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STUDIES ON ANTIFUNGAL ACTIVITY OF CINNAMON BARK OIL AND ITS ACTIVE CONSTITUENTS AGAINST *CHAETOMIUM GLOBOSUM KUNZE*, A DESTRUCTIVE CELLULOLYTIC FUNGUS

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ABSTRACT

Fungi causing biodeterioration of cellulose rich substances like paper and textiles are called cellulolytic fungi. They cause severe damage to our cultural heritage made of paper and textiles. A high fungal diversity has been reported in the air of Gorakhpur, including these cellulolyti fungi. The chemical fungicides used to control these fungi deface and destroy the objects and are non-biodegradable, toxic, non-ecofriendly, pollutive and have a carcinogenic risk too. In recent years, volatile constituents of higher plants, *i.e.* , many essential oils and their constituent terpenoids, have shown potent fungitoxic activity in their vapours. Of these, Cinnamon bark oil has been proved as a potent fungitoxicant against an array of fungi. Therefore, the present study has been done to investigate the antifungal activity of cinnamon (*Cinnamomum zeylanicm* Breyn) bark oil against *Chaetomium globosum* Kunze, a destructive cellulolytic fungus causing biodeterioration of paper manuscripts in Gorakhpur. The fungitoxicity was determined as minimum inhibitory concentration (MIC), minimum lethal concentration (MLC), inoculums density sustained and exposure duration for fungicidal action at MIC and hyper-MIC doses. This oil has been found effective against the test fungus. Cinnamic aldehyde has been identified as the active fungitoxic constituent of cinnamon bark oil.

KEYWORDS: Antifungal activity, Cinnamon bark oil, Active constituent, *Chaetomium globosum*, Cellulolytic fungi

INTRODUCTION

Microbial biodegradation of various cultural commodities made of paper, textile, wood and leather commonly occur everywhere in humid, tropical and sub-tropical countries of the world. These countries suffer the most by this calamity due to their hot and humid climate. Cultural heritage made of paper, textile, wood and leather, either movable or immovable, is subjected to biodegradation induced by these microbes. Of all the microorganisms, fungi are the most active ones in this process (Arroyo, 2007). In India, damage to cultural properties is enormous due to fungal biodeterioration of paper manuscripts and archival materials (Agrawal, 1995). A large number of fungi are known to degrade paper (Aranyanak, 1995). These fungi invading paper and other cellulose rich substances are called ***"Cellulolytic Fungi".*** Gorakhpur is located in the North-Eastern Uttar Pradesh of India, in the foot hills of Himalayas. It is characterized by high relative humidity and moderate temperature in most of the months (July to March), which is suitable for the growth of these cellulolytic fungi. A high fungal diversity has been reported in paper from Gorakhpur (Srivastava *et al.* 2007, 2011). Of these, *Chaetomium globosum* Kunze is one of the most frequently occurring fungus genus, which was selected as the *"test fungus"* in the present study.

The inappropriate use of synthetic fungicides to control these destructive fungi cause adverse effects on ecosystems and a possible carcinogenic risk (Research Council Board of Agriculture, 1987; Osman *et al*. 2003; Masuduzzaman *et al*. 2008; Siva *et al.* 2008). These synthetic fungicides are mostly non-biodegradable, heavily pollute the environment, adversely affect the non-target organisms and deface and destroy the cultural objects (Khan *et al*. 2010). Moreover, the fungi develop resistance against these fungicides, which in turn become ineffective (Zhonghua *et al*. 2005).

Therefore, there is an urgent need to develop new management system to reduce the dependence on synthetic fungicides. Recent trends favour the use of alternative substances derived from natural plant extracts to control these fungi. In recent years, volatile constituents of various higher plants, *i.e*., many essential oils and their constituent terpenoids, have shown potent fungitoxic activity in their vapours against a wide range of fungi (Pandey and Srivastava, 1995; Abd-Alla *et al.* 2001). These natural substances do not deface and destroy the objects including cultural properties, are biodegradable, eco-friendly, cause no pollution and non-toxic. Use of such volatiles for protection of stored foods against fungal infestation and also for controlling fungal diseases of crops has been suggested (Yaouba *et al*. 2010; Morandim *et al*. 2010; Ozcan *et al.* 2011; Mostafa *et al*. 2011; Barkat and Bouguerra, 2012; Naeini and Shokri, 2012; Shukla *et al.* 2012). A perusal of literature proves that of all these plants and their parts, cinnamon bark oil is a potent fungitoxicant against an array of fungi (Singh *et al.* 1995; Velluti *et al*. 2003; Jham *et al*. 2005; Singh and Maurya, 2005; Singh *et al*. 2007; Lopez-Malo *et al.* 2007; Bansod and Rai, 2008).

Therefore, the present investigation has been done with an aim to investigate the antifungal activities of vapours of essential oils extracted from Cinnamon (*Cinnamomum zeylanicum* Breyn) bark and its active constituents against *Chaetomium globosum* Kunze, a destructive cellulolytic fungus causing biodeterioration of paper manuscripts in Gorakhpur.

MATERIALS AND METHODS

Test Fungus

Chaetomium globosum Kunze was selected as the test fungus in the present study. It was isolated from deteriorated pages of Webster's New International Dictionary of the English Language, 1934 (Srivastava *et al.* 2011), infested unglazed papers of a book and a Ph.D. thesis (Srivastava, 2014). This fungus was examined by Direct Observation and was isolated by direct lifting with inoculation needle and by Standard Blotter Method (Neergaard and Saad, 1962) and Agar Plate Method (Czapek Dox Agar of Raper and Thom, 1949 and Streptomycin Rose Bengal Agar of Martin, 1950). The mixed culture was purified by streaking on PDA Medium.

Plant Material

Cinnamon (*Cinnamomum zeylanicum* Breyn) bark was obtained from local market of Gorakhpur.

Extraction of Essential Oil

Essential oil of the Cinnamon bark was obtained by hydrodistillation in Clevenger's apparatus followed by drying over anhydrous calcium sulphate.

Assessment of Antifungal Activity of Essential Oil

Antifungal activity of vapors of extracted essential oil was assessed by the inverted Petri plate technique (Rao and Srivastava, 1994).

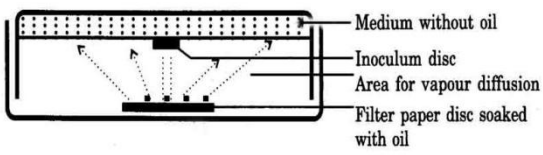

Fig. 1. Inverted Petri plate Technique

A 5 mm. diameter inoculums disc of the test fungus, cut from the periphery of the mycelial colony of a seven day old culture, was inoculated on 10 ml. Czapek Dox Agar medium in an 80 mm. diameter Petri dish. The dish was then inverted, and the requisite amount of oil in 0.5 ml. acetone, soaked on a 25 mm. diameter sterile filter paper disc, was placed inside the dish on its lid. Sterile distilled water, taken in place of oil in 0.5 ml. acetone, was used as control. Every experiment was repeated ten times and the average of results was recorded. The dishes were incubated at 25° ± 1 °C, and on 7th day, fungitoxicity was recorded as per cent inhibition of mycelial growth, calculated by the formula:

$$\% \text{ Mycelial Inhibition} = \frac{Gc - Gt}{Gc} \times 100$$

Where, Gc = Colony diameter of the control set,
Gt = Colony diameter of the treatment set.

The dose of vapors of essential oil was expressed as ppm (parts per million), *i.e.*, parts (volume) of oil per million parts of aerial volume inside the Petri dish available for diffusion of oil vapor, arbitrarily assuming that the given volume of oil volatilizes to produce an equal volume of vapor (Rao and Srivastava, 1994).

The Corning glass Petri dish (80 mm. diameter) used in this study had an average inner volume of 60 ± 2 ml., of which 10 ml. was occupied by the medium and 50 ml. medium-free aerial space was available for diffusion of oil vapour. The ppm dose of oil was calculated by progression as the amount of oil (µl) used per litre of of medium-free aerial space available for diffusion of oil vapour.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of essential oil vapour was determined by observing per cent inhibition of mycelial growth of the test fungus by progressively lower doses of oil, in

the range of 100 – 10 ppm. The minimum dose required for 100% inhibition (fungistatic/fungicidal) was recorded as the MIC (Garber and Houston, 1995).

Minimum Lethal Concentration (MLC)

The fungistatic/fungicidal nature of fungitoxicity was observed at the MIC and higher doses for determining the minimum lethal concentration (MLC), which was recorded as the minimum dose required for fungicidal action (Garber and Houston, 1995).

Nature of Fungitoxicity

For determining the nature of fungitoxicity of essential oil vapour, the treatment and control sets were prepared at MIC. After 7 days of incubation, the mycelial discs were removed from the Petri plates and re-inoculated on the fresh medium. The presence/absence of mycelia growth in the re-inoculated discs proved the fungistatic/fungicidal nature of the toxicity of vapours, respectively (Garber and Houston, 1995).

Inoculum Density Sustained

Inoculum density sustained by vapours of oil at MIC and hyper MIC doses was determined by increasing the number of inoculums discs in each assay dish of the treatment set in arithmetic progression of 2, up to a maximum of 24 discs (Rao and Srivastava, 1994).

Exposure Duration for Fungicidal Action

The Exposure Duration for Fungicidal Action was determined as the minimum duration of exposure to the MIC and higher doses of the oil vapour required for fungicidal action on one inoculums disc of test fungus (Rao and Srivastava, 1994).

Active Constituent of Oil

To study the active constituent of cinnamon bark oil, it was fractionated into non-aldehydic and aldehydic fractions (Rao and Srivastava, 1994). The aldehydic fraction was determined as cinnamic aldehyde by determination of the boiling point of the mixture of equal amounts of aldehydic fraction and a known sample of cinnamic aldehyde.

OBSERVATIONS

Table 1. MIC* and nature of fungitoxicity of *Cinnamomum zeylanicum* Breyn bark oil vapours against *Chaetomium globosum* Kunze

Concentration of Oil (ppm)	Per cent Mycelial Inhibition of *Chaetomium globosum* Kunze	Nature of Fungitoxicity** (at MIC)
10	82.6	+
20	100	-
50	100	-
100	100	-

* = **Minimum Inhibitory Concentration** (fungicidal/fungistatic)

** + = **Fungistatic Nature** (presence of mycelial growth in re-inoculated discs)

\- = **Fungicidal Nature** (absence of mycelia growth in re-inoculated discs)

Table 2. Inoculum density sustained (Number of inoculum discs of 5 mm. diameter inhibited) of the test fungus *Chaetomium globosum* Kunze and Exposure Duration for Fungicidal Action

Inoculum Density Sustained		Exposure Duration for Fungicidal Action	
At MIC dose (20 ppm)	At Hyper MIC dose (5 x MIC dose = 5 x 20 = 100 ppm)	At MIC dose (20 ppm)	At Hyper MIC dose (5 x MIC dose = 5 x 20 = 100 ppm)
2	24	48 hrs.	12 hrs.

RESULTS AND DISCUSSION

Data of Table – 1 reveal that minimum inhibitory concentration (MIC) of *Cinnamomum zeylanicum* bark oil vapours is 20 ppm dose, at which the oil shows fungicidal nature against the test fungus *Chaetomium globosum* causing biodeterioration of paper manuscripts in Gorakhpur. At 10 ppm dose also, it is effective (82.6% mycelia inhibition), but is fungistatic in nature and mycelial growth is present in re-inoculated discs. The nature of fungitoxicity reveals that at the same 20 ppm dose, the mycelial growth is absent in re-inoculated discs. Therefore, minimum lethal concentration (MLC) of the oil is also 20 ppm. Consequently, MIC and MLC, both values are 20 ppm against *Chaetomium globosum*.

Data of Table – 2 reveal that vapours of cinnamon bark oil can inhibit not more than two inoculum discs of *Chaetomium globosum* at MIC. However, at hyper MIC dose (5 x MIC), these vapours retain fungitoxicity for appreciably higher inoculums density and a maximum of 24 inoculum discs of 5 mm. diameter are inhibited. Exposure duration of the oil required at MIC is 48 hours and at hyper MIC is 12 hours.

The boiling point of aldehydic fraction was determined to be 246 ± 2° C, which was identical with that of a known sample of cinnamic aldehyde. Therefore, the active constituent of cinnamon bark oil is determined as cinnamic aldehyde.

CONCLUSION

It is therefore, concluded that vapour of essential oil of *Cinnamomum zeylanicum* Breyn bark is effectively toxic at very low dose against the selected test fungus – *Chaetomium globosum* Kunze, causing biodeterioration of paper manuscripts in Gorakhpur. Also, it can inhibit high inoculums density. Therefore, it is recommended for further detailed study under *in vivo* conditions to protect our cultural heritage in paper and textiles damaged by *Chaetomium globosum* and other cellulolytic fungi.

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