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COMPARISON OF ANTIFUNGAL PROPERTIES OF NEEM SEED OIL COLLECTED FROM DIFFERENT PARTS OF PAKISTAN

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Abstract

Oil extracted from neem (*Azadirachta indica*) seeds collected from five different localities of Pakistan was evaluated for their efficacy against three fungi viz., *Fusarium moniliforme, Macrophomina phaseolina* and *Rhizoctonia solani*. The neem oil sample from Karachi was the best among all the samples in checking the growth of *Macrophomina phaseolina* and *Rhizoctonia solani* @ 0.1% concentration compared to those from other localities.

Introduction

Plant extracts may be an alternative to currently used fungicides for controlling pathogenic fungi because they constitute a rich source of bioactive chemicals (Wink, 1993). *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium moniliforme* were found infecting a variety of host plants in Pakistan (Ghaffar, 1988). *Macrophomina phaseolina* is reported to produce charcoal rot of over 500 plant species; of which at least 72 different hosts has been recorded in Pakistan (Mirza & Qureshi, 1978; Sinclair, 1982; Shahzad *et al*., 1988). Similarly 64 hosts associated with *R. solani* (Shahzad & Ghaffar, 1990) and *F. moniliforme* with 20 hosts has been recorded in Pakistan. (Ghaffar, 1992; Mirza & Qureshi, 1978).

Use of neem for control of plant pathogenic fungi is known and has been amply documented. Khan *et al*., (1973) used neem cake against *R. solani*, *F. oxysporum*, *Alternaria tenuis, Helminthosporium nodulosum* and *Curvularia tuberculata*. Locke (1995) reported that in field *Alternaria alternata*, *Aspergillus niger* and *F. oxysporum* has been completely controlled by using 2-10% neem oil. According to *Kazmi et al*., (1993) 0.1% concentration of neem oil causes significant reduction on the growth of *Alternaria alternata* and *Aspergillus* species. Govindachari *et al*., (1998) also studied the antifungal activity of neem oil towards *Drechslera oryzae, F. oxysporum* and *Alternaria tenuis.* It was also found that dry neem seed extract completely inhibited the mycelial growth of *F. oxysporum* at all concentrations (Agbenin & Marley, 2006). According to Mirza *et al*., (2000) neem products were found highly effective at different life stages of *Phytophthora infestans*. Charmaine *at al*., (2005) reported anticandidal activity of neem extracts against *Candida* species. Present study reports the efficacy of neem seed oils, collected from different parts of Pakistan against three pathogenic fungal species viz., *Fusarium moniliforme, Macrophomina phaseolina* and *Rhizoctonia solani*.

Materials and Methods

Pure cultures of three common pathogenic fungi viz., *Fusarium moniliforme*, *Macrophomina phaseolina* and *Rhizoctonia solani* were maintained on Sabouraud Dextrose Agar medium at room temperature. Neem seed samples were collected from Karachi,

Hyderabad, Dokri, Shikarpur and Faisalabad. The neem seed kernels of each sample were powdered in an electric grinder and then extracted with hexane on a Soxhlet's extraction apparatus. The extract of each sample was then concentrated on a rotary evaporator at 30ºC and made solvent free to obtain neem oil. Each of the oil samples was evaluated for antifungal properties against the above fungal species by agar diffusion plate method. Required amounts of neem oil samples were dissolved in pure acetone separately and thoroughly mixed with melted Sabouraud dextrose agar to provide 0.1, 0.05 and 0.025% concentration. About 10 ml treated or untreated medium was poured into 70mm diam., sterilized Petri plates. Seven days old inoculum of each fungal species were placed in the centre of each Petri plate containing treated or untreated Sabouraud dextrose agar medium and incubated at room temperature for seven days. The average daily radial growth of the fungal colonies was measured with centimeter scale. Benlate, a standard fungicide was used for comparison and untreated medium was used as control. Each treatment was replicated three times. Comparison of difference in fungal growth in each species was made statistically by using Duncan's multiple range test ($p<0.05$).

Table 1. Growth of three fungal species in agar medium treated with neem seed oil collected from different parts of Pakistan at different concentrations.

Districts	Concentration	*F. moniliforme*	*M. phaseolina*	*R. solani*
	0.1	2.47 fgh	1.00 k	2.00 hi
Karachi	0.05	4.53 b	2.23 fg	3.37 cd
	0.025	4.50 b	3.10 bc	4.13 b
	0.1	2.87 efg	1.60 ij	2.50 fgh
Dokri	0.05	4.20 bc	2.13 fgh	2.93 def
	0.025	5.87 a	3.20 bc	3.43 cd
	0.1	2.27 ghi	2.57 def	2.27 ghi
Faisalabad	0.05	3.23 def	2.97 bcd	2.87 def
	0.025	3.87 bcd	3.07 bcd	3.27 cde
	0.1	2.27 ghi	1.87 ghi	2.10 hi
Shikarpur	0.05	2.63 fgh	2.13 fgh	2.90 def
	0.025	3.63 cde	3.30 b	3.20 cde
	0.1	1.83 hii	1.63 hij	2.73 efg
Hyderabad	0.05	3.20 def	1.83 ghi	3.27 cde
	0.025	3.20 bcd	2.77 cde	3.70 bc
	0.1	1.10 j	1.17 jk	1.13 j
Benlate (Fungicide)	0.05	1.57 ij	1.70 hi	1.83 I
	0.025	1.87 hij	2.30 efg	2.47 fgh
Control		4.47 b	4.11 a	4.67 a

Mean within columns followed by the same letter are not significantly different ($p>0.55$) according to Duncan's multiple range test. Average of three replications

Results and Discussion

Neem seed oils were evaluated against three fungal species viz., *Fusarium moniliforme*, *Macrophomina phaseolina* and *Rhizoctonia solani*. Results indicates that fungicide Benlate and neem oils collected from different localities, caused significant reduction in mycelial growth of above mentioned fungi (Table 1). The neem oil from Hyderabad was the most effective in checking the growth of *F. moniliforme* and its effect was quite near to that of Benlate (Fig. 1f). Neem seed oil from Karachi proved to be more effective than Benlate at 0.1% concentration against *M. phaseolina* (Fig. 1b). Similarly neem seed oil from Karachi was the best among all the samples in checking the growth of

R. solani at 0.1% concentration whereas not as effective as Benlate. Neem seed oil @ 0.1% controlled more than 50-80% growth of all the fungal species, however, the maximum growth inhibition was exhibited against *M. phaseolina*. Vir (1985) reported that 2.5 to 5.0% concentration of neem oil was less effective for growth inhibition of fungi viz., *F. moniliforme*, *Aspergillus niger*, *Drechslera rostrata* and *M. phaseolina*; however at 10% concentration, the oil gave 100% growth inhibition of all the tested fungi. Neem oil from Shikarpur @ 0.025% concentration was effective against *R. solani* whereas 0.05% concentration for controlling the growth of *F. moniliforme* compared to similar doses from other cities (Fig. 1e). According to Niaz & Kazmi (2006) neem seed extract at 0.1% concentration was as effective as Benlate against *R. solani* whereas neem cake extract was found most effective against *F. moniliforme*. It was also found that neem oil of Shikarpur amongst all oils successfully inhibited the mycelial growth of all fungi. Furthermore neem seed oil from Faisalabad @ 0.05% concentration showed remarkable antifungal activity against *R. solani* (Fig. 1d). Niaz & Kazmi (2005) also noticed that neem oil was quite effective against *Aspergillus* species. However, neem seed oil from Dokri did not show significant reduction in mycelial growth of fungi except 0.025% concentration of neem oil showed strong fungicidal effect toward *F. moniliforme* (Fig. 1c). The results are in close conformity with the finding of Kazmi *et al.*, (1995) who found that 0.1% neem oil showed significant suppression in the growth of *M. phaseolina*, *R. solani* and *F. moniliforme*.

Table 2. p x d x s Table of means for colony size (cm) (Ave. of 3 replicates).

Places (P)	*M. phaseolina*	*F. moniliforme*	*R. solani*	P-means
1%				
Karachi	1. 00d	2.47 ab	2.00 b	1.82
Hyderabad	1.60 bc	2.87 a	2.50 ab	2.32
Dokri	2.57 a	2.27 bc	2.27 ab	2.37
Shikarpur	1.87 b	2.27 bc	2.10 b	2.08
Faisalabad	1.63 bc	1.83 c	2.73 a	2.07
Benlate	1.17 cd	1.10 d	1.13 c	1.13
5%				
Karachi	2.23 b	4.53 a	3.17 a	3.31
Hyderabad	2.13 b	4.20 a	2.93 a	3.09
Dokri	2.87 a	3.23 b	2.87 a	2.99
Shikarpur	2.13 b	2.63 c	2.90 a	2.56
Faisalabad	1.83 b	3.20 b	3.27 a	2.77
Benlate	1.70 b	1.57 d	1.83 b	1.70
25%				
Karachi	3.10 a	4.50 b	4.13 a	3.91
Hyderabad	3.20 a	5.87 a	3.43 b	4.17
Dokri	3.07 a	3.87 c	3.60 ab	3.51
Shikarpur	3.30 a	3.63 c	3.20 b	3.38
Faisalabad	2.77 ab	3.87 c	3.70 ab	3.44
Benlate	2.30 b	1.87 d	3.47 c	2.21
0%				
Karachi	4.00	4.60 a	4.50 ab	4.37
Hyderabad	4.00	4.63 a	4.77 a	4.47
Dokri	4.17	4.00 b	4.90 a	4.36
Shikarpur	4.33	4.47 ab	4.47 ab	4.42
Faisalabad	4.03	4.67 a	4.70 a	4.47
Benlate	4.33	4.67 a	4.00 b	4.33
S-Mean	2.72	3.45	3.23	3.13

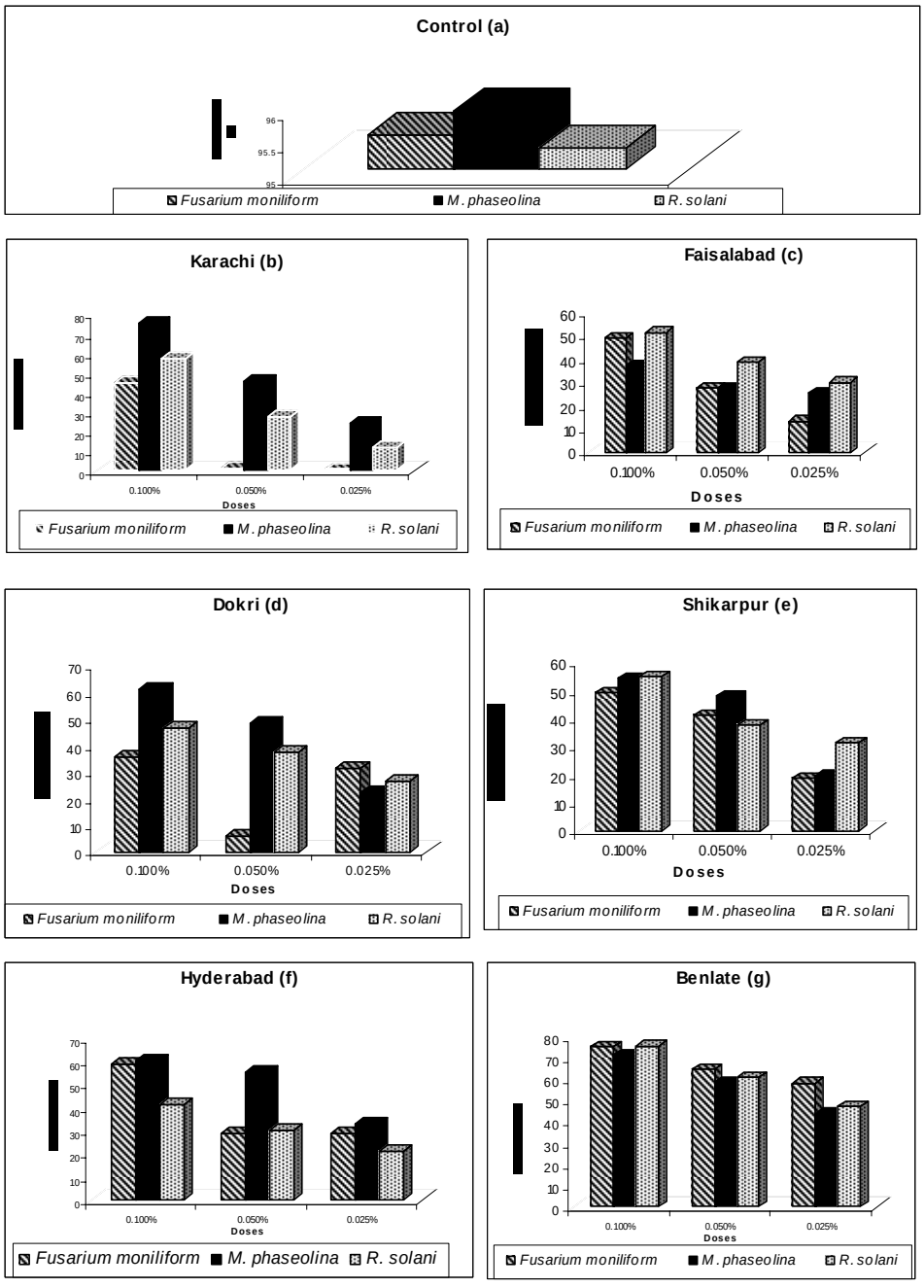

Fig. 1. Effect of neem oil of different localities on fungal species.

The statistical analysis of the data employing analysis of variance shown in Table 2 indicated that all the results were significant at 1% level. The oil samples from different localities and at different rates had significant effect on all the three fungal species. According to this study neem oil from different locations varied in checking the growth of test fungal species. This variation may be due to the difference in the quantity of the active ingredients in the oil samples. However, Benlate fungicide showed greater suppression at all doses level followed by neem oil of Shikarpur, Hyderabad, Faisalabad, Karachi and Dokri.

There is a need for further investigations on purified components of neem oil to have depth knowledge of the active ingredients responsible for controlling fungal species in Pakistan.

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