Optimization of microbial biomass production as biocontrol agent against root knot nematode on faba plants

Zeinat, Kamel M., [1]; Nagwa, M. Atef[1]; El-Sayed, S.A.[2] and Abd El-Wahab G.S.[3]

[1] Botany Department, Faculty of Science, Cairo University, Giza – Egypt
[2] Soils, Water, Environment Research Institute, Agricultural Research Center (ARC), Egypt
[3] National Organization for Drug Control and Research (NODCAR), Egypt
dr_mona_zaki@yahoo.co.uk

Abstract: Our objective was to optimize nutritional and environmental conditions of the isolated *Serratia marcescens* Ba-2 and *Pseudomonas fluorescens* Ba-11 for biomass production and to evaluate the bio-control agents against the root knot disease caused by *Meloidogyne incognita* on Faba bean plants under greenhouse conditions. Glycerol at 10.2 g/L and peptone as a nitrogen source were the most suitable for biomass and antagonistic efficiency of *S. marcescens* or *P. fluorescens* against *Meloidogyne sp*. Cultures of *S. marcescens* and *P. fluorescens* supplemented with 10 g/L peptone, reduced larvae to 91% and 95% respectively. Optimum biomass and antagonistic activity of either bacteria against larvae was at pH 7.6, and incubation temperature at 30°C. 100% reduction of larval density was achieved when *S. marcescens* or *P. fluorescens* cultures were shaken at 120 and 160 rpm respectively. *S. marcescens* and *P. fluorescens* were very effective as biocontrol agaents to reduce the root – knot nematodes. Our data also indicate a marked effect of the biocontrol agents and Rhizobia on the growth response of faba plants. The obtained results showed that both bacterial treatments significantly increased the growth parameters as well as shoot and root dry weights and number of pods. [Journal of American Science 2010; 6(6):245-255]. (ISSN: 1545-1003).

Keywords: Biological control, *Serratia marcescens*, *Pseudomonas fluorescens*, root-knot nematode, rhizobia.

1. Introduction

Root-knot nematodes are major pests that cause serious root damage of fruits and vegetables in temperate and tropical regions. Biological control of plant-parasitic nematodes with microbial agents is an alternative approach that received greater interest among nematologists in last decades, providing effective control against the target nematodes and avoiding environmental pollution.

In their review, Tian et al. (2007) stated that nematophagous bacteria exhibit diverse modes of action: these include parasitizing, producing toxins, antibiotics, or enzymes, competing for nutrients, inducing systemic resistance of plants, and promoting plant health.

Rhizobacteria are a subset of total rhizosphere bacteria which have the capacity, upon reintroduction to seed or vegetative plant parts (such as potato seed pieces), to colonize the developing root system in the presence of competing soil microflora (Kloepper et al. 1999). Fluorescent *Pseudomonas spp*. are among the most effective rhizosphere bacteria in reducing soil-borne diseases in disease – suppressive soils (Weller, 1988), where disease incidence is low, despite the presence of pathogens and environmental conditions conductive to disease prevalence. *Pseudomonas* isolates caused greater inhibitory effect on hatching and penetration of *M. incognita* than caused by isolates of *Bacillus* (Siddiqui et al., 2009). It was reported by Siddiqui et al. (2005) that *Pseudomonas fluorescens* CHAO mutant resulted in reduced biocontrol activity against the root-knot nematode *Meloidogyne incognita* during tomato and soybean infection. Exposure of root-knot nematode to culture filtrates of *P. fluorescens* under *in vitro* conditions significantly reduced egg hatch and caused substantial mortality of *M. javanica* juveniles (Siddiqui and Shaukat 2003). Ali (1996) found that the population density of nematode species was reduced by application of five bacterial isolates (*Arthrobacterium sp.*, *Bacillus sp.*, *Corynebacterium sp.*, *Serratia sp.*, and *Streptomyces sp.*). Reductions of nematode populations were ranged between 46% and 100%. Youssef et al. (1998) studied the potential of *Azotobacter chroococcum*, *Bacillus megatherium* and *Rhizobium lupine* for the control of *Meloidogyne incognita* infecting cowpea and tomato plants. They noticed number of both root galls and egg masses of *M. incognita* were decreased in soil treated with *Bacillus megatherium* and *Azotobacter chroococcum* except *Rhizobium lupine* treated soil. El-Sherif et al. (1999) studied the effect of culture filtrates of 5 isolates for their nematotoxic effect against plant parasitic nematode (*Bacillus sp. Corynebacterium sp., Serratia sp., Arthrobacterium sp.*, and *Streptomyces sp.*). The

authors determined the culture filtrate concentration as 0.1% to inhibit the hatching of the eggs and 0.6% to be highly toxic to the juveniles. The toxic effect of the filtrate varied with the different nematode species.

Our objectives were to investigate optimizing of nutritional and environmental conditions of the local isolates (*Pseudomonas fluorescens* Ba-11 and *Serratia marcescens* Ba-2) for biomass production. We aimed also to use the biomass greenhouse experiments as safe biological control agents of root-knot nematode disease.

2. Material and Methods

Bacterial strains, pathogen and plant:

Antagonists *Serratia marsescens* and *Pseudomones fluorescens* were previously isolated from a rhizosphere soil from a farm, in Cairo, Egypt. The isolates were identified by Kamel et al. (2009) and it were used as potential sources of antagonistic bacteria. *P. fluorescens* was cultured on King's medium (King et al., 1954), whereas *S. marsescens* was grown on nutrient broth media. Faba bean seeds (Giza 714) were obtained from the Agriculture Research Center, Giza, Egypt.

Nematode larvae extraction: Using Oosten brinks elutritor method (Goody, 1963).

Propagation of the selected bacterial isolates in shake flasks:

Conical flasks (250 ml capacity) containing 100 ml of nutrient broth medium were inoculated by one ml of the selected bacterial isolates, then incubated on GFL rotatory shaker (120 rpm) at 28°C for 48 h. Oxygen absorption rate (OAR) was 0.22 mMO_2/L/min. In these experiments different carbon and nitrogen sources were tested to study their effect on the growth of bacterial isolates and reduction percentage of population density of root-knot nematode larvae. The amount of nitrogenous and carbon compounds added to the propagation broth medium were calculated to give the final nitrogen and carbon concentration equal to 0.8 and 0.4 g/L respectively.

Potential antagonism of *Serratia marcescens* and *Pseudomonas fluorescens* against *Meloidogyne incognita* under greenhouse conditions.

Pot experiment was conducted to explore effectiveness of both *Serratia marcescens* and *Pseudomonas fluorescens* to reduce population density of root-knot nematodes larvae, under greenhouse conditions. Seeds of faba bean (Giza, 714) were sown in 30 cm pots containing autoclaved sandy loam soil (1:1). Five seeds were sown in each pot, then thinned to two plants/pot just 10 days after germination. Pots were divided into nine groups, each contained four replicates. Treatments were bio-agents of *Serratia marcescens* and *Pseudomonas fluorescens*, which were individually incorporated into the soil at dose rate of 20 ml/pot (10^9 cells/ml) every 10 days for four applications. *Rhizobium leguminosarum bv*. vaceae was used to inoculate faba bean seeds before planting using seed coating technique.

Meloidogyne incognita belongs to a group of nematodes that cause important crop losses in developing countries (Luc et al., (1990) and Sasser and Freckmann (1987). The present study as well as those of previous studies (Overbeek et al., 1997; Marschner et al., 1999; Tian et al., 2000; Shapiro Ilan et al., 2006

Pots were arranged in a complete randomized block design, watered and received the normal agricultural practices. Pots nematodes infested were received newly hatched second stage larvae of *M. incognita* at dose rate of 20 ml/pot (50 larvae/ml) after 10 days of planting.

Two months later, the plants in each pot were uprooted and the roots were gently separated from soil, washed with flow water and dried by pressing lightly between blotting paper. Average numbers of galls and rhizobia nodules were counted. Nematode larvae population density after harvest (Pf) were extracted from soil and counted using Oosten brink's elutritor technique. Reduction of nematodes population density in soil sample was calculated according to Tilton formula, as follows: Tilton formula =

$$\left(1-\frac{\text{Population density in the treated pot after application}}{\text{Population density in the treated pot before application}}\times\frac{\text{Population density of the control pot before application}}{\text{Population density of the control pot after application}}\right)\times 100$$

The growth response of faba bean (roots, shoot dry wt and number of pods/plant) were also recorded. Data were subjected to statistical analysis and means compared using the least significant difference (L.S.D. at P = 0.01).

Determinations of bacterial biomass dry weight: (White, 1954).

Determination of oxygen absorption rate (OAR): Cooper et al., (1944).

Determination of reducing sugars: Flood and Priestely method (1973).

Determination of glycerol: Was determined enzymatically in the fermented liquor using special kits according to Fossati & Prencipe (1982).

Statistical analysis calculations: Were achieved according to Gomez and Gomez (1984)

Results and Discussion

and Siddiqui et al., 2009) demonstrated that nutritional factors have great impact on growth and on the antagonistic activity of the antagonist against the pathogen. In the present study, *Serratia marcescens* and *Pseudomonas fluorescens* are capable of using different carbohydrates as a sole carbon source (Table 1). Maximum biomass and antagonistic efficiency were obtained when glycerol was used as a carbon source for both organisms. The percentage reduction in population density of nematodes larvae ranged from 91% to 93% when *S. marcescens* and *P. fluorescens* were grown on glycerol concentration of 10.2% and 12.2% respectively (Table 2). Our results agree with those reported by Daffy and Defago (1999) who found that the antagonistic agent *P. fluorescens* was stimulated by glycerol but were inhibited by glucose.

In the present study, organic nitrogen sources gave higher growth of both tested bacteria than the inorganic nitrogen sources (Table 3). Peptone and tryptone were the most effective sources, whereas, weak growth was obtained on ammonium acetate. Peptone also supported good level of antagonistic activity of *S. marcescens* and *P. fluorescens*, the reduction in population density of root-knot nematodes larvae of *Meloidogyne sp*. reached 90% and 94% respectively with peptone and 85% and 93.9% with tryptone-containing medium, respectively. These results could be attributed to NH^{3+} produced during the decomposition of peptone or tryptone which was the principal element responsible for the population density reduction of nematodes larvae. Similar results were reported by Walker, (1971) and Zavaleta et al., (1989), who reported that volatile substances produced by *S. marcescens* have capability to inactivate root-knot nematodes larvae. These nematotoxic volatile substances were produced by *S. marcescens* when the nitrogen source in the growth medium was organic in the form of amino group. Table 4 shows that the optimum biomass yields for both bacterial species were detected at 10 g/l peptone concentration. Increasing peptone concentration more than 10 g/l resulted in decreasing bacterial growth of *S. marcescens* and *P. fluorescens*. The highest percentage in nematode larvae by *S. marcescens* was obtained in peptone-containing medium at 6 g/1 whereas the nematicidal activity of *P. fluorescens* was recorded at 10 – 12 g/l peptone concentration. Increasing or decreasing the medium peptone concentration than 10 or 12 g/l, resulted in decreasing the reduction efficiency of the cells for larvae. This observation could be attributed to the number of viable cells as previously reported by Racke and Sikora, (1992), who found that the antagonistic activity of *Agrobacterium radiobacter* and *Bacillus sphaericus* against the nematode *Globodera pallida* was shown to be directly correlated with the number of colony forming units. Similarly results obtained by Weidenborner and Kunz (1993) revealed that the reduction of the concentration of peptone and yeast extract in the broth to 50 % increased the nematicidal activity of *P. fluorescens* to 70.8 %.

The data represented in Tables 5 and 6, show that the highest growth of the selected two strains was achieved in media buffered at pH 7.6. The same pH was also optimal for nematicidal activity of both tested bacterial strains. This is in accordance with the previous findings of Slininger and Shea - Wilbur (1995) who found that the antagonistic activity of *P. fluorescens* was very sensitive to the culture pH, and pH 7 was the optimum. Data in tables 5 and 6 show also that changing the pH value of the growth medium than pH 7.6 caused slight decrease in the efficiency of both tested strains to reduce nematodes larvae. These results could be attributed to the bacterial cells count, which was the principal element responsible for reducing the population density of nematodes larvae. Stirling and Sharma (1990) reported similar results, and they documented that increasing of bacterial cells number resulted in increasing numbers of cells attached to nematodes larvae.

Many authors demonstrated the importance of incubation temperature on growth and metabolic activities of *P. fluorescens* (Tu, 1994; Slininger et al., 1995) and *S. marcescens* (Pearson et al., 1997; Daffy and Defago, 1999). In this study, it has been found that good growth of either *S. marcescens* and *P. fluorescens* was obtained at incubation temperature ranged between 25°C to 35°C and the temperature 30°C could be optimal (Table 7). The results also clearly indicate that the efficacy of the bio-control agents was closely correlated with the suitable incubation temperature for the optimal bacterial growth. Sharp decrease in biomass yield and nematicidal activity was detected when either bacterial strains were incubated at 20°C or 40°C. These results are in line with those reported by Racke and Sikora (1992) who found that the antagonistic activities of *Agrobacterium radiobacter* or *Bacilltis sphericus* against *Globodera pallida* were directly correlated with the number of colony forming units. Significant reduction of root infection was recorded with bacterial density of $9.7x\ 10^9$.

Our study also represent that maximum biomass and nematicidal activity yield efficiencies of *S. marcescens* (Ba-2) and *P. fluorescens* (Ba-11) were achieved when medium volume to air ratio was 1:4 (Table 8). The increase in medium volume to air ratio has led to a decrease in cultural growth. This result agrees with that obtained by Yousten and Wallis (1987). Increasing of biomass dry weight and biomass yield efficiency with decreasing the medium volume

could be attributed to increase rate of oxygen absorption within the medium as reported by Karim et al., (1993). Similar results were obtained by Jaspe et al., (2000) on the effect of extra aeration on enzymatic activities and growth of *P. fluorescens*.

Table 9 elucidate the effect of oxygen absorption rate (OAR) on the biomass production of both *S. marcescens* and *P. fluorescens*. The reached data reveal that high efficiency (100%) of the tested strains to reduce the population density of nematodes larvae was achieved when *S. marcescens* (Ba-2) or *P. fluorescens* (Ba-11) were shaked on rotary shaker at 120 rpm (0.31 mMO_2/L/min.) and 160 rpm (0.52 mMO_2/L/min.), respectively. Accordingly, nutritional and environmental factors are needed to secure the optimal growth of both isolates, by culturing them on modified growth medium containing glycerol (10.2 g/1), peptone (10 g/1) and monobasic potassium phosphate (0.4 g/1) with pH value of 7.60 at 30°C for 48 h. To achieve maximum growth of *S. marcescens*, it should be aerated with 0.31 mMO_2/L/min. (OAR) within the growth medium (rotary shaker at 120 rpm, with working volume 50 ml) while, the optimal growth of *P. fluorescens* required 0.52 mMO_2/L/min on a rotary shaker at 160 rpm and the medium occupied 20 % of the flask.

Studies were conducted under greenhouse conditions to evaluate the biological control potential of *P. fluorescens* and *S. marcescens* as a soil treatment against *Meloidogyne incognita* infesting faba bean. Results in Table 10 indicate that populations of the nematode *Meloidogyne incognita* were affected by application of both bacterial isolates. The suppressive effect on the number of juveniles ranged between 77.2 % and 84.4 % during the growing season. The infectivity of the nematode was greatly affected in the presence of any of the bioagents, the number of galls per root system were significantly decreased, more prominently in pots inoculated with *Rhizobium leguminosarum*. The tested biocontrol agents not only reduced the infectivity of nematodes but also increased the number of rhizobia nodules on the root system.

These results are in a line with those reported by Zavaleta et al. (1989), who reported potentiality of *S. marcescens* to suppress root-knot larvae of *M. incognita*, they attributed this effect to the volatile substances produced during its metabolic activity. Eklund (1970) and Tian et al. (2007) confirmed that Pseudomonads, are natural inhabitants on the root surface and primary consumers of root exudates rich in amino acids which are converted to ammonia along the root to maintain a micro-zone around the growing roots that would be suppressive to pathogens. The reduction of root galls number may be due to that the majority of the encumbered juveniles were not able to penetrate the host root. Davis et al. (1988); Stirling and Sharma, (1990) and El-Nagar et al. (1998), reported that *Bacillus penetrans* not only prevents reproduction of the root-knot nematodes but also reduces the infectivity of the juveniles. Zaki et al. (2009) reported that *Pseudomonas* isolates caused greater effect on hatching and penetration of *M. incognita* that caused by isolates of *Bacillus*.

A limited number of bacterial spp. have been reported as biocontrol agents for nematode diseases. The bacterium *Bacillus penetrans* (Sayre, (1988); Brown et al. (1985a); Davis et al. (1988); Stirling and Sharma, (1990); Abou-Eid et al. (1997) and El-Nagar et al. (1998) and *Pasteura penetrans* Daudi et al. (1990); Oostendorp et al. (1991) and Liu et al. (1995) were recognized as antagonistic to phyto-nematodes including root-knot nematodes. Entomopathogenic strains of *S. entomophila* were used as a bio-control in New Zealand as they induce inhibition of larval feeding activity and larval death 1-3 months from the on set of infection (O'Callaghan and Jackson, (1993) and Villalobos et al. (1997)).

Results in Table 10 show an increase in the number of root galls in the pots group of *Meloidogyne + Rhizobia* treatment in comparison with the control treatment. El-Bahrawy and Salem (1989), concluded that *Rhizobia* had stimulatory effect on *M. incognita* infecting broad bean. On the other hand, our results showed a 54.35 % reduction in rhizobia nodules in comparison with control. This reduction could be attributed to the deleterious effect of *M. incognita* on the development of rhizobia nodules, or to the interference of root-knot nematodes with nitrogen fixation in legume hosts treated with *Rhizobium* (Sharma and Sethi, 1976 and Chahal and Chahal, (1987)).

Treating pepper seedlings, wheat plants, vitis vinifera, rice, potato roots or tomato plants with isolates of *P. fluorescens* reduced the nematode damage caused by *M. incognita* (Eapen et al. (1997); Brimercombe et al. (1999); Shanthi et al. (1998); Ramakrishnan et al. (1999); Mani et al. (1998) and Duponnois et al. (1999)).

Ali, (1996) and Mercer et al. (1992) suggested that chitinase of *S. marcescens* or *P. fluorescens* strains caused premature hatch of nematode eggs and could be used as an aid in the control of nematodes on sunflower plant.

Regarding the effect of bacterial agents on faba bean growth, the obtained results in Table 11 showed that all bacterial treatments significally ($P < 0.05$ and 0.01), increased the plant growth parameters

as compared with the control treatment (Mi, treatment) while. Mi + Rh treatment failed to cause significant increase. Results also, indicate that there was a marked effect of biocontrol agents (*S. marcescens* (Ba-2) or *P. fluorescens* (Ba-11)) and Rhizobia on the growth response of faba bean in either nematodes free pots or nematodes infested pots compared with that of control (Rh-treatment). Thus the application of *S. marcescens* resulted in increasing shoot, root dry weights and number of pods up to 28.40, 43.64 and 32.14 % over Rh-treatment, respectively. The corresponding figures for *P. fluorescens* were 46.80, 77.27 and 45.24 %, respectively.

The growth promotion of faba bean observed in the present study (Table 11) may be attributed to those bio-agents may benefit plant growth by providing growth factors or regulators or by producing toxic metabolites which may inhibit nematodes and exclude other deleterious microorganisms. The ability of *S. marcescens* and *P. fluorescens* isolated from soil and rhizosphere to produce biologically active compounds have been reported by several investigators (Daffy and Defago 1999; Siddiqui and Shaukat, 2003; Shapira-Ilan *et al*. 2006 and Burkett-Cadena *et al*. 2008 and others).

Consequently, the introduction of such bacteria in soils, or cultural practices aimed to increase the activity of native strains of these bacteria could greatly contribute to the efficiency of nematode biocontrol with *S. marcescens* and *P. fluorescens*.

Table 1. Effect of different carbon sources on the bacterial biomass of *S. marcescens* or *P. fluorescens* on *Meloidogyne sp. Larvae* (I , *S. marcescens*; II, *P. fluorescens*).

Carbon sources	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
Glucose	2.20	2.10	7.58	7.56	9.20	9.40	23.90	22.30	89	90
Fructose	1.70	2.10	7.10	7.57	9.00	9.20	18.90	22.80	89	90
Sucrose	2.00	2.40	7.57	8.12	8.90	8.90	22.47	26.90	89	93
Galactose	2.00	2.10	7.50	7.57	8.50	8.60	23.50	24.40	89	83
Xylose	0.70	1.20	6.89	6.30	7.30	7.30	09.58	16.40	79	83
Manitol	2.00	1.60	7.62	6.91	8.50	8.90	23.52	17.90	89	83
Maltose	1.60	2.00	7.00	7.45	7.90	9.50	22.80	24.20	89	90
Mannose	2.10	2.00	7.57	7.31	9.00	9.60	23.30	20.80	89	90
Raffinose	0.60	0.80	6.658	6.10	7.10	7.30	08.46	21.90	70	79
Glycerol	2.60	2.70	8.72	8.73	9.30	9.40	27.90	28.70	89	93

Table 2. Effect of different concentrations of glycerol on the bacterial biomass and antagonist of *S. marcescens* or *P. fluorescens* on *Meloidogyne sp. Larvae* (I , *S. marcescens*; II, *P. fluorescens*).

Glycerol conc. (g/L)	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
2.20	0.20	0.40	5.35	5.44	2.00	2.20	10.00	18.20	80	80
4.20	0.60	0.80	5.35	6.16	4.00	4.00	15.00	20.00	80	80
6.20	1.00	1.30	6.25	7.35	6.00	6.10	16.60	21.30	80	90
8.20	2.00	1.85	7.43	7.95	8.00	7.90	22.20	23.40	89	90
10.20	2.65	2.55	8.62	8.73	9.90	8.70	26.80	29.30	91	93
12.20	2.60	2.60	8.62	8.72	11.88	11.20	14.16	22.30	91	93
14.20	1.96	2.60	7.22	8.64	13.83	11.70	12.80	21.40	89	93

16.20	1.80	2.40	7.15	8.63	14.00	13.70	11.24	17.50	89	93
18.20	2.00	2.40	7.45	8.63	17.80	16.20	9.570	15.70	89	93
20.20	1.90	2.40	7.25	8.64	19.85	16.60	9.572	14.50	89	93

Table 3. Effect of different organic and inorganic nitrogen sources on the bacterial biomass and antagonistic effect of *S. marcescens* or *P. fluorescens* against larvae of *Meloidogyne sp* (I , *S. marcescens*; II, *P. fluorescens*).

Nitrogen source	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
Organic sources										
Urea	1.44	1.90	7.45	7.752	9.96	9.20	14.46	20.65	13.20	23.00
Peptone	2.54	2.88	8.81	8.712	10.00	10.00	25.40	28.80	90.00	94.00
Yeast extract	1.12	0.85	7.18	6.151	9.63	8.35	11.63	10.08	29.00	15.80
Tryptone	1.90	2.55	7.75	8.011	9.76	9.91	19.40	25.73	85.00	93.90
Amm. acetate	0.05	0.06	4.12	3.544	3.40	3.00	01.47	2.00	5.00	3.00
Inorganic sources										
Amm. chloride	0.60	0.70	6.650	6.892	8.04	7.30	7.40	9.60	14.00	10.00
Amm. sulphate	0.50	0.57	6.215	6.541	8.35	8.35	6.00	6.82	10.00	13.00
Amm. phosphate	0.62	0.80	6.711	6.910	9.85	8.00	6.29	10.00	13.90	11.00
Amm. nitrate	0.70	0.70	6.875	6.890	7.70	9.00	9.09	7.77	7.00	9.80
Potassium nitrate	1.20	0.65	7.115	6.710	9.85	8.85	12.18	7.34	15.00	26.00

Table 4. Effect of different peptone concentrations on the bacterial biomass and antagonistic effect of *S. marcescens* or *P. fluorescens* against larvae of *Meloidogyne sp*, (I , *S. marcescens*; II, *P. fluorescens*).

Peptone concentrations (g/L)	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
2	0.790	0.880	6.810	6.961	8.350	9.850	9.460	8.930	80	80
4	0.880	1.500	6.950	7.430	8.290	8.980	10.610	16.700	80	93
6	0.980	1.900	7.121	7.830	9.000	8.390	10.890	22.650	90	90
8	1.590	1.900	7.610	7.850	9.500	8.050	16.740	23.600	90	90
10	2.520	2.700	8.810	8.680	10.000	9.040	25.200	29.800	90	95
12	2.520	2.300	8.810	8.540	10.000	9.700	25.200	23.710	89	95
14	2.350	1.700	8.711	7.462	9.900	9.790	23.740	17.360	90	90
16	2.480	1.900	8.730	7.753	9.800	9.770	25.310	19.450	91	91
18	2.530	1.650	8.810	7.431	10.000	9.900	25.300	16.670	91	90
20	2.500	1.850	8.771	7.621	10.000	9.800	25.000	18.880	91	90

Table 5. Effect of the growth medium pH values on the biomass production of *S. marcescens* and antagonistic effect on *Meloidogyne* larvae.

Medium pH value		Biomass dry wt. g/l	Bacterial cells count (log no./ml)	Sugar consumption g/l	Biomass yield efficiency %	Nematode larvae reduction %
Initial	Final					
6.00	7.80	1.29	6.35	9.78	13.19	80
6.20	8.22	1.38	6.41	9.80	14.08	80
6.80	8.00	1.85	7.32	9.78	18.92	85
7.20	7.30	2.42	8.62	9.78	24.74	90
7.60	7.80	2.65	9.09	9.81	27.01	95
7.80	7.71	2.00	7.32	9.94	23.14	85
8.20	8.30	1.65	7.21	8.50	19.41	85
8.40	7.80	1.31	6.71	8.00	16.25	80
8.60	7.70	1.45	6.43	7.70	18.83	80

Table 6. Effect of the growth medium pH values on the biomass production of *P. fluorescens* and antagonistic effect on *Meloidogyne* larvae.

Medium pH value		Biomass dry wt. g/l	Bacterial cells count (log no./ml)	Sugar consumption g/l	Biomass yield efficiency %	Nematode larvae reduction %
Initial	Final					
6.00	7.40	7.32	6.55	8.30	15.90	80
6.20	7.13	1.39	6.45	8.40	11.50	80
6.80	7.51	2.03	7.85	8.30	24.45	88
7.20	7.58	2.62	8.71	9.00	27.58	95
7.60	7.42	2.85	9.21	9.00	31.67	100
7.80	7.68	2.35	8.32	8.30	27.65	92
8.20	7.66	2.00	7.41	8.30	24.09	88
8.40	7.78	1.05	6.32	8.00	13.13	81
8.60	7.80	1.00	6.21	7.60	13.16	82

Table 7. Effect of different incubation temperatures on the biomass production of *S. marcescens* or *P. fluorescens.* (I , *S. marcescens*; II, *P. fluorescens*).

Incubation temperature °C	Biomass dry wt. (g/l)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
20	0.82	0.75	6.92	6.74	8.95	8.78	9.16	8.54	80	80
25	2.45	2.65	8.63	8.79	9.80	9.42	25.00	28.13	90	90
30	2.65	2.85	9.09	9.31	9.00	10.00	29.40	28.50	95	100
35	2.55	2.31	8.74	8.55	9.75	9.45	26.15	24.44	86	89
40	1.23	1.57	6.12	6.61	8.50	8.75	14.47	17.26	70	76

Table 8. Effect of different medium working volumes on the biomass production of *S. marcescens* or *P. fluorescens.* . (I , *S. marcescens*; II, *P. fluorescens*).

Medium working volume (ml)	Biomass dry wt. (g/l)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
25	2.03	2.50	7.85	8.74	9.10	9.00	22.31	27.78	88	95
50	2.95	3.20	9.22	9.42	9.35	9.40	31.55	34.04	100	100
100	2.66	2.81	9.09	9.21	9.81	9.31	27.01	30.18	95	100
150	1.84	1.80	7.33	7.32	7.85	7.25	23.44	24.83	84	81

Table 9. Effect of different speed of shaking on the biomass production of *S. marcescens* or *P. fluorescens.* (OAR, Oxygen Absorption Rate ; I , *S. marcescens*; II, *P. fluorescens*).

Shaking speed (rpm)	(OAR) $mMO_2/L/min$		Biomass dry wt. g/l		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II	I	II
80	0.18	0.18	2.75	2.74	8.82	8.80	9.04	9.50	30.42	28.84	90	90
120	0.31	0.31	3.45	2.91	9.73	9.51	9.15	10.00	37.70	29.10	100	100
160	0.52	0.52	1.95	3.60	7.45	10.22	8.25	10.00	23.64	36.00	90	100

Table 10. Extended effect of *S. marcescens* or *P. fluorescens* on the root-knot nematode *Meloidogyne incognita* infecting faba bean, under greenhouse conditions. (Mi, *Meloidogyne incognita;* S., *Serratia marcescens*; P., *Pseudomonas fluorescens*; Rh, *Rhizobium leguminosarum;* Pi, initial population density of nematodes in siol,; Pf, nematodes population density at harvest).

Treatments	**Nematodes population density in soil**			**No. of galls/roots system**		**No. of rhizobia nodules/roots**		
	Pi	**Pf**	**Reduction %**		**Reduction %**		**Reduction %**	**Increase %**
Mi+S.+Rh	1000	950	77.2	27	74.04	53	0	15.22
Mi+P.+Rh	1000	830	80.1	23	77.9	61	0	32.61
Mi+S.	1000	750	82.0	18	82.7	0	0	0
Mi+P.	1000	650	84.4	10	90.4	0	0	0
Mi+Rh	1000	3900	0	115	0	21	54.35	0
S.+Rh	0	0	0	0	0	56	0	21.75
P.+Rh	0	0	0	0	0	67	0	45.7
Mi	1000	4100	0	104	0	0	0	0
Rh	0	0	0	0	0	46	0	0
LSD 0.01				16.6		13.65		
0.05				12.2		9.87		

Table 11. The growth response of faba bean as affected by different treatments of *S. marcescens* or *P. fluorescens* on the root-knot nematodes. (Mi, *Meloidogyne incognita;* S., *Serratia marcescens*; P., *Pseudomonas fluorescens*; Rh, *Rhizobium leguminosarum*).

Treatments	Nematodes population density in soil			No. of galls/roots system		No. of rhizobia nodules/roots		
	Pi	Pf	Reduction %		Reduction %		Reduction %	Increase %
Mi+S.+Rh	1000	950	77.2	27	74.04	53	0	15.22
Mi+P.+Rh	1000	830	80.1	23	77.9	61	0	32.61
Mi+S.	1000	750	82.0	18	82.7	0	0	0
Mi+P.	1000	650	84.4	10	90.4	0	0	0
Mi+Rh	1000	3900	0	115	0	21	54.35	0
S.+Rh	0	0	0	0	0	56	0	21.75
P.+Rh	0	0	0	0	0	67	0	45.7
Mi	1000	4100	0	104	0	0	0	0
Rh	0	0	0	0	0	46	0	0
LSD 0.01				16.6		13.65		
0.05				12.2		9.87		

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