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Full Length Article

Suppression of Brassicaceous Tissue on *Meloidogyne javanica* in a Rhizosphere

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Abstract

Root-knot nematodes are important pests of Solanaceous crops, especially tomato and egg-plant, in glasshouses and fields of Iran. Brassicaceous plants once incorporated into soil as green manures have recently been demonstrated to possess anti-phytopathogenic properties. The goal of the research was to evaluate the suppressive effect of chopped tissue of five Brassicaceous species (rapeseed, cabbage, garden cress, mustard greens and flixweed) on the reproduction and galling of *Meloidogyne javanica* on tomato as a host plant, and on the host plant growth properties. A pot experiment was conducted on seedlings of tomato cv. Super Chief-grown in 1kg steam-sterilized sandy loam soil. Inoculum used for artificial inoculation was 10 J_2/g soil of the nematode, and was considered at rates 0, 10, 20, 30, 40 and 50 g/kg soil of the Brassicaceous chopped tissue. The nematicide RUGBY® 10 G (Cadusafos) was used as a reference product at 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil. Two months after inoculation, the number of knots, egg masses and reproduction factor of the nematode, and growth properties of the host plant in treatments based on a completely randomized design (with descending order): control, nematode, nematode+nematicide, and nematode+Brassicaceous chopped tissue combinations were assessed. Results indicated that the combination nematode+Brassicaceous chopped tissue led to the suppressive effect on *M. javanica* activity. Tomato plant growth properties were greatly enhanced in treatments of the Brassicaceous chopped tissue. The least to the greatest level of the nematode activity was observed in rapeseed, garden cress, cabbage, mustard greens and flixweed treatments, respectively. © 2017 Friends Science Publishers

Keywords: Nematode; Amendment; Management; Cruciferous plant

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are economically the most damaging plant parasitic nematodes on a range of crops in subtropical climates (Perry *et al*., 2009). *Meloidogyne javanica* (Treub) Chitwood is a key pest of tomato (*Lycopersicon esculentum* Mill.), and is widespread in Iran (Gharabadiyan *et al*., 2013). In the country, plant-parasitic nematodes are successfully managed by application of nematicides. Nevertheless, some of the pesticides became unavailable recently or their supply is limited leaving growers with few alternative approaches for managing plant-parasitic nematodes. Therefore, there is an increased interest in non-chemical nematode management strategies.

Biofumigation has been suggested as a solution to manage plant parasitic nematodes (Monfort *et al*., 2007; Kruger *et al*., 2013). Most research on biofumigation has focused on using Brassicaceous plants (Poelman *et al*., 2009; Yim *et al*., 2016). Brassicaceous herbs have been used frequently as green manures for biofumigation, a pest-management strategy based on the release of biocidal volatiles during decomposition of soil-incorporated tissue. Glucosinolate compounds have been produced by Brassicaceous tissue when broken down to various allelochemics and incorporated into soil control soil-borne pests, insects and nematodes (Avato *et al*., 2013; Larkin, 2013). In addition, like other techniques based on amending soil with organic matter, it has important soil building properties, improving the soil nutrient status and water-holding capacity and increasing the presence and action of beneficial soil organisms, including those are antagonistic to plant-parasitic nematodes (McSorley, 2011).

According to the above, the scope of the research was to evaluate the effect of Brassicaceous chopped tissue as a control method against root-knot nematode, *M. javanica* activity on tomato seedling, as a host. A second objective was to determine effect of Brassicaceous chopped tissue as a soil amendment on the host growth properties in a glasshouse.

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Materials and Methods

Preparation of *M. javanica* Inoculum

The source of roots infected with root-knot nematodes was sampled from a population maintained in the fields of tomato cv. Walter in southern Tehran city, Iran. Extraction and preparation of *M. javanica* inoculum were applied according to the Hussey and Barker (1973) using the single egg mass method. According to the morphological and morphometrical characteristics of body and perineal pattern, the nematode was initially identified (Aydinli and Mennan, 2016). The egg mass of the species added to rhizosphere of tomato cv. local seedlings and then, the nematode was multiplied on the plants grown in steam-sterilized sandy soil in a glasshouse (10 h lighting and 25±2°C temperature). Eggs of the nematode were extracted by shaking the roots in a 1% NaOCl solution, and were incubated in water for three days at 25±2°C and then hatched second instar juveniles (J_2) were collected and counted (Gharabadiyan *et al*., 2013; Saeedizadeh, 2016).

Brassicaceous Tissue

Brassicaceous seeds were taken from Department of Plant Protection, Shahed University, Tehran; comprising five species, including rapeseed (*Brassica napus* L. cv. Okapi), cabbage (*B. oleracea* L. cv. Snow crown), mustard greens (*Brassica juncea* (L.) Czern. cv. Bard-1), garden cress (*Lepidium sativum* L. cv. local of Tehran) and flixweed (*Descurainia sophia* (L.) Webb ex Prantl cv. local of Tehran). The seeds were sown (300 seeds) in 40 L (80×50×10 cm) plastic pots filled with steam-sterilized sandy soil, individually; 45 days after sowing, the plants were collected from an experimental glasshouse (8 h lighting and 25±2°C temperature). Afterwards, soil was removed from the roots of the plants by rinse with tap water and drained on blotting paper. Then, the plants were chopped into approximately 0.5 cm pieces using a food processor and mixed thoroughly with the previously prepared soil. The soil was a steam-sterilized loamy sand (83% sand, 12% silt, 5% clay; 0.4% organic matter, pH 7.1). Six levels of chopped tissue, 0, 10, 20, 30, 40 and 50 g/kg soil, were utilized in treatments (Ploeg, 2007). In this experiment, the nematicide RUGBY® 10 G (Cadusafos), as a reference product, was added at 0, 0.5, 1, 1.5, 2 and 2.5 g per pot (Safdar *et al*., 2012).

Host Plant Material and Inoculation

To prepare the host plant seedlings, seeds of tomato (cv. Super Chief) were sown in the steam-sterilized loamy sand substrate, in the greenhouse. After a while, four-leaf seedlings were transferred to the pots, which were filled of 1000 g steam-sterilized loamy sand soil mixed with the nematicidal materials (Brassicaceous chopped tissue and the nematicide doses); and two days later, nematode inoculum (10 J_2/g soil) were individually applied to the pot in a volume of 5 mL suspension in a ring from around the plant to a depth of 3 cm.

To evaluate the effect of the nematicidal materials on growth properties of tomato seedling, a record of treatments were taken under the same conditions and without the nematode inoculum. For this purpose, four seeds of tomato (cv. Super Chief), as a host plant of the nematode, were sown in the pots, which were filled of 1000 g steam-sterilized loamy sand soil mixed with the nematicidal materials doses, in the greenhouse; and 1 week after germination thinning was done to save a single seedling per pot.

The pot containing one plant was represented one replication, and was kept in the glasshouse. The plants were irrigated as needed.

Evaluation of *M. javanica* Activity and Host Plant Growth Properties

Two months after inoculation, the nematode-inoculated plants were taken. The roots were investigated for galling; and soil samples were collected for the nematode population analysis. According to the proposed method of Hussey and Janssen (2002) the activity of *M. javanica* was evaluated as the number of egg masses and knots (galls) per root, and final population per pot. The roots were washed with tap water and drained on blotting paper. To specify the number of egg masses, the roots were divided into 3–4 cm parts, then, egg masses stained with Floxin solution B (0.15 g/L of water), bleached with lactophenol and counted under a dissecting microscope (Hussey and Janssen, 2002). For determination of nematode final population in the soil of the pot, a 100 g subsample of well mixed soil from each replication (pot) was processed by extraction method according to Jenkins (1964), known as centrifuge or sugar flotation method. The nematode suspension was collected and the number of nematodes was counted at 40x magnification in the suspension, and then it was applied to estimate the population of nematodes per 1 kg soil (pot). To estimate the number of juveniles and females inside the roots, 1 g subsample of well mixed chopped roots was macerated in a Waring blender and counts were done on the suspension thus obtained. The numbers of nematodes present in a root were calculated by multiplying the numbers of nematodes present in the subsample of the root, considering the total weight of the root. Reproduction factor was calculated, according to manipulated method of Walter *et al*. (1999), as follows: RF= (Pf-Pi)/Pi, which is RF: reproduction factor, Pf: final population of nematodes, and Pi: primary population of nematodes (the nematode inoculum).

To assess the effect of the nematicidal material on plant growth properties, two months after sowing seeds, plants were taken, rewetted, weighed fresh root and shoot, and measured length of stem.

Experimental Design and Statistical Analysis

This experiment was based on a completely randomized design on a greenhouse bench treatments for evaluation of *M. javanica* activity and tomato seedling growth properties, including the nematode (2 levels: applied and non-applied), the nematicide (6 levels: 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil), and the Brassicaceous chopped tissue (6 levels: 0, 10, 20, 30, 40 and 50 g/kg soil). The characters of 0, I, II, III, IV and V correspond to 0, 10, 20, 30, 40 and 50g/kg soil for Brassicaceous chopped tissue; and 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil for the nematicide (Cadusafos) in the text. The treatments were replicated four times. The pots were watered (distilled water) as needed and the experiment was terminated two months after inoculation of the nematode. The data of the nematode activity and seedling growth properties were then subjected to one way analysis of variance (ANOVA). Mean treatments were compared using a Duncan multiple range test (Steel and Torrie, 1980). All analyses were performed by using SAS software version 9.1.

Results

The incorporation of the Brassicaceous chopped tissue to the soil was reduced *M. javanica* activity (gall, egg mass and reproduction factor) in tomato (host) plant rhizosphere. Among the examples of the Brassicaceous material, the highest inhibitory effect on the nematode has been observed on rapeseed, cabbage, garden cress, mustard greens and flixweed, respectively (Figs. 1, 2 and 3). Thus, the applied the Brassicaceous tissue, exclusively, doses of flixweed was not capable to break the population development of the nematode (Fig. 3). Other examples of the Brassicaceous material have been leading the nematode reproduction factor to zero in the range of II and IV doses. In between, the reproduction factor of the nematode has been negative in dose III of rapeseed, and dose IV of garden cress, mustard greens and cabbage. There was a significant difference ($p \leq 0.01$) between the inhibition effect of the Brassicaceous chopped tissue and the nematicide (Cadusaphos) on the nematode activity (Figs. 1, 2 and 3). The number of gall and egg mass have been zero in the dose V of the nematicide; and a similar result was observed about rapeseed, cabbage, garden cress, mustard greens, too.

Related to comparison of tomato plant growth properties (root and shoot fresh weight, and stem length) under the doses of the nematicide and the Brassicaceous chopped tissue, the results showed the presence of the Brassicaceous chopped tissue in the rhizosphere of tomato seedling increased the quantity of growth properties of the seedling. The increase was more prominent in treatments of cabbage, mustard greens and flixweed. The treatments of the Brassicas, by increasing the dose used, the amount of growth properties had an increasing trend; so that the maximum weight of root and stem length was observed on dose V (50 kg/g soil). However, most of the growth properties of the doses of garden cress have been accessed on doses II and III. The results of the nematicide, in various doses, contained almost identical values; in other words, the nematicide have been ineffective on plant growth properties (Table 1).

Discussion

The ability of certain plants to suppress nematodes through the nematicidal activity of the secondary metabolites has been reported (Ntalli and Caboni, 2012). Research has furthermore proved that many Brassicaceous species show nematicidal capability on plant-parasitic nematodes (Monfort *et al*., 2007; Van Dam *et al*., 2009).

The suppressive effect of Brassicaceous species as green manures on soil-borne pathogens, weeds, and plant parasitic nematodes has been evaluated in laboratory, greenhouse, and field conditions (Larkin and Griffin, 2007; Larkin, 2013).

It has been studied on the incorporation of Bracecaceous material to control plant-parasitic nematode populations. The outcomes of the subjects showed that Bracecaceous species do have a nematicidal potential in soil (Zasada and Ferris, 2004; Hartz *et al*., 2005; Monfort *et al*., 2007; Roubtsova *et al*., 2007; Kago *et al*. 2013). In our trial, it was observed a clear nematicidal impact of amending soil with the Brassicaceous chopped tissue on *M. javanica* activity (gall, egg mass and reproduction factor) in the rhizosphere of tomato seedling. Roubtsova *et al*. (2007) and Everts *et al*. (2006) have reported similar results.

The plant species that generally are considered for biofumigation are found mostly in family Brassicaceae and include *B. oleracea* (broccoli, cabbage, cauliflower, kale), *B. rapa* (turnip), *Eruca sativa* (salad rocket, arugula), *Raphanus sativus* (radish), *B. napus* (canola, rapeseed) and various mustards, such as *Sinapis alba* (white mustard) and *B. juncea* (mustard greens) (Melakeberhan *et al*., 2006; Ploeg, 2007; Lopez-Perez *et al*., 2010; Edwards and Ploeg, 2014).

The level of nematode control from Brassicaceous plants was found to be different between their species (Hartz *et al*., 2005; Monfort *et al*., 2007). According to our findings, chopped tissue of rapeseed has shown the highest inhibitory effect on the *M. javanica* activity, while flixweed was the weakest in this instance. The results of cabbage, garden cress, mustard greens was located in the midst of the nematicidal effect (Figs. 1, 2 and 3). In other words, rapeseed, cabbage and garden cress generally being a good choice, and mustard greens and flixweed consistently ranking among the poorest choice for the nematode management. The suppressive effect of the plant tissues is possibly due to delaying or preventing a portion of the nematodes to reach the host roots. The survey offers important information for choosing a Brassicaceous amendment with the role of managing root-knot nematodes.

Table 1: Growth properties of treated tomato seedling with nematicidal materials (Mean±StE)

Trait	Dose* (g/kg soil)	Nematicidal material					
		Cabbage	Flixweed	Garden cress	Mustard greens	Rapeseed	Nematicide
Root fresh weight (g)	0	3.89±0.05abcd †	3.95±0.07abcd	4.05±0.4abcd	3.9±0.08abcd	4.02±0.08abcd	3.85±0.18abcd
	I	3.88±0.13abcd	3.9±0.06abcd	4.03±0.1abcd	3.83±0.09abcd	3.98±0.09abcd	4.08±0.9abc
	II	3.88±0.12abcd	3.93±0.14abcd	3.96±0.86abcd	3.83±0.14abcd	4±0.11abcd	4.15±0.47ab
	III	3.98±0.11abcd	3.98±0.09abcd	3.95±0.1abcd	3.88±0.09abcd	4.03±0.13abcd	4.02±0.7abcd
	IV	4.03±0.13abcd	3.97±0.08abcd	3.92±0.06abcd	3.98±0.09abcd	4.08±0.2abc	3.94±0.29abcd
	V	4.47±0.37a	4.48±0.35a	3.98±0.15abcd	4.49±0.33a	4.48±0.37a	3.92±0.19abcd
Shoot fresh weight (g)	0	35.09±1.71abcdefgh	34.95±0.35abcdefgh	35.8±1.04abcdefgh	34.92±0.91abcdefgh	36.3±1.22abcdefg	35.05±0.52abcdefgh
	I	35±0.82abcdefgh	34.75±0.75abcdefgh	35.4±1.76abcdefgh	34.75±1.11abcdefgh	35.75±1.31abcdefgh	34.97±1.51abcdefgh
	II	36.75±0.85abcdef	37±1.47abcdef	34.95±1.11abcdefgh	37.5±1.76abcde	36.5±1.04abcdefg	36.55±1.35abcdef
	III	37.25±1.31abcdef	37.2±1.47abcdef	34.78±1.29abcdefgh	36.75±1.03abcdef	36.75±1.38abcdef	34.63±0.92abcdefgh
	IV	37±1.08abcdef	37.1±0.41abcdef	36.81±1.75abcdef	37.25±1.31abcdef	37±0.91abcdef	36.65±1.08abcdef
	V	39.3±1.73a	39.5±1.71a	35.09±0.84abcdefgh	39±1.73a	39.7±1.75a	34.98±0.94abcdefgh
Length of stem (cm)	0	26.23±0.55abc	25.7±1.07abcde	27.2±1.1abc	25.8±0.95abcde	26.3±0.45abc	25.62±0.85abcde
	I	26.75±0.85abc	25.5±0.87abcde	27.5±2.1abc	25.5±1.94abcde	26.5±0.65abc	26.3±0.73abcd
	II	25.5±1.32abcde	27.3±0.61abc	27.25±0.85abc	27.5±1.04abc	26.5±0.87abc	25.9±1.45abcde
	III	27.5±0.65abc	26.5±0.65abc	26.95±1.65abc	27.5±1.71abc	26.5±0.65abc	26.4±0.95abc
	IV	27.5±0.65abc	28.75±1.03a	27.25±1.03abc	26±1.08abcd	26.25±1.25abcd	25±1.14abcde
	V	27.79±1.03ab	27.5±1.19abc	27.2±0.64abc	27.5±0.63abc	27.7±0.62abc	25.5±0.56abcde

†Means with different letters for each trait are significantly different (Duncan Test, $p \leq 0.01$; n = 4)
*Defined doses in the dose column correspond to 0, 10, 20, 30, 40 and 50g/kg soil for Brassicaceous chopped tissue; and 0, 0.5, 1, 1.5, 2 and 2.5g/kg soil for the nematicide (Cadusafos)

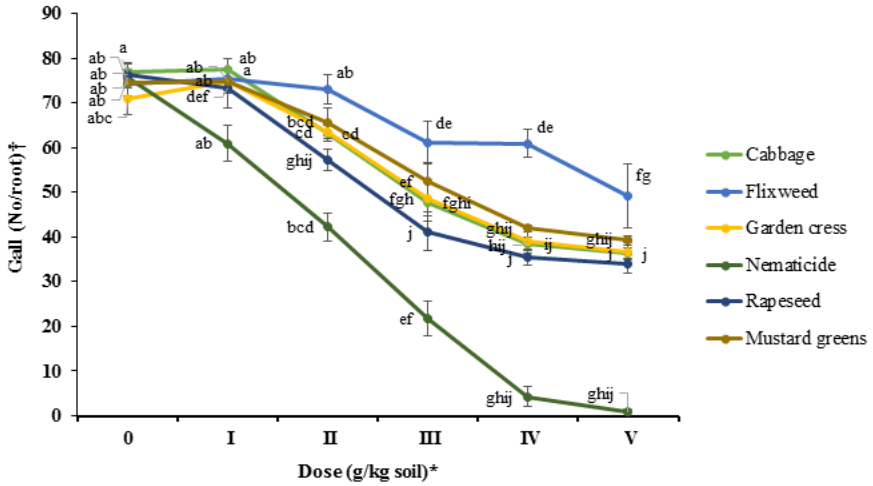

Fig. 1: Mean number of galls of *Meloidogyne javanica* on the roots of tomato treated with chopped tissue of Brassicaceous plants (rapeseed, cabbage, garden cress, mustard greens and flixweed) and the nematicide (Cadusafos). (Pooled data of the doses 0, I, II, III, IV, and V)
†Means with different letters on the curves are significantly different from each other (Duncan Test, $p \leq 0.01$; n = 4)
*Defined doses at the X axis correspond to 0, 10, 20, 30, 40 and 50g/kg soil for Brassicaceous chopped tissue; and 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil for the nematicide (Cadusafos)

The large net reduction in nematode populations after incorporations does support previous research that *Brassica* soil amendments do have potential as a biological control measure for *M. incognita* compared to the commercial standard (Riga, 2011).

Our results indicated, the Nematode + Brassicaceous chopped tissue combined was not efficient in preventing the development of the nematode as much as the nematicide for all the Brassicaceous materials. This proposes the Brassicaceous tissue had a nematostatic rather than a direct nematicidal effect, reducing the infectivity of the remaining *M. javanica* population, presumably by interfering with the nematode's ability to penetrate into the host roots.

In the application of Brassicaceous plants for the soil amendment to control root-knot nematodes, two topics should be taken into consideration. Firstly, Brassicaceous species used as a green manure may not be a suitable host for the nematode; and then, Brassicaceous species have not a negative effect on growth of the target plant.

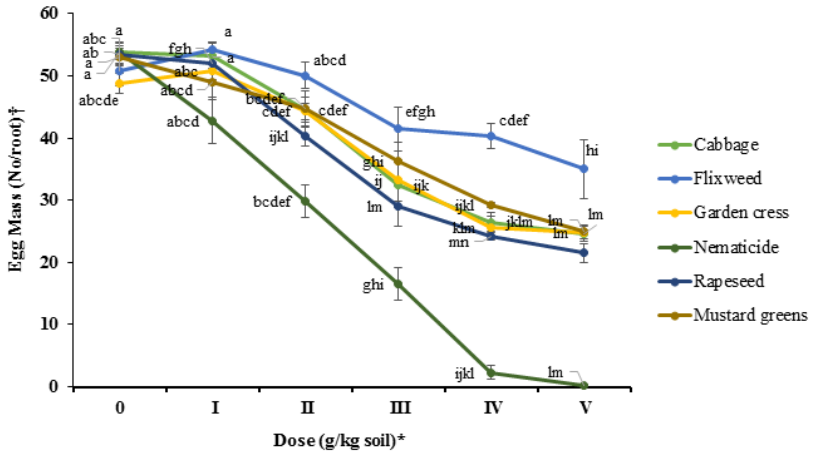

Fig. 2: Mean number of egg masses of *Meloidogyne javanica* on the roots of tomato treated with chopped tissue of Brassicaceous plants (rapeseed, cabbage, garden cress, mustard greens and flixweed) and the nematicide (Cadusafos). (Pooled data of the doses 0, I, II, III, IV, and V)

†Means with different letters on the curves are significantly different from each other (Duncan Test, $p \leq 0.01$; n = 4)

*Defined doses at the X axis correspond to 0, 10, 20, 30, 40 and 50g/kg soil for Brassicaceous chopped tissue; and 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil for the nematicide (Cadusafos)

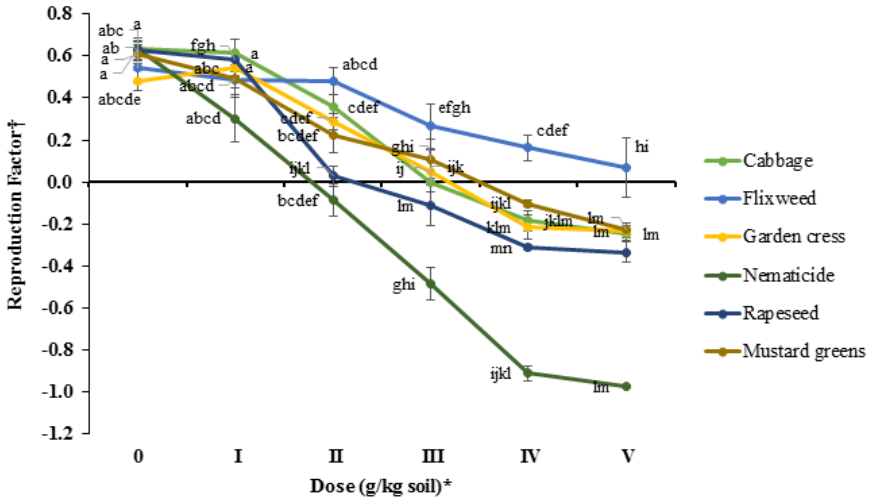

Fig. 3: Mean of reproduction factor of *Meloidogyne javanica* on the roots of tomato treated with chopped tissue of Brassicaceous plants (rapeseed, cabbage, garden cress, mustard greens and flixweed) and the nematicide (Cadusafos). (Pooled data of the doses 0, I, II, III, IV, and V)

†Means with different letters on the curves are significantly different from each other (Duncan Test, $p \leq 0.01$; n = 4)

*Defined doses at the X axis correspond to 0, 10, 20, 30, 40 and 50g/kg soil for Brassicaceous chopped tissue; and 0, 0.5, 1, 1.5, 2 and 2.5 g/kg soil for the nematicide (Cadusafos)

According to Edwards and Ploeg (2014) mustard greens (*B. juncea*) and turnip (*B. rapa*) were more often than good hosts for *M. incognita* and *M. javanica*, whereas most oil radish cultivars (*Raphanus sativus* ssp. *oleiferus*) were poor hosts. However, some oil radish cultivars were among the best hosts for *M. hapla*. The arugula (*E. sativa*) cv. Nemat was a poor host for all three nematode species tested.

In comparisons between use of *Brassica* green manure, seed meal or chemical fumigation in the field, 1- or 2-year *B. napus* green manure treatment suppressed disease nor enhanced growth or yield (Mazzola and Mullinix, 2005). Monfort *et al.* (2007) found that, there was no correlation between net change in nematode population densities (between cover crop harvest and incorporation and planting of the vegetable crop) and increased yields. In most places, increased yields were observed in treatments with low population densities at planting and/or treatments that

looked to deliver good effects on the vegetable crop other than nematode control, maybe an increase in available nutrients from the cover crops. In our study, the effects of the *Brassica* species amendments were also noted in seedling growth properties. Chopped tissue of cabbage, mustard greens and flixweed have caused the highest rate of growth of a tomato seedling, compared to the control (dose 0).

Other factors shown to greatly enhance the pest suppressive activity of Brassicaceous crops include a very thorough disruption of the plant tissue prior to soil incorporation and sufficient soil moisture at the time of tissue incorporation (Matthiessen *et al*., 2004). The potential for Brassicaceous amendment as part of an IPM approach consists of the role of the active compounds, in the direct suppression of nematodes, and also the secondary effect that can be expected during the application of the amendment in the soil. The secondary effect plays a very significant part in promoting microbial and other microorganism diversity in the soil, and therefore can be expected to have a positive impact on the stimulation of competition among soil-borne diseases in the rhizosphere. Further research is required to determine which Brassicaceous tissue amendment is more effective on the population of beneficial microbes in soil and determine the level of suppression of the various Brassicaceous tissues to hatching eggs of *M. javanica*. Furthermore, there is a need to evaluate the level of control for nematodes achieved with the incorporation of select *Brassica* amendments in combinations with varying rates of commercially available nematicides to determine if reductions in these pesticides can be accomplished in this type of management system.

Conclusion

It can be concluded that incorporation of the Brassicaceous chopped tissue to the soil reduced *M. javanica* activity in rhizosphere of tomato seedling. Results showed rapeseed, cabbage and garden cress generally being good amendment, and mustard greens and flixweed consistently ranking among the poorest amendment for the nematode management. The presence of the Brassicaceous chopped tissue, including cabbage, mustard greens and flixweed, in the rhizosphere of tomato seedling increased the quantity of growth properties of the seedling. The results of this trial, although variable, did show some promise of Brassica species as a biological control option in tomato production in greenhouses.

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