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EVALUATION OF ANTIFUNGAL PROPERTIES OF EXTRACTS OF *TAGETES PATULA* FOR THE CONTROL OF BUILDING FUNGI

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ABSTRACT

Growth and development of fungi causes microbial biodeterioration of building materials. Use of chemical fungicides cause environmental pollution, health hazards in humans and may also led to the resistance in the target organisms. Extracts of *Tagetes patula* were used as an antifungal agent of natural origin. In the present study, the pathogen (*Aspergillus niger*) was exposed to various concentrations (50, 100, 200, 300, 400 and 500 ppm) of methanolic, methanol-hexane, methanol-chloroform, ratio-chloroform, ratio-water, ratio-butanol, water-water and water-butanol extracts of flower of *T.patula* using poison food technique. All the employed concentrations except methanol-hexane and water-water extract suppressed the growth of *A.niger* at 50 ppm respectively.

INTRODUCTION

Building mycology deals with the study of fungi in and around the built environment which have direct and indirect effects on the health of the building fabric, its materials, structures, environments and occupants[1]. Fungi cause damage to the building structures, decorations and contents which eventually raise concerns for the indoor air quality and health of the building's occupants[2]. Illness associated with buildings have been classified as 'Sick Building Syndrome' and the common symptoms are fatigue, respiratory ailments such as wheezing, chest irritation, asthma, nasal congestion and eye irritation[3,4,5].Organic dust syndrome may be caused by a variety of biological agents including common species of fungi (eg. *Aspergillus* and *Penicillium* spp.).

For the management of fungal contamination in buildings certain chemical fungicides are being applied. The fungicides such as pentachlorophenol (PCP), tributyltin oxide (TBTO), have widely used in buildings, which is now restricted due to their harmful effect on human beings and mammals which causes adverse effects and pose residual toxicity[6]. In the present scenario, due to the increasing awareness of the pollutive residual, carcinogenic and phytotoxic effects of synthetic fungicides, the significance of indigenous products in fungi management are gaining popularity[7,8]. An alternative approach is the deployment of natural fungicides derived from plant products. Thus, the utilization of medicinal plant extracts as a safer fungicide is desirable. The presence of antifungal compounds in higher plants has long been recognized as an important factor to disease resistance[9]. Botanical pesticides and fungicides derived from plant products have been found to be eco-friendly, economic, target specific and easily biodegradable with little or no mammalian toxicity[10]. Antifungal potency of certain medicinal plants on building fungi have been reported by[11,12,13]. They found that the extracts isolated from *Tagetes* spp. were found to be effective against *Alternaria solani*, *Colletotrichum cocodes* and *Sclerotium cepivorum*.

Tagetes patula L. belonging to the family *Asteraceae* are small bushy plants commonly known as French marigolds. It is very popular as a garden plant and yields a strong aromatic essential oil (tagetes oil), which is mainly used in perfumes. It possess good medicinal properties such as antiseptic, as a modifier in hair lotions, flower head possess

stimulant and anti-helminthic properties. Occurrence of 18 active compounds, most of them terpenoids has been reported from these plants. The oil constituents isolated from the florets, leaves and stems, respectively were tageton, linalord, limonene, linalyiacetate and ocimene[14,15]. These compounds are known to exhibit antioxidant, antimycotic and analgesic activities[16]. The essential oil extracted by steam distillation from the capitula of Indian *Tagetes patula,* was evaluated for its antifungal properties and analysed by gas chromatography mass spectrometry[17].

In the present study, extracts derived from the flowers of *T. patula* were taken into consideration for the determination of their antifungal properties against building fungus.

MATERIALS AND METHODS

i) Preparation of Extract:

10 kg flowers of *T. patula* (marigold) were collected from the local market of Roorkee for the study. The plant material was shade dried at room temperature up to complete dryness and grinded into powdered form. The powdered material was divided into three parts (400 gm) each and were transferred into (2 l) of conical flask. The first part (400 gm) was macerated into methanol, second part macerated into distilled water and third part macerated into (methanol + distilled water) (900 ml+100 ml) ratio solution and kept overnight in conical flask to get solution of extraction. After 24 hours, these three parts were filtered separately through Whatman filter paper with the aid of Buckner funnel followed by concentration on Rotary Vacuum Evaporator to get their crude extracts viz., Methanol, Ratio (methanol + water) and Water extracts.

The isolated crude extracts (Methanol, Ratio, Water) were further fractioned according to nature of compounds with hexane, chloroform and partition between n-butanol and water.

ii) Fractionation of extracts:

The dried methanol extract was macerated in some quantity of hexane at 60-80% and kept for half an hour. After half an hour, solution was filtered to get two parts: soluble and insoluble. The soluble part was further evaporated through water bath to get crude extract i.e. Methanol-hexane extract.

The methanolic hexane residue or insoluble part (obtained after maceration with hexane at 60-80ºC) was macerated in chloroform and filtered. The soluble part was evaporated

on water bath at 100ºC and extracts were collected in Petri plates and stored in refrigerator. These extracts are Methanol-chloroform extract.

The insoluble part (residue) left in the Petri plate was macerated in n-butanol and water mixture (75 ml butanol+25 ml water) in a separating funnel. The whole mixture was shaken for 1 hour and kept for overnight to separate into two layers, i.e., one layer is of butanol and second is of water. The compound present in the methanol extract was dissolved in the butanol and water layer. After 24 hours, the two layers were not separated so collected as such and evaporated in water bath and stored in refrigerator. These extracts are Methanolic extracts.

In the same way, ratio extract was dissolved in same quantity of chloroform by maceration. After one hour it was filtered through Whatman filter paper. After filtration, the filtrate (soluble part) was evaporated on water bath at 100ºC and extracts were collected in Petri plates and stored in refrigerator. This extract is known as Ratio-Chloroform Extract.

The insoluble part of extract was macerated in water and butanol mixture (75 ml butanol+ 25 ml water) in separating funnel. The whole mixture was shaken for hour and kept for overnight to separate into two layers. After 24 hours, the two layers were separated and collected separately. Then these were evaporated on water bath at 100ºC, collected on Petri plate and stored in refrigerator. The extracts obtained were Ratio-water extract and Ratio-butanol extracts.

The third extract i.e. water extract was macerated in same quantity of chloroform by maceration. The water extract remained insoluble in chloroform. So, the extracted material was evaporated and then the residues were dissolved in n-butanol and water mixture in separating funnel.

The whole mixture was shaken for 1 hour and kept for 24 hours to separate into two layers. After 24 hours, the two layers were separated and collected separately in Petri plate and stored in refrigerator. These extracts are water-water extracts and water-butanol extracts respectively.

iii) Control of building fungi through plant extract

a) Collection of Building Fungi

To carry out the study, building fungi were collected in a scientific manner from different locations of the C.B.R.I. building.

b) Isolation of building fungi

The fungi collected were isolated and cultured on potato dextrose agar medium in Petri plates. The plates were incubated in a B.O.D. incubator at 25±1ºC for 7 days for mycelia growth. Based on the morphological characters, the fungi were identified with the help of standard monographs[18].

c) Screening of extracts for antifungal activity against test building fungi

All the obtained extract was screened for their antifungal activity against test fungus by adopting food poision method[19,20,21] at 50, 100, 200, 300, 400 and 500 ppm. The pure solvent was used for control study. The experiment was performed in triplicate.

RESULTS

Fungus causing deterioration in the institute building was identified as *Aspergillus niger* van Tieghem. The concentration showing complete inhibition is known as Minimum Inhibition Concentration (MIC). The minimum inhibition concentration was determined by taking six concentrations of each herbal extract, viz., 50, 100, 200, 300, 400, 500 ppm for the study. Each concentration was taken in sterilized culture medium inoculated with *A. niger* and incubated at 28°C for 48 hours. The colony diameters of *A. niger* growing on potato dextrose agar exposed with various extracts/components isolated from flowers of *T. patula* with different solvents were measured in mm. The results are recorded in Table -1. The table shows that no growth of *A. niger* occurred at concentration of 50 ppm in case of extract I, VIII i.e., Methanolic extract and Methanol-chloroform as they worked as an strong antifungal agent of herbal origin by inhibiting the growth of *A. niger* at 50 ppm (Fig. 1) while in case of extract II, i.e. Methanol-hexane minimum inhibition concentration was observed at 200 ppm i.e., at the higher concentration of extract (Fig. 1). Extracts IV and V, i.e., Ratio-chloroform and Ratio-water also showed strong antifungal property as they inhibited the growth of *A. niger* at 50 ppm (Fig. 2) Similar results were shown by extract III and VII, i.e., Water-butanol and Ratio-butanol (Fig. 3).

Extract VI, i.e., water-water showed weak inhibition as overgrowth of *A. niger* was observed even at maximum concentration of extract, i.e., 500 ppm.

The results showed substantial differentiation because different solvents have been used to make different extracts. Extracts I, III, IV, V, VI and VIII have shown promising antifungal potential against building fungus *A.niger* at (MIC 50 ppm) while extract II has shown antifungal properties at high concentration of 200 ppm. Whereas, minimum antifungal efficacy was depicted by extract VI.

Table 1.Antifungal activity of *Tagetes patula*

Diameter (mm) of fungal growth of *Aspergillus niger* at various concentration values are mean ± (S.E.)

S.No.	Extracts	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	Control	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
1.	Methanolic extracts	*						Methanol	38.0±0.2	34.7±0.2	28.2±0.2	21.0±0.6	18.9±0.6	15±0.3
2.	Methanol-hexane extract	16±1.3	7± 1.1	*				Hexane	39.4±0.4	33.6±0.6	26.8±0.5	12.0±0	9.4±0.4	5.8±0.5
3.	Water-butanol extract	*						Butanol	36.8±0.5	33.2±0.6	23.0±0.5	12.6±0.8	10.2±0.4	7.4±0.4
4.	Ratio-chloroform extract	*						Chloroform	40.0±0.6	32.6±0.6	26.2±0.3	14.2±0.6	9.4±0.2	8.0±0
5.	Ratio-water extract	*						Water	40.0±0.6	32.6±0.6	26.2±0.3	14.2±0.6	9.4±0.2	8.0±0
6.	Water-water extract	23± 1.5	17 ± 0.4	15± 0.7	10±0.5	8± 0.4	6± 0.3	Water	40.0±0.6	32.6±0.6	26.2±0.3	14.2±0.6	9.4±0.2	8.0±0
7.	Ratio-butanol extract	*						Butanol	36.8±0.5	33.2±0.6	23.0±0.5	12.6±0.8	10.2±0.4	7.4±0.4
8.	Methanol-chloroform extract	*						Chloroform	39.0±0	30.2±0.6	25.0±0	13.8±0.5	9.4±0.4	8.2±0.3

***MIC = Minimum Inhibition Concentration**

Fig.1. Inhibition of *A.niger* at 50 ppm (MIC)

a) Methanolic extract b) Methanol-hexane extract c) Methanol-chloroform extract

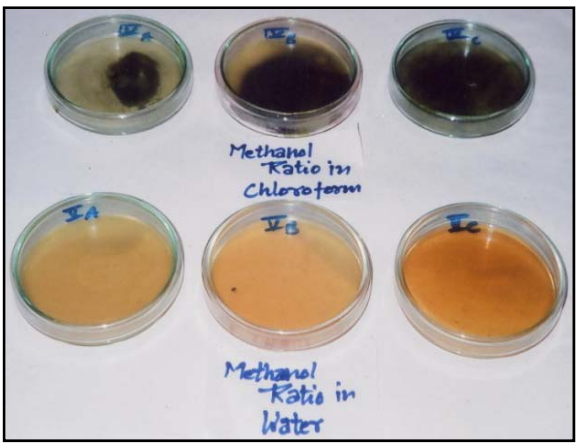

Fig.2. Inhibition of *A.niger* at 50 ppm (MIC)

a) Ratio-chloroform extract b) Ratio-water extract

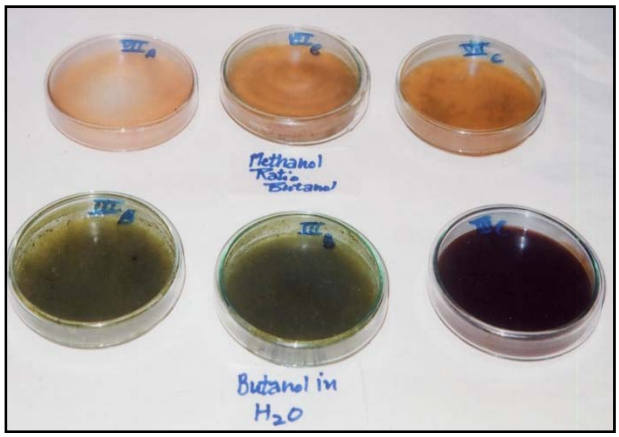

Fig.3. Inhibition of *A.niger* at 50 ppm (MIC)

a) Ratio-butanol extract b) Water-butanol extract

DISCUSSION

Utilization of extracts obtained from medicinal plant parts possessing medicinal properties has been encouraged by[22]. The variation in antifungal activity of the extracts in different solvents may be attributed to the different chemical nature of the solvents. [23]analyzed the phytochemical and mycostatic activity of leaf and flower extracts of *T.erecta* and *T.patula* and found them effective in inhibiting the growth of *Aspergillus niger*. [24]reported the efficacy of methanolic flower extracts of *Tagetes erectus* in suppressing the fungal growth of *Alternaria alternata*. Aqueous and methanol extracts of flowers of *T. erectus* were found effective in suppressing the growth of fungal pathogen *Aschochyta rabiei*[25].

CONCLUSION

The fungitoxic effects of the extract of flower of *T.patula* against *A.niger* indicates its efficacy as a source of natural fungicidal material. Extracts of *T.patula* in different solvents showed significant antifungal potency for inhibiting the mycelial growth of *A.niger* at (MIC 50 ppm). The findings of the present study confirm that the extracts of flower of *T.patula* contain ingredients that possess antifungal potential which are effective against *A.niger* (building fungus). Therefore, the use of the herbal compounds could be a significant step of using natural plant products under the current concepts of Integrated Pests and Disease Management.

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