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EFFECT OF CHEMICALS ON GERMINATION AND SEED BORNE MYCOFLORA OF WHEAT IN NEPAL

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ABSTRACT

In vitro seed borne fungal infections in wheat (*Triticum aestivum*) seeds were studied by blotter method on nine varieties/genotypes collected from Rampur, Chitwan; Khajura, Banke and Khumaltar, Lalitpur at Seed Quality Control Center, Harihar Bhawan, Lalitpur, during 2013 with the objective of determining fungal pathogens associated with wheat seeds and their management. Dithane M 45 (1.5g kg^{-1}), Vitavex (2g kg^{-1}) and Raxil (1g kg^{-1}) were used to treat wheat seeds of three varieties/genotypes from each location with high fungal infection. *Alternaria alternata, Bipolaris sorokiniana, Curvularia lunata, Cladosporium herbarum, Epicoccum nigrum* and *Bipolaris spicifera* were detected from untreated seeds. The fungicides were having significant effect in reducing percentage frequency of *Alternaria alternata, Bipolaris sorokiniana, Bipolaris spicifera,* and *Curvularia lunata* in the seeds of Khajura and Khumaltar locations, however they were non-significant among each other in the seeds of Rampur. Seed treatment with Dithane M 45 and Vitavex was significantly more effective for *Bipolaris sorokiniana* and *Alternaria alternata* as compared to Raxil. Thus, seed treatment with Dithane M 45 or Vitavex would be a good option for the management of seed borne pathogens.

Keywords: Blotter test, Pathogens, Genotypes, Fungicides

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INTRODUCTION

Wheat is the most widely cultivated cereal crop in the world and is the major winter cereal in Nepal. It is third most important staple crop in terms of area and production with productivity of 2.47 t ha^{-1}(MOAD, 2013). But the productivity is comparatively low than the other wheat growing countries. The lower productivity is due to diseases and poor management. Wheat is affected by as many as 120 different diseases, of which 42 are seed borne (Wiese, 1987). Some important seed borne diseases of wheat are, Karnal bunt *(Tilletia indica*), Head blight or scab *(Fusarium* spp.*),* Loose smut *(Ustilago tritici),* Tundu or ear cockle or yellow ear rot disease (*Clavibacter tritici* and *Anguina tritici*), Septoria blotch (*Septoria tritici*, *S. nodorum* and *S. avenae* f. sp. *triticea*), Spot blotch (*Bipolaris sorokiniana*), Tan spot (*Pyrenophora tritici-repentis*), Alternaria leaf blight (*Alternaria triticina*), Snow mold (*F. nivale*, *Stagonospora nodorum*) Leaf and glume blotch (*Stagonospora nodorum*), Ergot (*Claviceps purpurea*) and Black chaff or Bacterial streak (*Xanthomonas campestris* pv. *translucens*) (Majumder *et al.,* 2013).

The other common seed borne fungi isolated from wheat seeds are *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum* (Khan, 1992). These pathogens also affect the grain quality and human health. Barabara et al. (2004) reported that the quality and nutritional composition of wheat was reduced by fungal infection. Glutein content in Fusarious wheat was lower in comparison with glutein of healthy wheat (Dexter et al., 1996). *Bipolaris sorokiniana,* seed borne and seed transmitted in nature (Bazlur-Rashid, 1998) caused seedling blight, head blight, leaf blotch, foot rot etc and reduced 88.7% grains per ear (Hossain *et al.*, 2001). Similarly, *Fusarium* sp. a seed borne fungus, has significant constrains to wheat production by causing head blight, stem blight, grain rot etc.

Healthy seed plays an important role for successful cultivation and higher yield of crop (Rajput et al., 2005). Management of seed borne pathogens is very important to get the optimum yield of the crop and reduction in use of chemical fungicides. Protection of crop against diseases can be obtained by different management practices. Management of disease mainly aims to prevent disease epidemics and widespread of disease in host plant. This may be achieved through reducing the initial population of the pathogens and then decelerating the rate of increase in those reduced population. The use of chemicals, biological agents and botanical extracts to treat seed reduce the initial population of pathogen. Chemical seed treatments have been successfully used against seed borne pathogens, because very small amount of pesticides per unit area are required and their environmental impacts are quite low (Mathre et al., 1995). Fungicide seed treatments are also recommended to increase seed viability when higher germination rates are needed to

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meet seed quality standards (Gaska and Borges, 2004). Singh and Kumar (2008) reported that seed treatment with Vitavex (Carboxin) at 2.5 g kg^{-1} and Thiram at 3.0 g kg^{-1} of seed eradicated *Bipolaris sorokiniana* and *Alternaria tenuis* from wheat seed. The present study was carried out to identify the effective chemical against fungi prevailing in wheat seeds used commonly in Nepal.

MATERIALS AND METHODS

The study was carried out in mycology laboratory of Seed Quality Control Centre, Harihar Bhawan, Lalitpur, during August-December. Two hundred gram wheat seeds of 23 varieties and 17 genotypes were collected from research institutes of Nepal. Isolation and identification of seed borne fungi was done by blotter method described by International Seed Testing Association (Mathur and Kongsdal, 2003). Plastic petri-dishes were cleaned by washing with detergent solution, followed by rinsing several times in clean tap water. The petri-dishes were finally surface sterilized by rinsing with 4% NaOCl solution. Three pieces of blotting papers of nine cm diameter were moistened with distilled water and placed in petri-dishes. The accession number of the seed samples and date of incubation on each petri-dish were recorded.

Twenty gram seeds of each variety/genotype were treated with the recommended doses of fungicides. Dry seed treatment was done by mixing required amount of fungicide with seeds in a conical flask, followed by vigorous shaking manually for a few minutes. Untreated seeds were used as check.

Twenty five seeds per petri-dish were placed in equidistance, fifteen seeds in outer ring, nine in middle ring and one at the center. One hundred seeds formed one replication and four replications were maintained per varieties/genotypes. The petri-dishes were incubated at 20 ºC under alternate cycles of 12 hours near ultra violet light and darkness.

Five, seven and nine days after incubation, the seeds were observed under stereo-binocular microscope for the presence of fungi. A compound microscope was used for identification of fungi. Identification of the fungi was done based on their color, fruiting bodies, spore morphology and habitat characteristics as described by Mathur and Kongsdal (2003).

Germination of seed was recorded in the blotter test and germination percentage was calculated by applying following formula.

$$\text{Germination } \% = \frac{\text{number of seed germinated}}{\text{total number of seeds}} \text{X } 100$$

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Percentage frequency is the number of seeds out of one hundred in which at least a fungal species appears PF was calculated by using the following formula and mean were computed:

$$\text{Percentage frequency (PF)} = \frac{\text{No. of seeds on which fungus appears}}{\text{Total number of seeds}} \text{X } 100$$

RESULTS AND DISCUSSION

Germination of seeds

On an average seed treatment with Dithane M 45 improved germination percentage by 12.65% over control (Table 1). Similarly, germination percentage was increased by 9.79% in Raxil and 6.50% in Vitavax.

Table 1. Improvement in germination percentage of seeds through fungicide treatment over control

S.N.	Fungicides	Germination percentage over control			
		Rampur	Khajura	Khumaltar	Average
1	Dithane M 45	8.24	2.13	27.58	12.65
2	Raxil	6.96	1.96	20.46	9.79
3	Vitavex	6.47	1.71	11.32	6.50
	Mean	7.22	1.93	19.79	9.65

Javaid et al. (2006) found significant effect of Acrobat MZ, Dithane M 45, Aliette and Metalaxyl plus Mancozeb on seed germination and shoot biomass. Kabir et al. (2007) found that seed treatment with Vitavax 200 had highest germination over control. Schaafsma and Tamburic-

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Ilincic (2005) reported that emergence of wheat was greatest after seed treatment with Vitaflo 280 (Thiram + Carbathiin). Javaid et al. (2006) reported that Dithane and Metalaxyl plus Mancozeb can be used to disinfect the seeds prior to germination as these fungicides were very effective against the seed borne mycoflora of wheat.

Effect of seed treatment on seed borne fungi Rampur location

Effect of three fungicides, viz. Dithane M 45, Raxil and Vitavex was observed against seed borne fungi of wheat. All the fungicides reduced the incidence of seed borne fungi as compared to control (Figure 1). *Alternaria alternata, Bipolaris sorokiniana, Bipolaris spicifera, Curvularia lunata, Epicoccum nigrum* and *Cladosporium herbarum* were isolated from untreated wheat seeds and only *Alternaria alternata* and *Bipolaris sorokiniana* with Raxil and none with Dithane M 45 and Vitavex treatments. NL 1191 + Control had significantly highest frequency of *Alternaria alternata* (40.00%) and *Bipolaris sorokiniana* (22.75%). The fungi were more in seeds of Bhrikuti treated with Raxil, while, more in NL 1191 in control.

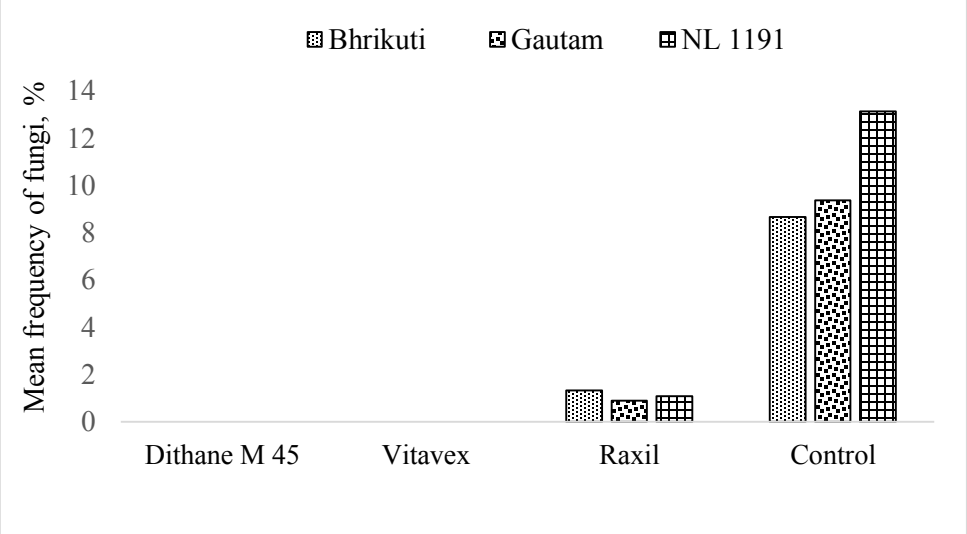

Figure 1: Varietal difference on percentage frequency of fungi in wheat seeds collected from Rampur after fungicide treatment

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Khajura location

Dithane M 45 eradicated all fungi from seeds, Vitavex all except *Alternaria alternata,* Raxil all except *Alternaria alternata* and *Bipolaris sorokiniana* and Control none (Figure 2). Percentage frequency of *Alternaria alternata, Bipolaris sorokiniana, Bipolaris spicifera, Curvularia lunata* and *Cladosporium herbarum* were significantly different among the varieties. Percentage frequency of *Bipolaris sorokiniana* was highest in BL 3978, followed by Bhrikuti and least in BL 4350.

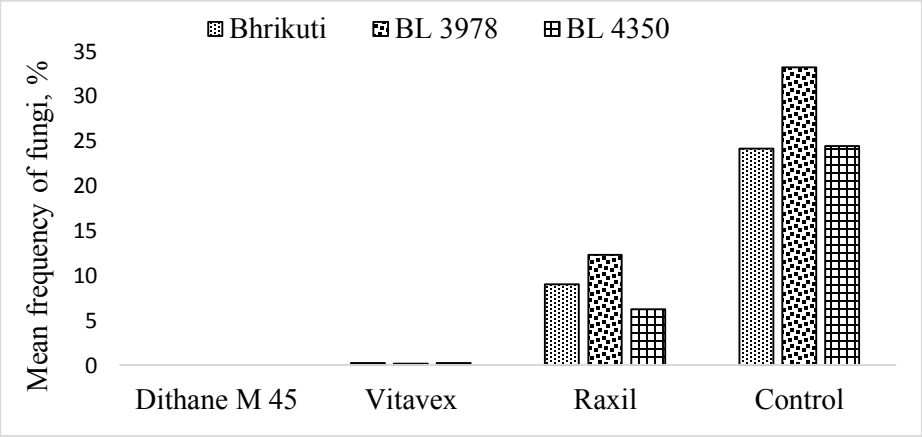

Figure 2: Varietal difference on percentage frequency of fungi in wheat seeds collected from Khajura after fungicide treatment

Khumaltar location

Percentage frequency of *Bipolaris sorokiniana* was highest in Bhrikuti + Control (23.25%), which was statistically at par with Annapurna 1 + Control (21.00%), but significantly higher than other treatments (Figure3). Percentage incidence was nil for Vitavex treated seeds in all varieties except *Alternaria alternata*. Percentage frequency of *Alternaria alternata, Bipolaris sorokiniana* and *Cladosporium herbarum* was significant among tested varieties Vitavex and Dithane M 45 appeared better to control seed borne fungi of wheat and *Alternaria alternata,* followed by *Bipolaris sorokiniana,* relatively resistant to the fungicides in Khumaltar.

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Seed borne fungi were high in Bhrikuti as compared to other varieties/genotypes, treated with Dithane M 45. Vitavex was significantly more effective in controlling seed borne fungi as compared to Dithane M 45 and Raxil. The fungi were more in RR 21 treated with Raxil and in control.

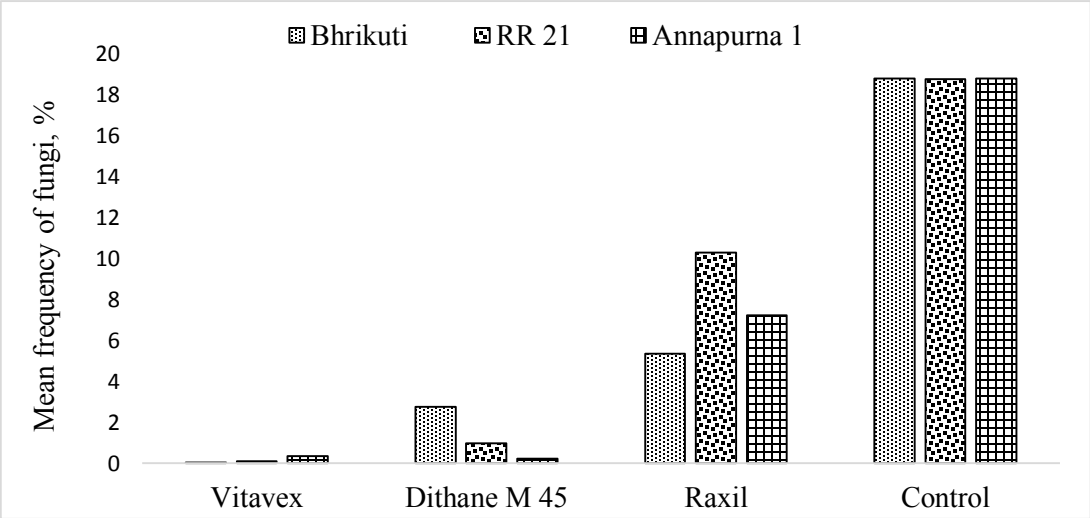

Figure 3: Varietal difference on percentage frequency of fungi in wheat seeds collected from Khumaltar after fungicide treatment

Mean percentage frequency of *Alternaria alternata* was highest in seeds from Khumaltar (30.92%), followed by Khajura (26.83%), and lowest in Rampur (8.25%). Similarly, highest incidence of *Bipolaris sorokiniana* was detected in seeds of Khajura (26.17%), followed by Khumaltar (8.90%) and lowest in Rampur (6.69%). The difference in percentage frequency of pathogens in different location may be due to different isolates of the fungi. The isolates of pathogen may have different reaction with the applied fungicides. The isolates of *Alternaria alternata* and *Bipolaris sorokiniana* present in seeds of Khumaltar and Khajura might have resistant reaction towards fungicides as compared to isolates of Chitwan.

Effect of all the fungicides was significant against the seed borne mycoflora of wheat. The result was similar to Singh and Kumar (2008). They treated wheat seeds with Vitavax (Carboxin) at 2.5 g kg^{-1} and Thiram at 3.0 g kg^{-1} of seed and reported eradication of *Bipolaris sorokiniana* and

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Alternaria tenuis from seed. Mogle and Maske (2012) reported that seed treatment of cowpea with Dithane M 45, reduced the seed mycoflora upto 60% and enhanced the seed germination percentage upto 90%. Javaid et al. (2006) reported that seed treatment of wheat with Dithane M 45 and Metalaxyl plus Mancozeb significantly reduced the incidence of *Dreschslera australiensis* and completely arrested the growth of *F. oxysporum*. Hall et al. (1978) screened fifty chemicals or combinations of chemicals and found that only those treatments containing Carboxin alone or in combination gave consistent control of the deep-seated type of diseases.

Applied fungicides inhibit fungal growth and development and also spore germination. Fungicides are absorbed by fungus as a result of exchange mechanism. This leads to accumulation and saturation of toxicants in fungal cell resulting in death of cell (Lukens, 1971). Wain and Carter (1977) reported that chemicals applied to the seed may inactivate the toxins and enzymes of the fungus resulting in alteration of development of the fungus. Fungicides may destroy the cell membrane of fungus causing leakage of cellular constituents leading to death of the cell. Wedding and Kendrick (1959) reported that Dithiocarbamate cause leakage of cellular constituents in *Rhizoctonia solani*.

CONCLUSION

Effect of all the fungicides was significant against the seed borne mycoflora of wheat, but Dithane M 45 and Vitavex were significantly more effective for *Bipolaris sorokiniana* and *Alternaria alternata* as compared to Raxil. Most of the wheat varieties/genotypes released or ready to be released carry markedly higher percentage of seed borne fungal pathogens. Therefore, for better germination and for the management of these pathogens, it is suggested to treat the seeds with fungicide Dithane M 45 or Vitavex to have better success in cultivation of wheat crop in Nepalese Agriculture.

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