seed with sodium hypochlorite indicates the farm establishment of the fungus inside the seed coat.

The location of *M. phaseolina* was studied in the seed of pumpkin by Sultana *et al.*, (1994). The seeds were dissected aseptically into four components *viz.,* seed coat, tegment, cotyledons, and embryo. The separated seed parts were incubated in soaked blotter. In the dotted spotted seeds, *M. phaseolina* was found significantly higher in the seed coat and tegment than in cotyledons and embryo. However, in the healthy looking seeds, less presence was observed in seed coat and tegment, and no any *M. phaseolina* was found in the cotyledons and embryo.

2.4.Seed transmission of *Macrophomina phaseolina*

Seed transmission of *M. phaseolina* was studied in various crops other than mungbean and blackgram by several researchers. In sunflower seed, *M. phaseolina* gets transmitted from seed to seedlings (Fakir *et al.*, 1976). Blotter method revealed that this fungus often prevented germination, killed the emerging radicle including discoloration of roots, hypocotyls and

cotyledons. The germination failure due to *M. phaseolina* infection in blotter test was highly correlated with high pre-emergence mortality in pot culture. From this investigation, they inferred that *M. phaseolina* could cause pre-emergence death of sunflower seedlings in the field.

According to Sinha and Khare (1977), when *M. phaseolina* infected cowpea seed, same event was appeared during germination in both blotter and in the soil. The seeds with heavy infection did not germinate and rotted completely. The mycelia of *M. phaseolina* encompassed the seed and pycnidia were observed. Incase of partial infection, the seed germinated but there was subsequent pre- and post-emergence rot, root rot, hypocotyls rot and stem rot. In the stem, the rot was observed as 'charcoal' appearance.

From the result of Chaudhary and Pal (1982), it was revealed that incase of sannhemp seeds, the growing on test clearly demonstrated that *M. phaseolina* seed transmitted. Infection became visible soon after the emergence of the cotyledonary leaves. Infected seedlings were not killed immediately. Firstly seedlings were blighted, the stem shriveled and blackened and eventually died. Numerous pycnidia developed on the blackened portion of the stem.

Singh and Singh (1982) reported that *M. phaseolina* is seed transmitted in sesame seed. Transmission study was done by blotter and water agar method. They were able to isolate the fungus from rotted, wilted, and healthy looking seedlings, which formed from infected seeds. Roots, stems, and fruits from mature and dried plants showed the presence of mycelia and microsclerotia. They also found only on rotted seeds and seedlings, and in lesions on stem pycnidia were frequently formed in blotter as well as in agar test.

Transmission of seed-borne *M. phaseolina* in sunflower was studied by Raut (1983). For assessment of pre- and post emergence losses from infected seed, incubated sprouted seed carrying *M. phaseolina* infection were

transferred singly from 3-5 days old blotter tests to the pot contained soil. These seeds showed severe pre- and post emergence mortality. Seed-borne inoculum caused infection of the primary root and cotyledons within two to three days of growth and subsequently under high temperature conditions resulted in pre-emergence death of seedlings or post-emergence mortality in the form of damping off, collar rot, root rot, and root and stem rot. The fungus did not cause systemic infection. Copious pycnidia and sclerotia were often formed on infected stem and on dead plant parts. When the infected un-germinated and germinated seeds and seedlings were plated on PDA, *M. phaseolina* was recovered.

Sharada and Shetty (1987) investigated the seed to seedling transmission of *M. phaseolina* in blackgram. They employed water agar, sand, and soil method, and found that *M. phaseolina* transmitted from seed to seedling. They also observed similar percentage of seed transmission in all the three methods. Seedlings were emerged after 4 days in all the methods. Circular and dark brown spots showed on the 16th day in cotyledonary nodal regions, and these spots gradually spread to the stem and leaves. Because of infection, some seedlings appeared to be stunted growth of plumule and radicle. Incase of heavy infection, seed rot was manifested. Furthermore, due to severe infection, 24 to 35 percent seed germination was restricted. It was also reported that, such un-germinated and rotted seed helped in the proliferation of *M. phaseolina* in the soil.

According to Abawi and Pastor-Corrales (1990), severely infected seeds of dry edible beans with *M. phaseolina*, generally failed to germinate or emerge. Abundant sclerotia and pycnidia were visible on the seed coat and cotyledonary tissue.

Seed transmission of *M. phaseolina* in pumpkin seed was investigated by Sultana *et al.*, (1994). They found most of the un-germinated seeds were covered with gray to black loose mycelia with minute black sclerotia. When

the infected plant parts placed on PDA medium, *M. phaseolina* was isolated. Typical charcoal rot symptom was observed in the basal part of some plants.

2.5. Effect of fungi on seed quality

Fungi produce toxic substances to plants, which affects on seed quality. Very little relevant information is available so far on the effect of *M. phaseolina* on seed quality of mungbean and blackgram. However, some pertinent reports on this aspect in various crops are found.

Maheshwari *et al.*, (1984) reported the effect of six fungi viz. *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, and *Fusarium moniliforme* on seed quality of cowpea seed. It was found that all fungi affected adversely on seed germination, root length, and shoot length to various degree. *A. nidulans* was the most effective inhibitor on seed germination by 38.78 percent, root length by 82.80 percent and stopped the plumule emergence completely, while *F. moniliforme* was least effective. They also reported that the quality deterioration of cowpea seed was responsible for certain amino acids, organic acids, and phenols, which were produced by seed-borne fungi.

The effects of various fungi were investigated by Singh (1984) on seed germination and seedling vigor of wheat seed. He found the maximum inhibition in seed germination was done by *Aspergillus terreus*, *A. niger*, and *A. flavus*. The highest reduction in both root and shoot growth was observed due to *Aspergillus terreus*, followed by *A. flavus*, *A. niger*, and *Helminthosporium sativum*. *Memnoniella echinata* and *Chaetomium globosum* retarded only the root growth.

Deteriorative abilities of some common storage fungi were studied in wheat by Ghosh and Nandi (1986). The fungi were used namely *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. restrictus*, *A. sydowi* and *Penicillium jenseni*. Due to these fungal invasions, a significant reduction was

noticed in germination, root length, and shoot length. They also reported that the decrease in germination and seedling growth due to the presence of diffusible toxic substances produced by these fungi.

Delouche and Baskin (1973) in Gupta *et al.*, (1993) developed an accelerated aging test (AA-test) to estimate the storability of seed. According to this test, the seeds were exposed to high humidity and high temperature. This condition promoted the growth of associated fungi with the seeds and caused deterioration. Seed germination, number of normal and abnormal seedlings provided the information about deterioration. The seeds, which produced more abnormal seedlings, were considered as low vigor and low storability. The study proved that the test could be used to predict seedling establishment in several crops.

Deterioration of seed quality of rice was reported by Purushotham *et al.*, (1996). They found considerable reduction in germination, root length and shoot length. The maximum inhibitory effect was showed by *Aspergillus flavus* in seed germination, root length, and shoot length followed by *A. glaucus* and *A. versicolor*.

2.6. Effect of fungi on carbohydrate and protein content of seed

Seed-borne pathogen deteriorates the seed quality not only in terms of viability, vigor and storability but also changes the biochemical properties like carbohydrate content, protein content etc. Vidhyasekaran *et al.*, (1973) recorded that the protein content in six varieties of rice was increased due to *Helminthosporium oryzae* infection and the increase in protein content ranged from 3 to 6 percent.

Bilgrami *et al.*, (1976) reported that due to *Aspergillus flavus* invasion, the seeds of mungbean and blackgram were lost their weight including changes in sugar, amino acid, and organic constituents. There was a decrease in sugar and amino acid contents but few amino acids and organic

acids were synthesized. The workers also indicated that the reduction in contents of sugar and certain acids obviously due to consumption of the fungus and the increasing was attributed by the breakdown of the seed protein.

Mukhopadhyay and Nandi (1976) estimated quantitatively the total carbohydrate content of the stem of healthy and *M. phaseolina* infected jute (*Corchorus capsularis*) plants of different ages. The total carbohydrate content in the tissues of healthy jute stems increased gradually with the age up to three and half months, and thereafter remained more or less unchanged. The corresponding increase in total carbohydrate content in the diseased plants was comparatively slow. Consequently, the total carbohydrate level of an infected plant was noticeably less compared to that healthy plant of the same age.

Deterioration in sugar, organic and amino acid content of 'Arhar' (*Cajanus cajan*) seeds infested by *Aspergillus flavus* was investigated by Sinha and Prasad (1977). Among the three soluble sugars viz. glucose, fructose and sucrose, two of them sucrose and fructose were utilized completely by the fungus. Correspondingly, concentration of some amino acids (glutamic acid, serin, leucine, and lysine) reduced in the infected seeds whereas others (glutamic acid and β-alanine) were showed increasing tendency during incubation. The authors explained increase in some of the amino acid contents by considering the process of protein breakdown accelerated by the concern fungal organisms whereas the decrease in some other amino acid contents likely to consumed by the fungi.

Bilgrami *et al.*, (1978) found a marked quantitative change in moong seeds (*Phaseolus aureus*) in carbohydrate like starch and in protein due to fungal infestation. They observed that the starch content was reduced and protein content was thrived. The probable cause of carbohydrate depleting

was the consumption by the fungi and the increasing of protein due to accumulation of fungal mycelium, which contains protein them self.

Sumbali and Mehrotra (1982) estimated the post infectional chemical changes in round gourds infected with *Geotrichum candidum*. Results indicated that there were both qualitative as well as quantitative changes in sugar content in response to infection. After 4 days of incubation, sucrose was disappeared and after 8 days, glucose was also disappeared but incase of fructose and ribose dawdling depletion was noticed. The declining of sugars was likely to be related to the breakdown of carbohydrates because of fungal enzymes.

Ghosh and Nandi (1986) found the reduction in carbohydrate content due to fungal invasion. The net losses were observed by 3.94, 4.99, 2.12, 0.64, 3.94, 3.08, and 0.83 percent in the grains inoculated with *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. sydowi*, *A. restrictus*, and *Penicillium jenseni*, respectively. The decrease was progressed when the storage period was prolonged.

Singh and Shukla (1987) recorded changes in amino acid in blackgram leaves infected with *Colletotrichum truncatum*. The concentrations of some amino acids like arginine, glutamic acid, histidine, lysine, methionine, phenyllalanine, threonine and valine were declined in case of diseased leaves as compared to healthy leaves. In contrast, the concentrations of cystine and proline were increased due to disease. They also reported that the decrease of some amino acid due to their utilization by the pathogen and accumulation of some amino acids in infected seeds due to host pathogen interaction, which was resulted because of the blockage of protein synthesis.

Biochemical changes due to *M. phaseolina* infection in lima bean plants were investigated by Jadeja and Patel (1989). The amino acid content was found by 3.38 and 15.00 mg/g in healthy and diseased stem respectively.

Effect of some seed-borne fungi namely *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, and *Fusarium moniliforme* on protein and carbohydrate content of sesame and sunflower seeds were studied by Saxena and Karan (1991). The protein and carbohydrate content was reduced due to infection of these fungi. The authors inferred that the reduction of protein and carbohydrate content was occurred because of consumption and conversion into carbon dioxide and water by the fungi. The probable cause of loss in protein content was hydrolysis of protein by the efficient enzyme in the organism.

Purushotham *et al.*, (1996) documented that fungal invasion in rice seed caused significant reduction in carbohydrate content. The maximum depletion in carbohydrate content was observed in *A. flavus* inoculated seeds followed by *A. versicolor* and *A. glaucus*. They indicated that rapid loss of carbohydrate due to utilization of fungi in respiration for energy requirement or due to increase in enzyme activity.

Sandhu *et al.*, (1998) found the levels of total sugars and starch always more in healthy stem and root tissues of cowpea as compared to *M. phaseolina* infected ones. The depletion in carbohydrate content in the infected tissues was caused by the activity of α-amylase and invertase enzymes.

2.7. Control of seed-borne diseases by seed treatment

The present recommended control measures against charcoal rot caused by *M. phaseolina* in mungbean and blackgram involves a set of strategies like suitable crop rotation, using uninfected seeds, and seed treatment with fungicide before planting (Watanasit and Thanomsub, 1995). However, seed treatment is the most convenient, effective, and cheap technology for controlling the disease (Neergaard, 1979).

2.7.1. Seed treatment by hot water

The seed-borne pathogen is killed by dipping the seeds in hot water for certain duration without hampering the germination ability. Indeed, no any hot water treatment method is recommended for controlling the charcoal rot disease of mungbean and blackgram yet. Nevertheless, this method has been proved to be effective against in multitude of seed-borne pathogens in several crops.

Successful control of *M. phaseolina* and *Fusarium equiseti* has been explored in cowpea seeds by hot water treatment by Sinha and Khare (1977). They used naturally infected seed with these two pathogens. Eight different temperatures *viz.* 42^{0}C, 44^{0}C, 46^{0}C, 48^{0}C, 50^{0}C, 52^{0}C, 54^{0}C, and 56^{0}C and four durations *viz.* 5, 10, 15, and 20 minutes for each temperature treatment. The most effective combination was 46^{0}C for 20 minutes, which was able to eliminate all *M. phaseolina* and *Fusarium equiseti* from cowpea seed without hampering the germination ability.

Gaur *et al.*, (1984) cited in Maude (1996) recorded that blackrot disease of mungbean caused by *Xanthomonas campestris* pv. *Phaseoli* could be controlled by hot water treatment at 52^{0}C for 20 minutes.

Tripathi *et al.*, (1987) eradicated *Ascochyta rabiei* from chickpea seeds when the seeds were treated at 55 to 60^{0}C for 6-12 hours. But more than 50 percent treated seeds lost their viability. Due to very long duration, the seeds could not able to keep their germinating ability.

According to Grondeau *et al.*, (1992) cited in Maude (1996), pea blight organism *Pseudomonas syringae* pv. *pisi* was successfully controlled by hot water treatment for 15 minutes at 55^{0}C to 60^{0}C.

2.7.2. Chemical seed treatment

There are some chemical seed treatment are recommended for controlling *M. phaseolina* in various crops including mungbean and blackgram.

For controlling dry root rot of mungbean caused by *M. phaseolina*, Grewal and Pal (1971) tested nine fungicides *viz.*, Demosan, Brassicol, Puraseed, Agrosan GN, Thiram, Spergon, Captan, Rhizoctol, and Vitavax in the field condition. The lowest disease incidence and highest germination and yield were found in Demosan treated plots.

In blackgram and soybean, Agarwal *et al.*, (1972) studied about the seed treatment to control *M. phaseolina*. According to their observation, Thiram or Captan was able to reduce the pathogen significantly but not completely.

Sinha and Khare (1977) reported that in case of both *in-vitro* and *in-vivo* trial the systemic fungicide Bavistin and Benomyl showed excellent control in cowpea seeds against *M. phaseolina*. Similarly, among non-systemic fungicides Ceresin dry, Difolatan and Thiram found to be most effective against this pathogen when applied as seed treatment.

In order to preventing colonization of *M. phaseolina*, Reddy and Subbaya (1981), screened five fungicides *in-vitro*. Out of five, four fungicides found to be effective which were Benlate, Thiram, Ceresin, and Vitavax.

Sharma (1988) treated french bean seed with eight fungicides viz. Benomyl, Carbendazim, Thiophanate methyl, Captan, Thiram, Captafol, Copper oxychloride and PCNB for controlling *M. phaseolina*. All fungicides reduced the pre and post emergence mortality but Benomyl, Carbendazim, and Thiophanate methyl showed excellent performance.

In Thailand, Watanasit and Thanomsub (1995) recommended Benomyl 50 WP at the rate of 2g per kg of seeds for controlling *M. phaseolina* in mungbean and blackgram seeds.

2.7.3. Biological control

Biocontrol of plant pathogens with biological antagonists has been considered as the safest and an effective alternative to chemical control. The biological antagonists control the pathogens either killing the pathogens through antibiosis or suppress the growth through competition for food and space. However, plenty of works have been done on biological control of plant pathogens especially the effect of different species of *Trichoderma* on various pathogens so far although works on *M. phaseolina* is very limited.

Elad *et al.*, (1983) unveiled the parasitism of *T. harzianum* or *T. hamatum* to *Sclerotium rolfsii* the soil borne plant pathogen. They recorded that the hyphae of *Trichoderma* sp. contact with *S. rolfsii* either producing appressorium like bodies or coiling around the hyphae, and then digest the cell wall enzymatically. Extra cellular fibril material was deposited between the interacting cells. In response to invasion, the pathogen produced a sheath matrix, which encapsulates the penetrating hyphae and the cell became without cytoplasm.

Ferrata and D'Ambra (1985) observed that although *T. harzianum* isolate showed low ability in coiling the hyphae of *S. rolfsii* but much powerful in penetrating and growing inside. An adverse effect was observed on *S. rolfsii* when they develop closely even without penetration.

Mutto *et al.*, (1986) reported that hyphae of *T. harzianum* developed in the medilla of sclerotia of *S. rolfsii*, growing on the inside of the cell walls and in the cell lumen, as a result the cytoplasm of the penetrated cells was degenerated sharply. The hyperparasite was going from cell to cell by lytic

perforation of the walls. Germ tubes of medullar cells were also parasitized with wall lysis and digestion of cytoplasm.

According to Upadhyay and Mukhopadhyay (1986), isolate of *T. harzianum* directly attacked and lysed the mycelia and sclerotia of *S. rolfsii* in dual culture. Hyphal coiling, invasion with haustoria like structures into hypha and sclerotia was noticed. When *T. harzianum* coated sorghum grain was planted in *S. rolfsii* infested soil, up to 88 percent disease was controlled in comparison to control.

Jacobs and Kamoen (1987) recorded that *T. harzianum* produced cell wall lysing enzymes, which had antagonistic property against plant pathogens. On the other hand, according to Davet (1987), mycoparasitic ability of *Trichoderma* depends on Chitinage and β-(1-3)-glucanase enzyme activity.

Siven and Chet (1989) recorded that two strains of *Trichoderma harzianum* could not able to parasitize colonies of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis*. Nevertheless, both strains of *T. harzianum* were found to be mycoparasitic when grew in liquid cultures containing laminarin, chitin, fungal cell walls or absolute carbon sources, both strains released extra cellular enzymes 1,3-glucanase and chitinase. These enzymes induced release of lytic enzymes with the hyphal cell wall of *F. oxysporum* and resulted degradation.

Calvet *et al.*, (1990) documented that non-volatile compounds released by *T. harzianum* isolates growing on cellophane discs over malt agar significantly inhibited growth of *F. oxysporum*. Lim and Teh (1990) observed that isolates of *T. harzianum*, *T. hamatum* and *T. koningii* inhibited the growth of *S. rolfsii* up to 67 percent in dual culture on malt agar and up to 100 percent using cellophane overlay technique at 28 ± 1.5^{0}C. Growth of the test organisms was inhibited by the production of both diffusible and volatile metabolites. The observed hyphal interactions were: hyphal coiling, by

producing appressoria and hooks and host cells appeared vaculation, granulation, coagulation, disintrigation and lysis.

Deshmukh and Raut (1992) reported that *Trichoderma harzianum* and *T. viride* grew over the colonies of *Fusarium oxysporum, F. monoliforme* (*Gibberella fujikuroi*), *Rhizoctonia bataticola* (*Macrophomina phaseolina*), *Colletotrichum gloeosporioides* (*Glomerella cingulata*) and *Curvularia lunata* (*Cochliobolus lunatus*). They observed *T. harzianum* to be more aggressive than *T. viride*. In pot trials, *T. harzianum* was found to be more effective against *F. oxysporum*.

Basher and Bharat (1994) studied on antagonistic potential of *T. harzianum* against *F. oxysporium* f. sp. *ciceri* of chickpea. From the study, *T. harzianum* was proved to be a potential antagonist. From the colony interaction, significant colony inhibition was observed. Iqbal *et al.*, (1995) observed that *T. harzianum*, *T. korangii*, and *T. viride* significantly suppressed the mycelial growth of *S. rolfsii* and overgrew on the pathogen with inhibition of growth up to 63.6 percent.

Haran *et al.*, (1996) recorded that *T. harzianum* produced 2β–1, 4-N-acetylglucosaminidases and 4-endochitinases which showed antagonistic activity against *S. rolfsii*.

Himani (1996) observed the antagonistic activity of *T. viride*, *T. harzianum*, and *T. koningii* against *F. udum*, the causal agent of wilt disease of pigeon pea.

However, the seed treatment with biological antagonists has been proved to be the very effective control measures of plant pathogens by various workers. According to Maite and Sen (1985), the viability of sclerotia in the soil reduced when *T. harzinium* mixed to the soil in a wheat bran formulation. Moreover, they also observed the additive effect when urea was added with *T. harzianum*.

Lifshitz *et al.*, (1986) unveiled that incidence of pre-emergence damping-off of pea caused by *Pythium* sp. could be reduced when the seeds were coated with the conidia of *T. harzianum* or *T. koningii*. Similarly, Dhedhi *et al.*, (1990) revealed that *T. harzianum* and *T. viride* showed adverse effect on the growth of *F. oxysporum* f. sp. *ciceris* the causal organism of vascular wilt of chickpea (*Cicer arietinum*). In 60-day-old plants, *Trichoderma* spp. checked the growth of *R. solani* and *Fusarium* spp.

Sharma and Singh (1990) reported that *T. harzianum* was most effective against *Sclerotinia scletotiorum* when added to sterilized and un-sterilized soils of pea in green house condition. Mycelial preparation of *T. harzianum* was found the most effective against *S. sclerotiorum*.

According to Farzana *et al.*, (1991), root infection of 30 and 60 days old soybean plant caused by root infecting fungi (*Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp.) can be significantly reduced after seed treatment with *Trichoderma harzianum*.

Singh (1991) recorded that *T. harzianum* paratisized the mycelia and sclerotia of *Sclerotinia sclerotium* caused white mould of peas and destroyed sclerotia within 15 days. Application of wheat bran culture of *T. harzianum* under field conditions gave significant control of the disease with increased yield. Mycelial preparations of *T. harzianum* were more effective than spore preparation.

Ehteshamul-Haque *et al.*, (1992) found the reduction of root rot infection of lentil caused by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. which were significantly decreased due to *Trichoderma harzianum* application. They also reported that the effectiveness of the antagonists was increased when they applied as a soil drench or as a seed dresser after multiplying the inoculums on wheat bran and rice grain.

Fernandez (1992) reported that application of *T. harzianum* was able to reduce the incidence of wheat and soybean pathogens viz. *Fusarium graminearum*, *Glomerella glycines* and *Macrophomina phaseolina* significantly. In addition, the incidence of other *Fusarium* spp. was also declined.

Kar and Mukhopadhyay (1992) unveiled that wilt complex of chickpea caused by mainly *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani* and *Sclerotium rolfsii* was effectively controlled by *T. harzianum* alone and in combination with fungicides. As a medium of *T. harzianum*, wheat bran sawdust found to be highly potential. In the field, integrated use of *T. harzianum* with fungicidal seed treatment resulted significant reduction of chickpea wilt along with increasing crop yield.

Sugha *et al.*, (1993) reported that the seedling mortality caused by *Sclerotium rolfsii* was reduced by 47.0 to 65.0 percent because of conidial coating of seeds with antagonists *T. harzianum* and *T. viride*.

Ehteshamul-Haque and Gaffar (1995) unfolded that soil borne infections of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. in 30 days old seedlings of soybean were significantly controlled when seed treatment was done with *T. harzianum*, *T. viride*, *T. hamatum*, *T. koningii* and *T. pseudokoningii*.

Sumitha and Gaikwad (1995) reported that *T. harzianum* produced a wide zone of inhibition against *F. udum* including complete inhibition of spore germination. The antagonist did not show any adverse effect on pigeon pea seed germination rather increased germination compared to untreated seeds. The seeds treated with antagonists produced longer roots and shoots in comparison to untreated seeds.

Field experiments were conducted by De *et al.*.(1996) in order to find out the comparative efficacy of bio-control agents and fungicides in controlling

chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Significant control of *F. oxysporum* f. sp. *ciceri* wilt by 30 to 45 percent was found because of seed treatment with *T. harzianum* and *T. viride*. Moreover, integration of biocontrol agents with carboxin showed significant increase in seed yield by 25.4 to 42.6 percent.

As a potential biocontrol agent against *F. oxysporium* the causal organism of foot and root rot of food legume, *T. harzianum* has been unveiled by Begum *et al.*, (1998). The interaction study between *T. harzianum* and *F. oxysporium* showed that *T. harzianum* inhibited the mycelial growth of *F. oxysporium*, grew over *F. oxysporium* along with lysing the mycelia of *F. oxysporium*. When the seeds of legume were coated with the conidia of *T. harzianum*, 92.3 percent *F. oxysporium* infection was declined compared to control. Furthermore, besides increasing 66.7 percent germination, *T. harzianum* did not show any detrimental effect on the seed.