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ANTIFUNGAL AND NEMATICIDAL ACTIVITY OF SELECTED LEGUMES OF PAKISTAN

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Abstract

The antifungal activity of legume seed extracts was tested against 6 fungi, viz., *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani and Candida glaberata*. The extracts showed moderate activity against different fungal strains. Nematicidal activity has also been carried out to evaluate their potential toxicity against juveniles of the root-knot nematode *Meloidogyne* spp. *In vitro* results showed that ethanolic extract of these legumes caused appreciable mortality of second stage juveniles of *Meloidogyne javanica* and *Meloidogyne incognita.* The concentrations used @ 1% and 0.5% were found more effective and produced significant results as compared to 0.25%, and 0.1%. The mortality rate increased with increasing exposure time for most of the extracts.

Introduction

Root-knot nematodes, *Meloidogyne* spp., are the major nematode pests of economic crops worldwide. The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced (Sasser, 1980). The damage caused by root-knot nematodes, represents one of the major obstacles for the production of an adequate food supply (Carter & Sasser, 1982). Use of conventional nematicides has been one of the most effective methods to reduce their populations. However there are concerns about the presence of residues of these toxic chemicals in the food supply and their consequent accumulation in human subcutaneous fat that could lead to adverse health effects including death. Further high costs and inconsistent results of these synthetic nematicide applications, together with reduced availability of nematicides as a result of increasing concerns for the environment and for public health, have led to ban on many effective nematicides in recent past and augmented interest in for alternative sources of effective and ecofriendly chemicals for nematode control (Viaene & Abawai, 1998; Khurma & Mangotra 2004).

The family *Leguminoase* is of considerable agricultural utility and agronomic potential however its biological activities remain unexplored. In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Zia-ul-Haq *et al*., 2007, 2008a,b; 2009a,b) we have screened the ethanolic extracts of selected legumes for their antifungal activity and to screen these legumes against the root-knot nematode *Meloidogyne* species.

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Materials and Methods

Preparation of crude extract: Seeds of legumes viz., desi and kabuli chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* (L.) Walp.), mash bean (*Phaseolus mungo*), mung bean (*Vigna radiate* (L.) Wilczek), and lentil (*Lens culinaris* Medik.) were procured from Department of Agronomy, Bahauddin Zakariya University, Multan. Samples of all the varieties were divided into groups for storage in stainless-steel containers at 4^{o}C prior to analysis. The seeds were ground to flour and extracted with 96% EtOH at room temperature. The combined ethanolic extract were filtered and evaporated under vacuum to obtain a thick gummy mass. All these extracts were tested for antifungal and nematicidal activity.

Antifungal bioassay: The antifungal activity was determined by the Agar Well Diffusion Method (Atta-ur-Rahman *et al*., 1991). In this method Griseofulvin was used as the standard drug. The crude extract was dissolved in DMSO (50 mg/5ml). Sterile Sabouraud's dextrose agar medium (5ml) was placed in a test tube and inoculated with the sample solution (400 µg/ml) kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29^{o}C and growth inhibition was observed and percentage growth inhibition was calculated with reference to the negative control by applying the formula:

$$\%\text{inhibition of fungal growth} = 100 - \frac{\text{linear growth and test (mm)}}{\text{linear growth in control (mm)}} \text{ X } 100$$

Miconazole and amphotericin B were used as standard drugs, while miconazole, amphotericin B and DMSO were used as positive and negative controls (Rashid *et al*., 2009).

Nematicidal activity

Culture preparation of root knot nematodes: Fresh egg masses of *Meloidogyne javanica* and *Meloidogyne incognita,* collected from stock culture maintained on tomato (*Lycopersicon esculentum*) root tissues were kept in water for egg hatching. The eggs suspension were poured on a cotton-wool filter paper and incubated at 28±2°C to obtain freshly hatched juveniles (J2). Juveniles collected within 48 h were used (Nazli *et al*., 2008).

Mortality test: Crude extracts were dissolved in water (passed through whatman filter paper No.1) to make dilutions of 1%, 0.5%, 0.25 and 0.1%.Experiments were performed under laboratory conditions at 28±2°C.Glass tubes 15 cm long and 8cm were taken for bioassay. Three ml were taken from all dilutions in each tube. The required amount of nematode suspension (100 freshly hatched second stage juveniles/3ml suspension) were poured in to tubes to each of which equal amount of plant extract had already been poured). Distilled water with nematode larvae was taken as control. The dead nematodes were observed under stereoscopic binocular microscope after 24 and 48 hours and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al*., 1989).

Results and Discussion

Medicinal plants play an important role for the management of different microbial infections because overmedication and long-term side effects of synthetic drugs have assumed alarming range. Effective, safe and cheap medicinal agents from plants may appear as potential alternatives for controlling microbial infections particularly the resistant cases (Nisar *et al*., 2010). The results indicated weak activity against tested microorganisms. However mash bean, mung bean and desi chickpea seed extracts indicated better activity as compared to legumes. As legumes are mainly used as food source so less work has been carried out on pharmacological properties of legume seed extracts. Legume seeds extracts has been scantly scanned for such type of activity

Different plant parts are being tested to identify the sources of nematicidal substances. However seeds have received only limited attention so far (Khurma & Mangotra, 2004). So the present study was also designed to carry out nematicidal activity of seeds of indigenous legumes of Pakistan.

Of the various legumes tested for nematicidal activity against larvae of *Meloidogyne javanica* and *Meloidogyne incognita* (Table 1), soybean appeared to be the most active legume, as it caused 88% mortality of the nematode larvae after 48 h exposure to its extract. Kabuli chickpea was found to be least active in its nematicidal activity, as it caused only 22% mortality after the exposure of 48.Black gram and green gram also exhibited moderate activity indicating 50 and 55% activity respectively. Lentil and desi chickpea pea exhibited almost similar activity i.e., 35% and 40% activity. Despite differences among investigated legumes, all legumes indicated time and concentration dependent activity. The activity was higher at high concentrations and increased with time. The results obtained in the present study are in agreement with the previous observations on the strong nematicidal potential of the *Leguminosae* seeds (Morris & Walker, 2002; Jourand *et al*., 2004). It is suggested that more seeds and other parts of plants belonging to this family, especially of wild variety, should be screened to identify the sources of nematicidal substances. The seeds with demonstrated high activity should be subjected to further investigation for possible application in nematode management.

The nematicidal activities of legumes would be a great help to prevent or at least reduce the root diseases in valuable plants, which cause serious losses to crop plants and adversely affect the botanical gardens and agricultural economy of our country. Research in this field would open door of future exploitation of indigenous resources and their commercialization in modern era.

Table 1. Antifungal bioassay.

Test organism	% inhibition						Standard
	Cowpea	Desi chickpea	Kabuli chickpea	Lentil	Mash bean	Mung bean	
Trichophyton longifusis	10	10	-	-	20	10	Miconazole70
Candida albicans	20	-	-	-	20	10	Miconazole110.8
Aspergilus flavus	10	10	-	10	10	10	Amphotericin20
Microsporum canis	20	20	-	10	20	-	Miconazole98.4
Fusarium solani	10	-	-	-	10	10	Miconazole73
Candida glabarata	10	10	-	-	-	-	Miconazole110.8

Table 2.Effect of Ethanolic extracts on mortality % of *Meloidogyne incognita* at different time intervals.

Legumes	% Mortality observed at different concentration								
	24 hour				48 hour				Control
	1	0.5	0.25	0.1	1	0.5	0.25	0.1	
Cowpea	22	17	8	3	34	18	10	4	3
Desi chickpea	30	16	8	5	40	18	10	6	5
Kabuli chickpea	10	6	2	2	20	6	4	2	2
Lentil	25	15	7	3	32	19	10	6	3
Mash bean	35	23	16	6	50	28	18	9	4
Mung bean	29	21	12	5	55	32	14	8	3
Soybean	50	38	32	25	85	49	38	33	2

Table 3. Effect of Ethanolic extracts on mortality % of *Meloidogyne javanica* at different time intervals.

Legumes	% Mortality observed at different concentration								
	24 hour				48 hour				Control
	1	0.5	0.25	0.1	1	0.5	0.25	0.1	
Cowpea	26	13	8	4	33	19	11	6	2
Desi chickpea	35	20	11	5	45	27	15	7	4
Kabuli chickpea	12	8	5	3	22	11	6	3	2
Lentil	30	17	8	4	35	20	12	6	3
Mash bean	34	18	12	4	48	26	17	5	3
Mung bean	32	23	11	6	50	34	16	10	3
Soybean	60	42	28	18	88	80	42	34	2

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