International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 1103-1105

Received: 04-09-2019
Accepted: 06-10-2019

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

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

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

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

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

In vitro bioefficacy of bioagents against *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate

KA Burgute, SJ Magar, MD Navale and AC Patil

Abstract
Pomegranate (*Punica granatum* L) is one of the important fruit crops grown in India. Fruit rot of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.,has becoming a major limiting factor in cultivation of pomegranate in some regions of India. The present investigation was carried out to test the *in vitro* bioefficacy of different bioagents against *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate were carried out in the Department of Plant Pathology, College of Agriculture, Latur during the year 2017-18. Among the different fungal and bacterial bioagents *T. hamatum* was found most effective with least mycelial growth (11.63 mm) and numerically highest mycelial inhibition (87.25%), followed by *T. virens* (15.46 mm and 82.73%), *T. harzianum* (15.76 mm and 82.55%), *T. koningii* (19.56 mm and 78.84%), *A. niger* (20.10 mm and 78.14%), *T. asperellum* (32.00 mm and 64.48%), *B. subtilis* (52.50 mm and 41.92%) and *P. fluorescens* (57.76 mm and 34.74%). The bioagents were evaluated *in vitro* against the pathogen *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate by dual culture technique. Among the *in vitro* evaluated all bioagents *T. hamatum, T. virens, T. harzianum* and *T. koningii* were found more efficient while *A.* niger, *T. asperellum, B. subtilis* and *P. fluorescens* were least effective against *Colletotrichum gloeosporioides* major fungal pathogen causing fruit rot in pomegranate.

Keywords: *Colletotrichum gloeosporioides*, bioagents, fungal, fruit rot, pomegranate

Introduction
Pomegranate (*Punica granatum* L.) is an ancient, delicious fruits consumed worldwide, gaining lot of attention of the world over, because of its high economic value and nutritional values. It is one of the important fruit crops in arid and semi-arid regions commercially important in both tropical and subtropical countries known for its drought tolerance which thrives well in dry tropical conditions with marginal soils of low fertility. Being the most adaptable subtropical fruit crop, its cultivation has increased rapidly creating its image as an important cash crop in global market. Globally India is ranked first in area and production. During 2015-16, pomegranate was cultivated over 2.09 lakh ha with an annual production of 24.42 lakh MT and productivity of 12.00 MT/ha in India (Anonymous, 2016) [2]. Maharashtra is the leading producer of pomegranate in India followed by Karnataka, Gujarat and Andhra Pradesh (Anonymous, 2013) [1]. Maharashtra considered as pomegranate basket of India contributes more than 70 per cent of the total area under pomegranate followed by Andhra Pradesh, Uttar Pradesh, Rajasthan, Gujarat and Karnataka which are the leading states; cultivating pomegranate commercially on a large scale.

However, the crop is under threat due to number of serious diseases such as bacterial blight (*Xanthomonas axonopodis* pv. *punicae*), wilt due to *Ceratocystis fimbriata*, anthracnose (*Colletotrichum gloeosporioides*) and leaf spot and severe fruit rotting due to *Alternaria alternata*, *Cercospora* sp., *Pseudocercospora* sp., *Drechslera* sp. and *Sphaceloma* sp. etc., are more or less equally important and harmful in some orchards and also take a heavy toll on the crop (Khosla and Bhardwaj, 2013). Among these; severe spotting and fruit rotting due to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc; remains hitherto unexplored but potentially dangerous pathogen on pomegranate and considered to be an emerging disease.

In recent years, there has been a major thrust on pesticide residue free organic pomegranate production. Taking the task into consideration, efficient bioagents need to be explored to fit into the management schedule. Use of bioagents for the management of various diseases of crop plants is eco-friendly and environmentally safe. Therefore, present investigation aimed to

evaluate bioagents (*in vitro*) against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc causing fruit rot in Pomegranate.

Material and Methods

Six fungal and two bacterial bioagents were evaluated *in vitro* against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc, applying Dual Culture Technique (Dennis and Webster, 1971) [4]. Seven days old cultures of the test bioagents and test pathogen (*Colletotrichum gloeosporioides*) 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.

The lists of bioagents used along with their types are given in Table.

Sr. No.	Name of Bioagents	Type of Pathogen
1.	*Trichoderma asperellum*	Fungal
2.	*Trichoderma harzianum*	Fungal
3.	*Trichoderma hamatum*	Fungal
4.	*Trichoderma koningii*	Fungal
5.	*Trichoderma virens*	Fungal
6.	*Aspergillus niger*	Fungal
7.	*Bacillus subtilis*	Bacterial
8.	*Pseudomonas fluorescens*	Bacterial

Observations on linear mycelial growth of the test pathogen and test bioagents 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) [3].

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

Results and Discussion

Results (Plate 1, Fig. 1 and Table 1) revealed that all the bioagents evaluated exhibited fungistatic / antifungal activity against *Colletotrichum gloeosporioides* causingfungal fruit rot in pomegranate and significantly inhibited its growth over untreated control. Of the six fungal antagonists tested, *Trichoderma hamatum* was found most effective and test pathogen recorded least linear mycelial growth (11.63 mm) with highest mycelial inhibition (87.25%) of the test pathogen. The second and third best antagonists found were *T. virens* and *T. harzianum*, which recorded mycelial growth of 15.46 mm and 15.76 mm, of the test pathogen respectively and inhibition of 82.73 and 82.55 per cent, respectively.

This was followed by *Trichoderma koningii* (col. dia.: 19.56 mm and inhibition: 78.84%), *Aspergillus niger* (col. dia.: 20.10 mm and inhibition: 78.14%) and *T. asperellum* (col. dia.: 32.00 mm and inhibition: 64.48%). The antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* were found least effective with 52.50mm and 57.76 mm linear mycelial growth and 41.92 and 34.74per cent mycelial inhibition.

These results are in conformity with the earlier findings of those workers who reported bioagents viz., *T. hamatum,T. virens, T. harzianum,* and *T. koningii* had significantly inhibited mycelial growth of *Colletotrichum gloeosporioides* infecting different crops (Mandhare *et al.*, 1996; Babu *et al.*, 2008; Gud and Raut, 2008; Prashant *et al.*, 2008; Jadhav *et al.*, 2009; Tasiwal *et al.*, 2008 and Vinod *et al.*, 2009) [13, 5, 9, 17, 10, 18, 19].

Bioagents *viz., T. asperellum, T. harzianum, T. hamatum, T. koningii, T. virens, A.* niger, *P. fluorescens* and *B. subtilis* were reported as efficient antagonists against many *Colletotrichum* spp. including *C. gloeosporioides* by several earlier workers (Pathania *et al.*, 2004; Kaur *et al.*, 2006; Mistry *et al.*, 2008; Gawade *et al.*, 2009; Watve *et al.*, 2009; Barhate *et al.*, 2012a; Bhujbal *et al.* 2015b) [15, 11, 14, 8, 20, 6, 7].

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

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T_1	*Trichoderma asperellum*	32.00	64.48 (53.41)
T_2	*T. harzianum*	15.76	82.55 (65.30)
T_3	*T. hamatum*	11.63	87.25 (69.07)
T_4	*T. koningii*	19.56	78.84 (62.61)
T_5	*T. virens*	15.46	82.73 (65.44)
T_6	*Aspergillus niger*	20.10	78.14 (62.12)
T_7	*Pseudomonas fluorescens*	57.76	34.74 (36.11)
T_8	*Bacillus subtilis*	52.50	41.92 (40.35)
T_9	Control (untreated)	90.00	00.00 (00)
	S.E. ±	0.46	0.55
	C.D. at 1%	1.27	1.50

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

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

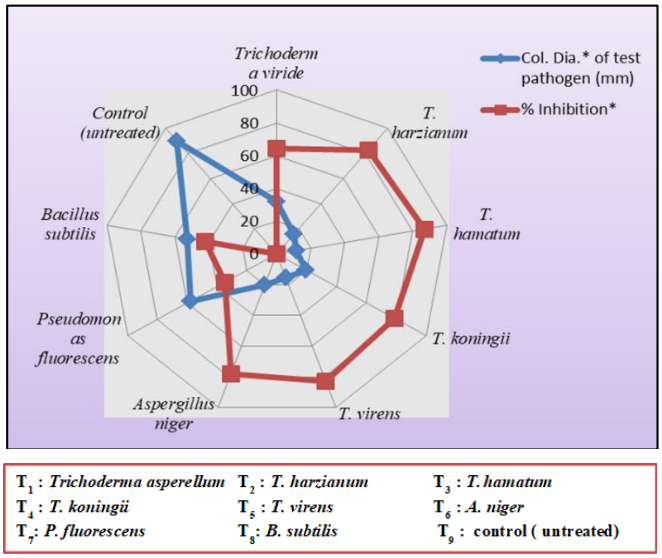

Fig 1: *In vitro* bioefficacy of bioagents against *C. gloeosporioides* causing fungal fruit rot in pomegranate

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

References

1. Anonymous Area under pomegranate cultivation in India and Maharashtra. National Horticultural Board, 2013, 62.
2. Anonymous. Area and production of horticultural crop-All India. National horticultural board, 2016.
3. Arora DK, Upadhyay RK. Effect of fungal staling growth substances on colony interaction. Pl. Soil. 1978; 49:685-690.
4. Dennis KL, Webster J. Antagonistic properties of species group of *Trichoderma* and hyphal interaction. Trans. British Mycol. Soc. 1971; 57:363-396.
5. Babu LJ, Gour HN, Pankaj S. Efficacy of phytoextracts and bioagents against *Colletotrichum gloeosporioides* causing banana fruit rot. J Mycol. Pl. Pathol. 2008; 38(3):635-637.
6. Barhate BG, Bidbag M.B, Ilhe BM, Shivagaje AJ. *In vitro* evaluation of fungicides and bioagents against *C.capsici* causing die-back of chilli. J Pl. Dis. Sci. 2012a; 7(1):64-66.
7. Bhujbal VB, Suryawanshi AP, Deshmukh MM, Kashid VS. Biological control of spinach anthracnoe disease caused by *C. dematium*. Sou. National Sym.: Prospects in diversity, diagnosis and management of the diseases of horticultural and field crops. COA, Badnapur. 2015b; 28:29-57.
8. Gawade DB, Suryawanshi AP. *In vitro* evaluation of fungicides, botanicals and bioagents against *Collectotrichum truncatum*, causing soybean anthracnose. Pl. Dis. Res. 2009; 24(2):120-123.
9. Gud MA, Raut SP. Control of mango anthracnose and stem end rot fungi by fungicides and bioagents. J of Maha. Agril. Universities 2008; 33(1):120-122.
10. Jadhav SN, Gadre UA, Kadam JJ, Joshi MS, Sawant UK. *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides* Penz. causing leaf spot of clove. J Pl. Dis.Sci. 2009; 4(1):104-106.
11. Kaur M, Sharma OP, Sharma PN. *In vitro* effect of *Ttrichoderma* species on *Colletotrichum capsici*, causing fruit rot of chilli. Indian Phytopath. 2006; 59(2):243-245.
12. Khosla K, Bhardwaj SS. Occurrence and incidence of important diseases of pomegranate in Himachal Pradesh. Pl. Dis. Res. 2013; 28(1):5-10.
13. Mandhare VK, Pawar BB, Kulkarni SR. Efficacy of fungicides against fruit spot of pomegranate. Pestology. 1996; 20(2):19-20.
14. Mistry DS, Sharma IP, Patel ST. Evaluation of fungicides, phytoextracts and bio-pesticides against die-back of chilli. J. Pl. Dis. Sci. 2008; 3(1):53-55.
15. Pathania N, Chandel SS, Singh SP. Screening of biocontrol agents against *Colletotrichum capsici* causing anthracnose of bell pepper. Seed Res. 2004; 32(1):111-112.
16. Penz. Fungi Agrum, 1884; 2:6.
17. Prashanth A, Satarddi AR, Naik M, Patil MB, Patil RS. Evaluation of fungicides, bioagents and botanicals against pomegranate anthracnose. Indian J. of Pl. Prot., 2008; 36(2):283-287.
18. Tasiwal V, Benagi VI, Hegde YR, Kamanna BC, Naik KR. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Karnataka J. Agric. Sci., 2008; 22(4):803-806.
19. Vinod T, Benagi VI, Yashoda RH, Kamanna BC, Naik KR. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by Colletotrichum gloeosporioides. Karnataka J of Agril.Sci. 2009; 22(4):803-806.
20. Watve YG, Diwakar MP, Kadam JJ. An evaluation of some bio-agents and plant extracts against leaf spot of Jatropa caused by *C. gloeosporioides* Penz. J Pl. Dis. Sci. 2009; 4(1):95-98.