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INVESTIGATING THE ANTIFUNGAL POTENTIAL OF PLANT DERIVED ESSENTIAL OILS AGAINST *Cochliobolus miyabeanus*

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ABSTRACT

The main objective of this study was to evaluate the antifungal activity of eight essential oils (EOs) against *Cochliobolus miyabeanus*. The essential oils had been extracted by hydro distillation in a Clevenger apparatus. Out of eight plant material used *Cinnamomum camphora* L. showed significant fungitoxic activity against *Cochliobolus miyabeanus* by poisoned food technique. Result of the present investigation showed that the EO of *Cinnamomum camphora* have a significant antifungal effect against *Cochliobolus miyabeanus* with a growth inhibition percentage of 100%.

KEYWORDS: Essential Oil, Antifungal, *Cochliobolus miyabeanus*, *Cinnamomum camphora* (L.), Clevenger, Poison Food Technique

Rice (*Oryza sativa* L.) is the most important cereal crop and serves as a primary calorie source for a large segment of the global population. It is grown in around 114 countries, with over 50 of those producing at least 100,000 tons (Reddy *et al.*, 2004). In India, more than half of the population depends on rice as a staple food. As one of the top rice-producing nations, India has approximately 44 million hectares allocated to rice farming, resulting in an annual yield of about 100 million tons. Rice cultivation spans nearly all Indian states, contributing roughly 42% to the nation's total food grain output and supporting the livelihoods of around 70% of its population (Prasad *et al.*, 2012). Fungi have a significant effect on rice quality, especially in conditions of high moisture and temperature before harvest. They can lead to various problems, such as seed abortion, seed rot, and seed necrosis, which may result in reduced or complete loss of seed germination and the emergence of diseases in subsequent stages of plant growth (Khanzada *et al.*, 2002).

A variety of synthetic antifungal drugs have been created to combat fungal infections. However, their application is often restricted due to a range of adverse effects on human health. The use of chemical and physical methods has led to considerable environmental risks and health concerns, such as toxic residues, hormonal imbalances, cancer risks, and reproductive toxicity (Pandey, 2003; Kumar *et al.*, 2007). The development of plant-based fungicides as alternatives to synthetic chemicals has become a top priority for scientists worldwide (Reddy, *et al.*, 2007). Numerous plant extracts and secondary metabolites, such as essential oils, tannins, alkaloids, and flavonoids, have shown promising in vitro antifungal properties against various fungal species (Anjorin *et al.*, 2013). The primary benefits of utilizing plant-derived antimicrobial products over synthetic chemicals include their lower toxicity to mammals, greater biodegradability, diverse mechanisms of action, and reduced occurrence of side effects (Raja, 2014). There has been an increasing interest in exploring essential oils as alternatives to traditional synthetic and chemical additives. These oils are eco-friendly, biodegradable, and do not leave harmful toxic residues in the environment (Abdel-Kader *et al.*, 2011; Yooussef *et al.*, 2016). The term "Essential Oil" (EO) was first coined in the 16th century by the Swiss physician Paracelsus von Hohenheim. Plant EOs are typically composed of intricate mixtures of natural compounds, which can be both polar and non-polar (Masango, 2005; Macwan, *et al.*, 2016). Furthermore, essential oils are well-known for their natural antifungal properties (Daferera *et al.*, 2000; Adjou *et al.*, 2012).

The main objective of the present investigation was to evaluate the antifungal activities of some essential oil against *Cochliobolus miyabeanus.*

MATERIALS AND METHODS

Plant Material

In the present study, plant materials were collected from local areas of Gorakhpur, Uttar Pradesh, India. The essential oils of 6 species of plants belonging to different families have been evaluated for their antifungal activity. (Table 1)

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Table 1: List of plant species tested for antifungal activity

S. No.	Plant Name	Part Used	Family
1.	*Cinnamonum camphora* L.	Leaf	Lauraceae
2.	*Curuma longa* L.	Leaves	Zingiberaceae
3.	*Tagetes erecta* L.	Whole Plant	Asteraceae
4.	*Putranjiva roxburghii* Wall.	Leaves	Euphorbiaceae
5.	*Polyalthia longifolia* Benth. & Hook. f.	Leaves	Annonaceae
6.	*Ocimum americanum* L.	Leaves	Labiatae
7.	*Murraya koenigii* L.	Leaves	Apiaceae
8.	*Callistemon lanceolatus* DC.	Leaves	Myrtaceae

Test Organism

The essential oils were tested against *Cochliobolus miyabeanus*. *Cochliobolus miyabeanus* (MTCC No., 2114) were ordered from Microbial Type Culture Collection (MTCC), Chandigarh. (Figure A, B, C)

Screening of essential oils for their antifungal activity

The antifungal activity of the essential oil was evaluated using the poisoned food technique (Adjou *et al.,* 2012). Initial concentrations of 1.0×10^3, 2.0×10^3, and 3.0×10^3 µL/L were created by mixing specific amounts of essential oil, which included 0.5% (v/v) Tween 80, into cooled molten PDA at 45°C. This mixture was then gently swirled in a sterile Erlenmeyer flask to ensure uniform distribution of the oil throughout the medium. Following this, 20 milliliters of the medium were carefully transferred into sterile Petri dishes (80 mm in diameter), taking precautions to avoid trapping air bubbles. For about one hour, the medium was allowed to solidify at room temperature (23 ± 2°C). Agar discs (5 mm in diameter) containing mycelia were carefully excised from the actively growing areas of a 5-day-old pure culture of the test fungus, *Cochliobolus miyabeanus*, using a sterile cork borer. These discs were then aseptically positioned at the center of Petri plates, while control plates, which contained media without plant extract, were inoculated using the same technique. Each concentration was tested in triplicate. The plates were incubated at 28 °C for fifteen days, and the colony diameter was measured on the 3rd, 4th, and 5th days. The experiments were performed in triplicate, and the inhibition rate was calculated using a formula derived from previous studies.

$$I= (D_T - D_t)/D_T \times 100$$

where, I is the inhibition rate of hyphal growth of tested fungi (%), D_T is the diameter of the hyphal growth area in the control set (mm) and D_t is the diameter of the hyphal growth area in the treated set (mm) (Schadler and George 2006; Jing *et al.,* 2014; Messgo-Moumene *et al.,* 2015) (Table 2, Graph 1)

All the results were analysed statistically.

Minimum Inhibitory Concentration (MIC)

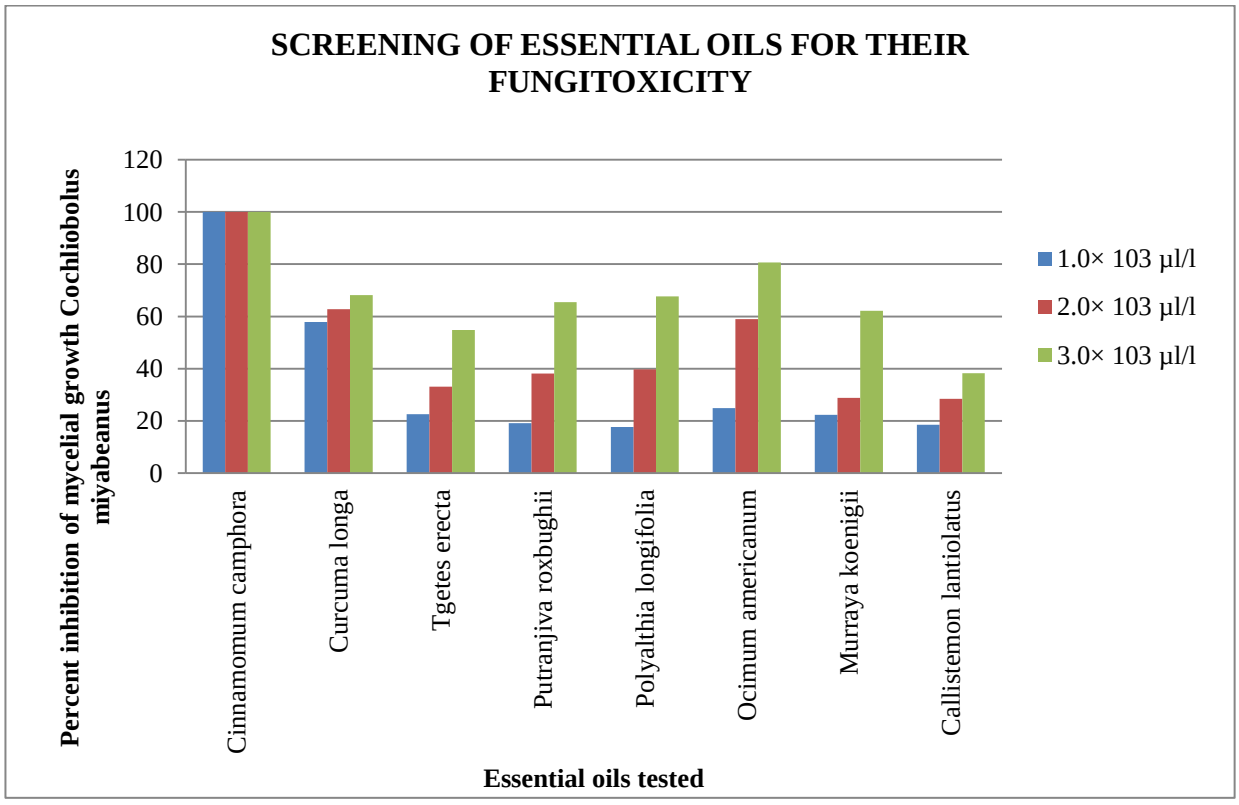
The minimum inhibitory concentration (MIC) of essential oil derived from *Cinnamomum camphora*, required to completely inhibit mycelial growth in the test fungus *Cochliobolus miyabeanus*, was determined using the Poisoned Food Technique (New, 1971). Specific volumes of the prepared extract were mixed into pre-sterilized Petri dishes containing 10 ml of molten PDA medium. Different concentrations of the essential oil were then added to the medium. The contents of the plates were thoroughly mixed in a circular motion to ensure uniform distribution of the extract. In the control groups, an equal volume of sterilized distilled water replaced the extract. The assay plates were incubated for six days at a temperature of 27 ± 2° C. Observations were recorded on the seventh day, indicate the percentage inhibition of mycelial growth. (Table 3, Graph 2)

Effect of storage

Essential oil was stored in airtight glass vials at room temperature. The antifungal activity of the stored extract was tested at intervals of 30 days upto 300 days. (Table 4).

Table 2: Screening of essential oils for their fungitoxicity

Plant Essential Oils	Concentrations	Percent Inhibition			
		3rd day	4th day	5th day	Mean ± SD
Cinnamomum camphora L. .	1.0×10^3 µl/l	100	100	100	100 ± 0.0
	2.0×10^3 µl/l	100	100	100	100±0.0
	3.0×10^3 µl/l	100	100	100	100±0.0
Curcuma longa L.	1.0×10^3 µl/l	55.5	58	60	57.83±2.25
	2.0×10^3 µl/l	60	63.4	65	62.8±2.55
	3.0×10^3 µl/l	65.67	68.8	70	68.15±2.23
Tagetes erecta L.	1.0×10^3 µl/l	20	22.32	25.3	22.54±2.65
	2.0×10^3 µl/l	29.9	33.36	36.2	33.15±3.15
	3.0×10^3 µl/l	51.56	54.7	58	54.75±3.22
Putranjiva roxburghii Wall.	1.0×10^3 µl/l	17	19.3	21	19.1±2.00
	2.0×10^3 µl/l	32.35	35	38	38.11±2.82
	3.0×10^3 µl/l	63	65.5	68	65.5±2.5
Polyalthia longifolia Benth. & Hook. f.	1.0×10^3 µl/l	15	18	20	17.66±2.51
	2.0×10^3 µl/l	37.67	39.6	42	39.75±2.16
	3.0×10^3 µl/l	65.1	68	70	67.7±2.46
Ocimum americanum L.	1.0×10^3 µl/l	21.7	25	28	24.9±3.15
	2.0×10^3 µl/l	55.56	59.2	62	58.92±3.22
	3.0×10^3 µl/l	78.6	80.46	83	80.68±2.20
Murraya koenigii L.	1.0×10^3 µl/l	19.6	22.3	25	22.3±2.7
	2.0×10^3 µl/l	25.1	29.2	32	28.76±3.47
	3.0×10^3 µl/l	59.2	62.2	65	62.13±2.90
Callistemon lanceolatus DC.	1.0×10^3 µl/l	16	18.2	21.4	18.53±2.71
	2.0×10^3 µl/l	25.2	28.1	32	28.43±3.41
	3.0×10^3 µl/l	35.3	38.2	41.2	38.23±2.95



Graph 1: Screening of essential oils against *Cochliobolus miyabeanus*

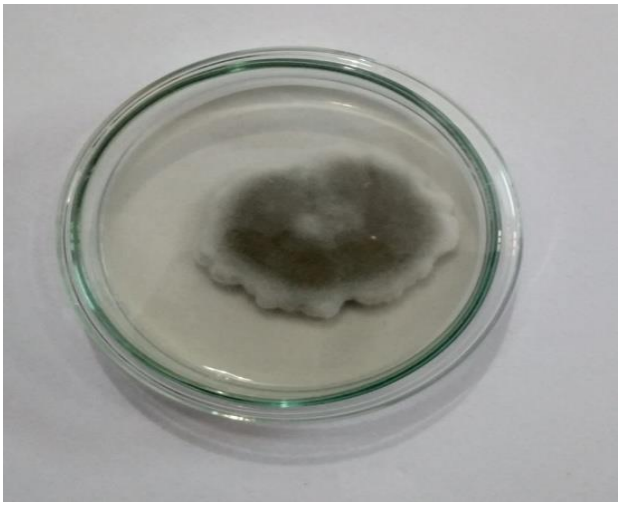


Figure A: Control (*Cochliobolus miyabeanus)*

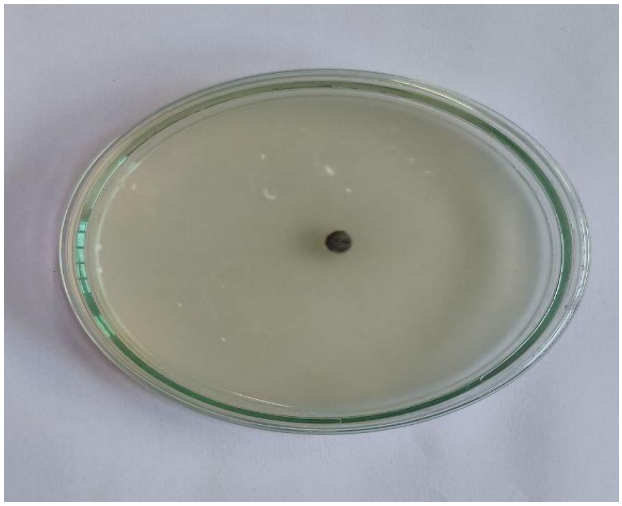
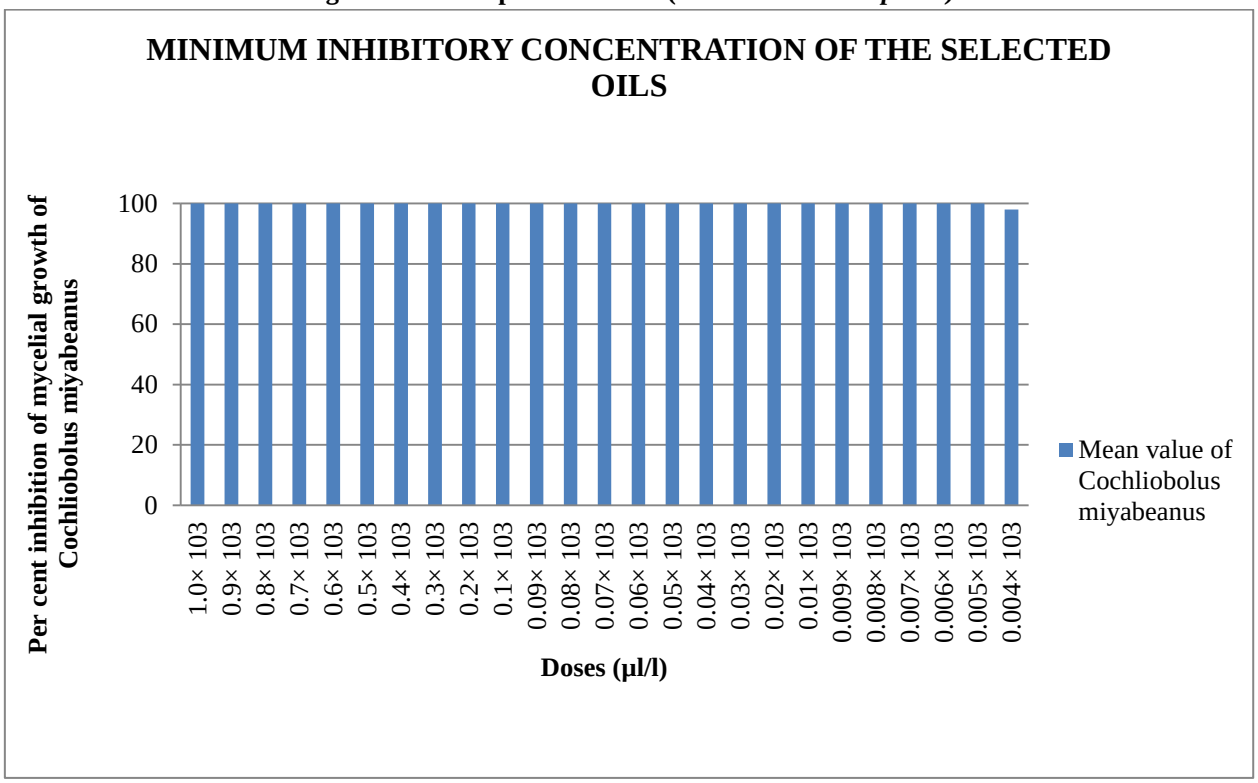


Figure B: Treatment (*Cinnamomum camphora*) on *Cochliobolus miyabeanus*

Figure C: Active plant material (*Cinnamonum camphora)*



Graph 2: Minimum inhibitory concentration of Cinnamomum *camphora* against *Cochliobolus miyabeanus.*

Effect of Temperature

A glass vial containing ten milliliters of essential oil was securely sealed. Five treatment sets were created, with each vial exposed to a different temperature: 40 °C, 60 °C, 80 °C, and 100 °C, for one hour in an oven. Once the hour was complete, the vials were taken out and allowed to cool to room temperature. The fungitoxicity of the essential oils treated at these temperatures was subsequently assessed against *Cochliobolus miyabeanus* at their respective minimum inhibitory concentrations (MIC). (Table 5).

Table 3: Minimum Inhibitory Concentration (MIC) of Essential oil of *Cinnamonum camphora*

Concentration of Essential oil (µl/l)	% inhibition of mycelial growth of *Cochliobolus miyabeanus*
1.0×10^3	100
0.9×10^3	100
0.8×10^3	100
0.7×10^3	100
0.6×10^3	100
0.5×10^3	100
0.4×10^3	100
0.3×10^3	100
0.2×10^3	100
0.1×10^3	100
0.09×10^3	100
0.08×10^3	100
0.07×10^3	100
0.06×10^3	100
0.05×10^3	100
0.04×10^3	100
0.03×10^3	100
0.02×10^3	100
0.01×10^3	100
0.009×10^3	100
0.008×10^3	100
0.007×10^3	100
0.006×10^3	100
0.005×10^3	**100**
0.004×10^3	90

Table 4: Effect of storage on fungitoxicity of Essential oil

Storage period (Days)	% inhibition of mycelial growth of *Cochliobolus miyabeanus*
30	100
60	100
90	100
120	100
150	100
180	100
210	100
240	100
270	100
300	100

Table 5: Effect of temperature on fungitoxicity of Essential oil

Temperature	% inhibition of mycelial growth of *Cochliobolus miyabeanus*
40°C	100
60°C	100
80°C	80
100°C	60

RESULTS

The present study investigated the in vitro antifungal activity of the eight essential oils against *Cochliobolus miyabeanus*. The data revealed that significant reduction in growth of *Cochliobolus miyabeanus* after an incubation period of 7 days at 28°C as observed with essential oils of *Cinnamomum camphora*, *Curcuma longa*, *Tagetes erecta*, *Putranjiva roxburghii*, *Polyalthia longifolia*, *Ocimum americanum*, *Murraya koenigii* and *Callistemon lanceolatus* and the oils showed significant differences in their efficacy are summarized in table 2. Out of eight different plant oils maximum inhibition of the pathogen was observed in *Cinnamonum camphora* (100±0.0%) followed by *Ocimum americanum* (80.68±2.20%), *Curuma longa* (68.15±2.23 %), *Polyalthia longifolia* (67.7±2.46%), *Putranjiva roxburghii* (38.11±2.82%), *Murraya koenigii* (62.13±2.90%), *Tagetes erecta* (54.75±3.22%) and *Callistemon lanceolatus* (38.23±2.95%) as compared to control. The MIC of the oil against *Cochliobolus*

miyabeanus is 0.005 µl/l concentration. The results are presented in Table 3 are based on the average of all the replication. The fungitoxicity of the oil was thermostabal up to 60˚C reported in table 4. The shelf life of the *Cinnamomum camphora* oil was found to be 300 days shown in table no.5.

DISCUSSION

The utilization of plant-based products and biocontrol agents for the management of plant diseases has become increasingly significant in recent times. This is attributed to their availability, antimicrobial properties, biodegradability, non- phytotoxic characteristics, and their capacity to enhance resistance in host plants. In this study, among the eight essential oils tested *in vitro* against *Cochliobolus miyabeanus*, *Cinnamomum camphora* demonstrated a notable inhibition of mycelial growth. Many essential oils are acknowledged for their antimicrobial effects (Derouiche, *et al.,* 2013). Previous studies have indicated that certain plants exhibit antifungal properties against a range of plant diseases (Wilson *et al.*, 1997; Al-Mughrabi *et al.,* 2001). Furthermore, secondary metabolites obtained from plants serve as valuable resources for the development of new eco-friendly bioherbicides (Batish, *et al.,* 2007). This study revealed that the antifungal efficacy of the oils increased with higher concentrations of essential oils. According to Nguefack *et al.,* (2008 and 2013), the essential oils from *C. citratus*, *O. gratissimum*, and *T. vulgaris* were particularly effective in suppressing the mycelial growth of *H. oryzae* and *A. alternata*, which are known to cause seed-borne diseases in rice. To our knowledge, this is the first report to of *Cinnamomum camphora* oils actively inhibits tha mycelial growth of *Cochliobolus miyabeanus in vitro*. The current research indicated that among the essential oils evaluated, *Cinnamomum camphora* oil was the most potent, completely inhibiting the mycelial growth of the pathogens even at a low concentration of 0.005µl/l, while other oils showed no effectiveness at this concentration *in vitro*. The biological property of an essential oil may change upon storage due to chemical modifications of its various elements (Turek and Stintzing 2012). The shelf life of the *Cinnamomum camphora* oil was found to be 300 days. The essential oil of *Cinnamomum camphora* have strong fungitoxicity, low MIC, thermostable nature, long shelf life, fungistatic/fungicidal nature against the test fungus. However, the potential use of essential oil to control seed borne fungal diseases requires a detailed analysis of their biological activity and dispersion in seed tissues and the development of formulations that inhibits the pathogens growth at non phytotoxic concentrations. In the future, this oil will be researched and evaluated in field conditions as natural fungicides.

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