CHAPTER IX

9. Control of *Macrophomina phaseolina* in Mungbean and Blackgram Seeds Through Seed Treatment

Seed is the richest source of attacking pathogens, which causes seed-borne diseases (Neergaard, 1979; Pal, 1996). Effective disease control methods depend on the specific nature and characteristics of the pathogen causing the disease. Seed treatment is probably the cheapest and often the safest method of direct plant disease control (Neergaard, 1979). Treatment of seed is a particularly elegant way of protecting the crop at an early stage, and its importance as such is undisputed. It gives the emerging plants outstanding early protection against a very wide range of fungal pathogens and pests, both in the root and the leaf region, and the quantities of active ingredient that are used relatively small. Suitable seed treatment applied directly to the seed disinfects the surface, kill any pathogens that are inside, and protect the roots, hypocotyls, and young plant against fungal attack. Seed treatment ensures that the crop emerges rapidly and of the desired density. It thus lays an important foundation for a high yield of good quality. Therefore, seed treatment is the most convenient, effective, and cheap technology for controlling the disease, which is also helpful in protecting the seed from soil-borne fungi in the early stage of the crop.

In general, seed treatment is provided by physical, chemical, or biological means. Physical seed treatment includes thermotherapy (such as hot water treatment, moist hot air treatment etc.), hot oil treatment, solar heat treatment, and so on. Physical seed treatment is unable to provide further protection of seed or emerging seedling after plantation from the infection of soil-borne and other pathogens, but this is eco-friendly seed treatment method because of involving no chemicals.

The chemical seed treatment includes the use of chemical fungicides in various ways to eradicate or reduce the population of pathogen or to protect seed and the emerging seedling from infection by the pathogen. Mode of chemical seed treatment is includes dipping, spraying, or dressing depending on the nature of pathogen and active ingredient formulation of fungicides. Nevertheless, the most widespread mode of chemical treatment is seed dressing; which is usually carried out by uniformly coating of the seed with the fungicide with utmost precision. For controlling disease, chemical seed treatment is very effective, but it is often very nonspecific in its effects, killing beneficial organisms, as well as pathogens, and it may have unwanted health, safety, and environmental risks.

The biological seed treatment involves the use of biological organisms to control the pathogen located in and/or on the seed. This seed treatment has been a successful approach for controlling seed-borne diseases for decades. Biological seed treatment is usually very specialized and uses specific microorganisms that attack or interfere with specific pathogens or types of pathogens. These organisms have little effect on other soil organisms, leaving the natural biology of the ecosystem more balanced and intact than using broad-spectrum chemical pesticides.

9.1. Physical seed treatment by hot water

9.1.1. An Overview

Thermotherapy is one of the most common methods of physical seed treatment for controlling certain plant diseases. Thermotherapy has also proved a wide range of applications in the type of host or plant parts treated, as well as in the type of pathogen to be killed (Grondeau and Samson, 1994). Hot water treatment has proved to be efficient technique as thermotherapy against various pathogenic microorganisms including some kinds of fungi. The basis for effective hot water treatment is temperature of the seed mass should

be raised quickly up to the lethal level for the infecting organism. This temperature should be maintained for a sufficient time to kill the pathogen but not the seed and then the process should be stopped quickly (e.g. by plunging the seed mass quickly into cold water) and the seeds dried (Maude, 1996). The principle of thermotherapy is described by Baker (1962) as "parasitic microorganisms often are killed, or viruses inhibited, at temperature time regimes only slightly injurious to the host." This phenomenon can be used to control diseases by eradicating, or at least sharply decreasing, the amount of primary inoculum that remains latent in vegetative plant parts and seeds during the quiescent period.

The margin of difference between the effective destruction of the pathogen and the risk of damaging the seed material is small and precise temperature control is needed (Tarr, 1972). Hot water treatment is not only most effective against superficial organisms but also has preventive properties and can reduce the incidence of internal pathogens (Maude, 1996). The treatment probably denatures the external tissues of seeds in addition to killing organisms but does not substantially affect the storage tissues, which provide a food base for the germinating seeds (Maude, 1996).

Hot water treatment represents an interesting means for controlling plant diseases because it is simple in principle, easy to use and not expensive. Moreover, it is eco-friendly because of absence of chemical residue. Its disadvantages include sometimes incomplete eradication of seed-borne fungi (Maude, 1983), seed damage (Baker, 1972), and the fact that only small amounts of seed can be treated in one time. Although hot water treatment is highly preventive against deep-seated infections of small seeds, but is ineffective against internal infections of larger seeds like *Ascochyta pisi* of pea (Maude, 1994). Nevertheless, the aims of thermotherapy are to reduce primary inoculum and thus, act as a preventive control of field diseases (Grondeau and Samson, 1994). Therefore, before application, a number of studies must be undertaken to find the combination of time and temperature

that is most adaptable to the plant material to be treated and the pathogen to be killed.

So far, no attempt has been made to control the seed-borne infection caused by *M. phaseolina* in mungbean and blackgram seeds through hot water treatment. A very few pertinent literatures are available to control *M. phaseolina* in other crops like cowpea. Sinha and Khare (1977) found most effective control of *M. phaseolina* in cowpea seeds by hot water treatment at 46^{0}C for 20 minutes. Therefore, the present study was undertaken on the basis of following objectives:

- to find out the appropriate temperature to control the seed-borne infection of *M. phaseolina*.
- to find out the suitable temperature, which is lethal only to the seed-borne *M. phaseolina* but not to the seed components.

9.1.2. Materials and Methods

9.1.2.1. Seed sample

Seed samples of mungbean and blackgram named as Chai Nat 60 and Uthong 2 respectively, were obtained from Chai Nat Field Crops Research Center. The mungbean and blackgram seeds were carrying 29.0 percent and 27.0 percent natural infection of *M. phaseolina* respectively, according to blotter method.

9.1.2.2. Seed treatment

Hot water treatment was carried out in a serological water bath controlled by thermostat (model: Memmert). Seven different temperatures viz. 50^{0}C, 52^{0}C, 54^{0}C, 56^{0}C, 58^{0}C, 60^{0}C and 62^{0}C including three durations 10, 15, and 20 minutes for each temperature treatment were employed. The

seeds were soaked in sterilized water for 10 minutes. Then 400 soaked seeds were wrapped in a thin and soft cloth following dipping in the water of water bath at the required temperatures for various durations. Before dipping the seed in water bath, the required temperature was adjusted. The control treatment was maintained with soaked seed in normal water but without treatment in hot water. After treating in exact duration, the seeds were immediately immersed in cold water 15 minutes.

9.1.2.3. Evaluation of hot water treated seed by blotter method

All the seeds were placed in sterilized Petriplate contained 3-layered moist Whatman no. 1 blotter paper. In each Petriplate, 10 seeds were placed. All the Petriplates with seeds were incubated under 12 hours alternating NUV light and darkness. After 7 days, the seeds were examined under stereo-binocular microscope for observing the infection of *M. phaseolina* including germination percentage. The data were recorded on the basis of 4 replications in each treatment while 10 Petriplate i.e. 100 seeds considered as one replication.

9.1.2.4. Plantation of hot water treated seed in *in-vivo* condition

The hot water treated seeds were evaluated in plastic pots filled with sterilized soil. The seeds were treated with effective temperature and durations found in blotter method. The seeds of both mungbean and blackgram were treated with hot water at 56^0C for 20 minutes. Two hundred treated seeds of each mungbean and blackgram and equal number of untreated seeds (but soaked with normal water for 20 minutes) for each category were planted in plastic pots (size 16cm x 12cm) with four replications using 25 seeds per pot. The pots were placed in the glass house and watering was done as usual. The germination and disease incidence was recorded until three weeks.

9.1.3. Results

9.1.3.1. Evaluation of hot water treated mungbean seed by blotter method

Results from hot water treatment of mungbean seed are presented as line graph in Figure 9.01. The most effective temperature and duration for complete reduction of *M. phaseolina* infection in mungbean seed were found to be 54^{0}C for 20 minutes and above. Below this temperature and duration i.e. 50^{0}C for 10 minutes, 15 minutes and 20 minutes; 52^{0}C for 10, 15 and 20 minutes; 54^{0}C for 10 and 15 minutes could not able to eliminate the *M. phaseolina* from the seed completely, although 52^{0}C for 20 minutes, 54^{0}C for 10 and 15 minutes were able to decline *M. phaseolina* infection partially (Figure 9.01).

For the best germination, the suitable temperature and durations were 54^{0}C for 20 minutes, 56^{0}C for 10, 15, and 20 minutes; and 58^{0}C for 10 and 15 minutes. After 58^{0}C for 15 minutes duration, the germination was depleted sharply with the increasing of temperature and period, albeit the infection did not appear any longer. Hence, in case of mungbean, for ensuring the highest germination and complete eradication of *M. phaseolina* infection, optimum temperature and duration were found as 54^{0}C for 20 minutes, 56^{0}C for 10 to 20 minutes and 58^{0}C for 10 to 15 minutes.

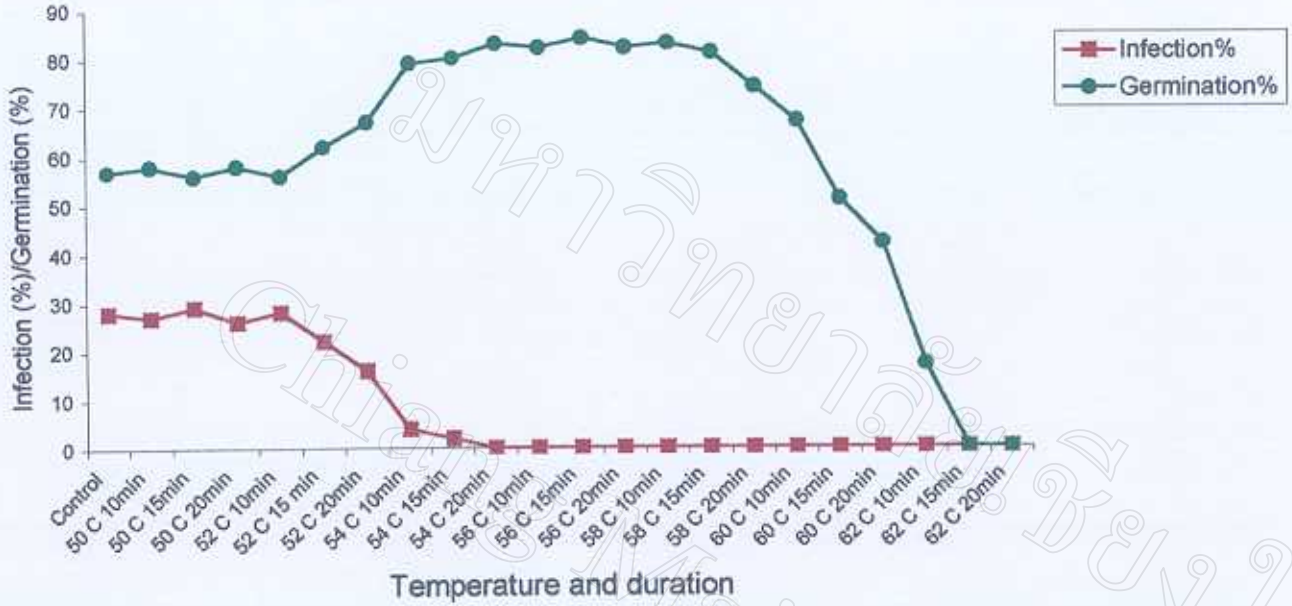

Figure 9.01: Effect of hot water treatment in different temperatures and durations for controlling seed borne *Macrophomina phaseolina* in mungbean seed.

9.1.3.2. Evaluation of hot water treated blackgram seed by blotter method

Figure 9.02 shows the results of hot water treatment of blackgram seeds in various temperatures and intervals. Regarding blackgram, 50^0C, and 52^0C in three durations like 10, 15 and 20 minutes could give significant change neither in germination nor in infection. Nevertheless, 54^0C and onwards provided infection declining including better germination. The absolute elimination of *M. phaseolina* infection appeared in 56^0C for 15 minutes and onwards. The maximum germination was found in 56^0C for 15 and 20 minutes, 58^0C for 10, 15 and 20 minutes. After 58^0C, the germination was coming down piercingly due to further temperature rising. Therefore, the most favorable temperature and duration for hot water treatment regarding blackgram seeds in order to complete removal of *M. phaseolina* infection including highest germination was revealed as 56^0C for 15 and 20 minutes, 58^0C for 10, 15 and 20 minutes.

9.1.3.3. Plantation of hot water treated seed in pot

Hot water treatment significantly reduced the diseased development and increased germination compared to untreated control (Table 9.01). In hot water treated seeds, 36.23 and 26.39 percent germination was increased in mungbean and blackgram respectively compared to untreated control treatment. Moreover, the treated seeds produced uniform and more vigorous seedlings (Plate 9.01 and 9.02). In the hot water treated seeds only 3.0 and 2.0 percent infection was appeared in mungbean and blackgram respectively which was decreased by 90.62 and 92.59 percent in mungbean and blackgram respectively, in comparison to control treatment. Finally, the healthy seedling production was increased by 37.31 and 30.43 percent in mungbean and blackgram respectively compared to control.

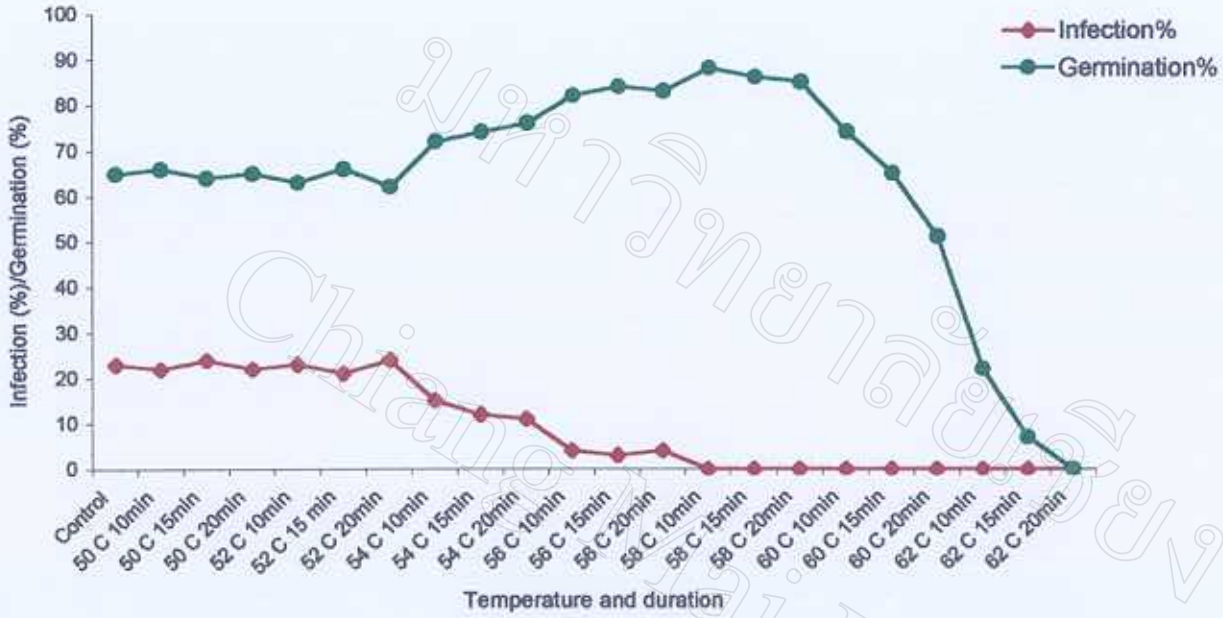

Figure 9.02: Effect of hot water treatment in different temperature and durations for controlling seed-borne *Macrophomina phaseolina* in blackgram seeds.

Table 9.01: Effect of hot water treatment on *Macrophomina phaseolina* infection and on germination when the seeds were planted in plastic pot with sterilized soil (mean of four replications).

Mungbean/ Blackgram	Germination (%)				Infection appeared (%)				Healthy seedlings (%)			
	Control	Hot water treated	Increase over control	LSD at 0.05	Control	Hot water treated	Decrease over control	LSD at 0.05	Control	Hot water treated	Increase over control	LSD at 0.05
Mungbean	69.0	94.0	36.23	5.95	32.0	3.0	90.62	6.87	67.0	92.0	37.31	7.23
Blackgram	72.0	91.0	26.39	5.95	27.0	2.0	92.59	4.64	69.0	90.0	30.43	5.66

Plate 9.01: Control treatment (without treated) and hot water treated (at most effective time and duration) seedlings of mungbean at the age of 8 days after plantation.

Plate 9.02: Control treatment (without treated) and hot water treated (at most effective time and duration) seedlings of blackgram at the age of 8 days after plantation.

9.1.4. Discussion

From the present investigation, it is unveiled that for mungbean and blackgram seed treatment so as to eradication of *M. phaseolina* infection as well as escalating of germination through hot water treatment is obviously effective. Incase of mungbean, the most impressive temperature and duration, which was lethal to *M. phaseolina* and enhance to maximum germination was found to be 54^{0}C for 20 minutes, 56^{0}C for 10, 15 and 20 minutes, and 58^{0}C for 10 and 15 minutes. Similarly, regarding blackgram, the suitable temperature and duration was 56^{0}C for 15 and 20 minutes, 58^{0}C 10, 15 and 20 minutes. However, for convenience, the recommended temperature, and period for hot water treatment can be 56^{0}C to 58^{0}C for 10 to 20 minutes for both mungbean and blackgram.

From the upshot of the present investigation, it is revealed that the hot water treatment in mungbean and blackgram seed not only eliminates the *M. phaseolina* infection but also improves the germination ability. Virtually when *M. phaseolina* infects the seed, the seed experiences germination reduction. Due to this reason, because of hot water treatment, the germination was increasing along with *M. phaseolina* infection reduction. In addition, when the hot water treated seeds planted in the pot, the seedlings were observed as more vigorous and uniform compared to control treatment. It is due to elimination of pathogen from the seed after hot water treatment.

Although pertinent literatures on hot water treatment in mungbean and blackgram for controlling *M. phaseolina* are not available, however, Sinha and Khare (1977) successfully controlled *M. phaseolina* infection in cowpea seeds by hot water treatment. They found 46^{0}C for 20 minutes was effective for *M. phaseolina* elimination in cowpea seeds. The effective temperature for cowpea and presently investing two crops (mungbean and blackgram) were not alike because these two types of seeds are not same. According to Grondeau and Samson (1994) the effective temperature and duration

depends on their seed structure, heat susceptibility of host such as moisture content, dormancy, age, vigor and conditions of external layers. In addition, they have reported that thermotherapy is not suitable for legume seed treatment. They mentioned thermotherapy was difficult for legumes like pea, bean, and soybean because a significant decrease in germination was found before the pathogen had been totally killed. Tripathi *et al.* (1987) also described that in chickpea seeds, *Ascochyta rabiei* was eradicated after 6-12 hours hot water treatment at 55 to 60^{0}C while more than 50 percent treated seed did not germinate. This finding does not support the outcome of present investigation. The probable reasons are the reported crops belong to legume but neither mungbean nor blackgram. Moreover, Tripathi *et al.* (1987) treated the seeds for very long period, which caused germination reduction. In the present investigation, duration of hot water treatment was maintained within 10 to 20 minutes, which did not show any adverse effect on germination of mungbean and blackgram seeds besides inducing germination. The germination increasing was due to reduction of pathogen as well as infection.

9.2. Chemical Seed Treatment by Seed-dressing Fungicides

9.2.1. An Overview

Chemical seed treatments with seed-dressing fungicides are applied to seeds to eliminate fungal inoculum and thereby to produce healthy seedling and crops. This may accomplish by killing or neutralizing seed-borne pathogens, and thus preventing transmission of disease. Chemicals are also applied to protect germinating seeds and emerging seedlings from soil-borne and air-borne pathogens. Very often eradicative and protective functions are necessary in a chemical seed treatment.

A considerable work has been employed for controlling *M. phaseolina* in different crops including mungbean and blackgram. However, very few chemicals are recommended in different countries for controlling *M. phaseolina* as seed-dresser. In Thailand, against this detrimental fungus in mungbean and blackgram only one chemical Benomyl 50 WP has been recommended so far (Watanasit and Thanomsub, 1995). So, there is a great need to conduct some work on chemical seed treatment of this fungus in mungbean and blackgram mainly to find out alternative chemicals for overcoming fungal resistance against prevailing fungicides and also to explore the efficacy of less hazardous products. In addition, to provide options to the farmers for the selection of fungicides based on cost-effectiveness and incase of one fungicide out of stock in the market, farmers can use another alternative. Therefore, the present investigation was undertaken with the following objective:

- To find out the effectiveness of the seed-dressing fungicides available in Thailand against *M. phaseolina* in mungbean and blackgram *in-vitro* and *in-vivo*.

9.2.2. Materials and Methods

9.2.2.1. *In-vitro* trial

Total six seed dressing fungicides namely Thiram, Metalexyl, Captan, Dithan M-45, Vitavax and Benlate were tested *in-vitro* in order to find out their relative efficacy against *M. phaseolina* causing charcoal rot dosease of mungbean and blackgram. These fungicides were weighed separately and mixed in the PDA medium under aseptic condition. The concentration of fungicides in the PDA medium was in three dosages like one was their normal recommended dose, another was below than their recommended dose, and other one was higher than their recommended dose (Appendix 9.01).

The fungicide mixed medium then poured in sterilized Petridish at the rate of 20ml per Petridish. When the fungicide mixed medium got solidified, a 3-day-old agar disk of 5mm diameter containing *M. phaseolina* (prepared as section 4.2.2) was placed in the middle of Petridish. For each fungicide, five Petridishes were prepared so as to keep five replications following the same manner. The control check was also maintained in the same way, but without pouring any fungicide in PDA. All the Petridishes were incubated under 12 hours alternating NUV light and darkness at 28^0C for 7 days. The radial growth of *M. phaseolina* was measured after 3 and 5 days. The radial growth regarding each fungicide was compared with control in order to find out the effectiveness of fungicide against *M. phaseolina*.

9.2.2.2. Seed treatment with screened effective fungicides

The seeds of mungbean (Chai Nat 60) and blackgram (Uthong 2) were treated with the effective fungicides (Benlate, Dithane M-45 and Thiram), which were found effective from *in-vitro* trial. For treatment, 0.15g fungicides were taken in a 250ml Erlenmayer flask containing 50g of seeds for each treatment. For uniform coating of fungicides on the seeds, the flasks were shaken for 15 minutes manually. Proper control with untreated seeds was maintained. The treated seeds along with untreated ones were used for evaluating the efficacy of fungicides after 24 hours of treatment.

9.2.2.3. Incubation of treated seeds by blotter method

All the treated and untreated seeds were analyzed by 'Blotter method'. In this method, three layers of blotter papers (Whatman no. 1) were soaked in sterilized water and placed on the sterilized glass Petridishes (9cm diameter). The randomly taken seeds were placed in 20 Petridishes using 10 seeds per Petridish. For each treatment, 4 replications were maintained while each replication contained 100 seeds. All the Petridishes with seeds were incubated at 28^0C under 12 hours alternating light and darkness. After

7 days the prevalence of *M. phaseolina* infection and germination of seeds were recorded.

9.2.2.4. Evaluation of treated seeds *in-vivo* (pot experiments)

The *in-vivo* experiment was conducted with the treated seeds with three effective fungicides including untreated control. The seeds of mungbean and blackgram were planted separately in plastic pot (size 12cm x 16cm) filled with sterilized soil. Two hundred seeds for each fungicidal treatment and 200 untreated seeds were shown in the plastic pots using 25 seeds per pot. Total 4 replications were maintained. After showing the seeds, the pots were kept in the glasshouse and watering was done whenever necessary. Germination and disease incidence was recorded until three weeks.

9.2.3. Results

9.2.3.1. *In-vitro* trial

The results (mean value of three dosages) of *in-vitro* trial of six seed-dressing fungicides namely Thiram, Metalexyl, Captan, Dithane M-45, Vitavax, and Benlate are presented in Figure 9.03. From this result it was found that among the 6 fungicides, only two viz. Benlate and Dithane M-45 were able to inhibit the growth of *M. phaseolina* absolutely when no any mycelial growth was appeared either after 3 or 5 days (Plate 9.03, 9.04, 9.05 and 9.06). Another fungicide Thiram could also able to reduce the growth of *M. phaseolina* encouragingly but not completely. Regarding Thiram, after 3 days and 5 days the radial growth of *M. phaseolina* was recorded by about 0.2cm and 0.5cm respectively (Plate 9.07 and 9.08). In case of remaining three fungicides viz. Metalexyl, Captan and Vitavax no any promising inhibition was noticed in neither 3 days nor 5 days of incubation. Albeit

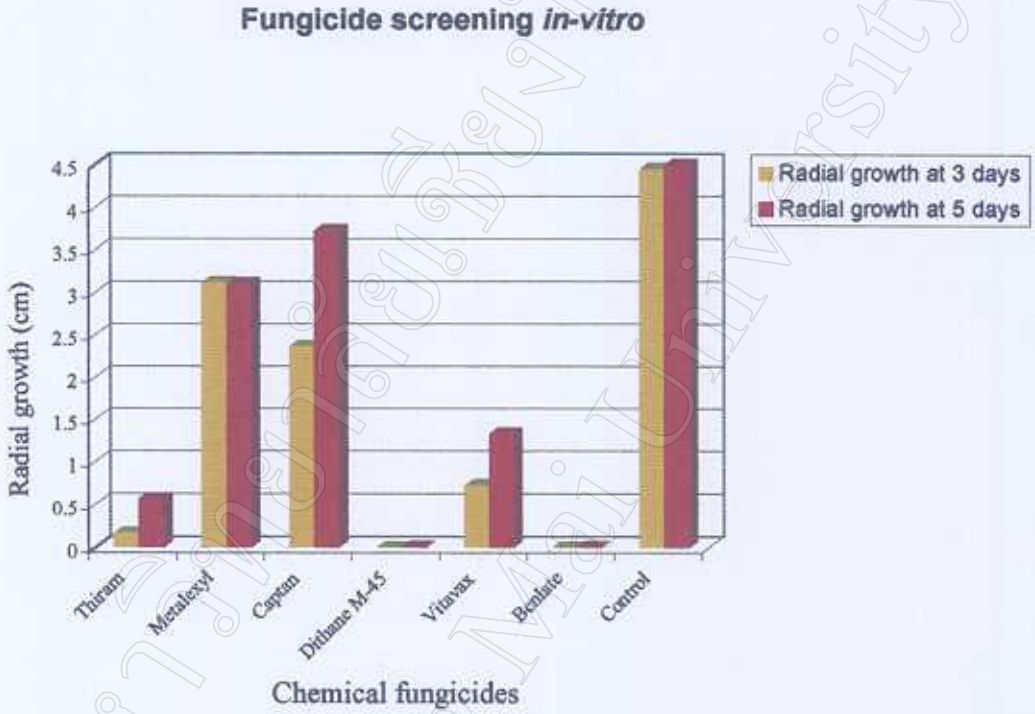

Figure 9.03: Percentage of decrease of radial growth of *Macrophomina phaseolina* over control in different fungicides.

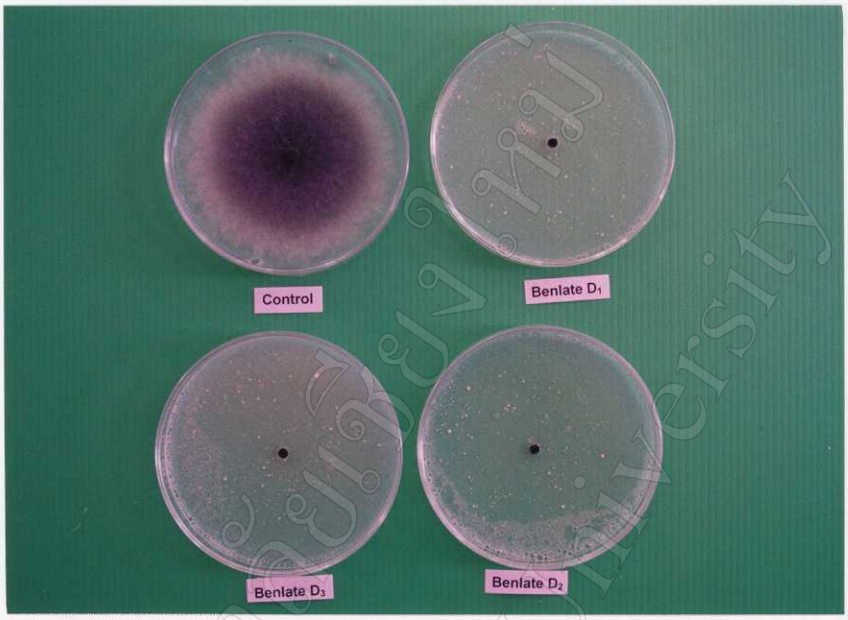

Plate 9.03: Radial growth of *Macrophomina phaseolina* on Benlate mixed PDA after 3 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

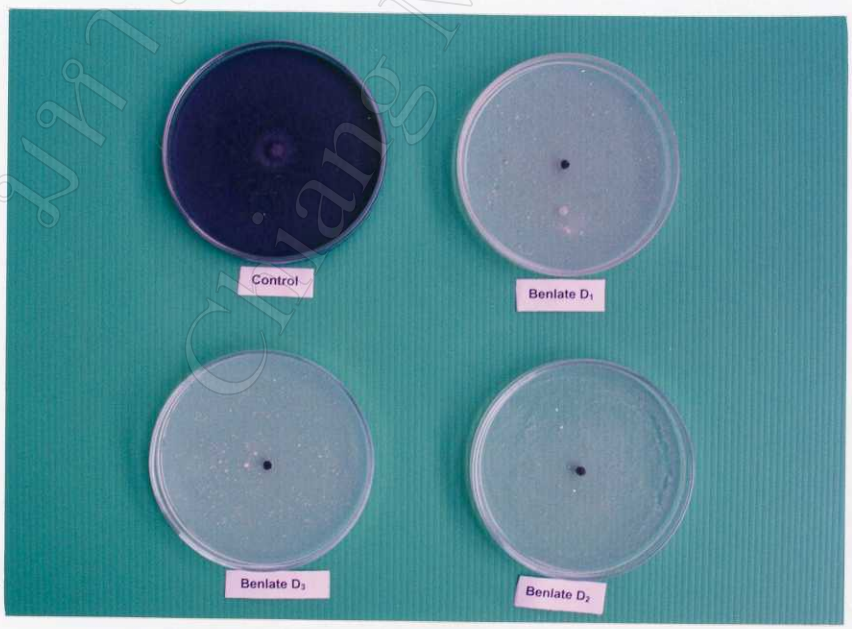

Plate 9.04: Radial growth of *Macrophomina phaseolina* on Benlate mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

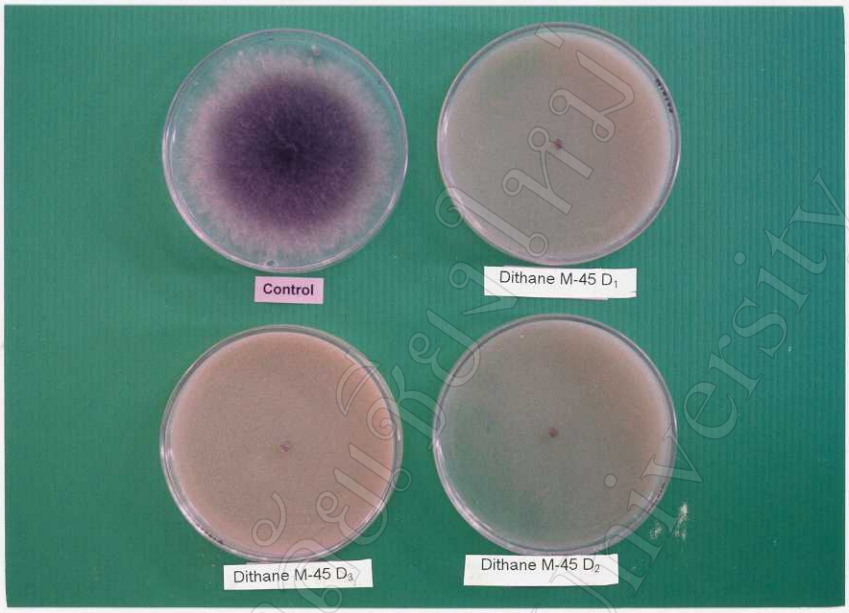

Plate 9.05: Radial growth of *Macrophomina phaseolina* on Dithane M-45 mixed PDA after 3 days of plating. D_1- below normal dose; D_2 - normal dose and D_3 - above normal dose.

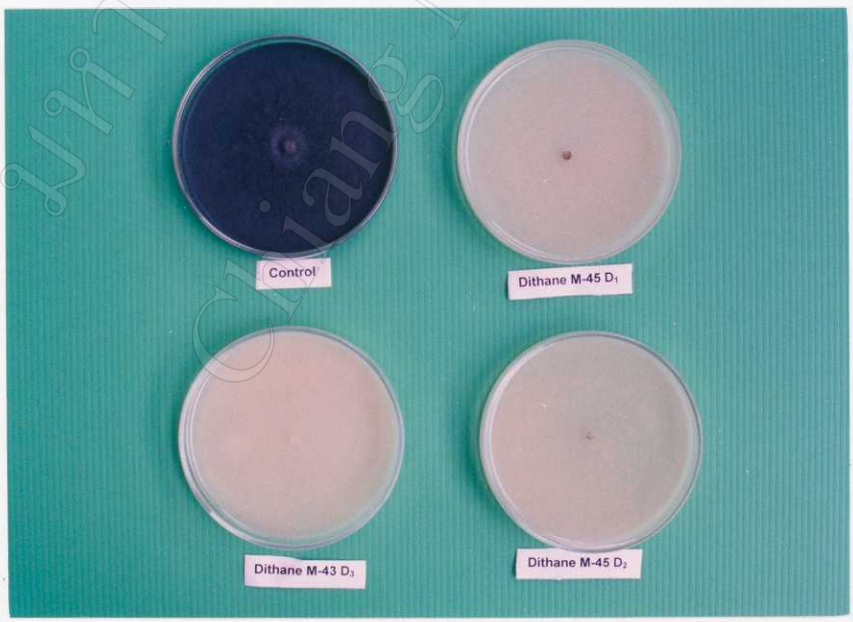

Plate 9.06: Radial growth of *Macrophomina phaseolina* on Dithane M-45 mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

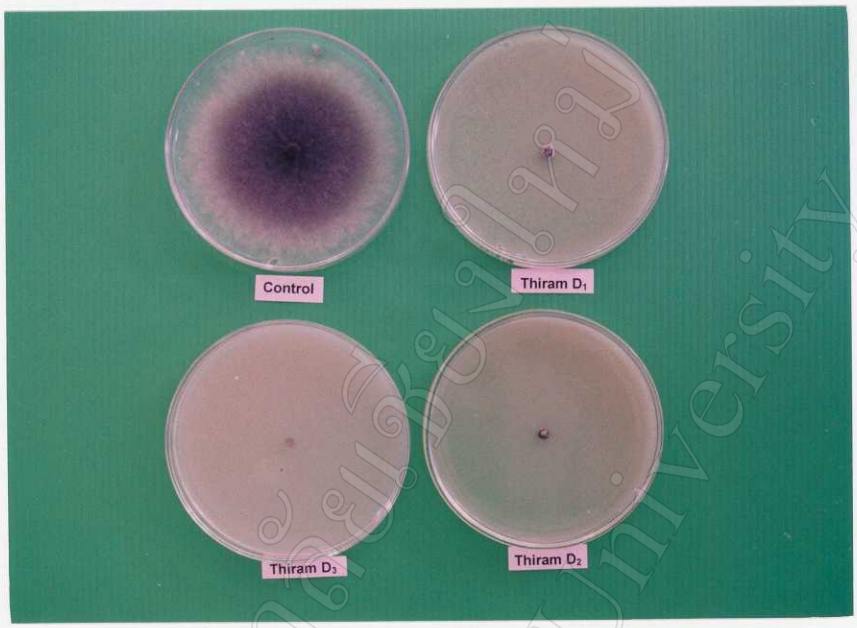

Plate 9.07: Radial growth of *Macrophomina phaseolina* on Thiram mixed PDA after 3 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

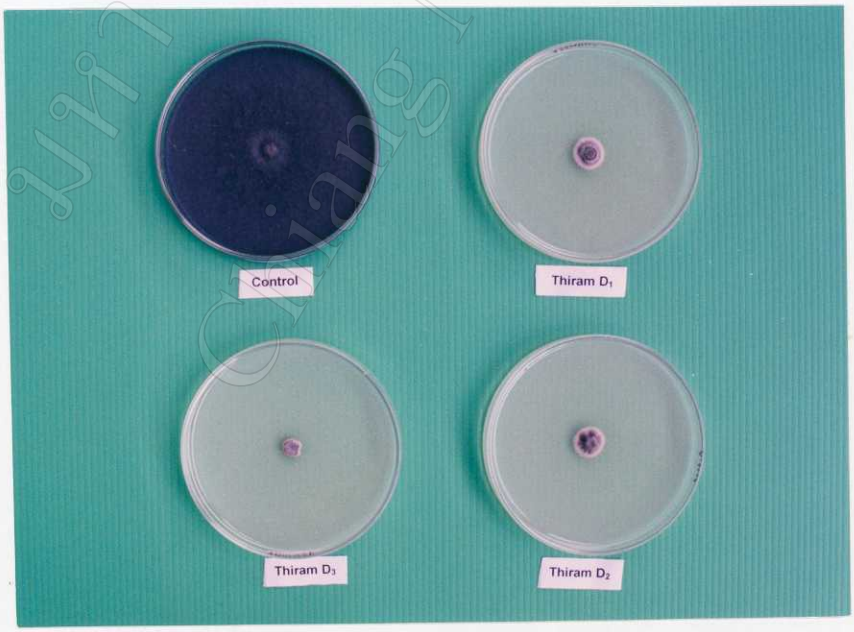

Plate 9.08: Radial growth of *Macrophomina phaseolina* on Thiram mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

Vitavax was able to keep the radial growth less than 1.0cm at 3 days of incubation, but in 5 days, it turned into nearly 1.4cm, which was not optimistic inhibition (Plate 9.09 and 9.10). With regard to inhibition by Captan and Metalexyl, nearly similar result was revealed. After 3 days of incubation, Captan and Metalexyl showed about 2.4cm and more than 3.0cm radial growth of *M. phaseolina* respectively. Moreover, after 5 days, their redial growth reached almost in 4cm (Plate 9.11, 9.12, 9.13 and 9.14).

9.2.3.2. Incubation of seeds with screened and effective fungicides by blotter method

After treatment of mungbean and blackgram seeds with screened and effective fungicides, the results on infection and germination percentages were presented in Table 9.02. From this result it was found that all three screened fungicides viz. Benlate, Dithane M-45 and Thiram were not only able to control the infection of *M. phaseolina* successfully but also could increase the germination ability significantly in both mungbean and blackgram. In the treated seed of mungbean and blackgram, regarding Benlate, Dithane M-45, and Thiram, no any infection was found whereas in control treatment 28.0 and 25.0 percent infection was evolved in mungbean and blackgram respectively. The germination increasing was appeared by 57.14 and 40.00 percent in mungbean and blackgram respectively when seed treatment was done by Benlate. Similarly, due to seed treatment with Dithane M-45, 55.36 and 41.54 percent germination was boost up in mungbean and blackgram respectively. In addition, regarding seed dressing with Thiram, 53.57 and 40.00 percent germination thriving was observed in munbean and blackgram respectively.

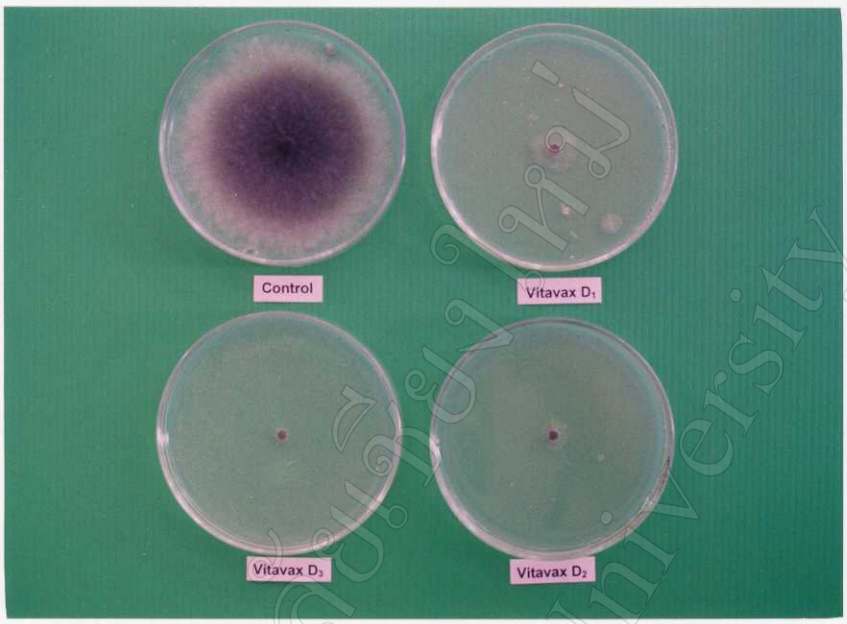

Plate 9.09: Radial growth of *Macrophomina phaseolina* on Vitavax mixed PDA after 3 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

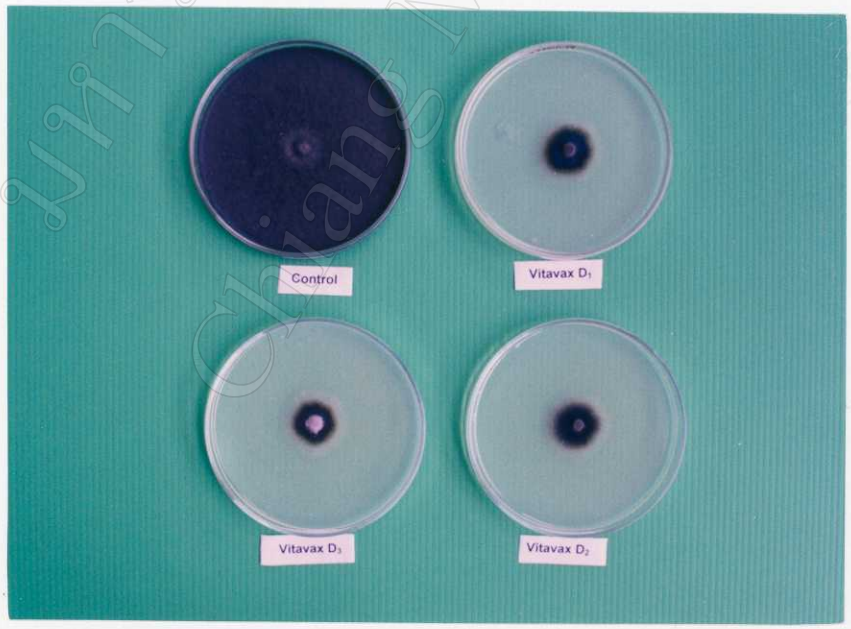

Plate 9.10: Radial growth of *Macrophomina phaseolina* on Vitavax mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

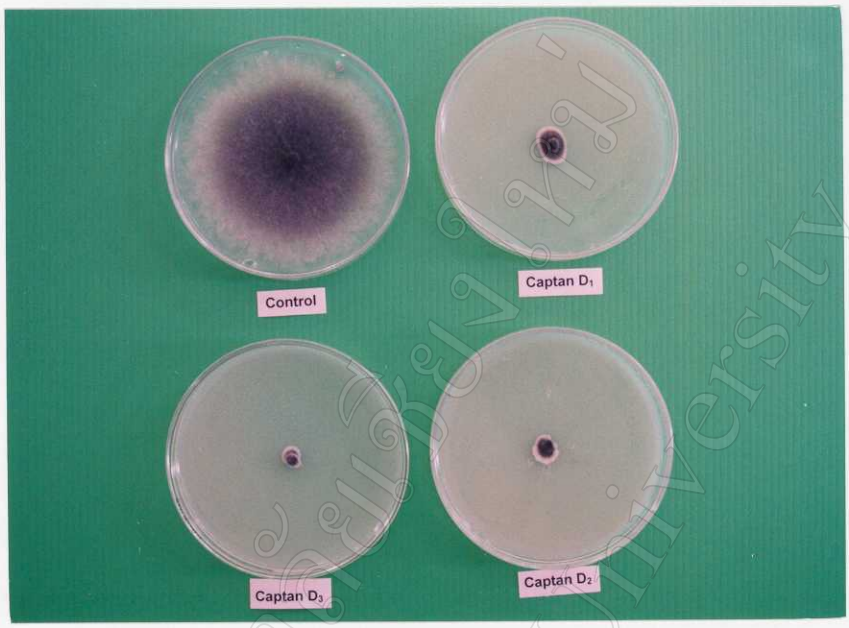

Plate 9.11: Radial growth of *Macrophomina phaseolina* on Captan mixed PDA after 3 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

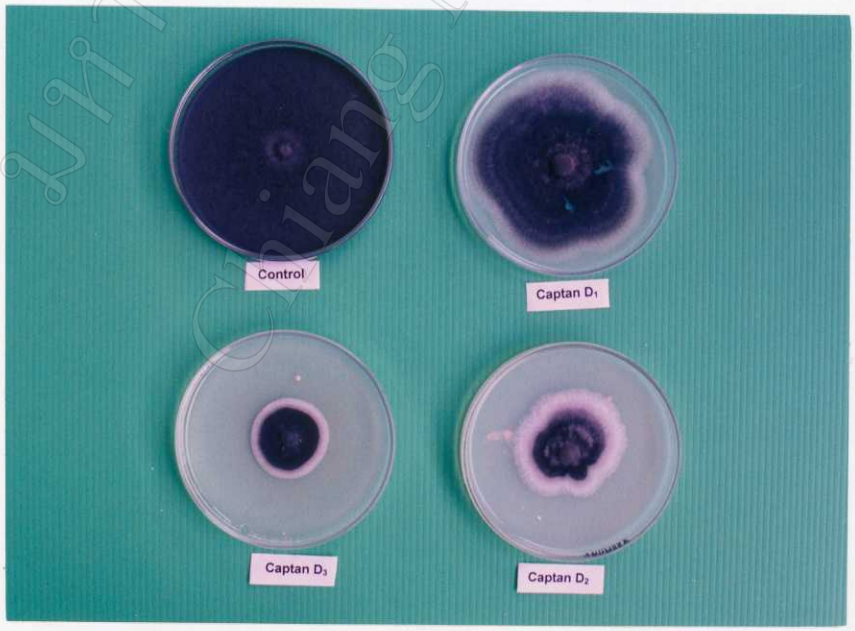

Plate 9.12: Radial growth of *Macrophomina phaseolina* on Captan mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

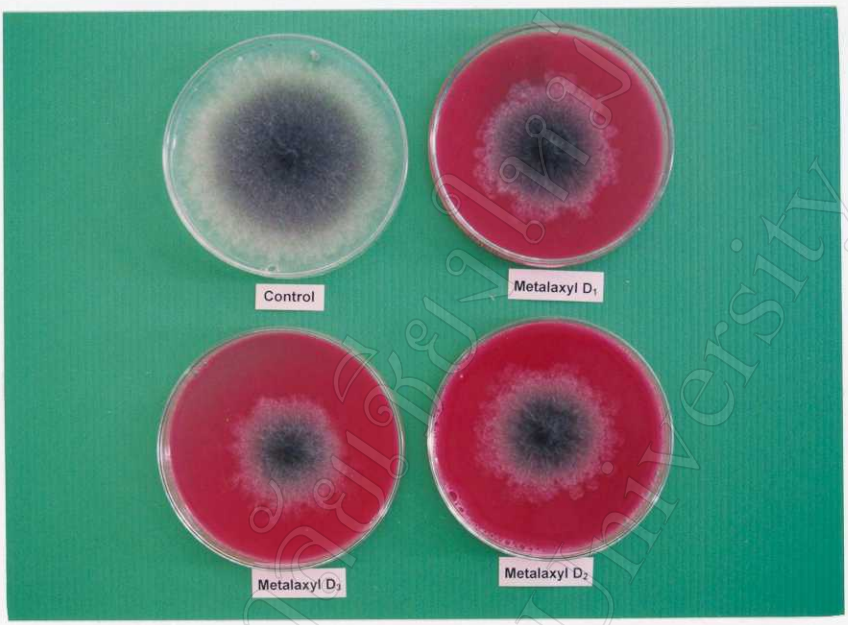

Plate 9.13: Radial growth of *Macrophomina phaseolina* on Metalexyl mixed PDA after 3 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

Plate 9.14: Radial growth of *Macrophomina phaseolina* on Metalexyl mixed PDA after 5 days of plating. D_1- below normal dose; D_2- normal dose and D_3- above normal dose.

Table 9.02: Effect of fungicidal seed treatment on *Macrophomina phaseolina* infection and germination according to blotter method with promising fungicides (results show the average of four replications).

Fungicides	Infection (%)		Germination (%)		Germination increased over control (%)	
	mungbean	Blackgram	mungbean	Blackgram	Mungbean	Blackgram
Benlate	0.0	0.0	88.0	91.0	57.14	40.00
Dithane M-45	0.0	0.0	87.0	92.0	55.36	41.54
Thiram	0.0	0.0	86.0	91.0	53.57	40.0
Control	28.0	25.0	56.0	65.0	-	-

9.2.3.3. Evaluation of treated seeds *in-vivo*

All the three tested fungicides (Benlate, Dithane M-45 and Thiram) significantly reduced the disease development and increased germination compared to that untreated control (Table 9.03). Complete control of disease was observed with Benlate and Dithane M-45 in both the tested crops. The significant disease control was also recorded in case of Thiram by 93.8 and 92.6 percent in mungbean and blackgram respectively. Again all tested three fungicides gave significantly higher germination than that of untreated control. The germination increased by 36.2 to 42.0 percent in mungbean and 26.4 to 33.3 percent in blackgram because of seed treatment. The highest germination (98.0 percent) was obtained in mungbean with Dithane M-45 followed by Benlate (97.0 percent) and Thiram (94.0 percent). In blackgram, Dithane M-45 and Benlated showed the highest germination (96.0 percent) followed by Thiram (91.0 percent). Due to seed treatment, significant number of healthy and vigorous seedlings was produced compared to untreated control (Plate 9.15, 9.16, 9.17, 9.18, 9.19 and 9.20). Increasing of healthy seedlings production after seed treatment was ranged from 37.3 to 46.3 percent in mungbean and 27.5 to 39.1 percent in blackgram (Table 9.03).

Table 9.03: Fungicidal seed treatment against *Macrophomina phaseolina* infection and germination planting the seed in plastic pot with sterilized soil (mean of four replications).

Name of Chemical	Germination (%)				Infected seedlings (%)				Healthy seedlings (%)			
	Mungbean	Increase over control	blackgram	Increase Over Control	mungbean	Decrease over control	blackgram	Decrease over control	mungbean	Increase over control	blackgram	Increase over control
Benlate	97.0	40.6	96.0	33.3	0.0	100.0	0.0	100.0	96.0	43.3	96.0	39.1
Dithane M-45	98.0	42.0	96.0	33.3	0.0	100.0	0.0	100.0	98.0	46.3	95.0	37.7
Thiram	94.0	36.2	91.0	26.4	2.0	93.8	2.0	92.6	92.0	37.3	88.0	27.5
Control	69.0	-	72.0	-	32.0	-	27.0	-	67.0	-	69.0	-
$LSD^{0.05}$	4.38	-	4.35	-	2.42	-	2.12	-	3.94	-	3.52	-

Plate 9.15: Comparison of control (untreated) and Benlate treated seedlings of mungbean at the age of 8 days after plantation.

Plate 9.16: Comparison of control (untreated) and Benlate treated seedlings of blackgram at the age of 8 days after plantation.

Plate 9.17: Comparison of control (untreated) and Dithane M-45 treated seedlings of mungbean at the age of 8 days after plantation.

Plate 9.18: Comparison of control (untreated) and Dithane M-45 treated seedlings of blackgram at the age of 8 days after plantation.

Plate 9.19: Comparison of control (untreated) and Thiram treated seedlings of mungbean at the age of 8 days after plantation.

Plate 9.20: Comparison of control (untreated) and Thiram treated seedlings of blackgram at the age of 8 days after plantation.

9.2.4. Discussion

From the *in-vitro* trial, it was inferred that among six fungicides (Thiram, Metalaxyl, Captan, Dithane M-45, Vitavex, and Benlate), Dithane M-45 and Benlate were found to be most effective against *M. phaseolina*. Another fungicide Thiram also showed promising inhibition on the radial growth of *M. phaseolina*.

After treating the seeds with these three screened fungicides (Dithane M-45, Benlate and Thiram), all fungicides were found highly effective and were able to eliminate the infection completely as well as improving germination according to blotter test and pot experiment compared to control.

The effectiveness of Benlate (Sinha and Khare, 1977; Reddy and Subbaya, 1981; Sharma, 1988; Watanasit and Thanomsub, 1995) and Thiram (Sinha and Khare, 1977; Reddy and Subbaya, 1981) against *M. phaseolina* was recorded earlier. However, Dithane M-45 was not mentioned so far. Although, Thiram was reported in various countries but in Thailand it was not recommended against *M. phaseolina* in mungbean as seed-dresser.

Therefore, Dithane M-45 and Benlate including Thiram can be used as seed treating chemical at the rate of 0.30 percent in order to control *M. phaseolina* in mungbean and blackgram.

9.3. Biological Seed Treatment by Biological Antagonists

9.3.1. An Overview

According to World Health Organization (WHO) estimates, approximately 7,50,000 people are taken ill every year with pesticide poisoning and up to 14,000 of these die in agony (Chengappa, 1989 in

Mukhopadhyay, 1994). Although the Third World uses one-sixth of the total pesticides produced globally, at least 37,500 people are poisoned yearly, 10,000 of them fatally. In the USA, some 300,000 farm-workers are affected by pesticide-related illness, such as giddiness, queasiness, pinpoint pupils, and severe skin rashes along with inflammations (Mukhopadhyay, 1994). Therefore, there is considerable public pressure and stress from environmental scientists to decrease emphasis on chemical control and use biological methods for controlling plant pests and diseases because chemical control of plant pests can be replaced by biological control. Pesticides are necessary at present but not a long-term solution to crop health. Besides their non-target effects and hazardous nature, pesticides become more expensive, and some are now loosing their effectiveness because of development of resistant strains. In this context, the increased emphasis on recourse to biological control of plant diseases is fully justified.

Biological methods mainly consist of using a microorganism by biological destruction, causing plant diseases without disturbing the ecological balance. The microorganisms used in biological control of plant diseases are termed as 'Antagonists' because it 'antagonizes', or interferes with, the target pathogen organism. 'Antagonism' is the balance wheel in nature. It operates through competition, parasitism, and antibiosis. Biological control is a natural phenomenon, nature's own way of keeping diseases from getting catastrophic.

Several antagonists have been found effective against plenty of plant pathogens, which cause destructive diseases of many plant crops. Many strains of *Trichoderma* have been considered as potential biocontrol agents against *M. phaseolina* in several hosts. Ehteshamul-Haque *et al.* (1992) reported that *Trichoderma harzinium* could significantly reduce root rot infection of lentil, which was caused by *M. phaseolina*. Farzana *et al.* (1991) found effectiveness of *Trichoderma harzianum* against *M. phaseolina* as a root infecting fungi of soybean. Antagonistic activity of *Trichoderma viride*, *T.*

harzianum and *T. koningii* was also reported against *Fusarium udum*, the causal agent of wilt of pigeon pea (Himani, 1996). Parasitic activity of *Trichoderma viride* on the sheath blight fungus of rice has been unveiled (Roy, 1977 in Mukhopadhyay, 1994). Harman *et al.* (1980) reveal that *Trichoderma hamatum* can effectively protect as fungicides to the seed and seedlings of radish and pea from *Pythium* spp. or *Rhizoctonia solani*.

In many crops, biological seed treatment provides longer protection to the crop compared to the fungicidal seed treatment. In addition, they offer benefits not obtainable with fungicidal seed protectants, especially the ability to colonize and protect the seed and germinating seedling as a whole (Mukhopadhyay, 1994). Harman *et al.* (1989) in Mukhopadhyay, (1994) observed excellent control of cotton, wheat, pea, and maize diseases by seed coating with *Trichoderma harzianium*. They noticed that biological seed treatments increased plant stand, reduced seedling mortality, and were as effective as the chemical fungicides. In India, biological seed treatment in tomato, potato, chickpea, lentil and peanut with *Trichoderma harzianum* and *Gliocladium virens* resulted an excellent protection against a wide range of pathogens like *Sclerotium rolfsii*, *Rhizotonia solani*, *Pseudomonas aphanidermatum* and *Fusarium oxysporum*, and the treatments were constantly as effective as or better than fungicidal seed treatment (Mukhopadhyay, 1989).

One unique feature of *Trichoderma spp.* as compared with classical fungicides, are their safety. *Trichoderma* preparations were feeding to cattle and no adverse effect was found (Mukhopadhyay, 1994). Bio Innovation AB, Sweden has been manufacturing *Trichoderma* preparation in the name of BINAB. No harmful consequence has been observed from feeding of BINAB to cattle in various forms, ranging from plain active ingredient to finished product, especially pellets (Richard and Highley, 1988 in Mukhopadhyay, 1994).

Despite the attractive possibility for controlling seed-borne and soil-borne fungi by using biological antagonists, very few works have been conducted and no any work has been done in Thailand regarding biological control of seed-borne *M. phaseolina* in mungbean and blackgram. Therefore, the efficacy and environmental concerns, as well as pathogen resistance to some pesticides, have encouraged research into biological control as an alternative approach or supplement to pesticides for protection against *M. phaseolina* in mungbean and blackgram with the following objectives:

- For screening the effective antagonists against *M. phaseeolina* of mungbean and blackgram *in-vitro*.
- To find out the efficacy of bio-control agents after seed dressing with the conidial suspension of effective biological antagonists.

9.3.2. Materials and Methods

9.3.2.1. Screening of effective antagonists in *in-vitro* against *M. phaseolina*

9.3.2.1.1. Isolation of *M. phaseolina*

Blackgram variety Uthong 2 was obtained from Chai Nat Field Crops Research Center, Thailand, which was carrying 24.0 percent natural infection of *M. phaseolina* according to blotter test. From this sample, 100 seeds were taken and surfaced sterilized by 10.0 percent sodium hypochlorite solution for three minutes. After that, the seeds were rinsed four times with sterilized distilled water. These seeds were transferred in sterilized glass Petridishes where three layers of soaked Whatman no. 1 blotter paper were placed earlier. In each Petridish, ten seeds were placed equidistantly in order to avoid contamination of incubated organisms. All the Petridishes were incubated at 25^0C under 12 hours alternating cycles of near ultraviolet light

(NUV) and darkness. After four days, the seeds, which manifested *M. phaseolina* symptoms like black sclerotia and pycnidia, were transferred singly in the center of other Petridishes containing 20ml sterilized solidified PDA medium. The transfer was done aseptically within laminar flow hood carefully to avoid contamination of other microorganisms. All seeded Petridishes were sealed with Nesco film and were kept under 12 hours alternating cycles of NUV light and darkness at 25^{0}C. After two days of incubation when the myceliua of *M. phaseolina* were grown, about 5mm disks were taken from the periphery of the colony by using a sterilized cork borer and then placed in an another sterilized PDA containing Petridish. These Petridishes were also sealed with Nesco film and incubated as before. After three days, the grown mycelia with blackish microsclerotia were used as pure culture in 'dual culture' against antagonists.

9.3.2.1.2. Collection and preparation of antagonistic agents

Six antagonistic agents namely *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *Pseudomonas aeruginosa*, *P. putida* and *Bacillus cereus* were obtained from the Royal Project Plant Pathology Laboratory, Chiang Mai, Thailand. All three *Trichoderma* spp. were transferred separately in sterilized PDA Petridishes and were kept for incubation as *M. phaseolina* incubation in 9.3.2.1.1. The bacterial antagonists (*Pseudomonas* spp. and *Bacillus* sp.) were mixed with 10ml sterilized distilled water individually and kept for three days. These fungal and bacterial antagonists were used in dual culture as antagonistic agents.

9.3.2.1.3. Dual culture

An agar disk of 5mm diameter was taken from the edge of 3-day-old PDA culture of *M. phaseolina* and was placed in the Petridish having 20ml solidified sterilized PDA, at 3cm apart from the periphery. Another agar disk of similar size and age of the test fungal antagonist (*Trichoderma* spp.) was

then placed 3cm away from the opposite side of the *M. phaseolina* disk. Bacterial antagonists (*Pseudomonas* sp. and *Bacillus* sp.) were streaked on PDA in the Petridish 3cm apart from agar disk of *M. phaseolina*. The control was maintained in the same manner without putting antagonists. For each antagonist, five pairing were prepared to maintain five replications. All the pairings including control were incubated under 12 hours alternating NUV light and darkness at 25^0C.

For quantifying antagonistic properties, the radius of *M. phaseolina* was measured at every 24 hours interval for five days till the radius of control reach to the edge of Petridish. Percent inhibition of radial growth (PIRG) of *M. phaseolina* was calculated by using the following formula:

$$PIRG = \frac{R1 - R2}{R1} x100$$

Where, R1 is the mycelial radius (in cm) of *M. phaseolina* in the control treatment (Figure 9.04); R2 is the mycelial radius (in cm) of *M. phaseolina* (Figure 9.05), which reflects antagonistic inhibition.

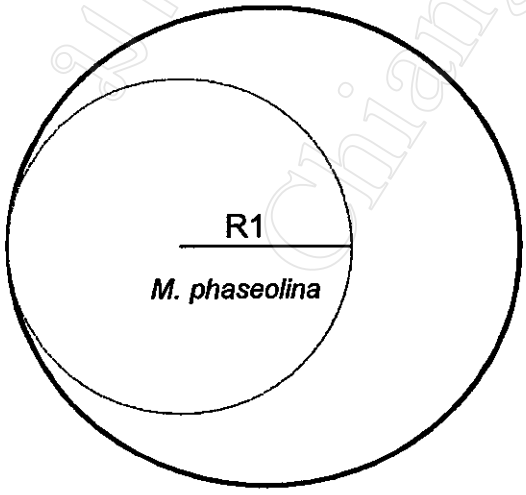

Figure 9.04: Radius of *Macrophomina phaseolina* as control

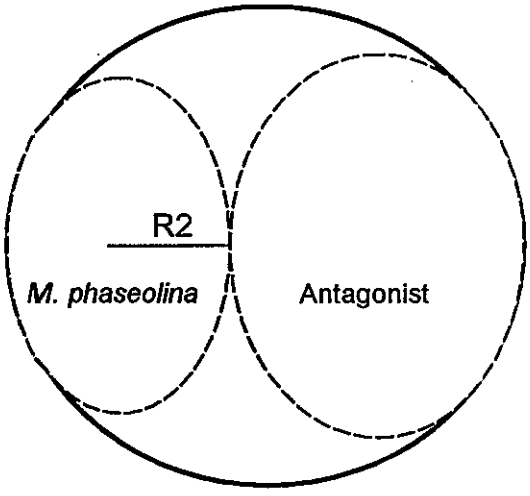

Figure 9.05: Radius of *Macrophomina phaseolina* after antagonism

After 7 days, a 5mm diameter of agar disk was taken from inhibited portion of *M. phaseolina*, and was transferred in the center of another sterilized PDA contained Petridish and incubated as before. After 3 days, the growing mycelia were observed and identified whether they were from *M. phaseolina* or from the respective antagonist.

9.3.2.1.4. Regression

Rate of inhibition of *M. phaseolina* by various antagonists was regressed against the age of incubation (in days).

9.3.2.1.5. Seed treatment with the screened antagonists

After *in-vitro* screening of antagonists, the effective antagonists were taken for seed dressing in order to observe the effectiveness of antagonists against *M. phaseolina* in mungbean and blackgram seeds (in the *in-vitro* screening, three species of *Trichoderma* i.e. *Trichoderma harzianum*, *Trichoderma hamatum* and *Trichoderma viride* found to be highly effective against *M. phaseolina* in Section 9.3.2.1.3).

9.3.2.1.5.1. Seed sample

For observing the effectiveness of screened antagonists against seed-borne *M. phaseolina*, one mungbean variety named Chai Nat 60 and one blackgram variety named Uthong 2 which were carrying respectively 29.0 and 24.0 percent natural infection of *M. phaseolina*, were taken.

9.3.2.1.5.2. Seed treatment

The screened fungal antagonists, who were incubated for seven days in PDA, were used for seed treatment. At first, the conidia of antagonists were collected from the PDA by scrapping with a sterilized knife and were mixed uniformly with 25ml sterilized distill water for each Petridish. The

spore concentration was 7.54 x 10^7 per ml. Thereafter, seeds of each variety were soaked for two hours with conidial suspension of antagonists separately. For control, seeds of each variety were soaked with sterilized distilled water for two hours.

9.3.2.1.5.3. Evaluation of treated seeds by blotter method

The treated seeds including control treatment were placed on 3-layered soaked Whatman no.1 blotter paper in sterilized Petridishes. In each Petridish, 10 seeds were placed equidistantly. After seven days, the appeared infection of *M. phaseolina* was examined including germination. The results of 10 Petridishes of each treatment were considered as one replication and total four replications were maintained for each treatment.

9.3.2.1.5.4. Evaluation of treated seeds *in-vivo* (pot culture)

The *in-vivo* experiment was conducted with the treated seeds with three effective antagonists (*Trichoderma* spp) including untreated control. The seeds of mungbean and blackgram were planted separately in plastic pot (size 12cm x 16cm) filled with sterilized soil. Two hundred seeds for each treatment and 200 untreated seeds were shown in the plastic pots using 25 seeds per pot. Total 4 replications were maintained. After showing the seeds, the pots were kept in the glasshouse and watering was done whenever necessary. Germination and disease incidence was recorded until three weeks.

9.3.3. Results

9.3.3.1. Screening of effective antagonists *in-vitro* against *M. phaseolina*

Results of dual culture for screening the effective antagonists against *M. phaseolina* in *in-vitro* were presented in Figure 9.06. From this figure it

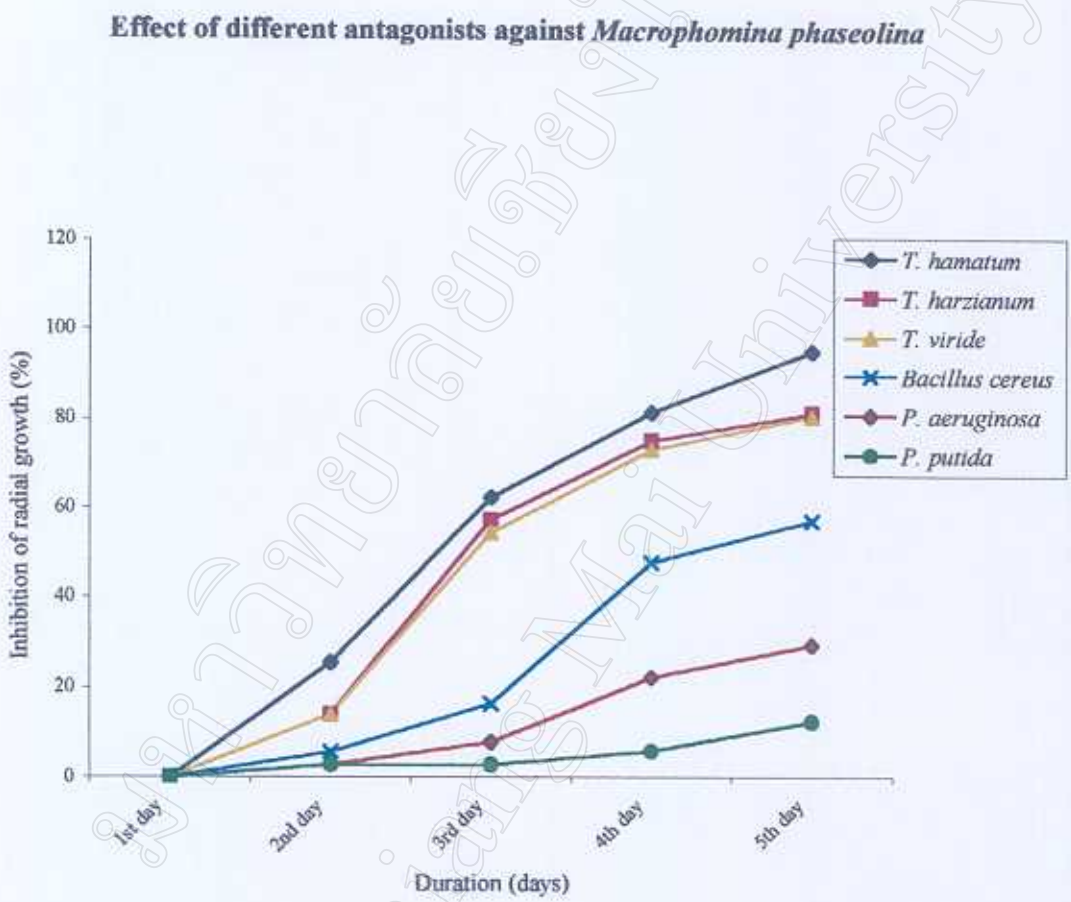

Figure 9.06: Inhibition percentage of radial growth of *Macrrophomina phaseolina* by different antagonists.

was revealed that all three *Trichoderma* sp. i.e. *Trichoderma harzianum*, *Trichoderma hamatum* and *Trichoderma viride* were found to be most effective among all (Plate 9.21 and 9.22). The bacterial antagonist i.e. *Pseudomonas* spp. and *Bacillus* sp. could not provide impressive effectiveness (Plate 9.23 and 9.24). Among three *Trichoderma* spp., *Trichoderma hamatum* provided the highest inhibition of *M. phaseolina*, which was more than 95 percent. The other two i.e. *Trichoderma harzianum* and *Trichoderma viride* showed almost similar inhibition, which was about 80 percent. On the other hand, among the bacterial antagonists, only *B. cereus* manifested about 60 percent inhibition while the other two like *P. aeruginosa* showed about 30 percent and 15 percent inhibition respectively.

All incubated agar disk from the *M. phaseolina* region, which reacted with *Trichoderma* spp., produced only respective *Trichoderma* mycelia when they cultured in PDA. No any *M. phaseolina* mycelium was observed. On the other hand, all disks from bacterial dual culture developed mycelia of *M. phaseolina* and no any bacteria was formed.

9.3.3.2. Regression

Figures 9.07, 9.08, 9.09, 9.10, 9.11, and 9.12 are showing the rate of inhibitions of *M. phaseolina* by different biological antagonists. The rate of inhibition by *T. hamatum* (Figure 9.07) was appeared as polynomial type of tendency with over 99.0 percent degree of determinations (R^2=0.9913). Linear fashion of inhibition was observed with very high degree of determination (R^2=0.9336) when inhibition rate of *M. phaseolina* was regressed against *T. harzianum* (Figure 9.08). Incase of *T. viride* (Figure 9.09), polynomial pattern of inhibition was showed with more than 96.0 percent degree of determination (R^2=0.9625). The inhibition rate was polynomially increased with above 96.0 percent degree of determination (R^2=0.9637) when inhibition was carried out against *Bacillus cereus* (Figure 9.10). Regarding *Pseudomonas aeruginosa* (Figure 9.11), also polynomial

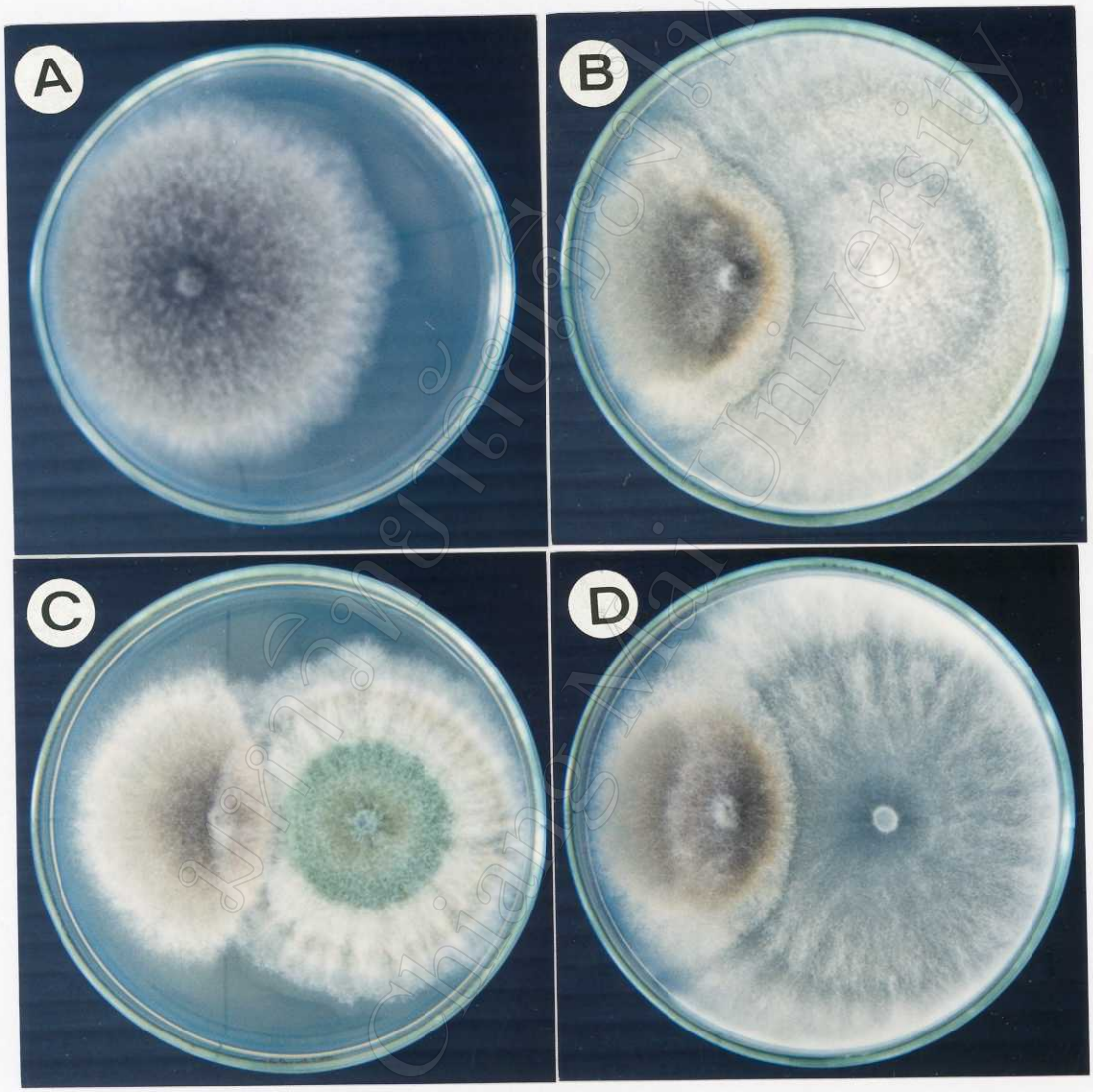

Plate 9.21: Control and dual culture of different *Trichoderma* spp. against *Macrophomina phaseolina* at 3rd day.

A. Radial growth of only *Macrophomina phaseolina* (control).
B. Dual culture of *Trichoderma harzianum* (right) against *Macrophomina phaseolina* (left).
C. Dual culture of *Trichoderma hamatum* (right) against *Macrophomina phaseolina* (left).
D. Dual culture of *Trichoderma viride* (right) against *Macrophomina phaseolina* (left).

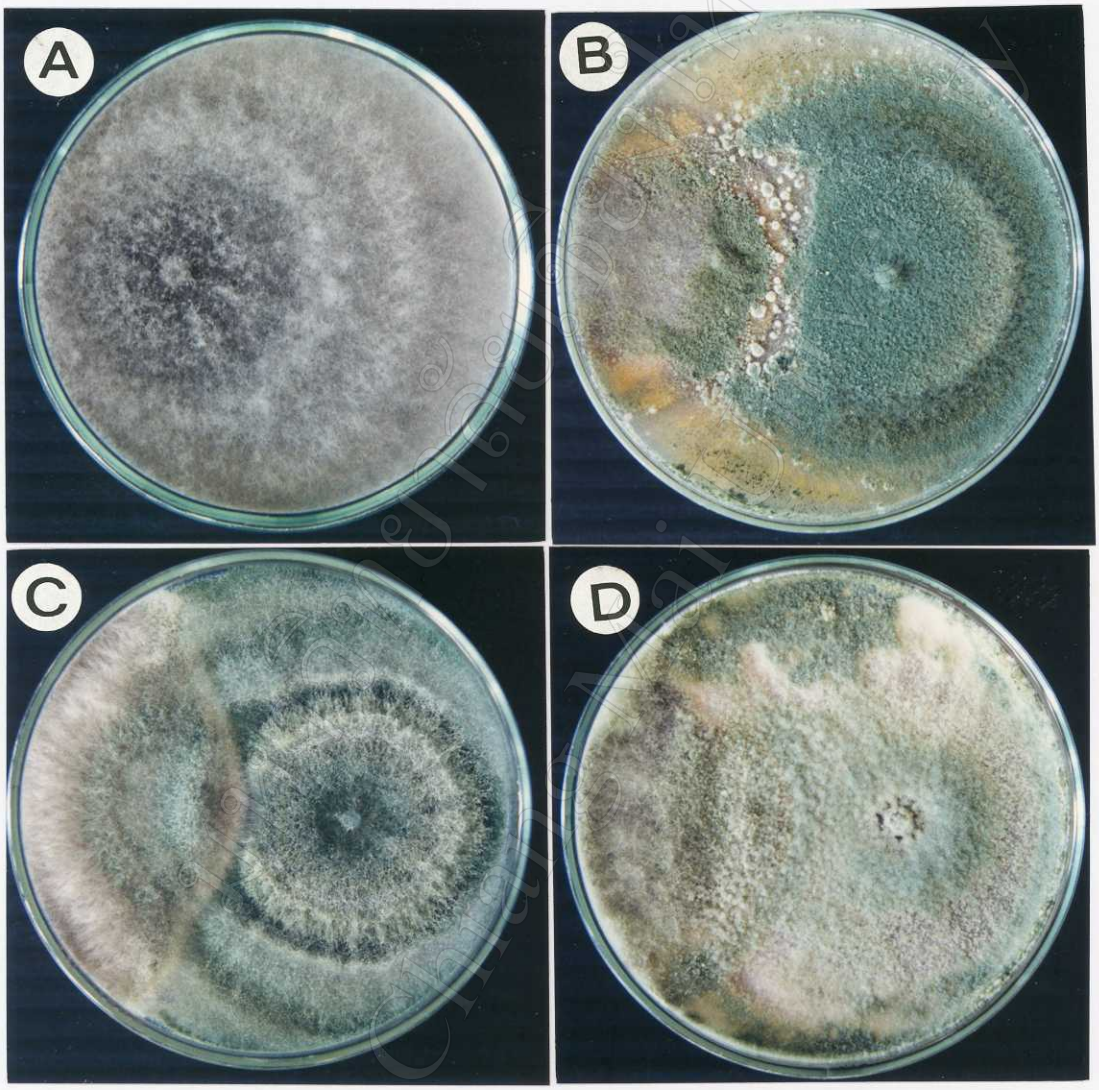

Plate 9.22: Control and dual culture of different *Trichoderma* spp. against *Macrophomina phaseolina* at 5th day.

A. Radial growth of only *Macrophomina phaseolina* (control).
B. Dual culture of *Trichoderma harzianum* (right) against *Macrophomina phaseolina* (left).
C. Dual culture of *Trichoderma hamatum* (right) against *Macrophomina phaseolina* (left).
D. Dual culture of *Trichoderma viride* (right) against *Macrophomina phaseolina* (left).

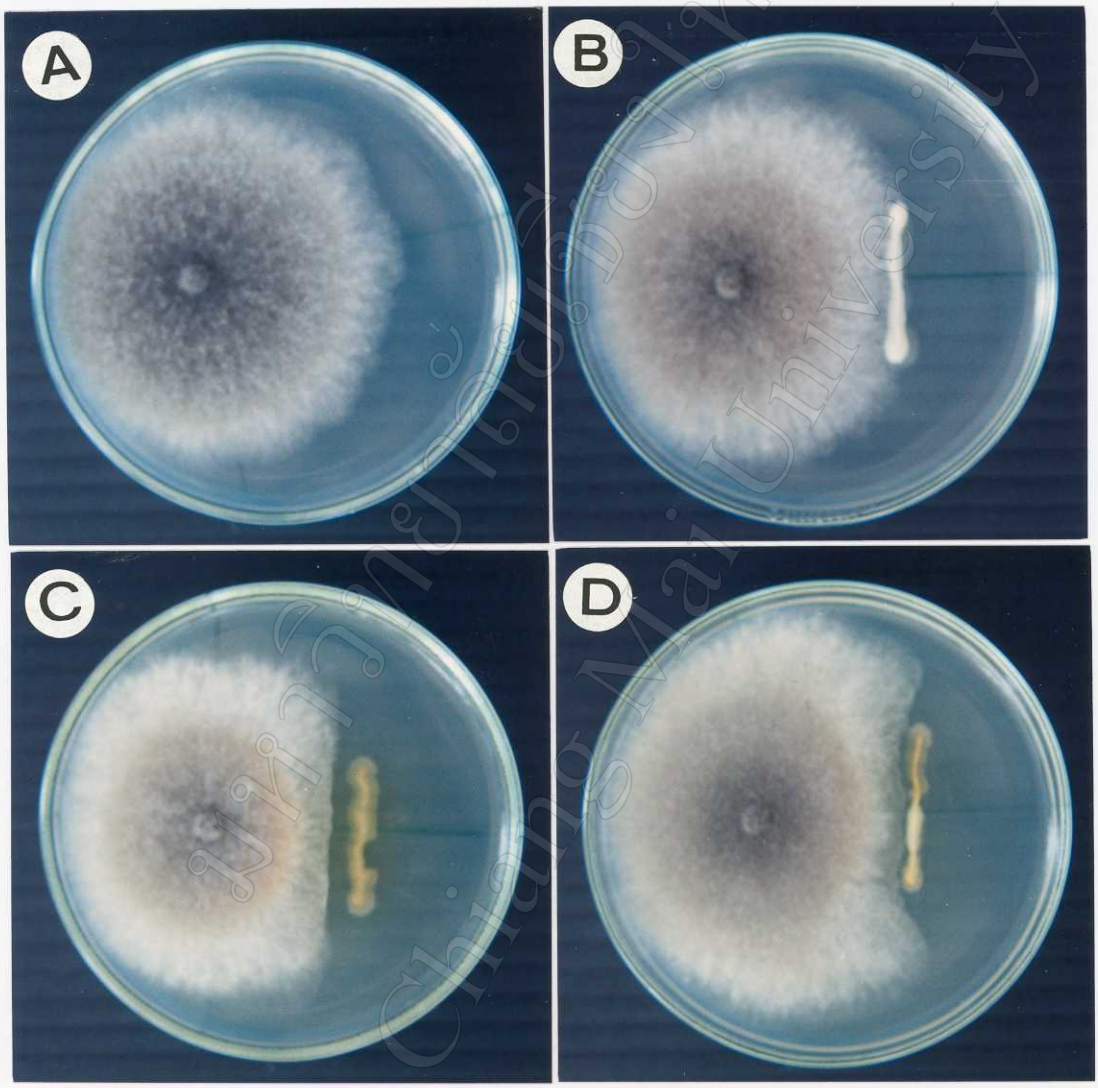

Plate 9.23: Control and dual culture of different bacterial antagonists against *Macrophomina phaseolina* at 3rd day.

A. Radial growth of only *Macrophomina phaseolina* (control).
B. Dual culture of *Bacillus cereus* (right) against *Macrophomina phaseolina* (left).
C. Dual culture of *Pseudomonas aeruginosa* (right) against *Macrophomina phaseolina* (left).
D. Dual culture of *Pseudomonas putida* (right) against *Macrophomina phaseolina* (left).

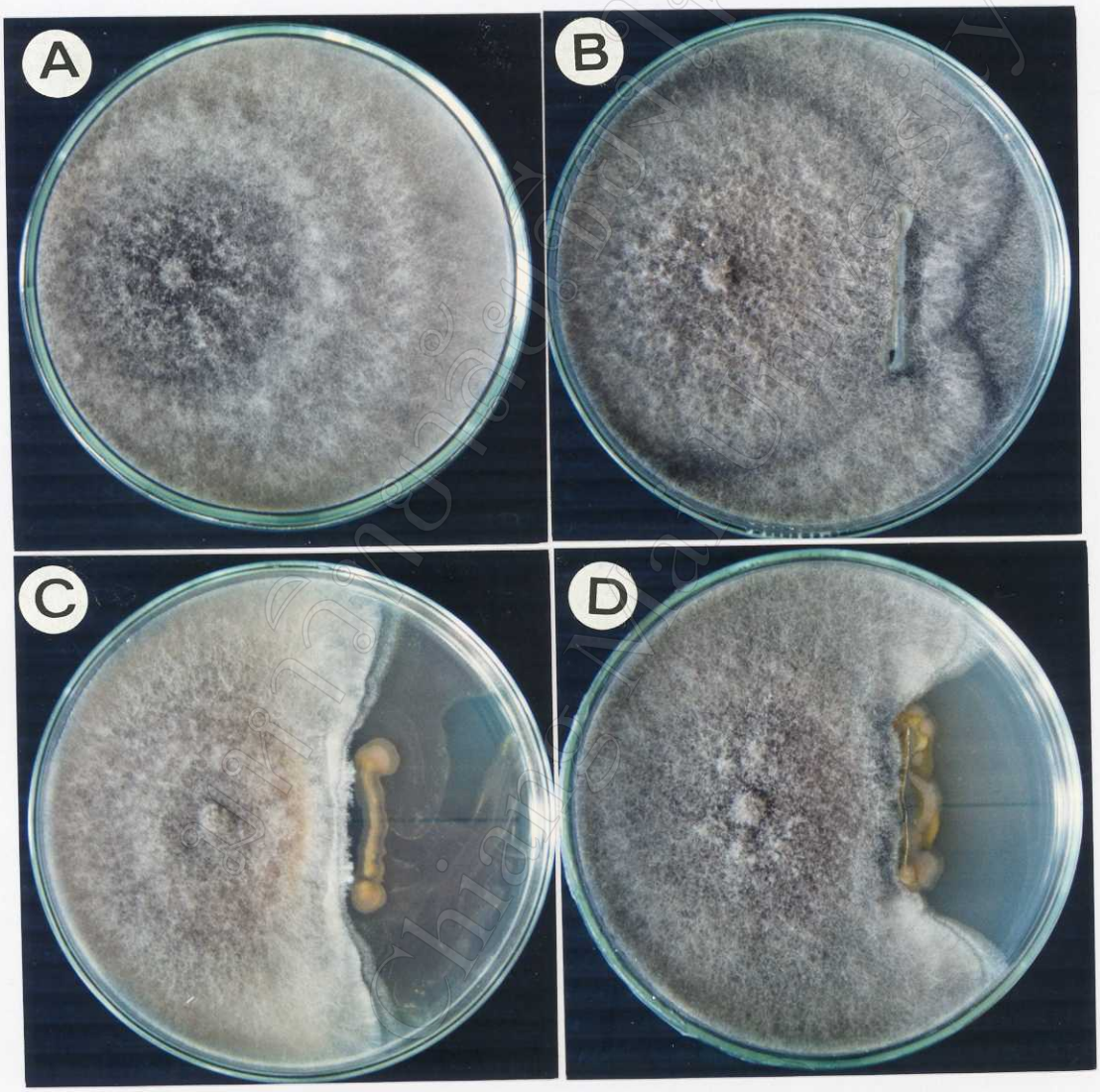

Plate 9.24: Control and dual culture of different bacterial antagonists against *Macrophomina phaseolina* at 5th day.

A. Radial growth of only *Macrophomina phaseolina* (control).
B. Dual culture of *Bacillus cereus* (right) against *Macrophomina phaseolina* (left).
C. Dual culture of *Pseudomonas aeruginosa* (right) against *Macrophomina phaseolina* (left).
D. Dual culture of *Pseudomonas putida* (right) against *Macrophomina phaseolina* (left).

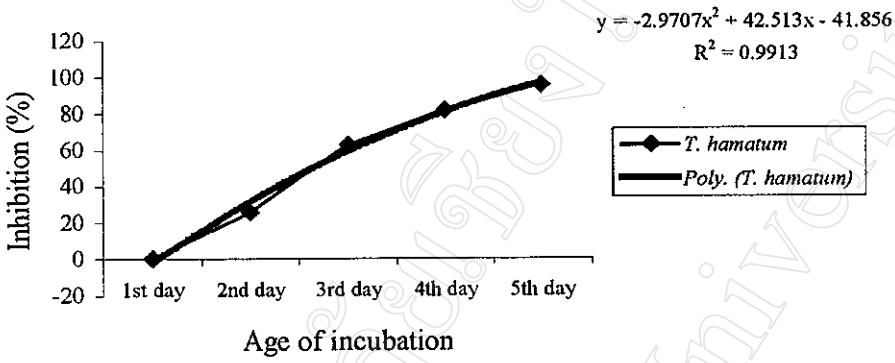

Figure 9.07: The trend of inhibition of *Trichoderma hamatum against Macrophomina phaseolina.*

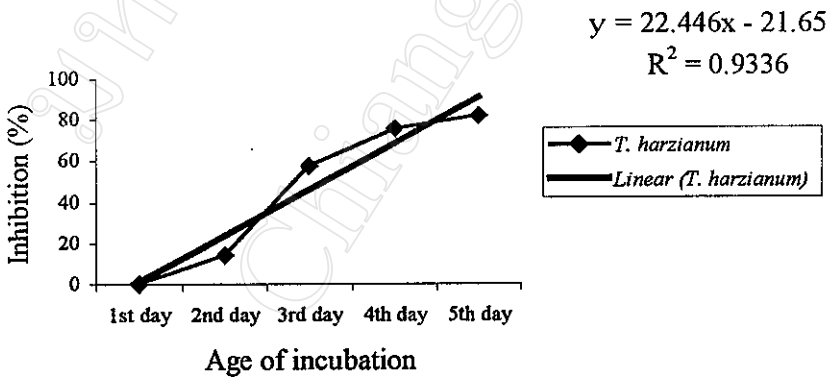

Figure 9.08: The trend of inhibition of *Trichoderma harzianum against Macrophomina phaseolina.*

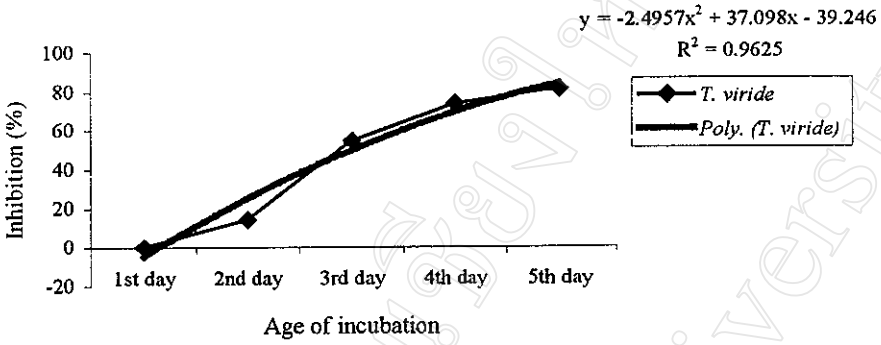

Figure 9.09: The trend of inhibition of *Trichoderma viride against Macrophomina phaseolina.*

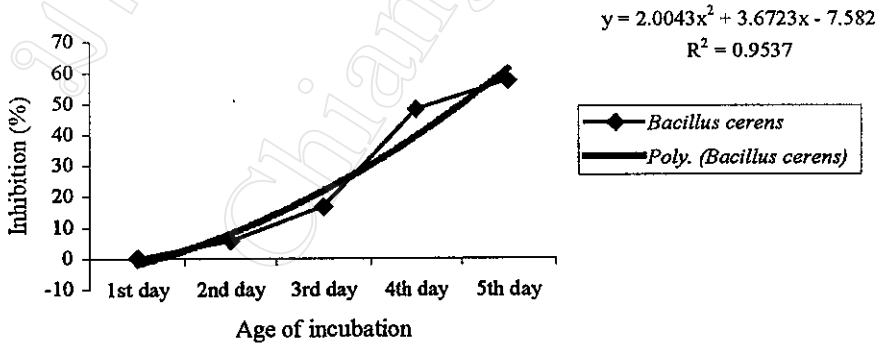

Figure 9.10: The trend of inhibition of *Bacillus cereus against Macrophomina phaseolina.*

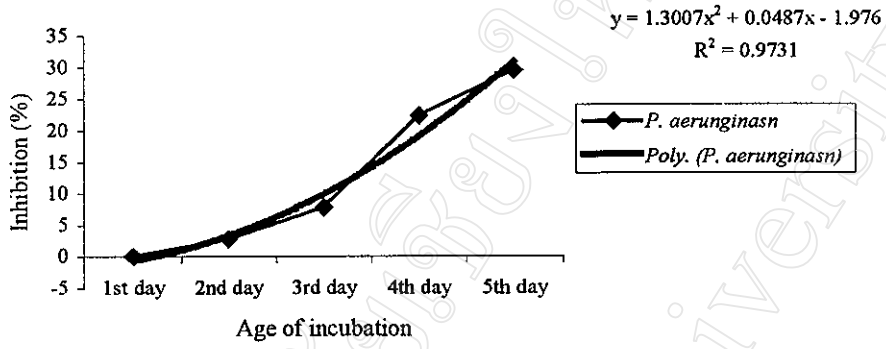

Figure 9.11: The trend of inhibition of *Pseudomonas aeroginosa* against *Macrophomina phaseolina.*

trend of inhibition was occurred with greater than 97.0 percent degree of determination (R^2=0.9731). Again polynomial tendency of inhibition was taken place incase of *P. putida* (Figure 9.12) with over 95.0 percent degree of determination (R^2=0.951).

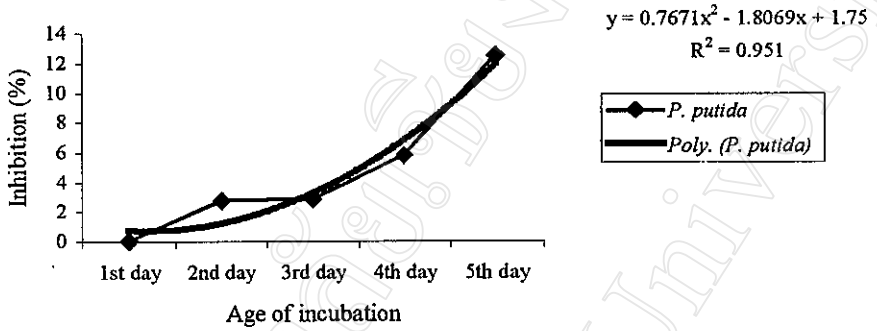

Figure 9.12: The trend of inhibition of *Pseudomonas putida against Macrophomina phaseolina.*

9.3.3.3. Seed treatment

9.3.3.3.1. Evaluation of treated seeds by blotter method

Table 9.04 showed the effect of seed coating with promising antagonists (*T. hamatum*, *T. harzianum* and *T. viride*) for controlling seed-borne *M. phaseolina* in mungbean and blackgram by blotter method. The results revealed that used all three *Trichoderma* spp. were able to control seed-borne *M. phaseolina* in mungbean and blackgram completely. After coating the seed with the conidial suspension of *Trichoderma* spp, no any infection was appeared either in mungbean or in blackgram. Simultaneously, the germination percentage was increased fascinatingly. In addition, from the treated seed of all antagonists, robust seedlings were produced.

Table 9.04: Seed coating with promising antagonists for controlling *Macrophomina phaseolina* in mungbean and blackgram (results show the average of four replications).

Antagonists	Infection (%)		Germination (%)		Germination increased over control (%)	
	Mungbean	Blackgram	Mungbean	Blackgram	Mungbean	Blackgram
Trichoderma harzianum	0.0	0.0	83.0	85.0	48.21	30.77
Trichoderma hamatum	0.0	0.0	85.0	88.0	51.79	35.38
Trichoderma viride	0.0	0.0	81.0	84.0	44.64	29.23
Control	28.0	25.0	56.0	65.0	-	-

Due to seed treatment with *T. hamatum*, 51.79 and 35.38 percent germination was augmented in mungbean and blackgram respectively. Incase of treatment with *T. harzianum*, germination increased by 48.21 and 30.77 percent in mungbean and blackgram respectively. Moreover, when seed treatment was done with *T. viride*, enhancement of germination was manifested by 44.64 and 29.23 percent respectively in mungbean and blackgram.

9.4.3.3.2 Evaluation of treated seeds in *in-vivo* (pot culture)

When the coated seed with conidia of three *Trichoderma* spp. were planted in the sterilized soil in plastic pot, infected seedling production was reduced almost completely (Table 9.05). Moreover, not only the germination was increased noticeably but also robust seedlings were produced from the treated seeds with all antagonists compared to control (Plate 9.25, 9.26, 9.27, 9.28, 9.29 and 9.30).

Table 9.05: Biological seed treatment with *Trichoderma* sp. against *Macrophomina phaseolina* infection and germination planting the seed in plastic pot with sterilized soil (mean of four replications).

Name of antagonists	Germination (%)				Infected seedlings (%)				Healthy seedlings (%)			
	Mungbean	Increase over control	blackgram	Increase over control	mungbean	Decrease over control	blackgram	Decrease over control	mungbean	Increase over control	blackgram	Increase over control
Trichoderma harzianum	96.0	39.13	95.0	31.94	2.0	93.75	2.0	92.59	94.0	40.30	93.0	34.78
Trichoderma hamatum	97.0	40.58	96.0	33.33	0.0	100.00	0.0	100.00	96.0	43.28	94.0	36.23
Trichoderma viride	93.0	34.78	92.0	27.78	2.0	93.75	2.0	92.59	92.0	37.31	90.0	30.43
Control	69.0	-	72.0	-	32.0	-	27.0	-	67.0	-	69.0	-
$LSD^{0.05}$	3.31	-	3.09	-	2.90	-	2.72	-	3.75	-	2.81	-

Plate 9.25: Comparison of control (untreated) and *Trichoderma harzianum* treated seedlings of mungbean at the age of 8 days after plantation.

Plate 9.26: Comparison of control (untreated) and *Trichoderma harzianum* treated seedlings of blackgram at the age of 8 days after plantation.

Plate 9.27: Comparison of control (untreated) and *Trichoderma hamatum* treated seedlings of mungbean at the age of 8 day after plantation.

Plate 9.28: Comparison of control (untreated) and *Trichoderma hamatum* treated seedlings of blackgram at the age of 8 days after plantation.

Plate 9.29: Comparison of control (untreated) and *Trichoderma viride* treated seedlings of mungbean at the age of 8 days after plantation.

Plate 9.30: Comparison of control (untreated) and *Trichoderma viride* treated seedlings of blackgram at the age of 8 days after plantation.

In mungbean, about 40.0 percent germination increased due to seed treatment with *T. harzianum* and *T. hamatum*. *T. viride* also increased germination about 35.0 percent. Similarly regarding blackgram, about 27.00 to 33.00 percent germination enhanced after seed treatment with three *Trichoderma* spp (Table 9.05).

In both mungbean and blackgram seeds, 92.59 to 100.00 percent infections was reduced as a result of seed treatment with conidial suspension of three *Trichoderma* spp. In addition to reduction of infection and increasing germination, *Trichoderma* treated seeds produced more vigorous seedlings compared to control treatment. Incase of mungbean and blackgram, the healthy seedling production ranged from 37.31 to 43.28 percent and 30.43 to 36.23 percent respectively (Table 9.05).

9.3.4. Discussion

The result of *in-vitro* screening of different fungal and bacterial antagonists revealed that only fungal antagonists (3 species of *Trichoderma*) could able to inhibit *M. phaseolina* outstandingly. The bacterial antagonists could not give impressive inhibition. Among three *Trichoderma* species, *T. hamatum* provided excellent inhibition, which was more than 95.0 percent. In case of the other two species of *Trichoderma*, i.e. *T. harzianum* and *T. viride* also found to be admirable antagonist against *M. phaseolina*.

The *in-vitro* inhibiting character of these *Trichoderma* species against different fungal pathogens has been widely reported by various authors. *T. harzianum* described as effective antagonist against *Macrophomina phaseolina* (Alagarsamy and Sivaprakasam, 1988; Deshmukh and Raut, 1992); *Sclerotium rolfsii* (Elad *et al.*, 1983; Upadhyay and Mukhopadyay, 1986; Ferrata and D'Ambra, 1985; Mutto *et al.*, 1986: Lim and Teh, 1990; Iqbal *et al.*, 1995; Haran *et al.*, 1996); *Fusarium*

oxysporum (Calvet *et al.*, 1990; Dhedhi *et al.*, 1990; Begum *et al.*, 1998; Deshmukh and Raut, 1992; Basher and Bharat, 1994); *F. moniliforme* (Deshmukh and Raut, 1992); *F. udum* (Sumitha and Gaikwad, 1995; Himani, 1996) *Colletortrichum gloeosporioides* (Deshmukh and Raut, 1992); *Curvularia lunata* (Deshmukh and Raut, 1992); *Rhizoctonia solani* (Elad *et al.*, 1980); *Sclerotinia sclerotium* (Sharma and Singh, 1990; Singh, 1991).

The *in-vitro* experiments of *T. hamatum* also showed impressive antagonistic effect against *Sclerotium rolfsii* (Chet and Baker, 1981; Elad *et al.*, 1983); *R. solani* (Elad *et al.*, 1983; Lim and Teh, 1990).

Similarly, *T. viride* gave incredible suppression against *M. phaseolina* (Alagarsamy and Sivaprakasam, 1988; Deshmukh and Raut, 1992); *Sclerotium rolfsii* (Deb, 1990; Iqbal *et al.*, 1995); *Fusarium oxysporum* (Dhedhi *et al.*, 1990; Deshmukh and Raut, 1992; Velikanov *et al.*, 1994); *F. solani* (Velikanov *et al.*, 1994); *F. molniliforme* (Deshmukh and Raut, 1992); *F. udum* (Mehta *et at.*, 1995; Himani, 1996); *R. solani* (Velikanov *et al.*, 1994); *C. gloeosporioides* (Deshmukh and Raut, 1992); *C. lunata* (Deshmukh and Raut, 1992); *Sclerotinia aclerotiorum* (Velikanov *et al.*, 1994); *Pythium* sp. (Velikanov *et al.*, 1994).

The evolving of *Trichoderma* mycelium instead of *M. phaseolina* from agar disks of 5 days old *M. phaseolina* growing portion (from dual culture) proved that all used three *Trichoderma* spp. were able to suppress the growth of *M. phaseolina* successfully. The mechanism of *Trichoderma* sp. against fungal pathogen was explained earlier by several scientists. Elad *et al.*, (1983) explained that the hyphae of *T. harzianum* and *T. hamatum* got contacted with the pathogen in two ways like producing appressorium like bodies or coiling around the hyphae followed by digestion of host cell walls enzymatically. Jacobs and Kamoen (1986), Sivan and Chet (1989) reported that *T. harzianum* produced cell wall

lysing enzymes, which worked as antagonistic property against plant pathogens. In dual culture, hyphal coiling, entry through haustorial-like structures, and direct entry of *T. harzianum* into the hyphae and sclerotia of pathogen have been reported (Upadhyay and Mukhopadhyay, 1986). Lim and Teh (1990) observed the inhibition of test organism was occurred by the production of both diffusible and volatile metabolites. They also observed hyphal coiling and production of appressoria and hooks by the *Trichoderma* spp. As a result, host cells exhibited vacuolation, granulation, coagulation, disintegration, and lysis.

When the inhibition percentage of *M phaseolina* was regressed against the age of incubation (in days) of various antagonists, the rate of inhibition showed polynomial pattern of growth in case of all antagonists except *T. harzianum*. *T. harzianum* showed linear type of tendency. In all cases of antagonists, very high (over 90 percent) degree of determination was found.

From the present investigation, it is revealed that mungbean and blackgram seed treatment with *T. hamatum*, *T. harzianum* and *T. viride* is not only able to reduce the seed-borne *M. phaseolina* infection but also increase the seed germination remarkably over the untreated control. The exciting effectiveness of seed Treatment with *Trichoderma* spp. against seed-borne *M. phaseolina* in various crops was widely supported by a variety of workers. There are reports that biological antagonists applied to seeds not only have the potential protecting the seed but also bring a protective effect against root infecting soil-borne pathogens being the initial colonizers of the root (Windels, 1981 in Hussain *et al.*, 1990). Alagarsamy and Sivaprakasam, (1988) found the reduction of seed mortality of cowpea due to *M. phaseolina* besides enhancing the growth of plant after pilleting the seeds with *T. viride*. Seed treatment of sunflower and mungbean with *T. harzianum* reduced charcoal rot disease successfully caused by *M. phaseolina* (Hussain *et al.*, 1990). Significant

reduction of *M. phaseolina* infection was found in soybean when seed treatment was done with *T. harzianum* (Farzana *et al.*, 1991). Root rot of lentil caused by *M. phaseolina* was controlled by *T. harzianum* (Ehteshamul-Haque *et al.*, 1992). Ehteshamul-Haque and Gaffar (1995) reported that *T. harzianum*, *T. hamatum* and *T. viride* can reduce *M. phaseolina* infection in soybean through seed treatment.