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The Potential of Mustard as a soil biofumigant against *Meloidogyne javanica* in-vitro and *in-vivo* on tomato

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ABSTRACT

The nematicidal activity of mustard plant against hatching, migration and mortality of the root-knot nematode *Meloidogyne javanica* was investigated *in-vitro* and in-vivo on tomato. In vitro test found that mixing sandy clay soil with mustard at 4% as a biofumigant significantly reduced the percentage of egg hatching at all different incubation periods compared to control treatment. Laboratory results also led to high percentage of larval mortality at the different intervals periods tested. Laboratory results confirmed the highest reduction in egg hatching and larval mortality obtained after 48 hrs incubation period.

Application mustard at 5% either before one week or 48 hrs. of nematode inoculation was the most effective treatment that significantly reduced all nematode parameters comparing to the check. The percent of chemical components i.e. total sugars, total amino acids and total phenols were markedly enhanced compared to positive and negative control. The highest percentage was obtained with mustard at 5% one week before nematode inoculation by 68.7, 57.3 and 45%, respectively .

Key words: Biofumigation; in-vivo; in-vitro; Mustard; Root-knot nematode, Meloidogyne spp.; Tomato (*Lycopersicon esculentum* Mill).

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp. are obligate endo-parasites and very damaging plant pests and they have been considered to be a limiting factor in crop production and agricultural productivity (**Ibrahim, 2011**). Most cultivated plant species are susceptible to root-knot nematode infection (**Sasser and Carter, 1985**). They attack more than 2000 species of plants almost all cultivated plants such as vegetables, ornamentals and …etc (**Agrios, 1997)**.

In Egypt, root-knot nematodes, *Meloidogyne* spp. are becoming a serious pests to the most vegetable crops especially tomato plants and cause severe yield losses especially in light soils in new reclaimed lands and infected plants suffer from vascular damages which disturb water and mineral uptake (**Netscher and Sikora, 1990; Abd-Elgawad and Aboul-Eid, 2001; Luc *et al*., 2005).**

Chemical nematicides are considered the most effective method in suppressing and controlling root-knot nematodes, but for its environmental pollution and its expensive price (**Adegbite and Adesiyan, 2001; Abd-Elgawad, 2008**); during the last decades, nematologists worldwide search the cheaper, safer and eco-friendly alternatives methods i.e. biological and cultural methods to control the plant-parasitic nematodes.

Biofumigation and modified biofumigation are a sustainable strategy to manage soil-borne pathogens, nematodes, insects, and weeds instead of methyl bromide in developing countries including Egypt **(Salem, 2012 and 2014).**

Until recently, almost universally these harmful nematodes have been controlled by applications of broad-spectrum, synthetic soil fumigants (i.e., methyl bromide, metam sodium, and 1,3-dichloropropene). These synthetic soil fumigants are highly toxic to pests but also to many beneficial soil organisms (**Schreiner *et al*., 2001; Cox, 2006).** In addition, many of these conventional soil fumigants exhibit vertebrate toxicity and

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other damaging environmental effects **(Cox, 2006).** Together, these negative environmental and human health concerns have driven a search for more benign alternatives **(Martin, 2003).**

We face this ecological problem in Egypt. However, concerns about the negative impact of synthetic nematicides on the environment and on general public health led to a re-evaluation of these products. For example, high use of the soil fumigant, methyl bromide and resulting contamination of ground, surface and drinking water in the Netherlands led to a ban on its use in the 1980s. Later, methyl bromide was listed as an ozone depleting compound at the 4th meeting of the Montreal Protocol in Copenhagen, **1992 (Salem, 2012).**

Brassicaceae produce glucosinolates which are β-D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups). Glucosinolates, classified as aliphatic, aromatic or indole forms, occur in all parts of the plant and degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase, which is stored separately in the cell, to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs), **(Salem *et al.,* 2012a and b).**

The present research aimed to use the mustard plant powder as a biofumigant eco-friendly material to suppress and control root-knot nematode, *M. javanica* on tomato plants under laboratory and greenhouse conditions.

MATERIALS & METHODS

In Vitro Experiment

These experiment was carried out under laboratory conditions in 250 ml conical flasks contains 100 g of sandy/clay mixture soil (2:1; v:v) amended with 4 g mustard powder (4%) and covered with 20 ml tap water to enhance the decomposing of mustard in soil. The flask has two openings, one of them covered with rubber cover and aluminum foil as shown in Fig. (1). A rubber tube was connected from the other pore to another small 50 ml conical flask covered with aluminum foil to limit the evaporation as shown in Fig. (1).

Figure (1): Effect of soil amended with mustard on the percentage of egg hatching and larval mortality of *M. javanica* under laboratory conditions

The small flask contain either 500 eggs and/or larvae in 100 ml tap water to determine its effect on the percentage of egg hatching and larval mortality at different intervals incubation period 24; 48; 72; 96 and 168 hrs. Egg hatching and larval mortality was calculated in 50 eggs as well as 50 larvae under stereomicroscope at magnification 100X.

In Vivo Experiment

Mustard (*Sinapis nigra*) as a powder was used in this study and mixed well with soil pots at three different doses i.e. 3%; 5% (before 48 hrs. and one week of nematode inoculation); and 7% (w/w). All doses were applied 48 hrs. before nematode inoculation, except 5% doses as it applied 48 hrs and one week before nematode inoculation. The mixture of sandy/clay soil amended with mustard powder at different doses was filled into plastic pots (15 cm in diam.). Three weeks-old tomato seedlings (*Lycopersicon esculentum* Mill cv. GS) were transplanted into pots (one plant/pot).

Pure culture of *M. javanica* was established from single egg masses on tomato plants under greenhouse conditions at 25±2ºC. Nematode species was identified according to the morphological characteristics of the female perineal patterns (**Taylor and Sasser, 1978**). Root-knot nematode eggs were extracted from heavily galled roots by using 1.5% sodium hypochlorite solution (NaOCl) technique as described by **Hussey and Barker (1973).** Two thousand nematode eggs were pipetting into three holes made around tomato root zone at the same time of transplanting, except the treatment of 5% one week before nematode inoculation. Each treatment replicated three times and the non-treated plants were served as control. Plants were arranged in a completely randomized block design in the greenhouse at approximately 25±2ºC. Plants were watered daily and fertilized weekly with 5 ml of 2 g/l N:P:K (20:20:20), International Egypt Company for Agricultural and Industrial Developing.

Two months after nematode inoculation, nematode and growth parameters were recorded. The recorded nematode parameters were: numbers of galls; gall index; number of egg masses/root system as well as number of juveniles in soil pots (**Goodey, 1957**). Root galling was estimated according to **Taylor and Sasser (1978)** whereas :

0= no galls or egg mass 1= 1-2 galls or egg mass 2= 3-10 galls or egg mass 3= 11-30 galls or egg mass 4= 31-100 galls or egg mass 5= more than 100 galls or egg mass. Egg-masses were stained prior to counting by dipping the infected roots in phloxine-B solution (0.015%) for 20 minutes as described by **Daykin and Hussey (1985).**

The determined growth parameters were: shoot and root length (cm), fresh shoot and root weights (g) as well as dry weight (g). Total amino acids (TAA) were determined in dry leaves following the method described by **Rosen (1957)** and total sugars were also estimated as described by **Dubois *et al*., (1956).**

Gas chromatography/mass spectrometry analysis (GC/Mass)

The mustard plant material, air-dried at room temperature for about one week, was subjected to hydrodistillation for 4 h according to the standard method using a Clevenger-type distillation apparatus (**Traboulsi *et al.,* 2002).** Plant components were determined by gas chromatography (GC) (Hewlett-Packard) coupled to an HP 5871A mass spectrometer detector and equipped with an on column DBI (30 m_0.20_0.05 µm). The temperature programme consisted of an initial temperature of 53ºC; hold 3 min-1; ramp rate 3ºC min-1, final temperature 220ºC; hold 65 min-1; column flow rate 0.6 ml d'He/mi constant. The injection temperature was 200ºC with an injection volume of 2 µl/min. The mass spectrometer settings were: electron impact ionization mode with 70 eV electron energy, scan mass range *m/z* 50–400. Detection temperature was 276ºC using the retention time and peak area as a mean of measure. Components were identified by comparing the GC retention and mass spectra with those reported in the literature. Pure essential oils of commercialorigin were kindly supplied by Jean-Marie Bessiere (Ecole Nationale Supérieure de Chimie de Montpellier, France). Each oil was separated from water with a Pasteur pipette, dried by filtration over anhydrous sodium sulphate and stored at -20°C in a sealed dark bottle until analysis. The Isothiocyanates yield (Table 1) was calculated relative to the mass of dry plant material

Table (1): Isothiocyanates, origin, structure, molecular weight, and concentration of isothiocyanates from *Sinapis alba*.

Major components of Isothiocyanates	Concentration ppm.	Structure of side chain R	Molecular weight
Lucanine 2	14.3	C27H30O16	440
-12octadeca dienoic acid,(Z) -2,3-bis[trimethyl silyl) oxy] proplyl ester	12.7	C27H45O4SI2	498
-15Hexa deca methyl-octasiloxane	12.3	C16H50O7SI8	578
-13teradeca methyl-Hepta siloxane	10.4	C14H44O6SI7	504
-11Dodecamethyl-Hexa-siloxane	9.2	C12H38O5SI6	430
-15octadeca trienoic acid,2,3-bis(tri methyl silyl) propyl ester,(z)	8.5	C27H52O4SI2	496
Ethyl isoallocholate	8.4	C26H44O5	436

Statistical Analysis

Data were statistically analyzed according to standard analysis of variance by a one way ANOVA with the software stat graphics (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.

RESULTS

In Vitro Experiment

Laboratory results revealed that the nematode eggs incubated in water and exposed to sandy/clay soil mixture amended with 4% mustard Fig. (1) was significantly reduced egg hatching of *M. javanica* at all intervals incubation period 24; 48; 72; 96 and 168 hrs. compared to control (mustard non-amended mixture soil) as illustrated in Fig. (2A). Results indicate that the percentage of egg hatching reduction was 88.5; 90; 81.4; 74 and 69.4%, respectively

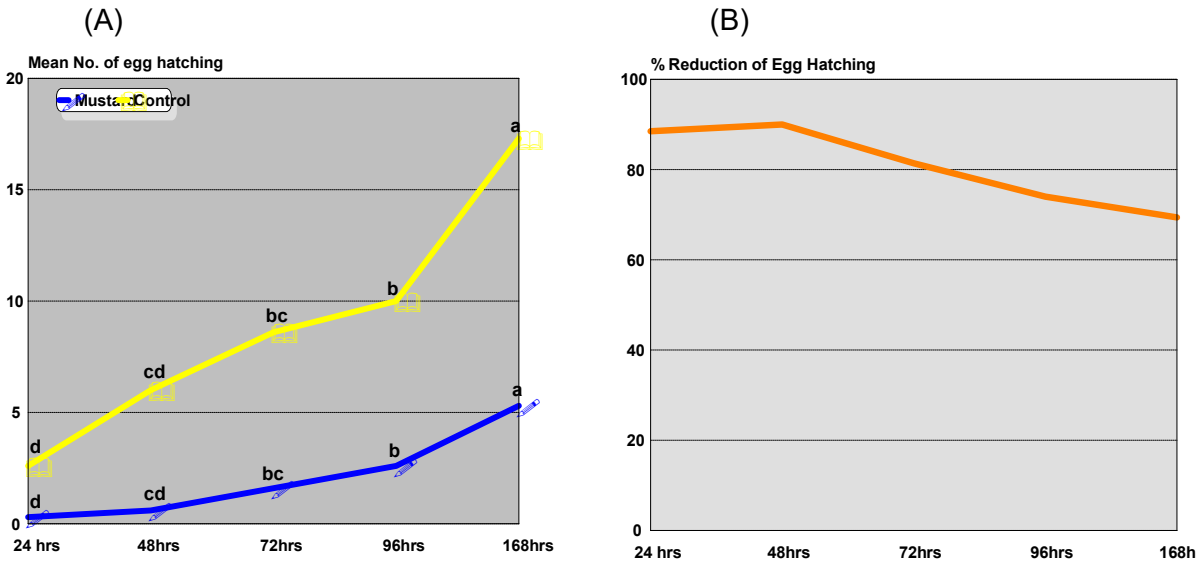

Figure (2): Effect of soil amended with mustard on the mean number (A) and percentage of egg hatching reduction (B) of *M. javanica* under laboratory conditions.

Results observed also that mustard amended with soil as a biofumigant led to high larval mortality at the different intervals incubation periods when compared to control as shown in Fig. (3A). The percentage of larval mortality recorded 94; 100; 90.5; 90.5 and 79.4%, respectively compared to control as shown in Fig. (3B). Laboratory results confirmed that at the incubation period of 48 hrs recorded the highest reduction in egg hatching the highest larval mortality.

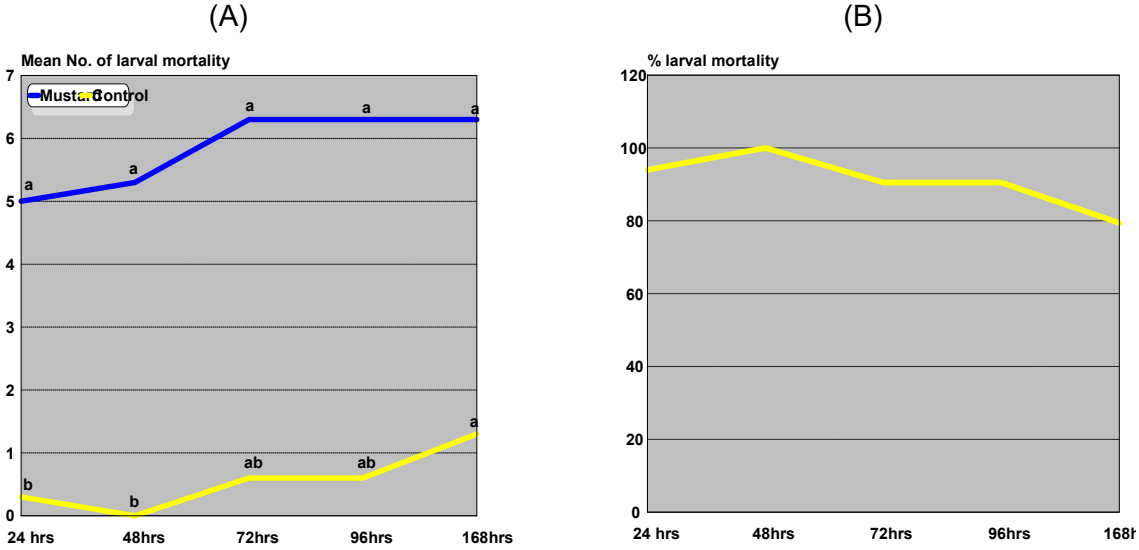

Figure (3): Effect of soil amended with mustard on the mean number (A) and percentage of larval mortality (B) of *M. javanica* under laboratory conditions.

In Vivo Experiment

Results of *in vivo* experiment revealed that the incorporation of soil pots with mustard powder at all different doses 3%, 5% (48 hrs and one week before nematode inoculation) and 7% of soil weight significantly reduced all related nematode parameters compared to treated plants with nematode alone. All nematode parameters i.e. number of galls/root system, root galling index, number of egg masses/root system as well as number of juveniles/250 g soil showed high reduction with mixing the soil pots with mustard at 5% one week before nematode inoculation

followed by 5% before 48 hrs nematode inoculations.

The maximum percentage of galls reduction was 96.8 and 96.7%, respectively, whereas the lowest reduction percentage of galls obtained at 7 and 3% by 90.4 and 90.5% as shown in Fig. (4).

Egg masses showed the same trend of results as mixing the soil pots with mustard at 5% either before one week or 48hrs was the most effective one in reducing the mean number of egg masses. The percentage of reduction recorded 97.4 and 95.9%, respectively as shown in Fig. (4). the lowest effect observed with the application dose 7% followed by 3% by 90.8 and 92.8%, respectively.

As a result to galls reduction, the root galling indices was significantly reduced at all used doses and application time compared to control as shown in Fig. (5).

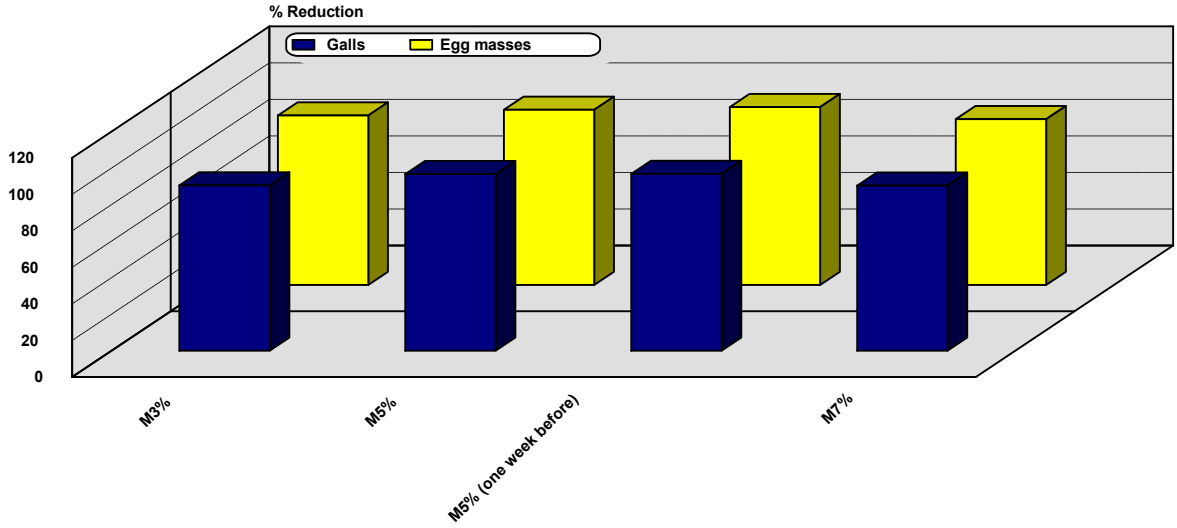

Figure (4): Percentage of galls and egg masses reduction of *M. javanica* in tomato roots grown in soil amended with mustard at different doses and application time

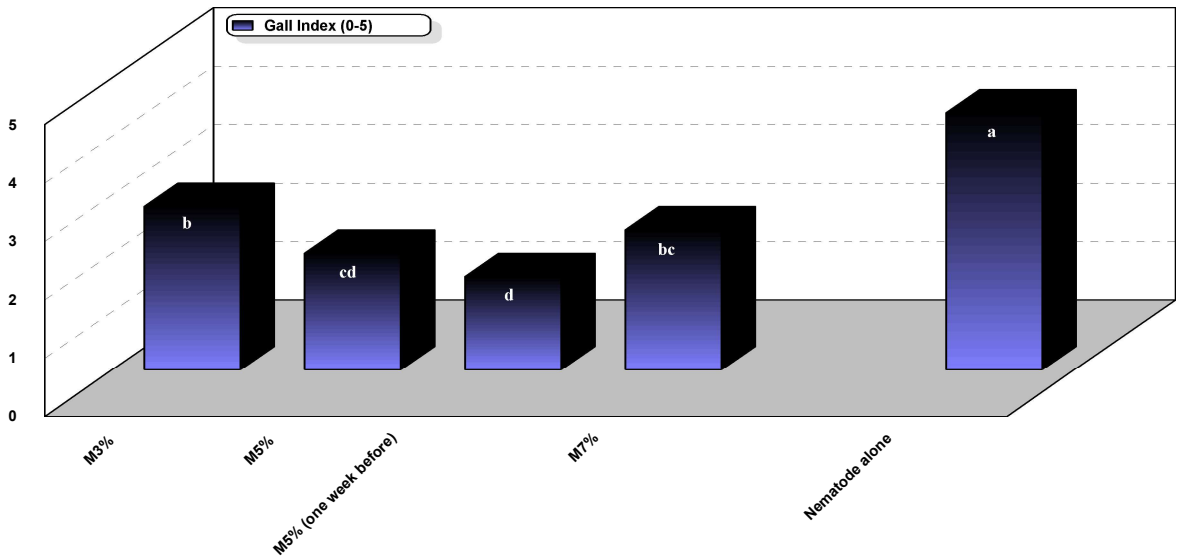

Figure (5): Root galling indices (0-5) of *M. javanica* as affected by amending soil with mustard at different doses and application time on tomato roots.

Soil amended with mustard at all tested doses appeared good results in suppressing nematode larvae, compared to mustard non-treated plants. Application mustard at 5% either before one week or 48 hrs was the most effective treatment. The percentage of reduction recorded 93.2 and 80%, respectively as shown in Fig. (6). Application of mustard at 3% was the lowest one by 66.5%.

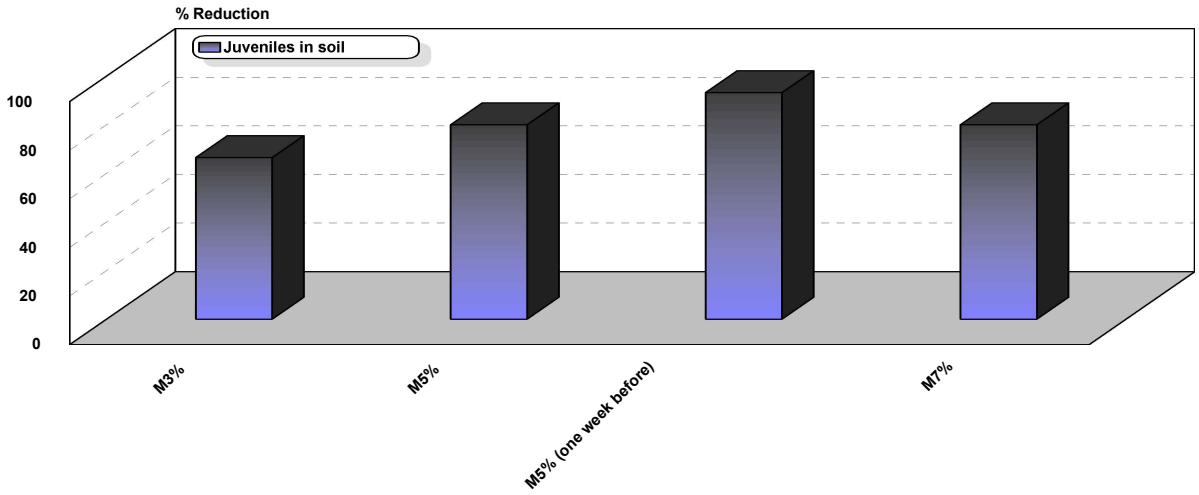

Figure (6): Percentage of reduction of *M. javanica* larvae in soil amended with mustard a different doses and application time.

The chemical components i.e. total sugars, total amino acids and total phenols were enhanced with all doses of mustard applied compared to plants treated with nematode alone as shown in Fig. (7).

Amending the mustard at 5% one week before nematode inoculation with soil pots encouraged the percent of all the chemical components compared to all the other treatments

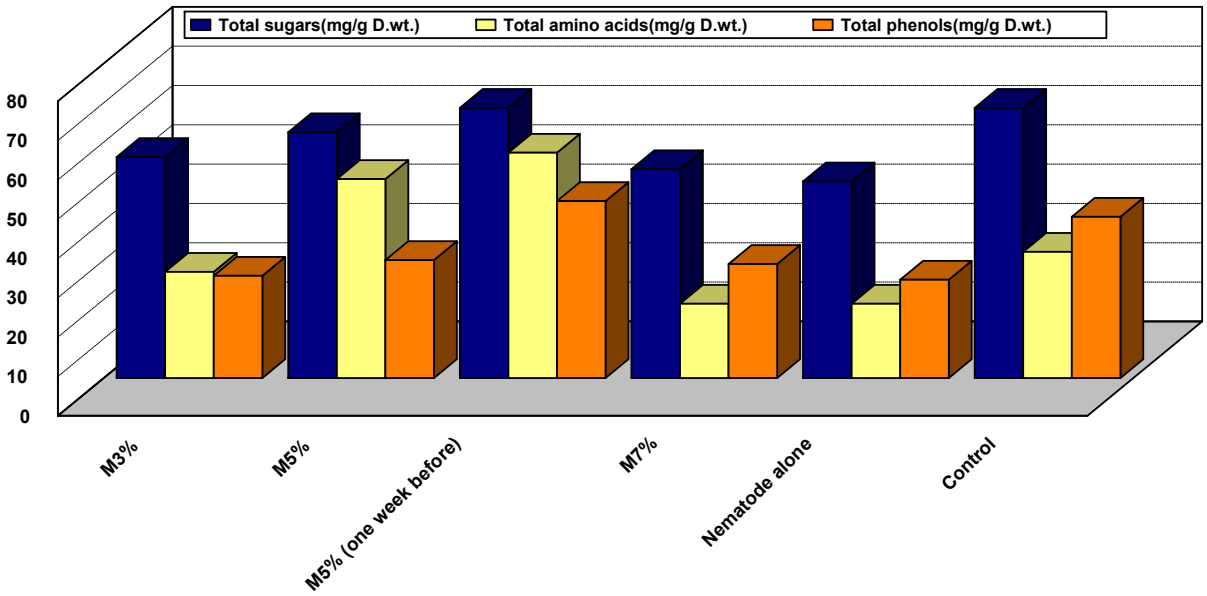

Figure (7): Effect of soil amended with mustard at different doses and application time on the percentage of chemical components in tomato plants infected with *M. javanica*.

DISCUSSION

The continuous use of chemical nematicides to control root-knot nematode

has had considerable environmental impact, and has resulted in the onset of resistance phenomena within some populations of nematode pests. This situation has led to an increased demand for environmentally-friendly products in order to reduce the effects of widespread nematicides utilization in crop protection (Salem, 2012; Salem et al.2012a; b). The use of natural products together with chemical nematicides at low dosage in the framework of integrated pest management programs could achieve the aims of reducing costs and limiting the environmental impact of crops. Several studies using natural products have demonstrated the possibility of their use to control pests and diseases. In the present study, the effects of a natural formulation on isothiocyanates were investigated. The present results revealed that, soil amended with mustard at all tested doses appeared good results in suppressing nematode larvae compared to mustard non-treated plants. Application mustard at 5% either before one week or 48 hrs was the most effective treatment. The formulation (Salem et al., 2012a), used at the dose of 2% emulsion in water, was obtained from vegetable oils of Brassica carinata added to meal obtained from the same species and Arabic gum. The meal contains glycosidic compounds whose enzymatic hydrolysis degradation products (isothiocyanates and nitriles) are well-known for their high cytotoxic activity (Lazzeri et al., 2004; Marciano et al., 2004). The experiments reported here were carried out in 2013 and under laboratory and field conditions (Personal communications).

This is consistent with glucosinolates, or their toxic breakdown products, acting as antagonists to nematodes (Zasada and Ferris, 2004; Salem et al.2012b.). Second, root-knot nematode infectivity was greatly affected (greenhouse experiment), and harmed, by the soil-incorporation of mustard, indicating that EPN infectivity was strongly impacted by the addition of mustard plant biomass. Thus, mustard green manures is powerful harmful to root-knot nematode.

Egyptian governments as well as other developing countries have restricted the use of synthetic soil fumigants such as methyl bromide, metam sodium, and 1, 3-dichloropropene, due to these chemicals' substantial environmental and human health risks (Salem, 2012). These concerns have led to an ongoing search for effective alternatives. In addition to Brassica and Sinapis mustard species, Sudan grass (Mojtahedi et al., 1993; Salem et al., 2012a; Salem et al., 2012b).

Mustards have been particularly attractive bio-fumigant candidates because of the broad activity of their toxic breakdown products against a range of soil pests (Brown and Morra, 1995; Kirkegaard et al., 1996; Zasada and Ferris, 2004). Biologically-active compounds are retained in waste-products following conversion of mustard seed to biofuels, forming an inexpensive and likely growing source of these soil amendments (Cohen and Mazzola, 2004).

The nematicidal effect of the tested mustard may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock et al.,1989; Salem, 2012a). The present in vitro study found that some of these medicinal plants extract were very effective against one or both nematodes at relatively low concentrations. The most promising extracts, their mode of action, and the effect of combinations of volatile oils. In vitro assay Isothiocyanates are released through enzymatic degradation of glucosinolates are effective on developmental stages of (RKN), and unaffected medicinal plant extracts on developmental of Meloidogyne. spp shown data. Addition, field applications of promising extracts should be conducted to verify their nematicidal effectiveness .

Biofumigation is the practice of using volatile chemicals released from decomposing plant material to suppress soil pathogens, nematodes, insects and germinating weed seeds. Brassicas are

mainly used for biofumigation. The decomposition of the plant tissues in these families releases isothiocyanates which are biocidal. Plants have different profiles of isothiocyanates, and stressing the plants increases the amount of isothiocyanates produced by mustard. Biofumigation has been used as an alternative to methyl bromide and other synthetic pesticides in horticulture and agriculture in general. It has also been used to reclaim soils infested with root-knot nematode. It is eco-friendly and adds organic matter to the soil. There is potential for this technique to be adopted in Egypt by mustard incorporation in soils and compost and horticulture farmers involved in organic farming and as a stored pest management technique. Finally, we are willing to put the recommendation of these results into practices; we should have an effort to educate Egyptian farmers about biofumigation since most farmers are not aware of this technique. There is a great need for local research into brassica that can be used for biofumigation. We adopted new and innovative technologies for a modified biofumigation that can suite farmers all over the world even in Africa, Europe, and Asia and taking into consideration the soil types. There is great need also to research on methods of incorporating the biofumigant plants into the soil as well as breeding for Brassica with high isothiocyanates content is an important demand nowadays.

REFERENCES

Abd-Elgawad, M. M. and Aboul-Eid, H. Z. (2001). Effects of oxamyl, insect nematodes and Serratia marcescens on a polyspecifc nematode community and yield of tomato. Egyptian Journal of Agronematology 5:79-89.

Abd-Elgawad, M. M. M. (2008). The current status of phytonematode management in Egypt with special reference to applicable nematicides. Egyptian Journal of Agronematology 6:33-46.

Adegbite, A. A. and Adesiyan, S. O. (2001). Efficacy of carbofuran on the performance of four nematode susceptible varieties of soybean (Glycin max (L.) Merrill). Tropical Oil Seeds J., 6: 11-23.

Agrios, G. N. (1997). Plant pathology, Academic press, New York, pp: 565-577.

Brown, P. D., Morra, M.J., (1995). Glucosinolate-containing plant tissues as bioherbicides. Journal of Agricultural and Food Chemistry 43, 3070–3074.

Cohen, M. F., Mazzola, M., (2004). A reason to be optimistic about biodiesel: seed meal as a valuable soil amendment. Trends in BioTechnology 22, 211–212.

Cox, C., (2006). Fumigant factsheet: metam sodium. Journal of Pesticide Reform 26, 12–16.

Daykin, M. E. and Hussey, R. S. (1985). Staining and histopathological techniques in nematology. Pp. 39-48 in Barker, K. R.; Carter, C. C. and Sasser, J. N., Eds. An advanced treatise in Meloidogyne, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Dubois, M., Gilles, A., Hamilton, J. K., Robers, P. A. and Smith, P. A. (1956). A colorimetric method for determination of sugar and related substances. Annal. Chem., 28: 350-356.

Duncan, B. (1955). Multiple ranges and multiple F. test. Biometriex, 11: 1-42.

Goodey, J. B. (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No.2, Min. Agric. Fish, Ed. London pp 47.

Ibrahim, I. K. A. (2011). Nematode Pests Parasitic on Agricultural Field Crops. Manshaat El-Maaref, Alexandria, 250pp.

Kirkegaard, J.A.,Wong, P.T.W., Desmarchelier, J.M., 1996. In vitro suppression of fungal root pathogens of cereals by Brassica tissues. Plant Pathology 45, 593.603

Knoblock, K., Weis, N., Weigant, R., (1989). Mechanism of antimicrobial activity of essential oils. 37th Ann. Cong. Med. Plant Res., Braunschweig, pp. 5–9.

Lazzeri L., G. Curto, O. Leoni and E. Dalla Valle, (2004). Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitwood. Journal of

Agricultural and Food Chemistry 52, 6703-6707.

Luc, M.; Sikora, R. A. and Bridge, J. (2005). Plant parasitic nematodes in subtropical and tropical agriculture. Pp. 871, 2nd Eds. CAB International, Wallingford, Oxon, UK.

Marciano P., M. Benetti, S. Odorizzi, L. Malaguti and L. Lazzeri, (2004). In vitro effects of glucosinolate degradation products on Sclerotinia spp. and Coniothyrium minitans. In : Proceedings of the First intErnational Symposium on "Biofumigation as Possible Alternative to Methyl Bromide". March 31 - April 1, 2004, Florence, Italy.

Martin, F.N., (2003). Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. Annual Review of Phytopathology 41, 325–350.

Mojtahedi, H.; Santo, G. S. and Ingram, R. E. (1993). Suppression of Meloidogyne chitwoodi with sudangrass cultivars as green manure. Journal of Nematology 25: 303–311.

Netscher, C., and R. A. Sikora. (1990). Nematode parasites of vegetables. Pp237-284 in M. Luc, R. A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agricultural. CAB International, Wallingford, UK.

Norton, D. C. (1978). Ecology of Plant Parasitic Nematode. John Willey and Sons, New York, p. 238.

Rosen, H. (1957). A modified ninhydrin colourimetric analysis for acid nitrogen. Arch. Biochem. Biophys., 67: 10-15.

Salem, M. F. (2012) . The Potential of Biofumigation in Solving Air Pollution in Developing Coutries. In D.G. Steyn and S. Trini Castelli (eds.), Air Pollution Modeling and its Application XXI, NATO Science for Peace and Security Series C: Environmental Security 4, DOI 10.1007/978-94-007-1359-8_124, Springer Science Business Media B.V. 2012.

Salem, M.F. (2014). Innovative approaches through modified biofumigation in controlling soil-borne pathogens and root-knot nematode. Aspects of Applied Biology 126, 5th International Symposium of Biofumigation, UK.

Salem, M. F.; Osman, Gamalat Y.; Hasab El–Nabi, S. E.; and Khalaf, Fatama M. A. (2012a). Effect Of Brassicous Natural Products on Meloidogyne spp. Management In Vitro. Egyptian Journal of Plant Disease and Plant Protection.

Salem, M. F., Osman, Gamalat Y,Hasab El – Nabi S. E., and Khalaf, Fatama M. A. (2012b). Effect Of Brassicaceous Natural Products on Meloidogyne spp. Management Under Greenhouse Conditions. Egyptian Journal of Plant Disease and Plant Protection.

Sasser, J. N. and Carter, C. C. (1985). An Advanced Treatise on Meloidogyne, Biology and Control. Vol I. North Carolina State University Graphics, 422 pp.

Schreiner, R.P., Ivors, K.L., Pinkerton, J.N., (2001). Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. Mycorrhiza 11, 273–277.

Taylor, A. L. and Sasser, J. A. (1978). Biology, identification and control of root-knot nematodes (Meloidogyne species). North Carolina State Univ. Graphics, USA. 111 pp.

Traboulsi, A. F., Taoubi, K., El-Haj, S., Bessiere, J. M., Rammal, S. (2002). Insecticidal properties of essential plant oils against the mosquito Culex pipiens molestus (Diptera: Culicidae). Pest Manage Sci, 58 (5), pp. 491–495.

Zasada, I.A., Ferris, H., (2004). Nematode suppression with brassicaceous amendments: application based upon glucosinolate profiles. Soil Biology and Biochemistry 36, 1017–1024.