Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Bacterial symbionts of entomopathogenic nematodes against plant pathogenic fungi

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ARTICLE INFO
Article History
Received: 20/7 /2023
Accepted:30 /9/2023
Keywords

Fusarium oxysporum, *Photorhabdus luminescens*, *Xenorhabdus nematophilus* and biological control.
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Abstract

Fungi, as one of the primary causes of plant diseases, significantly impede the development of agronomic crops. The use of synthetic pesticides has negative consequences for the environment. Bacterial symbionts of entomopathogenic nematodes are one of the most effective and eco-friendly biocontrol agents for treating plant diseases. This study's goal was to evaluate the effectiveness of cell-free filtrates of *Photorhabdus* and *Xenorhabdus* bacteria against plant pathogenic fungi. Cell-free filtrates of seven entomopathogenic nematodes bacteria (EPNsB) at 100% crude, non-diluted, and varying dilutions of the filtrates, such as 50% and 25%, were examined in vitro and under greenhouse conditions for their potential antifungal efficacy against three phytopathogenic fungi. *Photorhabdus luminescens* EGAP2 cell-free filtrate was able to provide the strongest antimycotic effect. All of the cell-free filtrates significantly reduced the incidence of fungal disease under greenhouse conditions. The most effective cell-free filtrate was created from *Xenorhabdus nematophilus*, and it reduced the disease incidence in *Sclerotium rolfsii*-infested soil to a minimum of 14.44 percent. The soil that was both infected with *Fusarium oxysporium* and treated with a filtrate from *P. luminescens* EGAP5 had the highest shoot and root dry biomass measurements, which increased by almost 200 percent and 100 percent, respectively, in comparison to the control. The data clarified that all seven symbiotic bacterial cell-free filtrates exhibited potent antifungal activities against the phytopathogenic fungi. For all filtrates, their antimycotic effect was concentration dependent. In conclusion, bacterial symbionts of entomopathogenic nematodes can be an optimal option for the biocontrol of plant pathogenic fungi.

Introduction

Agrochemicals are considered one of the most important pollutants in the world. Also, besides the high toxicity of most insecticides, fungicides and herbicides to most living organisms present in the environment, and also besides its clear negative effects on the environment. Entomopathogenic nematodes (EPNs) belong to two families; *Steinernematidae* and

Heterorhabditidae considered obligate parasites for many different pathogens. They can kill different pests with their bacterial symbionts, *Photorhabdus* sp. (Boemare *et al*., 1993) (Enterobacterales: Morganellaceae) and *Xenorhabdus* sp. (Thomas and Poinar, 1979) (Enterobacterales: Morganellaceae), which live in their intestine.

The associated symbiotic bacteria in the genera *Xenorhabdus* and *Photorhabdus* release many virulence factors, including complexes of toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds (Eleftherianos *et al*., 2010). Cultured *Photorhabdus* and *Xenorhabdus* contain bioactive, antimicrobial metabolites that have active effects against a wide range of microbial pathogens of animals and plants such as bacteria, oomycetes, and fungi (San-Blas *et al*., 2012). Another two peptides were isolated, namely cabanillasin and nemaucin; these peptides were isolated from *X. cabanillasii* and have shown significant bioactivity. Entomopathogenic bacteria called *Xenorhabdus* spp. produce secondary metabolites that have antibacterial potential and can be used in agricultural productivity. The strain *X. nematophila* TB culture's ability to prevent *Botrytis cinerea* (Persoon, 1794) (Helotiales: Sclerotiniaceae) plant pathogen growth was assessed. The mycelial growth of the pathogen was strongly inhibited (By 90%) by the cell-free filtrate of the TB culture.

This indicated that *X. nematophila* TB produces antimicrobial metabolites which have clear activity on plant pathogens, with great potential for control operations of tomato grey mould and pepper leaves scorch and can be used in integrated pests and disease control to reduce chemical uses (Fang *et al*., 2014). Bock *et al*. (2014) indicated to identified transcinnamic acid from *P. luminescens* (Thomas and Poinar, 1979) (Enterobacterales: Morganellaceae) and examined it against a pecan fungal pathogen, and also indicated to the inhibitory activity at a concentration of 148 to 200 g/mL. Ullah *et al*. (2015) indicated to isolated and characterized benzaldehyde (an aromatic aldehyde) which was isolated from *P. temperate* M102 (Fischer *et al*., 1999) (Enterobacterales: Morganellaceae).

Authors also tested activity of this molecule against three fungal phytopathogens, namely, *Phytophthora capsici* (Leonian, 1922) (Peronosporales: Morganellaceae), *Rhizoctonia solani* Kühn, 1858 (Pronicheva, 2014) (Cantharellales: Ceratobasidiaceae), and *Corynespora cassiicola* (Wei, 1950) (Pleosporales: Corynesporascaceae), show their ability to inhibit their growth in *in vitro* assays. Cell-free culture filtrates of three *Photorhabdus* strains (*Photorhabdus temperata*, *P. luminescens* (VS), and *P. luminescens* (K122)) can inhibit the vegetative growth of a variety of economically important phytopathogenic fungi, including *Fusicladium carpophilum* (Fisher,1961) (Pleosporales: Venturiaceae) (Peach scab), *Fusicladium effusum* (Gottwald, 1982) *(Capnodiales: Mycosphaerellaceae)* (Pecan scab), and *Monilinia fructicola (*Edwin and Fisher 1928) (Helotiales: Sclerotiniaceae) (Brown rot) (Hazir *et al*., 2016). The crude extracts of *P. luminescens sonorensis were evaluated* against two fungal pathogens, *Fusarium oxysporum* f.sp. asparagi (Cohen, 1946) (Hypocreales: Nectriaceae) and *Alteraria alternata* Keissl (Zitter and Wien, 1984 and Ruelas *et al.,* 2006) indicated to in vitro inhibition assays (Orozco *et al*., 2016). In this work, it was planned to examine the antagonistic effects of these bacteria against some fungi.

Materials and methods

The experiments were carried out in two phases, EPNs bacterial cell-free filtrate of *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P.*

luminescens (EGAP4), *P. luminescens* (EGAP5), *P. luminescens subsp. laumondii* HP88 and *X. nematophilus* were evaluated in the laboratory and in pot experiments to test their antagonistic effects on *Fusarium oxysporum* f.sp., *lycopersici* (Snyder and Hansen, 1940) (Hypocreales: Nectriaceae), *Fusarium solani* (Saccardo, 1881) (Hypocreales: Nectriaceae), and *Scloritium rolfsii* (Tu and Kimbrough,1978) (Atheliales: Atheliaceae).

1. Microorganisms:

Fusarium oxysporum f. sp., *lycopersici*, *Fusraium solani*, and *Scloritium rolfsii* from the culture collection of the Department of Plant Pathology, Faculty of Agriculture, Cairo University were used as test fungi to study the antimycotic activities of the isolated entomopathogenic nematodes bacteria (EPNsB) strains. *Photorhabdus luminescens* (EGAP1) MH368153, *Photorhabdus luminescens* (EGAP2) MH368154, *Photorhabdus luminescens* (EGAP3) MH368155, *Photorhabdus luminescens* (EGAP4) MH368156 and *Photorhabdus luminescens* (EGAP5) MH368157 and P. *luminescens subsp. laumondii* HP88 and *X. nematophilus* strains obtained from Pest Physiology Dept., Plant Protection Research Institute, Agricultural Research Centre, Egypt.

2. Soil:

Soil mixture, i.e., clay and sand (3:1). The clay soil was taken from a farm inside in the Agricultural Research Center, Dokki, Giza, Egypt.

3. Plant materials:

Thirty-day-old seedlings of the tomato cultivar E603 F1 (Logain) were kindly supplied by the seedling selling center in Qalubeia Governorate.

4. Cultural media:

Slants of Lauria Bertani (LB) Agar, Bertani (2004) were used for repeated sub-culturing and maintenance of EPNsB. According to Harold (1998), potato dextrose agar (PDA) was used for cultivating fungi. Submerged fermentation to produce bacterial secondary metabolites were performed using the yeast starch glucose (YSG) fermentation medium described by Sundar and Chang (1993).

5. Antimycotic activity of cell-free filtrates against three plant pathogens:

As an inoculum for a 50 ml culture, single colonies of EPNsB were introduced to 3 ml of LB media. The inoculum was placed in 250-ml Erlenmeyer flasks containing 50 ml of YSG medium and agitated for three days at 150 rpm for maximal aeration for 24 hrs. at 28 °C. Using a spectrophotometer set to 600 nm, the 72-hour-old growing bacterial cultures were calibrated to 4 x 107 cells mL. To obtain filtrates, they were then centrifuged at 10,000 rpm for 20 minutes at 40 °C. The obtained culture filtrates were thought to have a 100% concentration.

By adding the necessary distilled water aliquots, different dilutions of the filtrates (i.e., 50% and 25% of EPNsB-free filtrate) were created. A total of 100 ml of sterile, melted potato dextrose agar (PDA) medium was combined with 15 ml of bacterial filtrate from each dilution before being put into sterile Petri dishes. As controls, sterile PDA medium plates without bacterial filtrate were kept. On PDA media, five-mm mycelial disc plant pathogenic fungal cultures were positioned in the middle of Petri plates and incubated at 262 0C for the growth of the inoculation fungus. For each treatment, three replications were kept, and seven days after inoculation, the growth of the fungi was observed and documented.

The percentage of reduction in the mycelial growth of the pathogenic fungi was calculated by the following formula: [Inhibition Percentage (%) = (I–T/C) x 100]; where: I = inhibition zone of pathogen growth by antagonists; T = radial growth in the treatment; and C = radial growth in the control (Deans and Svoboda, 1990).

6. Evaluation of the EPNsB cell-free filtrates efficiency under greenhouse conditions:

Pots of 20 cm diameter and 20 cm depth were sterilized by immersion in formalin solution (0.5%) followed by aeration for 2 weeks. Every pot was filled with 4 kg of soil mixture, i.e., clay and sand (3:1). The soil mixture was sterilized by mixing it with a 0.5 percent formalin solution and covering it with polyethylene sheets. After 3 days, the covers were removed; ventilation continued for 14 days. The pathogenic fungi were grown on an autoclaved corn sand meal medium (Abd El-Ghany, 2001).

Flasks containing corn sand meal were inoculated with discs (Diameter 5 mm) taken from 7-day-old cultures of each successive fungi. The inoculated flasks were incubated at 252 C for 14 days. The sterilized soil was individually infested with the successive fungi at a rate of 5% of the soil's weight. The added inoculum was thoroughly mixed with the soil and regularly watered for ten days before planting to ensure the distribution of inoculum growth. Two ml of crude, non-diluted, cell-free filtrate of each EPNsB strain was applied to each seedling in the soil in spots (5 cm diameter and depth). Flasks containing corn sand meal were inoculated with discs (Diameter 5 mm) taken from 7-day-old cultures of each successive fungi. The inoculated flasks were incubated at 252 C for 14 days. The sterilized soil was individually infested with the successive fungi at a rate of 5% of the soil's weight. The added inoculum was thoroughly mixed with the soil and regularly watered for ten days before planting to ensure the distribution of inoculum growth. Two ml of crude, non-diluted, cell-free filtrate of each EPNsB strain was applied to each seedling in the soil in spots (5 cm diameter and depth). Four tomato seedlings thirty days old were planted both in each pot and each treatment contains four pots. Other groups of pots contained only pathogenic fungi and were kept as controls. The disease incidence was calculated after 45 days by dividing the number of wilted plants by the total number of plants in all pots and multiplying by 100, according to Haq *et al*. (2007). Data was analyzed by analysis of variance. The 45-day tomato seedlings were uprooted, fresh and dry roots and shoot weight were measured.

7. Statistical analysis:

Current experiments were conducted in triplicate and arranged in a random design. An arcsine transformation was used to normalise data presented as percentage values. Significant differences in the main effects were determined by the analysis of variance (ANOVA). Duncan's multiple range test (P 0.05) was used to determine significant differences between various treatments. Current analyses were conducted using the software package "Costat,". Results were recorded by means of three replications.

Results and discussion

1. Antimycotic activity of cell-free filtrates against three plantpathogens:

The crude non-diluted cell-free filtrates 100% and different dilutions of the filtrates i.e., 50%, and 25% of the seven symbiotic bacterial, *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), *P. luminescens* (EGAP5), *P. luminescens subsp. laumondii* HP88 and *X. nematophilus* were *in vitro* tested for their possible antifungal activity against three phytopathogenic fungi, *Fusarium oxysporum* f. sp., *lycopersici*, *Fusraium solani*, and *Scloritium rolfsii*.

All seven symbiotic bacterial strains exhibited potent antifungal activities against the phytopathogenic fungi. Therefore, this experiment was carried out to quantify the *in vitro* antifungal activities of diluted cell-free filtrates against the three phytopathogenic fungi. Fugal growth inhibition was expressed

as the inhibition percentage of the fungal growth on PDA agar amended with the cell-free filtrate compared with the full fungal growth without filtrate on PDA plates Table (1). Irrespective of the bacterial strain or the cell-free dilution rate, the growth of the examined fungal strains was inhibited by the metabolites produced by the EPNsB, with maximum inhibition recorded in plate cultures of *S. rolfsii*. Growth of this fungal strain was inhibited by 57.96%.

F. solani tolerated the cell-free filtrate introduced in PDA plates and grew at a 37.012% slower rate than on plain PDA plates. Growth of *F. oxysporum* on PDA was the least inhibited by the EPNsB cell-free filtrates, showing as low as 30.66% lower growth compared with control plates (Figure 1). Regardless of the test fungi and the filtrate dilution, the overall mean (±SE) percentage of fungal growth inhibition by the bacterial cell-free culture filtrates was significantly different (df = 2, F = 1029.61, P = 0.0000) according to the bacterial genotype (Figure 2).

The most potent antimycotic effect could be achieved using the EPNsB strain *P. luminescens* (EGAP2), while the lowest was observed in PDA plates supplemented with cell-free filtrate from *P. luminescens* (EGAP5). For all strains, their antimycotic effect was concentration-dependent, as the maximum inhibition was obtained using the crude, non-diluted filtrate (100%) and the lowest was recorded using the highly diluted ones (Figure 3 and Figures 4–10).

Table (1): Fungal growth inhibition by different dilutions of the EPNs bacterial cell-freefiltrate.

Bacteria species	Dilution %	*Scloritium rolfsii*	*Fusarium oxysporum*	*Fusraium solani*
***Photorhabdus luminescens* (EGAP1)**	25	39.33±0.6	8.6±0.8	23.5±0.7
	50	61.6±1.2	17.3±1.2	42±1.1
	100	72.8±1.7	62.15±2.5	44.8±2.8
***Photorhabdus luminescens* (EGAP2)**	25	47.3±2	10±1	32±1.4
	50	66±2.3	25.3±1.4	49±1.5
	100	76±2.6	71.5±1.7	49.1±2
***Photorhabdus luminescens* (EGAP3)**	25	37±1.2	7.5±0.5	21.8±1.4
	50	58.8±1.7	14.8±1.9	33.5±4.5
	100	69.3±1.2	58.6±3.1	40.1±3
***Photorhabdus luminescens* (EGAP4)**	25	44.16±2.6	9.3±1.2	29±1.5
	50	63.5±2.7	23.3±2.6	45±1.5
	100	72.5±2.7	69.7±0.9	47±1.6
***Photorhabdus luminescens* (EGAP5)**	25	33.8±1	8±0.2	22±2
	50	56.6±1.4	13±1.0	27.5±4.2
	100	67.8±1.6	56.8±2.1	38.5±3.9
***Photorhabdus luminescens subsp. laumondii* HP88**	25	38.8±1.5	9.1±0.6	24±1.5
	50	64±1.7	19.5±1.2	44±1
	100	74.6±1.2	64±2.6	47.8±1.8
Xenorhabdus nematophilus	25	41.5±2	8±0.5	27±1.3
	50	61.6±2.7	21±2.5	42.3±1.7
	100	69.8±2.3	65±1.7	44.3±2.4

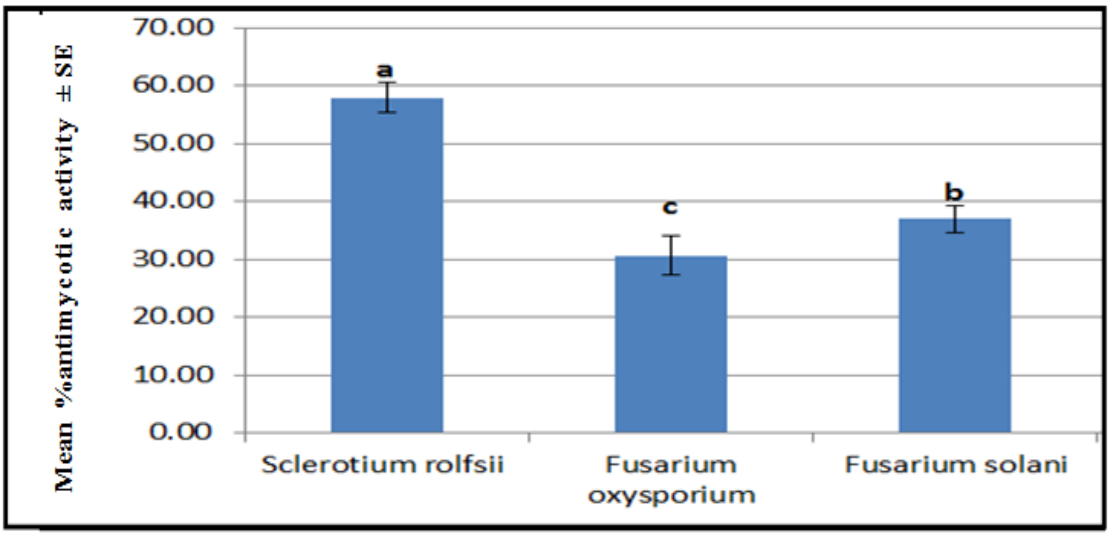

Figure (1): Mean % antimycotic activity as affected by three plantpathogens.

Bars with the same letter(s) are not significantly different (P<0.05).

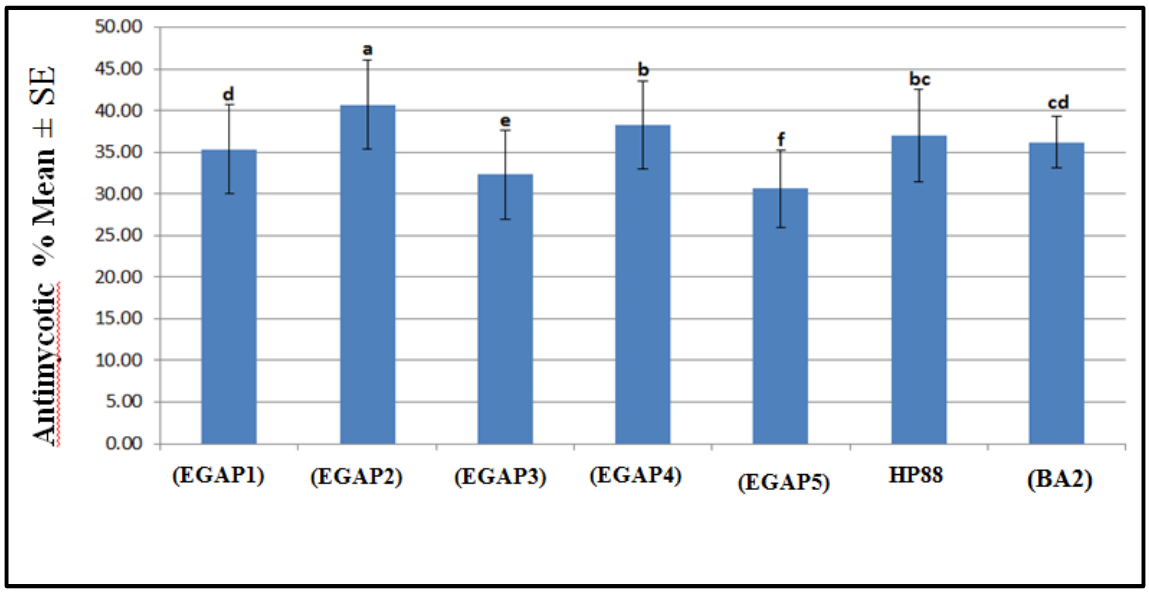

Figure (2): Mean % antimycotic as affected by different EPNsBstrains.

Bars with the same letter(s) are not significantly different (P<0.05).

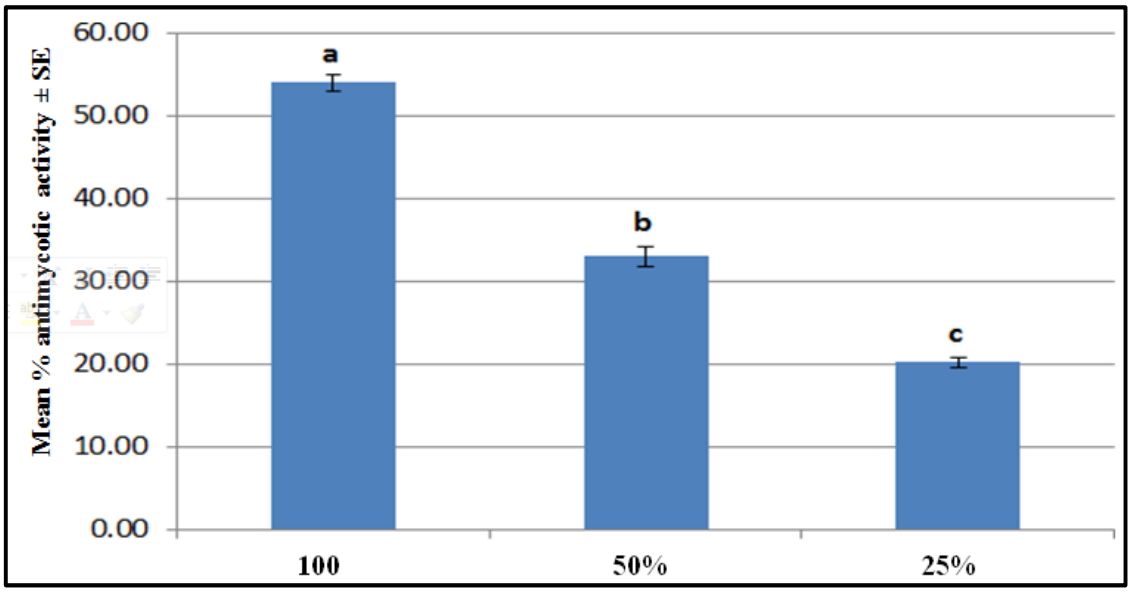

Figure (3): Mean % antimycotic activity as affected by different dilutions.

Bars with the same letter(s) are not significantly different (P<0.05)

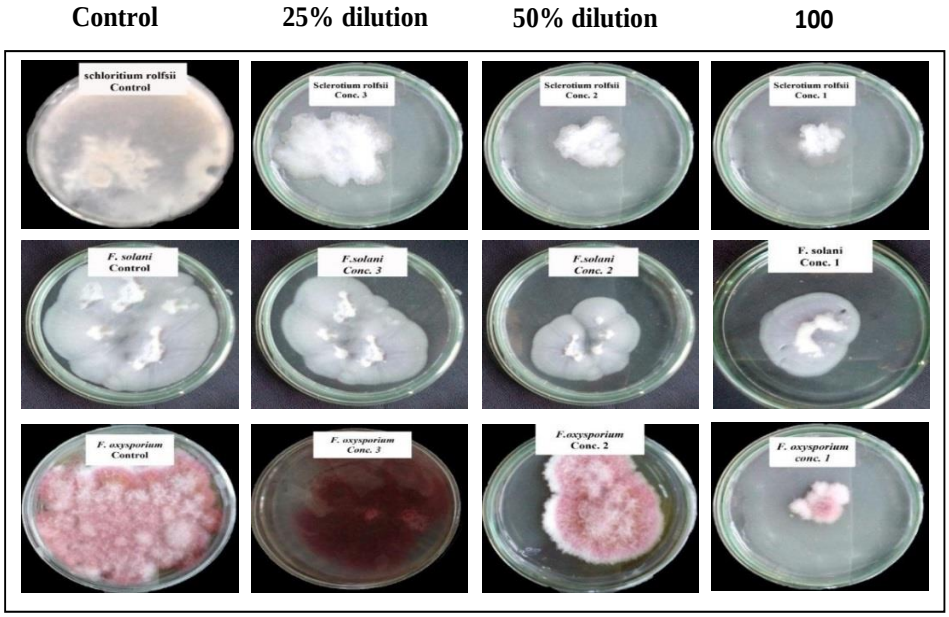

Figure (4): *In vitro* evaluation of cell-free filtrates (100, 50 and 25% dilution) from *Photorhabdus luminescens* (EGAP1) against three plant pathogenic fungi.

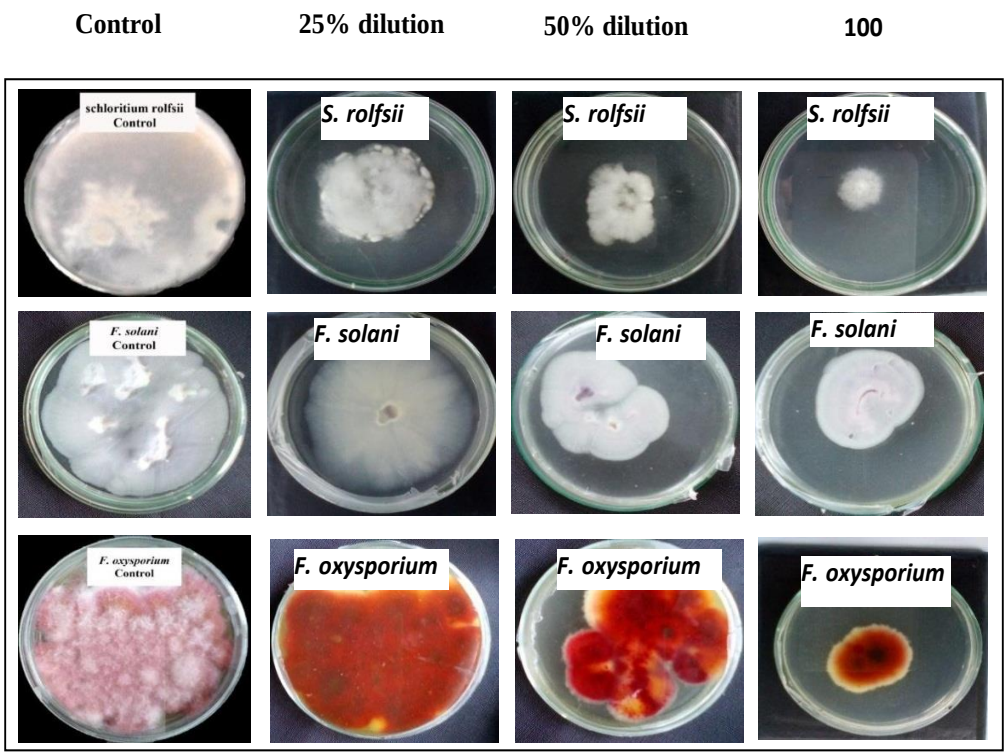

Figure (5): ***Photorhabdus luminescens*** **(EGAP2) cell- free filtrates against three plant pathogenic fungi.**

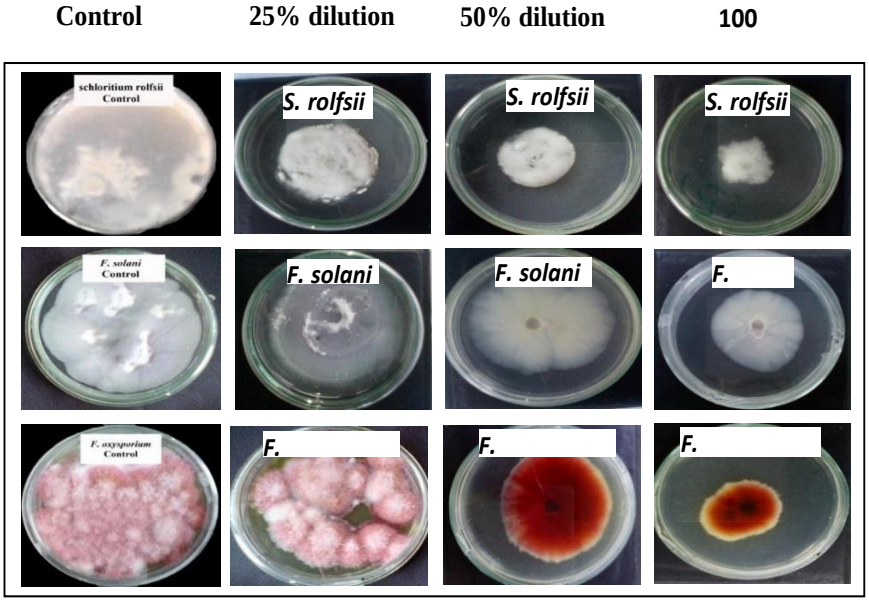

Figure (6): ***Photorhabdus luminescens*** **(EGAP3) cell- free filtrates against three plants.**

Control 25% dilution 50% dilution 100

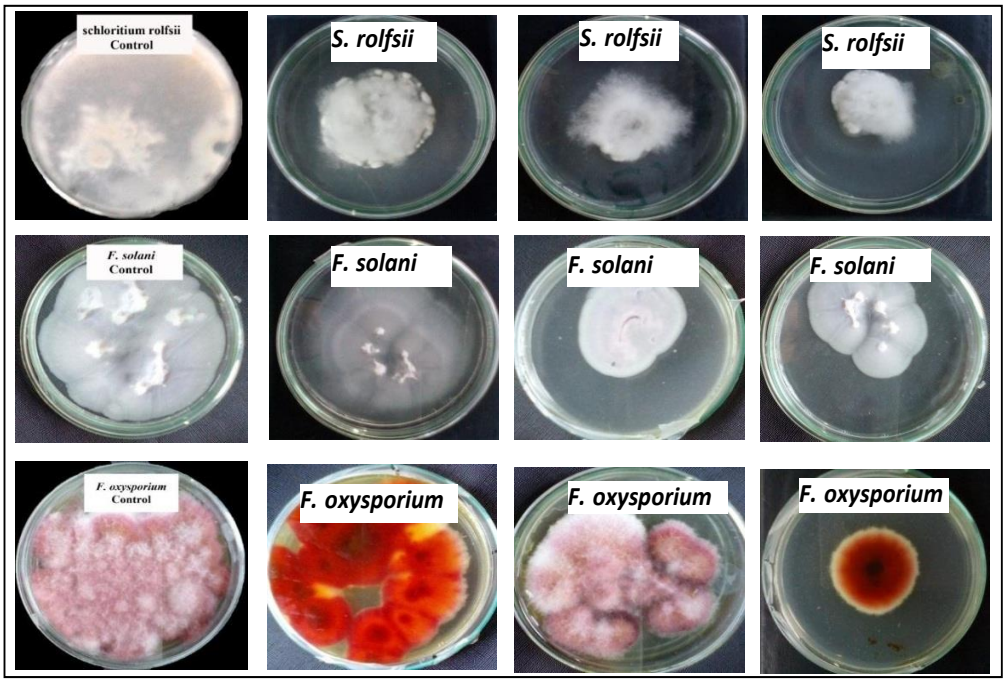

Figure (7): ***Photorhabdus luminescens*** **(EGAP4) cell- free filtrates against three plant pathogenic fungi**

Control 25% dilution 50% dilution 100

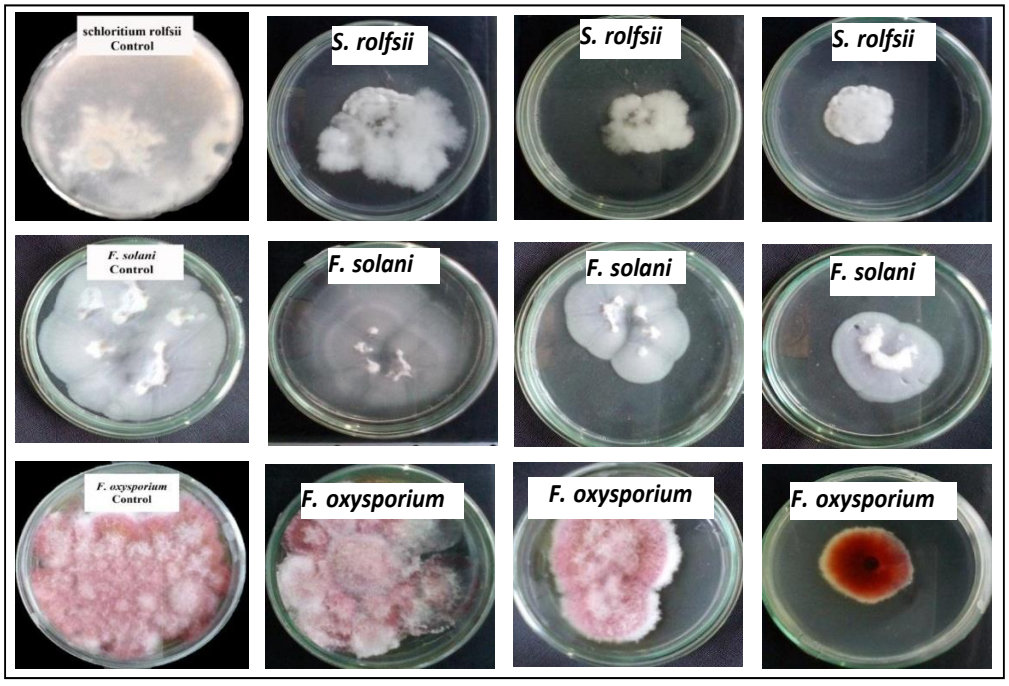

Figure (8): ***In vitro*** **evaluation of cell- free filtrates (100, 50 and 25% dilution) from** ***Photorhabdus luminescens*** **(EGAP5) against three plant pathogenic fungi.**

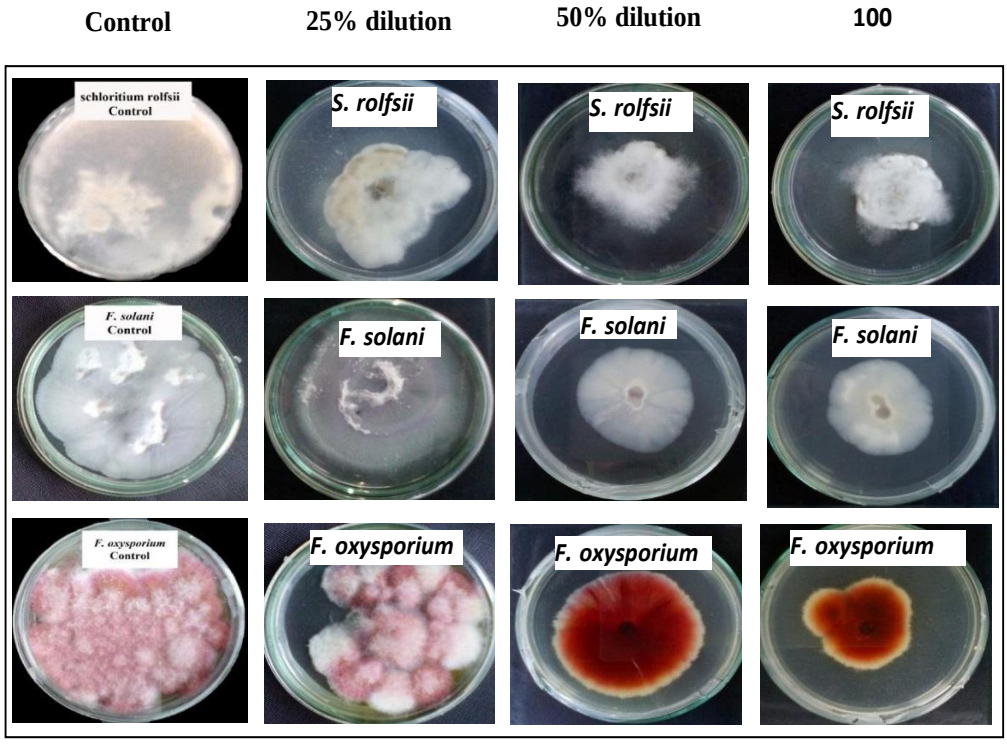

Figure (9): *In vitro* evaluation of cell- free filtrates (100, 50 and 25% dilution) from *Photorhabdus luminescens* subsp. *laumondii* HP88 against three plant pathogenic fungi.

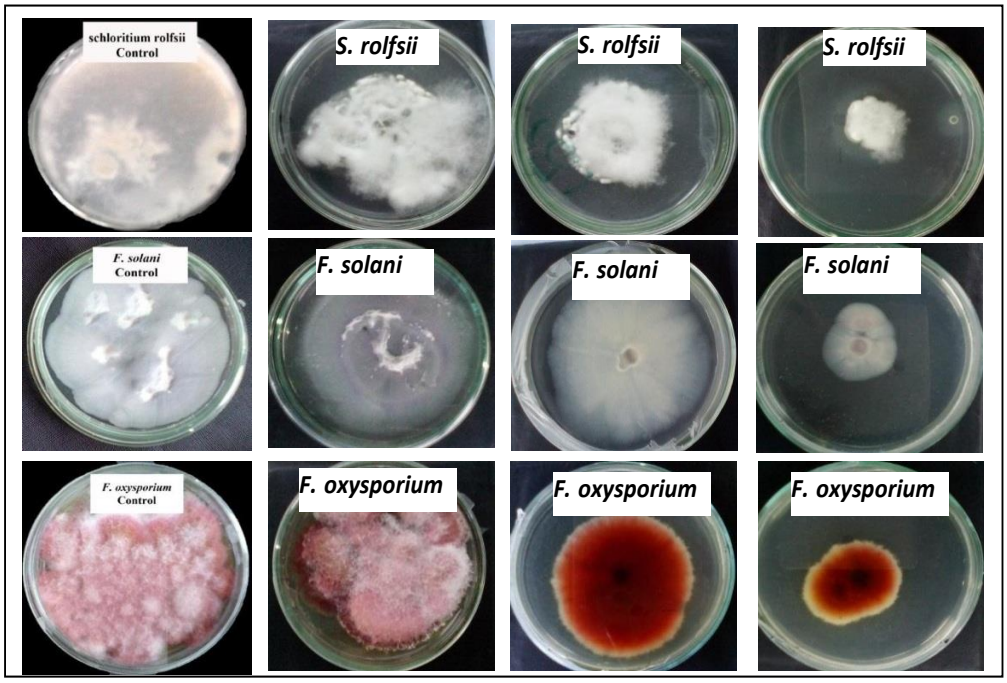

Figure (10): *In vitro* evaluation of cell- free filtrates (100, 50 and 25% dilution) from *Xenorhabdus nematophilus* BA2 against three plant pathogenic fungi.

2. Evaluation of the entomopathogenic nematodes bacteria (EPNsB) cell-free filtrates efficiency under greenhouse conditions:

In a greenhouse experiment, the potential use of the EPNsB strains to lessen tomato plant wilt caused *by F. solani*, *F. oxysporium*, and *S. rolfsii* was investigated. Application of bacterial cell-free filtrates to soil containing or lacking pathogenic fungi allowed for the measurement of the prevalence of wilt in plants as well as the measurement of root and shoot biomass in healthy plants (Table 2).

Control plants infested with *F. oxysporium* suffered from severe wilt incidence, with only 25% of seedlings surviving the fungal infestation. The incidence occurred in high percentages in the control plants infested with the phytopathogens *F. solani* and *S. rolsii as well*. More than 60 and 67% of control plants in the soil infested with *Sclerotium rolfsii* and *Fusarium solani* demonstrated root rot symptoms and wilt, respectively. However, all the applied cell-free filtrates from cultures of *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), *P. luminescens* (EGAP5), *P. luminescens subsp. laumondii* HP88, and *X. nematophilus* BA2 significantly decreased the incidence of the disease.

The most effective cell-free filtrate was prepared from the broth culture of *X. nematophilus*, by which the disease incidence was reduced to a minimum of 14.44 percent in the soil infested with *S. rolfsii*. Using such a strain decreased the disease incidence to minimal values of 25.6% and 27.8% in the soils infested with *F. solani* and *F. oxysporum*, respectively. The obtained results (Figure 11) refer to the superiority of *F. oxysporum* as a potent root rot fungus when compared with the other phytopathogens. This was clear not only in control plants but also with the application of the biocontrol EPNsB strains. With the application of these bacterial strains, the disease occurred in a higher range of 27.8–40% in the soil infested with *F. oxysporium* compared with a range of 25.55–32.22% in the soil infested with *F. solani* and 14.44–27.77% for plants grown with the EPNsB in the soil infested with *S. rolfsii*.

From the data illustrated in (Figure 12), significant differences were observed between plants supplied with the examined EPNsB with respect to their ability to survive the root rot disease caused by the examined fungi ($df = 7$, $F = 66.94$, $P = 0.0000$). However, plants receiving *X. nematophilus* demonstrated significantly higher survival rates compared with plants grown with *photorhabdus*. Plants in control pots infested with the three root rot fungi had the smallest fresh and dry root and shoot weights (Table 2). In contrast, remarkable improvements in plant biomass were recorded in pots that received filtrates from cultures of the examined strains. However, the highest shoot and root dry biomass were recorded in the soil infested with *F. oxysporium* and amended with a filtrate from the culture of the bacterial strain *P. luminescens* EGAP5. These plants showed >200% and 100% increases in shoot and root dry biomass compared with their control plants without cell-free filtrates, respectively.

Table (2): Root and shoot biomass and disease incidence in tomato seedlings.

Bacterial species	*Fungi infestation*	Disease incidence%	Fresh weight (g. plant ^{1})		Dry weight (g.plant^{-1})	
			Shoot	Root	Shoot	Root
***Photorhabdus luminescens* (EGAP1)**	***Fusarium oxysporum***	33.3	29.28	3.95	1.74	2.45
	Fusraium solani	26.39	10.06	4.23	1.84	2.68
	Scloritium rolfsii	20.83	9.38	3.98	1.65	2.53
***Photorhabdus luminescens* (EGAP2)**	***Fusarium oxysporum***	34.72	8.28	3.48	1.48	2.21
	Fusraium solani	29.00	9.15	3.82	1.68	2.41
	Scloritium rolfsii	25.00	9.82	4.11	1.76	2.61
***Photorhabdus luminescens* (EGAP3)**	***Fusarium oxysporum***	31.94	9.30	3.96	1.67	2.52
	Fusraium solani	26.39	7.49	3.11	1.39	2
	Scloritium rolfsii	25.00	9.77	4.14	1.82	2.54
***Photorhabdus luminescens* (EGAP4)**	***Fusarium oxysporum***	40.00	9.15	3.82	1.66	2.45
	Fusraium solani	27.22	9.51	4.03	1.74	2.50
	Scloritium rolfsii	23.30	9.19	3.85	1.68	2.44
***Photorhabdus luminescens* (EGAP5)**	***Fusarium oxysporum***	34.44	11.04	4.6	2.05	2.91
	Fusraium solani	25.55	9.97	4.2	1.76	2.67
	Scloritium rolfsii	27.78	9.12	3.87	1.67	2.4
***Photorhabdus luminescens* subsp. *laumondii* HP88**	***Fusarium oxysporum***	27.78	8.28	3.46	1.51	2.2
	Fusraium solani	32.22	8.56	3.63	1.63	2.31
	Scloritium rolfsii	22.22	8.24	3.45	1.57	2.19
Xenorhabdus nematophilus	***Fusarium oxysporum***	27.78	7.5	3.19	1.41	2
	Fusraium solani	25.55	9.17	3.81	1.63	2.45
	Scloritium rolfsii	14.44	10.01	4.21	1.82	2.68
Control	***Fusarium oxysporum***	75.33	4.27	1.91	0.9	0.39
	Fusraium solani	67.20	3.59	1.01	0.72	0.36
	Scloritium rolfsii	60.43	4.51	1.28	0.95	0.41

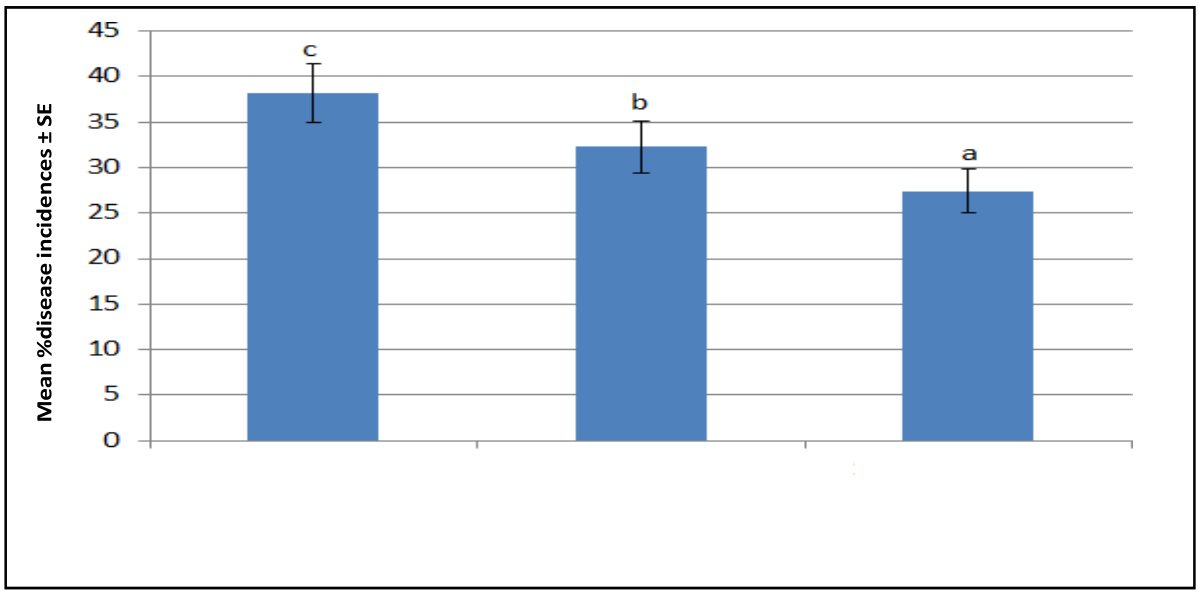

Figure (11): Mean disease incidence % as affected by three plantpathogens.

Bars with the same letter(s) are not significantly different (P<0.05).

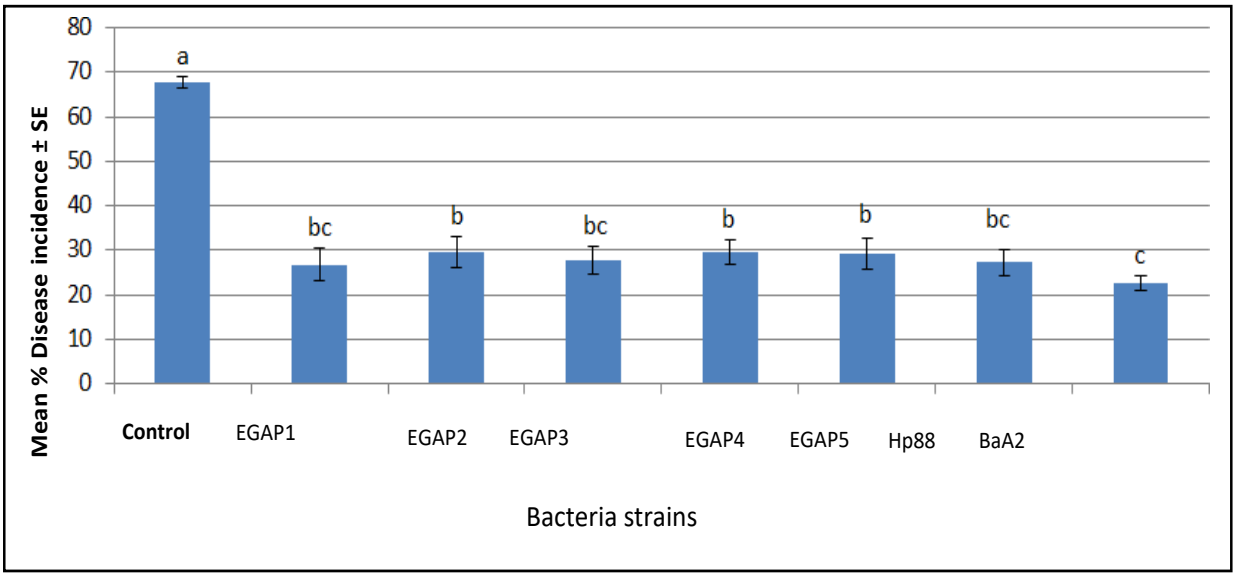

Figure (12): Mean disease incidence % as affected by different EPNsBstrains.

Bars with the same letter(s) are not significantly different (P<0.05).

The current study, the bacterial symbionts of the entomopathogenic nematode may offer a new source for natural products with applications not only in agriculture but also in the medical field. Although of the different importance of microbial natural products for human health, only a few bacterial genera have been mined for the new natural products needed to overcome the urgent threat of antibiotic resistance, antimycotic activity, and biocontrol agents. The present study indicated the different examples of bacteria that should be considered new sources of natural products, although the first natural products from *Photorhabdus* and *Xenorhabdus* bacteria have been known for almost 30 years and a huge variety of new compounds have been identified in the last 5–10 years (Bozhüyük *et al*., 2016). Exploitation of these producer strains, combined with advances in modern natural product research methodology, has the potential to open the way for a new golden era of microbial antifungal products. All cell-free filtrate of the seven symbiotic bacterial strains EGAP1, EGAP1, EGAP1, EGAP1, HP88, and BA2 has antimycotic activities against *S. rolfsii*, *F. solani*, and *F. oxysprium*, according to the results of in vitro and greenhouse experiments.

These results are in harmony with those obtained by Hazir *et al*. (2016), who showed that the cell-free culture filtrates of three *Photorhabdus* strains, *Photorhabdus temperata, P. luminescens* (VS), *and P. luminescens* (K122), can inhibit the vegetative growth of a variety of economically important phytopathogenic fungi, including *F. carpophilum* (Peach scab), *F. effusum* (pecan scab), *M. fructicola* (Brown rot), *G. cingulate* (anthracnose), and *Armillaria tabescens* (Root rot), *P. fructicola*. Similar findings were reported by Vanitha *et al.* (2010) regarding the fungistatic activity of EPNs bacteria against *Fusarium oxysporum* (Vanilla), *Alternaria solani* (tomato), *Sclerotium rolfsii* (Brinjal), and *Aspergillus niger* (Ggroundnut).

Data from the greenhouse pot experiment suggests the potential impact of the cell-free filtrate of the bacterial cultures on the occurrence of root rot disease in the soil infested by the three fungal phytopathogens. This was in parallel with the results of the pot experiment conducted by Haq *et al*. (2007) to evaluate the antagonistic effects of *X. nematophila* and *Xenorhabdus* spp. on *F. oxysporum* f. sp., *Lycopersici*. Both strains inhibited the fungus growth on agar plates, and in the greenhouse treatments, each bacterium reduced the disease incidence of tomato plants with significant increases in plant biomass.

The cumulative percent mean inhibition of fungal growth was also significantly different among the three pathogenic fungi tested, which indicates that the response of fungi is not uniform to the bioactive compounds of symbiotic bacterial isolates. *S. rolfsii* was more susceptible to the cell-free filtrates of bacterial isolates than *F. oxysprium*, which is less susceptible. This clearly explains the existence of diverse bioactive compounds in the cell-free filtrate with broad-spectrum activity against a wide range of pathogens. It was concluded that entomopathogenic nematodes bacteria (EPNsB) cell-free filtrate had a high virulence against *F. oxysporum* f. sp., *lycopersici*, *F. solani*, and *S. rolfsii*. The current study aimed to concentrate on the importance of entomopathogenic nematode bacterial symbionts as important and new sources of natural bioactive compounds that can be used in the biological control processes.

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