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Funaria Hygrometrica extracts with activity against plant pathogenic fungi *Alternaria species*

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Abstract

The *invitro* antifungal activity of *Funaria* was studied against test fungi *Alternaria* using disc diffusion and direct dilution methods. Extract treatments reduced the fungal growth several biomass ranging from 15 to 23%, The alcohol extract was evaluated for its short-term toxicity. Levels of activity against the test fungi; the alcohol extract exhibited maximum activity. Data showed that all the extracts showed variable antifungal activity. Among the various extracts, methanol extract, showed the antifungal activity resulting in 0–63% and 0-69% reduction in fungal biomass over corresponding control treatments, respectively. The implications of using the *Funaria* extracts in controlling *Alternaria species.* Therefore, products of bryophytes deserved to be reliable sources as biocontrol agents and may play significant roles for future practical applications in a socially and ecologically healthy crop management system.

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Introduction

The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Mohana *et al.*, 2011). Green plants represent a reservoir of effective chemo therapeutants and can provide valuable sources of natural pesticides. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. Alternaria fungus has about one hundred species, found in various places all over the world. Many of them are plant pathogens and cause disease in a wide range of hosts. Alternaria has an important place among species of this genus, as it depends on range of hosts including garden plants, field crops, vegetables, and ornamentals. The taxon is the principal causative agent of blight of ripe

tomatoes, brown necrotic lesions on foliage, black pit disease of potatoes.

Extracts of many plants have been reported to exhibit antifungal properties under laboratory (Parekh *et al.,* 2006; Buwa and Staden, 2006). Pathogenic fungi alone cause 20% reduction in the yield of major food and cash crops (Agrios, 2000). To avoid the implication of yield losses due to plant diseases, variety of control measures presently are in use. The chemical compounds are most commonly used for the controlling of plant diseases. No doubt the use of chemicals has been found very effective in controlling plant fungal diseases but some major problems threaten to limit the continued use of fungicides. Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest but are also indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens (Dellavalle *et al.* 2011).Fungal plant diseases represent an important cause of increased annual crop losses. More than 70% of all major crop diseases are caused by fungi (Agrios, 2005). *Alternaria alternata* causes leaf spots and blight on a large variety of agricultural and horticultural Moreover, *A. alternata* can also attack a several weeds and ornamental plants. There is also little doubt that sensitivity to *Alternaria* is an important factor in the induction of allergic rhinitis and asthma on immunodepressed patients, especially in children (Kuna *et al.,* 2011) Generally, the control of plant diseases and pests is well established with synthetic fungicides and other agricultural practices such as crop rotation inter-cropping and sanitation (Pretty, 2008). However, in the recent years the farmers all over the world have reported an efficacy decrease of the treatments with traditionally used fungicides to control early blight and other plant diseases (Fairchild *et al.,* 2013). Furthermore, the inappropriate use of fungicides, such as applying increased and more frequent units (Genet *et al.,* 2006) has resulted on the one hand in the occurrence of fungal resistance (Haouala, 2008) and on the other hand in hazardous effects in human and animal health and on the environment resulting in ecological imbalances (Pramila and Dubey, 2004).

Traditionally, because of their antimicrobial activity, mosses were used as a natural medicine in Indian culture. Today, mosses represent interesting tools for biotechnological use in medicine, agriculture, and pharmacology. However, although mosses are becoming increasingly important in many fields and moss is used as a model organism for antimicrobial studies. Little is known about moss-associated microorganisms, beneficial as well as pathogenic. The effect of mosses by fungi is a very frequent though generally neglected phenomenon. The bryophytes, including liverworts, hornworts, and mosses, are a diverse group of land plants that usually colonize habitats with moist or extremely variable conditions. One of their most important features is their life cycle, which involves alternation between a diploid sporophyte and a dominant, free-living haploid gametophyte generation. *Funaria hygrometrica* is a common type of water moss which grows on moist, shady, and damp soil. It can also be found on moist

walls and the crevices of rocks and places where recent fires have taken place. The plant body is green, soft, and upright. The rhizoids present in this species are multi-cellular and branched. They have oblique septa. The main axis of the plant, which is upright, bears a set of spirally arranged, sessile leaves having a clearly distinguishable midrib. At the apex of the main plant axis, the antheridium is borne. This is the male part of the shoot. A lateral branch from the main plant axis bears the female shoot archegonium at its meristem.

Materials and Methods

Isolation of pathogens from diseased plant tissues

Plant infected with disease found during the survey at field. Surface disinfestations of tissues selected for isolation work was done by 1 % sodium hypochlorite. The stems infected with disease were cut into small pieces and placed directly on acidified PDA (Mehrota R.S. and Aggarwal Ashok., 2003). After a day, colonies of fungi are visible which was further subjected for the identification of pathogen. The stems infected with disease were cut into small pieces and placed directly on acidified PDA (Mehrota R.S. and Aggarwal Ashok., 2003). .Microscopical examination of the pathogen revealed the pale brown to light brown conidia produced in long chain of 5, obclavate, short conical beak at the tip,smooth surface with several vertical and 8 tranverse septa which is confirmed as *Alternaria species.*

Preparation of plant extracts

Dried plant was powdered by using blender, and the powder was used for preparation of extracts in the organic solvent, viz methanol. The sample (powder) 100 g each was dissolved in 400 ml respective solvent using soaking method and allowed to stand at room temperature for few days. The extracts obtained with different solvents were filtered through Whatman filter paper No. 1. The medium (PDA) without any phytoextract served as the control. All the inoculated Petri dishes were incubated at 25±1°C. The radial growth of the test fungus was measured in all the treatments after three days and compared with the control (Tapwal Ashwani., *et al*, 2011). The petriplates were incubated at room temperature for the growth of Alternaria. After 3 days, blackish colony growth was observed in incubated petri dishes the per cent inhibition of fungal growth was estimated by using following formula (Vincet, 1927).

Identification of Isolated Fungi

The fungal isolate from growing culture was identified by lactophenol cotton blue staining. Microscopic examination was carried out after examining the colony characteristics, while the morphological and cultural characteristics were observed. The test fungus were grown and maintained on potato dextrose agar slants, following incubation for 5 days, the cultures were either utilized or stored until required. The organisms were sub cultured to obtain pure colonies and it was done once in every intervals.

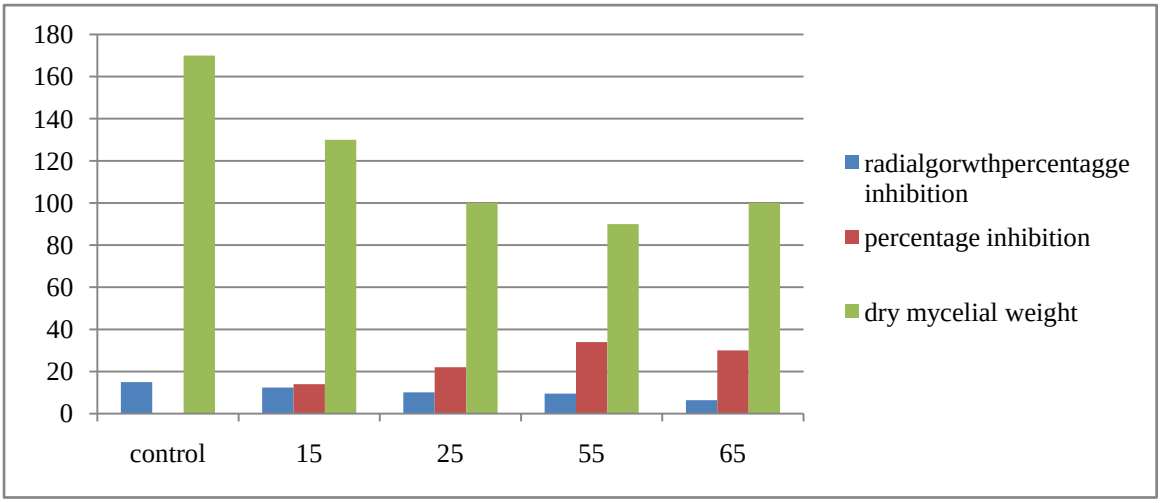
Antifungal assay of plant extracts

A volume of 1 ml of each extract (methanol) was aseptically poured in respective petri-plates followed by the addition of 9 ml of melted PDA and was swirled gently to

achieve thorough mixing of the contents. A petri-plate with PDA having no plant extract was used as control. After the solidification of the media, pure culture of the isolated fungi was then transferred aseptically onto the petriplates with plant extract using a sterile cork borer of 5.0 mm diameter upside down right at the centre. Distilled water was used as negative control. The plates were then incubated for 48 hours at 27°C. At the end of the incubation period the zones of inhibitions were measured to the nearest millimeter (Andrews *et al.*, 2001). The inhibition zone is the area surrounding the hole with no growth of inoculated fungi. For confirmation of the results each test was performed in triplicate. The petri plates were incubated at 27^{o}C and growth (diameter) of the tested fungi was measured after 24 hrs and 72 hrs respectively. The radial growth of the test fungus was measured in all the treatments after three days and compared with the control (Tapwal Ashwani., *et al.*, 2011).The rate of extraction of the fungicide from the disc is greater than the rate of diffusion, as the distance from the disc increases. Zone of inhibition of fungus growth around eachdisc is measured and the susceptibility is determined.

Treatments	**Mycelial growth** (mean) **after 48 hrs in mm**	**Inhibition percentage after 48**	**Mycelia dry weight after 48 hrs in mg**	**Mycelial growth** (mean) **after 72 hrs in mm**	**Inhibition percentage after 72 hrs**	**Mycelia dry weight after 72 hrs in mg**
control	15		170	15		170
15% methanol	12.4	14%	130	10	30%	110
25%	10.1	22%	100	9	45%	80
55%	9.5	34%	90	7	59%	70
65%	6.4	49%	70	5	67%	56

Table 1: Inhibition of mycelial growth at different concentration of methanolic plant extracts



(The inhibitory effects of the methanolic extract on mycelial growth of fungi after inoculation (percent inhibition after 48hrs)

The percent inhibition of mycelial growth was calculated using the formula:

Percent inhibition = C – T / C X 100

where C = Mycelial weight in control and

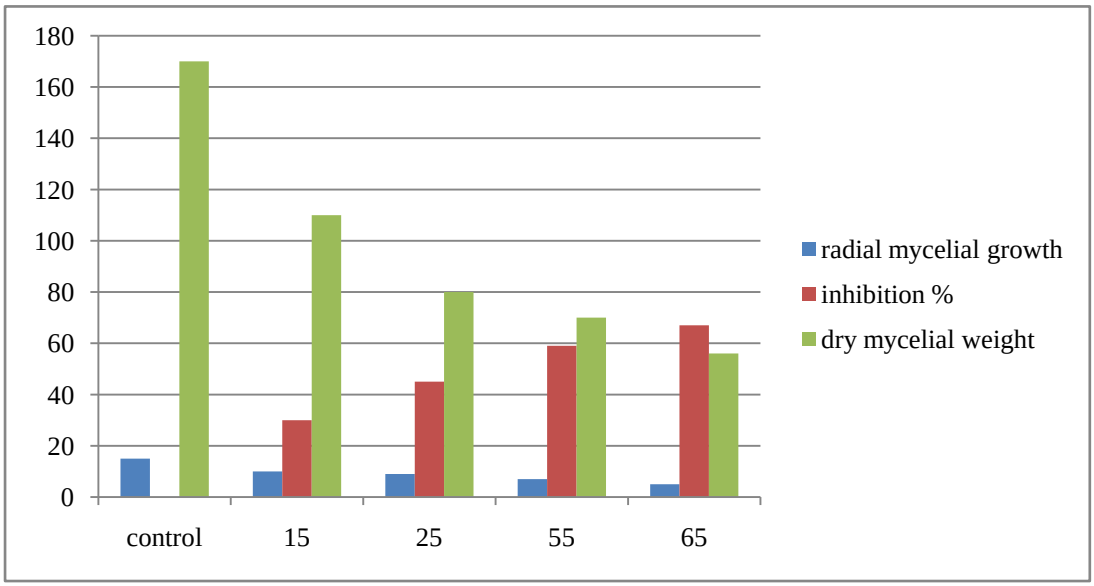
T = Mycelial weight in treatment.

In vitro efficacy of plant extracts on inhibition of *Alternaria alternata* against mycelial growth at different days of interval.

The inhibition of the growth of the pathogenic fungi is due to the active ingradients predominantly found in the plant. The present investigations are in line with the investigations carried out by other workers who infer that plant extracts in general have great potentiallity in the control of fungal diseases in commercially important crop plants. These leaf extracts could be suitable substitute for controlling fungal pathogens.

Treatments	**Mycelial growth (**mean) **after 48 hrs in mm**	**Inhibition percentage after 48**	**Mycelia dry weight after 48 hrs in mg**	**Mycelial growth** (mean) **after 72 hrs in mm**	**Inhibition percentage after 72 hrs**	**Mycelia dry weight after 72 hrs in mg**
control	15		170	15		170
15% methanol	14.4	9%	150	10	30%	140
25%	12.1	12%	130	9	35%	110
55%	11.5	14%	120	7	39%	98
65%	10.4	29%	100	5	57%	96

Table 2: Inhibition of mycelial growth at different concentration of aqueous Leaf extracts *(In vitro* screening of plant leaf extracts on *A.alternata)*



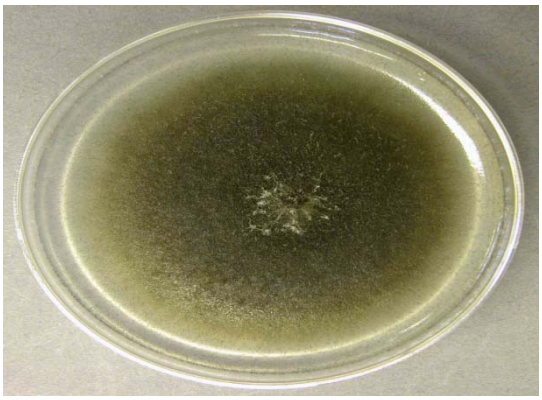
(The inhibitory effects of the methanolic extract on mycelial growth of fungi after inoculation (percent inhibition after 72hrs)

It may be concluded that keeping aside the environmentally hazardous commercial fungicides, these leaf extracts could be a suitable substitute for controlling the fungal pathogens. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects (Srivastava *et al*., 2000). The phyto-constituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000).

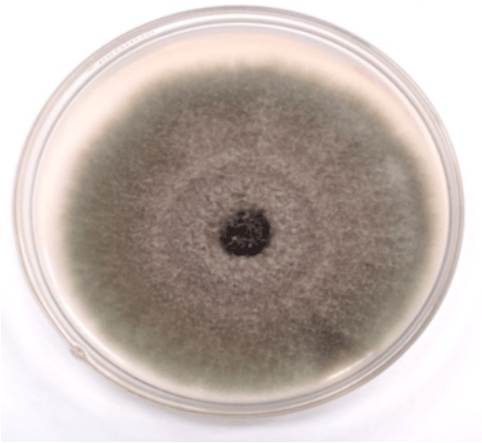
The differential extracts of plant demonstrated strong antifungal activity towards all the pathogenic fungi tested. The

highest activity was shown by methanol extract followed by water. All the solvent extracts showed activity against *fungus* .The differential and moderate activities were observed against pathogens but the results revealed that activity of methanol extract was greater in comparison to the synthetic standard antibiotic. The antifungal activity of extracts showed little variation and excellent reproducibility of zone of inhibition for selected pathogens. In *fungal* species, the inhibition zone diameter was found. The results were compared with the synthetic standard antibiotic. The methanol extract gave strong and promising results in comparison to the standard. The results also showed that different solvent extractions gave different results against the same pathogens.

Funaria plant



Diseased plant



Fungal growth of alternaria blight

Symptoms of alternaria blight

Botanical extracts with antifungal activity are being explored in order to make available the pesticides, which are easily biodegradable, selective, cheap and can be locally produced, especially for the farmers who cannot afford expensive synthetic pesticides. The results revealed that all of the tested plant extracts at given concentration inhibited the growth of pathogens Similar results on the efficacy of

plant extracts against *Alternaria* sp. have been reported by Baraka *et al*. (2011), Mishra and Gupta (2012), and Ravikumar and Garampalli (2013). These are the evidences from the earlier work that plants possess the pesticidial activity that can play a pivotal role in the management of the plant disease which are cheap, locally available, and biodegradable and environment friendly. This study shows optimistic results regarding the potential of plant species as sources of plant based products with activity against plant pathogenic fungi.

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