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In vitro evaluation of bioagents against *Alternaria alternata* causing *Alternaria* leaf blight disease of marigold

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Abstract
Five fungal and two bacterial bioagents/antagonists were tested *in vitro* against *A. alternata.* The results revealed *Trichoderma virens* was found most effective with highest mycelial growth inhibition (62.88%) of the test pathogen, followed by *T. harzianum* (60.63%) and *T. koningii* (52.88%). Rest of the bioagents significantly inhibited mycelial growth of test pathogen, except *Bacillus subtilis.*

Keywords: Bioagents, *Alternaria alternata*, leaf blight, Marigold

Introduction
Marigold is one of the most popular and commercial cultivated annual ornamental plants. Marigold was supposed to be originated from central and south America, especially Mexico. It occupied prominent place in floriculture, both in respect of area and production.
There are many diseases of marigold which is caused by several fungi, bacteria and viruses but among all diseases blight disease of marigold is most serious and destructive. *Alternaria* leaf blight of marigold has been reported to be caused by *Alternaria* spp. *viz, A. alternata, A. zinniae* and *A. tagetica*. The yield losses in the range of 50-60 per cent (Shome and Mustafee, 1966; Neher, 1989; Ratan and Shukla, 2000) [11, 8, 10] and 100 percent reduction in flower pigments due to *Alternaria* leaf spot caused by *Alternaria* spp. were reported (Mazumdar, 2000; Singh *et al.*, 2006) [6, 12]. There is severe disease occurrence of *Alternaria* leaf blight of marigold which is caused by *A. alternata* (Aktar and Shamsi 2012) [1].

Material and Methods
Five fungal bioagents *viz., T. harzianum*, *T. viride*, *T. koningii*, *T. hamatum*, *A. niger* and two bacterial bioagents *viz., B. subtilis*, *P. fluorescens* were evaluated against *A. alternarta* by Dual culture technique (Dennis and Webster, 1971) [3]. Seven days old culture of test fungus and test bioagents were used for the study. Disc of PDA along with culture growth of test fungus and test bioagents were cut out with cork borer and placed on petri plates containing PDA at equidistance and exactly opposite to each other and plates were incubated at 27 ± 1°C. PDA plates inoculated with only culture disc of test fungus were maintained as untreated control. Each treatment was replicated thrice.
Observations on mycelial growth of the test fungus and bioagents were recorded after 96 hrs and 168 hrs (after 7 days). Percent inhibition of test fungus over untreated control was calculated by formula given by Arora and Upaddhyay (1978) [2].

$$\text{Percent Growth Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,
C = Growth (mm) of test fungus in control plate.
T = Growth (mm) of test fungus in treated / intersecting plate.

Experimental details
Design: CRD
Replications: Three
Treatments: Eight

Table 1: Treatment Details

Tr. no.	Treatments	Tr no.	Treatments
T1	*Trichoderma harzianum*	T5	*Trichoderma koningii*
T2	*Trichoderma viride*	T6	*Pseudomonas fluorescens*
T3	*Aspergillus niger*	T7	*Bacillus subtilis*
T4	*Trichoderma hamatum*	T8	Control

Result and Discussion

The results (Table 2) revealed that the test bioagents significantly inhibited mycelial growth of *A. alternata*, over untreated control. However, *T. virens* was most effective with significantly least mycelial growth (33.4 mm) and highest mycelial growth inhibition (62.88%), followed by *T. harzianum* (33.63 mm and 62.33%, respectively, *T. koningii* (42.4 mm and 52.88% respectively, *T. hamatum* (51.26 mm mycelial growth and 43.04 mycelial growth inhibition), *A. niger* (55.33 mm and 38.52%, respectively *P. fluorescens* (67.34mm and 25.17%, respectively and *B. subtilis* (71.39 mm and 20.67%, respectively.

Table 2: *In vitro* efficacy of bioagents against *A. alternata*, causing *Alternaria* blight of marigold

Tr. No.	Treatments	Mean Colony Dia. (mm)* of pathogen		Inhibition (%)
		4th DAI	7th DAI	
1	*Trichoderma harzianum*	28.28	33.63	62.63 (52.31)
2	*Trichoderma hamatum*	37.31	51.26	43.03 (41.00)
3	*Trichoderma koningii*	38.33	42.40	52.88 (46.65)
4	*Trichoderma virens*	31	33.4	62.88 (52.46)
5	*Aspergillus niger*	31.47	55.33	38.52 (38.36)
6	*Bacillus subtilis*	38.16	71.39	20.67 (27.04)
7	*Pseudomonas fluorescens*	49.16	67.34	25.17 (30.11)
8	Control (Untreated)	66.21	90	00.00 (00.00)
S.E.±		-	0.53	0.53
CD (P=0.01)		-	2.19	2.19

*Mean of three replications. DAI= Days after inoculation
Figures in Parentheses are Arc sine transformed values

These result of the present study on antagonistic effects of the bioagents against *A. alternata* are in conformity with those reported earlier by several workers Thaware *et al.*, (2010) [13] reported that *T. harzianum*, followed by *T. viride* and *T. koningi* resulted highest mycelial growth inhibition of *A. alternata*. Similarly, *T. harzianum* was also reported effective against *A. alternata*. Naik *et al.*, (2010) and Panwar *et al.*, (2013) [7, 9] reported *T. koningi* and *T. harzianum* as efficiant antagonistic against *A. alternata*. The bioagents *viz.*, *T. virens*, *T. harzianum* and *T. koningii* were reported to significantly inhibit mycelial growth of *A. alternata* infecting various crops (Gohel *et al.*, 2011; Jakatimath *et al.*, 2017; Veeraghanti *et al.*, 2017; Wagh *et al.*, 2017) [4, 5, 14, 15].

Summary and Conclusion

Five fungal and two bacterial bioagents/antagonists evaluated *in vitro* were found antifungal/antagonistic against *A. alternata*. However, *Trichoderma virens* recorded least mycelial growth (33.4%) with highest inhibition (62.88%) of the test pathogen over untreated control. The second and third best antagonists found were *T. harzianum* and *Trichoderma koningii* which recorded mycelial growth, 33.63 and 42.40 mm and 62.63 and 52.88 percent inhibition, respectively. Rest of fungal antagonist also recorded significant inhibition of the test pathogen which was ranged from 38.52 to 43.04 percent. The bacterial antagonist, *Pseudomonas fluorescens* and *Bacillus subtilis* recorded 71.39 and 67.34 mm linear mycelial growth and 20.67 and 25.17 percent inhibition of test pathogen respectively.

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