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Study of efficacy of leaf extracts of some plants on germination and sporulation of fungi *Paecilomyces lilacinus*

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ABSTRACT

An experiment was conducted to evaluate the efficacy of leaf extract of five medicinal weed plant on growth and sporulation of nematophagous fungus Paecilomyces lilacinus.The effect of leaf extract was assessed at the different time of interval such as 24hours, 48hours, 72hours, 96hours and 120hours respectively. After 120hours, the maximum mycelial growth was observed in Amaranthus spinosus (8.83cm), minimum in Lantana camara (7.16cm).The maximum number of spores/cm^2 was observed in Veronica anagallis aquatica (5.9x10^6) and minimum in Gnaphalium perpureum (3.7x10^6).

Key words: Efficacy, Leaf extract, Medicinal weed plant and *Paecilomyces lilacinus*.

INTRODUCTION

Paecilomyces lilacinus is a naturally occurring fungus and has the capability to survive in many kind of soils throughout the world. *Paecilomyces lilacinus* was classified with the fungi imperfect or deuteromycetes. *Paecilomyces lilacinus* forms a dense mycelium which give rise to conidiophores. These bear phialides from the end of which spores are formed in long chains. Spores germinate when suitable moisture and nutrients are available.

Paecilomyces lilacinus is one of the effective biocontrol agent against phytonematodes. It protects the root system against disease caused by plant parasitic nematodes specifically root-knot nematode (*Meloidogyne* spp), reniform nematode (*Rotylenchulus reniformis*), banana nematode (*Rhadopholus similis*) and citrus nematode (*Tylenchulus semipenetrans*). These nematode infect the horticultural crop of economic importance. It is an antagonistic fungus colonizes on the root surface strongly parasitic to the egg and egg-masses and female of plant parasitic nematode. Fungal parasitisation can destroy upto 90% of eggs and 75-80% of egg masses or cysts. Before infecting a nematode egg *P. lilacinus* flattens against the egg surface and becomes closely appressed to it. *P. lilacinus* produces simple appressoria anywhere on the nematode egg shell either after a few hyphae grow along the egg surface, or after a network of

hyphae form on the egg. The presence of appressoria appears to indicate that the egg is to be infected. Appressorium is a simple swelling at the end of hypha closely appressed to the egg shell.

The nematicidal efficacy of plant leaves and *Paecilomyces lilacinus* found in controlling *Meloidogyne incognita* on orka and tomato. The addition of *P. lilacinus* without plant leaves increased plant dry weight and reduced root galling [11].

The assessment of nematophagous fungi and neem cake found compatible against *Heterodera cajani* on cowpea [9]. Integrated management of root-knot nematode in brinjal under field conditions, improved plant growth and considerable reduced gall index and also gave higher brinjal fruit yield over control [10].

Lantana camara
It belongs to the family Verbenaceae. Its foliage contains pentacyclic triterpenoids cause hepatotoxicity. It is not effected by pest or disease has low water requirement and is tolerant to heat. Extract of *Lantana camara* may be used for protection of cabbage against the aphid *Lipaphis erysimi*.

Gnaphalium purpureum
It belong to the family Asteraceae. It is annual or biennial weed with branching stem and dull green leaves.

Solanum nigrum
Commonly known as black night shade belong to family Solanaceae. All parts are poisonous containing solanine and other glycoalkaloids, the toxin are most concentrated in unripe green berries. The glycoalkaloid Solanin is extremely toxic and can be fatal.

Amaranthus spinosus
It belongs to the family Amaranthaceae. It is recommended for treating eruptic fever as a galactogogue and as a remedy for colic. The root and leaves are boiled and given to children as laxative.

Veronica anagallis aquatica
The root and leaf are alternative appetizer and diuretic. The leaves are used in the treatment of scurvy impurity of the blood. The plant is bruised and applied externally as a poultice on burn ulcer whitlows.

The evaluation of plant extracts of *Azadirachta indica, Cannabis sativus, Aegle marmelos, Achyranthus aspera* helped in controlling of *Alternaria* leaf spot of *Vicia faba*. It was recorded that *Azadirachta indica* inhibit the 46.2% of growth of *Alternaria alternata, Cannabis sativa* inhibit 36.2% of growth of *A. alternata, Aegle marmelos* inhibit 25.7% and *Achyranthus aspera* inhibit 15.5% of growth of *Alternaria alternate* [12]. The effect of aqueous leaf extracts of 8 allelopathic free species viz. *Acacia nilotica, Alstonia scholaris, Azadirachta indica, Eucaylptus citriodora, Ficus bengalensis, mangifera indica, Melia azedarach* and *syzygium cumini* on germination and seed borne mycoflora of wheat. It was recorded that extract of *A. indica* exhibited maximum toxicity against *A. alternata* and caused 77% and 60% reduction in fungal incidence [13]. The effect of 55 angiospermic plant extract on vegetative growth of *Fusarium moniliforme*. It was recorded that leaf extract of *Lawsonia inermis* showed maximum inhibition (60.65%) followed by root of *Asparagus racemosus* 50.59% [6]. The effect of methanolic extract

from root, stem and leaf of *Datura stramonium* on the vegetative and generative phases of the growth process of four fungi strains (*Fusarium semithectum, Fusarium colmorum, Ceratocystis ulmi* and *Rhizoctonia solani*) and four bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Bacillus subtilis*). It was recorded that methanol extract of green leaf export callus inhibited 22-23 mm growth of *B. subtilis* [1].

MATERIALS AND METHODS

Collection of plant material

For the purpose of isolation of leaf extracts five wild plants were selected. The plants were collected in an around Aligarh Muslim University campus. The taxonomic identification of the specimens was performed based on various morphological characters. The five wild plants selected were *Lantana camara, Gnaphalium purpureum, Solanum nigrum, Amaranthus spinosus, Veronica anagallis aquatica.*.

Plant extracts

Extracts were prepared from leaves of selected medicinal wild plants. The leaves were thoroughly washed in running tap water and sterile distilled water, air dried at 27^{o}C and ground to obtained extracts of each plant species the extraction was done by means of pestle and mortar. Water extract was obtained by adding each 30 g of leaves to 30 ml of distilled water (1:1 w/v).

Preparation of media

The fungus was grown on potato dextrose agar (PDA) media for the purpose of present study. PDA was prepared by using the following preparation.

Agar agar	-	20 gm
Dextrose	-	20 gm
Pealed potato	-	200 gm
Distilled water	-	1000 ml

In vitro test

In vitro test were carried out in sterile petridishes containing PDA. The effect of plant extracts on spore formation and radial growth of pathogen was determined using poisoned food technique [7]. Now the 10 ml of each plant extract was added to the 10 ml of PDA. Solution so obtained was autoclaved at 15 psi for about 15 min. Inocualtion of *Paecilomyces lilacinus* in petridishes was done by gently touching the needle tip with a 10 days old culture of *P. lilacinus* grown on PDA. The inoculated petridishes were kept in incubator at 27^{o}C ± 28^{o}C for the growth of fungus. The petridishes were observed at regular interval 24, 48, 72, 96 and 120 hours of time to check the colony formation of fungus. The diameter of fungal colony was measured in cm.

The counting of conidia was done by means of haemocytometer for this purpose one disc (1 cm) of each petridish was taken from 7 days old culture of *P. lilacinus*. The disc (1 cm) was washed in 2 ml of distilled water. For the collection of spores now one drop of solution was put on haemocytometer and spores were counted under microscope.

RESULTS AND DISCUSSION

In the present study the efficacy of five leaf extracts was evaluated against *Paecilomyces lilacinus*. Table 1 reveals that among the five plant leaf extracts tested all the leaf extracts showed an inhibitory effect on *P. lilacinus*. After 24 hrs, the minimum growth of colony

formation was observed in *Solanum nigrum* (0.5 cm), followed by , *Lantana camara, Gnaphalium purpureum* (0.6 cm), *Amaranthus spinosus* (1.33 cm). The maximum growth of 1.47 cm after 24 hrs was observed in *Veronica anagallis aquatica*. Further more it was observed that colony formation varied with time interval, in general it was observed that growth of colony formation increased with a increasing inoculation period. After 120 hrs the maximum growth was observed in *Amaranthus spinosus* (8.83). The minimum mycelial growth was observed in *Lantana camara* (7.16 cm) followed by *Veronica anagallis aquatica* (7.80 cm), *Solanum nigrum* (8 cm), *Gnaphalium purpureum* (8.50 cm). Furthermore the maximum number of spores/cm^2 were observed in, *Veronica anagallis aquatica* ($5.9x10^6$), followed by *Lantana camara* ($5.7x10^6$), and *Amaranthus spinosus* ($5.0x10^6$). The minimum number of spores/cm^2 were observed in by *Gnaphalium purpureum* ($3.7x10^6$), followed by *Solanum nigrum* ($4.6x10^6$).

Table 1: Effect of plant extracts on the mycelial growth and spore production in *Paecilomyces lilacinus.*

Name of plant	Diameter of mycelial growth (cm)					Number of spores/cm^2
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	
Control	2.73	3.83	5.83	8.0	10.0	$34.4 x10^6$
Amaranthus spinosus	1.33	2.30	5.67	8.00	8.83	$5.0 x10^6$
Gnaphalium purpureum	0.60	1.53	4.33	6.83	8.50	$3.7 x10^6$
Lantana camara	0.60	1.63	3.76	5.33	7.16	$5.7 x10^6$
Solanum nigrum	0.50	1.13	3.27	5.47	8.00	$4.6 x10^6$
Veronica anagallis aquatica	1.47	2.70	5.17	6.50	7.80	$5.9 x10^6$
L.SD (P=0.05)	0.421	0.485	0.517	0.576	0.485	5.938
L.SD (P=0.01)	0.598	0.690	0.735	0.820	0.690	8.445

The inhibitory effect of leaf extracts on colony formation and spore count might be attributed due to the presence of some antifungal ingredients [2-5,8].The present study conclude that leaf extracts of almost all the five tested plants have inhibitory effect on growth and sporulation of *P. lilacinus* on a varying degree.

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