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Original Research Article

In vitro Evaluation of Different Bio control Agents and Plant Extracts against *Alternaria* sp. Causing *Alternaria* blight in Red Pitahaya

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A B S T R A C T

The present experiment was conducted to test the efficacy of bio-control agents and plant extracts *in vitro* against *Alternaria* sp. causing *Alternaria* blightin red pitahaya first reported from Chhattisgarh. The efficacy of fungal and bacterial antagonist (*Trichoderma* and *P. fluorescence*) was evaluated through dual culture technique. Ten plant extracts *viz.*, Onion (*Allium cepa*), Duranta (*Duranta repens*), Ashoka (*Polyalthia longifolia*), Neem (*Azadirachta indica*), Bougainvillea (*Bougainvillea spectabilis*), Dhatura (*Datura* sp.), Tamarind (*Tamarindus indicus*), Cauliflower (*Brassica oleracea var. botrytis*), Caribbean copper plant (*Euphorbia* sp.) and Chilli (*Capsicum annum*) at 4% concentration were evaluated through poison food technique. Maximum inhibition percentage (71.23%) was recorded in T2 isolate of *Trichoderma* and P5 isolate of *Pseudomonas* (43.94%). Minimum inhibition percentage (54.36%) was recorded in T3 isolate of *Trichoderma* and P1 isolate of *Pseudomonas* (29.92). Among different plant extracts used, Ashoka (*Polyalthia longifolia*) showed best results to inhibit (47.35%) its growth.

Keywords

Plant extracts, Biocontrol, Red pitahaya, Inhibition percentage

Introduction

Dragonfruit (*Hylocereus undatus*) also known as red pitahaya is herbaceous, perennial, climbing vine cactus and a tropical fruit plant belongs to family Cactaceae. Dragonfruit tree is used in the embellishment as an ornamental vine in gardens and landscapes. It is also used as a natural colouring agent in the preparation of various drinks, juices, smoothies and pastries as a flavour enhancer. Unopened flower buds are cooked and eaten as vegetable. Major commercially cultivated species are *Hylocereus undatus*, *Hylocereus polyrhizus* and *Hylocereus costaricensis*, genus *Hylocereus* are native to Mexico, which were taken to Central America, perhaps by the Europeons (Morton, 1987). As reported from various countries, large number of fungi such as *Alternaria* sp., *Phomopsis* sp., *Bipolaris cactivera*, *Cladosporium cucumerinum* and *Colletotrichum gloeosporiodes* cause damage or loss to the plants and fruits of pitahaya (Ngoc *et al.*, 2017). Two species of fungi, *Phomopsis* sp. and *Alternaria* sp. have been found on the stems (Oeurn *et al.*, 2015). *Alternaria* sp. infects stems and fruits. In the spring, round reddish to orange lesions with dark red centers and no halo were observed

on 60% pitahayas stalks with an estimated loss of 10-20% on a farm on the Miami-Dade country farm in Florida. The lesions coalesce and formed larger diseased areas (Patel *et al.*, 2017). Since the disease was reported for the first time in Chhattisgarh not much work have been done in the management of *Alternaria* blight in red pitahaya.

Materials and Methods

Isolation of pathogen

Stems of red pitahaya showing typical blighted symptoms caused by *Alternaria* sp. were collected from the growing orchards from district Raipur (C.G.). Isolation process was done under aseptic condition in a laminar air flow. For fungal isolation, small segments of the diseased tissue were cut along with a portion of healthy stem (5 × 5 mm^2) with a sterilized blade and the surface of stem segments were sterilized in 0.1% sodium hypochlorite solution for 30s . The stem segments were rinsed three times in distilled sterile water, dried and placed on potato dextrose agar (PDA) medium in sterile petri plates and incubated for 7 days at 26 ± 1°C. After 7 days, fungal colony observed were identified and purified by hyphal tip method (Pathak, 1972).The pure culture obtained and maintained with the help of repeated sub culturing. The stock culture grown on potato dextrose agar (PDA) slants were stored at 5°C in refrigerator.

In vitro evaluation of *Trichoderma* isolates against the *Alternaria* sp.

Different *Trichoderma* isolates obtained from the Deptt. of Plant Pathology, College of Agriculture, IGKV, Raipur collected from different places of Chhattisgarh were evaluated to check their antagonism against the *Alternaria* sp. under *in vitro* conditions. Different isolates of *Trichoderma* was isolated from the soil rhizosphere and phylloplane of pitahaya plant by using *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). 5mm diameter discs of *Trichoderma* as well as the test pathogen were cut with the help of sterilized cork borer (5mm) from the 7 day old culture. The discs were placed on a petriplate containing PDA opposite to each other and 1.5cm away from plate boundary. Adequate control plate was maintained by placing only the pathogen in the culture medium. The plates were incubated at 26±1°C. Petri plates were observed after seven days of incubation to record the antagonistic interactions between the pathogen (*Alternaria* sp.) and the *Trichoderma* isolates. The percent inhibition (I) of the test pathogen was calculated after 7 days by using the formula (Vincent, 1947) as given below:

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

where,
I = Inhibition of pathogen growth,
C= Pathogen growth in control,
T= Pathogen growth in treatment.

In vitro evaluation of fluorescent *Pseudomonas* isolates against the *Alternaria* sp.

Different *Pseudomonas fluorescence* isolates obtained from the Deptt. of Plant Pathology, CoA, IGKV Raipur from different places of Chhattisgarh were evaluated *in vitro* to check their antagonism effect against the *Alternaria* sp. *Pseudomonas fluorescence* isolates were multiplied on King's B broth and incubated for 2 days at 26±1°C. Petri-plates having pre-sterilized peptone glucose agar medium (PGA) were inoculated with *Alternaria* sp.(center). The bipartite interactions were performed after a simple confrontation assay (Kotasthane *et al.*, 2017) and the plates were incubated at 26 ± 1°C. Petri plates were

observed after seven days of incubation to record the antagonistic interactions between the pathogen (*Alternaria* sp.) and the biocontrol agent (*Pseudomonas*). The percent inhibition (I) of the test pathogen was calculated after 7 days by using the formula as given below:

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

where,
I = Inhibition of pathogen growth,
C= Pathogen growth in control,
T= Pathogen growth in treatment.

In vitro evaluation of plant extracts against the *Alternaria* sp.

The effectiveness of the plant extracts against *Alternaria* sp. *in vitro* was determined by using the poison food technique. Ten plant samples were collected . Different plant parts were used (Bulb scales, young twigs, fruits, flowers) and 16g of each botanist (described below) were macerated with 40ml of distilled water in a mortar and pestle. Macerated biomass was stored overnight in culture tubes for the exudation of biochemicals. The macerated biomass was filtered with a muslin cloth. The filtered extracts were sterilized and stored at 4°C as a 40% stock solutions. At the center of the petri dishes was placed a 5mm diameter of an actively growing mycelium disc of the culture pathogen of 6-7days old culture. Plates containing 0.2% of mancozeb 75% WP fungicide in the PDA medium acted as a positive control and plates with PDA medium added with 2.2ml distilled water acted as a negative control, plates were incubated at 26±1°C and 3 replicates were maintained for each treatment. Radial growth of mycelium was measured after 7 days after inoculation. The results were compared with the negative control. The per cent inhibition of the fungus in the treatments was calculated using following formula:

Percentage of Inhibition = A - B / A ×100

Where A=Radius of pathogen in control plate, B=Radius of pathogen in treatment plate.

Results and Discussion

Data of experiment pertaining to *in vitro* efficacy of *Trichoderma* isolates against *Alternaria* sp. (Table 2) showed significante difference among all the isolates of *Trichoderma*. All the isolates of *Trichoderma* showed >60% inhibition except T3 and T8. Isolate-T2, T4 and T5 showed better inhibition %. Isolate T2 showed maximum inhibition of the mycelium growth of *Alternaria* sp. (71.23%) which was isolated from the phylloplane of red pitahaya, whereas minimum growth inhibition of mycelium was noticed in isolate T3 (54.36%).

The results obtained from this investigation were supported by findings of various workers. Akbari and Parakhi (2007) reported that *Trichoderma* isolates showed strong antagonism against *Alternaria alternata* compared to control

In vitro efficacy of fluorescent *Pseudomonas* against *Alternaria* sp. (Table 3) showed significant differences among all the isolates of *Pseudomonas*. Among these isolate P5 showed maximum inhibition (43.94%) in mycelium growth, whereas minimum growth inhibition of mycelium (29.92%) was observed in isolate P1.

The results obtained from this investigation were supported by findings of Maheswari and Krishna (2013) they reported *P. fluorescens* which significantly inhibits the growth of *A. alternata* as compared to control.

Table.1 List of plant extracts used in the experiment

S.N.	Plant species	Parts used
1.	Onion (*Allium cepa*)	Bulb scales
2.	Duranta (*Duranta repens*)	Young twigs with fruits
3.	Ashoka (*Polyalthia longifolia*)	Young twigs
4.	Neem (*Azadirachta indica*)	Young twigs
5.	Bougainvillea (*Bougainvillea spectabilis*)	Young twigs with flowers
6.	Dhatura (*Datura* sp.)	Whole plant
7.	Tamarind (*Tamarindus indicus*)	Young twigs with fruits
8.	Cauliflower (*Brassica oleracea var. botrytis*)	Leaves
9.	Caribbean copper plant (*Euphorbia* sp.)	Young twigs
10.	Chilli (*Capsicum annum*)	Young fruits
11.	Distilled water	-
12.	2% Mancozeb 75% WP	-

Table.2 *In vitro* efficacy of *Trichoderma* isolates against the *Alternaria* sp.

S.N.	*Trichoderma* Isolates	Mycelium diameter (mm)	Inhibition %
1	T1	32.00	63.17
2	T2	25.00	71.23
3	T3	39.66	54.36
4	T4	29.66	65.86
5	T5	26.33	69.70
6	T6	28.33	67.39
7	T7	27.33	68.55
8	T8	35.00	59.72
9	Control	86.90	
	C.D.(5%)	3.429	

Table.3 *In vitro* efficacy of fluorescent *Pseudomonas* isolates against the *Alternaria* sp.

S.N.	*Pseudomonas* Isolate	Mycelium diameter (mm)	Inhibition %
1	P1	61.66	29.92
2	P2	53.66	39.01
3	P3	58.00	34.09
4	P4	58.33	33.72
5	P5	49.33	43.94
6	Control	88.00	
	C.D.(5%)	3.547	
	SE(m)	1.139	

Table.4 Antifungal activity of aqueous plant extracts at 4% concentration after seven days of incubation

S.N.	Plant species used	Conc. in 4%	Mycelial growth (mm)	P. I. %
1	Distilled Water (negative control)	4	71.8	0
2	2% Mancozeb 75% WP (positive control)	0.2	0.00	100
3	Onion (*Allium cepa*)	4	63.40	11.69
4	Duranta (*Duranta repens*)	4	52.20	27.29
5	Tamarindus (*Tamarindus indicus*)	4	61.40	14.48
6	Ashoka (*Polyalthia longifolia*)	4	37.80	47.35
7	Azadirachta (*Azadirachta indica*)	4	60.20	16.15
8	Bougainvillea (*Bougainvilla spectabilis*)	4	67.00	6.68
9	Dhatura (*Datura* sp.)	4	38.60	46.23
10	Caribbean copper plant (*Euphorbia* sp.)	4	52.80	26.46
11	Chilli (*Capsicum annum*)	4	46.20	35.65
12	Cauliflower (*Brassica oleracea var. botrytis*)	4	63.00	12.25
	C.D.(5%)		2.902	
	SE(m)		1.017	

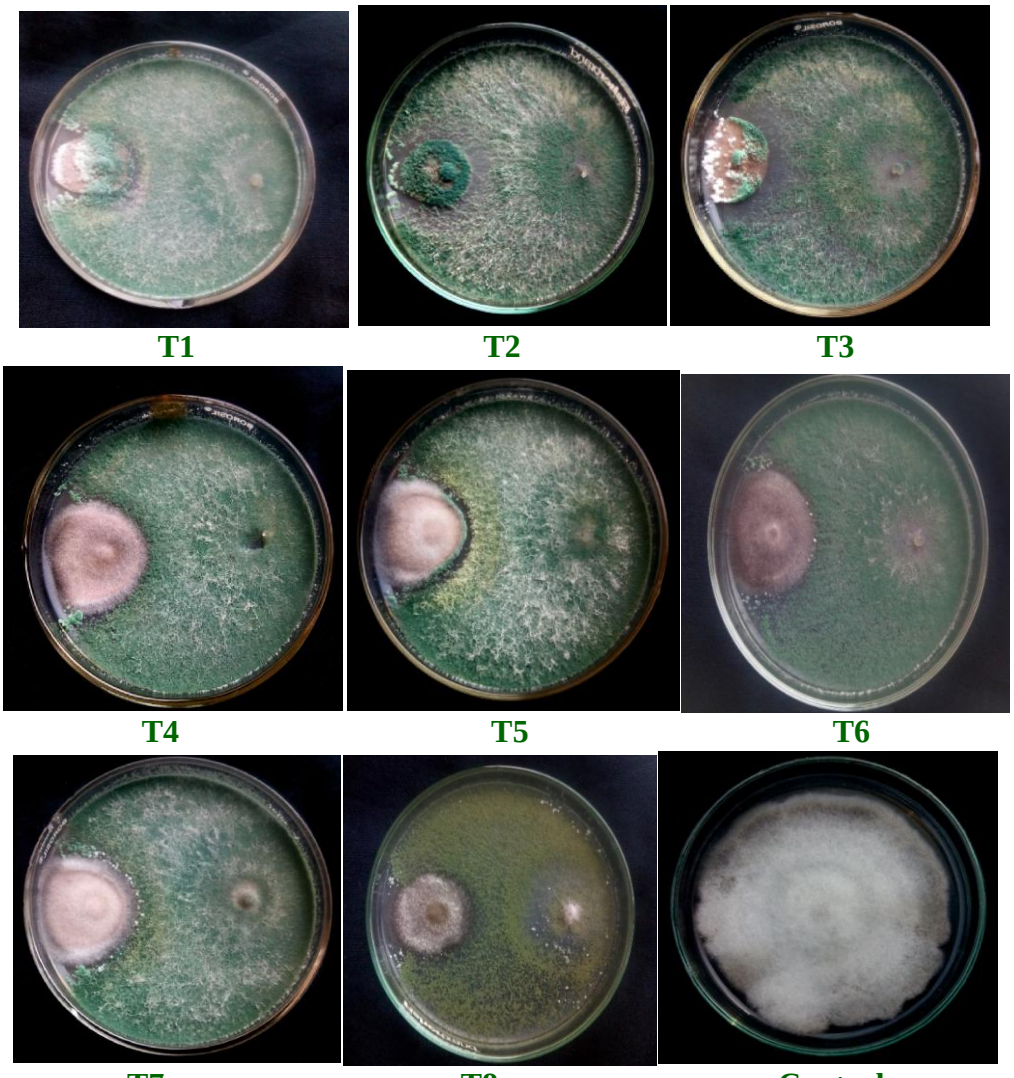

Plate.1 *In vitro* efficacy of *Trichoderma* isolates against the *Alternaria* sp.

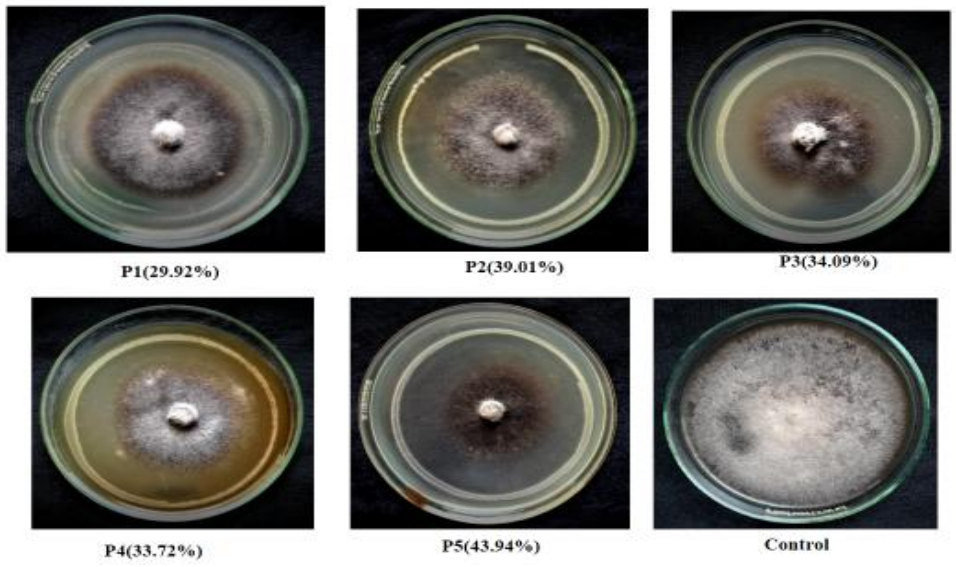

Plate.2 Efficacy of different isolates of fluorescent *Pseudomonas* against the *Alternaria* sp.

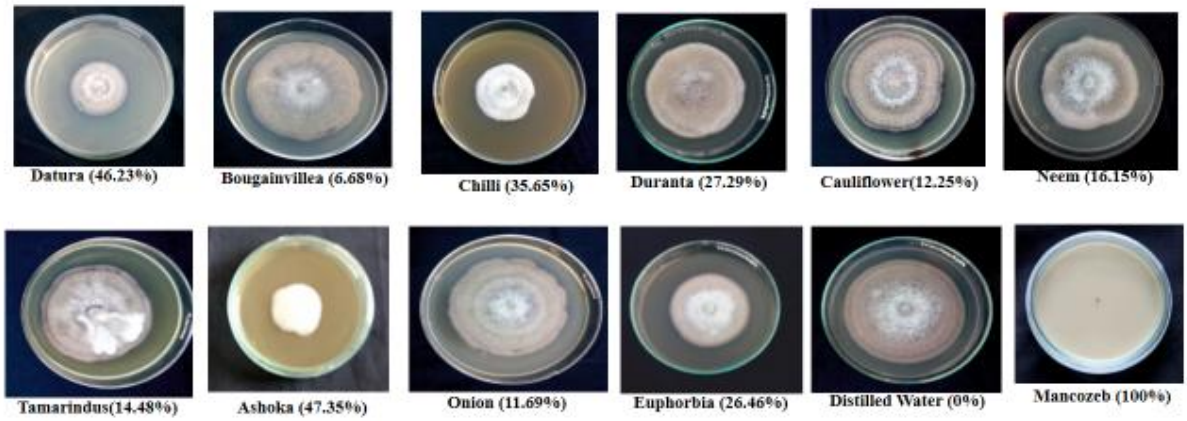

Plate.3 *In vitro* efficacy of isolates of fluorescent *Pseudomonas* against the *Alternaria* sp

***In vitro* evaluation of plant extracts against the *Alternaria* sp.**

In vitro efficacy of different plant extracts (4% concentration) against *Alternaria* sp. Showed significant difference in mycelium growth. Significantly higher inhibition % was observed in *Polyalthia* (47.35%) as compared to other treatments followed by *Dhatura* (46.23%), *Capsicum* (35.65%), *Duranta* (27.29%) and *Euphorbia* (26.46%). On the other hand, among the 10 plant extracts tested, most effective plant extract was found to be *Polyalthia* which exhibited minimum mycelium growth (37.80mm). However, maximum mycelium growth (67.000) was observed in *Bougainvillea* control plate (water) gained full growth in plate.

The results obtained from this investigation were supported by findings of Ravikumar *et*

al., (2013) they tested aqueous extracts of 39 plants selected from the local flora at 4% concentration in PDA (potato dextrose agar). Polyalthia extract was better and significantly reduced the mycelial growth of the *Alternaria solani*.

In conclusion, *Alternaria* blight of red pitahaya is an important disease caused by *Alternaria* sp. Under *in vitro* conditions, the disease was well controlled by biocontrol agents and plant extracts. The maximum mycelial growth inhibition was observed in *Polyalthia longifolia* plant extract, also *Trichoderma* isolate T2 and *Pseudomonas* isolate P5 were efficiently effective against *Alternaria* sp. *in vitro* condition.

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