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Effect of fresh chopped leaves of certain plants as biofumigants for management *Meloidogyne* spp. on tomato plants

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ABSTRACT

In this research four plants i.e. Moringa (*Moringa oleifera*), Cabbage (*Brassica aleracea*), Cassia (*Cassia nodosa*) and Chinaberry (*Melia azedaroch*) were mixed, at three levels 1, 2 and 4% of soil weight to manag root-knot nematodes, *Meloidogyne* spp. under greenhouse conditions. Results revealed that chapped leaves of the four evaluated plants significantly, reduced all related nematode parameters i.e. number of galls, egg masses, females and developmental stages / root system, eggs / egg mass and number of juveniles / 250 g soil, in comparing with nematode alone. The highest significant reduction in galls, egg masses and J2 / 250 g soil (93, 92.8 and 89.6%, respectively) obtained with moringa at 4% of soil weight, followed by cabbage chapped leaves at 4% (90.8, 88.9 and 85.8%, respectively), whereas the lowest one observed with cassia chapped leaves at 1%. Generally, the effective dose was 4% regardless the plant species and the effective plant was moringa regardless the rate significantly enhanced all vegetative plant growth parameters i.e. fresh shoot and root weight (g), dry shoot weight (g), plant height and root length (g). Results showed also all treatments significant increase antioxidant enzymes activity i.e. peroxidase and phenoloxidase, total sugars, total phenols and proline concentration. Membrane leakage showed also highly significant decrease with all treatment compared with the plant treated with nematode alone.

Key Words: Moringa, Root-knot nematode, *Meloidogyne* spp., biofumigants, fresh chopped leaves.

INTRODUCTION

Root-knot nematodes *Meloidogyne* spp. are obligate endo-parasites and very damaging plant pests which are considered to be limited factor in crop production and agricultural productivity (Bakr *et al*., 2011 and 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 most vegetable crops, especially tomato plants and cause severe yield losses in new reclaimed soil. Infected plants suffer from vascular damages which disturb water and mineral uptake (Abd-Elgawad and Aboul-Eid, 2001 and Luc *et al*., 2005). Chemical nematicides are considered the most effective method in suppressing and controlling root-knot nematodes, but its means environmental pollution and very 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. Recently, these harmful nematodes have been controlled using a brood-spectrum, of safety method i.e. beneficial soil organisms soil fumigants which exhibited vertebrate toxicity (Shreiner *et al*., 2001 and Cox, 2006). Control of nematodes has become

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increasingly different due to the removal of many nematicides and soil fumigants from the market because of their toxicity and adverse effects on the environment (Davies and Spiegil, 2011). Biofumigation is an alternative management strategy for controlling soil bonne diseases especially root-knot nematodes, *Meloidogyne* spp. Biofumigation was defined by several researchers that occurs when volatile compounds with pesticidal properties are released during decomposition of plant materials of which cruciferous plants belonging to Brassica spp. mustard (Griffiths *et al*., 2011 and Salem and Mahdy, 2015), Broccoli (Roubtsova *et a*l. 2007) and cabbage (Youssef and Lashin, 2013) that contains glucosinolate compounds as a toxic products (Brown and Morra, 1996 and Kirkegaard and Sarwar, 1998).

MATERIALS AND METHODS

In this experiment four plants i.e. moringa, cabbage, Cassia and chinaberry were mixed in nematode natural infested soil two weeks before tomato seedlings transplanting into pots (15 cm in diameter) as chopped fresh leaves. Each pot contain 2 kg soil contains 24000 freshly second stage juvenile of *Meloidogyne* spp. (12000 J2S/kg) Plants were mixed at three levels 1, 2 and 4% of soil weight. Pots watered and covered with transparent plastic sheets for two weeks. Pots were kept moist under greenhouse conditions to allow the amendments to decomposition. Numbers of live and dead juveniles were determined in 100 g soil, after soil covering for two weeks with transparent plastic sheets. Three weeks-old tomato seedlings cv. Beto-86 were transplanted into pots (one seedling / pot) after removing the plastic sheet. Six replicates were used for each particular treatment. Plants watered daily and fertilized once a week with nutrient solution obtained from the International Egypt Company for Agricultural and Industrial Developing, with 5 ml of 2 g/L (N: P: K, 20: 20: 20).

Fifteen days after tomato seedlings transplanting, plants were uprooted, and their roots carefully washed under running tap water larvae penetration rate determined after staining the roots with sodium hyochlorite-acid fuchsin stain. Roots transfers into a boiling acid fuchsin for 30 seconds as described by Byrd *et al*. (1983). Excess stain was removed by rinsing the stained roots in running tap water. Roots then placed in 20 – 30 ml glycerin acidified with a few drops of 5 N HCl, heated to boiling and then cooled and examined. Numbers of penetrated juveniles were counted under a stereomicroscope by pressing the root segments between microscopic glass slides.

Two months after tomato seedlings transplanting, plants were uprooted, and their roots were carefully washed under running tap water. Numbers of galls, egg masses, females, developmental stages / root system, eggs/egg mass, number of juveniles / 250 g soil, final nematode population (PF) as well as reproduction factor (RF) were determined.

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

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

RF=PF/PI (Sasser *et al*. 1984)

Egg masses were stained prior to counting by dipping the infected roots in Phloxin-B solution (0.15 g / L tap water) for 20 minutes as described by Daykin and Hussey (1985). Females and developmental stages/root system

were evaluated by submerging the roots in a beaker full of tap water at room temperature until they became softened. The roots were then washed through 250 and 500 µm sieves to separate the females and developmental stages from the root debris, counted under a stereo-microscope (Mahdy, 2002). Soil nematode population was enurmerated by extraction root-knot nematode juveniles (J2S) by using the tray modification of Baermann funnel as described by Barker (1985). Vegetative plant growth parameters i.e. plant height (cm), root & shoot length (cm), fresh root and shoot weight (g), dry root and shoot weight (g). Chemical components i.e. membrane leakage, antioxidant enzymes (Phenoloxidase and Peroxidase), total sugars, total phenols, and proline content, also were determined.

Statistical analysis:

The collected data were subjected to statistical analysis of using the F-test and means were compared by the LSD at 0.05 level of probability as described by Snedecor and Cochran (1967) and using Costat Software (2008) Version 6.40.

RESULTS

Data presented in Table (1) revealed that chopped leaves of the four evaluated plants mixed thoroughly with naturally infested soil as a biofumigants at 1, 2 and 4% of soil weight were significantly effective in reducing the penetration rate of second stage juveniles of *Meloidogyne* spp. into tomato roots after 15 days of tomato seedlings cultivation compared with plants cultivated in nematode infested soil only. The highest reduction percentage of penetrated larvae into tomato roots recorded with chopped leaves of moringa, cabbage and chinaberry at 4% as it reached 98.1, 97.4 and 96.2%, respectively.

Moringa at 4% recorded the highest percentage of dead larvae in 100 g soil by 89.3%, followed by cabbage (85.7%) at 4% and moringa at 2% (70.8%), whereas cassia and chinaberry at 1% were the least effective ones by 40%.

Data presented in Table (2) revealed that all chopped leaves of tested plants at all three doses significantly decreased all nematode parameters i.e. number of galls, egg masses, females, developmental stages / root system, eggs / egg mass and number of juveniles / 250 g soil compared with plants cultivated in naturally infested soil with nematode alone.

Concern the galls number, there are no significant differences between all chopped leaves of all evaluated plants at 4% in reducing number of galls, but there is a significant difference between them, and plants grown in soil infested with nematode alone.

Moringa gave the highest reduction in number of galls followed by cabbage and chinaberry at 4% of soil weight. Results also showed that all plants at all doses significantly reduced number of egg masses/root system compared with plants grown in nematode naturally infested soil as shown in Fig. (1). Moringa at 4% gave the highest reduction of eggs / egg mass followed by cabbage and chinaberry at 4%. The greatest second stage numbers inhibition recorded with moringa at 4%, followed by cabbage and chinaberry.

Figure (2) showed that all evaluated plants as a chopped leaf at doses of 1, 2 and 4% increased the reduction percentage of galls and egg masses as well as eggs / egg mass. The reduction percentage in these parameters ranged between 17.7 and 92.9%.

Table (1). Effect of fresh chopped leaves of some plants at different doses as biofumigants on nematode larvae penetration rate into tomato roots.

Treatments	Doses %	Mean number of penetrated larvae	% Reduction of penetrated larvae	% Larvae dead in 100 g soil
Cabbage	1	250.0d	90.4	58.3
	2	150.0f	94.2	50.0
	4	66.6h	97.4	85.7
Cassia	1	316.6b	87.8	40.0
	2	250.0d	90.4	46.0
	4	233.3e	91.03	60.0
Moringa	1	150.0f	94.2	66.7
	2	100.0g	96.2	70.8
	4	50.0i	98.1	89.3
Chinaberry	1	300.0c	88.5	40.0
	2	250.0d	90.4	50.0
	4	100.0g	96.2	66.7
C+ (nematode alone)		2600.0a	–	-

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Table (2). Effect of fresh chopped leaves of some plants at different doses as bio-fumigants for management *Meloidogyne* spp. on tomato plants.

Treatment	Doses %	Galls / root system	Egg masses / root system	Eggs / egg mass	Develop. stages / root system	Females / root system	J2/250 g soil	PF	RF
Cabbage	1	82.7bcd	45.3b	310.0bc	303.3cde	45.7b	350.0cd	14742.0b	1.23b
	2	55.0fgh	32.7b	240.0def	280.0cdef	33.7bcd	300.0cd	8181.7b	0.68b
	4	33.7ij	19.0b	208.3fg	180.0fg	19.0ef	250.0cd	4406.7b	0.37b
Cassia	1	98.3b	48.3b	370.0b	440.0b	48.3b	600.0b	18959.3b	1.60b
	2	72.7cdef	43.3b	301.7cd	373.3bc	43.3b	483.3bc	13963.5b	1.60b
	4	59.0efgh	33.7b	283.3cde	326.7cd	33.3 bcd	333.3cd	10240.5b	0.85b
Moringa	1	64.3defg	25.3b	283.3cde	296.7cde	25.3cde	333.3cd	7822.8b	0.65b
	2	52.7ghi	28.0b	233.3ef	220.0defg	28.0def	283.0cd	7063.4b	0.59b
	4	26.0j	13.0b	166.7g	130.0g	13.7f	183.3d	2394.1b	0.199b
Chinaberry	1	84.7bc	43.6b	316.7bc	323.3cd	44.3b	433.3bc	14608.7b	1.20b
	2	75.3cde	33.7b	275.0cde	280.0cdef	39.0bc	300.0cd	9886.5b	0.82b
	4	71.3hij	20.0b	233.3ef	210.0efg	20.0def	263.3cd	5159.3b	0.43b
C+ (nematode alone)		367.3a	180.0a	449.7a	680.0a	198.3a	1766.7a	83591.0	7.0a

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Moringa 4% + Nematode Nematode alone

Cabbage 4% + Nematode Nematode alone

Fig. (1). Effect of fresh chopped leaves of different plants as a biofumigants for management *Meloidogyne* spp.

The greatest reduction percentage of galls recorded with moringa at 4% by 92.9%, followed by cabbage and chinaberry at 4% by 90.8 and 88.8%, respectively. The same trend of results obtained with egg masses and eggs / egg mass as the highest reduction percentage of both parameters by 92.8 and 62.9%, respectively recorded with moringa at 4%.

Figure (3) revealed that moringa at 4% appeared effectiveness on the % reduction of J2S in soil pots by 89.6% and by 97.1% for both final population and reproduction factor, followed by cabbage and chinaberry at 4%.

Data presented in Table (3) showed that mixing the different treatments with soil pots significantly enhanced all vegetative plant growth characters i.e. fresh shoot and root weights (g), dry shoot weight (g), shoot and root lengths (cm) and plant height compared to untreated control plants. The greatest effect was recorded with the high application dose 4%, followed by 2%, whereas the lowest effect was recorded with 1%.

Results also showed that the highest growth enhancement noticed with applying moringa at 4%, followed by cabbage and chinaberry at 4%, the lowest one was cassia at 1%.

Data presented in Table (4) revealed that the effect of adding chopped leaves of four plants i.e. (cabbage, cassia, moringa and chinaberry) mixed thoroughly with naturally infested soil, as a biofumigants at 1, 2 and 4% of soil weight on chemical components of tomato leaves with root knot nematodes *Meloidogyne* spp.

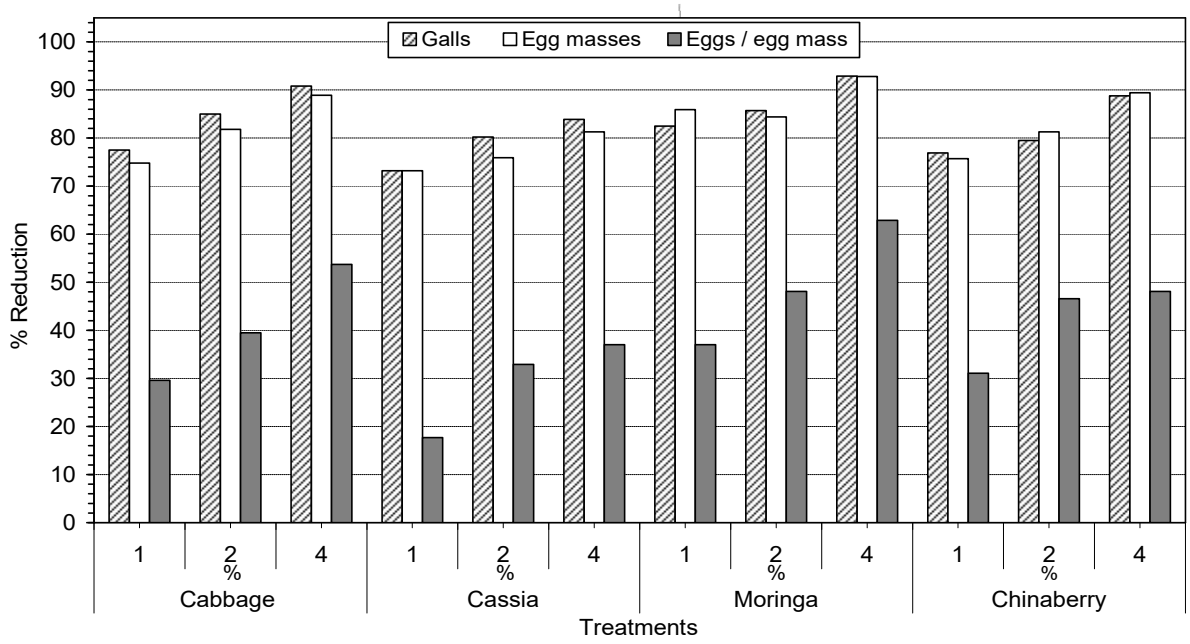

Fig. (2). Effect of fresh chopped leaves of some plants at different doses as a bio-fumigants on the number of galls, egg masses and eggs/egg mass of tomato plants.

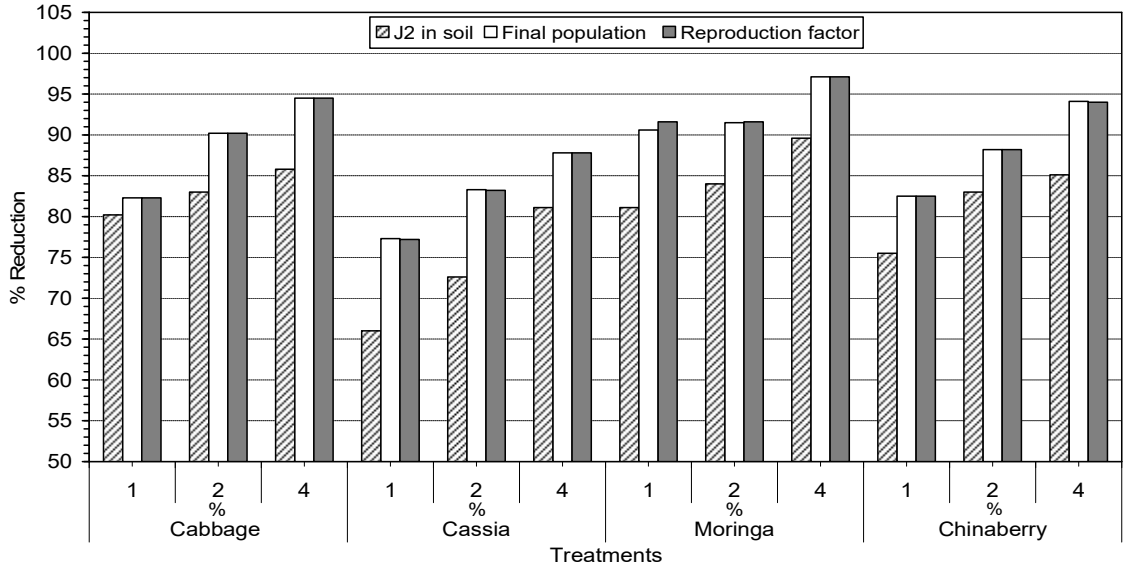

Fig. (3). Effect of fresh chopped leaves of some plants at different doses as a bio-fumigants on the number of J_2 / 250 g soil, final population and reproduction factor.

Table (3). Effect of fresh chopped leaves of some plants as bio-fumigants on growth characters of tomato plants infected with *Meloidogyne* spp.

Treatment	Doses %	Fresh root weight (g)	Root length (cm)	Fresh shoot weight (g)	Shoot length (cm)	Plant height (cm)	Dry shoot weight (g)
Cabbage	1	3.13bcde	11.7cde	19.0cdef	46.7bcd	58.33efg	7.5cde
	2	3.8abc	15.7abcd	20.5cd	52.3abc	69.7bc	8.23bc
	4	4.2a	17.7ab	24.8ab	57.0ab	73.7ab	9.2ab
Cassia	1	2.7de	11.33de	16.2efg	38.0cd	49.33h	6.33def
	2	3.0cde	13.7bcde	17.5defg	40.3cd	55.0gh	6.83def
	4	3.3bcd	15.7abcd	20.0cde	57.0ab	64.7cde	8.0bcd
Moringa	1	3.3bcd	12.3cde	19.8cde	36.3ab	68.7bcd	8.13bc
	2	3.8abc	16.0abc	21.3bcd	56.3ab	73.0abc	8.6bc
	4	4.3a	19.7a	27.3a	61.7a	81.33a	10.4a
Chinaberry	1	3.1cde	10.7e	11.96h	45.0bcd	55.7fgh	7.33cdef
	2	3.33bcd	15.7abcd	15.6fgh	49.0abcd	60.0defg	7.6cde
	4	4.0ab	17.7ab	22.9bc	52.0abc	73.7ab	8.3bc
C+ (nematode alone)		2.3e	11.33de	14.6gh	36.7de	48.0h	6.16f
C– (control)		3.8abc	10.3e	19.0cdef	50.0abcd	64.3cdef	7.7cd

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Table (4). Effect of fresh chopped leaves of some plants as bio-fumigants on chemical components of tomato plants infected with *Meloidogyne* spp.

Treatment	Conc. %	Membrane leakage (%)	Peroxidase (O.D. after 45 min)	Phenoxidase (O.D. after 2 min)	Total sugar (mg/g dr. wt)	Total phenols (mg/g dr.wt)	Proline g/g dr. wt)
Cabbage	1	81.01e	0.40fg	0.32g	28.08d	1.5h	208.9e
	2	67.2h	0.65de	0.37e	31.2c	3.1c	291.8d
	4	52.0k	0.99b	0.59b	37.4b	4.0b	540.3b
Cassia	1	95.4b	0.29h	0.17k	20.3f	1.3j	108.1g
	2	93.9c	0.38fg	0.29h	24.96e	1.5h	140.5f
	4	67.24h	0.63de	0.38de	28.08d	1.9f	291.8d
Moringa	1	76.1g	0.44f	0.33g	31.2c	2.8d	212.5e
	2	64.8i	0.69d	0.39d	35.88b	4.0b	342.2c
	4	45.6l	1.2a	0.65a	46.8a	4.3a	684.4a
Chinaberry	1	91.7d	0.3h	0.21j	20.3f	1.4i	212.5e
	2	67.2h	0.6e	0.35f	28.08d	1.6g	219.7e
	4	60.0j	0.896c	0.48c	31.2c	2.03e	345.8c
C+ (nematode alone)		97.7a	0.18i	0.11l	14.98g	0.8k	104.5g
C– (control)		75.8g	0.35gh	0.25i	11.2g	1.3j	140.5f

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Results showed that all treatments decrease membrane leakage compared to plants treated with nematode alone. The highest treatment was moringa chopped leaves at 4% in decreasing membrane leakage, followed by cabbage and chinaberry chopped leaves at 4% whereas the lowest one observed with cassia chopped leaves at 1%.

The activity of antioxidant enzymes was recorded in Table (4). All fresh chopped leaves of four evaluated plants recorded a highly significant increase in the activity of peroxidase and phenoloxidase enzymes. Moringa chopped leaves at 4% recorded the highest increase in peroxidase activity by 566.7 and 242.0% when compared with plants treated nematode alone and healthy plants without nematode, respectively. The highest value of phenoloxidase enzyme recorded by moringa at 4% followed by cabbage and chinaberry at 4%.

Concerning the total sugars and total phenols, the highly significant increasing in total sugars and total phenols were significant increasing in sugars and total phenols were recorded by moringa at 4% followed by cabbage at 4% when compared to the plants treated with nematode and plants without nematode. Results showed also all treatments increase proline concentration compared with plants treated with nematode alone. The highest increase in proline observed with moringa at 4% (554.9 and 387.1%) when compared to the plants treated with nematode and plants without nematode, respectively.

DISCUSSION

Results in this experiment indicated that all four botanicals were effective in reducing significantly all nematode parameters and significant increasing in plant growth which reflected a healthy plant compared to the untreated control. Moreover, analysis of the nematode parameters from soil and tomato roots showed that all botanicals were effective in a significant reduction of *Meloidogyne* spp. population considerably compared to control plants. The maximum reduction in nematode parameters and maximum increase in growth characteristics were by moringa at 4%.

The management of soil-borne pathogens and pests, including plant-parasitic nematodes, by amending soil with organic material is a well-known and long-practiced strategy and was reviewed by Lazarovits *et al*. (2001). The initial definition of “biofumigation” as a process referring to the breakdown of Brassica tissues, was expanded by Bello *et al*. (2004) to describe the process of biological decomposition of plant or animal byproducts, leading to the production of volatile compounds with disease and pest suppressive properties. It is now known that besides the brassicas, plants in the Caricaceae, Moringaceae, Salvadoraceae and Tropaeolaceae families also have biofumigant properties (Gouws, 2004 and Van Dam *et al*., 2009).

Bello *et al*. (2004) tested the biofumigant effect of a range of agro-industrial byproducts and livestock manures, in different doses and combinations, on the levels of *M. incognita* control. These authors concluded that the majority of materials could effectively be used. The decomposition of biofumigant plant tissues mainly releases isothiocyanates (ITCs), in addition thiocyanates, nitriles and oxozaliolinethiones (Fahey *et al*., 2001).

Glucosinolates (GCs) are sulphur containing stable and non-toxic compounds, but upon tissue disruption they come in contact with myrosinase (= thioglucosidase), an enzyme endogenously present in tissues, but stored in the cell walls or the cytoplasm, away from the glucosinolates (Poulton and Moller, 1993). The enzymatic hydrolysis of glucosinolates produces volatile isothiocyanates (ITCs), nitriles, and thiocyanates (Fenwick et al., 1983). The ITCs in particular, have general biocidal properties (Kirkegaard and Sarwar, 1998). Isothiocyanates also form the active ingredient of some synthetic nematicides (methyl isothiocyanate).

GCs are sulphur containing chemicals that are produced by plants as secondary metabolites (Agrios, 2005). GCs are relatively inactively against microbes. But when hydrolyzed, the ITCs in particular, are biocidal to nematodes (Sarwar *et al*., 1998).

Biofumigation is performed by incorporating the green manure into the upper part of the soil where the ITCs (isothiocyanates) are released (Matthiessen & Kirkegaard, 2006 and Jensen *et al*., 2010). These Glucosinolates (GLS) metabolic products are the factor of bioactivity and their fate in soil defines their residual life and thus efficacy. It must be noted that the ITCs originating for biofumigation are slowly released into the soil thus they might provide of a longer-term biological activity compared to their directly exposed to degradation synthetic analogs (Hanschen *et al*., 2015). ITCs can decline due to volatility, are adsorbed to soil with high organic matter due to their lipophilicity and they decompose because of biotic and abiotic soil properties (Gimsing & Kirkegaard, 2006; Gimsing et al., 2007; Poulsen *et al*., 2008; Gimsing *et al*., 2009). The ITCs degradation is faster in top soil than in sub soil (Gimsing *et al*., 2007). Nonetheless both chemical and natural ITC can also leach into the soil (Guo *et al*., 2003 and Laegdsmand *et al*., 2007).

To study the stability of GLS2 and respective nitriles as well as ITCs breakdown products, Raphanus sativus and Brassica juncea were incorporated in regulation, the improvement of soil organic matter and the suppression of weeds, all factors contributing to a holistic IPM approach in controlling plant parasitic nematodes (Kruger *et al*., 2013). Natural isothiocynates are toxic to organic of soil borne pest and pathogens, including nematodes (Ntalli and Caboni, 2017).

It was generally postured that the adverse influence of organic amendments on phytoparasitic nematodes in referred to numerous factors e.g. increasing host resistance to nematode infection and enhancement of growth performance. Courtency and Mallen (2008) illustrated the changing physical soil properties like cation exchanges, water retention and soil irrigation, producing chemicals during degradation like volatile fatty acids, organic acids, phenols, nitrogenous compounds, hydrogen sulphide (Zakie *et al*., 2004 ;Kesba & Al-Shalaby, 2008 and Bakr, R.A. (2017).

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