J. Agric. Res. Technol., 49 (2) : 256-261 (2024)

Efficacy of Certain Essential Oils Against the *Colletotrichum gloeosporioides* Causing Papaya Anthracnose Under *In vitro* Conditions

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(Received : 10.01.2024 Accepted : 12.02.2024)

Abstract

The study was conducted to evaluate the efficacy of various plant extracts against Colletotrichum gloeosporioides in papaya, utilizing a completely randomized design at the College of Agriculture, Pune-05 during the 2021-23 academic year. Among all the essential oils examined, cinnamon oil emerged as the most effective among the oil treatments, with a minimum mycelial growth of 42.00 mm (53.33% inhibition) at 1000 ppm concentration. In contrast, eucalyptus oil resulted in the highest radial mycelial growth of 62.5 mm (30.56% inhibition), which was on par with the ginger oil treatment (62.5 mm with 30.56% inhibition). The study indicates that the radial mycelial growth of Colletotrichum gloeosporioides decreases with rise in the concentrations of essential oils.

Key words : Essential oils, *Colletotrichum gloeosporioides*, Mycelial Growth, Inhibition.

The papaya (*Carica papaya* Linn), also known as "big melon" or "paw paw," originates from Tropical America and belongs to the Caricaceae family. It is currently cultivated globally, with India producing 5744 thousand hectares of papaya annually on a 149 thousand-hectare area. In the state of Maharashtra, 496.12 metric tons were produced on a 16,000-hectare area in the same year (Anonymous, 2021). Over the past few years, papaya has gained international recognition as a valuable commodity, both in its fresh and processed forms (Sankat and Maharaj, 1997). Referred to as the 'Fruit for Everyone,' papaya is known for its wholesome, revitalizing, and delicious qualities. Its exceptional nutritional value, economic importance, and medicinal attributes contribute to its prominence. Apart from its nutritional content, papaya is renowned for its therapeutic properties, antimicrobial activity, and antioxidant capacity (Bautista-Baños *et al*., 2013). The distinctive yellow color of papaya is attributed to the pigment 'caricaxanthin.' Additionally, papaya contains the valuable proteolytic enzyme papain, recognized for its diverse applications in both medicinal and industrial contexts. A 2022 study by NABCONS, conducted across five districts in three states with varying agro-climatic zones in India, revealed that papaya experiences postharvest losses of 6.59% throughout all stages of farm and market operations. Notably, a significant portion of these losses is linked to fungal diseases, with estimates indicating that these diseases alone contribute to over 50% of production losses (Demartelaere *et al*., 2017).

Papaya fruit experiences substantial post-harvest losses due to fungal infections, with various fungi identified as contributors to fruit rot, including *Colletotrichum gloeosporioides* (Penz.) Sacc, *Botryodiplodia theobromae* Pat., *Alternaria, Phomopsis, Fusarium, Aspergillus, Stemphylium* and *Pestalotiopsis* (Sawant and Gawai, 2011). *Colletotrichum gloeosporioides*, causing papaya anthracnose, particularly hinders storage and transit, impacting tropical regions where papayas are

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cultivated (Bolkan *et al*., 1976). These diverse fungal pathogens collectively present challenges in preserving papayas, maintaining quality, and ensuring marketability. Currently, chemical solutions are commonly employed to combat papaya diseases. However, prolonged use of these chemical pesticides has led to pathogen resistance, diminishing their effectiveness. Additionally, these chemicals contribute to increased Maximum Residual Limits (MRL), posing risks to human health and environmental pollution. In the contemporary era, there is a growing awareness regarding food and health, leading to a shift towards selecting residue-free or organic food options. Embracing eco-friendly management practices not only results in higher quality fruits with an extended shelf life but also positions organic produce at a premium in the market (Ademe *et al*., 2013; Dias *et al*., 2020; Vinod *et al*., 2023). Considering the aforementioned aspects, this study has been meticulously designed and implemented with the aim of evaluating various eco-friendly measures under in vitro conditions for the management of post-harvest diseases in papaya.

Material and methods

***In vitro* evaluation of essential oils :** Efficacy of essential oils listed in Table 1 was determined against the test fungal isolates Colletotrichum gloeosporioides using the poisoned food technique (Nene and Thapliyal, 1993; Prakash *et al*., 2015). These essential oils were collected from Pune local market. The required quantity of oil samples were dissolved in purified ethyl acetate separately (Radaelli *et al*., 2016). The pathogen was grown on potato dextrose agar medium prior to the setting of the experiment. Required quantity of dissolved individual oils was incorporated in the molten potato dextrose agar medium and mixed homogeneously before it was poured into the petri dishes at a temperature of 45-50 °C to get the desired concentration (500 ppm and 1000 ppm) of different oils. 20 ml of the poisoned medium was poured into each sterilized petri plates. The fungal disc of 5 mm was taken from the periphery of the 7 to 10 days old culture and was placed in the centre of the poisoned medium aseptically with help of sterilized cork borer and appropriate control was also maintained without adding any oils to compare the treatments. Petri dishes were then incubated at 28 ± 2°C and radial growth (diameter in mm) of each fungal mycelium was observed and recorded after seven days of incubation. Per cent inhibition of mycelial growth was computed after comparison with the control as per formula of Vincent (1927). Details of treatments are given below:

Table 1. Treatment details of essential oils

Treatment details	Essential oils	Botanical name
T_1 - Neem oil	Neem	Azadirachta indica
T_2 - Eucalyptus oil	Eucalyptus	Eucalyptus globulus
T_3 - Clove oil	Clove	Syzygium aromaticum
T_4 - Cinnamon oil	Cinnamon	Cinnamomum zeylanicum
T_5 - Lemon grass oil	Lemon grass	Cymbopogon citratus
T_6 - Ginger oil	Ginger	Zingiber officinale
T_7 - Control	-	-

Concentrations: 500 and 1000 ppm, Replications: three, Design: CRD

Observation recorded : Observations on radial mycelial growth were recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen was calculated after comparison with the control by applying the formula given by Vincent (1927). The data obtained was averaged and analysed statistically.

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

Where, I = Per cent growth inhibition, C = Growth (mm) in control after seven days and T = Growth (mm) in treatment after seven days

Results and Discussion

***In vitro* evaluation of essential oils against *Colletotrichum gloeosporioides*:** The findings presented in Table 2, regarding the in vitro impact of essential oils at a concentration of 500 ppm against *C. gloeosporioides*, unveiled significant differences in the growth of the pathogen. The average radial mycelial growth resulting from the application of different essential oils at 500 ppm ranged from 49.5 mm to 69.00 mm, with a corresponding inhibition in the fungus's growth ranging between 23.33% and 45.00%. Notably, among all the essential oils assessed in this study, cinnamon oil demonstrated the most potent effectiveness compared to the other oils, as it exhibited the least pathogen growth (45% inhibition) with a radial mycelial growth of 49.5 mm. Subsequently, clove oil exhibited a radial mycelial growth of 50.5 mm, resulting in a 43.89% reduction in the pathogen's growth. Nevertheless, the radial mycelial growth observed in the latter treatment did not display a statistically significant difference from the previous one. Therefore, it can be concluded that both cinnamon and clove oils demonstrated an equal level of effectiveness and most superior over rest of the treatments in suppressing the pathogen's growth. This was followed by lemon grass oil, which showed radial mycelial growth of 52.5 mm, with 41.67% inhibition in the growth of the pathogen. However, radial mycelial growth recorded in the lemon grass oil treatment did not differ significantly from the clove oil. Thus, lemon grass and clove oils were equally effective in inhibiting growth of the pathogen. In terms of efficacy, following the essential oils mentioned earlier, neem and ginger oils demonstrated significant effects on the mycelial growth of the pathogen. Neem oil resulted in a radial mycelial growth of 59.5 mm, while ginger oil showed a radial mycelial growth of 64.5 mm. Consequently, neem oil exhibited a 33.89% and ginger oil displayed a 28.33% reduction in mycelial growth of the pathogen in comparison to the control. Eucalyptus oil exhibited the least mycelial inhibition, registering a 23.33% reduction, accompanied by the highest mycelium growth of 69.00 mm. The findings of this study clearly indicate that, among the tested concentrations, the most effective inhibition of *C. gloeosporioides* growth was achieved with cinnamon and clove oils at a 500 ppm concentration. The control plates displayed the maximum radial mycelial growth of 90.00 mm.

Table 2. Inhibition of radial growth of Colletotrichum gloeosporioides with 500 and 1000 ppm concentration of essential oils

Treat-ment	Essential oil	Botanical name	500 ppm		1000 ppm	
			Mean radial growth* (mm)	Per cent Inhibition	Mean radial growth* (mm)	Per cent Inhibition
T_1	Neem	Azadirachta indica	59.50	33.89	51.00	43.33
T_2	Eucalyptus	Eucalyptus globulus	69.00	23.33	62.50	30.56
T_3	Clove	Syzygium aromaticum	50.50	43.89	49.00	45.00
T_4	Cinnamon	Cinnamomum zeylanicum	49.50	45.00	42.00	53.33
T_5	Lemon grass	Cymbopogon citratus	52.50	41.67	46.00	48.89
T_6	Ginger	Zingiber officinale	64.50	28.33	61.00	32.22
T_7	Control		90.00		90.00	
SE(m)±			0.6814		0.9258	
CD @1%			2.8686		3.8976	

*Mean of three replications.

The results presented in Table 2, which pertain to the in vitro impact of essential oils at a concentration of 1000 ppm on *C. gloeosporioides*, demonstrated significant variations in the pathogen's growth. The mean radial mycelial growth resulting from the application of various essential oils at this concentration ranged from 42.00 mm to 62.5 mm, with a corresponding inhibition in the fungus growth falling between 30.56% and 53.33%. Notably, among all the essential oils examined in this study at the 1000 ppm concentration, cinnamon oil emerged as the most effective over rest of the oil treatments, limiting the mycelial growth to 42.00 mm and achieving a remarkable 53.33% inhibition in the pathogen's growth. Following this, lemon grass and clove oils displayed radial mycelial growths of 46.00 mm and 49.00 mm, along with 48.89% and 45.00% inhibitions in the pathogen's growth, respectively. However, radial mycelial growth recorded in the latter treatment did not differ significantly from the former. Thus, lemon grass and clove oils were equally effective in inhibiting growth of the pathogen. This was followed by neem oil with 51 mm radial mycelial growth and 43.33% mycelial growth inhibition. Though the radial mycelial growth observed in the neem oil treatment did not show a significant difference from that in the clove oil treatment. Therefore, neem and clove oils exhibited an equal level of effectiveness in inhibiting the growth of the pathogen. Following the previously mentioned oils, ginger and eucalyptus oil ranked next in terms of their effectiveness. Ginger oil displayed a radial mycelial growth of 61.00 mm, demonstrating a notable 32.22% inhibition in the pathogen's growth compared to the control. In contrast, eucalyptus oil led to the highest radial mycelial growth, measuring 62.5 mm, and exhibited the lowest inhibition of pathogen growth, with only a 30.56% reduction when compared to the control. However, the radial mycelial growth observed in the eucalyptus oil treatment did not display a significant difference from that in the ginger oil treatment. Thus, it can be concluded that ginger and eucalyptus oils were equal and least effective in inhibiting the growth of the pathogen. In the control plates, the maximum radial mycelial growth observed was 90.00 mm (Plate 1).

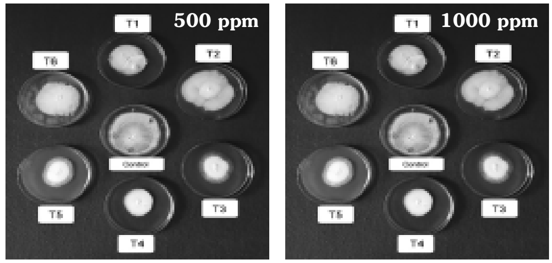

Plate 1. Efficacy of different essential oils against *C. gloeosporioides* at 500 and 1000 ppm concentration

In the course of the current study, a prominent and consistent pattern became evident, showing that elevated concentrations of essential oils correlated with a reduction in the radial mycelial growth of *Colletotrichum gloeosporioides*. Markedly, cinnamon oil consistently emerged as the most potent in inhibiting the radial expansion of *Colletotrichum gloeosporioides*, a trend observed at both the 500ppm and 1000ppm concentrations. Conversely, eucalyptus oil consistently demonstrated the least efficacy in curbing the pathogen's radial growth at both the 500ppm and 1000ppm concentrations.

The results obtained in the present investigation align with the findings of Barrera-Necha *et al*. (2008), who observed that essential oils from *Cinnamomum zeylanicum* and *Syzygium aromaticum* provided the most effective control compared to the other oils tested against *Colletotrichum gloeosporioides* isolated from papaya, causing anthracnose. Similarly, Maqbool *et al*. (2011), Darshan *et al*. (2015), Hernández Lopez *et al*. (2018), and

Burgute *et al*. (2019) found that the essential oil of cinnamon species was most effective against *C. gloeosporioides* on various host plants, including papaya.

Conclusion

In the evaluation of six essential oils, cinnamon (*Cinnamomum zeylanicum*) oil exhibited the greatest efficacy against both pathogens at concentrations of 500 and 1000 ppm, surpassing the effectiveness of the other essential oils that were examined. During the ongoing research, a clear and consistent pattern was observed, showing that higher concentrations of plant extracts and essential oils correlated with a decrease in the radial mycelial growth of *Colletotrichum gloeosporioides*.

Acknowledgment

The authors extend their sincere appreciation to Mahatma Phule Krishi Vidyapeeth Rahuri and the Department of Plant Pathology at the College of Agriculture Pune for generously providing essential research facilities, invaluable guidance, and unwavering support throughout the entirety of the research endeavour and manuscript preparation process.

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