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IN-VITRO EVALUATION OF PLANT EXTRACTS AND OILS AGAINST THE GROWTH OF *COLLETOTRICHUM MUSAE*

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ABSTRACT
Banana anthracnose caused by *Colletotrichum musae* is one of the major post harvest disease. Present research was aimed at studying the effective plant extracts and oils against *Colletotrichum musae*. Among the five plant extracts tested at three different concentrations *Solanum nigrum* leaf extract at all three concentrations (10 %, 15 %, 20 %), recorded the maximum inhibition of colony growth (79.21 %, 84.02 % and 90.41 % respectively). The next best plant extract was *Eupatorium* leaf extract at 15 % and 20 % produced least colony growth of pathogen followed by Citronella leaf extract and *Nerium oleander* leaf extract at both 15 and 20 % concentrations. Citronella leaf extract and *Nerium oleander* leaf extract @ 10 % recorded the least inhibition. Tulsi (*Ocimum*) leaf extract at all the three concentrations showed no inhibitory effect against pathogen. Among the oils tested against *Colletorichum musae* Cinnamon oil at all the tested concentrations completely inhibited the mycelial growth of fungus (100 %). Groundnut oil had moderate effect on inhibiting the fungus. Among all treatments *solanum nigrum* leaf extract, (10%, 15%, 20%) and cinnamon oil at all three concentrations effectively inhibits the mycelial growth of *Colletotrichum musae*.

INTRODUCTION

Banana (*Musa* sp) is one of the important fruit crops of the world as well as India. It is a good source of energy, minerals and vitamins and is one of the biggest single trade items in international fruit trade (Snehalatharani and Khan, 2009**).** Banana is susceptible to several diseases resulting in massive and extensive postharvest losses during transportation and storage (Basel *et al.*, 2002). Anthracnose, caused by the fungus *Colletotrichum musae* (Berk. & M.A. Curtis) Arx, is the most important postharvest disease of banana that can result in 30 to 40% losses of marketable fruit (Ranasinghe *et al.*, 2002). Anthracnose is a latent infection where fungal spores infect immature banana in the field but symptoms occur as peel blemishes as black or brown sunken spots of various sizes on fruit that may bear masses of salmon-colored acervuli with their associated conidia on the fruit peel after ripening (Ranasinghe *et al.*, 2005). Thus, any potential control measure which can effectively delays the symptoms of anthracnose infection would have an important role in extending the shelf life of banana fruit during storage. Synthetic fungicides e.g. benomyl and thiabendazole (TBZ) are the most commonly used synthetic fungicides for controlling postharvest diseases (Khan *et al.*, 2001). However, persistent use of these fungicides has resulted in the emergence of resistant strains of *Colletotrichum musae* (De Lapeyre de Bellaire and Chilin-Charles, 2008). In addition, there is concern that residues of chemical fungicides may cause health problems *viz.*, carcinogenic risk (Wilson *et al.*, 1997). The use of chemical fungicides to control postharvest diseases of fruit is scheduled to be phased out worldwide by year, 2020 under the terms of the Montreal Protocol (Anonymous, 2013). Therefore, there has been an increasing pressure on the banana industry to minimize the use of synthetic fungicides and discover sustainable non-chemical alternative fungicides for controlling postharvest diseases. So, the present study was therefore conducted to examine the effective plant extracts and oils against *Colletotrichum musae*.

MATERIALS AND METHODS

An experiment was carried out during 2013 at K. R. C. College of Horticulture, Arabhavi to evaluate different plant extracts against *Colletotrichum musae*. Plant extracts of *Eupatorium* (*Eupatorium odoratum* L.), *Solanum nigrum* (*Solanum nigrum*), Tulsi leaf extract (*Ocimum sanctum* L.), *Nerium* (*Neriumoleander* L.) and Citronella leaf extract (*Cymbopogan winterianus* L.) were tested for their inhibitory effect on the growth of *Colletotrichum musae* by poison food technique .

Fresh leaves sample were collected in all these five plant species and brought to the laboratory immediately, washed in sterile water to remove dust if any. By using pestle and mortar, the plant samples were crushed using sterile water under laminar air flow chamber after sterile water and filtered through muslin cloth, poured in conical flasks and tied with cotton plugs. The plant extract filterate was kept under refrigerator and used next day. Required quantity of the plant extract was mixed with potato dextrose agar medium and was poured into each sterilized petriplates. Three replications were maintained for each treatment following completely randomized design. Appropriate control was also maintained without adding any

plant extract to potato dextrose agar to compare the treatments. Seven days old fungal disc of 5mm was taken from the periphery of the culture and was placed in the centre of the poisoned medium aseptically and incubated at 28ºC for 7 days. Three replications were maintained for each treatment and the diameter of the colony was measured in 2 directions and the average was recorded after incubation for seven days.

The efficacy of oils *viz*., Cinnamon oil (0.1, 0.2, 0.3, 0.4 per cent), Ground nut oil (0.5, 1.0, 2.0 per cent) was tested by following "Poison food technique" suggested by Sharvelle (1961).

The pathogen was grown on potato dextrose agar medium prior to the setting of the experiment. Required quantity of individual oils was incorporated in molten potato dextrose agar to get the required concentration of different oils. 20 ml of the test poisoned medium was poured into each sterilized Petriplates. Seven replications were maintained for each treatment following completely randomized design. Appropriate control was also maintained without adding any oils to compare the treatments. Seven days fungal disc of 5 mm was taken from the periphery of the culture and was placed in the centre of the poisoned medium aseptically and incubated at 28 ^{0}C for seven days .Observations on radial colony growth was recorded at seven days and when maximum growth in control plates occurred. The efficacy of different oils was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula given by Vincent (1947).

RESULTS AND DISCUSSION

Results of the leaf extract revealed at 10 % concentration of *Solanum nigrum* extract recorded the least growth of pathogen (18.50 mm) followed by *Nerium oleander*(54.87mm) , Citronella leaf extract (57.25 mm), Eupatorium leaf extract (65.88 mm) and Tulsi leaf extract (76.75 mm). Of the five plant extracts tested at 15 % concentration, the leaf extract of *Solanum nigrum* recorded the least growth of the pathogen (14.38 mm) followed by Citronella leaf extract (31.75 mm), Eupatorium leaf extract (32.25 mm), *Nerium oleander* (38.00 mm) and tulsi leaf extract(61.75 mm).At 20 % concentration

Table 1: *In-vitro* evaluation of plant extracts at 10 per cent concentration against *Colletotrichum musae*

Treatment	Common name	Botanical name	Concentration (%)	Parts used	Radial growth of the pathogen(mm)	Percent inhibition of colony growth over control
T_1	Eupatorium	*Eupatorium odoratum*	10	Leaves	65.88	25.97
T_2	*Solanum sp.*	*Solanum nigrum*	10	Leaves	18.50	79.21
T_3	Tulsi	*Ocimum sanctum*	10	Leaves	76.75	13.76
T_4	*Nerium*	*Nerium oleander*	10	Leaves	54.87	38.34
T_5	Citronella	*Cymbopogan winterianus*	10	Leaves	57.25	35.67
T_6	Control				89.00	-
S. Em ±					0.82	-
CD at 1%					3.35	-

Table 2: *In-vitro* evaluation of plant extracts at 15 per cent concentration against *Colletotrichum musae*

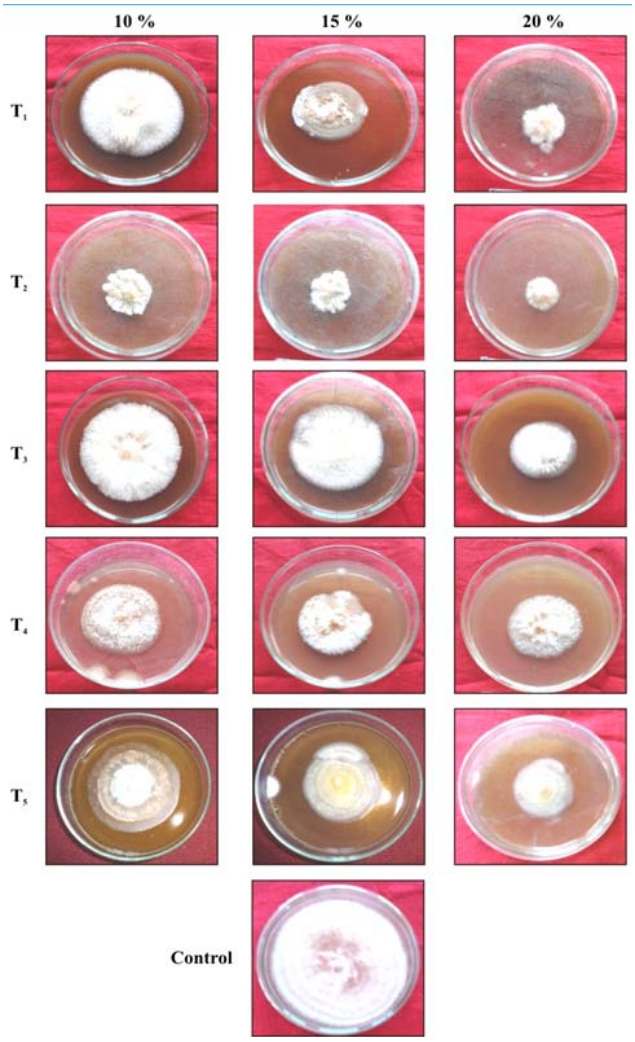
Treatment	Common name	Botanical name	Concentration (%)	Parts used	Radial growth of the pathogen (mm)	Percent inhibition of colony growth over control
T_1	Eupatorium	*Eupatorium odoratum*	15	Leaves	32.25	64.16
T_2	*Solanum sp.*	*Solanum nigrum*	15	Leaves	14.38	84.02
T_3	Tulsi	*Ocimum sanctum*	15	Leaves	61.75	31.38
T_4	*Nerium*	*Nerium oleander*	15	Leaves	38.00	57.77
T_5	Citronella	*Cymbopogan winterianus*	15	Leaves	31.75	64.72
T_6	Control				90.00	-
S. Em ±					0.58	-
CD at 1%					2.39	-

Table 3: *In-vitro* evaluation of plant extracts at 20 per cent concentration against *Colletotrichum musae*

Treatment	Common name	Botanical name	Concentration (%)	Parts used	Radial growth of the pathogen (mm)	Percent inhibition of colony growth over control
T_1	Eupatorium	*Eupatorium odoratum*	20	Leaves	19.75	78.05
T_2	*Solanum sp.*	*Solanum nigrum*	20	Leaves	8.63	90.41
T_3	Tulsi	*Ocimum sanctum*	20	Leaves	34.75	61.38
T_4	*Nerium*	*Nerium oleander*	20	Leaves	21.75	75.83
T_5	Citronella	*Cymbopogan winterianus*	20	Leaves	20.63	77.07
T_6	Control				90.00	-
S. Em ±					0.43	-

Table 4: Evaluation of different oils on the growth of *Colletotrichum musae* under *in-vitro* conditions

Treatments	Radial growth of the pathogenat different concentrations (mm)				Per cent inhibition of colony growth over control			
	0.1 %	0.2 %	0.3 %	0.4 %	0.1 %	0.2 %	0.3 %	0.4 %
T_1 Cinnamon oil	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
T_2 Groundnut oil	19.07	17.64	14.21	11.92	64.20	68.17	72.13	76.94
T_3 Control	53.28	55.42	51.00	51.71	-	-	-	-
S.Em ±	0.47	0.16	0.26	0.22	-	-	-	-
CD at 1%	1.95	0.68	1.08	0.89	-	-	-	-
CV (%)	5.27	1.82	3.23	2.75	-	-	-	-



T_1-Eupatorium leaf extract , T_2-*Solanum nigrum* leaf extract ,T_3-Tulsi leaf extract, T_4-*Nerium oleander* leaf extract, T_5-Citronella leaf extract

Plate 1: Evaluation of different plant extracts on the growth of *Colletotrichum musae* under *in vitro* conditions

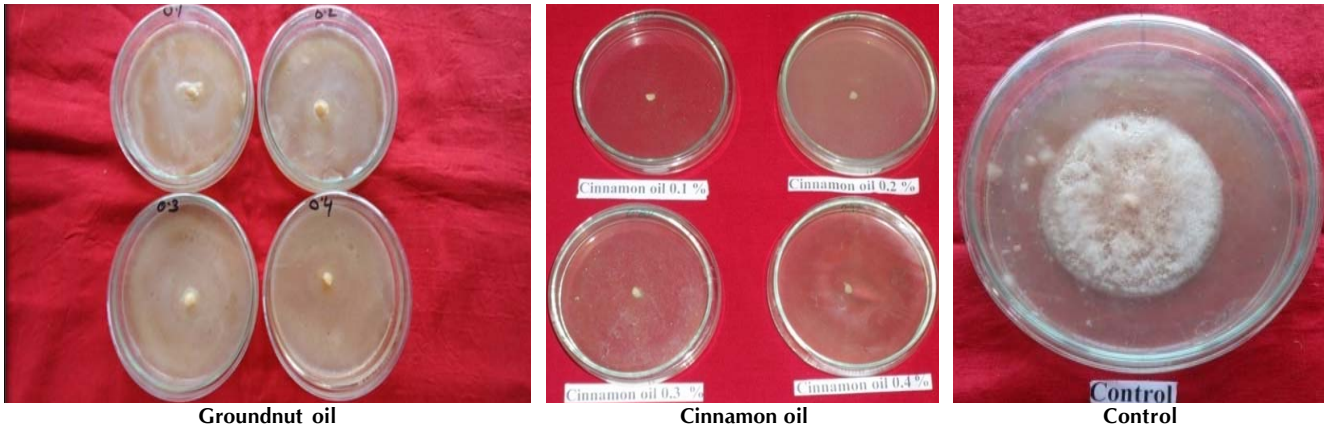
of leaf extract, the treatment *Solanum nigrum* extract recorded the least growth of pathogen (8.63 mm) followed byEupatorium leaf extract (19.75 mm) Citronella leaf extract (20.63 mm), *Nerium olendear*(21.75 mm) and Tulsi leaf extract (34.75 mm).

At 10 % concentration, the percent inhibition of colony growth over control was maximum in *Solanum nigrum*(79.21 %) followed by*Nerium oleander* (38.34 %), Citronella leaf extract (35.67 %) and Eupatorium leaf extract (25.97 %). The least inhibition of colony growth was recorded in tulsi leaf extract (13.76%). At 15 % concentration, the per cent inhibition of colony growth over control was maximum in *Solanum nigrum*(84.02 %) followed by Citronella leaf extract (64.72 %), *Eupatorium* leaf extract (64.16 %) and *Nerium oleander* (57.77 %). The least inhibition of colony growth was recorded in tulsi leaf extract (31.38 %). At 20 % concentration, per cent of inhibition of colony growth over control was maximum in *Solanum nigrum*(90.41 %) followed by Eupatorium leaf extract (78.05 %),Citronella leaf extract (77.07 %) *Nerium oleander*(75.83 %), and it was lowest in tulsi leaf extract (61.38 %).

In the present investigation, the mycelial growth of fungus was inhibited to a greater extent by *Solanum nigrum* leaf extract due to the presence of alkaloids, glycosides, lignins, tannins and terpenoid compounds like monoterpenes, sesquiterpenes, diterpenes or triterpenes. Probably these compounds get through the fungal cell wall/membrane and supress their growth or if these compounds deeply penetrated, might kill them completely. The effectiveness of *Solanum nigrum* leaf extracts is supported by Chakrabarthy *et al.,* (2004) where they reported that leaf extract of *Solanum nigrum* inhibited the growth of fungus.

The inhibitory effect of *Eupatorium* might be due to the presence of antifungal component in the leaf which inhibits the pathogen growth.The effectiveness of *Eupatorium* is supported by Sontaya (2007) where they reported that ethanol extracts of *Eupatorium odoratum* inhibited mycelial growth of *Fusarium oxysporium* and *Collectotrium capsici* by 52.9 % and 64.0 %, respectively. The effectiveness of citronella leaf extract might be due to the presence of antifungal components(geraniol, limonene, methyl isoeugenol, citronellol and citronellal) present in the leaf. Vijaykumar *et al.*, (2014) reported that citronella can inhibit the growth of fungus. Similarly, the antifungal principle present in the *Nerium oleander* leaves inhibit the growth of fungus.The ineffectiveness of tulsi leaf extract against *Colletotrichum gloeosporioides* is supported by the work of Patel and Joshi (2001), where in they reported that tulsi leaf extract was ineffective in inhibiting the mycelial growth of fungus.

Cinnamon oil at all the concentrations (0.1, 0.2, 0.3 and 0.4 %) completely inhibited the radial growth of pathogen as they exhibited 100 per cent inhibition of colony growth. In the present study, Groundnut oil @ 0.4 % was found to be moderately effective in inhibiting the growth of pathogen. The



Groundnut oil **Cinnamon oil** **Control**

Plate 2: Evaluation of different oils on the growth of *Colletotrichum musa*e under *in vitro* conditions

effectiveness of Cinnamon oil is also reported by Ranasinghe *et al*. (2002) who reported that Cinnamon oil could be used as antifungal agents to manage post harvest fungal diseases of banana caused by *Colletotrichum musae*. Maqbool *et al.,* (2010) reported that 0.4% cinnamon oil can inhibit the growth of *Colletotrichum musae*. The effectiveness of cinnamon might be due to the presence of cinnamaldehyde in cinnamon oil which is generally regarded as fungistatic. The groundnut oil treatment inhibited the growth of fungus moderately at high concentration of 0.3 % and 0.4 % (72.13 and 76.94 % respectively). At lower concentrations of 0.1% and 0.2%,per cent of inhibition of colony growth over control were 64.20% and 68.17% respectively.

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