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Research Article

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Effect of Botanicals and Bio agents on Growth of *Ceratocystis fimbriata* ELL. and Halst. Causing Wilt in Pomegranate

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ABSTRACT

Seven each of botanicals and four each of bioagents were evaluated in vitro against Ceratocystis fimbriata, the causal agent of wilt of pomegranate. Among four bioagents T. harzianum and T. viride showed maximum inhibition of the test fungus (100%) followed by P. fluorescens (42.33%), within four days and completely inhibited the perithecium production as well as grows over the pathogen.Among the plant extracts Allium sativum (32.96%) was found effective in inhibiting mycelial growth and Zingiber officinale on par (32.90%). Least growth inhibition of pathogen was obtained in Eucalyptus (11.47%).

Key words: *Botanicals, Bioagents, Wilt, Ceratocystis fimbriata,Trichoderma harzianum, Allium sativum*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae and pomegranate is a native of Iran. It is commercially an important fruit crop of both tropical and subtropical regions. In India, it is regarded as a "vital cash crop", grown in an area of 1, 16,000 ha with a production of 89,000 MT with an average productivity of 7.3 MT. Karnataka state has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 1, 29, 547 tonnes. Where this crop has spread across different districts viz., Bijapur, Bellary, Bagalkot, Koppal, Chitradurga, Belgaum, Davangere, Tumkur, Bangalore and Gulbarga. Pomegranate suffers from ten economically important diseases, among them bacterial blight or spot, fruit rot, anthracnose and wilt complex are severe and cause significant losses in recent years. Wilt caused by *Ceratocystis fimbriata* is the most severe disease in Karnataka which causes yellowing, drooping and death of pomegranate plant leading to loss to the farmers. It is pertinent to generate information on the efficacy of available and new botanicals and bioagents for managing the disease. Hence, the present study was undertaken to screen various botanicals and bioagentsin vitro to manage wilt, there is no residual effect as fungicides.

MATERIAL AND METHODS

a. Evaluation of plant extract

Plant based pesticides are relatively cheaper, safe and non-hazardous and they can be used easily and successfully against the plant pathogenic fungi. The present investigation was aimed to study the anti - fungal activity of some plant extracts. The following plant extracts were evaluated at 10, 20 and 30 per cent concentration.

S. No	Botanical name	Parts used	Common name
1.	*Azadirachtaindica* A. Juss	Leaf	Neem leaf extract
2.	*Allium sativum* L.	Bulb	Garlic bulb extract
3.	*Allium cepa* L.	Bulb	Onion bulb extract
4.	*Datura stromanium* L.	leaf	Datura leaf extract
5.	*Ocimum sanctum* L.	Leaf	Ocimum leaf extract
6.	*Eucalyptus manniferrae* L	Leaf	Eucalyptus leaf extract
7.	*Zingiber officinalae* L.	Rhizome	Rhizome extract

Preparation of cold aqueous extract

Fresh leaves of each test plant were collected and washed with tap water and then in distilled water. Then 10, 20 and 30 grams of fresh sample was crushed in a surface sterilized pestle and mortar by adding 50 ml sterile distilled water (1:1 w/v). The extracts were filtered through two layers of muslin cloth. Finally filtrate, thus obtained, was used as a stock solution. To study the anti-fungal mechanism of plant extract the poisoned food technique was followed as suggested by Nene and Thapliyal[1]. 50 ml of 10, 20 and 30 percent stock solution was mixed with 50 ml of sterilized molten PDA media respectively so as to get 10, 20 and 30 per cent concentration. The medium was thoroughly shaken for uniform mixing of the extract.

Twenty ml of medium was poured into each of the 90 mm sterilized Petriplates. Each plate was seeded with 0.5 cm mycelial discs taken from the periphery of twelve day old fungal culture and incubated at 25 ± 2^0C till the growth of colony touches the periphery in the control plate. The disc was placed upside down in the centre of the Petriplates, so that the mycelium was in direct contact with the mycelium poisoned with the requisite plant extract at required concentration. Three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs were inoculated into the centre of PDA plates without plant extracts. Mean colony diameter in each treatment was recorded by taking the diameter of the colony in two directions. Radial growth over control was measured and per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent[12].

$$I = 100 (C-T) / C$$

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

b. Evaluation of bio agents

Four bio agents were tested against *C. fimbriata* and they were collected from UAS, Dharwad. About 20 ml of PDA was poured into sterile Petriplates and allowed to solidify. From previously grown young cultures of both fungal bio agents and host pathogen 0.5 cm fungal disc of test fungus and respective bioagents were transferred aseptically to Petriplates simultaneously by leaving sufficient space in between two discs. In case of bacterium, mycelial discs of the fungus were kept at opposite ends and bacteria streaked at the center. Three replications were maintained for each treatment. The Petriplates were

incubated at 25±1^{0}C till the growth of colony touches the periphery in the control plate. Colony diameter of both the test fungus and bio agents were measured and per cent inhibition was calculated. Data were analyzed statistically.

RESULTS

Among bioagents tested (Table 1 and Plate 1), fungal bio agents were found better than bacterial bio agents. Among the different bio agents *T. harzianum* showed the maximum inhibition of the test fungus (100%) but remains on par with *T. viride* (100%). Whereas, *P. fluorescens* (42.33%) showed lower inhibitory effect over pathogen, while the *Bacillus subtilis* was recorded zero inhibition on pathogen growth.*T. harzianum* showed the maximum inhibition of the test fungus within four days and completely inhibited the perithecium production as well as grows over the pathogen (Table 2). *T. viride* was taken six days. *P. fluorescens* also inhibited perithecium production in eight days.*B. subtilis* was completely ineffective.

In vitro evaluation of plant extracts on growth of *C. fimbriata*

The antifungal activity of seven plantsextracts *viz.*, onion bulb, neem leaf, tulsi leaf, garlic cloves, datura leaf, ginger rhizome, nilagiri leaf were tested against *C. fimbriata* (Table 3 and Plate 2). Among them *A. sativum* (32.96%) was found effective in inhibiting mycelial growth followed by *Z. officinalis* (32.90%) which were on par with each other and these were significantly superior over all other plant extracts evaluated. Ocimum (22.27%) was the next best followed by neem (18.88%) and datura (18.60%) which were on par with each other. Least growth inhibition of pathogen was observed in eucalyptus (11.47%).The leaf extract at 30 per cent were significantly superior over 10%, 20%. ginger (47.96%) at 30% was the best and significantly superior over all other plant extracts. Next was garlic (35.74%) followed by ocimum (22.27%), neem (18.88%), datura (18.63%) and onion (15.55%). eucalyptus and onion were non effective at all concentrations.

Table 1: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by different bioagents

Sl. No	Bioagents	Percent inhibition over control
1	*Bacillus subtilis*	0.00 (0.00)
2	*Pseudomonas fluorescens*	42.33 (40.72)
3	*Trichoderma viride*	100 (90.00)
4	*Trichoderma harzianum*	100 (90.00)
Mean		55.56 (60.58)
SEm ±		0.30
CD @ 1%		1.23

Table 2: Effect of bioagents on growth and perithecial production of *Ceratocystis fimbriata*

S. No.	Bioagents	Growth of pathogen	Days taken for inhibition	Perithecium production
1	*Bacillus subtilis*	Present	No inhibition	+
2	*Pseudomonas fluorescens*	Absent	8	-
3	*Trichoderma viride*	Absent	6	-
4	*Trichoderma harzianum*	Absent	4	-

\- Absent
\+ Present

Table 3: *In vitro* evaluation of different plant extracts against the mycelial growth of *Ceratocystis fimbriata*

S. No	Plant extracts		Percent inhibition of mycelial growth (mm)			Mean
			Concentration			
	Botanical name	Common name and part used	10%	20%	30%	
1	*Allium cepa*L.	Onion (Bulb)	12.22 (23.25)	12.59 (23.26)	15.55 (23.67)	13.45 (23.39)
2	*Allium sativum* L.	Garlic (Clove)	28.33 (32.77)	34.81 (36.54)	35.74 (36.70)	32.96 (35.33)
3	*Azaradictaindica*A. Juss	Neem (Leaf)	10.73 (19.71)	20.18 (24.92)	25.73 (30.58)	18.88 (25.07)
4	*Datura stromanium* L.	Datura (Leaf)	10.92 (19.27)	18.69 (25.64)	26.29 (30.20)	18.63 (25.17)
5	*Eucalyptus manniferrae* L.	Nilgiri (Leaf)	9.25 (23.20)	12.03 (23.54)	13.14 (23.96)	11.47 (23.57)
6	*Ocimum sanctum* L.	Tulasi (Leaf)	17.77 (24.91)	22.40 (27.54)	26.66 (31.07)	22.27 (27.84)
7	*Zingiber officinalae* L.	Ginger (Rhizome)	23.51 (28.54)	24.25 (29.50)	47.96 (44.39)	32.90 (34.14)
Mean			16.10 (24.52)	20.70 (27.28)	27.29 (31.51)	21.50 (27.78)
Source					SE m ±	CD @ 1%
Plant extract (P)					0.45	1.70
Concentration (C)					0.29	1.12
P×C					0.78	2.96

Plate 1: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by different bioagents

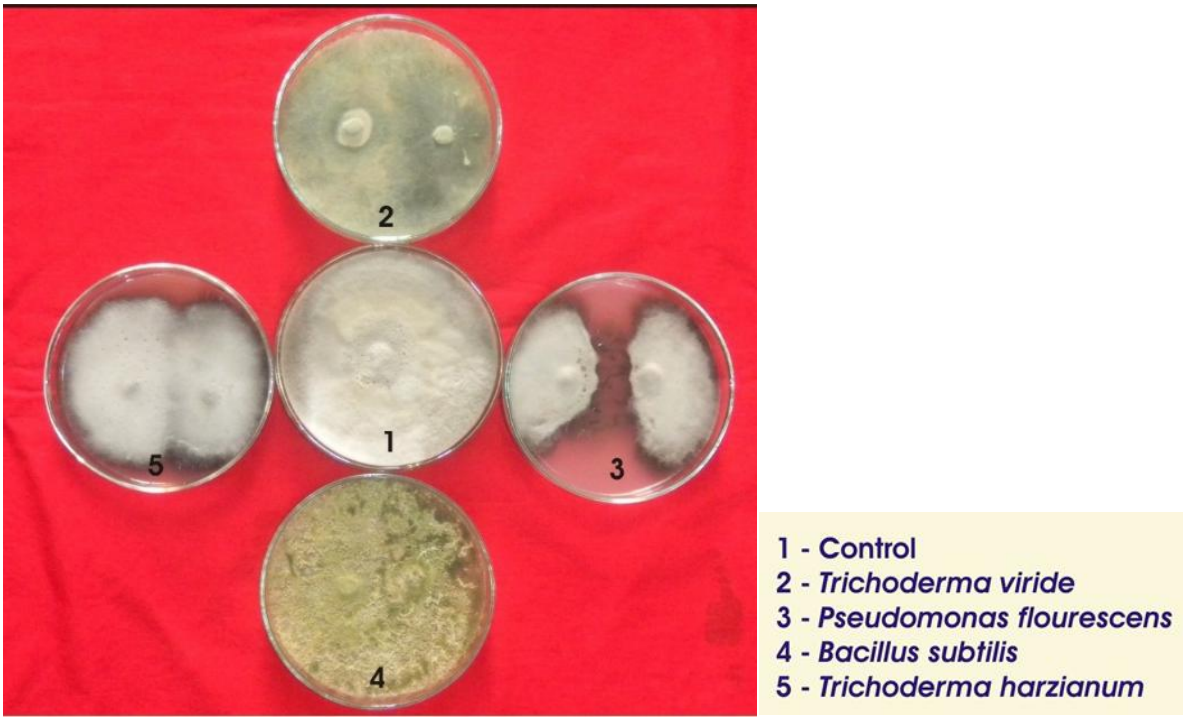


Plate 2: *In vitro* evaluation of different plant extracts against the mycelial growth of *Ceratocystis fimbriata*

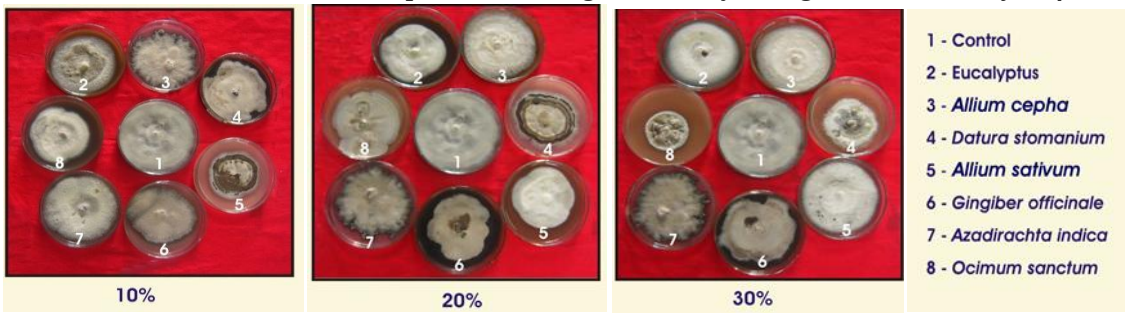


Fig. 1: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by different bioagents

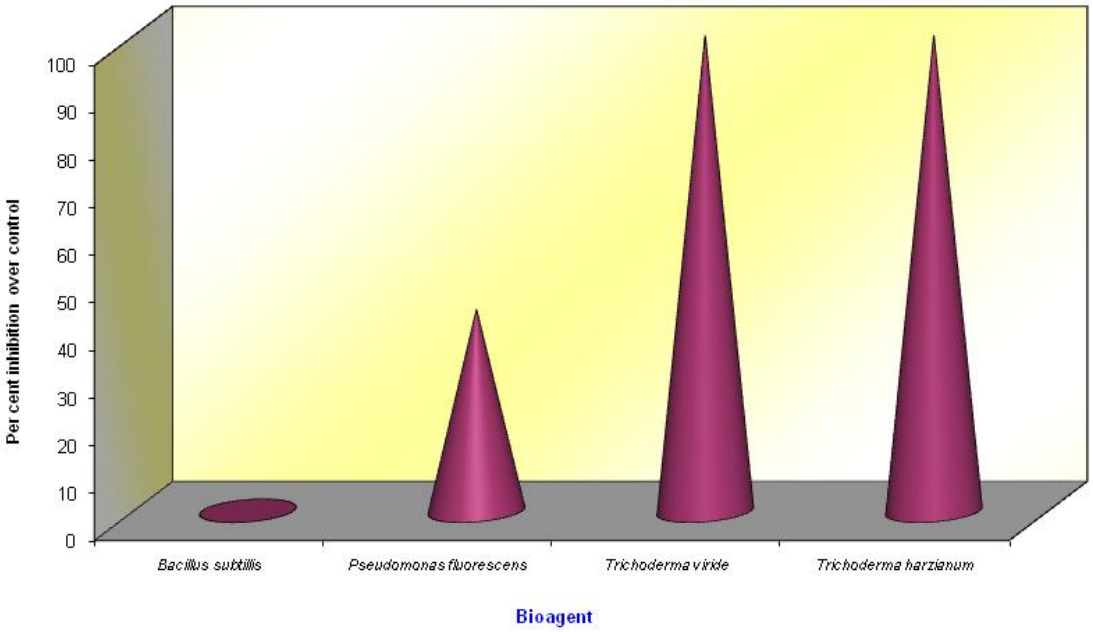
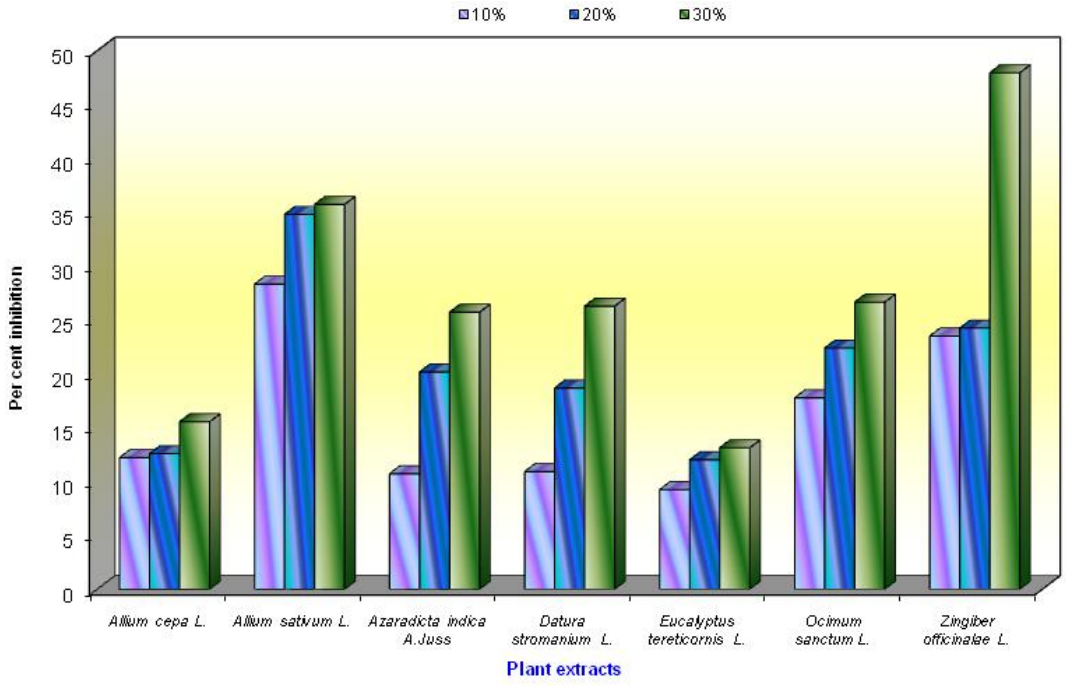


Fig. 2: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by plant extracts



DISCUSSION

In vitro evaluation of bioagents

The results of dual culture technique revealed that stronger antagonism was noticed in case of *T. harzianum* within four days and *T .viride* in six days. All the species of *Trichoderma s*howed more mycelial inhibition compared to bacterial antagonists. This can be attributed to higher competitive ability of *Trichoderma* spp. similar trend was observed earlier in *C. paradoxa* by Vijaya *et al*.,[11]. It was widely known that *T. harzianum* shows antagonistic behaviour towards *C. paradoxa*. The powerful antagonistic behaviour of the *T. harzianum* can be attributed to competition, parasitism, and antibiosis or by synergistic combination of these modes of action[13]. The mechanism involved in inhibition of the test fungus may be due to the release of antibiotic (viridian) produced by *T. viride*[2,4,5]. It may also due to coiling effect around the hyphae of the fungal pathogen[6,7]. Another possibility for reduction in mycelial growth of test fungus may be competition between test fungus and *T. viride* for nutrition and other growth factors[3,8]. It was due to the penetration of the antagonistic hyphae into hyphae of the pathogen at the place of contact as confirmed by Mukherji *et al*.[9]. The next best bio control agent in inhibiting test fungi was *P. fluorescens*, which inhibited mycelial growth of 42.33 per cent. It may be due to release of antibiotic substances produced by *P. fluorescens* like I) Phenozines II) Pyroluterin III) Pyrrolnitrin and also by competition[10], showed in sugar cane sett rot caused by *C. paradox*.

In vitro evaluation of plant extracts

In present investigation seven plant extracts were evaluated under *in vitro* condition against *C. fimbriata* to know the fungi toxic nature of their extracts. Though complete inhibition of the pathogen was not observed in any of the plant extract tested but considerable amount of inhibition was noticed in some of them. Among the seven plant extracts tested against *C. fimbriata*, Ginger at 30 per cent (47.96%) and Garlic at 10 per cent (28.33%), 20 per cent (34.81%) and at 30 per cent (35.74%) and Datura at 30 per cent (26.29%) and Neem at 30 per cent (25.73%) was significantly superior over all other plant extracts. Least growth was observed in case of *Eucalyptus* at all three concentrations. The mycelial growth of fungus was inhibited to greater extent by ginger followed by Garlic. Similar trend was observed earlier in *C. paradoxa* reported in sugarcane sett rot (Vijaya *et al*., 2006).

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