

Effect Of Antagonists Against *Ceratocystis fimbriata* ELL. and Halst. Causing Wilt in Pomegranate

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ABSTRACT

Wilt complex of pomegranate is a serious threatening in Karnataka, it is caused by *Ceratocystis fimbriata*. So two fungal and two bacterial antagonists were screened against *Ceratocystis fimbriata* viz., *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens*. In fungal antagonists both are effective and inhibited pathogen completely. The *T. harzianum* showed the maximum inhibition against fungus (100%) but remains on par with *T. viride* (100%). In bacterial antagonists *P. fluorescens* inhibited pathogen development but *B. subtilis* is not inhibited growth of fungus. The *P. fluorescens* (42.33%) showed lower inhibitory effect over pathogen, while the *Bacillus subtilis* was recorded zero inhibition on pathogen growth. On the whole, fungal bio agents were found better than bacterial bio agents. *T. harzianum* showed the maximum inhibition of the test fungus within four days and completely inhibited the perithecial production as well as grows over the pathogen. *T. viride* was taken six days. *P. fluorescens* also inhibited perithecial production in eight days. *B. subtilis* was completely ineffective in inhibition of pathogen growth as well as perithecial production.

Key words: *Ceratocystis fimbriata*, *Trichoderma harzianum*, *Viride*, *Perithecial* and Wilt

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family puniceae. Pomegranate is a native of Iran, where it was first cultivated in about 2000 BC. Area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, and drought tolerance, higher yield levels with excellent keeping quality and remunerative prices in domestic as well as export market. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status as well as on saline soil. Pomegranate is regarded as the “Fruit of Paradise”. It is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly. 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 ¹⁴. Among the different states growing pomegranate, Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh,

Gujarat and Rajasthan. 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 tonnes¹⁵. Where this crop has spread across different districts viz., Bijapur, Bellary, Bagalkot, Koppal, Chitradurga, Belgaum, Davangere, Tumkur, Bangalore and Gulbarga.

Successful cultivation of pomegranate in recent years has met with different traumas such as pest and diseases. Among diseases wilt complex caused by *Ceratocystis fimbriata* Ell. and Halst. is a major threat. At present, the crop is severely affected by wilt pathogen and day by day the wilting severity is increasing at faster rate. It was first noticed in two areas of the Bijapur district of India in 1990. Around 1993, rapid spread of this disease was observed in the entire Bijapur district. The cause was not identified until 1995, in 1996, the fungus *C. fimbriata* was isolated from discoloured stem, root, and branch tissues on wilted plants. Disease is characterised by the initial symptoms were yellowing and wilting of leaves on one to several branches leading to death of affected plants in a few weeks. Cross sections of diseased plants revealed brown discoloration in the outer xylem from roots to the main trunk¹³.

The disease is prevalent in parts of a Maharashtra, Karnataka, Andrapradesh, Gujarat and Tamil Nadu states¹². Despite many factors conducive for the high severity of disease, seedlings selection for planting, soil borne nature and also association with shot hole borer and plant parasitic nematodes. This might be the reason for the current rampant spread of the disease in south Indian states. This draws the attention of the present study to know the incidence of wilt complex in north Karnataka. The Bioagents are the natural enemies against plant pathogens, they are eco-friendly in nature and there is no health hazardous effect on mankind. Hence our study is directed towards biological control of wilt complex. Bioagents are also equally important components of the management. Therefore effort has been made in this regard to see the efficacy of some biological agents against the pathogen.

MATERIAL AND METHODS

Four bio agents were tested against *C. fimbriata* and they were collected from Dept. of Plant Pathology, 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 $25 \pm 1^{\circ}\text{C}$ 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 analysed statistically.

RESULTS

The competitive ability of antagonists (Plate 1) against *C. fimbriata* was studied by dual culture method, the results obtained are presented in Table 1 (Fig. 1). There are significant differences between the bio agents tested. On the whole, 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 peritheciium production as well as grows over the pathogen. *T. viride* was taken six days. *P. fluorescens* also inhibited peritheciium production in eight days. *B. subtilis* was completely ineffective.

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

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

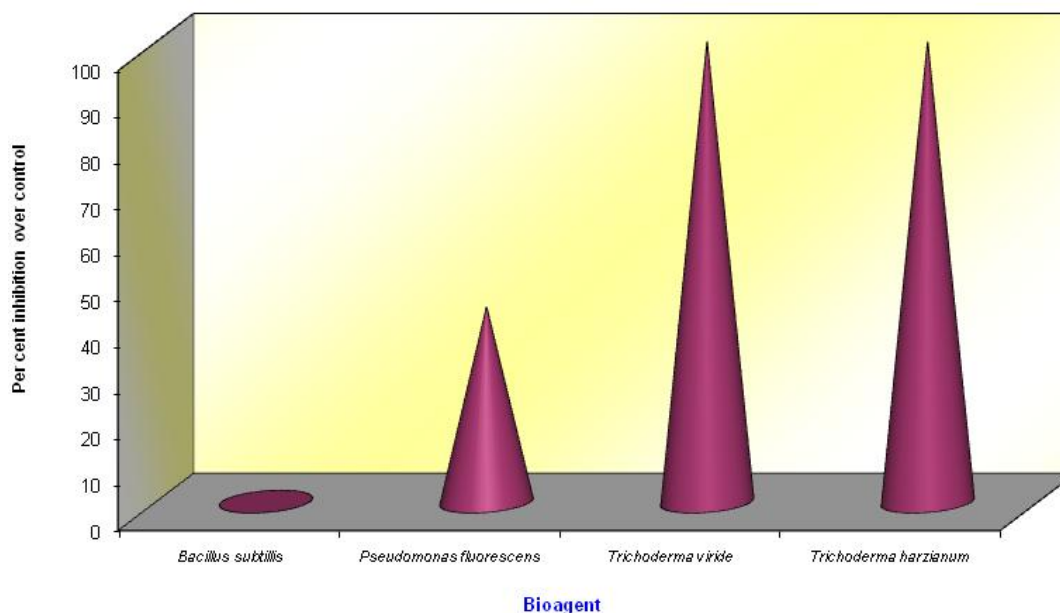
Table 2: Effect of bioagents on growth and perithecial production of *Ceratocystis fimbriata*

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

- Absent

+ Present

Plate 11: Per cent inhibition of mycelia growth of *Ceratocystis fimbriata* by different bioagents

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

DISCUSION

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* showed 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*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 action¹⁰. The mechanism involved in inhibition of the test fungus may be due to the release of antibiotic (viridian) produced by *T. viride*^{1,9,8}. It may also due to coiling effect around the hyphae of the fungal pathogen^{2,7}.

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^{6,5}. 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.*⁴. 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) Phenazines II) Pyroluterin III) Pyrrolnitrin and also by competition³, showed in sugar cane sett rot caused by *C. paradox*.

SUMMARY AND CONCLUSION

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 perithecia production as well as grows over the pathogen.

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