In-Vitro evaluation of phytoextracts against *Colletotrichum gloeosporioides* caused anthracnose disease of custard apple (*Annona squamosa* L.)

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Abstract— *The Custard apple or sugar apple (Annona squmosa L.) is one of India's oldest dry land fruit crop belonging to family Annonaceae and genus Annona. The extract of ten plants part was evaluated against leaf spots by poisoned food technique. Among them, extract of lantana leaves (Lantana camera L.) (81.91%) was proved excellent in inhibiting mycelial growth of the pathogen. Next best in order of merit was bougainvillea (Bougainvillea spectabilis Willd) leaves extract (58.60%), neem (Azadirachta indica Juss). leaves extracts (58.01%), subabul (Leucaena leucocephala Lam) leaves extract (57.68%), garlic (Allium sativum L.) clove extracts (55.20), ashoka (Polyanthia longifolia Sonn.) leaves extract (54.99%) and Simarouba (Simarouba glauca DC) leaves extract (54.78%).*

Keywords— In vitro, Colletotrichum gloeosporioides, Custard apple, anthracnose.

I. INTRODUCTION

Custard apple (*Annona squamosa* L.) is commercially important fruit crop of tropical and sub-tropical regions. This fruit is sometimes also considered as "poor man's rich food" in the arid zones of India and require dry climate with mild winter. The genus name, *Annona* is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus. Custard apple belong to family *Annonaceae* having 2n=14 and 16 chromosomes and is one of the finest fruits gifted to India by tropical America. Custard apple is commonly cultivated in Mexico, Philippines, New guinea, Malaysia, India and South America contries in the world. In India, it is found wildly and cultivated, especially in Andhra Pradesh, Punjab, Rajastan, Uttar Pradesh, Madhya Pradesh, Bihar, West Bengal, Assam, Tamil nadu. During 2021-22 (Anon, 2022), custard apple was cultivated over 47 million hectares with an annual production of 402 MT in India. Among the various diseases, fungal diseases play an important role to severe loss of custard apple production. Major fungal diseases are leaf and fruit spot (*Colletotrichum gloeosporioides)*, alternaria leaf spot (*Alternaria spp.),* cylindrocladium leaf spot (*Cylindrocladium colhounii* and *Cylindrocladium scoparium*), botryodiplodia rot *(Botryodiplodia theobromae*), black canker *(Phomopsis anonacearum*) and gliocladium rot (*Gliocladium roseum*) (Shamsi and Hosen, 2016).

II. MATERIAL AND METHODS

In recent years, many phyto-extracts are being used as fungi toxicant for the management of various plant diseases. The leaves extracts of the Neem (*Azadirachta indica* Juss), Ashoka (*Polyanthia longifolia* Sonn), Nilgiri (*Eucalyptus globulus* Labill), Ardusi *(Adhatoda vasica* Ness.) Lantana (*Lantana camera* L.), Simarouba (*Simarouba glauca* DC), Subabul (*Leucaena leucocephala* Lam.), Bougainvillea (*Bougainvillea spectabilis* Willd.), Garlic (*Allium sativum* L.), Onion (*Allium cepa* L.) were evaluated *in vitro* against *C. gloeosporioides* at 10%,20% and 30% concentrations through poisoned food technique.

The freshly collected plant materials from each plant species were washed thoroughly with tap water and then finally with sterilized distilled water and finally sterilized with 90 per cent methanol and air dried. Weighted plant parts were crushed in electrically operated mixture and grinder using 1:1 w/v amount of sterile distilled water and acetone for 100g of bulb and leaves separately (Singh and Majumdar,2001). The extract was homogenized for five minutes and filtered through two layer of sterilized muslin cloth and then the filtrate was centrifuged at 5000 rpm for 15 minutes. The clean supernatant was collected and was considered as cent per cent concentration (standard solution). This formed the standard plant extract solution (100 %). Phyto-extracts were tested for growth inhibition of the fungus by employing poisoned food technique.

For evaluation of antifungal activities of the plant extracts, desired concentrations (10, 20 and 30%) were obtained by adding appropriate amount of standard solution of plant extracts in 100ml PDA medium in conical flasks. Then 20 ml PDA mixed with such extracts were poured in sterilized Peri plates. A five mm disc of seven days old culture of the pathogen was cut by means of a sterilized cork borer and placed at the centre of the Petri plate. The plates were incubated at 27$\pm$2^{o}C. The medium without incorporating the plant extract was serve as control. Observation on radial growth of fungus was measured by averaging two diameter of colony at right to one another when the control treatment with pathogen reached full growth. Three plates were maintained for each treatment. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the following equation (Bliss, 1934).

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

Where,

PGI = Per cent growth inhibition

C = Average mycelial growth in control (mm)

T = Average mycelial growth in treatment

III. RESULTS AND DISCUSSION

The plant extracts inhibited the mycelial growth of *C. gloeosporioides* at three different concentrations (10%,20% and 30%) that differed significantly. Among the ten plant extracts, maximum of mycellial growth inhibition (81.91%) was recorded in lantana leaves extract which was significantly superior over other extracts. Next best in order of merit was leaves extract of bougainvillea (58.60%) followed by leaves extract of neem (58.01%) were found on par in mycelial growth inhibition of the pathogen.

At 30% concentration of plant extracts of maximum of mycellial growth inhibition (84.43%) inhibition of mycellial growth was recorded in lantana leaves extract which was significantly superior over other extracts. Next best in order of merit was leaves extract of bougainvillea (68.35%) followed by leaves extract of neem (66.27%). At 20% concentration of plant extracts of maximum of mycellial growth inhibition (81.64%) inhibition of mycellial growth was recorded in lantana leaves extract which was significantly superior over other extracts. Next best in order of merit was leaves extract of bougainvillea (57.07%). At 10% concentration, mycelial growth inhibition was 45.90 per cent garlic cloves extracts. Overall the maximum per cent inhibition was found in lantana leaves extracts (81.91%) while the least was found in ardusi leaves extracts (48.24%).

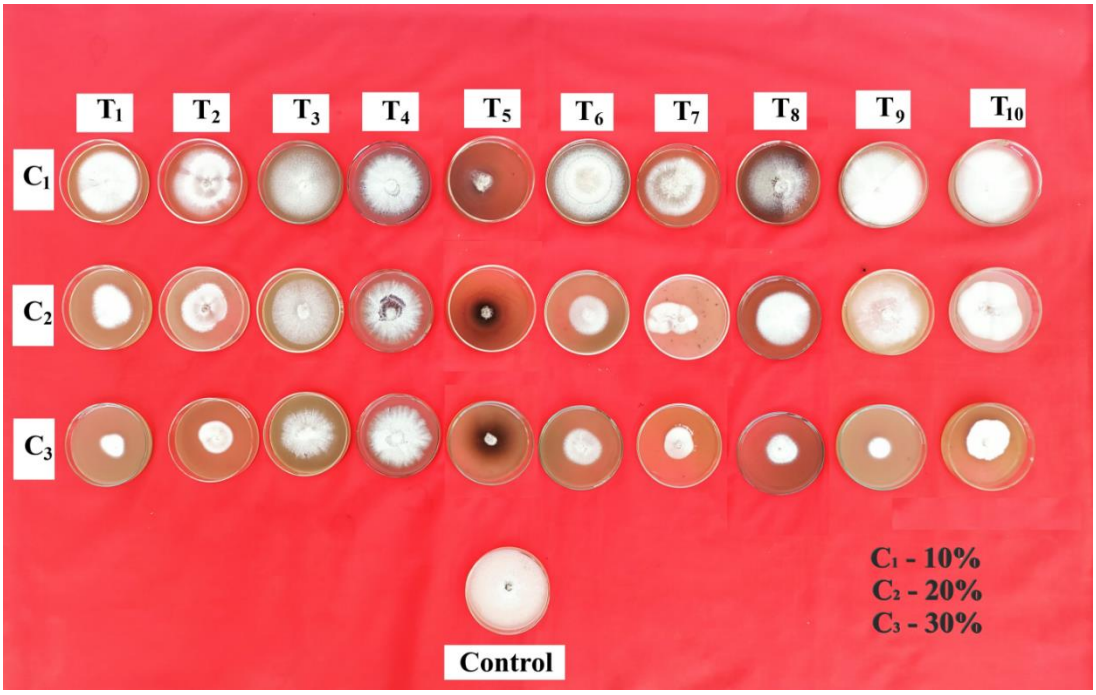
Study revealed that, most of the plant extracts showed fungi-static nature at higher concentration (30%). All the plant extracts showed $\geq$ 58% inhibition of mycelial growth, maximum was found in in lantana leaves extract (81.91%) while least inhibition of mycelial growth was noticed in bougainvillea leaves extract (58.60%). At 20 %concentration, two plant extracts namely lantana leaves extract, bougainvillea leaves extract showed more than 60% inhibition of mycelial growth. Similar effect of the test botanicals/phyto-extracts against *C. gloeosporioides* were reported to cause significant mycelial growth inhibition of *C. gloeosporioides*, earlier by several workers Anteneh *et al*. (2013) studied the antifungal activities of nineteen plant extracts against papaya anthracnose caused by *Colletotrichum gloeosporioides*. They found highest inhibition with ethyl acetate extracts of Lantana camara and showed strong activity against *C. gloeosporioides*. Ramani *et al*. (2015) revealed that the lantana leaves extract at 10 per cent solvent was effective for growth inhibition of *C. gloeosporioides.*

TABLE 1

EFFECT OF PHYTOEXTRACTS ON MYCELIAL GROWTH INHIBITION OF *COLLETOTRICHUM GLOEOSPORIOIDES*

Tr. No.	Phytoextract	Plant part used	Per cent Growth inhibition over control			Mean
			Concentration%			
			10	20	30	
T1	Neem	Leaves	47.81 (54.44)**	59.96 (74.44)	66.27 (83.33)	58.01 (70.7)**
T2	Ashoka	Leaves	48.45 (55.55)	57.31 (70.37)	59.21 (73.33)	54.99 (66.41)
T3	Nilgiri	Leaves	47.39 (53.7)	50.17 (58.51)	59.93 (74.44)	52.50 (62.21)
T4	Ardusi	Leaves	46.75 (52.59)	48.45 (55.55)	49.53 (57.40)	48.24 (55.18)
T5	Lantana	Leaves	79.67 (96.29)	81.64 (97.37)	84.43 (98.51)	81.91 (97.39)
T6	Simarouba	Leaves	47.81 (54.44)	57.07 (69.99)	59.45 (73.70)	54.78 (66.04)
T7	Subabul	Leaves	50.82 (59.62)	57.07 (69.99)	65.14 (81.85)	57.68 (70.49)
T8	Bougainvillea	Leaves	50.39 (58.88)	57.07 (69.99)	68.35 (85.92)	58.60 (71.06)
T9	Garlic	Clove	45.90 (51.11)	49.74 (57.77)	69.95 (87.77)	55.20 (65.49)
T10	Onion	Bulb	46.54 (52.22)	52.57 (62.41)	62.19 (77.77)	53.76 (64.19)
T11	Control	-	4.5 (0.00)	4.5 (0.00)	4.5 (0.00)	4.5 (0.00)
Mean			47.02 (53.53)	52.44 (62.41)	59.11 (72.18)	
			Treatment (T)	Concentration (C)		TxC
S.Em.±			0.327	0.171		0.566
C.D. at 5%			0.923	0.482		1.599
C.V.%			1.86			

Figures in parentheses are original values and outside are arc-sine transformed values



PLATES 1: *In vitro* effect of phyto-extracts on growth and inhibition of *C. gloeosporioides*

IV. CONCLUSION

The present study demonstrated that phyto-extracts possess considerable antifungal potential against Colletotrichum gloeosporioides, the causal agent of leaf and fruit spot in custard apple. Among the ten plant extracts evaluated, Lantana camara leaves extract consistently exhibited the highest level of mycelial growth inhibition across all concentrations, recording up to 84.43% inhibition at 30% concentration. Bougainvillea and neem leaf extracts were found next in efficacy, while extracts such as ardusi showed comparatively lower activity.

The findings clearly indicate that certain botanicals, particularly Lantana camara, can serve as effective, eco-friendly alternatives to synthetic fungicides for the management of anthracnose in custard apple. Since the antifungal activity of these extracts increased with concentration, their role in integrated disease management appears promising. However, further investigations under field conditions, along with standardization of formulations and application methods, are required before their large-scale use in commercial custard apple cultivation.

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