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Effects of Inorganic Fertilizers on Virulence of the Entomopathogenic Nematode *Steinernema glaseri* and Peanut Germination under Field Conditions

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Abstract: The use of entomopathogenic nematodes as safe biopesticidal alternatives to hazardous chemicals entails improving the prediction of their native efficacy against soil pests. The effect of ten inorganic fertilizers, used extensively in Egypt, on the virulence of indigenous *Steinernema glaseri* and peanut germination was examined herein. The nematode added either before or tank-mixed with 1%, 5%, and 10% concentrations of each fertilizer in a peanut field was sampled 1 and 7 days before and 1, 7, 14, 21, 28, 49, and 56 post-tank mixes to check for *S. glaseri* virulence via baiting soil with *Galleria mellonella* larvae. Phosphorus fertilizers had more adverse effects than others on *S. glaseri* virulence and peanut germination. Plots with only *S. glaseri* had high germination close to chlorpyrifos. Averages of insect mortality in soil samples of potassium, nitrogen: phosphorus: potassium (NPK), nitrogenous, and phosphorus fertilizers, and non-fertilized checks (nematode only) were 85.8, 83.8, 80, 69.2%, and 93.3% respectively. Using *S. glaseri* is preferred before fertilizing. Most 1% fertilizer concentrations are compatible with *S. glaseri* in tank mixes for short-term (1–7 days) insect control but may affect long-term control.

Keywords: biological control; *Steinernema glaseri*; inorganic fertilizers; peanut; Egypt

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1. Introduction

Peanut (*Arachis hypogaea* L.) cultivation ranks high in Egypt as an important edible oilseed and cash crop and a suitable plant for growth in the newly reclaimed regions of Egypt [1]. However, such reclaimed areas usually have various problems, especially with sandy soil, which is often deficient in plant nutrients but infested with various pests and pathogens. These problems also apply to other paramount crops such as tomato, potato, citrus, and strawberry that can provide increasingly foreign exchange revenue [2–5]. Moreover, the continuous expansion of cultivation into the newly reclaimed lands of Egypt implies the hazards of increasing and reproduction of pests and diseases. The notion is that peanut growth and yield is damaged by many pests, including insects that invade the newly planted area via plant materials, organic fertilizers, irrigation, infected seedlings, and machinery. Also, mulching virgin soil with fertile but possibly unhealthy silty soil from the Nile Valley to enhance soil quality before peanut cultivation may generally worsen the pest infection [6,7]. Thus, the peanut crop is eventually infected by many insect pests.

Some or all stages of important insect species are found in the soil to feed on peanut plants during different stages of growth. They can attack and damage the roots, stems, pod, husks and seeds of the peanut, which causes a significant decrease in the quantity and quality of the peanut yield. On the other hand, increasing dissatisfaction with hazardous synthetic insecticides has created a growing interest in safe alternatives such as entomopathogenic nematodes (EPN). As the soil is their native habitat, EPN have a marked

potential to control soil insects where there is protection from UV radiation and rapid desiccation. Their potential to recycle in their host populations could be further employed for long-term pest management. However, agricultural practices such as irrigation, tillage, and fertilization may affect the abundance and insecticidal activities of EPN [8]. The effect of inorganic fertilizers varied according to their composition, the EPN species exposed, and the duration of its exposure to the used fertilizer [9–11].

This work was carried out to estimate the effect of three concentrations for each of ten different inorganic fertilizers of extensive use in Egyptian reclaimed regions on indigenous *Steinernema glaseri* isolate Sg-EG in a peanut field. Our study estimated the effects of fertilizers on peanut germination and nematode virulence.

2. Materials and Methods

2.1. The Nematode

The local isolate designated as *Steinernema glaseri* Sg-EG was provided by the Plant Pathology Department, National Research Centre, Giza, Egypt. It was reared at approx. 25 °C on last instar greater wax moth larvae (*Galleria mellonella* L.), according to procedures described in [12]. The insect was cultured on artificial diet according to [13]. Using White traps [14], the number of nematode-infective juveniles (IJs) produced per larva was determined through dilution counts [12]. An average of 22477.35 ± 1024 IJs/insect larva was produced. After harvesting, *S. glaseri* was stored in tap water at 10 °C. The newly released, two-day-old IJs were used in our experiments.

2.2. The Fertilizers

The inorganic fertilizers that were selected for this study are commonly applied in Egypt and belong to the main nutrient sources (nitrogen, phosphorus, and potassium). Their names, formulae, nourishing elements, and degree of solubility are given in Table 1. As the recommended rate of fertilizer application in peanut fields varies according to the previously-planted crop, irrigation regime, growth stage, and soil requirement [1], we used three typically applied concentrations for each of the 10 fertilizers tested. These fertilizers are extensively used by Egyptian farmers. Each fertilizer material was grinded to ease its dissolution in water. Three concentrations 1%, 5%, and 10% of each fertilizer were prepared; i.e., 10, 50 and 100 g were weighted and dissolved in 1000 mL distilled water using a magnetic stirrer in a container to form stocks of fertilizer solutions for the treatments.

2.3. Field Experiment

We selected a field in which cutworms (*Agrotis ipsilon* Hufnagel), wireworms (*Ampedus nigricollis* Herbst), and mole crickets (*Gryllotalpa gryllotalpa* L.) were visually observed soon after harvesting Egyptian clover (*Trifolium alexandrinum* L.) at the beginning of April 2019 in the area dedicated to the present study. The soil of this field (about 2100 m^2 or 0.5 Feddan, at Mashtoul El-Souk Center, El-Sharkia governorate) was analyzed at Sothe il Department of the National Research Centre and resulted composed by 59.1% sand, 23% silt, 17.9% clay, 1.82 OM, 5.89% N, 1.2% P, 6.4% K (pH 6.8). Plots (30 m^2) of this field were planted with peanut seeds cv. Giza 5 on 22 April 2019. All plots were plane and evenly shaped. Each plot contained three beds (20-m long, 0.5-m thick); seeds were sown every 25 cm and arranged in one line per bed. The peanut seeds were removed from the shell and soaked for 12 h in water; then, they were wrapped in a moist paper towel with a plastic envelope for a couple of days in a warm place then seeded. Other agricultural practices, such as rhizobium inoculation, were carried out as recommended [1].

Healthy IJs were added with irrigation water in one-half of the planted area soon after seed-sowing (Table 2). One week after their addition (29 April 2019), three different concentrations of each fertilizer were chemigated to the whole area via drippers in two different applications. The first application distributed the fertilizers to that half of the area that had already been inoculated by *S. glaseri* soon after seeding; at the second application, the fertilizers were mixed with IJs via tank mixing and distributed to that half of the field

that was left not inoculated (Table 3). Low solubility fertilizers were first solved in special barrels before being added into the chemigation unit for drip irrigation; a drip line per bed contained 80 drips. For each fertilizer concentration, three beds or replicates were treated in each of the two applications. Six beds were used as controls, as follows: three beds were only inoculated by the IJs soon after seeding without fertilization, in the first type of application (Table 2), and three beds were only inoculated by the IJs a week after seeding without fertilization in the second type of application (Table 3).

About 80,000 healthy IJs per bed (equal 1000 IJs per plant or 240,000 IJs per plot for each concentration of the fertilizer) were applied by ferti-irrigation. Nematode inocula were thoroughly mixed with the solution of the tested fertilizer concentration in the chemigation unit; then, they were pumped by an electric motor (5 HP) to the soil through the dripping irrigation lines. The suction pump (12 cm diameter) took 30 min to pump out the contents of the chemigation unit (150 L) into the soil of a plot.

To assess the effect of the fertilizers on the virulence (power to kill *G. mellonella* larvae) of *S. glaseri* applied one-week before fertilizers, 1 kg soil was randomly collected from each bed, 1 and 7 days before and 7, 21, and 49 days after fertilization. Similarly, samples were collected from the other half area where IJs were tank-mixed with fertilizers seven days after seeding. They were taken just before and 1, 7, 14, 28, and 56 days post-chemigation (tank mixing). A soil sample was taken at 10–15 cm depth below the drippers of 3 plants in a line and mixed to form a composite sample. Samples were bagged, labelled and carefully taken to the laboratory for baiting with 5 live *G. mellonella* larvae per each sample in an assay chamber (plastic semi-cone cups, 12.8 cm diam., 9 cm height); three cups used as replicates per one treatment. After 7 days of baiting, the soil cups were examined for the insect larvae infested by EPNs [12]. The infested larvae were transferred to White traps to check for agreement with Koch's postulates [15]. Numbers of dead larvae were recorded to express the virulence of IJs exposed to inorganic fertilizers under field conditions.

Table 1. List and description of the tested inorganic fertilizers.

Common Name of Fertilizer	Main Nutrient Component (s)	Percentage of Component (s)	Solubility
Urea (CH_4N_2O)	Nitrogen source	Nitrogen 46.5%	high
Ammonium sulfate [$(NH_4)_2SO_4$]		Ammonia 20.6% Sulfur 24%	low
Ammonium nitrate (NH_4NO_3)		Ammonia 33% Mg 0.5%	high
Calcium nitrate [$Ca(NO_3)_2$]		Nitrogen 15.5% Calcium 19%	high
Single Superphosphate [$3Ca(H_2PO_4)_2H_2O + 7CaSO_4$]	Phosphorus source	Phosphoric acid 15%	low
Triple Superphosphate [$Ca(H_2PO_4)_2H_2O$]		Phosphoric acid 42% Sulfur 3%	low
Ammonium phosphate [$(NH_4)_3PO_4$]		Phosphoric acid 39% Nitrogen 18%	high
Potassium sulfate (K_2SO_4)	Potassium Source	Potassium oxide 48% Sulfur 18%	low
Potassium nitrate (KNO_3)		Potassium oxide 46% Nitrogen 13%	high
Nitrogen, phosphorus, and potassium or NPK (in equal amounts)	Combination	Nitrogen 20% Potassium 20% Phosphorus 20%	high

Table 2. Mean number and percentage mortality of *Galleria mellonella* last instar larvae exposed to soil samples taken one day before and 7, 21, and 49 days post-fertilization where *Steinernema glaseri* was added with irrigation water seven days before fertilization [+].

Treatments	Fertilizer Conc.	Mortality at One Day before Fertilization		Mortality at 7 Days Post-Fertilization		Mortality at 21 Days Post-Fertilization		Mortality at 49 Days Post-Fertilization		% Overall Average of Mortality *
		Mean	%	Mean	%	Mean	%	Mean	%	
Urea	10%	5	100	3.7 bc	73	3.33 cde	67	3 abcd	60	80
	5%	5	100	4 abc	80	4 abc	80	3 abcd	60	84
	1%	5	100	5 a	100	4.67 a	93	3.3 abcd	67	92
Ammonium sulfate	10%	5	100	3.3 bcd	67	3.3 cde	67	2.7 bcd	53	77
	5%	5	100	4 abc	80	3.3 cde	67	3.7 abc	73	84
	1%	5	100	4 abc	80	3.7 bcd	73	3.7 abc	73	85
Ammonium nitrate	10%	4.67	93	3.7 bc	73	3.3 cde	67	3.3 abcd	67	80
	5%	5	100	3.7 bc	73	3.3 cde	67	3 abcd	60	80
	1%	5	100	4 abc	80	3.7 bcd	73	3.7 abc	73	85
Calcium nitrate	10%	5	100	4 abc	80	3.3 cde	67	3.3 abcd	67	83
	5%	5	100	4.3 ab	87	2.7 ef	53	2.3 cde	47	77
	1%	5	100	5 a	100	4 abc	80	3.7 abc	73	91
Potassium sulfate	10%	5	100	4.3 ab	87	4 abc	80	2.3 cde	47	83
	5%	5	100	4.3 ab	87	3.7 bcd	73	2.3 cde	47	81
	1%	5	100	5 a	100	3.7 bcd	73	2.7 bcd	53	85
Potassium nitrate	10%	5	100	5 a	100	3.3 cde	67	2.7 bcd	53	84
	5%	5	100	5 a	100	4.3 ab	87	4 ab	80	93
	1%	5	100	5 a	100	4.3 ab	87	4.3 a	87	95
Single superphosphate	10%	4.67	93	3 cd	60	3 de	60	2.3 cde	47	72
	5%	5	100	3.3 bcd	67	2. 67	53	2.3 cde	47	73
	1%	4.67	93	4 abc	80	4 abc	80	3.3 abcd	67	84
Triple superphosphate	10%	5	100	2 d	40	2 f	40	1 e	20	60
	5%	5	100	2.7 cd	53	2.7 ef	53	1.3 e	27	67
	1%	5	100	3 cd	60	3 de	60	2.3 cde	47	73

Table 2. *Cont.*

Treatments	Fertilizer Conc.	Mortality at One Day before Fertilization		Mortality at 7 Days Post-Fertilization		Mortality at 21 Days Post-Fertilization		Mortality at 49 Days Post-Fertilization		% Overall Average of Mortality *
		Mean	%	Mean	%	Mean	%	Mean	%	
Ammonium phosphate	10%	5	100	3.3 bcd	67	2.7 ef	53	2 de	40	72
	5%	4.67	93	3 cd	60	3 de	60	2.7 bcd	53	73
	1%	5	100	3. 67 bc	73	3.7 bcd	73	2.3 cde	47	79
Nitrogen, phosphorus, and potassium or NPK (in equal amounts)	10%	5	100	3.7 bc	73	3.3 cde	67	3 abcd	60	80
	5%	5	100	4 abc	80	3.3 cde	67	2.7 bcd	53	80
	1%	5	100	5 a	100	3.3 cde	67	3.3 abcd	67	87
control	zero	5	100	5 a	100	4.3 ab	87	4 ab	80	93

[+] Mean of three replicates (cups), each has five insects. In a column, values with the same letter are not significantly ($p \leq 0.05$) different according to Duncan's New Multiple Range Test. * Overall average mortality considered samples taken at 7, 1 before and 7, 21, and 49 days post-fertilization.

Table 3. Mean number and percentage mortality of *Galleria mellonella* last instar larvae exposed to soil samples taken 7, 14, 28, and 56 days post-tank mixes as *Steinernema glaseri* was added with fertilizers seven days after seeding to all treatments [+].

Treatments	Fertilizer Conc.	Mortality at 7 Days Post-Chemigation		Mortality at 14 Days Post-Chemigation		Mortality at 28 Days Post-Chemigation		Mortality at 56 Days Post-Chemigation		Overall Average of Mortality % *
		Mean	%	Mean	%	Mean	%	Mean	%	
Urea	10%	4 abc	80	3.7 abc	73	3 bcd	60	3 abcd	60	75
	5%	4.3 ab	87	3.3 bcd	67	3.3 abcd	67	3 abcd	60	76
	1%	5 a	100	4.3 ab	87	4 abc	80	3.7 ab	73	88
Ammonium sulfate	10%	3.7 abc	73	3.3 bcd	67	2.7 cd	53	1.7 def	33	65
	5%	4.7 a	93	3.3 bcd	67	2.7 cd	53	2.7 abcde	53	73
	1%	4.7 a	93	3.7 abc	73	3.3 abcd	67	3 abcd	60	79
Ammonium nitrate	10%	3.7 abc	73	3.7 abc	73	2.7 cd	53	2.7 abcde	53	71
	5%	4.3 ab	87	4 abc	80	3.3 abcd	67	3 abcd	60	79
	1%	5 a	100	4.3 ab	87	3.3 abcd	67	3.3 abc	67	84

Table 3. *Cont.*

Treatments	Fertilizer Conc.	Mortality at 7 Days Post-Chemigation		Mortality at 14 Days Post-Chemigation		Mortality at 28 Days Post-Chemigation		Mortality at 56 Days Post-Chemigation		Overall Average of Mortality % *
		Mean	%	Mean	%	Mean	%	Mean	%	
Calcium nitrate	10%	4.3 ab	87	3.3 bcd	67	3 bcd	60	2.7 abcde	53	73
	5%	4.3 ab	87	3.7 abc	73	2.7 cd	53	2.7 abcde	53	73
	1%	5 a	100	4.3 ab	87	3.3 abcd	67	3.3 abc	67	84
Potassium sulfate	10%	5 a	100	3.7 abc	73	3.7 abc	73	2 cdef	40	77
	5%	5 a	100	3.7 abc	73	3 bcd	60	2.3 bcdef	47	76
	1%	5 a	100	4.3 ab	87	4 abc	80	2.7 abcde	53	84
Potassium nitrate	10%	5 a	100	5 a	100	3.3 abcd	67	3.3 abc	67	87
	5%	5 a	100	4.7 ab	93	4 abc	80	4 a	80	91
	1%	5 a	100	5 a	100	4.3 ab	87	4 a	80	93
Single superphosphate	10%	3.7 abc	73.3	3.7 abc	73	3.3 abcd	67	3.3 abc	67	76
	5%	3 bcd	60	3.3 bcd	67	2.7 cd	53	2.3 bcdef	47	65
	1%	3.3 bcd	67	4 abc	80	4 abc	80	3.3 abc	67	79
Triple superphosphate	10%	2.7 cd	53	2 d	40	2 d	40	1 f	20	51
	5%	2.3 d	47	2.7 cd	53	2.7 cd	53	1.3 ef	27	56
	1%	3.3 bcd	67	3 cd	60	3 bcd	60	2.3 bcdef	47	67
Ammonium phosphate	10%	2.7 cd	53	3.3 bcd	67	2.7 cd	53	2 cdef	40	63
	5%	3 bcd	60	3 cd	60	3 bcd	60	2.7 abcde	53	67
	1%	2.7 cd	53	3.7 abc	73	3.7 abc	73	2.3 bcdef	47	69
Nitrogen, phosphorus, and potassium or NPK (in equal amounts)	10%	4.3 ab	87	4 abc	80	3.7 abc	73	3.3 abc	67	81
	5%	4.7 a	93	4.7 ab	93	3.7 abc	73	3 abcd	60	84
	1%	5 a	100	5 a	100	4.3 ab	87	3.3 abc	67	91
Control	zero	5 a	100	4.7 ab	93	4.7 a	93	4 a	80	93

[+] Mean of three replicates (cups) each has five insects. In a column, values with the same letter are not significantly ($p \leq 0.05$) different according to Duncan's New Multiple Range Test. * Overall average mortality considered samples taken at 1, 7, 14, 28, and 56 days post-tank-mixing.

An evaluation of the potential of *S. glaseri* (Sg-EG) in protecting germination of seeds from subterranean insects was done. Numbers of germinated seeds were examined from 7 to 30 days after sowing. Seed germination was recorded in all fertilizers-treated plots and compared with the number of germinated seeds in three additional plots treated as follows: (A) 1 L/Feddan (1 Feddan = 4200 m^2) Chlorophan 48% EC (active ingredient: chlorpyrifos 48%, Kafer El-Zayat company (EL-Gharbia governorate, Egypt), Chlorophan was mixed with corn grits and then scattered 1 day-post seeding at sunset as chemical toxic bait, (B) *S. glaseri* (Sg-EG); (C) untreated not inoculated controls (Table 4).

We used a completely randomized design for data analysis to comply with the designed irrigation system. Data were subjected to analysis of variance via Microsoft Excel using its spreadsheet software for statistical analyses. Averages of fertilizer treatments in each of the two applications as well as averages of germinated peanut seeds were compared using Duncan's New Multiple Range Test.

Table 4. Means and percentages of germinated peanut seeds as *Steinernema glaseri* was added soon after seeding or in tank mixes with different inorganic fertilizers compared to untreated controls and chemical insecticide.

Treatments [+]	Fertilizers Concentration	Nematode in Tank Mixes		Nematode Soon after Seeding	
		Mean	% Germination	Mean	% Germination
Chlorophan 48%	Zero	77 a	96	78	98
Untreated Control	Zero	54.3 g	68	57.67	72
S. glaseri only	Zero	68.3 a–f	85	72.33	90
Urea	10%	62.3 b–e	78	69.0	86
	5%	61 b–g	76	67.0	84
	1%	68.7 a–e	86	70	88
Ammonium sulfate	10%	58 c–g	73	64.33	80
	5%	56.7 d–g	71	70	88
	1%	71.3 ab	89	68	85
Ammonium nitrate	10%	60 b–g	75	65.67	82
	5%	67.7 a–f	85	64	80
	1%	70.3 a–c	88	68	85
Calcium nitrate	10%	58.3 c–g	73	65.67	82
	5%	65 b–g	81	69.67	87
	1%	69.3 a–d	87	73.33	92
Potassium sulfate	10%	55.7 fg	70	69.33	87
	5%	65.7 b–g	82	71.67	90
	1%	65 b–g	81	68.33	85
Potassium nitrate	10%	64 b–g	80	70.67	88
	5%	62 b–g	78	70.67	88
	1%	68.7 a–e	86	68	85
Single Superphosphate	10%	56.3 e–g	71	63	79
	5%	57.7 c–g	72	60	75
	1%	65 b–g	81	67	84
Triple Superphosphate	10%	54 g	68	59. 67	74
	5%	54 g	68	59. 67	75
	1%	63.7 b–g	80	66	83

Table 4. *Cont.*

Treatments [+]	Fertilizers Concentration	Nematode in Tank Mixes		Nematode Soon after Seeding	
		Mean	% Germination	Mean	% Germination
Ammonium phosphate	10%	58 c–g	73	58. 67	73
	5%	57 d–g	71	57. 67	72
	1%	58.7 c–g	73	64	80
Nitrogen, phosphorus, and potassium or NPK (in equal amounts)	10%	58.7 c–g	73	62. 67	78
	5%	56.7 d–g	71	65.33	82
	1%	63.3 b–g	79	68	85

Mean of 80 plants replicated thrice and recorded up to 30 days after cultivation. In a column, values with the same letter are not significantly ($p \leq 0.05$) different according to Duncan's New Multiple Range Test. [+] *Steinernema glaseri* was applied in all treatments except Chlorophan 48% and untreated control.

3. Results

3.1. Nematode Virulence

Baiting with sentinel *G. mellonella* larvae to recover EPNs from soil samples was negative before the addition of *S. glaseri* (Sg-EG) in all treatments, thus indicating their absence in the area. Soil samples resulted positive to EPNs only after *S. glaseri* was exogenously added to the plots. The five *G. mellonella* larvae used as bait were all killed by IJs one day before fertilization (Table 2). A significant ($p \leq 0.05$) difference in *S. glaseri* virulence was found among fertilizer-treated beds, although insect mortality remained generally high 7 days after fertilization, especially at 1% fertilizer concentrations. *S. glaseri*, not exposed to any fertilizer, induced an overall average of *G. mellonella* larval mortality (93.3%) higher than those nematodes exposed to any type of fertilizers (Tables 2 and 3). Generally, all fertilizers in both types of applications reduced the virulence of the tested untreated EPNs over time and according to the concentration level. Both triple and single superphosphate, as well as ammonium phosphate (phosphorus fertilizers), showed the highest adverse effects on nematode virulence (Tables 2 and 3). The overall averages of insect mortality in soil samples treated with potassium, nitrogen: phosphorus: potassium or NPK (in equal amounts), nitrogenous, and phosphorus fertilizers (Table 1) were 86.9, 82.2, 83.2, and 72.6%, respectively, when IJs were inoculated soon after peanut seeding (Table 2). The corresponding values were 84.7, 85.3, 76.7, and 65.8%, respectively, when IJs were tank-mixed with fertilizers (Table 3). Thus, the overall averages of both treated areas were 85.8, 83.8, 80, and 69.2%, respectively, compared with 93.3% in the untreated controls of both *S. glaseri* applications. The averages of induced insect mortality across 1, 7, 14, 28, and 56 days post-tank-mixing (Table 3), resulting from data of all the three concentrations used for each fertilizer, in a descending order, are: 90.2% (potassium nitrate), 85.3% (NPK), 79.6% (urea), 79.1% (potassium sulfate), 77.7% (ammonium nitrate), 76.8% (calcium nitrate), 73.3% (single superphosphate), 72.4% (ammonium sulfate), 66.2% (ammonium phosphate), and 57.8% (triple superphosphate).

3.2. Germination Rate

The germination of peanut seeds was recorded from 7 to 30 days after seeding; germination was observed from the emergence of small leaves that started 1 day after germination. No significant ($F = 1.474$; F critical = 1.624; $p = 0.094$) difference in germination rate was found among treatments in which *S. glaseri* (Sg-EG) was applied soon after seeding. In these plots, germination ranged from 97.5% in Chlorophan-treated beds to 72.1% in untreated beds (neither EPN nor fertilizer) or in 5% ammonium phosphate-treated beds. Significant ($p \leq 0.05$) differences were found among those treatments in which *S. glaseri* was tank-mixed with fertilizers (Table 4). Beds treated with Chlorophan 48%, ammonium nitrate (1 and 5%), urea (1%), calcium nitrate (1%), potassium nitrate (1%), and only *S. glaseri*

(no fertilizer) had the highest percentages of germination without significant difference among them.

4. Discussion

The relative increase of peanut germination rate in treatments with favorable *S. glaseri* virulence implies nematode effectiveness against soil insects that feed on cultivated or germinated peanut seeds. This implication is supported by the fact that *S. glaseri* is quite effective on *A. ipsilon* [16], which was detected in many of the examined samples. Also, the mortality of bait insects in plots with IJ inoculation soon after peanut seeding (Table 2) is mostly higher than that in plots with later IJ inoculation (Table 3). This is likely because of the non-contact of IJs with the chemicals for 7 days. Therefore, combinations of the first application system are more interesting for the practice than the second application system. Jaffuel et al. [17] reported negligible pest control when rootworm attacking maize started a week before the EPN application. On the contrary, they found that well-timed application of alginate capsules (beads that encapsulate the EPNs) caused considerable reduction in the banded cumber beetle *Diabrotica balteata* Le Conte-inflicted root damage comparable ($p < 0.05$) to that of EPNs in water suspension.

Hence, our nematode application—via the EPN tank-mixing with fertilizers or separately through the drippers before the fertilizers—can also offer practical techniques that growers may apply, but with two different predictions to improve the EPN performance under similar conditions. As the methods apply EPNs via conserving their water regime, they support the government's interest in water conservation and pest management in the newly reclaimed areas in Egypt. Future research is warranted to document if these fertilizer concentrations affect EPN in a manner similar to that used herein and in soils cultivated with various crops.

Various studies [8,9,11,18,19] have measured the effect of different organic and inorganic fertilizers on nematode fitness, although previous research is scanty regarding EPN efficacy at improving seed germination of field crops. This is understandable, given the many hidden abiotic and biotic variables affecting not only EPN but also germination rate. Although an occasional aggregated distribution of soil insects was found in our experimental field [20], we preferred not to risk introducing exogenous insects in regular patterns to the plots, to avoid the possibility of their future spread and consequent damage. For instance, the introduction of little amount of the sole black cutworm *Agrotis ipsilon* (Hufnagel) to assess damages to corn plants, required special precautionary measures to prevent their post-experimental spread [19]. The insects detected in our experimental fields, such as black cutworm, wireworm, and mole cricket, can considerably damage valuable crops such as peanut [1], potato [21], tomato [22], citrus [23], and strawberry [24] in Egypt and elsewhere [25–27]. While wireworms and mole crickets usually live in the soil and feed on seeds, roots, crowns, and stalks of various plants, cutworm larvae may feed on plant leaves and sprouts, but their fourth instar often cause significant damage by severing young seedlings at the base. Few of the above-mentioned insects that were scattered in the soil samples did not show any significant results (data not shown). This is likely due to the cryptic nature of soil insects, or in this case, EPN hosts. Most surveys could isolate EPN from soil rather than naturally infected insects [5,8]. Hence, there is a tantalizing possibility of the effect the nematode might have on seed-feeding insects and consequently, the germination rate.

The present study suggests that nematode virulence against insects is lower in soil treated with phosphorus than in soil treated with potassium, NPK, and nitrogenous fertilizers. No research has formerly compared impacts of this specific variety of fertilizers on *S. glaseri*, though they are quite common in Egyptian agriculture. To ensure they are used in amounts proportional to soil fertility, we applied three different but commonly used, concentrations of each fertilizer. However, growers and stakeholders should be aware of the fact that many biotic and abiotic factors can impact nematode virulence under field conditions. These may comprise the soil microbiome (including the used EPN

species) and properties (e.g., texture, moisture, temperature, pH, organic matter content), cultivated plant genotypes, and traits of the used fertilizer such as its physical status, nutrient content, nutrient mineralization rate, and decomposition products [8,11,28–30]. For instance, these products may be directly toxic to EPNs; fertilizer application may raise biotic activity in terms of predation and parasitism on EPNs. In a biological process such as mineralization, its rate may vary with soil moisture, aeration, and temperature. Otherwise, fertilizers may lower entomopathogenic nematode activity by modifying the soil-physical status. Some or all of these factors may contribute to reducing nematode virulence [8,18,31]. However, our data indicate that certain fertilizers, such as those containing phosphorus, may have stronger effects on *S. glaseri* virulence. This finding is partially supported by Zhao et al. [28], who found that the addition of phosphorus caused a higher reduction of the soil nematode community over that of nitrogen. The basis for these different reactions require physiological investigation [9]. Eventually, the attributes of the used fertilizer [9,11], nematode species [32], and relevant settings [8,18,19] should be taken into consideration when assessing EPN efficacy.

In conclusion, our results are consistent with other research [9,11] regarding the reduction of EPN fitness caused by prolonged exposure to high concentrations of inorganic fertilizers. Nevertheless, these studies have not elucidated if the decreased nematode fitness may prevent plant damage under field conditions. In our study, the effect of fertilizers on nematode virulence was associated with a reduced seed germination rate in fields, thus indicating that high entomopathogenic activity by nematodes can have a positive effect on plant protection from soil insects. The present study indicates a feasible approach that growers and stakeholders may apply via chemigation to improve production practices in which EPNs and inorganic fertilizers are to be used.

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