Minufiya J. Agric. Res. Vol.37 No. 1: 29 - 43 (2012) "http://www.mujar.net"

ARE ORGANIC WASTES PLAY IMPORTANT ROLE IN REDUCING ROOT-KNOT NEMATODES POPULATION?

M. E. Mahdy[1], Mervat E. Sorial[1] and Neveen G. Magdy[2]

[1] Fac. Of Agric.; Agric. Botany Dept., Minuflya Univ.; Shebin El-Kom, Egypt.

[2] Nematode Research Dept., Plant Pathology Institute, Agric. Res. Centre, Giza, Egypt.

(Received: Dec. 20, 2011)

ABSTRACT: *In this research, three organic wastes namely: marjoram (Majorana hortensis L.); green tea and black tea (Camellia sinensis) were evaluated at three rates 10, 20 and 50 g/plant with two application methods as soil treatment powder and soil drench after soaking in water overnight, to study its effect on the population of root-knot nematodes, Meloidogyne spp. under naturally infested field soil cultivated with tomato. Results confirmed that all applied treatments either at the three rates or with the two application methods, significantly reduced all related nematode parameters i.e. number of galls, egg-masses and number of females/root system; number of eggs/egg-mass; number of second stage juveniles/250 g soil as well as the final nematode population (PF) and the reproduction factor (RF) when compared to plants treated with nematode only. Application of green tea at 50 g/plant recorded the highest effect in reducing the nematode population followed by the treatments of black tea at 50g/plant, and green tea at 20g/plant, while the lowest one was showed with marjoram at 10g/plant. The same trend of results were showed with plant growth parameters i.e. fresh and dry shoot & root weights; root length and plant height. Chemical constituent characters i.e. membrane leakage (ML %); total soluble sugars, total amino acids, total phenols as well as the antioxidant enzymes i.e. peroxidase, phenoloxidase and catalase recorded significant enhancement when compared with the positive and negative control. The highest enhancement in all chemical constituents found with the application of green tea as a powder at 50 g/plant. Results also revealed that all applied treatments saved the root cells in normal case and reduced the development of giant cells compared to the plants treated with nematode alone.*

Key words: *Antioxidant enzymes, Organic wastes, Green tea, Black tea, Marjoram, Root-knot nematodes, Tomato*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important solanaceous crops in Egypt either for local consumption or exportation. Tomato is considered as one of the highest nutrion crops because of its high contents of vitamin C as well as many chemical compounds and elements which are not found in the other solanceous crops.

Root-knot nematodes, *Meloidogyne* spp. are a dangerous obligate endoparasitie that feeds exclusively on the cytoplasm of living root cells (Abad *et al.*, 2003). *Meloidogyne* infects a large number of crops and causes severe losses in yield. The disease symptoms on infected plants include galls on the roots, stunted growth, wilting and increased susceptibility to the other pathogens (Williamson, 1998).

Tea is a very rich source of specific kind of antioxidant called flavonoids. The detoxifying affect of these antioxidants protects from free radicals. Antioxidants bind harmful oxygen-containing molecules called free radicals and peroxides that otherwise could damage

DNA, cell membranes and other cell components. Naturally occurring antioxidants found in most plants help in removing free radicals. Flavonoids are nutrient antioxidants found in most plant (green and black tea). Catechins are a type of flavonoids contained in the leaves of tea (Leung *et al.*, 2001). Catechins are very strong antioxidants even more powerful than vitamin A, C, E and beta carotene at combacting harmful free radicals and have for reaching positive effects (Lee and Lee, 2002).

The objective of this study was to reduce the harmful effect and infection of root-knot nematodes, *Meloidogyne* spp. by using some natural antioxidant materials as green and black tea as well as marjoram plants to improve tomato yield.

MATERIALS AND METHODS

These experiments were conducted at the experimental farm of Faculty of Agriculture, Minuofiya University, Shebin El-Kom to evaluate the effect of green and black tea as well as marjoram either as a soil treatment powder or as a soil drench application to control root-knot nematodes, *Meloidogyne* spp. and the response of tomato plants (*Lycopersicon esculentum* cv. GS) grown in nematode naturally infested soil.

The experimental design was a complete randomized block in three replicates per treatment. The experimental plot area was 12 m^2 as it included three rows, 5 m long and 60 cm in width. Tomato seedling cv.GS were germinated and transplanted on 18th and 20th of January when reached 15 cm (average height) in both seasons, respectively.

Soil samples were taken before seedlings transplanting and treatment applications to extract and count the nematode population at zero time according to Goodey (1957). Number of nematode population as initial population was estimated by 2000 J_2S/250 g soil.

The recommended doses of N, P and K chemical fertilizers were 300 kg superphosphate/fed., 250 kg ammonium sulphate/fed. and 150 kg potassium sulphate/fed. N and K fertilizers were added in two equal parts after 20 and 45 days from transplanting.

Green and black tea as well as marjoram was applied at two forms powder or soil drench. Three rates 10, 20 and 50 g/plant of each plant powder were used. One hundred gram of each plant was soaked overnight in tap water and tomato seedlings were irrigated with 20 ml/plant.

The tomato seedlings were subjected to 14 treatments as follows:

(1) Marjoram 10g+N (2) Marjoram 20g+N

(3) Marjoram 50g+N

(4) Marjoram drench+N

(5) Green tea 10g+N (6) Green tea 20g+N

(7) Green tea 50g+N

(8) Green tea drench+N

(9) Black tea 10g+N (10)Black tea 20g+N

(11) Black tea 50g+N

(12) Black tea drench+N

(13) Nematode (N) alone (14) Control

Control plants grown in separately free area from root-knot nematodes infection. Three plants were taken randomly from each treatment at 90 days after transplanting and the following plant growth parameters and chemical components data were recorded as follows:

Nematode parameters:

At the end of the experiment the following nematode parameters were recorded:

Number of galls, number of egg masses, number of eggs/egg mass,

number of females/root system, number of developmental stages/root system, number of juveniles/250 g soil (Goodey, 1957), final nematode population (PF) and the rate of nematode reproduction (RF). Egg-masses, were stained prior to counting by dipping the infected roots in 0.015% phloxine-B solution for 20 minutes as described by Daykin and Hussey (1985). Females were collected by cutting the root system of each plant in 2 cm pieces and submerging the roots in a beaker full of tap water for 4 days at room temperature until they became soft. The roots were then washed through 500 and 250 µm sieves to separate the females from the root debris (Mahdy, 2002).

Final nematode population (PF) was counted according to the equation:

*PF = (No. of egg masses X No. of eggs/egg mass) + No. of females + No. of developmental stages + No. of juveniles in soil

Rate of nematode reproduction (RF) was recorded according to Norton (1978) as:

*RF = PF/Pi (Pi = initial population)

Plant growth parameters:

Plant height; fresh and dry shoot as well as root weights and root length.

Chemical components:

Total soluble sugars (TSS); total phenols (TP) and total free amino acids (TFAA) were estimated in dried leaves according

to the methods described by Dubois *et al.*, (1956), Snell and Snell (1953) and Rosen (1957).

Antioxidant enzymes activity were estimated in plant fresh leaves according to Broesh (1954); Fehrman and Dimond (1967) and Bach and Oparin (1968), respectively.

Membrane leakage (ML) was determined following the method of Leopold *et al.* (1981). The percentage leakage of solutes was calculated as:

$$\% \text{ leakage of substances} = \frac{\text{Initial absorbance of bathing medium}}{\text{Final absorbance at the bathing medium}} \times 100$$

Anatomical studies:

Samples for anatomical studies were taken from the roots contain galls of each treatment at harvesting time. Samples were cut into suitable pieces in order to facilitate the exchange of different solutions. Samples were placed in formalin, alcohol, acetic acid (F.A.A.) for 36-48 hours. Then samples were washed, then dehydrated in ascending concentration of ethyl alcohol. Before infiltrations, samples were passed into increasing concentrations of xylol in absolute alcohol. Infiltration was completed in oven when pure melted wax was added. Samples were embedded in paraffin wax (O'Brien and Me Cully, 1981). Sections were microtomed at 15 microns. The double combination of safranin-light green stain, followed photomicrography was obtained. In order to obtain the different changes in gaint cells formed due to nematode infection and the effects of different treatments compared to the uninfected plants were drowning by means of camera lucida.

Statistical Analysis:

Data were analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics. Crop., Rockville, MD), Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out by Duncan's Multiple Range Test (Duncan, 1955) as given in the figures and tables.

RESULTS

Data presented in Figs. (1&2) indicated that all applied treatments either as powder or drench significantly reduced the mean number of galls, egg-masses/root system as well as number of eggs/egg-mass compared to the treatment of nematode alone. Application the tested plants as a powder form to soil was more effective in reducing nematode parameters than soil drench application. Results revealed that green and black tea at 50 g/plant as well as green tea at 20g/plant was highly significant treatments in reducing the number of galls/root system compared with nematode alone (Fig. 1).

Egg-masses and eggs were showed high significant decrease when plants were treated with green tea at 50 g/plant followed by black tea at 50g/plant and green tea at 20 g/plant when compared with nematode alone as shown in Fig. (2).

Results also reported that all applied treatments significantly reduced the mean number of juveniles in soil and the rate of nematode reproduction when compared with nematode alone as shown in Figs. (3&4). Concerning the second stage juveniles, treatment of green and black tea at 50 g/plant led to high significant reduction in juvenile numbers followed by green and black tea at 20 g/plant compared with nematode alone as shown in Fig. (3). Similar trend of results was obtained with that of the rate of nematode reproduction as it significantly reduced with the treatment of green and black tea at 50 g/plant followed by green tea at 20 g/plant compared with nematode alone (Fig. 4).

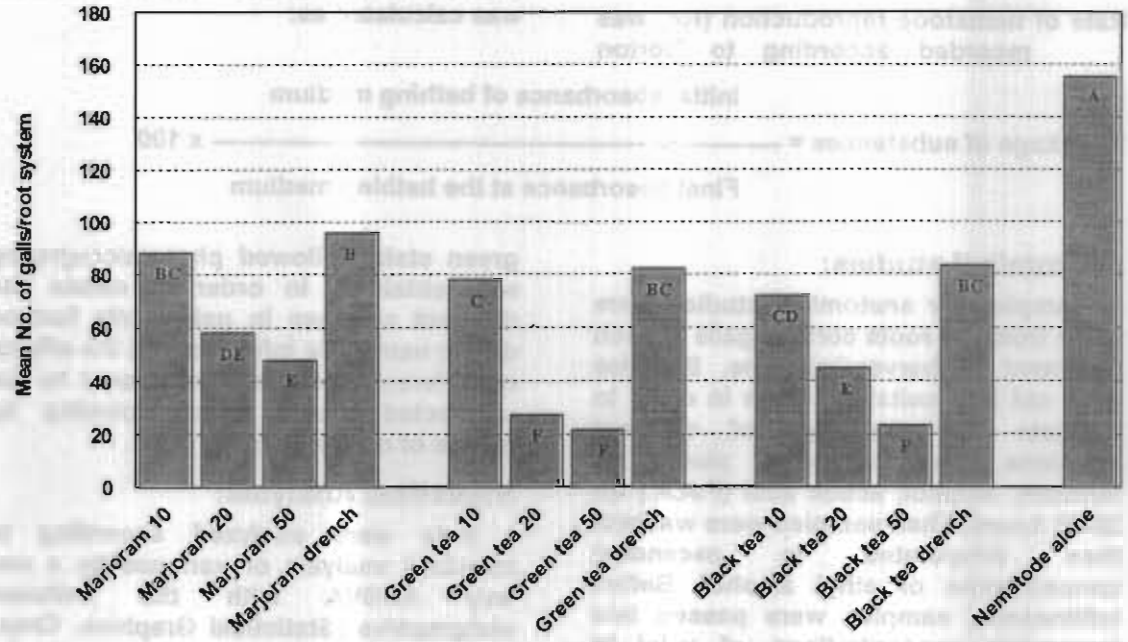

Figure (1): Effect of different organic wastes on number of galls of *Meloidogyne* spp. on tomato roots. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at $p \leq 0.05$.

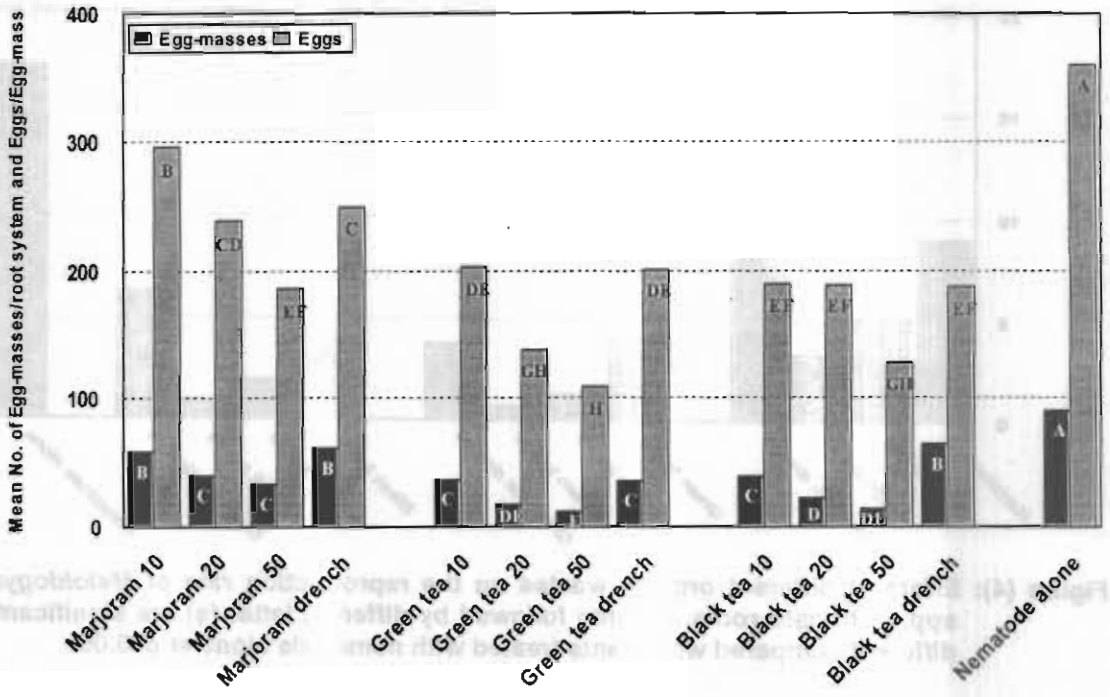

Figure (2): Effect of different organic wastes on number of egg-masses/root system and number of eggs/egg-mass of *Meloidogyne* spp. on tomato roots. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

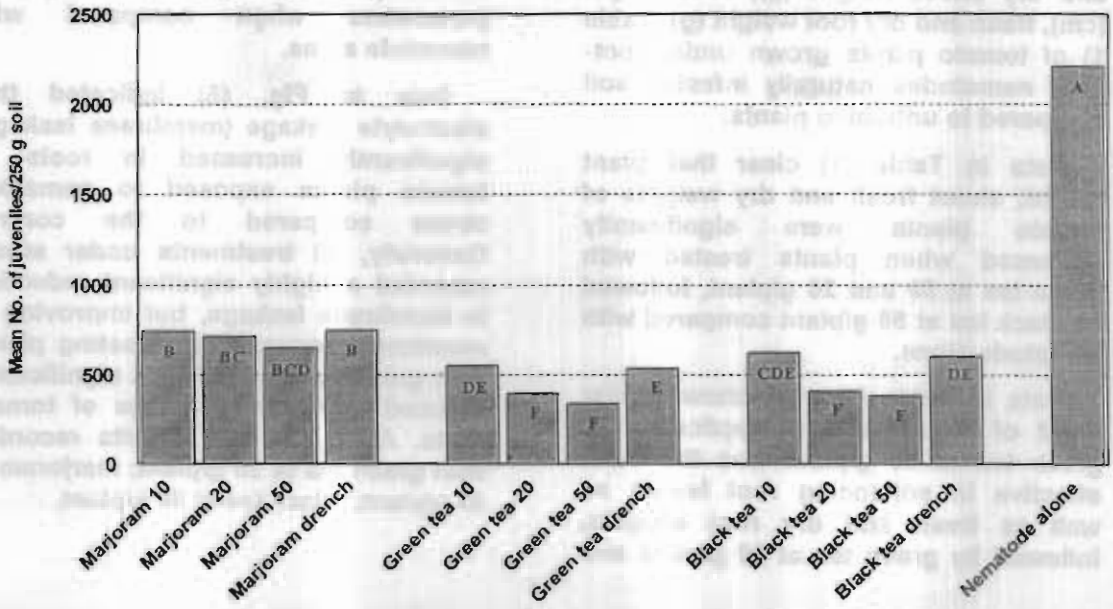

Figure (3): Effect of different organic wastes on number of *Meloidogyne* spp. juveniles/250 g soil. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

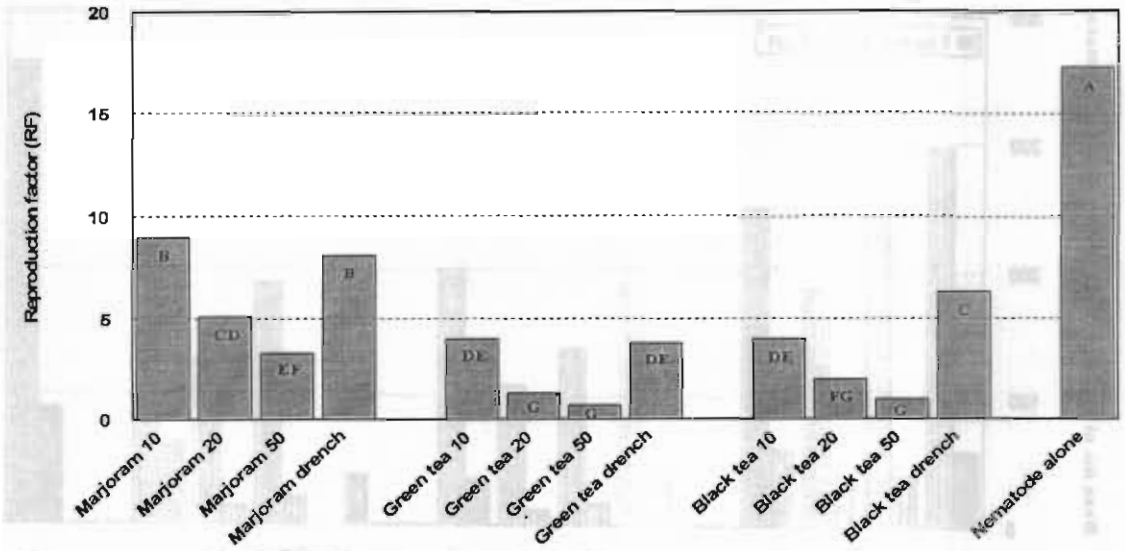

Figure (4): Effect of different organic wastes on the reproduction rate of *Meloidogyne* spp. in tomato roots. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at $p \leq 0.05$.

Results also showed that all applied organic wastes either as powder at three different rates or soil drenches, markedly enhanced the plant growth parameters i.e. plant height (cm), fresh and dry shoot weight (g); root length (cm), fresh and dry root weight (g) (Table 1) of tomato plants grown under root-knot nematodes naturally infested soil compared to untreated plants.

Data in Table (1) clear that plant height; shoot fresh and dry weights of tomato plants were significantly enhanced when plants treated with green tea at 50 and 20 g/plant, followed by black tea at 50 g/plant compared with nematode alone.

Data in Table (1) also show similar trend of results as the application of green tea at 50 g/plant was the most effective in enhancing root length as well as fresh and dry root weights, followed by green tea at 20 g/plant and black tea at 50 g/plant, respectively compared with nematode alone. Generally, results reported that all applied organic wastes as a powder at the three rates was positively effective in encouraging all the plant growth parameters when compared with nematode alone.

Data in Fig. (5) indicated that electrolyte leakage (membrane leakage) significantly increased in roots of tomato plants exposed to nematode stress compared to the control. Generally, all treatments under study, recorded a highly significant reduction in membrane leakage, but improving in membrane permeability. Treating plants with green tea at 50 g/plant significantly reduced membrane damage of tomato roots. Also, the best results recorded with green tea at 20 g/plant; marjoram at 50 g/plant; black tea at 50 g/plant.

Table (1): Effect of different organic waste applications on plant growth parameters of tomato plants grown under nematode naturally infested soil.

Treatments	Plant Height (cm)	Shoot Fresh weight (g)	Shoot Dry weight (g)	Root Length (cm)	Root Fresh weight (g)	Root Dry weight (g)
Marjoram 10 g +N	63.3 g	13.6 gh	0.79 h	13.2 gh	1.8 bc	0.08 cd
Marjoram 20 g +N	72.2 def	18.4 ef	1.02 g	15.0 fg	2.7 abc	0.17 b
Marjoram 50 g +N	73.8 de	21.0 de	1.14 f	16.0 ef	2.9 abc	0.18 b
Marjoram drench +N	63.0 g	13.0 gh	1.79 d	13.2 gh	1.8 bc	0.08 cd
Green tea 10 g +N	70.5 ef	16.6 fg	0.94 g	14.0 fg	2.4 abc	0.15 bc
Green tea 20 g +N	81.7 ab	26.6 abc	2.05 c	21.0 bc	4.0 ab	0.22 b
Green tea 50 g +N	82.4 ab	27.6 ab	2.40 b	23.0 ab	4.5 ab	0.36 a
Green tea drench +N	70.0 ef	16.0 fg	0.94 g	14.0 fg	4.5 ab	0.15 bc
Black tea 10 g +N	69.3 f	14.7 fg	0.82 h	13.8 fg	2.3 abc	0.08 cd
Black tea 20 g +N	76.3 cd	22.6 cd	1.60 e	18.0 de	3.0 abc	0.19 b
Black tea 50 g +N	79.5 bc	24.6 bcd	1.80 d	20.0 cd	3.8 ab	0.21 b
Black tea drench +N	69.0 f	14.0 gh	0.82 h	13.8 fg	2.3 abc	0.08 cd
Nematode alone	50.2 h	10.0 h	0.55 i	11.0 h	1.0 c	0.02 d
Control	85.6 a	30.0 a	2.75 a	24.8 a	5.0 a	0.40 a

* Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

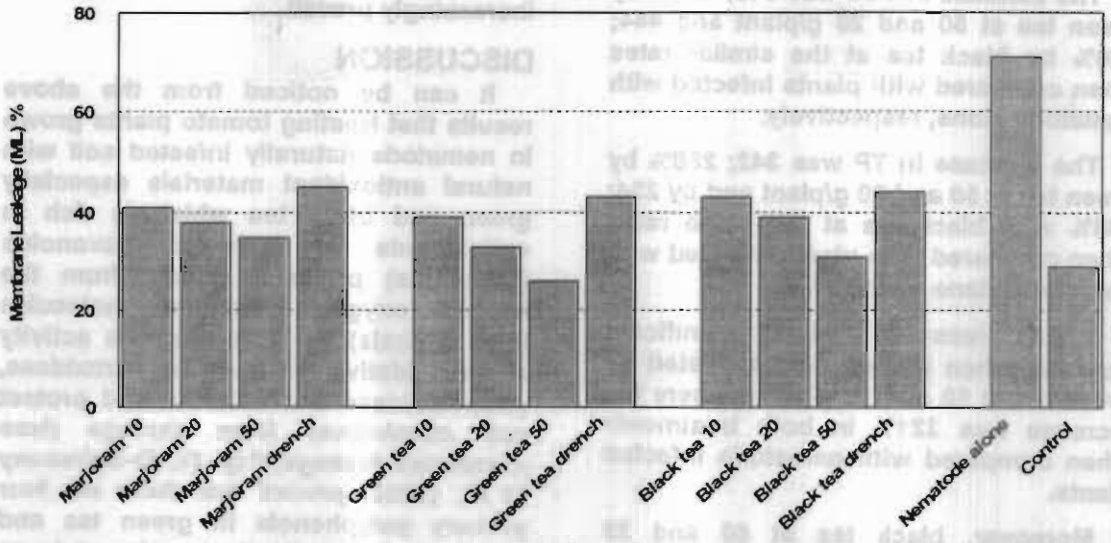

Figure (5): Effect of different organic wastes on membrane permeability (membrane leakage %) of tomato plant roots grown under nematode naturally infested soil. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

Data illustrated in Fig. (6) indicated that, tomato plants infected by root-knot nematodes, *Meloidogyne* spp. recorded highly significant depression in all tested antioxidative enzymes i.e. peroxidase, phenoloxidase and catalase. Whereas treating tomato plants by green tea at 50 g/plant recorded a highly significant induction in previous mentioned enzymes. These increases were estimated by 567, 184 and 128%, compared to the nematode alone treatment, respectively and by 240, 76 and 67% compared to the control plants. Moreover, green tea at 50 g/plant was the most effective in this respect followed by black tea at 50 and 20 g/plant followed by marjoram at 50 and 20 g/plant. Meanwhile, drench treatment of all materials was not effective in this respect. 50 and 20 g/plant50 and 20 g/plant.

Treating tomato plants by green and black tea at 50 and 20 g/plant recorded highly significant increases in TSS, TP and TFAA when compared either by plants infected by nematode alone or the control plants as shown in Fig. (7).

The increase in TSS was 545; 524% by green tea at 50 and 20 g/plant and 444; 386% by black tea at the similar rates when compared with plants infected with nematode alone, respectively.

The increase in TP was 342; 288% by green tea at 50 and 20 g/plant and by 234; 248% with black tea at the same rates when compared with plants infected with nematode alone, respectively.

TFAA recorded high significant increase when tomato plants treated by green tea at 50 and 20 g/plant, where the increase was 221% in both treatments when compared with nematode infected plants.

Moreover, black tea at 50 and 20 g/plant gave significant effect in inducing TFAA in nematode infected plants; the increase in TFAA was 197 and 135% when compared with the nematode infected plants, respectively. It can be mentioned that drench treatment either by green and black tea or marjoram had non-significant effect in this respect.

Data illustrated in Fig. (8, A) showed that tomato plants infected by nematode contained more numbers and big giant cells around the nematode head and small vascular tissues (xylem and phloem) when compared with the plants grown in healthy soil (Fig. 8, B). Meanwhile, plants treated with green tea at 50 g/plant showed a highly significant effect in reducing the nematode infection effects, very less number or no giant cells were shown in root section under this treatment as shown in Fig. (8, C), followed by black tea at 50 as shown in Fig. (8, D) and green tea at 20 g/plant (Fig. 8, E). The lowest effect was recorded with marjoram at 50 and 20 g/plant as shown in Fig. (8, F). Results showed that drench treatments with green and black tea as well as marjoram were not effective in this respect.

It can be noticed that as a parenchyma cell is transformed to a developing giant cell, the predominant control vacuole gradually disappears and small vacuoles increasingly prevail.

DISCUSSION

It can be noticed from the above results that treating tomato plants grown in nematode naturally infested soil with natural antioxidant materials especially green and black tea which is rich in antioxidants in form of flavonoids (Catechins) protected plants from the harmful oxygen containing molecules (free radicals) by increasing the activity of antioxidative enzymes i.e. peroxidase, phenoloxidase and catalase and protect cell membranes from damage (less membrane leakage, Fig. 5). El-Salmouny *et al.*, (2009) proved that there are four primary polyphenols in green tea and they are often collectively referred to as catechins and this reflect higher total phenols in infected plants treated with green and black tea.

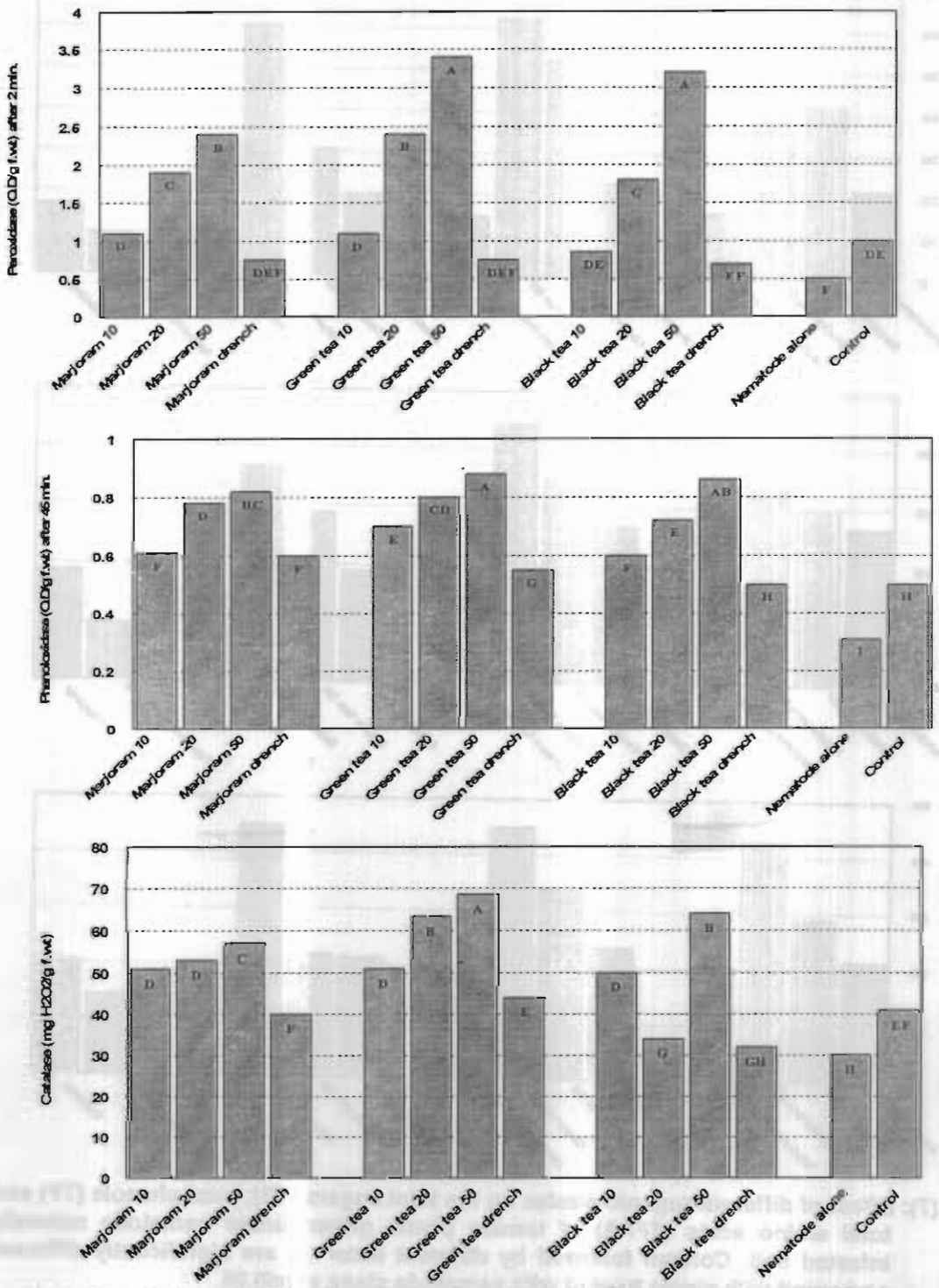

Fig. (6): Effect of different organic wastes application on the antioxidant enzymes activity of tomato plants grown under nematode naturally infested soil. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

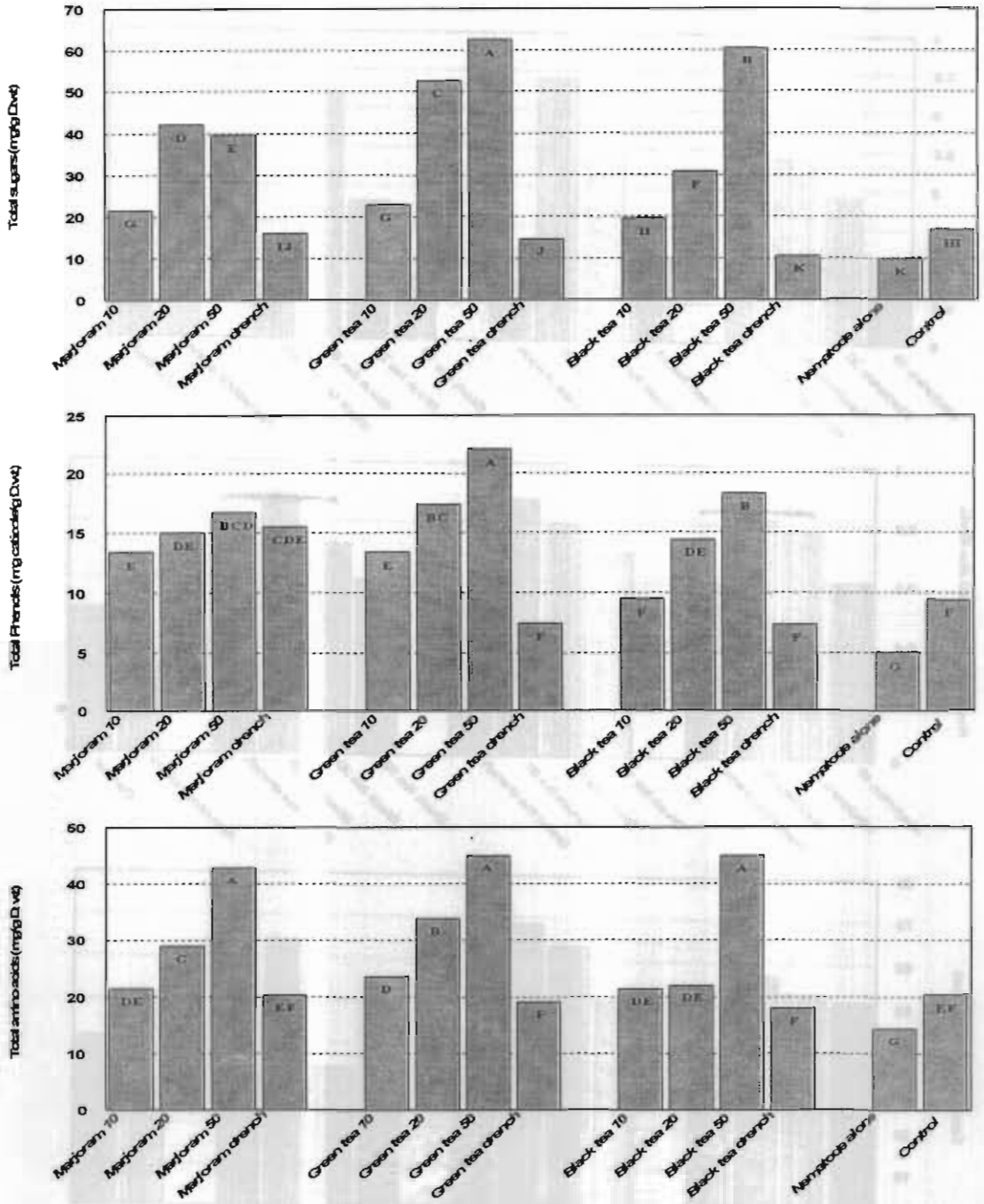

Fig. (7): Effect of different organic wastes on the total sugars (TS); total phenols (TP) and total amino acids (TFAA) of tomato plants grown under nematode naturally infested soil. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

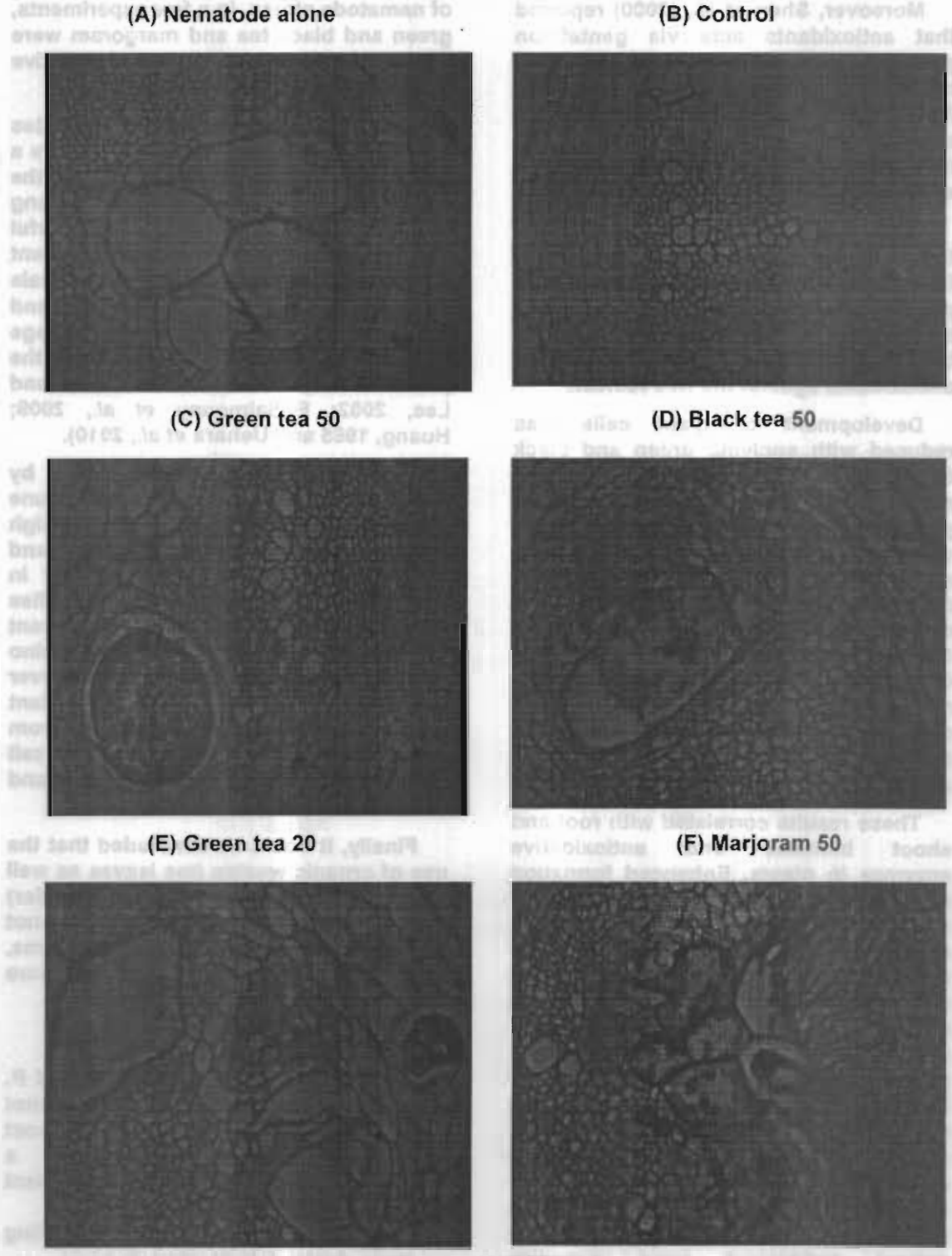

Figure (8): Effect of different organic wastes application on the development of giant cells (white arrow) of tomato roots infected with *Meloidogyne* spp (black arrow).

Moreover, Shen *et al.*, (2000) reported that antioxidants acts via gentathion peroxidase enzymes to protect lipid membranes from oxidant damage. Also Molinari and Miacola (1997) proved that antioxidant prevents propagation of free radical reactions (Ashour, 2009 and Yoda *et al.*, 2006).

Data of TSS, TP and TFAA indicated that, the plants treated with green and black tea showed a highly better performance as a reflection of increasing these components, this is may be due to the protection of plants by natural antioxidants against the free radicals.

Development of giant cells was reduced with applying green and black tea especially at the high rate 50 g/plant as tea contains several phenolic/polyphenolic components which are antioxidant in natural. Many studies have shown that tea possesses the ability to prevent oxidant induced cellular damage as reported by Lee and Sher, (1984); Matsuzaki and Hara, (1985). Graham (1992) and Alschuler (1998) who reported that green tea polyphenols have demonstrated significantly antioxidant and antimicrobial properties in numerous animal and *in vitro* studies.

These results correlated with root and shoot biomass and antioxidative enzymes in plants. Enhanced formation of reactive oxygen species (ROS) under stress conditions induced protective responses and cellular damage (Foyer and Noctor, 2005). The membrane damage and leakage of electrolytes due to the increase in O_2 and H_2O_2 concentrations which lead to lipid peroxidation (Uemura *et al.*, 2006; Hong-Bo *et al.*, 2008). The present results showed that nematode stress (biotic stress) cause a dramatic increase in electrolyte leakage in tomato roots indicating the occurrence of nematode stress. However application of some natural antioxidant plant materials reduced electrolyte leakage under nematode stress, indicating an alleviation of nematode stress. In a few experiments, green and black tea and margoram were also found to be effective preventative agent against the nematodes.

Green tea leaves contains flavonoides are nutrient antioxidants. Catechins are a type of flavonoides contained in the leaves of tea and are very strong antioxidants. These powerful antioxidants have been shown in recent studies to fight nematodes. Free radicals are highly reactive molecules and fragments of molecules that can damage the cell at the cellular level leaving the plants susceptible to diseases (Lee and Lee, 2002; El-Salmouny *et al.*, 2009; Huang, 1985 and Uehara *et al.*, 2010).

Treating infected tomato plants by green and black tea boosts the immune system because of the high concentrations of polyphenols and flavonoides. This is clearly noticed in anatomical and biochemical studies which recorded highly significant increase in total phenols, total free amino acids and total soluble sugars, moreover highly significant increase in antioxidant enzymes which protected plants from nematode infection or decrease giant cell formation (Williamson, 1998 and Williamson and Gleason 2003).

Finally, it could be concluded that the use of organic wastes (tea leaves as well as marjoram as a soil treatment powder) successfully controlled root-knot nematodes, and at the same time, encourage plants to produce much more of antioxidants enzymes.

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هل المخلفات العضوية تلعب دوراً هاماً في تقليل عشيرة نيماتودا تعقد الجذور؟

مجدى السيد مهدى[١] ، مرفت أدوارد سوريال[١] ، نيفين مجدى جلال[٢]

[١] قسم النبات الزراعى ــ كلية الزراعة ــ جامعة المنوفية ــ شبين الكوم ــ مصر

[٢] قسم بحوث النيماتودا ــ معهد أمراض النبات ــ مركز البحوث الزراعية ــ جيزة ــ مصر

الملخص العربى

في هذا البحث قد تم اختبار ثلاثة مخلفات عضوية وهى مخلفات البردقوش *Marjorana hortensis* والشاى الأخضر والشاى الأسود *Camellia sinensis* بثلاث جرعات وهى ١٠ ، ٢٠ ، ٥٠ جم/نبات وعلى صورتين الأولى على شكل بودرة جافة معاملة للتربة والثانية على شكل مستخلص مائى بعد نقع المخلفات في الماء لمدة ٢٤ ساعة وذلك لتقليل عشيرة نيماتودا تعقد الجذور *Meloidogyne* spp تحت ظروف حقل طماطم مصاب طبيعياً بالنيماتودا.

أثبتت النتائج أن كل المعاملات التي تم تطبيقها بجرعات مختلفة وبطرق إضافة مختلفة قد قللت معنويا من كل الصفات النيماتودية محل الدراسة متمثلة في عدد العقد النيماتودية وعدد أكياس البيض وعدد الاناث/ مجموع جذرى وعدد البيض/ كيس بيض وعدد الطور اليرقى الثانى/ ٢٥٠ جم تربه هذا بالإضافة إلى عشيرة النيماتودا النهائية ومعدل تكاثر النيماتودا وذلك مقارنة بالنباتات المعاملة بالنيماتودا فقط .

كذلك أظهرت النتائج أن إضافة الشاى الأخضر بمعدل ٥٠ جم/نبات كانت أكثر المعاملات فاعلية في تقليل عشيرة النيماتودا تلاها في التأثير الشاى الأسود بمعدل ٥٠ جم/نبات ثم الشاى الأخضر بمعدل ٢٠ جم/نبات. وكانت إضافة البردقوش بمعدل ١٠ جم/نبات من أقل المعاملات فاعلية في خفض عشيرة النيماتودا . وكذلك شوهدت نفس النتائج بالنسبة للصفات الخضرية مثل الوزن الطازج والوزن الجاف للمجموع الخضرى والجذرى وطول الجذر وطول النبات .

أشارت النتائج الى وجود تحفيز معنوى لبعض الصفات الكيماوية مثل نفاذية الجذور والسكريات الكلية والأحماض الأمينية الكلية والفينولات الكلية بالإضافة إلى أنزيمات الأكسدة مثل إنزيم البيروكسيديز والفينول أوكسيديز والكتاليز وذلك مقارنة بالنباتات المعاملة بالنيماتودا فقط أو النباتات الغير معاملة وكان أعلى تحفيز في الصفات الكيماوية قد شوهد مع إضافة الشاى الأخضر في صورة بودرة جافة بمعدل ٥٠ جم/نبات .

كذلك أظهرت النتائج التشريحية أن كل المعاملات المستخدمة قد حافظت على خلايا الجذور في الحالة العادية وقللت من تطور الخلايا العملاقة مقارنة بالنباتات المعاملة بالنيماتودا فقط .