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Impact of Certain Eco-Friendly Agents and Safety Commercial Nematicide Compared with The Chemical Nematicide Vydate for Controlling *Meloidogyne javanica* Infected Tomato Plants

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ABSTRACT

The main efforts of scientists to reduce the appliance of nematicides that are commonly found in the markets led to an increase in the demand for safe, effective and environment-friendly alternatives. Hence, the present research aims to test three safe and effective substances, Humic acid (HA) concentration of 1500 ppm; Mychorrhizal fungi, *Glomus mosseae* and the commercial nematicides Bio-zeid (*Trichoderma album*) half dose compared with the nematicide Vydate on root-knot nematodes *Meloidogyne javanica* infected tomato plants, as well as its effects on the vegetative characteristics of tomato plants. The obtained results revealed that all tested materials decreased *M. javanica* J_2 population density. The triple treatment (Humic acid + *G. mosseae* + Bio-zeid (half dose)) gave the best results, as it led to a decrease in the number of *M. javanica* J_2 by 88.0% more than that occurred by the chemical nematicide Vydate, which gave a reduction percentage of 87.1%. The triple treatment also led to a decrease in the numbers of root gall index by 80.0%. As well as triple treatment also improved the vegetative characteristics of tomato plants, it caused an increase in plant height, shoot weight and root weight by percentages (53.0, 29.0 and 148.1%) while contrariwise occurred by an appliance with Vydate (42.9, 21.4 and 92.6%), respectively, compared to the control. Thus, triple treatments can be recommended as a cheaper and safer alternative for plants and humans instead of chemical nematicides in integrated nematode control programs.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the vegetables rich in nutrients that are beneficial to the body, as it supports the health of the skin and heart as well as helps in losing weight, protects against cancer and maintains blood pressure and is of great economic importance because they contain carbohydrates, vitamins, sugars and proteins (Mukesh and Dhirendra, 2019). Tomatoes attack many pests, the most important of which are plant-parasitic nematodes, especially root-knot nematodes (*Meloidogyne* spp), which directly attack tomato roots and cause huge economic

losses, or act as vectors for many other pests that damage tomato plants (Shady, 2011); (El-Shennawy, and Abo Korah, 2016).

When humic acid entered plants at the early stages of development, promoted an increase in cell division and root development hastening establishment. In addition, humic acid acted as a natural chelator by enhancing the availability of iron thus increasing photosynthesis and sugar production translating into increased storage for defense (Abo-Korah, and Moussa, 2015). There is the most important need for an eco-friendly substitute for nematode management. Humic acid released during the decomposition of raw organic materials is one of many factors contributing to reducing nematode damage (Mcbride *et al.,* 2000). A new strategy for adjusting plant parasitic nematodes is based on the activation of the plant's own defense system through various biotic and abiotic agents such as humic acid (El-Sherif *et al.,* 2015).

Mycorrhizae, *Glomus mosseae*, lives with the roots of the plant as a symbiotic life, hence it facilitates the absorption of water; salts and nutrients by the plant, and thus increases its resistance to root-knot nematodes, as well as creating insufficient conditions for the development and reproduction of nematodes inside the roots (Abo-Korah, 2022).

Bio-zeid (*Trichoderma album*) inhibits nematode reproduction and leads to the death of juvenile plant-parasitic nematode which infected tomato plants, considered one of the most efficient bio-pesticides (Samak, and Abo-Korah, 2021).

The harmful effects of chemical control on human and animal health, as well as the plants, have promoted scientists to think about alternative & safe and harmless methods for the environment, hence the idea of the research under study.

MATERIALS AND METHODS

The potted experiment was conducted under field conditions at the experimental farm of the Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt. To shed light on the impact of three safety treatments Humic acid (HA) concentration 1500 ppm; Mychorrhizal fungi, *Glomus mosseae* and the commercial nematicides Bio-zeid (*Trichoderma album*) half dose dual or triad, in comparison with the chemical nematicide (Vydate) for controlling the root-knot nematode *M. javanica* which infected tomato plants and its connectedness with vegetative characteristics.

Nematode Culture:

Meloidogyne javanica larvae (J_2) were collected from pure culture reared on black nightshade, *Solanum nigrum* plants in the Nematode laboratory of the Entomology and Zoology Department of the Faculty of Agriculture, Menoufia University. Galled roots of the nightshade were washed in the water to clean them from the soil sticking to the roots, then the roots were cut into pieces 1 cm long and stirred in sodium hypochlorite NaOCl (0.5%) for 3 minutes and shacked well in sterile water to free eggs from egg masses (Kerry and Bourne 2002). Eggs were incubated in a modified Baermann funnel at 25 ± 1°C for 4 days to obtain second-stage juveniles (J_2) according to the method of (Gray, 1984) to use it in the experimental infection procedure (Chuixu *et al.,* 2013).

Preparation of Treatments:

Humic acid treatments were prepared and mixed with the experimental soil with concentration rates (1500 ppm). (Abo-Korah, and Moussa, 2015).

Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*) were introduced to the soil during planting 15 days earlier than nematode inoculation. Pots receiving endo-

mychorrhiza were each inoculated with (3g) of infested soil and (0.5 g) of the onion roots colonized by G. mosseae. Plants were thinned to one plant per pot after 5 days (Amin and Mostafa, 2000).

Bio-zeid bio-nematicide ($1x10^{10}$ vital spores per gram product of fungus, *Trichoderma album*) was applied at the rate of 0.2 g/plant. (Metwally *et al.,* 2019).

Vydate 24% L: Oxamyl is a chemical insecticide and nematicide: (2-(Dimethylamino)-N-[[(methylamino)carbonyl] oxy]-2-oxo ethanimidothioic Acid Methyl Ester-d6;). It is commonly sold under the trade name Vydate. It was applied at the rate of 3 ml /pot and added at the time of nematode inoculation (Abo-Korah, and Fathalla, 2022).

Experimental Preparation and Design:

The experiment was conducted in a tomato field (*Lycopersicon esculentum* var. Super strain B) selected for this study during the summer season from April, to June 2021. At transplanting time, the treatments were arranged as a completely randomized block design. The experiment was done in potted under felid conditions and each treatment consisted of (3) replicates. One seedling 30-day-old tomato, *Solanum lycopersicom* cultivar Super strain B was planted in a plastic pot 25 cm containing 3kg of sterilized clay-sand mixed soil (1:3 v/v). After seven days of seedlings adaptation, (1000 J_2 / 1 kg soil) of *Meloidogyne javanica* was added by pipette into three holes around each seedling. Treatments were applied dual or triad immediately after infection with nematodes.

Nematode Extraction and Numeration:

Each soil sample was carefully mixed, and an aliquot of 250 cm3 was processed for nematode extraction according to methods described by (Christie and Perry 1951) and (Southey 1970). About 300-400 ml. of water was added to the soil in a glass beaker (1000 ml) and the mixture was agitated by fingers, after a few seconds the suspension was poured onto a 60-mesh sieve and passing suspension was collected in another clean glass beaker. Materials caught on the 60-mesh sieve were discarded, while the collected suspension was then poured onto a 200 mesh sieve. Materials remaining on the sieve were thoroughly washed by a gentle stream of water into a 200 ml beaker. The resulting suspension containing nematodes was then transferred to a Modified Baermann pan fitted with soft tissue paper for the separation of active nematodes from debris and fine soil particles. After 72 hrs. nematode water suspension was collected and concentrated to 20 ml in a vial by using a 350-mesh sieve. An aliquant of 1 ml each of nematode suspensions was pipetted off, placed in a Hawksley counting slide and examined using a stereomicroscope.

At the end of the experiment, the roots are washed and the root gall index was computed according to (Taylor and Sasser 1978) as shown in Table (1).

Table 1: Rating scale levels of galls numbers and cultivar's resistance rating

Number of galls	Galling index
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5

Vegetative Characters:

At the end of the experiments, plant height (cm), shoot weight (g) and root weight (g) were measured and determined. The increase or decrease in plant vegetative characters was computed according to the following formula:

Increase or decrease % = treatment – Control / Control x 100

Statistical Analysis:

All obtained data were subjected to ANOVA test using a computer program (Costat, 2008) to determine Duncan's multiple range test and the LSD 5% (least significant difference). In addition, Abbott's formula was used to determine the increased percentages of vegetative characters.

Reduction percentages were counted according to Abbott's formula (1925).

$$\text{Corrected Mortality \%} = (1 - \frac{\text{No In Treatment After Treatment}}{\text{No In Control After Treatment}} \times 1oo$$

RESULTS AND DISCUSSION

Results in Table (2) revealed that triple treatment {Humic acid + *G. mosseae* +Vydate (half dose)} was the best treatment which reduced the number of root-knot nematodes juveniles after application by 30, 60 and 90 days to (249.0, 124.0 and 67.9), respectively, with an average of (146.9). Followed by tri treatment {Humic acid + *G. mosseae* + Bio-zeid (half dose)} which reduced the number of root-knot nematodes juveniles after application by 30, 60 and 90 days to (291.0, 147.3 and 91.0), respectively, with an average of (176.4). Compared with the chemical nematicide Vydate (full dose) which reduced the number of root-knot nematodes juveniles after application by 30, 60 and 90 days to (315.3, 158.3 and 96.0), respectively, with an average of (189.9). As for binary treatments {*G. mosseae* + Vydate (half dose)} reduced the number of root-knot nematodes juveniles after application by 30, 60 and 90 days to (435.0, 251.0 and 131.0), respectively, with an average of (272.3).

Table 2: Effect of combined treatments on the population density of root-knot nematode, *Meloidogyne javanica* infected tomato plants variety (Super strain B) under field conditions.

Treatments	Aver. no. of *Meloidogyne javanica* juveniles/ 100 g soil			
	Days post-treatments			
	30 Days	60 Days	90 Days	Overall mean
Humic acid+ Bio-zeid (half dose)	501.0 c	312.0 c	161.0 c	324.7 C
G. mosseae + Bio-zeid (half dose)	481.0 d	300.3 d	109.3 f	296.9 D
Humic acid+ *G. mosseae*	706.0 b	584.0 b	349.9 b	546.6 B
Humic acid + *G. mosseae* + Bio-zeid (half dose)	291.0 h	147.3 g	91.0 g	176.4 G
Humic acid + Vydate (half dose)	458.0 a	294.0 d	152.3 d	301.4 D
G. mosseae + Vydate (half dose)	435.0 f	251.0 e	131.0 e	272.3 E
Humic acid + *G. mosseae* +Vydate (half dose)	249.0 i	124.0 h	67.9 h	146.9 H
Vydate (full dose)	315.3 g	158.3 f	96.0 g	189.9 F
Control	1341.9 a	1609.3 a	1811.0 a	1587.4 A
LSD 5%	8.6	8.6	8.6	8.5

Means in each column followed by the same letter (s) are no significant differences at a 5% level.

Our results agree with (Nagachandrabose and Baidoo, 2021) who recorded that, humic acid stimulates the growth of roots by encapsulating the soil granules and chelating nutrients to provide them to the roots to absorb over long periods, thus maximizing their benefit of them. (Abo-Korah and Moussa, 2015) recorded that, humic acid facilitates the absorption of nutrients for the plant and thus leads to an increase in the plant's immunity against infection by parasitic nematodes, and it also improves the vegetative and fruiting characteristics of the plant.

The role of humic acid in overcoming the harmful effects of rust diseases in Faba bean plant may be due to the increase in chitinase activity (Abd El-Kareem, 2007).

Data in Table (3) and Fig. (1) showed that triple treatment {Humic acid + *G. mosseae* +Vydate (half dose)} gave the highest reduction percentage in root-knot *M. javanica* J_2 infected tomato plants with an overall mean by (90.0%), followed by triple treatment {Humic acid + *G. mosseae* + Bio-zeid (half dose)} with an overall mean by (88.0%) compared with chemical nematicide Vydate (full dose) which gave (87.1%). As for binary treatments {*G. mosseae* + Vydate (half dose)} gave a reduction percentage in root-knot *M. javanica* J_2 infected tomato plants with an overall mean of (81.6%), compared with control.

Table 3: Reduction percentage of *Meloidogyne javanica* infected tomato plants variety (Super strain B) under field conditions.

Treatments	Reduction %			
	30 Days	60 Days	90 Days	Overall mean
Humic acid+ Bio-zeid (half dose)	62.7	80.6	91.1	78.1
G. mosseae + Bio-zeid (half dose)	64.2	81.3	93.9	79.8
Humic acid + *G. mosseae*	47.4	63.7	80.7	63.8
Humic acid + *G. mosseae* + Bio-zeid (half dose)	78.3	90.9	94.9	88.0
Humic acid + Vydate (half dose)	65.9	81.7	91.6	79.7
G. mosseae + Vydate (half dose)	67.6	84.4	92.8	81.6
Humic acid + *G. mosseae* +Vydate (half dose)	81.4	92.3	96.3	90.0
Vydate (full dose)	76.5	90.2	94.7	87.1

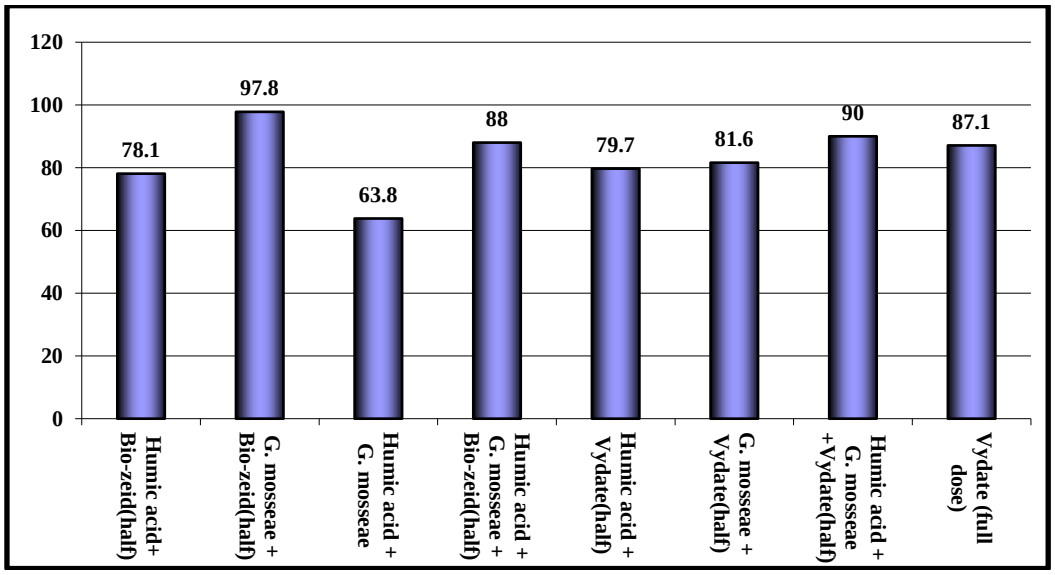

Fig 1: Effect of combined treatments on the average reduction percentage of *Meloidogyne javanica* infected tomato plants variety (Super strain B) under field conditions**.**

Our results agree with (Udo, *et al.,* 2022) Approved that, Vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus mosseae*) act on the root and make it anti-nematode due to the high level of amino acids and sugars in the roots of mycorrhizal plants, which increases the resistance of plants to nematodes (the amino acids Serine and Phenylalanine act as nematicides). (Abo-Korah, *et al.,* 2022) recorded that, *Glomus mosseae* penetrates the roots of the host and enters into the cells and multiplies with the presence of some outside the roots extending in the soil and works to facilitate the absorption of water, salts and nutrients to the roots and it works to improve the qualities of vegetative and fruitful tomatoes. (Sankaranarayanan and Hari, 2021) Approved that, such a reduction in nematode population resulted in increased growth of the plants. This reduction in the severity of disease caused by nematode in mycorrhizal plants might be due to the altered biochemical constituents in the host plant.

Table 4: Influence of combined treatments on some tomato plant characters and root gall index.

Treatments	Plant height cm	Shoot weight g	Root weight g	Root gall index
Humic acid+ Bio-zeid (half dose)	69.8 bc	76.0 cde	17.9 d	2.0 c
G. mosseae + Bio-zeid (half dose)	66.3 e	75.0 de	18.6 bc	2.0 c
Humic acid + *G. mosseae*	70.1 b	76.7 bcd	18.7 b	3.0 b
Humic acid + *G. mosseae* + Bio-zeid (half dose)	71.0 a	79.0 a	20.1 a	1.0 d
Humic acid + Vydate (half dose)	69.0 c	76.9 bc	18.0 cd	2.0 c
G. mosseae + Vydate (half dose)	67.7 d	73.9 e	18.9 b	2.0 c
Humic acid + *G. mosseae* + Vydate (half dose)	69.9 b	78.4 ab	19.7 a	1.0 d
Vydate (full dose)	66.3 e	74.3 e	15.6 e	1.0 d
Control	46.4 f	61.2 f	8.1 f	5.0a
LSD 5%	0.8	1.7	0.6	0.3

Means in each column followed by the same letter (s) are no significant differences at a 5% level**.**

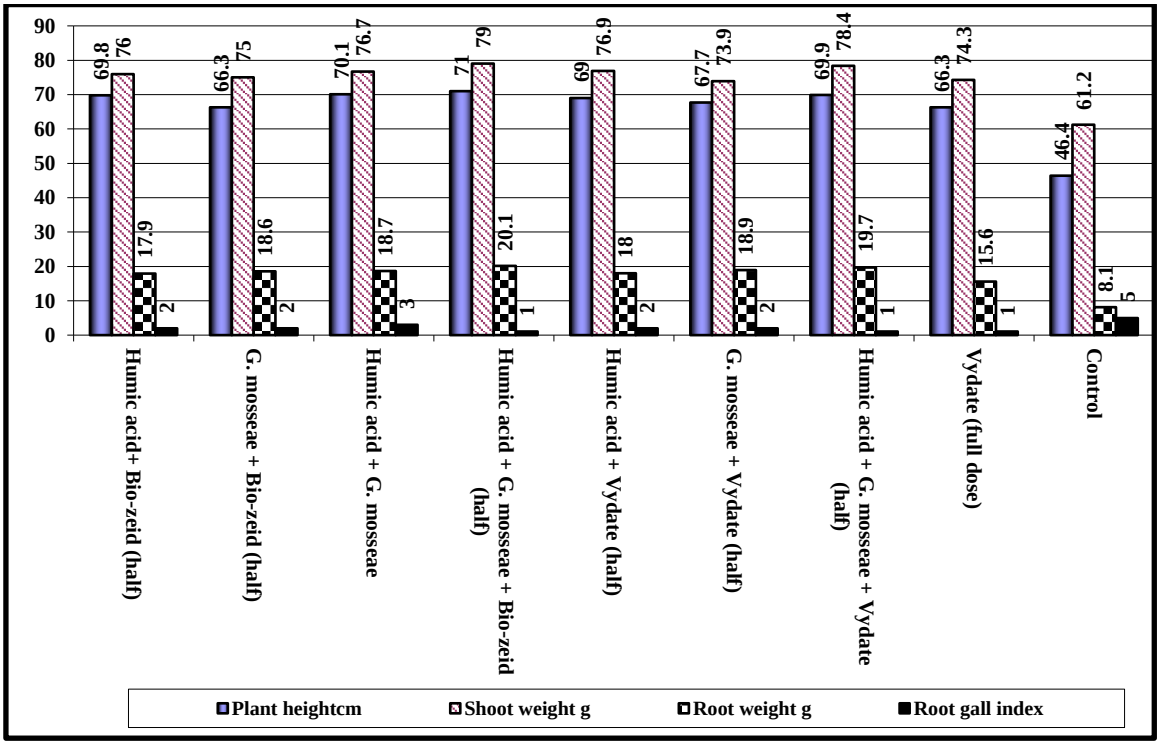

Fig 2: Effect of combined treatments on some tomato plant variety (Super strain B) characters and root gall index**.**

Data in Table (4) and Fig. (2) showed that significant differences were observed between all treatments. The ability of the combined treatments to improve the characteristics of the tomato plants, as {Humic acid + *G. mosseae* +Vydate (half dose)} record plant height (cm); shoot weight (g); root weight (g) and root gall index by (69.9, 78.4, 19.7 and 1.0), respectively, and {Humic acid + *G. mosseae* + Bio-zeid (half dose)} with (71.0, 79.0, 20.1 and 1.0), respectively. As for binary treatments {Humic acid + Bio-zeid (half dose)} by (69.8, 76.0, 17.9 and 2.0), respectively. Compared with Vydate (full dose) which gave (66.3, 74.3, 15.6 and 1.0), respectively. As for control gave (46.4, 61.2, 8.1 and 5.0) respectively.

Bio-zeid (*Trichoderma album*) inhibits nematode reproduction and leads to the death of juvenile plant parasitic nematode which infected tomato plants, considered one of the most efficient bio-pesticides (El-Deeb *et al.*, 2018). Bio-zeid application led to a decrease in the number of root-knot nematodes that infected tomato plants, and the rate of root-knot formation was significantly reduced and improved the vegetative and fruiting qualities of tomatoes (Samak and Abo-Korah, 2021).

Table 5: Increase or decrease percentages of some vegetative characters and gall index on tomato as influenced by treatment applications.

Treatments	Plant height %	Shoot weight %	Root weight %	Root gall index %
Humic acid+ Bio-zeid (half dose)	+50.4	+24.2	+120.9	-60
G. mosseae + Bio-zeid (half dose)	+42.9	+22.5	+129.6	-60
Humic acid + *G. mosseae*	+51.1	+25.3	+130.8	-40
Humic acid + *G. mosseae* + Bio-zeid (half dose)	+53.0	+29.0	+148.1	-80
Humic acid + Vydate (half dose)	+48.7	+25.6	+122.2	-60
G. mosseae + Vydate (half dose)	+45.9	+20.7	+133.3	-60
Humic acid + *G. mosseae* + Vydate (half dose)	+50.6	+28.1	+143.2	-80
Vydate (full dose)	+42.9	+21.4	+92.6	-80

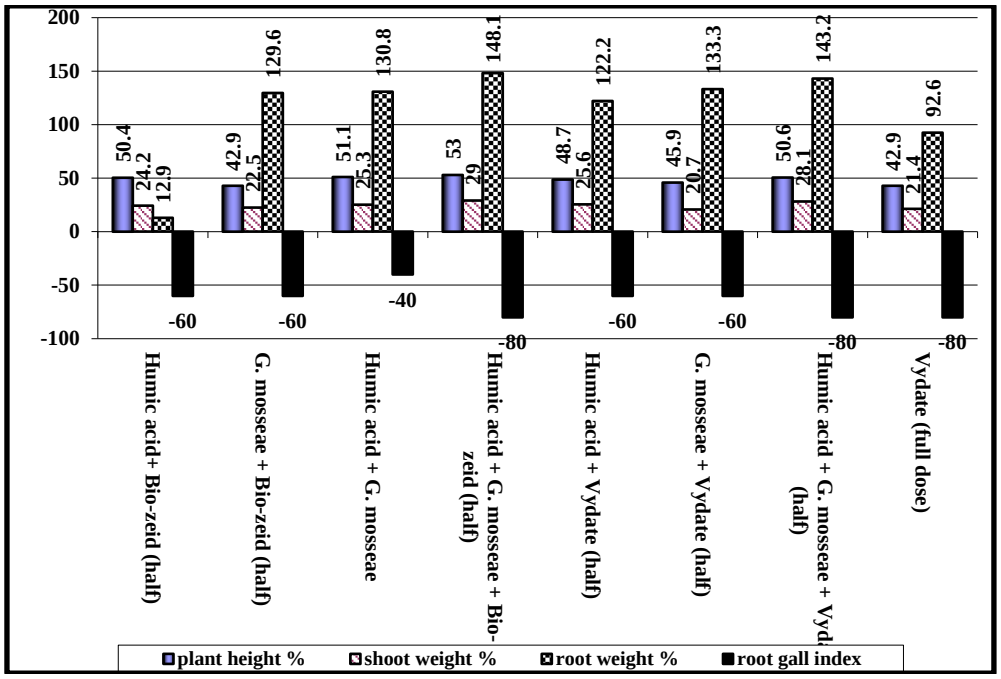

Fig 3: Increase or decrease percentages of some vegetative characters and gall index on tomato as influenced combined treatments applications.

Data in Table (5) and Fig. (3) showed that treble treatment {Humic acid + *G. mosseae* + Bio-zeid (half dose)}, gave the best results in improving the vegetative characteristics of tomato plants, which led to increased plant height (cm); shoot weight (g) and root weight (g) by (+53.0, 29.0 and +148.1%), respectively, but decrease root gall index by (-80.0%). followed by triple treatment {Humic acid + *G. mosseae* + Vydate (half dose)} increased by (+50.6, +28.1 and 143.2%), respectively, and decrease root gall index by (-80.0%). As for binary treatments {Humic acid + Bio-zeid (half dose)} increased by (+50.4, +24.2 and +120.9%), respectively. and decrease root gall index by (-60.0%). Compared with Vydate (full dose) which increased by (+42.9, +21.4 and +92.6%), respectively, and decrease root gall index by (-80.0%) compared with control. Chemical nematicides are widely used to control parasitic nematodes on vegetables, fruits and ornamental plants in Egypt, in spite of the expensive costs of other agricultural operations, not safe for humans, animals and plants (Barker and Koenning, 1998).

(Abo-Korah, 2022) recommended that, the use of triple treatment (saponins extract + algae + BioNematon half dose) as an integrated control program for root-knot nematodes *M. incognita* which infected cowpea plants practically considerably by its safety, inexpensive, and harmlessness, in addition, it also reduced root-knot nematodes *M. incognita* juveniles, mature females, root galls, and improved the vegetative properties of cowpea plants in greater proportions than the chemical nematicide Carbofuran. It is clear from the previous results that, the triple treatment {Humic acid + *G. mosseae* + Bio-zeid (half dose)} led to the elimination of root-knot nematodes *Meloidogyne javanica* juvenile that infect tomatoes by (88.0%), as well as improving the vegetative characteristics of tomato plants compared to the chemical pesticide Vydate, as it is safe for humans, animals and plants and less expensive than chemical nematicides.

Finally, it could be concluded that integrating several safety treatments can make an excellent model for nematode control and could be effectively used with other cultural, biological and chemical nematode management strategies.

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