CHAPTER VII

7. Effect of *Macrophomina phaseolina* on Viability, Vigor and Storability of Mungbean and Blackgram Seed

7.1. An Overview

The quality of seed is the major deciding factors in achieving production targets. Decreasing the viability, vigor and storability of seed might be caused by invasion of microorganism in the seed. Especially the pathogenic microorganisms, which are seed-borne, can reduce the seed germination greatly (Neergaard, 1979). After germination, seedlings can also be attacked by the seed-borne pathogen. Furthermore, during storage, quality of seed might deteriorate due to the infection of pathogen. Fungi are the organisms, which can affect on the seed quality leading to reducing viability, vigor and storability of seed. Fungi are known to produce toxic substances to plants, which affect on seed germination and seedling vigor adversely (Maheshwari *et al.*, 1984). Several seed-borne fungi inhibited the seed germination in rice, wheat, sorghum, and cowpea (Maheshwari *et al.*, 1984).

Macrophomina phaseolina has an important role on lowering seed quality in many crops like soybean (Gangopadhyay *et al.*, 1970), sunflower (Raut, 1983), sesame (Gupta and Cheema, 1990), sannhemp (Chaudhary and Pal, 1982) etc. It can also decrease the vigor and viability of mungbean (Nayak and Behera, 1994) and blackgram (Sharada and Shetty, 1987). In addition to reducing viability and vigor, it is suspected that as a seed-borne pathogen, this fungus worsens the storability of mungbean and blackgram. However, there is no any published paper on these aspects in mungbean and blackgram. Therefore, the present investigation was designed to meet the following objectives:

- to evaluate the extent of damage caused by the seed-borne fungus *M. phaseolina* to seed viability and vigor of mungbean and blackgram.

- to assess the effect of *M. phaseolina* infection on storability of mungbean and blackgram seeds.

7.2. Materials and Methods

7.2.1. Source of seed

Three mungbean varieties namely Chai Nat 36, Chai Nat 60 and Khampen Saen 2 and two blackgram varieties viz. Uthong 2 and Phitsanulok 2 were grown in the Chiang Mai University Experimental Farm. Newly harvested seed samples were used in the experiment.

7.2.2. Isolation and preparation of pure culture and inoculum of *Macrophomina phaseolina*

Seed sample of blackgram named as Phitsanulok 2 was obtained from Chai Nat Field Crops Research center, Thailand, which was carrying 26.0 percent natural infection of *M. phaseolina*. Fifty seeds were soaked with 10% sodium hypochlorite solution followed by washing with sterilized water for three to four times. The water around the seed was soaked by sterile blotter paper. Then the seeds were placed in sterilized 9cm-diameter Petriplates contained 3-layered moist Whatman no. 1 blotting paper at the rate of 10 seeds per plate. All the plates were kept under 12 hour alternating NUV light and darkness at about 25^0C. After 3 days, when the pycnidia and microsclerotia of *M. phaseolina* were observed on the seed coat and radicle, only these seeds were transferred in sterilized Petriplate containing about 20ml solidified PDA and the seeds were placed singly at the middle of the each plate. The plates were sealed by Nesco film to avoid contamination by other aerial microorganisms and were kept again under 12 hour alternating NUV light and

darkness at about 25^{0}C. After 3 days, when the plates were covered by the blackish mycelia and microsclerotia of *M. phaseolina*, these were used as pure culture. The mycelia and microsclerotia were harvested by adding 10ml sterilized water per plate and scrapping gently by a sterilized spoon by taking as minimum as possible PDA media. These fungal structures were blended for 2 minutes to get uniform suspension. The suspension was used as inoculum of *M. phaseolina*.

7.2.3. Inoculation of *Macrophomina phaseolina* inoculum

Newly grown seed samples of all five mungbean and blackgram varieties were inoculated by the freshly prepared inoculum of *M. phaseolina*. About one thousand seeds of each variety were surface sterilized with 10% sodium hypochlorite solution for 2 minutes followed by rinsing in sterilized distilled water for minimum three times. Half of each variety was used for control or check and remaining half was soaked for one hour by the inoculum suspension of *M. phaseolina,* which was prepared earlier as 7.2.2. The control seed samples were soaked with only sterilized water. All the seeds were dried under fan for overnight. These seeds were used for viability, vigor and storability test.

7.2.4. Viability test:

Viability test of seed was employed by Towel method (ISTA, 1976). In three layered moist germinating papers, fifty seeds were placed on two layered papers and another paper was covered and rolled. In each case, four replications were maintained and result of 100 seeds considered as one replication. All the rolls were kept in germinating chamber at 25^{0}C temperature. The inoculated and non-inoculated seeds were placed separately in the germinating chamber. After 6 days, data were taken on normal seedlings, abnormal seedlings, rotten or dead seeds, and hard seeds.

Isolation and culture was made from the dead or rotten seed and from infected plant parts to confirm the infection of *M. phasolina*.

7.2.5. Vigor test:

Seeds of each variety were germinated in germinating chamber as described in section 7.2.4. After 7 days, the vigor of seedlings was estimated by the mean of shoot length and root length in addition to dry weight of seedlings. Dry weight of seven days old seedlings was taken by drying the seedlings in air-dry oven at 60^0C for three days. The average of seedlings shoot and root length was estimated from 100 seedlings per replication. Altogether 4 replications were maintained for each variety.

7.2.6. Storability test:

Storability test was done by Accelerated Aging test (AA-test) developed by Delouche and Baskin (1973). By the AA-test, relative life span of seed in storage can be estimated. In this method, the seeds are exposed in high humidity and high temperature. This condition enhances the growth of fungi on storage seeds and cause deterioration. Therefore, we can get the storability of seeds.

For AA-test, about 10g of inoculated and non-inoculated seeds of each variety were distributed uniformly in two layers in 10x10cm trays. The trays were kept on the stand just above one cm from the surface of 50ml of water in a glass container. Caution was taken to avoid direct contact between water and seeds. The glass containers were sealed with their lids and placed in an accelerated aging chamber maintained at 41^0C temperature and 99 to 100% relative humidity. In each case, four replications were maintained and each replication contained results of 100 seeds. After 96 hours, seeds were removed and dried on paper towel on the table for two hours at room temperature. The aged seeds were tested by Paper Towel method

(ISTA, 1976) involving same procedure of viability test. After 7 days, data were taken on normal seedlings, abnormal seedlings, dead seeds, and hard seeds.

7.3. Results

7.3.1. Viability test

Almost three fold lower normal seedlings were produced in *M. phaseolina* inoculated seed with compared to healthy seeds in all mungbean and blackgram varieties.

7.3.1.1. Viability of mungbean

The effect of *M. phaseolina* in mungbean seeds has been presented in Table 7.01. From the result it was found that 63.0, 58.5, and 61.0 percent normal seedlings production were decreased in artificially inoculated seeds of Khampensaen 2, Chai Nat 36, and Chai Nat 60 varieties, respectively compared to healthy or un-inoculated seeds. On the other hand, the abnormal seedlings were increased by 56.5, 52.0, and 54.0 percent in Khampen Saen 2, Chai Nat 36, and Chai Nat 60 varieties, respectively, compared to healthy seeds. In healthy seeds, no any dead or rotten seeds were produced while the inoculated seeds produced 7.0, 7.0, and 6.5 percent dead or rotten seeds in the variety Khampensaen 2, Chai Nat 36, and Chai Nat 60 respectively. Due to artificial inoculation of *M. phaseolina*, significant difference of hard seed production was not found in any varieties of mungbean.

7.3.1.2. Viability of blackgram

In blackgram, 48.0 and 61.5 percent normal seedlings production were reduced in Uthong 2 and Phitsanulok 2 varieties, respectively due to *M. phaseolina* inoculation compared to healthy or un-inoculated seeds (Table 7.02). Similarly, increasing of abnormal seedlings were found by 39.5 and

Table 7.01: Effect of *Macrophomina phaseolina* on different variables of seed viability in mungbean seeds (mean of four replications).

Variables	Mungbean varieties											
	Khampensaen 2				Chai Nat 36				Chai Nat 60			
	Healthy or Control	Inoculated	Change over control (%) $\pm LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	Change over control (%) $\pm LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	Change over control (%) $\pm LSD^{0.05}$	Significant at p value
Normal seedlings (%)	93.5	30.5	-63.0 ±5.51	0.000	91.5	33.0	-58.5 ±3.05	0.000	94.0	33.0	-61.0 ±4.11	0.000
Abnormal seedlings (%)	2.5	59.0	+56.5 ±1.59	0.000	3.0	55.0	+52.0 ±4.50	0.000	4.0	58.0	+54.0 ±2.59	0.000
Dead, rotten seed (%)	0	7.0	+7.0 ±1.84	0.001	0	7.0	+7.0 ±1.83	0.001	0	6.5	+6.50 ±3.04	0.006
Hard seed (%)	4.0	3.5	-0.5 ±4.00	0.718	5.5	5.0	-0.5 ±3.05	0.638	2.0	2.5	+0.50 ± 3.04	0.637

Table 7.02: Effect of *Macrophomina phaseolina* on different variables of seed viability in blackgram seeds (mean of four replications).

Variables	Blackgram varieties							
	Uthong 2				Phitsanulok 2			
	Healthy or Control	Inoculated	(%) Change over control ± $LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	(%) Change over control ± $LSD^{0.05}$	Significant at p value
Normal seedlings (%)	85.85	37.5	-48.0±5.81	0.000	91.5	30.0	-61.5±3.05	0.000
Abnormal seedlings (%)	3.0	42.5	+39.5±1.59	0.000	2.5	56.0	+53.5±1.59	0.000
Dead, rotten seed (%)	0.0	8.0	+8.0±2.59	0.002	0.0	9.0	+9.0±1.84	0.000
Hard seed (%)	11.5	12.0	+0.5±4.00	0.727	6.0	5.0	-1.0±4.11	0.495

53.5 percent in the inoculated seeds of Uthong 2 and Phitsanulok 2 varieties respectively compared to un-inoculated seeds. The dead and rotten seeds were also observed because of *M. phaseolina* inoculation by 8.0 and 9.0 percent in the seeds of Uthong 2 and Phitsanulok 2 varieties respectively, whereas in the non-inoculated seeds of both varieties did not found any dead or rotten seeds. The difference of hard seed production was not significant in inoculated and non-inoculated seeds.

7.3.2. Vigor test:

In all varieties of mungbean and blackgram, the percentage of shoot length, root length and dry weight were found significantly lower due to *M. phaseolina* inoculation.

7.3.2.1. Vigor test of mungbean

In the inoculated seeds of the variety Chai Nat 36, chai Nat 60 and Khampen saen 2, the shoot length was decreased by 13.93, 26.94 and 25.30 percent respectively compared to un-inoculated seeds (Table 7.03). Moreover, root length of inoculated seed was also declined in the variety Chai Nat 36, Chai nat 60, and Khampensaen 2 by 19.20, 19.32, and 22.60 percent respectively in comparison with healthy or non-inoculated seeds. In addition to shoot and root length decreasing, dry weight of seedlings also reduced by 11.26, 15.65, and 10.03 percent in the variety Chai Nat 36, Chai Nat 60 and Khampensaen 2 respectively as a result of *M. phaseolina* inoculation.

7.3.2.2. Vigor test of blackgram

The two tested variety of blackgram, Uthong 2 and Phitsanulok 2 showed reduction of shoot length by 38.81 and 36.82 percent respectively because of seed inoculation by *M. phaseolina* inoculum (Table 7.04). The root length was also found decreased by 19.46 and 18.83 percent in the inoculated variety of Uthong 2 and Phitsanulok 2 respectively. Moreover, the dry weight

Table 7.03: Effect of *Macrophomina phaseolina* on different variables of vigor estimation in mungbean seeds (mean of four replications).

Variables	Mungbean varieties											
	Khampensaen 2				Chai Nat 36				Chai Nat 60			
	Healthy or Control ± $LSD^{0.05}$	Inoculated ± $LSD^{0.05}$	Decrease over control (%)	Significant at p value	Healthy or Control ± $LSD^{0.05}$	Inoculated ± $LSD^{0.05}$	Decrease over control (%)	Significant at p value	Healthy or Control ± $LSD^{0.05}$	Inoculated ± $LSD^{0.05}$	Decrease over control (%)	Significant at p value
Shoot length (cm)	15.06 ± 0.65	11.25 ± 0.65	25.30	0.000	13.99 ± 0.62	11.18 ± 0.62	13.93	0.000	15.37 ± 0.39	11.23 ± 0.39	26.94	0.000
Root length (cm)	10.53 ± 0.77	8.15 ± 0.77	22.60	0.002	10.26 ± 0.51	8.29 ± 0.51	19.20	0.001	10.25 ± 0.49	8.26 ± 0.49	19.32	0.001
Dry weight (g/100 seedlings)	3.89 ± 0.07	3.50 ± 0.07	10.03	0.000	4.53 ± 0.05	4.01 ± 0.05	11.26	0.000	4.41 ± 0.02	3.72 ± 0.02	15.65	0.000

Table 7.04: Effect of *Macrophomina phaseolina* on different variables of vigor estimation in blackgram seeds (mean of four replications).

Variables	Blackgram varieties							
	Uthong 2				Phitsanulok 2			
	Healthy or Control ± $LSD^{0.05}$	Inoculated ± $LSD^{0.05}$	Decreasee over control (%)	Significant at p value	Healthy or Control ± $LSD^{0.05}$	Inoculated ± $LSD^{0.05}$	Decrease over control (%)	Significant at p value
Shoot length (cm)	16.36 ± 0.70	10.01 ± 0.70	38.81	0.000	16.08 ± 0.21	10.16 ± 0.21	36.82	0.000
Root length (cm)	10.43 ± 0.37	8.4 ± 0.37	19.46	0.000	10.25 ± 0.57	8.32 ± 0.57	18.83	0.001
Dry weight (g/100 seedlings)	4.08 ± 0.05	3.52 ± 0.05	13.73	0.000	4.14 ±0.022	3.46 ± 0.022	16.42	0.000

of seedlings was also dwindled away by 13.73 and 16.63 percent in mungbean and blackgram seeds respectively compared to non-inoculated seeds.

7.3.3. Storability test:

From the storability or AA-test, the *M. phaseolina* inoculated seeds showed a drastic reduction on storability in all tested mungbean and blackgram varieties.

7.3.3.1. Storability of mungbean

In AA-test, the varieties Chai Nat36, Chai Nat 60, and Khampensaen 2 produced 24.0, 32.0, and 34.5 percent respectively, less normal seedlings due to seed inoculation with *M. phaseolina* (Table 7.05). On the other hand, production of abnormal seedlings were increased by 16.0, 25.0 and 27.5 percent in the inoculated Chai Nat 36, Chai Nat 60 and Khampensaen 2 variety respectively compared to non-inoculated or healthy seeds. The higher dead or rotten seeds were observed by 9.0, 7.0 and 7.0 percent more in the variety Chai Nat 36, Chai Nat 60 and Khampensaen 2 respectively as caused by *M. phaseolina* inoculation. However, The differences of hard seed production between inoculated and non-inoculated seeds were not significant.

7.3.3.2. Storability of blackgram

The blackgram variety, Uthong 2 and Phitsanulok 2 in inoculated state produced respectively 30.5 and 29.0 percent less normal seedlings (Table 7.06). Simultaneously, the inoculated variety Uthong 2 and Phitsanulok 2 raised 33.50 and 26.00 percent respectively more abnormal seedlings compared to non-inoculated seeds. The percentage of dead or rotten seed in the variety Uthong 2 and Phitsanulok 2 were found higher by 2.5 and 3.0 respectively. Nevertheless, the hard seed percentage was not considerably differed due to *M. phaseolina* inoculation in both the varieties of blackgram.

Table 7.05: Effect of *Macrophomina phaseolina* on different variables of storability estimation in mungbean seeds (mean of four replications).

Variables	Mungbean varieties											
	Khampensaen 2				Chai Nat 36				Chai Nat 60			
	Healthy or Control	Inoculated	Change over control (%) ± $LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	Change over control (%) ± $LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	Change over control (%) ± $LSD^{0.05}$	Significant at p value
Normal seedlings (%)	50.5	16	- 34.5 ± 3.04	0.000	43.5	19	- 24.5 ± 4.77	0.000	49.5	17.5	- 32.0 ± 2.59	0.000
Abnormal seedlings (%)	41.5	69	+ 27.5 ± 3.05	0.000	49	65	+16.0 ± 4.50	0.001	43.5	68.5	+ 25.0 ± 4.10	0.000
Dead, rotten seed (%)	5	12	+ 7.00 ± 1.83	0.001	3.5	12.5	+ 9.0 ± 4.10	0.006	3.5	10.5	+ 7.0 ± 4.10	0.012
Hard seed (%)	3.5	3	+ 0.5 ± 3.04	0.637	4	3.5	- 0.5 ± 3.04	0.637	3.5	3.5	0.0 ± 2.5	1.000

Table 7.06: Effect of *Macrophomina phaseolina* on different variables of storability in blackgram seeds (mean of four replications).

Variables	Blackgram varieties							
	Uthong 2				Phitsanulok 2			
	Healthy or Control	Inoculated	(%) Change over control ± $LSD^{0.05}$	Significant at p value	Healthy or Control	Inoculated	(%) Change over control ± $LSD^{0.05}$	Significant at p value
Normal seedlings (%)	67.5	37.0	-30.5 ± 4.77	0.000	67.5	38.5	-29.0 ± 5.51	0.000
Abnormal seedlings (%)	16.5	50.0	+33.5 ± 5.43	0.000	22.0	48.0	+26.0 ± 3.67	0.000
Dead, rotten seed (%)	4.0	9.0	+5.0 ± 2.25	0.005	5.0	8.0	+3.0 ± 1.83	0.014
Hard seed (%)	9.5	9.0	+0.5 ± 3.05	0.638	5.5	4.75	-0.75 ± 2.37	0.391

7.4. Discussion

From the result of viability test (Table 7.01 and Table 7.02), it was revealed that due to *M. phaseolina* infection three folds normal seedlings production were reduced compared to healthy check in all tested varieties of mungbean and blackgram. Similarly, in the inoculated seeds, abnormal seedlings were found to be terrifically higher in all tested varieties. In addition, 6.5 to 9.0 percent dead or rotten seeds were found to be manifested in inoculated tested varieties while dead or rotten seeds did not appear from any healthy seeds of tested varieties. However, no any significant difference was found incase of hard seed production because of *M. phaseolina* inoculation. The present result supported by various records of other researchers, where fungi reduced the viability of several crops such as *M. phaseolina* reduced the viability of mungbean seed (Nayak and Behera, 1994); six fungi (*Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus* and *Fusarium moniliforme*) declined the viability of cowpea seeds (Maheshwari *et al.*, 1984); *Aspergillus flavus*, *A. versicolor* and *A. glaucus* decreased the viability of rice seeds (Purushotham *et al.*, 1996) and *Aspergillus terreus*, *A. niger* and *A. flavus* inhibited the viability of wheat seeds (Singh, 1984).

The result of vigor test (Table 7.03 and Table 7.04) showed that the invasion of *M. phaseolina* in mungbean and blackgram seed resulted the inhibition of seedling vigor. The decrease of shoot length from inoculated mungbean seedlings ranged from 13.93 to 26.94 percent. The shoot length reduction was found higher in two blackgram varieties, which was 36.82 to 38.81 percent. The root length of inoculated mungbean seedlings was observed almost similar in all three varieties, which was ranged from 19.20 to 22.60 percent. In case of blackgram seedlings, root length declination in inoculated and non-inoculated seed was noticed as 18.83 to 19.46 percent. In addition, dry weight of seedlings, which was another important variable of vigor was varied from 10.03 to 15.65 percent in mungbean, and 13.73 to 16.42 percent in blackgram. Due to fungal invasion, vigor loss was found by the

fungi like *Aspergillus terreus*, *A. flavus*, *A. niger* and *Helminthosporium sativum* in wheat seeds (Singh, 1984); *Aspergillus flavus*, *A. versicolor* and *A. glaucus* in rice seeds (Purushotham *et al.*, 1996); and six fungi namely *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus* and *Fusarium moniliforme* in cowpea seeds (Maheshwari *et al.*, 1984).

From the result of AA-test, it was found that *M. phaseolina* reduced the storability of mungbean and blackgram seed greatly (Table 7.05 and Table 7.06). After accelerate aging in inoculated seeds, 32.0 to 34.5 percent and 29.0 to 30.5 percent normal seedling production were decreased in mungbean and blackgram respectively. Correspondingly, abnormal seedling found to be increased by 16.0 to 25.0 percent and 26.0 to 33.5 percent in mungbean and blackgram, respectively. Furthermore, in mungbean and blackgram seeds, dead or rotten seed was noticed by 7.0 to 9.0 percent and 3.0 to 5.0 percent, respectively. Nevertheless, hard seed production was not affected either in mungbean or blackgram seeds. When the seeds produce low normal seedlings after AA-test, it was considered as reduction in storability (Delouche and Baskin, 1973). In the present investigation, it is revealed that *M. phaseolina* reduced the storability of mungbean and blackgram obviously as we found significant less normal seedlings and high abnormal seedlings from inoculated seeds after AA-test.