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COMPARATIVE STUDY ON EFFECT OF PLANT EXTRACT AND CHEMICAL FUNGICIDES AGAINST SEED BORNE PATHOGENS OF CHICKPEA

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Abstract

The yield of chickpea (*Cicer arietinum*) is decreasing day by day due to some seed borne pathogen in Bangladesh. In this experiment, some plant extract and chemical fungicides were evaluated against seed borne pathogens of chickpea along with germination ability. A total of 15 seed samples of BARI Chola-9 were collected from different chickpea growing areas in Bangladesh. A significant variation was observed in germination percentage as well as pathogen association of the seeds. Seven seed-borne fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Fusarium* sp., *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata* were detected. The highest prevalence of seed borne pathogen in chickpea was found from Bianibazar upazila sample along with lowest germination percentage. After the seed treatment with plant extract and chemical fungicides, the lowest disease incidence was found in Bavistin 70 WP than other treatments. Thus, Bavistin 70 WP may be an efficient seed-treating chemical fungicide to control seed borne pathogens of chickpea.

Keywords: Chemical, plant extract, chickpea, fungicide, germination.

Introduction

Chickpea (*Cicer arietinum*) is one of the important pulse crops in Bangladesh. It belongs to the family Leguminosae (Fabaceae) and a self-pollinated crop. In Bangladesh, chickpea is the third major pulse crop after grasspea (Khesari) and lentil. It is ranked in 5th position in term of its production among the pulses in Bangladesh. The average yield of chickpea is 0.80 t ha^{-1} in Bangladesh (BBS, 2011). Recently, the average yield of chickpea has been declined due to various biotic and abiotic factors. Among the biotic factors, disease is playing a vital role in yield reduction. Chickpea is reported to be attacked by about 67 fungi, 3 bacteria, 22 viruses and mycoplasma and 80 nematodes (Nene *et al*., 1996). Mainly five seed borne fungi are common in chickpea seeds. Field fungi also associated with seed causes deterioration of quality resulting reduce viability and germination of seeds (Nene *et al*., 1996). The infection of chickpea seeds by *Fusarium* spp. and *Sclerotium rolfsii* caused poor germination and reduced vigor (Chaithra, 2009). Root rot caused by *Fusarium oxysporum, Fusarium solani* and *Sclerotium rolfsii* is considered as an important and destructive disease of pulse crops in Bangladesh as well as almost all legume growing countries of the world (Fakir, 1983). Some effective fungicides are available in the market but their number is very few (Andrabi *et al.*, 2011). Recently, different kinds of plant extracts are used to control the disease, which are eco-friendly, and, their use in crop protection is a practical in sustainable agriculture as alternative to chemical. In addition, some plant extracts, which have anti-microbial qualities and antagonistic effect to pathogens (Hossain *et al.*, 1993). Antifungal activities of garlic, neem, allamanda, biskatali, and vatapta have been reported by many researchers (Rahman *et al.*, 1999). Considering the value of chickpea production and its association with seed borne pathogens, this study was carry out to evaluate the efficacy of some plant extracts and chemical fungicides against seed borne pathogens of chickpea under laboratory conditions.

Materials and Methods

Collection and preparation of working sample: Chickpea seeds of BARI Chola-9 were collected from different locations of chickpea growing areas, namely; Gazipur sadar, Sunamganj sadar, Sylhet sadar, Golapganj upazila, and Bianibazar upazila in Bangladesh. Fifteen seed samples (500 g sample^{-1}) were collected from the farmer's field (3 samples from each location) following the International Rules for Seed Testing (ISTA, 2001). These seeds were

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mixed to get a composite sample. From each of the composite sample, 400 seeds were randomly taken from each sample and used as working sample for further studies.

Selected plant extract and chemical fungicides: In pursue of the objectives of the research, three chemical fungicides (Bavistin 70 WP, Iprozim 26 WP and Qubee 50 WP) and three plant extract (Neem leaf extract, Biskatali leaf extract and Marigold leaf extract) were selected. The chemical fungicides were purchased from the local market (Zindabazar, Sylhet). The plant extracts were prepared by blending the leaf of Neem, Biskatali and Marigold, respectively. Initially, 100 g of leaf were collected from each plant and thoroughly washed, air dried and macerated with 400 ml sterile water in a blender for 10 min. After blend, the suspension was filtered through double layered muslin cloth and then centrifuged at 4000 × g at 4^0 C for 30 min. The supernatant was collected through filter paper (Whatman No. 1) and used as working sample of leaf extract (1:4).

Detection of associated seed borne pathogens: The associated seed borne pathogens were detected using blotter method. Initially, three blotter paper (Whatman No.1) were soaked in sterilized water and placed at the bottom of plastic Petridish (9 cm × 9 cm). In each plate, 10 seeds were placed and incubated at $(22 \pm 2)^0$ C for 7 days by 12-hours cycle of alternate ultra violet (UV) light and darkness. After incubation, each plate was observed through stereo-binocular microscope. The associated pathogens were detected by observing their morphological growth characters.

Effect of seed treatment with plant extract and chemical fungicides: The effects of plant extract and chemical fungicides against the detected seed borne fungi were evaluated using blotter method. The seeds were treated with Bavistin 70 WP (2.5 g kg^{-1}), Iprozim 26 WP (2.5 g kg^{-1}), Qubee 50 WP (2.5 g kg^{-1}), Neem leaf extract (1:4), Biskatali leaf extract (1:4), Marigold leaf extract (1:4), respectively. For control, the seeds were treated with distilled water. All treated plates were incubated at $(22 \pm 2)^0$ C for 7 days. After incubation, each plate was observed through stereo-binocular microscope, Incidence of fungi was calculated using the following formula:

$$Disease\ incidence(\%) = \frac{Number\ of\ infected\ plants\ in\ each\ plot}{Total\ number\ of\ plants\ in\ each\ plot} \times 100$$

Experimental design and statistical analysis: The experiments were carried out in laboratory conditions using Completely Randomized Design (CRD) with three replications. The difference between the treatment means was evaluated using Duncan's Multiple Range Test (DMRT) following the procedure as described by Gomez and Gomez (1984).

Results and Discussion

Detection and prevalence of associated seed borne fungi: The detected and prevalence of associated seed borne fungi were shown in Table 1. Seven seed-borne fungi, namely; *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Fusarium* sp., *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata* were detected. These findings are partially agreed with Fakir (1983), who detected five seed borne fungi in chickpea seeds viz; *Fusarium oxysporum* caused wilt, *Macrophomina phaseolina* and *Rhizoctonia* sp. caused seed rot, *Sclerotium rolfsii* caused collar rot and *Ascochyta rabei* caused blight of chickpea. Rauf (2000) also recorded twenty-four seed borne fungi belonging to different genera using blotter method. The prevalence of *A. niger* ranged from 8.70% to 15.70%. The maximum prevalence (15.70%) of *A. niger* was recorded in the seed sample of Bianibazar upazila and minimum prevalence (8.70%) was observed in the seeds collected from Golapganj upazila. The prevalence of *A. flavus* ranged from 9.00% to 17.00%. The highest prevalence (17.00%) was found in the sample of Bianibazar upazila and the lowest (9.00%) was recorded in Gazipur. The highest prevalence (12.70%) of *Penicillium* sp. was found in sample of Bianibazar upazila, where the lowest (6.00%) prevalence was recorded in samples collected from Sylhet sadar. The highest occurrence of *Fusarium* sp. (16.70%) was recorded in samples of Bianibazar sadar and the lowest (7.00%) was recorded in Sylhet sadar. The highest (11.00%) occurrence of *B. cinerea* was found in the sample of Bianibazar sadar and the lowest (4.67 %) was recorded in sample of Gazipur sadar. The percentage of *R. stolonifer* ranged from 6.33% to 14.00%, whereas, the prevalence of *A. alternate* ranged from 4.33% to 16.70% in the seed sample. In both cases, maximum prevalence was recorded in the seed sample of Bianibazar upazila and minimum prevalence was observed in the seeds collected from Golapganj upazila. In Bianibazar upazila, there may be poor storage conditions

and not properly drying the seeds. Rahman and Ahmed (1985**)** detected seed-borne fungi of chickpea, where *Aspergillus* sp. was the most predominant and pathogenic fungi *Colletotrichum dematium* and *Fusarium* sp. were also prevalent.

Table 1. Prevalence of seed borne pathogen in chickpea seeds.

Sample	Prevalence (%) of seed borne fungi						
	A. niger	*A. flavus*	*Penicillium* sp.	*Fusarium* sp.	*B. cinerea*	*R. stolonifer*	*A. Alternate*
Gazipur	10.3 b	9.00 c	8.33 bc	8.67 bc	4.67 c	6.33 c	4.33 c
Sunamganj	12.0 ab	9.33 c	7.33 c	9.67 b	5.33 b	10.00 b	7.30 bc
Sylhet	11.70 b	10.30 bc	6.00 c	7.00 c	5.33 b	6.67 c	8.67 b
Golapganj	8.70 c	11.70 b	9.00 b	11.00 b	7.00 b	8.00 bc	6.67 bc
Bianibazar	15.70 a	17.00 a	12.70 a	16.70 a	11.00 a	14.00 a	16.70 a

Effect of seed treatments on germination of seeds at different sample: After seed treatment with plant extract and chemical fungicides, variation was found in germination of seeds at different sample as shown in Fig. 1. The germination percentage of chickpea seeds ranged from 77.70% to 83.00%. The highest germination (83.00%) was found in the sample of Gazipur followed by the Sunamganj (81.30%), Sylhet (79.30%) and Golapganj (78.30%) and the lowest (77.70%) was recorded in the samples collected from Bianibazar. These results partially supported by Morshed *et al.* (2014) who stated that the germination of chickpea were increased by treating seeds with Secure 600 WG (48.60%) followed by Provax 200 WP (44.40%) over control. Seed treatment with Carbendazim increased seed germination (71.24%), though it was at par with Carbendazim + Mancozeb (62.21%) and Mancozeb (61.46%) (Andrabi *et al.*, 2011).

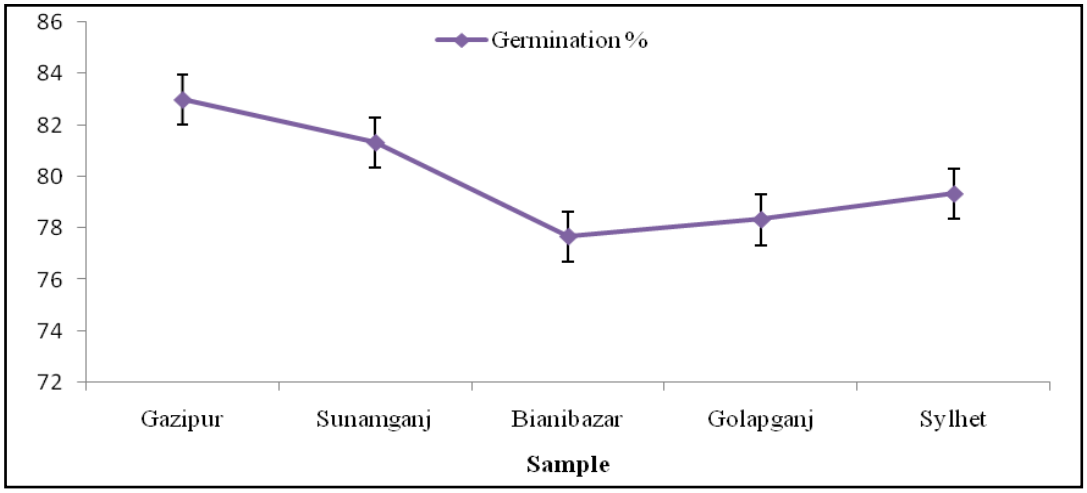

Fig. 1. Effect of seed treatments on germination of seeds at different sample.

Effect of seed treatments in controlling seed borne fungi: The effect of seed treatment with plant extract and chemical fungicides, also found significantly different as shown in Table 2. To control these seed borne fungi, all the seed treating agents appeared to be effective in comparison to the control. The highest incidence (20.70%) of *A. niger* was observed in control and the lowest (5.65%) was recorded in Bavistin 70 WP treated seeds. Similar trend of incidence was also observed in case of *A. flavus* and *Penicillium* sp. In case of *Fusarium* sp. Bavistin 70 WP showed lower incidence (4.67%) than other treatments. The highest incidence (17.70%) of *B. cinerea* was observed in control and the lowest (3.00%) was recorded in Bavistin 70 WP treated seeds. Minimum incidence (6.00%) of *R. stolonifer* was also observed in Bavistin 70 WP treated seeds. In case of *A. alternate*, the lower incidence (2.00%) was found in Bavistin 70 WP treated seeds than other treatments. Gaikwad and Sen (1987) reported that seed treatment with Bavistin (Carbendazim) and Cercobin (Thiophanate) were the most effective fungicides in inhibiting spore germination and growth of *F. oxysporum*. Fungicides like Derozal (Carbendazim) and TMTD (Thiram) gave the best control *in vivo* situation. Mugibon, Vitavax 200-NP and Homai 80 performed better *in vitro* condition

(Buchvarva *et al.,* 1989). Aurangzeb *et al.* (1998) reported that Carbendazim inhibited the mycelial growth of *F. moniliforme in vitro* and also controlled the disease.

Table 2. Effect of seed treatments on seed borne fungi of chickpea.

Treatments	Percentage of incidence						
	A. Niger	*A. flavus*	*Penicillium* sp.	*Fusarium* sp.	*B. cinerea*	*R. stolonifer*	*A. alternate*
Bavistin 70 WP	5.67 g	8.67 g	6.00 g	4.67 e	3.00 f	6.00 e	2.00 g
Iprozim 26 WP	8.67 f	11.00 f	10.00 f	11.70 d	9.00 b	9.00 de	5.00 f
Qubee 50 WP	11.30 e	12.00 e	13.70 e	11.70 d	7.67 c	11.00 cd	9.67 b
Neem leaf extract	13.30 d	14.70 d	15.70 d	16.30 c	6.67 d	15.00 b	8.67 c
Biskatali leaf extract	14.30 c	17.70 c	18.70 c	18.70 b	7.67 c	14.70 b	6.67 e
Marigold leaf extract	16.70 b	18.30 b	19.70 b	19.00 b	5.67 e	14.00 bc	7.67 d
Control	20.70 a	22.70 a	24.70 a	24.30 a	17.70 a	23.00 a	16.30 a

From this study, it may be concluded that a significant presence of seed-borne pathogens was present on the collected chickpea seeds from different locations. After seed treatments, the germination percentage of the chickpea seed was increased. Among all treatment with plant extract and chemical fungicides, Bavistin 70 WP gave the best results. So, Bavistin 70 WP may be a suitable seed treating agents to control seed-borne pathogens associated with chickpea. Further studies are carried out to examine the effectiveness of Bavistin 70 WP in field conditions.

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