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In-vitro evaluation of essential oil against the growth of *Colletotrichum gloeosporioides* (Banana anthracnose)

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Abstract
Anthracnose (*Colletotrichum gloeosporioides*) is one of the major constraints to production and productivity of worldwide. Toxicity to non-target organisms, pathogens resistance development, environmental contamination, are among the limitations for using the synthetic chemicals as control options. Although essential oils treatments are reported to be safe and effective in controlling of fungal development. The present study was conducted to investigate the effect of essential oils treatments on banana anthracnose disease management under *in vitro* condition.

Keywords: *In vitro,* essential oils, *colletotrichum gloeosporioides*

Introduction
Banana is one of the most widely grown tropical attributed due to problems in storage and transportation fruits, cultivated over 130 countries, along the tropics and subtropics. The wide consumption of banana is due to its sensory characteristics and the caloric contribution of vitamins and minerals, mainly potassium. During storage, banana fruits can develop many postharvest diseases that often affect the quality of the fruit. Anthracnose of banana is caused by the *Colletotrichum* species and is one of the most serious diseases of ripe banana. In the developing world, loses of harvested foods (up to thirty-seven percent) are attributed due to problems in storage and transportation.

Material and methods
The efficacies of seven essential oils *viz.*, Cinnamon oil (*Cinnamomum Zeylanicum*), Citronella oil (*Cymbopogon winterianus)*, Clove oil *(Syzygium aromaticum)*, Lemongrass oil (*Cymbopogon flexuosus)*, Pachouli oil (*Pogostemon cablin*), Rosemary oil *(Rosemarinus officinalis)* and Vetiver oil *(Chrysopogon zizanioides)* at 0.1, 0.2, 0.3, 0.4 per cent (v/v) was tested by poison food technique (Flack, 1907) [5].
Required quantity of individual essential oil was added at concentrations of 0.1, 0.2, 0.3, 0.4 per cent (v/v) to PDA medium and 20 ml of the test poisoned medium was poured into each sterilized Petri plates. Fungal agar disc of 5 mm was taken from the periphery of 10 days old culture and placed aseptically at the centre of the poisoned PDA medium. All treatments were replicated thrice and the data obtained was statistically analyzed. After seven days of incubation at 28 ^{0}C growth of the fungus was measured and calculated the per cent growth inhibition using the formula as suggested by Vincent (1947) [15].

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent inhibition
C = Radial growth of the pathogen in control plate
T = Radial growth of the pathogen in treatment plate

Result and discussion

Using poisoned food technique, the effects of different essential oils at a concentration from 0.1 - 0.4% in PDA medium on the growth of *Colletotrichum gloeosporioides* were studied. Radial growths (mm) of fungus in PDA plates were recorded and are presented in table. 1. Fungus *Colletotrichum gloeosporioides* failed to grow and complete growth was inhibited in plates supplemented with 0.2% of cinnamon oil or clove oil or lemongrass oil, but at 0.1% level growth inhibitions were only 30.30, 27.27 per cent and 4.93 per cents respectively for cinnamon oil, clove oil and lemongrass oil. At 0.4% tested, patchouli-, rosemary- and vetiver oil inhibited the fungus from 2.48 to 57.57 per cent only over control. In the recent year, more attention has been given on exploitation of natural products including essential oils to control decay and prolonging the storage life of fruits. In the vapour phase of essential oils will exhibit their bioactivities and is an advantage to use them as fungicides in the form of fumigants. Most of the essential oils have been reported to inhibit post-harvest fungi under *in vitro* conditions (Hidalgo *et al.* 2002) [7]. Complete inhibition of radial growth of pathogen was reported by Cinnamon oil, clove oil and lemongrass oil at 0.2, 0.3, and 0.4% respectively. The reason might be due to lipophilic nature of essential oils may facilitate in the penetration of lipid bilayer fungal membrane and cause membrane disruption (Ultee, *et al.* 2000; Gill *et al.* 2006) [14, 6]. Eugenol was found to disintegrate the cell membrane causing cell permeability leading to leakage of cell constituents (plasmolysis) and subsequently death of the fungus as shown by Gill, *et al.* (2006) [6]; Pinto, *et al.* (2009) [11]; Devi, *et al.* (2010) [3]. In the present study, Citronella oil up to 0.4% was found to be moderately effective in inhibiting the growth of pathogen. Use of Cinnamon oil as antifungal agent to manage post-harvest fungal diseases of banana caused by *Colletotrichum musae* has been reported (Ranasinghe *et al.* 2002) [12]. Similar results of inhibitory action of cinnamon oil (0.4%) on the growth of *Colletotrichum musae* has been observed by Maqbool, *et al.*, (2010) [10]. Rani and Thammaiah (2014) [13] reported that Cinnamon oil showed total inhibition (100%) of fungal growth at all concentration tested (1-4%). The fungal inhibitory action of cinnamon oil might be due to the presence of fungistatic cinnamaldehyde in the oil. Barrera-Necha *et al.* (2008) [8] found that *Cinnamomeus zeylanica* (cinnamom) and *Syzygium aromaticum* (clove) oils inhibited conidial germination of *C. gloeosporioides* at 50-250 µg/ml. Cinnamon oil applied in its volatile form also inhibited *in vitro* conidial germination of *C. gloeosporioides* and reduced the lesion diameter on pepper fruit inoculated with the fungus (Hong *et al.* 2015) [8]. The citronella oil treatment inhibited the growth of fungus moderately at high concentration of 0.3% and 0.4%. At lower concentrations of 0.1% clove oil, lemongrass, pacholi, rosemary oil, vetiver oil was also not effective on the fungus. While Pacholi, Rosemary, Vetiver at (0.2-0.4%) inhibition of mycelia growth of the fungus was more significant. Significant inhibition of mycelia growth by rosemary from 0.2 to 0.3% concentrations has been reported by Idris *et al.* (2015) [9]. Oil's chemical structure and their functional groups in particular phenolic moieties play important roles in determining their antimicrobial activities (Deans *et al.*, 1995; Dorman and Deans, 2000) [2, 4].

From the current study, we can conclude that essential oils have a significant effect on development of anthracnose disease, quality and shelf life of banana fruit. From all the concentration levels of essential oils, cinnamon, clove oil and lemongrass oil (100%) showed better results in all parameters.

Table 1: Evaluation of essential oil against growth of *Colletotrichum gloeosporioides* under *in vitro* condition

Essential oil	Percent inhibition over control			
	Concentration (%)			
	0.1	**0.2**	**0.3**	**0.4**
T_1-Cinnamom oil	30.30(33.63)	100.00(89.68)	100.00(89.68)	100.00(89.68)
T_2-Citronella	6.06(14.23)	83.34(65.90)	83.34(65.90)	87.5(69.29)
T_3-Clove oil	27.27(31.45)	100.00(89.68)	100.00(89.68)	100.00(89.68)
T_4-Lemongrass oil	4.93(12.73)	100.00(89.68)	100.00(89.68)	100.00(89.68)
T_5-Pacholi	6.81(15.09)	48.48(44.13)	50.00(44.99)	57.57(49.35)
T_6-Rosemary oil	16.29(23.78)	17.43(24.66)	20.07(26.60)	23.86(29.24)
T_7-Vetiver	4.54(12.30)	13.26(21.30)	14.39(22.25)	20.45(26.88)
	S. Em ±		CD at 1%	
Essential oil	0.43		1.23	
Concentration	0.33		0.93	
Essential oil X Concentration	0.87		2.47	

Figures in parenthesis are Arc sin values

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