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Invitro Evaluation of Botanicals and Bioagents against *Colletotrichum gloeosporoides* Causing Blight of Foxtail Palm

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A B S T R A C T

Foxtail palm (*Wodyetia bifurcata* L.), is a graceful palm species native to Australia, belongs to family Arecaceae. It is recognized as valuable addition by palm enthusiasts and landscapers to be used in a wide spectrum of landscapes. Leaf blight is one of the emerging and serious disease of foxtail palm. The first evidence of disease appeared as minute circular to irregular spots, which were light-dark brown in colour, lightly sunken surrounded by brown margin and yellow halo. Studies on *in vitro* evaluation of botanicals and bioagents against blight of the foxtail palm were carried out in the department of Plant Pathology, College of Agriculture, Bengaluru. Among the botanicals evaluated maximum per cent inhibition (65.60 %) was recorded in *Curcuma longa* followed by *Lantana camara* (49.75 %). Least mycelial inhibition of fungus was recorded in *Nicotiana tabacum* (17.09 %) showing its least effectiveness against *Colletotrichum gleosporoides*. Among bacterial bioagents *Bacillus subtilis* (36.85 %) showed evaluated maximum inhibition percentage which was followed by *Bacillus megaterium* (35.74 %). *Pseudomonas fluroscens* has shown least inhibition of 30.37 per cent against *C. gloeosporoides.* Among the fungal bioagents evaluated maximum (55.93 %) inhibition of mycelial growth was noticed in *Trichoderma harzianum* followed by *T. hamatum* (53.33 %) and *T. asperellum* (50.74 %).

Introduction

The foxtail palm (*Wodyetia bifurcata* L.), has one of the most stunning foliage displays of all palms. It is only plant in the genus, which was named in the honour of Wodyeti, an Australian bushman with vast traditional knowledge of foxtail palm's natural habitat. The species name *bifurcate*, comes from the

Latin meaning twice forked which refers to the unique shape of both the fruit and the fish tailed leaflets (Tadahal, 2012). The pale green arching fronds have leaflets extending outward in all directions from centre of the stem.

Upon reaching maturity foxtail palm forms a canopy of 8-10 leaves each, showcasing a distinctive foxtail like pattern. Palms provide unparalleled elegance and architectural statue in landscapes, their fruits, seeds, fronds (leaves) and stems are rich sources of food, cosmetic products, biofuels and timber. This palm can be grown in indoors as a potted plant by providing proper light, soil, temperature (Desai *et al.,* 1971).

This palm is adaptable to various climates and and is host of only a few fungal pathogens. However it is infected by bud rot, leaf blight, spots, fusarium wilt, Ganoderma bud rot diseases that badly damages its ornamental value. Palm leaflets and rachis often have pronounces spots and lesions, they can be induced by environmental or cultural conditions such as excess irrigation on leaves, high pH, potassium and iron deficiency, cold weather and compacted soils and poorly developed root system (Tariq *et al.,* 2020).

Probable causes of leaf spots and blight include *Bipolaris, Colletotrichum, Calonectria, Cercospora, Gliocladium, Exersohilum, Phaeotrichoconis, Stigmina, Pestalotiopsis, Pseudocercospora, Phyllachora.* Among the diseases that infect foxtail palm leaf blight is an emerging and serious disease of crop.

Leaf blights are more severe on palms in the juvenile stage of growth (Dev *et al.,* 2016). The symptoms initially as small, water-soaked, irregularly, circular, brown, slightly sunken necrotic lesions with a well-defined border, surrounded by a chlorotic halo that then turned various shades of yellow, grey, reddish-brown, brown or black, later these spots coalesced to give blighted appearance. Detailed studies on the blight disease have yet to be carried out. The causal agent of the blight disease has not been clear but it is presumed to be *Colletotrichum* spp. Therefore the current study was initiated to identify causal agent and to screen the bioagents and botanicals against blight disease in order to know the efficacy (Tandon and Chandra, 1962).

Materials and Methods

The efficacy of seven botanicals was assayed against the blight of the foxtail palm. Botanicals have been listed here.

Preparation of botanicals

Fresh leaf samples of botanicals were collected from different places in college of agriculture, Bengaluru. These leaf/kernels/clove samples were washed thoroughly using tapwater followed by sterile distilled water. Stock solution of each botanical was prepared by grinding 100g of sample with 100 ml sterile distilled water (1:1 w/v) using a clean pestle and mortar. A ground stock solution of all the extracts was collected by filtering through double layered muslin cloth followed by bacterial membrane filter assembly and were evaluated in lab conditions using Poison food technique.

The botanicals were tried at 5, 10 and 15 per cent concentrations. The calculated quantities of botanicals were thoroughly mixed in the molten medium before pouring into Petri plates so as to get the desired concentration of botanical separately. Twenty ml of fungicide amended medium was poured in each of 90 mm sterilized Petri plates and allowed to solidify. The plates were inoculated centrally with 8 mm disc of 10 days old young sporulating culture of *C. gloeosporoides.* Controls without botanicals were also maintained.

Dual culture method- bioagents evaluation

The fungal bioagents and test fungus were inoculated opposite to each other and placed two cm away from the periphery in a single petriplate. The bacterial bioagents were also streaked two cm away

from periphery inside the plate alongside of the test fungus. Three replications were maintained for each treatment and control was maintained by inoculating pathogen alone in petriplates. All the petriplates were incubated and observations were taken when pathogen attained maximum growth in control. The diameter of the pathogen colony was measured in two directions and average was recorded.

In this experiment six antagonists such as *Trichoderma harzianum, T. asperellum, T. hamatum, Bacillus subtilis, B. megaterium, Pseudomonas fluoroscens* were collected. The two experiments were conducted in Completely Randomised Design (CRD) with three replications in each treatment. The inoculated Petri plates were incubated at 25 ± 2°C. The colony diameters were measured after 10 days when the control plates were full of fungal growth. Per cent inhibition of growth was calculated by using formula given by Vincent (1947).

$$I = [(C - T)/ (C)] X 100$$

Where,

I=Per cent inhibition

C=Colony diameter in control

T=Colony diameter in treatment

Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and interpretation of data was carried out. The level of significance used in 'F' and 'T' test was P = 0.05 and P = 0.01.

Critical differences were calculated wherever 'F' test was significant. The values percent disease index was subjected to angular transformation according to the table given by Sundarraj *et al.,* (1972).

Critical difference (C.D.)

C.D. = S.Ed. × t values at error degrees of freedom t = tabulated t value at '5' per cent probability level

Results and Discussion

At 5 per cent concentration all the botanical extract were significantly inhibited the growth of pathogen to varying degrees as compared to control and the inhibition ranged from 8.89 to 54.07 per cent. *Curcuma longa* showed maximum per cent mycelial growth inhibition of 54.07 per cent followed by *Lantana camara* (45.19 %) and *Azadirachta indica* (42.96 %) and minimum inhibition was observed in *Caricae papaya* (8.89 %) and *Nicotiana tobacum* (10.37 %) among all tested botanicals. At 10 per cent concentration, the inhibition was in the range of 20.37 to 68.15 per cent. *Curcuma longa* showed 68.15 per cent inhibition over other treatments tested.

Second best botanical was found to be *Lantana camara* which exhibited 49.26 per cent growth inhibition, followed by *Azadirachta indica* and *Allium sativum* with 45.19 and 40.74 per cent growth inhibition respectively and significantly better than rest of the treatments. Whereas, *Nicotiana tobacum* was found to be comparatively less effective in inhibiting mycelial growth of test pathogen with 20.37 per cent.

At 15 per cent concentration also similar results were obtained as that of 5 and 10 per cent. All the botanicals significantly inhibited the mycelial growth of pathogen to varying degree as compared to control. From the different concentration levels, it was observed that botanicals inhibited the mycelial growth with a range of 22.96 to 72.96 per cent. The significant highest per cent mycelial inhibition was recorded in *Curcuma longa* of 72.96 per cent followed by *Lantana camara* (54.81 %). *Azadirachta indica* and *Ocimum sanctum* showed similar inhibition per cent of 45.93 and minimum inhibition was noticed in *Nicotiana tobacum* (22.96 %) among all tested botanicals.

Table.1 List of botanicals utilized in this study

SL No	Common Name	Botanical Name	Parts used
1	Neem	*Azadirachta indica*	Kernel
2	Lantana	*Lantana camara*	Leaves
3	Garlic	*Allium sativum*	Cloves
4	Turmeric	*Curcuma longa*	Rhizome
5	Papaya	*Caricae papaye*	Leaves
6	Tulsi	*Ocimum sanctum*	Leaves
7	Tobacco	*Nicotiana tabacum*	Leaves

Table.2 *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*

Treatment Number	Bioagents	Per cent inhibition of mycelial growth
T1	*Trichoderma hamatum*	53.33*(46.94)**
T2	*Trichoderma asperellum*	50.74(45.45)
T3	*Trichoderma harzianum*	55.93(48.43)
T4	*Bacillus megaterium*	35.74(36.73)
T5	*Bacillus subtilis*	36.85(37.40)
T6	*Pseudomonas fluorescens*	30.37(33.46)
	S.Em.±	0.54
	CD@1%	1.67
	CV%	2.27

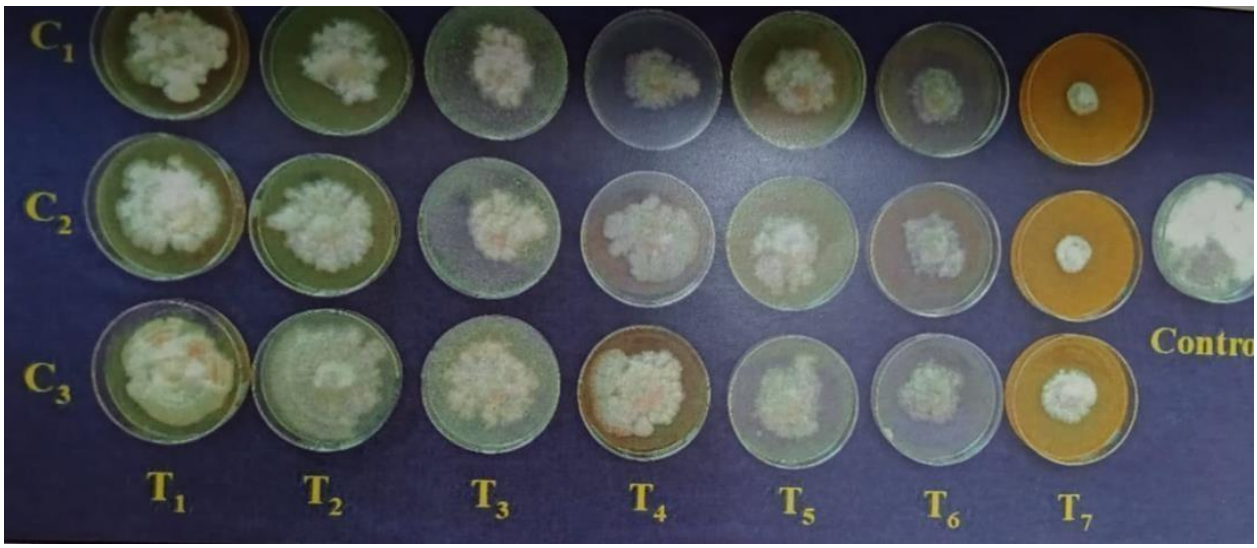
*Mean of three replications; **Figures in the paranthesis are arcsine transformed values

Table.3 *In vitro* evaluation of botanicals against *Colletotrichum gloeosporioides*

Treatment Number	Botanicals	Per cent Inhibition of mycelial growth at Different concentrations			Mean
		5%	10%	15%	
T1	*Ocimum sanctum* (Tulsi)	23.33* (28.90)**	27.78 (31.82)	45.93 (42.68)	32.35 (34.68)
T2	*Allium sativa* (Garlic)	25.19 (30.14)	40.74 (39.68)	41.85 (40.33)	35.93 (36.85)
T3	*Caricae papaya* (Papaya)	8.89 (17.35)	23.33 (28.90)	44.44 (41.83)	25.55 (30.38)
T4	*Lantana camara* (Lantana)	45.19 (42.26)	49.26 (44.60)	54.81 (47.79)	49.75 (44.88)
T5	*Nicotina tobaccum* (Tobacco)	10.37 (18.80)	20.37 (26.84)	22.96 (28.65)	17.90 (25.04)
T6	*Azadirachta indica* (Neem)	42.96 (40.98)	45.19 (42.26)	45.93 (42.63)	44.69 (41.97)
T7	*Curcuma longa* (Turmeric)	54.07 (47.36)	68.15 (55.67)	72.96 (58.70)	65.60 (53.79)
		Botanicals(B)	**Concentration(C)**	**Interaction(B*C)**	
S.Em.+-		**0.51**	**0.33**	**0.88**	
CD@1%		**1.46**	**0.95**	**2.52**	
CV%		**4.03**			

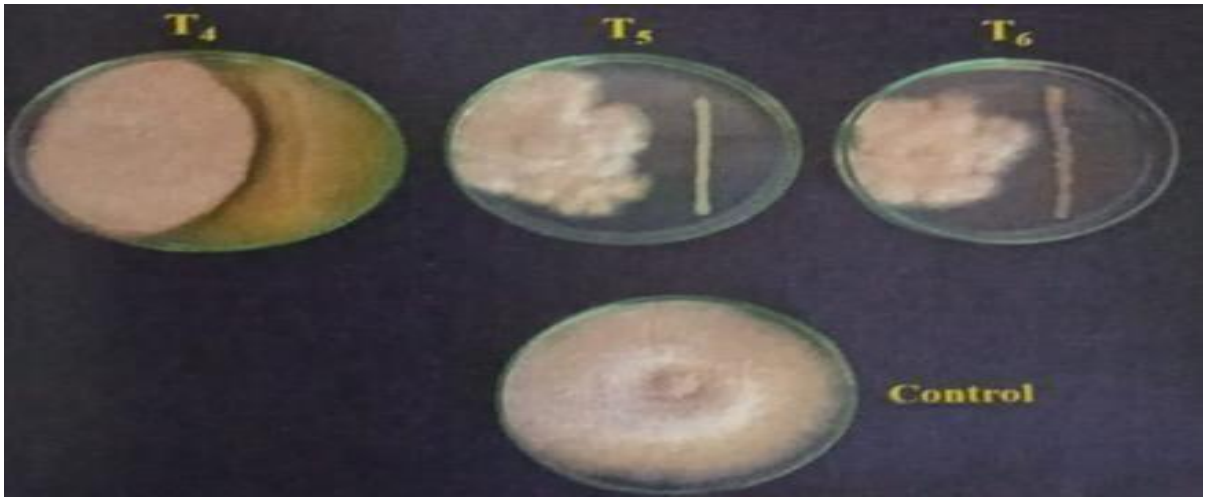
*Mean of three replications; **Figures in the paranthesis are arcsine transformed values

Plate.1 *In vitro* evaluation of botanicals against *Colletotrichum gloeosporioides*



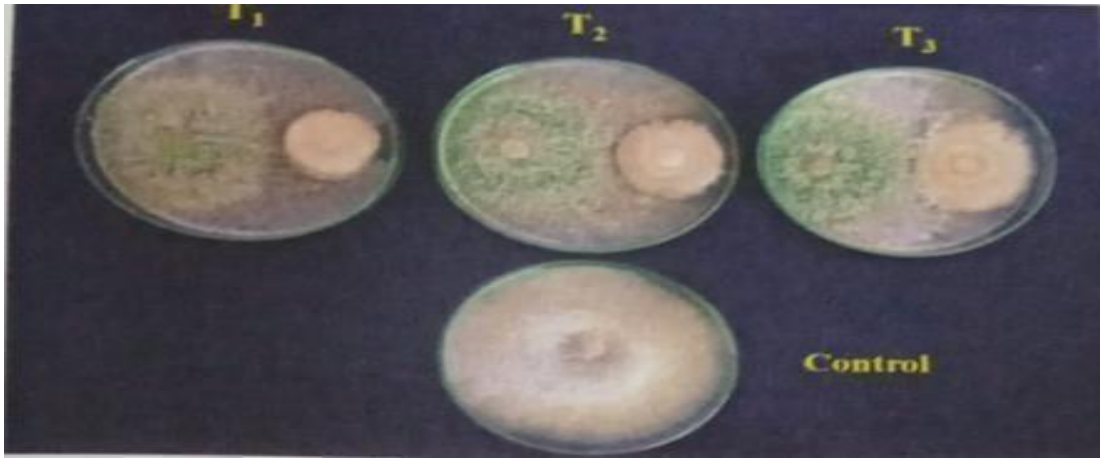
T1- *Nicotiana tobacum* (Tobacco) C1- 15 %
T2- Caricae papaya (Papaya) C2- 10 %
T3- Allium sativa (Garlic) C3- 5
%T4- Ocimum sanctum (Tulsi)
T5- *Azadirachta indica* (Neem)
T6- *Lantana camara* (Lantana)
T7-Curcuma longa (Turmeric)

Plate.2 *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*



T4- *Pseudomonas fluorescens*
T5- *Bacillus megaterium*
T6- *Bacillus subtilis*

Plate.3 *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*



T1-*Trichoderma harzianum*
T2- *Trichoderma hamatum*
T3- *Trichoderma asperellum*

Overall mean mycelial inhibition (65.60 %) was recorded in *Curcuma longa* followed by *Lantana camara* (49.75 %), both were effective in controlling the mycelial growth of pathogen. Least mean mycelial inhibition (17.09 %) was observed in *Nicotiana tobacum* showing its least effectiveness against *C. gloeosporioides*.

The maximum mean mycelial inhibition of 65.06 per cent was recorded with *Curcuma longa* due to presence of an alkaloid curcumin which has antifungal property.

The results were comparable with the findings of Kothikar (2017) and Desai *et al.,* (2019) that *Azadirachta indica* seed extract at 5 per cent concentration was found to be superior in inhibiting (74.69 %) leaf spot pathogen *Colletotrichum dematium* in turmeric and study conducted by Vinod *et al.,* (2009) showed that *Lantana camara* at 7.5 per cent found to be superior with 45.54 per cent mycelial inhibition followed by turmeric at 7.5 per cent with 40.73 % against *Colletotrichum gloeosporioides* causing papaya anthracnose.

Certain compounds due to their biodegradability and selective toxicity, are considered valuable for effectively managing diverse plant diseases (Mishra and Dixit, 1979; Tiwari and Mehrotra, 1968).

Among the different bioagents used against *Colletotrichum gloeosporoides*, *Trichoderma harzianum* has inhibited the growth of fungus with maximum extent (55.93 %) followed by *T.hamatum* (53.33 %), *T. asperellum* (50.74 %). Among bacterial bioagents, maximum inhibition of 36.85 per cent was observed with *Bacillus subtilis*.

In case of Trichoderma spp., it overgrew and suppressed the test fungus (*Colletotrichum gloeosporoides*) growth *in vitro.* Inhibition of the growth *C. gloeosporoides* appeared to be due to the lysis of pathogen cellwall by *Trichoderma* enzyme along with hyphal parasitism, coiling and penetration. The present study is in agreement with Sharma *et al.,* (2021), who found effectiveness of *T. harzianum* (89.26 %) and *Bacillus subtilis* (76.30 %) against *C. gloeosporioides*, and Patil *et al.,* (2010) who found *Trichoderma harzianum* as a very effective in reducing the growth of *C. gloeosporioides.*

Trichoderma spp. provided antagonistic activity against plant pathogenic fungi by competition for nutrients, mycoparasitism and antibiosis, *Trichoderma* spp. hyperparasite formed coil, hooks or appressorium like structures around the pathogen mycelium (Elad *et al.,* 1983). Begum *et al.,* (2008) observed that *Trichoderma* isolates coiled around

the hyphae of *C. truncatum*, which further restricts its growth and spread. The present study is also in agreement with Devamma *et al.*, (2012) and Deshmukh *et al.*, (2012).

Foxtail palm leaf samples affected by blight was collected from the field, isolation and identification was done and pathogenicity was proved for the *Colletotrichum* pathogen. Among the botanicals evaluated, maximum mean mycelial growth inhibition was recorded with *Curcuma longa* (65.60 %) and *Lantana camara* (49.75 %). Among different bioagents tested the higher 55.93 per cent inhibition of mycelial growth was observed in *T. harzianum* found to be statistically superior over other treatments.

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Declarations

Ethical approval

This article follows experimental guidelines and this research does not involve any human participants or animal performed.

Conflict of interest

The authors of present investigation declare that they have no conflict of interest.

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