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Efficacy of integrated microorganisms in controlling root-knot nematode *Meloidogyne javanica* infecting peanut plants under field conditions

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Abstract

Background: Peanut (*Arachis hypogaea* L.) is considered one of the most important legume and oil crops in Egypt. Root-knot nematodes *Meloidogyne* spp. is the most damaging nematodes of peanut. Losses can exceed 50% in severely infested soil. Application of nematicides is one of the primary strategies in controlling plant-parasitic nematodes. The nematicides are proven to be hazardous to the environment. A promising alternative is the use of microorganisms antagonistic to plant-parasitic nematodes. Some microorganisms that can grow in the rhizosphere such as *Azotobacter* and *Bacillus* bacteria and fungi, e.g., *Trichoderma* and *Paecilomyces* represent the front line of defense for roots against nematode attack and ideal for use as biocontrol agents.

Objectives: The main aim of this study was to evaluate the efficacy of application of yeast fungus *Saccharomyces cerevisiae* singly or combined with fusant Bas 8; (*Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* Amira strain); *Bacillus thuringiensis* strain code K, *Trichoderma harzianum*, or *Paecilomyces lilacinus* to control the root-knot nematode *Meloidogyne javanica* infecting peanut plant cv. Giza 6, and to estimate their yield under field conditions.

Results: Compared to the untreated control, all treatments exhibited variable potential inhibitory activities against root-knot nematode *M. javanica* and enhanced peanut yield production. The most nematode suppressive treatment was the single treatment of *Saccharomyces cerevisiae*, followed by *T. harzianum* either singly or combined with the yeast. Moreover, data indicated that application of the combined treatment of yeast plus *T. harzianum* gave the best results in improving peanut production, plant growth parameters, and seed nutrient contents.

Conclusion: It was concluded that integrated application of microorganisms could enhance peanut production and reduce the need for either chemical fertilizers or nematicides.

Keywords: Root-knot nematode, *Bacillus thuringiensis*, *Saccharomyces cerevisiae*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, Fusant Bas 8, Peanut plant

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Background

Peanut (*Arachis hypogaea* L.) is considered one of the most important legume and oil crops in Egypt. Its seeds have high nutritive value for humans, and their green leaf hay for feeding farm animals (Fageria et al. 1997). The cultivated area of peanut in Egypt has been recorded to be 190,000 hectare in the season 2017, (Anonymous 2017). Plant-parasitic nematodes are considered one of the major obstacles to the production of peanut crop. Root-knot nematodes *Meloidogyne* spp. is the most damaging nematodes in agriculture (Oka 2010). Several species of root-knot nematodes are pathogenic on peanut and cause considerable yield loss annually (Starr and Morgan 2002). Losses can exceed 50% in severely infested soil. Ibrahim and Mokbel (2009) reported the occurrence of *M. arenaria* on peanut plants in Alexandria and El-Behera governorates of Egypt. Many other workers reported the occurrence of *M. arenaria* and or *M. javanica* on peanut plants (Starr and Morgan 2002).

Application of nematicides is one of the primary strategies in controlling plant-parasitic nematodes. The nematicides have been proven to be carcinogenic, build up residues in the edible plants, and leak out into groundwater. Moreover, some of these chemicals are hazardous to livestock, plant, and beneficial fauna and flora of the soil. Due to constrain in the use of such nematicides, alternative methods for disease management are being applied (Osman et al. 2018).

A promising alternative is the use of microorganisms antagonistic to plant-parasitic nematodes. Some microorganisms that can grow in the rhizosphere such as *Azotobacter* and *Bacillus* bacteria, and fungi, e.g., *Trichoderma* and *Paecilomyces* represent the front line defense for roots against nematode attack and ideal for use as biocontrol agents. Biological control of plant-parasitic nematodes by using fungi and bacteria has been found to be a feasible option (Osman et al. 2018).

Bacteria that colonize roots are termed plant growth promoting rhizobacteria (PGPR). Controlling nematodes by using the rhizobacteria can be achieved by many mechanisms such as direct effects on egg hatching (Meyer et al. 2001), production of toxins (Ravari and Moghaddam 2015), plant growth promotion (Kloepper and Ryu 2006), induction of enzymes that modulate plant hormone levels, hydrolytic enzymes of proteins and glucanase, and antibiotics or antimicrobial products of low molecular weight (Sela et al. 1998). Other indirect effects include alteration of root exudates which make roots less attractive to nematodes. Another promising mechanism has been studied in detail is induced systemic resistance against plant pathogenic nematodes (Kloepper and Ryu 2006). A particular plant growth-promoting bacterium may have one or more of these mechanisms (Kerry 2000). Many studies confirmed the

toxicity of the *B. thuringiensis* against plant pathogenic nematodes and improving host production (Elkelany 2017; Osman et al. 2018; Saad et al. 2019).

The antagonistic fungi play an important role as bio-control agents for many plant-parasitic nematodes (Jatala 1986). *Paecilomyces lilacinus*, a saprophytic soil fungus has attracted many research workers due to its promising effect in parasitizing and controlling the population of *Meloidogyne* spp. and improving plant growth (Brand et al. 2010, Wagh and Pramanik 2014). The pathogenic fungus *P. lilacinus* has a high frequency of occurrence in the tropics, subtropics areas and can be found in most of agricultural soils.

Trichoderma is a genus of fast-growing fungi widely distributed in soil. *Trichoderma* spp. is known in plant agriculture, both for disease control and yield improvement even under axenic conditions (Harman 2006). The role of *Trichoderma harzianum* in controlling nematodes and improving plant growth was reported by many investigators (Olabiya et al. 2013; Osman et al. 2018).

In recent years, considerable attention has been paid to the application of antagonistic yeasts for controlling of different plant diseases and improving plant growth (Hashem et al. 2008; Karajeh 2013; Cunha et al. 2018). *Saccharomyces cerevisiae* is considered a promising yeast fungus for promoting plant growth of different crops (Ignatova et al. 2015). It became an effective alternative to chemical fertilizers safely used for human, animal, and environment (Omran 2000). The yeast fungus, *S. cerevisiae*, reduced infection of *M. incognita* on Egyptian hebane plant, *Hyoscyamus*, and increased its growth (Youssef and Soliman 1997).

The main aim of this study was to compare the efficacy of application of yeast fungus *S. cerevisiae* singly or combined with Fusant Bas 8; (*Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* Amira strain); *B. thuringiensis* (strain code K), *T. harzianum*, or *P. lilacinus* in controlling the root-knot nematode *M. javanica* infecting peanut plant, and estimate their yield under field conditions.

Materials and methods

Source of seeds

Seeds of peanut cv. Giza 6, which is susceptible to *M. javanica*, were obtained from the Department of Horticulture Research Centre, Ministry of Agriculture, Giza, Egypt.

Preparation of the experimental field and nematode identification

The experimental area was naturally infested with root-knot nematode *M. javanica*. The roots of peanut plants previously planted in the experimental field were collected, and the adult females were removed from their

egg masses. Adult females were collected to identify the nematode species by the morphological characteristics according to the female perineal pattern (Taylor and Sasser 1978). Initial population densities of *nematodes* were determined as described under experimental design and treatments.

Sources of used microorganisms

Five nematode-antagonistic microorganisms viz, *S. cerevisiae*, Fusant Bas 8 (produced by protoplast fusion technique between two parents, *Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* Amira strain and registered in Gene Bank); *B. thuringiensis* strain code K, *T.harzia-num*, and *P. lilacinus* were provided as follows:

- *B. thuringiensis* Bt. (strain code K) from Microbial Genetics Department, National Research Centre, Giza, Egypt. Lauria Bartani (LB) medium (Davis et al. 1980) was used for bacterial culture.
- Fusant Bas 8 (*Bacillus amyloliquefaciens* sub sp. plantarum) SA5, Microbiol Genetic Dept. NRC (GenBank accession number: KCU 29571.1). *Lysinibacillus sphaericus* Amira strain, Microbiol Chemistry Dept., NRC (GenBank accession number: KT 361851.1).
- Cultures of *T. harzianum* and *P. lilacinus* on liquid media, potato dextrose broth (PDB), from the laboratory of Plant Pathology Department, National Research Centre, Giza, Egypt.
- Dry active bread yeast (commercial product), containing the fungus (*Saccharomyces cerevisiae*), was carefully prepared before use by adding 100 g of the active bread yeast +20 ml Egyptian treacle +1 L of distilled water to prepare a solution to activate reproduction of yeast. Yeast solution was left standing at 38 °C for 1 hour before soil application (5 ml/hill).

Experimental design and treatments

Agricultural practices

Inorganic and organic fertilizers were applied according to the following schedule: potassium sulfate 60 kg/faddan and urea 50 kg/faddan 30 days after planting, followed by calcium nitrate 100 kg/faddan 50 days after planting.

This field experiment was carried out during June–October 2018, at Kafre Hakim village Giza Governorate, Egypt. The environmental conditions during this season were temperature (30–40 °C) and humidity (40–60%). The experimental area (naturally infested with *Meloidogyne javanica*) was divided into plots each comprising of rows of 5 m length and 50 cm width, and the distance between each plant was 15 cm. The experiment was set up in a completely randomized block design with eleven

treatments (ten of them representing microorganisms under investigation and one treatment representing positive control using the nematicide oxamyl). Each treatment was replicated three times. Methods of application and doses are shown in Table 1. Initial population densities of *M. javanica* juveniles were determined 1 week prior to planting time from 250 g subsamples of well-mixed soil from each row according to Barker (1985). Four months later, at harvest time, five plants were chosen at random from every row, carefully uprooted and the following data were recorded. (1) Peanut pods were hand harvested for yield estimation as follows: Peanut pods were collected, and the pods from each of the five rows taken were weight and their average weights were recorded. (2) For evaluation of nematode reproductive parameters, the numbers of root galls and egg masses/5 g roots were recorded. Final nematode soil population was determined according to Barker (1985). The nematodes were counted and an average of three counts was taken to determine the final population densities of *M.javanica* juveniles in soil and expressed as number of juveniles/250 g soil. Percentage nematode

Table 1 Treatments designed for controlling root-knot nematode, *Meloidogyne javanica*, infecting peanut plant cv.6 under field conditions

Treatments	Concentrations	Methods and time of applications
A: Single applications		
1-Infected untreated negative Control		
2-Oxamyl* positive control	6 ml/L 24% liquid	Foliar spraying two times. The first one after plants emerged and the second 15 days from the first spraying
3-Fusant Bas 8	2 × 10 ⁶ CFU/ml	7 ml/of the suspension/hill at planting
4- <i>Bacillus thuringiensis</i> Bt (strain code K)	2 × 10 ⁶ CFU/ml	20 ml/of the suspension/hill at planting
5- <i>Saccharomyces cerevisiae</i> yeast	5 ml/hill	At planting
6- <i>Paecilomyces lilacinus</i>	1 × 10 ⁹ CFU/ml	At planting
7- <i>Trichoderma harzianum</i>	10 ⁴ CFU/ml	10 ml of the filtrate/hill, at planting
B: Combined treatments (methods+concentration + time of application as individual treatments)		
8-Yeast+fusant Bas 8		
9-Yeast+Bt		
10-Yeast+ <i>P. lilacinus</i>		
11-Yeast+ <i>T.harzianum</i>		

Oxamyl* is a systemic nematicide: methyle-N-N-dimethyl-(N-9methyl) carbomycocyl-1-thioamiate

reduction was determined according to Henderson and Tilton formula (Puntener 1981) as follows:

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

where PTA is the population in the treated plot after application, PTB is the population in the treated plot before application, PCB is the population in the check plot before application, and PCA is the population in the check plot after application.

Methods of analysis

The soil nutrient status of the experimental field was subjected to analysis (Table 2) in the Department of Soil Sciences National Research Centre, Giza, Egypt according to (Chapman and Pratt 1961).

Nitrogen, phosphorus, and potassium in seeds were determined according to the methods described by (A. O. A. C 2000); nitrogen (%) was estimated by using Micro-Kjeldhl, then the seed protein content was calculated by multiplying total nitrogen content by 6.25 (A. O. A. C. 1990); phosphorus (%) was also estimated by using a spectrophotometer and micronutrients elements by using atomic absorption spectrophotometry.

Statistical analysis

Data were subjected to analysis of variance and means were compared according to Duncan (1951).

Table 2 Physical and chemical properties of the soil of the experiment

Components	
Silt %	20.2
Sand %	21.6
Clay %	58.0
Chemical analysis	
pH	8.0
Ec	1.2/ds m ⁻¹
Soluble ions in soil paste (meq/L)	
Ca ⁺⁺	4.8 meqL ⁻¹
Mg ⁺⁺	3.5 meqL ⁻¹
K ⁺	0.74 meqL ⁻¹
Na ⁺	1.3 meqL ⁻¹
Cl ⁻	1.24 meqL ⁻¹
Hco ₃ ⁻	5.5 meq ⁻¹
SO ₄ ⁻	5.6 meqL ⁻¹
Available elements (ppm)	
P	112 ppm
N	344 ppm
Organic matter%	2.2

Results

Effect of different treatments on nematode reproductive parameters

Under field conditions, the obtained data in Table 3 indicated that all treatments resulted in variable significant decreases in *Meloidogyne javanica* populations in soil and roots of peanut plants compared to the untreated control. The percentage reduction in *M. javanica* egg masses were decreased by 81.9%, 76.1%, 71.4%, 60.0%, and 59.1% by using each of yeast *Saccharomyces cerevisiae*, *Trichoderma harzianum*, *Bacillus thuringiensis*, *Paecilomyces lilacinus*, and the fusant Bas 8 respectively compared to untreated control. The combination of fusant Bas 8 with yeast produced the least significant reduction (40.0% and 44.7%) in galls and egg masses of *M. javanica* than single treatment (56.0% and 59.1%) respectively. However, the applications of combined treatments of yeast with *P.lilacinus* were significantly effective than *P. lilacinus* single treatment in reducing *M. javanica* population in soil. The combined treatment exhibited 84.2% reduction while the single treatment exhibited 66.6% reduction in *M. javanica* population in soil. Moreover, the applications of combined treatments of yeast with either *P.lilacinus* or *B. thuringiensis* were more effective than *P. lilacinus* or *B. thuringiensis* single treatments. These combined treatments exhibited 65.7% and 74.2% reduction in egg masses due to *P. lilacinus* and *B. thuringiensis* respectively. Compared with the nematicide oxamyl, the results showed the greatest percentage reduction 91.2%g in *M. javanica* population in soil versus untreated control.

Effects of different treatments on peanut yield

All treatments showed a significant increase in peanut yield compared to the untreated control (Table 3). The combined treatment with yeast plus *T. harzianum* exhibited the highest percentage increase 202.7% in yield as compared to untreated control. It was followed by application of single treatment of *P. lilacinus*, *B. thuringiensis*, *T. harzianum*, the fusant Bas 8, and yeast. The percentage increase in yields were 166.6%, 152.7%, 130.5%, 111.1%, and 100% respectively compared to untreated control. While the nematicide oxamyl exhibited 52.8% percentage increases in peanut production as compared with untreated control (Table 3).

Effects of different treatments on peanut growth parameters

Fresh and dry weight of 100 pods and weight of 200 seeds in 100 pods

The data in Table 4 revealed that all the treatments improved peanut pod characters and weight of 200 seeds in 100 pods except by application of the combined treatment of yeast plus the fusant Bas 8 (-9.2%) and the

Table 3 Effects of fusant Bas 8, *Bacillus thuringiensis*, *Saccharomyces cerevisiae*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, and oxamyl on peanut plants cv. Giza 6 infected with root-knot nematode *Meloidogyne javanica* under field conditions

Treatments	Initial pop. 250/g soil	Final pop.	% Red	Root galls/5 g roots		Egg masses/5 g roots		Peanut production	
				No.	% Red.	No.	% Red.	kg	% Inc.
Oxamyl (positive control)	901 a	140 e	91.2	42.0 fg	66.4	28.0 cd	73.3	5.5 e	52.8
Fusant Bas 8	907 a	253 d	84.3	55.0 d	56.0	43.0 c	59.1	7.6 cd	111.1
<i>Bacillus thuringiensis</i> (Bt)	908 a	256 d	84.1	42.0 fg	66.4	30.0 cd	71.4	9.1 bc	152.7
<i>Saccharomyces cerevisiae</i> (Yeast)	902 a	352 c	78.0	37.0 fg	70.4	19.0 f	81.9	7.2 d	100.0
<i>Paecilomyces lilacinus</i>	900 a	532 b	66.6	56.0 d	55.2	42.0 cd	60.0	9.6 ab	166.6
<i>Trichoderma harzianum</i>	904 a	242 d	85.0	36.0 g	71.2	25.0 ef	76.1	8.3 bc	130.5
Yeast+ usant Bas 8	898 a	239 d	85.00	75.0 c	40.0	58 b	44.7	4.9 ef	36.1
Yeast + Bt	903 a	247 d	84.6	45.0 ef	64.0	27 de	74.2	7.2 d	100.0
Yeast+ <i>P. lilacinus</i>	908 a	255 d	84.2	52.0 de	58.4	36 cd	65.7	8.6 bc	138.8
Yeast+ <i>T. harzianum</i>	900 a	270 d	83.1	37.0 fg	70.4	25 ef	76.2	10.9 a	202.7
Infected untreated (negative control)	907 a	609 a	-	125.0 a	-	105 a	-	3.6 f	-

Each value represents mean of five replicates

No. number, % Red. reduction, % Inc. % increase

Means followed by the same letter (s) within a column are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test

Initial pop. initial population of *M.javanica* juveniles, Final pop. final population of *M. javanica* juveniles

nematicide oxamyl (-19.4%) reduction in weight of 200 seeds in 100 pods compared to untreated control. The combined treatment of yeast plus *T. harzianum* resulted in the highest significant increase 107.9% and 129.5% in weight of 200 seed and dry weight of 100 pods respectively as compared to *T. harzianum* single treatment which exhibited 23.7% and 27.1% respectively.

Weight of dry shells of 100 pods

All the treatments whether single or combined significantly increased the dry shell weight of 100 pods

compared to untreated control. The greatest percentage increase 207.6% was recorded in *P. lilacinus* single treatment compared to untreated control. It was followed by 197.1% by application of yeast plus *T. harzianum* combined treatment compared to untreated control. However, the application of yeast plus *B. thuringiensis* resulted in the lowest percentage increase 36.2% in weight of dry shells compared to untreated control. However, the nematicide oxamyl exhibited a 143.2% increase in weight of dry shells compared with the untreated control (Table 4).

Table 4 Growth parameters of peanut plant infected by *M. javanica* and treated with fusant Bas 8, *Bacillus thuringiensis*, *Saccharomyces cerevisiae*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, and oxamyl under field conditions

Treatments	Fresh weight of 100 pods		Dry weight of 100 pods		Weight of 200 seed of 100 pods		Yield of dry shell of 100 pods	
	(g)	% Incr.	(g)	% Incr.	(g)	% change	(g)	% Inc.
Oxamyl (positive control)	427.1 ed	22.0	372.7 ef	20.1	189.3 l	-19.4	183.4 d	143.2
Fusant Bas 8	583.6 b	66.4	466.7 c	50.4	305.0 d	30.2	160.8 e	113.2
<i>Bacillus thuringiensis</i> (Bt)	606.2 cd	72.8	420.1 e	35.3	273.1 b	16.3	147.0 f	94.9
<i>Saccharomyces cerevisiae</i> (Yeast)	482.2 c	37.4	360.8 f	16.3	248.9 f	6.0	111.9 g	48.4
<i>Paecilomyces lilacinus</i>	600.3 b	71.1	485.7 c	56.5	253.7 f	8.0	232.0 g	207.6
<i>Trichoderma harzianum</i>	463.1 cd	32.1	394.4 e	27.1	290.5 e	23.7	103.9 g	37.7
Yeast + fusant Bas 8	381.8 ef	8.8	332.5 gh	7.2	213.4 g	-9.2	119.1 g	57.9
Yeast+ Bt	483.8 c	37.3	425.9 d	37.3	323.2 c	37.6	102.7 g	36.2
Yeast+ <i>P. lilacinus</i>	640.7 b	82.6	520.2 b	67.6	314.8 cd	34.0	205.4 c	172.4
Yeast+ <i>T. harzianum</i>	900.9 a	156.8	712.3 a	129.5	488.3 a	107.9	224.0 b	197.1
Infected untreated (negative control)	350.7 f	-	310.3 h	-	234.9 h	-	75.4 h	-

Each value represents mean of five replicates

% Red. % reduction, % Inc. % increase, % change % change of control

Means followed by the same letter(s) within a column are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test

Seed quality

Protein contents as well as some macro and micronutrients in peanut seeds are shown in Table 5. The results revealed that most of the treatments had relatively positive effects on nutritional status as compared to infected untreated control. Concerning seed contents of macro nutrients, the highest significant protein content (9.75), (9.63), and (9.13) were obtained in *B. thuringiensis*, yeast single treatments, and yeast combined with *T. harzianum* respectively as compared with infected untreated control. Moreover, the highest significant increases (1.56%), (1.54%), in N% value were recorded in *B. thuringiensis*, yeast single treatment respectively. It was followed by an insignificant increase (1.46%) in yeast combined with *T. harzianum* treatment.

Almost the same trend was observed in phosphorus content. The highest significant increase (0.86%), (0.73%), (0.67% and 0.67%) in P value were recorded in yeast combined with *T. harzianum*, oxamyl, yeast, and *B. thuringiensis* single treatments respectively compared to infected untreated control.

Generally, potassium contents were not significantly affected by any treatment (Table 5).

Regarding the seed contents of micro elements, data in Table 5 clarified that Zn concentration was significantly increased in all the treatments except insignificant differences were recorded in yeast combined with fusant Bas 8 treatment and oxamyl treatments. The highest significant increase 8.46 in Zn concentration was recorded in *B. thuringiensis* treatment, followed by 4.20 due to application of *P. lilacinus* compared to infected untreated control.

Concerning Mn content, Bt single treatment exhibited the highest value 1.18 in Mn content as compared to infected untreated control. It was followed by yeast combined

by either *P. lilacinus* or *T. harzianum*, then yeast single treatment. Insignificant differences were recorded in oxamyl and in yeast combined with fusant Bas 8 treatments in Mn content as compared to infected untreated control.

In contrast, all the treatments exhibited a significant decrease in copper content except *B. thuringiensis* single treatment recorded increase (5.38) in Cu content, followed by (2.98) significant increase due to yeast single treatment compared to untreated control (Table 5).

Discussion

The results of Table 3 can be discussed in the light of Hashem et al. (2008) who stated that the production of acetic acid, chitinases, and proteases by different yeast strains may result in a similar action in killing the nematode juveniles. It could be suggested that the accumulation of such enzymes in yeast plus either *P. lilacinus* or *B. thuringiensis* treatments may lead to synergistic effects on reducing egg masses compared to their single treatments. This hypothesis needs to be confirmed by future studies. The effects of the nematicide oxamyl against *M. javanica* in our study came in the same direction with Dramola et al. (2013), Osman et al. (2018) who recorded the efficacy of oxamyl as a carbamate nematicide in suppressing nematode population and increasing the crop yield. They attributed this effect to be due to starvation resulting from the inability of the nematodes to penetrate and initiate feeding sites on the roots and not due to acute toxicity of the nematicide. On the other hand, the nematicide oxamyl proved their potentiality in controlling *Meloidogyne* spp., by inhibiting cholinesterase enzyme in nematodes leading to increased levels of the neurotransmitter acetylcholine that end with paralysis of the nematode (Nordemeyer and Dickson 1990).

Table 5 Protein content, macro and micronutrients in peanut seeds in response to fusant Bas 8, *Bacillus thuringiensis* Bt, *Saccharomyces cerevisiae*, *Paecilomyces lilacinus*, and *Trichoderma harzianum* applications of peanut plants infected by *M. javanica*

Treatments	N%	Protein**	P%	K%	Zn (ppm)	Mn (ppm)	Cu (ppm)
Oxamyl (positive control)	1.35b	8.44f	0.73ab	1.64a	2.96j	0.62 h	1.40d
Fusant Bas 8	1.25b	7.81i	0.63bc	1.46a	3.08e	0.64 g	1.24 g
<i>Bacillus thuringiensis</i> (Bt)	1.56a	9.75a	0.67b	1.45a	8.46a	1.18a	5.38a
<i>Saccharomyces cerevisiae</i> (yeast)	1.54a	9.63b	0.67b	1.42a	3.00l	0.90C	2.98B
<i>Paecilomyces lilacinus</i>	1.04b	6.50j	0.55bc	1.44a	4.20b	0.88d	1.08j
<i>Trichoderma harzianum</i>	1.28b	8.00 h and	0.58bc	1.51a	3.02 h	0.72f	1.36f
Yeast+ fusant Bas 8	1.32b	8.25 g	0.52 cd	1.51a	2.96j	0.54i	1.10i
Yeast+Bt.	1.38b	8.63e	0.55bc	1.44a	3.04 g	0.84e	1.14 h
Yeast+ <i>P. lilacinus</i>	1.43b	8.94d	0.56bc	1.75a	3.32c	0.98b	1.06j
Yeast+ <i>T. harzianum</i>	1.46b	9.13c	0.86a	1.65a	3.06f	0.98b	1.38e
Infected untreated (negative control)	1.02b	6.38 k	0.42d	1.40a	2.96j	0.62 h	1.50c

Means followed by the same letter(s) within a column are not significantly ($P \leq 5.0$) different according to Duncan's multiple range test

Total protein content: protein content was determined by Kjeldal method for the calculation of all protein which equal nitrogen content multiplied by 6.25 (A.O.A.C. 1990)

**Calculated from nitrogen content

In a previous study in our laboratory, the nematicidal activities of the fusant Bas 8 and its parents (*Bacillus amyloliquefaciens*, *Lysinibacillus sphaericus*) were studied on *Meloidogyne incognita* on tomato plant under greenhouse conditions. Fusant Bas 8 proved to be more effective in reducing nematode reproduction and increasing tomato plant growth more than its parents (Soliman et al. 2017). The data in Tables 3 and 4 of the present study indicated that fusant Bas 8 single treatment exhibited significant reduction in galls and egg masses, and significantly increased peanut yield compared with untreated control. El-Hamshary et al. (2006), and Zaied et al. (2009) indicated that the protoplast fusion technique is a promising biotechnological approach to improve the potentiality of bacterial strains to control plant-parasitic nematodes and improve plant growth.

The toxicity observed by application of fusant Bas 8 and the nematicide oxamyl on the weight of 200 seeds of 100 pods compared to untreated control may be indirectly supported by the findings of Karajeh (2013), who indicated that the use of the organophosphate ester nematicide ethoprophos in controlling root-knot nematode *M. javanica* infecting cucumber plant resulted in phytotoxicity caused by stress on cucumber plants as evidenced by reduced growth compared with the other treatments and the untreated control. Moreover, Osman et al. (2017) reported that the chemical nematicide oxamyl plus amino green11 treatment resulted in a decrease in sunflower shoot weight compared to untreated control. Also, the combined treatment of another nematicide furfural plus amino green 11 exhibited a significant decrease in sunflower oil contents. The authors suggested that the applied dose levels were not appropriate. The mechanism of the observed phytotoxicity in the present study may be explained in the light of the fact that a possible synergistic toxic action of yeast plus fusant Bas 8 treatment or by application of nematicide oxamyl treatment at the applied dose levels. In fact, there has been evidence that application of yeast increased generation of H₂O₂ (Guo and Ohta 1994) and it could be suggested that these interactions may include accumulation of secondary metabolites such as hydrogen peroxide and oxygen free radicals leading to oxidative stress that inhibit plant growth parameters. However, this hypothesis needs to be confirmed by further studies.

Concerning yeast single or combined with *T. harzianum* treatments, our results came in the same direction of many previous studies (Karajeh 2013; Ferweez and Abd El-Monem 2018). Olabiyi et al. (2013), who indicated that application of *T. harzianum* T 22 isolate and, palm wine yeast single or combined treatment significantly increased the growth and yield of soybean and significantly reduced the soil population of nematode pests of soybean. The growth-enhancing effect of yeast application may be due

to their enrichment with growth factors such as cytokinins, (Ferguson et al. 1987) indole acetic acid IAA (Moller et al. 2016). In addition, yeasts may provide amino acids and vitamins as well as magnesium that improve photosynthesis (Mahmoud 2001). Ferweez and Abd El-Monem (2018) studied the enhancement of yield, quality, and profitability of sugar beet by application of yeast (*Saccharomyces cerevisiae* strain SC). Their results revealed that the root length and diameter respond significantly to the time of yeast application. These findings might be based on the fact that yeast is a source of phytohormones, enzymes, minerals that stimulate cell division through proteins and nucleic acid synthesis (Shehata et al. 2012).

Zhoua et al. (2018) speculated that the biological activity of yeasts may involve induction of resistance in plants, and also modify physical and chemical soil structure to be undesirable for pathogenic agents to grow. Karajeh (2013) indicated that a high concentration of phenolics in cucumber roots of *Saccharomyces cerevisiae* treated plants gives a clue to the ability of yeast to induce plant resistance. Furthermore, the effects of yeast on *M. javanica* might be due to the ability of yeast fungus to convert carbohydrate to ethyl alcohol and CO₂ that are highly toxic to nematodes (Azhar et al. 2017).

Improvement of plant growth and reduction of gall formation in *T. harzianum* treated plants in the present study (data presented in Tables 3, 4) agrees with Osman et al. (2018), who reported positive effects of *T. harzianum* in controlling root-knot nematode *M. incognita* and a mild increase in eggplant yield production. They attributed their results to a chitinolytic activity of the fungus which causes degradation in the chitin layer of nematode eggs. Selim et al. (2014) showed that *T. harzianum* isolate (T10) induced systemic resistance to tomato plants against the root-knot nematode, *M. javanica*, by increasing the accumulation of hydrolytic enzymes which affect nematode invasion.

Concerning the application of *B. thuringiensis* as a single treatment, our results are in harmony with many authors (Ravari and Moghaddam 2015; Osman et al. 2018), who reported that the spore/crystal proteins of *B. thuringiensis* strains can produce a number of toxins with different structure and mode of action against *M. incognita*. More supportive evidence to the efficiency of *B. thuringiensis* K. used in this study as a single application has been reported by Elkelany (2017). He showed significant nematicidal effects of the bacteria with improvement in eggplant yield. He refers to the potentiality of bacterial suspension to produce antimicrobial compounds and protease enzymes.

Application of the antagonistic fungi *P. lilacinus* single treatment improved peanut plant growth and reduced nematode *M. javanica* reproductive parameters as compared to untreated control (Tables 3, 4). The present data is concordant with previous findings recorded by several workers (Mostafa et al. 2015).

The mechanism of action of *P. lilacinus* against plant-parasitic nematode was suggested as a direct infection of sedentary stages in particular the eggs (Kiewink and Sikora 2006). The production of leucinotoxin, chitinases, proteases, and acetic acid by *P. lilacinus* has been associated with the infection process (Park et al. 2004).

Concerning macro and micronutrients in peanut seeds, the increase in protein content mainly due to the increase in N% in the same treatments indicates that both yeast and *B. thuringiensis* can provide plants with essential nutrient elements required for protein formation (Mekki and Ahmed 2005). The results of Mn content join with Mekki and Ahmed (2005), who reported that Mn concentration was increased by treatment with yeast singly or combined with a biofertilizer. They also considered yeast as a natural growth stimulator, due to its improvement of phosphorus and Mn uptake. Our results are in harmony with Korayem et al. (2016), who reported that N, P, Mn, and Zn contents were decreased with increasing nematode infestation, while K content was not affected in sunflower plants infected with *Meloidogyne arenaria*. It has been suggested that the nematode parasitism adversely affects the nutrition uptake of the plants that leads to reduced photosynthetic activities and reduction in chlorophyll contents (Robert 2015).

In general, microorganisms can play a significant role in making available nutrients to plants. They are capable of promoting plant growth through different mechanisms such as phosphate and potassium solubilization and siderophore production (Bashan and de-Bashan 2005). Osman et al. (2018) reported that *Bacillus thuringiensis* (Bt), a phosphate solubilizing bacteria had the potential to break down the insoluble phosphate compounds in the soil into soluble forms and make it available to roots, thus increasing nutrient acquisition and consequently the yield of the crop.

The translocation of nutrients from soil via roots as well as minerals uptake increases the size of the root system and consequently increases the root surface adsorption (Soliman et al. 2011). The appropriate supply of nutrients is very important and positively affects peanut plant yield and its components, (weight of pods, weight of seeds, and weight of shell) and seed quality as cleared in Tables 3, 4, and 5).

Conclusion

Compared to the untreated control group, all treatments exhibited variable potential activities against root-knot nematode *M. javanica* and peanut yield production as well as seed quality. The most nematode suppressive treatment was yeast (*Saccharomyces cerevisiae*) single treatment, followed by using *T. harzianum* single treatment and combined with yeast. Moreover, the application of the combined treatment of yeast plus *T. harzianum* gave the best result in improving peanut

yield and quality. Continuous application of microorganisms could enhance peanut production and reduce the need to chemical fertilizers and nematicides that may hurt both crop yield as well as the environment.

The authors recommend the application of combined treatment of yeast plus either *T. harzianum* or *P. lilacinus* for enhancement peanut production, seed quality, and controlling root-knot nematode *M. javanica* under field conditions; however, further studies are needed to investigate the optimum dosage forms of these biocontrolling agents that could achieve the best nematicidal activity as well as plant quantity and quality.

Abbreviations

PTA: Population in treated plot after application; PTB: Population in the treated plot before application; PCB: Population in the check plot before application; PCA: Population in the check plot after application; CFU: Colony-forming unit

Acknowledgements

The authors acknowledge the National Research Centre, Giza, Egypt, for providing the laboratory facilities and technical assistance.

Authors' contributions

OH suggested the point of research and designed the experimental work and wrote the manuscript. AH participated in the design and statistical analysis of the data. MM participated in field work and nematode laboratory investigations. EU participated in field work and nematode laboratory investigations. The authors read and approved the final manuscript.

Funding

This work was funded by a grant, code number 11030133 in House report from the National Research Centre, Giza, Egypt.

Availability of data and materials

The tested nematodes and antagonistic pathogens are available in the Egyptian environment and were identified in the laboratory.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 15 December 2019 Accepted: 17 June 2020

Published online: 17 August 2020

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