International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 1629-1632

Received: 25-11-2020
Accepted: 28-12-2020

SN Banne
Department of Plant Pathology, College of Agriculture, Latur Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Sunita J Magar
Department of Plant Pathology, College of Agriculture, Latur Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Shruti S Kadam
Department of Plant Pathology, College of Agriculture, Latur Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
SN Banne
Department of Plant Pathology, College of Agriculture, Latur Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

In vitro evaluation of bio control agents against *Alternaria alternata* (Fr.) Keissler, causing Leaf Blight disease of Chrysanthemum

SN Banne, Sunita J Magar and Shruti S Kadam

DOI: https://doi.org/10.22271/chemi.2021.v9.i1w.11462

Abstract
Biotic and abiotic stresses are major constraints in the production of chrysanthemum. Among biotic stresses apart from bacterial and viral diseases, many fungal diseases are of economic importance. Leaf blight was the most important and destructive disease of chrysanthemum. Leaf blight caused by *Alternaria alternata* (Fr.) Keissler a serious threat to successful cultivation of chrysanthemum. For the management of *Alternaria* leaf blight causing foliar disease, an experiment was conducted to study the efficacy of antagonistic organism against *Alternaria* blight. The bio-agents *i.e. Trichoderma asperellum, T harzianum, T hamatum, T Koningii, T lignorum, T virens, Aspergillus niger, Pseudomonas fluorescens* and *Bacillus subtilis* etc. were evaluated *in vitro*, found antifungal to *A. alternata*. However, *T. asperellum* was found most significant with highest mycelial growth inhibition (80.10%) of the test pathogen. The second and third most inhibitory antagonists found were *Aspergillus niger* and *T. koningii* with mycelia growth inhibition of 79.44 and 77.25%, respectively.

Keywords: Biocontrol, *Alternaria alternata*, *in vitro*, inhibition

Introduction
Chrysanthemum {*Dendranthema indicum* (= *Chrysanthemum indicum* L.)}, the golden flower (Chryos = golden, anthus = flower), is one of the most beautiful and perhaps the oldest flowering plants commercially grown in different parts of the World. Chrysanthemum belongs to the family Asteraceae. There has been constant demand for chrysanthemum flowers particularly from European markets during winter months and throughout the year in our Country.

In India, chrysanthemum is commercially grown in major states such as Karnataka, Tamilnadu, Maharashtra, Rajasthan, Madhya Pradesh and Bihar. In Maharashtra, chrysanthemum is grown on an area of 0.39 thousand ha with the production of 1.65 thousand tonnes loose flowers and 0.05 thousand tonnes cut flowers (Anonymous, 2018) [2]. In Maharashtra, the leading districts in floriculture production are Nasik, Ahmednagar, Thane, Pune, Satara, Sangli and Nagpur. However, Ahmednagar district is specialized as growing district of the Maharashtra (Tupe *et al*., 2017) [12].

However, it is difficult to get good quality exportable blooms, higher yields and long lasting post harvest life of the cultivars under open conditions. The most important factors responsible are the diseases like *Alternaria* leaf blight, *Septoria* leaf spot, Rust, Wilt, Bacterial blight and non-availability of leading varieties, which are resistant to biotic and abiotic stresses. Among these diseases, *Alternaria* leaf blight caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive diseases, commonly prevailing in almost all chrysanthemum growing pockets of India, which causes heavy losses under field as well as market conditions. Since, there are no sources of resistance available for the cultivation. So, it is necessary to have information regarding *in vitro* evaluation of bio-agents. The biological control is one of the viable propositions for management of such a pathogen. Therefore, the present investigation was undertaken for the management of leaf blight causing pathogen *in vitro* by dual culture technique to identify the new effective bio-control agents, which derive maximum benefit to the farmers.

Material and methods

A total of nine bio-agents (seven fungal and two bacterial), as detailed under treatments were evaluated *in vitro* against *A. alternata*, applying dual culture technique (Dennis and Webster, 1971) [6]. Seven days old pure cultures of the test pathogen and test bio-agents grown on PDA media were used for the study. Two 5 mm culture discs, one each of the test pathogen and the test bio-agent were cut out with sterilized cork borer and inoculated at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and the plates incubated at 27+2°C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as control. All the treatments were replicated thrice.

Experimental details

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

Treatment details

Tr. No.	Bio agents	Tr. No.	Bio agents
T_1	Trichoderma asperellum	T_6	T. virens
T_2	T. harzianum	T_7	Aspergillus niger
T_3	T. hamatum	T_8	Pseudomonas fluorescens
T_4	T. koningii	T_9	Bacillus subtilis
T_5	T. lignorum	T_{10}	Control (untreated)

Observations on radial mycelial growth / diameter of colony of the *A. alternata* were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the bio-agents, over untreated control was calculated (Arora and Upadhyay, 1978) [4].

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

Results and discussion

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

Results (Plate I, Table 1, Fig 1) revealed that, all the bio-agents evaluated, exhibited fungi static / antifungal activity against *A. alternata* and significantly inhibited its growth, over untreated control of the antagonists tested, *Bacillus subtilis* was found highest colony diameter (55.30 mm) of the test pathogen. The second highest colony diameter antagonists found was *Pseudomonas fluorescens* (45.81 mm). These were followed by *T. hamatum* (22.41 mm), *T. lignorum* (22.05 mm), *T. virens* (21.19 mm), *T. harzianum* (20.56 mm), *T. koningii* (20.47 mm), *Aspergillus Niger* (18.50 mm) and *T. asperellum* (17.19 mm), respectively.

Mycelial growth and inhibition of *A. alternata*

Results (Table 1, Plate I and Fig. 1) indicated that, mycelial growth inhibition of *A. alternata* of the antagonists tested, *T. asperellum* was found most significant with highest mycelial growth inhibition (80.10%) of the test pathogen. The second and third inhibitory antagonists found were *Aspergillus Niger* and *T. koningii* with and inhibition of 79.44 and 77.25%, respectively. These were followed by *T. harzianum* (77.15%), *T. Virens* (76.45%), *T. lignorum* (75.50%), *T. hamatum* (75.10%), *Pseudomonas fluorescens* (49.10%) and *Bacillus subtilis* (38.55%), respectively. Thus, the bio-agents *viz*, *T asperellum*, *Aspergillus Niger*, *T koningii*, *T harzianum* were found most potential antagonists against *A. alternata* (Fig. 4).

These results of the present study were in consonance with the earlier findings of those workers, who reported bio-agents *viz*, *T viride*, *A niger*, *T koningii*, *T. harzianum*, *T virens*, *T lignorum*, *T hamatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *A. alternata* and other phytopathogenic *Alternaria* spp. were reported earlier by several workers (Arun Kumar, 2008, Gohel *et al*., 2005, Ahire *et al*., 2012, Mumtaz *et al*., 2012, Apet *et al*., 2014, Hans and Sharma, 2017, Sarkar *et al*., 2017, Zade *et al*., 2018) [5, 7, 1, 9, 3, 8, 11, 13].

Table 1: *In vitro* bio efficacy of bio agents against *A. alternata*

Tr. No.	Treatments	Colony Dia. of test pathogen * (mm)	% Inhibition
T_1	Trichoderma asperellum	17.19	80.10 (63.50)
T_2	T. harzianum	20.56	77.15 (61.44)
T_3	T. hamatum	22.41	75.10 (60.06)
T_4	T. koningii	20.47	77.25 (61.51)
T_5	T. lignorum	22.05	75.50 (60.33)
T_6	T. virens	21.19	76.45 (60.96)
T_7	Aspergillus niger	18.50	79.44 (63.03)
T_8	Bacillus subtilis	55.30	38.55 (38.38)
T_9	Pseudomonas fluorescens	45.81	49.10 (44.48)
T_{10}	Control (Untreated)	90.00	0.00 (00.00)
--	S.E. ±	0.62	0.56
--	C.D. (P = 0.01)	1.86	1.67

* Mean of three replications, Dia.: Diameter

Conclusion

From the present study, it may be concluded that, in biological control, *T. asperellum* was found most significant found most effective in inhibiting the growth of leaf blight, causing *A. alternata*.

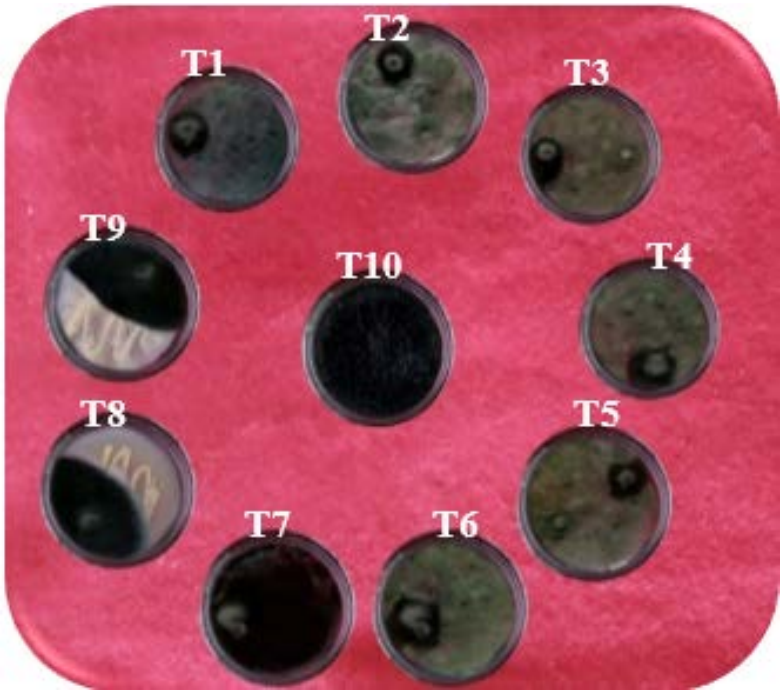

Plate I: *In vitro* efficacy of various bio-agents against *A. alternata*

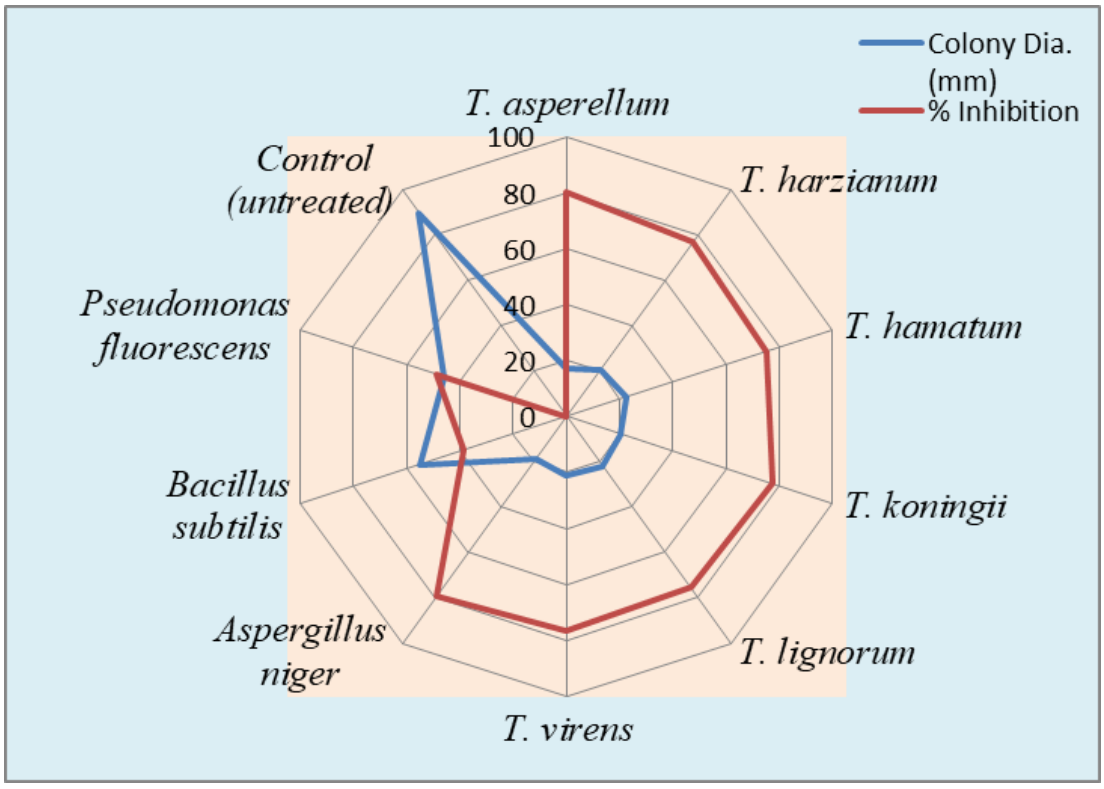

Fig 1: *In vitro* efficacy of bio-agents against *A. alternata*, causing chrysanthemum leaf blight

Acknowledgment
We are thankful to the Head of the Department of Plant Pathology, College of Agriculture, Latur for providing the laboratory facilities.

Reference

1. Ahire CS, Suryawanshi AP, Kuldhar DP, Badgujar SL, Dey U. In vitro evaluation of fungicides, botanicals and bio-agents against Alternaria alternata, causing blight of marigold. Paper presented in IPS (WZ) Nat. Symp. On "Microbial Consortium Apporoaches for plant health management", Dr. PDKV, Akola. 2012;30-31:50.
2. Anonymous. Area and Production of Horticultural Crops, 2018., www.Agricoop.nic.in.
3. Apet KT, Jagdale JS, Chavan PG, More AS, Mirza FN. Baig. In vitro evaluation of fungicides, botanicals and bioa gents against Alternaria alternata, causing leaf spot of gerbera, Trends Biosci. 2014;7(21):3374-3382.
4. Arora DK, Upadhyay RK. Effect of fungal staling growth substances on colony interaction Pl Soil. 1978;49:685-690.
5. Arun Kumar GS. Studies on leaf blight of chrysanthemum caused by Alternaria alternata (Fr.) Keissler M.Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, 2008.

6. Dennis KL, Webster J. Antagonistic properties of species group of Trichoderma and hyphal interaction Trans British Mycol. Soc. 1971;57:363396.
7. Gohel NM, Solanky KU, Patel SJ, Patel RV. Bio-control of Alternaria alternata causing leaf spot and fruit rot of chilli, J. Mycol. Pl. Pathol. 2005;35(3):357.
8. Hans raj, Sharma JN. In vitro evaluation of botanicals, bio-agents and fungicides against Alternaria alternata, causing mouldy core, core rot of apple, Int. J. Agril. Sci. 2017;9(49):4835-4840.
9. Mumtaz Baig, Sumia Fatima, Kadam VB, Yasmine Shaikh. Utilization of antagonis against seed borne fungi, An Int. J. Trends Life Sci. 2012;1(1):42-46.
10. Naik MK, Sen B. Bio-control of plant diseases caused by Fusarium spp. in biological control of plant diseases. Vol. 3 (ed. Mukherjji K.G.) Aditya books, Ansari Road.
11. Sarkar, Dipankar, Barhate BG, Joshi VR. (2017). Studies on leaf spot of chilli, Int. J. Pl. Prot. 1991;10(2):369-374.
12. Tupe SB, Perke DS, Korade VC. Costs, returns and profitability of chrysanthemum in Ahmednagar district of Maharashtra state Agric. Update, 2017;12(Techsear-2):389-392.
13. Zade SB, Ingle YV, Ingle RW. Evaluation of fungicides, botanicals and bioagents against Alternaria alternata, incitant of leaf spot of soybean, J. Pharma. Phytochem. 2018;7(5):1687-1690