In-vitro screening of antifungal activity of essential oils of some higher plants against *Chaetomium globosum* Kunze

Neeraj Srivastava

***Abstract*— During an investigation of Webster's Dictionary in Gorakhpur infested by cellulolytic fungi, Chaetomium globosum Kunze has been found as one of the most frequently occurring fungi, isolated from all the eight samples[1]. This fungus was selected as the test fungus in the present study. Essential oils of five plant parts viz. Cinnamomum zeylanicum Bark, Ageratum conyzoides Leaf, Aegle mormelos Leaf, Callistemon lanceolatus Leaf and Citrus aurantifolia Leaf were extracted by hydrodistillation in Clevenger's apparatus. Antifungal activity of volatile essential oils of these plant parts were determined in-vitro as per cent mycelia inhibition, by inverted Petri plate method. Cinnamomum zeylanicum (Cinnamon) Bark oil was found to inhibit the mycelial growth of this fungus completely, at the lowest dose. This oil was selected for further studies. The minimum inhibitory concentration (MIC) as well as the nature of fungitoxicity of the oil were determined. It is concluded that this oil can be used as a potent fungitoxicant at the place of chemical fungicides used, which deface and destroy the objects at which they are applied.**

***Index Terms*— Cinnamomum zeylanicum bark, essential oils, fungitoxicant, Chaetomium globosum, Cellulolytic fungi.**

I. INTRODUCTION

Plant volatiles exert potent bioregulatory action, particularly on microorganisms[2-3]. In recent years, volatile constituents of various higher plants ,*i.e.*, many essential oils and their constituents, terpenoids and alcohols have shown potent fungitoxic activity in their vapors[4-11]. Use of such volatiles for protection of stored foods against fungal infestation and also for controlling fungal diseases of crops has been suggested[12-13].

The climatic condition of Gorakhpur is characterized by high relative humidity and moderate temperature in most of the months (July to March). This climate is suitable for microbial biodeterioration of various commodities including cultural ones, made of paper, textile, wood and leather. 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[14]. In India, damage to cultural properties is enormous due to fungal biodeterioration of paper manuscripts and archival materials[15]. A large number of fungi are known to degrade paper[16]. A high fungal diversity has been reported in paper from Gorakhpur[17]. During an investigation of "Webster's New International Dictionary of the English Language (Second Edition, Unabridged, 1934), London" infested by fungi, 15 fungal genera were isolated from unglazed papers[18]. Out of these, *Chaetomium globosum* Kunze was one of the most frequent genus isolated from all the 8 samples. This fungus was selected as the test fungus in the present study.

Therefore, the present study was undertaken to collect plant samples and to test the *in-vitro* antifungal activity of their volatile essential oils against *Chaetomium globosum.*

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Neeraj Srivastava, Associate Professor, Post-Graduate Department of Botany, St. Andrew's (P.G.) College, Gorakhpur – 273001, India. Mobile: +91-9415362639

II. MATERIALS AND METHODS

A. Plant Materials: -

Five plant parts were collected from local and forest flora or obtained from local stockists. These were identified with the help of Floras (Hooker, 1872-1897; Duthie, 1960; T.N. Srivastava, 1976) and experts.

B. Extraction of Essential Oils:-

Essential oils of the five plant parts were obtained by hydrodistillation in Clevenger's apparatus.

C. Test Fungus:-

Chaetomium globosum Kunze was selected as the test fungus in the present study.

D. Antifungal Activity of Plant Essential Oils: -

Antifungal activity of vapors of extracted essential oils was assessed by the inverted Petri plate technique[13].

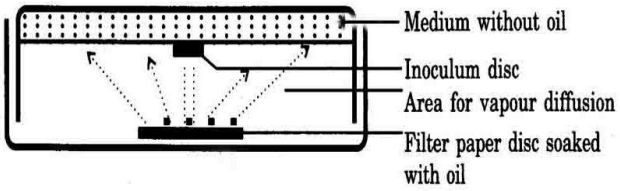

Fig. I : Inverted Petri plate Technique

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 day 7 , fungitoxicity was recorded as per cent inhibition of mycelia 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 oils 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[13].

E. MIC AND NATURE OF FUNGITOXICITY:-

The most effective oil selected on the basis of its fungitoxicity was chosen for its detailed study as minimum inhibitory concentration (MIC) and nature of fungitoxicity[19].

Minimum Inhibitory Concentration:

The minimum inhibitory concentration (MIC) was determined by observing per cent inhibition of mycelial growth of the test fungus by progressively lower doses of oil , in the range of 300 – 10 ppm. The minimum dose required for 100% inhibition (fungistatic/fungicidal) was recorded as the MIC. The fungistatic/fungicidal nature of fungitoxicity was observed[20] at the MIC and higher doses for determining the minimum lethal concentration (MLC), which was recorded as the minimum dose required for fungicidal action.

Nature of Fungitoxicity:

For determining the nature of fungitoxicity of essential oil vapors, the treatment and control sets were prepared at MIC. After 7 days 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 vapors, respectively.

III. OBSERVATIONS

Fig. II : WEBSTER'S DICTIONARY,1934

infested by fungi
(Source of the test fungus – *Chaetomium globosum)*

Fig. III : Deteriorated inner cover of the Dictionary

Fig. IV: Close up of infested inner page showing fungal colonies

Table – I
Comparative fungitoxicity of essential oil vapours of selected plant samples against *Chaetomium globosum* Kunze

Plant Species	Per cent Mycelial Inhibition of *Chaetomium globosum* Kunze per Dose of Oil		
	100 ppm	200 ppm	400 ppm
Aegle mormelos (L.) Correa (Family – Rutaceae) (Leaf)	79.8	82.0	100
Ageratum conyzoides L. (Family – Asteraceae) (Leaf)	68.2	72.2	100
Callistemon lanceolatus DC. (Family – Myrtaceae) Leaf	66.8	78.4	100
Cinnamomum zeylanicum Breyn (Family – Lauraceae) (Bark)	97.8	100	100
Citrus aurantifolia (Christm.) Swingle (Family – Rutaceae) (Leaf)	52.4	69.8	96

Table – II

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

Concentration (ppm)	Per cent Mycelial Inhibition	Nature of Fungitoxicity** (At MIC)
10	52.8	+
20	84.8	+
50	94	+
100	100	-
200	100	-
300	100	-

* = **Minimum Inhibitory Concentration**

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

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

IV. RESULTS AND DISCUSSION

Data of Table – 1 reveal that essential oil vapours of all the five plants are effective against the test fungus *Chaetomium globosum* Kunze at different doses. However, the essential oil vapour of *Cinnamomum zeylanicum* bark has been proved as the most potent one against the test fungus, by completely checking its mycelial growth at the lowest dose of 200 ppm. The least potent oil among those tested, was of the leaf of *Citrus aurantifolia* (Christm.) Swingle, which could not completely inhibit the fungal growth even at the highest dose of 400 ppm (maximum per cent mycelia inhibition recorded was 96%). Consequently, *Cinnamomum zeylanicum* bark oil was selected for further detailed study of its fungitoxic properties.

Data of Table – 2 reveal that minimum inhibitory concentration (MIC) of *Cinnamomum zeylanicum* bark oil vapours. It was recorded as 100 ppm dose, at which the oil showed fungicidal nature. At 10, 20 and 50 ppm doses also, it is effective, but is fungistatic in nature.

Therefore, the oil of *Cinnamomum zeylanicum* bark is recommended for further detailed study under *in- vivo* conditions to protect our cultural heritage in paper and textiles damaged by cellulolytic fungi like *Chaetomium globosum* etc.

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AUTHOR'S PROFILE

Name: **Dr. Neeraj Srivastava**

Present Position: Associate Professor,
Post-Graduate Department of Botany,
St. Andrew's P.G. College,
Gorakhpur – 273 001 (U.P.)
Mobile: +91-9415362639

Immediate Past Position:
Scientist (Botanist) in Botanical Survey of India
(Ministry of Environment & Forests, Govt. of India).

Educational Qualification:
1. B.Sc. (Botany, Zoology, Chemistry), 1984, First Div.
2. M.Sc. (Botany), 1986, First Div.
3. Ph.D. (Botany), 1992.

Fellow: F.B.S. (Fellow of the Indian Botanical Society).

Area of Interest: Antifungal Plant Products / Mycology & Plant Pathology.

Research Experience: **27 Years 7 Months** (January, 1987 till date), as follows:
1.For Ph.D. Degree: 5 Years 9 Months (January, 1987 to October, 1992).
2.Post-Doctoral: 21 Years 10 Months (November,1992 till date).

Teaching Experience: 20 Years 8 months, as detailed below:

(1) 5 Years 2 Months in the Dept. of Botany, University of Gorakhpur (1.1.1993 to 21.2.1998; as Young Scientist of D.S.T. and Pool Officer of C.S.I.R., New Delhi, in Lecturer pay scale).

(2) 15 Years 6 Months in the Dept. of Botany, St. Andrew's P.G. College, Gorakhpur (5.3.1999 till date):

(i) As Lecturer = 4 Years (5.3.1999 to 4.3.2003);
(ii)As Assistant Prof. = 5 Years (5.3.2003 to 4.3.2008);
(iii)As Associate Prof. = 6 Years 6 Months (5.3.2008 till date).

Fellowships Awarded:
1. J.R.F. and S.R.F. of CSIR, New Delhi.
2. Young Scientist of D.S.T., New Delhi.
3. S.R.A. (Pool Officer) of CSIR, New Delhi.

Research projects completed: 02
(Funded by D.S.T., New Delhi and U.G.C., New Delhi).

Research Publications:

(1) Res. Papers Published = 18 Nos. (In Journals like ***Mycological Research***, U.K.; ***Mycotaxon***, U.S.A.; ***Indian Phytopathology***; ***Proceedings National Academy of Sciences***, India; ***Journal of Plant Biochemistry & Biotechnology; International Journal of Biological Technology; International Journal of Research in Engineering and Bioscience; Journal of Living World and Vegetos).***

(2)Review Articles Published = 2 Nos.
Published by: i) Science Publishers, Inc., U.S.A. ; ii) SCI Tech Publishing LLC., Texas, U.S.A.

(3)Papers Presented in Conferences/Symposia = 13 Nos.
(including two International Conferences/Symposia, one of SAARC Countries and the other of Mycological Society of India).

Research projects completed/ongoing: 02 (Funded by D.S.T., New Delhi and U.G.C., New Delhi).
Research Supervision:
(1) One student has been awarded M. Phil. degree.
(2) Four students are working for Ph.D. degree and one of them has been awarded Rajiv Gandhi National Fellowship of U.G.C., New Delhi.
(3 One student has been awarded Young Scientist Fellowship of D.S.T., New
Delhi.

Workshop Attended: on November 1st , 2012 on "Biodiversity Conservation."
Attended: ***"Popular Lecture Series in Biotechnology"*** on December 5th & 6th , 2012, sponsored by Department of Biotechnology, Govt. of India.
Orientation Course Attended: One, organized by Academic Staff College, DDU Gorakhpur University and sponsored by U.G.C., New Delhi.
Refresher Courses Attended: Two, organized by Academic Staff College, DDU Gorakhpur University and funded by U.G.C., New Delhi.
Books Co-Authored : 4 Nos.
(1) A Textbook of Microtechnique and Biotechnology.
(2) Naveen Saral Botany, Vol. – I.
(3) Naveen Saral Botany, Vol. – II.
(4) Naveen Saral Botany, Vol. – III.
Scientific Articles in Magazines & News Papers : 3 Nos.
Contributions in the Corporate Life of the Institution Served/Serving:
1. Vice-President of Botanical Society.
2. Certificate of proficiency during ***"International Conference of Plant Physiologists of the SAARC Countries"***.
3. Member of Library Committee in Botanical Survey of India.
4. Drawing & Disbursing Officer (D.D.O.) in Botanical Survey of India.
5. Chaired as Judge in essay writing competition organized by Zoological Survey of India.
6. In-Charge of Departmental Herbarium of Botany Dept. in St. Andrew's P.G. College, Gorakhpur.
7. In-Charge of Departmental Library of Botany Dept. in St. Andrew's P.G. College, Gorakhpur.
8. Joint Secretary (Press & Publicity), St. Andrew's College Democratic Teachers Association.
9. In-Charge of Press & Publicity of Centenary Year & Foundation Week Celebration of St. Andrew's P.G. College, Gorakhpur, 2003.
10. Member, Publicity Committee of Annual Sports Meet, St. Andrew's P.G. College, Gorakhpur.
11. President, Botany Study Circle, St. Andrew's P.G. College, Gorakhpur.

Extra-Curricular Activities:
1. "A"-Certificate of National Cadet Corps (N.C.C.) .
2. Certificate of National Service Scheme (N.S.S.).
3. Radio talks on "All India Radio" (A.I.R.) concerning popular science.

DR. NEERAJ SRIVASTAVA.