Pak. J. Bot., 40(1): 397-401, 2008.

EFFECT OF DIFFERENT SEED OILS AND BENLATE FUNGICIDE ON *IN VITRO* GROWTH OF FOUR *DRECHSLERA* SPECIES

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Abstract

Effect of neem (*Azadirachta indica*) seed oil, Dill (*Anethum graveolens*) seed oil and benlate fungicide @ 0.1, 0.01, 0.001 & 1.0% concentration were tested against four species of *Drechslera* viz., *Drechslera rostrata, D. hawaiiensis, D. papendorfii* and *D. specifera*. Dill seed oil 1% was most effective against *D. rostrata* and *D. papendorfii* whereas 1% Neem seed oil inhibited the growth of *D. specifera* and *D. hawaiiensis*. All treatments significantly inhibited the growth of all tested fungi; however dill seed oil showed greater suppression at all dose level followed by neem seed oil and benlate fungicide.

Introduction

Essential oils are known to contain a natural cocktail of monoterpenes, diterpenes and hydrocarbons with a variety of functional groups showing antifungal (Daferera *et al*., 2000) and antimicrobial (Cowan, 1999; Hammer *et al*., 1990) activities. Essential oils with botanical toxicant have shown promising results in control of pathogenic fungi (Ghaffar, 1995; Easton *et al*., 1978). *Drechslera* species mostly isolated from the seed of wheat, rice, barley, maize produce leaf spot, foot rot, seedling blight, leaf blotch, southern leaf spot, stem spot, leaf streak and blight (Neergaard, 1977). *D. hawaiiensis* which produce leaf spot are rather frequent on seed of a wide range of Graminae (Chidambaram *et al*., 1977). Many research workers have carried out studies to find safe and economical control of plant diseases by using plant extracts. Dill seed oil showed antifungal activity against *Aspergillus niger, A. flavus, Alternaria alternata, Helminthosporium* sp., and *Fusarium solani* (Rizki *et al*., 1997). Dill seed oil revealed high effectiveness against the mold *Aspergillus niger, Saccharomyces cerevisiae* and *Candida albicans* (Jirovelz *et al*., 2003). Hall & Fernandez (2004) also studied the inhibitory effect of Dill seed oil at 10µl doses on *Penicillium digitatum.* Locke (1995) reported that in field *Alternaria alternata, Aspergillus niger* and *Fusarium oxysporum* has been completely controlled by using 2-10% neem oil. Dill seed oil was found to be highly effective for controlling the growth of *Fusarium graminerum, Penicillium citrinum* and *Aspergillus niger* at 6µl doses (Singh *et al*., 2005). Neem oil at 0.025% concentration has been found to inhibit the growth of *Aspergillus* species (Niaz & Kazmi, 2005). Dry neem seed extract gave 100% inhibition of mycelial growth of *Fusarium oxysporum* (Agbenin & Marley, 2006). Experiment has been carried out to define the effect of neem seed oil and dill seed oil for the control of *Drechslera* species *In vitro* as compare to benlate fungicide.

Materials and Methods

Seeds of neem (*Azadirachta indica*) and dill (*Anethum graveolens*) were pulverized to fine powder on electric grinder and preserved in glass jars separately. These powders were extracted with n-hexane (B.P. 60-80°C) by Soxhlet's extraction apparatus for eight

hours. The oils were obtained by removing n-hexane from the extract on rotary evaporator under reduced pressure. Each oil was tested for its antifungal properties against *Drechslera hawaiiensis, D. rostrata, D. specifera* and *D. papendorfii* by amending agar diffusion plate method (Nene & Thapliyal, 1979). All these four species were isolated from infested maize grain and their cultures were maintained on Potato Dextrose Agar. The required amount of oil was dissolved in acetone and thoroughly mixed with melted potato dextrose agar @ 0.001, 0.01, 0.1 & 1.0% concentration. Benlate fungicide was used at the same concentration for comparison and untreated medium was used as control. The media was poured into 70mm diam., Petri plates @ 10ml per plate and 5mm diam., disc of inoculum of fungal culture were placed in the centre of each Petri plate and incubated at room temperature (30±1°C). Radial growth of fungi was recorded after seven days. Each treatment was replicated three times and statistically analyzed at 5% level of significance.

Results and Discussion

Neem seed oil and dill seed oil caused significant reduction in the growth of *Drechslera rostrata, D. papendorfii, D. hawaiiensis* and *D. specifera*. The rate of growth inhibition was directly proportional to the concentration of tested oils in the medium. *D. specifera and D. papendorfii* were more susceptible to the neem seed oil at 0.1% and 1.0% concentration whereas *D. rostrata and D. hawaiiensis* showed greater suppression at 1.0% concentration (Table 1). All fungi revealed moderate effect at 0.001% dose. Among four species of *Drechslera*, *D. papendorfii* showed more sensitivity towards all concentration of neem seed oil (Fig. 1b). Govindachari *et al.,* (1998) noted fungicidal effect of neem seed oil on *D. oryzae*, *Fusarium oxysporum* and *Alternaria alternata;* and also found that *D. oryzae* was more susceptible for neem oil. Vir & Sharma (1985) also reported that 10% neem oil concentration gave 100% inhibition of *D. rostrata, Aspergillus niger* and *Macrophomina phaseolina*.

Remarkably, dill seed oil exhibited inhibition of mycelial growth of the tested fungi at all dose level whereas 0.1 and 1.0% concentration showed strong fungicidal effect. At 1.0% concentration, the inhibition in mycelial growth was 8.82% against *D. rostrata* and only 14.7% against *D. papendorfii* (Fig. 1c). Sridhar *et al*., (2003) also examined the inhibitory effect of dill seed oil on the growth of 20 fungal species including *D. oryzae*, which was found to be more potent. Fungicide benlate did not show significant reduction when compared with neem seed oil and dill seed oil. Dwivedi & Dubey (1993) analyzed the antifungal effect of some selected plant essential oils and fungicides including benlate and found that only dill seed oil showed highly inhibitory effect on *Aspergillus flavus*. It is also interesting to note that fungal growth was somewhat promoted after using benlate fungicide @ 0.01 and 0.001% concentration and only *D. papendorfii* was found to be more susceptible @ 0.01, 0.1 & 1.0% concentration (Fig. 1d). According to Kazmi *et al*., (1995) 0.1% neem seed oil was more effective against *Macrophomina phaseolina* than benlate. Kazmi *et al*., (1993) studied that dill seed oil was the most effective, causing total inhibition of the growth of *Aspergillus* spp., and *Alternaria alternata* compared to benlate at 0.025, 0.05 and 0.01% concentration.

Difference in efficacies of neem seed oil and dill seed oil was highly significant at 5% for all doses. Similarly efficacy on fungi was also highly significantly different at all doses (Table 1). The results showed that all oils were more effective and showed strong fungicidal activity against all tested fungi and well compared to fungicide benlate, moreover, dill seed oil could be considered as a source for natural antifungal material.

Table 1. Mean diameter of fungal colonies (cm) on agar medium with different concentration of oils and fungicide.

Treatments	Conc. (%)	Name of fungi			
		Drechslera rostrata	***D. papendorfii***	***D. specifera***	***D. hawiinesis***
Neem seed oil (Mean ± S.E)	1.0	1.3 ± 0.17321	0.1 ± 0.0000	1.1 ± 0.10000	1.2 ± 0.10000
	0.1	1.6 ± 0.10000	0.7 ± 0.21633	1.7 ± 0.20000	1.6 ± 0.20000
	0.01	1.9 ± 0.15275	1.4 ± 0.10000	2.4 ± 0.20000	2.0 ± 0.20000
	0.001	2.3 ± 0.17321 1	9 ± 0.26458	2.0 ± 0.20000	2.2 ± 0.26458
Dill seed oil (Mean ± S.E)	1.0	0.3 ± 0.05000	0.5 ± 0.10000	1.3 ± 0.36056	1.4 ± 0.10000
	0.1	0.7 ± 0.10000	0.8 ± 0.20000	1.5 ± 0.10000	1.6 ± 0.30000
	0.01	1.3 ± 0.10000	1.0 ± 0.20000	1.9 ± 0.10000	2.1 ± 0.26458
	0.001	1.7 ± 0.10000	1.6 ± 0.20000	2.3 ± 0.36056	2.2 ± 0.10000
Benlate (Mean ± S.E)	1.0	1.4 ± 0.17321	1.2 ± 0.17321	1.6 ± 0.17321	1.7 ± 0.10000
	0.1	1.8 ± 0.26458	1.5 ± 0.26458	1.9 ± 0.20000	2.0 ± 0.10000
	0.01	2.3 ± 1.47309	1.7 ± 0.17321	2.3 ± 0.10000	2.3 ± 0.10000
	0.001	3.3 ± 0.15275	2.5 ± 0.17321	2.5 ± 0.45826	2.5 ± 0.26458
Control (Mean ± S.E)		3 ± 0.0000	2.8 ± 0.10000	2.9 ± 0.15275	3.2 ± 0.20000

Table 2. Analysis of variance of oils, doses & fungi at different concentration.

	Source of variation	Sum of square	df	Mean of square	F	Sig./P
Oil	Between group	38.013	3	12.671	34.795	< 0.000
	Within group	55.354	152	0.364		
	Total	93.367	155			
Doses	Between group	27.656	3	9.219	35.013	< 0.000
	Within group	36.861	140	0.263		
	Total	64.517	143			
Fungi	Between group	8.405	3	2.802	5.012	< 0.002
	Within group	84.961	152	0.559		
	Total	93.367	155			

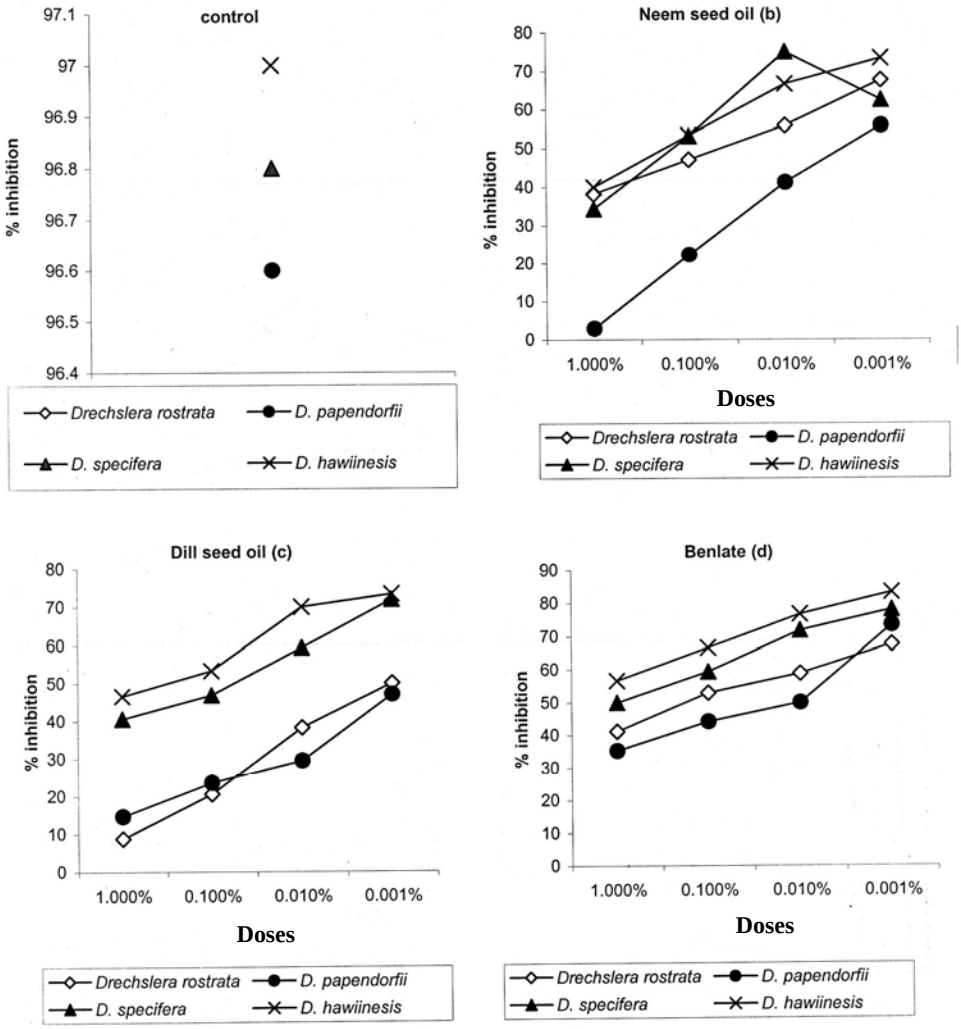

Fig 1. Effect of Neem seed oil, Dill seed oil and Benlate on radial growth of fungi.

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(Received for publication 5 October 2007)