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Original Research Article

Inhibitory effect of cinnamon (*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.) essential oil and its aldehyde constituents on growth and spore germination of phytopathogenic fungi

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ABSTRACT

Plant pathogens lead to severe damage of the agricultural crops, worldwide. The disease management is currently being done by the synthetic chemicals. In the present report, *Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm. oil was analyzed by gas chromatography-FID and GC/MS, which revealed (*E*)-cinnamaldehyde (40.6%), (*E*)-cinnamyl acetate (19.6%), linalool (10.2%) and benzaldehyde (3.6%) as marker constituents. Contact, volatile and spore germination inhibition assays were performed to determine the antifungal activities of *Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm. against *Rhizoctonia solani* AG 4, *Choanephora cucurbitarum* and *Bipolaris australiensis*. *C. tamala* oil showed complete fungal growth inhibition in poison food technique over concentration range of 500-560 ppm. Solid-phase microextraction analysis of drop diffusion assay revealed the presence of benzaldehyde in headspace of Petri-plate, and found to be one of the strongest antifungal compounds against tested phytopathogens. Similarly, an oil concentration of 500 ppm inhibits spore germination. Therefore, products containing such compositions can be used for the plant diseases management.

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Spore germination inhibition

1. Introduction

Natural volatiles occur as chemical constituents of various plants (vascular and non-vascular), microorganisms, fungi, insects, and marine organisms (Pare and Tumlinson, 1999; Pejin et al., 2011; Vicherová et al., 2020). Terpenoids class, synthesized as secondary metabolites from two different biogenetic pathways (mevalonic acid or MVA and methylerythritol phosphate or MEP), are the most common constituents reported from the plant kingdom. The biosynthesis is initiated by the formation of two universal precursors, isopentenyl diphosphate (IPP), and dimethylallyl diphosphate (DMAPP). Essential oils usually consist of a complex mixture of monoterpenes and sesquiterpenes, and oxygenated compounds such as alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, ethers, and esters (Dorman and Deans, 2000; Lubbe and Verpoorte, 2011). Besides, benzenoids, phenylpropanoids, and fatty acid derivatives are the other important classes identified in a number of plant materials (Dudarova et al., 2013; Pichersky et al., 2006). Essential oils have been frequently studied for their biological actions in nutritional, pharmaceutical, and agricultural sectors as a natural alternative (Bukvički et al., 2014; Erenler et al., 2018; Karan et al., 2018a; Karan et al., 2018b; Nikolić et al., 2013; Stringaro et al., 2018).

Plant diseases can cause great crop damage. Furthermore, plant pathogens have remained important constraints worldwide, on efforts to increase crop production, and productivity. The phytopathogenic effects ranged from mild symptoms to large losses of target agricultural crops. It has been noticed that pathogens infect a majority of medicinal and aromatic plants such as *Ocimum basilicum* L., *Cassia angustifolia* Vahl, *Withania somnifera* (L.) Dunal, etc. (Saroj et al., 2011; Saroj et al., 2012; Saroj et al., 2014) leading to the huge loss in terms of plant biomass and essential oils. Thus,

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the management of disease-causing organism is also a challenge to the mankind. This is mainly because their populations are variable in time, space and genotype (Strange and Scott, 2005). *Alternaria*, *Bipolaris*, *Botrytis*, *Pseudocercospora*, *Choanephora*, *Drechslera*, *Fusarium*, *Phoma*, *Rhizoctonia*, and *Verticillium* are among the most common groups that cause blights, rust, wilt, damping off, leaf spots, root rots, etc. (Alam et al., 2004; Saroj et al., 2011; Saroj et al., 2012; Saroj et al., 2014; Saroj et al., 2015).

Disease severity can be minimized by many ways like reduction of the pathogen's inoculum, inhibition of pathogenic virulence mechanisms, and by promoting crop diversity. Till date, disease management is solely based on application of synthetic fungicides such as Ridomill, Carbendazim, Bavistin, Blitox 50, etc. However, these synthetic chemicals have greatly contributed towards management from such losses. Moreover, the indiscriminate application has been identified with many side effects due to its residual toxicity, carcinogenicity, teratogenicity, hormonal imbalance, spermatotoxicity in humans (Bajaj and Ghosh, 1975; Omura et al., 1995), and resistance in pest, and fungal pathogens (Holmes and Eckert, 1999). Consequently, new pesticides including natural products are being discovered and developed. More importantly, they require to combat the evolution of fungal resistance (Copping and Duke, 2007). There has been a great interest in application of essential oils as natural substitutes for synthetic pesticides. In addition, spices and essential oils are known to exhibit significant antimicrobial activity (Wan et al., 1998). For instance, eugenol, a major component of *Syzygium aromaticum* essential oil (EO) showed inhibition at concentration of 150 µL/L, which suppressed mycelia production in *B. cinerea*, *Monilinia fructigena*, *Penicilllium expansum*, and *Phlyctema vagabunda*, whereas in combination with lecithin it protects stored apples from fungal infection (Amiri et al., 2008). Similarly, an eugenol rich EO from dried leaves of *C. tamala* was found effective against *Alternaria alternata* and *Curvularia lunata* as compared to the oil extracted from fresh cinnamon leaves (Heer et al., 2017). Cinnamon bark (*Cinnamomum verum*) and lemongrass (*Cymbopogon citratus*) essential oils showed 100% inhibition against *Rhizoctonia solani*, and *R. quercus-mongolicae* at 5 mg/paper disk (Lee et al., 2020).

In recent years, several studies on composition of essential oils and their action against various pathogenic fungi causing diseases in medicinal and aromatic plants have been reported (Bisht et al., 2011; Pragadheesh et al., 2013a; Pragadheesh et al., 2013b; Badawy et al., 2014; Elshafie et al., 2015; Matusinsky et al., 2015; Saroj et al., 2015; Lopez-Meneses et al., 2017; Perina et al., 2019; Saroj et al., 2019; Zheng et al., 2019; Parikh et al., 2020).

The genus *Cinnamomum* (family Lauraceae) is reported with twenty species in India. *Cinnamomum tamala* is a tree with medium height that has its wide distribution in the NorthWestern Himalaya, Sikkim, Assam, Mizoram and Meghalaya regions (Kirtikar and Basu, 2000). *Cinnamomum tamala* is the sole species cultivated widely for its Tejpat leaves in Bhimtal and adjoining areas of Kumaon in Uttarakhand, India (Gupta, 1968). It gives livelihood to the local people in the form of its dried leaves, which is marketed as a commodity under the segment 'spice and condiments'. Sometimes, the over production of cinnamon herbage creates imbalance in the supply chain. As a consequence, the market demand drops, which resulted in the loss of income of the farm community engaged in its cultivation. Therefore, in order to search for an alternative to minimize such losses, we have investigated the chemistry behind its aroma in the form of its EOs. In addition, the increase in survival tendency of microbes against exposure to the antimicrobial agents has prompted us to explore cinnamon as a new alternative natural agent against some selected plant pathogens. The present communication aimed to explore oil composition and antifungal action of volatile fraction of cinnamon oil and its active constituents against pathogenic fungi *Choanephora cucurbitarum*, *Bipolaris australiensis*, and *Rhizoctonia solani* AG 4. In addition, prospects of solid-phase microextraction (SPME) technique in investigation of vapor composition of Petri-plate headspace have also been reported and discussed.

2. Experimental

2.1. Plant materials and isolation of essential oil (EO)

Fresh leaves of *C. tamala* (Buch.-Ham.) T.Nees & Eberm. were collected in October 2020 from its natural habitat (Bhimtal, Uttarakhand; 1370 m above sea level; latitude 29.35°N, longitude79.56°E), India and identification was done by Dr. Subhash C Singh, Taxonomist in CSIR-CIMAP, Lucknow (specimen no. CIMAP LKO.12451). The fresh leaves were washed with distilled water and subjected to distillation using a Clevenger-type apparatus for 4 h. The collected oil was dried over anhydrous Na_2SO_4 and stored at 4 °C prior to analysis.

2.2. Gas chromatography and gas chromatography-mass spectrometry analysis

Gas Chromatography coupled with either flame ionization detection or mass spectrometry has been carried out as per our published methods (Pragadheesh et al., 2013b). A Varian CP3800 GC-FID and PerkinElmer Clarus 680 GC coupled with SQ8 C mass spectrometer fitted with 5% diphenyl column were used for the essential oil analysis. For GC analysis, a DB-5 fused silica capillary column of 30 m length and 0.25 mm i.d. with film thickness of 0.25 µm was used. The oven was programmed from 60 °C to 240 °C at the rate of 3 °C/min with 2 min isothermal time, to 280 °C at 5 °C/min. Hydrogen was used as carrier gas at constant flow rate of 1 mL/min, a split ratio of 1:40. The S/SL injector and detector (FID) temperatures were 270 °C and 300 °C, respectively.

For mass spectrometry analysis, an Elite-5 MS fused silica capillary column with dimensions similar to FSCAP

as above in GC analysis was used. The PSS injector temperature was kept at 250 °C with a split ratio of 1:50 and column oven programmed at the rate of 3 °C/min from 60 °C to 240 °C and increased to 270 °C with a final ramp rate of 5 °C/min. Helium was chosen as the carrier gas at a constant flow of 1 mL/min. Transfer line and source temperatures were 220 °C; ionization energy 70 eV; and mass scan range 40-450 amu. Compound identification was done using TurboMass NIST 2011 libraries version 2.3.0, Wiley registry of mass spectral data 9th edition, and by comparing with the mass spectral literature data (Adams, 2006).

2.3. Test standard compounds

(1*R*)-(+)-α-Pinene (Aldrich; 00306AE-256), (*R*)-(+)-limonene (Sigma-Aldrich; 10426AD-306), *p*-cymene (Aldrich; 11029KE-187), and benzaldehyde (Polyscience Corp.; S31240-216) was chosen for their antifungal activities (Pragadheesh et al., 2013a).

2.4. Test phytopathogens

Three plant pathogenic fungi like *Rhizoctonia solani* AG 4 causal organism of pyrethrum root rot (Alam et al., 2004), *Bipolaris australiensis* causal organism of senna pod rot (Saroj et al., 2011), and *Choanephora cucurbitarum* causal organism of withania wet rot (Saroj et al., 2012) were selected for antifungal activity. All three fungal species were well characterized and maintained in respective department in CIMAP, Lucknow, UP.

2.5. Poison food technique

The plant volatiles (EO) and pure standards were subjected to their antifungal activity studies by poisoned food technique (Knobloch et al., 1989; Pragadheesh et al., 2013a; Saroj et al., 2015). The plates (in agar) were prepared by adding different concentrations (0.4, 0.8, 1.2 and 1.5 mg/mL) of EO and standards like limonene, α-pinene, *p*-cymene into the PDA at 40-45 °C. In order to mix EO with PDA homogenously, a 0.1% Tween-80 was used. Plates with PDA only, served as control plate. Subsequently, all test plates were incubated at 25 °C depending upon the type of fungi: 24 h in case of C. *cucurbitarum*; 48 h for *R. solani* and 72 h for *B. australiensis*, respectively. For calculation of growth inhibition (%) of each fungus, a comparison of culture diameter between poisoned and non-poisoned Petri-plates was done using the formula as shown below (Eqn. 1):

Inhibition (%) = [(C-T)/C] × 100 (Eqn. 1)

Where

C = growth of mycelial colony in control plate

T = growth of mycelial colony in treated plate

2.6. Drop diffusion or Volatile Phase assay

Test on volatile activity was performed according to our previous reports (Pragadheesh et al., 2013a; Saroj et al., 2015). PDA (15 mL) was poured in glass Petri-plate of 90 mm diameter followed by a 5 mm disc of 5 days old culture of phytopathogens which was placed in the centre of Petri-plate. Sample amount of each of essential oil, benzaldehyde, α-pinene, limonene and *p*-cymene were taken (three different volumes; 4, 5 and 8 µL per sample per Petri-plate) for the volatile phase assay and each experiment was performed in triplicate. *C. tamala* essential oil was introduced aseptically onto the inner side of Petri-plate cover and immediately sealed with parafilm. The plates were incubated in the dark at 25°C. The mycelial growth of each fungus was measured at different time interval up to 72 h depending upon the fungal growth.

2.7. Detection of fungal growth inhibitors (compounds) in Petri-plate headspace in volatile assay using solid-phase microextraction (SPME) technique

Chemical constituents of EO, which can be volatilized at room temperatures within the headspace of Petri-plate were studied. The presence of each constituent was correlated quantitatively with the resultant observed growth inhibition using an SPME fiber. The fiber was composed of divinylbenzene/carboxen/polydimethylsiloxane coated phase of 50/30 µm thickness fitted in a manual SPME holder 24 GA (Supelco, Bellefonte, PA). The activated fibre was exposed to the headspace region of plant pathogen bearing Petri-plate at different time interval and then the fibre was desorbed into the heated GC injector. Furthermore, fungal growth inhibition was correlated with volatile fingerprint of headspace region as recorded in GC-FID and GC-MS.

2.8. Spore germination inhibition

Spores of *B. australiensis*, and *C. cucurbitarum* were harvested from two-week old cultures via, scraping in 5 mL sterile distil water. Spore suspensions were adjusted up to the concentration of 10^6 conidia/mL. The spore suspension of 100 µL was added in Eppendorf tubes having 900 µL potato dextrose broth (PDB) medium treated with EO at concentrations of 500, 600 and 800 ppm. The tubes were incubated at 25 °C for 16 h. To check the spore germination inhibition, 200 spores of each fungus were studied under the light microscope. Furthermore, the experimental design was similar to a previously published article (Badawy et al., 2014) except a few modifications.

3. Results and Discussion

3.1 Chemistry of volatile fraction of *Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.

Essential oil (EO) obtained from the leaves of *C. tamala* was analyzed using GC and GC/MS techniques (Fig. 1). The identified chemical constituents are listed in (Table 1). Accordingly, the separated EO contained more than 35 chemical constituents, of which 28 constituents were identified contributing to 90.3% of total oil

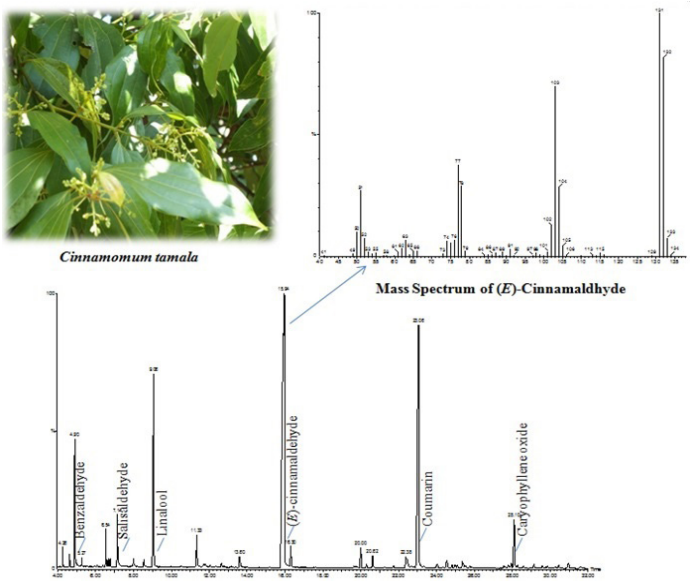

Fig. 1. Chromatogram depicting major constituents in essential oil extracted from *C. tamala* (Buch.-Ham.) T.Nees & Eberm.

Table 1
Composition of *Cinnamomum tamala* essential oil.

Constituents	RI(obs)	RI(rep)	EO % in FID	Modeb
Terpenoids				
α-Pinene	936	932	0.4	A, B, C
Camphene	952	946	0.3	A, B, C
β-Pinene	981	974	0.2	A, B, C
p-Cymene	1027	1020	1	A, B, C
Limonene	1031	1024	0.3	A, B, C
1,8-Cineole	1034	1026	0.2	B, C
cis-Linalool oxide (furanoid)	1075	1067	0.4	B, C
trans- Linalool oxide (furanoid)	1092	1084	0.4	B, C
Linalool	1104	1095	10.2	A, B, C
Borneol	1170	1165	0.2	A, B, C
Terpinen-4-ol	1179	1174	0.1	A, B, C
p-Cymene-8-ol	1181	1179	0.1	B, C
α-Terpineol	1195	1186	0.1	A, B, C
Bornyl acetate	1291	1287	0.9	A, B, C
α-Copaene	1381	1374	0.9	B, C
β-Bourbonene	1391	1387	0.1	B, C
β-Caryophyllene	1426	1417	0.2	B, C
Spathulenol	1590	1577	0.2	B, C
Caryophyllene oxide	1593	1582	2.5	B, C
Phenylpropanoids				
Hydroxycinnamaldehyde	1165	-	1.7	B, C
Methyl chavicol	1202	1195	0.1	B, C
(*Z*)-Cinnamaldehyde	1223	1217	0.6	B, C
(*E*)-Cinnamaldehyde	1281	1267	40.6	B, C
(*Z*)-Cinnamyl acetate	1392	1388	0.7	B, C
(*E*)-Cinnamyl acetate	1452	1443	19.6	B, C

Table 1 (continued)

Constituents	RI(obs)	RI(rep)	EO % in FID	Modeb
Benzenoids				
Benzaldehyde	963	952	3.6	A, B, C
Salisaldehyde	1046	1039	2.5	B, C
Others				
Coumarin	1444	1432	2.2	B, C
Total identified constituents			90.3	
Monoterpenoids			14.8	
Sesquiterpenoids			3.9	
Phenylpropanoids			63.3	
Benzenoids			6.1	
Others			2.2	

RI(obs) : Retention index observed on Equity-5 capillary column using homogeneous series of n-alkanes (C_6- C_{28} hydrocarbons, polyscience corp., Niles. IL), RI(rep) : Retention index reported as per Adams 2006, EO: essential oil, [b]A = GC co-injection with authentic sample. [b]B = GC/MS data matched with reported spectra in reference: RP Adams, 2006. [b]C = spectral matching with GC/MS library, <0.1% : not reported

composition. Phenylpropanoid class contributes 69.4% of the total identified constituents, while terpenoids constituted only 18.7% of the total profile. The marker phenylpropanoid constituents identified were (*E*)-cinnamaldehyde (40.6%) and (*E*)-cinnamyl acetate (19.6%). Among the identified terpenoids, linalool (10.2%) and caryophyllene oxide (2.5%) were found in significant proportions. Notably, coumarin (2.2%) was recorded as an important marker compound in the EO of this region. Furthermore, this finding has been well supported by some earlier reports from this region (Bisht et al., 2011; Chanotiya and Yadav, 2010).

3.2. The *in-vitro* antifungal activity

Antifungal activity of the isolated EO was assessed by mixing different oil concentrations in agar media plates or poison food and also using diffusion assay methods. Diffusion assay utilizes neat oil towards establishing activity against the target pathogens, if any. Furthermore, there is no direct contact of oil or its components with pathogens in this assay. More importantly, combination of these methods clearly gives an idea about oil components responsible for antifungal action in term of their volatility and non-volatile nature. In order to investigate how rapid a volatile component diffuses in headspace air of Petri-plate, a 50/30 µm thickness divinylbenzene/carboxen/polydimethylsiloxane coated SPME fiber was used to extract each individual followed by analysis in gas chromatographic system. The antimicrobial activity results are discussed below in detail.

3.2.1 Effect on mycelial growth inhibition (contact phase)

The antimicrobial potential of *C. tamala* EO was evaluated against the three pathogens causing severe damage to medicinal plants in Gangetic plains of India. *C. tamala* showed superior inhibition against *Choanephora cucurbitarum*, *Bipolaris australiensis*, and *Rhizoctonia solani* AG 4 where different oil concentrations resulted in complete reduction in fungal growth (Table 2, assay type: contact phase). In an earlier report, EO components such as cinnamaldehyde, and eugenol have been found to inhibit fungal growth, completely (Bullerman et al., 1977). Therefore, high proportion of aldehyde class of constituent such as (*E*)-cinnamaldehyde, benzaldehyde, salisaldehyde etc could lead to best antifungal activity as exhibited by *C. tamala* EO.

3.2.2. Drop diffusion assay

The inhibitory activity of pure benzaldehyde against *C. cucurbitarum*, *B. australiensis*, and *R. solani* AG 4 was similar to antifungal activity shown by EO in assay type contact phase. As shown in (Table 2), less growth inhibition (ca. 22%) of EO against *B. australiensis* at 5 µL/Petri-plate was recorded (assay type: volatile phase). In addition, a further 1.3-fold increase in EO concentration i.e., 6.5 µL/Petri-plate completely inhibited the growth of *B. australiensis*. Therefore, the results revealed that the higher EO concentration is required for growth inhibition of *B. australiensis* in vapour phase assay. Interestingly, benzaldehyde was the most exclusive constituents recorded in Petri-plate in SPME analysis. (*E*)-Cinnameldehyde was not recorded even in trace amount in SPME study, which indicates that this compound does not generate vapors at ambient temperature. Consequently, it could not give the desirable growth inhibition effect in diffusion assay. Notably, lower benzaldehyde concentrations in volatile assay did not change the inhibition pattern very much. Moreover, antifungal activity of pure benzaldehyde clearly suggests that the overall activity of EO in volatile phase was solely due to the presence of benzaldehyde. Similarly, findings on other compounds such as α-pinene, limonene, and *p*-cymene also indicated their moderate to low antifungal nature (Table 2, assay type: volatile phase).

Table 2
Anti-phytopathogenic potential of *Cinnamomum tamala* essential oil and its constituent against different plant pathogens

Assay type		Different samples with observed inhibition (%)								
		C. cucurbitarum			*B. australiensis*			*R. solani* AG 4		
		Observation (h)			Observation (h)			Observation (h)		
		18	21	24	24	48	72	21	24	48
Benzaldehyde	volatile$^{\Psi}$	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)
	contact*	-	-	-	-	-	-	-	-	-
Pinene	volatile$^{\Psi}$	63(1.1)	60(1.4)	25(1.1)	33(1.1)	20(1.1)	25(0.7)	57(1.1)	35(1.7)	18(1.3)
	contact*	50(1.1)	35(1.3)	38(1.8)	25(0.8)	0(0)	0(0)	25(0.8)	18(1.4)	22(1.0)
Limonene	volatile$^{\Psi}$	65(1.3)	50(1.8)	25(1.3)	0(0)	11(1.2)	6(1.1)	77(2.0)	65(0.8)	30(1.4)
	contact*	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	24(1.1)	0(0)	0(0)
p-Cymene	volatile$^{\Psi}$	80(1.8)	60(1.4)	49(1.0)	66(1.1)	33(1.1)	12(1.6)	100(0.8)	81(1.6)	42(1.6)
	contact*	62(1.7)	64(3.1)	70(1.1)	57(1.3)	21(0.5)	25(0.7)	25(0.5)	0(0)	0(0)
Essential oil	volatile$^{\Psi}$	100(0)	100(0)	100(0)	22(1.9)	18(1.8)	22(1.1)	100(0)	100(0)	100(0.5)
	contact*	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)
	MIC value(ppm)			400			500			450

$^{\Psi}$ Activity recorded at 5 µL/ Petri plate in volatile assay, * Activity recorded at 550 ppm in contact phase, MIC: Minimum inhibitory concentration, standard deviation reported in parentheses.

3.2.3. Effect on spore germination

The effect of EO on spore germination of *B. australiensis* and *C. cucurbitarum* was examined (Fig. 2). No germ tube formation was observed in microscopic studies in oil treated plates as compared to the non-treated or control plates. Moreover, MIC of EO was recorded as 500 ppm. Moreover, spore germination inhibition study was quite new and being reported for the first time for these fungi.

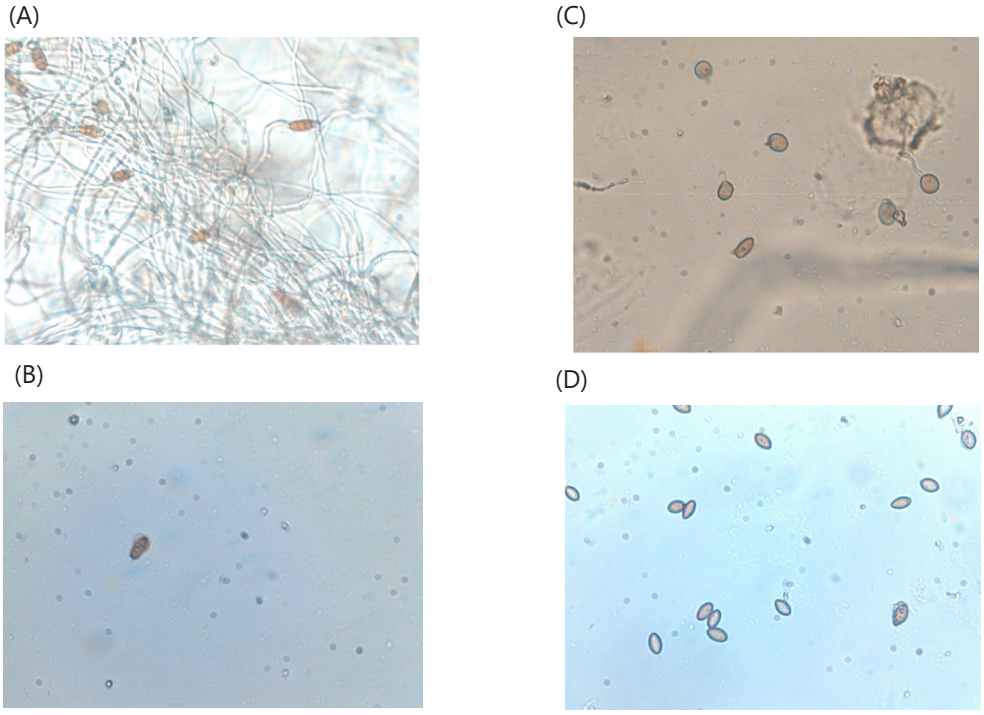

Fig. 2 Spore germination Inhibition assay of Cinnamon oil against *B. australiensis* (A control plate; B test plate) and *C. cucurbitarum* (C control plate; D test plate) against selected essential oils.

3.3. SPME analysis of Petri-plate headspace for volatile effects of *C. tamala* EO against plant pathogens

The application of solid-phase microextraction technique aids in understanding the proportion of different oil volatiles in headspace of Petri-plates at different time intervals, which can be correlated with the growth pattern of each plant pathogen. Using SPME, it is likely to associate its results with that of observed growth inhibition in volatile phase. We have observed that four major compounds such as α-pinene, linalool, *p*-cymene, and benzaldehyde were first detected in SPME within one h from complex mixture of essential oil. The area count of benzaldehyde reduced to less than half of its initial concentration after 24 h, whereas after 48 h, benzaldehyde proportion was wiped out completely from the headspace of *B. australiensis* containing experimental Petri-plate. Conversely, linalool remained in moderate proportion in vapor phase for up to 48 h and decreased gradually in course of this study. The extraction efficiency of SPME towards volatile constituents of EO in headspace of *B. australiensis*, and *C. cucurbitarum* Petri-plate are shown, separately (Fig. 3a & 3b). Interestingly, (*E*)-cinnameldehyde remained untraceable in all plates, as far as the area counts are concerned. This is probably due to the non-vaporising characteristics of (*E*)-cinnamaldehyde under ambient condition. The tendency of cinnamaldehyde to be transferred into vapor phase is very less as compared to linalool. The consistency in growth inhibition of *C. cucurbitarum* up to 24th h could be due to the availability of adequate proportion of volatile antifungal constituents such as linalool and many others in Petri-plate. Also, decrease in activity thereafter may be explained as the less volatility and less area counts of oxygenated monoterpenes/ phenylpropanoids available in headspace of Petri-plate to further inhibit the fungal growth. We conclude that antifungal activity of these essential oils was due to synergistic effect of monoterpenoids and phenylpropanoids as the major components. It is evident from an earlier report (Knobloch et al., 1989) that the class of constituents of essential oils could affect the antimicrobial activity as a whole; some constituents influenced greater potency, while some others lower, such as monoterpene hydrocarbons possessed lower antifungal and antibacterial activities as compared with oxygenated terpenoids/ phenylpropanoids. EO components such as cinnamaldehyde and eugenol are reported to inhibit fungal growth completely (Bullerman et al., 1977). Therefore, antifungal activity of the *C. tamala* oil could be due to the high proportion of (*E*)-cinnamaldehyde. In view of the present work, it has also been concluded that the cinnamon oil possesses strong spore germination inhibition potential.

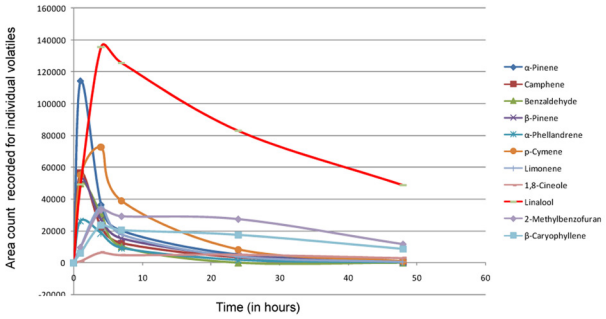

Fig. 3a. SPME volatility pattern of constituents of *C. tamala* essential oil in headspace of *Bipolaris australiensis* culture Petri-plate in drop diffusion assay.

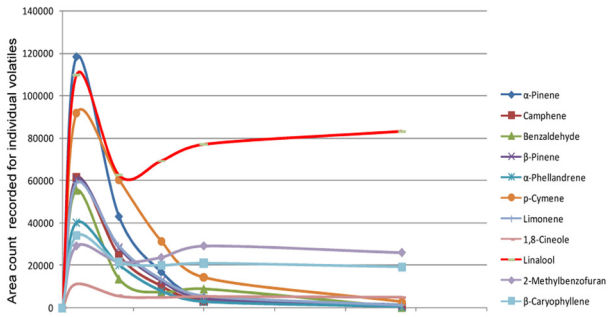

Fig. 3b. SPME volatility pattern of constituents of *C. tamala* essential oil in headspace of *C. cucurbitarum* culture Petri-plate in drop diffusion assay.

4. Concluding remarks

C. tamala EO possessed strong antifungal activity against the target plant pathogenic fungi in poison food technique. Besides, EO of *C. tamala* completely inhibits spore germination of sporulating phytopathogenic fungi at a concentration of 500 ppm. Solid-Phase microextraction technique provides valuable information in identifying the type of volatile individuals of a distinct sample having tendency to diffuese in vapor phase in headspace region of Petri-plate at ambient temperature. To the best of our knowledge, mycelial growth inhibition and spore germination inhibition behavior of chemically characterized EO and compounds like benzaldehyde has been reported for the first time. Since, essential oils are available at low cost and are less harmful to humans, these compositions are highly recommended for the treatment of medicinal plants from such phytopathogenic infestation. Importantly, the prospects to control plant diseases might be explored in field trials.

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Conflict of interest

The authors declare that there is no conflict of interest.

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