THE IMPACT OF SOLARISATION INTEGRATED WITH PLANT BIO-FERMENTATION ON ROOT KNOT NEMATODES

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ABSTRACT

The impact of different freshly/dried chopped medicinal or aromatic plant materials as an organic amendment in pot cultures, as well as integrated with solarisation under greenhouse conditions on the root knot nematodes population was evaluated. Results indicated that application of solarisation alone gave good control (72%) but when integrated with different plant materials, the control level increased to 95% with Allium sativum and 90% with Mentha microphylla and slightly less with other plant materials which ranged from 75 to 80%. The results of pot experiments revealed that the most significant effect on the number of nematodes was achieved with Tagetes patula followed by Pimpinella anisum, Melia azadirach and Origanium syriacum reaching 0.0, 1.2, 1.2 and 2.5/g of roots, respectively. Total control was obtained with Allium sativum. Origanium syriacum contained the highest amount of essential oil (6%). Results obtained indicated that integrated approach using solarisaion combined with plant materials could be the best alternative control for the root-knot nematodes.

Keywords: essential oils, integrated control, nematodes, *Meloidogyne*, organic amendment, plant extracts, solarisation

INTRODUCTION

Nematodes, or eelworms, are present in virtually all soil types. World-wide annual losses caused by plant-parasitic nematodes mainly root-knot nematodes (RKN) (*Meloidogyne)* and cyst nematodes *(Heterodera* and *Globodera)* (CN), are estimated at approximately US$100 billion. They attack a wide range of economically important crops of horticultural and field crops as well as forest systems. Nematodes control is very difficult and relies heavily on the use of soil fumigants with toxic and expensive nematicides. Agrochemicals have been playing a major role in meeting yield requirements in world food production. However, concern has arisen out of findings relating their use to human health and environmental problems. Some pesticides contain active ingredients that have been shown to act as hormone disruptors, possibly causing loss of fertility, carcinogenesis and mutagenesis. Widespread application to most cash crops means pesticides are present in the ecosystems, aquifers and water systems of the main agricultural areas. In the long-term, this could have repercussions on both the natural environment and human health (Dinham, 1993). Hence, there is an urgent need to find less toxic and more environment-friendly alternatives. Plant extracts which contain volatile compounds, especially essential oils, have been found to possess antimicrobial, insecticidal and nematicidal activity (Thackray *et al.*, 1990; Okoko *et al.,*, 1999) against bacteria (Digrak *et al*., 1999) and nematodes (Marban-Mendoza *et al.*, 1987; Ibrahim *et al.*, 2006). Certain plants are able to kill or repel pests, disrupt their lifecycle, or discourage them from feeding.

Methyl-bromide is effective against nematodes, but harmful to human, environment and beneficial organisms. Lebanon is committed to phasing out the use of this chemical and aimed at alternatives to methyl-bromide. One way of searching for such nematicidal compounds environmentally benign is to screen naturally occurring compounds in plants. Lebanese flora is known to be rich with medicinal and aromatic plants (Nehmeh, 1978). Volatile compounds from plants, especially essential oils, have been found to possess antimicrobial and insecticidal activity (Oka *et al.*, 2001; Ibrahim *et al.*, 2006). Only few oils and their components have been evaluated for their nematicidal effects.

When glucosinolates in cruciferous crop residues are hydrolyzed, they release biocidal compounds in the soil mainly isothiocyanates suppressing soil-borne pests and pathogens, a phenomenon named biofermentation or biofumigation (Vaughn, 2004). This term is now used whenever there is a release of volatile substances from the microbial degradation of any organic amendments resulting significant toxic activity toward a soil-borne disease (Luc *et al*., 1990; Vaughn, 2004). As a result of the decomposition of bio-products by soil organisms that increase rapidly in number to attack the freshly incorporated plant material, many gases are released (Sullivan, 2003). Sudan grass (Sorghum sudanense, Piber (Stapf) cultivars suppressed *M. chitwoodi* (Mojtahedi *et al.*, 1993). Ammonia, nitrates, hydrogen sulfide and a great number of volatile substances and organic acids can produce a direct nematicidal effect on eggs hatching or on the mobility of juveniles. Phenols and tannins are nematicides at certain concentrations (Bello *et al*., 2000). Soil solarisation is a hydrothermal process that occurs in moist soil which is covered by transparent plastic film and exposed to sunlight during the warm summer month (DeVay, 1991). The appeal of solarisation is that it is a non-chemical, inexpensive and simple technology, with apparently no major undesirable effects on the soil biological balance (Katan & DeVay, 1991).

This paper reports the impact of different medicinal and aromatic plant materials on root knot nematodes in pot experiments and when combined with solarisation under greenhouse conditions in different locations in Lebanon.

MATERIALS AND METHODS

Sample preparation and oil extraction

Plant materials were collected from different regions of Lebanon (Table 1). The essential oils were extracted by steam distillation (Traboulsi *et al.*, 2002). A 100-200g sample of fresh plant material for each plant species was used. Ethyl alcohol (99% pure) was used as solvent for the extraction process. One hundred ml of ethyl alcohol was used for each extraction run. Every run consisted of four extraction cycles, each lasting 20 min at 150ºC heating temperature and 50ºC cooling temperature. Each plant extract was reduced to a volume of 50 ml using a rotary evaporator (90 ºC) and stored at 4ºC until required. To determine the concentration of an essential oil in each plant extract the following formula was used: Yield (%)= (volume of essential oil/weight of dried test material)X100.

Soil used

The soil samples infested with root knot nematode were brought from three different greenhouses in Jiyeh (Chouf area), Khayzaran, Sarafand (south), Minyeh (north) and planted with tomato plants to determine the nematodes population level.

Impact of chopped plant materials on root knot nematodes using pot experiment

Fresh plant materials were chopped into small pieces and 200g were mixed thoroughly with approximately 1kg of infested soil, and then placed in 1.5 liter plastic pot. Each pot was planted with 2 weeks old tomato plant. Each treatment was replicated four times. The control was replicated four times and treated under the same conditions but without treatment. Pots were arranged on the bench in a randomised complete block design, and watered as needed. After one month of tomato growth, plant roots were carefully removed and fresh shoot length, shoot and root weight were recorded. Root galling was rated on scale of 0 to 5 where 0= no nematodes, 1=1-2 galls, 2=3-10 galls, 3=11-30 galls, 4=31-100 galls, 5=more than 100 galls per root system (Tayler & Sasser, 1978).

Greenhouse experiment

Several experiments were done in greenhouses (polytunnels) during 2005-2008 summers at different locations (Jiyeh, Khayzaran, Sarafand, Minyeh) of Lebanon. Four different plant materials were used in each greenhouse (Table 1). Each greenhouse was 35 meters long and 7 meters wide. It was divided into 20 plots; each plot measured 2.5 X 3 meters. One meter guard was between each plot. Control treatments were used without plant materials.

Preparation of the greenhouses and soil collection

Several polytunnels were propagated with vegetables and severely infested with the root-knot nematodes. The soil was ploughed at 30 cm depth and the remains of plant were removed and burned. Poultry amendment (750kg) was spread equally all over the greenhouses and mixed with the soil. Approximately 2 kg of soil samples were collected from each plot to determine the population initial (*Pi)* (Ibrahim *et al*., 2004). To each plot 2 kg of chopped plant materials were added and mixed with the 20 cm of soil top. Drip irrigation pipes were installed for each plot to keep the soil wet in order to help to increase the humidity and thus increase temperature inside the polytunnel The soil was well watered and covered with a tight transparent polyethylene film (50µm). Several thermometers were placed at different positions under cover at 20 cm depth. After four weeks of solarization the plastic cover was removed. Approximately 2 kg of soil samples were collected from each plot in order to determine population final (*Pf)*. Soil samples were colleted at two different depths, 0-15 and 15-30 cm.

Extraction and counting of nematodes

Extraction and nematodes counts were preformed as previously described (Ibrahim *et al*., 2004). Approximately, 1 kg of soil was collected and thoroughly mixed. A two 250g sub-samples were placed in separate sieve (53mm) with tissue paper. Then the sieve was put in a glass funnel. Distilled water was slowly added to moist the soil (Fenwick, 1949). After

24 hours the sieve was removed, the water was transferred into 250 ml cylinder and left for at least 30 min. The excess water was gently sucked out. The remaining 50 ml were thoroughly mixed and 2 ml suspension was transfer into a counting dish and the number of nematodes was counted in each soil sample. Reading was repeated twice, and the final number of nematodes present in 1 g of soil was calculated.

Assessment of the population level (*Pi/Pf*) using pot experiment

Two weeks old tomato (*Lycopersicum esculentum*) seedlings of were transplanted into pots. Each pot contained 650 g of soil from the soil samples used to determine the final population of nematodes in the different plots. Plants were left to grow under sunlight at 23-25ºC. Pots were arranged on the bench in a randomised complete block design, and watered as needed. After four weeks of growth, roots were removed carefully, washed and cleaned from soil. Roots were examined for nematodes infection (galls) and the number of galls was counted.

Statistical analysis

Statistical analysis of the results of “the degree of treatment” was done according to the analysis of variance in the Randomized Complete Block Design, and using the Duncan’s method for pairwise comparison.

RESULTS

Plant oil yields

The percentage of essential oil extracted either from the flowers, leaves, stems or seeds from different species of medicinal or aromatic plants are listed in Table 1. The highest concentration (6%) was detected from leaf extracts of *Origanium syriacum* L. The yields of the other plant species were less than 2%.

Soil temperature

Temperature level during solarisation under greenhouse condition was measured at 20 cm depth. At the beginning of each experiment the soil temperature was approximately 34-36 °C, but rose to 45-47 °C with occasional rise to 55°C at the end of August beginning of September.

Impact of different plant materials on root-knot nematodes in pot experiment

The impact of different plant materials on plant and root-knot nematodes development are presented in Table 2. All the treatments used had significant effect (P=0.05) on nematodes invasion except with *Cymbopogon citratus* and *Petrosilinum crispum* treatments in comparison to the control. The most significant effect on the number of galls was observed with *Tagetes patula* followed by *Pimpinella anisum* and *Origanium syriacum* where the number (galls) of nematodes were below 2.5/g of roots. Total control was obtained with *Allium sativum* extracts. Moreover, significant increase in the shoot and root weight was recorded in the pots treated with *Raphanus sativus*, *Pimpinella anisum*, *Tagetes patula* and *Origanium syriacum* by 139, 92, 88.6, 86.7% respectively. No significant increase of the root system, however, was noted in the treatments with *Cinnamoum zeylanicum* and *Cymbopogon citrates*. The gall index ranged from 0 to 4 in all treatments used, however, root gall development was totally suppressed in the treatment with *Allium sativum* (Table 2).

TABLE 1

Plant Origins, Plant Parts and the Percentage of Essential Oils

Common Name	Plant species	Harvest place	Plant part	Essential oil (%)
Lavander	Lavandula stoechas	*Bchemon* (Mount)	flowers	*0.8*
Myrtle	Myrtus communis	*Akkar*	leaves	*1.2*
Oregano	Origanium syriacum	*Mashgara (Bekaa)*	leaves	*6.0*
Mastic tree	Pistacia lentiscus	*Mashgara (Bekaa)*	leaves	*0.2*
Mint	Mentha microphylla	*Mashgara (Bekaa)*	leaves, flowers	*1.1*
Fennel	*Foeniculum vulgare*	Tabarja (South)	flowers	1.0
Sweet orange	*Citrus sinensis*	Bourghoulieh	leaves	0.25
Eucalyptus	*Eucalyptus*	Hazmieh (Mount)	leaves	0.4
Pine	*Pinus pinea*	Mashgara (Bekaa)	leaves	0.5
Garlic	Allium sativum	Market	cloves	0.3
Sage	*Salvia officinalis*	Mount Lebanon	leaves, stem, flowers	1.3
Anise	Pimpinella anisum	*Mount Lebanon*	*seeds*	2.6
Chamomile	Matricara discoidea	*Mount Lebanon*	*whole plant*	0.9
Chrysanthemum*	Chrysanthemum coronarium	Mount Lebanon	*whole plant*	-
Lemon grass	Cymbopogon citratus	Mount Lebanon	*leaves*	2.1
Marigold	Tagetes patula	Mount Lebanon	leaves, stem, flowers	1.3
Tayoun*	*Inula viscosa*	Chouf	leaves, flowers	-
Cinnamom	Cinnamoum zeylanicum	Mount Lebanon	barks	0.5
Pelargonium	Pelargonium graveolens	Mount Lebanon	leaves	0.8

* Oil content was not determined

TABLE 2

The Effect of Different Medicinal and Aromatic Plant Materials on Nematodes Population and Tomato Growth in Pot Experiment

Treatment	Latin name	Number of galls/ g of root	Number of galls/g roots	(%) Control	Plant height (cm)	Shoot weight (g)	Root weight (g)	Total plant fresh weight (g)	% of increase	Root gall index
Algae	*Ulva lacuca*	3.6	20.0	79.5a	14.3±2.2	2.3±1.1	5.5±4.6	7.8	47.1d	3
Anise	*Pimpinella anisum*	2.0	12.0	87.7a	21.2±1.3	4.2±1.2	6.0±4.2	10.2	92.4c	3
Garlic	*Allium sativum*	0.0	0.0	100a	21.4±2.9	3.3±0.5	5.2±1.9	8.5	60.3c	0
Chamomile	*Matricara discoidea*	9.0	33.4	65.9a	16.8±4.0	2.4±0.2	3.7±1.7	6.1	15.1e	4
Chrysanth-emum	*Chrysanthemum coronarium*	4.9	23.6	75.9a	18.8±1.6	3.5±0.4	4.8±0.7	8.3	56.6d	3
Cinnamom	*Cinnamoum zeylanicum*	15.4	50.4	48.5b	13.6±1.1	2.3±0.5	3.3±0.3	5.6	5.6e	4
Lemongrass	*Cymbopogon citratus*	29.1	64.2	34.4b	15.0±1.4	3.3±0.3	2.2±0.4	5.5	3.7e	4
Inula	*Inula viscosa*	3.5	17.00	82.6a	18.2±3.8	2.7±0.7	4.5±1.1	7.2	35.8d	3
Chinaberry	*Melia azedarach*	1.2	13.0	86.7a	19.5±8.8	3.9±1.6	6.0±3.5	9.9	86.7c	3
Marigold	*Tagetes patula*	1.2	7.0	92.8a	20±2.9	4.4±0.9	5.7±2.2	10	88.6c	2
Mint	*Mentha microphylla*	4.3	22.0	77.5a	12.1±4.5	3.1±1.1	5.1±3.1	8.2	54.7d	3
Parsley	*Petrosilinum crispum*	8.0	20.0	79.5a	11.2±1.3	2.5±0.9	2.9±2.1	5.4	1.8e	3
Pelargoniu m	*Pelargonium graveolens*	4.3	21.4	78.1a	16±1.0	2.5±0.2	4.9±0.5	7.4	39.6d	3
Pistacio	*Pistacia lentiscus*	26.8	88.5	9.6b	13.4±2.3	3.7±0.6	3.3±1.6	7.0	32.0d	4
Radish	*Raphanus*	6.0	33.3	66.0a	20.0±2.1	5.5±1.3	7.2±2.1	12.7	139.6c	4

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Table 2 (continued):

Sage	*Salvia officinalis*	4.8	27.0	72.4a	17.2±1.5	2.3±0.4	5.6±0.2	7.9	49.0d	3
Thyme	*Origanium syriacum*	2.5	15.5	84.1a	19.7±2.3	3.7±0.5	6.2±1.2	9.9	86.7c	3
Control		11.7	98.0	-	12.6±1.6	2.2±0.2	3.1±0.3	5.3	-	4

*Means followed by the same letter are not significantly different at *P*=0.05

The impact of solarisation alone or integrated with different medicinal or aromatic plant materials on root-knot nematodes population under greenhouse conditions

The impact of solarisation or solarisation integrated with different plant treatments on nematode population are presented in Table 3. Significant control was obtained with solarisation alone, but more effective control was achieved when it was integrated with plant materials. For example, when garlic was incorporated with solarisation treatment, the number of nematodes per gram of soil decreased by 23% (Table 3). The extraction result revealed that all the tested soil samples collected from greenhouses in different areas showed high number of nematode populations. The population level ranged from 2.6 to 12 nematodes/g of soil. The highest population initial (*Pi*) level was detected in the plots treated with inula (12nem/g of soil), whereas the lowest *Pi* (2.6nem/g soil) was recorded in plots treated with *Nerium oleander*. Although all the treatments showed significant (P=0.5) effect on nematodes popualtion with different plant extracts, the treament with humic acid alone caused little or no nematode control. The number of nematodes was significanlty reduced (P<0.5) from 12 nem/g soil to 2.3 nem/g soil when garlic-solarasation treatment was used. The highest reduction in the number of nematodes was observed in the plots treadted with methyle-bromide (0.1 nem/g of soil) followed by *Alluim sativum* (0.3 nem/g of soil) and mint (0.3 nem/g of soil).

TABLE 3

Impact of Soil Solarisation Alone or Integrated with Different Plant Materials on Root-Knot Nematodes Under Greenhouse Conditions in Jeyih, Khayzaran, Sarafand and Minyeh Regions

Greenhouse/ treatment	Number of nematodes/g soil		Ratio *Pf/Pi*	(%) of nematodes remaining	Control (%)
	Before treatment (*pi*)	After treatment (*pf*) (duration)			
SS+Adelfa	2.6	0.5a	0.19	19	81
SS+Algae	4.0	0.5a	0.12	12	88
SS+Chinaberry	7.9	2.0a	0.25	25	75
SS+Garlic	6.4	0.3a	0.05	5.0	95
Humic acid	6.3	4.2b	0.67	67	33
Inula	12	2.3a	0.19	19	81
Inula + Humic acid	11	1.5a	0.14	14	86
Mint	2.9	0.3a	0.10	10	90
Parsley	3.5	0.6a	0.17	17	83
Methyl-bromide	3.1	0.1a	0.03	3.0	97
Solarization alone	2.8	0.8a	0.28	28	72

DISCUSSION

It is well recognised that the development of an effective integrated pest management derived from plant or plant bioproducts have no or little adverse effect on plants, beneficial organisms or environment when applied to the plant or soil as alternative to conventional pesticides. Allelochemicals are plant-produced compounds that affect the behaviour of other organisms and thought to be toxins and secondary metabolites, which act as attractants or deterrents (Dodds, 1996; Brown & Morra, 1997). For example, sudangrass contains a chemical, called d'hurrin which is degraded into hydrogen cyanide, which is a powerful nematicide (Wider & Abawi, 2000). Results obtained from this study revealed that all plants tested contained compounds that showed nematicidal effect in both pot experiment and under greenhouse conditions. Several essential oil/plant extracts isolated from similar medicinal or aromatic plants had significant effects on the second stage juveniles of *M. incognita* even at the lowest concentration (1mg liter^{-1}) (Ibrahim *et al*., 2006). Oka *et al.*, (2000) reported that several essential oils from various plants which have shown promise as potential sources for new nematicides. Most of those plants were aromatic or culinary herbs that contained the nematicidal compounds such as carvacrol and thymol. Although over 20 major compounds of essential oils were identified (Ibrahim *et al*., 2006), only carvacrol, linalool, thymol and menthone were the most toxic against the J2 of *M. incognita* at very low concentrations (1mg liter^{-1}). Moreover, several of these oils immobilized juvenile root-knot nematodes and some also reduced hatching of eggs (Ibrahim *et al*., 2006).

The mode of action of nonhost plant residues on plant parasitic nematodes is not known. However, a study has shown that *Brassica* spp (*e.g*., rapeseed, mustard) as a result of enzymatic degradation release by-products which have nematode-suppressive effect by interfering with their reproductive cycles (Brown & Morra, 1997). Other mechanisms could also be responsible for the anti-nematodes activity as it has been shown with *Brassica* leaf manure (McLeod & Steel, 1999). Furthermore, rapeseed and sudan grass green manures grown prior to potatoes gave 86% control of the root-knot nematode in potatoes (Stark, 1995).

Tagetes spp have long been known to possess nematicidal activity (Reynolds *et al*., 2000; Ploeg, 2002). Nematodes are attracted to marigold roots but when they invade them, the root releases ozone killing them (Ogden, 1997). Moreover, French marigold (*Tagetes patula*) was shown not only to be the most effective type in lowering root-knot nematode populations (Ogden, 1997) but also in altering the hatching behaviour of *M .incognita* (Ibrahim, *et al.*, 2006). In this study marigold significantly (*P*=0.05) reduced the number of nematodes in pot experiment. Belcher and Hussey (1977) found that *T. patula* acted as a trap crop to *M. incognita*, but prevented giant cell initiation.

On the other hand, Park *et al*. (2005) reported that garlic and cinnamon essential oils also possess nematicidal activity. Minimum concentration of 1mg liter^{-1} of *Allium sativum* L. and *Foeniculum vulgare* L. significantly (*P=*0.05) decreased the emergence of juveniles of *M. incognita* to 7.6% and 25% respectively (Ibrahim *et al.,* 2006). In the present study total control was achieved with *Allium sativum* in pot experiment and 95% when used together with solarisation under greenhouse conditions.

The highest concentration of carvacrol (61%) was detected in *Origanium syriacum* L plant extracts (Ibrahim *et al.,* 2006). In this study *Origanium syriacum* gave 87% control against root knot nematodes. Some pure essential oils such as carvacrol, linalool, thymol and menthone have exhibited nematode-suppressive characteristics equivalent to cadusafos, a synthetic chemical pesticide (Ibrahim & Haydock, 1999). Similarly, plant extracts combined with solarization in this study gave control ranging from 80 to 95% and was comparable to Methyl-bromide (97%). Leaf powder of rock fleabane (*Inula viscose*) at a concentration of

0.1% in sand reduced hatching of second-stage juveniles of *Meloidogyne javanica* and the citrus nematode (*Tylenchulus semipenetrans*) while the stem-bulb nematodes (*Ditylenchus dipsaci*) were unaffected (Oka *et al.,* 2001). The current study has shown that leave extracts of the same plant combined with solarisation gave 81% control under greenhouse conditions.

Essential oils naturally occurring in *Mentha spicata*, *Mentha rotundiforila* and *Foeniculum vulgare* also exhibited nematicidal activity (Oka *et al*., 2000). In this study *Mentha microphylla* in combination with solarization gave 90% control of nematodes in greenhouses and 55% in pot experiment.

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