Biological Control Using *Streptomyces* sp. Kp109810 and Different Genotypes of Pepper (*Capsicum annuum* L.) on Root Rot Diseases

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Abstract: This study was carried out through two experiments in laboratory and greenhouse at the Agricultural botany department and Biological Control Center, Faculty of Agriculture, Suez Canal University. The isolated fungi, which caused root-rot of pepper plants, were identified as *Rhizoctonia solani* (R) and *Macrophomina phaseolina* (Mc) from infected pepper samples. The pathogenic fungi showed deformation, shrinkage, and collapse when observed by scanning electron microscopy (SEM) and *Streptomyces* sp. KP109810 (S) showed the presence of *PKSII* and *NRPS* genes by polymerase chain reaction (PCR) amplification. The extract was analyzed for chemical profiling by Thin-layer chromatography (TLC), and a unique pattern of secondary metabolites was observed. The strain was positive production for protease and chitinase while it was negative for cellulase. Antagonistic *Streptomyces* sp. KP109810 was subjected to a comprehensive *in vitro* screening for various plant growth promoting (PGP) traits. *Streptomyces* sp. KP109810 (S) showed strong antagonism against test fungi (R and Mc) which showed 84.6% and 78.7% maximum inhibition respectively, in dual culture plat assays as a result of diffusible compounds produced by (S), the inhibitory effect of volatile compounds was recorded up to 78.2% against (R) and 66.3% against (Mc) and multiple plant growth promoting attributes such as Indole Acetic Acid (IAA), phosphate solubilization activity, siderophore, NH_3 and HCN production. Five genotypes of pepper planted in greenhouse and treated with six treatments: control, (S), (R), (Mc), (S+R) and (S+Mc). Through mean performance analysis, results showed Saidah and SUPER MARD genotypes have tolerant ability to root rot diseases over rest studied genotypes, although there is a significant decrease in growth and yield under (R) and (Mc) treatments for all genotypes. Streptomyces sp treatment acts as biocontrol to these fungi by reducing the percentages of damping-off and root rot severity. There is a positive significant correlation between fruits weight/plant and all studied traits except root length under control and biotic stress conditions. Fruits weight / plant, fresh weight of root and shoot traits used as a good indicator to select root rot resistance genotypes of pepper as they have high values of heritability (h2) and high values of genetic advance as a percent of mean (GAM).

Keywords: Biocontrol, *Rhizoctonia*, *Macrophomina*, *Streptomyces*, Pepper, Genetic parameters, Genetic correlation

INTRODUCTION

Pepper (*Capsicum* spp.) is a fruit vegetable belongs to Solanaceae family. The growing in consumption of pepper has been detected in the last twenty years from 19 to 40 million tons yield and from 2.5 to about 3.8 million of hectares surface area over worldwide (Faostat, 2017), and for keeping this trend, the improvement of new high yielding and resistant varieties represents the effective strategy to protect pepper crop from biotic or abiotic stresses (Sarath Babu *et al*., 2011; Parisi *et al*., 2020).

Also, a lot of vegetables like pepper subjected to the attack by several plant pathogens that cause significant losses in quantity and quality traits. Root-rot and wilt diseases were detected by (Wu *et al*., 2008; Abdel-Naby, 2001; El_sharkawy, 2010) the most devastating plant diseases for pepper plants. *Phytophthora capsici*, *Fusarium solani*, *F. oxysporum*, *Verticillium* spp, *Rhizoctonia solani*, *Pythium aphanidermatum* and *Macrophomina phaseolina*are fungal pathogens that cause root rot diseases (Ling *et al*., 2010). Root rot pathogens affect all parts of the plant including stem, leaves, and fruits. The most obvious symptom of root rots is wilting and death of plants even when soil has enough moisture. However, in disease progression, the stem dries up and withers, die back occurs, leaves defoliate and the whole plant finally die (Babadoost and Islam, 2003). *R. solani* Kihn can cause several types of damage at multiple growth stages of pepper such as seed decay, pre and post- emergence damping off, wire stem, root rot and hypocotyl or tap root with necrotic spots (Wu *et al*., 2008; López *et al*., 2011). *M. phaseolina* is a harmful seed and soil borne pathogen and cause many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot, wilt, root rot and reddish-brown discoloration and black streaks can form in the pith and vascular tissues of the root and stem (Babu *et al*., 2007; Güney and Güldur, 2018).

It is difficult to control soil-borne pathogens because of the wide host range and specialized structures of resistant produced by some fungi that can survive in soil for long periods in the absence of their hosts. Efforts focused on further improvement of the production and productivity along with desirable traits of pepper through genetic manipulation which is important in different potential areas. The using of chemical compounds have led to side effects that may cause damage and pollution of environment, but restriction on the use of this fumigant has increased the risks for soil-borne pathogen outbreaks and has resulted in bigger efforts to develop chemical and non-chemical environmentally user-friendly alternative control methods like using beneficial microorganisms in the form of bio fertilizers to reduce the use of pesticides and chemical fertilizers which can provide more environmentally sound and economically feasible alternatives (Ahmad *et al*., 2008;

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Egamberdieva, 2009; Dantas *et al*., 2013; Ros *et al*., 2014; Law *et al*., 2017).

Plant growth promoting rhizobacteria (PGPR) is a group of naturally occurring, free living rhizosphere can actively colonize plant root that stimulate the plant growth either through direct or indirect mechanisms and its strong antimicrobial potential (Vessey, 2003; Franco-Correa *et al*., 2010; Kumar *et al*., 2017) actinomycetes are present extensively in the plant rhizosphere and produce various groups of natural bioactive metabolite and produces compounds such as ivermectin, tetracycline, streptomycin, nystatin; particularly *Streptomyces* show immense biocontrol action against a variety of phytopathogens and can decompose organic matters such as lignocellulose, starch, and chitin in soil (Wang *et al*., 2013; Ashokvardhan *et al*., 2014; Ser, 2015; Kumar *et al*., 2017).

The use of some statistical genetic analyzes such as heritability (h^2), high genotypic and phenotypic coefficient of variation (GCV-PCV) and genetic advance as percent of mean (GAM) was specified in some previous studies and a good tool for selection under environmental or pathological stresses (Zegeye *et al*., 2014).

The main objectives of this study were (i) to screen for various plant growth promoting (PGP) traits *of Streptomyces* sp. KP109810 and their antimicrobial activities against fungal pathogens (ii) to decrease using of fungicides in agriculture to produce high quality food by *Streptomyces* sp. KP109810 as a potential biocontrol agent against root-rot of pepper genotypes caused by *M. phaseolina* and *R. solani* and (iii) to study correlation and genetic variability for assessment of yield and its components in pepper genotypes.

MATERIALS AND METHODS

This study carried out through two experiments in laboratory and greenhouse at the Agricultural botany department and Biological Control Center, Faculty of Agriculture, Suez Canal University.

Microbial strains

Streptomyces sp. KP109810 was obtained from the Faculty of Science; Suez Canal University while the fungal strains were obtained from was obtained from Agric. Res. Cent., Ismailia used as antifungal against phytopathogenic fungi; *Rhizoctonia solani, Macrophomina phaseolina* and *Fusarium solani*.

***In vitro* antagonistic bioassay**

Streptomyces sp. KP109810 was evaluated according to the inhibition ability against phytopathogenic fungi on potato dextrose agar (PDA) plates using dual-culture *in vitro* assay (Chen *et al*., 2018). A plate with each target pathogen was used as the control. The colony diameters of the target pathogens were measured according to (Yun *et al*., 2018).

Effect of volatile compounds on the mycelial growth of phytopathogenic fungi *in vitro*

The dish containing the fermentation broth of *Streptomyces* sp. KP109810 strain was prepared and covered with dish containing a 6 mm disc of 7 days old culture of the tested phytopathogenic fungus which was placed in the middle of another PDA plates. Pathogen growth was measured and compared to control developed in an antagonist's absence according to (Velázquez-Becerra *et al.,* 2011).

Scanning electron microscopy

To detect the profound changes in the morphology of selected phytopathogens (*R. solani* and *M. phaseolina*). Hyphae were fixed and coated with gold by auto fine coater (JFC-1600) (Yuan, 1995). Then, the sample was viewed under scanning electron microscope (Jeol-jsm 5200).

Evaluation of plant growth promotion effect of *Streptomyces* sp. KP109810

Indole acetic acid (IAA) production

IAA production by *Streptomyces* sp. was carried out using the method by (Loper, 1986).

Phosphate Solubilization

Streptomyces sp. KP109810 was inoculating on Pikovskaya's agar medium containing tricalcium phosphate. The quantitative bioassay of P-solubilization was determined by (Jackson *et al*., 1973).

Siderophore production

Streptomyces sp. KP109810 was inoculated on Chrome azurol S (CAS) agar medium and incubated agar medium and incubated at 28°C for 7 days according to (Schwyn and Neilands, 1987). The appearances of orange zones were considered as siderophore production.

Ammonia Production

Streptomyces sp. KP109810 was cultured in 10 ml of peptone water and incubated at 30°C for 5 days. ammonia production was detected by adding Nessler's reagent (Cappuccino and Sherman, 2002).

Hydrogen Cyanide Production

Production of hydrogen cyanide by *Streptomyces* culture was observed by the method of (Reddy *et al.*, 2008).

Detection of antibiotic producing *Streptomyces* sp. KP109810 by TLC

Streptomyces sp. KP109810 was cultivated PDB medium for 8 days at 28°C with shaking condition. After incubation, it was centrifuged at 8000 rpm for 15 min at room temperature. The supernatants were collected for the solvent extraction. The extraction of antibiotic substances was done according to (Chung *et al*., 2012). Spots were visualized by UV irradiation (254 nm).

Detection of antimicrobial biosynthetic genes *PKSII* and *NRPS*

The amplification of *PKSII* and *NRPS* were performed using degenerate primer pairs KS-α (5′-TSGCSTGCTTCGAYGCSATC-3′)/KS-

β(5′TGGAANCCGCCGAABCCGCT-3′) (Metsä-Ketelä *et al*., 1999) for PKSII gene. and A3F (5′GCSTACSYSATSTACACSTCSGG-3′)/A7R (5′-SAS GTCVCCSGTSCGGTAS-3′) (Ayuso-Sacido and Genilloud, 2005) for NRPS gene. The PCR reaction mixture was applied according to (Das *et al*., 2018) protocol. The amplified products were analyzed in 1% (w/v) agarose gel. The desired bands are 600 bp for *PKSII* genes and 800 bp for *NRPS* genes.

Production of hydrolytic enzymes:

Observation of the protease activity by the appearance of clear halo zones around the bacterial colony on skim milk agar medium after incubation for 48h at 28°C as defined by (Perneel *et al*., 2007). Evaluation of chitinase activity by inoculating bacteria on chitin agar plates containing colloidal chitin, the appearance of the halo zone after 7 days incubation at 30°C is evidence of positivity (Frankowski *et al.*, 2001). The cellulase production was examined on M9 medium amended with yeast extract (1.2 g^{l-1}) and cellulose (10 g^{l-1}). The strain was inoculated onto the center of the plates and cultured at 30°C for 7 days, and the clearing zones around the colonies were considered positive for cellulase production (Zhou *et al.*, 2004).

Plant experiment

Plastic pots, 20 cm diameter and 35 cm depth were filled with soil, 200 ml of bacterial culture (10^8 CFU ml^{-1}) was mixed thoroughly with 2.8 kg of non-sterilized sandy soil for 2 min. Soil mixed with 200 ml saline solution served as positive and negative controls.. Soil infestation was performed using the inoculum of *Rhizoctonia solani* and *Macrophomina phaseolina* from infected pepper samples by the rate of 2% of soil weight (w/w), which was grown on sterilized sorghum medium (El_sharkawy, 2010). Each treatment was replicated in 6 pots. Control treatment was performed using the non-sterilized sandy soil mixed with the sterilized sorghum medium at the rate of 2% (w/w) and replicated six times as the rest of treatments. Pots were watered daily after inoculation. One week later, Three seedling one –month old of each five genotypes of pepper ; super mard (V1), Hybrid F1 Hot pepper Saidah (V2), Hot pepper Biskra (V3), F1 Sweet pepper Giro (V4), Hybrid pepper Top star (V5), were sown per pot. Percentages of post- emergence damping-off for root-rot plants were recorded at 15 days after sowing. On the other hand, severity was calculated at 90 days after sowing. Disease severity of root rot and the degree of root discoloration were rated using a numerical scale defined by (Aoyagi *et al*., 1998).

Pots were arranged in Complete Randomized Design (CRD) with six replications. At flowering and mature stages some growth traits are measured like; plant height (cm), root length, fresh shoot weight (gm), fresh root weight (cm), Dry shoot weight, Dry root weight, and fruits weight / plant (gm.).

Statistical analysis

Analysis of variance (ANOVA) of mean values of the samples from each replication was subjected in CRD to statistical analyses using a Computer program Costat software (version 6.311). Means were separated using LSD at $P<0.05$ to compare the effects of pathogenic stress and non-stress conditions on genotypes.

Estimation of Variance Components: The phenotypic, genotypic and environmental variances and coefficient of variations were calculated according to the formula suggested by (Singh, 1985).

Broad-Sense Heritability (h^2): was estimated according to (Allard, 1960).

Genetic Advance as Percent of Mean: was calculated to compare the extent of predicted advance of different traits under selection, using the following formula: GAM= GA x 100 /x Where, GAM= Genetic advance as percent of mean, x=Grand mean of the trait. GA = K.h²b. σp Where, GA= Expected genetic advance, k = the standardized selection differential at 5% selection intensity (K = 2.063), h^2b= Heritability in broad sense σp = Phenotypic standard deviation.

Estimation of correlation coefficients: Phenotypic and genotypic correlation coefficients were estimated using the standard procedure suggested by (Al-Jibouri *et al*., 1958).

RESULTS AND DISCUSSION

***In vitro* antagonistic bioassay**

The results showed an effective antagonism to *R. solani* (84.6%), *M. phaseolina* (78.7%) and *F. solani* (72.3%). *Streptomyces* sp. KP109810 showed the highest inhibitory activity against *R. solani* and *M. phaseolina* by 84.6% and 78.7%, respectively, (Table 1 and Fig. 1). They were selected for further experiments in search of potential strain that can be employed as bio-fertilizer strain. Our research results are parallel to some of the earlier studies where endophytic actinomycetes are reported as potential antifungal agents (Strobel, 2003; Verma *et al*., 2009; Kaur *et al*., 2014). Several isolates of streptomyces inhibited growth of *M. phaseolina* by 89.3%. Many studies have found *Streptomyces* species have antifungal activity and can reduce the growth in a number of phytopathogens in vitro, including *Collectotrichum gloeosporioides*, *Penicillium digitatum*, *Fusarium oxysporum*, *Alternaria brassicicola* and *Sclerotium rolfsii* (Khamna *et al*., 2009; Dean *et al*., 2012; Law *et al*., 2017; Singh, 2018).

Effect of volatile compounds on the mycelial growth of phytopathogenic fungi *in vitro*

The results showed that volatile compounds produced by *Streptomyces* sp. KP109810 reduced the growth of *R. solani* (78.2%), *M. phaseolina* (66.3%), and *F. solani* (61.5%), respectively (Table 1). Previous studies showed that streptomycete VOCs have antimicrobial activities against *R. solani* in vitro, in addition to species of *Aspergillus* and *Fusarium* (Wan *et al*., 2008; Wang *et al*., 2013).

Table (1): Inhibitory effect (%) of dual culture assay and volatile compounds of *Streptomyces* sp. KP109810 on growth of tested fungi *in vitro*

Pathogenic test fungi	*Streptomyces* sp. KP109810			
	Dual culture assay		volatile compounds	
	mm†	%‡	mm†	%‡
Rhizoctonia solani	12.3	84.6	17.4	78.2
Macrophomina phaseolina	17.1	78.6	26.9	66.3
Fusarium solani	22.3	72.3	30.8	61.5

†: mycelial growth, ‡: Percentage of fungal growth inhibition

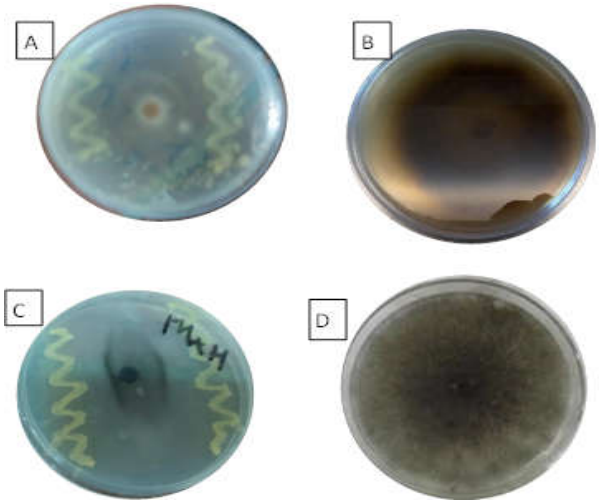

Figure (1): *In vitro* inhibition of *Streptomyces* sp. KP109810 on the mycelium growth of *Rhizoctonia solani* and *Macrophomina phaseolina* (A, C). Control, plate inoculated only with *Rhizoctonia solani* and *Macrophomina phaseolina* (B, D)

Scanning electron microscopy:

The effect of *Streptomyces* sp. KP109810 against *R. solani* and *M. Phaseolina* was confirmed by SEM (Figure 2). Lysis of fungal hyphae and broken cells were observed in *R. solani* (Fig. 2A*).* In contrast, those phenomena have not been found in R. solani hyphae from the control treatment (Fig. 2B). The *M. phaseolina* mycelia were collected from the inhibition zone periphery of a 7 days dual culture plate. Observations made under SEM showed that the mycelia were deformed and ruptured (Fig. 2C). In a research done by Patil *et al.* (2010) similar findings have been recorded for antagonism of *Streptomyces* against *R. solani* under SEM.

Characterization of PGPR and Extracellular enzymes production

The amount of IAA in the presence of tryptophan was 47.02 µg/mL. The selected strain also was tested to its ability to solubilize phosphate in the pikovskaya medium containing tricalcium-phosphate as indicated by producing clear zone around the colonies after 7 days of incubation. Phosphate solubilization index of this strain was 72.3 µg/m. This strain has a potential to mobilize insoluble inorganic phosphate to improve plant under low phosphate availability. Siderophore, Ammonia, and HCN production among the selected strain, it was found to be positive for the ammonia and HCN production but lacking the capacity to produce siderophores. Of the three tests for hydrolysis enzymes, the strain was positive for two enzymes protease and chitinase production while it was negative for cellulase production. There are many previous researches which demonstrate the ability of several *Streptomyces* species, such as *S. olivaceoviridis, S. rimosus, S. Rochei, S. griseoviridis,* and *S. lydicus*could produce IAA (Yandigeri *et al*., 2012; Harikrishnan *et al*., 2014). Hamdali *et al.* (2008) found that *Streptomyces cavourensis, Streptomyces griseus*, and *Micromonospora aurantiaca* could produce 83.3, 58.9, and 39 phosphate solubilization mg/100 ml, respectively. Almost all the *Streptomyces* species were also able to produce ammonia and hydrogen cyanide. HCN production plays an important role in plant disease suppression which is an important factor for phytopathogen antagonism. It has shown that endophytic actinomycetes isolated from Azadirachtaindica exhibited high levels of siderophore and IAA production (Verma *et al*., 2009). It is also noted that the production of plant hormones, phosphate solubilization and some other components produced by actinomycetes will interfere with plants as part of their colonization, which leads to enhancing plant growth, increasing resistance and modifying plant defense mechanisms (Singh *et al*., 2015; Goudjal *et al*., 2016).

In our study, *in vitro* evaluation showed that *Streptomyces* sp. produced lytic enzymes like chitinase and protease which might be responsible for strong antagonistic activity against pathogenic fungi observed. One of the potential biocontrol mechanisms for fungal phytopathogens may be due to the production of chitinase by antagonistic strain, because the cell wall of the fungi is made up of polysaccharides such as glucan and chitin (Patil *et al*., 2010; Kaur *et al*., 2013). Also, inhibition of *R. solani* and *M. phaseolina* mycelial growth by *Streptomyces* sp. KP109810 culture fungi may be refer to the production of extracellular compounds such as cell wall degrading enzymes and HCN, which cause damage and disintegration of the hyphae of pathogenic fungus as observed under SEM.

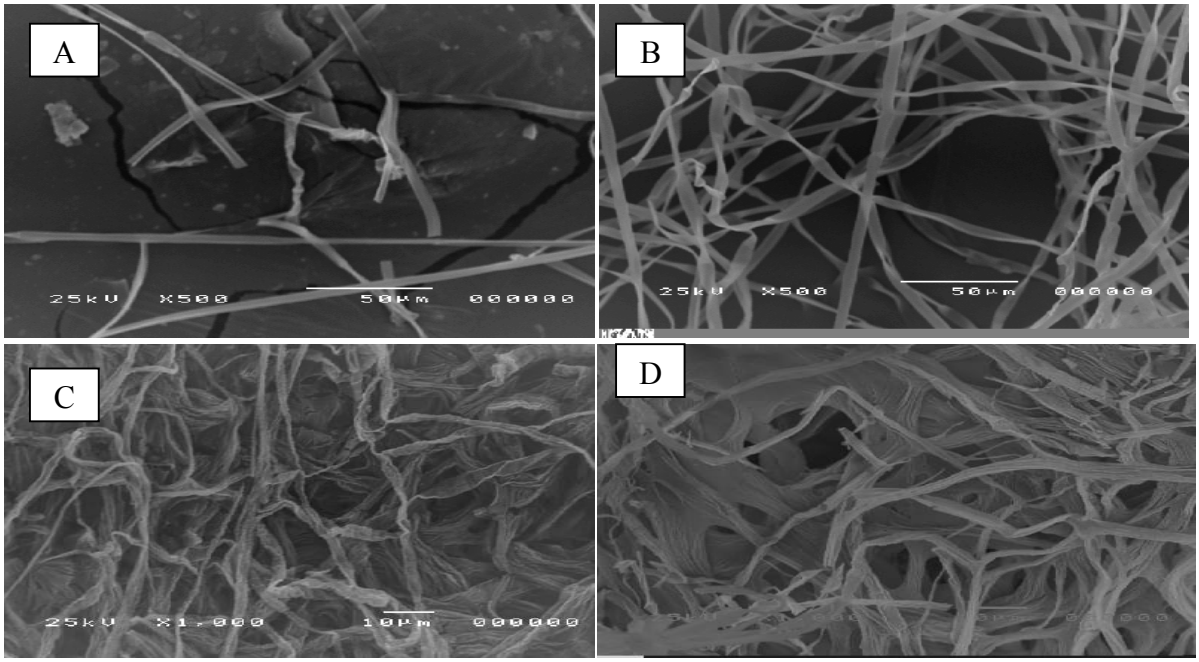

Fig. (2): SEM microphotographs of *Rhizoctonia solani* and *Macrophomina phaseolina* growth without the presence of antagonistic streptomyces (control, B and D); with *Streptomyces* sp. KP109810 (A and C)

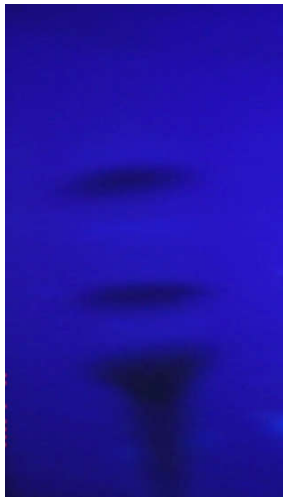

Fig. (3): TLC analysis of bioassay from the culture of *Streptomyces* sp. KP109810

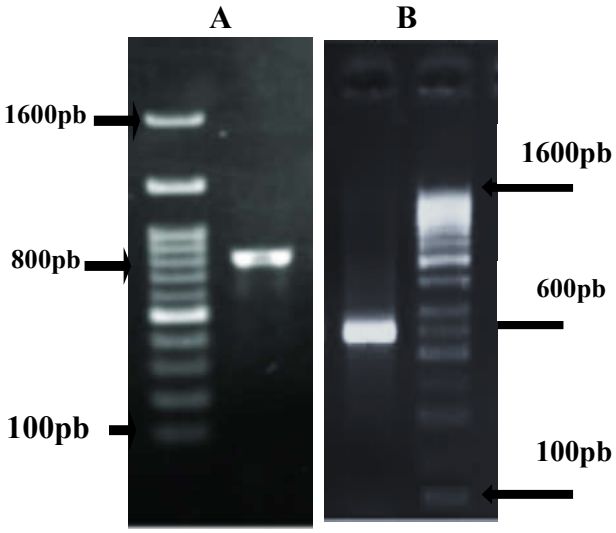

Figure (4): *Streptomyces* sp. KP109810 positive for *NRPS* gene amplification (A) showed the amplification of *PKSII* gene fragment (B) in Agarose gel photograph

Detection of antibiotic producing *Streptomyces* by TLC

In a thin-layer chromatography, the selected strain was subjected to analyse of different biologically active components in the crude extracts. *Streptomyces* sp. KP109810 indicated various bands and several components of the crude extract displayed UV absorbance (Fig. 3). The retardation factor (Rf) recorded values; 0.7, 0.48, and 0.29, as determined by co-migration with pure standards. Similar kinds of results were observed by Usha Kiranmayi *et al.* (2011), who obtained good yield of antimicrobial fractions while using ethyl acetate as an extracting solvent.

Evaluation of antimicrobial activity and screening of PKS-II and NRPS genes in selected actinomycetes

Streptomyces sp. KP109810 was evaluated for their biosynthetic potential to produce secondary metabolites by PCR screening of PKS-II, and NRPS genes, using degenerate primers previously reported. *Streptomyces* sp. KP109810 showed the presence of PKSII and NRPS genes at 800bp and 600bp respectively, (Fig. 4 A, B). Encheva-Malinova *et al* (2014) reported that almost all the strains of streptomyces possessed PKSII gene and NRPS gene among the 11 strains screened and the isolate PWS11 also showed the presence of NRPS gene and PKSII (Das *et al*., 2018).

Plant experiment

Effect of *Streptomyces* sp. KP109810 on the root rot disease caused by *R. solani* and *M. phaseolina*

The effect of the PGPR *Streptomyces* sp. KP109810 on the root rot disease caused by *R. solani* and *M. phaseolina* was evaluated under glasshouse conditions (Table 2). Generally, cv Giro was the most susceptible amongst tested varieties for the infection with *R. solani* and *M. Phaseolina* in terms of lowest survival, highest percentage of post-emergence and disease severity. Pertaining to the infection with *R. solani*, the tested *Streptomyces* significantly reduced the root rot disease incidence in pepper plants hybrids as compared with control treatment. cv Saidah showed the highest percentage of survival plants (100%) subsequent lowest post-emergence and severity index of 0.0%, 8.33%, respectively compared with respective values for control at 86.11, 13.89 and 36.11% followed by cv Super Mard F1 which showed the highest percentage of survival plants (100%) and subsequent lowest post-emergence and disease severity (0.0, 11 and 81%) compared with control (75.01, 24.99 and 30.21%). These finding are in harmony with those of (Pineda-Mendoza *et al*., 2018), which found that the application of the rhizobacterial strains to *Serrano chili* decreased the *R. solani*-related mortality rate in seedlings; particularly, S108 had the greatest effect potential as a bio fungicide to control *R. solani* in chili seedlings. Also, Güney and Güldür (2018) tested eight bacterial isolates for reducing post-emergence damping-off and disease severity of pepper seedlings hybrids inoculated with *R. solani*, and they also noticed that *Streptomyces* did not negatively effect on the germination of the host plant. Among the variety of secondary metabolites, *Streptomyces* often produces IAA, which can improve the plant growth by stimulating cell elongation and root growth (El-Tarabily, 2008). As for the antagonistic effect against *M. phaseolina*, *Streptomyces* sp. also showed significant reduction in the root rot disease incidence in pepper plants hybrids as compared with control treatment. cv Saidah recorded the highest percentage of survival plants (100%) and subsequent lowest Post-emergence and disease severity of 0.0% and 13.19% followed by cv Biskara which showed the highest percentage of survival plants (91.67%) and subsequent lowest post-emergence and disease severity of 8.34, 18.61%. compared with control at 77.78, 22.22 and 38.96% These results are in the same trend with (Yadav *et al*., 2014) who reported that *Streptomyces* sp. S160 reduced the incidence of charcoal root rot caused by *M. phaseolina* in chickpea in the greenhouse, similarly, *Streptomyces caeruleatus* strain ZL2 significantly reduced the root rot of tomato seedlings caused by phytopathogenic fungus species (Zamoum *et al*., 2015).

Table (2): Efficacy of *Streptomyces* sp. KP109810 on the incidence of root rot disease caused by *R. solani* and *M. phaseolina* in different pepper genotypes

Genotypes	Treatment	Control	S	R	Mc	S+R	S+Mc	L.S.D 0.05%
SUPER MARD F1	Post-emergence (%)	0.0^{c}	0.0^{c}	24.99^{a}	19.45^{ab}	0.0^{c}	11.11^{b}	9.93
	Survival (%)	100^{a}	100.0^{a}	75.01^{c}	80.56^{bc}	100.0^{a}	88.89^{b}	9.93
	DS (%)	0.0^{c}	0.0^{c}	30.21^{ab}	42.46^{a}	11.81^{bc}	18.06^{bc}	19.37
SAIDAH	Post-emergence (%)	0.0^{b}	0.0^{b}	13.89^{a}	16.67^{a}	0.00^{b}	0.00^{b}	7.89
	Survival (%)	100.0^{a}	100.0^{a}	86.11^{b}	83.33^{b}	100.0^{a}	100^{a}	7.89
	DS (%)	0.0^{c}	0.0^{c}	36.11^{a}	25^{ab}	8.33^{c}	13.19^{bc}	14.24
BISKRA	Post-emergence (%)	2.78^{c}	0.0^{c}	30.55^{a}	22.22^{ab}	11.11^{bc}	8.34^{c}	11.25
	Survival (%)	97.22^{a}	100.0^{a}	69.45^{c}	77.78^{bc}	88.89^{ab}	91.67^{a}	11.25
	DS (%)	2.22^{c}	0.0^{c}	48.33^{a}	38.96^{a}	22.64^{b}	18.61^{b}	11.15
GIRO	Post-emergence (%)	2.78^{c}	5.56^{c}	36.11^{a}	25^{ab}	19.45^{b}	22.22^{b}	11.72
	Survival (%)	97.22^{a}	94.44^{a}	63.89^{c}	75^{bc}	80.56^{b}	77.78^{b}	11.71
	DS (%)	3.61^{c}	2.90^{c}	47.22^{a}	45^{a}	25.28^{b}	21.39^{b}	6.01
TOPSTAR	Post-emergence (%)	0.0^{c}	2.81^{bc}	24.99^{a}	33.33^{a}	$5.56b^{c}$	11.11^{b}	9.49
	Survival (%)	100.0^{a}	97.2^{ab}	75.0^{c}	66.67c	94.44^{ab}	88.89^{b}	9.49
	DS (%)	0.0^{c}	0.0^{c}	43.06^{a}	40.63a	19.72^{b}	20.56^{b}	7.54

Means in the same row followed by different letters indicate significant difference (LSD at 0.05) **DS**: disease severity. **Ck**: Control without treatment, **S***: Sterptomyces* sp**., R**: *Rhioctonia solani,* **Mc**: *Macrophomina phaseolina*

Growth behaviors of different pepper genotypes under *M. phaseolina* and *R. solani* with and without *Streptomyces* sp. KP109810:

Biotic stress by *R. solani* (R) and *M. phaseolina* (Mc) reduce the growth characters as illustrated by decline in plant height, root length, fresh and dry weight of shoot and root traits of studied pepper genotypes except V1 and V2 genotypes which are considered a tolerant genotypes to R and Mc (Tables 3 and 4). *Streptomyces* sp treatment (S) caused significant increasing of growth characters for all genotypes especially V5. The combined of *Streptomyces* sp. and *R. solani* (S+R) and the combined of *Streptomyces* sp. and *M. phaseolina* (S+Mc) caused beneficial effect and reduce the harmful effect of biotic stress. The results may be illustrate the dual effect of bio-agent *Streptomyces* sp. which induced the increasing of growth regulators production (Gopalakrishnan, 2017) and antioxidant play a vital role in improving physiological and metabolic processes which produced by *Streptomyces* sp. (Borrero-López et al., 2019). Moreover, PGPR can enhance growth characters by many several mechanisms; like inhibition of phytopathogens by produce volatiles (Parisi *et al.,* 2020).

Yield characters: fruit number and Fruits weight/plant traits:

R. solani (R) and *M. phaseolina* (Mc) caused significant decreasing in mean values for fruit number and Fruit weight / plant which are sensitive traits in most studied genotypes (Tables 3 and 4) due to R and Mc. fungi are a harmful soil borne pathogens cause many symptoms such as seedling damping-off, root and stem rot which causing decreasing in quality and quantity of fruits yield, and this suggestion agree with (Wu *et al.,* 2008; El_sharkawy, 2010) While S+R treatment caused significant increasing in mean values of fruits weight / plant for most genotypes as V1 and V2 record (23% and 18%) over R treatment (30% and 3%) over yield under Mc treatment respectively and these results are agreement with Vurukonda *et al.* (2018). The increase of yield in both genotypes might be due to either healthy root system that absorb and supply adequate amount of raw nutrient (Numan *et al*., 2018).

Table (3): Mean values of plant height, fruits number, root length and fresh weight of shoot for five genotypes of pepper under six treatments

	Plant height (cm)					**Fruits number**				
	V1	**V2**	**V3**	**V4**	**V5**	**V1**	**V2**	**V3**	**V4**	**V5**
Con	34.33^{a}	38.00^{a}	32.33^{b}	25.00^{b}	28.00^{b}	5.33^{b}	5.33^{b}	4.67^{ab}	3.67^{ab}	5.33^{ab}
S	36.00^{a}	39.33^{a}	38.67^{a}	30.67^{a}	32.00^{a}	6.80^{a}	6.67^{a}	5.00^{a}	4.33^{a}	5.67^{a}
R	26.67^{bc}	32.67^{b}	29.67^{b}	24.33^{b}	25.67^{bc}	4.67^{cd}	3.67^{c}	2.67^{c}	2.67^{bc}	2.33^{c}
M	25.00^{c}	31.67^{b}	29.67^{b}	23.67^{b}	25.33^{c}	4.00^{d}	3.33^{c}	2.33^{c}	2.33^{c}	2.33^{c}
S+R	34.33^{a}	34.00^{b}	31.67^{b}	25.00^{b}	27.67^{bc}	5.67^{bc}	5.33^{b}	4.67^{ab}	3.00^{bc}	5.00^{ab}
S+M	28.33^{b}	33.00^{b}	31.00^{b}	24.67^{b}	27.00^{bc}	5.00^{bcd}	4.33^{c}	3.33^{bc}	2.67^{bc}	4.33^{b}
L.S.D. 0.05	2.5	2.38	2.89	1.54	2.56	1.54	1.61	1.46	1.03	1.05
	Root Length (cm)					**Shoot fresh weight (cm)**				
Con	13.33^{a}	10.67^{ab}	11.67^{a}	9.67^{a}	13.67^{a}	9.00^{a}	11.00^{b}	14.33^{a}	10.33^{ab}	10.00^{a}
S	10.00^{b}	12.00^{a}	10.67^{a}	9.67^{a}	11.00^{b}	8.67^{a}	13.67^{a}	11.00^{b}	11.33^{a}	7.33^{bc}
R	9.00^{b}	6.67^{d}	5.67^{c}	7.67^{b}	5.67^{cd}	8.33^{ab}	9.00^{d}	7.33^{cd}	7.00^{d}	7.00^{bc}
M	9.00^{b}	7.33^{c}	7.67^{b}	6.00^{c}	6.00^{d}	7.00^{b}	8.67^{d}	6.33^{d}	7.33^{cd}	5.33^{c}
S+R	9.67^{b}	8.67^{c}	10.33^{a}	8.00^{b}	10.67^{c}	8.67^{a}	9.33^{cd}	9.67^{bc}	8.33^{bcd}	8.33^{ab}
S+M	10.00^{b}	9.67^{bc}	10.33^{a}	8.00^{b}	9.67^{cd}	8.67^{a}	11.00^{bc}	10.67^{b}	9.33^{abc}	8.67^{ab}
L.S.D. 0.05	1.54	2.16	2.34	2.03	2.08	1.22	1.61	1.84	2.00	0.42

^{A,b}; different letters at the same column means significant difference between treatments

Table (4): Mean values of fresh and dry weight of root, dry weight of shoot and Fruits weight /plant for five genotypes of pepper under six treatments

	Root Fresh Weight (g)					Shoot Dry Weight (g)				
	V1	**V2**	**V3**	**V4**	**V5**	**V1**	**V2**	**V3**	**V4**	**V5**
Con	7.33^{a}	9.67^{a}	7.67^{a}	6.67^{a}	5.00^{a}	5.73^{a}	8.32^{a}	6.97^{a}	6.60^{a}	5.50^{a}
S	6.33^{ab}	10.00^{a}	7.33^{a}	6.00^{ab}	5.00^{a}	5.53^{a}	7.40^{ab}	5.77^{b}	5.80^{b}	5.43^{ab}
R	5.67^{b}	6.00^{bc}	3.00^{c}	3.33^{c}	3.00^{c}	5.11^{a}	5.40^{cd}	5.00^{c}	4.97^{cd}	3.67^{bc}
M	5.33^{b}	5.00^{c}	2.33^{c}	3.00^{c}	2.00^{d}	4.87^{a}	5.27^{d}	4.47^{d}	4.63^{d}	3.23^{c}
S+R	6.00^{b}	6.67^{b}	5.00^{b}	4.33^{bc}	4.67^{a}	5.43^{a}	6.40^{bc}	5.63^{b}	5.47^{bc}	5.17^{ab}
S+M	5.67^{b}	6.33^{bc}	5.00^{b}	3.67^{c}	4.00^{b}	5.40^{a}	5.47^{cd}	5.47^{b}	5.03^{cd}	5.17^{ab}
L.S.D. 0.05	1.22	1.61	1.84	2.00	0.42	1.16	1.02	0.46	0.68	0.82
	Root dry weight (g)					**Fruits weight /plant (g)**				
Con	3.40^{a}	4.30^{a}	4.00^{a}	3.40^{ab}	3.07^{a}	79.00^{b}	82.00^{b}	66.33^{b}	50.00^{b}	56.67^{a}
S	3.17^{ab}	4.67^{a}	3.97^{a}	3.63^{a}	2.99^{ab}	86.00^{a}	96.33^{a}	73.33^{a}	59.67^{a}	52.33^{a}
R	2.97^{b}	3.07^{b}	2.33^{c}	2.54^{c}	2.33^{d}	56.00^{d}	65.67^{cd}	31.67^{d}	32.33^{d}	28.00^{b}
M	2.93^{b}	2.73^{b}	2.18^{c}	2.41^{c}	2.13^{e}	50.33^{e}	70.67^{c}	34.00^{d}	34.67^{d}	32.33^{b}
S+R	3.10^{ab}	3.17^{b}	3.17^{b}	2.87^{bc}	2.88^{b}	69.00^{c}	77.33^{b}	62.00^{bc}	42.00^{c}	51.67^{a}
S+M	3.07^{b}	3.10^{b}	3.17^{b}	2.64^{c}	2.65^{c}	65.00^{cd}	72.67^{bc}	58.00^{c}	47.33^{bc}	50.67^{a}
L.S.D. 0.05	0.32	0.45	0.79	0.71	0.16	5.13	4.59	5.86	6.2	5.8

^{A,b}; different letters at the same column means significant difference between treatments

Variance components and genetic parameters:

PCV values are more than GCV values under studied conditions due to the environmental condition had an important role in the expression of these characters. GCV values ranged from 2.00 to 24.59 under control condition for dry weight of shoot and fruits weight/plant, respectively, from 6.93 in dry weight of shoot to 63.9 in fruits weight/plant under M stress and 4.61 in fruit number to 31.48 in fresh weight of shoot under R stress (Table 5). h2 values are excellent tools to know the progress which be achieved by breeding methods; high h2valuesunder control, R and Mc conditions are found in Plant height trait (93.78%, 88.46% and 93.03%) and root length trait (81%,77% and 77%) respectively. Fruits number, fresh and dry weight of root have moderate values of h2. While dry weight of shoot and fruit weight have low h2 values according to (Singh, 2001). R and M stress caused decreasing of h2 values. GAM values ranged from 1.06% in dry weight of shoot to 39.99% in fruits weight / plant under control, from 1.63 in fruit number to 56.78 in fresh weight of root under R stress. 8.77 in dry weight of shoot to 122.88 in fruits weight/plant under M stress. These quantitative traits are highly influenced by biotic stresses such as pathogenic fungi, significantly impact agricultural productivity and are known to change photosynthetic process (Samaniego-Gámez *et al*., 2016**)**. The selection for improving genotype based on plant height, fruits weight/plant, and fresh weight of root traits with a relatively high GAM increase the performance of the studied genotypes. Moreover; high GCV and PCV values coupled with high h2 and GAM in fruits number /plant and fruit

Weight can be improved through recurrent selection effectively due to larger gene effects for these traits and least effect of environment so, they (Dinakaran *et al.,* 2012; Usman *et al*., 2014; Zegeye *et al*., 2014)**.**

Table (5): Values of some genetic parameters; GCV, PCV, h2and GAM for growth and yield traits under control, R and Mc conditions

Traits	Plant height	Root length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Fruit weight
Control							
GCV	14.65	10.27	15.47	2	21.99	13.79	12.27
PCV	15.12	11.41	16.73	7.81	30.23	20.17	21.01
h^2	93.78	81.08	85.51	6.58	52.91	46.71	34.08
GAM	29.22	19.06	29.46	1.06	32.95	19.41	14.75
R stress							
GCV	15.02	20.38	7.15	6.97	31.48	9.78	26.38
PCV	15.97	23.17	17.05	10.14	35.95	13.44	35.08
h^2	88.46	77.42	17.59	47.32	76.67	52.99	56.54
GAM	29.1	36.95	6.18	9.88	56.78	14.67	40.86
M stress							
GCV	19.06	17.76	18.2	6.93	49.23	16.34	31.64
PCV	19.77	21.04	24.72	11.29	54.33	19.9	34.37
h^2	93.03	71.23	54.21	37.73	82.12	67.41	84.75
GAM	37.88	30.87	27.6	8.77	91.9	27.63	60.01

Association among Characters:

The phenotypic and genotypic correlations of fruits weight/ plant with other traits are indicated in Tables (6, 7 and 8) under three conditions the genotypic correlations were greater than phenotypic correlation in all the traits. Plant height showed positive significant correlation with all studied traits. While root length trait showed different responses of correlation under control condition while showed high positive significant correlation with all studied traits R stress comparing with control. Moreover, under Mc condition it showed negative significant correlation with all studied traits these results agree with (Rêgo *et al*., 2011). Although fruits number trait showed different forms of correlation with rest traits, fruits weight/plant showed positive significant correlation with all studied traits except root length trait. These findings depending on the nature and magnitude of genetic variability have of important value for planning efficient breeding program to improve the yield potential of genotypes. Information on the association of plant traits with yield is of vital important to breeder in selecting the best genotype (Singh, 2001; Teklu *et al*., 2014). An intense selection in the positive side for number of plant height, fresh and dry weight of shoot and root will improve yield since these traits expressed significant and positive correlation among themselves and with fruits weight/plant (Akbar *et al.,* 2011; Gidey *et al*., 2012).

Table (6): Values of the phenotypic and genotypic correlations of Fruits weight / plant with growth traits under control condition

		Control conditions					
		Root	**Shoot**	**Shoot dry**	**Root fresh**	**Root dry**	**Fruits**
Fruits number	**gr**	-0.66	0.13	-0.26	0.46	0.50	0.79
	pr	-0.24	0.12	-0.10	0.25	0.03	0.76
Root length	**gr**		-0.08	0.85	0.27	0.66	-0.38
	pr		-0.07	0.66	0.10	0.57	-0.17
Shoot fresh weight	**gr**			0.50	0.96	0.61	0.76
	pr			0.34	0.77	0.36	0.67
Root fresh Weight	**gr**				0.90	0.91	0.36
	pr				0.24	0.89	0.15
Shoot dry weight	**gr**					0.28	0.91
	pr					0.24	0.63
Root dry weight	**gr**						0.79
	pr						0.33

Table (7): Values of the phenotypic and genotypic correlations of fruits weight/plant with growth traits under R stress

		R stress					
		Root length	**Shoot fresh weight**	**Shoot dry weight**	**Root fresh weight**	**Root dry weight**	**Fruits weight / plant**
fruits number	**gr**	0.26	5.28	0.61	0.51	0.43	0.74
	pr	0.23	0.13	0.34	0.19	0.33	0.69
root length	**gr**		0.74	0.76	0.48	0.79	0.73
	pr		0.54	0.6	0.98	0.47	0.52
Shoot fresh weight	**gr**			0.64	0.59	0.81	0.64
	pr			0.62	0.91	0.53	0.24
Root fresh Weight	**gr**				0.78	0.98	0.47
	pr				0.72	0.96	0.38
Shoot dry weight	**gr**					0.63	0.12
	pr					0.58	0.08
Root dry weight	**gr**						0.48
	pr						0.41

Table (8): Values of the phenol typic and genotypic correlations of fruits weight /plant with growth traits under Mc stress

		M stress					
		Root length	**Shoot fresh weight**	**Shoot dry weight**	**Root fresh Weight**	**Root dry weight**	**Fruits weight / plant**
Fruits number	**gr**	-0.65	0.65	0.61	0.61	0.55	0.84
	pr	-0.49	0.47	0.48	0.46	0.38	0.82
Root length	**gr**		-0.63	-0.66	-0.4	-0.64	-0.97
	pr		-0.58	-0.6	-0.38	-0.61	-0.67
Shoot fresh weight	**gr**			0.94	0.83	0.5	0.78
	pr			0.51	0.74	0.47	0.65
Root fresh Weight	**gr**				0.58	0.98	0.85
	pr				0.5	0.92	0.66
Shoot dry weight	**gr**					0.43	0.86
	pr					0.4	0.61
Root dry weight	**gr**						0.85
	pr						0.59

CONCLUSION

Streptomyces sp. KP109810 and its antagonistic effects with *R. solani*, and *M. phaseolina* is considered ideal method for control root rot diseases of pepper. Through known the relationship between growth and yield traits and estimate some genetic parameters aid to obtain and /or select good genotypes have desirable traits which can growth and produce high yield under these stress conditions.

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المكافحة البيولوجية بواسطة *Streptomyces* sp. KP109810 وبعض التراكيب الوراثية المختلفة للفلفل (*Capsicum annuum* L.) على أمراض تعفن الجذور

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أجريت هذه الدراسة من خلال تجربتين معمل وصوبة زجاجية في قسم النبات الزراعي ومركز التحكم البيولوجي بكلية الزراعة جامعة قناة السويس. تم إخضاع *Streptomyces* sp. KP109810 (S) لفحص شامل في المختبر لخصائص تعزيز النمو المختلفة وبناءً على النتائج التي تم الحصول عليها، تم اختياره كمضاد للفطريات المسببة لأمراض تعفن جذور نبات الفلفل. *Rhizoctonia solani* (R) و *Macrophomina phaseolina* (Mc) . أظهرت *Streptomyces* sp. KP109810 (S) وجود جينات PKSII و NRPS عن طريق PCR ولوحظ بعض من نواتج التمثيل الغذائي الثانوية بواسطة TLC. أظهرت *Streptomyces* sp. KP109810 (S) تضادًا قويًا ضد فطريات الاختبار (R و Mc) حيث أظهر تثبيطًا بحد أقصى ٨٤.٦٪ و ٧٨.٧٪ على التوالي وفي قياسات طبق المزرعة المزدوجة نتيجة للمركبات القابلة للانتشار المنتجة بواسطة (S) ، تم تسجيل التأثير المثبط للمركبات المتطايرة حتى ٧٨.٢ ٪ مقابل (R) و ٦٦.٣٪ ضد (Mc) ودراسة بعض خصائص تعزيز نمو النبات المتعددة مثل (IAA) ، نشاط إذابة الفوسفات ، وانتاج حمض السيانيد و الأمونيا و خالبات الحديد. تجربة الصوبة الزجاجية تم زراعة خمسة تراكيب وراثية من الفلفل ومعاملتها بستة معاملات [الكنترول، (S)، (R)، (Mc)، (S + R) و (S + Mc)]. أظهرت النتائج انخفاضًا معنويًا في القيم المتوسطة لمعظم الصفات المدروسة لجميع التراكيب الوراثية للفلفل تحت معاملات R وMc بينما اظهر التركيبين Saidah (V2) وSuper Mard (V1) على التوالي القدرة على مقاومة أمراض تعفن الجذور. اثبتت المعاملة ببكتيريا *Streptomyces* sp. كاحد عوامل المكافحة البيولوجية كفاءة فى خفض نسبة موت البادرات وشدة الاصابة باعفان الجذور. كما أظهرت النتائج زيادة معنوية في القيم المتوسطة لمعظم صفات النمو والمحصول تحت معاملات (S) حيث ان هذه المعاملة نتج عنها تأثيرات تحفيزية وخفضت من الآثار الضارة للفطريات. يوجد ارتباط موجب بين صفة وزن الثمار/النبات ومعظم الصفات المدروسة بينما لديها ارتباط سالب مع صفة طول الجذر. طول النبات ووزن الثمار/نبات والوزن الغض للمجموع الخضري والجذري صفات لها درجة عالية من التوريث مقرونة بقيم عالية للتقدم الجيني GAM يمكن اعتبارهم من المقاييس الجيدة لانتخاب تراكيب وراثية مقاومة لأمراض تعفن الجذور في الفلفل.