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Suppression of root-knot nematode through innovative mustard biofumigation

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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 test confirmed that mixing the sandy clay soil mixture with mustard as 4% as a biofumigant significantly reduce the percentage of egg hatching at all different incubation periods 24, 48, 72, 96 and 168 h, compared to control treatment (un-amended mixture soil and eggs in free water). Results indicate that the percentage of egg hatching reduction was 88.5, 90, 81.4, 74 and 69.4%, respectively. Mustard mixed with soil as a biofumigant led to high percentage of larval mortality at the different intervals periods in vitro. The percentage of larval mortality was 94, 100, 90.5, 90.5, and 79.4%, respectively compared to control. Laboratory results confirmed that the highest reduction in egg hatching and larval mortality was obtained after incubation period for 48 h. In vivo experiment reveals that the incorporation of the soil pots with mustard at all different doses used 3, 5% (48 h before nematode inoculation, or soil infestation with nematode), and 5% (one week before nematode inoculation or 7% of soil weight) significantly reduces all the nematode parameters compared to plant treated nematode alone. All nematode parameters i.e. the number of galls per root system, gall index, number of egg masses per root system, as well as number of juveniles per 250g soil showed high reduction with mixing the soil pots with mustard at 5% (one week before nematode inoculation), followed by the same treatment for 48h before nematode inoculation. Mustard application, one week before nematode inoculation, reduced the nematode parameters by 97, 64, 97, and 93%, respectively, compared to control. 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. Finally, we have to conclude that this modified technology is an innovative and can be used efficiently to control Root-knot nematode under organic agriculture and Global GAP agricultural systems instead of these carcinogenic nematicides.

Introduction

Root-knot nematodes, Meloidogyne spp. are obligate endo-parasites and very damaging plant pests which are 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 and almost all cultivated plants such as vegetables, ornamentals. In Egypt, root-knot nematodes, Meloidogyne spp. are becoming serious pests to

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most vegetable crops, especially tomato plants, and cause severe yield losses in new reclaimed soils, especially the light types 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 controling root-knot nematodes, but means environmental pollution and is expensive in price (Adegbite and Adesiyan, 2001; Abd-Elgawad, 2008). During the last decades, nematologists worldwide searched for 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; Salem, 2014, Salem et al., 2015). Recently, these harmful nematodes have been controlled using 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 as well as many beneficial soil organisms (Schreiner et al., 2001; Cox, 2006). In addition, many of these conventional soil fumigants exhibit vertebrate toxicity and other damaging environmental effects (Cox, 2006). Together, these negative environmental and human health concerns have driven a search for more benign alternatives (Martin, 2003). Egypt faces this ecological problem. However, many have concerns about the negative impact of synthetic nematicides on the environment and general public health require a re-evaluation of these products. For example, the 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 (Salem, 2012; Salem et al., 2015).

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; Salem et al., 2012b). This investigation 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.

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

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Materials and methods

In Vitro Experiment

This experiment was carried out under laboratory condition in 250 ml conical flasks contains 100g of sandy/clay mixture soil (2:1; v:v) amended with 4 g mustard powder (4%) and covered with 20ml 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. A rubber tube was connected from the other pore to another small 50ml conical flask covered with aluminum foil to limit the evaporation (Figure 1). The small flask contain either 500 eggs and/or larvae in 100ml 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 h. 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 and mixed well with soil pots at three different doses i.e. 3%, 5% (before 48 hr and one week of nematode inoculation) and 7% (w/w). All doses were applied 48h before nematode inoculation, except 5% doses as it applied 48h 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 (NaClO) technique as described by Hussey and Barker (1973). Two thousand nematode eggs were pipetting into three holes made around the 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).

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 (Rosen et al., 1957) and total sugars (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 4h 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 3°C 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 commercial origin were kindly supplied by Jean-Marie Bessiere (Ecole Nationale Supérieure de Chimie de Montpellier, France). Each oil was sepa-

Table 4: Major components 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	$C_{27}H_{30}O_{16}$	440
12-octadeca dienoic acid,(Z) -2,3-bis(trimethyl silyl) oxy) proplyl ester	12.7	$C_{27}H_{45}O_4S_{I2}$	498
15-Hexa deca methyl-octasiloxane	12.3	$C_{16}H_{50}O_7S_{I8}$	578
13-teradeca methyl-Hepta siloxane	10.4	$C_{14}H_{44}O_6S_{I7}$	504
11-Dodecamethyl-Hexa-siloxane	9.2	$C_{12}H_{38}O_5S_{I6}$	430
15- octadeca trienoic acid,2,3- bis(tri methyl silyl) propyl ester,(z)	8.5	$C_{27}H_{52}O_4S_{I2}$	496
Ethyl isoallocholate	8.4	$C_{26}H_{44}O_5$	436

rated 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.

Statistical Analysis: Data were statistically analyzed according to a 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/

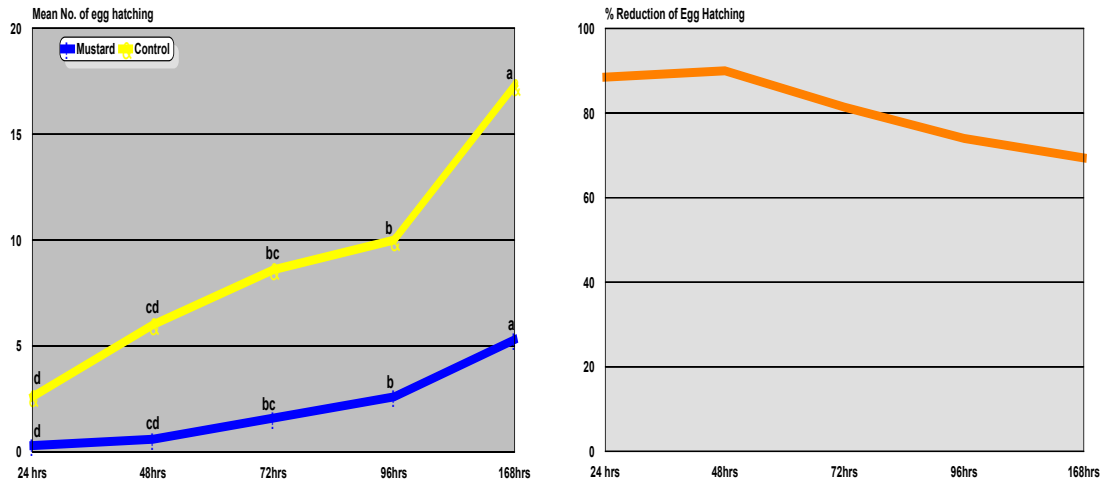

Figure 2: Effect of soil amended with mustard on the mean number (A) and percentage of egg hatching reduction (B) of M. javanica under laboratory condition

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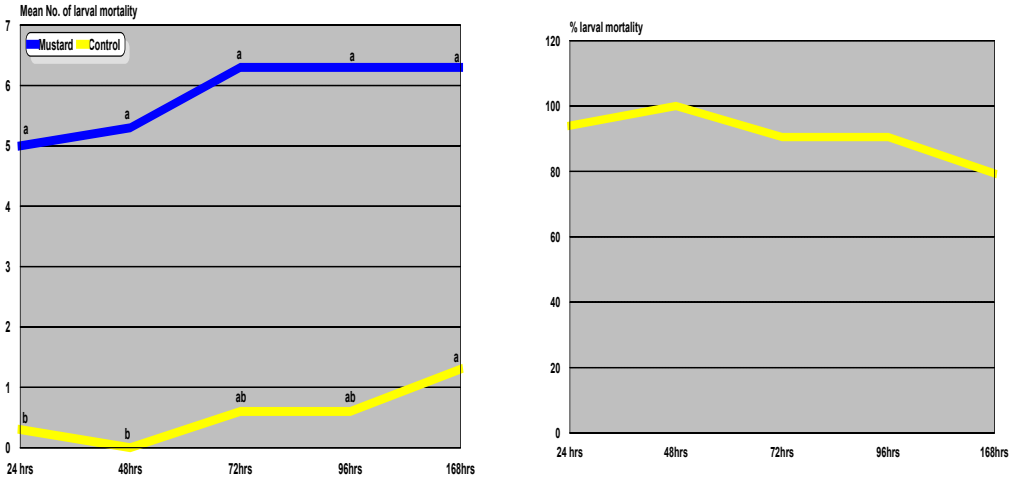

Figure 3:): Effect of soil amended with mustard on the mean number (A) and percentage of larval mortality (B) of M. javanica under laboratory condition

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 h, compared to control (Fig. 2A). Results indicate that the percentage of egg hatching reduction was 88.5, 90, 81.4, 74 and 69.4% respectively.

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 (Fig. 3A). The percentage of larval mortality recorded 94, 100, 90.5, 90.5 and 79.4%, respectively compared to control (Fig. 3B). Laboratory results confirmed that at the incubation period of 48h recorded the highest reduction in egg hatching the highest larval mortality.

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 h 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 48h 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

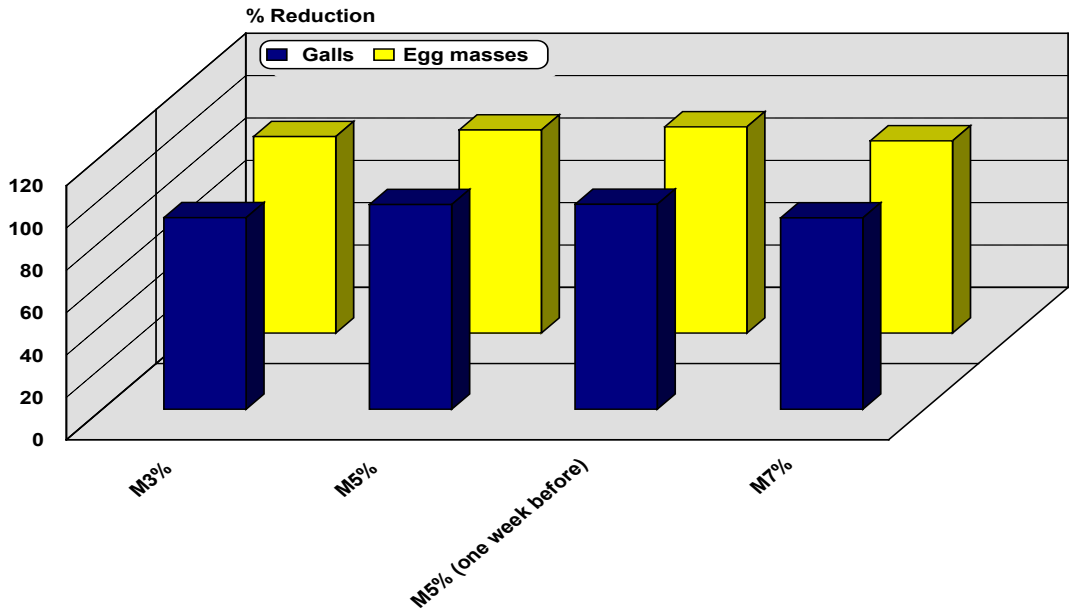

Figure 4: Percentage of galls and egg masses reduction of M. javanica in tomato roots grown in soil amended with mustard at different doses and application time

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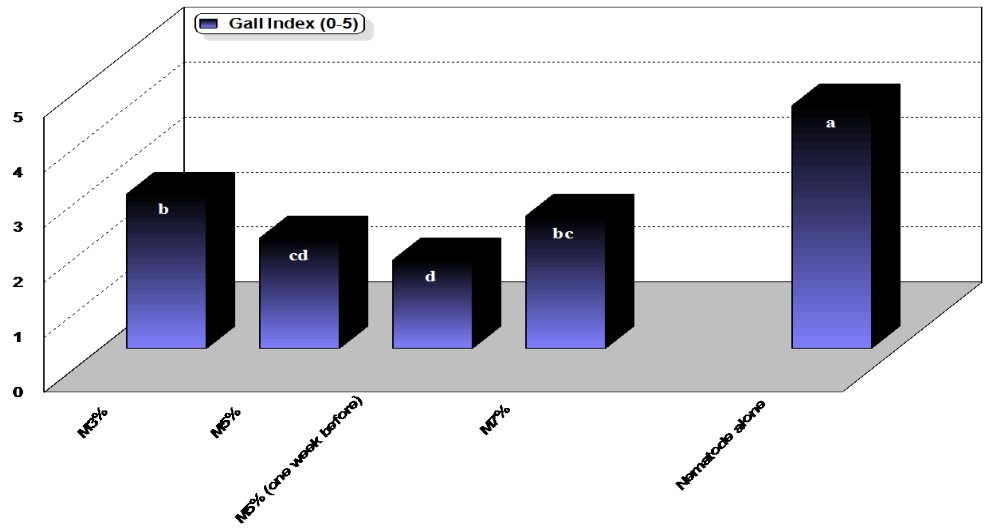

Figure 5: Root galling indices (0-5) of M. javanica as affected by amending soil with mustard at different doses and application time on tomato roots

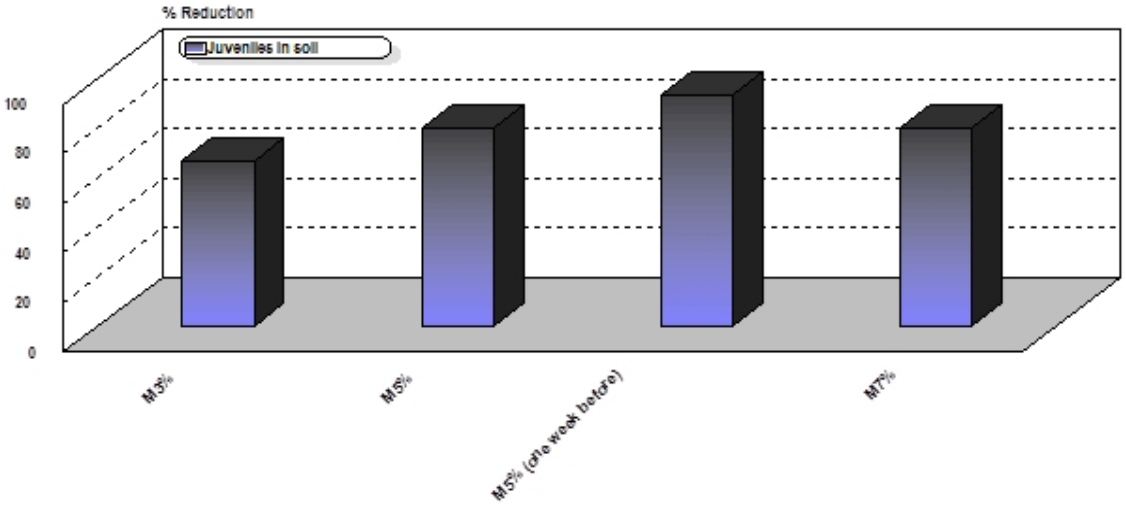

Figure 6: Percentage of reduction of M. javanica larvae in soil amended with mustard at different doses and application time

and 3% by 90.4 and 90.5%, respectively (Fig. 4). Egg masses showed the same trend of results as mixing the soil pots with mustard at 5% either before one week or 48h was the most effective one in reducing the mean number of egg masses. The percentage of reduction recorded 97.4% and 95.9%, respectively (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 (Fig. 5). Soil amended with mustard at all tested doses appeared to have good results in suppression nematode larvae compared to mustard non-treated plants. Application mustard at 5% either before one week or 48hr was the most effective treatment. The percentage of reduction in suppression nematode larvae recorded 93.2 and 80%, respectively (Fig. 6). Application of mustard at 3% was the lowest one by 66.5%.

The chemical components i.e. total sugars, total amino acids and total phenols were enhanced with all doses of mustard applied compared to p treated plants with nematode alone (Fig. 7). Amending the mustard at 5% one week before nematode inoculation with soil pots encouraged the percent of all the chemical com-

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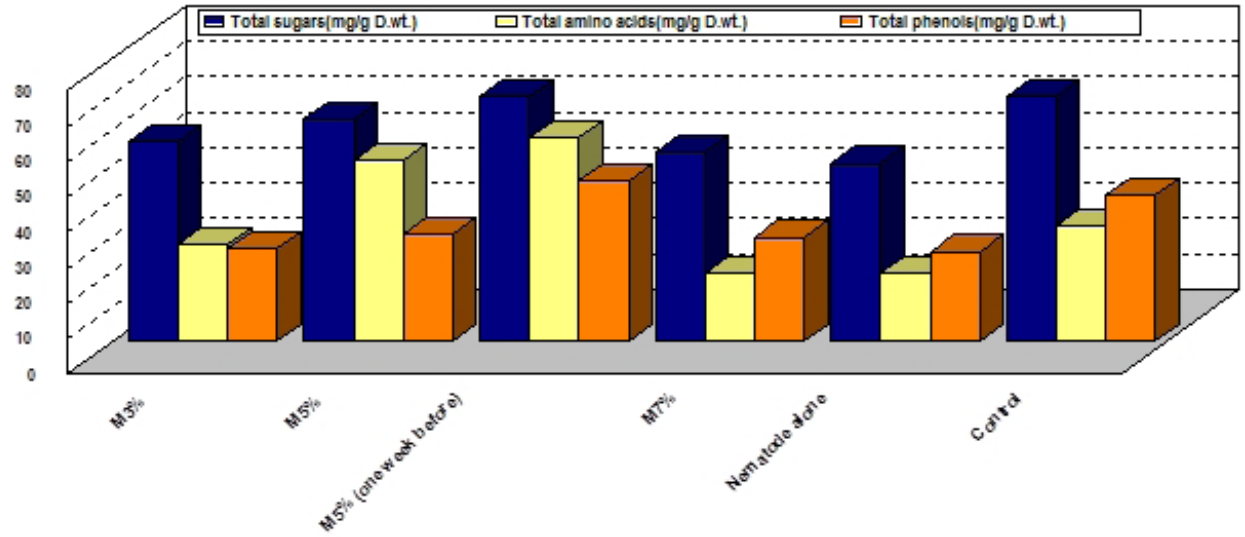

Figure 7: Effect of soil amended with mustard at different doses and application time on the percentage of chemical constituents in tomato plants infected with M. javanica

ponents compared to all the other treatments.

Discussions

The continuous use of chemical nematicides to control Root-knot nematode has 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 environment friendly products in order to reduce the effects of widespread nematicides utilization in crop protection (Salem, 2012; Salem et al., 2012a; 2012b.). 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 pollution impact on the 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. Our results reveal that, soil amended with mustard at all tested doses appeared to show good results in suppression nematode larvae compared to mustard non-treated plants. The application of mustard at 5% either before one week or 48hr 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). In 2013, the experiments reported that 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 manure is 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, such as Brassica and Sinapis mustard species and 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, Salem et al., 2015). 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; Salem et al., 2015). 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. 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. Modified or innovative biofumigation technology that firstly described worldwide by (Salem, 2014) 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 practice: we should create an effort to educate Egyptian farmers about this modified/innovative biofumigation since most farmers are not aware of this innovative 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 differences in soil type. 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.

Conclusions

This modified technology of biofumigation would be an innovative and can be used efficiently to control Root-knot nematode under organic agriculture and Global GAP agricultural systems instead of these carcinogenic nematicides.

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Conflict of Interests

The authors hereby declare that there is no conflict of interests.

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