Chapter VI

6. Seed Transmission of *Macrophomina phaseolina* in Mungbean and Blackgram

6.1. An Overview

Seed transmission of a pathogen implies that infected or contaminated seeds are a source of the pathogen for transmission to subsequent plantings (McGee, 1988); or else, the result of transferring inoculum from infected seeds to the germinating seeds and seedlings is seed transmission (Maude, 1996). Establishment or development of an infection within a seedling or subsequent plant is the last decisive link in the process of seed transmission, and this link can only be established if completion of the infection process has been positively demonstrated to the exclusion of other means of transmission (Neergaard, 1979).

Many species of fungi under various genera are transmitted by seed in several hundreds plant host. *Macrophomina phaseolina* has been reported as a seed transmitted fungus in many crop plants like blackgram (Sharada and Shetty (1987), soybean (Gangopadhyay *et al.*, 1970), dry edible bean (Abawi and Pastor-Corrales, 1990), pumpkin (Sultana *et al.*, 1994), sunhemp (Basu Chowdhary and Pal, 1982), cowpea (Sinha and Khare, 1977), sunflower (Fakir *et al*, 1976; Raut, 1983), sesame (Singh and Singh, 1982) and so on. The pathogen survives in soil mainly in the form of microsclerotia, which is the primary source of inoculum (Papavizas and Klag, 1975). *M. phaseolina* is a soil-borne as well as seed-borne fungus (Richardson, 1979).

Singh and Singh (1982) have shown that in sesame *M. phaseolina* is surface as well as internally seed-borne. The infection of *M. phaseolina* in sunnhemp seeds occures either in the form of microsclerotia, pycnidia or in

mycelia (Chaudhary and Pal, 1982). In sunflower, the seed-borne nature of this fungus is recorded (Chohan and Kaur, 1975; Raut, 1983) and caused severe pre- and post emergence losses (Fakir *et al.,* 1976). The cowpea seeds with awful infection resulted rotten of the seeds completely and the pathogen *M. phaseolina* finally grows on the seed coat (Sinha and Khare, 1977).

Among seed-borne pathogen of mungbean and blackgram, *M. phaseolina* is most important as they cause reduction of seed viability by seed and seedling rot. So far, no attempt has been made comprehensively to study the further development of this pathogen during seed germination, especially in mungbean and blackgram seeds. Hence, the present investigation was taken up considering the following objectives:

- to explore the mode of transmission of *M. phaseolina* in mungbean and blackgram seeds.
- to observe the effect of *M. phaseolina* on emerging seed and seedlings of mungbean and blackgram.

6.2. Materials and Methods

6.2.1. Seed source

Seed sample of mungbean and blackgram variety named as Chai Nat 60 and Uthong 2 respectively were obtained from Chai Nat Field Crops Research Center, Thailand. The seed samples were carrying natural infection of *M. phaseolina* which was revealed by blotter method (ISTA, 1976).

6.2.2. Methods of studying seed to seedling transmission

To determine how *M. phaseolina* transmits from seed to seedling, several types of seedling symptom tests were employed. The different types of tests are described below:

I. Blotter method

In this method, mungbean and blackgram seeds were plated in glass Petridishes (9cm diameter) on moist triple layer of Whatman no. 1 filter paper. Four hundred seeds were taken randomly from the each seed samples and were placed 10 seeds per Petridish equidistantly. Thereafter, all Petridishes were incubated at alternating conditions of 12 hours NUV and darkness. Observations were made on the transmission of *M. phaseolina* after 7 days of incubation.

II. Test tube agar method

From each mungbean and blackgram seed samples, 100 seeds were taken and soaked with 10 percent sodium hypochlorite solution for 3 minutes in order to disinfect their surface. After that, the seeds were washed with sterilized water three to four times for removing sodium hypochlorite from the seed. Then the seeds were placed on sterilized filter paper for soaking the excess adhering water with the seeds. Thereafter, the seeds were transferred singly under aseptic condition on Pyrex glass test tube (20cm x 2.5cm) contained 20ml of 2 percent water agar, which were previously autoclaved and solidified. All the test tubes were incubated under 12 hours alternating NUV light and darkness conditions. Data were taken after 7 days on the basis of *M. phaseolina* transmission.

III. Sand and soil method

Healthy and M. phaseolina infected seed selection: Mungbean and blackgram seed samples were incubated by blotter method (as 6.2.1.I.) to find out *M. phaseolina* infected seeds. After incubation of 3 days, the infected seeds with *M. phaseolina* infection and healthy seeds without any symptom were separated and used for following sand and soil method.

Sand method: In the plastic pot (16cm x 10cm) containing sterilized sand, *M. phaseolina* infected seeds were planted at the rate of 5 seeds per pot. Total 100 of each mungbean and blackgram seeds were sowed. In the same way, healthy seeds without infection were also planted as control.

Soil method: *Macrophomina phaseolina* infected seeds were transferred in plastic pots (16cm x 10cm) with sterilized soil at the rate of 5 seeds per pot. Like in sand method, 100 seeds from each sample were planted. Healthy seeds were also planted as control in the same manner.

In all methods, data on germination, seed rot, seedling mortality, symptoms appeared on seeds and seedlings including cotyledonary leaves were recorded. Isolations were made from root to tips in the infected plants subject to confirmation of *M. phaseolina* infection.

6.3. Results

6.3.1. Seed transmission of *M. phaseolina* in mungbean

I. Blotter method

Percentage of seed transmission of *M. phaseolina* was found by 29.75 and among them ungerminated seeds were 13.00 percent and infected cotyledon bearing seedlings were only 1 percent (Table 6.01). The radicle emerged out in 15.75 percent seeds, but before plumule initiation, the radicle turned into blemish appearance (Plate 6.01). Blackish pycnidia and

Table 6.01: Types of infection resulting from *Macrophomina phaseolina* infected mungbean and blackgram seeds in blotter method (Based on 400 seeds).

Mungbean/ Blackgram	Ungerminated seeds (%)	Seedlings with only blemished radicle (%)	Seedlings with infected cotyledon (%)	Total infected seeds (%)
Mungbean	13.00	15.75	1.00	29.75
Blackgram	9.00	13.00	2.00	24.00

Plate 6.01: *Macrophomina phaseolina* transmitted mungbean seed.

1) Ungerminated seed.
2) Very short blemished radicle.
3) Longer blemished radicle
4) Healthy seedling without infection.

microsclerotia formation were frequent on the blemish radicle and ungerminated seeds. The pycnidia and microsclerotia formation started within three to four days of seed plating. At the initial stage of development, pycnidia were formed on the seed coat (Plate 6.02). During the course of its development, the young pycnidia enlarged.

Many of the ungerminated seeds turned into charcoal like black appearances, where the pycnidia and microsclerotia of *M. phaseolina* were present. Some of the ungerminated seeds were covered with gray to black loose mycelia with minute microsclerotia (Plate 6.03). Seeds with heavy infection were rotted completely and could not germinate. When the infected plant parts like ungerminated seeds, blemished radicle, infected cotyledon, and stem were cultured in PDA, *M. phaseolina* grew in every cases.

II. Test tube agar method

Transmission of *M. phaseolina* from infected seed was observed by 26.00 percent (Table 6.02) in test tube agar method. In this method, ungerminated seeds, seedlings with blemished radicle and seedlings with infected cotyledons were found by 10.00 percent, 14 percent, and 2.00 percent, respectively (Plate 6.04). The disease symptoms of infected seeds and seedlings were almost similar to blotter method. In addition, pycnidia and microsclerotia formation was alike to blotter method. In this method, additional symptoms like circular and dark brown spots appeared within a week in cotyledonary regions, and gradually these symptoms spread to stem and leaves. Moreover, some ungerminated seeds were encompassed with heavy mycelial growth of *M. phaseolina* (Plate 6.05). On the other hand, *M. phaseolina* free seed produced healthy seedlings without any disease symptom (Plate 6.06). Nevertheless, the total *M. phaseolina* transmission was found by 4.00 percent, which was less compared to blotter method.

Plate 6.02: Pycnidia of *Macrophomina phaseolina* formation on the seed coat of mungbean. P- Pycnidium.

Plate 6.03: Pycnidia and microsclerotia of *Macrophomina phaseolina* on the un-germinated seed. P- Pycnidium; S- Microsclerotium.

Table 6.02: Types of infection resulting from *Macrophomina phaseolina* infected mungbean and blackgram seeds in Test tube agar method (Based on 100 seeds).

Mungbean/ Blackgram	Ungerminated seeds (%)	Seedlings with only blemished radicle (%)	Seedlings with infected cotyledon (%)	Total infected seeds (%)
Mungbean	10.00	14.00	2.00	26.00
Blackgram	7.00	12.00	3.00	22.00

Plate 6.04: *Macrophomina phaseolina* transmitted mungbean seed in test tube agar method.

1) Seedling with infected cotyledon.
2) Seedling with blemished radicle.
3) Ungerminated seed.

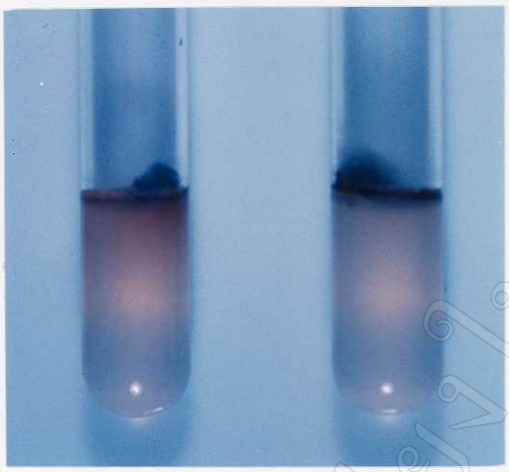

Plate 6.05: Un-germinated seed with heavy mycelia of *Macrophomina Phaseolina* observed in test tube agar method.

Plate 6.06: *Macrophomina phaseolina* transmitted seedling (left) and without transmitted healthy seedling (right) observed in test tube agar method.

III. Sand and soil method

From diseased seed, absolute healthy seedlings were steamed neither in sand nor soil method. After 7 days of transferring the infected seeds in pot, 57 and 72 percent seeds showed pre-emergence mortality in sand and soil methods, respectively (Table 6.03). When the ungerminated seeds dug up, the seeds were found as covered by microsclerotia and mycelia along with abundant pycnidia. Most of the seeds were rotted. In some seeds, radicles were started to appear but died before developing the plumule. The radicle and cotyledon infection were started within two to four days of plantation. Sixteen and 15 percent seedlings in sand and soil methods, respectively produced dark brown spot on the cotyledonary leaves due to *M. phaseolina* infection (Plate 6.07 and 6.08). These infections gradually spread to whole stems and leaves, and turned into yellowish color, which eventually caused the death of seedlings (Plate 6.09 and 6.10). Hypocotyls near the root and cotyledonary nodes showed infection about one week after sowing. Twenty-five and 13 percent seeds in sand and soil methods, respectively, produced apparently healthy seedlings, but within 21 days all of them died after demonstrating symptom (yellowing the leaves followed by wilting of the seedlings) of *M. phaseolina*. After 23 days of sowing, numerous pycnidia and microsclerotia of *M. phaseolina* were formed on all the dead seedlings. These abundant pycnidia and microsclerotia were formed on the outside of the stem and some microsclerotia formed also inside the stem. Due to this reason, the stem turned into black in color, charcoal appearance. This symptom also appeared in some of the ungerminated seeds. The post-emergence mortality was occurred in the form of damping-off, collar rot, root rot, and root and stem rot. The different parts of infected seedlings were cultured in PDA media, and in every cases *M. phaseolina* was recovered.

On the other hand, healthy seed produced robust seedlings in both sand and soil method and symptom of *M. phaseolina* were not appeared in any seedlings (Plate 6.11 and 6.12). All the plants from healthy seed remained green and vigorous.

Table 6.03: Percentage of seed to seedling transmission of *Macrophomina phaseolina* (sand and soil method, based on 100 seeds).

Methods	Mungbean/ Blackgram	Nature of seeds	Pre-emergence mortality	Post-emergence mortality (seedlings with symptom)	Post-emergence mortality (apparently healthy emerged seedlings which died within 21 days)	Absolute healthy seedlings
Sand	Mungbean	Healthy	0	0	0	100
		Diseased	59	16	25	0
	Blackgram	Healthy	0	0	0	100
		Diseased	55	19	26	0
Soil	Mungbean	Healthy	0	0	0	100
		Diseased	72	15	13	0
	Blackgram	Healthy	0	0	0	100
		Diseased	63	18	19	0

Plate 6.07: Transmission of *Macrophomina phaseolina* to the cotyledonary leaves of mungbean planted in sand at 4 days.

Plate 6.08: Transmission of *Macrophomina phaseolina* to the cotyledonary leaves of mungbean planted in soil at 4 days.

Plate 6.09: Spreading of infection due to *Macrophomina phaseolina* from the cotyledonary leaves to true leaves (left) and a healthy seedling without infection (right) at the age of 3 days .

Plate 6.10: Eventual death of seedling due to *Macrophomina phaseolina* infection (left) and a healthy seedling (right) at the age of 5 days.

Plate 6.11: In sand, *Macrophomina phaseolina* transmitted (left) and healthy seedlings (right) of mungbean at the age of 6 days.

Plate 6.12: In soil, *Macrophomina phaseolina* transmitted (left) and healthy seedlings (right) of mungbean at the age of 6 days.

6.3.2. Seed transmission of *M. phaseolina* in blackgram

I. Blotter method

In blotter method, 24.00 percent blackgram seeds showed transmission of *M. phaseolina* of which 9.00 percent could not get germinated and 13.00 percent noticed blemished radicle without plumule and in remaining 2.00 percent infected blakish cotyledons were observed (Table 6.01). Like mungbean, on the blemished radicle and ungerminated seed, which were died already, profuse microsclerotia and pycnidia were developed after 4 days of plating. Reculture of all infected seed and plant parts resulted presence of *M. phaseolina*.

II. Test tube agar method

The total seed transmission of *M. phaseolina* in blackgram was manifested by 22.00 percent in test tube agar method. In this method, 7.00 percent, 12.00 percent, and 3.00 percent seeds revealed as germination failure, seedlings with blemished radicle without plumule and seedlings with infected cotyledon, respectively (Table 6.02). On the infected parts of seedlings and seeds, copious sclerotia and pycnidia were exposed. Alike to mungbean, circular and dark brown colored infection was spread towards the stem and leaves from cotyledonary region. The presence of *M. phaseolina* was confirmed in all infected parts by reculturing in PDA media.

III. Sand and soil method

From the healthy seeds, neither pre-emergence mortality nor post-emergence mortality was observed. Correspondingly, from diseased seed, no any absolute healthy seedling was found in either sand or soil method. In sand and soil methods, 55.00 and 63.00 percent seeds respectively, were manifested pre-emergence mortality. When these seeds were taken away from soil, plenty of microsclerotia and pycnidia along with mycelia of *M.*

phaseolina were seen on seeds. The remaining seeds showed post-emergence mortality (Table 6.03). However, likewise mungbean, 19.00 and 18.00 percent seeds in sand and soil method revealed symptoms on cotyledonary leaves as brownish color, which ultimately spread on the stem and leaves turning them yellowish in color and in due course caused the death of whole plants. In addition, 26.00 and 19.00 percent seeds did not show any symptom during germination but after 21 days, all seedlings turned into yellowish color and eventually died within 23 days. On the dead seedlings, profuse microsclerotia and pycnidia developed. When the infected plant parts re-cultured, *M. phaseolina* was recovered.

6.4. Discussion

Results obtained from seed transmission studies carried by blotter and various growing on tests (test tube agar method, sand method and soil method) revealed that *M. phaseolina* could obviously be transmitted from seed to germinated seed and seedlings in both mungbean and blackgram. In all methods, almost similar events occur in case of mungbean and blackgram. In every trial, a considerable amount of seeds were found to be manifested germination failure among the infected seed by causing either seed rot or producing numerous sclerotia and pycnidia. Some of the dead seeds turned into charcoal appearance within seven days of plantation. Due to this reason, the disease caused by *M. phaseolina* designated as charcoal rot (Raut, 1983). All infected seedlings were died eventually followed by producing pycnidia and microsclerotia. When the infected plant parts were incubated in solidified PDA, in every case, *M. phaseolina* was recovered.

According to blotter method, a large number of infected seeds could not give rise the emergence of either radicle or plumule. Instead of that, huge pycnidia and microsclerotia along with mycelia were exhibited on the seed. Although some seeds were able to produce radicle without plumule but eventually that turned into a blemish appearance and ultimately caused death

of seedling. This type of transmission of *M. phaseolina* in blotter method has been reported by various authors in several crops. Sinha and Khare (1977) studied the seed transmission of *M. phaseolina* in naturally infected cowpea seed. They observed a number of pycnidia and mycelia on the seed. In addition, they noticed that heavily infected seeds could not germinate and eventually rotted. Singh and Singh (1982) recorded the transmission of *M. phaseolina* in sesame seed by blotter and agar method. They found the frequent pycnidia formation on rotted seedlings and in un-germinated seeds, which were resulted from the infected seed. Sultana *et al.*, (1994) studied transmission on this pathogen in pumpkin seed and observed minute black sclerotia with gray to black loose mycelium on the un-germinated seed. According to blotter test, in sunflower seed, *M. phaseolina* reduced germination, killed the germinating radicle and also caused discoloration of roots, hypocotyls and cotyledons (Fakir *et al.*, 1976). They also found abundant pycnidia and sclerotia on un-germinated seed.

Results obtained from seed transmission studies carried by test tube agar method revealed that most of the symptom appeared on the seed and seedlings were almost similar to blotter method. In test tube agar method, in some seedlings, a circular dark brown color spots exposed on the cotyledonary region of both mungbean and blackgram seedlings, and progressively they extended to stem and leaves. This event has been supported by Sharada and Shetty (1987) in case of blackgram. In addition, Singh and Singh (1982) observed significant germination failure, browning, and rotting of seedlings in sesame in test tube agar method. They also noticed some seedlings had initial vigorous growth but subsequently most of them became diseased.

In the present investigation, the results from sand and soil method did not show any healthy seedling neither in mungbean nor blackgram when cent percent *M. phaseolina* infected seeds were used. Likewise, all robust seedlings were produced when uninfected seeds were used in both sand and

soil methods in case of these two crops. The seed-borne pathogen infected the radicle and cotyledons of sprouting seeds within two to four days. The infection from cotyledonary leaves to stem and leaves started about within a week. The infected parts became brown and seedlings collapsed eventually. Meanwhile, copious pycnidia and microsclerotia developed on the dead tissues. The first true leaves were infected sometimes at the time of unfolding as a contact infection from infected cotyledons. The fungus attacked the radicle and the majority of seedlings died before plumule initiation. Numerous pycnidia and microsclerotia were formed on the outside of the stem in both mungbean and blackgram and gave it a charcoal rot appearance. The results thus indicated that *M. phaseolina* does not cause systemic infection in mungbean and blackgram. This finding agrees with Raut (1983), who found similar observation in sunflower. Chaudhary and Pal (1982) also found abundant pycnidia and sclerotia in dead stem of sannhemp while they studied transmission of *M. phaseolina*. Furthermore, the mode of transmission of *M. phaseolina* as observed in the present investigation is akin to those in blackgram (Sharada and Shetty, 1987), dry edible beans (Abawi and Pastor-Corrales, 1990), sesame (Singh and Singh, 1982), and pumpkin (Sultana *et al.*, 1994).

The pycnidial stage of *M. phaseolina* has been considered as an effective source of inoculum for seed infection. This pathogen forms numerous pycnidia on the dead stem and seed of mungbean and blackgram, and these usually drop in the soil, which cause proliferation of *M. phaseolina* in soil as well as becomes the source of inoculum for further infection cycles.