



Efficacy of nematicides, Tricuran-P (*Trichoderma harzianum* T-22) and chicken manure on cucumber root-knot nematode populations, plant growth and soil enzyme activities

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Abstract

Root-knot nematodes (RKNs), *Meloidogyne* spp., are globally important plant-parasitic nematodes with a very broad host range including cucumber. In this research, we evaluated the nematicidal efficacy of commercial Abamectin-CAP (Vertimec 1.8% EC) versus Iranian-produced Abamectin-IAP (Vertimec 2% EC) compared with Cadusafos (Rugby), *Trichoderma harzianum* T-22 (Tricuran-P), and chicken manure against the cucumber RKN, *Meloidogyne javanica*, in commercial greenhouses. We also analyzed the effect of the products and amendment on several soil enzymes because of their significant roles in increasing the rate of decomposition and release of plant available nutrients. The results showed highly significant differences among treatments. The highest reduction of second-stage juveniles (J2) in the soil was recorded for Abamectin-CAP and Abamectin-IAP (93–95%), followed by Tricuran-P (90%), Rugby (82%) and chicken manure (65%). Similar results were obtained for the number of J2 and eggs in the root (94%), root gall indexes (94%), egg mass indexes (74–79%), and reproduction percentage (5.4–8.3%) in the Abamectin-CAP and Abamectin-IAP treatments. Enzyme activity assays showed that Rugby and chicken manure both caused a significant decrease in urease activity, followed by Abamectin-CAP and Abamectin-IAP. The highest alkaline phosphatase activity was observed for Abamectin-IAP and Abamectin-CAP, whereas the highest acidic phosphatase activity was in the Abamectin-CAP treatment. The results form a basis for developing integrated pest management strategies for RKN in cucumber.

Keywords Abamectin · Cadusafos · *Trichoderma harzianum* · Chicken manure · Nematicide · *Cucumis sativus*

Introduction

Cucumber, *Cucumis sativus* L., is one of the most important vegetable crops worldwide and ranks fourth in global vegetable production (Huang et al. 2009; Vercelli et al. 2017). Cucumber is very popular among consumers for a wide range of food uses (e.g., salads, pickles and digestive aids) (Sadeghpour et al. 2023). Cucumber production is affected by various plant pathogens including plant-parasitic nematodes, of which the most destructive genus is *Meloidogyne* (root-knot nematode, RKN) (El-Marzoky et al. 2022). RKN has a high fertility rate, short generation time, and wide host range. The related hosts included more than 2000 plant species, such as vegetables, fruit trees, oil and fiber crops, grain crops, and forage crops (Oka et al. 2020), of which are considered as secondary hosts to nematodes

in addition to the weed hosts (Resquín-Romero et al. 2023). RKNs are sedentary endoparasites that attack the root, leading to host nutrient deprivation and impaired water transport, causing aboveground symptoms of stunting, wilting, chlorosis, and reduced crop yields (Resquín-Romero et al. 2023).

Conventionally, RKN control has been performed by soil chemical fumigation, but bans and restrictions on the use of most chemical fumigants, such as methyl bromide, 1,3-dichloropropene, chloropicrin or metam-sodium, have brought serious concerns regarding nematode control in intensive horticulture (Rodrigues et al. 2017). For this reason, the search for new efficient nematicides with different modes of action to the long-time used organophosphate and carbamate nematicides is presently a priority in nematological research (Li et al. 2021). In this regard, Abamectin is one of the suggested alternative biorational tools; this compound belongs to the avermectin group, macro-cyclic lactone metabolites produced via

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natural fermentation by an actinomycete, *Streptomyces avermitilis*. Abamectin is marketed under the trade name Vertimec EC1.8% (Hebei Enge Biotech), and its mixture contains more than 80% avermectin B1a and less than 20% avermectin B1b (Hoti et al. 2023). Abamectin is used as an insecticide, acaricide and nematicide on vegetables, fruits and field crops (Sasanelli et al. 2021). This pesticide is formulated as emulsifiable concentrate (EC) in Iran (Hoti et al. 2023). Abamectin is effective in controlling *M. javanica* and *M. incognita* in many crops including vegetables such as cucumber, pumpkin, and tomato (Zhang et al. 2023).

Nematode control strategies include a variety of methods such as removing infected roots, flooding the field, deep plowing the soil, using trap plants, steaming, and solarization (Gine et al. 2016; Roth et al. 2020; Sasanelli et al. 2021). Applying biological control agents, such as species of *Trichoderma* or *Gliocladium*, is another potential tool to suppress RKNs (Hesar et al. 2011, 2022; Dhillon et al. 2023). Moreover, the addition of organic fertilizers including chicken manure to the soil not only changes its physical and chemical properties, but also reduced the population of *M. javanica* in cucumber (Nasr Esfahani and Ahmadi 2014, 2020) and *M. incognita* in eggplant (Osman et al. 2018). Many researchers confirmed the efficacy of organic manures in managing RKNs associated with improvement of plant

growth and crop yield (Osman et al. 2018; Dhillon et al. 2023). The addition of chicken manure to the soil caused a change in nematode movement by altering soil physical properties, especially increasing the hydraulic conductivity and porosity of the soil (Forghani et al. 2021). Previous research documented that chicken manure and *T. harzianum* improved disease-control efficacy and crop yields and were economically as well as environmentally acceptable (Halalat et al. 2017; El-Remaly et al. 2022; Liu et al. 2023).

Soil enzymes play a key role in maintaining the ecology, physical and chemical properties, fertility and health of soils (Koçak 2020; Badawy et al. 2022). These enzymes regulate overall organic matter decomposition in the soil system, of which urease has an important role in the accessibility of N for plant growth in the N cycle (Halalat et al. 2017; Abdel-Nasser et al. 2018). Phosphomonoesterase, i.e., alkaline and acid phosphatases, are the most active soil phosphatases, hydrolyzing phosphomonoesters and in some cases phosphodiester to release assimilable phosphates for microbes and plants, playing an important role in organic P cycling (Abdel-Nasser et al. 2018; Uwituzi et al. 2022). To date, there are few studies asserting the effect of nematicide application on soil enzyme activities (Fig. 1).

The specific objective of this study was to evaluate the efficacy of commercial Abamectin-CAP (Vertimec 1.8% EC), Iranian-produced Abamectin-IAP (Vermectin®

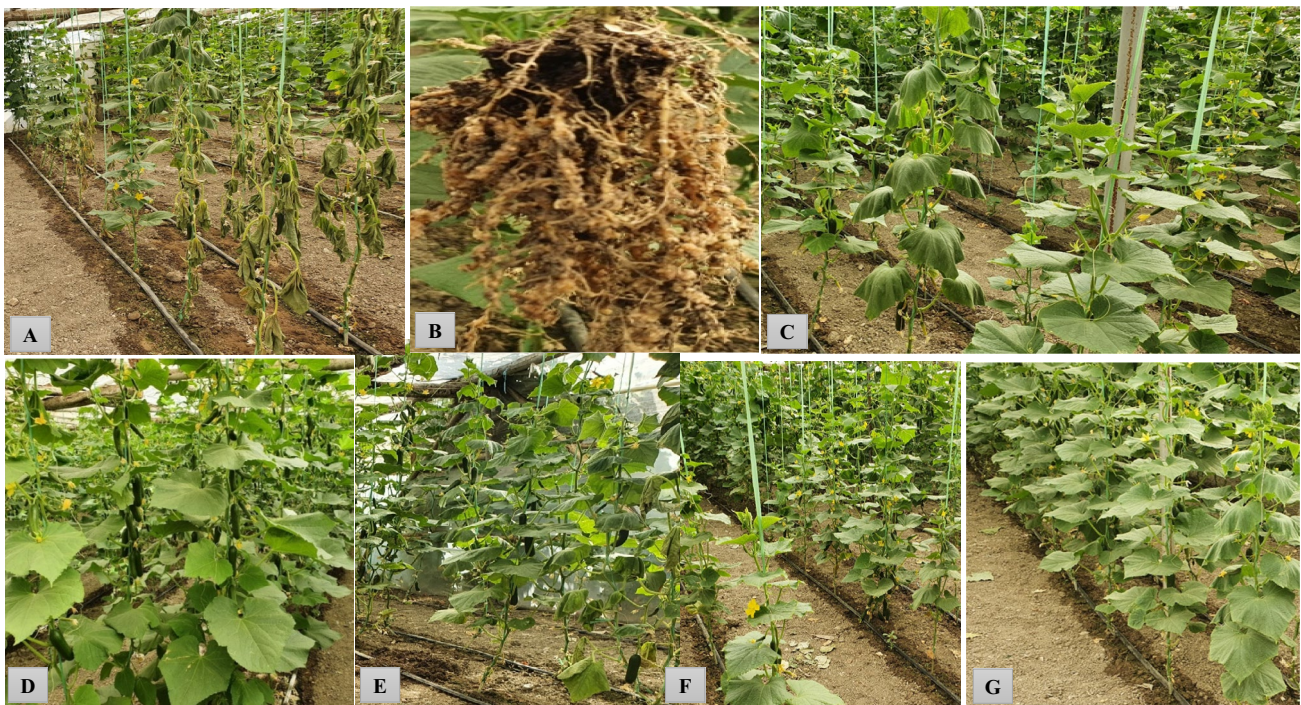


Fig. 1 Illustration of the effects of various soil treatments on cucumber plants in root-knot nematode infested soil in the greenhouse. A. Untreated controls; B. infected roots in untreated

controls; C. Tricurin-P (*Trichoderma harzianum* T22); D. Commercial Abamectin-CAP; E. Iranian Abamectin-IAP; F. chicken manure

2% EC), the acetylcholinesterase inhibitor nematicide Cadusafos (Rugby); *T. harzianum* T22 (Tricurán-P), and chicken manure against *Meloidogyne javanica*, the dominant RKN species on cucumber in Iran in infested commercial greenhouses in two successive years, 2020/21 and 2021/22. In addition, we analyzed crop biomass growth parameters and soil enzyme activities in all treatments.

Materials and methods

Experimental design and treatment application

Two trials were conducted in a loamy soil (36% clay, 39% sand, 25% silt) over the two consecutive growing seasons (2020/21 and 2021/22) in commercial greenhouses at localities in Dorcheh, Isfahan (latitude: 32°39'8.86" N; longitude: 51°40'28.63" E) in the central part of Iran. In this greenhouse production system, cucumbers are traditionally grown in a rotation with tomato and pepper, all of which are particularly susceptible to RKN (Khader et al. 2023; Li et al. 2020). The trials utilized natural RKN populations present in the soils. Environmental conditions were characterized by temperature 22–27 ± 2 °C, humidity 50–60%, and 16 h daylight.

Forty-day-old Nunhems cucumber seedlings produced by Rexvan (the Netherlands) were transplanted into the soil in the greenhouse with spacing of 0.5 m between plants and 1.0 m between rows. Plots were 30 m² in area (60 plants/plot) and arranged in a randomized complete block design with three replicates. Cucumber plants were amended through irrigation water with Knop's nutrient solution (10 mg FeCl₃; 0.25 g KH₂PO₄; 0.25 g KNO₃; 0.25 g MgSO₄•7H₂O and 1 g NaNO₃ per liter of tap water; Columbus Chemical Industries, Columbus, WI, USA). General pest and disease management followed suggested prescriptions (Nasr Esfahani et al. 2023).

At the same time, the treatments were applied in the greenhouse as follows: Cadusafos (30 g/m²; Rugby, FMC Corporation, Philadelphia, PA, USA); chicken manure (40 t/ha; Sepahan Poultry, Isfahan, Iran); and *T. harzianum* T22 (1.5 kg/ha Tricurán-P; Koppert, Bengaluru, India) were applied directly into the soil, whereas Abamectin-CAP (Vertimec 1.8% EC; Hebei Enge Biotech, Shijiazhuang, China) and Abamectin-IAP (Vermectin® 2% EC, Gyah Co., Tehran, Iran) were applied as a drench at 8 L/ha each (2.5 ml/plant). All of these applications were repeated as a drench thrice at an interval of 3 weeks. The cucumber plants were harvested 140 days after planting for further studies. The nutrient composition of the poultry litter was as follows (on a dry-weight basis): organic matter 73.6%; total nitrogen 3.61%; ash 48.4%; pH 7.50; EC 46.00 dS/m; P 1.99%; K 1.66%; Na 0.31%; Ca 7.09%; Mg 0.89%.

To determine the effect of the treatments on plant growth parameters, fresh and dry weights of stems and roots were measured with a digital scale, and the lengths and diameters of the stems and roots as well as root volumes (in an measuring cylinder of 1000 cc filled with tap water) for three plants per replicate were measured at the time of harvesting. Dry weights were determined after placing tissues in a dryer at 70 °C for 48 h (Tehrani et al. 2020; 2021).

Soil samples were taken at the beginning and at the end of each trial to determine RKN populations as well as soil fertility variables including soil enzymes. Details of the sampling process are described in subsequent sections. The soil samples were analyzed at the Esfahan Agriculture and Natural Resource Research and Education Center, Esfahan, AREEO, Iran.

Nematode analysis

Initial population densities of *M. javanica* were determined prior to planting from 200-g subsamples of well-mixed soil from each row according to Brennan et al. (2020). Randomized quadrat sampling from the related soils were taken up to a depth of 30 cm. The initial populations were recorded to be 8.9 and 6.4 J2/g for the first and second trial, respectively.

Three months after treatment application, five cucumber plants along with their roots were removed gently from the soil for each replicate. After washing the roots, the number of egg masses and gall index on each root were determined as described by El-Kelany et al. (2020). Gall index (GI) and egg mass index (EMI) of the root system of each plant were scored as follows: 0 = roots without gall or egg masses; 1 = presence of 1 to 2 galls or egg masses; 2 = presence of 3 to 10 galls or egg masses; 3 = presence of 11 to 30 galls or egg masses; 4 = presence of 31 to 100 galls or egg masses; and 5 = presence of more than 100 galls or egg masses on the root system (Taylor and Sasser 1978). To count the J2 per 3 g of root, the roots were cut into 1–2-cm pieces and poured into a blender. Then, about 300 ml of 0.9% sodium hypochlorite solution (10% commercial Vitex bleach) was added, roots were crushed at maximum speed for 40 s, and J2 were counted for each replication. Furthermore, the collected soil samples from around the roots were extracted by sieve and centrifuge (Jenkins 1964). The number of J2 per 200-g subsamples of well-mixed soil was counted as described by El-kelany et al. (2020) in 1 ml suspension using a counting slide under the light microscope at 10× magnification. Means were based on five counts and the population density expressed per g of soil per replication (El-Kelany et al. 2020; Khan et al. 2022; Moatamedi et al. 2018; 2023).

The nematode reproduction factor ($R_f = P_f/P_i$) was calculated by dividing the final (after 3 months) and initial (prior to planting) RKN population numbers. The data were transformed to $\sqrt{x+1}$ for analysis to meet the assumptions of normality and homoscedasticity, but original values are presented in the manuscript.

Morphological and molecular characterization of nematode species

Nematode identification was based on morphological and morphometric data of the adult females, perineal pattern, and J2 (Sasser & Carter 1985). Females were carefully excised from the cucumber root tissue, and the perineal patterns were cut according to Adam et al. (2007) and cleaned with lactic acid. Finally, the perineal patterns were mounted in glycerin on glass slides and viewed. J2 were isolated, killed and fixed using Hussey and Barker's methods (Hussey and Barker 1973; Ye et al. 2015). After preparing the slides, the characteristics of the J2 and the perineal pattern were examined using an Olympus microscope at 40× magnification (Hawk 2019; Taylor & Sasser 1978).

Molecular identification was based on polymerase chain reaction (PCR) using three *Meloidogyne* species-specific SCAR primer pairs: **ar**, **inc**, and **jav** (Table 1). The single egg masses were flash-frozen after collection and separately ground with a pestle and mortar. Genomic DNA was extracted using the DENAzist Plant DNA Isolation Kit (DENAzist, Mashhad, Iran) according to the manufacturer's instructions (Poursakhi et al. 2023; Qalavand et al. 2023). In this way, 500 µl of DG1 buffer and 5 µl from 2-mercaptoethanol to 30 mg were added to ground tissue and mixed and centrifuged the tube at 10,000 rpm for 2 min. The supernatant was transferred into a new spin column and centrifuged at 10,000 rpm for 1 min. Then after, 500 µl from DG2 solution was added into the spin column and centrifuged at 10,000 rpm for 1 min, following addition of 700 µl from DG3 solution into the spin column and centrifuged again. Thereafter, the spin column separated from its collecting tube and placed

it into a new 1.5-ml microfuge tube, following addition of 50 µl from DG4 solution onto the center of the spin column and centrifuged at 10,000 rpm for 2 min. Finally, the quantity and purity of total DNA were evaluated using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific). Then, based on the sequencing of the amplified regions and comparison with GenBank samples, the *Meloidogyne* species was determined (Mohammadbagheri et al. 2021; Nasehi et al. 2016; 2019; Maleita et al. 2021).

Soil enzyme assays

To determine enzyme activity, soil sampling was conducted by randomized quadrat sampling 3 months after treatment application, as described in Sect. "Nematode analysis". Urease activity was determined by adding 2 g of fresh soil to toluene, 20% urea, and 5 ml of citric acid buffer with a pH of 6.7, followed by incubation at 37 °C for 24 h (Javanshad et al. 2023). Then, 4 ml sodium phenol and 3 ml hypochlorite were added to determine the release of NH_4^+ -N by colorimetry at 578 nm (Cordero et al. 2019; Nasr-Esfahani et al. 2020; Qalavand et al. 2022).

For determination of acid and alkaline phosphatase activity, 5 ml of p-nitrophenyl phosphate substrate, 1 ml of toluene, and 5 ml of pH 5.0 acetic acid buffer were added to 1 g of fresh soil and the suspension was filtered and incubated at 37 °C for 12 h. Thereafter, 1 ml of the filtrated was taken and 100 mM boric acid buffer (pH 9.0), 0.5% 4-amino alternating pyridine (0.5 M, pH 5.0), and 2.5% potassium ferricyanide (0.5 ml) were added. The produced purine nucleoside phosphorylase (pNP) was determined colorimetrically at 570 nm (Liu et al. 2023). Three replicates of each subsample for each of the three enzymes were analyzed. Moreover, substrate-free and soil-free controls were added for each sample to account for non-enzymatic substrate hydrolysis (Ciarkowska et al. 2014).

Table 1 Species-specific SCAR (sequence-characterized amplified regions) primers used in polymerase chain reactions to identify *Meloidogyne* spp

RAPD marker	SCAR primer name	SCAR sequence (5' to 3')	SCAR Sequence (bp)	Target species	References
OPA12-420	ar	F TCGGCGATAGAGGTAATGAC	420	<i>M. arenaria</i>	Zijlstra et al. (2000)
	ar	R TCGGCGATAGACATACTAACT			
OPA06-420	inc	F CTCTGCCCAATGAGCTGCC	1200	<i>M. incognita</i>	Zijlstra et al. (2000)
	inc	R CTCTGCCCTCACATTAAG			
OPA01-700	jav	F GGTGCGCGATTGAACAGAGC	670	<i>M. javanica</i>	Randig et al. (2002)
	jav	R CAGGCCCTTCAGTGGAACTATAC			

Statistical analysis

To assess the normality of the obtained data, Kolmogorov–Smirnov and Shapiro–Wilk tests were used by using SPSS 20.0 software (IBM Corporation). The homogeneity of variances within the treatment was also determined using Bartlett's test (Gholamaliyan et al. 2021). Statistical analysis was performed via analysis of variance (ANOVA), and mean comparisons were performed using protected least significant difference (LSD) tests, with significant difference defined as $p < 0.05$ using SAS 9.1 software version 9.4 (SAS Institute, Cary, NC, USA) (SAS Institute 2004) (Mohammad et al. 2021; Hejazi et al. 2021; Monazzah et al. 2022).

Results

Identification of nematode species

Based on the results of morphometric studies, the characteristics of the J2, and the terminal patterns of the adult female nematode species in the greenhouse was determined corresponding to the description of Taylor and Sasser (1978) for *M. javanica*. In the molecular assays (Table 1), two SCAR primers, ar and inc, did not produce

any bands, but specific bands were amplified with the jav marker for *M. javanica*, which was a 670-bp fragment consistent with the results of Hawk (2019).

Effect of treatments on nematode population parameters

Based on the analysis of variance, a significant difference was observed at $p < 0.01$ between the number of J2 in the root, the EMI on the root, and the reproduction factor in both experiments (Suppl. Table 1). There was no significant difference in the number of J2 in the soil and in GI between the commercial Abamectin-CAP and Iranian-Abamectin-IAP. For all investigated traits, a significant interaction effect was observed at $p < 0.01$ between trial and treatment. In comparison of the two experiments, the reproduction factor in the soil was significantly lower in the first year than in the second one (Table 2). The number of J2 in the root, the EMI on the root, and the reproduction factor based on the eggs and J2 in the root were significantly lower in the first experiment than in the second one (Table 2).

In comparison, the lowest relative number of J2 in the soil was achieved using Abamectin-CAP followed by Abamectin-IAP, 95% and 93% reductions, respectively, as compared with untreated controls. In this regard, both treatments performed significantly better than other

Table 2 Means comparison of the effect of test and treatment on root knot nematode (*Meloidogyne javanica*) parameters on cucumber in the greenhouse

Test or treatment	J2 No. in the soil/g	J2 and egg No/3 g of root	Gall No./root	Egg mass inde×per 3 g of root	Reproduction factor of J2 in the soil (%)	Reproduction factor of eggs and J2 in the roots (%)
Means over all treatments for test 1 and 2						
Test 1	13.7 ^a ± 1.25	17.4 ^b ± 1.14	47.7 ^a ± 3.63	7.2 ^b ± 0.14	21.7 ^a ± 1.30	7.3 ^b ± 0.63
Test 2	13.4 ^a ± 1.42	27.6 ^a ± 1.36	46.3 ^a ± 2.74	8.4 ^a ± 0.24	20.6 ^b ± 1.87	11.6 ^a ± 1.42
Test × Treatment						
Test 1 Commercial Abamectin	3.8 ^{ji} ± 0.36	5.0 ⁱ ± 0.42	44.0 ^e ± 3.21	5.9 ^e ± 0.41	6.5 ⁱ ± 0.42	2.0 ^h ± 0.04
Iranian Abamectin	5.7 ^{gh} ± 0.25	10.3 ^h ± 1.47	90.0 ^b ± 4.14	6.7 ^d ± 0.25	8.2 ^{hi} ± 0.11	4.3 ^g ± 0.04
Tricurán-P	5.3 ^{hi} ± 0.14	13.1 ^g ± 1.75	56.0 ^d ± 2.21	7.1 ^d ± 0.35	9.7 ^{gh} ± 0.12	5.5 ^{fg} ± 0.12
Rugby	13.0 ^e ± 1.02	30.7 ^{bc} ± 1.52	26.0 ^f ± 1.96	8.3 ^c ± 0.63	22.4 ^e ± 1.74	12.9 ^{bc} ± 1.41
Chicken manure	16.1 ^d ± 1.45	16.9 ^f ± 1.36	29.0 ^f ± 1.88	5.3 ^e ± 0.42	24.6 ^d ± 1.68	7.1 ^e ± 0.09
Control (non-treated)	38.4 ^a ± 1.11	28.3 ^d ± 1.45	41.0 ^e ± 4.85	9.7 ^b ± 0.11	58.7 ^a ± 1.54	11.9 ^c ± 1.20
Test 2 Commercial Abamectin	2.9 ^j ± 0.22	48.7 ^a ± 1.48	96.0 ^a ± 4.72	5.7 ^e ± 0.55	4.4 ^j ± 0.14	20.4 ^a ± 1.42
Iranian Abamectin	4.0 ^{ji} ± 0.36	30.4 ^c ± 1.81	44.0 ^e ± 2.77	9.9 ^b ± 0.63	6.9 ⁱ ± 0.36	12.8 ^{bc} ± 1.14
Tricurán-P	7.2 ^g ± 0.42	16.2 ^f ± 1.14	66.0 ^c ± 3.44	8.7 ^c ± 0.47	11.0 ^g ± 1.75	6.8 ^{ef} ± 0.63
Rugby	10.1 ^f ± 1.11	13.6 ^g ± 1.23	18.0 ^g ± 0.69	6.8 ^d ± 0.85	15.4 ^f ± 1.36	5.7 ^f ± 0.54
Chicken manure	25.6 ^c ± 1.32	31.9 ^b ± 1.65	28.0 ^f ± 1.25	8.9 ^c ± 0.12	39.0 ^c ± 1.42	13.4 ^b ± 1.45
Control (non-treated)	30.4 ^b ± 1.57	25.1 ^e ± 1.44	26.0 ^f ± 1.42	10.7 ^a ± 0.46	46.6 ^b ± 1.14	10.5 ^d ± 1.42

The initial population was recorded to be 8.9 J2 per g of soil

Means having same letters in each column and table section are not significantly different according to LSD ($p < 0.05$)

treatments. The lowest relative number of J2 in the roots was obtained with Tricuran-P treatments at 94%, followed by Abamectin-IAP at 91% reduction, as was compared with untreated controls (Suppl. Table 2). In this regard, the two abamectin formulations performed significantly better than the controls (Table 2).

Similarly, the lowest GI was observed in the two abamectin formulations, both of which were significantly different from the other treatments and the untreated controls ($p < 0.01$). In fact, Abamectin-CAP and Abamectin-IAP treatments reduced the GI by 58% and 56%, respectively (Suppl. Table 2). Also, the lowest EMI values in the root were recorded in the application of Abamectin-CAP followed by Abamectin-IAP with 79% and 74% reduction, respectively ($p < 0.01$). The lowest percentage of J2 reproduction in the soil was in Abamectin-CAP and the highest one in the controls. In this regard, all treatments performed significantly better than the controls. The lowest reproduction factor of eggs and J2 in the root occurred in the treatment using Abamectin-CAP followed by Abamectin-IAP, and the lowest reproduction factor in the soil in the treatment of Abamectin-CAP followed by Abamectin-IAP with 5.4% -8.3%, respectively ($p < 0.01$) (Table 2).

Biomass growth parameters

Based on the results of variance analysis, a significant difference was observed in the fresh and dry weight traits of stem and root between the two experiments at $p < 0.01$, and in the root volume trait at $p < 0.05$ (Suppl. Table 3) (Table 3). There was no significant difference between the two experiments in the traits of diameter and length of stem and root. The treatments had a significant effect on all traits, except the length and diameter of the stem and the diameter of the root, at $p < 0.01$. In all traits, except length and diameter of stem and root, a significant interaction effect was observed at $p < 0.01$ between the two factors of test and treatment (Suppl. Table 3). In the comparison of the two experiments, the stem fresh weight was significantly higher in the first experiment than in the second one. The mean of stem dry weight, fresh and dry weight of roots and root volume in the second experiment were significantly higher than in the first one (Table 3).

The highest stem fresh weight was recorded in chicken manure treatment. In this regard, all treatments were significantly different from the non-treated controls. The highest dry weight of the stem was recorded in the application of Abamectin-CAP followed by the Abamectin-IAP treatment. Regarding the stem length, there was no significant difference among the treatments. The highest root fresh weight was recorded for Abamectin-IAP followed by Abamectin-CAP and the controls. In this regard, the treatments were not significantly different from the control.

The largest root volume was recorded for Abamectin-CAP followed by the Abamectin-IAP treatment, and the treatments were significantly different from the control. The highest root length was recorded for chicken manure and the lowest in the control (Table 3).

Activity of the selected soil enzymes

As indicated in Tables 4 and Suppl. Table 4, the treatments had significant effect on urease, alkaline phosphatase, and acid phosphatase enzyme activity. Among the treatments, Rugby and chicken manure caused a significant ($p = 0.05$) decrease in urease activity compared with the non-treated controls (Table 4). In contrast, the highest urease activity was in the untreated control followed by Abamectin-CAP, Abamectin-IAP, and Tricuran-P.

Phosphatase enzyme activities also underwent significant changes. The highest alkaline phosphatase activity was of Abamectin-IAP and Abamectin-CAP, followed by Tricuran-P, Rugby and chicken manure. Similar results were also observed for acid phosphatase (Table 4).

Discussion

Root-knot nematodes are a growing concern for vegetable producers because chemical nematicides are gradually reducing (Li et al. 2021). In this research, we confirmed *M. javanica* as the causal agent of root-knot of cucumber and analyzed the effect of soil management using chemicals, organic amendments, and biocontrol and found large and significant variations among the applied treatments. Abamectin acts by the avermectin mode of action which blocks the transmittance of electrical activity in nerves and muscle cells of the nematode larvae by stimulating the release and binding of gamma-amino butyric acid (GABA) at nerve endings (Beixing et al. 2018; Mumby et al. 2022). This causes an influx of chloride ions into the cells, which leads to hyperpolarization and subsequent paralysis of the neuromuscular systems and then death (Sasanelli et al. 2021). GABA has also been reported in the J2 of *Globodera rostochiensis* and *Meloidogyne incognita* (Khalil 2013; Hawk 2019; Li et al. 2022). Our results showed that the highest reductions of nematode population parameters were recorded in both Abamectin treatments, ranging from 93 to 95%, which is a highly desirable result as far as RKNs management is concerned.

Abamectin is a broad-spectrum insecticide and larvicide prepared from the bacterium *Streptomyces avermitilis* (Nasr Esfahani et al. 2014; El-Eslamboly et al. 2019), and in the management of RKNs, it offers effective control with minimal environmental pollution (Sasanelli et al. 2021). Abamectin is an effective nematicide that controls a wide

Table 3 Means comparison of the effect of test and treatment on plant biomass growth parameters on cucumber naturally infected with root knot nematode (*Meloidogyne javanica*) in the greenhouse

Test	Stem fresh weight(g)	Stem dry weight (g)	Stem diameter (cm)	Stem length (cm)	Root fresh weight (g)	Root dry weight (g)	Root diameter (cm)	Root volume (cm ³)	Root length (cm)
Test 1	396.4 ^a ± 23.5	45.1 ^b ± 2.52	1.04 ^a ± 0.08	263.4 ^a ± 21.3	21.5 ^b ± 2.03	5.02 ^b ± 0.32	1.01 ^a ± 0.05	34.7 ^b ± 2.32	27.8 ^a ± 2.03
Test 2	356.4 ^b ± 36.1	50.8 ^a ± 3.02	1.00 ^a ± 0.09	250.8 ^a ± 12.6	25.7 ^a ± 1.09	5.46 ^a ± 0.52	1.01 ^a ± 0.06	37.7 ^a ± 2.41	27.0 ^a ± 1.50
Test × Treatment									
Test 1 Commercial Abamectin	364.0 ^d ± 24.1	53.0 ^{cd} ± 4.02	1.02 ^a ± 0.11	245.4 ^{ab} ± 11.0	32.7 ^c ± 2.63	11.58 ^b ± 0.25	1.00 ^a ± 0.03	58.1 ^b ± 3.21	26.0 ^{ab} ± 1.03
Iranian Abamectin	248.0 ^f ± 11.6	31.3 ^{gh} ± 1.89	1.01 ^a ± 0.21	263.1 ^a ± 14.6	29.5 ^c ± 2.41	8.50 ^c ± 0.41	1.01 ^a ± 0.02	44.2 ^c ± 2.65	29.4 ^a ± 1.54
Tricurán-P	326.3 ^{de} ± 12.5	51.0 ^{cd} ± 3.60	1.03 ^a ± 0.22	269.2 ^a ± 22.3	15.9 ^e ± 1.01	2.51 ^e ± 0.06	0.94 ^a ± 0.01	23.8 ^{de} ± 1.21	30.6 ^a ± 1.63
Rugby	512.0 ^b ± 14.6	58.3 ^c ± 2.98	1.06 ^a ± 0.15	288.0 ^a ± 20.8	13.7 ^{gh} ± 1.06	1.50 ^f ± 0.21	1.04 ^a ± 0.04	17.7 ^{ef} ± 1.52	29.9 ^a ± 1.75
Chicken manure	508.3 ^b ± 22.6	41.7 ^{ef} ± 2.41	1.16 ^a ± 0.14	259.6 ^{ab} ± 30.2	21.1 ^e ± 2.02	3.40 ^d ± 0.14	1.14 ^a ± 0.06	52.8 ^b ± 3.21	32.4 ^a ± 1.74
Control	420.0 ^c ± 34.5	35.0 ^{gh} ± 1.96	0.93 ^a ± 0.09	254.9 ^{ab} ± 13.6	16.0 ^{fg} ± 1.32	2.60 ^c ± 0.09	0.91 ^a ± 0.02	11.6 ^{fg} ± 1.01	18.5 ^{bc} ± 1.01
Test 2 Commercial Abamectin	518.0 ^b ± 11.6	79.3 ^b ± 3.98	0.98 ^a ± 0.08	243.4 ^{ab} ± 12.3	19.5 ^{ef} ± 0.98	2.59 ^c ± 0.32	1.05 ^a ± 0.03	55.7 ^b ± 3.21	26.4 ^{ab} ± 1.62
Iranian Abamectin	193.3 ^e ± 21.6	37.0 ^{fs} ± 2.08	0.85 ^a ± 0.04	249.6 ^{ab} ± 11.6	43.8 ^a ± 3.21	8.60 ^c ± 0.41	0.90 ^a ± 0.01	27.5 ^d ± 1.98	29.7 ^a ± 1.96
Tricurán-P	315.3 ^c ± 32.1	47.0 ^{de} ± 2.69	1.02 ^a ± 0.12	266.2 ^a ± 10.9	38.3 ^b ± 1.96	13.91 ^a ± 1.02	1.02 ^a ± 0.06	66.3 ^a ± 4.25	26.9 ^a ± 1.33
Rugby	356.7 ^{de} ± 14.3	28.0 ^{hi} ± 1.85	1.01 ^a ± 0.03	279.4 ^a ± 10.9	14.7 ^{gh} ± 1.01	2.61 ^e ± 0.32	0.97 ^a ± 0.03	14.8 ^f ± 1.06	28.7 ^a ± 1.24
Chicken manure	594.7 ^a ± 17.3	90.3 ^a ± 5.62	1.17 ^a ± 0.04	255.0 ^{ab} ± 11.6	25.6 ^d ± 2.32	3.41 ^d ± 0.21	1.15 ^a ± 0.06	56.7 ^b ± 4.21	33.3 ^a ± 1.41
Control	160.3 ^e ± 19.4	23.3 ⁱ ± 1.30	0.97 ^a ± 0.04	210.9 ^b ± 12.4	12.3 ^h ± 1.20	1.65 ^f ± 0.11	0.94 ^a ± 0.07	5.2 ^e ± 0.21	17.0 ^c ± 1.52

Means having same letters in each column and table section are not significantly different according to LSD ($p < 0.05$)

Table 4 Comparison of the average effect of treatments on soil enzymes treated with various treatments including, commercial Abamectin, Iranian Abamectin, Tricurin-P, Rugby, Chicken manure and control on cucumber

Treatment	Enzymes					
	Urease $\mu\text{g/ml NH}_4/\text{g Soil}/2\text{ h}$		Alkaline phosphatase $\mu\text{gNPg}^{-1}\text{ h}^{-1}$		Acid phosphatase $\mu\text{gNPg}^{-1}\text{ h}^{-1}$	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Commercial Abamectin	2.95 ^a ± 0.21	2.63 ^a ± 0.14	66.33 ^a ± 3.21	61.69 ^a ± 2.36	52.87 ^{ab} ± 2.54	47.58 ^{ab} ± 1.90
Iranian Abamectin	2.33 ^c ± 0.32	2.07 ^c ± 0.20	67.19 ^a ± 2.98	62.49 ^a ± 2.52	59.16 ^a ± 2.36	53.24 ^a ± 2.63
Tricurin-P	2.87 ^b ± 0.18	2.55 ^b ± 0.14	27.36 ^b ± 1.41	25.44 ^b ± 1.41	43.45 ^{ab} ± 1.98	39.10 ^{ab} ± 1.47
Rugby	1.26 ^e ± 0.14	1.13 ^e ± 0.18	31.87 ^b ± 1.32	29.64 ^b ± 1.96	40.05 ^{ab} ± 1.48	36.04 ^{ab} ± 2.06
Chicken manure	1.26 ^e ± 0.21	1.10 ^e ± 0.09	34.06 ^b ± 1.98	31.68 ^b ± 1.98	37.85 ^b ± 2.50	34.06 ^b ± 1.49
Non-treated control	2.32 ^d ± 0.19	2.06 ^d ± 0.12	38.76 ^b ± 1.02	36.05 ^b ± 1.54	34.75 ^b ± 1.25	31.28 ^b ± 2.05

The means in each column, which have at least one letter in common, are not significantly different according to LSD ($p < 0.05$)

range of plant parasitic nematodes such as *Meloidogyne* spp., *Rotylenchulus reniformis* and *Tylenchulus semipenetrans* on different crops (Cabrera et al. 2009; El-Eslamboly et al. 2019; Hawk 2019; Oka et al. 2020). Abamectin contains four major components A1a, A2a, B1a and B2a and four minor components A1b, A2b, B1b and B2b (Beixing et al. 2018). According to Li et al. (2020), Abamectin LC₅₀ values of approximately 2 mg/L to the J2 stage of RKN and Abamectin microcapsule suspension were superior to an emulsifiable concentrate (EC) formulation. It has been suggested that Abamectin effects on female nematodes and egg masses may not match with effects on the gall index and or other parameters, which may be due to the attenuated or delayed development of adult females and reduction of their fecundity (Forghani and Hajihassani 2020).

We also found that Rugby can effectively reduce the RKN populations in the soil and in the roots. Rugby is an organophosphate insecticide used to control nematodes, ringworms, and soil insects in several crops including vegetables and fruit trees (Nasr Esfahani et al. 2014; Oka et al. 2020). The compound works by contact and will only control the mobile stages of the nematodes while they are active in the soil (Oka et al. 2020). Eggs in the soil and larvae that have already penetrated the roots are not controlled (El-Eslamboly et al. 2019). Rugby when correctly applied and incorporated will establish a barrier layer in the soil and will only provide protection for the below ground plant organs within that zone (Soltani et al. 2013). Rugby paralyzes nematodes by inhibiting the acetylcholinesterase enzyme and continuously stimulating the nerve fibers and destroys them by eliminating the possibility of movement and stopping them from feeding. There are several other reports on the efficacy of Rugby against *M. incognita* in cucumber (El-Eslamboly et al. 2019) and on olive (Soltani et al. 2013), and against the citrus nematode, *Tylenchulus*

semipenetrans, consistent to our results (Cabrera et al. 2009; Oka et al. 2020).

Our results also revealed that Tricurin-P could effectively reduce RKN populations to some extent compared with the other treatments including Abamectins, with almost similar results in terms of soil and root J2, GL, and EMI. Tricurin-P contains the antagonist fungus *Trichoderma harzianum*, a plant-associated fungus with a high adaptability in different soils and climates. *T. harzianum* can act symbiotically in plants, promoting plant growth and improving nutrient utilization efficiency and host resistance (Yao et al. 2023). Based on the production of cellulose and chitin decomposing enzymes and the release of plant growth hormones, especially auxin, the fungus is an ideal treatment against soil-pathogenic fungi and to increase plant growth (Zhang et al. 2022). It also increases plant resistance against environmental stressors (Wang et al. 2022; Khan and Tanaka 2023). Species of *Trichoderma* are able to reduce the damage by RKNs directly by parasitism, antibiosis, and paralysis via production of lytic enzymes (You et al. 2022; Wang et al. 2022). They also modify root morphology and/or rhizosphere interactions, which is advantageous for plant-growth. In addition, *Trichoderma* spp. able to induce host resistance against nematodes by activating hormone-mediated (salicylic and jasmonic acids and strigolactones, among others) plant-defense mechanisms (Bagheri et al. 2021; 2022; Khan and Tanaka 2023).

Amendments from organic sources such as compost, biochar, crop residues, livestock manure, and poultry litter have been introduced to significantly control root diseases and improve soil health, and thus, they serve as an alternative method for managing plant parasitic nematodes (Khader et al. 2023). We also found that chicken manure can reduce RKN populations to some extent compared with the other treatments. Similar results on RKNs have been reported by Kasim et al. (2021) on coffee; Izuogu

and Oyedunmade (2009) on fluted pumpkin (*Telfairia occidentalis*); and Auwal et al. (2015) on RKNs infecting rice. Chicken manures improve the soil capacity for holding nutrients and water, which improves plant vigor and therefore increases plant tolerance to nematodes; release specific compounds that may be nematicidal (Cole et al. 2020); stimulate microbial activities in the soil, including nematode antagonists; and indirectly stimulate nematode predators and parasites that depend on microbial activities (Collange et al. 2011; Kankam et al. 2014; Azim et al. 2018). Indeed, laboratory studies have shown that chicken manure controls the nematode by releasing toxic substances (Osman et al. 2018). However, such alternative nematode management strategies are unlikely to be as effective and fast-acting as nematicides. Although nematicides effectively reduce plant parasitic nematodes, other environmental and soil fertility issues arise. Therefore, sustainable management of plant-parasitic nematodes from addition of organic amendments to the soil remains an important consideration (Wachira et al. 2009).

Soil enzymes have been suggested as suitable indicators of soil quality because of their intimate relationship with soil biology, the ease with which they can be measured, and their rapid response to change in soil management and environment (Badawy et al. 2022). Soil enzymes can promote the transformation of matter and energy in soil, and the activity of soil enzymes has a close relationship with soil nutrients and their availability (Badawy et al. 2022). Acid phosphatase, alkaline phosphatase, urease, and arylsulfatase activities have been significantly correlated with redox potential (Uwituze et al. 2022). These enzymes work in biochemical processes of overall organic matter decomposition in the soil system, of which urease has an important role in the accessibility of N for plant growth in the N cycle (Abdel-Nasser et al. 2018). In our study, application of Abamectins and Tricuran -P increased urease activity in the soil, which is a positive impact of these particular treatments. Other studies (Micuti et al. 2017; Badawy et al. 2022; Uwituze et al. 2022) showed inconsistent effects of abamectin treatments on soil urease activity.

Phosphomonoesterase is the most active soil phosphatase hydrolyzes phosphomonoesters and in some cases, phosphodiesteres to release assimilable phosphates for microbes and plants, playing an important role in organic P cycling (Abdel-Nasser et al. 2018; Uwituze et al. 2022). In our study, soil application of both Abamectins increased alkaline and acidic phosphatase activity. This result is consistent with the findings of other studies, which showed increased alkaline phosphatase activity following nematicide application (Curtright and Tiemann 2021; Uwituze et al. 2022).

Beside the RKN population reduction associated with the various treatments, there were also positive effects on plant growth parameters. The highest stem fresh and dry weights, stem diameter, root length and volume were in chicken manure followed by Abamectin-CAP, Abamectin-IAP, Tricuran-P, and Rugby. In another study, Abamectin recorded an increase in plant shoot and root systems length and weight (Khalil 2013). Kankam et al. (2014) showed that poultry manure significantly reduced nematode populations and significantly increased carrot yield. Contrary to our report, application of a *Trichoderma* species and chicken manure was ranked third and fourth in the management of *M. incognita* and plant growth, respectively (Ramazani 2013). According to Li et al. (2021), incorporation of chicken manure with *T. harzianum* is acceptable for improving the effectiveness of RKN control and eggplant yield. In another study, a significant reduction in root galls and improved growth yield on soil amended with organic manure was associated with the addition of poultry litter in a cucumber establishment (Osman et al. 2018, 2023).

Many alternatives to the use of chemical pesticides have been evaluated for their effectiveness in suppressing nematode population and for environmental compatibility. Unfortunately, most of the environmentally benign chemical products that have recently introduced in the market are not that effective in controlling nematode damage to cucumbers (Daramola et al. 2013). This research showed that Abamectins (1.8% EC, and 2% EC), Tricuran-P, and chicken manure can be considered for incorporation into an integrated pest management strategy for replacing chemical nematicides to achieve sustainable agriculture.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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