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Research Article

IN VITRO EVALUATION OF BIOAGENTS AGAINST WILT OF POMEGRANATE CAUSED BY *CERATOCYSTIS FIMBRIATA*

KERAKALAMATTI M.*[1], MESTA R.K.[1], RUDRESH D.L.[1], LOKESH M.S.[2], KIRAN KUMAR K.C.[1] AND RAGHAVENDRA G.[1]
[1]Department of Plant Pathology, College of Horticulture, University of Horticultural Sciences, Bagalkot, 587104, Karnataka, India
[2]Department of Plant Pathology, College of Horticulture, Munirabad, 583233, University of Horticultural Sciences, Bagalkot, 587104, Karnataka, India
*Corresponding Author: Email - madhushri199456@gmail.com

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Abstract- Pomegranate (*Punica granatum* L.) is one of the important fruit crops, nowadays is highly threatened by the wilt caused by *Ceratocystis fimbriata*. Eleven bioagents were evaluated *in vitro* against *Ceratocystis fimbriata*. Among the bio agents tested, *Trichoderma harzianum, Trichoderma isolate* 1 and *Trichoderma* isolate 5 recorded the maximum percent inhibition of mycelial growth (100%). It was found significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma* isolate 4, *Trichoderma virens*, *Trichoderma* isolate 2 and *Pseudomonas fluorescens*. Whereas, the minimum parasitic activity was noticed in case of *Pseudomonas putida* (43.12%), *Paecilomyces lilacinus* (44.98%), *Trichoderma* isolate 3 (48.94%) and *Bacillus subtilis* (55.00%).

Keywords- *Pomegranate, Wilt, Ceratocystis fimbriata, Bioagents, Inhibition*

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Introduction

Pomegranate (*Punica granatum* L.) is an attractive, highly prized, nutrient rich fruit and is a long lived drought tolerant plant. Arid and semiarid zones are popular for growing pomegranate trees. Pomegranate belongs to the family Lythreceae, having 2n=16 number of chromosome and it is native to Iran. The nutrient dense, antioxidant rich fruit has been revered as a symbol of health, fertility and eternal life. The basket of pomegranate was chosen as a symbol of plenty for the 18th International Horticultural Congress, held in 1970 [1]. However, in the recent past pomegranate cultivation has been highly threatened due to incidence of wilt (*Ceratocystis fimbriata*) disease. In some orchards diseased plants were died due to wilt in patches, thereby indicating the spread of the disease from an infected to an adjacent healthy orchard. Splitting of root or vertical sections of diseased plant parts showed dark grayish brown streaks or distinct starburst like black discoloration in vascular and adjoining cortex tissues. Blue stains were also observed on the stem during the present investigation. Management of plant disease caused by fungi is very difficult with chemicals alone which seems to be ineffective and uneconomical. Therefore, present study was undertaken to know the efficacy bio agents against *Ceratocystis fimbriata* causing wilt of pomegranate.

Material and Methods

The efficacy of different bioagents were tested against *Ceratocystis fimbriata* through dual culture technique. About 20 ml of PDA was poured into sterile Petri plates and allowed to solidify. From previously grown young cultures of 0.5 disc of test fungus and respective bioagents were transferred aseptically to Petri plates simultaneously by leaving sufficient space in between two discs. In case of bacterial bio agent, mycelial discs of the fungus were kept at opposite ends and bacteria streaked at the center. Three replications were maintained for each treatment. The Petri plates were incubated at 25±1°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 percent inhibition was calculated. Data were analyzed statistically.

Results

The competitive ability of antagonists against *C. fimbriata* was studied by dual culture method and the results obtained are presented in [Table-1], [Fig-1] and [Plate-1]. There was a significant difference between the bioagents tested with respect to percent inhibition of mycelial growth of *C. fimbriata*. Among the bio agents tested, *Trichoderma harzianum*, *Trichoderma* isolate 1 and *Trichoderma* isolate 5 recorded the maximum percent inhibition of mycelial growth (100%). It was found significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma* isolate 4, *Trichoderma virens*, *Trichoderma* isolate 2 and *Pseudomonas fluorescens*. Whereas the minimum parasitic activity was noticed in case of *Pseudomonas putida* (43.12%), *Paecilomyces lilacinus* (44.98%), *Trichoderma* isolate 3 (48.94%) and *Bacillus subtilis* (55.00%).

Table-1 *In vitro* evaluation of bioagents against *Ceratocystis fimbriata* through dual culture technique

SN	Bioagent	Percent inhibition of mycelial growth (mm)
	Bacillus subtilis	55.00(47.85) *
1	*Pseudomonas fluorescens*	70.00(56.76)
2	*Pseudomonas putida*	43.12(41.00)
3	*Trichoderma virens*	90.62(72.30)
4	*Trichoderma harzianum*	100.00(90.00)
5	*Paecilomyces lilacinus*	50.00(44.98)
6	*Trichoderma isolate* -1	100.00(90.00)
7	*Trichoderma isolate* -2	81.25(64.31)
8	*Trichoderma isolate*-3	56.87(48.94)
9	*Trichoderma isolate*-4	92.50(77.08)
10	*Trichoderma isolate*-5	100.00(90.00)
S. *Em*±		2.5
CD @ 1%		7.34

**Values in parenthesis are arc sine transformed values*

Discussion

Biological control offers an environmentally friendly and safe technology to control the plant pathogens.

Plate-1 Inhibition of mycelial growth of *Ceratocystis fimbriata* by different bio agents

It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for future and can be successfully utilized especially within the frame work of integrated disease management system [2]. Among the bio agents tested, *Trichoderma harzianum*, *Trichoderma* isolate 1 and *Trichoderma* isolate 5 recorded the maximum percent inhibition of mycelial growth (90.00%). It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma* isolate 4 (77.08%), *Trichoderma virens* (72.30%), *Trichoderma* isolate 2 (64.31%), *Pseudomonas fluorescens* (56.76%), *Trichoderma* isolate 3 (48.94%) and *Paecilomyces lilacinus* (44.98%). Whereas, the minimum parasitic activity was noticed in case of *Pseudomonas putida* and *Bacillus subtilis* which inhibited 41.00 and 47.85 percent of *C. fimbriata* colony. *Trichoderma harzianum* inhibits enzymes necessary for pathogens to penetrate plant surfaces. Mechanism involved in inhibition of the test fungus may be due to the release of antibiotic (viridian) produced by *T. virens* [3]. *T. harzianum* showed antagonistic behaviour towards *C. paradoxa* [4]. *T. viride* was found significantly superior which inhibited 73.50 percent of mycelial growth of *C. paradoxa* [5]. Another possibility for reduction in mycelial growth may be competition between *C. fimbriata* and *T. viride* for nutrition and other growth factors. It was due to the penetration of the antagonistic hyphae into hyphae of the pathogen at the place of contact [6]. The bio agents *T. longibrachiatum, T. koningii, T. hamatum, T. harzianum* and one bacterial antagonist *P. fluorescens* were completely inhibited *Ceratocystis paradoxa*, causing sett rot of sugarcane [7]. *T. harzianum* and *T. viride* showed maximum inhibition of the *C. fimbriata* (100%) followed by *P. fluorescens* (42.33%), where completely inhibited the perithecium production and could grow over the pathogen [8]. Bioagents against *C. fimbriata* in which *Trichoderma harzianum*-55 recorded the maximum percent inhibition of mycelial growth (76.00%) [9]. It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma viride*-27 (70.33%), *Trichoderma viride* (PDBC) (67.00%), *Trichoderma viride* (64.00%), *Trichoderma harzianum*-1 (54.67%), *Trichoderma harzianum*-2 (51.00%), *Pseudomonas fluorescens* (45.00%). The minimum parasitic activity was noticed in case of *Bacillus subtilis*-1 and *Bacillus subtilis*-2 which inhibited 40.67 and 41.67 percent of *C. fimbriata* colony.

Application of research: Use of bioagents is an ecofriendly and important component of management for plant disease caused by fungi. The research findings of in vitro evaluation of bioagents against wilt of pomegranate caused by Ceratocystis fimbriata is further helpful for in vivo studies to manage the disease.

Research Category: Plant Pathology

Abbreviations: C.D: Critical difference, S.Em: Standard Error of Mean

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***Research Guide or Chairperson of research: Professor Dr R. K. Mesta**
University: University of Horticultural Sciences, Bagalkot, 587104, Karnataka
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Study area / Sample Collection: College of Horticulture, University of Horticultural Sciences, Bagalkot, 587104, Karnataka

Cultivar / Variety name: Pomegranate (*Punica granatum* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
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References

[1] Singh Ranjit (1985) *Fruits, Fourth Edition, National Book Trust, India*, 135-137.
[2] Muthamilan M. and Jeyarajan R. (1996) *Indian J. Mycol. Pl. Path*., 26, 204-209.
[3] Dennis C. and Webster J. (1971) *Transact. British Mycol. Soc*., 57, 25-39.
[4] Whips J.M. (1992) *Bio. Sci. Tech*., 2, 3-24.
[5] Joshi S.N. (1999) *M.Sc. (Agri.) Thesis, Univ. Agril. Sci., Dharwad*.
[6] Mukherji S., Tripathi H.S. and Rathi Y.P.S. (2000) *J. Mycol. Pl. Path*., 31, 213-215.
[7] Vijaya H.K., Kulkarni S. and Hegde Y.R. (2007) *J. Agric. Sci*., 20 (1), 62-64.
[8] Sonyal S. (2010) *M.Sc. (Agri.) Thesis, Univ. Agril. Sci., Dharwad, India.*
[9] Somu R. (2017) *Ph.D. (Hort.) Thesis, Univ. Hort. Sci., Bagalkot, India.*