Plant Archives Volume 20 No. 1, 2020 pp. 2153-2160 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IMPACT OF SOME GENETICALLY IMPROVED RHIZOBACTERIA IN CONTROLLING *MELOIDOGYNE INCOGNITA* AND TWO WEEDS INFECTING *SOLANUM LYCOPERSICUM* SEEDLINGS UNDER GREENHOUSE CONDITIONS

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Abstract

Biological control is considered as an alternative strategy to chemical tool for controlling either nematodes or weeds. So, two pot experiments were carried out to evaluate the role of two native isolated bacteria *Bacillus cereus, Pseudomonas aeruginosa* and their three fusant strains namely (fusant7, fusant 20 and fusant 35) as biocontrol agents against root knot nematode *Meloidogyne incognita* and two weeds *Portulaca oleracea* and *Echinochloa crus-galli* infecting *Solanum lycopersicum*. Revealed results on nematode population, weeds and *S. lycopersicum* seedlings growth were recorded seven weeks after nematode inoculation. The results exhibited that, all bacterial strains suspension at the rates of ($1.25x10^7$ and $2.50x10^7$cuf/ml) significantly suppressed *M. incognita* parameters, decreased fresh and dry weights of weeds and improved *S. lycopersicum* growth. In general, undiluted concentration of all bacterial suspensions was more effective than diluted one. Additionally, fusants were more effective than their parents in reducing nematode population and controlling both weeds. Combination of two strains was more effective than the individual application in controlling nematode and *P. oleracea* weed. But, it was less effective in controlling *E. crus-galli* weed. *P. aeruginosa* was more effective than *B. cereus* in suppress nematode and weeds parameters. The fusant 7 was more effective than all other treatments in suppressing *M. incognita* J_2 in soil, galls and eggmasses /root system by 91.30%, 92.50% and 90.91% reduction, respectively as compared to control. Also, fusant 7 was the highly effective strain in decreasing the fresh and dry-weights of *E. crus-galli*, by 75.87% and 75.55%, respectively and of *P. oleracea*, by 84.68% and 86.17% consecutively as compared to unweeded control treatment. This in turn reflected on *S. lycopersicum* seedlings by improving growth parameters. In conclusion, the genetically improved bacterial strains could be used as biological alternative safe method to chemical pesticides for suppressing root knot nematode reproduction and weed seedlings growth.

Key words: Biological control, genetically improved rhizobacteria, *Solanum lycopersicum*, root knot nematode, Weeds.

Introduction

Tomato (*Solanum lycopersicum* L. family, Solanaceae) is the second nutritive vegetable crop ranking next to potato all over the world (Tetteh *et al.,* 2011). *S. lycopersicum* is a vital source of minerals and antioxidants such as carotenoids, lycopene, vitamins C and E as well as phenolic compounds which are important in human nutrition and protect against certain cancers and other diseases (Adalid *et al.*, 2004). Several agents are responsible in *S. lycopersicum* yield losses. The major factors are plant parasitic nematode especially the root knot nematodes and weed infestations that are reducing quality and crop value.

Root-knot nematode *Meloidogyne* spp. infect and damage a wide range of important crops particularly vegetables in tropical and subtropical countries (Osman

et al., 2012). The most important species adapted to warm and tropical climates are *Meloidogyne incognita, M. javanica* and *M. arenaria*. The most characteristic symptom of *Meloidogyne* spp. infection is the formation of root galls which alter the whole physiology of the infected plant, destruct the root vascular system, altering the nutrient and water attraction and the whole root system becomes stubby. The infested plant seems to be chlorotic, stunted and unthrifty (Archana and Saxena, 2012). Additionally, other pathogens such as fungi and bacteria may easily attack plants through the nematode injuries resulted in disease complex and finally the quality and quantity of the yield are drastically decreased (Hussey and McGuire 1987; Nelson 2005; Bagheri *et al*., 2013).

Weeds are known as one of the major constraints in agricultural production. They affect crop yield and quality by competing with them on light, nutrients, water, space and interfere with the distribution of fertilizers application (Kremer and Kennedy, 1996). Weeds cause great economic losses in crop productivity that reaches to 34% (Oerke, 2006). Weed management aims to manipulate the competitive balance in favorable manageable levels between crop and undesirable weeds (Bond and Grundy, 2000).

Currently, the most effective means for controlling plant parasitic nematodes and weeds are chemical pesticides, which have been responsible for the contamination of groundwater, soils and food products as well as threaten human health and finally nematodes and weeds become resistant to these chemical pesticides (Yamashita and Viglierchio, 1987; Jabran *et al.,* 2015). Recently, many scientists developed the effective nonchemical methods for the management of both nematode and weeds (Siddiqui 2000; Siddiqui and Ehteshamul-Haque, 2000; Siddiqui and Shaukat 2005; Jabran *et al.,* 2015; El-Rokiek *et al.,* 2018; El-Wakeel *et al.,* 2019).

Biological control using microorganisms and their natural products promises to be a new approach for controlling plant parasitic nematodes and weeds. The most studied microorganisms are the plant growth promoting rhizobacteria. Some of rhizobacteria colonize the tissues of living economic plants and enhance the plant growth as a growth promoter or reduce the damage from soil borne plant pathogens (Kloepper *et al.,* 1980). There are several possible mechanisms involving in the prevention of nematode reproduction by rhizobacteria: enzymes that affect the external structural components in one or more developmental stages of nematodes and metabolic by-products that may be lethal to nematode organs and affect nematode behavior or may modify the plant-parasite recognition process. The most prominently studied enzymes are proteases which are directly affect nematodes infective stages and cause significant damage to their cuticle and the chitinase which are responsible in degrading nematode eggshell. Moreover, some rhizobacteria can induce systemic resistance in plant against nematodes (Hasky-Gunther *et al.,* 1998; Huang *et al.,* 2005; Siddiqui and Shaukat, 2005; Niu *et al.,* 2006, 2007).

Since 1990 scientists have increasingly focused on bacteria which have potential efficiency as a microbe-based herbicide (Li and Kremer, 2006; Mejri *et al.,* 2013; Yang *et al.,* 2014). This group of microorganisms has been realized to suppress weed seedling growth by colonizing weed roots and localize their metabolite production, thus minimizing potential deleterious effects on the growth of desirable plants (Kremer and Souissi, 2001; Li and Kremer, 2006). The crude extracts of *Pseudomonas aeruginosa* strain CB-4 have high inhibition activity on *Digitaria sanguinalis* (Yang *et al.,* 2014).The seeds of *Phalaris minor* inoculated with *Bacillus subtilis* strain SYB 101 caused 70.8 and 80.7% decrease in root and shoot dry weight of weed seedlings and induced 136.8 and 316.6% increase in root and shoot dry weight of wheat (Phour and Sindhu, 2018).

Recently, for maximize the potentiality of such rhizobacterial strains against plant pathogens, researchers rely on the biotechnological approaches to create genetically superior strain combines all the desired properties and increase the production of such toxins or enzymes via induced protoplast fusion between different promising bacterial strains. Yari *et al.,* (2002) reported that the concentration of ä-endotoxin of *B. thuringiensis* fusion was 1.48 times more toxic than the wild type. El-Hamshary *et al.,* (2004) found that under greenhouse conditions the fusant strain from *P. fluorescens* and *P. aeruginosa* proved to be more effective than its parental strain in reducing *M. incognita* reproduction and enhanced sunflower plant growth. Zaied *et al.,* (2009) found that the fusion between *Serratia* and *Pseudomonas* strains resulted in high mortality levels on nematodes if compared with the parental strains due production of antibiotic, chitinoltic enzymes, chitinases and bacteriocin more than their parents.

The objectives of this greenhouse initial study are to evaluate the nematicidal and herbicidal potentials of the two native bacterial strains *Bacillus cereus* and *Pseudemonas aeruginosa* in comparison with their fusants against root knot nematode *Meloidogyne incognita*, broad leaved weed *Portulaca oleracea* and grassy weed *Echinochloa crus-galli* as well as *Solanum*

lycopersicum plant growth under greenhouse conditions. This greenhouse test is an important step in documenting the effectiveness and host specificity of deleterious bacteria and the most efficient treatments of this study will be applied in the next field experiment.

Materials and Methods

Bacterial strains

Two rhizobacterial isolates originating from the rhizospheric soil of *S. lycopersicum* plants collected from different area of Giza governorate, Egypt were selected for this study. They were identified based on 16S rRNA sequence analysis in the Gen Bank database as *Bacillus cereus* GEs (Accession No. LC215052) and *Pseudomonas aeruginosa* GEs (Accession No. LC215048).A protoplast fusion technique was performed between them according to Yari *et al.*, (2002) to construer new genetically improved strains namely (fusant 7, fusant 20 and fusant 35) combined all the desired properties from the parents.

All bacterial strains were stored on slants of Complete Medium and maintained in Nematode Lab., Plant Pathology Department, National Research Centre, Egypt.

Growth conditions

a. Complete Medium (CM):

Agar Agar 15 g, Peptone 5 g, Meat Extract 3 g and Distilled water 1000 ml

b. Luria broth Medium (LB) Davis *et al.*, 1980:

Tryptone 10 g, Yeast extract 5 g, NaCl 5 g, Distilled water up to 1000 ml of Preparation of bacterial inoculation

For each bacterial strain, a conical flask (250 ml) containing 100 ml of LB broth medium was inoculated and incubated at 28-30 °C with shaking at 150 rpm for 48 hrs. prior to application. Each ml distilled water contain $2.50x10^7$ cfu.

Nematode inoculum

Meloidogyne incognita J_2 were extracted according to Hussey and Barker, (1973) from the pure culture maintained on eggplants roots in the greenhouse of the Plant Pathology Department National Research Centre. Each one ml distilled water contain 1000 J_2.

Weeds targeted in this study are

Broad-leave weed *Portulaca oleracea* (Purslane) and grassy weed *Echinochloa crus-galli* (Barnyard grass**)** seeds obtained from Agricultural Research Centre, Egypt.

Pot experiments

Two pot experiments were conducted during two successive summer seasons of 2017 and 2018 at the greenhouse of the National Research Centre. Plastic pots (20cm in diameter) were filled with equal amounts of sandy clay soil (1:1w/w). All pots were infested with the same weight of weeds seeds (0.1 g) *i.e. P. oleracea* as a broad leaved weed and *E. crus-galli* as a grassy weed. Weed seeds were mixed thoroughly at 2cm depth from the soil surface. Instantly, three weeks old *Solanum lycopersicum* (Tomato) seedlings cv. Alisa were transplanted (two seedlings/pot). Before inoculation of *M. incognita* J_2 two *S. lycopersicum* seedlings were thinned to one seedling/pot. After two weeks from transplanting, all pots inoculated with 2000 *M. incognita* J_2 and the bacterial suspension strains at the same time as follow:

a. Six treatments were inoculated with 2ml from the genetically improved rhizobacteria at the dose of $1.25x10^7$cuf/ml (diluted concentration).

1. Fusant 7
2. Fusant 20
3. Fusant 35
4. *Bacillus cereus*
5. *Pseudemonas aeruginosa*
6. *Bacillus cereus* + *Pseudemonas aeruginosa*

b. Six treatments were inoculated with 2ml from the aforementioned bacterial strains at the dose of $2.5x10^7$cuf/ml (undiluted concentration).

c. Control treatment nematodes inoculation without bacterial suspension.

All treatments were applied in randomized complete block design with 5 replicates under greenhouse condition at 30C° ± 5ºC and watered as needed.

Recorded data

Seven weeks after nematode inoculation, *S. lycopersicum* plants in the five replicates were gently uprooted and the roots were washed and cleaned from the adhering soil particles. The second- stage juveniles (J_2) in 200g soil were extracted by sieving and decanting technique (Barker, 1985) and examined under a light microscope using a Hawksley counting slide. Number of galls and egg masses were determined from the whole root system and indexed according to Sharma *et al.*, (1994). The fresh and dry weights of each weed separately were detected and the percentage of reduction was calculated. Lengths, fresh and dry weight of shoots

and fresh weight of root systems of *S. lycopersicum* plants were recorded as well as number of flowers and leaves.

Statistically Analysis

All obtained data were subjected to proper statistical of variance according to Snedecor and Cochran (1980) using Assistant program version 7.6 beta. The means values were compared using Duncan, (1955) Multiple Range Test at $P \leq 0.05$ level.

Results

Results in Table 1 showed that all microbial inoculums significantly suppressed *M. incognita* population densities in soil and *S. lycopersicum* roots. Generally, the high concentration achieved the highest percentage reduction in all nematodes parameters. The fusants were more effective than their parents individually or in combination in reducing J_2 in soil and nematode counts (number of galls and eggmasses / root system). Combined parent strains were more effective than the individual application. Fusant 7 was the highly effective in suppressing nematode counts.

The recorded percentage reductions in *M. incognita* J_2 in soil due to the undiluted bacterial suspensions ($2.5x10^7$cfu/ml) ranged from 91.30 to 53.04%.While in case of the diluted ($1.25x10^7$cfu/ml) it ranged from 68.70 to 29.57%. For root galls the % reductions due the undiluted concentration ranged from 92.50 to 26.67% and from 80.83 to 12.50% reduction in diluted concentration. While the undiluted concentration resulted in 90.91 to 45.45 % reduction in eggmasses/root system and the diluted one showed 84.42 to 44.16% reduction Table 1. The fusant 7 ranked the first in suppressing J_2 in soil and *M. incognita* recoded parameters (root galls and eggmasses /root system) by 91.30%, 92.50% and 90.91%, respectively due to the undiluted concentration and by 68.70%, 80.83% and 84.42% reduction, consecutively due to the diluted one (Table 1).

Results presented in table 2 indicated the positive performance of the evaluated bacterial strains in suppression of *E. crus-galli* and *P. oleracea* growth parameters. It is obvious that all the bacterial inoculums significantly decreased the fresh and dry weights of the target weeds as compared to the unweeded control treatment.

The highest concentration of each strain achieved the highest percentage of reduction in fresh and dry weights of both weeds. Results revealed that *B. cereus* was more effective in reducing *E. crus-galli* grass weed growth than *P. aeruginosa*. In contraire, *P. aeruginosa* was more effective than *B. cereus* in reducing *P. oleracea* seedlings growth. The individual application of the wild types was more effective than their combination with significant difference in controlling *E. crus-galli* grass

Table 1: Effect of *Bacillus cereus*, *Pseudomonas aeruginosa* and their fusants on *Meloidogyne incognita* reproduction under greenhouse conditions (Average of two seasons).

Treatments	Conc.	No. of J_2 in 200g soil	% Red.	No. of galls /root system	% Red.	Galls Index	No. of eggmasses/ root system	% Red.	Egg mass Index
Fusant 7	Diluted Conc. $1.25x10^7$cfu/ml	36cb	68.70	23b	80.83	5	12 b	84.42	4
Fusant 20		42ed	63.48	44d	63.33	6	25 c	71.43	5
Fusant 35		47e	59.13	72 e	40.00	8	18 e	76.62	4
B. cereus		81ih	29.57	86 f	28.33	8	43 e	44.16	6
P. aeruginosa		76h	33.91	105g	12.50	9	39 e	49.35	6
B. cereus + *P. aeruginosa*		67 g	41.74	79 fe	34.17	8	31 d	59.74	6
Fusant 7	Undiluted Conc. $2.5x10^7$cuf/ml	10a	91.30	9 a	92.50	3	7 a	90.91	3
Fusant 20		31b	73.04	18 b	85.00	4	8 a	89.61	3
Fusant 35		42 ed	63.48	31 c	74.17	6	18 b	76.62	4
B. cereus		54f	53.04	75	37.50	8	42 e	45.45	6
P. aeruginosa		47 e	59.13	88 f	26.67	8	26 dc	66.23	5
B. cereus + *P. aeruginosa*		40dc	65.22	73 e	39.17	8	25 c	67.53	5
Untreated control		115 i	0.00	120h	0.00	9	77 f	0.00	8

*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test. % Red.= Reduction over control Gall index, Egg mass index: 1 = no galls or eggmass, 2 = 1 - 5, 3 = 6 - 10, 4 = 11 - 20, 5 = 21 - 30, 6 = 31-50, 7 = 51- 70, 8 = 71 - 100 and 9 >100 galls or eggmass / plant (Sharma *et al*., 1994).

weed. Whereas, the combination of parents was more effective in controlling of *P. oleracea* weed than individual application. The fusants were more effective than their parents in decreasing fresh and dry weights of both grass and broad leaved weeds. Also, the fusant 7 undiluted treatment caused the maximum decrease in fresh and dry weights of *E. crus-galli* grass weed by 75.87% and 75.55% reduction, respectively, and 84.68% and 86.17% reduction in fresh and dry weight of *P. oleracea*, respectively as compared to untreated control.

Results presented in table 3 revealed that all rhizobacterial suspension induced significant increase in *S. lycopersicum* plant growth parameters (lengths, fresh and dry weights of shoots and fresh weight of roots as well as number of leaves and flowers) as compared to control untreated. The undiluted concentration was more effective than the diluted one. The recorded results in Table 3 indicated that the fusant 7 was the superior treatment and caused the greatest increase in *S. lycopersicum* plant growth parameters. The increase in *S. lycopersicum* length, fresh and dry weights of shoot system reached to 121.28, 169.87 and 118.52% in, respectively due to the undiluted concentration and 57.45, 81.37 and 143.20% consecutively, due to the diluted one as compared to control (Table 3). As shown in table 3 the untreated *S. lycopersicum* root systems showed relative increase in the fresh weight than all other treatments, while the fusant 7 resulted in the least one. Also, Table 3 showed that fusant 7 undiluted suspension gave the greatest increase in numbers of *S. lycopersicum* flowers than all other treatments. A significant difference was observed between the recoded number of leaves taking in consideration that un-diluted fusant 7 gave the highest number of leaves.

Discussion

Recently, many studies have been undertaken to investigate the effects of using microorganisms as biocontrol agents against plant parasitic nematodes and weeds. Rhizobacteria which grown in soil rhizosphere provides front line defense for plant roots against nematode attack. Our results revealed that treatments with *P. aeruginosa*, *B. cereus* and their fusants had the abilities to suppress root knot infection on *S. lycopersicum* and significantly increased shoot and root parameters as well as numbers of flowers and leaves. These findings are supported by other works of (Siddiqui, 2000; Siddiqui and Ehteshamul-Haque, 2000; Siddiqui and Shaukat, 2005; Prakob *et al.*, 2009) who reported that *P. aeruginosa* suppressed root knot nematode *Meloidogyne* spp. directly by producing toxins and lytic enzymes and indirectly by enhancing defense mechanism leading to induced systemic resistance in plants. Oka *et al.*, (1993) found that exposure of *M. javanica* second stage juveniles to *B. cereus* in soil inhibited the penetration of the juvenile into *S. lycopersicum* roots. They also

Table 2: Effect of *Bacillus cereus and Pseudomonas aeruginosa* and their fusants on fresh and dry weights of *Echinochloa crus-galli* and *Portulaca oleracea* weeds (Average of two seasons).

Treatments	Conc.	*Echinochloa crus-galli*				*Portulaca oleracea*			
		Fresh weight (g/pot)	% Red.	Dry weight (g/pot)	% Red.	Fresh weight (g/pot)	% Red.	Dry weight (g/pot)	% Red.
Fusant 7	Diluted Conc. $1.25x10^7$cfu/ml	14.73 ba	73.02	1.53 a	74.02	20.14 b	75.51	1.05 b	84.72
Fusant 20		17.94 c	67.14	2.01 a	65.87	20.96 b	74.51	1.1 a	83.99
Fusant 35		25.98 d	52.41	2.91 b	50.59	41.70 g	49.29	3.15 a	54.15
B. cereus		38.08 fe	30.24	4.27 dc	27.50	39.43 f	52.05	2.95 ba	57.06
P. aeruginosa		39.10 f	28.38	4.41 dc	25.13	37.62 e	54.26	2.64 ba	61.57
B. cereus + *P. aeruginosa*		47.25 h	13.45	5.22 e	11.38	31.00 d	62.31	2.09 ba	69.58
Fusant 7	Undiluted Conc. $2.5x10^7$cuf/ml	13.17 a	75.87	1.44 a	75.55	12.60 a	84.68	0.95 a	86.17
Fusant 20		14.71 ba	73.05	1.64 a	72.16	13.30 a	83.83	1.05 a	84.72
Fusant 35		16.98 cb	68.90	1.93 a	67.23	36.34 e	55.81	2.55 ba	62.88
B. cereus		26.14 d	52.12	2.98 b	49.41	29.42d	64.23	2.27ba	66.96
P. aeruginosa		35.50 e	34.97	3.98 c	32.43	25.41c	69.10	1.77ba	74.24
B. cereus + *P. aeruginosa*		42.87 g	21.47	4.81 ed	18.34	25.24 c	69.31	1.73ba	74.82
Untreated control		54.59 i	0.00	5.89 f	0.00	82.24 h	0.00	6.87 c	0.00

*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test. % Red.= % Reduction.

Table 3: Effect of *Bacillus cereus and Pseudomonas aeruginosa* and their fusants on plant growth parameters of *S. lycopersicum* (Average of two seasons).

Treatments	Conc.	Shoot System						Root System				No. of Flowers	No. of Leaves
		length (cm)	% Inc.	fresh weight (gm)	% Inc.	dry weight (gm)	% Inc.	length (cm)	% Inc.	fresh weight (gm)	% R.		
Fusant 7		37.00 d	57.45	12.46c	81.37	1.97 e	143.20	17. 33 de	92.56	5.13bc	18.57	3.33cd	8.67 cd
Fusant 20		36.75 de	54.38	12.07 c	75.69	1.96 ef	141.98	17.00 de	88.89	4.37 c	30.63	2.00 e	8.00 ef
Fusant 35		34.00 ef	44.68	7.80de	13.54	1.50 h	85.19	16.00 ef	77.78	5.74ab	8.89	2.00e	7.33 fg
B. cereus		31.00 g	31.91	8.20 d	19..36	1.73 fg	113.58	14.67 g	63.00	5.17 bc	17.94	3.33 cd	8.00 ef
P. aeruginosa		32.50 fg	38.29	8.60 d	25.18	1.58 gh	95.06	12.33 h	37.00	3.27 ef	48.10	4.00 bc	9.67bc
B. cereus + *P. aeruginosa*		32.78fg	39.49	8.16d	18.80	1.77 ef	118.5	16.0 0ef	77.78	3.40 ef	46.03	3.00 de	8.33 de
Fusant 7		52.00 a	121.28	18.54 a	169.87	3.48 a	118.52	29.33 a	225.89	2.09 g	66.83	8.00 a	11.33 a
Fusant 20		48.00 b	104. 26	16.39b	138.57	3.30 ab	307.41	24.67 b	274.11	3.23 ef	48.73	4.33 bc	9.67 bc
Fusant 35		40.50 c	72.34	18.27a	165.94	3.12bc	285.19	28.67 a	211.11	3.12 ef	50.48	5.00b	9.67bc
B. cereus		33.50 fg	42.55	8.47d	23.29	1.50 h	85.19	15.00 fg	66.67	4.37c	30.63	3.00 de	8.33de
P. aeruginosa		35.25 de	50.00	12.73c	85.30	3.05 c	276.54	18.17 d	101.89	2.99 f	52.54	4.33 bc	9.33bc
B. cereus + *P. aeruginosa*		34.75 de	45. 74	11.63c	69.29	2.59 d	219.75	20.67 c	229.67	3. 90 c	38.10	3.67cd	10.00b
Untreated control			.....	6.87e	.....	0.81 i	.....	9.00 i	.....	6.30a	.....	0.00f	7.67 g

*Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test. % Red.= % Reduction.

suggested that the ammonia released during protein degradation by the bacterium may contribute significantly to the recorded nematicidal activity. Additionally, Gao *et al.,* (2016) found that *B. cereus* can produce two nematicidal compounds identified as sphingosine and phytosphingosine which inhibit nematode reproduction by destroying the genital area.

Our results table 1 demonstrated that the fusants were more effective in suppressing root knot nematode than their parents individually or in combination. This result is in conformity with the early studies of Yari *et al.,* 2002; El-Hamshary *et al.,* 2004; Zaied *et al.,* 2009; Elkylany 2017; Abdel-Salam *et al.,* 2018; Soliman *et al.,* 2018 who reported that fusants from the different bacterial strains exhibited nematicidal potential more than their wild types and produced antibiotic, chitinoltic enzymes, chitinases and bacteriocin more than the parents.

The results table 2 of this study are in consistent with the previous reports of Patil (2014) and Lakshmi *et al.,* (2015) who found that inoculation with *B. cereus* caused 34% and 17% inhibition of root and shoot length of target weed due to the production of sodium vanillate and 2-aminobenzoic acid, while *P. aeruginosa* inhibited root and shoot length of the same weed by 38% and 23% respectively, due to the HCN production. Our work is in conformity with that reported by Kremer and Kennedy (1996) who concluded that rhizobacteria control strategy is to regulate the development of weeds before or coincident with emergence of crop plants. Therefore, do not necessarily eradicate weeds problem but significantly suppress early growth of weeds and allow the development of crop plants to effectively compete with weakened weed seedlings. This novel ecologically based weed management option using rhizobacteria may become a powerful alternative or addition to traditional weed control programs.

All bacterial strains showed a promoting effect on the growth of *S. lycopersicum* plant. These were obvious by the significant increase in length, fresh and dry weights of shoot system as well as numbers of flowers and leaves as compared to infected untreated plants. The improving the *S. lycopersicum* growth parameters may be related to the growth regulatory effect of rhizobacterial strains with inhibition of nematode and weeds infections (Table 3). These are in agreement with the findings of Adesemoye *et al.,* (2008) who reported that *P. aeruginosa* increased the dry biomass of *S. lycopersicum* by 31% over control. Only fresh weight of root systems showed the reverse trend this may be explained by the increased numbers of galls due nematode infection 120 galls in untreated plants as compared to 9 and 23 galls in plants treated with the fusant7 undiluted and diluted concentrations, respectively.

Conclusion

The genetically improved bacterial strains (fusants 7, 20 and 35) from the two native isolated strains *Bacillus*

cereus and *Pseudomonas aeruginosa* via protoplast fusion technique were found to be more significant than its parents in suppressing root knot nematode, *Meloidogyne incognita* reproduction and decrease *Echinochloa crus-galli* and *Portulaca oleracea* weeds growth as well as enhance *S. lycopersicum* plant growth parameters. So, in the future it could be used as a promising and safe control product alternative to chemical pesticides in a large scale production system.

Acknowledgement

This research was funded by house project (No11090408) of National Research Centre, Egypt.

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