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เชื้อรา *Macrophomina phaseolina* ที่ติดมากับเมล็ดถั่วเขียว : ผลที่มีต่อความมีชีวิต ความแข็งแรง และความสามารถในการเก็บรักษา

Seed-borne *Macrophomina phaseolina* in Mungbean: Effect on Seed Viability, Vigour and Storability

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Abstract : *Macrophomina phaseolina* is a causal organism of charcoal rot disease of mungbean being a seed-borne fungus. To know the actual detrimental effect of this fungus on seed quality of mungbean, the experiment was undertaken by comparing inoculated and uninoculated seeds. Artificially inoculated seeds of three mungbean varieties with *M. phaseolina*, showed three fold lower normal seedlings in comparison to non-inoculated seeds. The mean shoot length, root length and dry weight of seedlings, which implies the seedling vigour, were also found significantly reduced in every inoculated mungbean variety. Accelerated aging test (AA-test) showed almost three times less normal seedlings in inoculated seeds compared to control in all varieties, which indicates that *M. phaseolina* can reduce the storability of mungbean seed greatly. In AA-test, the proportion of dead and rotten seeds in the inoculated treatments appeared to be two to three folds higher than that of control treatment. However, no significant change in hard seed formation was found in both viability test and in the AA-test.

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บทคัดย่อ: *Macrophomina phaseolina* เป็นเชื้อราสาเหตุโรคเน่าดำของถั่วเขียวและสามารถติดมากับ เมล็ดพันธุ์ได้ เพื่อหาผลกระทบของเชื้อราชนิดนี้ต่อคุณภาพของเมล็ดพันธุ์ถั่วเขียว การทดลองจึงได้มีการคลุกเมล็ดถั่วเหลือง เปรียบเทียบกับเมล็ดที่ไม่ได้คลุกเชื้อ ผลการเปรียบเทียบโดยวิธีการวัดความแข็งแรงของเมล็ดคือหาจำนวนต้นอ่อน ปกติที่งอกได้ วัดความยาวของยอด ความยาวของราก และน้ำหนักแห้งของต้นอ่อน พบว่าจำนวนต้นอ่อนปกติ จากกลุ่มเมล็ดติดเชื้อมีน้อยกว่ากลุ่มเมล็ดที่ ไม่ติดเชื้อถึง 3 เท่า นอกจากนั้นยังมีความแตกต่างของความยาวยอด และรากและน้ำหนักแห้ง อย่างมีนัยสำคัญทางสถิติ ในการทดสอบความสามารถในการเก็บรักษาของเมล็ดพันธุ์ทั้ง 2 กลุ่ม โดยวิธีการเร่งอายุ (AA-test) ปรากฏว่าเมล็ดกลุ่มที่มีการติดเชื้อมีอัตราการตายของเมล็ดมากกว่า กลุ่มที่ไม่มีการติดเชื้อ 2 ถึง 3 เท่า อย่างไรก็ตามจากการทดลองพบว่าเมล็ดแข็งที่พบในทั้ง 2 กลุ่ม ไม่มีผลตอบสนองต่อการทดสอบคุณภาพของเมล็ดโดยรวม

Index words : Mungbean, *Macrophaning phaseoline*, postharvest disease

Introduction

Among the pulses, mungbean (*Vigna radiata*) is one of the major crops containing high protein and carbohydrate. It has also the ability to fix the atmospheric nitrogen in soil, which enriches the soil quality. The successful production of crops including mungbean is impeded by various growth limiting and reducing factors. Several plant pathogenic fungi are known to produce substance toxic to plants, which affect adversely on germination and seedling vigour (Neergaard, 1979). Infection of *Aspergillus niger*, *Curvularia* sp, *Fusarium equiseti*, *Fusarium oxysporum*, *Penicillium crustosum* and *Phoma glomerata* results in the reduction in seed germination and seedling vigour in blackgram (Singh and Chohan, 1977). Similarly, six seed-borne fungi namely *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus* and *Fusarium moniliforme* were found to inhibit seed viability and thereby retarding the growth of root and shoot of cowpea at different degree (Maheshwari *et al.*, 1984).

Mungbean carries a high incidence of *Macrophomina phaseolina* that causes reduction in seed viability and seedling vigour. Moreover, it is also suspected that this fungus declines the storability of mungbean. It is a seed-borne disease and generally called 'charcoal rot'. Usually, the seed viability is determined by Paper Towel Method (ISTA, 1976) and seed vigour is assessed by the average shoot length, root length and dry weight of seedlings raised in germinator. For storability test, Delouche and Baskin (1973) developed an accelerated aging (AA) test to estimate the relative life span of seed in warehouse storage. The seeds are exposed to high humidity and high temperature. These conditions promote the growth of fungi on seeds and cause deterioration. For mungbean, exposing of seeds at 41^{0}C temperature and 99 to 100 percent relative humidity for 96 hours is almost equivalent to store the seed for one year (Rose and Minalo, 1972). Therefore, the present investigation was designed to evaluate the extent the damage caused to seed viability, vigour and storability of mungbean due to the invasion by the seed-borne fungus *M. phaseolina*.

Materials and Methods

Seed sample:

The three-mungbean varieties Khampensaen 2 , Chai Nat 36 and Chai Nat 60 were grown in the Chiang Mai University experimental farm and newly harvested seed samples were used in the series of experiments.

Isolation and pure culture of *M. phaseolina*:

Seed sample of blackgram named as Uthong 2, which was obtained from Chai Nat Field Crops Research Center, Thailand, was carrying about 24.0 percent M. phaseolina according to blotter test. Fifty seeds were soaked with 10% sodium hypochlorite solution followed by washing with sterilized water for three times. Then the seeds were placed equidistantly at the rate of 10 seeds per plate, in sterilized 9 cm-diameter Petriplates, containing 3-layered moist Whatman no. 1 blotting paper. After 3 days, the seeds with well-noticed pycnidia and sclerotia of M. phaseolina on seed coat and radicle were transferred in sterilized Petriplate containing about 20 ml solidified potato dextrose agar (PDA) Difco. In the middle of the plate, one seed was placed. The plates were sealed by Nesco film to avoid contamination by other aerial microorganisms. After 3 days, when the mycelium of *M. phaseolina* was spread around the seed, 5mm diameter of PDA medium were cut by sterilized cork borer and placed on the middle of another sterilized Petriplate containing PDA. These plates were kept under 12-hour alternating light and darkness after sealed by Nesco film. After 3 days, the blackish mycelia and sclerotia of *M. phaseolina* grown profusely in the plates were used as pure culture.

Inoculation of seed:

Newly grown seeds of the three varieties of mungbean were inoculated by the pure culture of *M. phaseolina*. About one thousand seeds of each variety were surface sterilized with 10% sodium hypochlorite solution for 2 minutes followed by rinsing with sterilized distilled water for 3 to 4 times. Half amount of seed of each variety was used for control or check and remaining half was infested with earlier prepared mycelia and sclerotia of *M. phaseolina*.The mycelia and sclerotia were separated from PDA media by scrapping with a sterilized knife. Then scrapped fungal structures were dissolved with sterilized water at 50 ml water per Petriplate. The seeds were soaked for one hour in this suspension. The control seeds were also soaked with sterilized water in the same way for one hour. All the inoculated and uninoculted or control seeds were dried under fan for overnight.

Viability test:

Viability test of seed was employed by Paper Towel Method (ISTA, 1976). Fifty seeds were placed on two layered moist germinating papers, covered with another moist germinating paper, and then rolled. Four such rolls each containing 50 seeds were placed as four replicates. All the rolls were kept in germinating chamber (Temperature 30 ^{0}C

and Relative humidity 85%). 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.

Vigour test:

The vigour of seedling was estimated by the mean shoot length, root length, and dry weight of 7 days old seedlings from inoculated and non-inoculated seeds. The seedlings were raised in the germinator with Paper Towel Method (ISTA, 1976). The average of seedlings shoot and root length was estimated from 50 seedlings per replication. Dry weight of seven days old seedlings was taken after drying the seedlings in air-dry oven at 60 ^{0}C for three days. In every case, four replications were maintained.

Storability test:

It was done by Accelerated Aging test (AA-test) outlined in the AOSA's Vigour Testing Handbook (1983). About 10 g of inoculated and non-inoculated seeds of each variety were distributed uniformly in two layers in 10x10 cm trays. The trays were kept on the stand just above one cm from the surface of 50 ml of water in a glass container. Caution was taken to avoid direct contact of water and seeds. The containers were sealed with their lids and placed in an accelerated aging chamber maintained at 41 ^{0}C temperature and 99 to 100% relative humidity. 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 the same procedure as the viability test. After 7 days, data were taken on normal seedlings, abnormal seedlings, dead seeds, and hard seeds.

Results and Discussion

Viability:

From the viability test, it was observed that *M. phaseolina* reduced the production of normal seedlings and increased the abnormal seedlings including dead and rotten seeds remarkably (Table 1). Most of the abnormal seedlings contained typical symptom of charcoal rot disease. Infections started from cotyledonary leaves and spread later to the stem and downwards, which ultimately caused the death of the plant. The incidence of hard seed was not affected by *M. phaseolina* in any varieties.

From Table 1, it is evident that in the variety Khampensaen 2, Chai Nat 36 and Chai Nat 60, normal seedlings were decreased by 63.0, 58.5, and 61.0 percent, respectively, over the control due to *M. phaseolina* inoculation. Similarly, the increase in abnormal seedlings due to *M. phaseolina* infestation over the control (no inoculation) was in the order of 56.5, 55.0, and 58.0 percent in the variety Khampensaen 2, Chai Nat 36 and Chai Nat 60. No dead or rotten seeds were found from non-inoculated seeds of any varieties of mungbean, while inoculated seeds of Khampensaen 2, Chai Nat 36 and Chai Nat 60 were encountered 7.0, 7.0 and 6.5 percent, respectively.

This is in conformity with earlier reports

on various crops like sunflower (Singh and Prassad, 1988), *Comos boppinatns* (Srivastava and Gupta, 1981), and cowpea (Maheshwari *et al.* 1984). Besides, Nayak and Behera (1994) have also reported the germination reduction and seedling rot in blackgram due to *M. phaseolina* in India.

Vigour:

Vigour test was determined by the shoot length, root length, and dry weight of seedlings. The mean root length and shoot length were decreased considerably due to *M. phaseolina* infection. Significant decline in dry weight of seedlings was also observed. Because of *M. phaseolina* infection, growth of seedlings was retarded.

In the variety Khampensaen 2, Chai Nat 36 and Chai Nat 60, shoot length was reduced by 25.30, 13.93, and 26.94 percent respectively due to *M. phaseolina* infestation in comparison to healthy check. Similarly, the reduction of root length was observed in the order of 22.60, 19.20, and 19.32 percent. Furthermore, the dry-weight of infected seedlings was also lower than that of healthy ones by 10.03, 11.26, and 15.65 percent, respectively (Table 2).

Decline in seedling vigour due to *Aspergillus nidulans* was also recorded earlier in cowpea seeds, while reduction of root length was observed by 82.8 percent including the complete inhibiting of plumule emergence (Maheshwari *et al.* 1984).

Table 1 Effect of *Macrophomina phaseolina* on different variables for estimation of seed viability in mungbean seeds.

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 (%)	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 2 Effect of *Macrophomina phaseolina* on different variables for determining of seedling vigour variables.

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 3 Effect of *Macrophomina phaseolina* on different variables for estimation of storability in blackgram seeds.

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	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

After accelerated aging, the mycelia and sclerotia of *M. phaseolina* were grown profusely on the inoculated seeds. In the towel test, it was observed that the production of normal seedlings was reduced drastically in the inoculated seeds. On the contrary, the abnormal seedling production was increased greatly in the inoculated seeds in all three varieties. The dead or rotten seeds were also found significantly higher in the *M. phaseolina* infested seeds. No significant difference was noticed in proportion of hard seed between inoculated and non-inoculated seeds in any variety.

The number of normal seedlings decreased by 34.5, 24.5 and 32.0 percent in the variety Khampensaen 2, Chai Nat 36 and Chai Nat 60 respectively due to *M. phaseolina* infestation compared to healthy seeds. In the corollary, the proportion of abnormal seedlings increased by 27.5, 16.0, and 25.0 percent respectively. Another manifestation of the invasion of *M. phaseolina* was higher percentage of dead or rotten seeds, which was in the order of 7.0, 12.5, and 7.0 percent higher than control in the same varieties (Table 3).

Conclusion

Macrophomina phaseolina can decrease the seed quality of mungbean by reducing viability, vigour and storability. The *M. phaseolina* infested seed might be spread disease in the field. Therefore, to keep the quality of seed, appropriate, practical, and overall effective control measure like seed treatment should be explored.

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References

Association of Official Seed Analyst. Seed Vigour Testing Handbook. 1983. Contribution No. 32 to the Handbook on Seed Testing.

Delouche, J.C. and C.C. Baskin. 1973. Accelerated aging technique for predicting relative storability of seed lots. Journal of Seed Technology. 1:427-452.

ISTA. 1976. International Rules for Seed Testing. Inter national Seed Testing Association. Seed Sci. & Technol. 4:3-177.

Maheshwari, R.K.; S.K. Mathur and A. Mathur. 1984. Effect of seed-born fungi on seed viability and seedling vigour. Indian Phytopathology. 37:773-774.

Nayak, B. K. and N. Behera. 1994. Effect of *Macrophomina phaseolina* on seed germination and seedling rot in *Phaseolus mungo*. J. Mycopathol. Res. 32(1):71-74.

Neergaard, P. 1979. Seed Pathology Vol. II. The Macmillan Press, London and Barings.

Ross, E.F., and J.R. Manalo. 1972. Testing vigour of beans following unfevourable storage conditions. Horticulture Science. 6:347-348.

Singh, I., and J.S. Chohan. 1977. Seed borne fungi in blackgram (*Phaseolus mungo*) in Punjab. Indian J. Mycol. Plant. Path. 6:80. *In* Diseases and insect pests of mungbean and blackgram- a bibliography, AVRDC. February 1988. Se ries no-5.

Singh, B. K. and T. Prassad.1988. Effect of seed quality on germination and growth seed micoflora in sunflower (*Helianthus annuus*). Indian Bot. Soc. 67:316-318.

Srivastava, R.N. and J.S. Gupta.1981. Seed mycoflora from Indian seed lots of *Cosmos bippinatns* and their control. Indian Phytopathology. 34:383-385.