Short Research Article

Efficacy of certain plant extracts against the *Colletotrichum gloeosporioides* causing papaya anthracnose under in vitro conditions

Abstract

The study aimed to assess the effectiveness of various plant extracts against *Colletotrichum gloeosporioides* in papaya. This investigation employed a completely randomized design and took place at the College of Agriculture in Pune-05 during the academic year 2021-22.Among the six plant extracts tested, garlic (*Allium sativum*) was found to be superior to the rest, exhibiting the minimum mycelium growth (5.30 mm) and the highest percentage inhibition (94.11%) at a 20% concentration. However, the drumstick leaf (*Moringa oleifera*) extract was the least effective, with the maximum radial mycelial growth of 44.00 mm (51.11% inhibition) observed. The study indicates that the radial mycelial growth of *Colletotrichum gloeosporioides*decreases with rise in the concentrations of plant extracts and essential oils.

***Keywords**: Plant Extracts, Colletotrichum gloeosporioides, Mycelial Growth, Inhibition*

1. Introduction

A "big melon" or "paw paw" are other names for the papaya (*Carica papaya* Linn). Tropical America is where it originated. It's from the Caricaceae family. Papaya is currently grown all over the world. 5744 thousand ha of papaya are produced annually in India on an area of 149 thousand ha. Maharashtra produced 496.12 MT in the same year on an area of 16,000 Ha (Anonymous, 2021). The papaya fruit has gained significant international prominence in recent years, serving as a valuable commodity in both its fresh and processed forms (Sankat and Maharaj, 1997). Often referred to as the 'Fruit for Everyone' papaya stands out for its wholesome, revitalizing, and delectable qualities. Today, it is acknowledged for its exceptional nutritional value, economic significance, and medicinal attributes. Beyond its nutritional content, papaya is renowned for its therapeutic properties, antimicrobial activity and antioxidant capacity. (Bautista-Baños*et al.*, 2013).The vibrant yellow hue of papaya is due to pigment 'caricaxanthin'. Papaya also contains a valuable proteolytic enzyme, papain which has gained recognition for its diverse applications in both medicinal and industrial contexts.A recent study conducted by NABCONS in 2022, spanning five districts in three states across different agro climatic zones in India, revealed that postharvest losses in papaya amounted to 6.59% across all stages of farm and market operations. Notably, a substantial portion of these losses can be attributed to fungal diseases, with estimates suggesting that these diseases alone may account for more than 50% of production losses (Demartelaere*et al.*, 2017).

Post-harvest losses resulting from fungal infections are high in papaya fruit. A variety of fungi have been identified as culprits in causing fruit rot in papaya such as *Colletotrichum gloeosporioides* (Penz.) Sacc, *Botryodiplodiatheobromae* Pat., *Alternaria*, *Phomopsis, Fusarium, Aspergillus, Stemphylium,* and *Pestalotiopsis* (Sawant and Gawai, 2011).Papaya anthracnose (caused by *Colletotrichum gloeosporioides*) stands as a significant hindrance during storage and transit, and its impact extends to many other tropical regions where papayas are cultivated (Bolkan *et al.*, 1976). These diverse fungal pathogens collectively pose challenges to papaya preservation, quality maintenance and marketability.Presently, diverse chemical solutions are utilized to counteract diseases in the papaya industry. However, the prolonged use of these chemical pesticides has led to the development of resistance in pathogens, rendering the chemicals less effective. Moreover these chemicals results in an increase in Maximum Residual Limits (MRL), posing a hazard to human health and contributing to environmental pollution. In the current era, there is a growing awareness among people about food and health. Consequently, there is a shift towards choosing residue-free or organic food options. Embracing eco-friendly management practices not only yields better 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 aspects discussed earlier, this study has been meticulously designed and executed with the objective of assessing various eco-friendly measures under *invitro* conditions for managing post-harvest diseases in papaya.

2.Material and methods

2.1*In vitro* evaluation of plant extracts

The antifungal properties of various plant extracts listed in table 1 were evaluated against*Colletotrichum gloeosporioides in vitro*, applying poisoned food technique (Nene and Thapliyal, 1993).Fresh and healthy leaves of selected plant species were collected from the field and campus of College of Agriculture, Pune and brought to the laboratory for further studies. The leaves were first washed under running tap water to remove dirt material adhering to the surface. One hundred gram leaves from each sample were then grounded with sterile water (100 ml) in a mixer grinder. After thorough grinding, the extract was first filtered through muslin cloth and then through Whatman filter paper number 1. The extract was then used as standard plant extract solution of 100% concentration or 1:1 ratio. The inhibitory effects of aqueous extract of the botanicals were assessed against the pathogens by poisoned food technique.10 and 20 ml of plant extract was mixed in 90 and 80 ml of PDA medium, to prepare 10% and 20% of plant extract containing medium. A mycelial disc of fungus, 5 mm in diameter was cut from periphery of 7 to 10 days old culture of fungus with the help of sterilized cork borer and aseptically inoculated onto the medium; each set was replicated three times. The control was run side by side by using only sterilized PDA medium. The Petri plates were incubated at 28 ± 2°C temperature in BOD incubator (Jagtap *et al*., 2013). After incubation for seven days the diameter of the colony was measured in two directions and the average was recorded, also the per cent growth inhibition over control was calculated as per formula of Vincent (1927).

Details of treatments are given below:

Treatment details

Table 1: Treatment details of plant extracts

Treatment	**Treatment details**	**Common Name**	**Botanical name**	**Plant part used**
T1	Drumstick Extract	Drumstick	*Moringa oleifera*	Leaf
T2	Neem Extract	Neem	*Azadirachtaindica*	Leaf
T3	Tulsi Extract	Tulsi	*Ocimumsanctum*	Leaf
T4	Garlic Extract	Garlic	*Allium sativum*	Clove
T5	Custard apple Extract	Custard apple	*Annona reticulate*	Leaf
T6	Ghaneri Extract	Ghaneri	*Lantana camara*	Leaf
T7	Control	-	-	-

Concentrations: 10 and 20 per cent, 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

T = Growth (mm) in treatment after seven days

3.Results and discussion

3.1*In vitro* evaluation of plant extracts against *Colletotrichum gloeosporioides*

The outcomes of the study, which investigated the impact of various plant extracts at a 10% concentration on the colony diameter and percentage inhibition in mycelial growth, are presented in Table 2. The data pertaining to the effects of different plant extracts at this concentration clearly demonstrated significant inhibition of *Colletotrichum gloeosporioides*growth in comparison to control. The mean radial mycelial growth of the pathogen varied between 16.00 mm and 63.00 mm, with a percentage growth inhibition ranging from 30% to 82.22%. Notably, among all the plant extracts tested at a 10% concentration, garlic clove extract stood out as significantly superior over rest of the plant extracts. It exhibited the least radial mycelial growth of 16.00 mm, accompanied by the highest mycelial growth inhibition of 82.22% when compared to the control. In terms of effectiveness, the ghaneri leaf extract (*Lantana camara*) at a 10% concentration demonstrated the next highest efficacy, restraining the radial mycelial growth of the pathogen to 25.00 mm, resulting in a mycelial growth inhibition of 72.22%. Following this, the custard apple leaf (*Annona reticulata*) and neem leaf (*Azadirachta indica*) extracts exhibited mean colony diameters of 35.00 mm and 41.00 mm, accompanied by mycelial growth inhibitions of 61.11% and 54.44%, respectively. Subsequently, in order of effectiveness, the tulsi (*Ocimum sanctum*) leaf extract displayed a mean radial growth of 42.00 mm, with a mycelial growth inhibition of 53.33%. Nevertheless, the mean radial mycelial growth recorded for the tulsi leaf extract did not differ significantly from that of the neem leaf extract, indicating that these two extracts were equally effective in inhibiting the pathogen's growth. Among all the plant extracts examined in this study, the drumstick leaf (*Moringa oleifera*) extract was the least effective, as it led to a maximum radial mycelial growth of 63.00 mm and the lowest mycelial growth inhibition, which amounted to 30% in comparison to the control. In the control plates, the maximum radial mycelial growth observed was 90.00 mm.

The results concerning the impact of various plant extracts, administered at a higher concentration of 20%, on colony diameter and the percentage of inhibition in mycelial growth of *Colletotrichum gloeosporioides* are documented in Table 3. Notably, the data related to the effect of these different plant extracts clearly highlight their significant capacity to impede the growth of *C. gloeosporioides*.The mean radial mycelial growth of the pathogen varied within the range of 5.3 mm to 44.00 mm when exposed to various plant extracts at a 20% concentration. This resulted in a substantial mycelial growth inhibition spanning from

51.11% to an impressive 94.11%. Remarkably, among all the plant extracts examined at this higher concentration, the garlic clove extract stood out as notably superior over rest of the extracts in inhibiting the pathogen's growth. This is evidenced by the smallest recorded radial mycelial growth of 5.3 mm and the highest mycelial growth inhibition, reaching an impressive 94.11%, when compared to the control.In terms of efficacy, tulsi (*Ocimum sanctum*) and ghaneri leaf (*Lantana camara*) extracts were the next most effective plant extracts. These extracts significantly limited the radial mycelial growth of the pathogen to 14.00 mm and 25.00 mm, resulting in impressive mycelial growth inhibitions of 84.44% and 72.22%, respectively. Following closely in effectiveness were custard apple (*Annona reticulata*) and neem (*Azadirachta indica*) extracts, which exhibited mean radial growths of 29.5 mm and 34.00 mm, accompanied by mycelial growth inhibitions of 67.22% and 62.22%, respectively. Among all the plant extracts examined in this study at a 20% concentration, the drumstick leaf (*Moringa oleifera*) extract was the least effective. It allowed for the maximum radial mycelial growth of 44.00 mm and the lowest mycelial growth inhibition, amounting to 51.11%, when compared to the control. In the control plates, the maximum radial mycelial growth observed was 90.00 mm (Plate 1).

In the current study, a clear trend emerged, demonstrating that as the concentration of plant extracts increased, the radial mycelial growth of *Colletotrichum gloeosporioides*decreased. Garlic clove extract exhibited the highest efficacy in reducing the radial growth of *Colletotrichum gloeosporioides*, a finding consistent at both the 10% and 20% concentrations. On the other hand, moringa leaf extract was consistently the least effective at both the 10% and 20% concentrations in curbing the pathogen's radial growth.

Similar type of results were reported by Landero *et al*. (2013) who conducted a study evaluating the antifungal effectiveness of different plant extracts against postharvest anthracnose in papaya. Their findings revealed that garlic and cinnamon extracts exhibited the most promising results in inhibiting *in vivo* fungal growth compared to other extracts tested.Similar observations were made by Carreon *et al*. (2023) for *Colletotrichum gloeosporioides*and *Botrytis cinerea* affecting papaya and strawberry fruits. Additionally, Shinde *et al*. (2016) reported the effectiveness of the same extract against postharvest fungal diseases in papaya and mango caused by *Colletotrichum gloeosporioides* and *Alternaria alternata*.

Table 3. Inhibition of radial growth of *Colletotrichum gloeosporioides*with 10% and 20% concentration of plant extracts

Treatment	Plant	Botanical Name	10%		20%	
			Mean radial growth* (mm)	Per cent Inhibition	Mean radial growth* (mm)	Per cent Inhibition
T1	Moringa	*Moringa oleifera*	63.00	30.00	44.00	51.11
T2	Neem	*Azadirachta indica*	41.00	54.44	34.00	62.22
T3	Tulsi	*Ocimum sanctum*	42.00	53.33	14.00	84.44
T4	Garlic	*Allium sativum*	16.00	82.22	5.30	94.11
T5	Custard apple	*Annona reticulata*	35.00	61.11	29.50	67.22
T6	Ghaneri	*Lantana camara*	25.00	72.22	25.00	72.22
T7	Control		90.00		90.00	
	SE(m)±		**1.0000**		**0.6755**	
	CD @ 1%		**4.2099**		**2.8439**	

*Mean of three replications.

4. Conclusion

In an *in-vitro* assessment using the poisoned food technique, six plant extracts and six essential oils were tested against *Colletotrichum gloeosporioides*. Among the six plant extracts, garlic (*Allium sativum*) clove extract demonstrated the highest effectiveness against both pathogens at concentrations of 10% and 20%, outperforming the other plant extracts tested.Throughout the ongoing investigation, a noticeable and consistent trend emerged, indicating that increased levels of concentration of the plant extracts were associated with a reduction in the radial mycelial growth of *Colletotrichum gloeosporioides*.

10 %   20 %

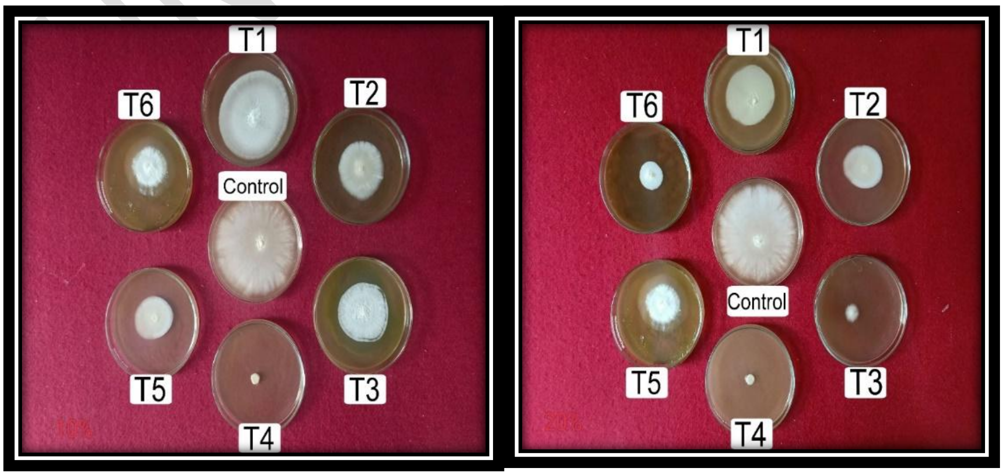

Plate 1: Efficacy of different plant extracts against *C. gloeosporioides*at 10% and 20% concentration

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