

EFFECT OF BIOCONTROL AGENTS ON DAMPING-OFF AND ROOT-ROT DISEASES OF QUINOA (*CHENOPODIUM QUINOA* WILLD) SEEDLINGS

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Quinoa, recently introduced to Egypt and is considered as an important crop as it can grow and give considerable yield in new reclaimed salt-affected soils. Isolation trials from rotted quinoa seedlings collected in Egypt (Fayoum governorate) yielded *Fusarium solani*, *Alternaria* sp., *Acremonium* sp. and *F. oxysporum*. All the isolated fungi were pathogenic and caused damping-off and root-rot diseases. The efficiency of *Streptomyces* strains as a biocontrol agent against the pathogenic fungi was investigated under laboratory and greenhouse conditions. *Streptomyces toxytricini* showed the highest inhibition zone of the three strongest pathogenic fungi. *S. toxytricini* produced indole acetic acid (IAA) and exhibited optimum phosphate solubilization. *Streptomyces* strains mixtures reduced damping-off incidence, root-rot incidence and severity as well as significantly increased the number of viable seedlings under greenhouse conditions. Increase of phenolic content as well as activity of peroxidase and chitinase enzymes in *Streptomyces*-treated plants proved the biocontrol-induced systemic resistance in quinoa seedlings against a mixture of three pathogenic fungi; *F. solani*, *Acremonium* sp. and *F. oxysporum*.

Keywords: *Fusarium solani*, *Acremonium* sp., *F. oxysporum*, actinomycetes, quinoa seedlings

Quinoa (*Chenopodium quinoa* Willd), a plant belonging to family Amaranthaceae, has been cultivated in the Andean region in South America for thousands of years. Quinoa grown primarily for its edible seeds, has been introduced elsewhere as a source of starch and high-quality proteins. Because quinoa has a high nutritive value and desirable agricultural traits as tolerance to drought and salinity, it has gained increase demand recent years in Egypt under various stress conditions (Barakat et al., 2017; Eisa et al., 2017 and Mahmoud, 2017).

Since quinoa has been introduced to Egypt a short time ago; there is little information about their associated diseases under Egyptian conditions. Downy mildew caused by the pathogen *Peronospora farinosa* f. sp. *chenopodii* is the most widespread and potentially destructive global disease of quinoa (Danielsen and Munk, 2004). There is scant knowledge about other quinoa pathogens that infect seeds or seedlings and lead to decreased crop production. These diseases are considered potential production constraints, particularly when the crop is introduced in areas outside its traditional growing regions (Danielsen et al., 2003). Root-rot and damping-off diseases for young quinoa seedling have been detected in five different Egyptian governorates (Giza, Fayium, Ismailia, Beheira and Monufia) (El-Moity et al., 2015). Damping-off is a disease that leads to the decay of germinating seeds and young seedlings, representing one of the most important yield constraints both in nurseries and fields (Lamichhane et al., 2017). *Ascochyta caulina*, *Fusarium avenaceum*, *Fusarium* spp., *Alternaria* spp. and *Pythium* spp. were isolated from infected parts of quinoa seedlings and were proved that, they were the causal agents of damping-off in greenhouse experiments (Dřímalková and Veverka, 2004).

Plant disease management is done mainly by means of chemicals. Various chemical pesticides have been reported to be effective against a wide range of pathogens, but not considered as long-term solution because of concerns about health and environmental hazards, expensiveness, residue persistence, pest resurgence, and elimination of natural enemies. Therefore, the need for alternative method has become vital. The development of biological control for plant diseases is accepted as a durable and eco-friendly alternative for agrochemicals. Actinomycetes are one of the most promising sources of biocontrol agents at present gaining increased attention in the field of biological control. The secondary metabolites produced by actinomycetes play a vital role in plant growth promotion as well as suppression of pathogen growth and development in host plant (Senthilraja, 2016 and Olanrewaju and Babalola, 2019). The main aims of this study were, therefore, the identification of pathogenic fungi causing damping-off disease of quinoa seedlings, as well as seedling protection with bioagents as an ecofriendly disease management.

MATERIALS AND METHODS

1. Source of Quinoa Seed

Quinoa var. Real seeds were obtained from the quinoa project of Plant Department, Faculty of Agriculture, Ain Shams University, Egypt and Migual Lillo Institute, Argentina.

2. Isolation of Fungi Causing Damping-off Disease

Quinoa seedlings showing the damping-off disease symptoms were collected from quinoa field under reclaimed soil conditions at Wady El-Rayan, Fayoum governorate. The diseased seedlings were taken in plastic bags, brought to the laboratory for isolating the disease-causing fungi. Isolation was done from 100 small root pieces, cut from adjoining areas of diseased and healthy areas of the plants. Root pieces were washed under tap water for about 30 min to remove any dirt or soil particle. The root pieces were dipped in 0.5% NaCl for about 2 min and then passed from two washes of distilled sterile water for 2-3 min each. The treated root pieces were dried completely and then transferred to Petri dishes containing sterilized potato-dextrose agar (PDA) medium with five pieces per plate. All the plates were kept at 25±1°C for 7 days. Fresh growth of the fungi was transferred to freshly prepared PDA medium for sub culturing. The growth was sub-cultured/multiplied whenever needed during the entire study. Isolated fungi were identified by studying their typical mycelial growth produced on the PDA medium and conidial morphology using standard diagnostic keys. *Fusarium* sp. and *Acremonium* sp. (*Cephalosporium*) were frequently isolated and identified according to Booth (1971), Gams (1975) and Domsch et al. (2007), respectively. Purified isolates were maintained on PDA medium at 4°C for further studies.

3. Pathogenicity Test

The pathogenicity test for most predominant isolated fungi was done. Inoculum of each fungal isolate was prepared using barley medium in polyethylene bags, each containing 200 g medium as described by Singleton et al. (1992). Each fungal inoculum or mixture of them was mixed with 1 kg of steam sterilized sandy loam soil in plastic pots. Twenty quinoa seeds were sown per pot containing infested soil. Number of healthy and infected plants were recorded after 15 and 30 days from sowing from pots containing infested and un-infested soil.

4. Source of Streptomyces Strains and Inoculum Preparation

Four of *Streptomyces* sp. strains (21SA, 25SA, 26SA and 28SI) were obtained from Microbiology Department, Agriculture Faculty, Cairo University as well as one strain of *Streptomyces toxytricini*, which showed antibacterial potent in a previous study (Hassan et al., 2018).

5. Evaluation of Streptomyces Strains *In Vitro*

5.1. As antifungal activity

The five strains of *Streptomyces* were evaluated as antifungal against *Fusarium solani*, *F. oxysporum*, and *Acremonium* sp. *in vitro*. The strains were streaked on one side of a Petri dish (1 cm from the edge of the plate) with PDA medium. Eight mm diameter mycelial disc of *F. solani*, *F.*

oxysporum and *Acremonium* sp. seven-days-old culture were placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran et al., 1997). The plates were incubated at room temperature (28°C) for 4 days and the zone of inhibition was measured. Growth inhibition percentage was calculated using the formula:

$$n = (a - b)/a \times 100$$

where 'n' is the growth inhibition percentage, 'a' is the fungal growth radius of a control culture (in cm) and 'b' is the distance of the fungal growth in the direction of actinomyces (in cm). For each isolate, three replicates (plates) were maintained.

5.2. *In vitro* plant growth-promoting properties of potent *Streptomyces* sp.

Streptomyces strains were grown in flasks (250 ml) containing 100 ml of glycerol nitrate agar media broth, for 48 h on a rotary shaker at 28±2°C. Cells were removed by centrifugation at 800 g for 10 min at 4°C and washed in sterile water. The pellet was transferred in a small amount of sterile distilled water and then diluted with an adequate amount of sterile distilled water to obtain a bacterial suspension of 10⁸ cfu ml⁻¹ (Thompson, 1996). For bacterization of quinoa seeds were surface sterilized with 2% sodium hypochlorite solution for 30 s and rinsed in sterile distilled water and dried overnight under a sterile air steam. Ten milliliters of bacterial inoculum (10⁸ cfu ml⁻¹) were put in a Petri dish. Then, 100 mg of carboxymethylcellulose was added as adhesive material. One gram of quinoa seeds was soaked in 10 ml of bacterial suspension for 12 h and dried overnight in sterile Petri dish. Plant growth-promoting activity of bacterial isolates was assessed based on the seedling vigor index by the standard roll towel method (ISTA, 1993). The vigor index was calculated by using the formula as described by Abdul Baki and Anderson (1973):

$$\text{Vigor index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination (\%)}$$

6. Indole Acetic Acid Production

The five strains were investigated for their ability to produce indole acetic acid (IAA). Each isolate was grown on glycerol nitrate agar broth medium containing tryptophan (1.0 mg/L) and incubated in a shaker at 30°C and 160 rpm for 48 h. Next, bacterial culture was centrifuged at 10000 rpm for 15 min, and 1 ml of culture filtrate was mixed with 1 ml of Salkowskis reagent (1.5 ml of FeCl₃.6H₂O 0.5 M solution, in 80 ml of 60% H₂SO₄) and the mixture was incubated at room temperature for 30 min, presence of pink color indicates that isolate can produce IAA. Meanwhile, IAA concentration for each tested strain was quantified spectrophotometrically at 550 nm by comparing with IAA standard curve (Gordon and Weler, 1951).

7. Phosphate Solubilization

Capacity of five strains to solubilize phosphate in the form of

calcium phosphate was checked qualitatively by using glucose yeast extract agar (GYA) medium containing per 1 L distilled water; 10 g glucose; 2 g yeast extract and 15 g agar. In addition, two other solutions were prepared separately; first 5 g K_2HPO_4 was dissolved in 50 ml distilled water and second 10 g $CaCl_2$ in 100 ml distilled water. These two solutions were added to 1 L CYA just before pouring medium to plates (Beneduzi et al., 2008). Each isolate was grown in GY broth for 24 h., and then 10 ml of bacterial culture were dropped in each plate and inculcated for 7 days at 28°C. Isolates which showed clear halos around their colonies were considered as phosphate solubilization.

8. Potential of *Streptomyces* Strains to Elicit Systemic Resistance Against Damping-off and Root-rot Diseases

A series of greenhouse experiment was conducted to induce resistance against damping-off pathogens by using *Streptomyces* strains. Treatments were arranged in a randomized complete block design with eight replications consisting of ten seeds per treatment. The following treatments were included in the experiment: (1) seeds treated with *Streptomyces* strains; (2) seeds treated with *Streptomyces* strains and challenge inoculated with mixture of *Fusarium solani*, *F. oxysporum*, and *Acremonium* sp., 15 days after planting (50 g sand-maize medium containing 10^3 cfu g^{-1} medium in each pot); (3) plants inoculated with the pathogens 15 days after sowing; and (4) non-treated plants. Pots with sterilized potting soil were sown with 15 seeds per each pot. Three replications were maintained in each treatment; each replicate consisted of eight pots and kept in the greenhouse with daily watering. The disease assessment was done periodically by examining and recording damping-off after 15 and 30 days from sowing date. Percentage of root-rot disease incidence was assessed 45 days after sowing. Disease severity (D.S.) was estimated visually by assessing necrotic regions on the roots and hypocotyls using rating scale of 0-5 described according to Filion et al. (2003).

$$D.S. = \frac{\sum (ab) \times 100}{AK}$$

where 'a' is the number of diseased plants having the same degree of infection, 'b' is the degree of infection, A is the total no. of examined plants, K is the highest degree of infection and D.S. is the disease severity.

Plant height and dry weight were determined. Four plants were sampled from each replication of the treatment separately for biochemical analysis. Fresh roots were washed in running tap water and homogenized with liquid nitrogen in a pre-chilled mortar and pestle. The homogenized root tissues were stored at -70°C.

9. Biochemical Changes Associated with *Streptomyces* Strains

9.1. Phenols determination

Root samples (1 g) of quinoa plants grown in pot experiment and obtained from different treatments and control (20 days after inoculation) were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70°C (Zieslin and Ben-Zaken, 1993). One ml of the metabolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteu reagent (1N) and the solution was kept at 25°C. The absorbance of the developed blue colour was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol mg protein⁻¹

9.2. Enzymes activity

9.2.1. Peroxidase

Root samples (1 g) of quinoa plants, grown in pot experiment and obtained from different treatments and control (20 days after inoculation), were homogenized in 2 ml of 0.1M phosphate buffer, pH 7.0 at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H₂O₂. The reaction mixture was incubated at room temperature (28±2°C). The changes in absorbance at 420 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min⁻¹ mg protein⁻¹ (Hammersmidt et al., 1982).

9.2.2. Chitinase

Root samples (1 g) of quinoa plants grown in pot experiment and obtained from different treatments and control (20 days after inoculation) were homogenized with 0.2 M Tris HCl buffer, pH 7.8 containing 14 mM B-mercaptoethanol at a rate of 1/3 (w/v). The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used to determine the enzyme activity, according to the colourimetric method suggested by Monreal and Reese (1969) using 1% colloidal chitin. Chitinase activity was measured by the release of N-acetyl-D-glucosamine (NAG) from colloidal chitin, and expressed as acetylglucosamine released/g fresh weight tissue/60 min.

10. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and were calculated for mean separation analyzed and subjected to Duncan's multiple range tests and comparison after analysis of variance (Duncan, 1955).

RESULTS AND DISCUSSION

1. Isolation of the Damping-off and Root-rot Causal Fungi

Isolated fungi from infected quinoa seedlings were identified as *Fusarium solani*, *F. oxisporum*, *Acrimonum* sp. and *Alternaria* sp. Percentage of isolated fungi occurrence were recorded in fig. (1), where *F.*

solani was the dominant pathogen with 48%, followed by *F. oxysporum* with 23%, then *Acremonium* sp. with 20% and *Alternaria* sp. with 9%.

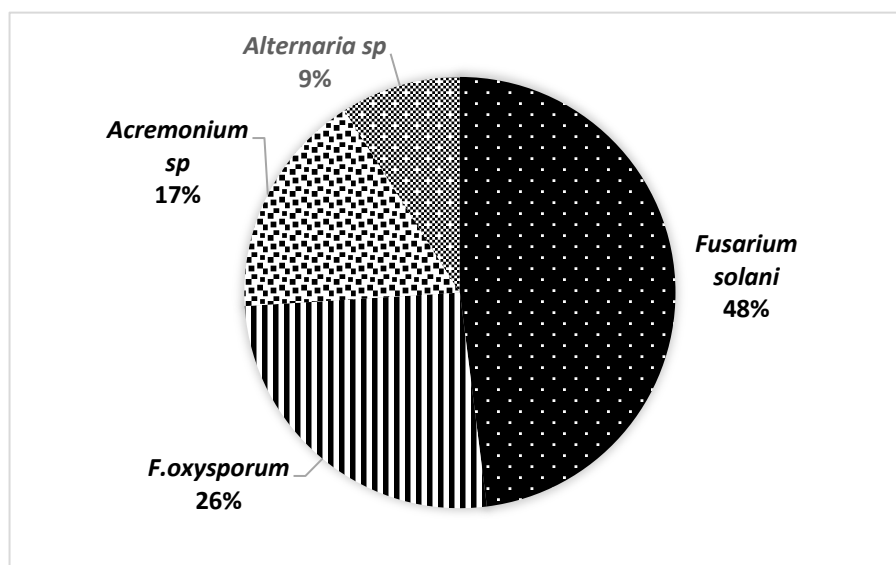


Fig. (1). Percentage of fungi isolated from quinoa seedlings grown in Wady El-Rayan, El-Fayoum governorate.

2. Pathogenicity Test

Results in table (1) show that all the tested fungi were pathogenic and caused pre- and post-emergence damping-off in quinoa seedlings, except *Alternaria* sp. didn't show pre-emergence damping-off. The mixture of isolated fungi has strongest pathogenic effect and had participated to cause disease complex. Three pathogenic fungi recorded pre- and post-emergence damping-off to quinoa seedlings with significant differences among them. *F. oxysporum* was recorded the lowest pre-emergence damping-off, whereas *Alternaria* sp. was recorded the lowest post-emergence damping-off for seedlings, while the highest values of pre and post damping-off were recorded with *F. solani*. The highest root-rot incidence of quinoa plants was recorded with *F. solani* after 45 days from sowing. The results agree with a previous study that refer to soil-borne pathogens; such as *Rhizoctonia solani* and *Macrophomena phaseolina* as aggressive causal organism for root-rot and damping-off diseases for young quinoa seedlings from five different Egyptian governorates (Giza, Fayium, Ismailia, Beheira and Monufia) (El-Moity et al., 2015).

Table (1). Effect of some soil-borne fungi on the incidence of pre- and post-emergence damping-off as well as survival of quinoa plants.

Fungal isolates	Damping-off (%)		Survival (%)	Root rot (%)
	Pre-emergence	Post-emergence		
<i>Fusarium solani</i>	33.3a	28.6a	38.1d	78a
<i>F.oxysporum</i>	08.7c	25.1b	66.2c	16c
<i>Acremonium sp.</i>	12.5b	17.8c	69.7b	32b
<i>Alternaria sp.</i>	00.0d	09.0d	91.0a	11d

3. Evaluation of Streptomyces Strains *In Vitro*

3.1. As antifungal activity

Among the five *Streptomyces* strains tested for their efficacy to inhabit the mycelial growth, three strains, *Streptomyces* sp. (26SA), *Streptomyces* sp. (25SA) and *Streptomyces toxytricini* exhibited the maximum inhibition for mycelial growth of *F. solani*, *F. oxysporum* and *Acremonium* sp. when compared with other tested strains *in vitro* (Table 2), due to antifungal substances released by the actinomycetes into the culture medium (Singh et al., 2017). In addition, *Streptomyces* sp. (25SA), *Streptomyces* sp. (26SA) and *Streptomyces toxytricini* showed the highest degree in vigor index increase of quinoa in the roll towel assay (Table 2). Actinobacteria are called plant growth-promoting rhizobacteria (PGPR). Direct promotion of plant growth by PGPR involves several mechanisms; such as production of plant growth regulators (Yang et al., 2013).

Table (2). Antagonistic effects of *Streptomyces* strains on the growth of *F. solani*, *F. oxysporum* and *Acremonium* sp. as well as their potential for plant growth promotion.

<i>Streptomyces</i> sp. strains	Inhibition zone (mm) ^a			Vigor index ^b
	<i>F. solani</i>	<i>F. oxysporum</i>	<i>Acremonium</i> sp.	
<i>Streptomyces</i> sp. (21SA)	09.5d	13.7c	14.5e	1023d
<i>Streptomyces</i> sp. (25SA)	23.5b	22.4b	21.6b	1152c
<i>Streptomyces</i> sp. (26SA)	25.3a	22.4b	19.6c	1233b
<i>Streptomyces</i> sp. (28SA)	22.9c	22.4b	18.7d	1054d
<i>Streptomyces toxytricini</i>	23.7b	25.2a	22.6a	1435a
Untreated	00.0e	00.0d	00.0f	652e

*Significant at 0.01 level of probability.

- a.) Inhibition of pathogens is expressed as the distance (mm) between fungal mycelium and bacterial colony on potato dextrose agar (PDA); each value is the mean of three replicates.
b) Vigor index = (Mean root length + Mean shoot length) × Germination (%)

3.2. As IAA production and phosphate solubilization

All *Streptomyces* strains showed significant production of IAA (Table 3) that ranged from 15.4 to 38.5 µg/ml. The strain *Streptomyces toxytricini* produced the highest concentration of IAA that was 38.5 µg/ml. Moreover, three out of five strains; i.e. *Streptomyces* 25SA, *Streptomyces* 26SA and *Streptomyces toxytricini* showed phosphate solubilization ability. Production of growth hormone; such as IAA and solubilization phosphate by actinomycetes has also been reported by Amaresan et al. (2018).

Table (3). Determination of IAA and phosphate solubilization for *Streptomyces* sp. strains

<i>Streptomyces</i> sp. strains	IAA (µ/mg)	Phosphate solubility ability
<i>Streptomyces</i> sp. (21SA)	15.4	-
<i>Streptomyces</i> sp. (25SA)	35.2	+
<i>Streptomyces</i> sp. (26SA)	30.2	+
<i>Streptomyces</i> sp. (28SA)	19.6	-
<i>Streptomyces toxytricini</i>	38.5	+

4. In Vivo Screening of Selected Antagonistic Isolates

Data in table (4) indicate that five strains; i.e. *Streptomyces* sp. (21SA), *Streptomyces* sp. (25SA), *Streptomyces* sp. (26SA), *Streptomyces* sp. (28SA) and *S. toxytricini* were effective in reducing damping-off of quinoa seedlings caused by *Fusarium solani*, *F. oxysporum* and *Acremonium* sp. *in vivo*. The degree of reduction of damping-off disease varied according to *Streptomyces* strains and the pathogen. However, the fungicides rizolex-T seed treatment performed the best reduction in damping-off caused by the three pathogens. Data in table (4) also indicate that, there were varied effects of the tested *Streptomyces* strains on seedling survival in pathogen non-infested soil. *S. toxytricini* increased the seedling survival by 84% as well as dry weight by 17 mg. Results also indicated that the quinoa seed germination test in Petri-plates could be used for evaluation the biocontrol agents against root-rot pathogen. Actinomycetes are considered as a source of novel biocontrol and plant growth promoting agent in general (Chaurasia et al., 2018 and Vurukonda et al., 2018).

Table (4). *In vivo* screening of selected antagonistic strains against *F. solani*, *F. oxysporum* and *Acremonium* sp. of quinoa and their effect on seedling dry weight.

<i>Streptomyces</i> sp. strains	Infested soil								Non-infested soil	
	<i>F. solani</i>		<i>F. oxysporum</i>		<i>Acremonium</i> sp.		Mixture		Survived seedlings (%)	Dry weight (mg)
	Survived seedlings (%)	Dry weight (mg)	Survived seedlings (%)	Dry weight (mg)	Survived seedlings (%)	Dry weight (mg)	Survived seedlings (%)	Dry weight (mg)		
<i>Streptomyces</i> sp. (21SA)	72c*	9c	78b	10c	76d	12b	73dc	9c	78b	13b
<i>Streptomyces</i> sp. (25SA)	82b*	15a	82ab	16a	84b	14a	80b	14a	81ab	15ab
<i>Streptomyces</i> sp. (26SA)	81b	11b	84a	13b	83b	15a	76c	11b	84a	16a
<i>Streptomyces</i> sp. (28SA)	70c	8c	75c	11c	78d	12b	69e	8c	78b	12b
<i>Streptomyces toxytricini</i>	86a*	13ab	85a	13b	80c	14a	83a	11b	84a	17a
Rizolex ^a -T	86a	14a	85a	13b	87a	14a	85a	13a	78 b	12b
Untreated ^b	46d	5d	42d	5d	38e	4c	37f	5d	59c	6c

*Significant at 0.01 level of probability. a) Seeds were treated with rizolex-T at rate of 2 g/kg seeds. b) Seeds were treated with 0.01% MC only.

Based on the *in vitro* and *in vivo* assessment, three strains; *Streptomyces* (25SA), *Streptomyces* (26SA) and *Streptomyces toxytricini* were evaluated for further studies of assessing their efficacy against damping-off disease under greenhouse conditions.

5. Efficacy of *Streptomyces* Strains to Elicit Systemic Resistance Against Damping-off and Root-rot Diseases Under Greenhouse Conditions

Data in table (5) indicate that seeds treated with *Streptomyces* strains individual or in combination and challenge inoculated with mixture of *F. solani*, *F. oxysporum* and *Acremonium* sp. induced significant disease protection compared with untreated treatment. The three *Streptomyces* strains significantly reduced damping-off incidence as well as root-rot incidence and severity (Table 5). In the present study, the results illustrate that, mixture of the three *Streptomyces* strains reduced pre-emergence by 6.5% and post-emergence by 9.8%, root-rot incidence by 25.2% and severity by 1.2 compared with untreated treatment and rizolex-T as a standard treatment (Table 5 and Fig. 3). These results agree with Sadeghi et al. (2017), who reported that, two *Streptomyces* isolates with the highest antagonistic activity, strains C 201 and C 801, reduced seedling damping-off of cucumber caused by *Phytophthora drechsleri* by 77 and 80%, respectively, in artificially infested soils under greenhouse conditions. Moreover, figs. (2 and 3) illustrate that, the three *Streptomyces* strains have significant increase

plant height and dry weight when applied individually or in combination. These results agree with the previous study of Singh et al. (2017) in which the authors illustrated the exploitation of actinomycetes in agricultural practices as biocontrol agents or biofertilizers for damping-off disease management and tomato crop improvement. Generally, plant growth-promoting organisms facilitate the plant growth directly by either assisting improved nutrition acquisition (nitrogen, phosphorus, potassium and essential minerals) or phytohormone production (IAA, cytokinins, gibberellins, etc.), or suppression of plant disease (Amarean et al., 2018). Application of a mixture of three *Streptomyces* strains has resulted in much more intensive plant growth promotion and disease reduction when compared to strains tested individually (Fig. 2). This might be due to different mode of action for PGPR and related to sufficient root colonization and efficiency of biocontrol (El- Hadidy, 2012).

Table (5). Effect of *Streptomyces* strains as seed treatment on the incidence of quinoa damping-off and root-rot diseases under greenhouse conditions.

<i>Streptomyces</i> strains	Damping-off (%)		Survival plants (%)	Root-rot incidence (%)	Disease severity
	Pre-emergence	Post-emergence			
<i>Streptomyces</i> sp. (25SA)	8.1b	13.6b	78.3c	37.bc	2.0bc
<i>Streptomyces</i> sp. (26SA)	7.4c	13.9b	78.7c	39.5b	2.3b
<i>Streptomyces toxytricini</i>	7.2c	12.4c	80.4bc	34.3c	1.7c
Mixture	6.5d	9.8d	83.7b	25.2d	1.2d
Rizolex ^b T	5.3e	7.9e	86.8a	21.2e	0.8e
Untreated ^c	14.2a	23.6a	62.2d	58.3a	2.9a

Means within the same column followed by the same letter are not significantly different according to Duncan's

b) Seeds were treated with rhizolex-T at rate of 2 g/kg seeds. c) Seeds were treated with 0.01% MC only. multiple range test ($P \geq 0.05$).

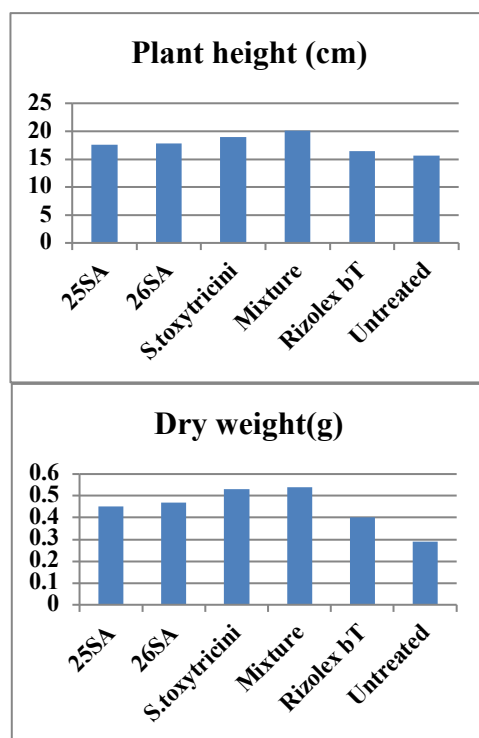


Fig. (2). Effect of *Streptomyces* strains on plant height and dry weight of quinoa plants.



Fig. (3). Efficiency of the mixture of *Streptomyces* strains on root-rot suppression and growth promotion.

6. Biological Changes Associated with *Streptomyces* Strains

In the present study, seed treatment with *Streptomyces* strains resulted in increasing accumulation of phenolic substances in response to infection by the pathogen. The maximum phenolic content was observed in *Streptomyces* sp. (26SA) and *S. toxytricini* pretreated plants challenge inoculated with the pathogens. The higher amounts of phenolics were noticed even on 10th day after the pathogens challenge. In plants inoculated with the pathogens alone, the phenolic content declined to the initial level on the 10th day after inoculation. Plants treated with *Streptomyces* strains alone have increased content of phenolics compared to untreated plants (Table 6). Phenolic compounds may be fungitoxic in nature and may increase the mechanical strength of the host cell wall. Phenolic compounds, including flavonoids have important roles in plant defense against phytopathogens (Treutter, 2006). Soil inoculation with *Phytophthora drechsleri* and/or treatments with *Streptomyces* significantly resulted in increasing the accumulation of phenolic content in leaves of cucumber 12 h after inoculation compared to the control (Sadeghi et al., 2017). Table (7) indicates that, quinoa seeds treated with *Streptomyces* strains; *Streptomyces* sp. (26SA) and *S. toxytricini* induced plants to synthesize defense-related enzymes; peroxidase and chitinase. Whereas an additional increase in the synthesis was observed when pretreated plants challenged by inoculation with a mixture of pathogens; *F. solani*, *F. oxysporum* and *Acremonium* sp. The activity reached the maximum level on the 3rd day after pathogens challenge and thereafter the activity remained at higher levels throughout the experimental period of 10 days. In plants treated with the pathogens alone, increased activity of peroxidase and chitinase was observed for period of 5 days and thereafter declined drastically in plant (Table 7). Treatment with the selected *Streptomyces* isolates enhanced the activities of defense related enzymes (Sunpapao et al., 2018) and increased activity of polyphenol oxidase and peroxidase enzymes (Sadeghi et al., 2017). As well as *Streptomyces felleus* YJ1 has strong antagonistic activity and ability to synthesize enhanced level of defense enzymes such as superoxide dismutase, peroxidase, poly phenol oxidase and phenylalanine ammonia lyase against *Sclerotium sclerotiorum* in oilseed rape under greenhouse conditions (Cheng et al., 2014).

Table (6). Effect of *Streptomyces* strains on phenolic compounds in quinoa plants inoculated with mixture of pathogens; *F. solani*, *F. oxysporum* and *Acremonium sp.*

<i>Streptomyces</i> strains	Total phenols ($\mu\text{g catechol mg protein}^{-1}$)					
	Without pathogen			With pathogen		
	Period per days			Period per days		
	0	5	10	0	5	10
<i>Streptomyces sp. (25SA)</i>	47	48	48	45	53	55
<i>Streptomyces sp. (26SA)</i>	55	52	50	58	90	86
<i>Streptomyces toxytricini</i>	52	50	50	55	88	85
Untreated	40	40	40	42	63	40

Table (7). Effect of *Streptomyces* strains on peroxidase and chitinase activity in quinoa plants inoculated with mixture of *F. solani*, *F. oxysporum* and *Acremonium sp.* pathogens

<i>Streptomyces</i> strains	Peroxidase activity ($\text{min}^{-1} \text{mg protein}^{-1}$) ^a						Chitinase activity ($\text{min}^{-1} \text{mg protein}^{-1}$) ^b					
	Without pathogen			With pathogen			Without pathogen			With pathogen		
	Period per days			Period per days			Period per days			Period per days		
	0	5	10	0	5	10	0	5	10	0	5	10
<i>Streptomyces sp. (25SA)</i>	1.6	1.8	1.6	1.6	2.0	1.8	2	2	2	2	2.4	2.0
<i>Streptomyces sp. (26SA)</i>	1.8	2.1	1.8	1.6	2.6	2.4	2	2	2	2	3.0	2.4
<i>Streptomyces toxytricini</i>	1.8	2.3	1.9	1.6	2.4	2.2	2	2	2	2	2.8	2.6
Untreated	1.6	1.6	1.6	1.6	1.6	1.6	2	2	2	2	2.4	2.0

a) Peroxidase activity was determined in 1 g of quinoa roots and expressed as changes in the absorbance $\text{min}^{-1} \text{mg protein}^{-1}$ (Hammersmidt et al., 1982)

b) Chitinase activity was determined in 1 g of quinoa roots according to the colourimetric method suggested by Monreal and Reese (1969)

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تأثير عناصر المقاومة الحيوية على أمراض موت وأعفان الجذور لبادرات الكنوة

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في هذه الدراسة تم عزل *Fusarium solani*, *Alternaria* spp, *Acremonium* sp. من بادات الكنوة بمحافظة الفيوم بجمهورية مصر العربية. حيث أظهرت اختبارات قدرة تلك العزلات الفطرية على إحداث أمراض موت البادرات وأعفان الجذور لبادرات الكنوة. تم دراسة فاعلية عزلات من الإستربتومييسيس كعوامل مكافحة حيوية في المعمل والصوبة. أظهرت العزلة *Streptomyces toxytricini* أعلى درجات تضاد لأعلى ثلاث فطريات مرضية، كما أظهرت إنتاجها لإندول حامض الخليك وتيسير الفوسفات. تم تقييم حيوية القدرة التضادية لعزلات الإستربتومييسيس. أوضح استخدام خليط من عزلات الإستربتومييسيس لخفض نسبة وشدة موت البادرات وعفن الجذور بالإضافة لزيادة معنوية لحيوية البادرات تحت ظروف الصوبة. أوضحت نتائج تقييم عزلات الإستربتومييسيس لإحداث المقاومة المستحثة لنبات الكنوة ضد الفطريات الممرضة من خلال زيادة المحتوى الفينولي ونشاط إنزيمات البيروكسيداز والشيتينيز في النباتات المعاملة بعزلات الإستربتومييسيس.