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STUDIES ON THE EFFICACY OF CHEMICAL AND NON CHEMICAL TREATMENTS TO CONTROL MYCOFLORA ASSOCIATED WITH CHILLI SEED

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Abstract

A total 19 genera and 38 species of fungi were isolated by using standard blotter and deep freezing method from chilli seeds. Of these *Aspergillus flavus, A. niger, A. fumigatus, Alternaria alternata, Drechslera hawiinesis, Fusarium moniliforme, F. oxysporum* and *F. solani* were more frequently isolated. Seed treatments with 8 fungicides viz., Metalaxyl + Mancozeb (72% w/w), Mancozeb (80% w/w Dithiocarbamate), Aliette (80% WP Fosetyle aluminium), Derosol (60% WP Carbendazim), Ridomyl Gold (68% WP), Thiophonate methyl (70% WP), Antracol (70%WP Propineb) and Copperoxychlorite (50% WP) and four seed powders of herbicides viz., asafoetida (*Ferula assafoetida*), black cumin (*Nigella sativa*), neem (*Azadirachta indica*) and mustard (*Brassica campestris*) were used @ 0.5%, 0.15% & 0.25% . Out of these 8 fungicides; Ridomyl Gold @ 0.15 & 0.25% inhibited the growth of all fungi whereas asafetida and *Nigella sativa* powder @0.25% were found to be more efficacious however showed little fungicidal activity toward *Fusarium moniliforme.*

Introduction

In Pakistan chilli (*Capsicum annum* L.) is an important vegetable crop planted over an estimated area of 47.3 thousand hectars with an annual production of 69.5 thousand tones, with an average yield of 1902 kg/ha (Anon., 2006). Chilli is susceptible to several diseases including root and collar rot produces by *Phytophthora capsici* (Ahmed *et al*., 1989; Saleem *et al*., 1996; 1998; Hussain *et al*., 1990; Than *et al*., 2008). Anthracnose or die-back and fruit rot caused by *Collectotrichum* spp., (Khaleeque & Khan, 1991; Sultana *et al*., 1992; Amusa, 2004). *Fusarium* spp., produces wilt, root rot and powdery mildew caused by the fungus *Leveillula taurica* (Hafeez, 1986; Mushtaq & Hashmi, 1997). Plant product with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent fungal and bacterial growth (Sagdic *et al*., 2003; Sridhar *et al*., 2003; Lanciotti *et al*., 2004).

Thagaraja & Honso (1996) reported antifungal activity of asafoetida against *Rhizopus sporus, Mucor dimorphosphorous, Penicillium commune* and *Fusarium solani*. Siddiqui *et al*., (1996) also studied the inhibition effect of asafetida on *A. flavus*. Antifungal activity of asafetida have been also found effective against *Microsporeum gypseum* and *Trichophyton* (Houghton *et al*., 2006). According to Sitara *et al*., (2008) 0.15% *Nigella sativa* oil possess a remarkable antifungal activity against *A. flavus, Fusarium moniliforme, F.oxysporum, F.nivale, F. semitectum, Drechslera hawiiensis* and *Alternaria alternata*. The aqueous extract of *N. sativa* seeds has also been shown inhibitory effect on *Candidus albican In vivo* (Khan *et al*., 2003). Neem is valuable plant sources of medically useful compound and has antimicrobial activities. According

to Agbenin & Marley (2006) dry neem seed extract completely inhibited the mycilial growth of *F. oxysporum* at all concentration. Agbenin *et al*., (2004) reported that using neem seed powder, also controlled *Fusarium* spp.. Niaz *et al*., (2008) also analyzed that 1% neem seed oil inhibited the growth of *Drechslera specifera* and *D. hawiinesis*. Sitara *et al*., (2008) showed that at 0.15% concentration mustard oil had antifungal properties towards *Fusarium oxysporum* and *F. nivale*. According to Bowers & Locke (2000) soil populations of *Fusarium oxysporum* were lowest after 3 to 7 days of incubation when the soil was treated with 5 and 10% aqueous emulsion of mustard extract. Experiments were therefore carried out to examine the mycoflora of chilli seeds and to study the comparisons between fungicides and herbicides.

Materials and Methods

For the detection of seed borne fungi of chilli seed standard blotter and deep-freezing method by ISTA techniques was used. Sodium hypochlorite (10%) was used for surface sterilization of seeds while eight fungicides viz., Metalaxyl + Mancozeb (72%), Mancozeb (72% w/w Dithiocarbamate), Aliette (80% WP Fosetyl aluminium), Derosol (60% WP Carbendazim), Ridomyl Gold (68% WP), Thiophonate methyl (70% WP), Antracol (70% WP Propineb) and Copper Oxychlorite (50% WP) and four powder of herbicides viz., neem, asafoetida, mustard and black cumin were used @ 0.5%, 0.15% & 0.25%.

The eight fungicides and powder of four herbicides were applied on seed in conical flask separately. All treated seed were plated @ 25 seeds/ plate on 3 layers of moistened blotter in 9cm 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 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. The fungi were identified upto species level after reference to Barnet & Hunter (1972), Booth (1971), Ellis (1971) & Nelson *et al*., (1983).

Results and Discussion

In blotter method, a total 19 genera and 38 species of fungi were isolated. *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Alternaria alternata*, *Drechslera hawiinesis*, *Trichoderma* sp., *Phoma betae*, *Fusarium moniliforme*, *Aspergillus candidus* and *Alternaria tenussima* were more frequent in order of prevalence (Table 1). Incidence of *Aspergillus* species was found to be dominant on chilli seeds. These results fully supported the results obtained by Kiran *et al*., (2005) and Tripathi *et al*., (2008) on same seed whereas in deep freezing method *Fusarium moniliforme, F. oxysporum, F. solani, F. nivale, A. fumigatus* and *Drechslera hawiinesis* were more frequently isolated. Sultana *et al*., (1988) reported that infection percent of *Fusarium moniliforme* and *Alternaria alternata* were generally higher in the deep-freezing method. Hashmi (1989) also observed that, of the 222 samples of capsicum, 64.0% samples were infected by *Fusarium moniliforme* and 60.8% by *Alternaria alternate*.

Table 1. Percentage occurrence of fungi after different treatments of fungicides by blotter method.

Name of fungi	Blotter method		Metalaxyl + Mancozeb (72%)			Mancozeb (80% W\W)			Aliette (80% WP)			Derosol (60% WP)		
	Nst	St	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
Aspergillus niger	60.25	46.5	9.75	3.5	1.5	3	1.75	0.75	3	2	1	0	0	0
A.flavus	70.75	47.75	4	2	0.75	3.75	2.75	0	2.25	1.5	0	10.8	5.5	5
A.candidus	37.25	14.25	6.25	1.5	0	2	0	0	0	0	0	9	4	0
A. terrus	27.25	12.5	0	0	0	0	0	0	0	0	0	0	0	0
A. sulphrus	36.5	13	0	0	0	0	0	0	0	0	0	0	0	0
A. fumigatus	53.5	33.25	4	0	0	0	0	0	0	0	0	7.25	5.75	4.75
A. tamari	35.5	20.75	0	0	0	0	0	0	2	0	0	0	0	0
Alternaria alternata	50.25	35.25	10	2	0.5	2.5	0	0	0	0	0	9	4.25	2
A. tenussinia	36.75	31.5	6.25	0	0	0	0	0	0	0	0	0	0	0
A. pori	25.25	15	0	0	0	0	0	0	0	0	0	0	0	0
A. solani	31.5	19.5	0	0	0	0	0	0	0	0	0	0	0	0
Phoma beta	39.5	23	6	0	0	0	0	0	0	0	0	4.25	0	0
P. lingam	31.75	19.75	0	0	0	0	0	0	0	0	0	0	0	0
Phomopsis	32.25	18.25	0	0	0	0	0	0	0	0	0	5	0	0
Phyllosticta sp	16	5.5	0	0	0	0	0	0	0	0	0	0	0	0
Chaetomium globossom	33.75	17.75	8.25	0	0	0	0	0	0	0	0	4.75	1	0
C. gracile	13.75	7	0	0	0	0	0	0	0	0	0	0	0	0
C. distortum	12	5.75	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium caldosporoides	26.25	6.5	0	0	0	0	0	0	0	0	0	6.75	5	3.5

Table 1. (Cont'd.).

Sr#	Name of fungi	Blotter method		Metalaxyl + Mancozeb (72%)			Mancozeb (80% W\W)			Aliette (80% WP)			Derosol (60% WP)		
		Nst	St	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
20.	*Curvularia pallescens*	14	6.75	0	0	0	0	0	0	0	0	0	0	0	0
21.	*C. lunata*	11	4.25	0	0	0	0	0	0	0	0	0	0	0	0
22.	*C. robusta*	18	55	0	0	0	0	0	0	0	0	0	0	0	0
23.	*Drechslera hawiinesis*	50.5	26	8.75	1.5	0	2	1.5	0	0	0	0	7.75	0	0
24.	*D. specifera*	32.75	19.5	0	0	0	1.5	0	0	0	0	0	0	0	0
25.	*D. poae*	29.5	10.5	8.25	0	0	0	0	0	0	0	0	0	0	0
26.	*Rhizopus sp*	32.5	21.75	0	0	0	0	0	0	0	0	0	0	0	0
27.	*Rhizoctonia solani*	30.5	19	0	0	0	0	0	0	0	0	0	0	0	0
28.	*F. monilifrme*	38.5	16	12.3	3	0	3.5	0	0	1	0	0	6.5	4.75	3.5
29.	*F. oxysporum*	29.75	10.75	9.5	0	0	4.25	0	0	0	0	0	0	0	0
30.	*F. nivale*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31.	*F. solani*	0	0	7	1	0	0	0	0	0	0	0	6.25	4.75	3.75
32.	*Helminthosporium valutinum*	15.5	6.25	4	2	0	0	0	0	0	0	0	0	0	0
33.	*Nigrospora sp*	19	9.5	0	0	0	0	0	0	0	0	0	1.5	0	0
34.	*Cercospora sp*	26.5	14.75	0	0	0	0	0	0	0	0	0	0	0	0
35.	*Cephaliophora irreglaris*	14.25	8	0	0	0	0	0	0	0	0	0	0	0	0
36.	*Myrothecium straiatispor*	20	6	0	0	0	0	0	0	0	0	0	0	0	0
37.	*M. brachysporum*	19.5	8	0	0	0	0	0	0	0	0	0	0	0	0
38.	*Collectotrichum capsci*	27	12.25	9.5	1.75	0	0	0	0	0	0	0	4.25	0	0
39.	*Trichoderma*	39.75	20.5	10	1	0	0	0	0	0	0	0	2.5	0	0

Table 1. (Cont'd.).

Sr#	Name of fungi	Ridomyl Gold (68%WP)			Thiophonate methyl (70%WP)			Antracol (70%WP)			Copper Oxychlorite (50%WP)		
		0.50	**0.15**	**0.25**	**0.50**	**0.15**	**0.25**	**0.50**	**0.15**	**0.25**	**0.50**	**0.15**	**0.25**
1.	*Aspergillus niger*	1.5	0.5	0	8.25	4.5	2.25	9.25	6.25	4.25	11.3	7.8	3.75
2.	*A.flavus*	0.5	0	0	4.5	2	0	8.5	4	2.75	9.5	4.8	4
3.	*A.candidus*	0	0	0	0	0	0	0	0	0	8	6.8	0
4.	*A. terrus*	0	0	0	0	0	0	0	0	0	0	0	0
5.	*A. sulphrus*	0	0	0	0	0	0	0	0	0	0	0	0
6.	*A. fumigatus*	0	0	0	3.5	2	0	0	0	0	0	0	0
7.	*A. tamari*	0	0	0	0	0	0	7	0	0	0	0	0
8.	*Alternaria alternata*	0	0	0	4	2.25	0	8.25	3.75	0	6.75	5.8	3
9.	*A. tenussinia*	0	0	0	0	0	0	0	0	0	0	0	0
10.	*A. pori*	0	0	0	0	0	0	7.5	0	0	0	0	0
11.	*A. solani*	0	0	0	0	0	0	0	0	0	0	0	0
12.	*Phoma beta*	0	0	0	0	0	0	0	0	0	5	3	0
13.	*P. lingam*	0	0	0	0	0	0	0	0	0	0	0	0
14.	*Phomopsis*	0	0	0	0	0	0	0	0	0	0	0	0
15.	*Phyllosticta sp*	0	0	0	0	0	0	0	0	0	0	0	0
16.	*Chaetomium globossom*	0	0	0	0	0	0	0	0	0	7.75	0	0
17.	*C. gracile*	0	0	0	0	0	0	0	0	0	0	0	0
18.	*C. distortum*	0	0	0	0	0	0	0	0	0	0	0	0
19.	*Cladosporium caldosporoides*	0	0	0	7.25	0	0	0	0	0	10	0	0

Table 1. (Cont'd.).

Sr#	Name of fungi	Ridomyl Gold (68%WP)			Thiophonate methyl (70%WP)			Antracol (70%WP)			Copper Oxychlorite (50%WP)		
		0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
20.	*Curvularia pallescens*	0	0	0	0	0	0	0	0	0	0	0	0
21.	*C. lunata*	0	0	0	0	0	0	0	0	0	0	0	0
22.	*C. robusta*	0	0	0	0	0	0	0	0	0	0	0	0
23.	*Drechslera hawiinesis*	0	0	0	0	0	0	0	0	0	5.25	5.3	2.25
24.	*D. specifera*	0	0	0	0	0	0	0	0	0	5.25	0	0
25.	*D. poae*	0	0	0	0	0	0	0	0	0	0	0	0
26.	*Rhizopus sp*	0	0	0	0	0	0	0	0	0	0	0	0
27.	*Rhizoctonia solani*	0	0	0	0	0	0	0	0	0	0	0	0
28.	*F. monilifrme*	1.5	0	0	9.75	0	0	11.3	10	8	14.3	7.5	3.75
29.	*F. oxysporum*	0	0	0	0	0	0	0	0	0	8.25	5.5	4.25
30.	*F. nivale*	0	0	0	0	0	0	0	0	0	0	0	0
31.	*F. solani*	0	0	0	0	0	0	10.5	7.25	0	7.25	0	0
32.	*Helminthosporium valutinum*	0	0	0	0	0	0	0	0	0	3.75	0	0
33.	*Nigrospora sp*	0	0	0	4.25	0	0	0	0	0	0	0	0
34.	*Cercospora sp*	0	0	0	0	0	0	0	0	0	0	0	0
35.	*Cephaliophora irreglaris*	0	0	0	0	0	0	0	0	0	0	0	0
36.	*Myrothecium straiatispor*	0	0	0	0	0	0	0	0	0	0	0	0
37.	*M. brachysporum*	0	0	0	0	0	0	0	0	0	0	0	0
38.	*Collectotrichum capsci*	0	0	0	0	0	0	0	0	0	7.5	4.5	0
39.	*Trichoderma*	0	0	0	0	0	0	0	0	0	5.75	2.5	0

Eight fungicides and four herbicides were used to control the fungus associated with the seed of chilli @ 0.5%, 0.15% & 0.25% concentration. Results showed that out of all fungicides, Ridomyl Gold (68% WP) @ 0.25% concentration completely controlled the fungi; however, 0.15% dose also suppressed the growth of all fungi whereas *A. niger* showed only 0.5 % growth. This result is in close conformity with the findings of Sitara & Shahida (2007) who found that Ridomyl Gold was effective @ 0.3% concentration against all fungi except *A. niger*. Fungicides Mancozeb, Aliette, Thiophonate methyl @ 0.25% also controlled all isolated fungi whereas *A. niger* gave 0.75%, 1% & 2.25% mycelial growth respectively. Aliette @0.15% showed better result as compare to Mancozeb and Thiophonate methyl at same doses. It is also interesting to note that Derosol (60% WP) completely inhibited the growth of fungi at all doses in blotter and deep freezing method.

In deep freezing method three fungicides viz., Ridomyl Gold, Thiophonate methyl and Mancozeb @ 0.25% concentration completely controlled all fungi whereas Thiophonate methyl and Aliette were effective for all fungi nevertheless the growth of *A. niger* and *A. flavus* increased to some extent (Table 2). It is also noted that fungi viz., *Phoma lingam, Phomopsis* sp., *Phyllosticta* sp., *Cheatomium gracile, C. distortum, Curvularia pallescens, C. lunata, C. robusta, Rhizopus* sp., *Rhizoctonia solani, Cercospora* sp., *Cephalophora irregularis, Myrothecium straiatispor* and *M. brachysporum* were completely inhibited by all fungicides in blotter and deep freezing methods. The growth of *Fusarium nivale* and *Nigrospora* sp., promoted only in deep freezing method.

Antifungal activity of asafoetida, *Nigella sativa*, neem and mustard seed powder were analyzed by blotter and deep freezing method against all isolated fungi. The results revealed that asafoetida @ 0.25% showed positive response for all fungi except the growth of *F. moniliforme* (Table 3). Siddiqui *et al*., (1996) also reported antifungal activity of asafoetida oil against A. *flavus*. According to Sitara *et al*., (2008), asafoetida oil @ 0.1% & 0.15% significantly inhibited the growth all tested fungi except A. *flavus*. Antifungal activity of *Nigella sativa* was most significantly effective @ 0.25 %; however, it exhibited no fungicidal activity against A. *flavus* and *Fusarium moniliforme*. The oil extract of *Nigella sativa* showed antimicrobial effect *In vivo* towards *Staphylococcus aurous*, *Pseudomonas aeroginosa* and *Candida albicans* (Hanafy & Hatem, 1991; Mashhadian & Rakshandeh, 2005). Neem oil was effective @ 0.15% and 0.25%; nevertheless the growth of *A. flavus, A. niger, Fusarium moniliforme* and *F. solani* were not reduced. Ishrat *et al*., (2008) found that 0.1% concentration of neem oil was effective against for *Macrophomina phaseolina* and *Rhizoctonia solani*. Kazmi *et al*., (1995) also noted that 0.1% neem seed oil was more effective against *Macrophomina phaseolina*. Mustard oil @ 0.25% concentration also showed significant growth reduction in all isolated fungi, moreover, the growth of *Aspergillus flavus, A. niger, Alternaria alternata, F. moniliforme* and *F. solani* was somewhat repressed or promoted. Kazmi *et al*., (1993) also reported fungistatic activity of mustard oil, most significantly against *Alternaria alternata* as compare to other fungi.

Table 2. Percentage occurrence of fungi after different treatments of fungicides by deep freezing method.

Sr#	Name of fungi	Blotter method		Metalaxyl + Mancozeb (72%)			Mancozeb (80%W\W)			Aliette (80%WP)			Derosol (60%WP)		
		Nst	St	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
1.	*Aspergillus niger*	41.5	31.25	2.75	1.5	0.5	1.5	1	0	2.5	1.5	1.5	0	0	0
2.	*A.flavus*	37.25	25	2.5	1	0.5	2	1.5	0	1.25	0.5	0	7.5	4	1.5
3.	*A.candidus*	20.25	7.5	4.75	0.5	0	1.75	0	0	0	0	0	6.25	2	0
4.	*A. terrus*	18.25	8	0	0	0	0	0	0	0	0	0	0	0	0
5.	*A. sulphrus*	20	11.75	0	0	0	0	0	0	0	0	0	0	0	0
6.	*A. fumigatus*	40.5	30.25	2	0	0	0	0	0	0	0	0	4	3.75	3.5
7.	*A. tamari*	28.25	13.75	0	0	0	0	0	0	1.5	0	0	0	0	0
8.	*Alternaria alternata*	34.75	23	3.75	1.25	0.25	1.25	0	0	0	0	0	1.75	4.25	0
9.	*A. tenussinia*	31	24.5	4.75	0	0	0	0	0	0	0	0	0	0	0
10.	*A. pori*	25.75	14.25	0	0	0	0	0	0	0	0	0	0	0	0
11.	*A. solani*	27.25	15.75	0	0	0	0	0	0	0	0	0	0	0	0
12.	*Phoma beta*	36.5	18.75	3.5	0	0	0	0	0	0	0	0	3	0	0
13.	*P. lingam*	27.25	14	0	0	0	0	0	0	0	0	0	0	0	0
14.	*Phomopsis*	29.75	13.5	0	0	0	0	0	0	0	0	0	0	0	0
15.	*Phyllosticta sp*	10	5	0	0	0	0	0	0	0	0	0	0	0	0
16.	*Chaetomium globossom*	20	9.25	4.5	0	0	0	0	0	0	0	0	3	0.5	0
17.	*C. gracile*	9.25	4	0	0	0	0	0	0	0	0	0	0	0	0
18.	*C. distortum*	7	4	0	0	0	0	0	0	0	0	0	0	0	0
19.	*Cladosporium caldosporoides*	14.5	3.75	0	0	0	0	0	0	0	0	0	3.75	3.75	3

Table 2. (Cont'd.).

Sr#	Name of fungi	Blotter method		Metalaxyl + Mancozeb (72%)			Mancozeb (80% W\W)			Aliette (80% WP)			Derosol (60% WP)		
		Nst	St	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
20.	*Curvularia pallescens*	8.75	4.25	0	0	0	0	0	0	0	0	0	0	0	0
21.	*C. lunata*	6.25	3.75	0	0	0	0	0	0	0	0	0	0	0	0
22.	*C. robusta*	10.75	3.75	0	0	0	0	0	0	0	0	0	0	0	0
23.	*Drechslera hawiinesis*	38	22.25	4.5	0.75	0	0.75	0.5	0	0	0	0	6.25	0	0
24.	*D. specifera*	33	20	0	0	0	1.25	0	0	0	0	0	0	0	0
25.	*D. poae*	18.75	8.25	8.25	0	0	0	0	0	0	0	0	0	0	0
26.	*Rhizopus sp*	23	13.75	0	0	0	0	0	0	0	0	0	0	0	0
27.	*Rhizoctonia solani*	25	16	0	0	0	0	0	0	0	0	0	0	0	0
28.	*F. monilifrme*	72.5	46.5	6.5	1	0	2	0	0	1	0	0	3.75	3	3.75
29.	*F. oxysporum*	68.75	46	4.5	0	0	1.75	0	0	0	0	0	0	0	0
30.	*F. nivale*	43.25	22.5	0	0	0	0	0	0	0	0	0	0	0	0
31.	*F. solani*	52	31	3.5	0.5	0	0	0	0	0	0	0	3.75	0	4.75
32.	*Helminthosporium valutinum*	10	4.75	2	0.75	0	0	0	0	0	0	0	0	0	0
33.	*Nigrospora sp*	13.5	6.75	0	0	0	0	0	0	0	0	0	0	0	0
34.	*Cercospora sp*	21	11	0	0	0	0	0	0	0	0	0	0	0	0
35.	*Cephaliophora irreglaris*	8	2.5	0	0	0	0	0	0	0	0	0	0	0	0
36.	*Myrothecium straiatispor*	10.5	5	0	0	0	0	0	0	0	0	0	0	0	0
37.	*M. brachysporum*	13	5	0	0	0	0	0	0	0	0	0	0	0	0
38.	*Collectotrichum capsci*	17.5	10.5	3.25	0.5	0	0	0	0	0	0	0	0	0	0
39.	*Trichoderma*	29.5	21.25	4.5	0.75	0	0	0	0	0	0	0	8	0	0

Table 2. (Cont'd.).

Sr#	Name of fungi	Ridomyl Gold (68%WP)			Thiophonate methyl (70%WP)			Antracol (70%WP)			Copper Oxychlorite (50%WP)		
		0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
1.	*Aspergillus niger*	0.75	0.25	0	5	3.75	0	10.3	5	3	7.25	3.5	2.5
2.	*A.flavus*	0	0	0	2.25	0.75	0	3.5	2.75	1.75	5.25	3	3
3.	*A.candidus*	0	0	0	0	0	0	0	0	0	4.25	4	0
4.	*A. terrus*	0	0	0	0	0	0	0	0	0	0	0	0
5.	*A. sulphrus*	0	0	0	0	0	0	0	0	0	0	0	0
6.	*A. fumigatus*	0	0	0	2	0	0	0	0	0	0	0	0
7.	*A. tamari*	0	0	0	0	0	0	0	0	0	0	0	0
8.	*Alternaria alternata*	0	0	0	3.25	0	0	4.5	3.25	0	3.75	3.5	1.75
9.	*A. tenussinia*	0	0	0	0	0	0	0	0	0	0	0	0
10.	*A. pori*	0	0	0	0	0	0	0	0	0	0	0	0
11.	*A. solani*	0	0	0	0	0	0	0	0	0	0	0	0
12.	*Phoma beta*	0	0	0	0	0	0	0	0	0	0	0	0
13.	*P. lingam*	0	0	0	0	0	0	0	0	0	0	0	0
14.	*Phomopsis*	0	0	0	0	0	0	0	0	0	0	0	0
15.	*Phyllosticta sp*	0	0	0	0	0	0	0	0	0	0	0	0
16.	*Chaetomium globossom*	0	0	0	0	0	0	0	0	0	0	0	0
17.	*C. gracile*	0	0	0	0	0	0	0	0	0	0	0	0
18.	*C. distortum*	0	0	0	0	0	0	0	0	0	0	0	0
19.	*Cladosporium caldosporoides*	12	0	0	2.5	0	0	0	0	0	0	0	0

Table 2. (Cont'd.).

Sr#	Name of fungi	Ridomyl Gold (68% WP)			Thiophonate methyl (70% WP)			Antracol (70% WP)			Copper Oxychlorite (50% WP)		
		0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25	0.50	0.15	0.25
20.	*Curvularia pallescens*	0	0	0	0	0	0	0	0	0	0	0	0
21.	*C. lunata*	0	0	0	0	0	0	0	0	0	0	0	0
22.	*C. robusta*	0	0	0	0	0	0	0	0	0	0	0	0
23.	*Drechslera hawiinesis*	0	0	0	0	0	0	0	0	0	4.5	4.5	1.5
24.	*D. specifera*	0	0	0	0	0	0	0	0	0	0	0	0
25.	*D. poae*	0	0	0	0	0	0	0	0	0	0	0	0
26.	*Rhizopus sp*	0	0	0	0	0	0	0	0	0	0	0	0
27.	*Rhizoctonia solani*	0	0	0	0	0	0	0	0	0	0	0	0
28.	*F. monilifrme*	1	0	0	6.25	0	0	11	9.75	4.25	14.3	9.3	5
29.	*F. oxysporum*	0	0	0	0	0	0	0	0	0	11	3.5	4.75
30.	*F. nivale*	0	0	0	0	0	0	0	0	0	0	0	0
31.	*F. solani*	0	0	0	0	0	0	14.5	7	0	0	0	0
32.	*Helminthosporium valutinum*	0	0	0	0	0	0	0	0	0	0	0	0
33.	*Nigrospora sp*	0	0	0	0	0	0	0	0	0	0	0	0
34.	*Cercospora sp*	0	0	0	0	0	0	0	0	0	0	0	0
35.	*Cephaliophora irreglaris*	0	0	0	0	0	0	0	0	0	0	0	0
36.	*Myrothecium straiatispor*	0	0	0	0	0	0	0	0	0	0	0	0
37.	*M. brachysporum*	0	0	0	0	0	0	0	0	0	0	0	0
38.	*Collectotrichum capsci*	0	0	0	0	0	0	0	0	0	3.75	2.5	0
39.	*Trichoderma*	0	0	0	0	0	0	0	0	0	2.75	0	0

Table 3. % Occurrence of fungi after different treatments (herbicides) by blotter method.

S.No	Name of fungi	Asafoetida			Kalongi			Neem			Mustard		
		0.50%	0.15%	0.25%	0.50%	0.15%	0.25%	0.50%	0.15%	0.25%	0.50%	0.15%	0.25%
1.	*Aspergillus flavus*	2	1	0	3.25	1.75	0.5	3.75	3	2.25	7	5.5	2.5
2.	*A.niger*	1.5	0.5	0	2.5	1.75	0	5	4	3	6.25	5.75	4
3.	*A.fumigatus*	0	0	0	0	0	0	2.5	1.5	0	4.5	3	0
4.	*A. candidus*	0	0	0	0	0	0	0	0	0	4.5	0	0
5.	*Alternaria alternata*	0.5	0	0	1.5	1	0	2	1.25	0	6	4	2
6.	*A. tennius*	0	0	0	0	0	0	2.5	0	0	2.5	0	0
7.	*A.solani*	0	0	0	0	0	0	0	0	0	4.75	0	0
8.	*A. tenussinia*	0	0	0	1.75	0	0	0	0	0	4	0	0
9.	*F. monilifrme*	2.25	1.5	0.75	2.5	1.5	1	4.5	1.75	1	5.75	4.5	2.25
10.	*F. solani*	0	0	0	0	0	0	2.5	2	1.25	4	2.25	1
11.	*F. oxysporum*	0	0	0	0	0	0	2	0	0	4.5	2.5	0
12.	*Cladosporium cladosporidi*	0	0	0	0	0	0	0	0	0	2	0	0
13.	*collectotrichum*	0	0	0	0	0	0	0	0	0	3.5	0	0

Table 4. % Occurrence of fungi at different treatments (deep freezing method).

S.No	Name of fungi	Asafoetida			Kalongi			Neem			Mustard		
		0.50%	0.15%	0.25%	0.50%	0.15%	0.25%	0.50%	0.15%	0.25%	0.50%	0.15%	0.25%
1.	*Aspergillus flavus*	1.5	0.5	0	2	1.25	0	3	2	1.75	4.5	4	1.5
2.	*A.niger*	1	0	0	1.75	0.75	0	4	3	2.25	6	4.75	2.25
3.	*A.fumigatus*	0	0	0	0	0	0	0	0	0	3	1.75	0
4.	*A. candidus*	0	0	0	0	0	0	0	0	0	3.5	0	0
5.	*Alternaria alternata*	0	0	0	1	0	0	1.5	0	0	3	2.25	0
6.	*A.solani*	0	0	0	0	0	0	0	0	0	3	0	0
7.	*A. tenussinia*	0	0	0	1	0	0	0	0	0	0	0	0
8.	*F. monilifrme*	1.75	0.5	0.25	2.25	1	0.75	3.5	1.5	0.5	4.5	2.75	1.25
9.	*F. solani*	0	0	0	0	0	0	2	1.25	0.5	3	1.25	0.5
10.	*F. oxysporum*	0	0	0	0	0	0	0	0	0	3.5	0	0
11.	*collectotrichum*	0	0	0	0	0	0	0	0	0	2.25	0	0
12.	*Drechslera hawiinesis*	0	0	0	0	0	0	0	0	0	2	0	0

In deep freezing method, asafoetida powder @ 0.5 & 0.15% was effective against for all isolated fungi; however *Aspergillus flavus*, *A. niger* and *Fusarium moniliforme* were not controled whereas 0.25% concentration also inhibited the growth of all fungi except F. moniliforme. Remarkably *Nigella sativa* powder @ 0.25% controlled all fungi; however *Fusarium moniliforme* showed little growth (Table 4). Neem seed powder also inhibited the growth of same fungi as in blotter method. Mustard powder @ 0.15% controlled *F. oxysporum* whereas in blotter method the growth of *Fusarium oxysporum* was somewhat promoted. It is also noted that 0.50% mustard powder inhibited the growth of *Drechslera hawiinesis* in blotter method nevertheless the growth was not much reduced in deep-freezing method. Sumbali & Mehrotra (1980) also analyzed the control of *Macrophomina phaseolina* by using mustard oil. Metalexyl + Mancozeb, Derosol, Copper Oxychlorite, and Antracol were not effective @ 0.5% concentration whereas Mancozeb, Aliette and Ridomyl Gold reduced infection percent at same dose level (Fig. 1a). Infection percent is high in Mustard seed powder @ 0.5 & 0.15% concentration whereas growth inhibited in *Asafoetida* and *Kalongi* @ 0.25% concentration. All herbicides were effective at 0.25% concentration and compete well with fungicides (Fig. 1b).

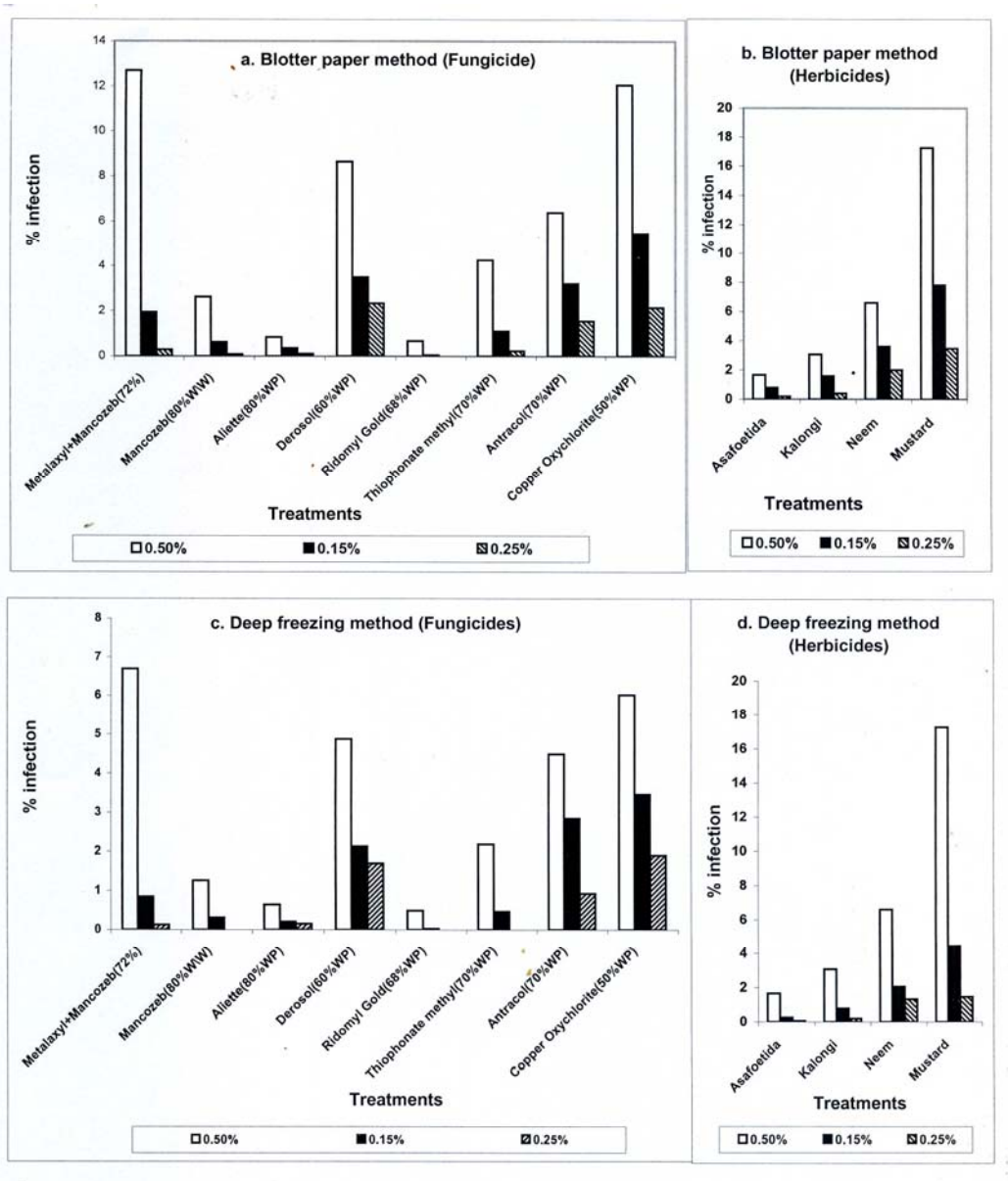

Fig. 1. Effect of fungicides and herbicides on the growth of fungi in blotter and deep freezing method.

Table 5a. Mean and standard error of fungicides and herbicides (Blotter paper method).

Sr.No	Treatments	0.5 Mean ± Std. Error	0.15 Mean ± Std. Error	0.25 Mean ± Std. Error
1.	Metalaxyl + Mancozeb (72%)	12.692 ± 2.662	1.948 ± 0.594	0.282 ± 0.175
2.	Mancozeb (80%W\W)	2.615 ± 0.864	0.615 ± 0.359	0.076 ± 0.076
3.	Aliette (80%WP)	0.846 ± 0.432	0.358 ± 0.253	0.1025 ± 0.1025
4.	Derosol (60%WP)	8.641 ± 2.094	3.487 ± 1.1931	2.333 ± 0.925
5.	Ridomyl Gold (68%WP)	0.666 ± 0.369	0.051 ± 0.051	0
6.	Thiophonate methyl (70%WP)	4.256 ± 1.6032	1.1025 ± 0.572	0.230 ± 0.230
7.	Antracol (70%WP)	6.384 ± 2.249	3.205 ± 1.4570	1.538 ± 0.951
8.	Copper Oxychlorite (50%WP)	12.052 ± 2.658	5.461 ± 1.6033	2.153 ± 0.837
9.	Asafoetida	1.666 ± 0.826	0.8 ± 0.4700	0.2 ± 0.2
10.	Kalongi	3.066 ± 1.220	1.6 ± 0.728	0.4 ± 0.2894
11.	Neem	6.6 ± 1.856	3.6 ± 1.3444	2.0 ± 0.9904
12.	Mustard	17.26 ± 1.528	7.8 ± 2.240	3.466±1.312

Table 5b. Mean and standard error of fungicides and herbicides (Deep freezing method)

Sr.No	Treatments	0.5 Mean ± Std. Error	0.15 Mean ± Std. Error	0.25 Mean ± Std. Error
1.	Metalaxyl + Mancozeb (72%)	6.692 ± 1.456	0.8461 ± 0.2611	0.128 ± 0.075
2.	Mancozeb (80%W\W)	1.256 ± 0.418	0.307 ± 0.187	0
3.	Aliette (80%WP)	0.641 ± 0.329	0.205 ± 0.1608	0.153 ± 0.153
4.	Derosol (60%WP)	4.871 ± 1.362	2.128 ± 0.807	1.692 ± 0.757
5.	Ridomyl Gold (68%WP)	0.487 ± 0.328	0.025 ± 0.025	0
6.	Thiophonate methyl (70%WP)	2.179 ± 0.191	0.461 ± 0.390	0
7.	Antracol (70%WP)	4.487 ± 2.128	2.846 ± 1.344	0.923 ± 0.550
8.	Copper Oxychlorite (50%WP)	6.02 ± 2.150	3.461 ± 1.249	1.897 ± 0.800
9.	Asafoetida	1.666 ± 0.826	0.2666 ± 0.1817	0.066 ± 0.066
10.	Kalongi	3.066 ± 1.220	0.8 ± 0.438	0.2 ± 0.2
11.	Neem	6.6 ± 1.856	2.066 ± 0.987	1.333 ± 0.728
12.	Mustard	17.266 ± 1.528	4.466 ± 1.6814	1.466 ± 0.735

In deep freezing method fungicides @ 0.25% concentration showed low infection percent compare to blotter method. Metalaxyl + Mancozeb & Aliette also suppressed the fungal growth @ 0.15% concentration (Fig. 1c). *Asafoetida* and *Kalongi* posses strong antifungal activity at all doses levels followed by neem and mustard (Fig. 1d).

Statistical analysis of fungicides and herbicides revealed that Ridomyl Gold (68% WP) was found to be most effective at all dose levels followed by Mancozeb (80% W/W), Aliette (80% WP) and asafoetida @ 0.25% inhibited mycelial growth of fungi as compare to other treatments in blotter paper method (Table 5a) whereas in deep freezing method Mean & Std. error showed that Ridomyl Gold, Mancozeb and Thiophonate methyl controlled all fungal flora asafoetida possess strong fungicidal effect @ 0.25% (Table 5b). Analysis of variance to compare fungicides and herbicides at 0.01 level of significance; showed significant differences at all level i.e., 0.50% ($p<0.01$), 0.1% ($p<0.01$) & 0.25% ($p<0.01$). The results showed that fungicide Ridomyl Gold (68% WP) and herbicide asafoetida @ 0.25% were more effective and showed strong fungicidal activity towards isolated fungi.

References

Agbenin, O.N. and P.S. Marley. 2006. *In vitro* assay of some plant extracts against *Fusarium oxysporum* f.sp. *Lycopersici* causal agent of tomato wilt. *Journal of Plant Protection Research*, 46(3): 215-220.

Agbenin, O.N., A.M. Emeeheebe and P.S. Marley. 2004. Evaluation of Neem seed powder for *Fusarium* wilt and *Meloidogne* control on tomato. *Archives of Phytopathology and Plant Protection*, 37(4): 319-326.

Ahmed, S.M. and A. Iqbal. 1989. Root and Collar-rot of chillies caused by *Phytophthora capsici* (Van Breeda As Haan) waterhouse. A new record for Pakistan. *J. Agr. Res*., 27: 155-156.

Amusa, N.A., I.A. Kehinde and A.A. Adegbite. 2004. Pepper (Capsicum frutescens) fruit anthracnose in humid forest region of South-western Nigeria. *Nutrition & Food Science*, 34(3): 130-134.

Anonymous. 2006. *Agriculture Statistics of Pakistan*, 2005-2006. Ministry of Food and Agricultural and Livestock, Govt of Pakistan, Islamabad.

Barnet, H.L. and B.B. Hunter. 1972. *Illustrated genera of imperfect fungi*. The American Phytopathological Society, St. Paul, Minnesota, 218 pp.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Myco. Inst. Kew, Surrey, England, 237 pp.

Bowers, J.H. and J.C. Locke. 2000. Effect of botanical extracts on population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the green house. *Plant Dis.*, 88: 300-305.

Ellis, M.S. 1971. *Dematiacious Hhypomycetes* (C. M. I., Kew. Surrey, England). 608pp.

Hafeez, A. 1986. *Plant Diseases.* Khurseed Printers (Pvt) Ltd. Islamabad PARC, pp. 552.

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

Hashmi, M.H. 1989. Seed borne mycoflora of *Capsicum annum L. Pak. J. Bot*., 21(2): 302-308.

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.

Hussain, A., M.N. Ahmed and A.S. Akhter. 1990. Protect chillies crop from *Phytophthora*, a new disease in Pakistan. *Capsicum Newsletter*, 8-9: 59.

Kazmi, A.R., I. Niaz and G. Jilani. 1993. Evaluation of some plant extract for antifungal properties. *Pak. J. Phytopathol.*, 5(1-2): 93-97.

Kazmi, S.A.R., S. Shahzad and I. Niaz. 1995. Effect of neem oil on *In vitro* growth of root infecting fungi. *Pak. J. Bot.*, 27(1): 217-220.

Khaleeque, M.I. and S.M. Khan. 1991. Fungi associated with fruit rot and die back diseases of Chillies in Faisalabad. *Pak. J. Phytopathol*., 3: 50-52.

Khan, M.A., M.K. Ashfaq, H.S. Zuberi, M.S. Mahmood and A.H. Gilani. 2003. The *In vivo* antifungal activity of the aqueous extract from *Nigella sativa* seed. *Phytother Res. Fe*., 17(2): 183-6.

Kiran, R.D., K.J.P. Narayana and M. Vijayalakshmi. 2005. Aflatoxin B1 production in chillies (*Capsicum annum* L.) kept in cold storage. *African Journal of Biotechnology*, 4(8): 791-795.

Lanciotti, R., G. Anotti, A. Patrigani, N. Belleti, M.E. Guerzoni and F. Gardini. 2004. Use of natural aroma compound to improve shelf life of minimally processed fruits. *Trends in Food science and Technology*, 15: 201-208.

Limonard, T. 1966. A modified blotter test for seed health. *Neth. Pl. Path.*, 72: 319-321.

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

Mirza, J.I., S.H.I. Ahmed, N. Ayub and R.H.C. Strang. 2000. *In vitro* antifungal activity of Neem products against *Phytophthora infestens. Pakistan Journal of Biological Science*, 3(5): 824-828.

Mushtaq, M. and M.H. Hashmi. 1997. Fungi associated with disease of capsicum in Sindh, Pakistan. *Pak. J. Bot*., 29(2): 217-222.

Nelson, P.E., T.A. Toussoun and W.F.O. Marassas. 1983. *Fusarium* species. An *Illustrated Manual for Identification*. The Pennsylvania State University Press, 193 pp.

Niaz, I., U. Sitara and S. Qadri. 2008. Effect of different seed oils and benlate fungicide on *In Vitro* growth of four *Drechslera* species. *Pak. J. Bot*., 40(1): 397-401.

Niaz, I., U. Sitara, S.A.R. Kazmi and S. Qadri. 2008. Comparison of antifungal properties of neem seed oil collected from different parts of Pakistan. *Pak. J. Bot*., 40(1): 403-408.

Sagdic, O., A.G. Karahan, M. Ozcan and G. Ozcan. 2003. Effect of some spices extracts on bacterial inhibition. *Food science and Technology International*, 9: 353-359.

Saleem, A., M. Ansar, K. Hamid and F.F. Jamil. 1998. Effect of Physical parameters on the incidence of root and collar rot disease in Chillies. *Pak. J. Bot*., 30(1): 39-43.

Saleem. A., M.H. Bokhari, K. Hamad and M. Ansar. 1996. Mycoflora associated with root and collar rot disease of chillies in different districts of the Punjab (Pakistan). *Pak. J. Bot*., 9: 80-84.

Siddiqui, R.R., H. Ahmed, S.S. Chaudhary, A.F.M. Ehteshamddin and S. Shireen. 1996. Antimicrobial activity of essential oils Part 11. *Pak. J. Sci. Ind. Res*., 34: 1-4.

Sitara, U., I. Niaz, J. Naseem and N. Sultana. 2008. Antifungal effect of essential oils on *In Vitro* growth of pathogenic fungi. *Pak. J. Bot*., 40(1): 409-414.

Sridhar, S.R., R.V. Rajagopal, R. Rajavel, S. Masilamani and S. Narasiman. 2003. Antifungal activity of some essential oils. *J. Agric. Food Chem.*, 51: 7596-7599.

Sultana, N., A.K. Khanzada and M. Aslam. 1992. A new caused of fruit rot of chilies in Pakistan. *Pak. J. Sci. Ind. Res*., 35: 491-492.

Sultana, N., S.A.J. Khan and A.K. Khanzada. 1988. Studies on seed borne mycoflora of chillies and control of fruit rot disease. *Pak. J. Sci. Ind. Res*., 31(5): 365-368.

Sumbali, G. and R.S. Mehtotra. 1981. Evaluation of some fixed oils for the control of certain temperature fruit rot fungi. *India Phytopathol*., 33(3): 517.

Than, P.P., H. Prihastuti, S. Phoulivong, P.W.J. Taylor and K.D. Hyde. 2008. Chilli anthracnose disease caused by *Collectotrichum* species. *J. Zhejiang. Uni. Sci*., 9(10): 764-778.

Thyagaraja, N. and A. Hosono. 1996. Effect of spice extract on fungal inhibition. *Lebensmittel – Wissenchaftund – Technologie*, 29: 286-288.

Tripathi, N.N., A. Asthana and S.N. Dixit. 2008. Toxicity of some terpenoids against fungi infesting fruits and seeds of *Capsicum annum* L., during storage. *Journal of Phytopathology*, 110(4): 328-335.

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