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Effect of some biofertilizers and biofungicides applications on control onion root-rot disease

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Abstract

Five biofertilizers and biofungicides namely, (Cerialien, Biogen, Nitrobein, Phosphoren and Potassiumag) and (Rhizo-N, Bio-Arc, Plant-guard, Biozied and T-34) were used to evaluate their ability to protect onion plants (Giza 6 Mohassan var.) against root rot diseases, which mainly caused by *Fusarium oxysporum* f.sp. *cepae* causing Fusarium basal rot, *Pyrenochaeta terrestris* causing pink root rot and *Sclerotium cepivorum* causing white rot disease as well as improving growth and yield of onion under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. Data clearly showed that the tested biofungicides decreased the disease severity of onion bulb root rot as compared with the check treatment. The treated soil with different biofungicides significantly decreased the disease severity of onion root rot diseases compared with the control. T-34 biocontrol at the rate of 2 and 3 g/kg soil was the most effective biofungicide in minimizing disease severity caused with the tested fungi followed by Biozied and Rhizo-N at the same concentrations during 2019/2020growing seasons. Also, Treated transplants with commercial biofertilizers *i.e.* Cerialien, Biogen, Nitrobein, Phosphoren and Potassiumag at 3 g/Kg soil and planted in infested soil with tested pathogenic fungi caused the highest reduction of the tested pathogenic fungi under greenhouse. As mean treated transplants with Nitrobein gave the greatest reduction of root rot diseases caused by *F. oxysporum* f. sp. *cepae*, *P. terrestris* and *S. cepivorum*, when used under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. Moreover, all these treatments significantly increased growth parameters *i.e.* fresh bulb weight, dry bulb weight and bulb diameter as compared with the check treatment.

Keywords: onion, *Allium cepa*, root rot, biofertilizers, biofungicides.

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1. Introduction

Onion (*Allium cepa* L.) is a species of the alliaceae family, the largest commercial crop in the world and used for food and medicine since ancient times (Marrelli et al. 2019; Yang et al. 2019; Hossain et al. 2017; Khan et al. 2017). China, India, USA, Turkey, Japan, Spain, Brazil, Poland, and Egypt are the largest countries of the world producing onion (FAO, 2018). Egypt's production of onion reached 2.4 million tons (FAO, 2017). In Egypt, Onion to the second important cash crop after rice. The consumption is attributed to several factors, which mainly heavy promotion that links flavor and health and the popularity of onion-rich ethnic foods. The litle production of onion is due to effects of fertilizers, growing unsuitable varieties and different root-rots fungi diseases under the agro climatic conditions of an area. Onion plants are infected with a wide spectrum of fungal diseases as they are 23 diseases as surveyed (Conn et al., 2012). In Egypt, onion plants are most infected with fungal diseases than any other diseases due to climatic conditions which are suitable to infect the plant. In Egypt, Onions suffered from the fungal root-rots the most prevalent and dangerous root disease all over the country and world wide**.** However, yield losses reached 75-80% in case of soil born fungi (Tjamos et al., 2010). This work aimed to study the effect of some bio-fertilizers and biofungicides on control onion root rot disease.

2. Materials and methods

This work was carried out in the Research Laboratory and Farm of Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt.

2.1 Isolation and identification of the causal pathogens

Survey for root-rot diseases were conducted during season 2016/2017 at different localities in Menofeia, Assiut, Sohag and Luxor on onion plants. Samples of diseased plants were taken into the laboratory to isolate the causal pathogens. The infected roots were excised and carefully washed with tap water to remove any adhesive soil. Small segments of the infected roots were superficially sterilized in 70% ethyl alcohol for 2 minutes. Then, the fragments were left to dry on sterilized filter papers then placed on PDA plates and incubated at 27 0c for seven days. The isolated fungi were purified using the single spore or the hyphae tip technique (Dhingra and Sinclair, 1985). The purified fungi were identified according to fungal morphological and microscopical characteristics as described by Barnett and Hunter (1986), Booth (1977) and confirmed by Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut branch), Egypt. The obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further studies. The frequency of the isolated fungi was calculated separately for each of the collected samples. Stock cultures were routinely sub-cultured on fresh slant every month.

2.2 Pathogenicity tests

Pathogenic capability of 40 isolates was carried out on onion transplants (Giza 6 Mohassan cv.) under greenhouse conditions, Faculty of Agriculture, Al-Azhar University, Assiut Egypt during

2016/2017 growing season. Pots (30 cm diameter) were sterilized by immersing in 5 % formalin for 15 minutes, and then left to air dry for 10 days. A mixture of clay soil and sand (1:1 w/w) was also sterilized with the same solution and covered with polyethylene sheet for 15 days, then left uncovered to remove the residual of formalin for 10 days. The pots were filled with the sterilized soil (5 kg/pot).

2.3 Inoculum preparation

The fungal inoculum was grown in 250 ml plastic jars containing the following substrate per jar (75 g grain barley, 25 g coarse sand and 25 ml tap water to cover the mixture in jars). The jars were autoclaved at 121°C for 30 minutes, left to cool, then inoculated with the tested fungi and incubated at 25°C for 15 days to obtain sufficient growth of each fungus. Then, sterilized plastic pots in 5% formalin solution (30 cm in diameter) were filled with sterilized soil (5 kg/ pot). After that, the inoculum was mixed with the soil at the rate of 2% (w/w) of soil, and then irrigated three times a week before transplanting to ensure even distribution and growth of each particular fungus. Other sterilized pots were filled with sterilized soil and un-inoculated with the tested fungi were kept as control. Five transplants were planted in each pot and a set of three replicates were used for each particular treatment. The disease assessment was estimated. Percentages of infection and diseases severity were recorded after 120 days from transplanting date. The arbitrary (0-5) disease index scale as described by Bayraktar et al. (2010) was adopted. Where: 0 = no disease, 1 = 1–20% diseased, 2 = 21–40% diseased, 3 = 41–60% diseased, 4 = 61–80% diseased, and 5 = 81–100% diseased. Disease severity index (DSI) was calculated according to Ichielevich-Auster et al. (1985) as follows:

$$\text{Disease severity index} = \sum (\text{disease severity scale} \times \text{numbers of plants at each scale}) / \text{total number of plants.}$$

2.4 Effect of certain biofertilizers on incidence of root rot disease under greenhouse conditions

The experiment was carried out in winter during 2018/2019 and 2019/2020 growing seasons using five different types of bio-fertilizers (Table 1) as a substitution of chemical fertilization namely; (Cerialien, Biogen, Nitrobein, Phosphoren and Potassiumag). These bio-fertilizers were obtained from the bio-fertilization unit, Agricultural Research Centre Ministry of Agriculture, Giza, Egypt as microorganisms in peat moss carrier substrate. The sterilized pots which used in this experiment were filled with the sterilized soil of clay and sand, (1:1 v/v).

Table 1: Bio-fertilizers used to control root rot diseases of onion.

Bio-fertilizers	Bio- components
Cerialien	*Azosperillium* sp
Biogen	*Azotopacter* sp. + *Azosperillium* sp
Nitrobein	*Azotopacter* sp. + *Azosperillium* sp
Phosphoren	As phosphorus solubilizing bacteria at (10^8 cfu/g) *Bacillus megaterium var. phosphaticum*
Potassiumag	*Bacillus verculanes*, *Bacillus megaterium var. phosphaticum*

The inoculum of *Fusarium oxysporum* f.sp. *cepae*, *Pyrenochaeta terrestris* and *Sclerotium cepivorum* were added at the rate of 2% of soil weight and mixed with the upper surface of the soil, irrigated and left 7 days for fungal growth. Then the fresh preparation of each biofertilizer was mixed separately in the soil at the rates of (1, 2 and 3 g/kg soil). The transplants of onion were prepared and immediately planted in the infested or non-infested soil with the tested fungi (5 transplants/pot). Three pots were used for each treatment. Disease incidence was assessed as the percentages of diseased transplants as mentioned before.

2.5 Effect of commercial biofungicides on controlling root rot disease of onion under greenhouse conditions

Five commercial bio-products, *i.e.* (Bio-Zeid, Plant guard, Bio-Arc, Rhizo-N and T34 Biocontrol). Table (2) which obtained from Organic and Bio-technology (Sadat city, Menofeia, Egypt) under Supervision of Biological Control and Microorganisms Unit, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. While, T34 biocontrol provided by Shoura Agrochemical Company .These biofungicides were used to evaluate their efficiency on controlling root rots of onion. Three concentrations of each tested commercial biofungicide *i.e.* 1, 2 and 3 g/kg soil were used. Plastic pots (30 cm in diameter) were filled with sterilized soil and mixed with the inoculum of the pathogenic fungi *F. oxysporum* f.sp . *cepae* , *P. terrestris* and *S. cepivorum* at the rate 2% (w/w) of clay soil, one week before adding biofungicides and planting, while the pots were filled with sterilized soil and infested with the pathogenic fungi only served as a control. The commercial biofungicides were added and distributed in the infested soil at the time of transplanting. The transplants of onion were prepared and immediately covered in the infested or non-infested soil with the tested fungi (5 transplants /pot). Three pots were used for each treatment. Disease incidence was assessed as the percentages of diseased transplants as mentioned before. Also, the growth parameters were recorded.

Table 2: Biofungicides used as bio-agents against the causal pathogens.

Biofungicides	Bio-agents (density/ml)
Bio-Zied	(*Trichoderma album*),$10x10^6$spores/g
Bio-Arc	(*Bacillus megaterium*) $25x10^6$ cell/g
Plant guard	(*T. harzianum*) $30x10^6$ spores/g
Rhizo-N	(*B. subtilis*) $30x10^6$ cell/g
T34 biocontrol	(*T. asperellum*) $12x10^6$ spores/g

2.6 Statistical analysis

Data collected were subjected to the statistical analysis according to the standard methods recommended by Gomez and Gomez (1984) using the computer program (Costat). The differences between the mean values of various treatments were compared by Fisher’s LSD.

3. Results and Discussion

3.1 Isolation and identification of the associated fungi with onion root rot disease

Different fungal isolates *i.e.* *S. cepivorum*, *F.* oxysporum f.sp. *cepae*, *P.terrestris*, *Botrytis allii*, *Aspergillus niger*, *F. moniliforme*, *F.semitectium*, *F. proliferatum*, *F. verticillioides*, *F. anthophilium* and *Penicillium* sp. were isolated from onion root rots collected from different Localities in Assiut, Sohag, Menofeia and Luxor governorates, Egypt during 2016 and 2017 growing seasons. The fungal isolates were identified by using the morphological features of mycelium and spores as described by Barnet and Hunter (1986) and Booth (1977) and confirmed by Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut branch), Egypt. Data in Table (3) indicated that the highest percentage of occurrence was recorded by *S. cepivorum*, *F. oxysporum* f. sp. *cepae*, *Botrytis allii* and *P. terrestris*. These fungi were the most dominant fungi in all locations as their frequencies were 35, 26.3, 10 and 6.6 % respectively. Also, *Aspergillus niger*, *F. proliferatum* and *F. semitectium* reached it 4 , 3.4 and 3.4 % respectively, followed by *F. anthophilium*, *F. verticillioides* and *F. moniliforme* 3.2 , 3 and 2.9% respectively, while *Penicillium* sp exhibited the lowest ones, 2.2 % of the total count of fungi. These results are in agreement with those obtained by Ali (2015) who mentioned that isolated fungi were, identified as *S. cepivorum*, *A. niger*, *F. oxysporum*, *P. chrysogenum*, *B. allii*, and *Py. terrestris* from onion plants and rhizosphere soil of onion plants from different locations of Assiut governorate.

Table 3: Occurrence and frequency of root-rot fungi isolated from diseased onion plants collected from different governorates, Egypt.

Governorate	Location	Isolated fungi											Total
		F1	F2	F3	F4	F5	F6	SCL	Py	AS	B.a	Pn	
Menofeia	Ashmoun 1	20	5	-	-	-	-	10	5	5	-	-	45
	Ashmoun 2	5	-	-	-	-	5	15	10	-	5	-	40
	Total	25	5	-	-	-	5	25	20	5	5	-	90
	Frequency %	29.4	5.8	-	-	-	5.8	29.4	18	5.8	5.8	-	100
Assiut	AL-Azhar Univ.	10	3	3	3	-	-	10	-	-	6	5	40
	Dronka	40	-	-	-	-	-	5	-	-	3	2	50
	El-Fath	5	-	-	-	-	-	38	-	-	7	-	50
	Vegetable markets	3	2	3	2	٥	-	20	15	15	10	5	80
	Total	58	5	6	5	5	-	73	15	15	26	12	220
	Frequency %	26.3	2.2	2.7	2.2	2.2	-	33.1	7	7	11.9	5.4	100
Sohag	Tahta	5	-	5	5	5	5	15	-	-	5	-	45
	Shandaweel	15	-	-	-	3	2	20	-	-	5	-	45
	El Munsha	15	3	-	-	-	-	20	-	5	-	-	43
	Total	35	3	5	5	8	7	55	-	5	10	-	133
	Frequency %	26.3	2.2	3.8	3.8	6	5.3	41.3	-	3.8	7.5	-	100
Luxor	Armant	10	-	5	5	5	5	10	-	-	5	-	45
	Isna	5	5	-	-	-	-	20	-	-	6	-	36
	Total	15	5	5	5	5	5	30	-	-	11	-	81
	Frequency %	18.5	6.2	6.2	6.2	6.2	6.2	37	-	-	13.5	-	100
Total		138	18	16	15	18	17	183	35	20	52	12	524
Frequency %		26.3	3.4	3	2.9	3.4	3.2	35	6.6	4	10	2.2	100

F1: *F. oxysporum* f.sp. *cepae*, F2: *F. proliferatum*, F3: *F. verticillioides*, F4: *F. moniliforme*, F5: *F. semitectium*, F6: *F. anthophilium*, SCL: *S. cepivorum*, Py: *Py. Terrestris*, AS: *Aspergillus niger*, Pn: *Penicillium* sp., B.a: *Botrytis allii*.

The fungus *F. oxysporum* showed the highest frequency, mean frequency rate, was 31% followed by *S. cepivorum* (21%) and *A. niger* (20%). While, *P. terrestris* had the lowest frequency, mean frequency rate was 5%, followed by *P. chrysogenum,* and *B. allii*. The occurrence and frequency of the isolated fungi were differed from one location to another. These differences are probably due to the environmental conditions such as moisture, temperature and soil type, dissemination factors of fungi in different locations and agricultural practices. The isolated fungi were purified, identified and the most frequently isolated fungi *i.e. S. cepivorum*, *F. oxysporum* f.sp. *cepae, P.terrestris*, *Botrytis allii* and *Aspergillus niger* were used for further studies.

3.2 Pathogenicity tests

Forty fungal isolates were tested to study their pathogenic capabilities on onion plants (Giza 6 Mohassan cv.) under greenhouse conditions during 2016/2017 growing season. Data presented in Table (4) illustrated that all tested fungal isolates were able to infect onion plants caused root rot diseases with different degrees of disease severity. Data showed that *F. oxysporum* f.sp. *cepae* (No. 4) gave the highest percentage of disease severity, followed by *P. terrestris* (No. 24) and *S. cepivorum* (No. 15) These isolates were the most virulent among all the tested isolates. In which, they recorded (95.8, 95.7 and 93.4%) disease severity, respectively. According to obtained data, *F. oxysporum* f. sp. *cepae* (No. 4), *P. terrestris* (No. 24) and *S. cepivorum* (No. 15) were selected for further studies under lab and greenhouse conditions. These results are in harmony with those reported by Mahdy et al. (2018) who found that inoculating onion bulbs (Giza 20 cv.) with 14 isolates of *Fusarium* indicated that the fourteen tested isolates were pathogenic to onion plants.

Table 4: Pathogenicity tests of 40 fungal isolates on onion plants (Giza 6 Mohassan cv.) under greenhouse conditions during 2016 growing season.

Isolate number	Fungal isolate	Disease Severity (%)	Isolate number	Fungal isolate	Disease Severity (%)
1	*F. oxysporum*	30	21	*Py.terrestris*	35.1
2	*F. oxysporum*	43.3	22	*Py.terrestris*	86.9
3	*F. oxysporum*	41.6	23	*Py.terrestris*	88.2
4	*F. oxysporum*	95.8	24	*Py.terrestris*	95.7
5	*F. oxysporum*	20.2	25	*Py.terrestris*	19.6
6	*F. oxysporum*	24.8	26	*Py.terrestris*	37.1
7	*F. oxysporum*	53.5	27	*Py.terrestris*	37.1
8	*F. oxysporum*	86.2	28	*Py.terrestris*	56.1
9	*F. oxysporum*	31.6	39	*Py.terrestris*	75.1
10	*F. oxysporum*	24.1	30	*Py.terrestris*	12.1
11	*S. cepivorum*	48.5	31	*B. allii*	10.8
12	*S. cepivorum*	84.5	32	*B. allii*	64.5
13	*S. cepivorum*	89.8	33	*B. allii*	46.5
14	*S. cepivorum*	38.8	34	*B. allii*	32.4
15	*S. cepivorum*	93.4	35	*B. allii*	54.4
16	*S. cepivorum*	23.6	36	*A. niger*	12.8
17	*S. cepivorum*	19.7	37	*A. niger*	12.8
18	*S. cepivorum*	90.4	38	*A. niger*	33.4
19	*S. cepivorum*	32.2	39	*A. niger*	45
20	*S. cepivorum*	74.5	40	*A. niger*	18.9
Un-infested soil		0			
LSD at 0.05		3.86			

As for virulence of each one of the isolates on bulbs and seedlings of onion, *F. oxysporum* caused severe basal rot and damping-off as a highly virulent species were also confirmed by Shalaby et al. (2013) who mentioned that screening trials of the fields infested with onion white rot disease resulted in eight isolates of *S. cepivorum* and pathogenicity of these isolates showed varied degrees against onion transplants Giza 20 cv., ranging from the most aggressive (100%) in case of isolate Sc2 to the lowest degree (75%) for isolate Sc1. Strong pathogenic variations between *S. cepivorum* isolates. Kafi (2009) who showed that evaluate the influence of the inoculum sources with *Pyrenochaeta terrestris* inoculum (transplants and soil) resulted in 100% infection and significantly reduced the root development, leaf growth and bulb weight of cv. Kamlin yellow when assessed two months after transplanting and at maturity.

3.3 Effect of different biofertilizers on incidence of root rot of onion plants under greenhouse conditions

Different biofertilizers *i.e.* (Cerialien, Biogen, Nitrobein, Phosphoren and Potassiumag) with different concentrations (1, 2 and 3 g/kg soil) were evaluated to study theis effectivenes on incidence of onion root rot diseases caused by *F. oxysporum* f.sp. *cepae*, *P. terrestris* and *S. cepivorum* were tested under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. It was clear from data in Table (5) that all the tested biofertilizers were effective in reducing the disease severity of onion root rot disease. The disease severity decreased as biofertilizers concentration increased. The treated soil with different biofertilizers significantly decreased the disease severity of onion root rot diseases compared with the control. Nitrobein, followed by Phosphoren with all tested concentrations (1, 2 and 3 g/kg soil) gave the highest effect in minimizing the disease severity caused by *F. oxysporum* f.sp. *cepae.* Also, Biogen at 2 and 3 g/Kg soil revealed higher effects in decreasing disease severity caused with the same fungus. Meanwhile, Nitrobein at 3 g/kg soil was the most effective biofertilizer in minimizing disease severity, followed by Phosphoren with the same concentration during 2019/2020 growing season. On the other hand, Nitrobein, followed by Phosphoren each at 2 and 3 g/kg soil were the best biofertilizers, which reduced disease severity caused with *S. cepivorum*. Also, Biogen and Cerialien at 3 g/kg soil revealed the higher effect in reducing disease severity, while Potassiumag was less effective in controlling root rot diseases caused by *S. cepivorum.* It was shown from the same Table that Nitrobein, Biogen and Phosphoren with all tested concentrations were the most effective biofertilizers in reducing disease severity caused by *P. Terrestris*, when applied as soil treatment. Also, Potassiumag 3 g/kg soil revealed the highe effect in reducing disease severity, while Cerialien came in the last with the same concentrations. Also, data revealed that the bio-fertilizers reduced disease incidence caused by the tested fungi. Under greenhouse conditions, Nitrobein, Phosphoren and Biogen was more effective on the tested fungi. In this respect, Hassouna et al. (1998), Bhardwaj et al. (2014) and Dhir (2017) stated that, *Azotobacter* *brasilensis* and *A. chrococcum* were very effective against the infection with *R.solani* and *F. oxysporum*. Phosphoren was effective than Microbin in reducing pod of peanut. This effect was attributed to the decrease of population density in the rhizosphere (Zeidan, 2000). The same trend was recorded by Emara (2005) using Rhizobacterin and Phosphoren. Also, Brown (2012) and Zaghloul et al. (2007) observed that *Azotobacter* besides the N-fixation was able to produce growth substances and fungal antibiotics, the response of the crops to the inoculation could be attributed to the substances produced by the organisms. Also, Chung and Wu (2000) recorded the efficiency of *Bacillus megaterium* var. *phosphaaticum* to control root-rot caused by *R.solani* and the mycelia growth was generally reduced, where some isolates were able to cause a significant reduction in the damping-off of the plants. Also, Potassiumag containing *Bacillus verculanes* was suppressive compared with the control.

Table 5: Effect of using different biofertilizers on controlling bulb rot of onion diseases under greenhouse conditions during 2019/2020 growing seasons.

Biofertilizer (A)	Rate of application (g/kg soil) (B)	Disease severity (%)					
		F. oxysporum		*Py. Terrestris*		*S. cepivorum*	
		2019	2020	2019	2020	2019	2020
Cerialien	1	38.5	36.2	34.8	32.4	65.5	62.4
	2	30.6	31.1	29.9	30.4	53.7	54.8
	3	23.3	24	19.6	19.2	27	25.5
Biogen	1	27.4	30.2	15.8	14.2	35.2	33.8
	2	22.1	24.3	10.3	9.6	32.2	31.1
	3	12.9	11.5	8.1	7.8	22.8	25.4
Nitrobein	1	18.5	16.2	11.8	14.4	26.6	28.4
	2	11.4	10.4	12.7	10.7	19.6	19.2
	3	4.6	5	3.5	3.1	15.1	14
Phosphoren	1	22.9	23.1	18.1	17.7	36.5	34.5
	2	18.5	19.5	14.5	15	24.1	26.1
	3	5.2	5.9	8.5	9.2	17	17.8
Potassiumag	1	35.7	37.6	32.8	28.7	72.1	71.1
	2	32.2	34.6	24.8	27.6	67.8	64.2
	3	19	21.4	10	9.8	59	47.3
Check		99.65	99.99	94.55	92.14	100	100
L.S.D. at 5%	A	2.39	0.82	2.23	2.88	2.70	2.78
	B	0.59	0.98	0.98	1.63	2.59	1.12
	A×B	1.44	4.88	2.40	3.99	6.35	2. 74

3.4 Effect of biofertilizers on growth parameters

The effect of soil treatments with different biofertilizers *i.e.* (Cerialien, Biogen, Nitrobein, Phosphoren and Potassiumag) with different concentrations (1, 2 and 3 g/kg soil) on the growth parameters in infected soil during 2019 and 2020 growing seasons was studied. Data shown in Table (6) that the pathogens infested potted plantlets treated with different biofertilizers showed significant differences in terms of Fresh bulb weight, dry bulb weight and bulb diameter than untreated plantlets. The maximum Fresh bulb weight, dry bulb weight and bulb diameter was recorded using Nitrobein, Phosphoren and Potassiumag at concentration 3 g/kg soil. From data presented in the same Table, it could be noticed that these treatments also promoted the growth parameters of onion plants in infested soil with *F. oxysporum* f.sp. *cepae* as compared with the control treatment during 2019 and 2020 growing seasons. In this respect, Nitrobein followed by Phosphoren and Potassiumag proved to be the most effective in increasing Fresh bulb weight, dry bulb weight and bulb diameter as compared with other treatments and control. As for, Biogen with all concentrations tested were found to be the most effective in increasing the bulb diameter more than other biofertilizers in infested soil with *F. oxysporum* f.sp. *cepae* during 2019 and 2020 growing seasons. The same data Table (6) showed that the highest dosage of all biofertilizers exhibited higher increasing of all growth parameters in infested soil with *P. terrestris* as compared to their lower dosages and the check treatment. Nitrobein, Biogen and Phosphoren recorded higher increasing of Fresh bulb weight, dry bulb weight and bulb diameter more than other biofertilizers in infested soil with *P. terrestris*. The lowest growth parameters were observed in infested soil and untreated with any biofertilizers during 2019 and 2020 growing seasons. Regarding the effect of biofertilizers on growth parameters in infested soil with *S. cepivorum*, highest fresh bulb weight, dry bulb weight and bulb diameter has been observed in treated soil with Phosphoren, Nitrobein and Biogen with all concentrations tested. However, Intermediate increases in Fresh bulb weight, dry bulb weight and bulb diameter were obtained with Cerialien and Potassiumag at concentration 3 g/Kg soil as compared with other biofertilizers in infested soil with *S. cepivorum* and untreated ones during 2019 and 2020 growing seasons.

Table 6: Effect of different concentrations of biofertilizers on Fresh bulb weight , dry bulb weight and bulb diameter of bulb rot of onion Giza 6 Mohassan, cv. under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons.

Biofertilizer (A)	Rate of pplication (g/kg soil) (B)	2018/2019									2019/2020								
		F. oxysporum			*Py. Terrestris*			*S. cepivorum*			*F. oxysporum*			*Py. Terrestris*			*S. cepivorum*		
		Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter
Cerealein	1	22.1	10.3	4.8	31.4	16.2	5.1	37	19.3	5.18	23.3	10.4	4.6	31.1	16.4	5.1	32.3	15.8	5.1
	2	31.4	16.2	5.9	47.2	27	5.9	39.7	24.5	6.9	36.8	14.1	6	35.2	25.9	6.2	36.7	23.3	6
	3	34.7	16.5	6.3	48.8	37	6.8	49.7	32.3	8.4	35.2	25.9	6.2	50	37	6.6	48.9	33	7.4
Biogeain	1	40.5	24.2	9.1	40.5	23.5	6.6	36.8	23.5	5.1	39.2	23.2	8.8	46.6	28.1	6.7	39.2	24.3	6.2
	2	54.8	34.6	9.7	53.8	36.9	6.9	45.1	24.5	6.6	53.3	36.3	10.3	53.3	37.3	7	42.1	28.1	6.6
	3	80.1	54.3	11.9	83.9	47.1	9.1	64.2	38.7	9.1	78.7	54	11.6	82.1	46.2	8.8	63	46.2	9.5
Nitrobein	1	53.8	47.2	6.6	50.7	24.8	6.8	40.4	28.5	6.3	50.5	43.8	7.5	48.8	34.7	7.5	39.7	27	6.4
	2	83.9	53.2	9.1	54.8	46.9	7	63.1	39.1	8.7	82.3	57.7	7.5	55.2	43.8	7.7	62.5	41.9	8.8
	3	102	73.3	10.4	96.1	73.1	10.6	65	44.5	10.3	100	77.1	10.6	98.5	76.1	11.2	65.2	46.2	10.6
Phosphoren	1	36.8	33.5	7.7	38.3	23.2	5.9	54.8	36.9	6.6	48.8	27	6.6	41.7	26.8	6.5	55.3	36.2	6.6
	2	65.9	47.1	9.1	51.8	36.2	6.3	83.9	47.1	9.1	64.8	46.2	9.5	48.8	33.8	6.6	82.1	46.2	9.5
	3	97.3	71.7	10.3	63.1	39.1	8.7	97.3	71.7	10.7	99.9	73.9	10.4	62.5	41.9	9.5	95.7	76.9	10.9
Potassiumag	1	45	24.8	6.6	36	22.3	5.3	34.6	16.8	4.8	41.6	26.8	6.7	36.9	24.4	5.4	33.1	17.7	4.8
	2	63.8	37.3	8.8	39.9	24.8	6.25	38	23	6	60	33	6.6	37	26.8	6.4	38.3	23.3	6.7
	3	83.7	57.1	9.1	54.8	38	7.7	47.1	30.2	7.7	82.3	56.2	9.5	53.3	39.3	6.9	37.2	32.5	6.4
Check		16.9	10.3	3.7	10.3	5.2	4	5.9	4.4	2.9	16.9	9.22	4.03	14.1	5.8	3.6	10.1	3.6	2.2
.S.D. at 5%	A	3.94	2.02	1.46	3.07	2.55	1.45	5.59	3.55	1.81	2.53	2.21	1.46	2.89	1.53	1.51	7.69	3.69	2.16
	B	2.59	1.35	0.94	4.09	2.21	1.58	1.54	1.20	1.52	2.11	1.66	1.14	2.63	2.42	1.03	2.99	1.87	1.64
	A×B	6.35	3.30	2.31	8.95	6.45	3.49	3.78	2.94	3.73	5.17	4.07	2.79	7.69	7.06	3.02	7.32	4.58	4.02

Such results are in agreement with those reported by Arfaoui et al. (2006), El-Mohamady and Ahmed (2009) and Zeidan et al. (2012). The enhancement of plant growth parameters and yield components might be due to ability of biofertilizers to provide plant by nutritional requirements and plant growth regulators and vitamins secured. Isolates of *Azotobacter* and *Azospirillum* produce IAA and possessed phosphorus solublization capability of *R. solani* growth as well as production, ACC deaminase, siderophore, salicylic acid, hydrogen cyanide, cellulase, chitinase and α-1,3-glucanase (Zarrin et al., 2009).

3.5 Effect of commercial biofungicides on control onion root rot diseases under greenhouse conditions

The efficacy of Rhizo-N, Bio-Arc, Plant-guard, Biozied and T-34 as biofungicides on controlling root rot disease of onion transplants, Giza 6 mohassan cv. was studied under greenhouse conditions during 2019 and 2020 growing seasons. Data presented in Table (7) indicated that all the tested biofungicides were effective in reducing the disease severity of onion root rot diseases. The disease severity decreased as biofungicidal concentration increased. The treated soil with different biofungicides significantly decreased the disease severity of onion root rot diseases compared with the control. T-34 biocontrol at the rate of 2 and 3 g/kg soil was the most effective biofungicide in minimizing disease severity caused with *F. oxysporum* f.sp. *cepae*, *P. terrestris* and *S. cepivorum*, followed by Biozied and Rhizo-N with the same concentration during 2019 and 2020 growing seasons. These findings were in agreement with those previously obtained by Abou-Zied et al. (2016) and El-Naggar et al. (2018) reported that biocides *i.e.* Biozied, BioArc, Plantguard and Rhizo N were significantly effective in controlling root rot disease incidence.

Table 7: Effect of different biofungicides on control onion root rot diseases under greenhouse conditions during 2019/2020 growing seasons.

Biofungicide (A)	Rate of application (g/kg soil) (B)	Disease severity %					
		F. oxysporum		*Py. Terrestris*		*S. cepivorum*	
		2019	2020	2019	2020	2019	2020
Rhizo-N	1	23.8	23.6	17.6	19.5	36.5	36
	2	13.8	13.6	9.6	8.9	19.8	19.5
	3	5.2	5.9	3.3	4.4	10.7	11.6
Bio-Arc	1	27.4	30.2	15.8	14.5	39.9	38.1
	2	16.6	15.2	12.8	11.5	25.7	20.5
	3	10.1	10.7	6.5	7.6	13.7	10.7
Plant-guard	1	24.2	24.6	14	15.4	40.7	42.5
	2	18.8	19.5	13	13.8	32.2	33.8
	3	10.3	11.4	7.4	9.2	15.1	14
Biozied	1	13.8	14.1	9.6	9.2	20.7	21.3
	2	9.6	10.4	7.8	8.5	18.8	19.4
	3	4.8	5	2.9	3.1	8.1	7
T-34 biocontrol	1	10.7	9.9	4.8	3.3	13.4	12.2
	2	5.1	4.3	3.7	3.1	7.8	7.8
	3	2.2	2.4	1.1	2	6	5.8
Check		99.6	99.9	94.5	92.1	100	100
L.S.D. at 5%	B	1.95	1.3	2.49	2.41	2.07	2.9
	R	0.98	0.81	0.94	1.27	2.03	1.34
	B×R	2.41	1.98	2.31	3.13	4.9	3.3

Recently, chemical control is faced with many difficulties especially what concerned with their efficacy, selectivity, toxicity and general impact on the environment (Burrows et al., 2007; Nawar, 2005). As well as the harmful side effects of the fungicides on human and environment led to searching new means or bio-agents with low toxicity and side effects that can effectively replace the fungicides in controlling plant diseases. Therefore, Bio-Arc (Bacillus megaterium), Bio-Zied (Trichoderma album) as biocides play a very useful role as effective and safe means in controlling root-rots. In this respect, similar results were obtained by Chavan et al. (2004) on the positive efficacy of treating with bio-agents, i.e. several strains of bacterial bio-agents including *Bacillus megaterium* and also by *Trichoderma* spp., to control damping-off of safflower.

3.6 Effect of biofungicides on growth parameters

The effect of soil treatments with different biofungicides *i.e.* Rhizo-N, Bio-Arc, Plant guard, Biozied and T34 biocontrol with 3 rates on the growth parameters in infected soil during 2019 and 2020 growing seasons was studied. Data in Table (8) illustrated the effect of different treatments on the Fresh bulb weight, dry bulb weight and bulb diameter of Giza 6 mohassan cv. as compared with the control treatment. All the biofungicides at three different concentrations showed the highest increase of all growth parameters as compared with the control treatment during 2019/2020 growing seasons. The obtained results proved that all biofungicides with all concentrations tested significantly increased the growth parameters as compared to control. In this concern, T34 biocontrol followed by Biozied, Rhizo-N, Bio-Arc and Plant-guard each at 3 g/ kg soil were found to be the most effective in increase of all growth parameters of onion transplants in infested soil with *F.oxysporum* f.sp. *cepae*, *P. terrestris* and *S. cepivorum* as compared with the control treatment.

Table 8: Effect of biofungicides on Fresh bulb weight, dry bulb weight and bulb diameter of onion plants Giza 6 Mohassan cv. under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons.

Biofungicide (A)	Rate of pplication (g/kg soil) (B)	2018/2019									2019/2020								
		F. oxysporum			*Py. Terrestris*			*S. cepivorum*			*F. oxysporum*			*Py. Terrestris*			*S. cepivorum*		
		Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter	Fresh Bulb weight	Dry Bulb weight	Bulb diameter
Rhizo-N	1	53.8	47.2	6.7	38	23.5	5.9	40.4	28.5	5.2	48.8	43.8	6.7	41.8	24.4	5.4	42.1	28.1	6.2
	2	67	49.2	9.1	59.3	36.9	8.2	45.1	34.5	6.9	62.3	46.2	9.5	67	36.8	8.9	49.7	35.3	6.9
	3	83.9	74.1	10.3	74.8	39.1	8.9	64.2	38.7	9.1	82.3	48.6	10.6	73.3	39.3	9.4	63	44.2	9.5
Bio-Arc	1	43.8	27.7	6.6	36.8	23.3	5.3	37	19.3	5.18	39.2	27.8	6.6	41.6	28.1	5.1	32.2	17.7	5.1
	2	57.2	36.9	7.7	54.8	34.8	7.3	39.7	30	6.6	42.2	37.6	6.6	55.3	36.2	6.7	39.3	34.3	6.6
	3	74.4	42.9	9.1	63.1	38	8.6	49.7	32.3	8.4	73.5	46.2	9.5	52.5	31.9	8.8	49.2	37.5	8.4
Plant-guard	1	43.6	23.2	4.9	34.6	16.8	4.81	34.6	16.8	4.8	53.8	27	4.8	33.1	17.7	4.8	33.1	15.8	4.8
	2	52.1	36.2	6	38	24.5	6.9	38	27.5	6	24	37	6.05	38.3	23.2	6.6	36.7	33.3	6.7
	3	58.1	40.4	6.9	47.1	30.2	8.4	47.1	30.2	7.7	61.4	38.2	7.7	47.2	33	7.4	48.9	33	7.4
Biozied	1	66.8	53.5	8.7	50.3	40.2	6.6	54.8	36.9	6.3	60.5	53.2	8.8	45.9	36.8	6.7	55.3	36.2	6.6
	2	83.9	57.1	11	63.8	47.2	8.7	63.1	39.1	8.7	80.8	56.3	10.3	69.2	37	9.6	62.5	41.9	8.8
	3	87.3	81.7	11.2	83.7	47.1	9.1	64	44.5	10.1	82.4	76.9	11.4	82.3	46.2	9.5	64.2	46.2	9.6
T-34	1	70.5	54.8	9.4	41.8	32.3	7.4	66.8	23.5	6.6	68.6	56.8	9.5	48.8	43.8	9.7	69.2	37	7.6
	2	84.8	67.3	10.7	84.8	57.3	9.7	73.9	47.1	9.1	83.3	67.7	10.5	83.3	57.7	9.9	82.3	46.2	9.5
	3	95.5	86.3	11.4	102	77.6	10.9	90.3	71.7	10.3	93.3	80.1	11.5	99.8	76.1	11.2	88.8	76.9	10.6
Check		16.9	16.9	10.3	3.7	10.3	5.2	4	5.9	4.4	2.9	16.9	9.2	4	14.1	5.8	3.6	10.1	4.3
.S.D. at 5%	A	3.66	2.94	1.49	2.52	3.19	1.1	5.59	3.55	1.86	2.94	2	1.52	3.22	1.83	1.31	7.69	3.69	2.16
	B	1.45	1.43	1.05	1.66	1.84	0.91	2	1.8	0.98	1.98	1.53	0.89	2.13	1.8	0.83	2.15	1.78	0.85
	A×B	4.52	3.52	2.58	4.06	4.5	2.24	4.9	4.41	2.41	4.85	3.75	2.19	5.22	4.41	2.04	5.28	4.36	2.1

These findings were in agreement with those previously obtained by Abou-Zied et al. (2016) and El-Naggar et al. (2018). reported that biocides *i.e.* Biozied, BioArc, Plantguard, Rhizo N and clean root that reflect on the increase in plant height, bulb diameter, bulb weight and total bulb yield compared with untreated after 90 days from sowing. Plant growth promoting Rhizobacteria can promote plant growth and development either directly and indirectly. Direct stimulation includes biological nitrogen fixation, producing phytohormones like auxins, cytokinines and gibberellins, solubilizing minerals like phosphorus and iron, production of enzyme and induction of systemic resistance. While, indirect stimulation is basically related to biocontrol, including antibiotic production chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for niches within rhizosphere (Castro et al., 2009; Vanloon, 2007; Zahir et al., 2004). Generally, growth promotion resulted by the biocontrol agents which may be due to antagonistic fungi and bacteria in plant root zone.Root zone antagonistic fungi and bacteria are able to generate a wide array of secondary metabolites which can have a positive influence on plant growth, enhancing the availability of minerals nutrients, improving nitrogen fixation, decreasing susceptibility to frost damage, improving plant health through the biocontrol of phytopathogens, inducing systemic plant resistance and facilitating plant establishment growth and development.

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