

Biological and chemical control of *Fusarium* root rot in tomato plants at flowering stage.

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Abstract: A common soil-borne plant pathogen called *Fusarium* sp. causes ailments including root rot. A secure method to avoid the issues associated with fungal diseases that reduce agricultural production is biological control of plant disease. Eight *Trichoderma* isolates were isolated from soil surrounding healthy tomato roots in various parts of Dakahlia Governorate, The most successful isolate of *Trichoderma* was *Trichoderma atrovirde*, which had a 91.89% percentage inhibition against *Fusarium equiseti* after being tested using a dual culture method. *T. atrovirde* treatments enhanced tomato plant growth parameters compared with control plants or plants that were simply infected, as well as an increase in photosynthetic pigments and relative water content was observed in response to *T. atrovirde* treatment. Additionally, decreased in proline content, saturation water deficit and disease incidence percentage (40.55%) were also detected, compared to plants that were solely infected (74.21%). Based on the obtained results, *Trichoderma atrovirde* could be used as a biological control against root rot disease of tomato plants.

Keywords: Biological control, Mycoparasitism, *Trichoderma*, *Fusarium*, root rot and tomato plants.

1. Introduction

Tomato is one of the most widely consumed vegetables, both nationally and internationally [1]. It can lower the risk of cancer, cardiovascular disease, and osteoporosis, among other ailments, and offers a variety of nutritional and physiological advantages [2]. Unfortunately, several pathogens frequently cause a major reduction in production and quality [3]. One of the most distinct diseases is root rot caused by *Fusarium* sp. infection [4]. Controlling such diseases currently depends mainly on two basic methods as chemical fungicide application, which may present hazards to human health and increase environmental pollution [5]. Since chemical fungicides have been shown to be detrimental to soil and the environment, biological treatment of fungal disease is seen as a workable option that is also harmless for the environment [6]. Increasing regulations and restrictions of chemical pesticides or failed attempts at control have increased interest in biological control. Biological control by microorganisms that antagonize pathogens is

particularly attractive because diseases caused by soil pathogens are difficult to control with certain fungicides [7]. Further, rhizosphere-competent fungi and bacteria can regulate pathogens or produce growth-stimulating factors [5]. Microorganisms isolated from specific plant roots or rhizospheres may be better adapted such plants than organisms isolated from other plant species [8].

T. atroviride, are frequent saprophytes that lives in soil and has no negative effects on the environment or human health [9].

The objectives of this study were to evaluate the efficiency of bio-control as *Trichoderma* sp. and chemical control (fungicide Rizolex-T 50%) in control root rot of tomato caused by *Fusarium* sp. under in vitro and pots conditions.

2. Materials and methods

Isolation and purification of fungal isolates from soil:

Different isolates of *Fusarium* were isolated from rhizosphere of infected tomato plants according to the method of [10].

Effect of *Trichoderma* isolates against *Fusarium* sp. pathogen in vitro:

Using a dual culture approach, the antagonistic potential of the *Trichoderma* spp. fungal isolates was evaluated in vitro against *Fusarium*. *Trichoderma* spp. isolates and *Fusarium* sp. were both cultured on PDA medium for six days before being employed as an inoculum. 5 mm-diameter *Trichoderma* spp. discs from each isolate were infected in a Petri dish with PDA medium on one side and *Fusarium* sp. inoculum on the other (5 mm in diameter). Each treatment consisted of three replicates, with infected plates containing just *Fusarium* sp. serving as the control. The measured pathogen showed linear development after five days of incubation at 28° C ±1. Percentage of growth inhibition was calculated using the following formula [11] :

$\% \text{Reduction of growth} = (\text{growth in control} - \text{growth in treatment} / \text{growth in control}) \times 100$

Pot experiment:

The pot experiment was carried out in the greenhouse at Faculty of Science, Mansoura University, to evaluate the potential of the tested biological control (*Trichoderma* sp.) and chemical control (fungicide Rizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) to control root rot of tomato caused by *Fusarium* sp. Twenty cm diameter clay pots each containing sterilized clay: sand: peat moss (1:1:1) were infested with inoculum of *Fusarium* sp. (2% w/w). The pots were kept for one week with regular irrigation to facilitate growth and colonization of the causal agent in the soil. Then, tomato plants were transplanted to the infested soil.

Six sets were arranged in complete randomize plot design with ten pots replicates as following:

1. Control (Normal treatment) (C).
2. *Fusarium* sp. treatment (pathogen) (P).
3. *Trichoderma* sp. treatment (T).
4. Rhizolex-T (fungicide) (F).
5. *Trichoderma* sp. + *Fusarium* sp. treatment (T+P).
6. Rhizolex-T + *Fusarium* sp. treatment (F+P).

Fungicide:

The fungicide Rizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) applied as seed dressing at the rate of 3g/kg soil.

Application of *Trichoderma* sp.

After seven days from the pathogen infestation in the soil, tomato seedlings that were 28 days old were transplanted into pots. There were three tomato seedlings in each pot [12]. When tomato plants were transplanted into the pots, the antagonistic isolates were added to the contaminated soil (at a rate of 1% w/w). For each treatment, three replicas (three pots) employed.

Disease assessment

After 45 days of planting, a disease assessment was determined. For each treatment, three plant samples were uprooted and checked for signs of root rot. Using the following formula, percentages of disease incidence (DI) were calculated:

$DI (\%) = (\text{No. of diseased plants} / \text{Total no. of assessed plants}) \times 100$

2.7. Disease severity % (DS%)

Disease severity was determined according to a 0-5 scale with minor modification [13].

Where: 0=0, 1= 0≤10, 2=10≤25. 3 =25≤50, 4=50≤75 and 5=75≤100%. it was calculated as recommended by Liu and Lu [14].

2.7. Disease severity% = $(\sum n \times r / N5) \times 100$

Where: n= number of plants in each numerical rate, N= total number of plants, multiplied by the maximum numerical rate r= 5.

Measurements

During flowering stage these measurements were determined :

- **Growth parameters:** Shoot fresh and dry weight, root fresh and dry weight, number of leaves, shoot and root length.
- **Relative water content (RWC%)** as described by [15] and calculated according to the following equation:

$$RWC = (FM - DM) / (TM - DM) \times 100$$

Where: **FM** is leaf fresh mass, **TM** is the fresh mass at full turgor, **DM** is leaf dry mass. Dry mass was determined after drying the leaf samples at 80°C for 24h and TM determination, leaves were rehydrated by immersing the

petiole in distilled water in a beaker sealed with parafilm. Full rehydration was achieved in 24–48 h in complete darkness at 2–4°C.

- **Saturation water deficit (SWD%)** was calculated according to [16] from the following equation:

$$\text{SWD\%} = 100 - \text{RWC\%}$$

- **Photosynthetic pigments:** (Chlorophyll a, Chlorophyll b and Carotenoids) were determined spectrophotometer according to the spectrophotometer method recommended by [17].

- **Proline content:** Free proline was determined according to [18]. One gram of macerated fresh tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid, and then filtered through filter paper whatman No.2. Two ml of the filtrate were mixed with 2 ml glacial acetic acid and 2 ml of freshly prepared acid ninhydrin

reagent (1.25g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid). The reaction mixture was extracted with 4 ml toluene, mixed vigorously in a test tube for 15–20 second. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance was recorded at 520 nm using toluene as a blank. The proline concentration was determined using a standard curve and calculated on a dry matter basis as mg proline 100 g⁻¹ fresh matter.

Data was analyzed by statistical variance (ANOVA) using the statistical Analysis System (CoStat). Means separated by Duncan's Multiple Range Test at $P \leq 0.05$ levels [19]

Results

In vivo effect of *Trichoderma* on tomato plants infected with *Fusarium* sp.:

Screening for antagonistic potential of *Trichoderma* isolates against *Fusarium* sp. (dual culture experiments):

Trichoderma isolates of various grades significantly suppressed *Fusarium* sp. radial development (Table 1, Figure 1). *Trichoderma* isolates were able to reduce *Fusarium*'s mycelial growth by a percentage (41.44–91.89%). T5 isolation showed the highest level of inhibition (91.89%), which was followed by isolates T1 (63.80) and T7 (61.64%). Isolate T4 showed the lowest level of inhibition (41.44%). In order to determine the species level of the isolate T5, which had the strongest antagonistic

effect on the tested phytopathogenic fungus (Fig. 1)

Table (1): Antagonists effect of *Trichoderma* isolates on the growth of *Fusarium* sp. under in vitro conditions

<i>Trichoderma</i> isolates	<i>Fusarium</i> liner growth (mm)	Growth inhibition (%)
Control	87.6a	0.00
T1	31.7g	63.8
T2	43.1e	50.80
T3	34.2f	60.95
T4	51.3b	41.44
T5	7.13h	91.89
T6	49.7c	43.26
T7	33.6g	61.64
T8	48.1d	45.09
LSD at 5%	0.16	-

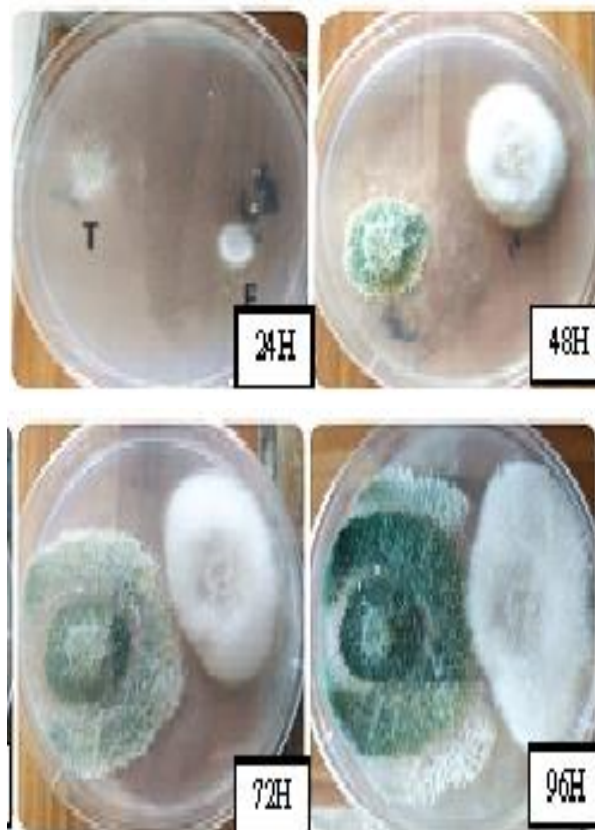


Fig (1): Effect of *Trichoderma* (T) antagonists on the mycelial growth of *Fusarium* sp. (F) under vitro conditions

Disease incidence (DI)

Obtained data represented in Table 2 and Fig. 2 showed that the disease incidence (DI) was very high (74.21%) in tomato plants infected with *Fusarium* sp.- As for the treatment with *Trichoderma* decreased DI to 40.55%, while the treatment with Rhizolex-T as fungicide, found a decrease in DI to 44.11%. Occurrence of the DI was substantially reduced

by treating infected tomato plants, using *Tricoderma* alone comparing to Rhizolex-T as fungicide.

Disease severity (DS)

In the same line, data in Table 2 and Fig. 2 revealed that disease severity (DS) recorded the highest percentage (67.40%) in plants infected with *Fusarium* sp. than the control plants. When the plants treated with *Tricoderma* the DS decreased to 30.72% and plants treated with Rhizolex-T as fungicide decreased just to 33.51%, which recorded high percentage in comparing to treating with *Tricoderma* alone to the control.

Table (2): Effect of *Tricoderma* on DI and DS of tomato plants infected by *Fusarium* sp.

Treatments	Disease incidence(DI)%	Disease severity (DS)%
Control	0.00d	0.00d
T	0.00d	0.00d
F	0.00d	0.00d
P	74.21a	67.40a
P+T	40.55c	30.72c
P+F	44.11b	33.51b
LSD at 5 %	0.14	0.19
T: <i>Trichoderma</i> F: fungicide (Rhizolex-T) P: pathogen		

Values with the same letter in the column are not statistically different, according to Duncan's

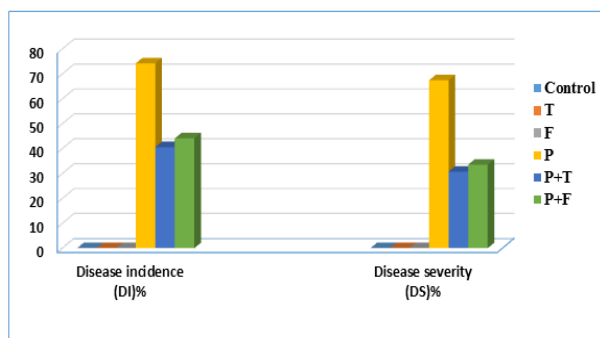


Fig (2): Effect of *Tricoderma* on DI and DS of tomato plants infected by *Fusarium* sp.

Table (3): Fresh and dry weight of tomato shoot and root in soil infested with *Fusarium* sp. as affected by biocide and chemical fungicide treatment.

Stages	Treatment	Shoot		Root	
		Fresh weight g/plant	Dry weight g/plant	Fresh weight g/plant	Dry weight g/plant
Flowering	Control	12.1b	1.32bc	1.43b	0.29b
	T	14.72a	2.22a	2.11a	0.43a
	F	9.33c	1.35b	1.12c	0.24bc
	P	2.41e	0.27d	0.42e	0.06d
	P+T	8.66c	1.26bc	1.05c	0.22bc
	P+F	6.53d	1.16c	0.85d	0.17c
	LSD at 5 %	2.02	0.18	0.13	0.07
T: <i>Trichoderma</i> F: fungicide (Rhizolex-T) P: pathogen					
Values with the same letter in the column are not statistically different, according to Duncan's multiple range test					

Growth parameters:

Data presented in Table 3 and Fig. 3 indicated the effect of fungicide and biocide on the incidence of *Fusarium* infection in tomato plant, which infected plants with root rot disease during flowering stages. Both shoot and root fresh and dry weights were significantly affected by the application of the chemical fungicide and biocide despite the increase in growth parameters in biocide compared with control plants during growth stages. The *Trichoderma* as biocide was superior in comparison with fungicide treatment (Rhizolex-T) in decreasing the disease incidence to as low as 40.55% as indicated in Table (2). The minimum disease incidence was recorded with plants treated with the *Trichoderma* isolate, which supported good growth of roots and shoots as well as increased fresh and dry weight of tomato plant during growth stages (vegetative, flowering and yield).



Fig 3: Growth parameters of tomato plants in soil infested with *Fusarium* sp. as affected by biocide and chemical fungicide treatment. Where T: *Trichoderma*, F: fungicide and P: pathogen

The effect of *Trichoderma* as biocide and Rhizolex-T as chemical fungicide on plant length of shoot and root, leaves number and branches number of tomato under biotic stress of root rot disease during flowering stage were presented in Table 4. All treatments under investigation significantly increased growth parameters and alleviating the biotic stress of root rot on tomato plants comparing with untreated plants. Healthy plants treated with *Trichoderma* recorded the highest mean values

of the traits during the flowering stage. All parameters recorded a significant increase comparing to the control. As for the plants infected with root rot disease, it was found a decrease in mentioned vegetative traits, but infected plants treated with *Trichoderma* scored an increase comparing to the infected one followed by the plants treated with Rhizolex-T. So, from the data it was found that *Trichoderma* was the most superior in plants infected with root rot disease.

Table (4): Plant length of shoot and root, leaves and branches number of tomato plants in soil infested with *Fusarium sp.* as affected by biocide and chemical fungicide treatment.

Stages	Treatments	Plant length cm		Number of leaves/plant	Number of branches /plant
		Shoot	Root		
Flowering	Control	35.00b	12.00b	33.00c	8.00b
	T	44.00a	15.00a	39.00a	10.00a
	F	30.00d	10.00c	28.00d	7.00bc
	P	14.00f	5.00e	22.00e	5.00d
	P+T	32.00c	9.00cd	37.00b	6.00cd
	P+F	27.00e	8.00d	34.00c	6.00cd
	LSD at 5%	1.37	1.01	1.63	1.62
T: <i>Trichoderma</i>		F: fungicide (Rhizolex-T)		P: pathogen	
Values with the same letter in the column are not statistically different, according to Duncan's multiple range test.					

Relative water content and saturation water deficit:

During flowering stage, statistical differences ($p \leq 0.05$) was obtained between treatments under investigation for the relative water content and saturation water deficit (Table 6). From the data, it can be noticed a significant effective on RWC and SWD%, the un-infected plants treated with *Trichoderma* recorded the highest significant amount of RWC and lowest of SWD followed by the untreated plants. So, under the same line it could be found that the infected plants treated with *Trichoderma* recorded the highest mean value of RWC and lowest of SWD comparing to the infected plants.

Table (6): Relative water content and saturation water deficit of tomato plants in soil infested with *Fusarium sp.* as affected by biocide and chemical fungicide treatment.

Treatments	RWC%	SWD%
Control	93.75b	6.25d
T	94.80a	5.21e
F	81.81c	18.19c
P	77.50e	22.50a
P+T	80.71d	19.29b
P+F	80.41d	19.59b
LSD at 5%	0.34	0.48
T: <i>Trichoderma</i> F: fungicide (Rhizolex-T) P: pathogen		

Photosynthetic pigments content:

It is shown from the data presented in Table 5, the effect of *Trichoderma* as biocide and Rhizolex-T as chemical fungicide on chlorophyll a, b, carotenoid and total pigments. All treatment under investigation increased significantly the photosynthetic pigments content. The increase in mentioned parameters was more pronounced with plants treated with *Trichoderma* comparing to the untreated plants with high significant effect. In the same way, the infected plants recorded a decrease in photosynthetic pigments content, but infected plants treated with *Trichoderma* increased the previously parameters comparing to the infected one followed by Rhizolex-T.

Proline mg/100 g:

Effect of *Trichoderma* as biocide and Rhizolex-T as chemical fungicide treatment on proline content of un-infected and infected tomato plants with root rot disease as illustrated in Fig (4). From the data, it was found a decrease in proline content in untreated plants

comparing to the infected one. Plants infected with root rot disease recorded high content of proline comparing to the un-infected one. Plants treated with *Trichoderma* and

rizolex-T increased the content of proline, while pathogen plants decrease the proline

content comparing to the control which recorded the lowest value.

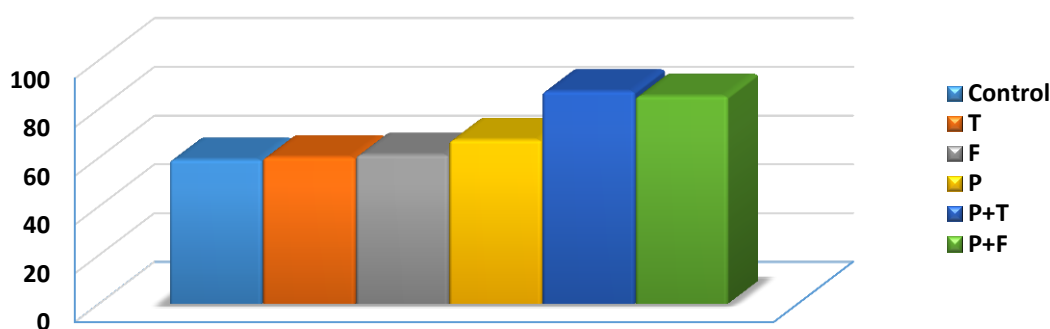


Fig (4): Proline (mg.100 g⁻¹) of tomato plants in soil infested with *Fusarium* sp. as affected by biocide and chemical fungicide treatment

Table (5): Photosynthetic pigments content of tomato plants in soil infested with *Fusarium* sp. as affected by biocide and chemical fungicide treatment.

Treatments	Chlorophyll a mg/g FW	Chlorophyll b mg/g FW	Carotenoids mg/g	Total pigments mg/g FW
Control	0.652b	0.422b	0.286b	1.360b
T	0.668a	0.488a	0.294a	1.450a
F	0.638c	0.363c	0.273c	1.274c
P	0.591f	0.306f	0.199f	1.096f
P+T	0.622d	0.334d	0.253d	1.209d
P+F	0.612e	0.316e	0.244e	1.172e
LSD at 5%	0.003	0.003	0.007	0.007
T: <i>Trichoderma</i> F: fungicide (Rhizolex-T) P: pathogen				
Values with the same letter in the column are not statistically different, according to Duncan's multiple range test				

DISCUSSION

Trichoderma sp. is widely used as bio-fungicide in agriculture induction of plant defense and mycoparasitism (killing of one fungus by another) are considered to be the most important mechanisms of *Trichoderma*-mediated biological control. *Trichoderma* species was found to be an effective biological control agent for protecting a number of crop plants from damaged induced by *Fusarium oxysporum* under greenhouse condition as indicated in the study by [20-22]. Different *Trichoderma* spp employ various pathogen-inhibiting strategies, including mycoparasitism through the secretion of hydrolytic enzymes, antibiosis through the production of secondary metabolites, competition for space and nutrients, promoting plant growth, and inducing systemic plant resistance mechanisms [23].

In dual in vitro research, antagonistic ability of *Trichoderma* isolates showed inhibitory effect on *Fusarium* sp. growth, the causal pathogen of tomato root rot disease, ranging from 41.44 to 91.89%. The present study

showed that *Trichoderma* sp. was more successful in inhibition of *Fusarium* sp. growth in vitro [24]. Prior to now, a dual culture technique was used to assess *Trichoderma* sp. potential to stop *Fusarium* sp. growth [25]. The defense mechanisms of *Trichoderma* sp. against fungi that cause plant damage were described in numerous studies, for example, Shrinkhala et al. [26] indicated that *Trichoderma* sp. grew more quickly, causing a shortage of nutrients and space that reduced *Fusarium*, which was cause of tomato withering.

Obtained results showed also that *Trichoderma* significantly reduced the DI of root rot on tomatoes with a reduction in disease ranging from 74.21 to 40.55% over time. *Trichoderma* can be used to control a large number of soil-borne fungi *F. solani* and *Pythium* spp. [27]. In the present study, the plant height was also increased in plants treated with *T. atroviride*, and this may be due to suppress the infection, encouragement of plant resistance, high nutrient uptake, and promotion of plant development.

In a pot experiment, two soil treatments (Rizolix-T and *T. harzianum*) that had been independently inoculated with strain of soilborne *Fusarium* were compared. *T. atroviride* enhanced plant development as evidenced by raised plant dry and fresh weight, length, and branch count, and caused the greatest decrease in disease incidence in soil injected with *Fusarium*. Rizolix-T performed less effective. [28] showed in vitro and in vivo the efficiency of native *Trichorema* isolates to enhance the growth and yield of tomatoes and control *Fusarium* rot disease. In comparison to other isolates and untreated controls, plants treated with *Trichoderma* sp. had significant stimulating effects on plant height (by 44cm) and dry weight (by 2.22g). In this experiment, plant height and fresh and dry weights were significantly higher in all treatments, with the exception of soil treated with *Trichoderma*.

In the connection, tomato plants treated with *Trichoderma* showed improved net photosynthetic rates when compared to the untreated control. In comparison to FOL-infested plants, [29] discovered that *Trichoderma* sp. boosted the net photosynthetic rate, stomatal conductance, and transpiration rates in cherry tomato plants. Increased chlorophyll content, which directly affects plants' photosynthetic activity, was the cause of an increase in net photosynthetic rate. This was in line with [30], They showed that *Trichoderma* treatment accelerated plant development by increasing leaf chlorophyll levels, which in turn boosted photosynthetic activity. [31] also noted that *Trichoderma* sp. stimulated metabolism and the activities of stress resistance enzymes. They noted that plant chlorophyll content is a key measure of photosynthetic activity and discovered that *Trichoderma* sp. treated plants had significantly higher levels of chlorophyll, nitrate nitrogen, root activity, total root absorption area, and root specific surface area. In their study, [32] found that the cape gooseberry plants' stomatal conductance, leaf water potential, growth parameters, total chlorophyll, carotenoid and proline contents were all improved after treatment with *Trichoderma* sp. increased relative water content in the leaves and roots, chlorophyll content, and root activity in wheat seeds, according to Zhang [33].

4. References

1. Renna, M.; Durante, M.; Gonnella, M.; Buttarò, D.; D'Imperio, M.; Mita, G. and Serio, F. (2018).
2. Collins, E. J.; Bowyer, C.; Tsouza, A. and Chopra, M. (2022). Tomatoes: an extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. *Biology*, **11**(2): 239.
3. De Curtis, F.; Lima, G.; Vitullo, D. and De Cicco, V. (2010) Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Protection*, **29**: 663- 670.
4. Nirmaladevi, D.; Venkataramana, M.; Srivastava, R. K.; Uppalapati, S. R.; Gupta, V. K.; Yli-Mattila, T.; Clement Tsui, K. M.; Srinivas, C.; Niranjana, S. R. and Chandra, N. S. (2016). Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. *Scientific reports*, **6** (1): 1-14.
5. Akrami, M. and Yousefi, Z. (2015). Biological control of *Fusarium* wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi. In *Biological Forum*. **7** (1): 887. *Research Trend*.
6. . Saad, A. M. A.; Hashem, M.; Mostafa, Y. S.; Nafady, N. A. and Abo-Elyousr, K. A. M. (2019). Biological control of root rot in lettuce caused by *Exserohilum rostratum* and *Fusarium oxysporum* via induction of the defense mechanism. *Biological Control*; **128**: 76–84. *stress in crops. Microbiological research*, 2020. **235**: p. 1-10.
7. Moussa, A. L.; Morsy, E. M.; Shaltout, A. M. A. and Fahmy, S. S. (2007). Efficiency of some bacterial strains for controlling limb rot diseases of peanut in sandy soil. 12th Conference of Microbiology, Cairo, Egypt, March, 18-20.
8. Cook, R. J. (1993). Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology*, **31**, 53-80.
9. Ghazalibiglar, H.; Hampton, J. G.; van ZijldeJong, E. and Holyoake, A. (2016). Evaluation of *Paenibacillus* spp. isolates

- for the biological control of black rot in Brassica oleracea var. capitata (cabbage). *Biocontrol Sci Technol*; **26**:504–515. <https://doi.org/10.1080/09583157.2015.1129052>.
10. Davet, P. and Rouxel, F. (2000). Detection and isolation of soil fungi. Science Publishers, Inc.
 11. Elrazik, A. A. A.; Hassan, M. and Koch, E. (2009). Powder formulations of *Bacillus subtilis*, *Trichoderma* spp and *Coniothyrium minitans* for biocontrol of Onion White Rot. *Arch Phytopathol Plant Protect*, **42**(2):142–147.
 12. Selim, M. E.; Khalifa, E.; Amer, G. A.; Ely-kafrawy, A. A. and El-Gammal, N. A. (2015). Evaluation and characterization of some Egyptian *Fusarium oxysporum* isolates for their virulence on tomato and PCR detection of (SIX) effector genes. *J Bioprocess Biotech*, **05**:1–6.
 13. Shahzad, S. and Ghaffar, A. (1992). Root rot and root knot disease complex of mungbean and its biological control. pp. 349-256. Department of Botany, University of Karachi, Karachi-75270, Pakistan.
 14. Liu, H. Y. and Lu, Y. F. (1995). Chen, W.J.; Predictive equations for basal metabolic rate in Chinese adults: A cross-validation study. *J. Am. Diet. Assoc.*, **95**(12): 1403-1408.
 15. Dijkstra, P. (1989). Cause and effect of differences in specific leaf area, in: H. Lambers, M.L. Cambridge, H. Konings, T.L. Pons (Eds.), *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants*, SPB Academic Publishing, The Hague, 1989, pp. 125–140.
 16. Weatherly, P. E. and Barrs, C. (1962). Are-examination of relative turgidity technique for estimating water deficits in leaves. *Aust. J. Bio. Sci.*, **15**:413-42.
 17. Harborne J. B. (1984). Photochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, London, Pp132.
 18. Bates, L. S.; Waldren, R. P. and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, **39**(1): 205-207.
 19. Gomez, K. A., and A. A. Gomez, (1984). “Statistical Procedures for Agricultural Research”. John Wiley and Sons, Inc., New York, pp:680.
 20. Marzano, M.; Gallo, A. and Altomare, C. (2013). Improvement of biocontrol efficacy of *Trichoderma harzianum* vs. *Fusarium oxysporum* f. sp. *Lycopersici* through UV-induced tolerance to fusaric acid. *Biol control*; **67**: 397-408.
 21. Redda, E. T.; Ma, J.; Mei, J.; Li, M.; Wu, B. and Jiang, X. (2018). Antagonistic potential of different isolates of *Trichoderma* against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*. *European Journal of Experimental Biology*, **8**(2): 1-8.
 22. Tkalenko, H. M.; Borzykh, O. I.; Horal, S. V.; Barvas-Hremiakova, K. M. and Janse, L. A. (2020). Screening new *Trichoderma* isolates for antagonistic activity against several phytopathogenic fungi, including *Fusarium* spp. *Agricultural Science and Practice*, **7**(3): 14-25.
 23. Chao, W. and Zhuang, W. (2019). Evaluating effective *Trichoderma* isolates for biocontrol of *Rhizoctonia solani* causing root rot of *Vigna unguiculata*. *J. Integr. Agric.*, **18**: 2072–2079.
 24. . Sallam, N., Eraky, A. M. and Sallam, A. (2019). Effect of *Trichoderma* spp. on *Fusarium* wilt disease of tomato. *Molecular biology reports*, **46**(4): 4463-4470.
 25. Nwankiti, A. O. and Gwa, V. I. (2018). Evaluation of antagonistic effect of *Trichoderma Harzianum* against *Fusarium oxysporum* causal agent of white Yam (*Dioscorea rotundata* Poire) Tuber Rot Trends Tech Sci Res, **1**(1): TTSR. MS. ID.555554.
 26. Shrinkhala, M.; Bimala, P.; Chetana, M. and Suraj, B. (2019). In-vitro evaluation of biocontrol agents against soil borne plant pathogens. *J Nepal Agricult Res Council*, 5:68 –72.
 27. Ngo, B. H.; Vu, D. N. and Tran, D. Q. (2006). Analyze antagonist effects of *Trichoderma* spp. for controlling southern stem rot caused by *Sclerotium rolfsii* on peanut. *Plant Protection*, **1**:12 –14.

28. Sundramoorthy, S. and Balabasker, P. (2013). Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Applied Microbiology and Biotechnology*, **1(3)**: 36- 40.
29. Hasan, Z. A. E.; Mohd Zainudin, N. A. I.; Aris, A.; Ibrahim, M. H. and Yusof, M. T. (2020). Biocontrol efficacy of *Trichoderma asperellum*-enriched coconut fibre against *Fusarium* wilts of cherry tomato. *Journal of Applied Microbiology*, 129 (4): 991–1003. DOI: <https://doi.org/10.1111/jam.14674>.
30. Harish, S.; Kavino, M.; Kumar, N.; Saravanakumar, D.; Soorianathasundaram, K. and Samiyappan, R. (2008). Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. *Applied Soil Ecology*, **39 (2)**: 187–200. DOI: <https://doi.org/10.1016/j.apsoil.2007.12.006>.
31. Mei, L. I.; Hua, L. I. A. N.; Su, X. L.; Ying, T. I. A. N.; Huang, W. K.; Jie, M. E. I. and Jiang, X. L. (2019). The effects of *Trichoderma* on preventing cucumber *Fusarium* wilt and regulating cucumber physiology. *Journal of Integrative*
32. Chaves-Gomez, J. L.; Chavez-Arias, C. C.; Cotes Prado, A. M.; Gomez-Caro, S. and Restrepo-Diaz, H. (2019). Physiological response of cape gooseberry seedlings to three biological control agents under *Fusarium oxysporum* f. sp. *physali* infection. *Plant Disease*, **104 (2)**: 388–397. DOI: <https://doi.org/10.1094/pdis-03-19-0466-re>.
33. Zhang, F.; Ge, H.; Zhang, F.; Guo, N.; Wang, Y.; Chen, L.; Ji, X. and Li, C. (2016). Biocontrol potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum* in soybean. *Plant Physiology and Biochemistry*, **100**: 64–74. DOI: <https://doi.org/10.1016/j.plaphy.2015.12.017>.