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Efficacy of VAM fungi and antagonistic bacteria against onion basal rot incited by *Fusarium oxysporum* f.sp. *cepae*

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Abstract
The study was undertaken to investigate the biocontrol potential of the native antagonists for the efficient usage of *Pseudomonas fluorescens*, *Trichoderma viride* and *Glomus mosseae* for the successful biological management of *F. oxysporum* f.sp. *cepae* causing basal rot of onion. In general all the native *Trichoderma* spp. tested significantly inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *cepae*. However, among the isolates, the isolate Tv_5, showed the maximum inhibition and significantly inhibited the growth of *F. oxysporum* f.sp. *cepae* (15.42 mm), which was 82.86 per cent reduction on the growth of the pathogen when compared to control. Among the *Pseudomonas* isolates, Pf_2 produced significantly the minimum mycelial growth (23.32 mm) accounting for 80.82 per cent reduction on the mycelial growth of *F. oxysporum* f.sp. *cepae* over control. The results obtained on the efficacy of combined application of antagonists and *G. mosseae*, soil application with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_2) plus soil application of *G. mosseae* treatment (T_7) recorded the minimum wilt incidence (4.82 %). Similarly, the same treatment recorded the maximum growth parameters such as shoot length (35.42 cm), root length (5.0 cm) and bulbs (6.0). The control recorded the minimum growth parameters such as shoot length (18.62 cm), root length (2.5 cm) and number of bulbs per plant (1.0).

Keywords: Onion basal rot, VAM, bacterial antagonist

Introduction
Onion (*Allium cepa* L.) is one of the important pungent, edible crops grown in India. Onions have medicinal properties because it contains starch, sugar, some protein, and vitamins A, B and C (Baloch, 1994) [2]. It is also used in the treatment of many diseases such as coronary heart diseases (Lanzotti, 2006) [18], cancers (Shutenko *et al*., 1999) [28] and diabetes (Sheela *et al*., 1995). The basal rot disease of onion caused by *Fusarium oxysporum* f.sp. *cepae* (Hans) is the most destructive disease and causes yield losses in all growing areas of the world (Coskuntuna and Ozer, 2008) [5]. Onion basal rot was first observed in Ohio, USA (Clinton, 1915) [4]. In India, the occurrence of this disease was first reported from Rajasthan (Ilhe *et al*., 2013) [13]. In Tamil Nadu, this disease was first observed by Ramakrishnan and Eswaramoorthy (1982) from Coimbatore district. Onion basal rot considered as one of the most important soil-borne diseases of onion, causes severe losses in productivity both in the field and in storage condition (Coskuntuna and Ozer, 2008) [5]. Yield loss up to 50 per cent has been recorded in susceptible cultivars (Everts *et al*., 1985) [10] with 90 per cent losses during the seedling stage. Biological control using fungal and bacterial antagonists has been suggested as a possible control of *Fusarium* basal rot disease of onion (Coskuntuna and Ozer, 2008) [5]. Several researchers have observed improved disease control using various biocontrol organisms such as *Trichoderma* sp. (Adekunle *et al*., 2001) [1] and *Pseudomonas* sp. (Rashmi Srivasta *et al.,* 2010) as they have antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al*., 2004) [32]. Besides these organisms, the Arbuscular mycorrhizal fungi (AMF) have also been reported in combating the soil-borne diseases by inducing plant defense proteins i.e. PR proteins (Pozo *et al*., 2002; Van Loon *et al*., 2006) [22]. Hence keeping these points in view the present study was planned to test the integrated effect of these antagonists for the management of onion basal rot.

Materials and Methods
Isolation of *Fusarium oxysporum* f.sp. *cepae*
The pathogen was isolated from the infected bulbs of onion by tissue segment method (Rangaswami, 1958) [23]. The infected portions of bulb were cut into small pieces using

sterilized scalpel and these were surface sterilized with 1.0 per cent sodium hypo chloride for one minute and washed in three changes of sterile distilled water and then placed on Petri dish containing Potato Dextrose Agar (PDA) medium. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of fungus. The hyphal tips of fungi grown from the plates were transferred aseptically to PDA slants for maintenance of the culture. The pathogens were identified based on their cultural and morphological characters**.**

Isolation of native antagonists from rhizosphere soil
***Trichoderma* spp.**
Rhizosphere soil samples collected from ten different locations were used for the isolation of *Trichoderma* isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983) [8]. These *Trichoderma* cultures were purified by single hyphal tip method and used for the studies.

Isolation of native antagonistic bacteria
Antagonistic bacteria were isolated from the rhizosphere soil collected from different onion growing areas of Tamilnadu by serial dilution method on King's B medium, incubating at room temperature for 24 h. Colonies with characteristics of *Pseudomonas* sp., were isolated individually and purified by streaking them on King's B medium.

Efficacy of antagonists against *F. oxysporum* f.sp. *cepae* (*in vitro*)
Effect of antagonists on mycelial growth
The antagonistic activity of bio control agents against *F.oxysporum* f.sp. *cepae* was tested by dual culture technique (Dennis and Webster, 1971) [6]. At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium a 9 mm mycelial disc obtained from five day old culture of *Trichoderma* spp. was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a 9 mm culture disc of *F.oxysporum* f.sp. *cepae* was placed and incubated. A control was maintained by inoculating *F.oxysporum* f.sp. *cepae* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for three days. In case of *P. fluorescens* one cm long streak was gently made onto the medium using two days old culture. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927)

Per cent inhibition (I) = C-T/C × 100

Where, C- mycelial growth of pathogen in control
T- Mycelial growth of pathogen in dual plate
I- inhibition per cent

Preparation of talc-based formulations
***Pseudomonas* sp.**
Different *Pseudomonas* isolates were maintained in KB broth and incubated for 48 h at 28 ± 2ºC in a rotary shaker at 150 rpm. Four hundred ml of 72-h-old bacterial culture in their respective medium with a population of 9 x 10^8 cfu / ml were mixed with 1 kg of talc containing 15 g of calcium carbonate and 10 g of carboxy methyl cellulose. Moisture content of the product was reduced to 20 per cent by shade drying and it was packed in polythene bags. The population of bacteria was 2.5 to 3 x 10^8 cfu /g of talc powder, at the time of application (Vidhyasekaran and Muthamilan, 1995).

***Trichoderma* sp.**
Different *Trichoderma* isolates were grown in yeast molasses medium and it was incubated for 15 days and then the content was drained. The fungal biomass was removed and this was mixed with one kg of talc at 1:2 ratio. The mixture was shade dried. Carboxyl methyl cellulose was added to the talc @ 5 g/kg of talc. The materials were packed in polythene bags. Sealed and incubated at room temperature. After 20 days of storage the samples were drawn at ten days intervals and the *Trichoderma* sp. population was assessed by dilution plate methods.

Isolation of AMF from onion rhizosphere soil
The rhizosphere soil collected from different onion growing fields were examined for the presence of AM fungal spore by wet sieving and decanting method described by Gerdemann and Nicolson (1963) [11] followed by sucrose centrifugation (Smith and Skipper, 1979). These spores were cleaned of soil particles by sucrose density gradient centrifugation method and washed with distilled water. The spore suspension was observed under stereo zoom microscope and morphologically similar spores were separated into groups, mounted and identified. The AM fungi were identified based on the Manual for Identification of VA Mycorrhizal Fungi (Schenck and Perez, 1990) [24] and recorded.

Mass production of *G. mosseae* inoculum
For mass production of *G. mosseae* inoculum the methodology suggested by Kumutha *et al.* (2010) was followed. A trench (3m × 1m × 0.3m lbh) lined with back polythene sheet was used as plant growth tub. 500 kg of vermiculite and 50 kg of sterilized soil was mixed and packed in the trench up to a height of 20 cm. To this ten kg of mother inoculum of *G. mosseae* containing 400-450 spores per 100 g soil was spread 2-5 cm below the surface of vermiculite. Surface sterilized maize seeds were sown and applied with 20 gm of urea, super phosphate and 10 gm of muriate of potash per trench. Further 10 gm of urea was applied twice on 30 and 45 DAS. Thus the stock plants were grown for eight weeks and de-topped. The inoculum was prepared by collecting the vermiculite in the pit along with root bits infected with *G. mosseae*. Thus, approximately 55 kg of inoculum could be produced from one square meter area and used for the field studies. The propagules in soil-based culture consisted of both spores and (400-450 spores per 100 g soil) chopped, colonized root fragments.

Effect of combined application of antagonist on plant growth and basal rot incidence of onion (pot culture)
Strelized soil was mixed with the pathogen inoculums @ 5per cent (W/W) level and filled in 30cm earthern pots. The most effective soil application dosages identified as earlier experiment alone were used for testing the efficacy of soil application of the antagonists. The antagonists meant for soil application were applied to the pots and incorporated well. The onion bulbs were planted in pot soil mixed with the

innoculum of *F.o.*f.sp.*cepae* alone served as a control. Carbendazim @ 0.1% was used for comparison. The experiment was conducted with three replication in a randomized block design with five bulbs per pot. All the observations *viz.*, plant growth parameters, bulb rot incidence, population of the antagonist and population of pathogen were recorded.

Treatment schedule

T_1 – Soil application of *T.viride* @ 5 g kg^{-1} of soil
T_2 – Soil application of *P.fluorescens* @ 5 g kg^{-1} of soil
T_3 – *Glomus mosseae* 50 g kg^{-1} of soil
T_4 – SA of *T.viride* @ 5 g kg^{-1} + SA of *P.fluorescens* @ 5 g kg^{-1}
T_5 – SA of *T.viride* @ 5 g kg-1 + *Glomus mosseae* 50 g kg^{-1} of soil
T_6 – SA of *P.fluorescens* @ 5 g kg^{-1} + *Glomus mosseae* 50 g kg^{-1} of soil
T_7 – SA of *T.viride* @ 5 g kg^{-1} + SA of *P.fluorescens* @ 5 g kg^{-1} + *Glomus mosseae* 50 g kg^{-1} of soil
T_8 – Carbendazim 50% WP @ 0.1 %
T_9 – Control

Results and Discussion

Antifungal activity of *Trichoderma* sp. against mycelial growth of *F.oxysporum* f.sp. *cepae* in *in vitro* (Dual culture technique)

In general all the native *Trichoderma* spp. tested significantly inhibited
the mycelial growth of *F.oxysporum* f.sp. *cepae* (Table 1). However, among the isolates, the isolate Tv_5, showed the maximum inhibition and significantly inhibited the growth of *F.oxysporum* f.sp. *cepae* (15.42 mm), which was 82.86 per cent reduction on the growth of the pathogen when compared to control. This was followed by the isolates Tv_3 and Tv_9 in the decreasing order of merit, which inhibited the growth of *F. oxysporum* f.sp. *cepae* by 81.98 and 79.86 per cent over control. The least growth inhibition of the pathogen (44.17%) was exhibited by the isolate Tv_7.

The results of the present study correspond with Ilhe (2013) [13] who observed that *T. viride* was found to be most effective in inhibiting the growth of *F. oxysporum* f.sp. *cepae*. Hacer Handan and Oktay (2015) [12] observed that *T. harzianum* 16 and 23 strains showed significant inhibition of mycelial growth of the pathogenic strains of *F. oxysporum*. Both *T. harzianum* strains produced volatile and non-volatile metabolites that inhibited growth of *F. oxysporum* strains. Narayan Prasad Verma *et al.* (2018) showed variation in the antagonistic activities of *Trichoderma* sp. isolates against the tested *Fusarium* sp. Smilar such findings were given by Patel, (2017). Also, inhibition of the pathogen may be attributed to the production of secondary metabolites (such as glioviridin, viridin and gliotoxin) the antagonists (Inbar *et al.*, 1994). Several studies (Jayalakshmi *et al.*, 2009; Muhammad and Amusa, 2003; Shabir *et al.*, 2013) [26] reported that inhibition of some soil borne pathogens, including *Fusarium* spp. by *Trichoderma* species could probably be due to the secretion of extracellular cell wall degrading enzymes such as chitinase, β-1, 3- glucanase, β-1, 6-glucanase, protease, cellulase and lectin, which help mycoparasites to colonize their host.

Efficacy of native bacterial isolates against *F.oxysporum* f.sp. *cepae* (Dual culture)

The results presented in Table 2 revealed varying degree of antagonism by the isolate of *Pseudomonas* against *F.oxysporum* f.sp. *cepae*. Among the *Pseudomonas* isolates, Pf_2 produced significantly the minimum mycelial growth (23.32 mm) accounting for 80.82 per cent reduction on the mycelial growth of *F. oxysporum* f.sp. *cepae* over control. This was followed by isolate Pf_4 which recorded 78.22 per cent reduction on the mycelial growth over control. The isolate Pf_{10} was the least effective among *Pseudomonas* isolates as it recorded the minimum percent inhibition control. Intesar Ali Mezeal (2014) [14] showed that different isolates of *P. fluorescens* showed the highest of growth inhibition against *R.solani* and *F. oxysporum.* Boukerma *et al.* (2017) [3] reported that the potential of *P. fluorescens* PF15 and *P. putida* PP27 showed significant inhibition of the *F.oxysporum* f.sp. *lycopersici* in tomato. Malathi (2015) reported that, Pf 12 and Pf 27 of the *Pseudomonas* isolates were found to be the most effective in inhibiting the growth of *F. oxysporum* f. sp. *cepae.* Many strains of *Pseudomonas* have been found to produce broad spectrum antibiotics including phenazine, pyrrolnitrin, pyoverdine and 2,4 diacetylphloroglucinol (Gardener *et al.,* 2000), lytic enzymes such as chitinases and β-1,3- glucanases which degrade fungal chitin (Velazhahan *et al.,* 1999) [30, 31], siderophore (Loper, 1988), HCN (Ahl *et al.*, 1986) and induced systemic resistance (Van Peer *et al.,* 1991).

Effect of combined application of antagonists and *G. mosseae* on basal rot incidence of onion (Pot trial)

The results obtained on the efficacy of combined application of antagonists and *G. mosseae* is furnished in Table 3. Among the treatments, soil application with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_2) plus soil application of *G. mosseae* treatment (T_7) recorded the minimum wilt incidence (4.82 %). This was followed by the treatment (T_4) with soil application of *T. viride* (Tv_5) + *P. fluorescens* (Pf_5) which recorded (7.05 %) at par results with carbendazim 0.1 % in reducing the basal rot incidence. The control recorded the maximum disease (45.87%) incidence.

The combined application of biocontrol agents treatment (Soil application of *T. viride* @ 2.5 kg / ha. + Soil application of *P.fluorescens* 2.5kg/ ha + Soil application of *G. mosseae* @ 12.5 kg /ha) was more effective than individual treatments, which might be due to the additive and interactive effect of the bio agents. The observations made in the present study are in accordance with the findings of Tayal *et al.* (2011) [29] and Sathiyasivanathamoorthy (2017). Shiva Yendyo *et al.* (2018) also showed that the combined application of native isolates of *Trichoderma* spp. and *P. fluorescens* reduced the disease incidence of *Ralstonia* wilt of tomato.

Effect of combined application of antagonists and *G. mosseae* on plant growth promotion of onion (Pot trial)

The results revealed that the efficacy of combined application of antagonists and *G. mosseae* is furnished in Table 4. Among the treatments, soil application with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_{52}) and soil application of *G. mosseae* treatment (T_7) recorded the maximum parameters such as shoot length (35.42 cm), root length (5.0 cm) and bulbs (6.0). This was followed by the treatment (T_4) with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_2) which recorded at par results. The control recorded the minimum growth parameters such as shoot length (18.62 cm), root length (2.5 cm) and number of bulbs per plant (1.0).

Similar observations were made by Ngullie and Daiho (2013) [21] who recorded reduced incidence of seedling rot in both

greenhouse and field condition from combination of *T. viride* + *P. fluorescens*. Malathi (2015) reported that increase the growth factors shoot length, root length and number of bulbs with Pf 12 + Pf 27 + TH 3 in the field experiment.

Enhanced plant growth by the siderophore producing strains of fluorescent *Pseudomonas* was reported (Dutta *et al*., 2005). *P. fluorescens* might have stimulated plant growth by improving uptake of minerals into the host plants particularly phosphate (Kloepper *et al*., 1980), siderophore mediated iron uptake (Jurkevitch *et al.*, 1988) [16], association with N_2 fixation (Hong *et al*., 1991), production of IAA (Dubeikovsky *et al.*, 1993) [7], promotion of mycorrhizal function (Garbaye, 1994) and solubilizing nutrients such as phosphorus (Whitelaw, 2000) *Trichoderma* spp. are known to produce large quantities of fungistatic metabolites sucha as trichodermin, dermin, trichoviridin, trichobrachin, chitinase, β-1,3 glucanase, protease etc. (Elad *et al*., 1982; Bruchkner *et al*., 1990) [9] which were active against many soil borne pathogens. Thus, the results of the present study and the earlier reports have confirmed that the growth promoting substances produced by *P. fluorescens*, *T. viride* (Tv_3+Pf_5) and *G. mosseae* would have exerted a synergism in promoting the growth parameters of onion.

Table 1: Antifungal activity of *Trichoderma viride* against mycelial growth of *F.oxysporum* f.sp. *cepae* in *in vitro* (Dual culture technique)

S. No.	Isolates	Mycelial growth of *F.oxysporum* f. sp. *cepae* (mm)	Per cent (%) inhibition over control
1.	Tv_1	35.78^{g}	60.24
2.	Tv_2	47.12^{i}	47.64
3.	Tv_3	16.21^{b}	81.98
4.	Tv_4	38.21^{h}	57.54
5.	Tv_5	15.42^{a}	82.86
6.	Tv_6	23.72^{e}	73.64
7.	Tv_7	50.24^{j}	44.17
8.	Tv_8	28.82^{f}	67.97
9.	Tv_9	18.12^{c}	79.86
10.	Tv_{10}	20.21^{d}	77.54
11.	Control	90.00^{k}	-

*****Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Table 2: Antifungal activity of *Pseudomonas fluorescens* against mycelial growth of *F.oxysporum* f.sp. *cepae* in *in vitro* (Dual culture technique)

S. No.	Isolates	Mycelial growth of *F.oxysporum* f.sp. *cepae* (mm)	Percent (%) inhibition over control
1.	Pf_1	35.73^{d}	60.10
2.	Pf_2	23.32^{a}	80.82
3.	Pf_3	48.55^{i}	46.05
4.	Pf_4	29.72^{b}	78.22
5.	Pf_5	49.62^{j}	44.86
6.	Pf_6	41.81^{f}	53.54
7.	Pf_7	31.75^{c}	64.72
8.	Pf_8	38.98^{e}	56.68
9.	Pf_9	46.12^{h}	48.75
10.	Pf_{10}	42.35^{g}	52.94
11.	Control	90.00	-

*****Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Table 3: Effect of combined application of antagonists and *G. mosseae* on basal rot incidence of onion (Pot trial)

Tr. No.	Treatments	Basal rot incidence (%)			% disease increase over control		
		30 DAS	45 DAS	At harvest	30 DAS	45 DAS	At harvest
T_I	SA of *Trichoderma viride* (5) @ 5g/kg of soil	5.23^{f} (13.22)	8.21^{f} (16.65)	9.59^{f} (18.03)	76.42	72.63	79.15
T_2	SA of *Pseudomonas fluorescens*(2)@ 5g/kg of soil	6.52^{g} (14.79)	9.54^{g} (17.99)	12.81^{g} (20.97)	70.60	68.02	72.07
T_3	SA of *Glomus mosseae* @ 50g/kg of soil	7.95^{h} (16.37)	10.67^{h} (19.06)	15.62^{h} (23.27)	64.15	64.43	65.94
T_4	SA of *Trichoderma viride* (5) @ 5g/kg of soil + SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil	2.45^{b} (9.00)	4.12^{b} (11.71)	7.05^{b} (15.39)	88.95	86.26	84.63
T_5	SA of *Trichoderma viride* (5) @ 5g/kg of soil + SA of *Glomus mosseae* @ 50g/kg of soil	4.81^{d} (12.66)	6.05^{d} (14.23)	7.12^{d} (15.47)	85.93	80.06	84.47
T_6	SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil + SA of *Glomus mosseae* @ 50g/kg of soil	4.52^{e} (12.27)	7.28^{e} (15.65)	8.84^{e} (17.29)	78.31	79.83	80.72
T_7	SA of *Trichoderma viride* (5)@ 5g/kg of soil + SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil+ SA of *Glomus mosseae* @ 50g/kg of soil	0.00	2.45^{a} (9.00)	4.82^{a} (12.68)	100.00	91.83	89.49
T_8	Carbendazim 50% WP@0.1%	3.12^{c} (10.17)	5.82^{c} (13.96)	7.50^{c} (15.89)	94.95	87.26	84.63
T_9	Control	10.00^{i} (18.43)	15.34^{i} (23.05)	45.87^{i} (42.63)	---	---	---

*****Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Table 4: Effect of combined application of antagonist and *G. mosseae* on Biometrics of onion (Pot trial)

Tr. No.	Treatments	Shoot length (cm)	Root length (cm)	Number of bulbs /plant
T_1	SA of *Trichoderma viride* (5) @ 5g/kg of soil	32.52^{e}	4.2^{c}	4.0^{c}
T_2	SA of *Pseudomonas fluorescens*(2)@ 5g/kg of soil	30.18^{f}	4.0^{e}	4.0^{c}
T_3	SA of *Glomus mosseae* @ 50g/kg of soil	29.15^{g}	3.8^{f}	3.0^{d}
T_4	SA of *Trichoderma viride* (5) @ 5g/kg of soil + SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil	34.28^{b}	4.5^{b}	5.0^{b}
T_5	SA of *Trichoderma viride* (5) @ 5g/kg of soil + SA of *Glomus mosseae* @ 50g/kg of soil	33.72^{c}	4.2^{c}	4.0^{c}
T_6	SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil + SA of *Glomus mosseae* @ 50g/kg of soil	32.18^{d}	4.3^{d}	4.0^{c}
T_7	SA of *Trichoderma viride* (5)@ 5g/kg of soil + SA of *Pseudomonas fluorescens* (2) @ 5g/kg of soil+ SA of *Glomus mosseae* @ 50g/kg of soil	35.42^{a}	5.0^{a}	6.0^{a}
T_8	Carbendazim 50% WP@0.1%	32.15^{e}	4.2^{c}	4.0^{c}
T_9	Control	18.62^{h}	2.5^{g}	1.0^{e}

*****Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Reference

1. Adekunle AT, Cardwell KE, Florini DA, Ikotun T. Seed treatment with *Trichoderma* species of control of damping off of cowpea caused by *Macrophomina phaseolina*. Biochem. Sci. Technol. 2001; 11:449-457.
2. Baloch AF. Vegetable crops. Horticulture.National Book Foundation. Manza printing corporation, Islamabad. 1994, 633.
3. Boukerma L, Benchabane M, Charif A, Khelifi L. Activity of plant growth promoting rhizobacteria (PGPRs) in the biocontrol of tomato *Fusarium* wilt. *Plant Protect. Sci*. 2017; 53:78-84.
4. Clinton GP. Notes on plant diseases of Connecticult. Conn. Agr. EXP. Stat. Ann. Rpt. 1915; 6:421-451.
5. Coskuntuna A, Ozer N. Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. *Crop Prot.* 2008; 27(5):330-336.
6. Dennis L, Webstar J. Antagonistic properties of species-groups of *Trichoderma*. The production of non volatile antibiotics. *Trans. Bri. Mycol. Soc.* 1971; 57:25-39.
7. Dubeikovsky AN, Mordukhova EA, Kochethov VV, Polikarpovo FY, Boronin AM. Growth promotion of black currant soft wood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indole-3-acetic acid. *Soil Biol. Biochem*. 1993; 25:1277-1281.
8. Elad Y, Chet I. Improved selective media for isolation of *Trichoderma* or *Fusarium* spp. *Phytoparasitica.* 1983; 11:55-58.
9. Elad Y, Chet I, Boyle P, Henis Y. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*-scanning electron microscopy and fluorescent microscopy. *Phytopathology*. 1982; 72:85-88.
10. Everts KL, Schwartz HF, Epsky ND, Capinera JL. Effects of maggots and wounding on occurrence of *Fusarium* basal rot of onion in Colorado. *Plant Dis.* 1985; 69(10):878-882.
11. Gerdemann JW, Nicolson TH. Spores of mycorrhizal endogone extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 1963; 46:235-244.
12. Hacer Handan Altinok, Oktay Erdogan. Determination of the *In vitro* Effect of *Trichoderma harzianum* on Phytopathogenic Strains of *Fusarium oxysporum*. *Nat. Bot. Horti. Agro. bo.* 2015; 43(2):494-500.
13. Ilhe M, Musmade NA, Kewade SB. Studies on basal bulb rot of onion caused by *Fusarium oxysporum* f.sp. *cepae*. Internat. J Plant Protec. 2013; 6(2):364-366.
14. Intesar Ali Mezeal. Study biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* and *Fusarium oxysporum* causing disease in tomato (*Lycopersicon Esculentum* L.). Indian Journal of Fundamental and Applied Life Sciences. 2014; 4(4):175-183.
15. Jayalakshmi V, Venkatesan S. Effect of biocontrol agents on cassava tuber rot under field conditions. Indian. J Plant Prot. 2006; 34(1):105-107
16. Jurkevitch E, Hadar Y, Chen Y. Involvement of bacterial siderophores in the remedy of lime induced chlorosis in peanut. Soil Sci. Soc. Am. J. 1988; 52:1032-1037.
17. Kavi Sidharthan V, Rashmi Aggarwal N, Surenthiran, Shanmugam V. Selection and characterization of the virulent *Fusarium oxysporum* f. sp. *lycopersici* isolate inciting vascular wilt of tomato. Int. J Curr. Microbiol. App. Sci. 2018; 7(2):1749-1756.
18. Lanzotti V. The analysis of onion and garlic. *J Chromatogr. A.* 2006; 11:213-220.
19. Malathi S, Mohan S. Analysis of genetic variability of *Fusarium oxysporum* f. sp. *cepae* the causal agent of basal rot on onion using RAPD markers. Archives of Phytopathology and Plant Protection. 2012; 45(13):1519-1526.
20. Nandakumar R, Babu S, Vishwanath R, Sheela J, Raguchander T, Samiyappan R. A new bio formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biocontrol.* 2001; 46:493-510.
21. Ngullie M, Daiho L. Efficacy of biocontrol agents in controlling *Rhizoctonia solani* naga king Chilli (*Capsicum chinense* Jacq.). Journal of Experimental Biology and Agricultural Sciences. 2013; 1:197-201.
22. Pozo MJ, Cordier C, Dumas gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C. localized versus systemic effect of arbuscular mycorrhizal fungi on defense responses to *Phytopthora* infection in tomato plants. J Exp. Bot. 2002; 53:525-534.
23. Rangaswami G. An agar blocks technique for isolating soil micro organisms with special reference to pythiaceous fungi. Science and Culture. 1958; 24:85.
24. Schenck NC, Perez Y. Manual for Identification of VAM Mycorrhizal Fungi. INVAM, University of Florida, Gainesville, USA., 1990.

25. Schenck NC, Ridings WH, Cornell JA. Interaction of two vesicular arbuscular mycorrhizal fungi and *Phytophthora parasitica* of two citrus root stocks, in Proceedings of the third North American Conference on Mycorrhizae (Oregon: Corvallis), 1977, 9.
26. Shabir R, Rubina L, Ebenezer JK, Talat MA, Shabir AG, Waseem AD *et al*. Eco-friendly management of root-rot of chilli caused by *Rhizoctonia solani* kuehn. Afr. J Agric. Res. 2013; 8(21):2563-2566.
27. Shabir-U-Rehman WA, Dar SA, Ganie, Javid A, Bhat GH, Hassan Mir, Rubina Lawrence *et al*. Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f sp. *ciceris* causing wilt of chickpea. African Journal of Microbiology Research. 2013; 7(50):5731-5736.
28. Shutenko Z, Henry Y, Pinard E, Seylaz J, Potier P, Berthet F *et al*. Influence of the antioxidant quercetin *in vivo* on the level of the nitric oxide determinated by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. *Biochem. Pharm.* 1999; 57:199-208.
29. Tayal P, Kapoor R, Bhatnagar AK. Functional Synergism among *Glomus fasciculatum*, *Trichoderma viride* And *Pseudomonas Fluorescens* on *Fusarium* wilt in tomato. Journal of Plant Pathology. 2011; 93(3):745-750.
30. Velazhahan R, Samiyappan R, Vidhyasekaran P. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzyme. J Plant Dis. Prot. 1999; 106(3):244-250.
31. Velazhahan R, Samiyappan R, Vidhyasekaran P. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzyme. J Plant Dis. Prot. 1999; 106(3):244-250.
32. Zaidi NW, Pramila N, Singh US. Biological control of plant pathogens: Status in India. *In:* Singh, S.P. and Singh, S.B. (Eds.), Eco-Agriculture with Bio augmentation: An emerging concept, DASP, Lucknow, 2004, 21-52.