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# การควบคุมเชื้อรา *Macrophomina phaseolina* ที่ติดมากับเมล็ดพันธุ์ถั่วเขียวด้วยสารฆ่าเชื้อราคลุกเมล็ด

# Control of Seed-borne *Macrophomina phaseolina* of Mungbean by Seed Treatment with Fungicides

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**Abstract :** Six different fungicides viz. Benlate, Dithane M-45, Captan, Metalexyl, Thiram and Vitavax were evaluated against seed-borne *Macrophomina phaseolina* of mungbean in *in vitro*. Among them, Benlate, Dithane M-45 and Thiram found to be highly effective. Thereafter, these three fungicides were evaluated as seed dresser of mungbean against *M. phaseolina* in blotter method and in pot with sterilized soil. In both blotter and pot, the three fungicides were able to control seed-borne *M. phaseolina* effectively including increase germination significantly.

**บทคัดย่อ :** จากการนำสารฆ่าเชื้อรา 6 ชนิด ได้แก่ Benlate, Dithane M-45, Captan, Metalaxyl, Thiram และ Vitavax มาคลุกเมล็ดเพื่อทดสอบผลต่อการควบคุมเชื้อรา *M. phaseolina* ที่ติดมากับเมล็ดพันธุ์ถั่วเขียว พบว่าสารฆ่าเชื้อราที่มีประสิทธิภาพสูงในการยับยั้งการเจริญของเชื้อรา *M. phaseolina* บนอาหาร PDA ได้แก่ Benlate, Dithane M-45 และ Thiram และหลังจากนำเมล็ดที่ผ่านการคลุกด้วยสารฆ่าเชื้อราทั้ง 3 ชนิดนี้ มาตรวจสอบผลต่อเชื้อราที่ติดมากับเมล็ดและความงอกของเมล็ด โดยวิธีเพาะบนกระดาษชื้นและปลูกในกระถาง พบว่าสารทั้ง 3 ชนิดมีประสิทธิภาพสูงในการควบคุมเชื้อรา *M. phaseolina* ที่ติดมากับเมล็ดได้และช่วยเพิ่มเปอร์เซ็นต์ความงอกของเมล็ดอย่างมีนัยสำคัญ

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*Macrophomina phaeolina*

## Introduction

*Macrophomina phaseolina* is one of the most serious seed-borne fungi of mungbean, which causes charcoal rot disease. The fungus is seed-borne as well as soil-borne in nature (Nath, 1970; Neergaard, 1979; Grewal, 1988). A considerable work has been employed for controlling *M. phaseolina* in different crops including mungbean. However, very few chemicals are recommended in different countries for controlling *M. phaseolina* as seed-dresser. In Thailand, against this detrimental fungus in mungbean only one chemical Benomyl 50 WP has been recommended so far (Watanasit and Thanomsub, 1995). So, there is a great need to conduct some works on chemical seed treatment of this fungus in mungbean mainly to find out alternative chemicals for overcoming fungal resistance against prevailing fungicides and also to explore the efficacy of other products. In addition, to provide options to the farmers for the selection of fungicides based on cost-effectiveness and in case of one fungicide out of stock in the market, farmers can use another alternative. Therefore, the present investigation was undertaken with the following objectives to find out the effectiveness of the seed-dressing fungicides available in Thailand against *M. phaseolina* in mungbean *in-vitro* and *in-vivo*.

## Materials and Methods

***In-vitro* trial:**

Total six seed dressing fungicides namely Thiram, Metalexyl, Captan, Dithan M-45, Vitavax and Benlate were taken for *in-vitro* trial. These fungicides were weighed separately and mixed in the PDA (potato dextrose agar) medium under aseptic condition. The concentration of fungicides in the PDA medium was in three dosages viz. one was their normal recommended dose, another was below than their recommended dose, and other one was higher than their recommended dose (Table 1).

**Table 1 Amount of different fungicides mixed with liquid PDA.**

| Name of fungicides | Weight of fungicides (gm) | Which was mixed with 100ml liquid PDA | |
|---|---|---|---|
| | Below normal dose | Normal dose | Above normal dose |
| Thiram | 0.076 | 0.086 | 0.100 |
| Metalexyl | 0.700 | 0.850 | 1.000 |
| Captan | 0.150 | 0.170 | 0.200 |
| Dithane M-45 | 0.150 | 0.170 | 0.200 |
| Vitavax | 0.073 | 0.100 | 0.126 |
| Benlate | 0.073 | 0.113 | 0.153 |

The fungicide mixed liquid 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* (isolated earlier from infected seed) 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 (near ultra violet) light and darkness at 28°C 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*.

**Seed treatment with screened effective fungicides:**

The seeds of mungbean variety Chai Nat 60 (carrying 29.75 percent *M. phaseolina* according to blotter method) were treated with the effective fungicides (Benlate, Dithane M-45 and Thiram), which were found the most effective from *in-vitro* trial. For seed treatment, 0.15g fungicides were taken in a 250ml erlenmayer flask containing 50g of seeds. 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.

**Evaluation of treated seeds *in-vivo* (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°C under 12 hours alternating light and darkness. After 7 days, the prevalence of *M. phaseolina* infection and the germination of seeds were recorded.

**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 were planted in plastic pots (size 12cm x 16cm) filled with sterilized soil. Two hundred seeds for each fungicidal treatment and 200 untreated seeds were sown in the plastic pots using 25 seeds per pot. Total 4 replications were maintained. After sowing the seeds, the pots were kept in the glasshouse and watering was done whenever necessary. Germination and disease incidence was recorded until three weeks.

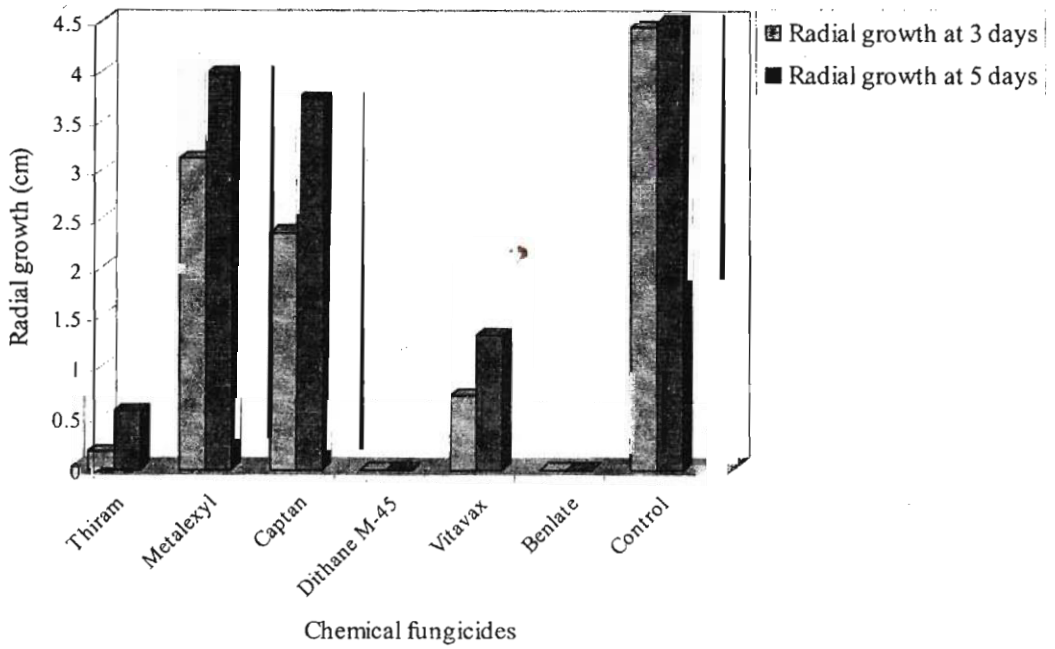


**Figure 1 Percentage of decrease radial growth of *Macrophomina phaseolina* over control in different fungicides.**

days (Plate 1 and 2). Another fungicide Thiram could also be 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 0.2 cm and 0.5 cm respectively (Plate 3). In case of remaining three fungicides viz. Metalexyl, Captan and Vitavax, no any promising inhibition was noticed in neither 3 nor 5 days of incubation. Albeit Vitavax was able to keep the radial growth less than 1.0 cm at 3 days of incubation, but at 5 days, it turned into nearly 1.4 cm, which was not optimistic inhibition (Plate 4). With regard to inhibition by Captan and Metalexyl, nearly similar result was revealed. After 3 days of incubation, Captan and Metalexyl showed about 2.4 cm and more than 3.0 cm radial growth of *M. phaseolina* respectively. Moreover, after 5 days, their redial growth reached almost in 4 cm (Figure 6 and 7).

**Incubation of seeds with screened and effective fungicides by blotter method:**

After treating the seeds with screened and effective fungicides, the results on infection and germination percentages are presented in Table 2. 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 the treated seed with Benlate, Dithane M-45, and Thiram, no any infection was found whereas in control treatment 28.0 percent infection was evolved. Due to seed

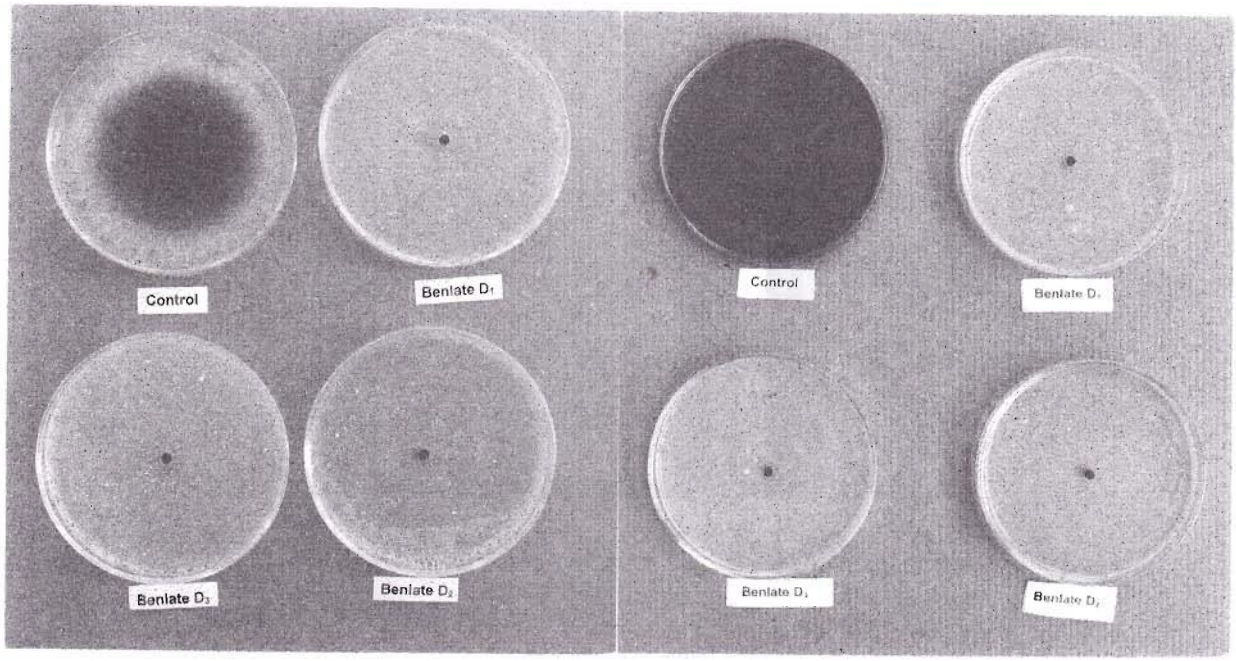


**Figure 2** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Benlate mixed PDA media at 3rd day (left) and 5th day (right).

$D_1$ - below normal dose; $D_2$ - normal dose; $D_3$ - above normal dose.

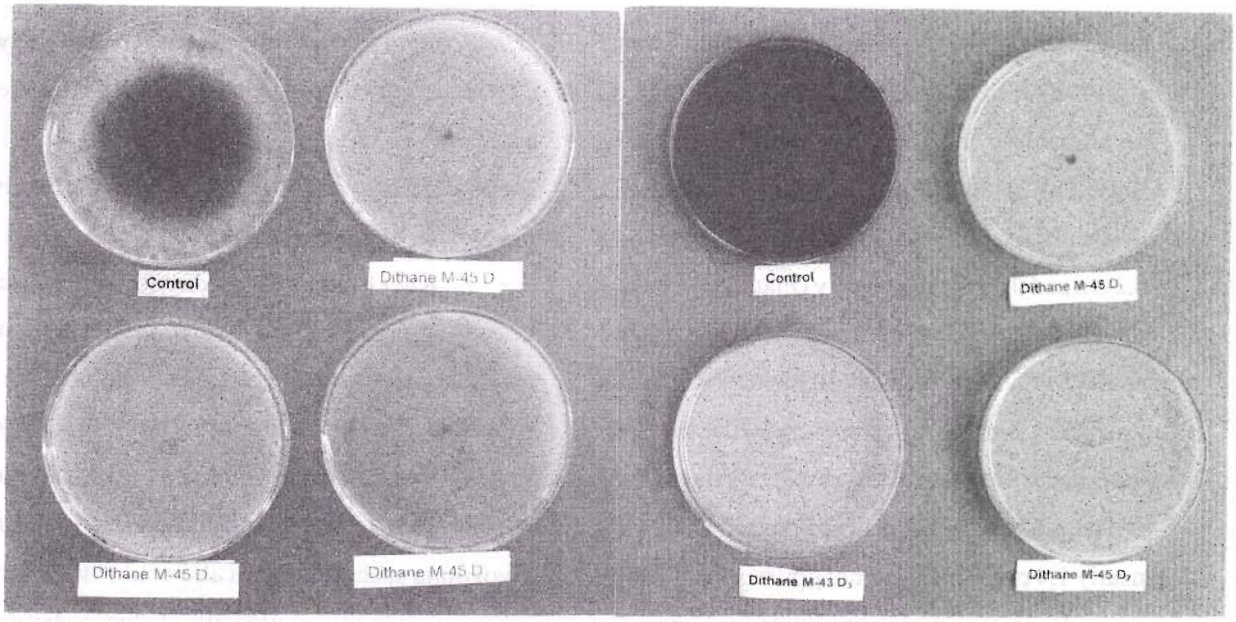


**Figure 3** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Dithane M-45 mixed PDA media at 3rd day (left) and 5th day (right).

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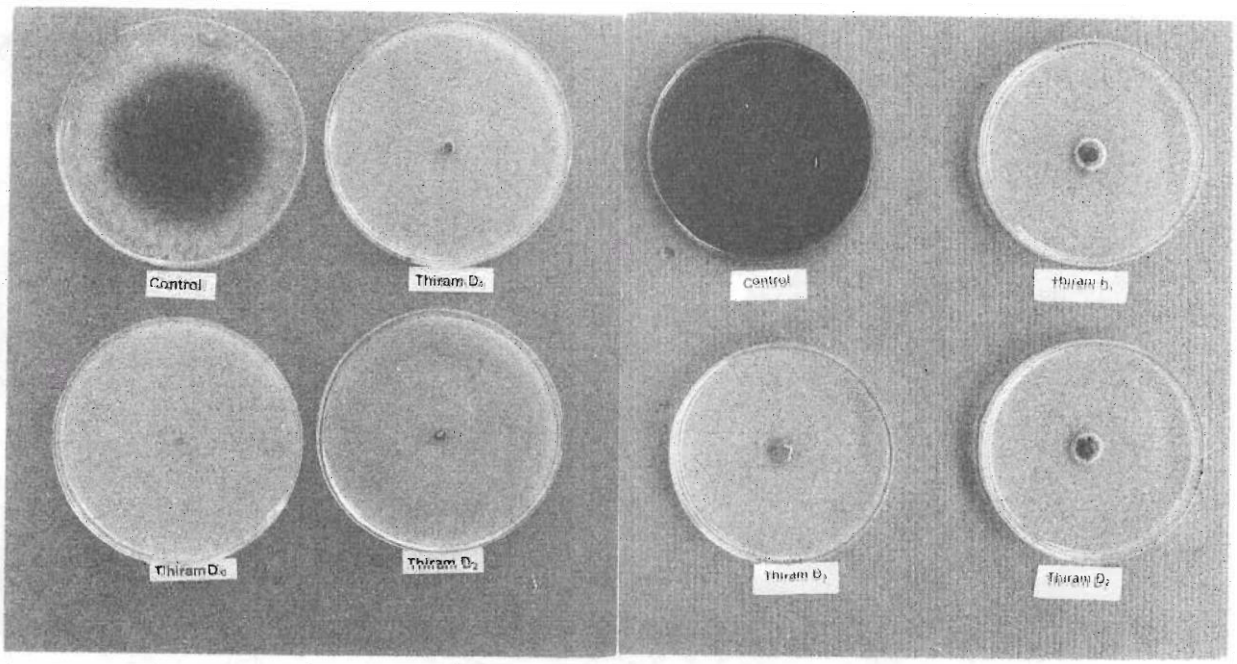


**Figure 4** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Thiram mixed PDA media at 3rd day (left) and 5th day (right).

$D_1$ - below normal dose; $D_2$ - normal dose; $D_3$ - above normal dose.

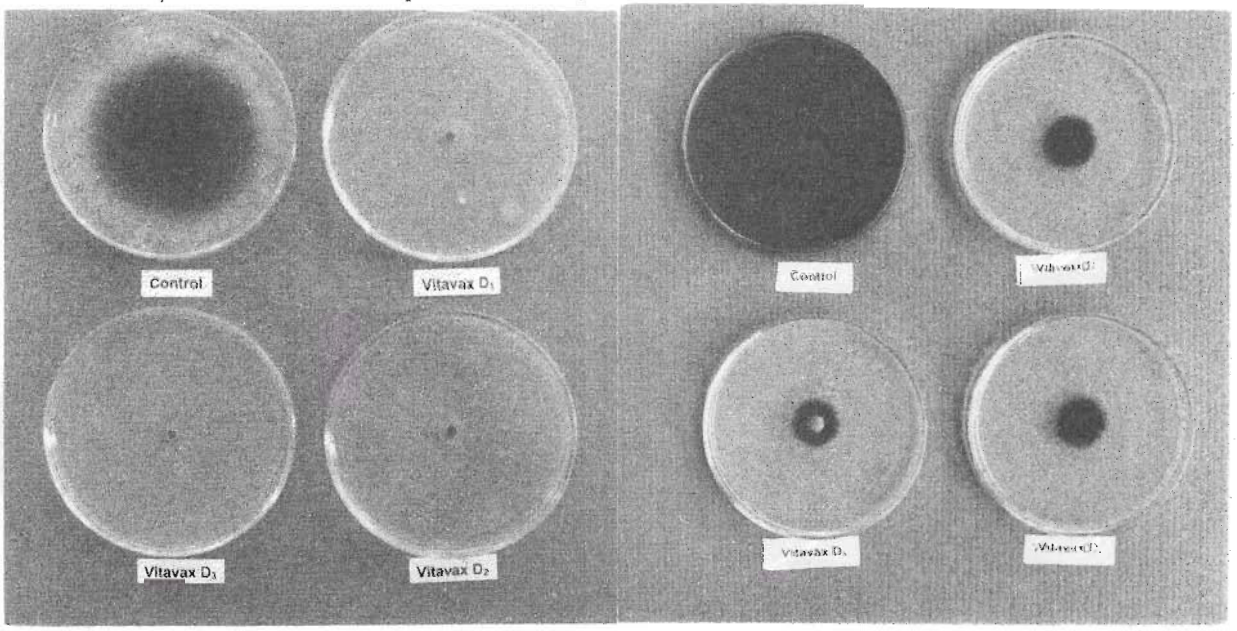


**Figure 5** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Vitavax mixed PDA media at 3rd day (left) and 5th day (right).

$D_1$ - below normal dose; $D_2$ - normal dose; $D_3$ - above normal dose.

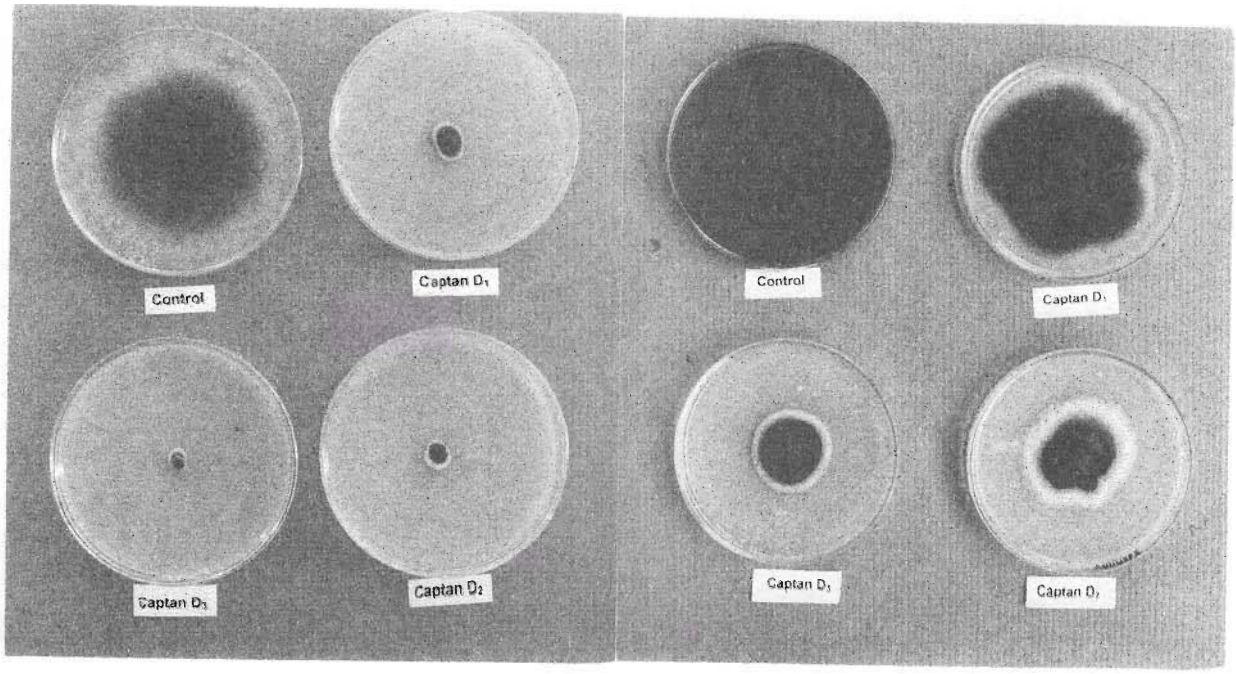


**Figure 6** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Captan mixed PDA media at 3rd day (left) and 5th day (right).

$D_1$ - below normal dose; $D_2$ - normal dose; $D_3$ - above normal dose.

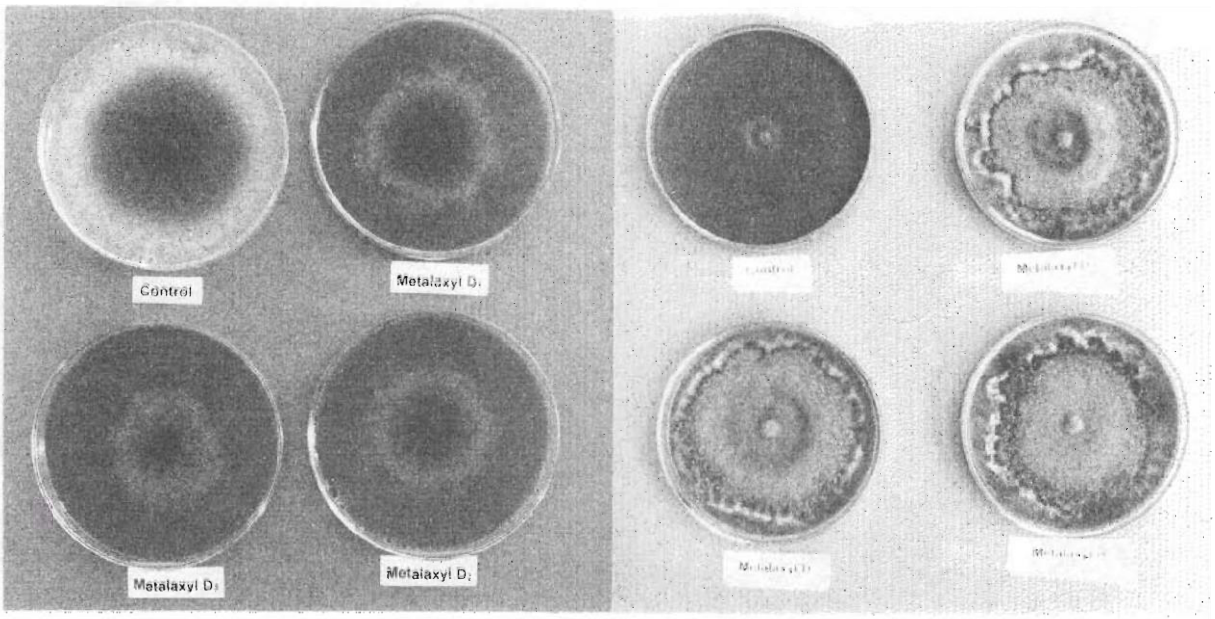


**Figure 7** Mycelial growth of *Macrophomina phaseolina* in only PDA media (control) and in Metalaxyl mixed PDA media at 3rd day (left) and 5th day (right).

$D_1$ - below normal dose; $D_2$ - normal dose; $D_3$ - above normal dose.

**Table 2 Effect of fungicidal seed treatment on *Macrophomina phaseolina* infection and germination according to blotter method with promising fungicides**

| Fungicides | Infection (%) | Germination (%) | Germination increased over control (%) |
|---|---|---|---|
| **Benlate** | **0.0** | **88.0** | **57.14** |
| **Dithane M-45** | **0.0** | **87.0** | **55.36** |
| **Thiram** | **0.0** | **86.0** | **53.57** |
| **Control** | **28.0** | **56.0** | **-** |

results show the average of four replications

treatment with Benlate, Dithane M-45 and Thiram 57.14, 55.36 and 53.57 percent germination increasing was appeared respectively.

**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 untreated control (Table 3). Complete control of disease was observed regarding Benlate and Dithane M-45. The significant disease control was also recorded in case of Thiram by 93.8 percent. Again all tested three fungicides gave significantly higher germination than that of untreated control and the germination increased by 36.2 to 42.0 percent because of seed treatment. The highest germination (98.0 percent) was obtained with Dithane M-45 followed by Benlate (97.0 percent) and Thiram (94.0 percent). Due to seed treatment, significant number of healthy and vigorous seedlings was produced compared to untreated control (Plate 7). Increasing of healthy seedling production after seed treatment was ranged from 37.3 to 46.3 percent.

## Discussion

From the *in-vitro* trial, it was revealed 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 to be highly effective and were able to eliminate the infection completely as well as improved germination in comparison to control. The observation in blotter and pot experiment found to be similar.

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.

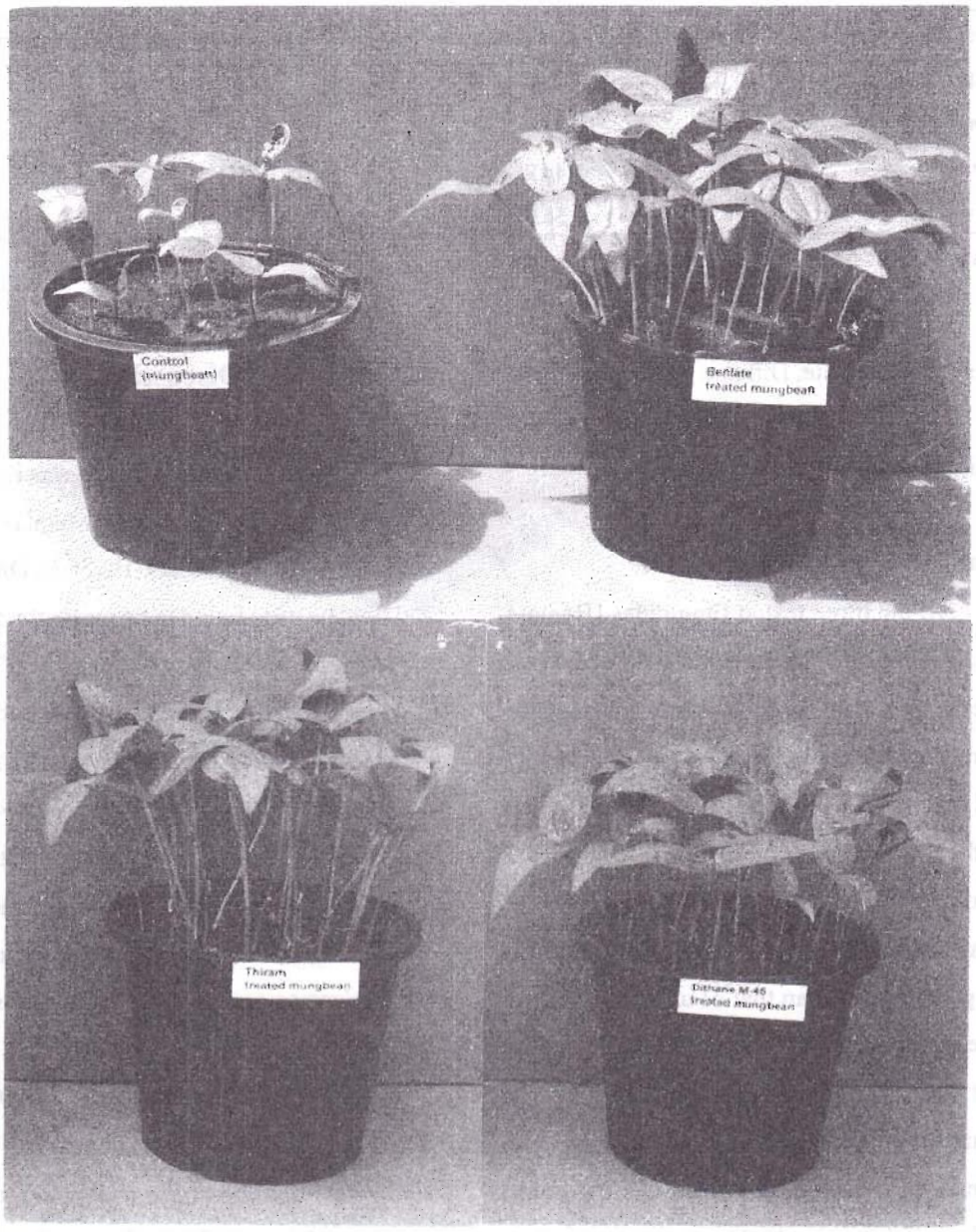


**Figure 8** Control and Benlate, Dithane M-45 and Thiram treated mungbean seedlings after 8 days of planting.

**Table 3 Fungicidal seed treatment against *Macrophomina phaseolina* infection and germination planting the seed in plastic pot with sterilized soil**

| Name of Chemical | Germination (%) | Increase of germination over control (%) | Infected Seedlings (%) | Decrease of infected seedlings over control (%) | Healthy Seedlings (%) | Increase of healthy seedling over control (%) |
|---|---|---|---|---|---|---|
| Benlate | 97.0 | 40.6 | 0.0 | 100.0 | 96.0 | 43.3 |
| Dithane M-45 | 98.0 | 42.0 | 0.0 | 100.0 | 98.0 | 46.3 |
| Thiram | 94.0 | 36.2 | 2.0 | 93.8 | 92.0 | 37.3 |
| Control | 69.0 | - | 32.0 | - | 67.0 | - |
| $LSD_{0.05}$ | 4.38 | - | 2.42 | - | 3.94 | - |

mean of four replications.

## Conclusion

From the present investigation it can be inferred that 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. However, before making any recommendation to the farmer, comprehensive research including field trial is needed.

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