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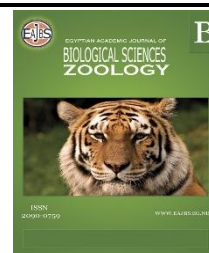


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Potential Efficacy of Bioformulations of *Trichoderma* and *Bacillus* in the Management of Peanut Root-Knot Nematode, *Meloidogyne arenaria*

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ABSTRACT

The peanut root-knot nematode, *Meloidogyne arenaria* is one of the most damage species of nematodes to peanut crop that cause significant losses. The efficacy of some commercial bio-based formulations of *Trichoderma* (Biocontrol T34) and *Bacillus* (Rhizo-N, Phosphoren and Potassiumag) for suppression of *M. arenaria* on peanut was evaluated under greenhouse conditions. The rates of tested of formulations (2.5, 5 and 10g plant⁻¹) had a significant ($P \leq 0.05$) direct or indirect effect on each of the *M. arenaria* criteria and parameters of plant capacity for growth and production, compared to untreated control and the nematicide (Fothiazate 10%). Results showed that biocontrol T34 achieved the best significant ($P \leq 0.05$) of nematode reduction at 10g plant⁻¹ (92.881%), with 3.117% increase in plant weight. In exchange for 62.268% in Phosphoren and 7.239% in potassiumag. With percentages decrease in the final population amounted to 3.117%, 7.376% and 33.73% of the nematicide. It could be concluded that the application of commercial bio-based formulations of *Trichoderma* and *Bacillus* was useful in suppressing root-knot nematode, *M. arenaria*, especially when added at the beginning of the growing season.

INTRODUCTION

Plant-parasitic nematodes (PPNs) constitute one of the most important determinants of agricultural crop productivity, especially in developing countries (Krif *et al.*, 2020). Their presence, which is often difficult to notice, has not been taken seriously except in recent periods (El-Sagheer, 2019), especially in leguminous crops such as peanut, *Arachis hypogaea* (Fabaceae) (Taborda 2019). Peanut ranks fourth place globally among oil crops in terms of

production area (Rai *et al.*, 2016). In addition to its use in the production of human and animal food, recently, studies related to peanuts as a renewable source of biofuels have gained more attention (Antmen 2019). According to some earlier studies, a large number of nematode genera parasitizes on peanuts in different climatic zones, within different parts of the plant (Ahmad *et al.*, 2021).

The peanut root-knot nematode, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 is one of the important nematode pests that cause significant damage to peanuts and other crops, particularly in warmer regions (Dickson and Waele 2005). Since the early 1950s, plant nematodes have been effectively controlled on a variety of agricultural crops and in numerous regions of the world chemical nematicides (El-Senousy *et al.*, 2023; Wu *et al.*, 2021). However, their usage has been constrained due to their high expense and the associated hazards to the environment and human health (Seong *et al.*, 2021). In the modern agricultural pest management system, efforts have been focused on bringing pathogen intensity below the critical level by adopting other methods and methods of control, including bioformulations of antagonistic bioagents (Hendy *et al.*, 2023; Josephraj Kumar *et al.*, 2022), which offers one of the alternatives to address even partially the issue of excessive use of chemical pesticides to reduce plant diseases in addition to being a safe and inexpensive method economically (Lahlali *et al.*, 2022). The outcomes of laboratory and field studies carried out in various locations across the globe demonstrate effectiveness of some pure isolates of microorganisms in the suppression of nematodes, e.g., *Trichoderma asperellum* (Almeida *et al.*, 2022), *T. harzianum* (Yang *et al.*, 2021), *Bacillus subtilis* (Manju and Sankari Meena 2022) and *B. megaterium* (Aballay *et al.*, 2017) as well as their strong capacity to adapt to various climatic and soil conditions (Poveda *et al.*, 2020). Therefore, the current study aimed to evaluate the efficacy and optimize of some proven commercial bio-based formulates of *Trichoderma* and *Bacillus* for the suppression of peanut root-knot nematode under greenhouse conditions.

MATERIALS AND METHODS

Propagation of *Meloidogyne arenaria* in Pure Culture:

A pure stock culture of the peanut root-knot nematode, *Meloidogyne arenaria* was prepared from naturally infected peanut plants roots collected from an infested field in Minya Governorate. Individual egg-masses with their mature females were removed from root tissues. Each female was placed in a small glass capsule containing fresh water. The females, from which egg-masses were taken, preserved in 4% formaldehyde solution in glass capsules. Each female was identified to species based on perineal pattern according to Taylor and Netscher, (1974). The identified species was *M. arenaria* race 1 and its freshly hatched second stage juveniles from egg-masses (about 500 juveniles) were transferred to 30 cm pots filled with steam sterilized sandy loam soil which planted with a seedling of peanut, *Arachis hypogaea* cv. Giza 4 at 45 days old. Inoculated pots were arranged in a greenhouse and watered when needed. After three months of inoculation, infected roots were chopped and used as sources of inoculation in greenhouse.

Greenhouse Experiment:

At natural growing season, seedlings of peanut cv. Giza 4 were planted in pots (50 cm diam.) filled with mixture of clay: sand (1: 3, v: v.) after one week, plants were and treated with 3000 freshly hatched second stage juveniles /pot, poured into three holes in the soil around the base of the plant stem (Montasser *et al.*, 1986; May and June 2022). After seven days of nematode inoculation, the pots were randomly divided according to the treatments and concentrations. Seedlings were drenched separately with 20 ml of the tested bio-compounds with three concentrations (Table 1) as soil drench around plants. Treatments were replicated three times. The pots were maintained at 32 ± 5 °C and watered as needed.

Table 1. Tested bio-compounds against peanut root-knot nematode, *Meloidogyne arenaria*.

Trade name	Active ingredient	Form	Application rate
Biocontrol T34	<i>Trichoderma asperellum</i> strain T34 (910x10 ⁶ spores/g)	Powder	2.5, 5 and 10g plant ⁻¹
Rhizo-N	<i>Bacillus subtilis</i> (30x10 ⁶ cell/g)	Powder	
Phosphoren	<i>Bacillus megaterium</i> (10 ⁸ cfu/g)	Powder	
Potassiumag	<i>Bacillus verculanes</i> + <i>Bacillus megaterium</i> (10 ⁸ cfu/g)	Powder	
Jubitar-X (Nematicide)	Fothiazate 10% (Organophosphorus)	Granule	1 g plant ⁻¹ (12.5 kg acre ⁻¹)

Data Collection:

After 50 days of nematode inoculation, the plants were gently removed from the pots. The whole plant's weight and number of pods were measured. The galls and egg- masses of the whole root systems of plants were counted. The roots were stained with acid fuchsin and examined for counting the number of developmental stages and females in the root to estimate root-knot fecundity and final nematode population, as described by (Bybd Jr *et al.*, 1983). Egg-masses and eggs/egg-mass were extracted by using method as described by (Hussey and Barker 1974) by sodium hypochloride (NaOCl). The gall index (GI) was scored according to Sharma *et al.*, (1994), as follows: 1 = no galls, 2 = 1 to 5 galls, 3 = 6 to 10 galls, 4 = 11 to 20 galls, 5 = 21 to 30 galls, 6=31 to 50 galls, 7 = 51 to 70 galls, 8 = 71 to 100 galls, and 9 = >100 galls per root system. The juveniles were extracted from the soil of each individual plant in their respective pots by Cobb's sieving and decanting method along with the Baerman funnel (Seinhorst 1956). Nematode final population (P_f) estimated by the following formula:

$$P_f = [\text{no. Egg/masses} \times \text{no. Eggs / Egg - masses}] + [\text{Developmental stage/root}] + [\text{Juveniles in soil}] + [\text{Adult females/root}].$$

The reproduction rate of (Build -up) was calculated by dividing the final population (P_f) by the initial one (P_i). The percentage of nematode reduction calculated by the following formula:

$$\text{Reduction\%} = \left(\frac{\text{Number of nematode in control} - \text{Number of nematode in treatment}}{\text{Number of nematode in control}} \right) \times 100$$

The percentage reduction or increase in growth parameters was calculated by the following formula:

$$\text{Reduction/increase \%} = \frac{C-A}{C} \times 100$$

Where, C is value of control, A is value of treatment.

Statistical Analyses:

In pots experiment the Randomized Complete Design was used. All the data were subjected to Analysis of Variance (ANOVA) using SPSS package (V. 21.0). The means were compared according to Duncan's multiple range tests at $P \leq 0.05$ (Duncan 1955) and L.S.D. at 5 % level of significance. The correlation and regression equations were determined using regression analysis by Pearson correlation type.

RESULTS**Effects of Tested Bio-Compounds Against Peanut Root-Knot Nematode, *Meloidogyne arenaria* under Greenhouse Conditions:**

The tested compounds had a significant ($P \leq 0.05$) direct or indirect effect on each of the *M. arenaria* criteria and parameters of plant capacity for growth and production, compared

to the nematicide and untreated control (nematode only).

Galling Index:

According to the galls formation of the peanut root-knot nematode as measured by the galling index, all infected plants by *M. arenaria* showed gall development on peanut roots at the end of the experiment. All of the bio-compounds tested lowered the formation of galls on peanut, and their gall index values were significantly lower than those of the untreated control (Table 2 and Fig. 1). The lowest formed galls and galling index were observed associated with biocontrol-T34 at the highest rate of 10g plant⁻¹ (57, 7), followed by the medium rate of 5g plant⁻¹ (63, 7), and the lowest rate of 2.5g plant⁻¹ (67, 7), compared to the nematicide (8, 3) and untreated control (143, 9), respectively. A Rhizo-N compound with a similar pattern in the effect of the tested rates on the formed galls (85, 93 and 98) was ranked the second, with a mean of 8, as the galling index for all rates. While no significant difference was observed between the medium and the lower rate, compared to Potassiumag compound, which achieved (93, 85), respectively, with a mean of 8 as the galling index for all three rates. In contrast to the lower rate of potassiumag, where a clear significant difference ($P \leq 0.05$) was observed in formed galls mean and galling index (102 and 9) compared to untreated control (143, 9), (Fig. 2). Generally, the structure of the galls formed in the plants treated with Phosphoren appeared to be larger in size than the other treatments and somewhat similar to the control. Also, relating to the progress of the experiment, it was observed that the development of formation galls in the Phosphoren treatments was faster than the other treatments. While the galls associated with Potassiumag treatments were smaller in size and less juicy than those associated with Phosphoren and Bio control T34; respectively.

Table 2: Efficacy of some biocompounds in suppression of peanut root-knot nematode, *M. arenaria* under greenhouse conditions

Treatments	Rate (g)	Nematode criteria						**GI	P _f	*RR	Reduction %
		Galls	Juveniles in soil (250 g)	DS	Females	No. of egg-masses/root	No. of eggs/egg-mass				
Bio control T34	2.5	67±1.50 ^e	1345±3.50 ⁱ	90±1.50 ^f	26±0.88 ^{ij}	21±0.58 ^{ef}	199±1.00 ^e	7	5539	1.846	88.732
	5	63±0.67 ^e	1274±2.91 ^j	77±2.96 ^{fg}	22±0.88 ^{jk}	20±1.00 ^{ef}	190±0.88 ^e	7	5179	1.726	89.464
	10	57±1.45 ^f	1018±2.65 ^k	71±1.20 ^{fg}	19±1.33 ^k	14±0.33 ^f	175±7.5 ^l	7	3500	1.167	92.881
Rhizo-N	2.5	98±2.00 ^{cd}	1495±4.00 ^f	100±2.08 ^f	31±3.46 ^h	25±0.00 ^{ef}	216±4.50 ^e	8	7039	2.346	85.681
	5	93±0.50 ^{cd}	1476±2.50 ^g	105±2.33 ^f	30±0.88 ^{hi}	29±0.50 ^e	209±1.00 ^e	8	7567	2.522	84.608
	10	85±2.00 ^d	1428±3.00 ^h	101±2.00 ^f	24±0.88 ^{jk}	20±2.00 ^{ef}	202±2.00 ^e	8	5593	1.864	88.622
Phosphoren	2.5	117±2.04 ^b	1805±2.61 ^b	120±4.00 ^e	96±3.50 ^b	84±0.50 ^b	277±1.00 ^c	9	25076	8.359	48.988
	5	111±3.08 ^{bc}	1827±0.58 ^b	112±7.00 ^{ef}	90±0.33 ^b	78±3.50 ^{bc}	270±2.50 ^{cd}	9	22915	7.638	53.384
	10	105±4.50 ^c	1731±8.66 ^d	100±0.00 ^f	81±1.76 ^d	63±7.50 ^c	265±5.00 ^{cd}	9	18548	6.183	62.268
Potassiumag	2.5	102±2.37 ^c	1797±5.58 ^{bc}	157±3.00 ^b	55±0.00 ^e	46±1.00 ^d	369±9.48 ^b	8	18983	6.328	77.246
	5	93±3.00 ^{cd}	1750±0.76 ^c	140±0.00 ^c	48±2.50 ^f	40±0.00 ^{de}	247±7.00 ^{cde}	8	11818	3.939	75.96
	10	85±0.00 ^d	1609±4.50 ^e	130±0.00 ^d	42±1.50 ^g	38±2.50 ^e	200±5.25 ^e	9	11186	15.200	7.239
Jubitar-X	1.00	8±0.33 ^g	351±0.50 ^j	40±0.00 ^g	11±1.00 ^l	9±1.00 ^g	174±6.00 ^f	3	1968	0.656	95.998
Control (Nematode only)		143±3.28 ^a	3656±157.27 ^a	971±3.56 ^a	123±3.21 ^a	104±2.89 ^a	424±2.65 ^a	9	9	-	-
P		0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	-	-	-	-
LSD		4.594	139.185	24.287	4.821	5.9357	4.899	-	-	-	-

Each value represents the mean ±SE of three replicates. Galls represents No. of galls/root system. ** Gall index (GI) was scored according to Sharma *et al.* (1994). DS represents Developmental stages successful in penetrating the root. *RR represents the reproduction rate of nematode. Small letters represent significance between all concentrations in all treatments. Values followed by the same letter (s) in a column do not significantly differ according to according to Duncan's multiple range tests, LSD ($P \leq 0.05$).

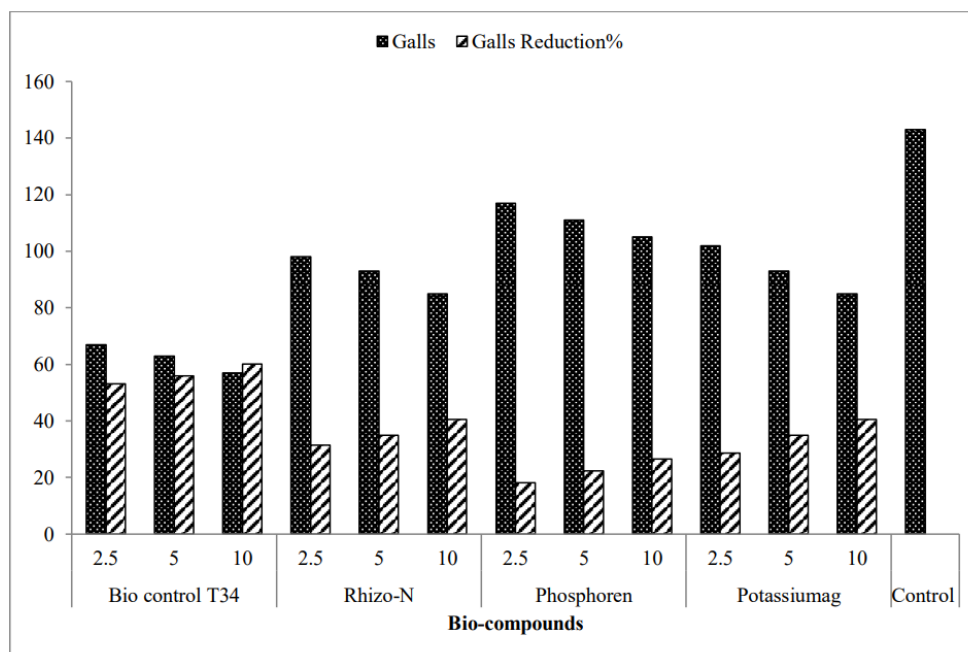


Fig. 1: Reduction of galls formation as a percentage of the infected control, showed the ability of the tested compounds to achieve a significant reduction percentage compared to the infected untreated control.

The Fecundity of *Meloidogyne arenaria*:

The fecundity of peanut root-knot nematode was evaluated depending on the number of both egg- masses/root, eggs/egg-mass, nematodes in roots (developmental stage and females), juveniles in soil, final population and reproduction rate of (build- up) as illustrated in **Table 2**. In affecting the numbers of juveniles in soil compared to the untreated plants, the best significant effect at the number of juveniles in soil and reduction percentages from the control were noted in higher rate of (10g plant⁻¹) of Biocontrol-T34 (1018, 72.16), Rhizo-N (1428, 60.94), Potassiumag (1609, 55.99) and Phosphoren (1731, 52.65); respectively. Although there was a significant difference between the medium and lower rate compared to the untreated control, there was no significant difference between the effects of these two rates in all the tested compounds. As for the ability of nematode to penetrate of the root as developmental stages, the Biocontrol-T34 compound achieved the best significant effect at the three tested rates in descending rank (90, 77 and 71) with reduction percentages from the untreated control of (90.73, 92.07 and 92.69); respectively. Followed by Rhizo-N compound with the same pattern of rate, and Phosphoren (10g plant⁻¹). While the results varied significantly in the effects of the tested compounds on the rate of egg-masses formation and its reduction. Where the results indicated that, the rate of 10g plant⁻¹ of Biocontrol-T34 (14, 86.54) and Rhizo-N (20, 80.77) compounds had the best effects compared to control (104). Followed by Biocontrol-T34 and Rhizo-N at 5 and 2.5g plant⁻¹. While the effects of Potassiumag and Phosphoren were consistent across all tested rates. As a comparison between the structure and the size of the formed egg-masses, during the experiment, it was observed that all the tested compounds had a remarkable effect compared to the untreated control and nematicide (Table 2).

On the other hand, the egg-masses associated with the Phosphoren treatments were larger in size, and the gelatinous matrix was less opaque than that of the other treatments and the control, particularly in Potassiumag, where the features of the egg-masses appeared clearer and darker. On the contrary, the effect of the tested compounds on the ability of nematodes to produce eggs was less affected than the other fecundity criteria, where the mean number of formed eggs ranged from 175 at 10 g of Biocontrol-T34 to 277 in 2.5 g of Phosphoren (**Fig.2**).

Finally, as a result of the nematode criteria, the Biocontrol-T34 compound recorded the best significant effect on final populations (3500, 5539 and 5179), reproduction rates (1.167, 1.726 and 1.846) and reduction percentages (92.881, 88.732 and 89.464), from the untreated control at three tested rates 10, 5 and 2.5g plant⁻¹; respectively. With deficiency rate of 3.12%, 7.27% and 6.53 of the nematicide (Fig.3). While Rhizo-N achieved the second rank with same pattern, and deficiency rate were 7.38%, 10.32% and 11.39%. Moreover, Potassiumag compound at the three tested rates recorded a medium effect compared to other treatments and control in same criteria. Phosphoren compound was ranked the last in effect on the final population (11186, 18983 and 11818), reproduction rates (3.939, 6.328 and 15.200) and reduction percentages (7.239, 7.724 and 7.559), from the untreated control at three tested rates; 10, 5 and 2.5g plant⁻¹, respectively (Table 2 and Fig.2).

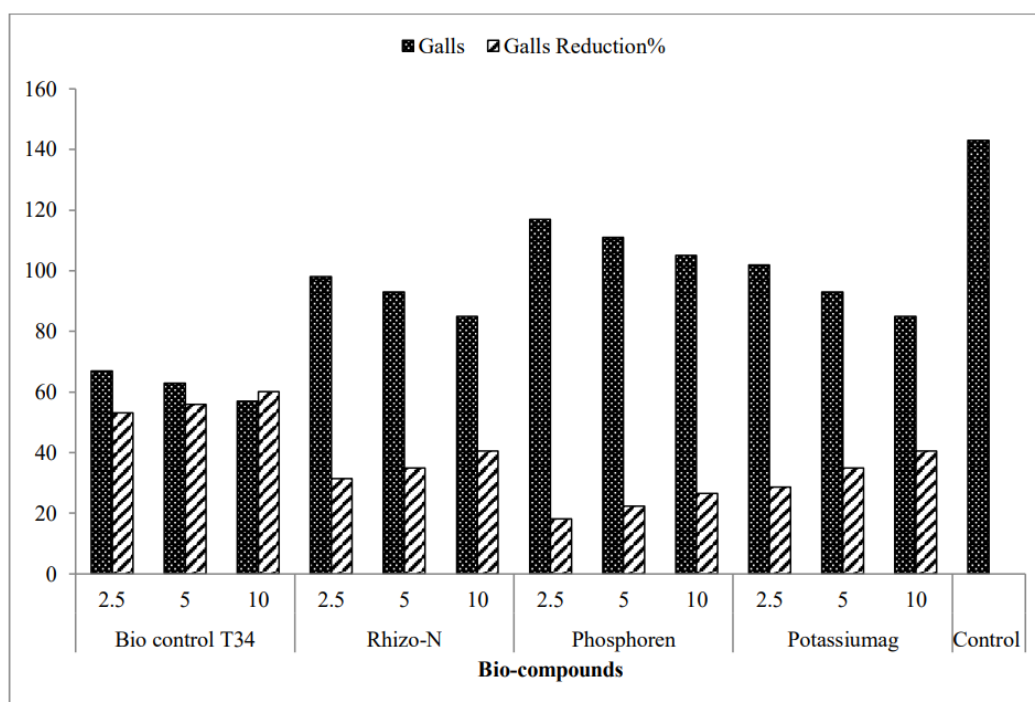


Fig. 2: Reduction% in the criteria fecundity of peanut root-knot nematode as affected by tested bio-compounds.

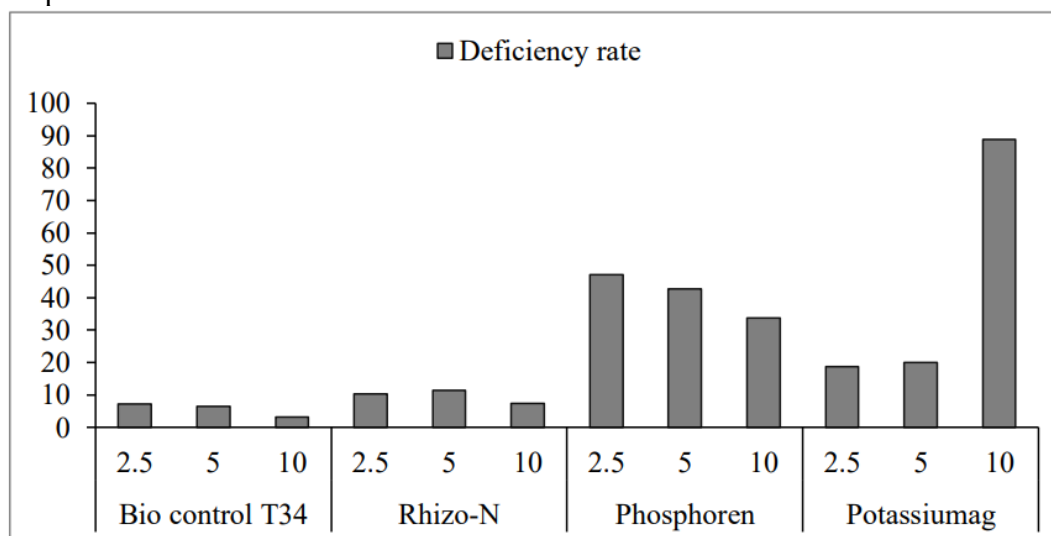


Fig. 3: The difference in the effectiveness rate as a percentage of the nematicide (Jubitar-X). The graph depicted the significant difference in the effects of the tested compounds as a percentage of nematicide effects.

Response of Peanut Growth Parameters to *M. arenaria* controlled by Biocompounds Under Greenhouse Conditions:

The data presented in Table 3, indicates the significant ability ($P \leq 0.05$) of all the tested compounds to have a positive varying degree of effect on the parameters of growth and production of peanuts. On the contrary, the ability of compounds to affect nematode criteria, the best results were observed with the Phosphoren compound in the three tested rates (10, 5 and 2.5 plant⁻¹). Where the increase percentages in both total fresh weight of the plant were 91.298, 84.289 and 76.686 and 5%, respectively, and in pods were 156.25, 118.75 and 143.75; respectively. Followed by Potassiumag treatments at 10g (62.094 and 93.75) and at 5g (50.950 and 93.75) and Rhizo-N at 10g (54.864 and 68.75); respectively. While, Biocontrol-T34 compound achieved the least significant effect at all tested rate of 10g (32.258 and 56.25), at 5g (21.194 and 31.25) and at 2 and 5g (11.899 and 18.75), respectively (Table 3).

On the other hand, the results showed that was a significant inverse correlation between the reduction in the formed galls and the percentage increase in the total fresh plant weight ($P = 0.003$ and $r = -0.777$) and the percentage increase in the number of pods ($P = 0.009$ and $r = -0.718$) (Fig.4).

Table 3: Response of peanut growth parameters to *M. arenaria* controlled by bio-compounds under greenhouse conditions.

Treatments	Rate (g)	Plant parameters			
		Fresh weight(g)	Increase %	No. pods	Increase %
Bio control T34	2.5	263.88±2.73 ^m	11.899	19±0.34 ^g	18.75
	5	285.8±2.08 ^l	21.194	21±0.68 ^f	31.25
	10	311.89±2.39 ^k	32.258	25±0.67 ^e	56.25
Rhizo-N	2.5	322.38±2.73 ^j	36.706	23±0.58 ^f	43.75
	5	342.88±1.36 ^h	45.399	26±0.37 ^e	62.5
	10	365.2±3.27 ^f	54.864	27±0.88 ^e	68.75
Phosphoren	2.5	416.66±1.97 ^d	76.686	35±0.69 ^c	118.75
	5	434.59±1.66 ^c	84.289	39±0.58 ^b	143.75
	10	451.12±3.29 ^b	91.298	41±0.58 ^a	156.25
Potassiumag	2.5	334.94±2.35 ⁱ	42.032	26±0.97 ^e	62.5
	5	355.97±2.24 ^g	50.950	31±0.58 ^d	93.75
	10	382.25±1.2 ^e	62.094	31±0.77 ^d	93.75
Jubitar-X	1.00	476.12±1.81 ^a	101.89	43±0.43 ^a	168.75
Control (Nematode only)		235.82±0.47 ⁿ	-	16±0.58 ^h	-
<i>P</i>		0.0000 ***	-	0.0000 ***	-
LSD		6.495	-	1.731	-

Each value represents the mean of three replicates. * Increase % represents growth's percentage increase as compared to the control. Small letters represent significance between all concentrations in all treatments. Values followed by the same letter (s) in a column do not significantly differ according to Duncan's multiple range tests, LSD ($P \leq 0.05$).

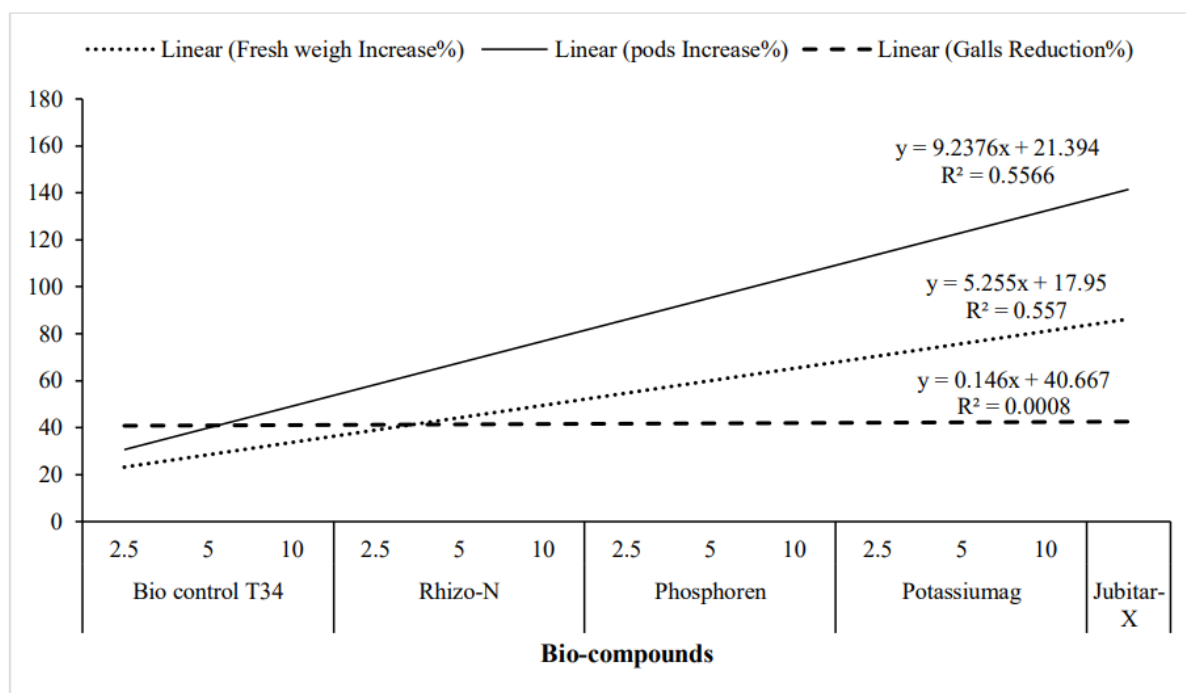


Fig. 4: Correlation and regression equations between the ability of the tested bio-compounds to suppress root-knot nematodes and the response of peanut growth and production. showed the significant inverse correlation between the reduction in the galls formation and the increase percentage in the total fresh plant weight ($P = 0.003$ and $r = -0.777$) and the percentage increase in the number of pods ($P = 0.009$ and $r = -0.718$), where r represents the correlation coefficient, P represents significance at $P \leq 0.05$.

DISCUSSION

This study provided evidence for the ability of some non-intended commercial bio-based formulates, to reduce the ability of root-knot nematode, *M. arenaria* to cause damage to the peanut crop. In comparison to the nematicide and untreated control (Nematode only), the investigated bio-compounds exhibited a significant ($P \leq 0.05$) direct or indirect influence on each of the peanut root-knot nematode, *M. arenaria* criteria and measures of plant capacity for growth and yield. These results were in line with previous study (Sikora *et al.*, 2018).

The effect was differed based on the nature of the composition of the tested bio-compounds and applied rate. According to our results, the compound based on *Trichoderma asperellum* strain T34 (Bio control T34) achieved the best effect on the ability of nematode to reproduce at the three tested rates. There have been several documented mechanisms for *Trichoderma* as biocontrol agent against plant pathogens (Mukhtar *et al.*, 2013). Antibiosis, competition, enzymatic hydrolysis, parasitism, and systemically generate of d resistance are key factors (Mukhtar *et al.*, 2021). As well as trichokonins, trichodermin, and trypsin-like protease, which are derived from *Trichoderma* spp., are only a few examples of the biocontrol substances and activators that have been shown to have nematocidal. Also, *Trichoderma* produces conidia that may adhere to various worm stages by the use of highly branching conidiophores. This process was frequently linked to the development of appressorium-like structures and fungus coiling (Sharon *et al.*, 2007). The current study indicated the significant effect of Biocontrol-T34 (*T. asperellum* strain T34) compound at 10g plant^{-1} in the effect on each of the formed galls, number of developmental stages in root and number of females, by reducing percentages (60.14%, 72.16% and 84.55); respectively. This decrease might be attributed to bio-agents' high rhizosphere competence because of their ease in colonizing roots and potential reduction in nematode feeding grounds. The bulk of the juveniles may not have

been able to reach the host roots, which would explain the decrease in root galling (Affokpon *et al.*, 2011). The present results indicated that both compounds Rhizo-N (*Bacillus subtilis*) and Potassiumag (*B. verculanes* and *B. megaterium*) had medium effect in suppressing nematodes. These findings were consistent with those investigations (Prakob *et al.*, 2009). According to previous study, the primary mechanisms of *Bacillus* sp. for controlling root-knot nematodes included direct parasitism on eggs and juveniles production of extracellular antibiotic metabolites or catabolic enzymes (proteases, chitinases, glucanases, etc.) incentivization of host defenses induction of systemic plant resistance and release of some potential nematocidal volatile substances (Ann 2013). In contrast to its limited ability to suppress nematodes, the Phosphoren (*B. megaterium*) compound achieved the best results in increasing peanut growth production, followed by Potassiumag. Another study indicated the effectiveness of these species in as growth-promoting to raising the vigor and nutrition of plants, and the potential to fight against plant pathogens (Prabhukarthikeyan *et al.*, 2022). This effect is due to the nature of the *B. megaterium* mechanism, which includes the ability to increase the uptake and transport rates of nutrients in the roots of the host plant, increased levels of non-enzymatic antioxidants and activity of enzymes involved in the ntioxidant system in the host plants. Which could help the redox state recover when there is an infection, also increase the non-enzymatic antioxidants (Chi *et al.*, 2022).

CONCLUSIONS

Based on the ability of commercial bio-based formulations of *Trichoderma* and *Bacillus* to reduce the ability of root-knot nematodes to cause damage to the peanut plants by a significant percentage compared to nematicide. The current study recommended the possibility of applying the commercial bio-compounds as an effective alternative on the long term to reduce the damage of nematodes, especially when added at the beginning of the growing season.

Declarations:

Ethical Approval: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Author's Contributions: All authors are equal in contribution.

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Availability of Data and Materials: Data presented in this study are available on fair request from the corresponding author.

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