DOI : 10.5958/0974-0112.2022.00014.7

Indian J. Hortic. 79(1), March 2022: 91-98

In vitro efficacy of essential oils against *Colletotrichum gloeosporioides*, the causal agent of Anthracnose

Gajanan Gundewadi, Shalini Gaur Rudra*, Robin Gogoi[1], Haritha Bollinedi[2], S.K. Singh[3] and R.R. Sharma

Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi - 110 012, Delhi, India

ABSTRACT

***Colletotrichum gloeosporioides* Penz, the anthracnose disease causing fungal pathogen, accounts for up to 50% of spoilage of fresh fruits and vegetables. The present study explored a non-chemical approach for controlling postharvest anthracnose disease. Antifungal activities of essential oils from thyme (*Thymus vulgaris* L), betel leaf (*Piper betle*), cinnamon (*Cinnamomum verum*) and oregano (*Origanum vulgare*) were determined against *C. gloeosporioides* through in vitro poisoned food technique. Partial or complete inhibition of the pathogen was recorded using oils from thyme, betel leaf, cinnamon, and oregano at 100-400 ppm levels. Regarding the EC50 values, the antifungal activity of cinnamon oil was highest, followed by oregano, thyme and betel leaf. Characterization through GC-MS yielded the major constituents from thyme oil as thymol, cymene; caryophyllene in betel leaf oil; trans-cinnamaldehyde in cinnamon oil and carvacrol in oregano oil. The findings suggest that natural plant-based essential oils can emerge as promising agents for preventing spoilage of horticultural commodities from anthracnose disease.**

Keywords: Essential oils, Anthracnose, Thyme, Betel Leaf, Cinnamon, Oregano

INTRODUCTION

Colletotrichum gloeosporioides Penz, is the dreaded pathogen causing anthracnose disease in horticultural crops, which poses great threat to global horticulture. Anthracnose may cause upto 50% losses of fresh fruits and vegetables (Barrera-Necha *et al*., 1; Bosquez-Molina *et al*., 3) in tropical conditions prevalent in developing countries. *Colletotrichum gloeosporioides* belong to the Phyllachoraceae family of the Ascomycota division. It is known to infect a variety of fruits including mango, papaya, guava, avocado, dragon fruit etc. The highly perishable nature of these fruits makes it necessary to devise solutions that can reduce spoilage caused by anthracnose disease. Traditionally, the control of *C. gloeosporioides* is accomplished by the use of fungicides. The risks of environmental pollution, fungicidal toxicity, advancement in fungicidal resistance in pathogens against conventional pesticides and effect of chemical additives on human health, have led to the emergence of alternative strategies for reducing postharvest disease (Sivakumar and Bautista-Banos, 12).

In recent times, natural plant based essential oils have emerged as promising alternatives for protecting the horticultural commodities from postharvest infections (Dutra et al., 4; Sivakumar and Bautista-Banos, 12). Essential Oils (EOs) are aromatic and volatile secondary metabolites produced in various aromatic plants and may be extracted from leaves, bark, flowers, fruits, seeds, peels. These volatile EOs are environmentally safe and are known as "reduced-risk" pesticides, hence they have earned the generally considered as safe (GRAS) status for both human health and environment. Essential Oils are composed of an array of chemical compounds of which specific active components exhibit antimicrobial activity against several postharvest pathogens at minuscule doses without compromising the environment or consumer's well-being. However, apart from the major bioactive components identified for antimicrobial activity, minor components present in whole EOs also play critical roles and often have synergistic effects. Ultee *et al.* (14) illustrated the synergistic relation between carvacrol and its successive precursor *p*-cymene against the spoilage by *Bacillus cereus*. These compounds are predominantly found in oregano and thyme. The mode of action revealed that *p*-cymene induces swelling of the bacterial cell membranes. As*p*-cymene enables transportation of carvacrol inside the cell, they produce a synergetic effect. Similar findings have reported carvacrol, the major chemical compounds in oregano EO and thymol as synergistic compounds as they demonstrated

*Corresponding author: gaurshalini@gmail.com

[1]Division of Plant Pathology, ICAR-IARI, New Delhi, Delhi, India.
[2]Division of Genetics, ICAR-IARI, New Delhi 110 012, Delhi, India.
[3]Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, Delhi, India.

higher efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Lambert *et al.,*9). Thus, compared to pure synthetic compounds, the use of whole essential oils from tropical plants as eco-friendly techniques can solve the existing problems and boost the potentiality of fruit market. Considering the above cited points, the present investigation was conducted with the objective to screen essential oils under *in vitro* conditions to obtain the Minimum Inhibitory Concentration (MIC) and inhibition percentage against *Colletotrichum gloeosporioides*.

MATERIALS AND METHODS

Essential oils : Thyme (*Thymus vulgaris* L), Betel leaf (*Piper betle*), Cinnamon (*Cinnamomum verum*) and Oregano (*Origanum vulgare*) provided by Greenleaf Extraction Pvt. Ltd., Cochin, Kerala, India; were screened for their antimicrobial potential. The culture of fungal species *Colletotrichum gloeosporioides* was obtained from ITCC, Plant Pathology, IARI, New Delhi. Reagents and culture medium for pathological assay were obtained from Himedia, Mumbai, India. Experimental solutions were prepared by using ultra-pure Milli-Q water. Reference chemical carbendazim 50% WP was obtained from Crystal Crop Protection Pvt. Ltd. for use as positive control.

Gas Chromatography-Mass Spectroscopy analysis of these four essential oils was performed by using Focus-DSQ GC/MS (Thermo Scientific) equipped with a single quad and capillary column (30m × 0.25 mm, with the film thickness 0.25 µm). Temperature programming was conditioned at 60°C to 260°C with a column temperature of 60°C and holding time for 5 min. The temperature of the injector and detector were kept at 260°C and 230°C. Major carrier gas helium was used at the flow rate of 1 mL/min. Sample (0.4 µL) was injected with a split flow of 20 mL/min by microsyringe. Electron impact mode was kept at 70 eV with the mass range of 30-400 a.m.u at 1 scan/s. The composition of these four essential oils was identified and confirmed by using their retention time and standard library (Dutra *et al.,* 4).

Antimicrobial activity of essential oils was carried out by using poisoned food technique (Gundewadi *et al.,* 6). The selected essential oils at a desired final concentration were incorporated into a molten agar with the media temperature 40°C and mixed well. The fungal mycelia inoculum (disc of 3 mm dia) was sourced from profusely growing conidia and placed on the test Petri plates (Fig. 1), which was followed by sealing with parafilm strip to prevent contamination. In the control plates, sterilized water was incorporated while the positive control plates were prepared by adding Carbendazim 50% WP in molten agar to obtain a desired concentration of 0.1%. The inoculated plates were incubated at 28°C upto 9 days.

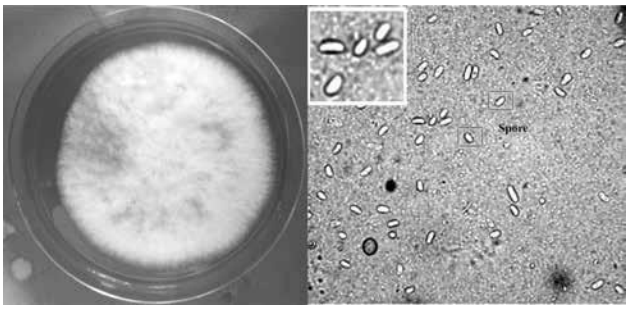


Fig. 1. Mycelia (a) and spores (b) of *Colletotrichum gloeosporioides* fungus

The radial mycelium growth (mm) of *C. gloeosporioides* was determined by measuring the diameter of the fungal colony in three different directions in all the treated plates at the different incubation time periods. Percent inhibition in radial growth was calculated using the standard formula (Gundewadi *et al.,*6):

$$\text{Inhibition Percentage (\%)} = \frac{(C-T)}{T} \times 100 \quad (1)$$

where, C is the fungal conidial growth in control plates (mm); T is the fungal conidial growth in treatments (mm).

EC_{50} value (Effective concentration for 50% inhibition), the lower and upper fiducial limits at 95% confidence level and *Chi*-square values were calculated using Probit analysis. The growth rate of the *C. gloeosporioides* mycelial disc on PDA media incorporated with essential oils at varying concentrations (50 to 400 ppm) was noted each day. The rate of growth of the disc was compared with control on the basis of first order kinetics equation (2).

$$\frac{G_i}{G_o} = \exp (K * D) \quad (2)$$

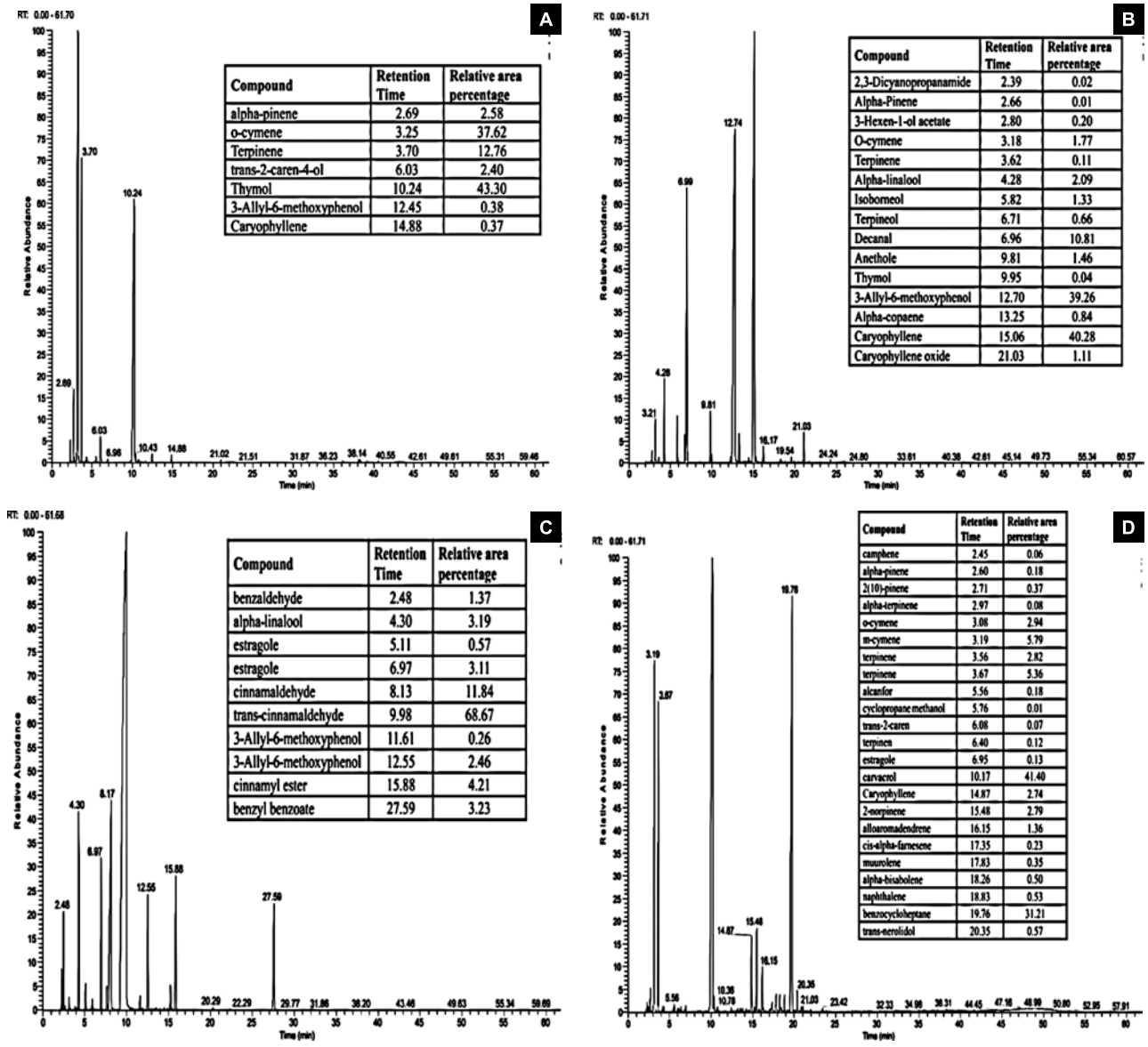
where, G_i is the diameter of mycelia disc on day i and G_o is the mycelia growth diameter in control media plates on the corresponding day

The experiment was structured out in a CRD with four replications. The results were analyzed using analysis of variance (ANOVA) and the comparison among treatment means was done using Tukey's test (post-hoc analysis) at a significance level of <0.05. All analyses were conducted using SPSS Statistics 17.0 (IBM, New York, New York, USA).

RESULTS AND DISCUSSION

The chromatogram for thyme oil obtained by GCMS revealed the presence of major compounds namely, o-cymene (37.62%, RT = 3.25 min), terpinene

(12.76%, RT = 3.70 min), thymol (43.30%, RT = 10.24 min), together revealing 99.41% composition of the thyme oil (Fig.2A). Bosquez-Molina *et al.,*(3)also reported the most dominant compounds of thyme oils to be thymol (50.48%), *p*-cymene (24.79%), linalool (4.69%), γ-terpinene and (4.14%). Similar findingswere reported by Nikolic *et al.,*(12) for *Thymus vulgaris* oil (thymol:49.10 % and p-cymene 20.01%). In case of betel leaf oil, the composition of major compounds wasdecanal (10.81%, RT = 6.96 min), 3-allyl-6-methoxyphenol (39.26%, RT= 12.70 min) and caryophyllene (40.28%, RT = 15.06 min), with a total of 99.99% of the betel leaf oil (Fig.2B). Basak and Guha(2) however enlisted the composition of *Piper betle* cv. *tamluk mitha* leaf essential oil as chavibetol (22.0%), estragole (15.8%), β-cubebene (13.6%), chavicol (11.8%), and caryophyllene (11.3%) and determined their efficacy against *Pencillium expansum* spores. In cinnamon essential oil, the chromatograph showed prominent peaks at retention times 4.30, 8.13, 9.98 and 15.88 minutes corresponding to α-linalool (3.19%), cinnamaldehyde (11.84%), *trans*-cinnamaldehyde (68.67%) and cinnamyl ester(4.21%) respectively (Fig.2C). Similar findings were also reported by Firmino *et al.,* (5) depicting (E)-cinnamaldehyde,



Compound	Retention Time	Relative area percentage
alpha-pinene	2.69	2.58
o-cymene	3.25	37.62
Terpinene	3.70	12.76
trans-2-caren-4-ol	6.03	2.40
Thymol	10.24	43.30
3-Allyl-6-methoxyphenol	12.45	0.38
Caryophyllene	14.88	0.37

Compound	Retention Time	Relative area percentage
2,3-Dicyanopropanamide	2.39	0.02
Alpha-Pinene	2.66	0.01
3-Hexen-1-ol acetate	2.80	0.20
O-cymene	3.18	1.77
Terpinene	3.62	0.11
Alpha-linalool	4.28	2.09
Isoborneol	5.82	1.33
Terpineol	6.71	0.66
Decanal	6.96	10.81
Anethole	9.81	1.46
Thymol	9.95	0.04
3-Allyl-6-methoxyphenol	12.70	39.26
Alpha-copaene	13.25	0.84
Caryophyllene	15.06	40.28
Caryophyllene oxide	21.03	1.11

Compound	Retention Time	Relative area percentage
benzaldehyde	2.48	1.37
alpha-linalool	4.30	3.19
estragole	5.11	0.57
estragole	6.97	3.11
cinnamaldehyde	8.13	11.84
trans-cinnamaldehyde	9.98	68.67
3-Allyl-6-methoxyphenol	11.61	0.26
3-Allyl-6-methoxyphenol	12.55	2.46
cinnamyl ester	15.88	4.21
benzyl benzoate	27.59	3.23

Compound	Retention Time	Relative area percentage
camphene	2.45	0.06
alpha-pinene	2.60	0.18
2(10)-pinene	2.71	0.37
alpha-terpinene	2.97	0.08
o-cymene	3.08	2.94
m-cymene	3.19	5.79
terpinene	3.56	2.82
terpinene	3.67	5.36
alcanfor	5.56	0.18
cyclopropane methanol	5.76	0.01
trans-2-caren	6.08	0.07
terpinen	6.40	0.12
estragole	6.95	0.13
carvacrol	10.17	41.40
Caryophyllene	14.87	2.74
2-norpinene	15.48	2.79
alloaromadendrene	16.15	1.36
cis-alpha-farnesene	17.35	0.23
muurolene	17.83	0.35
alpha-bisabolene	18.26	0.50
naphthalene	18.83	0.53
benzocycloheptane	19.76	31.21
trans-nerolidol	20.35	0.57

Fig. 2. GCMS profile of (A) Thyme (*Thymus vulgaris* L); (B) Betel Leaf (*Piper betle)*; (C) Cinnamon (*Cinnamomum verum)* and (D) Oregano (*Origanum vulgare*) Essential Oils

cinnamoyl (E)-acetate, and eugenol at 68.7%, 71.2%, and 6.33%, respectively as the major components of cinnamon oil.

Similarly, oregano oil recorded constitution as: *m*-cymene (5.79%, RT = 3.19 min), terpinene (5.36%, RT = 3.67 min), carvacrol (41.40%, RT = 10.17 min), benzo-cyclo-heptane (31.21%, RT = 19.76 min) giving a total of 99.79 % of the oil composition as shown in Fig. 2D.

The hydrophobic property of EO has been known to induce mechanical disruption and ion leakage from the cell membrane due to the imbalance in H^+ and K^+ ion gradients leading finally in collapse of cellular compartmentalization and death of fungi (Sivakumar and Bautista-Baños, 12). The effect of thyme oil on *C. gloeosporioides*is presented in Table 1. The MIC and $C_{max}$of thyme essential oil under *in vitro* condition were observed to be50 ppm and 200 ppm, respectively (Figure 3A). While control treatment showed no inhibition (0%), treatments with EO of 50 ppm and 100ppm showed significant mycelial growth inhibition (0.37% & 91.11%, respectively). The EO concentrations of 200, 400, 800 and 1000 ppm recorded complete inhibition (100%) after prolonged incubation period (9 days) of *C. gloeosporioides*. In a similar study, thyme and Mexican lime essential oil were employed for controlling *C. gloeosporioides* in papaya fruit (Bosquez-Morila *et al.,* 3). 600 ppm thyme oil concentration was reported to show complete inhibition of *C. gloeosporioides* and *Rhizopus stolonifer*. Present study reveals that thyme oil can play an important role in inhibition of fungal flora associated with post-harvest fruits spoilage and in particular, *C. gloeosporioides*.

In Indian folklore medicine, betel leaf is popular as a mouth freshener, antiseptic and is applied on wounds and lesions for its healing properties. Besides, chemo-preventive effect of betel leaves have been demonstrated on lowering of benzopyrene induced forestomach papillomas in Swiss mice (Sripadha, 13). The betel leaf oil mainly contained caryophyllene and 3-Allyl-6-methoxyphenol (Fig. 2B). Sripadha (13) have also reported 3-Allyl-6-methoxyphenol as the major phenolic compound in betel leaf oils. In case of betel leaf oil, the MIC andC_{max} essential oil under *in vitro* conditions were found to be 50 ppm and 400 ppm, respectively. The 50 ppm, 100 ppm and 200 ppm concentrations were found to inhibit the mycelial growth by 0.37 %, 64.07 % and 73.33 % respectively as compared to control (Table 1).

Betel leaf oil at concentrations of 400, 800 and 1000 ppm demonstrated complete inhibition (100%) after 9th day of incubation of *C. gloeosporioides* (Fig. 3B). It showed reasonably good antimicrobial activity against *C.gloeosporioides* under *in vitro* conditions. Similar findings on antimicrobial activity of betel leaves have been reported by Pawar *et*

Table 1. Effect of thyme and betel leaf oils on mycelial growth inhibition (%) of *Colletotrichum gloeosporioides*

Essential oil	Concentration (ppm)	Mycelial growth inhibition (%)				Mean
		Incubation period (days)				
		3	5	7	9	
Thyme	50	90.74±0.05^{b}	74.07±0.05^{b}	47.22±0.00^{b}	0.37±0.05^{a}	53.10
	100	97.41±0.00^{c}	92.41±0.06^{c}	90.56±0.07^{c}	91.11±0.00^{b}	92.87
	200	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100
	400	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100
Control	Positive Control	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100
	Negative Control	68.89±0.08^{a}	27.04±0.49^{a}	0.00±0.00^{a}	0.00±0.00^{a}	23.98
	Mean	92.84	82.25	72.96	65.25	
Betel leaf	50	77.04±0.05^{b}	54.44±0.08^{b}	26.30±0.12^{b}	0.37±0.05^{a}	39.54
	100	96.44±0.00^{c}	78.33±0.04^{c}	63.52±0.19^{c}	64.07±0.12^{b}	75.59
	200	100±0.00^{d}	88.15±0.05^{d}	73.70±0.05^{d}	73.33±0.00^{c}	83.80
	400	100±0.00^{d}	100±0.00^{e}	100±0.00^{e}	100±0.00^{d}	100
Control	Positive Control	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100
	Negative Control	68.89±0.08^{a}	27.04±0.49^{a}	0.00±0.00^{a}	0.00±0.00^{a}	23.98
	Mean	90.39	74.66	60.59	56.30	

All data are means of three replicate samples. Means with different uppercase letters within a column are significantly different at $p<0.05$

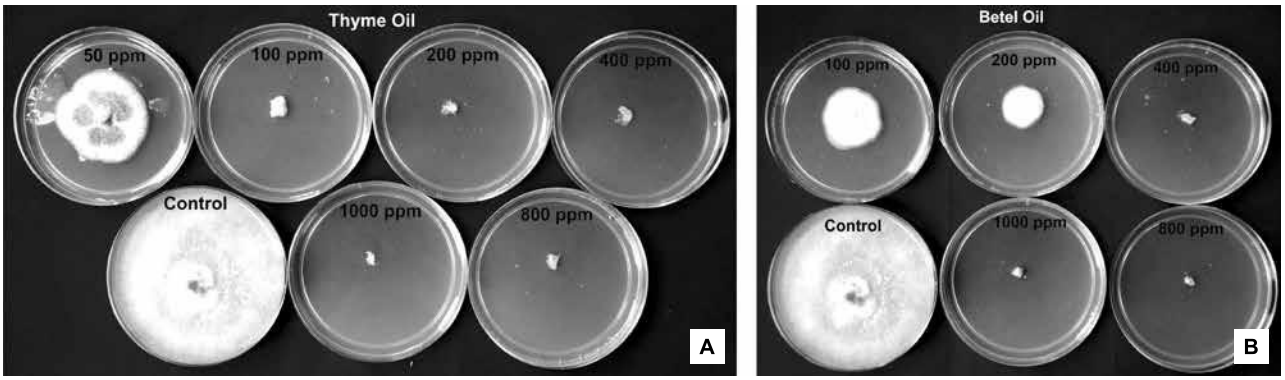


Fig. 3. Effect of (A) Thyme oil and (B) Betel leaf oil on percent inhibition of *Colletotrichum gloeosporioide*

al.,(11) who investigated the effect of ethyl acetate, hexane and ethanol-methanol extracts of betel leaves againstc*Aspergillus niger, wild Aspergillus sp.* and *Rhizopus sp.* They reported effective antifungal activity of the 50µL ethyl acetate extract. Reports on use of betel leaf oil for antimicrobial purposes are however limited (Pawar *et al.,* 11). This is the first report on use of betel leaf oil for control of *Colletotrichum gloeosporioides* responsible for the devastating anthracnose disease in subtropical and tropical fruits. Use of essential oil derived from same agro-climatic region as the area of fruits production can provide impetus for the adoption of technology.

In the case of cinnamon essential oil which is rich in trans-cinnamaldehyde, the MIC and C_{max} were found to be 50 ppm and 100 ppm, respectively. The concentration of 50 ppm and 100 ppm inhibited the mycelial growth by 30.74 % and 93.70 % respectively (Table 2). Cinnamon oil at 200, 400, 800 and 1000 ppm concentrations showed complete inhibition of *C. gloeosporioides* even after 9th day of incubation (Fig. 4A). Similarly, in the case of oregano essential oil which is rich in carvacrol and benzo-cycloheptane, the MIC and C_{max} of thyme essential oil under *in vitro* conditions were found to be 50 ppm and 200 ppm, respectively. The concentration of 50 ppm and 100 ppm in growth media could inhibit the mycelial growth by 31.11 % and 58.15 % respectively

Table 2. Effect of Cinnamon and Oregano oils on mycelial growth inhibition (%) of *Colletotrichum gloeosporioides*

Essential oil	Concentration (ppm)	Mycelial growth inhibition (%)				Mean
		Incubation period (days)				
		3	5	7	9	
Cinnamon	50	87.78±0.08^{b}	58.89±0.24^{b}	46.30±0.58^{b}	30.74±0.71^{b}	55.93
	100	97.78±0.08^{c}	96.30±0.00^{c}	94.81±0.00^{c}	93.70±0.00^{c}	95.65
	200	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100±0.00^{c}	100
	400	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100±0.00^{c}	100
Control	Negative Control	81.11±0.08^{a}	43.33±0.22^{a}	29.63±0.12^{a}	0.00±0.00^{a}	38.52
	Positive Control	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100±0.00^{c}	100
	Mean	93.33	79.70	74.15	64.89	
Oregano	50	82.96±0.26^{b}	70.37±0.29^{b}	54.44±0.16^{b}	31.11±0.08^{b}	59.72
	100	98.01±0.00^{c}	79.63±0.05^{c}	71.85±0.05^{c}	58.15±0.05^{c}	77.41
	200	100±0.00^{c}	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100
	400	100±0.00^{c}	100±0.00^{d}	100±0.00^{d}	100±0.00^{d}	100
Control	Negative Control	69.63±0.12^{a}	34.07±0.09^{a}	8.89±0.08^{a}	0.00±0.00^{a}	28.15
	Positive Control	100±0.00^{d}	100±0.00^{d}	100±0.00^{c}	100±0.00^{c}	100
	Mean	91.77	80.68	72.53	64.88	

All data are means of three replicate samples. Means with different uppercase letters within a column are significantly different at $p<0.05$

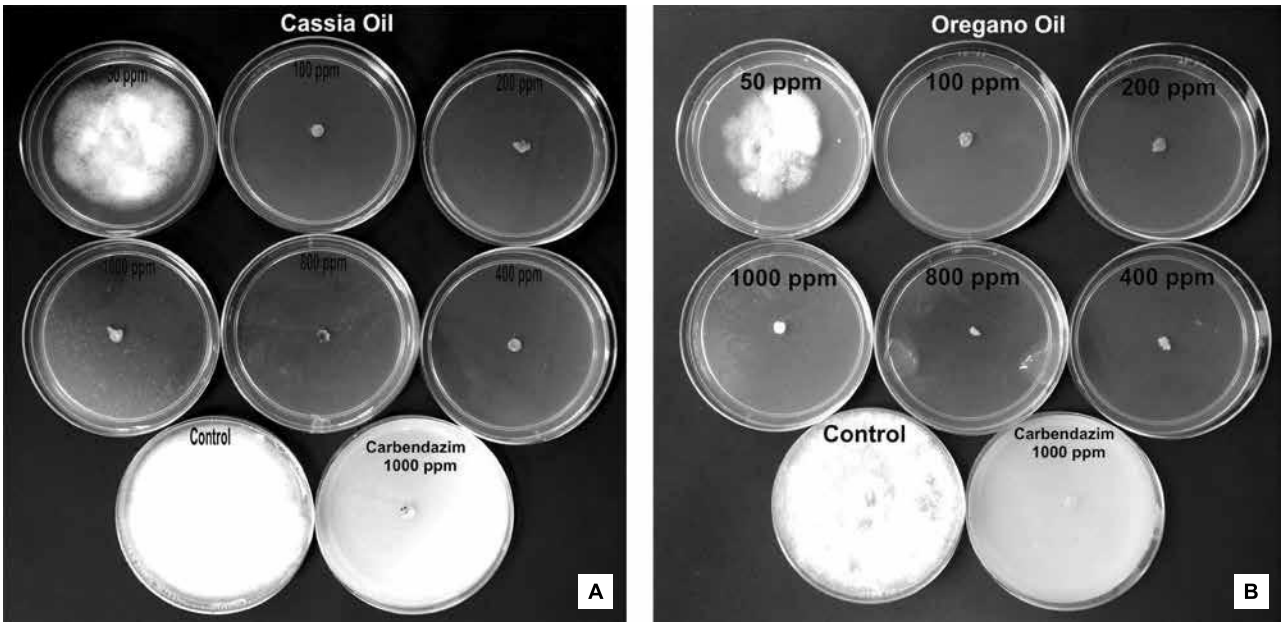


Fig. 4. Effect of (A) Cinnamon oil and (B) Oregano oil on percent inhibition of *Colletotrichum gloeosporioides*

as compared to control (Table 2). The observed effectiveness for the oils was found higher than the reports by previous researchers. Idris *et al.,* (8) investigated the use of essential oils such as basil, cinnamon, and rosemary for control of anthracnose disease caused by *C. musae* in banana. According to their findings, cinnamon oil at 250, 500 and 750 ppm concentrations showed radial growth inhibition of 72.34%, 100%, and 100% respectively after 7th day of incubation. Barrera-Necha *et al.,*(1) reported that *Cinnamomum zeylanicum* essential oil at 200 ppm completely inhibited the conidial germination and growth of *C. gloeosporioides* under *in vitro* conditions while working for controlling postharvest anthracnose disease in papaya. Most of the investigations suggested that cinnamon oil at a concentration of 200-300 ppm is sufficient to inhibit the *C. gloeosporioides* under *in vitro*.

Oregano oil at 200, 400, 800 and 1000 ppm levels recorded complete inhibition (100%) upto 9th day of incubation (Fig. 4B). The concentration of 50 ppm and 100 ppm in growth media could inhibit the mycelial growth by 31.11 % and 58.15 % respectively as compared to control (Table 2). Several researchers have documented the antifungal effect of essential oil from oregano (Dutra *et al.,*4; Lambert *et al.,* 9).

Based on the antifungal activity of EO against C. gloeosporioides on 9th day of incubation, ED50 values, determined using probit analysis were in the following order: cinnamon oil (58.243 ppm)> oregano oil (73.657 ppm)> thyme oil (77.425 ppm)> betel leaf oil (110.726 ppm) (Table 3). Our findings are in the IC_{50} values range reported by Xie *et al.,*(15) for *Origanum vulgare* (79.1 µg/mL); *Thymus vulgaris* (130.1 µg/mL); *Cinnamomum zeylanicum* (96.9 µg/ mL) against white-rot fungus *Trametes hirsuta* and brown-rot fungus *Laetiporus sulphureus*. Fiducial limits also confirmed that the effectiveness of essential oils against *C. gloeosporioides* (Table 3).

The rate of growth of *C. gloeosporioides* in media containing EOs is reported in Table 4. The k values in terms of mycelia growth per day were found to be the lowest for oregano followed by cinnamon, thyme and betel leaves in increasing order. These findings are in agreement to the MIC, ED_{50} and C_{max} values recorded against *C. gloeosporioides* mycelia in this study.

EOs have been reported to have antifungal activity against several postharvest pathogens in tropical fruits and vegetables and are considered to be safer for environment. In this work, the antifungal activities of four essential oil namely thyme, betel leaf, cinnamon, and oregano essential oils were evaluated against *C. gloeosporioides,* the causal agent of anthracnose disease in tropical and sub-tropical fruits. EOs above 400 ppm were found to completely inhibit the growth of *C. gloeosporioides*. The most effective essential oils for reducing the growth of tested pathogen was cinnamon oil followed by thyme, oregano and betel leaf oil at all tested concentrations. Therefore, essential oils could be used as a potential source of eco-friendly botanical fungicides for fruits during storage and transit. However further studies are required for large scale

Table 3. Probit analysis for effective dose (ED_{50}) and fiducial limits of EO against *Colletotrichumgloeosporoides*

Essential Oil	Incubation period (days)	ED_{50}	95% FL (Lower-Upper)	χ2 (df=4)	Sig
Thyme	3	10.761	0.565 - 22.834	0.639	0.959
	5	22.727	10.981 – 33.128	3.475	0.482
	7	49.938	41.897 - 56.669	2.359	0.670
	9	77.425	65.747 – 89.496	8.665	0.070
Betel leaf	3	24.914	11.150 - 34.728	5.730	0.972
	5	46.596	35.324 - 56.841	5.067	0.281
	7	85.657	53.371 - 118.446	14.353	0.006
	9	110.726	56.102 – 184.974	38.707	0.000
Cinnamon	3	14.108	1.915 – 25.819	0.944	0.918
	5	39.741	29.790 – 47.293	4.762	0.313
	7	48.102	28.740 – 61.217	7.442	0.114
	9	58.243	35.521- 76.024	12.612	0.013
Oregano	3	17.983	4.401 – 29.085	4.962	0.291
	5	35.326	5.657 – 55.075	10.587	0.032
	7	51.278	24.010 – 70.042	11.575	0.021
	9	73.657	52.093 – 95.754	13.436	0.079

Table 4. First order kinetics of different essential oil treatments for zone of inhibition of *Colletotrichum gloeosporioides*

Essential oil	Intercept (A)	Rate of growth, k (cm/ day)	R^2	Variance explained
Control	0.031	0.230	0.947	89.600
Thyme	0.054	0.136	0.838	70.199
Betel	0.005	0.171	0.926	85.715
Cinnamon	0.043	0.119	0.959	91.915
Oregano	0.036	0.104	0.958	91.344

trials in the context of sustainable postharvest disease management. Besides, the flavor tainting aspects of specific essential oils need to be considered for each fruit in particular.

AUTHORS' CONTRIBUTION

Conceptualization of research (SGR, RRS, SKS), Designing of the experiments (RG, SGR, GG), Contribution of experimental materials (RG, HB), Execution of field/ lab experiments and data collection (GG, RG, HB). Analysis and Interpretation of data (GG, SGR, RG). Preparation of the manuscript (GG, SGR).

DECLARATION

The authors declare no conflict of interest in this study.

ACKNOWLEDGMENTS

The authors are thankful to Post Graduate School, ICAR-Indian Agricultural Research Institute for supporting the research through fellowship to Gajanan Gundewadi. We acknowledge the support of Greenleaf Extractions Pvt. Ltd., Cochin, Kerala, India for providing the essential oils.

REFERENCES

1. Barrera-Necha, L.L., Bautista-Baños, S., Flores-Moctezuma, H.E., Estudillo, A.R. 2008. Efficacy of essential oils on the conidial germination, growth of *Colletotrichum gloeosporioides* and control of postharvest diseases in papaya (*Carica papaya* L.). *Plant Path. J.* **7**: 174-78.

2. Basak, S. and Guha, P. 2015. Modelling the effect of essential oil of betel leaf (*Piper betle* L.) on germination, growth, and apparent lag time of *Penicilliumexpansum* on semi-synthetic media. *Intl. J. Food Microbiol.* **215**: 171-78.

3. Bosquez-Molina, E., Ronquillo-de Jesús, E., Bautista-Baños, S., Verde-Calvo, J.R., Morales-López, J. 2010. Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. *Postharvest Biol. Technol.* **57**: 132-37.

4. Dutra, T.V., Castro, J.C., Menezes, J.L., Ramos, T.R., do Prado, I.N., Junior, M.M. and de Abreu Filho, B.A. 2019. Bioactivity of oregano (*Origanum vulgare*) essential oil against *Alicyclo bacillus spp*. *Ind. Crops Prod.* **129**: 345-49.

5. Firmino, D.F., Cavalcante, T.T., Gomes, G.A., Firmino, N., Rosa, L.D., de Carvalho, M.G. and Catunda Jr, F.E. 2018. Antibacterial and anti-biofilm activities of *Cinnamomum sp*. essential oil and cinnamaldehyde: Antimicrobial activities. *Sci. World J.* 1-5

6. Gundewadi, G., Sarkar, D.J., Rudra, S.G. and Singh, D. 2018. Preparation of basil oil nanoemulsion using *Sapindus mukorossi* pericarp extract: Physico-chemical properties and antifungal activity against food spoilage pathogens. *Indl. Crops Prod.* **125**: 95-104.

7. Hyldgaard, M., Mygind, T. and Meyer, R.L. 2012. Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. *Frontiers Microbiol.* 3: 1-12.

8. Idris, F.M., Ibrahim, A.M., Forsido, S.F., 2015. Essential oils to control *Colletotrichum musae in vitro* and *in vivo* on banana fruits. *Am.-Eurasian J. Agric. & Envt. Sci.* **15**: 291-302.

9. Lambert, R.J.W., Skandamis, P.N., Coote, P.J. and Nychas, G.J. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Applied Microbiol.* **91**: 453-62.

10. Nikolic, M., Glamoclija, J., Ferreira, I.C., Calhelha, R.C., Fernandes, Â., Markovic, T., Markovic, D., Giweli, A. and Sokovic, M. 2014. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L. *Indl. Crops Prod.* **52**: 183-90.

11. Pawar, S., Kalyankar, V., Dhamangaonkar, B., Dagade, S., Waghmode, S. 2017. Biochemical profiling of antifungal activity of Betel leaf (*Piper betle* L.) extract and its significance in traditional medicine. *J. Advanced Res. Biotechnol.* **2**: 1-4.

12. Sivakumar, D. and Bautista-Baños, S. 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Prot.* **64**: 27-37.

13. Sripradha, S. 2014. Betel leaf-the green gold. *J. Pharm. Sci.* **6**(1): 36.

14. Ultee, A., Kets, E.P.W. and Smid, E.J. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied Envt. Microbiol.* **65**: 4606-10.

15. Xie, Y., Wang, Z., Huang, Q. and Zhang, D. 2017. Antifungal activity of several essential oils and major components against wood-rot fungi. *Indl. Crops Prod.* **10**: 278-85.

Received : June, 2020; Revised : February, 2022;
Accepted : March, 2022