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DUAL MANAGEMENT OF ROOT- KNOT NEMATODE *MELOIDOGYNE INCOGNITA* AND WEEDS INFESTED *SOLANUM LYCOPERSICUM* WITH PROTOPLAST FUSANTS OF *BACILLUS CEREUS* AND *PSEUDOMONAS AERUGINOSA*

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Abstract

The application of rhizobacteria and their genetically improved bacterial strains is a modern approach to manage pests. So, two successive field experiments were applied to reveal the dual effect of two wild bacteria namely *Bacillus cereus* and *Pseudomonas aeruginosa* and their genetically improved bacterial strains *viz*. fusant 7, fusant 20 and fusant 35 as a safe method to control root-knot nematode and weeds infested *Solanum lycopersicum*. The recorded results improved that oxamyl and three genetically aforementioned bacterial strains scored the highest inhibitory effect on *Meloidogyne incognita* reproduction by reducing the numbers of J_2 in soil, galls and egg masses in *S. lycopersicum* roots as compared untreated control at 70 Days after transplanting (DAT) and at harvest. Concerning to weeds, two hand hoeing and metribuzin herbicide scored the highest deleterious effect on broad leaved weeds. Whereas, clethodim herbicide, two hand hoing and metribuzin came in the first rank in controlling broad leaved weeds. The three applied fusants came in the second rank to these mechanical and chemical applications in both seasons at 70 DAT and at harvest as compared to unweeded check. Fusants were more effective than their wild parents individually or in combination in controlling weeds. Additionally, individual application of wild bacterial strains was more effective than combined application. Dual pest management of nematodes and weeds actually reflected on yield quality (TSS) as well as yield and its attributes. Meanwhile, metribuzine herbicide and three investigated fusants (7, 35 and 20) achieved the highest increment in yield reached to 175.08, 155.23, 150.79 and 117.46, successively.

Key words: Management, root- knot nematode, weeds, protoplast fusants, *Bacillus cereus, Pseudomonas aeruginosa*, *Solanum lycopersicum*.

Introduction

Solanum lycopersicum L. (Tomato) is a most popular vegetable crop next to potatoes all over the world. Biotic and abiotic stresses negatively affect on *S. lycopersicum* vegetative growth which consequently reflect on yield and crop production (Chaudhary *et al.,* 2012). Biotic stress agents such as viruses, bacteria, nematodes, weeds and arachnids additionally induce plant mortality and reduce plant vigor (Singla and Krattinger, 2016). Rhizosphere is a thin film of soil which surround the plant roots where plant uptake its nutrients. Moreover, rhizosphere is the location of many chemical, physiological and biological activities. The bacteria which derived from the plant rhizosphere are known as rhizobacteria. These types of bacteria have been designated as plant-growth-promoting rhizobacteria (PGPR). PGPR interact with plant roots and directly or indirectly affect on the growth of this plant to reduce biotic and a biotic stresses. Additionally, the induction of plant growth is achieved by different mechanisms such as antibiosis, systematic resistance and competitive omission (Tripathi *et al.,* 2012). The most dominant known genera of PGPR are *Pseudomonas* and *Bacillus* spp. which can be applied as eco-friendly management tools (Grover *et al.,* 2011; Vejan *et al.,* 2016). The chemical pesticides have a toxic and harmful effect on human, animals and the surrounding environment. So, many scientists exert great efforts to develop the management system by reducing the

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pesticide dependency in controlling weeds and nematodes. Many advantages have been proven by using microorganisms as a biological control agent such as high selectivity, low production costs and it's a non polluted eco-friendly practice (Bolton and Elliot, 1989; Crump *et al.,* 1999; Flores-Vargas and O'Hara, 2006). The protoplast fusion technique is applied to produce genetically engineering microbial strains such as rhizobacteria. These genetically improved rhizobacterial strains is a modern approach which recently applied as a biocontrol agent to manage pests such as nematodes and weeds.

Root-knot nematodes are one of the major plant parasitic nematodes on *S. lycopersicum*. Considering its damage potential, a field experiment was piloted for the management of root-knot nematode, *Meloidogyne incognita* in tomato. Managing nematode chemically causes serious ecological and human health problems; Innovate for alternative eco-friendly control methods are increasingly needed (Weller, 1988). In this regard, El-Hamshary *et al.,* 2004 improved that the fusant strain between *P. fluorescens* and *P. aeruginosa* was more effective than their parental strains in reducing different nematode parameters as well as enhanced plant growth. Also, the fusants between *Serratia and Pseudomonas* induced high mortality levels against *M. incognita* when compared with the parental strains under laboratory conditions (Zaied *et al.,* 2009). *Bacillus thuringiensis* fusants have 1.48 times more δ-endotoxin than their parents (Yari *et al.,* 2002). Elkelany, 2017 found that fusant from *Anoxybacillus flavithermus* and *B. pumilus* strains was more effective in reducing *M. javanica* eggplant than their parents. Evaluate the nematicidal effect of *B. licheniformis*, *P. aeruginosa* and two from their fusant products *viz*. F28 and F40, for controlling root knot nematode, *M. incognita* on *S. lycopersicum* and eggplants under greenhouse conditions. The analyzed results indicated that both fusant products F28 and F40 were the highly effective treatments in decreasing *M. incognita* reproduction (El-Nagdi *et al.,* 2019).

Weeds reduce *S. lycopersicum* yield by competing on nutrients, water, light, space causing reduction in tomato yield reach to 53-67% (Sanok *et al.,* 1979) as compared to weed free check. Although chemical herbicides are effective in controlling weeds but these are highly expensive and negatively affect on human and animal health. Moreover, weeds seem to be resistant to these reapplied herbicides. The modern agricultural studies are directed to achieve organic agriculture through the application of safe and effective approaches such as allelopathy and mulching in controlling weeds (El-Metwally and El-Wakeel, 2019; El-Wakeel *et al.,* 2019). Biological control is also a safe modern approach that achieved weed management such as *B. pumillus* which was applied as bioherbicide formulations (Japan Tobacco Inc., 1998). Likewise, Kim and Kremer, (2005) reported the selective bioherbicidal effect of *B. megaterium* that inhibited the growth of *Ipomoea hederacea* Jacq but it had no effect on lettuce crop. Also, (Phour and Sindhu, 2018) ensured that *B. subtilis* strain SYB 101 can be applied as a selective bioherbicide which inhibited the growth of *Phalaris minor* and stimulated growth of wheat plants. Concerning to *Pseudomonas* spp., its possible to mention that two strains of rhizospheric *Pseudomonas* strain exhibited its suppression effect on germination and growth of *Bromus tectorum* grassy weed (Kennedy *et al.,* 1991). Likewise, strain G2-11 of *P. fluorescens* that inoculated to wheat and soybean crops improved its selective bioherbicidal activity by promoting the growth of agricultural crops and inhibiting the growth of associated weeds such as *Ipomea* sp. and *Convulvolus arvensis* (Li and Kremer, 2006). Similarly, Yang *et al.,* (2013) recorded high inhibition effect of *P. aeruginosa* crude extract strain CB-4 on *Digitaria sanguinalis*

Our recent study (Ameen *et al.,* 2020) reported that genetically improved bacterial strains through protoplast fusion technique namely (fusant 7, fusant 20 and fusant 35) at 2.50×10^7 cfu. improved high progress as a biocontrol agents in controlling nematodes and weeds under green house condition. So, this work aimed to assess the efficiency of the approved superior treatments in the previous study *i.e. Bacillus cereus* and *Pseudomonas aeruginosa* as a wild bacterial strains and their genetically improved strains through protoplast fusion technique namely (fusant 7, fusant 20 and fusant 35) comparing to mechanical and chemical practices against root-knot nematode (*Meloidogyne incognita*) and weeds as well as their effect on *Solanum lycopersicum* growth parameters and yield under field conditions.

Material and Methods

Bacterial strains

Two bacterial strain isolated from the Egyptian soil and had nematicidal activity against plant parasitic nematode were identified based on 16S rDNA sequence analysis in the Gene Bank database nucleotides as *Bacillus cereus* GEs (Accession No. LC215052) and *Pseudomonas aeruginosa* GEs (Accession No. LC215048.1) were realized by Ameen *et al.,* 2002. 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

Table 1: Common, trade and chemical names, dose and time of application of the applied pesticides treatments.

Pesticides treatments	Common name	Trade name	Chemical name	Dose	Time of application
Nematicide	Oxamyl	Vydate 24% liquid	N,N-dimethyl-2-methylcarbamoyloxyimino 2-(methylthio) acetamide	7.5L/ha	Immediately pre planting
Herbicides	Metribuzin	Sencor 70% WP	4-amino-6-*tert*-butyl-3-methylsulfanyl-1,2, 4-triazin-5-one	700 g/ha	Two weeks after transplanting
	Clethodim	Select super 240EC	[(*E*)-*N*-[(*E*)-3-chloroprop-2-enoxy]-*C*-ethylcarbonimidoyl]-5-(2-ethylsulfanylpropyl) -3-hydroxycyclohex-2-en-1-one	0.06 to 0.36 kg ai/ha	Post emergence foliar application at 30 DAT

35) combined all the desired properties from the parents.

Media and growth conditions

• 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 contains 2.50×10^7 cfu.

Field experiments

Two field experiments were conducted during two successive summer seasons of 2018 and 2019 at Mansoria village, Giza Governorate, Egypt. The experimental field area was divided into plots. The plot area was 22.5m^2. Each plot contained four ridges 3.75m length and 1.5m in width. The experiment was set up in a completely randomized block design with four replicates. The soil of the experimental site clay loam texture with 1.23% organic matter, 0.15% total nitrogen and pH of 7.5. *Solanum lycopersicum* seedlings Elisa cv. were transplanted at the first week of April in both seasons with 70cm in distance between them. All cultural practices were applied according to the recommendations of the Egyptian Ministry of Agriculture. Flooding irrigation was applied according to standard recommendations.

Both experiments include 11 treatments to evaluate their efficiency in controlling nematodes and weeds. These applied treatments include biological control treatments using bacterial strains and recommended chemical as well as mechanical treatments for comparison which were as follow:

1. Bacillus cereus
2. *Pseudomonas aeruginosa*
3. *Bacillus cereus* + *Pseudomonas aeruginosa*
4. Fusant 7
5. Fusant 20
6. Fusant 35
7. Oxamyl nematicide
8. Clethodium herbicide
9. Metribuzin herbicide
10. Two hand hoeing at 30 and 50 DAT
11. Untreated control

Biological control treatments using bacterial strains were inoculated to the soil twice (at transplanting and 30 DAT at 2.50×10^7cuf/ml. 10 ml of each bacterial filtrate is inoculated in the rhizosphere around each *S. lycopersicum* roots.

Recorded data

1. On *Meloidogyne incognita*:

Initial population densities of *M. incognita* were determined one week prior to transplanting time from 250 g, subsamples of well mixed soil from each plot according to Barker (1985).

After 70 and 120 Days after transplanting (DAT) *S. lycopersicum* plants were carefully uprooted and roots were washed thoroughly with running tap water. The data on nematode parameters were recorded as: numbers of galls and egg-masses per root system, second stage juveniles (J_2) per 5g roots of tomato. The second stage juveniles J_2 in soil (250 g) was extracted using sieving and decanting technique (Barker, 1985). The number of J_2 in roots was count in 5g roots using incubation method (Young, 1954). All numbers of nematodes were counted under a light microscope.

The percentage reductions of the root-knot nematode populations in soil (J_2) induced by the treatments were determined according to the formula of Handerson and Tilton (Puntener, 1981):

$$\text{Nematode reduction (\%)} = [1- (PTA/PTB \times PCB/PCA)] \times 100$$

Where; PTA = Population in the treated tomato plant after application,

PTB = Population in the treated tomato plant before application,

PCB = Population in the check tomato plant before application,

PCA = Population in the check tomato plant after application.

The Percentage nematode reduction (J_2 in roots, galls and egg-masses) were calculated with respect to untreated control (nematode only).

$$\text{Nematode reduction (\%)} = \frac{\text{untreated control-treated}}{\text{untreated control}} \times 100$$

2. On weeds:

Weed samples were collected randomly from 1m^2 in each plot at 70 DAT and at harvest. Collected weeds were classified into two groups *i.e.*, annual broad leaved and grassy weeds. Fresh weight for each group was recorded. The constant dry weight of weeds was recorded after drying in a forced draft oven at 70°C for 72 h.

3. On *Solanum lycopersicum* crop:

• Vegetative growth:

At 70 DAT, samples of five *S. lycopersicum* plants were taken from the central area of each plot for to determine plant height, number of branches, number of leaves, fresh weight and dry weight.

• Yield traits:

• Fruit quality:

Total soluble solids (TSS %) parameter using a hand refractometer was determined as a measure to fruit quality.

• Three samples of *S. lycopersicum* fruits were collected at harvested periodically along 45 days that five plants were taken from each plot to determine number of fruits /plant, weight of fruits/plant, yield of fruits kg/m^2, yield of fruits ton/fed and yield increment was determined according to this equation

$$\text{Yield increment (\%)} = \frac{\text{untreated control-treated}}{\text{untreated control}} \times 100$$

Statistical analysis

All recorded data were subjected to analysis of variance (ANOVA) according to Gomez and Gomez, (1984) using CoStat Software Program Version 6.303 (2004) and LSD at 0.05 level of significance was used for the comparison between means.

Results

***Meloidogyne incognita* parameters**

Biological control and chemical applied practices significantly ($P \leq 0.05$) suppressed *M. incognita* reproduction by reducing the numbers of J_2 in soil, galls and egg masses in tomato roots as compared to untreated infected plants at 70 DAT and at harvest (120 DAT) were recorded in table 2 and 3. At mid-season (70 DAT), the nematicidal effects of biological control applied of fusants bacterial strains (7, 35 and 20) were more effective in reducing nematode parameters than their parental wild bacterial strains (*B. cereus* and *P. aeruginosa*) in some cases. These treatments were recorded percentages reduction in galls (85.2, 71.6 & 72.7%) and egg-masses (87.5, 72.2 & 75%), respectively, as compared to untreated control (Table 2).

At harvest in both seasons, fusant 20 product was recorded the highest nematode reduction in J_2 in soil (86.6%), galls (81.7%) and egg-masses (84.6%) as compared to fusant 7 and fusant 35. Fusant 35 was more effective in suppressing *M. incognita* J_2 in roots by 80.9% followed by fusant 20 than fusant 7 which resulted in 74.1 and 70.9% nematode reduction, respectively (Table 3). It is clear that there was synergistic effect

Table 2: Effects of the applied treatments on *Meloidogyne incognita* parameters infested tomato plants at 70 DAT (Average of two seasons).

Treatments	Initial population	J_2 in soil	% Red.	J_2 in roots	% Red.	No. of galls/ root system	% Red.	No. of egg masses /root system	% Red.
B. cereus	150.0	102.0	78.74	138.0	33.65	27.0	69.31	14.0	80.55
P. aeruginosa	133.0	161.0	62.15	107.0	48.55	32.0	63.63	22.0	69.44
B. cereus + *P.aeruginosa*	128.0	159.0	61.16	158.0	24.03	27.0	69.31	20.0	72.22
Fusant 7	120.0	100.0	73.94	100.0	51.92	13.0	85.22	11.0	87.50
Fusant 20	116.0	80.0	78.43	132.0	36.53	25.0	71.59	20.0	72.22
Fusant 35	85.0	88.0	71.34	41.0	80.28	24.0	72.72	18.0	75.00
Oxamyl	113.0	69.0	80.91	30.0	85.57	13.0	85.22	8.0	84.72
Clethodim	118.0	160.0	57.60	80.0	61.53	31.0	64.77	16.0	77.77
Metribuzin	105.0	198.0	41.03	147.0	29.32	24.0	72.72	16.0	77.77
Two hand hoeing	94.0	304.0	0.00	203.0	2.40	90.0	-	69.0	4.17
Control	96.0	307.0	-	208.0	-	88.0	-	72.0	-
LSD at 5%	3.7	5.2	-	2.2	-	1.7	-	1.1	-

Table 3: Effects of the applied treatments on *Meloidogyne incognita* parameters infested tomato plants at harvest (Average of two seasons).

Treatments	Initial population	J_2 in soil	% Red.	J_2 in roots	% Red.	No. of galls/ root system	% Red.	No. of egg masses /root system	% Red.
B. cereus	150.0	471.0	84.20	419.0	63.21	263.0	66.83	100.0	67.15
P. aeruginosa	133.0	515.0	80.52	308.0	72.95	187.0	76.41	113.0	81.62
B. cereus + P.aeruginosa	128.0	394.0	84.51	468.0	58.91	179.0	77.42	150.0	75.60
Fusant 7	120.0	317.0	69.94	331.0	70.93	200.0	74.77	119.0	80.65
Fusant 20	116.0	310.0	86.55	295.0	74.10	145.0	81.71	20.0	84.55
Fusant 35	85.0	283.0	83.25	218.0	80.86	163.0	79.44	95.0	79.10
Oxamyl	113.0	219.0	90.25	205.0	82.00	103.0	87.01	74.0	87.96
Clethodim	118.0	563.0	75.99	379.0	75.50	187.0	76.41	138.0	77.56
Metribuzin	105.0	424.0	79.68	418.0	63.30	272.0	65.59	219.0	64.39
Two hand hoeing	94.0	1805.0	0.00	1122.0	1.49	785.0	1.01	602.0	2.11
Control	96.0	1908.0	-	1139.0	-	793.0	-	615.0	-
LSD at 5%	3.3	5.4	-	4.5	-	2.4	-	2.8	-

between the parents. Also, Results showed that combined parental strains (*B. cereus + P.aeruginosa*) were more effective than when applied individually in some case. Oxamyl was recorded the highest nematode reduction in previous nematode criteria compared to two herbicides (Tables 2 and 3). The treatment of two hand hoeing did not give any effect compared to untreated control at 70 DAT and at harvest (120 DAT).

Weeds growth

The dominant weeds in the investigated area of the experimentation in both seasons, were mostly grassy, *i.e. Echinochloa colonum*, (L.), *Cynodon dactylon* and *Dactyloctenium aegyptium*, (L.), in addition to a few broad-leaved weeds *i.e. Portulaca oleracea* (L.), *Anagallis arvensis* (L.) and *Hibiscus trionum* (L.).

As shown in table 4 fresh and dry weight of grassy, broad and total weeds significantly inhibited as a response to most applied management practices as compared to unweeded control treatment at 70 DAT. In this regard, clethodium herbicide, two hand hoeing and metribuzin herbicide were considered as the most efficient treatments in controlling grassy weeds which expressed in fresh and dry weight depression of the mentioned weed groups. Three investigated fusants (7, 35 and 20) and bacterial strain (*Bacillus cereus* and *Pseudemonas aeruginosa*) came in the second rank, successively. Additionally, It was observed that individual applications of wild bacterial strains were effective than the combination between them, as compared to unweeded control treatment. Two hand hoeing, metribuzin herbicide, fusants (7 and 20) as well as *P. aeruginosa* gave the lowest fresh and dry weight of broad leaved weeds, consequently.

Table 4: Effect of the applied treatments on fresh and dry weight of grassy, broad leaved and total weeds at 70 DAT (Average of two seasons).

Treatments	Fresh weight (g/m^2)			Dry weight (g/m^2)		
	Grassy weeds	Broad leaved weeds	Total weeds	Grassy weeds	Broad leaved weeds	Total weeds
B. cereus	376.85	312.67	689.52	43.11	41.19	84.30
P. aeruginosa	532.04	211.74	743.78	61.33	27.92	89.25
B. cereus + P.aeruginosa	717.15	366.82	1083.97	84.78	48.30	133.08
Fusant 7	143.08	70.93	214.01	16.81	9.48	26.29
Fusant 20	375.04	184.44	559.48	40.70	24.56	65.26
Fusant 35	205.96	260.59	466.55	24.18	34.11	58.29
Oxamyl	1085.53	738.13	1823.66	119.45	88.51	207.96
Clethodim	17.67	759.40	777.07	2.54	99.70	102.24
Metribuzin	35.10	59.63	94.73	3.83	7.92	11.75
Two hand hoeing	22.67	42.00	64.67	3.11	5.31	8.42
Control	1307.00	749.04	2056.04	178.92	98.26	277.18
LSD at 5%	14.16	9.80	17.76	2.42	2.76	5.13

Table 5: Effect of the applied treatments on fresh and dry weight of grassy, broad leaved and total weeds at harvest (Average of two seasons).

Treatments	Fresh weight (g/m^2)			Dry weight (g/m^2)		
	Grassy weeds	Broad leaved weeds	Total weeds	Grassy weeds	Broad leaved weeds	Total weeds
B. cereus	517.67	746.25	1263.92	101.30	97.78	199.08
P. aeruginosa	602.37	689.44	1291.81	124.09	88.22	212.31
B. cereus + *P.aeruginosa*	708.56	971.15	1679.71	146.12	127.22	273.34
Fusant 7	474.74	262.26	737.00	98.93	36.00	134.93
Fusant 20	540.55	498.37	1038.92	109.86	67.11	176.97
Fusant 35	453.39	578.59	1031.98	83.20	76.22	159.42
Oxamyl	2702.15	1694.12	4396.27	498.44	212.85	711.29
Clethodim	35.53	1763.48	1799.01	7.54	235.00	242.54
Metribuzin	52.70	185.63	238.33	12.27	24.67	36.94
Two hand hoeing	40.00	58.00	98.00	9.14	7.40	16.54
Control	3230.69	1841.63	5072.32	665.85	244.89	910.74
LSD at 5%	32.65	34.49	42.45	5.86	4.59	6.78

All chemical, mechanical and biological control applied practices significantly decreased fresh and dry weight of grassy, broad leaved and total weeds at harvest as compared to unweeded control treatment (Table 5). Concerning with grassy weeds, clethodium, two hand hoeing as well as metribuzin herbicides scored the lowest fresh and dry weight of weeds survey in the investigated area. These recommended superior treatments followed by biological control treatments with fusants (35, 7 and 20) and wild bacterial strains (*B. cereus* and *P. aeruginosa*), consequently as compared to unweeded control treatment. Regarding to broad leaved weeds, two hand hoeing and metribuzin herbicide scored the highest level of control that represented in lowest fresh and dry weight. Biological control which represented in fusants (7, 20 and 35) as well as wild strains (*P. aeruginosa* and

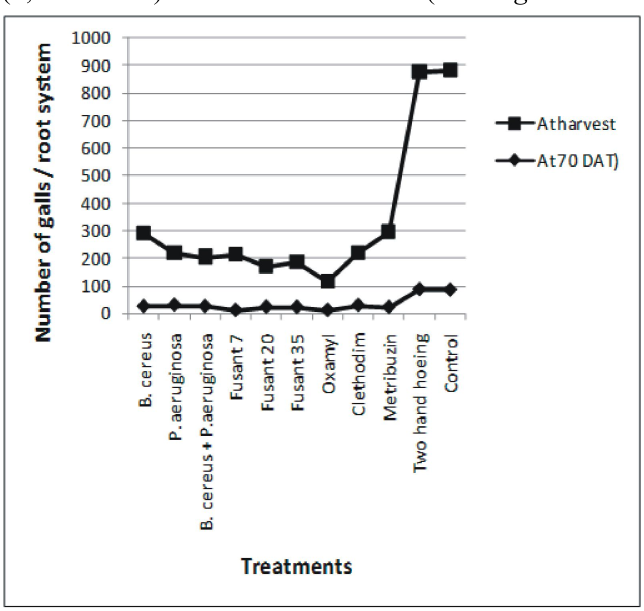

Fig. 1: Number of galls/root system at 70 DAT and at harvest.

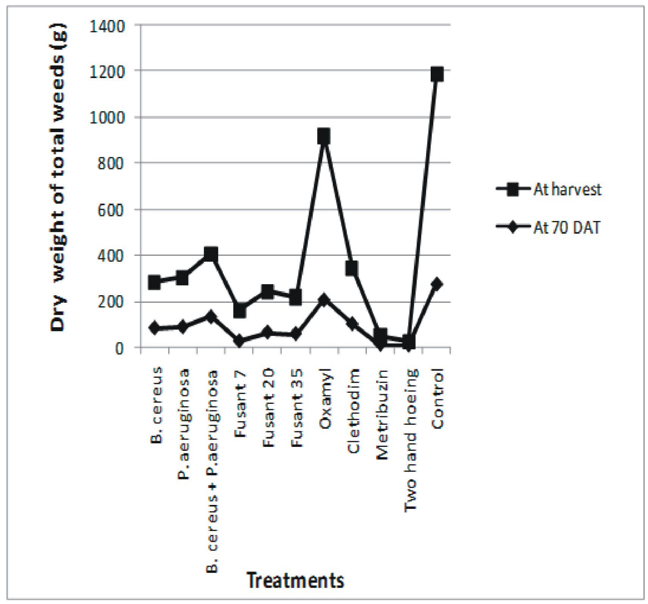

Fig. 2: Total weeds dry weight at 70 DAT and at harvest.

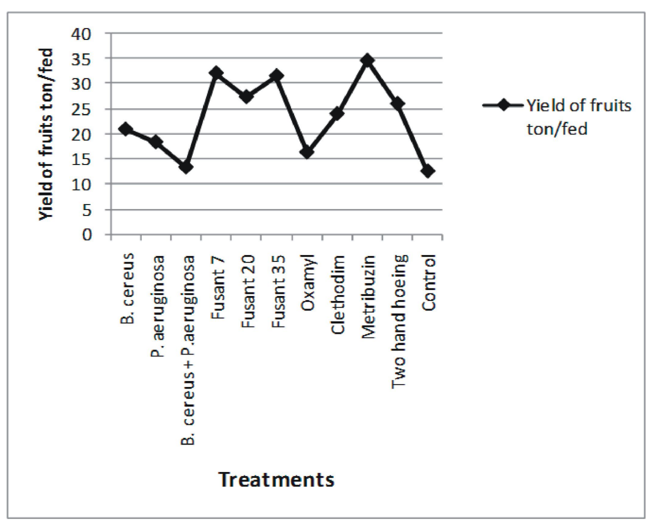

Fig. 3: Yield of fruits (ton/fed) (Average of both seasons).

Table 6: Effect of the applied treatments on *S. lycopersicum* growth parameters at 50 DAT (Average of two seasons).

Treatments	Plant height (cm)	No. of branches / plant	No. of leaves / plant	Fresh weight / plant (g)	Dry weight / plant (g)
B. cereus	52.0	2.0	22.7	56.94	21.89
P. aeruginosa	51.0	1.7	22.0	54.38	20.93
B. cereus + P.aeruginosa	44.0	1.7	21.0	41.76	16.15
Fusant 7	55.0	3.0	24.0	69.32	26.48
Fusant 20	54.2	3.0	24.0	64.60	24.77
Fusant 35	55.4	3.0	24.0	71.69	27.38
Oxamyl	45.5	2.7	21.0	39.00	14.00
Clethodim	52.3	2.7	22.7	57.38	22.00
Metribuzin	58.2	3.3	24.3	72.12	27.55
Two hand hoeing	53.0	1.7	23.7	62.27	23.55
Control	36.0	1.7	16.7	34.67	13.33
LSD at 5%	2.2	N.S.	1.6	4.43	1.66

B. cereus), successively as compared to unweeded control treatment.

Total weeds responded in the same trend at 70 DAT and at harvest. The recorded results in tables 4 and 5 ensured that two hand hoeing and metribuzin herbicide were the ideal treatments in controlling total weeds associated with *S. lycopersicum* in the investigated area. Biological control applied practices of fusants bacterial strains (7, 35 and 20) and wild bacterial strains (*B. cereus* and *P. aeruginosa*) followed the aforementioned effective treatments, successively.

Generally, It is obvious that all the inoculated bacterial strains either parents or fusants are significantly decreased both weeds and nematode pests as compared to untreated control (Fig. 1, 2). Meanwhile, the fusants were more effective than their wild parents individually or in combination in controlling weeds. Additionally, individual application of wild parents bacterial strains were more effective than combined application. Fusant 7 was the most efficient bacterial strain in controlling total weeds at the two ages in both seasons.

• *Solanum lycopersicum*

a. Vegetative growth:

The applied management practices positively reflected on the recorded vegetative growth parameters (plant height, number of branches and number of leaves as well as fresh and dry weight /plant) as shown in table 6. Most applied management practices significantly increased all growth parameters except number of branches. Metribuzin herbicide, fusant bacterial strains (35, 7 and 20) as well as hand hoeing gave the maximum values of *S. lycopersicum* vegetative growth as compared to unweeded check. It is worth to mention that fusant bacterial strains achieved vegetative growth values higher than it wild bacterial strains (*B. cereus* and *P. aeruginosa*) with. In addition, individual application of wild parents scored vegetative growth values higher than the application in combination.

Table 7: Effect of the applied treatments on *S. lycopersicum* yield attributes (Average of three collections and average of two seasons).

Treatments	% TSS	No. of fruits /plant	Weight of fruits /plant (Kg)	Yield of fruits kg/m^2	Yield of fruits ton/fed	Yield increment (%)
B. cereus	4.50	39.33	3.67	4.99	20.96	66.35
P. aeruginosa	4.30	36.67	2.74	4.38	18.40	46.03
B. cereus + P.aeruginosa	4.30	35.00	1.99	3.19	13.38	6.19
Fusant 7	5.40	57.00	4.79	7.66	32.16	155,23
Fusant 20	4.70	44.00	4.31	6.52	27.40	117.46
Fusant 35	4.90	52.00	5.12	7.52	31.60	150.79
Oxamyl	3.50	33.00	2.91	3.89	16.37	29.92
Clethodim	3.80	40.00	4.08	5.73	24.05	90.87
Metribuzin	4.80	57.33	5.16	8.26	34.66	175.08
Two hand hoeing	4.30	40.00	4.25	6.20	26.04	106.67
Control	3.10	30.00	1.76	3.00	12.60	-
LSD at 5%	0.32	2.8	1.07	1.55	2.52	-

b. Yield and its attributes

All chemical, biological and mechanical applied practices significantly increased yield quality (% TSS) as well as yield and its attributes (number of fruits/plant, weight of fruits/plant, yield of fruits/m^2 and yield of fruit/fed.) were illustrated in table 7 and fig. 3. Fusant 7 and Fusant 35 scored the highest %TSS followed by metribuzin herbicide. Whereas, metribuzin herbicide came in the first rank in improving yield and its attributes parameters. The investigated bioagents *i.e.* fusnts (7, 35 and 20) came the second rank after chemical herbicide. These superior treatments scored yield increment ton/fed reached to 175.08, 155.23, 150.79 and 117.46%, successively.

Discussion

Our study focused on evaluating the dual suppression effect of safe biocontrol agents on nematodes and weeds in comparing with chemical and mechanical recommended methods. Herein, the present result revealed that the genetically improved Bacteria *viz*. fusant 7, fusant 20 and fusant 35 that intergeneric through protoplast fusion between *B. cereus* and *P. aeruginosa* were more effective in controlling root-knot nematode (Tables 2 and 3). These are in agreement with the findings of Abdel-Salam *et al.,* 2018 who revealed that the *intergeneric* protoplast fusion technique between *B. amyloliquefaciens* and *L. sphaericus* enhanced the production of the enzyme chitinase which increased the percentage mortality of *M. incognita* J_2. In accordance with nematicidal activities, Bas 8 which produced high chitinase induced complete mortality of *M. incognita* J_2 *in vitro* test after 24 h. Under greenhouse conditions, Bas 8 resulted in the higher decrease in nematode reproduction on tomato plants than its parents singly or in combination and increased tomato growth. Rhizobacteria can colonize the plant roots and secrete extracellular enzymes that stimulate plant growth against pathogens, including the root-knot parasite nematodes. Additionally, rhizobacteria secrete proteases for suppression of plant pathogenic nematodes by several mechanisms. Proteases and chitinases that play an important role in degradation of the nematode cuticle and serve as nematicidal factors for biocontrol of nematode populations. (Ali *et al.,* 2002). The alkaline protease, BLG4, produced by *Bacillus laterosporus* reduced about 57% of nematode activity (Tian *et al.,* 2007). Other study revealed that production of chitinase, chitosanase and protease by *Pseudomonas aeruginosa*, *Paenibacillus polymyxa*, *Lysinibacillus sphaericus*, *Bacillus cereus*, *Bacillus subtilis* and *Achromobacter xylosoxidans* has effectively suppressed *M. incognita* juveniles, inhibited egg hatching and reduced nematode growth and activity (Soliman *et al.,* 2019). Additionally, *Pseudomonas* or *Bacillus* spp. could colonize the rhizosphere of plants in, on or around plant tissues, stimulate plant growth and reduce nematode populations by antagonistic behavior. The application of some of these bacteria recorded promising results. The bacteria antagonistic to plant parasitic nematode in the rhizosphere provide the frontline defense for roots against nematode attack (Yang *et al.,* 2013). They act by producing toxins and enzymes that suppress nematode reproduction, egg hatching and juvenile survival as well as direct killing of nematodes (Siddiqui and Mahmood, 1999). Different attempts had used the protoplast fusion technique to obtain more efficient nematicidal bacterial strains as reported by El-Hamshary *et al.,* 2004, the intergeneric fusants between *P. fluorescens* and *P. aeruginosa* were more effective than its parental strains in reducing nematode as well as enhancing plant growth. Also, the intergeneric fusants between *Serratia* and *Pseudomonas* induced high mortality levels against *M. incognita* (Zaied *et al.,* 2009).

Although chemical application of either metribuzin or clethodim herbicides scored the highest control of broad-leaved and grassy weeds, successively, the genetically improved bacterial strains (fusant 7, fusant 20 and fusant 35) afforded really expressive values (Tables 4 and 5) in controlling both weeds as compared to weedy check. Karadeniz *et al.,* 2006 reported that *B. cereus* could produce indole-3-acetic acid, zeatin, giberelic acid and abscisic acid. All these substances are plant growth hormones that affect on plant depending on their concentrations (Pasqual, 2001). The literature ensured that *Bacilus pumillus* can be applied as a bioherbicidal agent which may be accompanied with the production of indole-3-acetic acid (Kang *et al.,* 2006). However, Kim and Kremer, 2005 and Karadeniz *et al.,* 2006 reported the deleterious effect of *Bacillus megaterium* on *Ipomoea hederacea* Jacq. growth through the production of phytotoxins. On the other hand. Caryalho *et al.,* 2007 ensured that it had no effect on lettuce crop. In addition, Egamberdieva *et al.,* 2008 concluded that *B. cereus* bacterial strains had the ability to produce indole-3-acetic acid (IAA) growth promoter. IAA is dose-dependent that is toxic to plant when used at inhibitory concentration (above 10^{-4}M) that can be applied as a herbicide (Taiz and Zeiger, 2006; Caryalho *et al.,* 2007). Anyway, the recorded results seem in accordance with Japan Tobacco Inc. 1998 that reported the use of *B. pumilus* as a bioherbicide. Whereas, the hydro-cyanic acid content of *P. fluorescens* WSM3455 strain may be responsible for the suppression in growth of *Raphanus raphanistrum* L. and *Lolium erigidum Guad* (Flores-Vargas and O'Hara, 2006). In this regard, Li and Kremer, 2006 ensured that G2-11 strain of *P. fluorescens* achieved suppression effect on *Ipomea* spp. and *Convolvulus arvensis* associated to wheat and soybean which are promoted. This inhibitory effect may be related to the bacterial phytotoxic metabolites and enzymes. Additionally, Flores-Vargas and O'Hara, 2006 attributed the inhibitory effect of *P. fluorescens* WSM3455 strain on *Raphanus raphanistrum L. and Lolium Erigidum Guad* to the hydro-cyanic acid.

As the metribuzin herbicide achieved the highest deleterious effect on total distributed in the investigated area, it reflected on *S. lycopersicum* yield scoring the highest *S. lycopersicum* yield. The beneficial effect of *B. cereus* to *S. lycopersicum* growth and yield improvement is given in tables 6 and 7. Kang *et al.,* 2015 and Kuan *et al.,* 2016 revealed that *Bacillus* spp. had the ability to convert P and N complex form to simple available form. Moreover, *Bacillus* spp. secret phosphatase and organic acids that acidifies the surrounding which is favorable for the conversion of inorganic phosphate into free phosphate (Kang *et al.,* 2015). However, some *Bacillus* spp. have the ability to liberate ammonia from nitrogenous organic matter (Hayat *et al.,* 2010). So, *Bacillus* spp. act as a biofertilizers beside its role as plant growth promoting hormones and enzymes as mentioned before that all act to increase plant growth and yield. It is worthy to mention that the selective effect of bacterial strains as a bioherbicide may be related to the release of phytotoxic compounds, bacterial metabolites, enzymes and growth regulators (Li and Kremer, 2006). The variation among plant species differ in response to these bacterial metabolites (Caryalho *et al.,* 2011). Cyanide production is the characteristic of *Pseudomonas* spp. Cyanide act as plant a biocontrol agent that can be considered as a plant growth promoter as well as plant growth inhibition (Martinez-Viveros *et al.,* 2010). Although two hand hoeing mechanical treatment deleteriously most efficient treatment in controlling weeds associated with *S. lycopersicum*, but didn't achieve the highest yield as nematodes pest was not affected by this treatment and seems to equal control treatment.

Conclusion

Twice inoculation of genetically improved bacterial strains (fusant 7, fusant 35 and fusant 20) to the soil at 2.50×10^7cuf/ml has effectively suppressed *M. incognita* nematode and weeds infested S. *lycopersicum* crop as compared to control. These aforementioned applied strains are more effective biocontrol agents than their wild bacterial strains (*B. cereus* and *P. aeruginosa*). Fusants under investigation increased S. *lycopersicum* yield, this increment reached to (117.46 to 155.26%). So, the three investigated fusants can be applied in both nematode and weed safe management strategies and provide an alternative approach to chemical nematicides and herbicides.

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