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EVALUATION OF ANTIFUNGAL ACTIVITY OF BOTANICAL EXTRACTS AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) PENZ. AND SACC. INCITING ANTHRACNOSE DISEASE IN MANGO

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ABSTRACT

Extracts of twelve plant species were evaluated at three different levels of concentrations (5, 10 and 15%) for their antifungal activity under *in vitro* condition against *Colletotrichum gloeosporioides*. The results from all experiments proved that extracts of all plants possessed fungistatic or fungicidal properties on growth of *Colletotrichum gloeosporioides* which increased at higher extract concentrations. In general, better antifungal effect was observed with leaf extracts of *Mentha cordifolia* which had strong growth inhibition of *Colletotrichum gloeosporioides* (29.33% and 42.78%) at 5 and 10 per cent concentration respectively and *Eucalyptus spp.* showed highest growth inhibition (63.48%) at 15 per cent concentration. Leaf extract of *Piper betle*, *Datura stramunium* and *Mentha cordifolia* exhibited a moderate action at 5, 10 and 15 per cent concentration respectively. The leaf extract of Fenugreek (*Trigonella foenumgraeum*) recorded lowest mean percent growth inhibition for all the concentration (4.41, 10.74 and 11.52 per cent).

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INTRODUCTION

Mango (*Mangifera indica* L.) belonging to the family Anacardiaceae is an important fruit crop in India and other tropical and sub-tropical countries. Mango is popularly known as 'King of fruits' or 'apple of tropics' (Prabakar *et al.*, 2008). It has been considered as esteemed fruit (Adhikary *et al.*, 2013). Mango is currently rated as the world's third most important fruit crop in the tropics preceded by Citrus and Banana (Silimela, 2003). India is the leading producer of mango in the world and it shares around 56 per cent of total global production (Kamle *et al.*, 2013). India shares 5.2 per cent of total mango exports in the world. In India, mango is grown in all the states, about in 2.5 million ha area with a production of 18 million metric tons with an average productivity of 7.2 metric tons ha^{-1}. Mango is valued for its colour and enjoyed for its taste and nutritional value in dietary fiber, vitamin C and especially the high content of β-carotene and other carotenoid sources of pro-vitamin A (Zheng *et al.*, 2013).

Among the diseases mango anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the most serious pre and postharvest disease and responsible for the losses up to 60 per cent (Devamma *et al.*, 2012; Pandey *et al*, 2012 and Pasuvaraji *et al.*, 2013). The disease is found in both sub-tropical and tropical mango producing countries (Kefialewa and Ayalewb, 2008). It affects both vegetative and reproductive structures. Initial infection starts from leaves and spreads to flowers causing blossom blight, which destroys inflorescence (flower panicles) leading to considerable reduction in fruit set and yield loss (Sundravadana *et al.*, 2007). Pathogen causes the infections on stems, leaves and young inflorescences are manifested as sub-circular or angular black lesions which enlarged and coalesced frequently to destroy leaf edges or entire inflorescences. Lesions often coalesce to form large necrotic areas frequently along the leaf margins severely affecting the growth of the leaves which often become dry and fall out giving the older leaves a 'shot hole' appearance. Under favourable conditions conidia are dispersed that invade young twigs causing twig dieback in some cases. Relative humidity above 95 per cent and temperature ranging from 20 to 30ºC for 12 hours are essential for infection and development of *C. gloeosporioides* on mango fruit (Kamle *et al.*, 2013). Black spots appear on the older fruits. If younger fruits are infected they drop down from the trees (Mehrotra and aggrawal, 2010).

Some of the fungicides have been found to control anthracnose in mango effectively. Moreover, their application may not be eco-friendly because of their detrimental effects on living organisms and plants. Compounds of plant origin have been proved to be possible alternative of fungicides. Therefore an attempt was made to find out some plant species which could show antifungal activity against *C. gloeosporioides*.

MATERIALS AND METHODS

Isolation and maintenance of pathogen

The fungus *C. gloeosporioides* was isolated from the samples collected from nursery

in Central Research Farm, Orissa University of Agriculture and Technology, Bhubaneshwar. A small section of anthracnose infected leaf was surface sterilized with 0.1% $HgCl_2$ and washed thoroughly with sterile distilled water. It was then inoculated on Potato Dextrose Agar (PDA) medium and incubated at 27±1°C for seven days. The culture was purified with "single spore isolation" (Choi *et al.*, 1999). The pathogen was identified as *Colletotrichum gloeosporioides* based on morphological characters and maintained on Potato Dextrose Agar (PDA) slants under controlled temperature.

Pathogenicity of *C. gloeosporioides*

Pathogencity of the isolated organism was proved in *in vivo* condition by pin prick (wound inoculation) method. Healthy one year old Dasheri mango grafts were chosen for this purpose (Bhuvaneswari and Rao, 2001). The inoculum suspension (10^5 conidia ml^{-1}) from ten days old culture was prepared in sterile distilled water. Leaves and twigs were washed in tap water, followed by surface sterilized with 0.1% mercuric chloride for 30 seconds and rinsed four times with sterile distilled water. Wounds were made on the leaves and twigs with the help of a sterile needle. Sterile cotton pad of about 5.0 sq. cm was dipped in spore suspension and swabbed over the wounded surface of the leaves and twigs. Similarly control plants were rubbed with cotton swab and rinsed with sterile distilled water for comparison. After inoculation, the seedlings were covered with polythene bags for two days to ensure high humidity by spraying sterile distilled water to provide congenial conditions for conidial germination and infection. The lesions were observed on the inoculated leaves within eight to ten days of inoculation. The fungus was reisolated from the infected leaves showing typical symptoms and its identity was confirmed.

Preparation of cold aqueous extract

Twelve plant species belonging to different families were collected to test their antifungal activity against *Colletotrichum gloeosporioides* (Table: 1). Fresh plant leaves were washed first in tap water and then in distilled water. Hundred gram of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v) to get hundred per cent concentration. The extract was filtered through two layer of muslin cloth. Finally filtrate thus obtained was used as stock solution.

Effect of botanicals on mycelial growth of *C. gloeosporioides*

The antifungal activity of plant extract was studied by employing poisoned food technique developed by Nene and Thapliyal (1982). Five ml, ten ml and fifteen ml of stock solutions were mixed with 95, 90 and 85 ml of sterilized molten PDA media, respectively so as to get 5, 10 and 15 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract and sterilized by autoclaving at 0.733 kg/cm^2 (10 psi) pressures for 20 min. After sterilization Streptomycin sulphate at the rate of 250 mg was added per litre of media to avoid bacterial contamination.

Twenty ml of poisoned medium was poured into each of the 90 mm sterile petriplates. Each plate was inoculated with five mm mycelial discs from periphery of actively growing zone of ten days old culture were cut out with cork borer and one such disc was placed at the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27 ± 1°C temperature for ten days and radial growth was taken when maximum growth was occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the formula suggested by Vincent (1947). Further, angular transformations were made for data and analysed statistically.

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

RESULTS AND DISCUSSION

All the botanical extracts evaluated at different level of concentration (5, 10 and 15%) for the efficacy of their antifungal activity against *Colletotrichum gloeosporioides* were found inhibitory and significantly inhibited the growth of the test pathogen over untreated control (Table 2 and Fig. 1). The percent inhibition of fungal growth was calculated as compared to growth in control. However, the highest percent of inhibition was achieved by leaf extracts of *Mentha cordifolia* 29.33 per cent and 42.78 per cent at 5 and 10 per cent concentration respectively. This was followed by *Piper betle* (22.85%) and *Momordica charantia* (22.41%) at 5 % concentration and *Datura stramunium* (40.96%) and *Eucalyptus spp.* (40.15) at

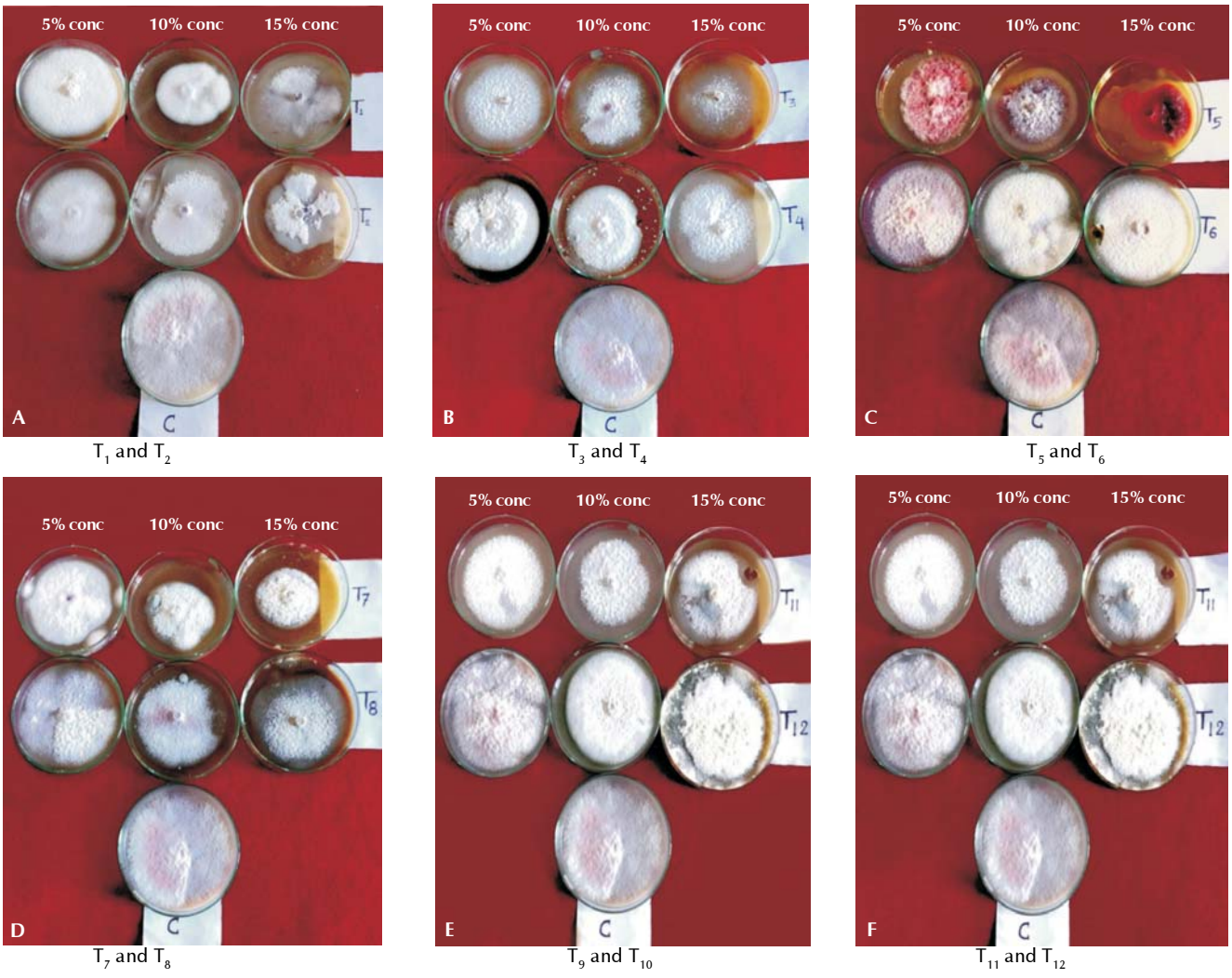
Table 1: Plant extracts used in the experiment

Sl. no.	Common name	Botanical name	Plant part used	Family
1.	Maxican poppy (Satyanashi)	*Argemone maxicana* L.	Leaves	Papaveraceae
2.	Bitter gourd (Karela)	*Momordica charantia* L.	Leaves	Cucurbitaceae
3.	Mint (Pudina)	*Mentha cordifolia* L.	Leaves	Lamiaceae
4.	Neem	*Azadirachta indica* Adv. Juss	Leaves	Meliaceae
5.	Neem	*Azadirachta indica* Adv. Juss	Oil	Meliaceae
6.	Fenugreek (Methi)	*Trigonella foenumgraecum* L.	Leaves	Fabaceae
7.	Jimson weed (Dhatura)	*Datura stramonium* L.	Leaves	Solanaceae
8.	Nagaeri	*Lantana camera* L.	Leaves	Verbenaceae
9.	Eucalyptus (Neelgiri)	*Eucalyptus spp.*	Leaves	Myrtaceae
10.	Indian Beech (Karanz)	*Pongamia pinnata* L.	Leaves	Fabaceae
11.	Betelvine (Paan)	*Piper betle L.*	Leaves	Piperaceae
12.	Arak / Madar	*Calotropics gigantean* (L.) W. T. Aifon	Leaves	Apocynaceae

Table 2: Effect of different plant extracts on growth inhibition of *C. gloeosporioides*

Treatment	Plant extract	Plant part used	Percent inhibition at different level of concentration (%)		
			5	10	15
T_1	*Argemone maxicana*	Leaf	15.59(23.06)	31.59(34.12)	42.07(40.42)
T_2	*Momordica charantia*	Leaf	22.41(28.42)	37.63(37.81)	47.59(43.62)
T_3	*Mentha cordifolia*	Leaf	29.33(32.77)	42.78(40.85)	61.96(51.92)
T_4	*Azadirachta indica*	Leaf	22.19(28.07)	32.33(34.63)	42.19(40.50)
T_5	*Azadirachta indica*	Oil	21.26(27.45)	29.63(32.97)	42.11(40.46)
T_6	*Trigonella foenumgraeum*	Leaf	4.41(12.00)	10.74(19.13)	11.52(19.84)
T_7	*Datura stramunium*	Leaf	16.33(23.83)	40.96(39.79)	44.89(42.07)
T_8	*Lantana camera*	Leaf	11.74(20.03)	29.22(32.71)	38.37(38.28)
T_9	*Eucalyptus spp.*	Leaf	20.22(26.71)	40.15(39.31)	63.48(52.83)
T_{10}	*Pongamia pinnata*	Leaf	12.37(20.58)	27.00(31.26)	29.78(33.06)
T_{11}	*Piper betle*	Leaf	22.85(28.55)	24.19(29.45)	35.15(36.35)
T_{12}	*Calotropics gigantea*	Leaf	10.74(19.14)	14.07(21.99)	20.67(26.99)
T_{13}	Control		0	0	0
Mean		24.22	32.84	38.86	
SEm ±		0.980	1.225	1.019	
CD at 5%		2.860*	3.577*	2.973*	

* Figures in parentheses indicate angular transformed values



T_1 and T_2 | T_3 and T_4 | T_5 and T_6

T_7 and T_8 | T_9 and T_{10} | T_{11} and T_{12}

T_1 = *Argemone maxicana* leaves; T_2 = *Momordica charantia* leaves; T_3 = *Mentha cordifolia* leaves; T_4 = *Azadirachta indica* leaves; T_5 = *Azadirachta indica* oil; T_6 = *Trigonella foenumgraecum* leaves; T_7 = *Datura stramunium* leaves; T_8 = *Lantana camera* leaves; T_9 = *Eucalyptus spp.* leaves; T_{10} = *Pongamia pinnata* leaves; T_{11} = *Piper betle L.* leaves; T_{12} = *Calotropics gigantean* leaves; C = Control

Figure 1: Efficacy of different plant extracts against mycelial growth of *Colletotrichum gloeosporioides* under *in vitro* condition.

10 per cent concentration respectively. The leaf extract of *Eucalyptus spp*. showed maximum inhibition of mycelial growth (63.48%) at 15 per cent concentration which was statistically superior over leaf extract of *Mentha cordifolia* (61.96%) at the same concentration. The results are in agreement with the findings of Bussaman *et al*. (2012) who recorded maximum inhibition of spore germination of *C. gloeosporioides* by Chloroform extract of *Mentha cordifolia* 2.5 per cent concentration. Pandey *et al*. (2009) reported highest growth inhibition in all isolates of *Colletotrichum gloeosporioides* by leaf extract of *Moras alba* and *Azadirachta indica*. Extracts of *Momordica charantia* (22.41%), *Azadirachta indica* Leaf and oil (22.19% and 21.26%), *Eucalyptus spp*. (20.22%) and *Piper betle* (22.85%) showed analogous results for growth inhibition at 5 per cent concentration. Extracts of *Datura stramunium* (40.96%) and *Eucalyptus spp*. (40.15%), *Argemone maxicana* (31.59%) and leaf extract of *Azadirachta indica* (32.33%) and oil of *Azadirachta indica* (29.63%) and leaf extract of *Lantana camera* (29.22%) and *Pongamia pinnata* (27.00%) were at par to each other of growth inhibition at 10 per cent concentration. Extracts of *Argemone maxicana* (42.07%), *Azadirachta indica* (42.19% and 42.11%) and *Datura stramunium* (44.89%) showed akin pattern of growth inhibition at 15 per cent concentration. The results are coincided with the findings of earlier workers, *viz*. Anand and Bhaskaran (2009), Banginwar *et al*. (2012) and Kuberan *et al*. (2012). Leaf extract of *Trigonella foenumgraeum* recorded least growth inhibition of 4.41, 10.74 and 11.52 per cent at 5, 10 and 15 per cent concentration respectively. However, *Colletotrichum falcatum* was almost completely inhibited up to 90.00 per cent by 20 per cent leaf extract of *Trigonella foenumgraeum* (Mishra and Behera, 2011) and Jayakumar *et al*. (2007). This study suggested that leaf extract of *Trigonella foenumgraeum* is not effective against *C. gloeosporioides*. According to the performance of top three botanical extracts we may conclude that these can be applied to control *Colletotrichum gloeosporioides* after repetitive evaluation in the field.

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