INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-4, Issue-1, Jan-Mar-2014
ISSN 2231-4490
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Coden : IJPAES www.ijpaes.com
Received: 10th Jan-2014 *Revised: 13th Jan-2014* *Accepted: 18th Jan-2014*
Research article

EVALUATION OF LEAF EXTRACT OF SOME MEDICINAL WILD PLANTS ON THE GROWTH AND SPORULATION OF *PAECILOMYCES LILACINUS*

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ABSRACT-: *In vitro* experiment was conducted to determine the effect of leaf extracts of five medicinal wild plant viz, *Chenopodium album*, *Eclipta prostrata*, *Euphorbia pulcherima*, *Oxalis corniculata* and *Stellaria media* on the growth and sporulation of fungus *Paecilomyces lilacinus*. The effect of the leaf extracts of these medicinal wild plant was noted at the different time interval such as, 24hrs, 48hrs, 72hrs, 96hrs and 120hrs. After 120 hrs the maximum mycelial growth was observed in *Oxalis corniculata* and minimum in *Euphorbia pulcherima*. However maximum number of spores/cm^2 was recorded in *Eclipta prostrata* and minimum in *Stellaria media*.
Keywords-: Leaf extract, Medicinal plants, *Paecilomyces lilacinus*.

INTRODUCTION

Medicinal plants as a group, comprise approximately 8,000 species and account for around 50% of all the higher flowering plant species of India. India possesses almost 8% of the estimated biodiversity of the world with around 0.126% million species. India is one of the 12 mega biodiversity centers with 2 hot-spot of biodiversity in Western Ghat and North-Eastern Region.

Chenopodium album

It is the fast growing weedy plant belonging to the family Chenopodiaceae. Plant bears seeds which are high in protein, vitamin A, calcium, phosphorus and potassium.

The leaves contain considerable amount of soluble oxalate that interact with calcium and induces hypocalcemia. The herb is laxative, anthelmintic and cardiotonic. The leaves are anti-scorbutic, and used to treat round and hookworm infection. The juice is used for treating burns. A decoction of the aerial parts mixed with alcohol is rubbed on the body affected by arthritis and rheumatism [1, 2].

Eclipta prostrata

Commonly known as false daisy belongs to the family Asteraceae. The herb contains wedelactone and dimethyl wedelactone. The herb is used in skin disease. The plant juice in combination with aromatic is administered for jaundice. The shoot extract shows antibiotic activity against *E. coli*. Root is applied externally as antiseptic to ulcer and wound in cattle. Rhizome is used against rheumatism, bronchial catarrh, ulcer and whooping cold in children.

Euphorbia pulcherima

It is a small tree or shrub belonging to the family Euphorbiaceae. Plant has milky juice or latex. Phytochemicals such as germanical, bamyrin and pseudotasosterol are present in latex. Pulcherrol is present in the stem. Extract of leaves, stem, flowers, fruit exhibited moderate antibacterial effects on *Micrococcus pyogenes*, *E. coli*. The plant is used as emetic and cathartic.

Oxalis corniculata

Commonly known as creeping wood sorrel also called as procumbent yellow sorrel or sleeping beauty. It belongs to the family Oxalidaceae. The leaves of wood sorrel are quite edible with a tangy taste of lemon. The entire plant is rich in Vitamin C. The leaves contain the flavonoids, vitexin, isovitexin. The plant is the source of unani drug. The drug is considered to be cooling refrigerant, antiscorbutic, astringent, appetizing, and useful in fever and dysentery.

Stellaria media

Commonly known as chickenwort, craches, winter weed, belongs to the family Caryophyllaceae. The chickweed is said to be useful in inflammation of the digestive, renal, respiratory, and reproductive tracts.

It has been known to soothe severe itchiness even where all other remedies have failed. In excess doses chickweed can cause diarrhoea and vomiting. It is also useful in severe inflammation of skins such as erysipelas, scald and burns, haemorrhoids, eczema and inflammations of eyes. The herb can be employed dried or fresh, or in the form of powder, extract, decoctions, fomentation, poultice or ointment. The plant is employed in plaster used for broken bones and swellings. *Paecilomyces lilacinus* is one of the effective biocontrol agent against phytonematodes. It protects the root system from the disease caused by plant parasitic nematodes specifically root-knot nematode (*Meloidogyne* spp), reniform nematode (*Rotylenchulus reniformis*), burrowing nematode (*Rhadopholus similis*) and citrus nematode (*Tylenchulus semipenetrans*). It is an antagonistic fungus that colonizes on the root surface and is strongly parasitic to the egg and egg-masses and female of plant parasitic nematode. Fungal parasitism can destroy up to 90% of eggs and 75-80% of egg masses or cysts of nematodes. Rao [15] studied the management of *M. incognita* by a bare root dip treatment of tomato seedling in plant leaf extract mixed with *Paecilomyces lilacinus* spores. It was shown that 5 or 10% of castor leaf extract increased mycelial growth, sporulation and propagule density of *P. lilacinus* on root and show increased colonization of the bio-agent on the root of tomato offering enhanced biological protection with the consequent effect of increased parasitisation of eggs of *M. incognita*. [5, 6].

The efficacy of bioagents *Trichoderma viride* and *T.harzianum* and plant extracts of *Allium sativa* and *Azadirachta indica* and fungicides on the mycelium growth of *Sclerotinia sclerotiorum* was recorded. Effect of extracts of neem cake, groundnut cake, gingelly cake on stimulating the germination and subsequent lysis of sclerotia [10].

MATERIAL AND METHOD

Collection of plant material

For the purpose of isolation of leaf extracts five medicinal 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. *Chenopodium album, Eclipta prostrata, Euphorbia pulcherima,Oxalis corniculata, Stellaria media.*

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 described by Nene & Thapliyal [11]. 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

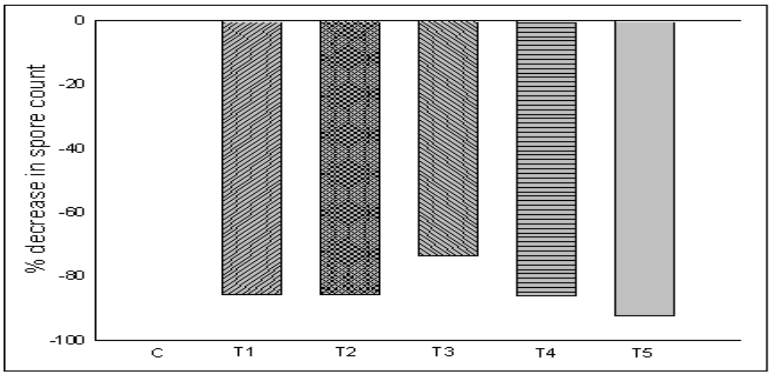
In the present study the efficacy of five leaf extracts was evaluated against *Paecilomyces lilacinus*. The result revealed that even after 24 hrs no mycelial growth was observed by using *Oxalis* leaf extracts. The minimum growth of colony formation was observed in *Chenopodium album* (0.5 cm) followed by *Euphorbia pulcherima, Eclipta prostrata,* (0.6 cm).

The maximum growth of 1.13 cm after 24 hrs was observed in *Stellaria media.* Senguttuvan [18] reported the antifungal activity of root and leaf extract of medicinal plant *Hypochaeris radiacata* against *Paecilomyces lilacinus, Trichoderma viride, aspergillus niger, Aspergillus fumigants, verticillium lecani, candida albicans* and *Penicillium* sp. and found that *P. lilacinus* and *T. viride* are more resistance than other fungi. From our experiment it has been observed that colony formation varied with time interval in general and growth of colony formation increased with a increasing inoculation period.

After 120 hrs the maximum growth was observed in *Oxalis* (9.17 cm), although the initial colony formation after 24 hrs in *Oxalis* was nil. This may be due to some antifungal agents present in *Oxalis* in the beginning and their depletion with time. Leaf extract of *Ageratum conizoies, Albutilon indicum, Chenopodium album, Delonix regia* and *Ocimum sanctum* inhibited 16.50 – 50.20% growth of *C. falcatum,* 7.10-24.3% growth of *Acremonium* sp., 12.75 – 46.30% *Fusarium moniliforme* var. *subglutinans* 5.30 – 15.0% of *U. scitaminea* and 2.53 – 12.80% of *C. paradoxa* Ramjilal [14]. Jalal and Ghaffar [4] reported antifungal activity of plants extracts of *Allium cepa* (onion), *Calotropis procera* (Akk), *Chenopodium album* (Bathu), *Chenopodium murale* (Karund), *Azadirachta indica* (Neem) and *Cannabis sativa* (Bhang) against *Macrophomina phaseolina, Alternaria radicina, Helminthos-porium tusricum* and *Ascochyta rabiei*. Water extracts of Asteraceae members showed strongest effect on reduction in growth of *A. niger* than the species of certain other families [13]. Our finding showed the minimum mycelial growth in *Euphorbia pulcherima* (8.17 cm), followed by *Stellaria media* (8.83 cm), *Chenopodium album* (8.97 cm), *Eclipta prostrata* (9 cm). Furthermore the maximum number of spores/cm^2 were observed in *Eclipta prostrata* (8.8x10^6) followed by *Euphorbia pulcherima* (5.0x10^6), *Chenopodium album* (5.0x10^6). The minimum number of spores/cm^2 were observed in *Stellaria media* (2.6x10^6) followed by *Oxalis corniculata* (4.8x10^6) (Table-1). Rao [16] studied integration of *Paecilomyces lilacinus* with neem leaf suspension for the management of root-knot nematodes on egg plant. It was recorded that the aqueous neem leaf suspensions supported the growth of *P. lilacinus* and result indicate significant increase in the colonization of *P. lilacinus* on roots, increased the percentage parasitization of egg of *M. incognita* by *P. lilacinus*.

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 (C)	2.73	3.83	5.83	8.0	10.0	34.4 x10^6
Chenopodium album (T1)	0.50	2.30	6.00	7.00	8.97	5.0 x10^6
Euphorbia pulcherima (T2)	0.60	1.30	3.87	6.33	8.17	5.0x10^6
Eclipta prostrata (T3)	0.60	0.97	4.33	8.03	9.00	8.8 x10^6
Oxalis corniculata (T4)	0	1.60	4.37	7.80	9.17	4.8 x10^6
Stellaria media (T5)	1.13	4.50	7.30	8.0	8.83	2.6 x10^6
LSD (P=0.05)	0.46	0.69	0.66	0.49	0.37	5.50x10^6
LSD (P=0.01)	0.66	0.98	0.95	0.69	0.53	7.50x10^6



Extract of 3 month old fresh leaves sorghum plant showed 73.74% disease control, reduced disease severity to 76.45% caused by the summer squash mosaic on *Cucurbita* pepo [20]. Qasem Aau-Blan [12] found that extract of *Chenopodium murale, Falearia vulgaris, Ranunculus asiaticus* and *Sisymbrium irio* were the most toxic to *A. solani; Anagallis arlensis, Atriptex leucoclada, Crepis aspera, Notobaris syriaca, Rumex crispa* toxic to *H. sativum; Ranunculus asiaticus, S. oleraceous* and *Mercurialis annua* toxic to *R. solani.* The above result are in agreement with the previous finding of Rao [16], Rifia [17], Domsch [3], Martha [7, 8] and Mazumdar [9]. Present study reveal that leaf extract of *Eclipta prostrata* showed the maximum mycilial growth and number of spores/cm^2. It has been concluded that leaf extract of *Eclipta prostrata* along with *P. lilacinus* can be used for the management of plant parasitic nematode [20].

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