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ANTIFUNGAL EFFECT OF ESSENTIAL OILS ON *IN VITRO* GROWTH OF PATHOGENIC FUNGI

UZMA SITARA, ISHRAT NIAZ, JAWED NASEEM AND NASREEN SULTANA

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

Abstract

Essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) were evaluated for their antifungal activity @ 0.5, 0.1 and 0.15% against eight seed borne fungi viz., *Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, F. nivale, F. semitectum, Drechslera hawiinesis* and *Alternaria alternata*. Ridomyl gold (MZ 68%WP) was used for comparison. All the oils extracted except mustard, showed fungicidal activity of varying degree against test species. Of these oils, Asafoetida oil @ 0.1% and 0.15% significantly inhibited the growth of all test fungi except *A. flavus* and *Nigella sativa* oil @ 0.15 was also effective but showed little fungicidal activity against *A. niger* followed by neem, Ridomyl gold and mustard oils.

Introduction

In world crop production, preharvest losses due to fungal disease may amount to 12% in developing countries (Lee *et al.*, 2001). Chemicals used in the control of diseases pollute the atmosphere and affect the properties of medicinal plants. To avoid the hazardous effects of chemicals, natural products of some plants have been used to control plant disease (Rahber-Bhatti, 1986; Bowers & Locke, 2000; Momin *et al.*, 2001). Fungal species of the genera *Aspergillus, Fusarium, Alternaria* and *Drechslera species* have been considered to be major plant pathogens world wide (Ghafoor & Khan, 1976; Mirza & Qureshi, 1978). *Aspergillus* and *Fusarium species* produce mycotoxins in food besides causing seedling blight, seed rot, kernel rot, stalk rot, wilt and stunt (Blat, 1969; Thiel *et al.*, 1991; Fandohan *et al*., 2003).

The essential oils and their constituents have been found effective as antifungal agent (Daferera *et al.*, 2000; Sridhar *et al.*, 2003). The oil extract of *Nigella sativa* showed *In vitro* and *In vivo* antimicrobial effect against *Staphylococcus aureus, Pseudomonas aeroginosa* and *Candida albicans* (Hanafy & Hatem, 1991; Mashhadian & Rakhandeh, 2005). The inhibitory effects of aqueous extract of seed of *Nigella sativa* against *C. albicans* have also been shown *In vivo* (Khan *et al*., 2003). Several reports have been made on the fungicidal properties of neem oil (Singh *et al.*, 1980; Kazmi *et al.*, 1995). Locke (1995) reported that in field *Alternaria alternata, Aspergillus niger and Fusarium oxysporum* has been completely controlled by using 2-10% neem oil. It is observed that mustard seed oil also showed antifungal activity (Nielsen & Rios, 2000; Dhingra *et al.*, 2004). Houghton *et al*., (2006) reported antifungal activity of asafoetida against *Microsporeum gypseum* and *Trichophyton interdigitale*.Thyagaraia & Hosono (1996) also studied the inhibition effect of asafoetida on *Rhizopus sporus, Mucor dimorphosphorous, Penicillium commune* and *Fusarium solani.* The present investigations were undertaken to find out effectiveness against *Aspergillus niger, A. flavus, Fusarium moniliforme, F. nivale, F. oxysporum, F. semitectum, Alternaria alternata* and *Drechslera hawiinesis*.

Materials and Methods

Seeds of mustard (*Brassica campestris*), black cumin (*Nigella sativa*), neem (*Azadirachta indica*) and Asafoetida (*Ferula asafoetida*) were ground into a fine powder in an electric grinder and oils were extracted with n-hexane on Soxhlet's extraction apparatus at 30°C. The agar diffusion plate method (Nene & Thaplliyal, 1979) was used to test seeds oils for the antifungal properties against eight fungal species viz, *Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, F. nivale, F. semitectum, Alternaria alternata and Drechslera hawiinesis.* Required amount of oils extracts were dissolved in pure acetone and thoroughly mixed with melted potato dextrose agar to provide 0.5, 0.1 and 0.15% concentration. About 10 ml treated or untreated medium were poured into Petri plate (70 mm diameter). Fungicide Ridomyl gold (MZ 68% WP) was used for comparison and untreated medium was used as control. Seven days old fungal cultures, maintained in the laboratory, were placed in the center of each Petri plate. There were three replicates of each treatment. The inoculated Petri plates were incubated at 28±2°C and radial growth in cm was recorded after 7 days of incubation and data were analyzed statistically to observe the difference among various treatments.

Results and Discussion

Antifungal activity of asafoetida (*Ferula asafoetida*), black cumin seed (*Nigella sativa*), neem (*Azadirachta indica*) and mustard (*Brassica campestris*) oils were determined against eight fungi viz., *Aspergillus niger, A.flavus, Fusarium moniliforme, F. oxysporum, F. nivale, F. semitectum, Alternaria alternata, Drechslera hawiensis.* The results were compared with a commercial fungicide (Ridomyl Gold MZ 68%WP). These essential oils were tested by agar diffusion plate method caused significant reduction in the growth of above mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium. Results showed that asafoetida and *Nigella sativa* oils possess a remarkable antifungal activity against all tested fungi. Asafoetida oil completely inhibited the growth of all tested fungi except *A. flavus* at 0.15% whereas *A.flavus* and *A.niger* at 0.1% showed moderate antifungal activity (Table 1). Antifungal activity of asafoetida oil against *Aspergillus niger* and *A. flavus* have been reported by Siddiqui *et al.,* (1996). *Nigella sativa* oil at 0.15% was most significantly effective; however, it exhibited no fungicidal activity against *A. niger*. *D. hawiensis, A. alternata* and *F. moniliforme* were completely inhibited at 0.1% and 0.15% concentration (Fig. 1d).

Mustard oil was not as effective as others, whereas only at 0.15% concentration; the oil had antifungal property towards *F.oxysporum* and *F. nivale* (Fig. 1d). Fungicide Ridomyl Gold showed most significant result at 0.15% compared to mustard oil and possesses strong fungicidal effect against all fungi, however, fungal growth was either somewhat suppressed or promoted at 0.1% & 0.5% (Fig. 1c). Sitara & Shaida (2007) controlled same fungi by using Ridomyl gold at 0.2% and 0.3%. Neem oil showed greater suppression in the growth of *D.hawiinesis* and *A.alternata* at 0.15% & 0.1% concentration whereas 0.5% did not show antifungal property (Fig. 1d). According to Niaz & Kazmi (2005) neem oil was quite effective for *Aspergillus* spp. Vir & Sharma (1985) found antifungal activity in neem oil against *Alternaria alternata* and *Aspergillus* spp. Sinniah *et al.,* (1973) also studied the toxicity of neem oil on *Aspergillus* spp.

Table 1. Mean diameter of colonies (cm) of fungi on Potato Dextrose Agar (PDA) amendment with different concentratrion of essential oils and fungicide.

Treatments	Conc. (%)	Name of fungi							
		Aspergillus niger	*A. flavus*	*F. moniliforme*	*F. oxysporum*	*F. nivale*	*F. semitectum*	*D. hawiiensis*	*Alternaria alternata*
Neem oil (Mean ± S.E)	0.5	3.2±0.577	3.7±0.1732	0.33±0.5774	1.3±0.4354	1±0.000	1.33±0.1528	0.4±0.5292	0.86±0.5508
	0.1	2.9±0.1155	3±0.2000	0	0.66±1.1547	0.5292	0.93±0.9018	0	0.26±0.4619
	0.15	2.4±0.5292	2.03±0.4509	0	0.6±1.0392	0.26±0.4169	0.66±1.1547	0	0
Mustard oil (Mean ± S.E)	0.5	3.03±0.057	3±0.000	3.06±0.1155	3.1±0.1000	2.83±0.0577	3.06±0.1155	2.36±0.4041	2.1±0.6557
	0.1	2.6±0.5292	2.63±0.3512	2.53±0.0577	1.86±0.3215	2.03±0.0577	2.8±0.1000	1.9±0.1000	1.86±0.0577
	0.15	2.06±0.6928	2.26±0.4619	1.93±0.1155	1±0.5774	1±0.000	2±0.000	1.2±0.000	1.4±0.1732
Nigella Sativa oil (Mean ± S.E)	0.5	1.33±1.52757	0.33±0.0577	0.1±0.1000	0.23±0.2517	1±0.000	0.16±0.2887	0	0
	0.1	0.33±0.5774	0.16±0.577	0	0.06±0.1155	0.260±0.4619	0	0	0
	0.15	0.16±0.2887	0	0	0	0	0	0	0
Asafoetida oil (Mean ± S.E)	0.5	1±0.000	0.6±0.3606	0	0	0.56±0.4041	0	0	0.5±0.5000
	0.1	0.5±0.000	0.46±0.1528	0	0	0	0	0	0
	0.15	0	0.16±0.2887	0	0	0	0	0	0
Ridomyl Gold (Mean ± S.E)	0.5	2.5±0.5000	2.6±0.1732	3.6±0.1000	2±0.000	2±0.1155	2.7±0.1000	0.66±0.3512	1±0.2000
	0.1	1.93±0.9074	2.2±0.2646	1.53±1.3429	1±0.000	0.56±0.6028	2.33±0.2887	1±0.000	0.5±.5000
	0.15	0.33±0.5774	1.16±1.0408	0.83±1.4434	0.33±0.5774	0.33±0.5774	0.66±0.5774	0	0.33±0.5774
Control		4.3±0.1732	4.26±0.2517	4.06±0.1155	2.76±0.2517	3.26±0.2517	3.73±0.3786	2.9±0.3606	3.43±0.4041

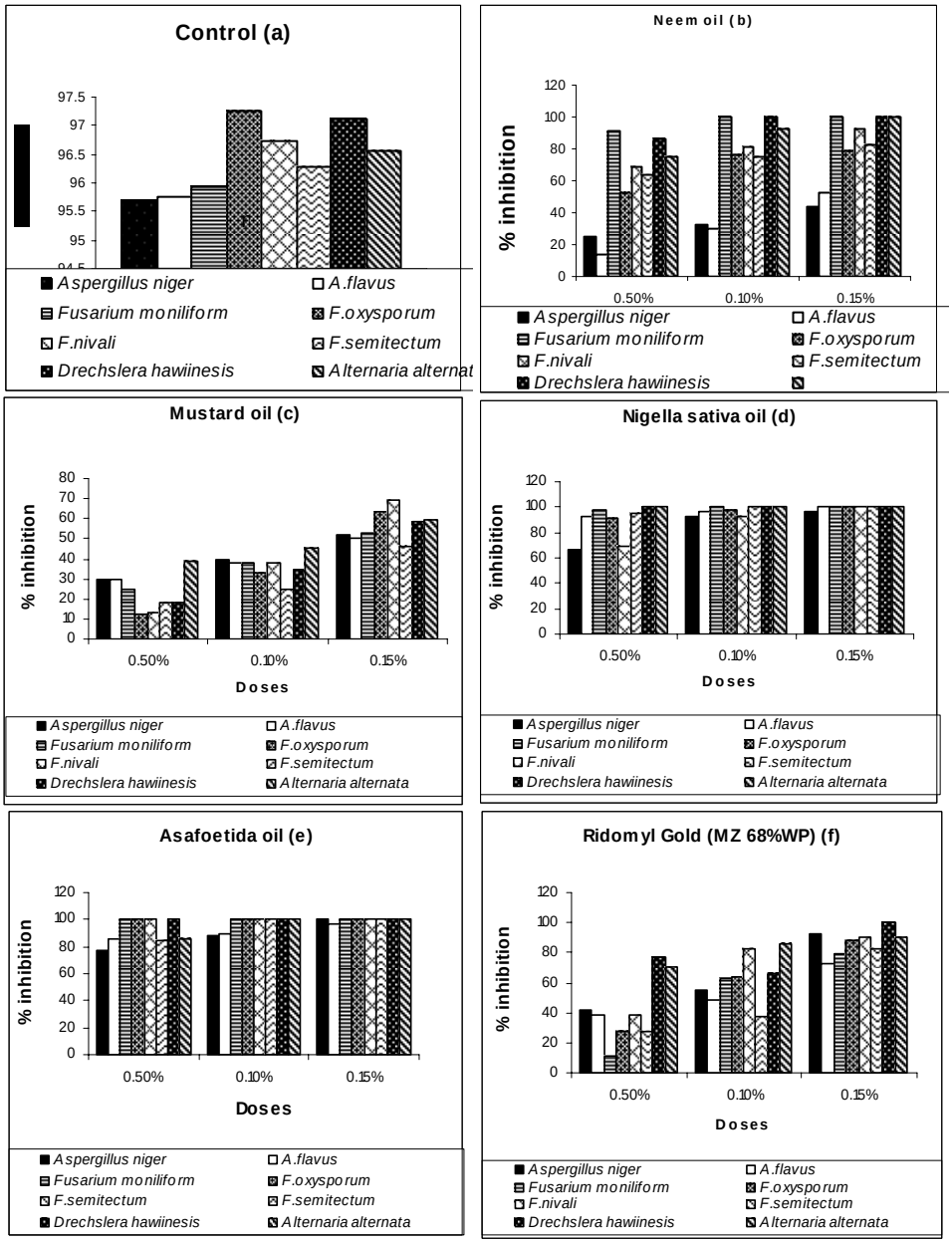

Fig. 1. Effect of different essential oils and fungicides on radial growth of fungi.

By using ANOVA, it was found that efficacies of all oils are highly significant at 5% for all doses ($F= 70.418$, $p<0.001$) similarly dose level ($F= 83.9$, $p< 0.001$), as well as efficacy of fungi ($F= 103.45$, $p< 0.001$) are also highly significantly different. According to this study asafoetida was found to be most effective at 0.15% and 0.1% for controlling the colony growth of fungi. *Nigella sativa* also showed strong fungicidal activity at

0.15%. Neem oil revealed moderate effect. All oils were more effective and well compared to fungicide except mustard oil. Our data on the antifungal properties of oils suggest that these oils should be examined further to evaluate its potential as a natural fungicide.

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