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Effect of some fungicides on seed mycoflora, germination, viability and their persistence in treated seeds

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A B S T R A C T

The effect of some fungicides on seed mycoflora, germination, seed viability and their persistence in treated seeds of mungbean, mustard and rice was studied in vitro. The highest appearance of pathogens viz., *Alternaria alternata* was recorded in seeds of mungbean (*Phaseolus aureus* Roxb.)*, A. brassicae* in mustard (*Brassica campestris* L.) and *Drechslera oryzae* in rice (*Oryza sativa* L.). Propineb 70WP and prochloraz 45EC considerably reduced the appearance of *A. alternata* and flusilazole 40EC reduced *Fusarium sp.* in mungbean seed than untreated control. A marked reduction in population of *A. brassicae* by difenoconazole 25EC and flusilazole 40EC was observed in mustard seeds. Inhibitory effect of fungicides on seed germination of all tested seeds was observed with prochloraz 45EC and flusilazole 40EC. There was increase in the plumule length of mungbean seeds with prochloraz 45EC and difenoconazole 25EC over untreated control, whereas sharp reduction in plumule length was recorded in rice seeds irrespective of fungicide treatments. The seeds treated with prochloraz 45 EC and flusilazole 40EC maintained their viability up to 360 days. Irrespective of nature of inoculum, the seeds treated with prochloraz 45EC persisted longer than that of other fungicides.

Introduction

Seeds as a basic unit of propagation carry a wide range of microorganisms externally or internally, become active under favourable conditions resulting extensive damage to seeds and diseases on crops raised from them. Most of the food crops grown are propagated by seed and these crops are infected by many harmful seed borne diseases (Neergard 1977). Seed treatment with fungicides not only controls the seed-borne diseases but also improves seed health, plant stand and crop yield (Tanweer 1982). In any seed production programme, storage of seeds from harvest to next planting season is of prime importance respect to seed viability and seed vigour under normal storage conditions. Fungicide treatment are required to be stored for several months and stability of fungicides' effect without affecting the seed health adversely. Present investigation was carried out to assess the effect of seed treatment with some fungicides on seed mycoflora, seed germination, viability of seeds and their persistence on treated seeds under normal storage conditions.

Materials and Methods

One hundred gram (100g) each of unsterilized mungbean, mustard and rice seeds were treated with 0.25% propineb 70 WP (w/w) {Polymeric Zinc Propylene (dithiocarbamate)}, 0.15% prochloraz 45EC {1-N-Propyl-N-[2-(2,4,6-trichlorophenoxy) ethyl] carbamoyl

imidazole}and difenoconazole 25EC {Cis, trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2yl]phenyl, 4-chlorophenyl ether}and 0.10% flusilazole 40 EC (v/v) {1-[bis(4-fluoro phenyl)(methylisilyl methyl)]-1H1,2,3-triazole}in 250ml Erlenmeyer conical flasks by shaking using a wrist action shaker to ensure uniform coating of the fungicide on the seed surface.

Study of seed mycoflora

The treated seeds were air dried overnight and were plated on half strength Potato dextrose agar (PDA) medium @ 16 seeds per Petri plate. The untreated seeds of respective crops served as control. The experiment was replicated thrice. The plated seeds were incubated at 25°C in a BOD incubator for 5 days under 12 hr alternate light and dark conditions. Different fungal colonics appearing on the agar plate were observed under compound microscope and identified using relevant keys and monographs (Subramanium 1971; Neergard 1977). The percentage of each fungal genus on the tested seeds was calculated by using the following formula-

$$\text{\% appearance of the frequency} = \frac{\text{No. of seeds yielding particular fungus}}{\text{Total no. of seeds observed}} \times 100$$

Effect of seed treatment on seed germination, radicle and plumule growth

Twenty seeds from each treatment were placed on double layered moist blotting paper into the Petri plates with four replications and incubated in seed germinator at 27°C at room temperature with suitable untreated control seeds. The germination and growth pattern of radicle and plumules and any abnormalities developed there on in the germinated seeds were periodically observed. The germination behaviour/nature of each fungicide treated seeds was recorded on the basis of 80 randomly observed seeds. The Vigour Index of seed was calculated by using the formula of Varadarajan and Rao (2002)

Vigour Index = Percent germination of seed x (Root length + Shoot length).

Seed viability test

The effect of fungicide treatments on seed viability as a function of storage period was studied by preserving the treated seeds in seed box at room temperature. The samples were drawn periodically (90 days interval) from the treated seed lots that were allowed to germinate on moist blotting paper placed in Petri plates. The rate of reduction in germination (%) of seeds as a function of storage periods over control was observed and recorded.

Persistence of fungicides in treated seeds

Fungicide treated seeds were preserved in separate seed box under room temperature and were assayed periodically for the persistence of fungicide on the treated seed. Microsclerotia of *Macrophomina phaseolina*, mycelial fragments of *Sclerotium rolfsii*, multicelled conidia of *Drechslera oryzae* and single celled microconidia of *Fusarium udum*, were used as inoculum. Twenty ml PDA medium presterilized and cooled at 45°C was mixed with inoculum of respective pathogen and was poured aseptically into sterilized 90 mm diameter petriplates. Treated seeds were placed and gently pressed at the centre of each plate,

replicated thrice and incubated at 28 ± 1°C for 48 hr. The seeds without fungicide treatment were used for control. The diameter of inhibition zone developed was measured and was considered as the criteria for estimation of persistence of fungicides (Horsfall 1956). The gradual reduction in diameter of inhibition zone was considered an index for reduction in persistence of fungitoxicity on/in treated seeds.

Results and Discussion

The mungbean seeds without fungicide treatment yielded *A. alternata* (14.1%) and *Curvularia lunata* (13.4%) and a low in festation by *Fusarium* sp. (4.0%). Propineb reduced the appearance of *A. alternata* to 6.8% followed by prochloraz (2.7%) whereas flusilazole reduced *Fusarium* to the extent of 1.0 % over untreated control (4.0%) (Table 1). Six genera of fungi were identified in mustard seeds, the *A. brassicae* being the most predominant (19.5%) followed by *A. alternata* (16.0%). *A. brassicae* in mustard seeds was reduced to 9.4 % and 8.9% by difenoconazole and flusilazole, respectively over untreated control. However, *D. oryzae* (16.0%) was most predominant untreated rice seeds followed by *C. lunata* (14.5%) and *F. moniliforme* (14.0%). Propineb reduce the population of *Alternaria* sp. (1.5%) significantly. The present observations are in agreement with that of Lal and Singh (1997) and Sahu and Jena (1997) on mung bean. On the other hand a high frequency of *A. alternata* and *A. brassicae* has been reported on mustard seeds (Ghosh & Das, 1999). However, Ahamad and Srivastava (2002) isolated the genera *Alternaria, Aspergillus, Curvularia, Cladosporium, Fusarium* and *Rhizopus* from mustard seeds among which *A. brassicola* was the most pathogenic. Similarly, Sagar *et al*. (2005) observed *Aspergillus* sp, *Fusarium* sp., *D. oryzae*, *Curvularia* sp., *Magnaporthe* grisea, *Alternaria* and *Rhizopus* sp. from rice seeds. Blazej *et al* (1995) reported successful using control of organisms like *Fusarium* sp., *Drechslera oryzae*, *Alternaria* and *Cladosporium* sp. prochloraz. Difenoconazole and flusilazole had been successfully used for seed treatment of rice to control several seed - borne pathogens (Previero *et al.* 1997).

Effect of fungicidal seed treatment on germination, radicle and plumule length of seeds of different crops

It appeared that (Table 2) seeds treated with prochloraz and flusilazole reduced 8.3 % and 13.8% germination over untreated control. Prochloraz 45EC and difenoconazole had no adverse effect on plumule length whereas propineb and flusilazole treated seeds caused poor development of root system showed strong inhibition in germination of mustard seeds compared to untreated ones. In case of rice seeds, the prochloraz treated seeds exhibited highest percentage inhibition (27.0%) over control followed by propineb (16.2%) and flusilazole (16.2%) respectively. Decrease in radicle and plumule length was noted in almost all treatments with very high per cent disease in treated seeds. Present findings are in accordance with the observation of Goulart *et al*. (1999). Considerable reduction in root vigour of fungicide treated seeds have been reported in mungbean (Siddiqui & Arif-uz-Zaman 2004). All treatments reduced the vigors index of seeds and centre, flusilazole and kprochlora more so

Table 1.
Effect of fungicidal seed treatment on seed mycoflora

Pathogens	Percentage of seeds yielding fungi					
	Propineb 70 WP	**Prochloraz 45 EC**	**Difenoconazole 25 EC**	**Flusialzole 40 EC**	**Control**	**CD (P=0.05)**
Mungbean						
A. alternata	6.8 (2.6)	7.2 (2.7)	9.2 (3.0)	8.5 (2.9)	14.1 (3.8)	0.189
C. lunata	7.2 (2.7)	8.3 (2.9)	8.7 (3.0)	8.4 (2.9)	13.4 (3.7)	0.378
Fusarium sp.	1.4 (1.2)	2.5 (1.6)	1.5 (1.2)	1.0 (1.0)	4.0(2.0)	0.063
Mustard						
A. brassicae	10.7 (3.3)	13.2 (3.6)	9.4 (3.1)	8.9 (3.0)	19.5 (4.4)	0.158
A. alternata	9.5 (3.1)	12.5 (3.5)	6.7 (2.6)	7.4 (2.7)	16.0 (4.0)	0.032
C. lunata	5.8 (2.4)	6.8 (2.6)	7.8 (2.8)	7.4 (2.7)	10.0 (3.2)	0.095
F. moniliforme	5.2 (2.3)	4.9 (2.2)	2.4 (1.5)	2.1 (1.4)	6.0 (2.4)	0.095
Rice						
Alternaria sp.	1.5 (1.2)	3.0 (1.7)	3.7 (1.9)	3.1 (1.8)	6.0 (2.4)	0.126
C. lunata	7.2 (2.7)	10.4 (3.2)	12.9 (3.6)	12.2 (3.5)	14.5 (3.8)	0.095
F. moniliforme	10.2 (3.2)	11.5 (3.4)	6.4 (2.5)	5.8 (2.4)	14.0 (3.7)	0.063
D. oryzae	10.7 (3.3)	11.2 (3.3)	8.2 (2.9)	7.3 (2.7)	16.0 (4.0)	0.063
T. padwikii	7.6 (2.8)	7.1 (2.7)	5.7 (2.4)	4.9 (2.2)	9.0 (3.0)	0.126

The figures in the parentheses are square root transformed value

Table 2 .
Effect of fungicidal seed treatment on germination, radicle and plumule length of test seeds

Fungicides	Germination (%)			Radicle length (mm)			Plumule length (mm)			Vigour index		
	Mung bean	**Mustard**	**Rice**	**Mung bean**	**Mustard**	**Rice**	**Mung bean**	**Mustard**	**Rice**	**Mung bean**	**Mustard**	**Rice**
Propineb 70 WP	87.5a (69.3)	82.7b (65.5)	77.5c (61.7)	54.9b	44.1b	41.0a	16.6c	32.9b	42.0b	625.6	636.8	643.2
Proc hloraz 45 EC	82.3b (65.1)	77.2c (61.4)	67.5d (55.2)	40.2d	28.9e	20.1b	35.7a	22.8d	26.4	625.1	398.8	313.8
Difenoconazole 25 EC	87.5 (69.3)	84.3b (66.7)	85.0b (67.2)	45.7c	38.8c	39.1a	36.1a	36.8b	37.4c	715.7	637.6	650.2
Flusilazole 40 EC	77.5 (61.7)	75.2d (60.2)	77.5c (61.7)	29.0e	33.5d	44.4a	14.5c	30.8c	31.0d	337.1	483.3	584.3
Control	90.0 (71.5)	86.0a 68.0)	92.6a (74.2)	67.7a	50.8a	45.7a	28.6b	41.7a	68.8a	866.7	795.2	1122.9
SEm (±)	0.86	0.61	0.751	0.83	0.650	3.06	0.78	1.32	0.68	-	-	-
CD (P=0.05)	2.60	1.84	2.263	2.46	1.930	9.23	2.32	3.92	2.02	-	-	-

Data sharing same letter did not differed statistically; Figures in the parentheses are angular transformed value

than other test fungicides.

Table 3.

Viability of fungicide treated rice seeds at different storage periods

Fungicides	Germination (%) at different times (days)				
	0	90	180	270	360
Propin eb 70 WP	98.7	27.5	5.0	5.0	0.0
	(84.9)*	(31.6)	(12.9)	(12.9)	(0.4)
Prochloraz 45 EC	96.0	100.0	95.0	90.0	90.0
	(78.6)	(90.0)	(77.1)	(71.6)	(71.6)
Difenoconazole 25EC	98.7	77.5	25.0	20.0	10.0
	(83.8)	(61.7)	(30.0)	(26.5)	(18.4)
Flusilazole 40 EC	100.0	100.0	95.0	95.0	80.0
		(90.0)	(77.1)	(77.1)	(56.8)
Control	(90.0)	100.0	95.2	95.0	95.0
		(90.0)	(77.4)	(77.1)	(77.1)
Mean	98.7	81.0	63.0	61.0	53.0
	(85.5)	(72.6)	(54.9)	(53.2)	(44.9)
	Fungicides	Duration	Fungicides x Duration		
SEm (±)	0.51	0.51	0.861		
CD (P= 0.05)	1.01	1.01	2.435		

*Figures in the parentheses are angular transformed value;

Viability of fungicidal treated seeds as a function of storage period

The results (Table 3) showed a marked adverse effect on the viability of rice seeds in case of propineb and difenoconazole treated seeds as compared to control, whereas no adverse effect on viability was observed with prochloraz and flusilazole. The germinability of untreated seeds decreased marginally by 5% upto a period of 360 days of storage, whereas 0 to 95 % germination was observed in treated seeds, the lowest being in propineb 70 WP where the treated seeds completely failed to germinate at 360 days. Treatment with prochloraz resulted in 90% seed germination at 360 days, compared to 96% at 0 days of treatment. Similarly, seed treated with flusilazole gave 90% seed germination at 360 days of storage. This corroborates the earlier report that germination (%) of fungicide treated seeds decreases with increase in storage period (Gupta & Singh 1990; Jakhar *et al.* 2003).

Table 4.

Persistence of fungicides in treated seeds under different storage periods

Fungicides	Diameter of inhibition zone (mm) at different time (days)																					
	0				90				180				270				360					
	MP	SR	DO	FU	MP	SR	DO	FU	MP	SR	DO	FU	MP	SR	DO	FU	MP	SR	DO	FU		
Propineb 70 WP	23.7	23.0	21.7	23.3	20.7	17.3	16.7	18.0	9.0	7.0	6.0	8.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Prochloraz 45 EC	60.3	52.3	49.3	44.0	55.3	48.7	42.3	37.3	38.0	30.0	27.0	25.0	29.0	24.0	19.0	20.0	21.0	18.0	14.0	13.0		
Difenoconazole 25 EC	32.3	33.7	27.7	22.7	27.7	28.7	23.3	14.7	14.0	15.0	12.0	7.0	6.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0		
Flusilazole 40EC	47.3	44.3	41.0	33.7	43.7	37.3	36.7	27.7	33.0	29.0	27.0	25.0	25.0	20.0	18.0	22.0	21.0	14.0	12.0	9.0		
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Mean	32.7	30.7	27.9	24.7	29.5	26.4	23.8	19.5	18.8	16.2	14.4	13.0	13.2	10.4	7.4	8.4	8.4	6.4	5.2	4.4		
	Fungicides	Duration		F X D	Fungicides	Duration		F X D	Fungicides	Duration		F X D	Fungicides	Duration		F X D						
SEm (±)	0.40	0.40		0.89	0.42	0.42		0.93	0.44	0.44		0.97	0.38	0.38		0.84						
CD (P=0.05)	1.1	1.1		1.1	1.2	1.2		2.64	1.2	1.2		2.8	1.1	1.1		2.38						

MP- *Macrophomina phaseolina*; SR- *Sclerotium rolfsii*; DO- *Drechslera oryzae*; FU- *Fusaium udum*

Persistence of fungicides in treated seeds

It appeared that (Table 4) persistence of prochloraz and flusilazole was more in treated seeds as compared to seeds treated with propineb and difenoconazole. Irrespective of nature of inocula used (*M. phaseolina*, *S. rolfsii, D. oryzae* and *F. udum*), both propineb 70 WP and difenoconazole disappeared from the treated seeds between 180 and 270 days of storage, whereas prochloraz 45EC and flusilazole persisted in treated seeds even at 360 days. It has been convincingly established earlier that persistence of toxicity of a fungicide in treated seeds were governed by storage period, physical condition of storage environment and the nature of seed container (Lakshmi & Gupta 1997). The diameter of inhibition zone also depends on physico-chemical properties of fungicides and nature of test organism including inocula.

Literature Cited

Ahamad Sahid Srivastava M. 2002 Fungi associated with Ethiopian mustard seeds, pathogenicity and chemical control. *Annals of Agricultural Research* **23**: 748-51.

Blazej J. 1995 Influence of the use of fungicides on the sowing value of faba bean seeds. *Materialy Sesji Instytutu Ochrony Roslin* **35**:157-59.

Ghosh SK Das N. 1999 Effect of fungicides on seed mycoflora and seed germination of mustard and cauliflower. *Journal of Mycopathological Research* **37**: 37-39.

Goulart ACP Fialha WFB Fujino MT. 1999 Technical viability of soybean seed treatment with fungicides before storage. *Boletin de Pesquisa EMBRAPA Agropecuaria Oeste* **2**: 41.

Gupta A Singh D. 1990 Viability of fungicide treated seeds of mungbean and cowpea in storage. *Seed Research* **18**: 70-76.

Horsfall JG. 1956 Principles of fungicidal action. Waltham, Chronica Botanica, 279pp.

Jakhar SS Tuhan JC Khard RRS. 2003 Effect of fungicide treatment and container on pearlmillet seed storability. *Indian Phytopathology* **56**(4): 484-85.

Lakshmi BS Gupta JP. 1997 Persistence of thiophanate-methyl on stored soybean seed. *Indian Phytopathology* **50**: 358-61.

Lal ML Singh DB. 1997 Seed mycoflora of green gram. *Madras Agricultural Journal* **84**: 681-85.

Neergard P.1977 Seed Pathology, Vol 1. The Macmillan press Ltd., London, 835pp.

Previero CA Soave J Groth D. 1997 Effect of chemical seed treatment on physiological and health quality of the seeds of *Brachiaria brizantha* cv. *Marandu*. *Fitopathologia Brasileira* **22**: 25-29.

Sagar SD Hegde YR Kulkarni S Rao MSL. 2005 Biocontrol of seed mycoflora of rice. *Annals of Biology* **21**: 217-20**.**

Sahu AK Jena N. 1997 Seed mycoflora of green gram (Phaseolus aureus Roxb.) cultivars of Orissa and their impact on seed germination. *Journal of Mycopathological Research* **35**:93-97.

Siddiqui ZS Arif.uz-Zaman. 2004 Effect of benlate systemic fungicide on seed germination, seedling growth, biomass and phenolic contents of *Vigna radiata* (L.) Wilczek and *V. mungo* (L.) Hepper. *International Journal of Biology and Biotechnology* **1**: 543-47.

Subramaniam CV. 1971 Hyphomycetes, Indian Council of Agricultural Research, New Delhi 1, 903pp.

Tanweer A. 1982 Effect of new fungicide on viability of rice and sorghum seeds. *Pestology* **6**: 9-10.

Varadarajan K Rao JSP. 2002 Effect of ethereal on seed germination and seedling growth of different genotypes of blackgram [(*Vigna mungo* L.) Hepper] under stimulated moisture stress. *Indian Journal of Physiology* **7**: 295-97.