International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 397-402

Received: 27-01-2018
Accepted: 28-02-2018

Somu R
Department of Plant Pathology, College of Horticulture, UHS, Bagalkot, Karnataka, India

RK Mesta
Department of Plant Pathology, College of Horticulture, UHS, Bagalkot, Karnataka, India

Kiran Kumar KC
Department of Plant Pathology, College of Horticulture, UHS, Bagalkot, Karnataka, India

Correspondence
Somu R
Department of Plant Pathology, College of Horticulture, UHS, Bagalkot, Karnataka, India

Effect of non systemic, systemic fungicides and bio control agents against wilt of pomegranate caused by *Ceratocystis fimbriata*

Somu R, RK Mesta and Kiran Kumar KC

Abstract
Pomegranate is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly. In recent years pomegranate plants are affected by many disease among them wilt caused by *Ceratocystis fimbriata* is the most destructive disease in pomegranate growing areas of the world. Use of fungicide or effective strain of biological control helps in controlling the diseases. *In vitro* screening serves as guide for field testing. Hence the present study was conducted to know the efficacy of different fungicides and biological control *in vitro*. Among seven non-systemic fungicides tested cymoxanil + mancozeb, mancozeb and captan completely inhibited the mycelial growth and least inhibition was recorded in copper hydroxide and chlorothalonil at all concentrations tested. Among thirteen systemic fungicides tested, 100 per cent inhibition was shown by all the fungicides at all concentrations tested expect in triadimefon and azoxystrobin. Triadimefon inhibited the mycelia growth completely at 0.2 and 0.3 per cent whereas at 0.1 per cent there was 76.66 per cent inhibition. The least mycelial growth inhibition was observed in azoxystrobin at all concentration. The maximum reduction in colony growth was observed in *Trichoderma harzianum*-55 which was very effective when compared to all other bioagents tested. Whereas, the minimum parasitic activity was noticed in case of *Bacillus subtilis* 1 and 2.

Keywords: systemic, non-systemic fungicides, bio control, pomegranate

Introduction
Pomegranate (*Punica granatum* Linnaeus.) called as "Fruit of paradise" is an ancient fruit belonging to the family Lythraceae. In India, pomegranate crop is being cultivated over an area of 143 thousand hectare with production of 1774 thousand tonnes with an average productivity of 10.75 tonnes/ha (Anonymous, 2015) [1]. It is one of the most adaptable subtropical minor fruit crop and its cultivation is increasing very rapidly. The fruit is very much liked for its cool and refreshing juice. The arils of the well matured fruit are consumed as such and also in processed form like juice or concentrate, syrup and jelly. The fresh juice contains moisture, total sugar, pectin, carbohydrate, acidity (as citric acid), minerals like calcium, phosphorus, iron, magnesium and vitamins (Dutta ray *et al.*, 2014) [6]. Seeds with fleshy portions of sour pomegranates are dried and marketed as 'Anardana', which is used as a condiment and for souring curries. However, pomegranate cultivation is facing several constraints and among them wilt caused by *Ceratocystis fimbriata* Ellis and Halst. is a major tailback in its successful cultivation. At present, the crop is severely affected by wilt pathogen and day by day the disease is increasing at faster rate. It was first noticed in two areas of the Vijayapura district of Karnataka, India in 1990 around 1993, rapid spread of this disease was observed in the entire Vijayapura district. The cause was not identified until 1995. In 1996, the fungus *C. fimbriata* was isolated from discolored stem, root and branch tissues on wilted plants. Disease is characterized by the initial symptoms *viz.*, yellowing and wilting of leaves on one to several branches leading to death of affected plants in a few weeks. Cross sections of diseased plants revealed brown discoloration in the outer xylem from roots to the main trunk (Somasekhara and Wali, 1999) [20]. Use of fungicide and bio control agent is one of the method of controlling the diseases or crop in the absence of resistant cultivars or when there is a sudden outbreak of disease. Hence, fungicide and bio control agent would continue to be the major tools of integrated disease management (IDM). Evaluation of different fungicides and bio control agents *in vitro* is a handy tool to screen a large number and thus can serve as guide for field testing. Hence the present investigation was aimed to identify suitable fungicides and bio controls against mycelial growth of *Ceratocystis fimbriata*.

Material and Methods

An experiment was conducted during 2014-17 at College of Horticulture, Bagalkot in the Department of Plant Pathology to find out the effective fungicide and bio control against *Ceratocystis fimbriata in-vitro*. The experiment was designed in Complete Randomized Design (CRD) with three replications.

Efficacy of non-systemic and systemic fungicides against *C. fimbriata* under *in vitro*

Twenty fungicides were tested *in vitro* against *C. fimbriata*. The fungicides were tested at 0.1, 0.2 and 0.3 per cent concentrations. Details of the fungicides, which were tested against *C. fimbriata* under *in vitro* experiment.

List of non-systemic fungicides

Sl. No.	Common name	Chemical name	Trade name
1	Copper oxy chloride	Dicopper chloride trihyroxide	Blue copper 50%WP
2	Copper hydroxide	Copper hydroxide	Kocide 77% WP
3	Cymoxanil + mancozeb	1. 2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)acetamide	Curzate 8% + 64% WP
4	Captan	N-trichoromethylthio- cyclohexane-1,2-ficarboxide (3aR,70s)-2-[(trichloromethyl)scelfonyl]-3ª,4,7,7atetrahdro- 1H-isoinfole-1,3(2H)-dione	Captan 50% WP
5	Chlorothalonil	Tetrachloroisophthalonitrate	Kavach 75% WP
6	Mancozeb	Manganese ethylene bis dithiocarbomate plus zinc	Dithane M- 45 75%WP
7	Propineb	Zinc propylenebisdithiocarbamate	Antracol 70%WP

List of systemic fungicides

Sl. No.	Common name	Chemical name	Trade name
1	Azoxystrobin	Methyl (E)-2-((6-(2-cyano phenoxy)-4-Pyrimidinyl)oxy)-alpha-(Methoxy Methylene) benzene acetate.	Amistar 25%SC
2	Carbendazim	2-(methoxy-carbomyl) benzimidazole	Bavistin 50% WP
3	Triadimefon	1-(4-Chlorophenoxy)-3,3-dimethyl-1-1H-(1,2,4-triazole-1-4)-2-butanone	Bayleton
4	Flusilazole	1-((Bis(4 fluorophenyl)methylsilyl)methyl)-1H-1,2,4-triazole	Nustar 40% EC
5	Tebuconazole	1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol	Folicur 250% EC
6	Tebuconazole + Trifloxystrobin	1. 1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol Methyl methoxyimino(alpha-(1-(alpha,alpha,alpha-trifluoro-3-tolyl)ethylideneaminooxy)-2-tolyl)acetate	Nativo 75% WG
7	Fenamidone + Mancozeb	1. (5S)-3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-4H-imidazol-4-oneManganese ethylene bis dithiocarbomate plus zinc	Sectin 10% + 50% WP
8	Difenoconazole	trans, cis-3-chloro-4- [4-methyl-2-(1H-1, 2, 4-triazole-1-group methyl)-1, 3-dioxapentane-2 group] phenyl-4 chlorophenyl ether	Score 25%EC
9	Thiophanate Methyl	Dimethyl 4, 4'-(*O*-phenylene)bis (3-thioallophanate)	Roko 70% WP
10	Propiconazole	1-{2-(2,4-dichlorophenyl)-4-1 propyl-1-1, 3-dioxolan-2-yl} -1H-1, 2,4-triazole	Tilt 25% EC
11	Propiconazole + Difenoconazole	1-{2-(2,4-dichlorophenyl)-4-1 propyl-1-1, 3-dioxolan-2-yl} -1H-1, 2,4-triazole trans, cis-3-chloro-4- [4-methyl-2-(1H-1, 2, 4-triazole-1-group methyl)-1, 3-dioxapentane-2 group] phenyl-4 chlorophenyl ether	Taspa 50% EC
12	Hexaconazole	(RS)-2-(2,4,-diclorophenyl)-1-(1H-1,2,4-trizol-1-yl)-hexan-2-ol (C14H17Cl2N3O)	Contaf 5% EC
13	Tricyclazole	5-methyl-1,2,4-triazol[3,4-f] fenzo-1,3-triazole; Methyl-1,2,4-trizole(3,4-f) fenzothiazolo	Baan 75%WP

Poison food technique was followed to test the efficacy of the above mentioned fungicides. The pathogen *C. fimbriata* was grown on PDA medium in Petriplates for fifteen days prior to setting the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient and whole product present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized Petriplates. Mycelial disc of 0.5 cm was taken from the periphery of ten day old culture and placed in the center and incubated at 25 ± 1^0C till growth of the fungus touched the periphery in control plate. Suitable checks were also maintained without addition of any fungicide, three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$I = 100 (C-T) / C$

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Efficacy of fungal and bacterial bio-agents against *C. fimbriata* under *in vitro*

Nine bio agents were tested against *C. fimbriata*. About 20 ml of PDA was poured into sterile Petriplates and allowed to solidify. From previously grown young cultures of both fungal bio agents and host pathogen 0.5 cm fungal disc of test fungus and respective bioagents were transferred aseptically to Petriplates simultaneously by leaving sufficient space in between two discs. In case of bacterium, mycelial discs of the fungus were kept at opposite ends and bacteria streaked at the center. Three replications were maintained for each treatment. The Petriplates were incubated at 25 ± 1^0C till the growth of colony touches the periphery in the control plate. Colony diameter of both the test fungus and bio agents were measured and per cent inhibition was calculated. Data were analyzed statistically.

Result and Discussion

The results obtained from the experiment are analyzed statistically and the data is presented in the tabular form and discussed here under.

Efficacy of non-systemic fungicides against *C. fimbriata* under *in vitro*

The results presented in Table 1 revealed that, there was a significant difference between the non-systemic fungicides with respect to per cent inhibition of mycelial growth. Cymoxanil + mancozeb, mancozeb and captan were found to be most effective and significantly superior over all other treatments which inhibited 100 per cent growth of the fungus. The next best treatment was propineb and copper oxy chloride which inhibited 95.43 and 94.81 per cent growth of the fungus respectively. However, there was least inhibition in copper hydroxide and chlorothalonil at all concentrations.

Among the concentrations, 0.3 and 0.2 per cent was found significantly superior over 0.1 per cent concentration in inhibition of growth of the fungus. Cymoxanil + mancozeb, mancozeb and captan inhibited the mycelia growth completely at 0.1, 0.2 and 0.3 per cent concentration. Copper oxy chloride and propineb inhibited the mycelia growth completely at 0.2 and 0.3 per cent whereas at 0.1 per cent there was 84.44 and 86.29 per cent inhibition respectively. Copper hydroxide inhibited the mycelia growth of fungus by 55.55, 87.03 and 88.51 per cent at 0.1, 0.2 and 0.3 per cent concentrations. Chlorothalonil at 0.1, 0.2 and 0.3 per cent inhibited the mycelia growth by 43.70, 43.70 44.07 per cent and found least effective among the fungicides tested.

In vitro evaluation of fungicides provides preliminary information regarding its efficacy against a pathogen with in a shortest period of time and therefore serve as guide for further field testing. In the present study, among seven contact fungicides *viz*., cymoxanil + mancozeb, mancozeb and captan recorded the maximum inhibition of (100 %) of mycelial growth at all tested concentrations (0.10 %, 0.20 % and 0.30 %). Mancozeb inactivates the sulfhydryl groups of amino acids and enzymes of fungal cell, resulting in disruption of lipid metabolism, respiration and production of adenosine triphosphate. The captan reacts with sulfhydryl groups ultimately, reduces fungal spore germination, growth, and oxygen uptake. Similar results were obtained by several workers Vijaya *et al*. (2007) [24] reported thiram was found best followed by captan at both the concentrations of 0.1 and 0.2%, whereas copper oxychloride was least effective against *C. paradoxa*. Somasekhara (2009) [19] tested various fungicides and reported *C. fimbriata* was completely inhibited by the fungicides mancozeb and Ziram. Sharma *et al.* (2010) [18] observed complete inhibition of *C. fimbriata* by mancozeb (0.2%) and captan (0.2%). Sonyal (2010) [21] reported that copper oxychloride was more effective than other fungicides at 0.1, 0.2 and 0.3 per cent against *C. fimbriata*. Apet *et al.* (2015) [2] observed 77.07 per cent mycelial growth inhibited by captan against *C. paradoxa*. Chaudhari *et al.* (2016) [4] reported copper oxy chloride and mancozeb were found most effective at 0.2, 0.25 and 0.3 per cent against *C. fimbriata*. Khan *et al.* (2017) [8] reported that among non-systemic fungicides Thiram (74.35 %) recorded the maximum inhibition of mycelial growth followed by copper oxy chloride (70.52 %) against *C. fimbriata*. Raja (2017) [16] reported captan, mancozeb, ziram, thiram and zineb recorded the maximum inhibition of mycelial growth at all concentrations tested against *C. fimbriata*.

Table 1: *In vitro* evaluation of non-systemic fungicides against the mycelial growth of *Ceratocystis fimbriata*

Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
	Concentration			
	0.1%	0.2%	0.3%	
Copper oxy chloride	84.44(66.79)*	100.00(89.63)	100.00(89.63)	94.81(82.02)
Copper hydroxide	55.55(48.18)	87.03(68.93)	88.51(70.19)	77.03(62.43)
Cymoxanil + mancozeb	100.00(89.63)	100.00(89.63)	100.00(89.63)	100.00(89.63)
Mancozeb	100.00(89.63)	100.00(89.63)	100.00(89.63)	100.00(89.63)
Chlorothalonil	43.70(41.37)	43.70 (41.37)	44.07(41.59)	43.82(41.45)
Captan	100.00(89.63)	100.00(89.63)	100.00(89.63)	100.00(89.63)
Propineb	86.29(68.27)	100.00(89.63)	100.00(89.63)	95.43(82.51)
Mean	81.43(70.50)	90.10(79.78)	90.37(79.99)	87.30(76.75)
Source			SEm ±	CD @ 1%
Fungicides (F)			0.24	0.77
Concentration (C)			0.13	0.50
F × C			0.34	1.34

*Values in parenthesis are arc-sine transformed values

Efficacy of systemic fungicides against *C. fimbriata* under *in vitro*

The per cent inhibition of mycelial growth of fungus in different systemic fungicides is presented in Table 2. The mycelial growth of the fungus was inhibited 100 per cent by carbendazim, flusilazole, tebuconazole, tebuconazole + trifloxystrobin, fenamidone + mancozeb, difenoconazole, thiophanate methyl, propiconazole, propiconazole + difenoconazole, hexaconazole and tricyclazole at 0.1, 0.2 and 0.3 concentrations. Triadimefon inhibited the mycelia growth completely at 0.2 and 0.3 per cent whereas at 0.1 per cent there was 76.66 per cent inhibition. Least inhibition was found in azoxystrobin with 22.96 per cent, 25.55 per cent and 30.74 per cent at 0.1, 0.2 and 0.3 per cent concentrations, respectively.

In the present investigation all the systemic fungicides expect azoxystrobin and triadimefon tested showed cent per cent inhibition of mycelial growth of *C. fimbriata* at 0.1 % concentration. Triadimefon inhibited the mycelia growth completely at 0.2 per cent whereas azoxystrobin was ineffective even at 0.3 per cent concentrations. The efficacy of the triazoles fungicides may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence causes irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of triazoles, which inhibited the biosynthesis pathway in fungi (Nene and Thapliyal, 1973) [13]. These findings are supported by earlier workers Vijaya *et al*. (2007) [24] who reported carbendazim and propiconazole were most effective in complete inhibition of the *C. paradoxa* at 0.05 and 0.1% concentrations. Somasekhara (2009) [19] observed complete inhibition of mycelial growth of *C. fimbriata* by

carbendaizm and propiconazole. Sharma *et al.* (2010) [18] revealed complete inhibition of the *C. fimbriata* by carbendarzim (0.1%), and propiconazole (0.1%). Sonyal (2010) [21] observed complete inhibition shown by propiconazole, tricyclazole at all concentrations tested. Kishore and Bhardwaj (2011) [9] revealed that 100 per cent inhibition of *C. fimbriata* in case of carbendazim (0.05%) and propiconazole (0.15%). Poussio *et al.* (2012) [14] observed no mycelial colony growth in Topsin-M at any dose, followed by Nativo and Alliate, as compared to control. Balaganur (2016) [3] found carbendazim and combi product of tebuconazole 50% + trifloxystrobin 25% WG were effective at all the concentrations tested.

Table 2: *In vitro* evaluation of systemic fungicides against the mycelial growth of *Ceratocystis fimbriata*

Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
	Concentration			
	0.1%	0.2%	0.3%	
Azoxystrobin	22.96(28.62)*	25.55(30.35)	30.74(33.66)	26.42(30.87)
Carbendazim	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Triadimefon	76.66(61.12)	100.00(89.73)	100.00(89.73)	92.22(80.19)
Flusilazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Tebuconazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Tebuconazole + Trifloxystrobin	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Fenamidone + Mancozeb	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Difenoconazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Thiophanate Methyl	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.69)
Propiconazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Propiconazole + Difenoconazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Hexaconazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Tricyclazole	100.00(89.73)	100.00(89.73)	100.00(89.73)	100.00(89.73)
Mean	93.28(82.83)	94.27(85.16)	94.67(85.42)	93.74(84.47)
Source			SEm ±	CD @ 1%
Fungicides (F)			0.11	0.48
Concentration (C)			0.06	0.23
F × C			0.22	0.84

*Values in parenthesis are arc-sine transformed values

Chaudhari *et al.* (2016) [4] revealed that hexaconazole and tricyclazole completely inhibited colony growth of *C. fimbriata* at 0.05%, 0.1%, 0.15% concentrations. Khan *et al.* (2017) [8] reported that among systemic fungicides tested, cent per cent inhibition of mycelial growth was recorded in propiconazole followed by hexaconazole (94.65 per cent). Raja (2017) [16] noted that carbendazim, hexaconazole, thiophanate methyl, propiconazole and tebuconazole showed 100 per cent inhibition at all concentrations tested.

Efficacy of fungal and bacterial bio-agents against *C. fimbriata* under *in vitro*

The antagonistic microorganisms like *T. harzianum*-55, *T. viride*-27, *T. viride* (PDBC), *T. viride*, *T. harzianum*-1, *T. harzianum*-2, *P. fluorescens*, *B. subtilis* -1 and *B. subtilis* -2 were evaluated for their antagonistic effect against *C. fimbriata* under *in vitro* conditions by dual culture technique. Inhibition zone in mm was recorded and the per cent inhibition was calculated and the results thus obtained are presented in the Table 3.

There was a significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *C. fimbriata.* Among the bio-agents tested, *Trichoderma harzianum*-55 recorded the maximum per cent inhibition of mycelial growth (76.00%). It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma viride*-27 (70.33%), *Trichoderma viride* (PDBC) (67.00%), *Trichoderma viride* (64.00%), *Trichoderma harzianum*-1 (54.67%), *Trichoderma harzianum*-2 (51.00%), *Pseudomonas fluorescens* (45.00%). Whereas, the minimum parasitic activity was noticed in case of *Bacillus subtilis* 1 and 2 which inhibited 40.67 and 41.67 per cent of *C. fimbriata* colony.

Biological control is a potential non-chemical and eco-friendly means for plant disease control by reducing harmful effects of a pathogen through the use of other living entities. It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyarajan, 1996) [12]. In the present study, among fungal antagonist *Trichoderma harzianum*-55 recorded maximum inhibition of (76.00%) mycelia growth of *C. fimbriata* followed *Trichoderma viride*-27 which inhibited to the extent of 70.33 per cent, among bacterial antagonist *P. fluorescens* inhibited 45.00 per cent and least inhibition was found with *B. subtilis*-1 (40.67%). It was widely known that *T. harzianum* shows antagonistic behavior towards *C. paradoxa*. The powerful antagonistic behaviour of the *T. harzianum* can be attributed to competition, parasitism, and antibiosis or by synergistic combination of these modes of action (Whips, 1992) [25]. The mechanism involved in inhibition of the test fungus may be due to the release of antibiotic (viridian) produced by *T. viride* (Karthikeyan, 1996) [7]. It may also due to coiling effect around the hyphae of the fungal pathogen (Chet *et al*., 1981) [5]. Another possibility for reduction in mycelial growth of test fungus may be competition between test fungus and *T. viride* for nutrition and other growth factors (Mukherji and Garg, 1988) [10]. It was due to the penetration of the antagonistic hyphae into hyphae of the pathogen at the place of contact as confirmed by Mukherji *et al.* (2000) [11]. The next best bio-control agent in inhibiting test fungus was *P. fluorescens*, which may be due to release of antibiotic substances produced by *P. fluorescens* like phenozines, pyroluterin, pyrrolnitrin and also by competition (Vidyasekharan and Muthamilan, 1995) [23]. The findings are in accordance with Talukder *et al.*

(2007) [22] who reported that *T. harzianum* was found effective antagonist against *C. paradoxa*. Sharma (2009) [17] reported that *T. viride* bioformulation resulted in 86.6% growth inhibition of *C. fimbriata* under *in vitro* condition. Rahman *et al.* (2009) [15] reported that *T. harzianum* IMI-392432 has the most potential to control the *C. paradoxa.* Sonyal *et al.* (2010) [21] reported that among bio-agents evaluated *in vitro* against *C. fimbriata*, *T. harzianum* and *T. viride* showed maximum inhibition of the test fungus (100%) followed by *P. fluorescens* (42.33%). Apet *et al.* (2015) [2] observed that *T. viride* was most effective followed by *T. harzianum. T. hamatum* and *P. fluorescens.* Balaganur (2016) [3] recorded *P. putida* isolates, UHSPs 2, *T. harzianum*, UHSTh 5, UHSTh 43 and UHSTh 48 and *B. subtilis* isolates Bs-3b and Bs-27 were effective against *C. fimbriata.* Khan *et al.* (2017) [8] reported among the different bio agents tested against *C. fimbriata*, *T. harzianum* was found to be the most effective with the highest inhibition of mycelial growth (88.77 %) followed by *T. viride* (86.60 %) and *P. fluorescens* (66.33 %). *Bacillus subtilis* was found less effective with (54.88 %) inhibition. Raja (2017) [16] revealed that *T. harzianum* (Th-R) and *T. viride* (Diamond) recorded maximum inhibition of 100 per cent mycelial growth of *C. fimbriata* followed *P. fluorescens* (Platinum), while least effective was *B. subtilis* (BS-1).

Table 3: *In vitro* evaluation of bioagents against *Ceratocystis fimbriata* through dual culture technique

Sl. No.	Bioagents	Per cent inhibition of mycelial growth
1	*Trichoderma harzianum*-55	76.00 (60.67)*
2	*Trichoderma viride*-27	70.33(57.00)
3	*Trichoderma viride* (PDBC)	67.00 (54.94)
4	*Trichoderma viride*	64.00 (53.13)
5	*Trichoderma harzianum*-1	54.67 (47.68)
6	*Trichoderma harzianum*-2	51.00 (45.57)
7	*Pseudomonas fluorescens*	45.00 (42.13)
8	*Bacillus subtilis* -1	40.67 (39.62)
9	*Bacillus subtilis*-2	41.67 (40.21)
	S.Em±	0.33
	CD @ 1%	0.99

*Values in parenthesis are arc-sine transformed values

Reference

1. Anonymous. Horticultural statistics at a glance. Ministry of Agrico and Farmer welfare Government of India. 2015, 43.
2. Apet KT, Sayyad AS, Wagh SS, Chavan PG. Bioefficacy of fungicides, bioagents and phyotextracts against *Ceratocystis paradoxa*, causing pineapple disease of sugarcane. Research Journal of Agriculture Science. 2015; 6(6):1266-1270.
3. Balaganur AL. Molecular characterization of *Ceratocystis fimbriata* and its associated defense functions in pomegranate during pathogenesis. *M.Sc (Hort.) Thesis*, University of Horticultural Sciences. Bagalkot, 2016, 93.
4. Chaudhari VG, Kshirsagar P, Tirmali AM. Studies on wilt complex disease of pomegranate (*Punica granatum* L.). Advanced Life Science. 2016; 5(3):747-755.
5. Chet I, Harkan GE, Baker R. *Trichoderma hamatum*: It's hyphal interactions with *Rhizoctonia solani* and *Pythium* spp., Microbiology and Ecology. 1981; **7**:29-38.
6. Dutta Ray SK, Takawale PV, Chatterjee R, Hnamte V. Yield and quality of pomegranate as influenced by organic and inorganic nutrients. The Bioscan. 2014; 9:617-620.
7. Karthikeyan A. Effect of organic amendments, antagonistic *Trichoderma viride* and fungicides on seed and collar rot of groundnut. Plant Disease Research. 1996; 11:72-74.
8. Khan IHS, Ravindra H, Ekbote1 S, Narayaswamy H, Narayanaswamy P, Pradeep S. Bio efficacy of fungicides and bio agents against *Ceratocystis fimbriata* ell. and halst. causing wilt disease of pomegranate. International Journal of Current Microbiology and Applied Science. 2017; 6(6):2902-2907.
9. Kishore K, Bhardwaj SS. *In vitro* evaluation of fungicides and plant extracts against wilt and fruit rot pathogen of pomegranate. Plant Disease Research. 2011; 26(2):175-180.
10. Mukherji KG, Garg KL. Biocontrol of plant diseases. CRC press Inc., Florida, USA. 1988; 1:1-211.
11. Mukherji S, Tripathi HS, Rathi YPS. Integrated management of wilt of Frenchbena (*Phaseolus vulgaries* L.). Journal of Mycology and Plant Pahtology. 2000; 31:213-215.
12. Muthamilan M, Jeyarajan R. Integrated management of sclerotium root rot of groundnut involving *Trichoderma harzianum*, rhizobium and carbendazim. India Journal of Mycology and Plant Pahtology. 1996; 26:204-209.
13. Nene YL, Thapliyal PN. Fungicides in Plant Disease Control. Third edition, Oxford and IBH Publishing Co, Private Litmited, New Delhi, India. 1993, 311-348.
14. Poussio GB, Fateh FS, Kumar L, Kazmi MR, Jiskani MM, Abro MA *et al*. Influence of different fungicides and plant extracts against *Ceratocystis* sp. associated with mango death (MSD). South-Asian Journal Multidisciplinary Studies. 2012; 3(2):1-10.
15. Rahman MA, Begum MF, Alam MF. Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease of sugarcane. Mycobiology. 2009; 37(4):277-285.
16. Raja. Investigations on wilt of pomegranate caused by *Ceratocystis fimbriata* Ell. and Halst. in karnataka**.** Ph.D (Agri.) Thesis, University of Agricultural Sciences Raichur. 2017, 85-105.
17. Sharma KK. Vascular wilt of pomegranate caused by *Ceratocystis fimbriata* Ellis and Halsted and its control. 5th International Conference on Plant Pathology in the Globalized Era, IARI, New Delhi, India, 2009, 240.
18. Sharam KK, Sharma J, Jadahv VT. Etiology of pomegranate wilt and its management. Fruit Vegetable and Cereal science and Biotechnology. 2010; 4(2):125-130.

19. Somasekhara YM, Ravichandran NG, Jain RK. Biological management of root knot nematode (*Meloidogyne incognita*) infesting pomegranate (*Punica granatum* L.) in Karnataka. Paper Presented In: 2nd *Inter. Symp.* Pomegranate and minor including Mediterranean Fruits, University of Agricultural Sciences, Dharwad, 2009; 23-27:143-144.
20. Somasekhara YM, Wali. New record of *Ceratocystis fimbriata* causing wilt of pomegranate. Plant Disease. 1999; 83(4):400.
21. Sonyal S. Studies on pomegranate wilt complex. M.Sc. (Agri.) Thesis, University of Agricultural Sciences. Dharwad, 2010, 87.
22. Talukder MI, Begum F, Azad MMK. Management of pineapple disease of sugarcane through biological means. Journal of Agriculture and Rural Development. 2007; 5:79-83.
23. Vidyasekharan P, Muthamilan M. Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Disease. 1995; 79:782-786.
24. Vijaya HK, Kulkarni S, Hegde YR. Chemical control of sett rot of sugar cane caused by *Ceratocystis paradoxa*. Karnataka Journal of Agriculture Science. 2007; 20(1):62-64.
25. Whips JM. Status of biological disease control in horticulture. Biological Science and Technology. 1992; 2:3-24.