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ผลของการใช้น้ำร้อนต่อเชื้อรา *Macrophomina phaseolina* และความงอกของเมล็ดพันธุ์ถั่วเขียว

Hot Water Treatment of Mungbean Seeds: Effects on Seed-borne *Macrophomina phaseolina* and on Germination

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Abstract : Hot water treatment was done in mungbean seeds in order to control seed-borne *Macrophomina phaseolina*. Total seven different temperatures viz. 50°C, 52°C, 54°C, 56°C, 58°C, 60°C and 62°C and three durations viz. 10, 15, and 20 minutes for each temperature treatment were employed. Among them, the suitable temperature and duration for best germination and complete eradication of *M. phaseolina* found to be 54°C for 20 minutes, 56°C for 10, 15, and 20 minutes; and 58°C for 10 and 15 minutes. After 58°C for 15 minutes duration, the germination was sharply decreased with the increasing of temperature and period although the infection did not appear any longer. However, for convenience, the recommended temperature and period for hot water treatment in order to control seed-borne *M. phaseolina* can be 56°C to 58°C for 10 to 15 minutes.

ทคัดย่อ : จากการนำเมล็ดถั่วเขียวแช่ในน้ำร้อนเพื่อกำจัดเชื้อรา *Macrophomina phaseolina* ที่ติดมากับเมล็ด โดยใช้อุณหภูมิน้ำร้อนทั้งหมด 7 ระดับ ได้แก่ 50°C, 52 °C, 54 °C, 56 °C, 58 °C, 60 °C และ 62 °C และแช่เมล็ดเป็นเวลานาน 10, 15 และ 20 นาที พบว่าอุณภูมิที่เหมาะสมของน้ำร้อนในการกำจัดเชื้อราได้อย่างสมบูรณ์และช่วยเพิ่มความงอกของเมล็ดให้สูงขึ้นมาก ได้แก่ ที่อุณหภูมิ 54 °C นาน 20 นาที อุณหภูมิ 56 °C นาน 10, 15 และ 20 นาที และที่อุณหภูมิ 58 °C นาน 10 และ 15 นาที การใช้อุณหภูมิที่สูงกว่า 58 °C นานกว่า 15 นาที พบว่าแม้สามารถกำจัด

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เชื้อราได้ดีแต่ทำให้ความงอกของเมล็ดลดลงมาก อย่างไรก็ตามในการใช้น้ำร้อนกำจัดเชื้อรา *M. phaseolina* เพื่อมิให้เกิดปัญหาเกี่ยวกับความงอกควรแนะนำให้ใช้น้ำร้อนที่อุณหภูมิ 56 °C-58 °C นาน 10 - 15 นาที

Index words : โรคเมล็ดถั่วเขียว, seed borne disease, Mungbean

Introduction

Thermotherapy is one of the most common methods of physical seed treatment for controlling certain plant diseases. Hot water treatment has proved to be efficient technique as thermotherapy against various pathogenic microorganisms including some kinds of fungi. It is not only most effective against superficial organisms but also has preventive properties and can reduce the incidence of internal pathogens (Maude, 1996). It represents an interesting means for controlling plant diseases because it is simple in principle, easy to use and not expensive. Moreover, it is eco-friendly because of absence of chemical residue.

So far, no attempt has been made to control the seed-borne infection caused by *M. phaseolina* in mungbean seeds through hot water treatment. A very few pertinent literatures are available to control *M. phaseolina* in other crops like cowpea. Sinha and Khare (1977) found most effective control of *M. phaseolina* in cowpea seeds by hot water treatment at 46°C for 20 minutes. Therefore, the present study was undertaken to explore the suitable temperature and duration of hot water for mungbean, which is lethal only to the seed-borne *M. phaseolina* but not on the germination.

Materials and Methods

Seed sample:

Seed sample of mungbean variety named as Chai Nat 60 was obtained from Chai Nat Field Crops Research Center, which was carrying 29.75 percent natural infection of *M. phaseolina* according to blotter method.

Seed treatment:

Hot water treatment was carried out in a water bath controlled by thermostat (model: Memmert). Seven different temperatures viz. 50°C, 52°C, 54°C, 56°C, 58°C, 60°C and 62°C including three durations viz. 10, 15, and 20 minutes for each temperature treatment were employed. At first, the seeds were soaked in sterilized water for 10 minutes. Then 400 soaked seeds were wrapped in a thin and soft cloth following dipping in the water of water bath at the required temperatures for various durations. Before dipping the seed in water bath, the desired temperature was adjusted. The control treatment was maintained with soaking the seeds in normal water but without treatment in hot water. After treating in required duration, the seeds were immediately immersed in cold water for 15 minutes.

Evaluation of hot water treated seed by blotter method:

All the seeds were placed in sterilized petriplate contained 3-layered moist Whatman No. 1 blotter paper. In each petriplate, 10 seeds were placed. All the petriplates with seeds were incubated under 12 hours alternating light and darkness. After 7 days, the seeds were examined under stereo-binocular microscope for observing the infection of *M. phaseolina* including germination percentage. The data were recorded on the basis of 4 replications in each treatment while 10 petriplate i.e. 100 seeds were considered as one replication.

Plantation of hot water treated seed in *in-vivo* condition:

The hot water treated seeds were evaluated in plastic pots filled with sterilized soil. The seeds were treated with effective temperature and durations found in blotter method. The seeds were treated by hot water at 56°C for 20 minutes (which was found to be effective for controlling *M. phaseolina* by blotter method). Two hundred treated seeds with equal number of untreated seeds (but soaked with normal water for 20 minutes) for each category were planted in plastic pots filled with sterilized soil (size 16cm x 12cm) maintaining four replications using 25 seeds per pot. The pots were placed in the glass house and watering was done as usual. The germination and disease incidence was recorded until three weeks.

Results

Evaluation of hot water treated mungbean seed by blotter method:

Results from hot water treatment are presented as line graph in Figure 1. The most effective temperature and duration for complete reduction of *M. phaseolina* infection in mungbean seed were found to be 54°C for 20 minutes and above. Below this temperature and duration, i.e. 50°C for 10, 15 and 20 minutes; 52°C for 10, 15 and Hot water treatment in mungbean seeds.

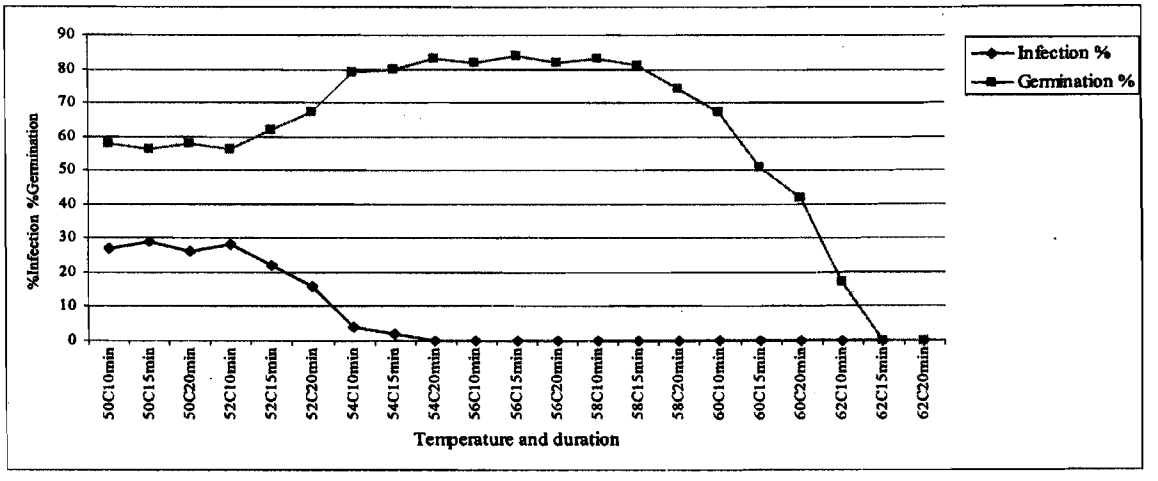

Figure 1 Effect of hot water treatment in different temperatures and durations for controlling seed borne *Macrophomina phaseolina* in mungbean seed.

20 minutes; 54°C for 10 and 15 minutes could not able to eliminate *M. phaseolina* completely although 52°C for 20 minutes, 54°C for 10 and 15 minutes were declined *M. phaseolina* partially (Figure 1).

Plantation of hot water treated seed in pot:

Hot water treatment significantly reduced the diseased development and increased germination compared to untreated control (Table 1). In hot water treated seeds, 36.23 percent germination was increased compared to untreated control treatment. Moreover, the treated seeds produced uniform and more vigorous seedlings (Figure 2). In the hot water treated seeds only 3.0 percent infection was appeared which was decreased by 90.62 percent in comparison to control treatment. Finally, the healthy seedling production was increased by 37.31 percent compared to control.

Discussion

From the present investigation, it is unveiled that for mungbean seed treatment so as to eradication of *M. phaseolina* infection as well as escalating of germination through hot water treatment is obviously effective. The most impressive temperature and duration, which was lethal to *M. phaseolina* and enhanced the maximum germination was found to be 54°C for 20 minutes, 56°C for 10, 15 and 20 minutes, and 58°C for 10 and 15 minutes. However, for convenience, the recommended temperature, and period for hot water treatment can be 56°C to 58°C for 10 to 15 minutes.

From the upshot of the present investigation, it is revealed that the hot water treatment in mungbean seed can not only eliminate the *M. phaseolina* infection but also able to improve the germination ability. Virtually when *M. phaseolina* infects the seed, the seed experiences germination reduction. Due to this reason, because of hot water treatment, the germination was increasing along with *M. phaseolina* infection reduction. In addition, when the hot water treated seeds planted in the pot, the seedlings were observed as more vigorous and uniform compared to control treatment. It is due to elimination of pathogen from the seed after hot water treatment.

Table 1 Effect of hot water treatment on *Macrophomina phaseolina* infection and on rmination of mungbean when the seeds were planted in plastic pot with sterilized soil (mean of four replications).

Germination (%)				Infection appeared (%)				Healthy seedlings (%)			
Control	Hot Water Treated	Increase over control	LSD at 0.05	Control	Hot Water Treated	Decrease over control	LSD at 0.05	Control	Hot Water Treated	Increase over control	LSD at 0.05
69.0	94.0	36.23	5.95	32.0	3.0	90.62	6.87	67.0	92.0	37.31	7.23

Figure 2 Seedlings from hot water treated seed and control (without hot water treatment) of mungbean after 8 days of planting.

Although pertinent literatures on hot water treatment in mungbean for controlling *M. phaseolina* are not available, however, Sinha and Khare (1977) successfully controlled *M. phaseolina* infection in cowpea seeds by hot water treatment. They found 46°C for 20 minutes was effective for *M. phaseolina* elimination in cowpea seeds. The effective temperature for cowpea and presently investing crop mungbean were not alike because these two types of seeds are not same. According to Grondeau and Samson (1994) the effective temperature and duration depends on their seed structure, heat susceptibility of host such as moisture content, dormancy, age and vigor, conditions of external layers. In addition, they reported that thermotherapy is not suitable for legume seed treatment. They mentioned thermotherapy was difficult for legumes like pea, bean, and soybean because a significant decrease of germination was found before the pathogen had been totally killed. Tripathi *et al.* (1987) also described that in chickpea seeds, *Ascochyta rabiei* was eradicated after 6-12 hours at 55 to 60°C while more than 50 percent treated seed did not germinate. This finding does not support the outcome of present investigation. The probable reasons are the reported crops belong to legume but not mungbean. Moreover, Tripathi *et al.* (1987) treated the seeds for very long period, which caused germination reduction. In the present investigation, duration of hot water treatment was maintained within 10 to 20 minutes, which did not show any adverse effect on germination of

mungbean seeds besides inducing germination. The germination increasing was due to reduction of pathogen as well as infection.

Conclusion

For complete eradication of seed-borne *M. phaseolina* from mungbean seed, hot water treatment at 56^{0}C to 58^{0}C for 10 to 20 minutes might be recommended. However, before making final recommendation to the farmers, comprehensive research and field trial is needed.

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