INT. J. BIOL. BIOTECH., 9 (4): 435-444, 2012.

EFFECT OF BOTANICAL PRODUCTS ON GERMINATION AND MYCOFLORA OF MAIZE SEEDS

Ishrat Niaz and Shahnaz Dawar

Department of Botany,University of Karachi, Karachi-75270, Pakistan.

ABSTRACT

Seed treatment with seed powder of *Nigella sativa* and *Ferula assafoetida* showed antifungal activities and reduced the seed borne fungi on maize. Maize seeds at 8 and 16% moisture when treated with seed powder of *Nigella sativa* and *F. assafoetida* @ 0 .5, 1.0 and 2.0 % and stored at 25 and 35 ºC showed a reduction in occurrence of fungi on maize seeds with the increase in storage time and concentration of botanical products.

Key words - Maize seeds, *Nigella sativa* seed powder, *Ferula assafoetida* powder, germination, fungal mycoflora, moisture and temperature.

INTRODUCTION

Different seed Protectants (mercurial and non-mercurial's) are used for the last many years but the grain treated with these preservative can not be used for human consumption because of their toxicological effects. Therefore, there is a need to find certain seed dressing materials which are safe for human consumption and at the same time could check the fungal growth. For the preservation of high moisture grain different organic acid and their related compound have been studied (Sauer, 1973; Lavermicocca *et al.,* 2003). For the control of seed borne fungi different volatile organic acid and their related compound have been studied (Sauer, 1973; Sauer and Burroughs, 1974; Herting and Drury, 1974; Sauer *et al.,* 1975; Nash and Easson, 1977; Nash, 1978; Vandegraft *et al.,* 1975). Black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) also have antifungal activity and reduced the seed borne fungi. (Hanafy and Haten, 1991; Houghton *et al.,* 2006; Khan *et al.,* 2003; Morsi, 2000; Mashhadian and Rakhshandeh, 2005; Nash, 1978). Experiments were carried out to study the fungal activity in spoilage of maize seeds (grains) under different storage conditions and its control.

MATERIALS AND METHOD

Maize seeds with 8 and 16 % moisture content were treated with, black cumin (*Nigella sativa* seeds powder and *Ferula assafoetida* powder @ 0.5, 1 and 2% w/w and stored in a glass jar for 90 days at 25 and 35°C. Untreated seeds were used as control. At 0 day and after 30, 60 and 90 days interval germination and mycoflora associated with maize seed were detected by using blotter method (Annon., 1993; Wallace and Sinha, 1962).

RESULTS AND DISCUSSION

Nigella sativa (Black Cumin)

Maize seeds with 8 and 16% moisture levels were treated with *Nigella sativa* seed powder @ 0.5, 1.0 and 2% w/w and stored for 90 days at 25 and 35 °C. Untreated seeds served as control. Mycoflora and germination of maize seeds were tested at 0, 30, 60 and 90 days by using blotter method (Anon., 1993)

Germination of maize seed decreased with the increase in storage time in non treated and treated seeds with *N. sativa* seed powder (Fig. 1-4) ($P<0.001$). Seed treatment with 2% *N. sativa* seed powder was better for the germination of maize seed. Infection of *A. alternata* was observed only in non treated seeds where as seed treatment with *N. sativa* at different concentration completely inhibited the infection of *A. alternata* (Table 1). Infection of *A. flavus, A. fumigatus, A. niger* ($P<0.001$). *F. nivale, F.oxysporum, Penicillium* and *Rhizopus* species. was highest in untreated seeds. *N. sativa* powder @ 2% was most effective in the inhibition of storage fungi (Table 1) while *N. sativa* powder was not effective against infection of *A. niger* ($P<0.001$).

Table 1. Effect of *Nigella sativa* powder in the control of fungal infection on maize seed at different moisture and temperature during storage.

8 % moisture

Temperature °C	25 °C												35 °C											
	0 day			30 days			60 days			90 days			0 day			30 days			60 days			90 days		
Dose (%)	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2
FUNGI	INFECTION (%)																							
Alternaria alternata	18	-	-	19	-	-	20	-	-	10	-	-	20	-	-	27	-	-	20	-	-	13	-	-
Aspergillus candidus	22	-	-	24	-	-	27	-	-	20	-	-	22	-	-	25	-	-	29	-	-	17	-	-
A.flavus	25	13	7	30	16	10	30	23	17	16	7	3	31	26	-	32	4	-	32	9	-	13	-	-
A.fumigatus	20	17	9	21	16	4	27	18	15	15	6	-	22	15	7	33	20	9	33	16	10	21	11	-
A.niger	27	14	10	29	20	19	30	18	17	20	13	9	30	18	12	32	22	17	35	27	15	12	16	10
A.wentii	25	-	-	27	-	-	30	-	-	17	-	-	30	-	-	37	-	-	40	-	-	19	-	-
F.moniliforme	16	-	-	22	-	-	27	-	-	19	-	-	16	-	-	18	-	-	22	-	-	12	-	-
F.nivale	17	7	6	19	12	9	22	17	15	12	8	4	20	17	11	25	20	16	29	18	14	17	7	3
F. oxysporum	18	11	7	21	13	7	22	16	8	16	5	-	20	14	9	21	13	6	25	14	9	16	4	-
F.semitectum	21	7	-	25	9	-	27	16	-	16	-	-	22	8	-	25	13	-	26	14	-	13	4	-
Penicillium sp	38	18	6	48	21	10	50	14	-	22	13	-	39	21	4	42	17	8	44	12	-	20	-	-
Rhizopus sp	16	10	-	21	17	13	25	10	4	18	7	-	16	-	-	20	13	7	22	7	2	14	-	-

LSD 0.05 = 2.0934 (days); 1.4802 (temp); 2.0934 (dose)

Cont'd.

16 % moisture

Temperature °C	25 °C												35 °C											
	0 days			30 days			60 days			90 days			0 days			30 days			60 days			90 days		
Dose (%)	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2
FUNGI	INFECTION (%)																							
Alternaria alternata	20	-	-	27	-	-	-	30	-	-	-	16	-	-	-	45	22	17	-	49	21	12	-	50	43	10	-	36	17	13	-
Aspergillus candidus	21	16	1	-	16	12	-	-	16	10	-	-	12	7	-	-	60	34	21	-	65	23	17	-	67	51	22	-	29	17	7	-
A.flavus	35	20	17	-	36	32	19	-	40	33	21	-	21	18	7	-	51	40	23	-	52	25	16	-	55	40	27	-	39	16	6	-
A.fumigatus	27	11	9	-	30	16	11	-	31	14	10	-	13	7	2	-	55	-	-	-	56	-	-	-	57	-	-	-	19	-	-	-
A.niger	59	41	20	11	60	22	18	13	65	32	21	10	39	13	9	-	56	27	21	10	57	27	19	-	57	27	13	7	21	16	2	-
A.wentii	61	-	-	-	62	-	-	-	62	-	-	-	40	-	-	-	59	-	-	-	60	-	-	-	62	-	-	-	22	-	-	-
F.moniliforme	44	21	-	-	46	31	-	-	49	25	-	-	32	19	-	-	57	27	-	-	59	25	-	-	59	28	-	-	29	13	-	-
F.nivale	27	13	7	-	23	17	4	-	20	7	3	-	13	4	1	-	35	21	12	-	36	14	10	-	39	19	11	-	16	7	2	-
F. oxysporum	18	11	3	-	20	7	-	-	21	7	2	-	13	3	1	-	22	16	8	-	25	12	9	-	27	29	14	-	13	11	-	-
F.semitectum	40	19	10	-	41	17	4	-	42	25	7	-	21	4	2	-	42	19	13	-	55	27	17	-	56	24	16	-	22	17	6	-
Penicillium sp	59	-	-	-	60	-	-	-	62	-	-	-	27	-	-	-	60	-	-	-	65	-	-	-	66	-	-	-	47	-	-	-
Rhizopus sp	21	13	-	-	27	10	-	-	30	13	9	-	13	6	-	-	100	33	24	-	95	35	21	-	96	30	16	-	29	20	4	-

LSD 0.05 = 1.3344 (days); 0.9435 (temp); .3344 (dose)

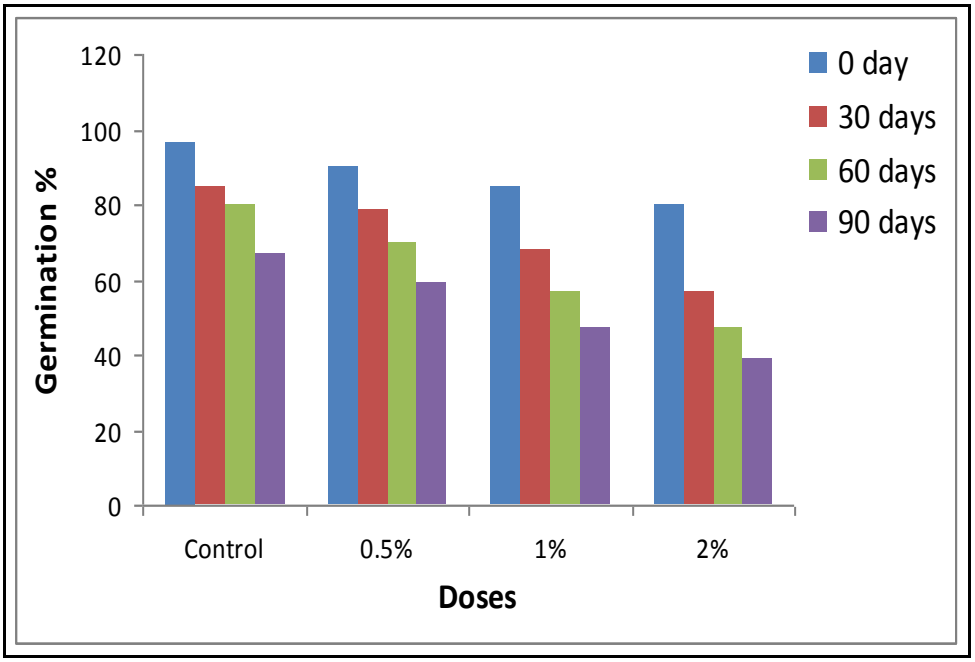

Fig. 1. Effect of *Nigella sativa* on seed germination at 8% moisture stored at 25˚C. $LSD_{0.05}$ = 2.729 (days); 2.729 (dose); F value = 243.2417 (days); 150.7698 (dose)

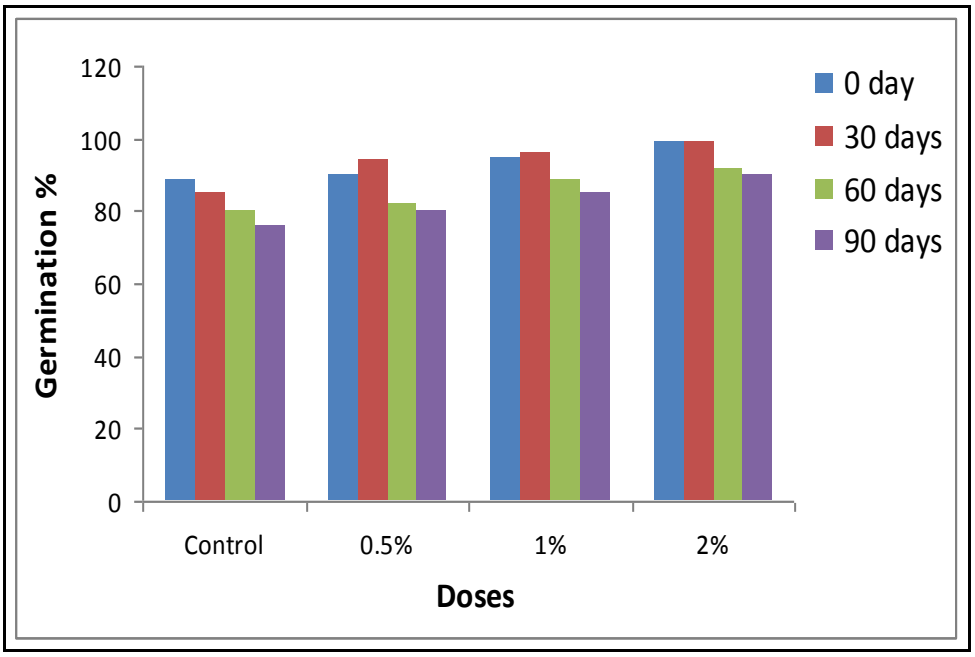

Fig. 2. Effect of *Nigella sativa* on seed germination at 16% moisture stored at 25˚C. $LSD_{0.05}$ = 2.067 (days); 3.220 (dose); F value = 19.5095 (days); 34.8216 (dose)

Ferula assafoetida

Maize seeds with 8 and 16 % moisture levels were treated with *Ferula assafoetida* powder @ 0.5, 1.0 and 2% w/w and stored at 25 and 35 °C for 90 days. Germination and infection of fungi was observed by blotter method at different intervals (Anon., 1993; Wallace and Sinha, 1962).

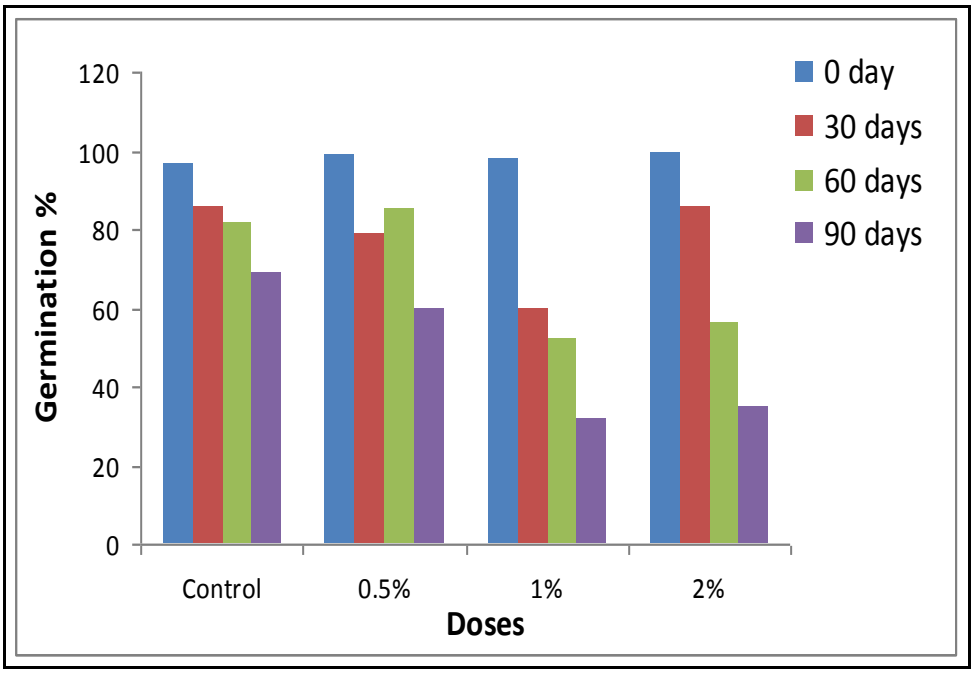

Fig. 3. Effect of *Nigella sativa* on seed germination at 8% moisture stored at 35˚C. $LSD_{0.05}$ = 2.462 (days); 2.462 (dose); F value = 564.6365 (days); 151.0498 (dose)

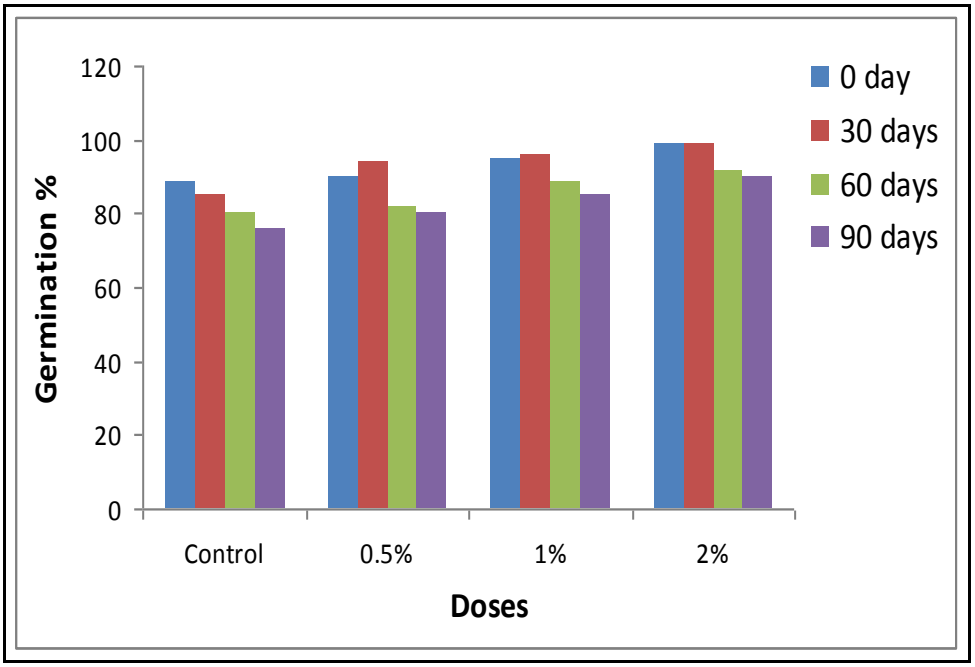

Fig. 4. Effect of *Nigella sativa* on seed germination at 16% moisture stored at 35˚C. $LSD_{0.05}$ = 2.523 (days); 2.523 (dose); F value = 415.0045 (days); 41.9728 (dose)

Significant increase in germination was observed when seeds were treated *F. assafoetida* powder used @ 2% w/w where as in non treated seeds of maize germination of seed decreased with the increase in time (Fig. 5- 6). Infection of fungi was highest in non treated seeds with 8 and 16% moisture levels and stored at 25 and 35 °C (Table 2) where as infection of fungi decreased with the increase in *F. assafoetida* concentration. Seed treatment with *F. assafoetida* powder @ 2% w/w completely controlled the infection of fungi (Table 2). It was also noted that

infection of *A.niger, Drechslera hawiiensis, F. moniliforme, F. oxysporum, F.semitectum* was completely inhibited in seeds with 8% moisture level however at 16 % moisture level. *F. assofoetida* completely inhibited the growth of *A.fumigatus, A.niger, D. hawiensis, F. moniliforme, F. oxysporum, F.semitectum* at 25 and 35 °C (Table 2) ($P<0.001$).

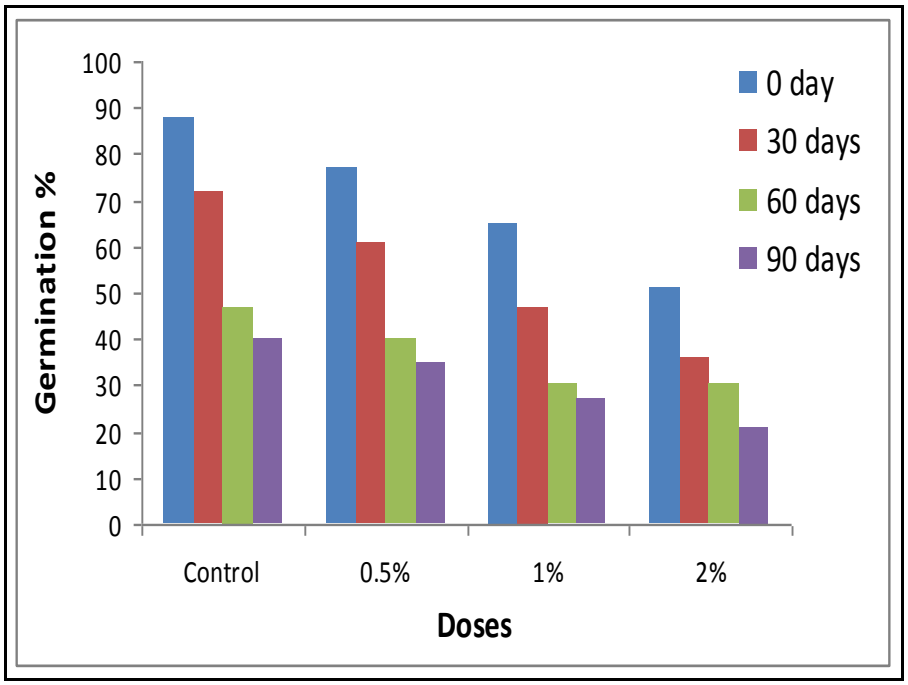

Fig. 5. Effect of *Ferula assafoetida* powder on seed germination at 8% moisture stored at 25˚C. $LSD_{0.05}$ = 1.219 (days); 1.219 (dose); F value = 254.7125 (days); 115.1791 (dose)

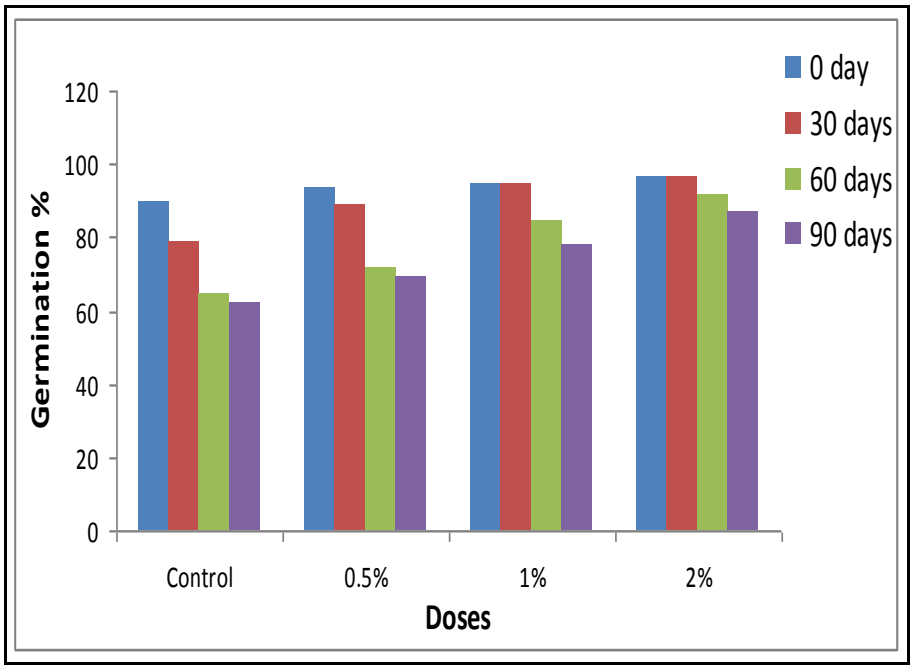

Fig.6. Effect of *F. assafoetida* powder on seed germination at 16% moisture stored at 25˚C. $LSD_{0.05}$ = 2.058 (days); 2.058 (dose); F value = 173.8367 (days); 138.8163 (dose)

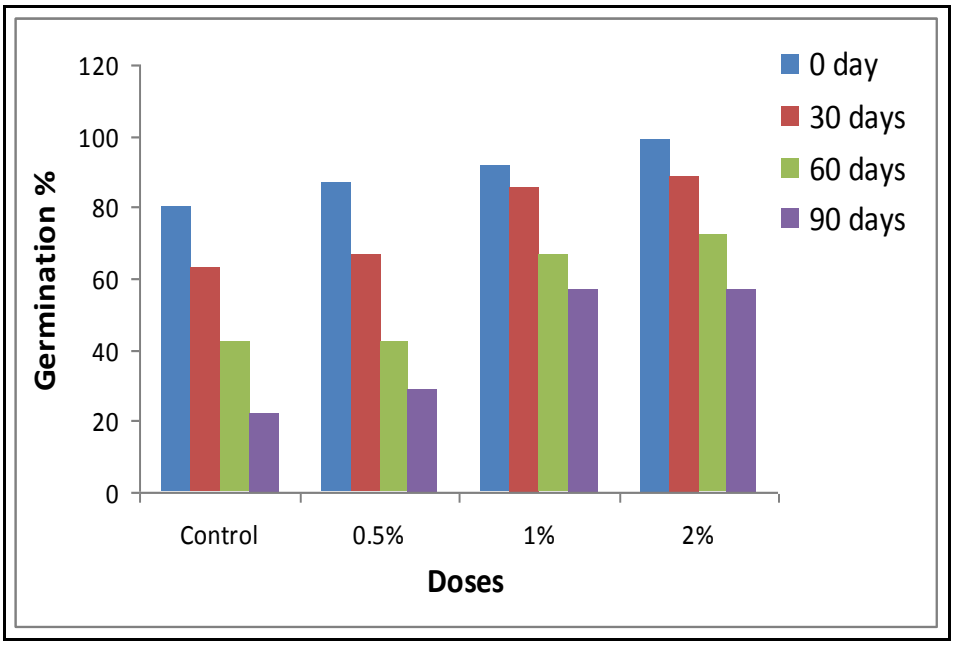

Fig. 7 Effect of *F. assafoetida* powder on seed germination at 8% moisture stored at 35˚C. $LSD_{0.05}$ = 2.302 (days); 2.302 (dose); F value = 704.5027 (days); 295.0534 (dose)

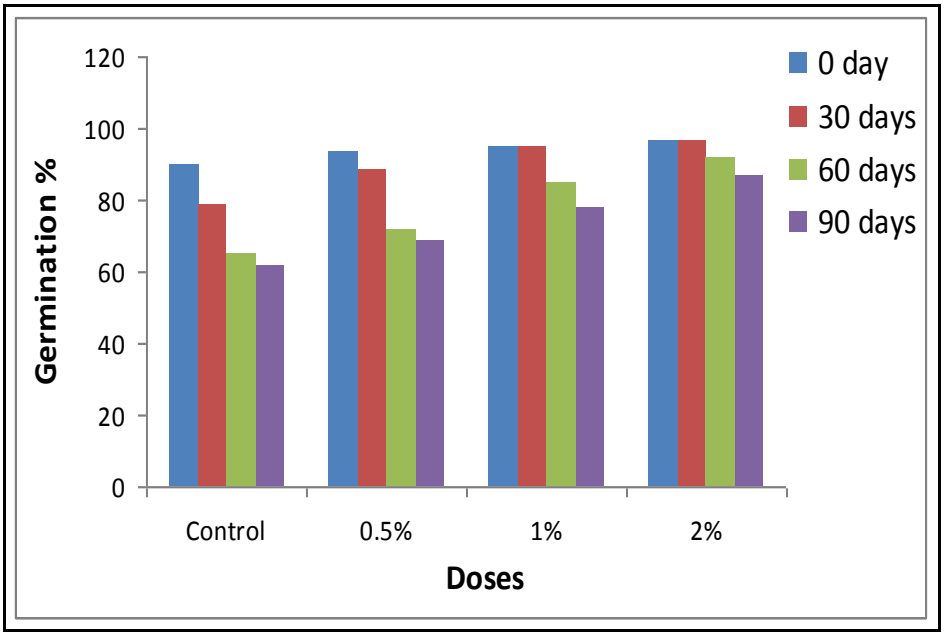

Fig. 8. Effect of *F. assafoetida* powder on seed germination at 16% moisture stored at 35˚C. $LSD_{0.05}$ = 2.804 (days); 2.804 (dose); F value = 157.6483 (days); 39.1428 (dose)

DISCUSSION

For the preservation of high moisture grains, different organic acid and their related compound have been studied (Sauer, 1973; Sauer and Burroughs, 1974; Hertirg and Drury, 1974; Sauer *et al.*, 1975; Nash and Easson, 1977; Nash, 1978). Wilson *et al.* (1979) also found volatile organic acid such as propionic acid for the preservation of mold growth on corn stored under high moisture conditions.

Table 2. Effect of *Ferula assafoetida* powder in the control of fungal infection in maize seed at different moisture and temperature during storage.

8 % moisture

Temperature °C	25 °C												35 °C											
	0 day			30 days			60 days			90 days			0 day			30 days			60 days			90 days		
Dose (%)	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2
FUNGI	INFECTION (%)																							
Alternaria alternata	22	15	-	-	19	13	-	-	12	7	-	-	10	7	-	-	40	30	-	-	45	26	-	-	46	9	-	-	17	4	-	-
Aspergillus candidus	18	12	10	-	16	10	-	-	-	-	-	-	-	-	-	-	20	16	9	-	21	4	-	-	-	-	-	-	-	-	-	-
A.flavus	61	23	21	-	40	20	13	-	31	16	11	-	12	10	-	-	80	60	39	-	70	45	30	-	55	30	21	-	16	13	-	-
A.fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	16	-	-	17	9	-	-	13	9	4	-	9	3	2	-
A.niger	27	-	-	-	24	-	-	-	19	-	-	-	13	-	-	-	55	-	-	-	57	-	-	-	29	-	-	-	20	-	-	-
A.wentii	20	-	-	-	18	-	-	-	13	-	-	-	9	-	-	-	22	-	-	-	42	-	-	-	20	-	-	-	17	-	-	-
F.moniliforme	21	-	-	-	17	-	-	-	13	-	-	-	4	-	-	-	24	-	-	-	20	-	-	-	17	-	-	-	12	-	-	-
F.nivale	27	14	-	-	20	10	-	-	13	9	-	-	12	2	-	-	39	17	-	-	29	12	-	-	25	10	-	-	17	10	-	-
F.oxysporum	42	-	-	-	31	-	-	-	27	-	-	-	21	-	-	-	70	-	-	-	65	-	-	-	59	-	-	-	32	-	-	-
F.semitectum	25	-	-	-	18	-	-	-	12	-	-	-	6	-	-	-	69	-	-	-	51	-	-	-	41	-	-	-	30	-	-	-
Penicillium sp	22	18	15	-	19	12	-	-	12	7	3	-	11	4	-	-	59	40	30	19	49	39	22	-	37	22	-	-	27	20	17	-
Rhizopus sp	27	14	-	-	-	10	-	-	-	-	-	-	-	-	-	-	77	61	35	17	60	22	-	-	40	22	-	-	-	-	-	-

LSD 0.05, = 1.314 (days); 0.929 (temp); .34 (dose)

Cont'd.

16 % moisture

Temperature °C		25° C												35 °C									
		0 day			30 days			60 days			90 days			0 day			30 days			60 days			90 days	
DOSE IN % FUNGI		C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2	C	.5	1	2
		INFECTION (%)																					
Alternaria alternata	50	14	10	-	56	18	17	-	71	21	20	-	42	9	2	-	55	40	9	-	69	13	-	-	81	14	12	-	50	17	-	-
Aspergillus candidus	38	16	8	-	44	13	-	-	49	10	6	-	30	4	1	-	48	12	-	-	56	16	-	-	59	19	11	-	37	14	8	-
A.flavus	22	7	3	-	32	9	2	-	41	6	2	-	27	8	-	-	35	21	10		47	7	-	-	57	16	-	-	30	13	-	-
A.fumigatus	21	-	-	-	30	-	-	-	33	-	-	-	18	-	-	-	22	-	-	-	35	-	-	-	45	-	-	-	17	-	-	-
A.niger	40	-	-	-	32	-	-	-	38	-	-	-	15	-	-	-	46	-	-	-	51	-	-	-	55	-	-	-	36	-	-	-
A.wentii	22	-	-	-	30	-	-	-	33	-	-	-	12	-	-	-	37	-	-	-	42	-	-	-	46	-	-	-	26	-	-	-
F.moniliforme	27	-	-	-	30	-	-	-	30	-	-	-	13	-	-	-	27	-	-	-	30	-	-	-	32	-	-	-	17	13	-	-
F.nivale	27	-	-	-	29	7	-	-	36	4	-	-	7	2	-	-	40	12	-	-	44	17	-	-	49	21	-	-	21	-	-	-
F. oxysporum	35	-	-	-	44	-	-	-	51	-	-	-	28	-	-	-	77	-	-	-	79	-	-	-	82	-	-	-	31	-	-	-
F.semitectum	55	-	-	-	62	-	-	-	71	-	-	-	37	-	-	-	76	-	-	-	80	-	-	-	88	-	-	-	69	-	-	-
Penicillium sp	42	16	-	-	49	19	-	-	53	21	12	-	21	9	-	-	60	23	10	-	61	17	7	-	66	33	10	-	51	13	-	-
Rhizopus sp	79	12	13	-	82	72	22	-	80	21	10	-	34	18	12	-	79	32	17	-	79	21	-	-	60	18	12	-	54	12	-	-

LSD 0.05 = 1.139 (days); 0.805 (temperature); 1.139 (dose)

In the present studies maize seeds with 8 and 16 % moisture levels treated with different doses of *Nigella sativa* seeds and *F. assafoetida* powder and stored at 25 and 35˚C for 90 days. It was observed that with the increase in dose, infection of mold decreased and maintained the germination of seeds. Several edible botanical extracts have been reported to have antifungal activity. *Nigella sativa* seeds and *F. assafoetida* powder @ 2 % was effective. However, no fungicidal activity was observed against *A. niger, A. alternata, A. candidus, D. hawiiensis* and *F. moniliforme* were significantly inhibited @ 1, 2 % w/w. Khan *et al.,* (2003) also reported the antifungal activity of *N. sativa* seed against *Candida albicans.* Sitara *et al.* (2008) used essential oil extract of seeds of neem, mustard, *N. sativa* and *Ferula assofoctida* for antifungal activity against seed borne fungi like *A. niger* and *A. flavus,* they found that the oil extract except mustard seed showed fungicidal activity against test fungi (Table 2).

Present results showed that most of fungi isolated from maize seeds are toxigenic which spoil the seeds/grain and also produce mycotoxin which produce harm to human being. Experiments on reduction of mycotoxigenic fungi were conducted like use of, *N. sativa* seed powder and *F. assofoetida* powder during storage. There is therefore need to minimize fungal contamination and mycotoxin production in large scale by using different control measures for obtaining the good quality of seeds for human consumption and for farmers.

REFERENCES

Anonymous (1993). International rules for seed testing. *Seed Science and Technol*., 21:1-288.

Hanafy, M.S. and M. E. Haten (1991). Studies on the antimicrobial activity of *Nigella sativa* seed. *J. Ethnopharmacol,* 34: 275- 278.

Herting, D. C. and E.E. Drury (1974). Antifungal activity of volatile fatty acids on grains. *Cereal Chem.,* 51(1): 75.

Houghton. P.J., K.M. Ismail, L. Maxia and G. Appendino (2006). Antidermatophytic prenglated coumarins from *asafoetida. Planta Med.,* 72 DOI; 10.1055/S – 949741.

Khan, M. A., M.K. Ashfaq, H.S. Zuberi, M. S. Mahmood and A. H. Gilini (2003). The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seed. *Phytother Res. Fe.*, 17 (2): 183- 186.

Lavermicocca, P., F. Valerio and A. Visconti (2003). Antifungal activity of phenyllacetic acid against moulds isolated from bakery products. *Applied and Environmental Microbiology*, 69(1): 634-640.

Mashhadian , N. V. and H. Rakhshandeh (2005). Anti bacterial and antifungal effect of *Nigella sativa* extract against S. *aureus, P. aeroginosa* and *C. albicans. Pak J. Med. Sci.*, 21(1): 47- 52.

Morsi, N.M. (2000). Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics resistant bacteria. *Acta Microbiol pol*., 49: 63-74.

Nash, M. J. and D. L. Easson (1977). Prevention of moisture hay propionic acid. *J. Stored Product Res*., 13: 65-75.

Nash, M. J. (1978). *Crop conservation and storage in cool temperature climates*. Pergamon Press, Oxford

Sauer, D. B. (1973). *Grain preservatives for high moisture food grains.* Report from U.S. Grain marketing Research Centre, Agricultural Research Services, U.S. Dept. of Agriculture, Manhatton, Kansas, U.S.A.

Sauer, D. B. and R. Burroughs (1974). Efficacy of various chemicals as grain mold inhibitor. *Trans. ASAE*., 17 (3): 557- 559.

Sauer, D. B., T.O. Hudges, R. Burroughs and H.H. Converse (1975). Comparision of propionic acid and methylene-bis- propionate as grain preservative. *Trans ASAE*., 18(6): 1162-1164.

Sitara, U., I. Niaz and J. Naseem (2008). Antifungal effect of essential oils an *in vitro* growth of pathogenic fungi. *Pak. J. Bot.*, 40(1): 409-414.

Vandegraft, E.E., C.W. Hesseltine and O.L. Shotwell (1975). Grain preservatives: effect on aflatoxin and ochratoxin production. *Cereal Chem*., 52:79-84.

Wallace, H.A.H. and R. N. Sinha (1962). Fungi associated with hot spots in farm stored grains. *J. Can. Plant Sci.*, 42: 130-141.

Wilson, D.M., W.W. Mcmillian and N.W. Widstrom (1979). Field aflatoxin contamination of corn in South Georgia. *J. Am. Oil Chemists. Soc.*, 50:79.

(*Accepted for publication October 2012*)