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ANTIFUNGAL POTENTIAL OF *CINNAMOMUM TAMALA* (TEJPAT) LEAF ESSENTIAL OIL AGAINST *CURVULARIA LUNATA*, A DESTRUCIVE CELLULOLYTIC FUNGUS

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ABSTRACT

Curvularia lunata (Wakker) Boedijn is a destructive cellulolytic fungus of Class – Hyphomycetes of Fungi Imperfecti. This mold fungus causes biodeterioration of our cultural heritage in paper manuscripts and books in libraries etc. Its occurrence has been reported from different libraries of the world and India, including Gorakhpur. Chemical fungicides used to control this fungus not only deface and destroy the objects, but are toxic, non-ecofriendly, non-biodegradable, highly pollutive and many of them have carcinogenic and other harmful effects too. *Cinnamomum tamala* T. Nees & C.H. Eberm. (*Tejpat* in Hindi), Family – Lauraceae contains volatile essential oils in its leaves having antimicrobial properties. In the present investigation, the fungitoxic efficacy of vapour of essential oil extracted from *Cinnamomum tamala* leaves has been tested against *Curvularia lunata*. Different parameters used to determine the antifungal potential of the oil *in vitro* are Minimum Inhibitory Concentration (MIC), Minimum Lethal Concentration (MLC) and inoculum density sustained at MIC and hyper MIC doses (fungicidal or fungistatic). It is concluded that this essential oil is effective against the test fungus – *Curvularia lunata* and can be recommended for further *in vivo* investigations. It is also suggested that *Cinnamomum tamala* leaf oil should be tested *in vitro* and *in vivo* against strains of *Curvularia lunata* causing Allergic Bronchopulmonary Diseases and Allergic Fungal Sinusitis in humans in order to explore the possibility of its use as a chemotherapeutic agent.

KEYWORDS: **Natural fungicide, Fungitoxic potential, *Cinnamomum tamala* (*Tejpat*), Essential oil, *Curvularia lunata*, Cellulolytic fungus.**

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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.[1] A large number of fungi are known to degrade paper.[2] These fungi invading paper and other cellulose rich substances are called *"Cellulolytic Fungi".* The growth of molds on paper containing cellulose is of frequent occurrence when the level of relative air humidity is high or when books become wet due to water leaks in libraries.[3] About 5,000 volumes of books were reported to be infected by molds in Virginia State Library and Archives, U.S.A.[4] However, majority of library and archival conservators have no training in microbiology.[5] In India, damage to cultural properties by fungal biodeterioration is enormous. Paper manuscripts and paintings are damaged by fungi, including *Curvularia* and other fungal genera. 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.[6-8] Of these, *Curvularia lunata* (Wakker) Boedijn 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 causes adverse effects on ecosystems and a possible carcinogenic risk too.[9-12] These synthetic fungicides are mostly non-biodegradable, heavily pollute the environment, adversely affect the non-target organisms and deface and destroy the cultural objects.[13] Moreover, the fungi develop resistance against these fungicides, which in turn become ineffective.[14] 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.[15-20] These natural substances do not deface and destroy the objects such as our 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.[21-27] A perusal of literature proves that of all, various plant parts of *Cinnamomum* spp. of Family – Lauraceae (*eg. C. tamala, C. zeylanicum, C. camphora etc.*) have shown significant fungitoxicity against an array of fungi.[18-20, 28-37] Indian Cassia lignea, *Cinnamomum tamala* Nees & Eberm. (in Hindi – *Tejpat*) of Family – Lauraceae is an evergreen tropical tree. It is mainly used as condiment for flavouring food and widely used in pharmaceutical preparations because of its hypoglycemic, stimulant and carminative properties.[36-37] The leaves of this tree have clove like taste and pepper like odour, and are used as spice. Essential oil of leaves of this plant has excellent antimicrobial properties.[38] Therefore, as a part of our ongoing research programme[17-20], the present investigation has been done with an aim to evaluate the antifungal activities of vapours of essential oil extracted from *Cinnamomum tamala* Nees & Eberm. against *Curvularia lunata* (Wakker) Boedijn, a destructive cellulolytic fungus causing biodeterioration of paper manuscripts in Gorakhpur.

MATERIALS AND METHODS

(i) Test Fungus : Curvularia lunata

Curvularia lunata (Wakker) Boedijn was selected as the test fungus in the present study, causing biodeterioration of paper manuscripts in Gorakhpur.[6-8] This fungus was examined by Direct Observation and was isolated by direct lifting with inoculation needle and by Standard Blotter Method[39] and Agar Plate Method.[40-41] (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.

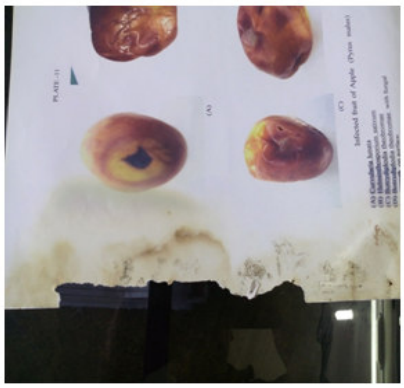

Figure 1A
Paper infested by cellulolytic fungi (Source of the test fungus – Curvularia lunata)

Figure 1B
Close up of infested portion of paper showing colonies of cellulolytic fungi

Figure 2A
Paper infested by cellulolytic fungi (Source of the test fungus – *Curvularia lunata)*

Figure 2B
Close up of infested portion of paper showing colonies of cellulolytic fungi

(ii) Plant Material

Cinnamomum tamala (Tejpat) leaves (Voucher Specimen No. 2014/109) were purchased from local market of Gorakhpur, Uttar Pradesh, stored for about four months and their identity was confirmed[42].

Figure 3
Cinnamomum tamala leaves : Source of Essential Oil (Voucher Specimen No. 2014/109)

(iii) Extraction of Essential Oil

The powdered leaves of *C. tamala* were subjected to hydro-distillation in a Clevenger apparatus for 6 hours in accordance with European Pharmacopoeia procedure[43] to get yellow volatile oil (2.3% yield) having characteristic odour and sharp taste.

Figure 4
Clevenger Apparatus for extracting essential oils

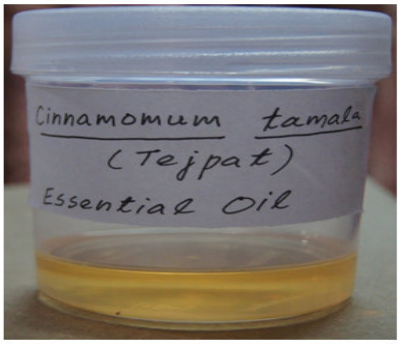

Figure 5
Essential oil of Cinnamomum tamala leaves

(iv) Assessment of Antifungal Efficacy of Essential Oil

In order to determine the antifungal efficacy of the volatile essential oil, inverted Petri plate technique was used.[44]

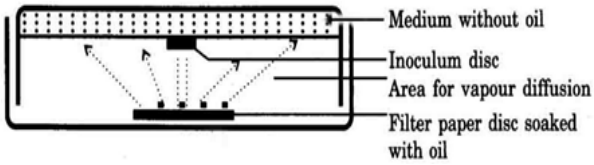

Figure 6
Inverted Petri plate Technique

A 5 mm. diameter inoculum disc of the test fungus, cut from the periphery of the mycelial colony of a seven days old pure 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 in 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 three times and the average of results was recorded. The dishes were incubated at 25° ± 1 °C, and on the 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 = Average colony diameter of the control set,

Gt = Average colony diameter of the treatment set.

The dose of vapours 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 vapour, arbitrarily assuming that the given volume of oil volatilizes to produce an equal volume of vapour.[44] 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 of the amount of oil (µl) used per litre of medium-free aerial space available for diffusion of oil vapour.

(v) 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. (100, 50, 20 and 10 ppm, respectively). The minimum dose required for 100% inhibition (fungistatic/fungicidal) was recorded as the MIC. 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.[45]

(vi) 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.[45]

(vii) 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 mycelial growth in the re-inoculated discs proved the fungistatic/fungicidal nature of the toxicity of vapours, respectively.[46]

(viii) Inoculum Density Sustained

Inoculum density sustained by vapours of oil at MIC and hyper MIC doses was determined by increasing the number of inoculum discs (of 5 mm. diameter each) in each assay dish of the treatment set in arithmetic progression of 2, up to a maximum of 22 discs.[44]

Table 1

MIC* and nature of fungitoxicity of essential oil vapours of Cinnamomum tamala Nees & Eberm. leaves against Curvularia lunata (Wakker) Boedijn

Concentration of Oil (ppm)	Per cent Mycelial Inhibition of *Curvularia lunata* (Wakker) Boedijn	Nature of Fungitoxicity** (at MIC)
10	86.4	+
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 mycelial growth in re-inoculated discs)*

Table 2

Inoculum density sustained (Number of inoculum discs of 5 mm. diameter inhibited) of the test fungus Curvularia lunata (Wakker) Boedijn 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 = 5x20 ppm = 100 ppm)	At MIC dose (20 ppm)	At Hyper MIC dose (5 x MIC dose = 5 x 20 ppm = 100 ppm)
4	22	48 hrs.	12 hrs.

RESULTS

Data of Table 1 reveal that minimum inhibitory concentration (MIC) of *Cinnamomum tamala* leaf essential oil vapours is 20 ppm dose. Data of Table 2 reveal that vapours of Cin*namomum tamala* leaf essential oil can inhibit not more than four inoculum discs of *Curvularia lunata* at MIC dose of 20 ppm. However, at hyper MIC dose of 100 ppm (5 x MIC dose), these vapours retain fungitoxicity for appreciably higher inoculum density and a maximum of 22 inoculum discs of 5 mm. diameter are inhibited. Exposure duration of the oil required for fungicidal action at MIC dose is 48 hours and at hyper MIC dose is 12 hours.

DISCUSSION

At MIC dose of 20 ppm, the oil shows fungicidal nature (100% mycelial inhibition) against the test fungus *Curvularia lunata* causing biodeterioration of paper manuscripts in Gorakhpur. At 10 ppm dose also, it is effective (86.4% mycelia inhibition), but is fungistatic in nature and mycelial growth is present in re-inoculated discs. This oil can inhibit high inoculum density at hyper MIC dose of 100 ppm.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 of the oil are 20 ppm against *Curvularia lunata*.Also, only vapour and not the oil comes in contact with the fungus. Therefore, the object infested by these cellulolytic fungi is not defaced and destroyed. This oil is obtained from natural source and is non-toxic, non-pollutive and eco-friendly too.

CONCLUSION

It is therefore, concluded that vapour of essential oil of *Cinnamomum tamala* Nees & Eberm. (in Hindi – *Tejpat*) leaves is effectively toxic at very low dose of 20 ppm against the selected test fungus – *Curvularia lunata* (Wakker) Boedijn, causing biodeterioration of paper manuscripts. As such, the oil is recommended for further detailed study under *in vivo* conditions to protect our cultural heritage in paper and textiles damaged by *Curvularia lunata* and other cellulolytic fungi.

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FUTURE PROSPECTS OF THE PRESENT INVESTIGATION

Strains of *Curvularia lunata* are reported to cause Allergic Bronchopulmonary Diseases and Allergic Fungal Sinusitis in humans. Therefore, it is suggested that *Cinnamomum tamala* leaf essential oil should be tested *in vitro* and *in vivo* against these human pathogenic strains in order to explore the possibility of its use as a chemotherapeutic agent.

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