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ANTIFUNGAL EVALUATION OF MEDICINAL PLANTS TO CONTROL THE GROWTH OF MOULD FUNGI ON BUILDING MATERIAL

Mamta Chauhan* Padma Singh

Kanya Gurukul Campus, Gurukul Kangri Vishwavidyalaya, Haridwar – 249404.

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***Correspondence for Author**
Dr. Mamta Chauhan
Kanya Gurukul Campus, Gurukul Kangri Vishwavidyalaya, Haridwar – 249404

ABSTRACT

To control the growth of building biodeteriorating fungi require the use of botanical fungicides which can be derived from medicinal plants instead of chemical fungicides. These chemical fungicides are mainly compound of copper, cadmium, mercury and arsenic. The present study was designed to evaluate the antifungal activity of plant extracts of *Emblica officinalis* and *Tagetes erecta* against *Aspergillus flavus* and *Penicillium chrysogenum* isolated from various sites of buildings in Haridwar. The antifungal activity was assayed by passion food method. In case of *T. erecta* methanol (extract) showed maximum activity comparatively ethanol extract of *T. erecta* whereas ethanol extract of *E. officinalis* showed highest activity in comparison of methanol. Essential oil of *E. officinalis* gave best activity then essential oil of *T. erecta* against *A. flavus* and *P. chrysogenum*. Maximum dry mycelium weight of *A. flavus* and *P. chrysogenum* was recorded after 20 and 25 days respectively at incubation of 28°C.

KEY WORDS: Building fungi, *Aspergillus flavus*, *Penicillium chrysogenum*, Possion food method, Dry mycelium weight.

INTRODUCTION

Microorganisms are responsible for the deterioration of building material, most frequently involved in biodeterioration of building materials are bacteria, fungi, cyanobacteria and algae. Microorganisms will not grow if there are no nutrients or carbon sources [8]. Fungi are essential to survival of global ecology but they may pose a significant threat to the occupant's health when they grow in buildings. Some favorable conditions like a favorable temperature (0-25°C), nutrients, oxygen and water plays a significant role in the growth of mould fungi.

The growth of mould fungi on building material is an early indication of increased humidity and moisture levels. Problems caused by mould fungi are mainly discoloration, odor and health disadvantage [6]. The commonest moulds, which are found in buildings are *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Trichoderma* spp., *Fusarium* spp., *Rhizopus* spp. etc. Bathrooms, kitchens, basements, balconies are most often affected by mould contamination. Building is a long lasting product in comparison of other utility goods and different kinds of water damages caused by several reasons may be construction errors, errors made during repair, or incorrect use. The performance of the building material depends on many factors coating, material, structure, environment, services etc. [21]. The moisture conditions connected with temperature and exposure time are the most important factor for development of fungal growth and damage of building materials. Common sources of moisture damage are: roof leakage, flooding, leaking services, and spillage and construction moisture. Factor affecting moisture transfer includes temperature, air, moisture content of the surrounding air and materials are the most important factor for transferring in moisture [5]. Several studies have shown that people living and working in damp or water damaged buildings have an increased risk of infections, adverse health effects, respiratory problems [7, 16] asthma and allergy [4] some fungi cause skin infection. The most common type of the fungal disease that is present in the buildings is the *SICK BUILDING SYNDROM (SBS)* [3]. The other most common complication arising due to fungal growth in the buildings is the production of various toxins (secondary metabolites) by the fungi which are known as mycotoxins. Some of these can be released toxic gas, depending on the species and substrate on which it grows. For example, a fungus growing on wallpaper release the highly toxic gas arsine, from arsenic containing pigments [9]. *Aspergillus* species are found to cause the serious manifestations called "*Aspergillosis*" and the production of the fungal balls referred as "*Aspergilloma"* [11].

In India over 2600 plant species have been considered useful in the traditional system of medicine like Ayurveda, Unani, Siddha and home remedies. Many plants have been used because of their antimicrobial traits and the antimicrobial properties of plants have been investigated worldwide [19]. Medicinal plants represent a rich source of antimicrobial agents plants are used medicinally in different countries and are a source of many potent and drugs [18]. Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to those extracts, which are placed in contact with them. Essential oils are volatile, liquid, and insoluble in water highly soluble in alcohol, ether, vegetables and

mineral oils. Essential oils are complex mixture of different chemical compounds. The term essential indicated that the oil is derived from essence of plant or active constituents of plants [12].

During the past several years, numerous antifungal components have been evaluated for use in the management of fungal infection. Here we focused our investigation on antifungal potential of medicinal plants. The aim of present investigation was to develop ecofriendly fungicides which has to be used for the management of building fungi. These fungi were isolated from different locations in Haridwar.

MATERIALS AND METHODS

Description of sampling sites- Three different locations in Haridwar will be selected for the present study like-

1. Industrial building (BHEL)
2. Religious building (Dakshmandir)
3. Educational building (G.K.U.)

Collections of building materials

The fungal sample were collected from three different locations i.e. industrial (building), religious (building) and educational (building). The sites or walls which have been continuously moist or damaged for a long period of time were chosen for the sampling. The moist spots on the walls were scratched with the help of sterile spatula and then transfer in a sterile polythene bags and stored at 4°C in refrigerator.

Isolation of building fungi

For the isolation of building fungi, Sabouraud Dextrose Agar medium (SDA) was prepared. The building fungi were isolated with the help of serial dilution method [22] and direct plate method [23].

Identification of building fungi (Barnett, 2003)

The fungus identified with the help of Lactophenol cotton blue staining method. Place a drop of lactophenol cotton blue on a clean glass slide. Transfer a small tuff of the fungus, preferably with spores and spore bearing structures, into the drop using a flamed cooled needle. Gently tease the material using the two mounted needles. Mix gently the stain with the mould structures. Place a cover glass over the preparation taking care to avoid trapping air

bubbles in the stain. Then the identification of the fungal colonies were done using the macroscopic and the microscopic features [2].

Inoculum preparation

Inoculums of test organisms were prepared by picking 5mm disc cut with the help of sterile cork borer from 5 to 10 days old culture grown on SDA medium. These colonies were then transfer in 5 ml of SD broth and incubated at 25°C for 3-5 days. The turbidity of the culture was compared to 0.5 McFarland standards to get 10^8 CFU/ml.

Mc Farland standards

To standardized the inoculums density for a susceptibility test. A 0.5 McFarland standard was prepared as described in NCCLS [14]. 1% solution of sulfuric acid was prepared by adding 1ml of concentrated H_2So_4 to 99 ml of water and mixed well. A 1.175% solution of barium chloride was prepared by dissolving 2.35 g of dehydrated barium chloride ($BaCl_2.H_2O$) in 200 ml of distilled water. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 1% 99.5 ml sulfuric acid solution and mixed well. This solution is dispensed into tubes which are sealed tightly and stored in the dark at room temperature.

Dry mycelium weight of test fungi

Inoculate a Sabourd Dextrose agar plate with the test organism for the preparation of a seed plate. Incubate at 25°C for 3-5 day in an inverted position. The equal quantity of SDA broth (20 ml) was taken in every flask. Plug the flasks with cotton-plugs and autoclave at 15 lb/ 121°C for 15 minutes. A mycelia disc of 5mm diameter of the isolates of test organism was inoculated into flask. Prepare three replicates for each treatment and these flasks were incubated at 25°C for 30 days. After 5 days filter the mycelia growth through preweighed Whatman's filter paper 42. Filter paper along with mycelia were dried in an electric oven at 60°C for 48 hours. Measure growth of mycelia at the intervals of 5days to 30 days. The dry mycelia weight of the fungal growth was calculated by deducting the weight of filter paper from mycelia weight +filter paper [13].

Test plants

The present work on two medicinal plants viz; *Emblica officinalis* and *Tagetes* erecta. These plants were collected from Haridwar and an adjoining area for their fungicidal study and were identified with the help of taxonomic literature, standard flora and herbarium at Gurukul Kangri Vishwavidyalaya, Haridwar, India.

Preparation of solvent extracts

Thoroughly washed mature leaves of all the test plants were shade dried and then powdered with the help of a blender. 50g of the powder was filled in the thimble and extracted with organic solvent i.e. ethanol and methanol using a Soxhlet apparatus for 48 h. After 48 h the extract was further evaporated to dryness at 40°C in rotavapour under vacuum. When all the solvent was evaporated the extract was preserved at 4°C in airtight brown bottle until further use [10].

Extraction of essential oils

Emblica officinalis, Tagetes erecta oils were selected for their important antifungal activity. Essential oils of *Emblica officinalis* and *Tagetes erecta* were purchased from local herbal shop in Dehradun (India).

Preparation of different concentrations

Three different concentrations (100mg/ml, 200mg/ml, 300mg/ml) of the extracts were prepared in particular solvent in which they were extracted. Different concentrations of the essential oils were prepared in di-methyl sulphoxide (DMSO).

Poison food technique (Nene and Thapliyal,1971)

Satish *et. al.* (2007) was adopted for the poison food method with little modification. In this technique different solvent extracts i.e. ethanol, methanol and distilled water which obtained from leaves of five test plants. .5gm each of extract was dissolved in .5 ml ethanol and pour in to 250 ml medium to achieve 1000 µg/ml concentration of the extract in the medium, autoclaved and poured (20ml) into 90 mm glass Petri dishes and allowed to solidification. After solidification of the medium, 5mm diameter disc of 7 days old culture of the test organisms were placed at the center of the Petri plates. Three replicates were maintained for each treatment. The 250 ml media containing ethanol served as control. The plates were incubated at 22±1°C for seven days [17].

RESULTS AND DISCUSSION

Dry mycelium weight

Dry mycelium weight of *A. flavus* and *P. chrysogenum* have been shown in **Table 1, 2 and Graph 1,2**. Dry mycelium weight of *Aspergillus flavus* and *Penicillum chrysogenum* was plotted as a graph between mycelium weight and incubation time at 28°C. In *Aspergillus flavus* maximum dry mycelium weight was recorded at 20 days of incubation, whereas in

Penicillium chrysogenum it was recorded at 25 days of incubation. Mohan and Ravesha [13] reported antifungal evaluation of some plant extract against *Fuasrium solani* and *Aspergillus flavus* by dry mycelia weight, spore germination and poisoned food method.

Table 3 and fig. 3-6 shows the results of ethanol and methanol extract of *E. officinalis* and *T. erecta*. Ethanol extract of *E. officinalis* showed best activity against both test fungi in comparison of ethanol extract of *T. erecta*. Whereas methanol extract of *T.* erecta showed good activity in comparison of methanol extract of *E. officinalis*. Afifi [1] reported that three different plant extract of *Anethum graveolens, Cymbopogon citrates* and *Juniperus oxycedrus* were evaluated to inhibit the fungal growth of stucco ornaments in the Ribate of Mostafa Pasha (Azdomor AI Salehy) that belonged to the Ayyubid period. The fungal species *Fusaroum oxysporum, Aspergillus niger, Alternaria alternata* were isolated from different sites of stucco ornaments. To our knowledge, no reports are available on the antifungal activity of ethanol and methanol extract of *E. officinalis* and *T. erecta* against building biodeteriorating fungi.

Table 4 and fig. 1, 2 shows the results of essential oils of *E. officinalis* and *T. erecta*. Essential oil of *E. officinalis* gave excellent activity then essential oil of *T. erecta* against *A. flavus* and *P. chrysogenum.* Vidya Sagar [20] reported that the volatile oil of *Tagetes Patula* Linn. Showed good activity against *Fusarium solani* and *Aspergillus niger*. Yang and Clausen [24] evaluate the antifungal activity of natural product for use on wood. Seven essential oils were evaluated for their ability to inhibit growth of *Aspergillus niger, Trichoderma viridi* and *Penicillium chrysogenum* on southern yellow pine stakes that were either dip treatment or vapors exposure, were compared in both Petri dish test chamber and tank test chamber. Thyme and Egyptain geranium oil inhibited growth of all test organisms for 20 weeks. Likewise, dill weed oil vapors inhibited all test fungi for 20 weeks.

Table-1- Dry mycelium weight of *Aspergillus flavus* taken on different days (Average of three replicates).

S. No.	Days	dry weight of mycelium (gm)±SD
1	5	0.261±.001
2	10	0.287±.001
3	15	0.301±.002
4	20	0.363±.003
5	25	0..435±0.00
6	30	0.398±0.001

gm- gram, SD- Standard deviation

Table-2-Dry mycelium weight of *Penicillium chrysogenum* taken on different days (Average of three replicates)

S.No.	Days	dry weight of mycelium (gm) ±S.D.
1	5	0.310±0.00
2	10	0.325±.002
3	15	0.355±.001
4	20	0.396±0.002
5	25	0.381±.002
6	30	0.369±0.00

gm- Gram, SD- Standard deviation

Table 3- Activity of (ethanol, methanol) extract of *E. officinalis* and *T. erecta* against *A. flavus* and *P. chrysogenum* by poison food method

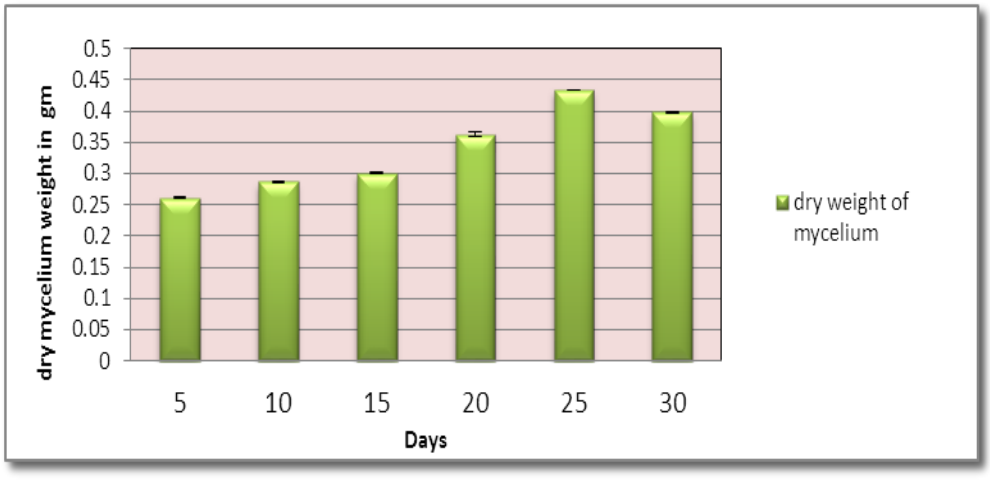
	E. officinalis		*T. erecta*	
Extracts	***Aspergillus flavus***	***Penicillium chrysogenum***	***Aspergillus flavus***	***Penicillium chrysogenum***
Ethanol	+	+	++++	++++
Methanol	+++	+++	++	++
Control	-	-	-	-

+ (excellent), ++ (best), +++ (very good), + (good), - (poor)

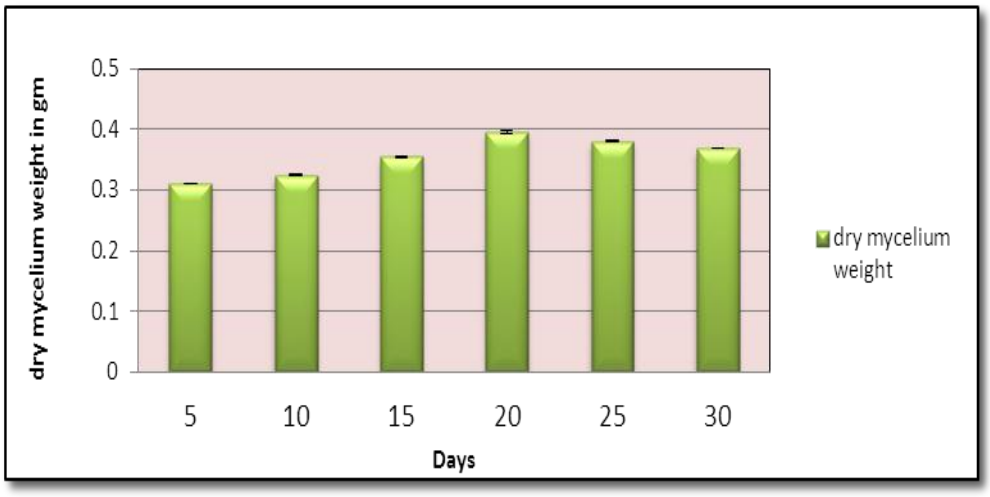
Table 4- Activity of essential oils of *E. officinalis* and *T. erecta* against *A. flavus* and *P. chrysogenum* by poison food method

Fungi	***Emblica officinalis***	***Tagetes erecta***	**Control**
Aspergillus flavus	+	++++	-
Penicillium chrysogenum	++	+++	-

++++ (excellent), +++(best), ++(very good), +(good), -(poor)



Graph- 1- Dry mycelium weight of *A. flavus* on different days



Graph- 2- Dry mycelium weight of *P. chrysogenum* on different days

E. officinalis *T. erecta*

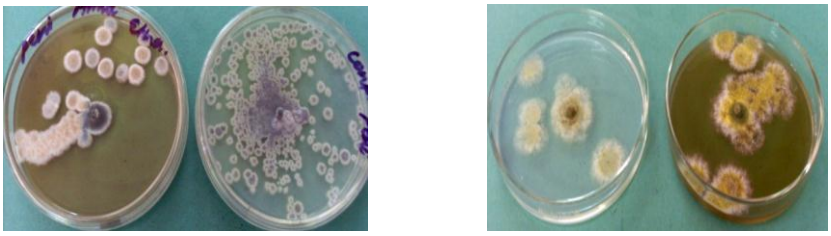
A. flavus Control *A. flavu*

Fig.-1- Activity of essential oil of *E. officinalis* and *T. erecta* against *A. flavu*

E. officinalis *T. erecta*

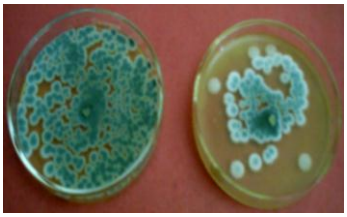
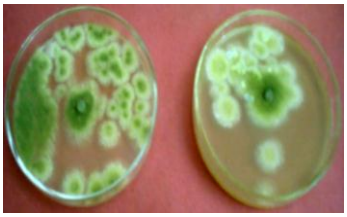
P. chrysogenum Control *P. chrysogenum*

Fig. -2- Activity of essential oil of *E. officinalis* and *T. erecta* against *P.chrysogenum*



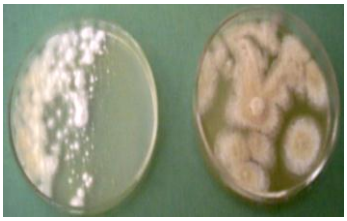
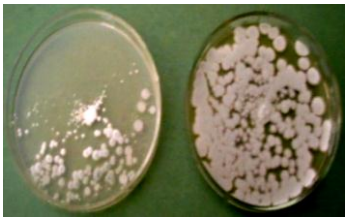
P. chrysogenum control *A. flavus* control

Fig.-3- Activity of ethanol extract of *E. officinalis* against *P. chrysogenum* & *A. flavus*

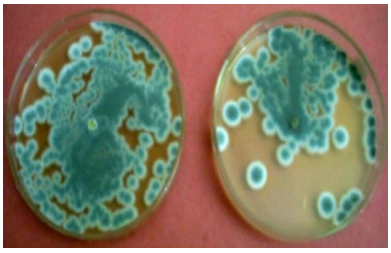
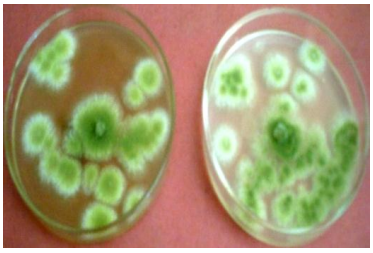
P. chrysogenum control control *A. flavus*

Fig. 4- Activity of Methanolic extract of *E. officinalis* against *P. chrysogenum* and *A. flavus*

A. flavus control *P. chrysogenum* control

Fig. 5- Activity of Methanolic extract of *T. erecta* against *A. flavus* and *P. chrysogenum*

Control *P. chrysogenum* *A. flavu* control

Fig. 6- Activity of Ethanolic extract of *T. erecta* against *P. chrysogenum* and *A.flavus*

CONCLUSION

A conclusion derived from the data presented in this research work is that building material supporting fungal growth must be remediated so as to ensure a healthy indoor environment. As the results is much promising to achieve the goal to develop biosafe botanical fungicides hence the findings of the present investigation are an important step towards the building protection strategies through plant extract formulations.

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