

เชื้อรา *Macrophomina phaseolina*, ที่ติดมากับเมล็ดถั่วเขียวและถั่วเขียวพิวดำ : ผลต่อความงอกและการเปลี่ยนแปลงทางชีวเคมี

Seed-borne *Macrophomina phaseolina* in Mungbean and Blackgram: Effect on Germination and Some Biochemical Changes

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Abstract: In the naturally infected seeds of mungbean and blackgram incited by *Macrophomina phaseolina* were assayed in order to find out the effect on germination, carbohydrate content, and protein content. Due to infection, the germination was reduced greatly in both mungbean and blackgram. While the infection was less, the seed germination was higher and less germination was found in case of severe infection. In biochemical studies, carbohydrate content was greatly depleted in the infected seeds. In contrast, protein content was found increased due to *M. phaseolina* infection in both mungbean and blackgram seeds.

บทคัดย่อ: ผลของเชื้อรา *Macrophomina phaseolina* ที่ติดมากับเมล็ดถั่วเขียวและถั่วเขียวพิวดำ ต่อความงอกของเมล็ดพันธุ์และการเปลี่ยนแปลงด้านชีวเคมีในส่วนของปริมาณคาร์โบไฮเดรตและ โปรตีนภายในเมล็ด พบว่า เมล็ดถั่วที่มีเชื้อติดมากทำให้ความงอกของเมล็ดลดลง โดยในพันธุ์ที่เชื้อติดมาน้อยความงอกของเมล็ดลดลงน้อยกว่าในพันธุ์ที่มีเชื้อติดมามาก ส่วนการเปลี่ยนแปลงทางชีวเคมีของเมล็ด พบว่าในเมล็ดที่มีการติดเชื้อ ปริมาณคาร์โบไฮเดรตลดลง แต่ปริมาณ โปรตีนเพิ่มขึ้นเมื่อเปรียบเทียบกับเมล็ดที่ไม่ติดเชื้อ

Index words : โรคหลังการเก็บเกี่ยว เชื้อราในเมล็ดถั่วเขียว

Pastharvest disease, Seed-borne disease, Mungbean, Blackgram

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Introduction

The fungus *Macrophomina phaseolina* is most frequently found associated with mungbean and blackgram as a seed-borne pathogen. This fungus infecting many crops and the disease caused by this fungus is generally called 'charcoal rot' (Burman and Lodha, 1996; Dhar and Sarbhoy, 1989). In mungbean and blackgram, it reduces not only viability and vigour of seed but also declines the quality of sprouts by producing brown to black spots on them. Seed deterioration by fungi is quite common (Bilgrami *et al.*, 1976). Mungbean and blackgram are substantially rich in protein and carbohydrate content (Nine, 1986). However, information on deterioration in germination and nutritive value such as protein and carbohydrate content in mungbean and blackgram seeds was lacking. Therefore, the present investigation was undertaken to assess the exact losses in germination, carbohydrate content, and protein content due to *M. phaseolina* infection in mungbean and blackgram seeds.

Materials and Methods

Seed samples:

For studying effect of *M. phaseolina* on seed germination, eight seed samples were obtained from different crop research institutes in Thailand. Among them, seven varieties and elite lines of mungbean, which were Chai Nat 60, Chai Nat 36,

Chai Nat 72, Khampeng saen 2, MoO 1, No.1, and No.2, and the remaining two of blackgram were Uthong 2 and Phitsanulok 2. The samples were kept at 4°C in a refrigerator till they were used for various studies.

For studying effect of *M. phaseolina* on carbohydrate and protein content, variety Chai Nat 60 was taken for mungbean and Uthong 2 was taken for blackgram, which were carrying natural infection.

Incubation and detection techniques for observing the effect on germination:

Seed samples were analyzed by 'Blotter method' following International Rules for Seed Health Testing (ISTA, 1993). In this method, three layers of blotter papers named Whatman no.1 were allowed to soak in sterilized water and were placed on sterile glass Petridish of 9 cm diameter. The seeds were taken randomly and 400 seeds were placed using equidistantly 10 seeds per Petridish. Findings of ten Petridishes i.e. 100 seeds were considered as one replication. The experiment was arranged in a completely randomized design.

The Petridishes with seeds were incubated at 20°C under 12 hours alternating cycles of near ultra violet (NUV) light and darkness. After 7 days of incubation, the germination and the prevalence of *M. phaseolina* was detected and recorded on the basis of their growth character of the incubated seeds

in blotter under stereo-binocular microscope, and was confirmed after preparing slides and examining under the compound microscope. Simultaneously, the germination of infected seed was observed.

Infected and healthy seed separation for carbohydrate and protein analysis, and sample preparation:

The seed samples were separated by Blotter method following the same manner. In this case, one hundred seeds of each variety were plated in blotter paper and just after two days, the seeds bearing with pycnidia, mycelia and sclerotia of *M. phaseolina* were separated. About 20 seeds healthy seeds and 20 infected seeds of each variety were dried at 65°C in air-oven for 3 days. Thereafter, the seeds were grinded finely in a ball mill keeping separate of two varieties, and healthy and infected seeds. The finely grinded samples were used for carbohydrate and protein analysis.

Carbohydrate and protein content estimation:

Total carbohydrate content in the seed samples was analyzed by Anthrone method (Morris, 1948 in Yoshida *et al.*, 1976). For protein content estimation, first, the nitrogen content was determined by micro-Kjeldahl method (Yoshida *et al.*, 1976) and then the percentage of protein content was calculated by the following formula:

$$\% \text{ protein} = 6.25 \times \% \text{ nitrogen in the sample}$$

Results and Discussion

Effect of *Macrophomina phaseolina* on seed germination of mungbean and blackgram:

Macrophomina phaseolina was isolated and recovered by Blotter method, confirming that the pathogen is obviously associated with mungbean and blackgram seed. The incidence of *M. phaseolina* and its effect on germination is presented in figure 1. From this investigation, it is revealed that *M. phaseolina* is a major cause of germination reduction. The frequency of *M. phaseolina* in different varieties ranged from about 2.00 percent to 30.00 percent. After 72 hours of incubation, the symptoms of *M. phaseolina* infection such as microsclerotia, pycnidia, whitish mycelia, and blemished radicle were appeared. These symptoms led the seeds or seedlings to die eventually. No plumule was emerged. In some cases, the seed could not germinate and plenty of pycnidia and microsclerotia were produced on the surface of seed including mycelia. It was also noticed that when the infected part of seedling like plumule, radicle, seed coat or cotyledon got touched with the other healthy seedlings, the healthy seedlings became infected and produced the same symptoms of *M. phaseolina* infection.

From this study, it is evident that *M. phaseolina* is a major seed-borne fungi which frequently get associated with seeds of mungbean and blackgram. It was observed that higher the

incidence of *M. phaseolina* the lower was the germination percentage or vice versa (Figure 1). Therefore, it can be inferred that the presence of *M. phaseolina* reduces germination of mungbean and blackgram greatly.

The presence of *M. phaseolina* in mungbean and blackgram causes not only germination reduction but also reduces the quality

of sprouts. Blemishness of sprout is responsible for quality deterioration. Because of this, Blackgram of Thailand is often complained by importers (Chainnuvati *et al.*, 1987; Pichitporn and Thavarasook, 1990; Putasamai and Surin, 1988). In soybean (Gangopadhyay *et al.*, 1970) and sunflower (Raut, 1983; Fakir *et al.*, 1976) seeds, the effect of *M. phaseolina* was reported and described similar symptoms.

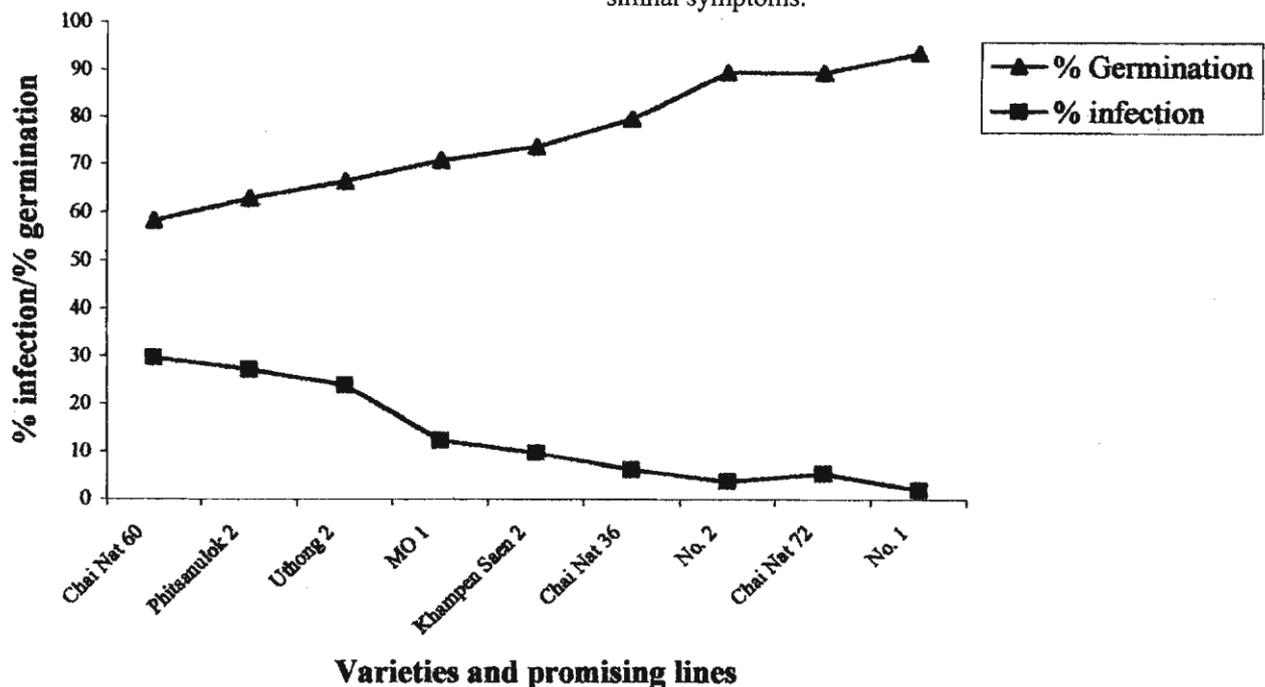


Figure 1 Percent infection of *Macrophomina phaseolina* and germination of seeds in different mungbean and blackgram varieties and promising lines.

Carbohydrate content:

The percentage of carbohydrate content in healthy and *M. phaseolina* infected mungbean and blackgram seeds were showed in Table 1. In the diseased seeds of both mungbean and blackgram, carbohydrate content declined greatly. Maximum carbohydrate reduced in diseased mungbean seeds

compared to healthy ones. In healthy and infected mungbean seeds, the carbohydrate content was found by 22.50 and 8.25 percent respectively. On the other hand, in blackgram seeds, carbohydrate content depleted from 22.25 percent to 12.25 percent due *M. phaseolina* infection. From this result, it is clearly observed that this remarkable reduction of carbohydrate content in mungbean and blackgram

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seeds absolutely caused by *M. phaseolina* infection. Saxena and Karan (1991) found the reduction in carbohydrate content in sesame and sunflower seeds due to infection of seed-borne fungi such as *Alternaria alternata*, *Aspergillus flafus*, *Aspergillus niger* and *Fusarium moniliforme*. They indicated as the probable causes of carbohydrate reduction were due to utilization and conversion into carbon dioxide and water by storage fungi. Different types of carbohydrates like fructose, glucose, sucrose, and ribose were greatly reduced and sometimes disappeared while *Geotrichum candidum* infected in round gourd (*Citrullus vulgaris* var. *fistulosus*) (Sumbali and Mehrotra, 1982). They described that the decrease in sugars in the infected round gourd was likely to be related to the breakdown of carbohydrates by fungal enzymes or host carbohydrates being used as substrate. Sandhu *et al.* (1998) reported that the levels of total sugars and starch were always more in healthy stem and root tissues as compared to plants infected by *M. phaseolina*. Decrease in carbohydrate levels has been ascribed to its utilization for the growth and multiplication of the pathogen and movements of photosynthates to the infection site. Sinha and Prasad (1977) observed a considerable decrease in carbohydrate contents (three soluble sugars viz. glucose, fructose and sucrose) of arhar

(*Cajanus cajan*) seeds because of *Aspergillus flavus* infection. They reported in the infected seed, glucose and fructose were completely utilized by the infecting fungi. Similarly, Srichuwong (1992) found less carbohydrate in the *Colletotrichum truncatum* infected soybean seeds in comparison to healthy ones. He mentioned the probable cause of carbohydrate depletion was using carbohydrate as a substrate or energy source, resulting in chemical break down of nutrients.

Protein content:

The change in protein content due to *M. phaseolina* infection is presented in Table 2. The result shows, owing to *M. phaseolina* infection, protein content in seeds of mungbean and blackgram were increased. The protein content was uplifted from 22.05 to 28.77 percent in mungbean seeds, because of infection. Correspondingly, the upsurge in protein content revealed by 27.13 to 32.81 percent from healthy to diseased blackgram seeds. The increasing level of protein in infected mungbean and blackgram seeds appeared to be almost similar. Increasing the amount of protein content in the *M. phaseolina* infected seed can be described as the additional protein was added from the fungal mycelium. Although reports regarding the effect of

Table 1 Percentage of carbohydrate content in healthy and diseased seeds of mungbean and blackgram.

Mungbean		Blackgram	
Healthy	Diseased	Healthy	Diseased
22.50	8.25	22.25	12.25
LSD = 0.683; Significant at P value 0.000.			
<small>0.05</small>			

M. phaseolina infection on protein content in mungbean and blackgram are not available, but in some studies, which were undertaken in other crops, and regarding other fungi, were showed similar results. Bilgrami *et al.* (1978) found higher protein in mungbean seed because of fungal infection. Vidhyasek *et al.* (1973) reported in rice grain due to *Helminthosporium oryzae* infection, the protein content was increased. Similarly, in lima bean stem, because of *M. phaseolina* infection, higher protein

content was explored (Jadeja and Patel, 1989). In soybean, due to *Colletotrichum truncatum* infection, the protein concentration was thrived (Srichuwong, 1992). Sinha and Prasad (1977) when studied the deterioration of arhar (*Cajanus cajan*) seeds by *Aspergillus flavus*, they observed some amino acids like glutamic acid and α -alanine were higher in the infected seeds. They also explained, as the cause of amino acid increase was fungal organism itself, which contained amino acid.

Table 2 Percentage of protein content in healthy and inoculated seeds of mungbean and blackgram.

Mungbean		Blackgram	
Healthy	Diseased	Healthy	Diseased
25.05	28.77	27.13	32.81
LSD = 0.190; Significant at P value 0.000.			
0.05			

Conclusion

Owing to *M. phaseolina* infection in mungbean and blackgram seeds, the seed viability and seed quality like carbohydrate and protein content can be declined greatly. Although, protein content can be increased due to some amino acids thriving because of fungal mycelium, nevertheless, further research is needed to identify the amino acid, which causes increasing of protein content in diseased seed in order to know their adverse effect on human consumption.

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