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EFFICACY OF FUNGICIDES, SODIUM HYPOCHLORITE AND NEEM SEED POWDER TO CONTROL SEED BORNE PATHOGENS OF MAIZE

UZMA SITARA AND SHAHIDA AKHTER

Pesticide Research Institute,
Southern-Zone Agricultural Research Centre, Pakistan Agricultural Research Council,
Karachi University Campus, Karachi-75270, Pakistan.

Abstract

Using standard blotter and deep freezing techniques, 7 genera and 11 species of fungi viz., *Aspergillus niger, A. flavus, A. wentii, Chaetomium* sp., *Drechslera* sp., *Fusarium chlamydosporum, F. oxysporum, F. moniliforme, F. semitectum, F. nivale, Nigrospora sp., Phoma* sp. and *Rhizopus* sp. were isolated from maize seeds. Seed treatment with fungicides viz., Antracol (70% WP), Aliette (80% w/ w), Ridomyl Gold (MZ 68% WP), Neem seed powder @ 0.1%, 0.2% & 0.3% and Sodium hypochlorite @ 10% were used. No adverse effects were observed on the germination of seeds in blotter method whereas the germination was reduced due to dead/frozen embryo in deep-freezing method. Ridomyl Gold was found to be effective against seed borne mycoflora of maize followed by Aliette, Neem seed powder, Antracol and Sodium hypochlorite.

Introduction

In Pakistan maize is third important cereal crop after wheat and rice. It accounts for 4.8% of the total cropped area and 3.5% of the value of agricultural out put. It is planted over an estimated area of 981.8 thousand hectares with an annual production of 2797.0 thousand tones, with an average yield of 2849 kg/ha (Anon, 2005). Maize is attacked by more than 60 diseases (Anon., 1980). *Fusarium moniliform* produces gibberela ear rot, kernel rot, stalk rot, seedling blight, seed rot, wilt and stunt (Kocharek & Kommedhol, 1966; Ullstrup, 1978; Ochar *et al*., 1987; Leon & Pandey, 1989; Thiel *et al*., 1991). *Aspergillus flavus* becomes systemic and produces aflatoxin and virescen in seedling of maize and damage stored corn (Blat, 1969). *Sclerotinia sclerotiorum* produces sclerotium blight in maize (Ahmed *et al*., 1987). Maize is also infected by downy mildew pathogen (Adenle & Cardwell, 2000; Ajala *et al*., 2003; Ahmed *et al*., 2006). *Fusarium* spp., invade more than 50% maize grain before harvest and produce mycotoxins (Bakan *et al*., 2002). The rank of fungi is second after insects as the cause of deterioration and loss of maize (Ominski *et al*., 1994). In Pakistan the most common seed borne fungi isolated from maize seeds are *Alternaria* spp., *Aspergillus* spp., *Curvularia* spp., *Helminthosporium maydis, Monilia spp., Penicillium* spp., *Rhizopus* spp. and *Trichoderma* spp. (Ghafoor & Khan, 1976). Seed borne fungi can be controlled by treatment with fungicides (Crosier & Patrick, 1946; Siddiqui & Zaman, 2004). Experiments were conducted to study the efficacy of some fungicides, Sodium hypochlorite and Neem seed powder against seed borne mycoflora of maize.

Materials and Methods

Seeds of maize were tested by ISTA techniques using blotter and deep freezing methods for the detection of seed borne fungi. Sodium hypochlorite (10/%) was used for

surface sterilization of seeds while fungicides viz., Antracol (70% WP), Aliette (80% w/w), Ridomyl Gold (MZ 68% WP) and Neem seed powder were used in addition to find their efficacy as a fungicide @ 0.1%, 0.2% & 0.3% in reducing seed borne mycoflora.

The fungicides and Neem seed powder were applied on seeds in conical flasks separately. The seeds treated with fungicides, Neem seed powder and Sodium hypochlorite (10%) were plated @ 10 seeds / plate on 3 layers of moistened blotter in 9 cm glass Petri plates, incubated at 25±1°C in alternate cycle of 12 hours light and 12 hours darkness for 7 days. In deep-freezing method (Limonard, 1966) the treated and untreated seeds in Petri plates were incubated for one day at 25±1°C and then in deep freezer at -4°C for 24 hours. After deep-freezing the Petri plates were taken out and incubated for 7 days at 25±1°C. In both methods the growth of fungi were observed after 7 days and isolated on Potato Dextrose Agar (PDA) slant. The fungi were identified up to species level after reference to Barnet & Hunter (1972), Booth (1972), Ellis (1970) & Nelson *et al*., (1983).

Results and Discussion

In blotter method the fungi isolated and identified were *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. candidus*, *Rhizopus* sp., *Phoma* sp., *Cheatomium* sp., and *Nigrospora* sp., as compared to control. These results fully supported the results obtained by Orisi *et al*., (2000). Sodium hypochlorite 10% completely controlled the growth of *Phoma* sp., and *Chaetomium* sp., (Table 1). The growth of *A. niger* was not reduced with Antracol and Neem seed powder whereas Aliette was effective @ 0.2% and 0.3%. Ridomyl Gold @ 0.3% controlled all fungi except *A. niger* which showed only 2% growth. The growth of *A. flavus* was not reduced by Sodium hypochlorite, Neem seed powder and fungicides (Antracol and Aliette), while 0.3% Ridomyl Gold completely controlled this fungus (Fig.1). For the control of seed borne mycoflora Ridomyl Gold was found to be the most effective followed by Neem seed powder, Aliette, Antracol and Sodium hypochlorite. The rest of the fungi viz., *A. fumigatus*, *A. candidus*, *Rhizopus* sp., *Phoma* sp., *Chaetomium* sp., and *Nigrospora* sp., showed a positive response of fungicides tested and Neem seed powder. Aziz (1988) reported that powder of neem provided protection to maize grain against fungi.

In deep freezing method, the fungi isolated were *A. flavus*, *A. niger*, *A. fumigatus*, *A. wentii*, *Drechslera* sp., *Fusarium nivale*, *F. oxysporum*, *F. chlamydosporum*, *F. moniliforme* and *F. semitectum*. The most dominant fungi were *Aspergillus* and *Fusarium* species as reported by Askun (2006) and Fandohan *et al*., (2003) on the same seed. The result revealed that Aliette, Ridomyl Gold and Neem seed powder completely controlled the growth of *A. fumigatus*, *A. wentii*, *Drechslera* sp., *Fusarium nivali*, *F. oxysporum* and *F. moniliform* (Table 2). Agbenin *et al*., (2004), reported that *Fusarium* spp., was also controlled by using Neem seed powder, however in the present study, *A. flavus*, *A. niger*, *F. semitectum* were not controlled by Antracol, Aliette and Neem seed powder whereas Ridomyl Gold (0.3%) gave complete reduction in infection of all fungi except *F. semitectum* (1%) (Fig. 2). Only *Drechslera* sp., was fully controlled by Sodium hypochlorite (Table 2). Ridomyl Gold was found to be effective in all doses in both blotter and deep freezing method for the control of seed borne fungi, followed by Aliette, Neem seed powder, Antracol and Sodium hypochlorite. Deep-freezing method was also found best for isolation of *Fusarium* spp. These results are in close conformity with the findings of Khanzada *et al*., (1988); Bilgrami & Ghaffar, (1993); Hussein *et al*., (2002).

Table 1. % Occurrence of fungi after different treatments (Blotter method).

S. #	Name of fungi	Control	NaOCl	Treatments											
				Antracol (70% WP)			Aliette (80% w/w)			Ridomyl gold (68%WP)			Neem seed powder		
			10%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%
1.	*Aspergillus niger*	80	83.75	37.5	42.5	52.5	70.1	86.25	97.5	88.75	95.1	97.5	33.75	47.5	65.1
2.	*A. flavus*	46	76.1	63.04	65.21	67.39	28.39	71.73	93.47	82.6	95.65	0	71.73	8.43	86.95
3.	*A. fumigatus*	17	82.36	75.1	0	0	0	0	0	0	0	0	0	0	0
4.	*A. candidus*	4	75.1	0	0	0	0	0	0	0	0	0	0	0	0
5.	*Rhizopus* sp.	6	83.33	0	0	0	0	0	0	0	0	0	0	0	0
6.	*Nigrospora* sp.	7	85.71	0	0	0	0	0	0	0	0	0	0	0	0
7.	*Phoma* sp.	9	0	0	0	0	0	0	0	0	0	0	0	0	0
8.	*Chaetomium* sp.	13	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. % Occurrence of fungi after different treatments (Deep freezing method).

S. #	Name of fungi	Control	NaOCl	Treatments											
				Antracol (70% WP)			Aliette (80% w/w)			Ridomyl gold (68%WP)			Neem seed powder		
			10%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%	0.10%	0.20%	0.30%
1.	*Aspergillus. flavus*	46	32.6	47.82	39.13	34.78	34.78	26.08	13.04	0	0	0	97.82	50.00%	26.08%
2.	*A. niger*	38	78.94	60.52	47.36	15.78	36.84	31.57	26.31	15.78	5.26	0	23.68	18.42	13.15%
3.	*A. fumigatus*	27	25.92	18.51	11.11	7.4	0	0	0	0	0	0	0	0	0
4.	*A. wentii*	23	56.52	0	0	0	0	0	0	0	0	0	0	0	0
5.	*Drechslera* sp.	7	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	*Fusarium chlamydosporum*	39	51.28	25.64	20.51	17.94	10.25	0	0	0	0	0	23.07	17.94	0
7.	*F. oxysporum*	39	69.23	15.38	0	0	0	0	0	0	0	0	0	0	0
8.	*F. moniliforme*	36	41.66	16.66	5.55	0	0	0	0	0	0	0	0	0	0

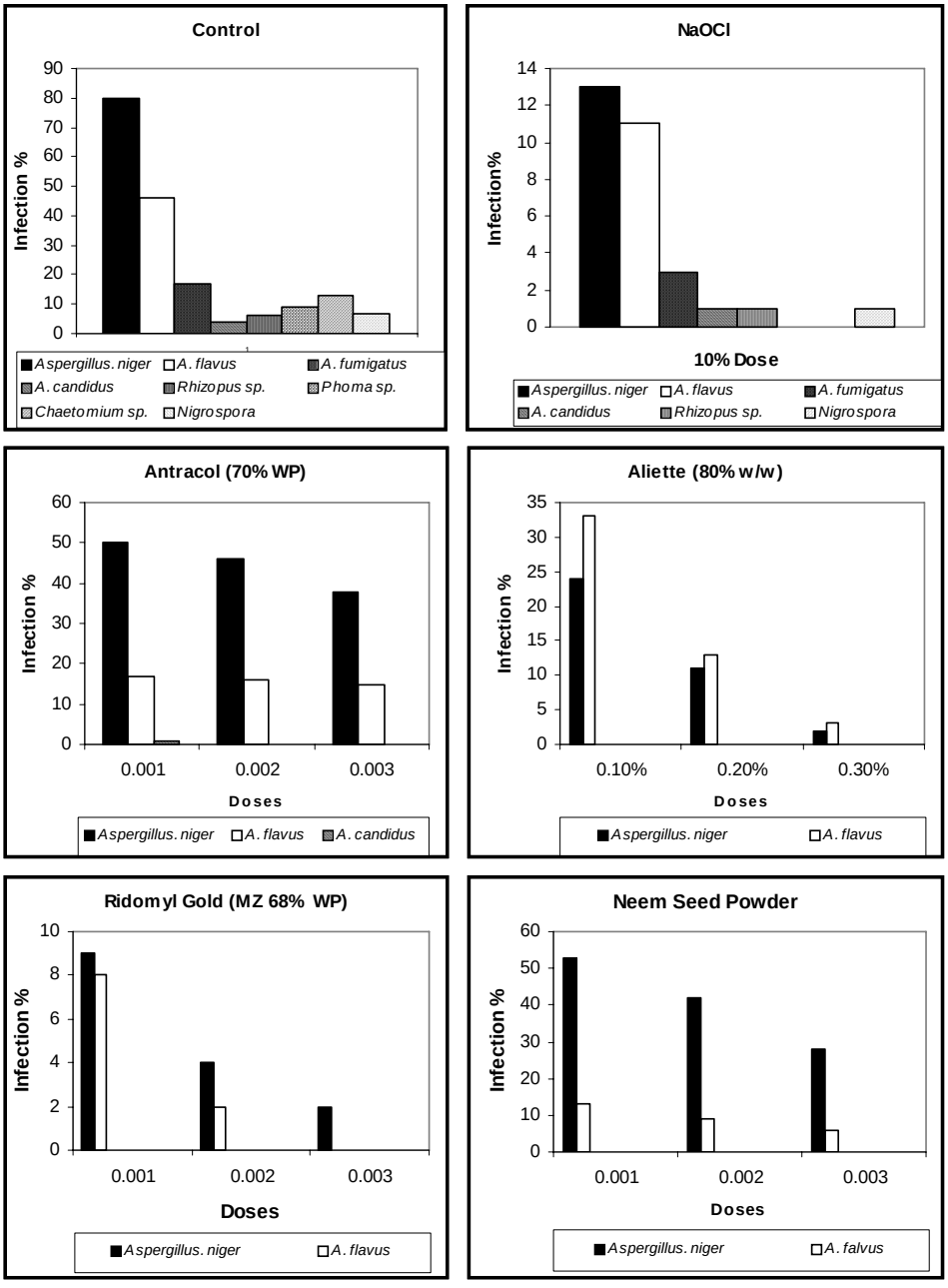

Fig. 1. Infection of seed borne fungi of Maize (Blotter method).

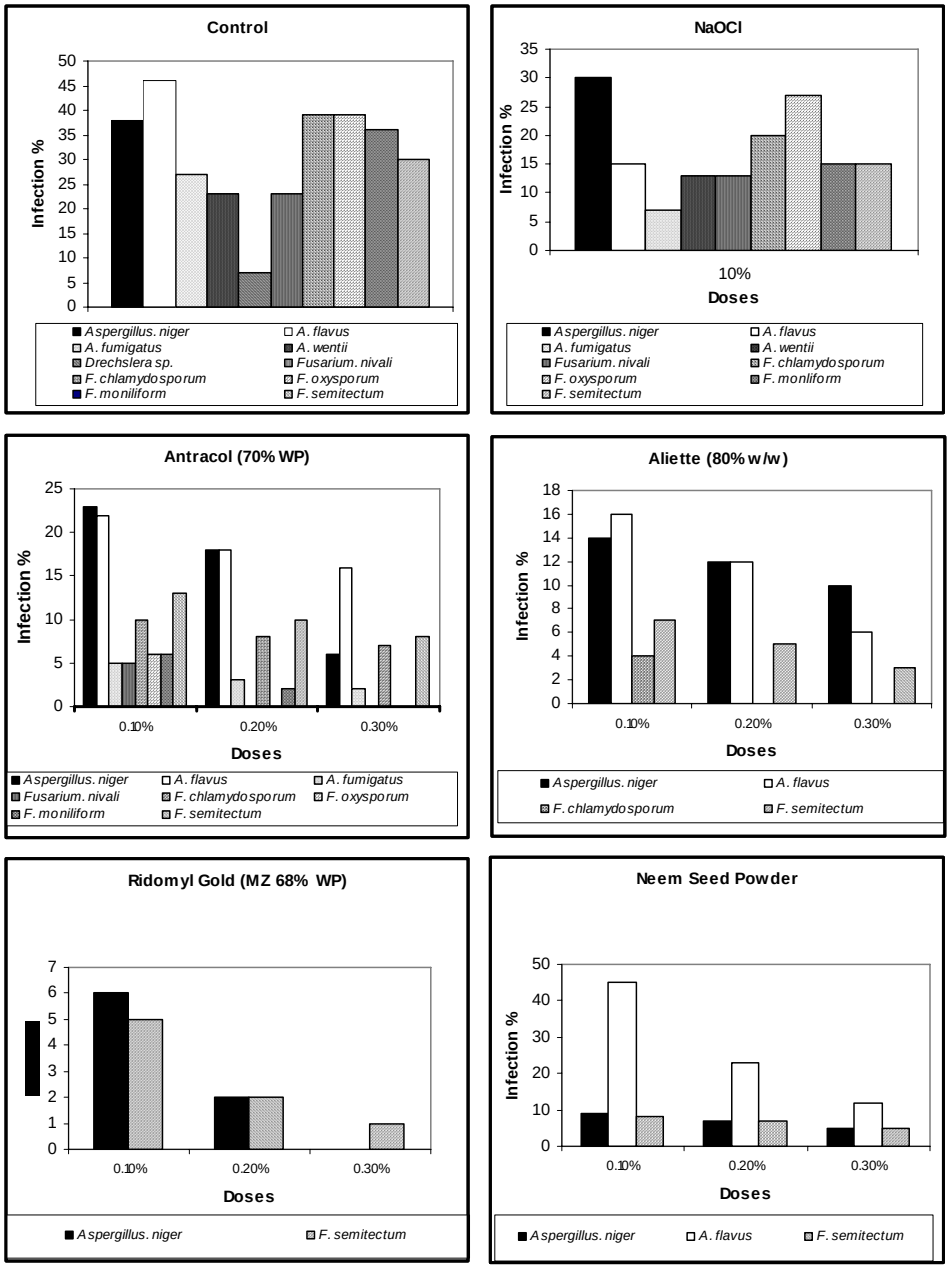

Fig. 2. Infection of seed borne fungi of Maize (Deep freezing method).

Table 3. Analysis of variance comparisons of fungicides with NaOCl* (Blotter Method).

S. #	Doses	Treatments								F Value	P Value
		Antracol (70% WP)		Aliette (80% w/w)		Ridomyl gold (MZ 68% WP)		Neem seed powder			
		Mean	St. Dev	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev		
1.	0.1%	8.50	17.78	7.13	13.41	2.12	3.94	8.25	18.65	1.51	0.221
2.	0.2%	7.75	16.44	3.00	5.58	0.75	1.49	6.37	14.74	**2.41	0.068
3.	0.3%	6.63	13.72	0.62	1.19	0.25	0.71	4.25	9.82	***3.41	0.019

*10% NaOCl was taken as standard in comparison with three concentration of the fungicides
Mean = 22.75
St. Dev = 26.77
**Significant at 0.05
***Highly significant at 0.05

Table 4. Analysis of variance comparisons of fungicides with NaOCl* (Deep Freezing Method).

S. #	Doses	Treatments								F Value	P Value
		Antracol (70% WP)		Aliette (80% w/w)		Ridomyl gold (MZ 68% WP)		Neem seed powder			
		Mean	St. Dev	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev		
1.	0.1%	9.000	8.124	4.100	6.226	1.100	2.331	7.100	13.932	**3.90	0.008
2.	0.2%	5.900	7.279	2.900	5.043	0.400	0.843	4.400	7.321	***7.98	0.000
3.	0.3%	3.900	5.343	1.900	3.479	0.100	0.316	2.200	4.022	***14.29	0.000

*10% NaOCl was taken as standard in comparison with three concentration of the fungicides
Mean = 15.500
St. Dev = 8.746
**Significant at 0.05
***Highly significant at 0.05

By using ANOVA, efficacies of fungicides, Neem seed powder showed highly significant result (α=0.05) as compared to Sodium hypochlorite (10%). In blotter method 0.2% and 0.3% doses are significant (Table 3) while in deep freezing method all doses (0.1%, 0.2% and 0.3%) are highly significant at 0.05% (Table 4). It was observed that in blotter method fungicides, Neem seed powder and Sodium hypochlorite has no adverse effects on germination of seeds specially Ridomyl Gold controlled the fungi and gave 100% seed germination while in deep freezing method it provided very low germination due to frozen or dead embryo.

The deep freezing method was best for the isolation of deep seated and pathogenic fungi viz., *Fusarium* spp. while blotter method was found suitable for germination test and for isolation of *Aspergillus* spp. Ridomyl Gold, Aliette and Neem seed powder were found to be most effective for the control of fungi associated with maize seeds.

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