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In vitro bioefficacy of bioagents against *Alternaria alternata* causing fungal fruit rot in pomegranate

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Abstract
In vitro bioefficacy of different bioagents against *Alternaria alternata* causing fungal fruit rot in pomegranate were carried out at the Department of Plant Pathology, College of Agriculture, Latur during the year 2017-18. Among the different fungal and bacterial bioagents *T. harzianum* was found most effective with least mycelial growth (14.66 mm) and numerically highest mycelial inhibition (82.11%), followed by *T. virens* (16.66 mm and 80.07%), *T. hamatum* (18.66 mm and 77.96%), *T. asperellum* (21.33 mm and 75.44%), *A. niger* (29.33 mm and 66.18%), *P. fluorescens* (43.00 mm and 53.14%), *B. subtilis* (52.00 mm and 41.74%) and *T. koningii* (52.33 mm and 41.01%). The bioagents were evaluated *in vitro* against the pathogen *Alternaria alternata* causing fungal fruit rot in pomegranate by dwell culture technique. Among the *in vitro* evaluated all bioagents *T. harzianum, T. virens, T. hamatum,*and *T. asperellum* were found more efficient while *A.* niger, *P. fluorescens, B. subtilis* and *T. koningii,* were least effective against *Alternaria alternata* major fungal pathogen causing fruit rot in pomegranate.

Keywords: *Alternaria alternata,* bioagents, fungal, fruit rot, pomegranate

Introduction

Pomegranate (L.) is an important fruit crop of arid and semiarid regions of the World. India is one of the leading producers of pomegranate in the World. Maharashtra is the leading producer of pomegranate in India followed by Karnataka, Gujarat and Andhra Pradesh (Anonymous, 2013) [2]. Pomegranate is regarded as the "Fruit of Paradise". The most popular varieties in India are Ganesh, Mridula, Arakta, Bhagwa (Kesar). Successful cultivation of pomegranate in recent years has met with different problems such as pests and diseases. Among the various fungal diseases, fruit rot disease majorly caused *Alternaria alternata* (Fr.) Keissler., is one of the most serious disease of pomegranate, remaining latent in early stages of fruit development, internal rotting and reducing fruit quality to a greater extent. The pathogen *A. alternata* responsible for fruit rot was first reported in India by Madhukar and Reddy (1976) [14] and subsequently from USA, Mexico (Farr *et al.*, 2007) [8]. Fungal fruit rot of pomegranate caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive disease of pomegranate (*Punica granatum*) inflicting considerable quantitative and qualitative losses. Mostly the disease occurred on leaves and fruits but causes more damages to fruit of pomegranate. Considering the economic importance of the fruit crop as well as disease, present investigation was undertaken for *in vitro* bioefficacy of bioagents against *A. alternata* causing major fungal fruit rot in pomegranate. *In vitro* study was conducted in the laboratory at Department of Plant Pathology, College of Agriculture, Latur.

Material and methods

Six fungal and two bacterial bioagents were evaluated *in vitro* against *Alternaria alternata,* applying Dual Culture Technique (Dennis and Webster, 1971) [7]. Seven days old cultures of the test bioagents and test pathogen (*A. alternata*) grown on PDA were used for the study. Two 5 mm culture discs, one each of the test pathogen and test bioagent were cut out with sterilized cork borer and placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and three plates were incubated at 27 ± 2^0 C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as untreated control. Pure cultures of formulations of biocontrol agents viz., *Trichoderma asperellum*, *T. harzianum*, *T. hamatum*, *T. koningii*, *T. virens, Aspergillus Niger*, *Bacillus* *subtilis* and *Pseudomonas fluorescens* were obtained from the Department of Plant Pathology, College of Agriculture Latur, VNMKV, Parbhani; maintained and multiplied on appropriate culture media and used for present studies.

Observations on linear mycelial growth of the test pathogen and test bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test bioagent, over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978) [4].

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in Control plate - Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and discussion

Results (Plate 1, Fig. 1 and Table 1) revealed that all the bioagents evaluated exhibited fungistatic / antifungal activity against *A. alternata* causing fungal fruit rot in pomegranate and significantly inhibited its growth over untreated control. Of the six fungal antagonists tested, *Trichoderma harzianum* was found most effective and test pathogen recorded least linear mycelial growth (14.66mm) with highest mycelial inhibition (82.11%) of the test pathogen. The second and third best antagonists found were *T. virens* and *T. hamatum*, which recorded mycelial growth of 16.66mm and 18.66mm, of the test pathogen respectively and inhibition of 80.07 and 77.96 per cent, respectively.

This was followed by *Trichoderma asperellum* (col. dia.: 21.33 mm and inhibition: 75.44 %), *Aspergillus niger* (col. dia.: 29.33 mm and inhibition: 66.18%) and *T. koningii* (col. dia.: 52.33 mm and inhibition: 41.01 %). The antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were found least effective with 43.00 mm and 53.14 mm linear mycelial growth and 52.00 and 41.74 per cent mycelial inhibition.

These results are in conformity with the earlier findings of those workers who reported bioagents viz., *T. asperellum*, *T. harzianum*, *T. koningii* and *T. hamatum* had significantly inhibited mycelial growth of *A. alternata* infecting different crops (Gohel *et al*., 2011; Akbari and Parakhia, 2007; Hudge *et al*., 2009; Waghunde *et al*., 2009; Balai and Ahir, 2011, Rajput *et al*., 2011; Apet *et al*., 2014) [10, 1, 11, 17, 5, 15, 3].

The fungistatic / antifungal action exerted by the species of *Trichoderma* and *A. alternata* and other species of *Alternaria* were reported by several workers, *A. macrospora* infecting cotton (Gholve *et al*., 2014) [9], *A. lini* infecting linseed (Charpe *et al*., 2014) [6], *A. solani* infecting tomato (Kharbhari *et al*., 2008) and *A. burnsii* infecting cumin (Vihol *et al*., 2009) [16].

The fungistatic / antifungal action exerted by the species of *Trichoderma* and *A. niger* against *A. alternata* and other species of *Alternaria* may be attributed to their production of volatile and non-volatile substances, cell wall degrading enzymes (glucanases, B1, 3 glucanase), the phenomenon of competition, lysis and antibiosis.

In conclusion, all the, bio agents evaluated *in vitro* were found fungistatic / antifungal against *A. alternata*. However, bioagents viz., *T. harzianum*, *T. virens* and *T. hamatum* were most efficient with significantly highest inhibition of mycelial growth of the *A. alternata* causing fungal fruit rot in pomegranate.

Table 1: *In vitro* bioefficacy of bioagents against *A. alternata* causing fungal fruit rot in pomegranate

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T_1	*Trichoderma asperellum*	21.33	75.44 (60.29)
T_2	*T. harzianum*	14.66	82.11 (64.97)
T_3	*T. hamatum*	18.66	77.96 (62.00)
T_4	*T. koningii*	52.33	41.01 (39.82)
T_5	*T. virens*	16.66	80.07 (63.48)
T_6	*Aspergillus niger*	29.33	66.18 (54.44)
T_7	*Pseudomonas fluorescens*	43.00	53.14 (46.80)
T_8	*Bacillus subtilis*	52.00	41.74 (40.24)
T_9	Control (untreated)	90.00	00.00 (00)
	S.E. ±	0.66	0.85
	C.D. at 1%	1.80	2.33

*: Mean of three replications, Dia.: Diameter,
Figures in parentheses are arcsine transformed values

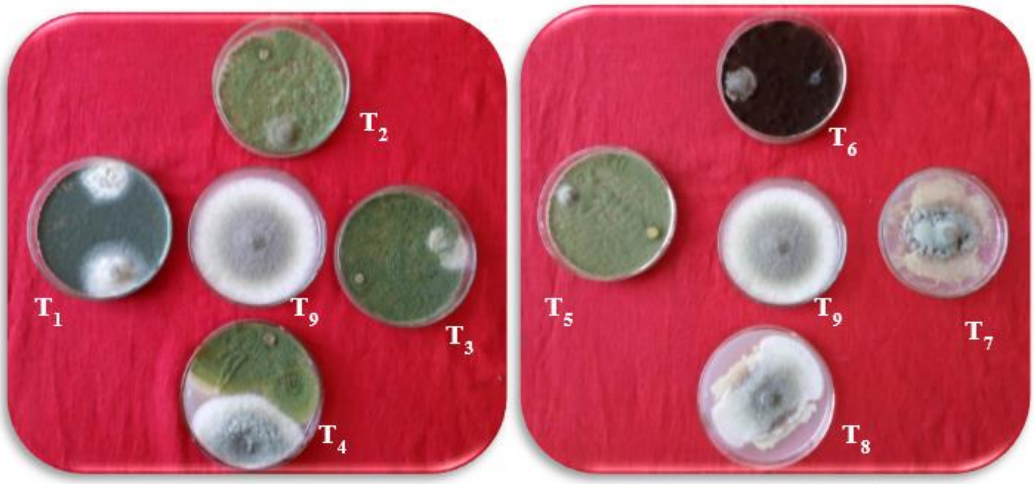
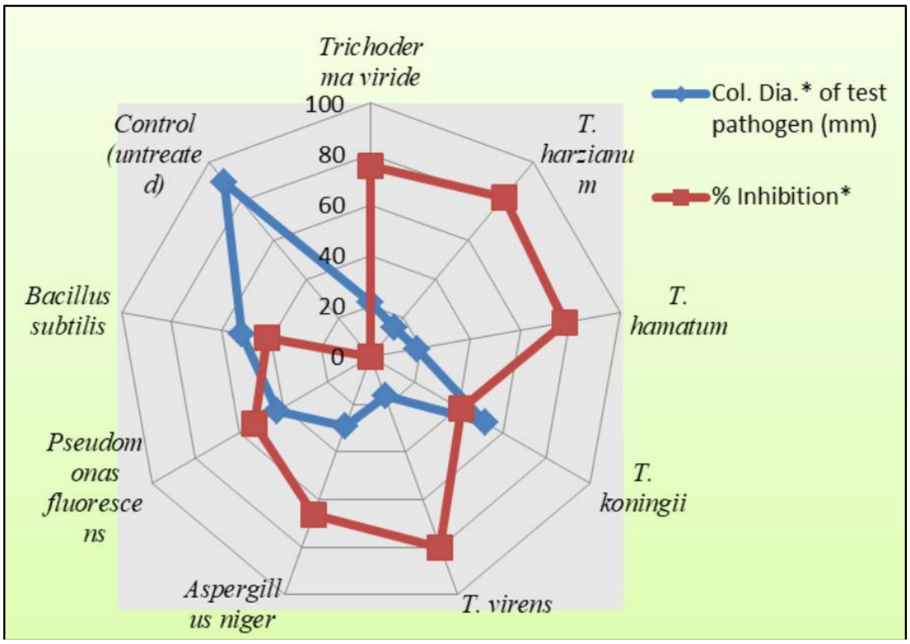


Plate 1: *In vitro* bioefficacy of bioagents against *A. alternata* causing fungal fruit rot in pomegranate



T1: Trichoderma asperellum	T2: T. harzianum	T3: T. hamatum
T4: T. koningii	T5: T. virens	T6: A. niger
T7: P. fluorescens	T8: B. subtilis	T9: control (untreated)

Fig 1: *In vitro* bioefficacy of bioagents against *A. alternata* causing fungal fruit rot in pomegranate

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