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In vitro evaluation of bio control agents against seed borne *Alternaria carthami* and *Alternaria alternata* causing leaf spot disease of safflower

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Abstract
Biotic and abiotic stresses are major constraints in the production of safflower. Among biotic stresses apart from bacterial and viral diseases, many fungal diseases are of economic importance. Leaf spots were the most important and destructive disease of safflower. Leaf spots caused by *Alternaria carthami* Choudhury and *Alternaria alternata* are a serious threat to successful cultivation of safflower. For the management of leaf spot causing seed borne pathogens an experiment was conducted to study the efficacy of antagonistic organism against seed borne leaf spot causing pathogens of safflower. The bioagents *i.e. Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum, Trichoderma koningii, Pseudomanas fluorescens* and *Bacillus subtillius* each @ 0.6 per cent were evaluated in vitro were found antifungal to *A, carthami* and *A. alternata*. However, *Trichoderma harzianum* was found most effective and recorded significantly highest mycelial growth inhibition of *Alternaria carthami* and *Alternaria alternata* of about 81.48 % and 83.70 % respectively over untreated control. The second and third best bioagents/antagonists found were *Trichoderma viride* and *Trichoderma hamatum* which recorded mycelial growth inhibition of *Alternaria carthami* 77.04 %, 70.74 % and *Alternaria alternata* 79.26 %, 74.08 % respectively.

Keywords: Biocontrol, *Alternaria carthami*, *Alternaria alternata. In vitro,* inhibition

Introduction
Carthamus tinctorius L. is commonly known as Safflower is one of the important rabi oilseed crops of the world. It is popularly called as '*Kardai*' in Marathi grown for its much valued edible oil having world-wide acceptability for its health benefits especially to heart patients which belong to family Asteraceae or Compositae with the chromosome number of 2n=24. In India the crop is grown in medium to heavy deep vertisols of Deccan plateau and extended to Uttar Pradesh, West Bengal, Madhya Pradesh, Malwa Plateau. In India it is cultivated over an area of 81.00 (000) and production of 45.1 (000 ton) with an average productivity of 557 kg/ha during 2017-18. (Anonymous 2018a) [2] India ranks first in the world in respect of acreage accounting for about 36% of the world total. Maharashtra and Karnataka states are major producers which contribute more than 90% of India's production whereas, productivity is highest in West Bengal (1000 kg/ha) followed by Bihar (805 kg/ha) and Karnataka (719 kg/ha) (Dambal and Patil, 2017) [8]. In Maharashtra, the safflower was grown on 32.7 (000 ha) area with total production of 15.7 (000 tones) and productivity 481 kg/ha during the year 2017-18 (Anonymous 2018b) [3]. In Maharashtra the crop is extensively cultivated in Hingoli, Osmanabad, Parbhani, Latur, Jalana, Solapur and Ahmednagar district.

The potential yield of this crop is affected by a number of seed borne disease, out of these *Alternaria carthami* and *Alternaria alternata* are a serious seed borne diseases. Production and productivity of safflower in India is less, when compared to other countries because of biotic and abiotic factors. Biotic factors which include diseases caused by fungi, bacteria, virus and parasitic nematodes are the main reasons for low yield. Among the biotic agents, major diseases are caused by fungal pathogens *viz., Alternaria* leaf spot (*Alternaria carthami*), *Cercospora* leaf spot (*Cercospora carthami*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *carthami*), *Ramularia* leaf spot (*Ramularia carthami*), Root rot (*Phytophthora drechsleri)* and Rust (*Puccinia carthami*). Minor diseases include Bacterial leaf blight (*Pseudomonas syringae*), viral diseases; Cucumber mosaic virus, Lettuce mosaic virus, Tobacco mosaic virus

and Root knot nematode (*Meloidogyne incognita*). Among the diseases, leaf spots were the most important and destructive disease of safflower. Leaf spots caused by *Alternaria carthami* Choudhury and *Alternaria alternata* are a serious threat to successful cultivation of safflower. The disease was reported for the first time from India by Choudhury (1944) [7] at Pune. Up to 50 per cent seed yield loss was recorded due to this disease (Indi *et al.,* 1987) [12]. This disease plays an important role in safflower cultivation and causes 25-60 per cent yield loss every year (Singh and Prasad, 2005) [17]. Some times as high as 80-90%, when the disease appears at early stage of crop growth (Krishna Prasad, 1988) [13]. Pathogen free seed is the basic requirement for disease free crop. However, safflower productivity has remained virtually stagnate over recent decades because of its susceptibility to various seed borne diseases. Seed borne fungi are generally known to affect adversely the seed germination and vigour of the seedling. The biological control is one of the viable propositions for management of such a pathogen (Naik and Sen, 1991) [14]. Therefore, the present investigation was undertaken for management of seed borne leaf spot causing pathogen *in vitro* by dual culture technique.

Material and Methods

Seven fungal antagonists *viz., Trichoderma viride, T. harzianum, T. koningii, T. hamatum, T. virens, T. longibrachitum, T. lignorum* and two bacterial antagonists *P. fluorescens* and *B. subtlis* were evaluated *in vitro* against *Alternaria alternata, Aspergillus flavus* and *A. niger* applying Dual Culture Technique (Dennis and Webster, 1971) [9]. Seven days old cultures of the test bio-agents and test fungus (*Alternaria alternata, Aspergillus flavus* and *A. niger*) grown on (PDA, NA) were used for the study. Discs (5 mm dia.) of culture growth of the test fungus and bio-agents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bio-agents were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates aseptically and plates were incubated at 26 ± 2 °C. PDA plates inoculated only with culture discs of the test fungus were maintained as untreated control and all the treatments were replicated thrice.

Experimental details

Design: CRD (Completely Randomized Design)
Replications: Three
Treatments: Seven

Treatment details

Treat. No.	Bioagents
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Trichoderma hamatum*
T4	*Trichoderma koningii*
T5	*Pseudomanas fluorescens*
T6	*Bacillus subtilis*
T7	Control

Observations on linear mycelial growth of the test pathogen and test bio-agent 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 bio-agent over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978) [4].

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

Results and Discussion

Radial mycelial growth / colony diameter of *A. carthami*

Results (Table 1, PLATE I and Fig. 1) indicated that all the bio-agents evaluated exhibited antifungal activity against *A. carthami* and significantly inhibited its mycelial growth, over control. Radial mycelial growth of *A. carthami* was ranged from 16.67 mm to 53.67 mm as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly least mycelial growth (16.67 mm), these were followed by *T. viride* (20.67 mm), *T.hamatum* (26.33 mm), *T. koningii* (28.33 mm), *P. fluorescens* (43.67 mm) and *B. subtilis* (53.67 mm), respectively.

Mycelial growth and inhibition of *A. carthami*

Results (Table 1, PLATE I and Fig. 1) indicated that mycelial growth inhibition of *A. carthami* was ranged from 40.37 per cent to 81.48 per cent as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly highest mycelial growth inhibition (81.48 %), these were followed by *T. viride* (77.04 %), *T. hamatum* (70.74 %), *T. koningii* (68.52 %), *P. fluorescens* (51.48 %) and *B. subtilis* (40.37 %).

Result (Table 1) revealed that bioagents tested were found effective against *A. carthami* and significantly inhibited its mycelial growth, over control. Fungal bioagents *viz.,T. harzianum,T. viride,* and *T. hamatum* were reported efficient antagonists against *A. carthami,* these results were in agreement with the finding of several workers. Similar result were reported on other crops like tomato by Babu *et al*. (2000) [5], in safflower by Gaikwad and Behere (2001) [10], Taware *et al.,* (2014) [18], in cotton by Ramegowda *et al.,* (2007) [16].

Table 1: *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *Alternaria carthami*

Treat. No.	Bioagents	Mean colony diameter (mm)*	Inhibition (%)
T1	*Trichoderma viride*	20.67 (27.02)	77.04
T2	*Trichoderma harzianum*	16.67 (24.04)	81.48
T3	*Trichoderma hamatum*	26.33 (30.87)	70.74
T4	*Trichoderma koningii*	28.33 (32.16)	68.52
T5	*Pseudomanas fluorescens*	43.67 (41.36)	51.48
T6	*Bacillus subtilis*	53.67 (47.10)	40.37
T7	Control	90 (71.57)	-
	S.E.±	0.68	
	CD at 5%	2.08	
	CV	3.04	

* Mean of three replications

Radial mycelial growth / colony diameter of *Alternaria alternata*

Results (Table 2, PLATE 2 and Fig. 2) indicated that all the bio-agents evaluated exhibited antifungal activity against *A. alternata* and significantly inhibited its mycelial growth, over control. Radial mycelial growth of *A. alternata was* ranged from 14.67 mm to 51.00 mm as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly least mycelial growth (14.67 mm), these were followed by *T. viride* (18.67mm), *T.hamatum* (23.33 mm), *T. koningii* (27.00 mm), *P. fluorescens* (40.00 mm) and *B. subtilis* (51.00 mm), respectively.

Mycelial growth and inhibition of *Alternaria alternata*

Results (Table 2, PLATE 2 and Fig. 2) indicated that mycelial growth inhibition of *A. alternata* was ranged from 43.33 per cent to 83.70 per cent as against control (90.00 mm). However, *T. harzianum* was found most effective with significantly highest mycelial growth inhibition (83.70 %), these were followed by *T. viride* (79.26 %), *T. hamatum* (74.08 %), *T. koningii* (70.00 %), *P. fluorescens* (55.56 %) and *B. subtilis* (43.33 %). Similar results were obtained by Amaresh (2000) [1] in sunflower, Babu *et al.* (2000) [5] in tomato, Ghosh *et al.* (2002) [11] in gerbera, Ramegowda *et al.* (2007) [16] in cotton, Raj hans and Sharma (2017) [15] in apple, Bhosale *et al*. (2018) [6] in groundnut.

Table 2: *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *Alternaria alternata.*

Treat. No.	Bioagents	Mean colony diameter (mm)*	Inhibition (%)
T1	*Trichoderma viride*	18.67 (25.59)	79.26
T2	*Trichoderma harzianum*	14.67 (22.51)	83.70
T3	*Trichoderma hamatum*	23.33 (28.86)	74.08
T4	*Trichoderma koningii*	27.00 (31.30)	70.00
T5	*Pseudomanas fluorescens*	40.00 (39.21)	55.56
T6	*Bacillus subtilis*	51.00 (45.58)	43.33
T7	Control	90 (71.57)	-
	S.E.±	1.18	
	CD at 5%	3.58	
	CV	5.00	

* Mean of three replications

Conclusion

From the present study, it may be concluded that in biological control, *Trichoderma harzianum* found most effective in inhibiting the growth of leaf spot causing seed borne pathogens *Alternaria carthami and Alternaria alternata*.

Plate 1: *In vitro* evaluation of bioagents against *A. carthami*

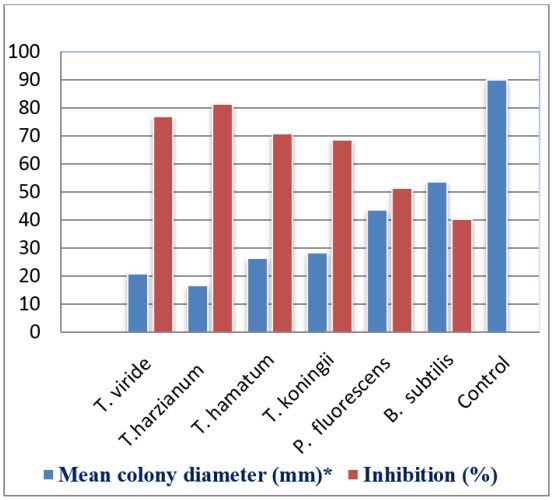

Fig 1: *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *A. carthami*

Plate 2: In vitro evaluation of bioagents against *A. alternata*

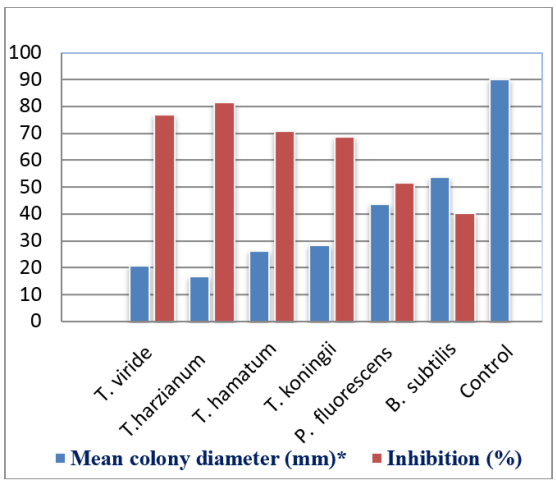

Fig 2: *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *A. alternata*

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