Plant Environment Development 3(2):19-23, 2014 (July)
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e-mail: plant.environ.dev@ru.ac.bd

Print ISSN 1994-1501
Online ISSN 2311-3529

Effects of Fungicides and Botanicals on Seed Germination and Seed Mycoflora of Chickpea and Mungbean

Mst. F. Mahal*

Department of Botany, University of Rajshahi,
Rajshahi - 6205, Bangladesh

*Corresponding author's e-mail: mini_mahal@yahoo.com

Abstract

In present investigation fungicides and botanicals were tested in chickpea and mungbean seeds to test their efficacy for seed germination and reduction of seed mycoflora. Seed germination percentage was significantly ($p \leq 0.05$) increased with both the treatments. Captan (1.2%) was more effective in comparison with Dithane M-45 (1.4%) and seed germination was gradually increased with increase the doses of fungicides. Seed mycoflora also significantly ($p \leq 0.05$) varied with the treatments. *Fusarium oxysporum* was the most dominant fungi in chickpea and *Aspergillus flavus* in mungbean for all cases. Complete elimination doses of the seed mycoflora were 1.2 and 1.4% for Captan and Dithane M-45, respectively. Plant extracts increased germination percentage over control and showed different levels of antifungal activity against seed mycoflora of both pulse seeds. Among the extracts, *Allium sativum* showed promising result in controlling of seed mycoflora of mungbean seeds. Besides this, *F. oxysporum* was remarkably controlled with the all types of extracts.

Keywords: Pulse seeds, Fungicides, Botanicals, Blotter test, Seed germination, Seed mycoflora.

INTRODUCTION

Pulses are an important crop and specially honored as a cheap protein source in comparison with high cost animal protein. They are a good source of carbohydrates, vitamins and protein, providing more than half of plant protein in human diets in some areas of the semiarid tropics (Singh *et al*., 1997; Tuan and Phillips, 1992). Every seed is a potential harbor of a wide variety of mycoflora containing both pathogenic and saprophytic microorganisms, both externally and internally (Utobo *et al.* 2011). These mycoflora deteriorate seed quality, affect viability and reduce germination of seeds, resulting in the production of abnormal seedlings (Paul, 1989; Bateman and Kwasna, 1999 and Khanzada *et al*., 2002). In case of severe fungal infection the seed completely deteriorates and the grain may become unsuitable even for animal consumption due to production of mycotoxic substances by seed fungi.

Several fungicides have been recommended for control of seed-borne fungi of pulse crops. However, the average Bangladeshi farmer cannot afford increasing cost of synthetic chemicals, besides chemical methods of disease management can affect the beneficial microbial population present in the ecosystem. Plant extracts is an

important sources of new agrochemicals and non-selective pesticides for control of plant diseases (Gulter, 1988; Tripathi and Dubey, 2004). Plant extracts and essential oils show antifungal activity against a wide range of fungi (Masoko *et al.*, 2007; Abd-Alla *et al*., 2001). Therefore, the present investigation was conducted in order to find out the effect of fungicides and botanics on seed germination and seed mycoflora of chickpea and mungbean.

MATERIALS AND METHODS

Materials used

The seed samples of chickpea and mungbean were collected from Beena Seed House, Harogram Bazer, Rajshahi during June - August 2012. The chemical fungicides viz., Captan and Dithane M-45 were collected from authorized agrochemical shops at local market in Rajshahi, Bangladesh. Plant materials were collected from Rajshahi University campus and local market in Rajshahi.

Treatment with fungicides

Fungicides viz., Captan (N-tri-chloromethyl thio-4 cyclohexene1, 2-dicarboximide) and Dithane M-45 (Manganese ethylene bisdithio carbamate plus zinc) were prepared at the rate of 0.3, 0.5, 0.7, 0.9, 1.0 and 1.2, 1.4% on the basis of the seed weight. Twenty gram of seeds was taken in 250 ml conical flask and 50 ml of each percentage of fungicide was added separately. The flasks were then shaken properly and kept for 30 minutes. After that the treated seeds were soaked with blotting paper. Then fungicides treated these seeds were applied for blotter test to determine seed germination and seed mycoflora.

Preparation of plant extracts and treatment

Plant materials such as fresh leaves of *Azadirachta indica* (neem), clove of *Allium sativum* (garlic) and rhizome of *Gingiber officinale* (ginger) were thoroughly washed in tap water. Fifty gram of each plant material were mixed with distilled water (w/v, 1:1) and crushed thoroughly in a mortar with pestle and then passed through two layers of cheese cloth. The extracts were centrifuged at 3000 rpm for 20 minutes and stored in a refrigerator at 4°C until used. Seeds were treated with the prepared plant extracts separately by dipping method for 30 minutes. The treated seeds were soaked on blotting paper and placed on moist blotter to determine seed germination and seed mycoflora.

Detection of seed germination percentage and seed mycoflora on blotter test method

The detection of seed germination percentage and seed associated mycoflora were carried out by the blotter test as recommended by the International Seed Testing Association (ISTA-1966) with some modifications (Basak and Mridha, 1985; Basak et al.1991). Blotting papers were soaked in sterile distilled water and three layers were placed in each Petri dish. One hundred seeds were taken at random as per ISTA rules. Out of 100 seeds, 25 seeds per petridish in case of mungbean and 10 seeds in case of chickpea were placed. The petridishes were then incubated at 28±2°C under defused light for seven days. Treated and control seeds were evaluated for recording percentage of seed germination with the following the formula:

Germination (%) = (Number of seeds germinated/Total number of seeds used) ×100

After seven days of incubation, the plated seeds were examined under a stereoscopic binocular microscope for the observation and enumeration of fungi. The emerging fungi on blotter were subcultures on PDA plate for identification following the keys outlined by Gilman, 1957; Booth, 1971; Subramanian, 1971; Ellis, 1971 and Alexopoulos and Mims 1979.

Statistical analysis of data

A completely randomized design was used. Analysis of the variance (ANOVA) was done accordingly and significant differences among the treatments means were identified by least significant differences (LSD) test at 5% level.

RESULTS AND DISCUSSION

Effect of fungicides on seed germination and incidence of seed mycoflora

Seed germination percentage of chickpea and mungbean were significantly ($p \leq 0.05$) varied with the treatments of different doses of fungicides and increased compare to control (Table 1). The maximum seed germination percentage of chickpea (96 and 93%) and mungbean (96 and 94%) was recorded at 1.2 and 1.4% doses of captan and dithane-M 45 and the lowest was at 0.3% dose of tested both fungicides. Captan was most effective than dithane-M 45 to increase seed germination percentage of both pulses. In another study, Umesh and Maske (2012)

observed that benomyl, dithane M-45 and bavistin effectively increased the germination percentage of cowpea. These findings completely agree with the present observation.

Table 1: Effect of fungicides and botanicals on seed germination of chickpea and mungbean in blotter test after 7 days of incubation at 28±2°C.

Teatment	Dose (%)	Seed germination[a] (%)	
		Chickpea	Mungbean
Captan	0.3	88	89
	0.5	88	92
	0.7	92	92
	0.9	94	93
	1.0	96	96
	1.2	96	96
F value ($LSD_{p\leq0.05}$)	12.48 (1.75)*		
Dithane-45	0.3	84	84
	0.5	84	87
	0.7	86	89
	0.9	88	91
	1.0	88	93
	1.2	91	94
	1.4	93	94
F value ($LSD_{p\leq0.05}$)	18.96 (01.50)*		
Botanicals			
Azadirachta indica	1:1	91	97
Zingiber officinale	1:1	80	94
Allium sativum	1:1	83	90
F value ($LSD_{p\leq0.05}$)	3.70 (10.82)*		
Control	79	79	

a =Mean of three replications.
*= Significant at 5% level

Seed mycoflora of chickpea and mungbean showed significantly ($p\leq0.05$) varied with the treatments (Table 2 and 3). In blotter test recovered fungi were identified as *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium* sp. in chickpea and in case of mungbean it was *A. flavus*, *F. oxysporum*, *Penicillium* sp. and *Curvularia* sp. Among them *F. oxysprum* (13, 17 and 23%) and *A. flavus* (11, 13 and 20%) were the most dominant fungi in chickpea and mungbean, respectively for both treatments and control. Both the fungicides significantly ($p\leq0.05$) reduced the number of mycoflora of the seeds of chickpea and mungbean (Table 2 and 3). It was observed from the experiment that 1.2% of Captan and 1.4% dose of dithane M-45 were completely eradicate all the fungi from the seeds of chickpea and mungbean, respectively and 0.3% dose of both fungicides was less effective. *A. flavus* and *Penicillium* sp. were totally controlled at 1.2% of Captan and 1.4% of dithane M-45 treated chickpea whereas it was 1.0 and 1.4% in mungbean. *Curvularia* sp. recorded less frequent fungus and totally control at 1.0% of captan and 1.2% of dithane M-45 in mungbean. On the other hand, *F. oxysprum* was completely controlled at 1.0% of Captan and 1.4% of dithane M-45 treated chickpea and in case of mungbean seeds it was 1% and 1.2%, respectively. In similar study Singh *et al*. (2002) reported that Captan, Dithane M-45, Vitavax and Bavistin were effective in controlling of *Fusarium* sp. on mungbean.

Table 2: Effects of fungicides and botanicals in controlling seed mycoflora of chickpea in blotter test after 7 days of incubation at 28±2°C.

Teatment	Dose (%)	Incidence of seed mycoflora[a] (%)		
		Aspergillus flavus	*Fusarium oxysporum*	*Penicillium* sp.
Captan	0.3	9	13	11
	0.5	9	9	9
	0.7	8	5	7
	0.9	5	5	5
	1.0	2	0	2
	1.2	0	0	0
F value ($LSD_{p\leq0.05}$)	36.69 (1.58)*			
Dithane-45	0.3	11	17	14
	0.5	10	14	11
	0.7	8	10	8
	0.9	6	7	6
	1.0	5	5	3
	1.2	2	2	1
	1.4	0	0	0
F value ($LSD_{p\leq0.05}$)	51.50 (1.44)*			
Botanicals				
Azadirachta indica	1:1	0	5	0
Zingiber officinale	1:1	3	3	0
Allium sativum	1:1	0	4	6
F value ($LSD_{p\leq0.05}$)	0.34 (5.93)*			
Control	13	23	17	

a =Mean of three replications.
*= Significant at 5% level

Effect of plant extracts on seed germination and incidence of seed mycoflora

Seed germination percentage of chickpea and mungbean were significantly ($p\leq0.05$) varied with the different plant extracts (Table 1). The maximum seed germination (%) was recorded as 91 and 97% with *A. indica* leaf extract treated chickpea and mungbean and the minimum was

80% with *G. officinale* treatment and 90% with *A. sativum*. It was remarkably noticed that seed germination percentage increased with the used plant extracts compare to control. These results are complete agreement with Bansal and Gupta's observation (2000) who reported many plant extracts increase seed germination by decreasing of *F. oxysporum* incidence.

Table 3: Effects of fungicides and botanicals in controlling seed mycoflora of mungbean in blotter test after 7 days of incubation at 28±2°C

Teatment	Dose (%)	Incidence of seed mycoflora[a] (%)			
		Aspergillus flavus	*Fusarium oxysporum*	*Penicillium* sp.	*Curvularia* sp.
Captan	0.3	11	8	8	7
	0.5	8	6	6	5
	0.7	5	4	4	3
	0.9	2	1	3	2
	1.0	0	0	0	0
	1.2	0	0	0	0
F value ($LSD_{p\leq0.05}$)		69.44 (1.02)*			
Dithane -45	0.3	13	10	8	7
	0.5	11	6	7	5
	0.7	8	5	5	4
	0.9	7	4	3	2
	1.0	5	2	2	2
	1.2	2	0	1	0
	1.4	0	0	0	0
F value ($LSD_{p\leq0.05}$)		42.76 (1.18)*			
Botanics					
Azadirachta indica	1:1	3	0	6	3
Zingiber officinale	1:1	4	0	3	0
Allium sativum	1:1	0	0	0	0
F value ($LSD_{p\leq0.05}$)		4.40 (1.74)*			
Control		20	13	11	12

a =Mean of three replications.
*= Significant at 5% level

Plant extracts also showed significant ($p\leq0.05$) difference of antifungal activity in controlling of seed mycoflora of both pulses (Table 2 and 3). In chickpea seeds the incidence of *A. flavus* was successfully controlled treated with *A. sativum* and *A. indica* extracts and *A. indica* and *Z. officinale* totally controlled *Penicillum* sp. (Table 2). On the other hand, it was remarkably noticed that *F. oxysporum* was not controlled with the used plant extracts. In case of mungbean seeds, seed mycoflora were successfully controlled with *A. sativum* extract while *Curvularia* sp. and *F. oxysporum* were totally controlled with *Z. officinale* extract but *F. oxysporum* was totally controlled with the extracts (Table 3). These results completely agree with the observation of Suratuzzama *et al*. (1995) who obtained good effect of *Z. officinale* extract in controlling of *Pyricularia oryzae* and *Curvularia lunata*.

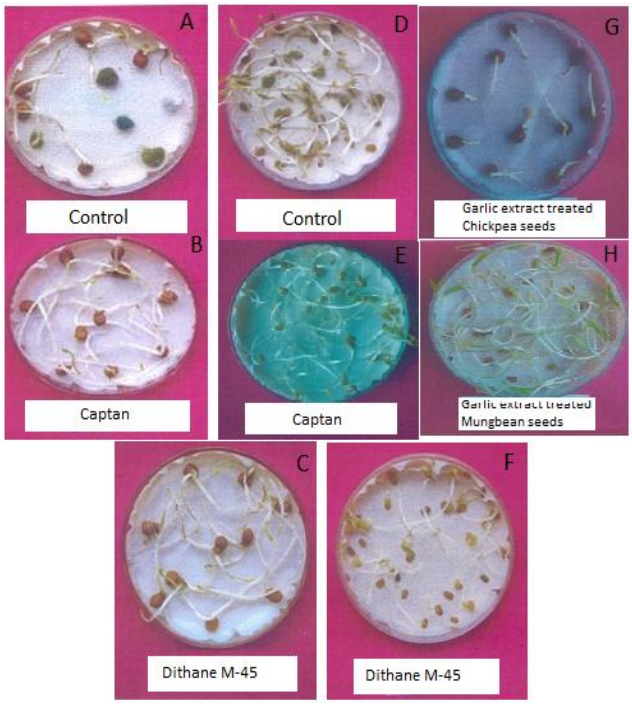

Fig. 1: A and D. Infected chickpea and mungbean seeds in control; B, C & G and E, F & H. complete elimination of seed mycoflora with Captan and Dithane M-45 and Garlic extract, respectively.

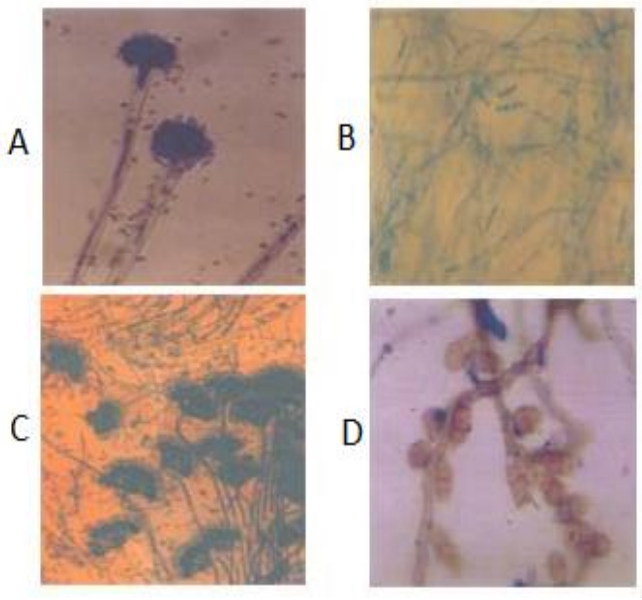

Fig. 2: A. *Aspergillus flavus* B. *Fusarium oxysporum* C. *Penecillium* sp. and D. *Curvularia* sp. which are recovered from chickpea and mungbean seeds.

From the results it was observed that fungicides and botanicals both played effective role on seed germination by controlling seed mycoflora. *A. sativum* showed promising result in controlling of seed mycoflora of pulses. So this extract can be used in controlling of seed mycoflora instead of chemical fungicides to safe environment.

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