



# Management of the root-knot nematode, *Meloidogyne javanica*, with non-chemical products on greenhouse-grown tomatoes

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Article Info.	Abstract
Article type:	In this research, the efficiency of Tricuran-P (Trichoderma harzianum) (8 kg/ha); Formaycin
Original article	Gold Px 21% (Pakgostar Com., Iran) for the non-chemical management of the Meloidogyne
Article history: Received 28 Feb. 2025 Received in revised form 22 May 2025 Accepted 26 May 2025 Available Online 30 May	<i>javanica</i> at the greenhouse level at three concentration rates (6, 8, and 10 L/ha); Nitroxin (5 L/ha); EM (5 L/ha); Fenamiphos (Nemacur® 400CE es) (15 gr/m2); and Phytohumic (10 L/ha) along with irrigation water was used in comparison with the infected and non- inoculated ones in a completely randomized design at three-time intervals of every three weeks, and the experiment was repeated twice. The results indicated that the fewest $J_2$ in the soil were found with Nemacur (5.5) and Tricuran-P (5.8), while the most were in Formycine 6 (21.1) and Nitroxin (20.0). Nemacur (13.4) and EM (14.5) had the lowest number of eggs
2025 <b>Keywords:</b> Fenamiphos, Nemacur, Nitroxin, Phytohumic, Tricuran-P.	and $J_2$ in the root, while Formycine 6 (1.96) had the highest. At the same time, the lowest number of root galls was in Formaycin 10 (3.5) and EM (3.7), and the highest ones were in Tricuran-P (0.5), Nitroxin (4.8), and Phytohumic (4.8), respectively. Nemacur also had the lowest number of egg masses (0.4). Increasing the concentration of Formaycin led to a decrease in the number of egg masses. Nemacur (110) and Tricuran-P (116), respectively, had the lowest $J_2$ reproduction factor in the soil. Cumulatively, as it was shown, Formaycin 10 L/ha can control the RKNs and can compete relatively compared to Fenamiphos (Nemacur) and other relevant bio-fertilizers in this research.
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#### Introduction

Tomato (*Solanum lycopersicum*), Solanaceae family is one of the important vegetable crops cultivated all over the world. Tomato is one of the oldest plants cultivated and known by the people of Peru, which was planted there in the fifth century BC and used as food (Seid et al., 2015). With the discovery of the new continent, tomatoes, potatoes and tobacco were brought to Spain. While in Europe, they believed that the tomato fruit is poisonous, so they planted it only as an ornamental

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plant. Italians were the first to realize the nutritional value of tomatoes and used them widely. In England (in the 18<sup>th</sup> century) it was used as a soup seasoning and flavoring. Canned tomatoes were prepared whole or sliced in metal cans in the state of Pennsylvania in 1847. This action led to the growth of tomato cultivation and consumption as much as possible. When the tomato was brought to France, it was called Love Apple. This plant was called tomatel in Mexico, which was later called tomato in Spain and Orange. In many parts of Europe, it is known as American tomato, which in the end was

called tomato with changes in all of Europe. Now tomato is known all over the world with the general name Tomato (Roth et al., 2021). Root knot nematodes (Meloidogyne spp.) with a wide range of hosts are one of the main problems limiting factor for the growth and production of agricultural crops, which annually cause irreparable damage to these products all over the world (Ali et al., 2019; Nasr Esfahani, 2007). This nematode genus is one of the most important plant parasitic nematodes, and is of high economic importance. The damage and losses of RKNs on agricultural products has been reported between 25 and 50% globally, depending on the species and population of RKNs, different plants reactions to nematode show various attacks (Mahdikhani et al., 2018; Oka et al., 2020). RKNs are more predominant in tropical and subtropical regions. RKNs have at least 90 species in the world, of which 4 species are more geographically distributed and economically important; including M. javanica, M. incognita, M. arenaria, M. hapla (Ali et al., 2019). M. arenaria, is more prevalent in temperate regions. M. hapla; in dry and wet areas and slightly acidic soils, M. incognita; causes more damage in hot and dry areas and *M. javanica* active in the soils with higher pH, and *M*. javanica causes disease and most aggressive in cucumber and tomato plants. RKN (Nasr Esfahani et al., 2023; Sadeghi et al., 2024a). In general, management of RKNs is considered difficult due to its wide host range, short generation and high fertility rate (Moatamedi et al., 2023). Several control measures have been used to manage RKNs including; cultural practices, bio-control, resistant sources and chemical methods of control (Qalavand et al., 2022; Qiao et al., 2012). The most common one being the use of chemical pesticides. For instance, Abemactin SC2% is available now, which is actually an insecticide, acaricide (miticide) that is obtained from the fermentation of Streptomyces, and it has been introduced in the market under the name of acaricide Austin. and the brand name of Vertimec® Pro (Nasr Esfahani et al., 2023; Gianfreda & Rao, 2010). Effective Micro-Organisms (EM) consists of common aerobic and anaerobic micro-organisms; photosynthetic bacteria, lactobacillus, Streptomyces, Actinomycetes, yeast, etc. will improve the structure of the soil, increase its fertility and radically improve biological diversity simultaneously (Liang et al., 2009). Moreover, EM suppress the soil-borne pathogens, fixes the nitrogen in soil and enhances nutrient uptake. At the same time accelerates the decomposition of organic waste, residues and composting, increases beneficial minerals in organic compound (El-Kelany et al., 2020). Thus, enhances the activities of indigenous microorganism and boosts the strength of plants and yield of the crops

(Wang et al., 2008). EM works by being dominant over other soil microbes. As a result, this encourages the bulk of the other microbes in the soil to follow them and in doing so suppress the activity of the smaller group of negative or opportunistic microbes (Nasr Esfahani et al., 2020). Effective microorganisms can help to improve and maintain the soil chemical and physical properties (Joshi et al., 2019). Due to the wide diversity within the species of Meloidogyne spp., these characteristics are approximate and can lead to doubt in the identification of Meloidogyne species. For this reason, molecular methods based on chromosomal DNA, mitochondrial DNA and ribosomal DNA are also used to identify the species and races of the Meloidogyne genus and other plant pathogens, in addition to morphological, morphometric, cytological, ecological and pathogenic characteristics on different hosts (Ghasemi et al., 2014; Naderi et al., 2020; Moatamedi et al., 2018; Peymani et al., 2022). Therefore, in this direction and according to the importance of the subject, in the implementation of this project, taking into account the problems and dilemmas of nematicides, in this research, the efficiency of Farmasin Gold PX 20% for the non-chemical management of the M. javanica at the greenhouse conditions in three concentration rates (6, 8 and 10 L/ha); Nitroxin (5 L/ha); EM (5 L/ha); Fenamiphos (Nemacur, Bayer) (15 g/m<sup>2</sup> ai); Tricuran-P (Trianum-P) (Trichderma harzianum T-22) (8 kg/ha); and Phytohumic (10 L/ha) on population parameters of M. javanica on tomato plant along with irrigation water were analyzed. In addition, determining the effect of treatments on growth indicators were taken into account.

### **Materials and Methods**

### **Reproduction of nematode species**

For the implementation of this experiment, a pure population of RKN, *M. javanica* available and approved by the Plant Protection Research department at Center for Agricultural Research, Isfahan, AREEO, Iran were used for the initial inoculum. The nematodes in the section were sufficiently multiplied by several cycles of transplanting on the susceptible tomato cultivar "Cloud Red" to infested soil in 2kgs sterile pots. In order to prepare the necessary inoculum, the infected roots were washed with a gentle stream of water and divided into 2 cm pieces and poured into a centrifuge 10,000 rpm for a min containing in 200 mL of distilled water, 5 mL of 5% sodium hypochlorite and mixed for 30 seconds. Then, the obtained suspension was washed twice with distilled water on a 325 mesh sieve. In order to determine the population density, the number of eggs and  $J_2$ , by mixing and homogenizing the nematode suspension, one mL of the suspension was poured into the counting container three times, and then the number of nematode  $J_2$  and eggs was counted using binoculars (Nasr Esfahani et al., 2020).

### Planting tomatoes in the greenhouse

Tomato seedlings were raised in the 96 cell- trays filled with the sterile culture medium composition of peatmoss and coco-peat (1:1) with tomato seeds, "Spadana" variety produced by Roxvan Netherlands Company, at 25-30 °C, RH 50% and light for 16 hrs. After watering, the trays were covered with black plastic for optimal germination for 5 days. The seeds germinated and continue to grow as the above conditions. During this period, the necessary care was taken to transfer the young tomato seedling into the two-kg pots to manage any pests and or diseases. After about four weeks, when the seedlings grown enough, they were transferred into the related pots. In this regard, the tomato seedlings were gently uprooted from the trays along with the soil around the roots and placed in the related pots accordingly. Necessary care was taken here so as not to damage the roots. Simultaneously with the transfer of seedlings, the pots were inoculated with 2000  $J_2$  of the nematode, M. javanica in the related soil pots.

In this formula, n is the number of evaluation times, i is the evaluation time, yi and ti are the average severity of the disease and time in the previous evaluation, yi + 1 and ti + 1 are the average severity of the disease and time in the current evaluation, respectively. The effectiveness of the treatments in reducing the disease was calculated using the following formula for the averages compared to the sprinkled control.

### **Experimenting in the greenhouse**

One week after transplanting, treatments were carried out in comparison with inoculated and non-inoculated controls. The bio-fertilizer as treatments including Nitroxin (*Azospirilium* and *Azotobacter rhizobacteria* in combination) 5 L/ha; Effective Micro-Organisms (EM) (EcoWarehouse Ltd, Ngātīmoti New Zealand) consists of common aerobic and anaerobic micro-organisms; photosynthetic bacteria, *lactobacillus, Streptomyces*, Actinomycetes, yeast, etc., 10 L/ha to the soil; solid biological fungicide *T. harzianum* T-22 (8 kg/ha Tricuran-P; Koppert, Bengaluru, India); Phytohumic (10 L/ha); and Formycine PX 20% (a disinfectant product, made of organic acids based on carboxylic acid) at three concentrate rates of 6, 8 and 10 L/ha compared to Fenamiphos (Nemacur® 400CE es) (15 gr/m<sup>2</sup>); AMVAC CHEMICAL CORPORATION USA. granule, at the recommended dosages in the greenhouse. All of these applications were repeated as a drench thrice at an interval of 3 weeks, in three replicates (three pots with 3 plant each) in the greenhouse condition. The experiment was repeated twice in the greenhouse. After 70 days passing of the experiment following the implication of the treatments, tomato seedling plants along with their roots were removed from every replicate soil and according to the level of nematode contamination and The mention includes: counting the number of root nodes (galls) and egg-mass, the number of eggs and J<sub>2</sub> in three g of roots and the number of J<sub>2</sub> in 200 g of soil, and then the final population and reproduction of the nematode were determined (Moatamedi et al., 2020).

### **Biomass growth parameters**

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 a measuring cylinder of 1000 cc filled with tape 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 hours (Qalavand et al., 2023; Sadeghi et al., 2024b).

### **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).

### Results

### Variance analysis of the nematode traits

According to the results of variance analysis of nematode traits, the year had no significant effect on the all evaluated traits except changes percent of  $J_2$  in the soil. There was a significant difference among the used treatments. There was also a significant interaction between year (Experiments repeated twice in two years) and treatment in all traits except gall and egg-mass number (Table 1).

### Variance analysis of the nematode traits on biomass parameters

According to the results of variance analysis of the nematode traits, the year had no significant effect on all evaluated traits except root volume. There was a significant difference among the used treatments. There was a significant interaction between year and treatment in all traits except stem length, leaf length, stem diameter, and root fresh weight (Table 2).

				Source of v	variation				
	Year (Y) [D.F=1]		Error [D.F=2]	Treatment (	T) [D.F=9]	YT [D	.F=9]	Error [D.F= 38]	- C.V%
Trait	Mean square (M.S)	P value	Mean square (M.S)	Mean square (M.S)	P value	Mean square (M.S)	P value	Mean squar e (M.S)	C. V 70
No. of J <sub>2</sub> in 1 gram soil	26.4 <sup>ns</sup>	0.1199	3.8	1372.8**	< 0.0001	183.9**	< 0.0001	7.6	23.0
No. of egg and $J_2$ in root	0.4 <sup>ns</sup>	0.7337	2.4	$239.7^{**}$	< 0.0001	$21.4^{**}$	< 0.0001	1.1	22.2
Gall No.	$0.002^{ns}$	0.7868	0.025	$18.844^{**}$	< 0.0001	0.733 <sup>ns</sup>	0.4765	0.753	24.6
Egg-mass No.	0.00 <sup>ns</sup>	1.0000	2.50	24.73**	< 0.0001	0.27 <sup>ns</sup>	0.9398	0.71	22.2
Reproduction factor of $J_2$ in soil	11.7 <sup>ns</sup>	0.1199	1.7	475.1**	< 0.0001	82.3**	< 0.0001	3.4	21.9
Reproduction factor of egg and $J_2$ in root	1.8 <sup>ns</sup>	0.7334	11.8	951.2**	< 0.0001	106.9**	< 0.0001	5.4	21.1
Changes % of J <sub>2</sub> in soil	667.6**	0.0013	0.9	47534.4**	< 0.0001	8213.0**	< 0.0001	67.5	15.7
Changes % of egg and $J_2$ in root	11.3 <sup>ns</sup>	0.1081	1.4	951.2**	< 0.0001	106.8**	< 0.0001	3.0	22.0

ns, \* and \*\* not significant and significant at p<0.05 and p<0.01, respectively.

Table 2. V	/ariance	analysis	in relation	to the	evaluated	traits	of biomass	parameters	effected	by ro	ot knot	nematode,
Meloidogyn	ıe javani	ca.										

	Source o	f variation							
	Year (Y)	Year (Y) [D.F=1]		Error [D.F=2] Treatment (T) [D.F		YT [D.F=	9]	Error [D.F=38]	- C.V%
	Mean square	P value	Mean square	Mean square	P value	Mean square	P value	Mean square	- C. V %
Trait	(M.S)		(M.S)	(M.S)		(M.S)		(M.S)	
Stem length	10.8 <sup>ns</sup>	0.2092	3.2	305.1**	< 0.0001	5.5 <sup>ns</sup>	0.9701	18.3	21.1
Root length	1.49 <sup>ns</sup>	0.1405	0.26	58.14**	< 0.0001	$25.59^{**}$	< 0.0001	1.25	10.3
Stem fresh weight	0.13 <sup>ns</sup>	0.7435	0.95	261.81**	< 0.0001	4.99**	0.0002	1.00	11.2
Stem dry weight	0.15 <sup>ns</sup>	0.1689	0.03	5.14**	< 0.0001	0.14*	0.0122	0.05	16.5
Leaf length	1.55 <sup>ns</sup>	0.5250	2.66	40.71**	< 0.0001	5.93 <sup>ns</sup>	0.6352	7.60	20.9
Root volume	$2.14^{*}$	0.0462	0.11	23.42**	< 0.0001	3.53**	0.0003	0.75	16.2
Stem diameter	0.016 <sup>ns</sup>	0.7699	0.144	2.699**	0.0063	0.797 <sup>ns</sup>	0.5118	0.858	18.6
Root diameter	2.59 <sup>ns</sup>	0.4585	3.12	7.55**	< 0.0001	$2.86^{*}$	0.0263	1.17	18.4
Root fresh weight	0.05 <sup>ns</sup>	0.8818	1.63	31.56**	< 0.0001	1.34 <sup>ns</sup>	0.3255	1.12	17.4
Root dry weight	$0.006^{ns}$	0.5296	0.011	$1.050^{**}$	< 0.0001	0.411**	< 0.0001	0.010	13.6

ns, \* and \*\* not significant and significant at p<0.05 and p<0.01, respectivel.

### Effect of the year on the assessed attributes of nematode parameters

In the comparison the effect of year on the assessed attributes, number of  $J_2$  in the soil, Reproductive factor of  $J_2$  in the soil, changes percent of  $J_2$  in the soil, and

root volume in the first year (12.6, 280, 180, and 5.84, respectively) significantly was higher than the second year (11.3, 251, 152, and 4.87, respectively) (Table 3) (Fig. 1 A-F).

Table 3. Effect of year on the assessed traits on root knot nematode, Meloidogyne javanica parameters.

	yea	ar
Trait	1 <sup>st</sup>	$2^{nd}$
No. of $J_2$ in 1 gram soil	12.6ª	11.3 <sup>b</sup>
Reproduction factor of $J_2$ in soil	280ª	251 <sup>b</sup>
Changes % of J <sub>2</sub> in soil	180 <sup>a</sup>	152 <sup>b</sup>
Root volume	5.84ª	4.87 <sup>b</sup>

Means in each row, having same letter, are not significantly different according to LSD test (p<0.05).



Fig. 1. Representing the state of the tomato plant in the transaction of treatments applied with root knot nematode.

### Effect of the treatment on the assessed attributes of nematode parameters

Comparing the effect of treatment on the number of  $J_2$ in the soil indicated that the highest number of  $J_2$  in the soil was relative to the infected control (29.5), and the lowest one to the non-inoculated control (0.0). Among the used treatments, the lowest number of  $J_2$  in the soil was of Nemacur (5.5), followed by Tricuran-P (5.8) and Formycine 10 (8.8), and the highest to Formycine 6 (21.1) and Nitroxin (20.0). Increasing the concentration of Formycine significantly led to reducing the number of  $J_2$  in the soil. There was no significant difference between Formycine 8 (14.2) with EM (15.9) and Phytohumic (13.0) (Table 4) (Fig. 1 A-F). The highest number of eggs and  $J_2$  in the root was in the infected control (100) and the lowest one to the non-inoculated control (0.0). Among the used treatments, the lowest number of eggs and  $J_2$  in the root was relative to Nemacur (13.4) and EM (14.5), and the highest one to Formycine 6 (96.1). Increasing the concentration of Formycine significantly led to reduce in the number of eggs and  $J_2$  in the root. There was no significant difference between Nitroxin (70.0) with Tricuran-P (84.0) and Phytohumic (58.4) (Table 4) (Fig. 1 A-F). The highest gall number was relative to the infected control (5.0) and Tricuran-P (5.0), and the lowest one to the non-inoculated control (0.0). Among the used treatments, the lowest gall number was relative to Formycine 10 (3.5) and EM (3.7), and the highest to Tricuran-P, Nitroxin, and Phytohumic (5.0, 4.8, and 4.8, respectively). Increasing the concentration of Formycine significantly led to reducing gall numbers. There was no significant difference among different concentrations of Formycine (Table 4) (Fig. 1 A-F). Comparing the effect of treatments on egg-mass number indicated that the highest egg-mass number was relative to the infected control, Formycine 6 and 8, and Phytohumic (5.0), and the lowest one to the non-inoculated control (0.0)significantly. Among the used treatments, the lowest egg-mass number was relative to Nemacur (4.0). Increasing the concentration of Formycine significantly led to reduce egg-mass number significantly (Table 4). Comparison the effect of treatments on the reproductive factor of J<sub>2</sub> in the soil indicated that the highest reproductive factor of  $J_2$  in the soil was relative to the infected control (590), and the lowest one to Nemacur (110) and Tricuran-P (116) significantly. Increasing the concentration of Formycine significantly led a reduced reproductive factors of  $J_2$  in the soil. There was no significant difference between different Formycine 8 (284) with Phytohumic (260), Nitroxin (350), and EM (318) (Table 4) (Fig. 1 A-F). The effect of treatment on reproductive factor of eggs and J<sub>2</sub> in the root indicated that the highest reproductive factor of eggs and J<sub>2</sub> was relative to the infected control (667), Formycine 6 (640), and Nitroxin (643), and the lowest one to EM (97) and Nemacur (90). Increasing the concentration of Formycine significantly led to reduce reproductive factor of eggs and  $J_2$  in the root (Table 4). Whereas, change percent of J<sub>2</sub> in the soil indicated that the highest change percent of  $J_2$  in the soil was relative to the infected control and Nitroxin (490% and 478% increase, respectively), and the lowest one to Nemacur and Tricuran-P (10% and 16% increase, respectively). Increasing the concentration of Formycine significantly led to a decrease in change percent of  $J_2$  in the soil (Table 4) (Fig. 1 A-F). The change percent of eggs and  $J_2$  in root indicated that the highest change percent of eggs and J<sub>2</sub> in the root was relative to the infected control and Formycine 6 (567% and 541% increase, respectively), and the lowest one to EM and Nemacur (3.3% and 10.5% decrease, respectively). Increasing the concentration of Formycine significantly led to a reduced change in percent of eggs and J<sub>2</sub> in the root (Table 4). There was no significant difference between Nitroxin (470) and Tricuran-P (460) (Table 4) (Fig. 1 A-F).

, Treatment	Trait	No. of J <sub>2</sub> in 1 gram soil	No. of egg and J <sub>2</sub> in 3 g. root	Gall No.	Egg- mass No.	Reproduction factor of J <sub>2</sub> in soil	Reproduction factor of egg and $J_2$ in root	Changes % of $J_2$ in soil	Changes % of egg and J <sub>2</sub> in root
Inoculated contr	ol	29.5ª	100.0 <sup>a</sup>	5.0ª	5.0ª	590ª	667ª	490 <sup>a</sup>	567ª
Non-inoculated control		$0.0^{\mathrm{g}}$	$0.0^{g}$	0.0°	0.0°	-	-	-	-
Formycine 6		21.1 <sup>b</sup>	96.1ª	4.5 <sup>ab</sup>	5.0ª	422 <sup>b</sup>	640 <sup>a</sup>	322 <sup>b</sup>	541ª
Formycine 8		14.2 <sup>cd</sup>	49.2 <sup>d</sup>	4.0 <sup>ab</sup>	5.0 <sup>a</sup>	284 <sup>cd</sup>	328 <sup>d</sup>	185 <sup>d</sup>	228 <sup>d</sup>
Formycine 10		8.8 <sup>e</sup>	37.3 <sup>e</sup>	3.5 <sup>b</sup>	4.5 <sup>ab</sup>	177 <sup>e</sup>	248e	76 <sup>f</sup>	148e
Nitroxin		20.0 <sup>b</sup>	70.0 <sup>bc</sup>	4.8 <sup>a</sup>	4.8 <sup>a</sup>	350 <sup>bc</sup>	643ª	478 <sup>a</sup>	470 <sup>b</sup>
$\mathrm{EM}^*$		15.9°	14.5 <sup>f</sup>	3.7 <sup>b</sup>	4.5 <sup>ab</sup>	318°	97 <sup>f</sup>	218°	-3.3 <sup>fg</sup>
Phenamiphos (Nemacur)		5.5 <sup>f</sup>	13.4 <sup>f</sup>	4.2 <sup>ab</sup>	4.0 <sup>b</sup>	110 <sup>f</sup>	90 <sup>f</sup>	10 <sup>g</sup>	-10.5 <sup>g</sup>
Tricuran-P		5.8 <sup>f</sup>	84.0 <sup>b</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	116 <sup>f</sup>	560 <sup>b</sup>	16 <sup>g</sup>	460 <sup>b</sup>
Phytohumic		13.0 <sup>d</sup>	58.4°	4.8 <sup>a</sup>	5.0ª	260 <sup>d</sup>	390°	160 <sup>e</sup>	290°

Table 4. Effect of treatments on the assessed traits on nematode, Meloidogyne javani	ca parameters.
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Means in each column, having same letter, are not significantly different according to LSD test (p<0.05).

## Effect of the treatment on the assessed attributes of biomass parameters

The highest stem length was observed in the noninoculated control and Nemacur (33.4 and 32.8 cm, respectively), and the lowest one in Tricuran-P and the infected control (10.7 and 13.4 cm, respectively). By increasing the concentration of Formycine, stem length increased. There was no significant difference between different concentrations of Formycine 10, Nitroxin, EM, and Phytohumic. In addition, the infected control had no significant difference with Formycine 6, Nitroxin, Tricuran-P, and Phytohumic (Table 5) (Fig. 1 A-F). The highest root length was in the non-inoculated control (19.3 cm). By increasing the concentration of Formycine, the root length increased. There was no significant difference between the infected control and Formycine 6. Formycine 8 and 10 and EM had no significant difference together. In addition, there was also no significant difference between Nitroxin and Phytohumic (Table 5) (Fig. 1 A-F). The highest stem fresh weight was in the non-inoculated control and Nemacur (21.02 and 20.70 g, respectively), and the lowest one in the infected control (1.72 and 1.42 g, respectively). By increasing the concentration of Formycine, stem fresh weight increased. There was no significant difference among Formycine 6, Nitroxin, and Phytohumic. In addition, Formycine 8 did not show significant difference with Formycine 10 and EM (Table 5). Whereas, the highest stem dry weight was observed in the non-inoculated control (3.24 g), and the lowest one in Tricuran-P, the infected control, and Formycine 6 (0.52, 0.58, and 0.76 g, respectively). By increasing the concentration of Formycine, stem dry weight increased. Formycine 6 had no significant difference with Nitroxin and EM. The highest leaf length was observed in the non-inoculated control and Nemacur (17.3 cm), and the lowest the infected control (9.3 and 10.5 cm, respectively). By increasing the concentration of Formycine, leaf length increased. The infected control had no significant difference with Formycine 6, Nitroxin, EM, and Phytohumic. The highest root

volume was in the non-inoculated control (9.20 cm<sup>3</sup>). By increasing the concentration of Formycine, root volume increased. The non-inoculated control had no significant difference with Formycine 10. Formycine 6 had no significant difference with Formycine 8, Nitroxin, EM, Nemacur, and Phytohumic. The highest stem diameter was observed in the non-inoculated control (6.06 mm), and the lowest in the infected control (3.67 and 3.93 mm). By increasing the concentration of stem diameter increased, Formycine, but this enhancement was not significant. There was no significant difference among Formycine, Nitroxin, EM, Nemacur, and Phytohumic. Whereas, the highest root diameter was observed in the non-inoculated control (8.79 mm) and the lowest one in Tricuran-P and the infected control (4.17 and 3.83 mm). By increasing the concentration of Formycine, the root diameter increased. There was no significant difference among different concentrations of Formycine, EM, and Nemacur. The highest root fresh weight was in the noninoculated control (10.40 g). By increasing the concentration of Formycine, root fresh weight increased. There was no significant difference among Formycine 6 and 8, Nitroxin, EM, and Phytohumic (Table 5). Whereas, the highest root dry weight was observed in the non-inoculated control (2.51 g). By increasing the concentration of Formycine, the root dry weight increased. There was no significant difference among Formycine 6, Nitroxin, EM, and Phytohumic (Fig. 1 A-F).

Table 5. Effect of treatments on the assessed traits of biomass para	meters.
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Trait Treatment	Stem length (cm)	Root length (cm)	Stem fresh weight (g)	Stem dry weight (g)	Leaf length (cm)	Root volume (ml <sup>3</sup> )	Stem diameter (cm)	Root diameter (cm)	Root fresh weight (g)	Root dry weight (g)
Inoculated control	13.4 <sup>de</sup>	9.1e	1.42 <sup>h</sup>	0.58 <sup>g</sup>	10.5 <sup>de</sup>	3.65 <sup>d</sup>	3.93 <sup>cd</sup>	3.83 <sup>e</sup>	3.78 <sup>e</sup>	0.38 <sup>f</sup>
Non-inoculated control	33.4 <sup>a</sup>	19.3ª	21.02 <sup>a</sup>	3.24 <sup>a</sup>	17.3 <sup>a</sup>	9.02 <sup>a</sup>	6.06 <sup>a</sup>	8.79 <sup>a</sup>	10.40 <sup>a</sup>	2.51 <sup>a</sup>
Formycine 6	16.0 <sup>cd</sup>	9.0 <sup>e</sup>	4.93 <sup>g</sup>	0.76 <sup>fg</sup>	11.2 <sup>cde</sup>	6.17 <sup>bc</sup>	5.00 <sup>bc</sup>	6.33 <sup>bcd</sup>	6.48 <sup>d</sup>	0.72 <sup>e</sup>
Formycine 8	19.8 <sup>bc</sup>	12.6 <sup>e</sup>	7.80 <sup>ef</sup>	1.02 <sup>e</sup>	14.0 <sup>bc</sup>	7.00 <sup>b</sup>	5.33 <sup>b</sup>	6.33 <sup>bcd</sup>	7.55 <sup>cd</sup>	0.91 <sup>d</sup>
Formycine 10	20.0 <sup>bc</sup>	13.4 <sup>c</sup>	8.85 <sup>b</sup>	1.13 <sup>e</sup>	14.8 <sup>ab</sup>	8.20 <sup>a</sup>	5.33 <sup>b</sup>	7.33 <sup>b</sup>	9.36 <sup>b</sup>	1.10 <sup>c</sup>
Nitroxin	15.8 <sup>cd</sup>	10.8 <sup>d</sup>	5.85 <sup>g</sup>	0.87 <sup>ef</sup>	12.8 <sup>bcd</sup>	6.02 <sup>bc</sup>	5.00 <sup>bc</sup>	5.67 <sup>cd</sup>	6.55 <sup>d</sup>	0.65 <sup>e</sup>
$\mathrm{EM}^*$	19.2°	12.9°	7.22 <sup>f</sup>	0.92 <sup>ef</sup>	$11.0^{cde}$	5.35°	5.33 <sup>b</sup>	6.67 <sup>bcd</sup>	6.48 <sup>d</sup>	0.73 <sup>e</sup>
Phenamiphos (Nemacur)	32.8ª	16.4 <sup>b</sup>	20.70 <sup>a</sup>	3.14 <sup>b</sup>	17.3ª	7.00 <sup>b</sup>	5.83 <sup>b</sup>	6.17 <sup>bcd</sup>	8.34 <sup>bc</sup>	1.66 <sup>b</sup>
Tricuran-P	10.7 <sup>e</sup>	5.4 <sup>f</sup>	1.72 <sup>h</sup>	0.52 <sup>g</sup>	9.3°	2.83 <sup>e</sup>	3.67 <sup>d</sup>	4.17 <sup>e</sup>	2.28 <sup>f</sup>	0.20 <sup>g</sup>
Phytohumic	15.4 <sup>cd</sup>	10.8 <sup>d</sup>	5.80 <sup>g</sup>	1.34 <sup>d</sup>	12.5 <sup>bcd</sup>	5.98 <sup>bc</sup>	5.04 <sup>bc</sup>	5.85°	6.55 <sup>d</sup>	0.61 <sup>e</sup>

Means in each column, having same letter, are not significantly different according to LSD test (p<0.05).

### Comparison the interaction between year and treatment on the nematode parameters

Interaction between year and treatment on the number of J<sub>2</sub> in the soil demonstrated that the highest number of  $J_2$  in the soil was relative to the infected control in both years (30.0 and 29.0, respectively), with no significant difference with Formycine 6 (23.1) and Nitroxin (22.0) in the second year. The lowest number of  $J_2$  in the soil was relative to the non-inoculated control in both years (0.0). Among the applied treatments, the lowest number of J<sub>2</sub> in the soil was relative to Nemacur and Tricuran-P in both years and Formycine 10 in the second year. In both years, increasing the concentration of Formycine caused reduction in the number of  $J_2$  in the soil. In application of Formycine 10, Nitroxin, and EM, different results were obtained in both years in viewpoint of the number of  $J_2$  in the soil, but in the other treatments, the results of the first and second years were similar (Table 6). Interaction between year and treatment on the number of eggs and J<sub>2</sub> in root showed that the highest number of eggs and J<sub>2</sub> in root was relative to Formycine 6 in the first year (111.8), and the infected control in both years (100.0) and Tricuran-P in the second year (99.3) were in the next class. The lowest number of eggs and  $J_2$  in the root was relative to the non-inoculated control in both years (0.0). Among the applied treatments, the lowest number of eggs and J<sub>2</sub> in the root was relative to EM and Nemacur in the first year (12.2 and 9.1, respectively), and second year (16.8 and 17.7, respectively). In both years, increasing the concentration of Formycine caused to significantly reduction of the number of eggs and  $J_2$  in the root. In application of Formycine 6, Tricuran-P. and Phytohumic, different results were obtained in both years in viewpoint of the number of eggs and  $J_2$  in the root, but in the other treatments, the results of the first and second years were similar (Table 6). Interaction between year and treatment on gall number showed that the highest gall number was relative to the infected control in both years, Formycine 6 in the first year, Nitroxin in both years, and Phytohumic in the first years (5.0) with a significant difference to the non-inoculated control in both years, Formycine 10 in the first year, and EM in the second year, but they had no significant difference with other treatments in both years. The lowest gall number was relative to the non-inoculated control in both years (0.0). Among the used treatments, the lowest gall number was relative to Formycine 10 in the first year (3), and EM in the second year (3.3). In the first year, increasing the concentration of Formycine caused a significant reduction of gall numbers. In application of all treatments, the results of the first and second years were similar (Table 6). Whereas, interaction between year and treatment on egg-mass number showed that there was not any significant difference among the used treatments and except in the non-inoculated control that did not have any egg-mass, the number of egg-mass in all treatments statistically was similar. Interaction between year and treatment on reproductive factor of  $J_2$  in the soil showed that the highest reproductive factor of J<sub>2</sub> in the soil was relative to the infected control in both years (600 and 580, respectively), and the lowest reproductive factor of  $J_2$  in the soil was relative to Nemacur and Tricuran-P in both years and Formycine 10 in the second year. In both years, increasing the concentration of Formycine caused a significant reduction of reproductive factor of J<sub>2</sub> in the soil. In application of Formycine 8 and 10, Nitroxin, and EM, different results were obtained in both years in viewpoint of reproductive factor of  $J_2$  in the soil, but in the other treatments, the results of the first and second years were similar (Table 6). Whereas, interaction between year and treatment on reproductive factor of eggs and  $J_2$  in the root showed that the highest reproductive factor of eggs and J<sub>2</sub> in the root was relative to Formycine 6 in the first year (745), and the infected control in both years (667), Nitroxin in both years (608 and 678, respectively), and Tricuran-P in the second year (662) were in the next class. The lowest reproductive factor of eggs and J<sub>2</sub> in the root was relative to EM and Nemacur in the first year (82 and 61, respectively) and second year (111 and 118, respectively). In both years, increasing the concentration of Formycine caused a significant reduction of reproductive factor of eggs and  $J_2$  in the root. In application of Formycine 6, Tricuran-P and Phytohumic, different results were obtained in both years in viewpoint of reproductive factor of eggs and J<sub>2</sub> in the root, but in the other treatments, the results of the first and second years were similar. Interaction between year and treatment on change percent of J<sub>2</sub> in the soil showed that the highest change percent of J<sub>2</sub> in the soil was relative to the infected control and Nitroxin in the first year (500 and 467%, respectively) and second year (480 and 489%, respectively), and the lowest change

percent of  $J_2$  in the soil was relative to Nemacur in the first and second years (without change and 21%, respectively). Change percent of  $J_2$  number in the soil in application of Tricuran-P in the first year decreased (-7.6%). In both years, increasing the concentration of Formycine caused a significant reduction of change percent of  $J_2$  in the soil. In application of Formycine 10, EM, and Tricuran-P, different results were obtained in both years in viewpoint of change percent of  $J_2$  in the soil, but in the other treatments, the results of the first and second years were similar (Table 6). Whereas, interaction between year and treatment on change percent of eggs and  $J_2$  in the root showed that the highest change of eggs and  $J_2$  in the root was relative to Formycine 6 in the first year (645) and the infected control in both years (567) was in the next class. The lowest change percent of eggs and J<sub>2</sub> in the root was relative to EM and Nemacur in both years. Change percent of eggs and J<sub>2</sub> in root in application of EM and Nemacur in the first year decreased (-18 and -39%, respectively). In both years, increasing the concentration of Formycine caused a significant reduction of change percent of eggs and J<sub>2</sub> in root. In application of Formycine 6, Nemacur, Tricuran-P, and Phytohumic, different results were obtained in both years in viewpoint of change percent of eggs and  $J_2$  in the root, but in the other treatments, the results of the first and second years were similar (Table 6).

		No.	No. of						Change
	Trait	of $J_2$	egg	Gall	Egg-	Reproduction	Reproduction	Changes	% of
Year x	Treatment	in 1	and $J_2$	No.	mass	factor of $J_2$ in	factor of egg	% of $J_2$	egg an
I cui A	Treatment	gram	in root	110.	No.	soil	and $J_2$ in root	in soil	J <sub>2</sub> in
		soil							root
	Inoculated control	30.0ª	100.0 <sup>b</sup>	5.0ª	5.0ª	600 <b>a</b>	667 <sup>b</sup>	500.0ª	567 <sup>b</sup>
	Non-inoculated control	$0.0^{\mathbf{f}}$	0.0 <sup>j</sup>	$0.0^{d}$	0.0 <sup>b</sup>	-	-	-	-
	Formycine 6	19.1 <sup>b</sup>	111.8ª	5.0ª	5.0ª	400 <sup>b</sup>	745ª	300.0 <sup>b</sup>	645ª
	Formycine 8	12.2 <sup>d</sup>	47.2 <sup>e</sup>	4.0 <sup>abc</sup>	5.0 <sup>a</sup>	264 <sup>d</sup>	318 <sup>e</sup>	175.0°	218 <sup>e</sup>
$1^{st}$	Formycine 10	11.7 <sup>d</sup>	36.3 <sup>f</sup>	3.0°	4.0 <sup>a</sup>	233 <sup>d</sup>	238 <sup>f</sup>	133.0 <sup>e</sup>	138 <sup>g</sup>
year	Nitroxin	18.0°	67.0 <sup>d</sup>	4.7ª	4.6 <sup>a</sup>	328°	608 <sup>b</sup>	467.0ª	456°
•	$\mathrm{EM}^*$	12.6 <sup>d</sup>	12.2 <sup>hi</sup>	$4.0^{\text{abc}}$	4.0 <sup>a</sup>	251 <sup>d</sup>	82 <sup>gh</sup>	151.1 <sup>de</sup>	-18 <sup>ij</sup>
	Phenamiphos (Nemacur)	5.0 <sup>e</sup>	9.1 <sup>hij</sup>	4.0 <sup>abc</sup>	4.0 <sup>a</sup>	100 <sup>e</sup>	61 <sup>h</sup>	0.0 <sup>gh</sup>	-39j
	Tricuran-P	4.6 <sup>e</sup>	68.7 <sup>d</sup>	5.0ª	5.0 <sup>a</sup>	92 <sup>e</sup>	458 <sup>d</sup>	-7.6 <sup>h</sup>	358 <sup>d</sup>
	Phytohumic	13.0 <sup>d</sup>	73.2 <sup>cd</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	260 <sup>d</sup>	488 <sup>cd</sup>	160.0 <sup>d</sup>	388°
	Inoculated control	29.0ª	100.0 <sup>b</sup>	5.0ª	5.0ª	580ª	667 <sup>b</sup>	480.0ª	567 <sup>b</sup>
	Non-inoculated control	$0.0^{\mathbf{f}}$	0.0 <sup>j</sup>	$0.0^{d}$	0.0 <sup>b</sup>	-	-	-	-
	Formycine 6	23.1 <sup>ab</sup>	80.3°	4.0 <sup>abc</sup>	5.0ª	444 <sup>b</sup>	536°	344.0 <sup>b</sup>	436
	Formycine 8	16.2 <sup>cd</sup>	51.2 <sup>e</sup>	$4.0^{\text{abc}}$	5.0ª	304°	338 <sup>e</sup>	195.0°	238e
$2^{nd}$	Formycine 10	6.0 <sup>e</sup>	38.3 <sup>f</sup>	4.0 <sup>abc</sup>	5.0ª	120e	258 <sup>f</sup>	20.0 <sup>fg</sup>	158 <sup>g</sup>
year	Nitroxin	22.0 <sup>ab</sup>	73.0 <sup>cd</sup>	4.9ª	5.0ª	372 <sup>b</sup>	678 <sup>b</sup>	489.0ª	484°
-	$\mathrm{EM}^*$	19.2 <sup>b</sup>	16.8 <sup>ghi</sup>	3.3 <sup>bc</sup>	5.0ª	384 <sup>b</sup>	111 <sup>gh</sup>	284.0 <sup>b</sup>	12 <sup>hij</sup>
	Phenamiphos (Nemacur)	6.0 <sup>e</sup>	17.7 <sup>gh</sup>	4.3 <sup>abc</sup>	4.0 <sup>a</sup>	120 <sup>e</sup>	118 <sup>gh</sup>	20.0 <sup>fg</sup>	18 <sup>hi</sup>
	Tricuran-P	7.0 <sup>e</sup>	99.3 <sup>b</sup>	5.0ª	5.0ª	140 <sup>e</sup>	662 <sup>b</sup>	40.0 <sup>f</sup>	562 <sup>b</sup>
	Phytohumic	13.0 <sup>d</sup>	43.7 <sup>e</sup>	4.7 <sup>ab</sup>	5.0ª	260 <sup>d</sup>	291 <sup>e</sup>	160.0 <sup>d</sup>	191 <sup>f</sup>

Table 6. Interaction of year and treatment on the assessed traits on nematode, Mela	oidogyne javanica parameters.
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Means in each column, having same letter, are not significantly different according to LSD test (p<0.05). \* (EM) consists of common aerobic and anaerobic micro-organisms; photosynthetic bacteria, lactobacillus, Streptomyces, Actinomycetes, yeast, etc.

### Comparison the interaction between year and treatment on the biomass parameters

Interaction between year and treatment on stem length demonstrated that the highest stem length was relative to the non-inoculated control and Nemacur in both years (33.4, 32.7, 33.3, and 33.0 cm, respectively). The lowest stem length relative to the infected control in the second year (12.0 cm), had no significant difference with the infected control in the first year, Formycine 6 in both years, Nitroxin in both years, and Phytohumic in both years. In both years, increasing the concentration of Formycine caused tem length to increase. In application of all treatments, the results of the first and second years were similar (Table 7). Whereas, interaction between the year and treatment on the root length demonstrated that the highest root length was relative to the noninoculated control in both years (19.3 cm), Nemacur in the first year (18.7 cm), and Formycine 10 in the second year (17.0 cm). The lowest root length was relative to Tricuran-P in the second year (7.7 cm) had no significant difference with the infected control in the first year (9.0 cm), Formycine 6 in the second year (8.7 cm), and Tricuran-P in the second year (7.7 cm). In both years, increasing the concentration of Formycine caused increase in the root length. In application of Formycine 8 and 10, EM, Nemacur, and Tricuran-P, different results were obtained in both years in viewpoint of root length, but in the other treatments, the results of the first and second years were similar. Interaction between year and treatment on stem fresh weight demonstrated that the highest stem fresh weight was relative to the noninoculated control in the first and second years (21.40 and 20.65 g, respectively) and Nemacur in both years (20.20 and 21.20 g, respectively) and the lowest stem fresh weight was relative to the infected control in both years (1.90 and 0.93 g, respectively). In both years, increasing the concentration of Formycine caused enhance in stem fresh weight. In application of Formycine 8 and Tricuran-P, different results were obtained in both years in viewpoint of stem fresh weight, but in the other treatments, the results of the first and second years were similar (Table 7). Whereas, interaction between year and treatment on stem dry weight demonstrated that the highest stem dry weight was relative to the non-inoculated control in the first and second years (3.30 and 3.19 g, respectively) and Nemacur in both years (3.15 and 3.13 g, respectively) and the lowest stem dry weight was relative to the infected control in the second year (0.48 g) had no significant difference with the infected control in the first year (0.68 g), Formycine 6 in the first year (0.65 g), Nitroxin in the second year (0.80 g), and Phytohumic in the second year (0.79 g). In both years, increasing the concentration of Formycine caused enhance in stem dry weight. In application of all treatments, the results of the first and second years were similar. Interaction between year and treatment on leaf length demonstrated that the highest leaf length was relative to the non-inoculated control and Nemacur in the first and second years (17.4, 17.3, 17.2, and 17.3 cm, respectively) that had significantly difference only with the infected control in both years, Formycine 6 in both years, Nitroxin in the second year, EM in both years, and Phytohumic in the second years. The lowest leaf length was relative to Formycine 6 in the first year (9.7 cm), had no significant difference with the infected control in both years, Nitroxin in the second year, EM in both years, and Phytohumic in the second year. In both years, increasing the concentration of Formycine caused enhance in leaf length. In application of all treatments, the results of the first and second years were similar Interaction between year and treatment on root volume showed that the highest root volume was relative to the non-inoculated control in both years (9.03 and 9.02 cm<sup>3</sup>, respectively), and Formycine 10 and Nemacur in the first year (8.7 and 9.0 cm<sup>3</sup>, respectively) that had no significant difference with Formycine 8 in the first year and Formycine 10 in the second year. The lowest root volume was relative to the infected control in the second year (3.30 cm<sup>3</sup>). In both years, increasing the concentration of Formycine caused to enhance root volume. In application of Formycine 6 and 8, and Nemacur, different results were obtained in both years in viewpoint of root volume, but in the other treatments, the results of the first and second years were similar. Interaction between year and treatment on stem diameter demonstrated that the highest stem diameter was relative to the non-inoculated control in both years (6.07 and 6.05 mm, respectively), and Nemacur in the second year (6.00 mm) had significantly difference only with the infected control in both years, and Nitroxin and Phytohumic in the second year. The lowest stem diameter was relative to the infected control in both years (3.87 and 4.00 mm, respectively). In both years, increasing the concentration of Formycine caused enhance in stem diameter. In application of all treatments, the results of the first and second years were similar. Whereas, interaction between year and treatment on the root diameter showed that the highest root diameter was relative to the non-inoculated control in both years (8.74 and 8.84 mm, respectively) and Formycine 10 in the second year (8.67 mm) that had no significant difference with EM and Tricuran-P in the first year and Formycine 8 in the second year (7.00 mm). The lowest root diameter was relative to the infected control in both years (4.33 and 3.33 mm, respectively). In both years, increasing the concentration of Formycine caused enhance in the root diameter. In the application of Formycine 10, different results were obtained in both years in viewpoint of root diameter, but in the other treatments, the results of the first and second years were similar. Interaction between year and treatment on the root fresh weight showed that the highest root fresh weight was relative to the noninoculated control in both years (10.41 and 10.38 g, respectively), and Formycine 10 in the first year (10.30 g) had no significant difference with Formycine 8 and Nemacur in the first year (8.80 and 9.17 g, respectively). The lowest root fresh weight was relative to the infected control in both years. In both years, increasing the concentration of Formycine caused to enhance the root fresh weight. In application of Formycine 8 and 10, different results were obtained in both years in viewpoint of root fresh weight, but in the other treatments, the results of the first and second years were similar. Whereas, interaction between year and treatment on the root dry weight showed that the highest root dry weight was relative to the non-inoculated control in both years (2.58 and 2.44 g, respectively), and Nemacur in the second year (2.38 g) with a significant difference with all treatments in both years. The lowest root dry weight was relative to the infected control in both years. In both years, increasing the concentration of Formycine caused to enhance in the root dry weight. In application of Formycine 8 and 10, EM, and Nemacur, different results were obtained in both years in viewpoint of root dry weight, but in the other treatments, the results of the first and second years were similar (Table 7).

Trait Year × Treatment		Stem length (cm)	Root length (cm)	Stem fresh weight (g)	Stem dry weight (g)	Leaf length (cm)	Root volume (ml <sup>3</sup> )	Stem diameter (cm)	Root diameter(cm)	Root fresh weight (g)	Root dry weight (g)
1 <sup>st</sup> year	Inoculated control	14.8 <sup>ef</sup>	9.0 <sup>ef</sup>	1.90 <sup>jk</sup>	0.68 <sup>ef</sup>	11.0 <sup>def</sup>	4.00 <sup>fgh</sup>	3.87°	4.33 <sup>cd</sup>	4.07 <sup>fg</sup>	0.44 <sup>f</sup>
	Non- inoculated control	33.4ª	19.3ª	21.40ª	3.30ª	17.4ª	9.03ª	6.07ª	8.74ª	10.41ª	2.58ª
	Formycine 6	15.0 <sup>ef</sup>	9.3 <sup>ef</sup>	4.47 <sup>hi</sup>	0.65 <sup>ef</sup>	9.7 <sup>f</sup>	7.00 <sup>bc</sup>	4.67 <sup>abc</sup>	6.00 <sup>bc</sup>	6.70 <sup>de</sup>	0.75 <sup>de</sup>
	Formycine 8	19.7 <sup>cde</sup>	9.7 <sup>de</sup>	7.70 <sup>ef</sup>	0.95 <sup>de</sup>	13.3 <sup>a-f</sup>	8.00 <sup>ab</sup>	5.67 <sup>ab</sup>	6.33 <sup>b</sup>	8.80 <sup>abc</sup>	1.16 <sup>b</sup>
	Formycine 10	20.3 <sup>cde</sup>	10.3 <sup>cde</sup>	9.10 <sup>de</sup>	1.12 <sup>cd</sup>	14.3 <sup>a-e</sup>	8.70 <sup>a</sup>	5.67 <sup>ab</sup>	6.67 <sup>b</sup>	10.30 <sup>a</sup>	1.24 <sup>b</sup>
	Nitroxin	18.0 <sup>def</sup>	11.7°	6.00 <sup>gh</sup>	0.94 <sup>de</sup>	13.8 <sup>a-f</sup>	6.70 <sup>bcd</sup>	5.67 <sup>ab</sup>	5.33 <sup>bc</sup>	6.97 <sup>de</sup>	0.67 <sup>de</sup>
	$\mathrm{EM}^*$	19.3 <sup>cde</sup>	14.3 <sup>b</sup>	6.43 <sup>fg</sup>	0.94 <sup>de</sup>	$11.0^{def}$	6.00 <sup>cde</sup>	5.67 <sup>ab</sup>	7.00 <sup>ab</sup>	7.17 <sup>cde</sup>	0.83 <sup>cd</sup>
	Phenamiphos (Nemacur)	32.7ª	18.7ª	20.20ª	3.15ª	17.3ª	9.00 <sup>a</sup>	5.67 <sup>ab</sup>	6.33 <sup>b</sup>	9.17 <sup>ab</sup>	0.94°
	Tricuran-P	25.7 <sup>bc</sup>	14.0 <sup>b</sup>	13.10 <sup>c</sup>	1.60 <sup>b</sup>	16.0 <sup>abc</sup>	$4.67^{efgh}$	5.00 <sup>abc</sup>	7.00 <sup>ab</sup>	6.73 <sup>de</sup>	0.66 <sup>e</sup>
	Phytohumic	17.3 <sup>def</sup>	11.6 <sup>c</sup>	6.02 <sup>gh</sup>	0.91 <sup>de</sup>	13.4 <sup>a-f</sup>	6.73 <sup>bcd</sup>	5.64 <sup>ab</sup>	5.13 <sup>bc</sup>	6.87 <sup>de</sup>	0.57 <sup>de</sup>
2 <sup>nd</sup> year	Inoculated control Non-	12.0 <sup>f</sup>	9.2 <sup>ef</sup>	0.93 <sup>k</sup>	0.48 <sup>g</sup>	10.0 <sup>ef</sup>	3.30 <sup>hi</sup>	4.00°	3.33 <sup>d</sup>	3.50 <sup>g</sup>	0.31 <sup>f</sup>
	inoculated control	33.3ª	19.3ª	20.65ª	3.19 <sup>a</sup>	17.2ª	9.02ª	6.05ª	8.84ª	10.38ª	2.44ª
	Formycine 6	17.0 <sup>def</sup>	8.7 <sup>ef</sup>	5.40 <sup>gh</sup>	0.86 <sup>de</sup>	12.8 <sup>b-f</sup>	5.33 <sup>def</sup>	5.00 <sup>abc</sup>	6.33 <sup>b</sup>	6.27 <sup>e</sup>	0.65 <sup>e</sup>
	Formycine 8	19.7 <sup>cde</sup>	15.0 <sup>b</sup>	5.40 <sup>gh</sup>	1.10 <sup>cd</sup>	13.7 <sup>a-f</sup>	6.00 <sup>cde</sup>	5.00 <sup>abc</sup>	7.00 <sup>ab</sup>	6.30 <sup>e</sup>	0.70 <sup>de</sup>
	Formycine 10	20.0 <sup>cde</sup>	17.0 <sup>a</sup>	8.60 <sup>e</sup>	1.13 <sup>cd</sup>	16.3 <sup>ab</sup>	7.70 <sup>ab</sup>	5.33 <sup>abc</sup>	8.67ª	8.43 <sup>bcd</sup>	0.96°
	Nitroxin	13.7 <sup>ef</sup>	$10.0^{cde}$	5.70 <sup>gh</sup>	$0.80^{def}$	11.7 <sup>c-f</sup>	5.33 <sup>def</sup>	4.33 <sup>bc</sup>	6.00 <sup>bc</sup>	6.13 <sup>e</sup>	0.64 <sup>e</sup>
	$\mathrm{EM}^*$	19.0 <sup>cde</sup>	11.5 <sup>cd</sup>	8.00 <sup>ef</sup>	0.89 <sup>de</sup>	$11.0^{\text{def}}$	4.70 <sup>efg</sup>	5.00 <sup>abc</sup>	6.33 <sup>b</sup>	5.80 <sup>ef</sup>	0.63 <sup>e</sup>
	Phenamiphos (Nemacur)	33.0ª	14.0 <sup>b</sup>	21.20ª	3.13ª	17.3ª	5.00 <sup>ef</sup>	6.00 <sup>a</sup>	6.00 <sup>bc</sup>	7.50 <sup>bcde</sup>	2.38ª
	Tricuran-P	23.0 <sup>cd</sup>	7.7 <sup>f</sup>	10.70 <sup>d</sup>	1.39 <sup>bc</sup>	13.7 <sup>a-f</sup>	6.00 <sup>cde</sup>	5.67 <sup>ab</sup>	5.33 <sup>bc</sup>	6.93 <sup>de</sup>	0.66 <sup>e</sup>
	Phytohumic	13.4 <sup>ef</sup>	9.9 <sup>cde</sup>	5.68 <sup>gh</sup>	0.79 <sup>def</sup>	11.6 <sup>c-f</sup>	5.23 <sup>def</sup>	4.43 <sup>bc</sup>	6.04 <sup>bc</sup>	6.23 <sup>e</sup>	0.65°

Means in each column, having same letter, are not significantly different according to LSD test (p<0.05).

### Discussion

Management measures of plant parasitic nematodes include all kinds of physical methods such as removing the infected roots, flooding, freezing, steaming and deep plowing of the soil, using trap and inhibitor plants, soilsolarization etc (Hassan et al., 2013; Nasr Esfahani et al., 2023; Westerdah et al., 2020). Moreover, implication of bio-control substances using fungi, bacteria, protozoa, predator nematodes, and other biological agents is another useful tool to manage plant parasitic nematodes (Sacchi et al., 2021; Storelli et al., 2020). However, the main methods are the use of resistant cultivars, crop rotation and chemical control (Roth et al., 2020; Sasanelli et al., 2021). The chemical control of nematodes practically started in 1950, and the application of nematicides was usually implicated before planting in the form of soil fumigation. Some nematicides are also applied along with irrigation water around the plant (Li et al., 2020; Cabrera et al., 2009; El-Marzoky et al., 2022). The use of chemical pesticides is one of the effective tools in increasing the production rate, and thus increasing the productivity in the agricultural sector (Hawk, 2019; Roth et al., 2020). Pesticides causing disorder in the nematode body, such as deactivating the nervous system, preventing penetration into the root and paralyzing, disrupting the movement toward the host root and preventing the hatching eggs leading reduction of the damages caused by the nematode (Moatamedi et al., 2018). Considering the harmful environmental and side effects of pesticides and their chemical hazard in consumption of fresh vegetable crop products such as cucumbers and tomatoes etc, it is important to point out the amount of pesticides used that remain in the soil for a long period, and is gradually absorbed by the plant, so the right choice of pesticide and the dosage used is important critically (Orisajo et al., 2008). In this regard, in this research, it was showed that there is a considerable reduction in nematode parameters using applied related treatments including the number of  $J_2$  in the soil,  $J_2$  and eggs in the root, galls and egg-masses in the root in comparison to inoculated and non-inoculated controls significantly. Among the related treatments, the lowest number of  $J_2$  changes in the soil was of Nemacur (10%) and Trianum-P (16%), followed by Formycine 10, and the highest ones in the Formycine 6 (478%) and Nitroxin (322%), and the rest stood intermediately including Phytohumic and EM. An increase in the concentration of Formycine led to a significant decrease in the number of  $J_2$  in the soil. Our results are partially consistent with Mardani et al. (2024) on cucumber in which the efficacy of Abamectin, Tricuran- P and biofertilizers on cucumber RKN populations and plant growth was analyzed, indicating that the addition of biofertilizers to the soil, not only changes physical and chemical properties of the soil, but also affects the population of microorganisms, including nematodes. At the same time, Nasr Esfahani et al. (2023) also found similar results on Pomegranate, Punica granatum infected with RKNs, M. javanica and M. incognita, and Hussein et al. (2018) on biological seed treatment to management of RKNs accordingly including Rodrigues et al., (2017) on physiological quality of seed and control of *Meloidogyne javanica* in watermelon plants. Furthermore, increasing the tolerance of T. harzianum T-22 to DMI pesticides including Metconazole, Ipconazole, Hexaconazole, and Prochloraz. enables the combined utilization of biological and chemical control strategies against related plant diseases (Wang et al., 2024). Regarding the comparison of the reduction percentage and or increase in the number of eggs and J<sub>2</sub> in the root, it was found that the highest percentage of eggs and J<sub>2</sub> changes in the root is related to the infected control (490%) and Nitroxin (478%) increase, and the lowest one in Nemacur (10%) and EM (16%) followed by Formycin 10. Furthermore, the effect of applied treatments on the number of eggs and J<sub>2</sub> changes showed that the highest one in the root was related to the infected control (567%) and Firmicin 6 (541%) increase, and the lowest ones were related to EM (3.3%)and Nemacur (10.5%) decrease, respectively. At the same time, increasing the concentration of Formycine led to a significant decrease in the percentage of changes of eggs and  $J_2$  in the roots. No significant difference was observed between Nitroxin (470%) and Trianum-P (460%). These findings are almost parallel with Mardani et al. (2024) and Nasr Esfahani et al. (2023) reports, as far as reproduction factor rate of target nematode is concerned. Ghorbani et al. (2022) also reported that Nitroxin bio-fertilizer could effectively control the fusarium wilt of chickpea. The analysis of the number of galls in the root also showed that the highest number of galls was in the infected control, and the lowest number in the non-inoculated ones. Among the treatments used, the lowest number of

galls was related to Formycine 10 and EM, and the highest one was related to Nitroxin and Phytohumic. Here, too, the increase in the concentration of Formycine decreased the number of galls in the root. Although, the number of galls in the root is not the criterion here, because, it is possible that the larva enters the root in the early stages, but is unable to reproduce due to the effect of various treatments (Brennan et al., 2020). Here, the evaluation criterion is more focused on the number of  $J_2$  in the soil and the eggs and  $J_2$  on the plant root (Roth et al., 2020; Sasanelli et al., 2021). In this regard, based on the searches in the literature, it seems that the results of this research are the first report on the effectiveness of Formycine Gold PX 20% in significantly reducing the population parameters of the RKNs, M. javanica. Therefore, according to our results, it is necessary to conduct more research in this issue. Biomass parameters were also affected in interaction between various implicated treatments and RKN pathosystem accordingly. According to our results, there was a significant difference among the used treatments in terms of biomass parameters. In this regard, the highest stem length was observed in Nemacur (59%) and Formycine 10 (33%) in comparison to the infected controls, and the rest stood intermediately including Formycine 8, EM and Phytohumic, respectively. By increasing the concentration of Formycine, stem length was also increased. The same trend was followed for the other biomass parameters accordingly. For instance, the highest root length was in Nemacur (55%) and Formycine 10 (46%), and the lowest ones in Trianum-P in comparison to the infected controls, respectively, and the rest stood in between intermediately. These results indicate that, almost all the implicated treatments had a significant effect in nematode population potential reduction and biomass growth parameters, based on their effective compositions by virtue of which could maintain the tomato plants accordingly. In this regard, there are several reports which indicate that implication of any bio-fertilizer in the soil against RKNs not only causes reduction in nematode population parameters, but also enhances the increased growth response based on their chemical properties simultaneously. For instance, El-Remaly et al. (2022) on bio-management of RKNs on cucumbers using biocidal effects of some brassicaceae crops, and Dhillon et al. (2022) on management of RKNs, M. incognita with mustard and neem cake application for sustainable production of cucumber reported almost similar results as per in our findings. El-Eslamboly et al. (2019) also by algal application as a biological control method on M, incognita on cucumber; El-Kelany et al. (2020) on M, incognita of eggplant using some growth-promoting Rhizobacteria and Chitosan; and Osman et al. (2018) on eggplant using nematicide, fertilizers, and microbial agents supported our findings in this issue. Effective Micro-Organisms (EM) consists of common aerobic and anaerobic micro-organisms; photosynthetic bacteria, lactobacillus, Streptomyces, Actinomycetes, yeast, etc.; Trianum-P consisting biological fungicide T. harzianum T-22 and other related species; and Nitroxin (Azospirilium and Azotobacter rhizobacteria combination) are the beneficial and effective microorganisms in agricultural products (Joshi et al., 2019; Battaglia et al., 2024). They are the most common soilamending fungi and bacteria as plant growth-enhancing the acidify at their surroundings by secreting organic acids such as gluconic acid, citric acid, and fumaric acid, and as a result, they are able to dissolve insoluble compounds and increase the availability of phosphorus, iron, manganese, and magnesium to the target pathogens (Gianfreda et al., 2010; Sharon et al., 2001). Mardani et al. (2024). EM as the mixed cultures of beneficial naturally-occurring organisms also can be applied as inoculants to increase the microbial diversity of soil ecosystem (Waldrop & Firestone, 2004). EM will improve the structure of the soil, increase its fertility and radically improve biological diversity, suppress soil-borne pathogens, fixes the nitrogen in soil and enhances nutrient uptake, accelerates the decomposition of organic waste, residues and composting, increases beneficial minerals in organic compound, enhances the activities of indigenous microorganism and boosts the strength of plants and yield of crops (Liang et al., 2009; El-Kelany et al., 2020; Wang et al., 2008). EM works by being dominant over other soil microbes. As a result, this encourages the bulk of the other microbes in the soil to follow them and in doing so suppress the activity of the smaller group of negative or opportunistic microbes (Nasr Esfahani et al., 2020). Moreover, effective microorganisms can help to improve and maintain the soil chemical and physical properties (Joshi et al., 2019). At the same time, Kabir, et al. (2024) found that Trichoderma afroharzianum T22 induces host transcriptome and endophytic microbiome leading to growth promotion in sorghum plants.

### Conclusion

These findings in our research are the first report on the efficiency of some of the bio-fertilizers including Formaycine Gold PX 20% as a novel product, Nitroxin, (EM) Effective Micro-Organisms and Phytohumic on root knot nematodes, M. javanica population potential and biomass growth parameters in comparison to Fenamiphos (Nemacur). It was shown that almost all the related treatments were effective against M. javanica and biomass growth parameters based on their composition relatively. Of which, Formaycine Gold PX 20% at the rate of 10 L/ha thrived better than others in this issue, not only on *M. javanica* population reduction, but also on biomass growth parameters simultaneously. It should be denoted that, it is a primarily study and need to be evaluated on some other related crops and other root knot nematodes species by virtue of which can be suggested for the management of this particular plant parasitic nematodes in an integrated pest management for reduction of the damages caused these serious plant parasitic nematodes and maintenance of the biomass growth parameters.

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### **Conflict of interest**

The authors declare that there are no conflicts of interest present.

### **CRediT** author statement

M. Rafiee: Obtaining resources, conducting the project.
M. Olia: Supervision, Conceptualization, Methodology,
Data curation, Review, & Editing. M. Nasr-Esfahani;
Supervision, Review, & Data curation. P. Mashayekhi
and A. Nasr Esfahani; Soil analysis, Data curation,
Review, & Editing.

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