

The use of biofumigation crops as an alternative to Methyl Bromide for the management of the root-knot nematode in greenhouse cucumber production

Garabed Haroutunian

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The use of biofumigation crops as an alternative to Methyl Bromide for the management of the root-knot nematode in greenhouse cucumber production

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Résumé

Les nématodes à galles demeurent l'un des problèmes les plus graves des agriculteurs de serres du Liban et du Moyen-Orient. Dans le passé, la fumigation du sol au bromure de méthyle a été considérée comme la meilleure mesure de contrôle contre ces nématodes. Toutefois, à la lumière de l'élimination globale du bromure de méthyle, l'accès aux alternatives efficaces et durables est devenu une nécessité essentielle.

L'objectif de cette étude est d'évaluer l'efficacité des deux engrais verts, le radis huileux (*Raphanus sativus oleifera*) et à la roquette (*Eruca vesicaria sativa*) appliquées avec ou sans bâche en plastique, séparément et en combinaison avec Oxamyl, dans la gestion des nématodes à galles en cultures de concombres de serre. En outre, cette étude vise à évaluer la faisabilité financière de la technique de biofumigation avec les deux cultures utilisées et de la rentabilité finale de ces engrais verts en termes de taux coût-profit par rapport au bromure de méthyle.

A cette fin, trois expériences sur le terrain ont été menées dans des serres commerciales, situées sur la zone côtière du Liban.

Dans les deux expériences A et B, des résultats cohérents ont été enregistrés en termes de production de rendements significativement plus élevés et de la réduction des populations de nématodes avec les engrais verts utilisés avec ou sans bâche en plastique, par rapport au témoin non traité.

Quant aux résultats obtenus par les engrais verts par rapport au bromure de méthyle, dans l'expérience A, le rendement produit par le bromure de méthyle était significativement plus élevé que celui du radis huileux avec bâche en plastique. Cependant, la différence n'était pas significative entre la réduction de la population de nématodes résultant du bromure de méthyle et celle du radis huileux avec bâche en plastique. Les différences du rendement ainsi que la réduction des nématodes étaient significatives entre le bromure de méthyle et le radis huileux sans bâche en plastique.

Dans l'expérience B, aucune différence significative n'a été observée dans le rendement ni la réduction de la population de nématodes entre aucun des deux engrais verts utilisés avec bâche en plastique et le bromure de méthyle. Ces différences étaient

significativement en faveur du bromure de méthyle uniquement lorsque le radis huileux a été utilisé sans bâche en plastique.

Dans l'expérience C, où Vydate (Oxamyl) a été appliqué à la dose de 1 litre par 1,000 m^2 à tous les traitements, il n'y avait pas de différence significative dans le rendement ni la réduction de la population de nématodes entre chacun des deux engrais verts utilisés avec ou sans bâche en plastique et le bromure de méthyle.

L'analyse coût-profit menée sur tous les traitements appliqués dans les 3 expériences a montré que dans tous les cas, tous les traitements appliqués, qu'ils soient chimiques, non-chimique (avec ou sans bâche en plastique) ou en association ont produit des bénéfices nets supérieurs au bromure de méthyle, même lorsque le rendement produit par le bromure de méthyle était significativement plus élevée.

L'utilisation du plastique pour couvrir les radis huileux a généralement produit de meilleurs résultats en termes de production de rendements plus élevés, une meilleure réduction des populations de nématodes à galles dans le sol, ainsi conduisant à des augmentations raisonnables des profits nets.

A la base de ces résultats et à la lumière de l'élimination globale du bromure de méthyle, on peut conclure que l'utilisation du radis huileux et la roquette comme engrais verts avec bâche en plastique peut être considéré comme un outil alternatif pour la gestion des nématodes à galles dans la production de concombres de serre dans les conditions libanaises.

Abstract

Root-knot nematodes remain one of the most serious problems faced by greenhouse farmers of Lebanon and the Middle East region. In the past, soil fumigation with methyl bromide has been considered as the best control measure against root-knot nematodes. However, in the light of the global phase out of methyl bromide, finding efficient and viable alternatives is an essential necessity.

The objective of this study is to evaluate the efficacy of the two biofumigation crops Oil Radish (*Raphanus sativus oleifera*) and Arugula (*Eruca vesicaria sativa*) applied with and without plastic tarp, separately and in combination with Oxamyl in the management of the root-knot nematodes in greenhouse cucumber crops. Additionally, this study aimed at assessing the financial feasibility of the biofumigation technique with the two cops used and the final profitability of these green manure crops in terms of cost to benefit ratio as compared to methyl bromide.

For this purpose, three field experiments were carried out in commercial greenhouses situated on the coastal zone of Lebanon.

In both experiments A & B consistent results were recorded in terms of production of significantly higher yields and reduction of nematode population with the biofumigation crops used whether with or without plastic cover, as compared to untreated fallow.

As to the results achieved by the biofumigation crops with respect to methyl bromide, in experiment A yield produced by methyl bromide was significantly higher than oil radish with plastic cover. However, no significant difference was found between reduction of nematode population resulting from methyl bromide and oil radish with plastic cover. Difference was significant between methyl bromide and oil radish without plastic cover in both yield and reduction of nematodes.

In experiment B no significant difference was observed in neither yield nor reduction of nematode population between any of the two biofumigation crops used with plastic cover and methyl bromide. These differences were significantly in favor of methyl bromide only when oil radish was used without plastic cover.

In experiment C where Vydate (Oxamyl) was incorporated at the rate of 1 liter per 1,000 m2 to all treatments, there was no significant difference in neither yield nor reduction of nematode population between any of the two biofumigation crops used with or without plastic cover.

Cost-benefit analysis made on all treatments applied in the 3 experiments showed that in all cases, all treatments applied, whether chemical, non-chemical (with or without plastic cover) or in combination have produced higher net profits than methyl bromide, even when yield produced by methyl bromide was significantly higher.

Use of plastic for covering oil radish has generally produced better results in terms of production of higher yields, better reduction of soil populations of root-knot nematodes and leading to reasonable increases of net profits.

Based on these findings and in the light of global phase out of methyl bromide, it can be concluded that the use of oil radish and arugula as biofumigation crops with plastic cover can be considered as an alternative management tool for the root-knot nematode in greenhouse cucumber production under Lebanese conditions.

Mots Clés

Alternatif

Bâche en plastique

Bénéfices nets

Biofumigation

Bromure de méthyle

Concombre

Coût-profit

Engrais verts

Eruca vesicaria sativa

Liban

Nématodes à galles

Oxamyl

Population

Radis huileux

Raphanus sativus oleifera

Rendement

Roquette

Serre

Vydate

Keywords

Alternative
Arugula
Biofumigation
Boss
Control
Cost-benefit
Cucumber
Eruca vesicaria sativa
Greenhouse
Green manure
Lebanon
Management
Meloidogyne incognita
Methyl Bromide
Mulch
Nematode
Net profit
Oil radish
Oxamyl
Plastic
Population
Raphanus sativus oleifera
Root gall
Root-knot
Tarp
Temperature
Variety
Vydate
Yield

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GENERAL INTRODUCTION

The total agricultural area of Lebanon is 247,939 hectares, 42% of which is irrigated and 40% of Lebanese work in agriculture and related activities (FAO, 2000). Greenhouse production is a major component of the overall Lebanese agriculture. In 2001, the total area of greenhouses in Lebanon reached around 2,000 hectares, of which vegetable production occupied 92%, cut flowers 5% and strawberries 3% (UNDP-Methyl Bromide Alternatives Project-Ministry of Environment of Lebanon, 2001). More than 80% of the 33,781 greenhouses in Lebanon are located along the coastline, which stretches over 225 kilometers from North to South on the East side of the Mediterranean (UNDP-Methyl Bromide Alternatives Project-Ministry of Environment of Lebanon, 2001).

Due to Lebanon's warm and humid climate and short winter seasons, *Meloidogyne incognita* (root-knot Nematode) is a major factor limiting vegetable production. Whereas some tolerant varieties of tomato exist and are widely used by farmers, all cucumber cultivars (*Cucumis sativus L.*) remain susceptible to *M. incognita*, and therefore cucumber production in Lebanon is strongly associated with pre-plant soil fumigation. Methyl Bromide was almost exclusively used for this purpose throughout many years. However, despite of its efficiency in controlling of a wide range of soil borne pests and pathogens in high-value horticultural crops, this fumigant was found to be as one of the most powerful Ozone Depleting Substances (ODS). It was reported that the Bromine atom it contains acts quickly in the stratosphere to break down sixty times as much Ozone as a Chlorine atom from CFC emissions. Methyl Bromide released from human activities was found responsible for 5 to 10% of the total Ozone depletion in the earth's stratospheric layer (MBTOC, 1997). Based on these considerations, in 1997 parties of the Montreal Protocol for the protection of the Ozone layer agreed to phase out Methyl Bromide and replace it with ozone friendly, safe and viable alternatives throughout the world. It was also decided to start gradual decrease immediately and achieve complete phase out of Methyl Bromide by year 2005 in the developed counties, and by year 2015 in Article 5 countries, among which is Lebanon (UNEP, 2001).

Having ratified the Montreal Protocol back in the year 1993, Lebanon was committed to its resolutions. Therefore, following the implementation of a demonstration project on the

efficiency of various chemical and non-chemical methods as alternatives to Methyl Bromide, the Ministry of Environment of Lebanon launched two large-scale projects in the year 2002, in coordination with the United Nations Development Programme (UNDP) and the United Nations Industrial Development Organization (UNIDO), having as objective to replace Methyl Bromide with environment friendly and viable alternatives by the year 2008, seven years before the 2015 deadline.

The most common soil-borne problems necessitating Methyl Bromide fumigation in Lebanon are nematodes, fungal diseases such as Fusarium and Verticillium wilt and weeds. However, nematodes are by far farmers' fiercest enemies. Among a variety of plant parasitic nematode species, *Meloidogyne incognita* (also referred to as Southern root-knot nematode) is the most wide-spread and ravaging. Root-knot nematodes cause extensive damage to a wide variety of economically important crops (Sasser, 1980). Along with *M. javanica* and *M. arenaria*, *M. incognita* is considered as one of the most important species in terms of both its worldwide geographical distribution and very large host range, reaching up to 3,000 different plant species (Lamberti, 1979).

In addition to the egg and adult stages, the life cycle of *Meloidogyne incognita* comprises four juvenile stages and four molts. The first stage juvenile develops in the egg, where the first molt usually occurs. Infective second-stage juveniles (or J2) emerge free from the egg into the soil or plant tissue, penetrate the rootlets and start feeding on the host plant by establishing feeding cells and consuming photosynthate produced in the leaves to supply their energy demands (Bergeson, 1966; Kochba and Samish, 1971). This results in the increase of root weight and decrease of shoot weight, thus shifting the root-to-shoot balance (Fortnum *et al*., 1991). Due to the fact that second stage juveniles of the root-knot nematodes enter the roots, create a permanent feeding site, become sedentary before molting and stay there for the rest of their life cycle, this specie is classified as sedentary endoparasite. The second, third and fourth molts occur producing the third, fourth and fifth or adult stages, respectively.

Root-knot nematodes have very small sizes, infective second stage juveniles varying between 0,3 and 0.5 mm in length. Females can reach up to 1.3 mm. Their bodies are externally coated with a transparent layer of cuticle (Sasser & Carter, 1985). They are able

to move easily from infested fields to clean soils through a variety of means, like equipment and machinery, transplants, organic manure, irrigation, and farm animals.

Symptoms of root-knot nematode attack are clearly visible as formation of galls on the roots and wilting of the upper parts of the infected plants. Moreover, soil-borne diseases can more easily penetrate in roots infected by nematodes, thus causing damage superior to the one caused by the nematode itself. Furthermore, Lebanon's warm and hot climate, short winter seasons, light sandy soils, and high soil moisture enhance rapid multiplication of this species.

Due to the impending phase out of Methyl Bromide, finding alternative nematode management option has become an imperative necessity. While most chemical nematicides are either less effective, or too expensive, and associated with a negative impact on the environment and public health (Braun and Supkoff, 1994), there is increased interest in non-chemical nematode management strategies. Therefore, soil biofumigation with the use of green manure crops was among the alternatives proposed by the Lebanese Ministry of Environment in the production of vegetable crops (Methyl Bromide Alternatives-Lebanon, 2001).

Many studies indicate that plants of the Brassicaceae family have the potential of replacing fumigant nematicides in the production of a large variety of crops. Growing trap crops in nematode infected soils triggers nematode eggs to hatch and induces the larvae to enter the crop wherein they are unable to reproduce (Hafez & Sundararaj, 1999). Green manure crops also increase soil organic matter and nutrient levels, thus increasing soil productivity (Davis *et al*., 1996). Incorporation of nematode resistant crops in rotation with sugar beet is the most economical method of sugar beet cyst nematode management. It has the potential of reducing nematode population considerably, as it improves soil structure and retains nutrients in the soil (Redondo and Villarias, 1991).

McLeod *et al*. (2001) have found that three weeks after inoculation, more *M. javanica* were developed into mature females in tomato than in eight Brassica crops tested. Ploeg and Stapleton (2001) stated that there is significant interaction between soil temperature, treatment time needed, and effect of soil amendment with chopped broccoli leaves for nematode control. They found that whereas at 20°C adding broccoli to the soil had very little

effect on *M. incognita* and *M. javanica* infestation and root galls of melon, at 30-35°C dramatical reduction of infestation and galling was observed.

Among many other Brassica plants, arugula (*Eruca sativa*) possesses biofumigant and trap crop qualities, and it has recently gained popularity as a potential alternative to Methyl Bromide. In a study conducted to determine the effects of arugula on three different glasshouse populations of *Meloidogyne hapla*, egg and egg mass production occurred at a normal rate in all nematode infected tomatoes, but no eggs were produced in more than 80% of arugula plants twenty-eight (28) days after nematode inoculation (Melakeberhan *et al*., 2006). Fourteen to fifteen weeks after sowing, *Eruca sativa* and *Raphanus sativus* were found as poor non-host species to the root-knot nematode (Curto *et al*., 2005). Pattison *et al*. (2006) also found that *Raphanus sativus* (oil radish) and *Sinapis alba* (white mustard) are consistently resistant to nematode reproduction and the formation of root galls.

Brassica varieties with high glucosinolate content are likely to suppress soil borne pests when provided with adequate moisture, which increases the release of isothiocyanates in the soil (Morra and Kirkegaard, 2002). All fifteen Brassica green manure crops tested have substantially lowered *M. javanica* population levels, with significant treatment effects at both application rates of ten and twenty g/kg soil. *M. javanica* has produced more eggs on tomato than on most of the Brassica crops. However, no correlation was found between higher concentrations of the dominant glucosinolates (progoitrin [2-hydroxy-3-butenyl], glucobrassicanapin [4-pentenyl] and gluconasturtiin [2-phenylethyl]) of the Brassica green manure crops and low egg production. This lead to conclude that mechanisms other than glucosinolate-derived toxicity are involved in the nematicidal activity of Brassica green manure crops (McLeod and Steel, 1999).

Potter *et al*. (1998) had also found that despite the suppression of 56.2-95.2% of the exposed root lesion nematodes (*Pratylenchus neglectus*) through soil amendment with Brassica leaf tissues, little of this nematicidal effect of Brassica leaves could be related to either total glucosinolate content or any individual glucosinolate, as determined by HPLC analysis. While total glucosinolate levels had a non-significant relationship with nematode mortality, levels of 2-phenylethyl glucosinolate was significantly correlated with nematode suppression ($P < 0.001$). Amendment of soils with equimolar levels of purified 2-phenylethyl isothiocyanate resulted in comparable levels of nematode mortality, which lead Potter *et al*.

to conclude that 2-phenylethyl glucosinolate had a role in the suppressive impact of Brassica green manure crops.

Further investigation of glucosinolates and their hydrolysis derivatives resulting from Brassica plants and particularly the isothiocyanates revealed that in-vitro pure glucosinolate had no effect on the root-knot nematode *Globodera rostochiensis*, while a mixture of 2-phenylethyl glucosinolate (1 mg/ml) and myrosinase (25%) caused 100% mortality within eight hours (Serra *et al*., 2002).

Several ornamentals and flowers were also used as green manure crops with satisfactory results. Chrysanthemum (*Chrysanthemum coronarium*) has significantly reduced *M. incognita* and *M. javanica* infection of tomato roots both in the greenhouse and microplots. Green plant parts have been more effective than flowers. Chrysanthemum has preserved its nematicidal activity even when applied as a dried material. An aqueous extract of Chrysanthemum has inhibited hatching of both *M. incognita* and *M. javanica* from eggs and egg-masses (Bar-Eyal *et al*., 2006).

The nematicidal and nematostatic characteristics of *Tagetes* species have also been subject to many studies. Sellami and Zemmouri (2001) found that mortality of *M. incognita* Chitwood juveniles increases at higher concentrations of aqueous extracts from different parts of *Tagetes erecta* plants. Egg hatching was also inhibited by 80%. Intercropping a susceptible crop (tomato) with *Tagetes erecta* reduced nematode population in the soil and root galling as well. In a similar study, Antoon Ploeg (2000) found that while galling and *M. incognita* populations were high when tomato followed tomato, they were very low when tomato was planted after pre-cropping with *Tagetes patula* (marigold) for eight weeks.

Some *Crotalaria* species have also been investigated for their susceptibility to *M. incognita* and *M. javanica* as compared to a sensitive host (tomato). Nematode invasion rates on *Crotalaria* spp. did not exceed 7.17% for *M. incognita* and 5.25% for *M. javanica*, while reaching 97% on tomato. Moreover, the inoculated J2 which invaded tomato plants developed into adult females, while those on *Crotalaria* spp. rarely developed beyond the third stage, confirming that all *Crotalaria* spp. evaluated are non-host or poor host plants to nematodes (Germani and Plenchette, 2005). In another study, Jourand *et al*. (2004) found that an aqueous extract from leaves of *Crotalaria virgulata* subsp. *grantiana* had nematostatic effect on second-stage juveniles of *M. incognita* at the concentration of one (1)

mg/ml (w/v). Furthermore, freeze-dried aqueous extract from *C. grantiana* leaves added to a sterile sandy substrate at the same concentration prevented *M. incognita* infestation of susceptible tomato plants.

Biofumigation with the use of green manure crops of the Brassica family may be an effective management practice to reduce populations of plant parasitic nematodes, thus having the potential to replace fumigant nematicides (Mojtahedi *et al*., 1991; McFadden *et al*., 1992; Spak *et al*., 1993; Angus *et al*., 1994). However, a better understanding of the actual effects of green manure crops on the root-knot nematodes is needed in order to elaborate improved management techniques.

In the past years, extensive research has been carried on the efficiency of biofumigation in the management of plant parasitic nematodes on plants of the Solanaceae family, which have a certain degree of resistance or tolerance towards these nematodes. However, little information is available on the applicability of this technique in the production of susceptible vegetable crops, such as cucumbers under greenhouses. Similarly, literature is rich in studies on the effects of different types of plastic mulches on nematode populations in the soil in different treatment methods varying from soil solarization to chemical fumigants (Singh, N.D. 1975; Wang, D. 1999, Ou, L.T. *et al*., 2005;), but in general, very limited data is available on the effect of covering soil treated with green manure crops with plastic on the management of nematodes through biofumigation. No such literature was found on any such effect of covering soil treated with oil radish specifically. Since biofumigation is a recent non-chemical plant protection management practice introduced in the Middle East and Mediterranean countries, no information is available on the efficacy of these crops on nematode management in these regions. In this research, two different crops of the Brassica family suitable for greenhouse production in Lebanon were identified as potential green manure crops. The efficacy of each crop, a local cultivar of arugula (*Eruca vesicaria sativa*) and a commercial cultivar of oil radish (*Raphanus sativus oleifera* cv. Boss), was investigated in terms of suppression of the root-knot nematode (*M. incognita*) population in the soil, and gall formation on the roots of cucumber crops in two consecutive growing seasons. Furthermore, the effect of covering the green manure crops with plastic tarp after their incorporation to the soil was tested. In addition, the effect of incorporation of a low dose of a chemical nematicide (Oxamyl-Vydate) to soil treated with each green manure crop was

studied. Finally, the effect of each treatment on total yield of cucumber was investigated, and a cost benefit analysis was done in order to explore the profitability of each treatment method as compared to Methyl Bromide.

In the light of the global phase out of Methyl Bromide and the recommendation made by the Lebanese Methyl Bromide Alternatives Project regarding the adoption of biofumigation as an alternative, in addition to the lack of adequate knowledge on the proper application method of this technique, scientific evaluation of the efficiency and cost effectiveness of biofumigation in Lebanon is an important area of research. In case where biofumigation (either alone or combined with low doses of some chemicals) is found a reliable and cost effective method, many small-scale farmers of Lebanon and the region would adopt it as a sound alternative to Methyl Bromide.

Therefore, the objective of this study was to evaluate the efficacy of two biofumigation crops (with and without plastic tarp) separately and in combination with Oxamyl in the management of the root-knot nematodes in greenhouse cucumbers.

Methodology followed in the design of the experiments was based on a practical and applied research approach, in order to facilitate the adoption and application of biofumigation by farmers. For this purpose, all materials used in the experiments such as seeds of the two biofumigation crops and the cucumber variety, irrigation equipment and the plastic tarp were selected according to their availability in the local market.

CHAPTER I

Efficiency of Oil Radish (*Raphanus sativus oleifera*) and Arugula (*Eruca vesicaria sativa*) in Controlling *Meloidogyne incognita* in Greenhouse Cucumber Crops With and Without Plastic Tarp.

INTRODUCTION

Root-knot nematodes are a group of semi-sedentary endoprasitic nematodes which form specialized feeding cells in the roots of their host plants and utilize the photosynthate produced in leaves for their energy needs (Kochba and Samish, 1971). As a result of such parasitism, root weight increases while shoot weight declines (Fortnum *et al*., 1991). Root-knot nematodes attack a wide range of horticultural crops causing severe damage reaching up to 25% yield loss. *Meloidogyne incognita* and *M. javanica* are the most abundant nematode species in parts of Europe, Africa, South America and the Caribbean (Trudgill *et al*., 2000). They are one of the most serious problems of greenhouse crop production in Lebanon as well. In the last decades soil fumigation with Methyl Bromide has been the most common method of nematode control in the country (Abou-Jawdah *et al*., 2000). However, due to increasing environmental concerns, Methyl Bromide is being phased out globally in accordance with the requirements of the Montreal Protocol for the preservation of the Ozone layer. Most countries will ban Methyl Bromide by year 2015 (UNEP 1992). In addition, the impacts of many pesticides on the environment and human health are currently being re-evaluated (Obenauf, 2004).

Therefore, more emphasis is currently put in the development of environment friendly, efficient and sustainable alternative techniques (Katan *et al*., 1976; Dufur *et al*., 2002). Some chemicals produced by certain plants have the potential of managing some pests and nematodes (Oka *et al*., 2006). Many chemicals having nematicidal properties have been isolated mainly from plants of the *Asteraceae* family (Gommers and Bakker, 1988). Besides having the great advantage of being safe for the environment and public health (Chitwood, 2002), these phytochemicals have shown nematicidal activity at low concentrations in-vitro (Uhlenbroek and Bijloo, 1958 & 1959). However, effective nematode management with the use of such phytochemicals in field applications could not be achieved

(Gommers and Bakker, 1988). It is most likely because of this that none of these chemicals have been developed into large scale commercial nematicides (Oka *et al*., 2006), except some formulations of neem (*Azadirachta indica* A. Juss.) extracts, which are commercially available and used mostly to control some insects and nematodes in IPM systems (Mojumdar, 1995). Nematicidal and fungicidal properties have been attributed to elecampane (*Inula viscosa*, syn. *Cupularia viscosa*, *Dittrichia viscosa*) (Asteraceae), which is a widespread plant in the Mediterranean countries. On lettuce, Oka *et al*. (2006) found that leaf extracts of this plant reduced *M. javanica* populations and gall index at concentrations as low as 0.01%. However, only a slight nematicidal effect was observed in the field (Oka *et al*., 2006).

The effect of many antagonistic fungi and bacteria has also been evaluated. Gautam *et al*. (1995) have assessed the effects of the fungus *Paecilomyces lilacinus* and the bacterium *Bacillus subtilis* separately and in combination with the common water hyacinth *Eichhornia crassipes* as green manure on the root-knot nematode. Both treatments of *P. lilacinus* and *B. subtilis* have increased tomato yields. However, best results were obtained when both of these biocontrol agents were used together in combination with water hyacinth as green manure (Gautam *et al*., 1995).

In the past decade growing interest was shown towards using cover crops in general and Brassicaceae plants as green manures for the control of soil fungi, nematodes, and other soil-borne pests in sustainable vegetable production systems (Lazzeri *et al*., 1993; Buskov *et al*., 2002; Davis *et al*., 1996). It has been found that the biocidal activity of these plants is due to the presence of certain organic compounds in their cells called glucosinolates, which in the presence of the myrosinase enzyme also released by the plants, are hydrolyzed into biologically active compounds such as thiocyanates, isothiocyanates, nitriles, epithionitriles (Fahey *et al*., 2001).

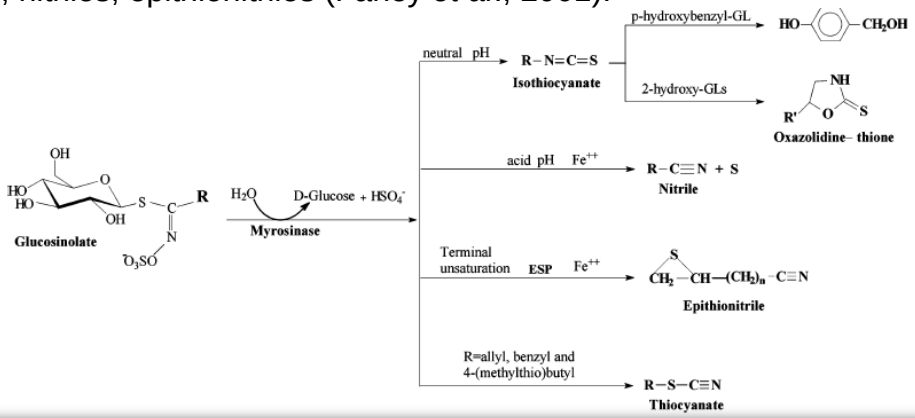


Figure # 1:
Degradation of glucosinolates into different organic compounds

Reproduction of the root-knot nematode in plant roots depends on the ability of the host plant to supply the necessary habitat and energy for the development of the nematode (Fortnum *et al*., 2000).

A good cover crop grown for the management of plant-parasitic nematodes has non-host or poor host status for the target nematodes, and has suppressive effect on the nematode population in the soil when applied as green manure (Viaene and Abawi, 1998).

Several cover crops have suppressed major nematode pests efficiently (Brodie *et al*., 1970; McSorley and Dickson, 1995; Rodriguez-Kabana *et al*., 1989; Wang *et al*., 2002). Cereal cover crops such as rye (*Secale cereale*) and oat (*Avena sativa*) have decreased *M. incognita* populations better than certain leguminous cover crops like lupine (*Lupinus angustifolius*), crimson clover (*Trifolium incarnatum*) and hairy vetch (*Vicia villosa*) during the winter season in Florida (Wang *et al*., 2004).

Various studies on the host status of different Brassicaceae plants to *M. incognita* have produced diverse results. An experiment on the life cycle of *Meloidogyne* on some Brassicaceae plants has confirmed the good host status and the resulting poor nematicidal activity of these plants (Mojtahedi *et al.*, 1991; McLeod & Steel, 1999, McSorley & Frederick, 1995).

Other experiments have shown considerable differences between root penetration and development potentials of *M. incognita* and *M. javanica* stage two juveniles in resistant Brassicaceae plants and other susceptible crops such as cucumber (*Cucumis sativus*), pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) (Haynes & Jones, 1976, Khan & Khan, 1991).

It has also been found that while *Meloidogyne* completes its life cycle in four weeks on garden tomato, the same process takes fourteen weeks on *Eruca sativa* cv. Nemat, and it does not complete at all on *Raphanus sativus* cv. Boss. Therefore, *Raphanus sativus* cv. Boss and *Eruca sativa* cv. Nemat are classified as non-host and poor-host species, respectively (Curto *et al*., 2005).

Melakeberhan *et al*. (2006) have found that production of eggs and egg masses in all tomato plants infected with *M. hapla* has occurred normally, whereas no eggs were produced in more than 80% of the *Eruca sativa* plants tested after twenty-eight (28) days of inoculation. This has shown that *Eruca sativa* hinders the development and reproduction of

M. hapla, and therefore it has the potential of being used as a trap crop for the management of *M. hapla*.

Another laboratory experiment has shown that although the sugarbeet cyst nematode *Heterodera schachtii* was able to penetrate certain oil radish varieties tested (Nemex and Pegletta), reproduction was significantly suppressed and no cysts were developed over the duration of the experiment (Gardner and Caswell-Chen, 1993).

A study has found that differentiation of *H. schachtii* into males or females depends on the ability of the host plant to produce certain essential amino acids (Grundler *et al*. 1991). A study investigating host suitability of several plants has found that vetiver grass has shown clear resistance to both *M. javanica* and *M. incognita* race two (Fourie *et al*., 2007). Among six crucifer plants tested in Spain, *Eruca vesicaria* and *Raphanus sativus* have been the least suitable hosts for *Meloidogyne arenaria* and *M. incognita,* respectively (Liebanas & Castillo, 2004).

In-vitro trials have shown a clear nematicidal effect of glucosinolate degradation products (GLDPs) of *Eruca sativa* cv. Nemat, *Raphanus sativus* cv. Boss, and *Brassica juncea* sel. ISCI 99 on *M. incognita*. Results have indicated that GLDPs of these same plants also have an immobilization effect on J2 larvae of *Meloidogyne incognita* (Lazzeri *et al*., 2004). In another laboratory experiment incorporation of fresh broccoli (*Brassica oleracea*) tissue at a 10% ratio (20 g of broccoli tissue in 200 g of soil) to the top 10 cm of soil in 50 cm glass tubes has reduced *Meloidogyne* J2 populations by 57 to 80% in three weeks. Better results (up to 96% reduction) were obtained by mixing a much lower ratio (2%) of broccoli tissue throughout the length of the glass tube (Roubstova *et al*., 2007).

However, several bio-assay experiments on Brassica tissues have shown that the actual conversion ratio of glucosinolates into isothiocyanates may be very low, and that it is difficult to establish a distinct relationship between the glucosinolate content of these tissues and their pest suppressive effect in general (Angus *et al*., 1994; Lazzeri and Manici, 2001; Harvey *et al*., 2002), and nematostatic features in specific (Kirkegaard and Matthiessen, 2004). Therefore, the extent to which nematode control achieved through biofumigation can be properly attributed to the isothiocyanate gases released remains unclear.

Many studies have confirmed that the use of plastic mulch generally increases soil fertility and improves yields of various crops (Johnson *et al*., 1979; Sumner *et al*., 1978;

Katan *et al*., 1987), including cucumbers grown in nematode infested fields (Coates-Beckford *et al*., 1997). The application of many chemical and non-chemical nematode management techniques necessitates a plastic cover. Plastic is generally used either to increase soil temperatures or to maintain volatile fumigants or gases released by some chemicals or crops in the soil for longer periods. For this purpose, clear or transparent plastic is used to cover either the entire soil or the planting beds (Saha *et al*., 2007).

1,3-Dichloropropene and Chloropicrin are good examples of liquid volatile fumigants requiring plastic cover (Ou *et al*., 2005). Other non-volatile chemicals like Metam Sodium quickly convert into methyl isothiocyanate (MITC) when applied in moist soils, and therefore plastic cover is needed to trap the MITC released in the soil (Smelt *et al*., 1989). Wang *et al*. (1999) have found that virtually impermeable films (VIF) are 190 times less permeable to cis- and trans-1,3-Dichloropropene than regular polyethylene films (Wang *et al*., 1999).

Many scientists have referred to soil solarization as one of the most efficient non-chemical methods of soil treatment for nematode control (McGovern and McSorley, 1997; Chellemi *et al*., 1993; Heald and Robinson, 1987; McSorley and McGovern, 2000; McSorley and Parrado, 1986).

Soil solarization alone or in association with certain chemical nematicides and fungicides has been recommended by many researchers as a sound alternative to Methyl Bromide for the control of soil-borne pests and pathogens (De Vay and Stapleton, 1998; Lamberti *et al*., 2000). The effects of the use of various types of plastic mulches on nematodes through soil solarization have been studied. In general, transparent or clear plastic mulches have proven more effective in heating the soil than black mulches (Katan, 1981). Through a field experiment in Jamaica, Coates-Beckford *et al*. (1998) found that at average monthly air temperatures above 26 ^{o}C and daily sunshine exceeding seven hours, covering the soil with plastic mulches of suitable thickness increases cucumber yields and suppresses the population of phytoparasitic nematodes throughout the growing period (Coates-Beckford *et al*., 1998).

Lamberti *et al.* (2000) have reported that covering the soil with plastic for eight weeks at soil temperatures varying between 35 and 45 ^{o}C at 15 cm depth did not increase lettuce yields significantly in Italy, and had little effect on *M. incognita*. Whereas incorporation of Fenamiphos at 15 kg of ai/ha with solarization increased lettuce yields and reduced root gall

index significantly in a period of four weeks. Similar results were obtained on tobacco. Increasing rates of Fenamiphos and longer solarization periods have always produced better results (Lamberti *et al*., 2000).

However, results of nematode control with soil mulching have often been inconsistent and even contradictory. Hankin *et al* have found that covering the soil with plastic is an ineffective measure to control nematodes in low temperatures (Hankin *et al*., 1982). Others have reported that solarization does not have a long lasting suppressive effect on nematodes as compared to Methyl Bromide (Overman and Jones, 1986).

Singh (1975) has reported that the use of plastic mulch even in combination with chemical nematicides has not enhanced the effect of these chemicals on plant parasitic nematodes (Singh, 1975). Furthermore, taking into account the positive effect of higher soil temperatures caused by mulching on root growth and development, Johnson *et al*. (1981) have assumed that plastic mulches account for providing nematodes with additional feeding sites, thus increasing their populations. However, they have found that plastic mulches reduce crop losses and increase yield of vegetable crops (Johnson *et al*., 1981). While several studies have shown that covering the soil with mulch for solarization has no effect on *M. incognita* (Stapleton and De Vay, 1983; Barbercheck and Von Broembsen, 1986; Duncan *et al*., 1992), many others have found that a large variety of plant-parasitic nematodes including some *Meloidogyne* species can be effectively controlled by soil solarization (Siti *et al*., 1982; Porter & Merriman, 1983; Lamondia & Brodie, 1984). It was also found that soil solarization with plastic mulch improves plant growth as effectively as different nematicides or solarization-nematicide combinations (Stapleton & De Vay, 1986; Stapleton *et al*., 1987). Plastic mulching of the soil has also reduced the population densities of different plant-pathogenic nematodes in cucumber crops significantly (Coates-Beckford *et al*., 1997).

Around a decade later, McSorley has proposed to use solarization in combination with other non-chemical nematode control measures such as cover crops. Although he has found that whereas solarization and a poor host cowpea variety (*Vigna unguiculata* (L.) Walp) used alone were not effective, their combination was as effective as Methyl Bromide in reducing root-knot nematode populations and increased pepper yields even more than Methyl Bromide (McSorley, 1998).

Stapleton and Duncan (1998) have found that incorporating residues of broccoli and other cruciferous plants to the soil before covering it for solarization can control *M. incognita* even at sub-lethal temperatures (Stapleton, J.J. & Duncan, R.A., 1998). Ploeg and Stapleton (2001) have demonstrated that covering the soil with plastic mulch increases soil temperature, and found a strong correlation between soil temperature levels and the effect of soil amendment with broccoli residues on *M. incognita*.

Bello *et al*. (2004) have studied the efficacy of various sources of organic material as potential biofumigation crops. They have found that most crops studied can effectively control *Meloidogyne* spp. In general, they have recommended using material with a C/N ratio between eight and twenty (20), at an application rate of fifty (50) tons of material per hectare. They have mainly stressed on the necessity of covering the soil with plastic for at least fifteen (15) days and application of biofumigation only at soil temperatures above 20°C (Bello *et al*., 2004) for satisfactory results.

Lopez-Perez *et al*. (2005) have studied the effect of soil temperature on the efficiency of three plant residues and chicken manure in root-knot nematode suppression. They have found that residues of broccoli, tomato, and melon plants have all reduced galling and root-knot infestation of the subsequently grown susceptible tomato cultivar at soil temperatures of 25 °C and 30 °C, but not at 20 °C (Lopez-Perez *et al*., 2005). These results correspond to the earlier findings of Ploeg and Stapleton (2001) (Ploeg and Stapleton, 2001) and with recommendations by Bello *et al*. (2004) (Bello *et al*., 2004). Furthermore, through the experiment mentioned above, Lopez-Perez *et al*. have found that at a soil temperature of 25 °C, residues of broccoli have reduced root-knot population more than tomato and melon, but at 30 °C residues of all three plants have been equally effective. Finally, chicken manure used alone without addition of any plant residue has also reduced galling and *M. incognita* infestation at 30°C (Lopez-Perez *et al*., 2005).

Two field experiments were carried out in Lebanon to assess the efficiency of oil radish (*Raphanus sativus oleifera*) and arugula (*Eruca vesicaria sativa*) in the control of *M. incognita* in greenhouse cucumber crops, in addition to the effect of covering these two biofumigation crops with plastic tarp for 15 days (experiment A) and 10 days (experiment B).

MATERIALS AND METHODS

Two field experiments (A & B) were conducted during consecutive autumn growing seasons in two adjacent greenhouses located in the coastal area of Northern Mount Lebanon. History of heavy root-knot nematode infestation was the main criterion for the selection of both greenhouses.



Figure # 2:
Heavy infestation of the previous tomato crop with root-knot nematodes

Experiment A

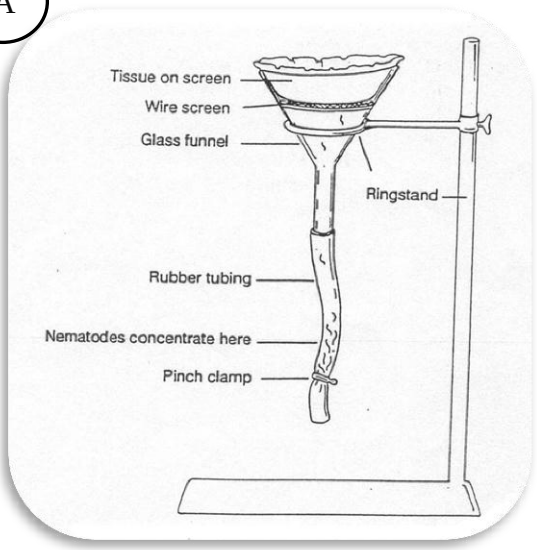
The first experiment was conducted in a greenhouse with an area of 336 m^2 (8 x 42 meters) covered with low density polyethylene (LDP) sheets of 200 microns thickness. During the previous (fall) season, the greenhouse was planted with a commercial tomato variety. The tomato crop was completed by mid-July, immediately after which the soil in the greenhouse was plowed and chemical fertilizer (NPK 17:17:17) was applied at the rate of 150 kgs per dunum (1 dunum=1000 m^2). The total greenhouse area of 336 m^2 was divided into sixteen (16) plots of 4.75 x 4.00 (19 m^2) each for the application of the following four treatments with four replicates for each treatment (as shown in figure # 10, page 34):

1. Control (untreated fallow)
2. Methyl Bromide
3. Oil radish (*Raphanus sativus*) variety “Boss” without plastic cover
4. Oil radish (*Raphanus sativus*) variety “Boss” with plastic cover

An initial soil analysis was done immediately following plowing after the end of the previous tomato crop for the confirmation of infestation of the soil with root-knot nematodes. For this purpose, a W shaped sampling pattern recommended by Hafez S. was followed, whereby five individual core samples of 500 grams were taken from the intended cucumber crop's root zone at a depth of 25 cm from each of the sixteen (16) plots, after removing the top 5 cms of soil (Hafez, S., 1997). Every five core samples taken from each plot were mixed together and a composite representative sample of each treatment area was thus prepared for analysis. A final sample can be considered as representative if it contains a minimum of four individual cores for each acre (Hafez, S., 1997), which is equivalent to one individual core for every 1,000 m^2. Therefore, samples prepared as described above were representative since they contained one individual core sample for every 3.8 m^2 of each treatment area. Representative samples thus formed were analyzed at the laboratory of the Ministry of Environment of Lebanon through a combination of Cobb's sifting and gravity method (Cobb, N.A., 1918) and the Baermann funnel technique (Baermann, G., 1917). This method was preferred due to the availability of the needed sieves and equipment in Lebanon, as well as to the fact that it minimizes the disadvantages of each method when applied separately. Furthermore, this combination method is found ideal for extraction of nematodes from relatively small soil samples. It usually results into clear samples containing less silt and debris, and allows recovery of both sluggish and active nematodes (Dela Cruz, *et al.,* 2005).

Representative soil samples were washed, and nematodes were extracted from the soil suspensions by differential sieving with the use of 60, 200 and 400 mesh sieves (250-µm, 74-µm and 38-µm aperture respectively). Sieving through the 400 mesh screen was repeated three times in order to collect any small sized nematodes that might have been passed through the sieve the first two times. Collected nematode suspensions were then placed on the Baermann funnels which were filled with water, and kept in normal room temperature of 27 ^{o}C. After 48 hours 10 ml of water containing the root-knot nematodes having migrated to the bottom of the funnels were collected in beakers, and identification and count of these nematodes took place at room temperature (Baermann, G., 1917; Barker, K.R., 1985).

A



B



C



Figure # 3 (A, B, C):
The Baermann funnel apparatus

An overhead mini-sprinkler system was installed in the greenhouse for the irrigation of the eight oil radish plots of treatments three and four. On August 9th, the oil radish (*R. sativus* spp. *oleifera*, variety "Boss") was sown at the rate of 3 kg per dunum. Six weeks later, on September 18th, when the height of green manure crop reached 35-40 cm, it was plowed and incorporated into the soil with a rotary tiller. Four plots were covered with transparent polyethylene films (50 microns), and another four plots were left uncovered.



Figure # 4:
Growth of the oil radish crop before plowing and incorporation to the soil



Figure # 5:
Plowing and incorporation of the oil radish crop to the soil by tractor

On the same day, four plots were treated with Methyl Bromide at the rate of 100 kg/ dunum, and covered with the same type of polyethylene sheet.



Figure # 6:
Methyl Bromide fumigation and covering of the oil radish crop with plastic

Soil temperature was recorded under each of the four replicated areas treated with oil radish and covered with plastic at 30 cms depth, in order assess any correlation between the effect of oil radish and heat on the nematode population. Readings were taken on daily basis at 16:00 hours (when soil temperature would presumably be at its peak), during the entire period of two weeks when the soil was covered with plastic (from 18 September until 2 October).

Fifteen days later, on October 3rd the plastic films were removed from all covered plots and the soil was aerated for three days, after which a drip irrigation system was installed to incorporate ten plantation rows in the greenhouse.

Meanwhile, 1,000 seeds of a Parthenocarpic cucumber variety (Thiram treated Noble F1 produced by De Ruiter Seeds) were sown in plastic pots on September 25th. Eleven (11) days after planting, on October 6th, 840 cucumber seedlings were transplanted in the greenhouse in ten rows at an inter-row spacing of 45 cms, according to the experimental design previously developed.

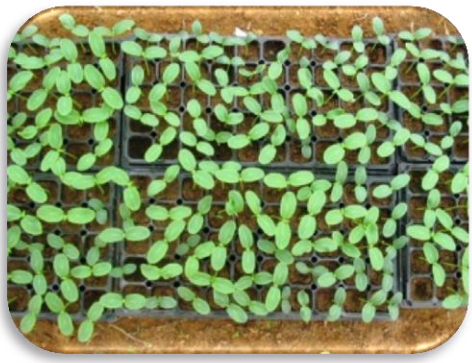


Figure # 7:
Germination of the Parthenocarpic cucumber seeds

Usual cultural practices were followed throughout the growing season in terms of irrigation, fertilization and pest control in all treatment areas identically. Irrigation was carried out daily through the drip irrigation system, generally in early mornings in order to avoid rapid evaporation of water. While the frequency of irrigation was kept constant during the entire growing season, the length of each irrigation session gradually increased throughout the season as needed. A 20-20-20 NPK + micronutrients compound soluble fertilizer was applied through the drip irrigation system starting fifteen (15) days after transplantation and was continued regularly throughout the season. Finally, chemical pesticides were used for the control of common pests and diseases such as spider mites, powdery and downy mildews equally on all treatment plots.

Collection of data initiated as early as the preliminary stages of soil preparation with soil sampling and analysis for the detection, identification and counting of root-knot nematodes in soil samples taken from each of the sixteen (16) treatment areas. This operation was first done immediately after soil preparation, following the end of the previous tomato crop (pre-treatment) on August 2nd. A second soil analysis was performed directly after the application of the different treatments (post-treatment) on October 6th. The actual cost of application of each treatment was calculated in all details, and the market value of the crop resulting from each treatment area was recorded for the evaluation of the cost/benefit analysis (Chapter III).

Following transplantation of the cucumber seedlings to the greenhouse, ten plants were marked in the middle of each treatment area and the yield resulting from these plants was recorded. For marking these plants the following procedure was followed: Each of the five plantation rows in every treatment area contained nine plants in length. The two middle rows of each treatment area were selected for harvest, and the five middle plants in each of these two rows were marked. The first two and the last two plants of each row were considered as buffers, and therefore were not marked, and harvest from these plants was not recorded.



Figure # 8:
Treatment areas (1 to 16) and plants marked (in red) for yield data collection

The plant vigor and quality of resulting yield were also evaluated. Furthermore, the actual market value of the yield was recorded for every harvest. Finally, immediately after the last harvest at the end of the season on December 16th, five plants were freed from the soil from each treatment area, and the roots were washed. Root galls were assessed using 0-10 scale, with 0 = no galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small and some big galls, 5 = 25% of roots severely galled, 6 = 50% of roots severely galled, 7 = 75% of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, 10 = plant and roots dead (Zeck, 1971).

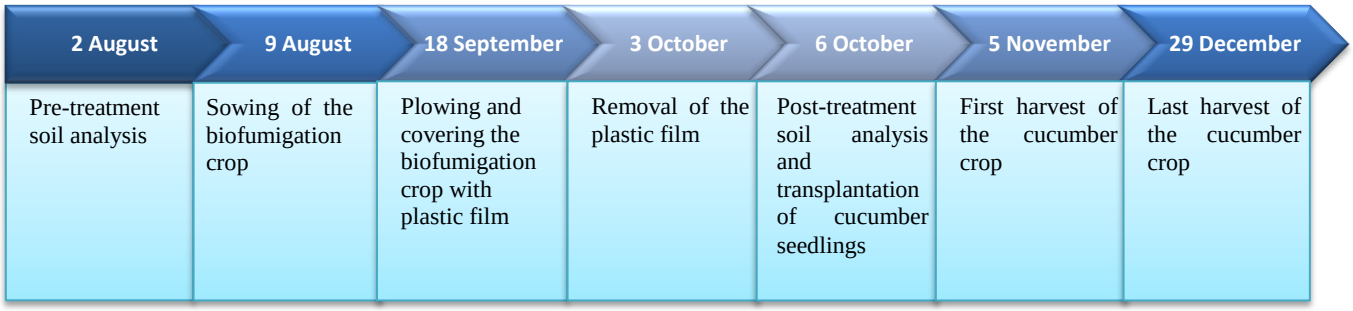
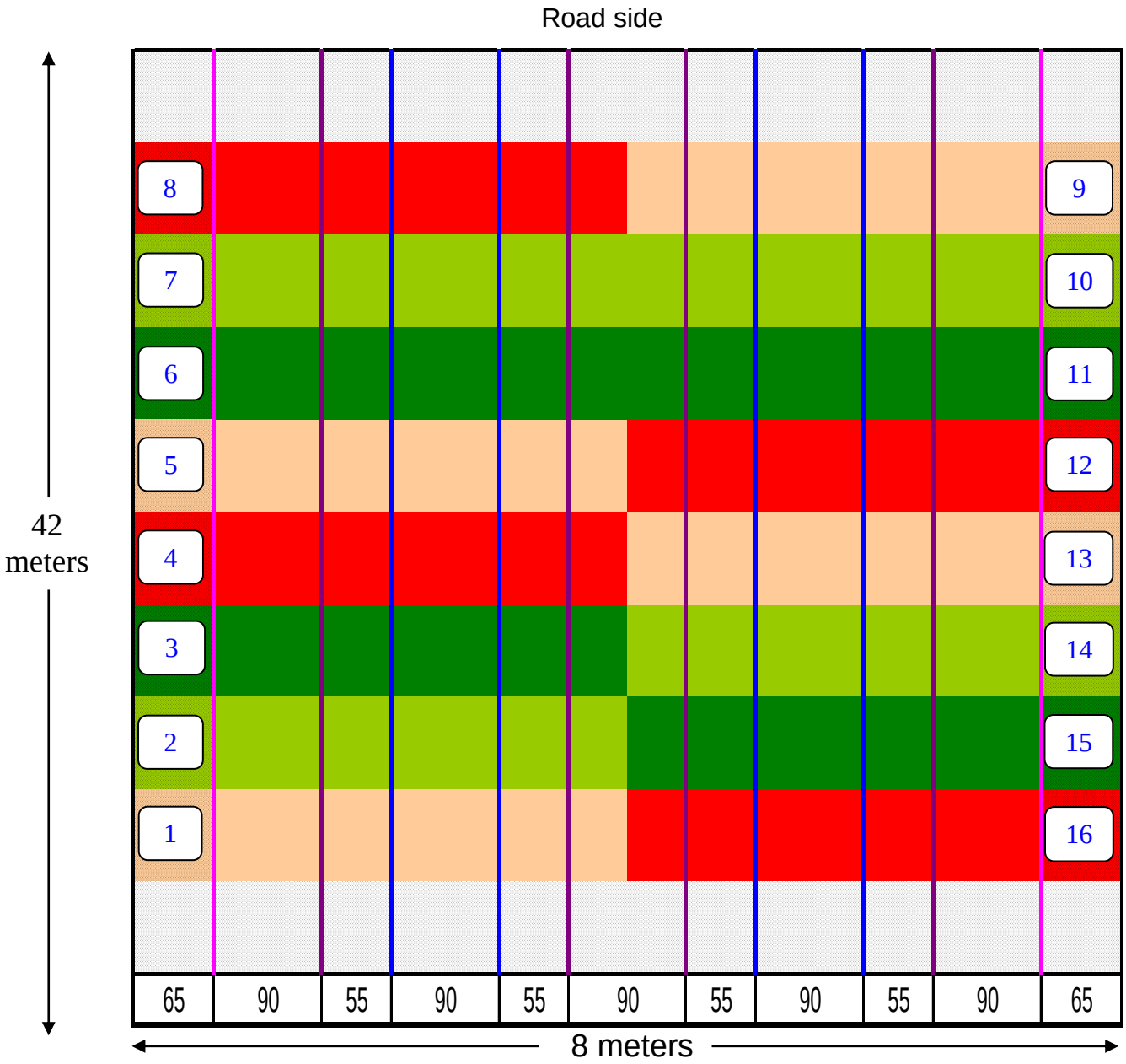


Figure # 9:
Timeline of operations (Experiment A)

Experimental design (Experiment A)



Legend

- Buffer zone
- Lines included in the buffer zone
- Lines of soil sampling
- Lines of harvest

Treatment areas

	Untreated Fallow (Control)
	MeBr
	Oil radish without plastic cover
	Oil radish with plastic cover

Figure # 10:
Experimental design (Experiment A)

Experiment B

The second experiment (B) initiated immediately after the end of the following summer season in a greenhouse having the same area and adjacent to the one used in experiment A. The previous tomato crop was removed and the soil was plowed after the incorporation of preparatory organic and chemical fertilizers at usual rates. The total area of the greenhouse was divided into twenty (20) plots of 3.80 x 4.00 (15.20 m^2) for the application of the following five treatments with four replicates each (as shown in figure # 13, page 37):

1. Control (untreated fallow)
2. Methyl Bromide
3. Oil radish (*Raphanus sativus* spp. *oleifera*) variety "Boss" without plastic cover
4. Oil radish (*Raphanus sativus* spp. *oleifera*) variety "Boss" with plastic cover
5. Arugula (*Eruca vesicaria* spp. *sativa*) with plastic cover

Initial soil samples were taken immediately after soil plowing following the end of the previous tomato crop for the confirmation of significant infestation of the soil with root-knot nematodes. For this purpose, five soil samples of 500 grams were taken from each of the twenty (20) plots from a depth of 25 cms. Every five samples were mixed together and a representative sample of each plot was analyzed at the laboratory of the Ministry of Environment of Lebanon through a combination of Cobb's sifting and gravity method (Cobb, N.A., 1918) and the Baermann funnel technique (Baermann, G., 1917), as per details mentioned earlier (refer to experiment A).

An overhead mini-sprinkler system was then installed for the irrigation of the eight oil radish and four arugula plots.

On September 9th, both biofumigation crops (*R. sativus* and *Eruca vesicaria*) were sown in twelve (12) treatment areas in the greenhouse at the rate of 3 kg per dunum (1 dunum=1000 m^2). Six weeks later (October 15th), when the height of the green manure crops reached 35-40 cm, they were plowed and incorporated into the soil with a rotary tiller. Four plots with *R. sativus* were covered with fifty (50) microns of transparent polyethylene films, while four others were left uncovered. Similarly, the four plots of *Eruca vesicaria* were covered with the same type of plastic films.

In parallel, four different plots were treated with Methyl Bromide at the usual rate of 100 kg. per dunum, and covered with polyethylene on October 15th. After ten days, plastic was removed from the covered biofumigation and Methyl Bromide treated plots, and the soil was aerated for three days, after which a drip irrigation system was installed to incorporate ten plantation rows in the greenhouse.



Figure # 11:
Growth of the oil radish & arugula crops before plowing and incorporation to the soil

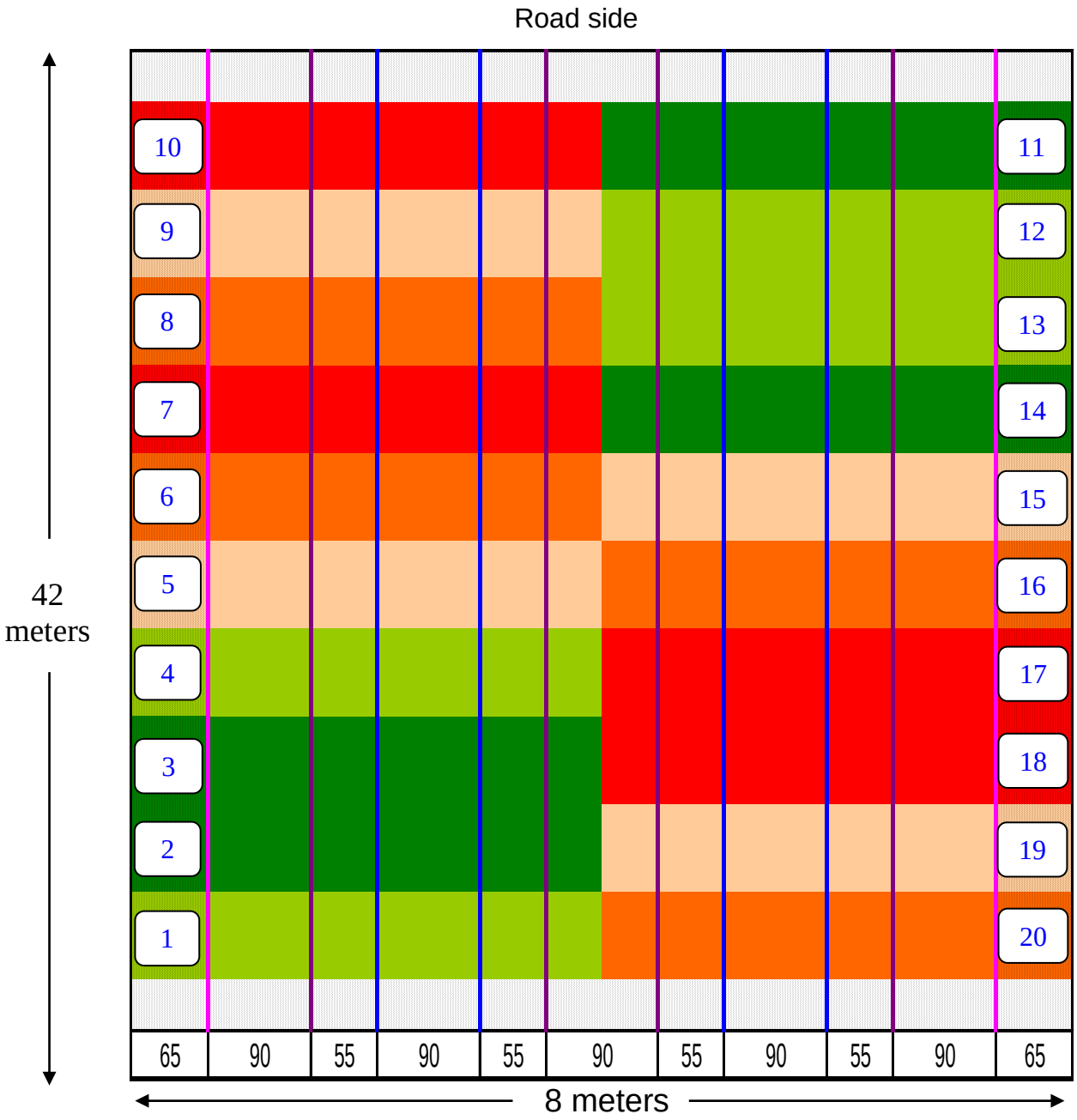
At this stage, soil samples were taken again from each of the twenty treatment plots for nematode analysis. Eight hundred and forty seedlings of a Parthenocarpic winter variety cucumber (Thiram treated Noble F1 produced by De Ruiter Seeds) were transplanted into the greenhouse on 28 October at an inter-row spacing of 45 cms. Common cultural practices were followed throughout the growing season in terms of irrigation, fertilization and pest control in all treatment plots identically.

Soil analysis was carried out to determine pre and post application population levels of the root-knot nematode in the different treatments. Furthermore, cucumber yields were recorded for each treatment throughout the growing season, in addition to the cost of each treatment and the actual market value of the yield produced from each treatment plot, for the evaluation of the cost/benefit analysis. Finally, root gall indexing was performed at the end of the season on April 16th, using a 0-10 scale as described previously.

1 September	9 September	15 October	25 October	31 October	21 December	16 April
Pre-treatment soil analysis	Sowing of the biofumigation crops	Plowing and covering the biofumigation crops with plastic film	Removal of the plastic film	Post-treatment soil analysis and transplantation of cucumber seedlings	First harvest of the cucumber crop	Last harvest of the cucumber crop

Figure # 12:
Timeline of operations (Experiment B)

Experimental design (Experiment B)



Legend

- Buffer zone
- Lines included in the buffer zone
- Lines of soil sampling
- Lines of harvest

Treatment areas

	Untreated Fallow (Control)
	MeBr
	Oil radish without plastic cover
	Oil radish with plastic cover
	Arugula with plastic cover

Figure # 13:
Experimental design (Experiment B)

RESULTS AND DISCUSSION

Two greenhouse experiments were conducted in two adjacent greenhouses on the effect of oil radish (*Raphanus sativus* spp. *oleifera*) variety “Boss” and arugula (*Eruca vesicaria* spp. *sativa*) as green manure crops with and without plastic cover on the cucumber crop (Figure # 9, page 34 & figure # 11, page 37). In both experiments, nematode population, root gall index and fruit yield were assessed in each treatment. Additionally, in experiment A, soil temperatures were recorded for the evaluation of any association between the effect of oil radish and heat on the nematode population.

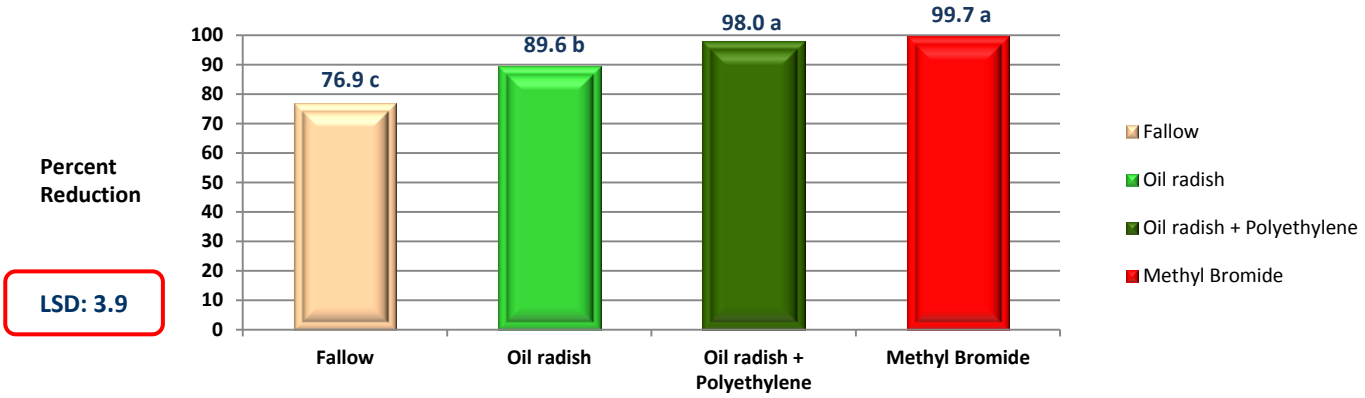
Nematode Population

In experiment A, all treatments significantly reduced nematode population in comparison with the untreated fallow (76.9%). The highest percent reduction in nematode population was observed in the Methyl Bromide treatment (99.7%) followed by the plots planted with oil radish covered with plastic (98%), with no significant difference between these two treatments. However, nematode population reduction in both treatments (Methyl Bromide or oil radish covered with plastic) was significantly higher than the plots planted with oil radish without plastic cover (89.6%).



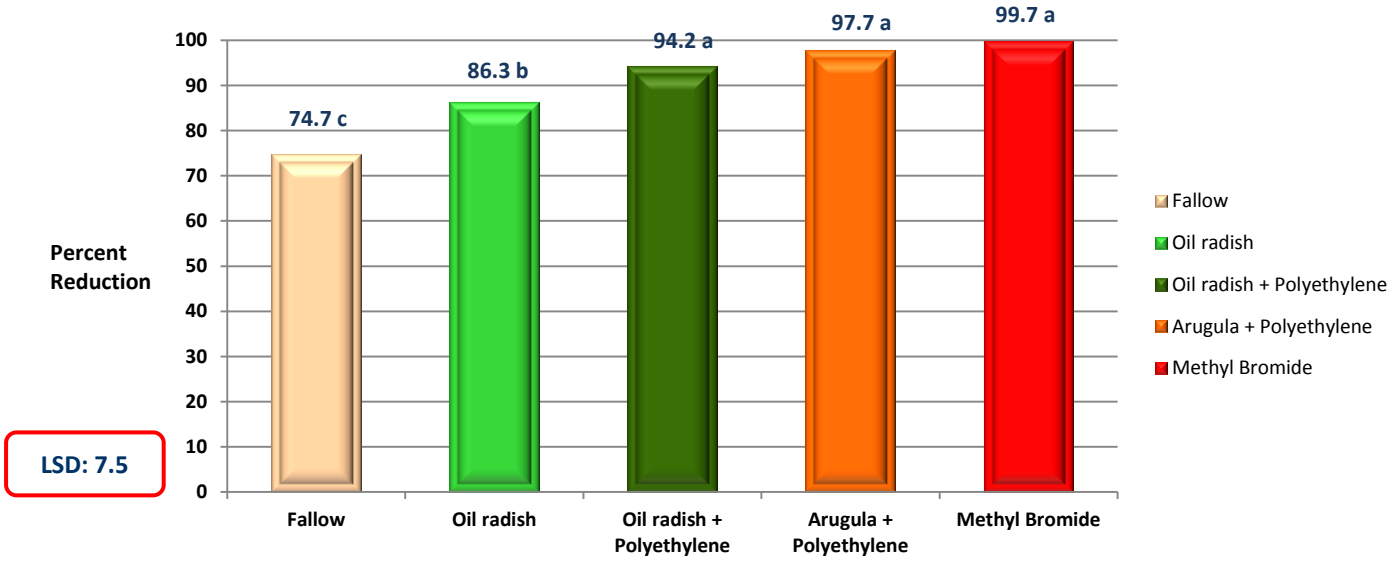
Figure # 14:
60 days after transplantation of cucumber plants (Experiment A):
- Death of cucumber plants due to nematodes in plot # 5 (untreated fallow)
- Healthy cucumber plants still in production in plot # 6 (oil radish with plastic)

Chart # 1.1: Percent reduction of the soil population of root-knot nematodes (Experiment A)



In experiment B, the highest reduction was once again achieved with Methyl Bromide (99.7%). In plastic covered arugula or oil radish plots, *M. incognita* population was decreased by 97.7% and 94.2%, respectively, whereas in uncovered oil radish plots, population reduction was only 86.3%. In the untreated fallow, nematode population reduction was as low as 74.7%.

Chart # 1.2: Percent reduction of the soil population of root-knot nematodes (Experiment B)



In both experiments A and B, all treatments significantly reduced nematode population in comparison with the untreated fallow. Furthermore, there was no significant difference between the percent reduction of nematodes achieved by any of the two green manure crops (oil radish and arugula) covered with plastic and Methyl Bromide. When covered with 50 microns transparent polyethylene film immediately following their incorporation to the soil, both oil radish and arugula have been as effective as Methyl Bromide in reducing nematode population in the soil.

Generally, in both experiments A and B both biofumigation crops used significantly reduced nematode population as compared to untreated fallow (control). This result is conform to findings of other studies describing oil radish and arugula as non-hosts (or least poor hosts among a variety of biofumigant crops tested) to root-knot nematodes, causing decline of soil populations by starvation at the first place (Curto *et al*., 2005; Liebanas & Castillo, 2004; Pattison *et al*., 2006). Results of our experiments also match other studies having found that these biofumigation crops are able to control nematodes (Buskov *et al*., 2002; Mojtahedi *et al*., 1991; McFadden *et al*., 1992; Spak *et al*., 1993; Angus *et al*., 1994), and more specifically those having associated the nematostatic effect of biofumigation crops to the release of glucosinolates which soon convert into isothiocyanates in the soil and thus suppress nematode populations (Morra and Kirkegaard, 2002).

In both experiments A and B, plastic cover has significantly increased the efficiency of oil radish and lead to better nematode control. These results indicate that plastic cover enhances the effectiveness of the biofumigation crops used in terms of nematode control. It is to note that there was remarkable difference in the initial soil populations of nematodes in plots treated with covered and uncovered oil radish in each of these two experiments. In fact, the average initial infestation with root-knot nematodes in all eight plots treated with oil radish (covered and uncovered) at the site selected for experiment A was considerably more severe than in plots treated similarly at the site where experiment B was conducted, as shown in table # 1.1 below. Furthermore, whereas in experiment B the average initial infestation in plots treated with oil radish and covered with plastic was lower (865) than in plots treated with oil radish and kept without cover (3,475), in experiment A plots treated with oil radish and covered with plastic had considerably higher average initial infestation (12,430) than those of with oil radish without cover (7,310).

Table # 1.1: Pre-treatment counts of initial populations of root-knot nematodes in experiments A & B)

Rep.	Treatment	Pre-Treatment counts	
		Experiment A	**Experiment B**
1	OR	4,520	700
2	OR	4,320	1,360
3	OR	12,440	8,760
4	OR	7,960	3,080
1	OR + PE	12,160	1,810
2	OR + PE	16,520	810
3	OR + PE	10,640	230
4	OR + PE	10,400	610

Extensive research has been carried out in the past years on the use and benefits of plastic mulch (tarp) for nematode control. However, most of these studies have evaluated the efficiency of various types of plastic tarps (thickness, color, length of covering period) on raising soil temperatures for solarization purposes (Katan, 1981; Lamberti *et al*., 2000), or their effects on the distribution of different molecules of certain chemical fumigants in the soil (Saha *et al*., 2007; Smelt *et al*., 1989). Practically no literature has been found concerning research on the possibility of enhancement of the biofumigation effect on nematode populations through covering soil treated with green manure crops with plastic tarp. This is probably due to the fact that generally, use of plastic tarps in the implementation of the biofumigation technique is not common practice, because of the relatively high cost of such an application, especially in large scale open-field production schemes, such as potato, sugar beet or carrot croppings. Instead, the usual trend is either compacting the top soil with the use of specific machinery (rollers), or creating a reasonably impermeable layer of soil through overhead irrigation of the treated land for the prevention of the dissipation of the isothiocyanates released.

Our results indicate that use of plastic cover with oil radish significantly enhances the effectiveness of the isothiocyanates, leading to a better control of the root-knot nematode. The beneficial effect of the plastic is not related to the initial infestation levels. Furthermore, decreasing the covering period from 15 to 10 days does not negatively affect the efficiency of the plastic in the control of the root-knot nematode.

Temperature Effect

Soil temperature records registered at 30 cms depth in the four replicated areas treated with oil radish and covered with plastic varied between 20 and 28 ^{o}C over the entire period when soil was covered with plastic (from 18 September until 2 October). The highest temperature reached was 28 ^{o}C (replicate 2). Table # 1.2 below summarizes the values recorded. It is to note, that the highest temperature reached in each of the four replicated areas persisted for either 24 hours (replicates 2 and 3 - 28 and 27 ^{o}C respectively) or a maximum of two days (replicates 1 and 4 - 27 and 25 ^{o}C respectively).

Table # 1.2: Soil Temperatures in the four replicated areas treated with oil radish covered with plastic (Experiment A)

Date	Replicate 1	Replicate 2	Replicate 3	Replicate 4
18-Sep	23	21	21	20
19-Sep	25	22	23	21
20-Sep	25	25	25	23
21-Sep	26	25	26	23
22-Sep	27	26	26	24
23-Sep	27	28	27	25
24-Sep	26	27	26	25
25-Sep	25	25	25	24
26-Sep	25	24	24	23
27-Sep	25	24	23	22
28-Sep	24	23	23	22
29-Sep	24	23	23	21
30-Sep	24	23	23	20
01-Oct	24	23	22	20
02-Oct	24	22	22	20

	Highest temperature recorded in each replicate

Through a laboratory experiment on the effect of temperature on the root-knot nematode *M. incognita*, Wang & McSorley have determined that while 100% of J2 were killed when exposed to a temperature of 42 ^{o}C for only 13.8 hours, no mortality was recorded at temperatures below 38 ^{o}C, no matter how long was the exposure period extended. Furthermore, the minimum temperature found effective on the J2 population was 39 ^{o}C, but a period of 47.9 hours was needed to kill 100% of J2 at this temperature (Wang & McSorley, 2008).

In another field experiment, Lamberti *et al.* have found that temperatures of 35 ^{o}C and 45 ^{o}C had little effect on *M. incognita* and did not increase lettuce yields in Italy (Lamberti *et al.* 2000).

Thorough literature review reveals that research on the correlation of the temperature effect with biofumigation is not abundant. However, most of the few available studies indicate that soil amendment with biofumigation crops does not have any suppressive effect on root-knot nematodes below soil temperatures of 20 ^{o}C (Ploeg and Stapleton, 2001; Bello *et al*., 2004; Lopez-Perez *et al.*, 2005).

On the other hand, many studies have associated temperature with root-knot nematode control through soil solarization. Most of these studies indicate figures varying between 35 and 38 ^{o}C as lethal temperature for *Meloidogyne incognita*.

Results of our experiments indicate that use of plastic mulch to cover soil treated with oil radish has always increased the effectiveness of this biofumigation crop and lead to a better control of the root-knot nematode. Findings of the experiments mentioned above (Wang & McSorley, 2008; Lamberti *et al.* 2000) and soil temperature levels recorded in experiment A, lead us to conclude that the additional effect of plastic cover on the root-knot nematode in this experiment was not due to the increase of soil temperature (since temperature levels achieved were far from the lethal temperature levels), rather than the capacity of the plastic in maintaining the isothiocyanates released by the oil radish crop in the soil for longer periods. Without the presence of these isothiocyanates, even the highest temperature recorded at any of the four replicates would not be sufficient alone to suppress the root-knot nematode population. This assumption is also concomitant with the outcome of the research conducted by Stapleton and Duncan, who found that covering the soil treated with residues of broccoli and other cruciferous plants can control *M. incognita* even at sub-

lethal temperatures (Stapleton and Duncan, 1998), as well as with the findings of Morra and Kirkegaard, who have associated the nematostatic effect of biofumigation crops to the release of high concentrations of glucosinolates, which soon convert into isothiocyanates in the soil and thus suppress nematode populations (Morra and Kirkegaard, 2002).

Our results also indicate that oil radish variety “Boss” and arugula fit the definition of Viaene and Abawi (1998) of a good cover crop due to the suppressive effect they showed on the nematode population in the soil when applied as green manure.

Finally, the above findings confirm that both oil radish variety “Boss” and arugula can suppress root-knot nematode populations in greenhouse production of cucumbers in Lebanon, acting first as a poor host by preventing buildup of root-knot nematode populations, as well as through their characteristics of a biofumigant green manure crops producing glucosinolates (biocides) when incorporated to the soil.

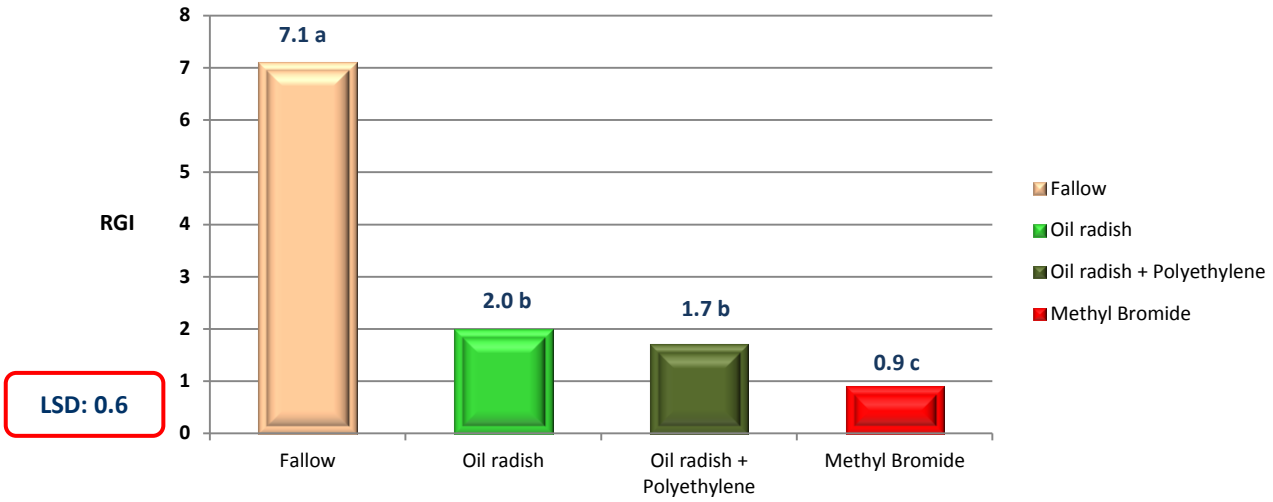
Root Gall Index

In experiment A root galling was significantly reduced by application of all treatments compared to fallow. The lowest root gall index was observed in the plots treated with Methyl Bromide (0.9). There was a significant reduction in the oil radish planted plots either covered with plastic or left uncovered compared to fallow. The gall reduction was less in plots planted with oil radish with or without plastic cover when compared to the untreated fallow plots (1.7 and 2.0 respectively). The highest galling was found in the roots of untreated fallow plots (7.1).



Figure # 15:
Experiment A
Galls on roots taken from
the untreated fallow (A) & oil radish with plastic cover (B)

Chart # 1.3: Effect of different treatments on the cucumber root gall index at harvest (Experiment A)



In experiment B root galling results were also quite similar to those of experiment A. On a scale of 1-10 the least root galling has occurred on plant roots taken from plots treated with Methyl Bromide (0.7). Galling on plant roots resulting from plastic covered arugula and oil radish plots was 1.7 and 2.4, respectively. Non-covered oil radish plots resulted into a root galling of 4.7, while roots taken from untreated fallow plots showed a gall index of 9.5.

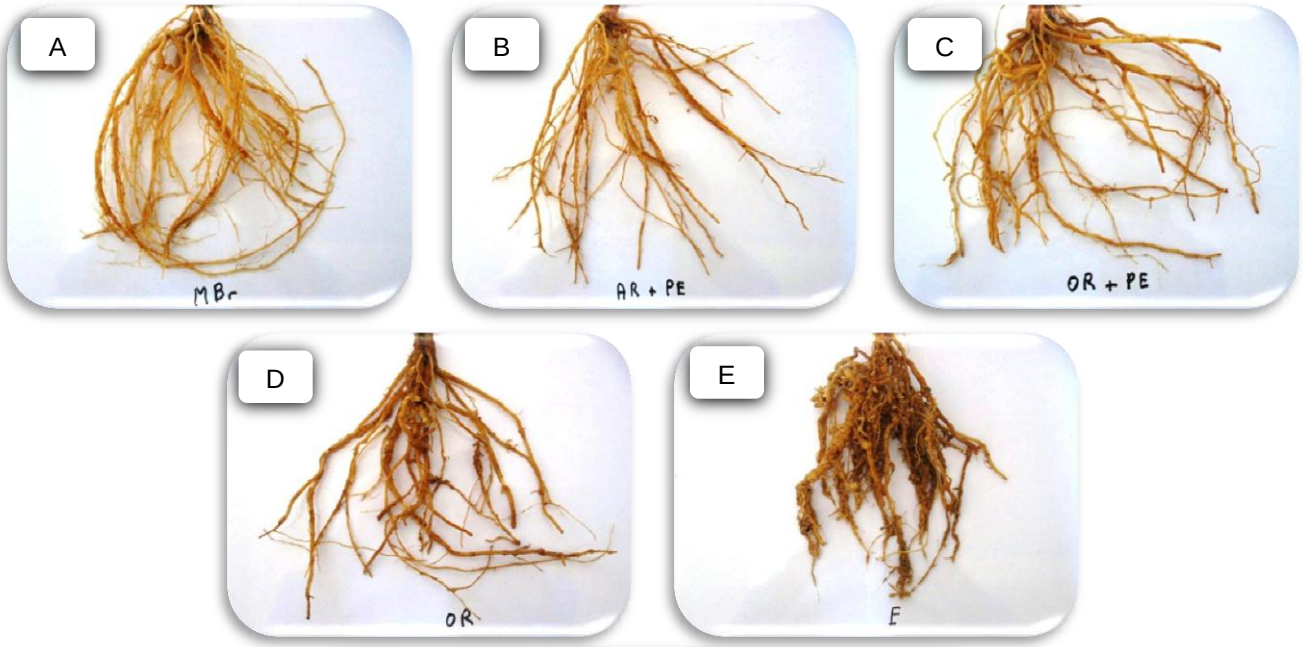
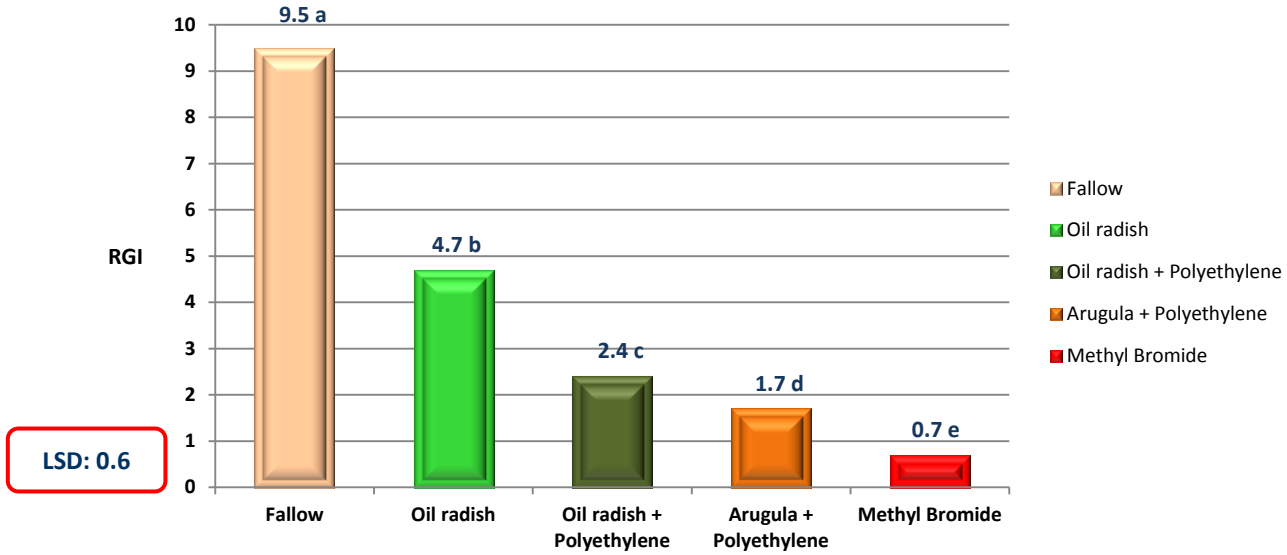


Figure # 16 (A, B, C, D, E):
Experiment B
Galls on roots taken from the different treatments:

- Methyl Bromide (A)
- arugula with plastic cover (B)
- oil radish with plastic cover (C)
- oil radish without plastic cover (D)
- untreated fallow (E)

Chart # 1.4: Effect of different treatments on the cucumber root gall index at harvest (Experiment B)



Both experiments A & B revealed similar results. Both biofumigation crops used significantly decreased root galls as compared to the untreated control. These results confirm the findings of several earlier studies classifying arugula and oil radish as non-host or poor host species to the root-knot nematode (Melakeberhan *et al*., 2006; Curto *et al*., 2005; Pattison *et al*. 2006). The significant decline of root galls under the influence of these biofumigation crops justifies also the reduction of the soil population of the root-knot nematodes discussed earlier. Obviously, when there are no juveniles (J2) or active juveniles proliferating in the soil, the root gall formation on the roots of the crop will definitely reduce.

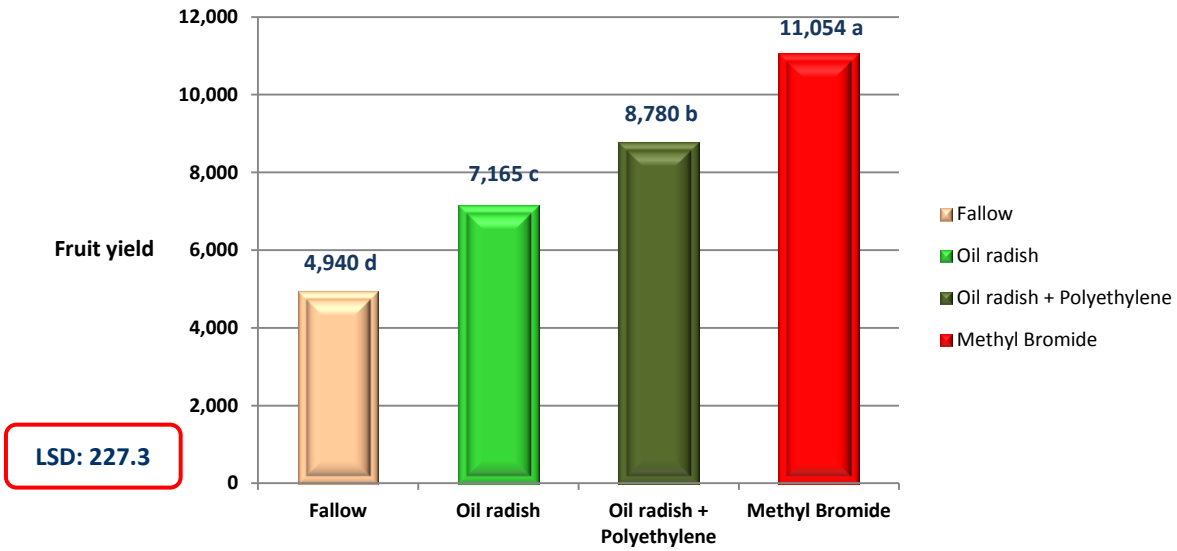
These results further confirm that oil radish variety “Boss” and arugula can be considered as good cover crops and cause decline of the root-knot nematode population in the soil and on the roots of greenhouse cucumbers under Lebanese conditions.

Fruit Yield

In experiment A where the total yield was based on the harvest of 10 plants per plot, the highest cucumber yield was obtained from plots treated with Methyl Bromide (11,054 grams), followed by oil radish covered with plastic (8,780 grams). Oil radish without cover yielded 7,165 grams, while yield resulting from untreated plots was as low as 4,940 grams.

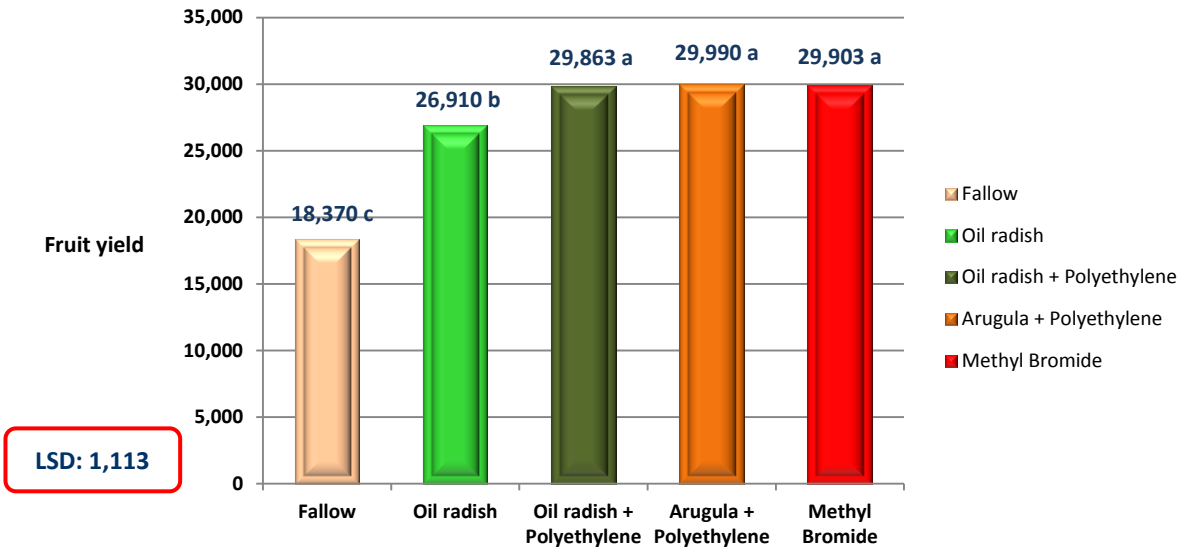
In this experiment, all treatments increased cucumber production significantly as compared to the untreated fallow (control).

Chart # 1.5: Effect of different treatments on cucumber fruit yield (Experiment A)



In experiment B, the highest cucumber yield was obtained from plots treated with arugula covered with plastic (29,990 grams), while Methyl Bromide treated plots yielded 29,903 grams. Yield resulting from plastic covered and uncovered oil radish plots was 29,863 grams and 26,905 grams respectively. Untreated fallow plots yielded only 18,368 grams. In this experiment as well, all treatments increased cucumber production significantly as compared to the untreated fallow (control). However, unlike in experiment A, there was no significant difference in experiment B between the yield produced by the plastic covered oil radish and arugula and Methyl Bromide.

Chart # 1.6: Effect of different treatments on cucumber fruit yield (Experiment B)



Results of both experiments A and B with respect to yield obtained from the different treatments confirm findings of several previous studies having stated that reduction of soil population of the southern root-knot nematode in the soil as well as in root tissues with resulting significant reduction of root gall formation, help the plant to restore its photosynthates otherwise consumed by the root-knot nematodes (Bergeson, 1966; Kochba and Samish, 1971). Our results are also concomitant with the outcomes of the research conducted by Fortnum *et al*. (1991), who have found that the southern root-knot nematode alters the normal metabolic pathway of the plants, resulting in the increase of root weight and decrease of shoot weight, thus shifting the root-to-shoot balance.

Therefore, it can be assumed that the adequate control of the southern root-knot nematode through both biofumigation crops used in our study have enhanced normal plant metabolism, restored the root-to-shoot balance and consequently increased the cucumber fruit yield. Both experiments A and B have revealed consistent results in terms of production of significantly higher yield with oil radish and arugula as compared to untreated fallow, especially when covered with plastic. Hence, use of these two biofumigation crops with plastic can be considered as an alternative management tool for the root-knot nematode in greenhouse cucumber production under Lebanese conditions.

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CHAPTER II

Effect of Two Green Manure Crops in Combination with the Non-Fumigant Nematicide Oxamyl on the Southern Root-Knot Nematode in Greenhouse Cucumber Crops.

INTRODUCTION

Meloidogyne incognita and *M. javanica* are the most devastating nematodes in the world, and constitute respectively sixty-four and twenty-eight percent of all plant parasitic nematode populations occurring in tropical countries (Sasser, 1979). These two root-knot species along with *M. hapla, M. arenaria* and *M. chitwoodi* are considered the most serious due to their wide host range and worldwide distribution. They can thrive in a very broad range of soil temperature and moisture conditions, causing serious damage to crops in terms of both yield quantity and quality (Sasser, 1979). Some researchers have reported that *M. javanica* is even more damaging than *M. incognita* (Arens and Rich, 1981; Barker *et al*., 1981).

Incorporation of organic matter into soils has been a common practice since the early days of agriculture. This technique has produced many beneficial effects on the physical and chemical properties of the soil, such as increase of fertility, enhancement of microbial activity, pest management, and, consequently, improved crop growth (Garcia-Alvarez *et al*., 2004, Widmer and Abawi, 2000; Kinloch and Dunavin, 1993; Prot *et al*., 1992; Viaene and Abawi, 1998). Organic materials used for the control of nematodes have been extremely varied, including animal manures, green manures, composts, oil seed cakes, agro-industrial residues such as citric peelings, rice husks, tea and coffee, starch, sugar cane molasses, as well as residues of shellfish with high cellulose and chitin content (Cook and Baker, 1983; Hoitink, 1983; D'Addabbo, 1995).

In the recent years, among a large variety of organic materials that have been recognized as biofumigants, crop residues in general and those of the *Brassicaceae* family in particular have become of special interest. It was found that when incorporated into the soil, crops of the *Brassica* spp. release aldehydes and isothiocyanates, which have a clear suppression effect on the plant parasitic nematodes (Garcia-Alvarez *et al*., 2004). All parts

of *Brassica* plants contain glucosinolates, which alone are not harmful (Sang *et al*., 1984). However, in broken tissue they are hydrolyzed by the enzyme myrosinase to break down into various forms of isothiocyanates having nematicidal, fungicidal and bactericidal properties (Olesen Laresen P., 1981; Ettlinger and Kjaer, 1968). The most common form of isothiocyanates is methyl isothiocyanate (MITC), which is a break down product of metam sodium, an effective nematicide (Lear B., 1956). The efficacy of isothiocyanates in suppressing nematodes, soil-borne diseases and weeds has been demonstrated by many researchers (Boydston and Vaughn, 2002; Brown *et al*., 1991; McFadden *et al*., 1992; Ploeg and Stapleton, 2001).

In a study on the host status of different cultivars of oilseed radish (*Raphanus sativus oleifera*) and white mustard (*Sinapis alba*) Gardner & Caswell-Chen (1994) have found that all cultivars tested were susceptible to *M. incognita* and *M. javanica* if planted when soil temperatures are high, but they reduced root penetration and consequent reproduction of *Heterodera schachtii* in the cool season under low soil temperatures (Gardner & Caswell-Chen, 1994). Similarly, Roberts *et al*. (1981) have found that *M. incognita* was unable to penetrate roots of the winter wheat crop when soil temperatures were below 16°C (Roberts *et al*., 1981).

Other plants, such as sudangrass, clover and flax, contain different levels of cyanogenic compounds within their tissues (Pederson *et al*., 1996; Schroder, 1977; Seigler, 1976; Harrington, 1966). When used as green manure crops, these plants release hydrogen cyanide (HCN) in the soil (Widmer and Abawi, 2000), which has been found efficient in reducing root galling and egg production of *M. hapla* (Abawi and Laird, 1994), and *M. chitwoodi* population (Mojtahedi *et al*., 1993a). Through a greenhouse experiment Widmer and Abawi (2002) have found that when incorporated into the soil, different hybrids of sudangrass suppress *M. hapla* at various degrees. A direct relation has been drawn between the amount of cyanide (CN) content of the leaves of each sudangrass cultivar and *M. hapla* suppression. Furthermore, the existence of a negative correlation between cyanide (CN) concentration levels in the soil and root galling has been confirmed (Widmer and Abawi, 2002).

Plants of *Crotalaria* spp. have been reported to suppress a wide range of plant parasitic nematodes such as reniform nematode, soybean cyst (*Heterodera glycines*),

stubby root (*Paratrichodorus minor*), sting (*Belonolaimus longicaudatus*), dagger (*Xiphinema americanum*) and burrowing (*Radopholus similis*) nematodes (Wang *et al*., 2005; Wang *et al*., 2003; Rich *et al*., 1989; McSorley *et al*., 1994; Bridge, 1996). Many studies have confirmed that several plants of this family such as *Tagetes* spp., *Crotalaria spectabilis* and *Ricinus communis* suppress root-knot nematode populations as well (Hackney and Dickerson, 1975; Rodriguez-Kabana, 1992).

African marigold (*Tagetes erecta*) applied alone or in combination with other treatments has significantly reduced root-knot damage and increased yield of bean seeds in Tanzania (Ijani *et al*., 2000). It has also reduced populations of the lesion nematode *Pratylenchus penetrans* by 98% when grown in rotation with tomato (Alexander and Waldenmaier, 2002). In another experiment Ploeg (2002) has found that *Tagetes* spp. consistently increase tomato yields, with results comparable to soil fumigation with the use of chemical nematicides (Ploeg 2002). *Tagetes erecta* has produced similar results with taro (*Colocastia esculenta*) when grown as a cover crop and incorporated into the soil (Sipes and Arakaki, 1997), or when intercropped with soybean (El-Hamawi *et al*., 2004). It has also been possible to control root-lesion nematodes through crop rotations with *Tagetes patula* in tobacco (Reynolds *et al*., 2000), and potato (Ball-Coelho *et al*., 2003) crops in Canada.

Cold aqueous extracts (20% w/v) of *T. erecta* plants have suppressed juvenile *M. incognita* populations and reduced gall formation on tomato roots significantly (Natarajan *et al*., 2006). Extracts prepared from stems and whole plants have been more effective than those prepared from roots (Siddiqui and Alam, 1988).

In a pot experiment conducted by Wang *et al*. (2006) for the determination of the effect of sunn hemp (*Crotalaria juncea*) and sorghum sudangrass (*Sorghum bicolor* x *S. bicolor* var. sudanense) on okra yields and nematode populations, both crops used have significantly increased okra yields and decreased root galling. Sunn hemp has produced significantly less root galling of okra than sorghum sudangrass. While sorghum sudangrass has suppressed the total numbers of both plant parasitic and non-parasitic nematodes, Sunn hemp has slightly reduced the aggregate number of nematode population, but has caused a drastic decrease in the numbers of plant-parasitic nematode taxa, allowing mycophagous and bactivorous nematodes to thrive (Wang *et al*., 2006). In a similar experiment, velvet bean, sunn hemp, and cowpea significanlty reduced *M. incognita*

numbers from 45% of the total initial population to 2.7%, 0.6% and 1.0% respectively in okra (Wang *et al*., 2007).

Biofumigation is a relatively new nematode management technique, which has gained popularity since the late 1990s. The first scientific article on biofumigation was published by Angus *et al*. in 1994 (Angus *et al*., 1994). It has been defined by several researchers as “the action of volatile compounds with pesticidal properties released during the biodegradation of organic matter (plant material or animal by-products) in the soil, and acting as fumigants for the control of plant pathogens” (Kirkegaard *et al*., 1993; Halbrendt, 1996; Kirkegaard and Sarwar, 1998; Garcia-Alvarez *et al*., 2004). Biofumigation has been listed among the possible alternatives to Methyl Bromide suggested by the Methyl Bromide Technical Options Committee (MBTOC) of the Montreal Protocol (MBTOC, 1997).

The main difference between incorporation of soil with organic matter and biofumigation relies in the state of the organic matter used. According to the new concept of biofumigation, applied organic matter must be in its first phases of decomposition in order to produce the requested biofumigant effect, which is not produced by stabilized organic manures (such as compost or “mature” manure) (Bello *et al*., 2002; Bello *et al*., 2003). It has been also recently found important to retain the gases released through the decomposition of the organic matter for at least two weeks in order to have an efficient management of the existing pathogens (Garcia-Alvarez *et al*., 2004). Extensive research carried in the past few years under field and laboratory conditions has shown that biofumigation decreases plant parasitic nematodes and soil fungi, while enhancing the growth of natural enemies of some pathogens as well as certain saprophagous and bacteriophagous nematodes (Abawi and Widmer, 2000; Riegel and Noe, 2000; Chavarria-Carvajal *et al*., 2001). These results confirm the association of the biological activity of soils to pathogen suppression (Werner and Dindal, 1990; Gamliel and Stapleton, 1993; Scow *et al*., 1994). Many researchers attribute the pest suppression effect of biofumigation to the release of volatile gases or to the changes of the pathogenic-antagonistic microorganisms ratio in the soil (Chen *et al*., 1987; De Brito Alvarez *et al*., 1995). In a greenhouse study on the effect of soil amendment with different cultivars of rapeseed, Mojtahedi *et al*. (1991) have found a direct relationship between the glucosinolate content of the plants used and control of *M. chitwoodi*. They also noticed that substances released from the rapeseed

plants during their decomposition were toxic to the tomato seedlings grown (Mojtahedi *et al*., 1991; Mojtahedi *et al*., 1993b). Through an experiment conducted under laboratory conditions, PiedraBuena *et al*. (2006) have found that byproducts of pepper, strawberry and tomato plants with and without manure have all controlled *M. incognita* populations with mortality rates reaching 100%. Similarly, with only one exception, all materials used have considerably lowered the root gall index of the tested tomato plants (PiedraBuena *et al*., 2006).

Rapeseed green manure has significantly reduced galling in squash plants (Crow *et al*., 1996). Oil radish (*Raphanus sativus* L. cv. Trez) and rapeseed (*Brassica campestris* L. cv. Humus) have been used as green manure crops to control *M. chitwoodi* in potato fields of Idaho-USA. Despite the increase of potato yield and considerable reduction in *M. chitwoodi* population, complete control with the use of any of these green manure crops alone could not be achieved. (Al-Rehiayani and Hafez, 1997; 1998). It was found that the efficacy of green manure crops may be improved by their use in combination with a biocontrol agent. In fact, through an experiment conducted in 1999, Al-Rehiayani *et al*. have found that when incorporated with a biocontrol agent (*Bacillus megaterium*), green manure crops may provide an adequate management of *Meloidogyne chitwoodi* in potato crops without the use of any chemical nematicide (Al-Rehiayani *et al*., 1999).

Vydate (Oxamyl)

Oxamyl is a chemical of the Carbamate family, commonly used to control nematodes, insects and mites. Being a systemic pesticide, it is among the few nematicides that can be applied pre-plant and post-plant. The fact that Oxamyl can effectively protect the roots from nematode attacks even when applied to the foliage has led many scientists to assume that either some metabolites of Oxamyl or Oxamyl itself is translocated to the roots through the vascular system of the treated plants (Radewald *et al*., 1970; Potter and Marks, 1976; Wright *et al*., 1980). However, only extremely small quantities of Oxamyl were detected in roots of foliar treated plants (Bunt and Noordink, 1977; Harvey *et al*., 1978; Wright *et al*., 1980). This has made it more probable that the nematostatic effect of Oxamyl in plant roots is due to a metabolite rather than Oxamyl itself.

Through a laboratory experiment on the nematostatic activity of Oxamyl and one of its metabolites, N,N-Dimethyl-1-Cyanoformamide (DMCF) on *M. incognita* juveniles, McGarvey *et al*. (1984) have found that at high concentrations of 4,000 µg/ml Oxamyl is lethal to nematodes exposed to it for periods exceeding forty (40) minutes, but shows only reversible nematostatic effects at low concentrations of below 500 µg/ml and short periods of exposure. The much weaker and reversible effects of DMCF at both high and low concentrations have led to assume that protection of plant roots from nematodes is more due to Oxamyl rather than any of its metabolites, even when Oxamyl is present at very low concentrations in the roots after its translocation from the foliage of the treated plants (Wright *et al*., 1980; McGarvey *et al*., 1984).

Several formulations of Oxamyl are available and suitable for different application methods (Desaeger and Csinos, 2006). Its nematicidal properties have been known for many years (Radewald *et al*., 1970). Recently Oxamyl has received considerable interest as a possible alternative to Methyl Bromide, which is being phased out for ozone protection considerations (Anonymous, 1992). It has been specifically used in the production of vegetable crops under greenhouses as a mid-season post-plant drip application following a pre-plant treatment with 1,3-Dichloropropene and/or metam sodium. Such combinations have resulted into improved plant vigor and fruit quality in tomato and pepper crops (Desaeger *et al*., 2004). Liquid and granular formulations have shown similar effective results in the control of plant parasitic nematodes (Rhoades, 1981).

Through a field experiment extending over two consecutive growing seasons Desaeger and Csinos (2006) have found that Oxamyl has reduced the root-knot nematode population and increased eggplant yields following fumigation with 1,3-Dichloropropene + Chloropicrin, but not following Methyl Bromide and non-treated control. Oxamyl has also increased squash yields following metam sodium and 1,3-Dichloropropene + Chloropicrin applications by 30% and 35% to 75%, respectively. Additional at-plant and post-plant applications of Oxamyl have always resulted into a more uniform reduction of the root galling index and yield increase following treatments with 1,3-Dichloropropene, 1,3-Dichloropropene + Chloropicrin, metam sodium and metam sodium + Chloropicrin. It was noticed that Oxamyl had not improved yield when root-knot nematode population was not significant (Desaeger and Csinos, 2006).

Oxamyl has effectively controlled nematodes and weevil in pepper in Puerto Rico. Foliar applications of Oxamyl on a local pepper cultivar have resulted into high yield, normal stand and good control of the reniform nematode (*Rotylenchus reniformis*) (Cruz *et al*., 1984). Oxamyl applied at two different rates of 0.56 and 1.12 kg/ha has in both cases produced lower root gall index in *Capsicum annum* roots and significantly higher yields as compared to untreated plots (Acosta *et al*., 1987). Oxamyl has also reduced *M. incognita* populations significantly and increased the percentage of marketable tomato yield by up to 27% (Singh, 1975)

Application of granular Oxamyl at the rate of 2 lb/acre at planting has remarkably reduced the population of *M. arenaria* larvae and increased potato yields in Alabama, USA. Higher rates resulted neither additional yield increase nor further reduction of larval populations of *M. arenaria* (Rodriguez-Kabana *et al*., 1981). In another similar experiment Rodriguez-Kabana and King (1985) have found that the lower rate of Oxamyl has produced the higher ratio of peanut yield increase to the amount of nematicide applied. Oxamyl was found as effective as Aldicarb and Fenamiphos in reducing *M. arenaria* larvae in the soil. (Rodriguez-Kabana and King, 1985; Rodriguez-Kabana and King, 1979). Both Carbofuran and ethoprop have been less effective than Oxamyl (Rodriguez-Kabana and King, 1985). In general, the efficacy of the systemic nematicides used in controlling *M. arenaria* on peanuts was not further enhanced by deep incorporation of these chemicals into the soil (Rodriguez-Kabana and King, 1979). Oxamyl has been effective in reducing carrot yield losses caused by *M. hapla* in New York (Gugino *et al*., 2006), and in Canada (Vrain *et al*., 1979; Belair, 1984).

In a greenhouse experiment near Baghdad, several chemical nematicides tested including Oxamyl have all increased yields of tomato by 47-51% and of eggplant by 36-38%. Oxamyl has also decreased both *M. javanica* populations in the soil and plant infection percentages, and delayed root invasion till 150-180 days after transplantation. However, Oxamyl was found less effective in controlling nematodes than other nematicides such as Fenamiphos and Cadusafos (Stephan, 1995). In contrary with these findings, Rodriguez-Kabana *et al.* (1980) have reported that Oxamyl applied at different rates has been more effective in reducing *M. arenaria* populations and increasing peanut yields as compared to Phenamiphos (Rodriguez-Kabana *et al*., 1980). Both granular and liquid formulations of

Oxamyl have significantly reduced population levels of the sting nematode *Belonolaimus longicaudatus* and increased pepper yields in Florida (Rhoades, 1981).

Despite economic and environmental concerns, chemical nematicides are still widely used for root-knot nematode management, since other management practices such as crop rotation have been proven ineffective due to the wide host range of these species (Katan, 1981; Ngundo and Taylor, 1974; Saka, 1990; Smittle and Johnson, 1982; Wyatt *et al*., 1980). Furthermore, availability of root-knot resistant varieties is limited to a small number of vegetable crops, such as certain cultivars of tomato, and high soil temperatures usually tend to break this resistance (Oka *et al*., 2006). Additionally, frequent cropping of these resistant cultivars might lead to the proliferation of virulent nematode populations (Tzortzakakis and Gowen, 1996; Williamson *et al*., 1992).

In the western states of the US where 64% of the national potato production is grown, crop rejection due to *M. chitwoodi* infection is a serious issue. Therefore, fields with *M. chitwoodi* history are generally treated with pre-plant fumigant or non-fumigant nematicides or with combinations of both (Ingham *et al*., 2007). Several chemical nematicides have been used alone and in combinations aiming to reduce infection of potato tubers to acceptable levels (Ingham *et al*., 2000). None of Metam Sodium, Aldicarb or Oxamyl applied alone through the drip irrigation system has been efficient in reducing potato tuber infection. However, pre-plant metam sodium treatment followed with two or three foliar applications of Oxamyl has reduced infection to below 10% (Ingham *et al*., 2007). Miller (1976) has found that use of chemical pesticides in combination with mulch may increase yields more than each component alone.

Extensive research exists on soil biofumigation with the use of green manure crops for the management of plant parasitic nematodes in vegetable crops. Many plants of the brassica family have been reported to suppress populations of root-knot spp. However, application of this technique has certain limitations. Biofumigation cannot be fit to every cropping system, and transportation of plant residues to the fields in order to be incorporated remains unpractical or very expensive (Bello *et al*., 2000). Incorporation of organic material into the soil or biofumigation without the use of any chemical nematicides has produced inconsistent results (Oka *et al*., 2006). Therefore, evaluation of the

effectiveness and economics of this approach alone and in combination with chemical nematicides remains an important area of research.

The objective of the current study was to investigate the efficacy of addition of the non-fumigant chemical Oxamyl to two biofumigant crops (*Raphanus sativus oleifera* and *Eruca vesicaria sativa*) on the control of *M. incognita* in greenhouse cucumber crops.

MATERIALS AND METHODS

A field experiment (C) was conducted during the autumn season in a commercial greenhouse of 7 x 42 meters (294 m^2) located in the coastal area of Northern Mount Lebanon. The greenhouse was selected upon history of heavy root-knot nematode infestation, which was further confirmed through soil analysis carried out with the Baermann funnel technique following the harvest of the previous tomato crop in early September.

After the incorporation of preparatory organic and chemical fertilizers at regular rates (specify rates), the soil was plowed with a tractor, and the total area was divided into twenty-four (24) rectangular plots of 3.3 x 3.5 meters each for the application of six treatments in four replicates for each (as shown in figure # 20, page 71). The six treatments were:

1. Control (untreated)
2. Methyl Bromide + Vydate (Oxamyl)
3. Vydate
4. Oil radish (*Raphanus sativus*) variety “Boss” without plastic cover + Vydate
5. Oil radish (*Raphanus sativus*) variety “Boss” with plastic cover + Vydate
6. Arugula (*Eruca vesicaria sativa*) with plastic cover + Vydate

Prior to the treatments, five soil samples of 500 g were taken in each plot at a depth of 25 cm of each of the twenty-four treatment areas. Every five samples taken from each of the twenty-four treatment areas were mixed together and a representative sample of each plot was analyzed at the laboratory of the Ministry of Environment of Lebanon through a modified Baermann funnel technique, as described in Chapter I.

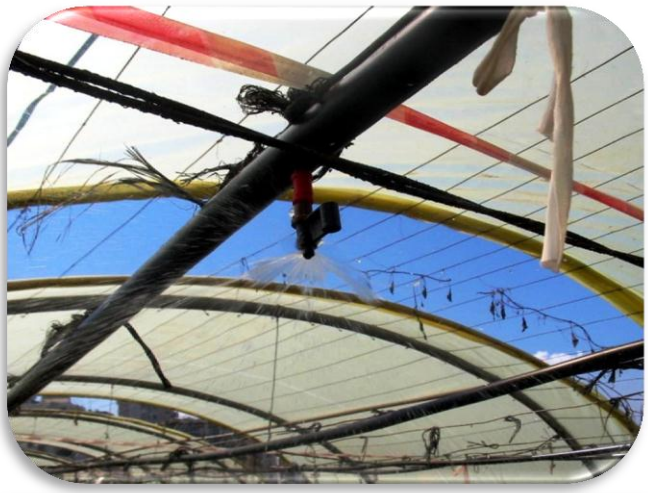


Figure # 17:
The overhead mini-sprinkler system in operation

An overhead mini-sprinkler system was installed for the irrigation of the twelve treatment plots in preparation for the sowing of the oil radish and arugula crops, which was carried out on September 10th at the rate of 3 kg/1,000 m^2.

After thirty-seven days (on October 17th) when the height of the green manure crops reached 35-40 cm they were plowed and incorporated into the soil with a rotary tiller. Four replicated areas planted with oil radish and four others planted with arugula were covered by fifty microns thick transparent polyethylene sheet, whereas four replicated oil radish plots were kept uncovered. Four other areas were treated with Methyl Bromide at the usual rate of 100 kg/1,000 m^2, and covered with the same type of polyethylene.



Figure # 18:
Plowing of the green manure crops with small tractor

Ten (10) days later (on October 27th) the plastic films were removed from all twelve covered plots and the soil was aerated for four days. On November 2nd, the soil was surface plowed and representative soil samples were taken once again from each of the twenty-four treatment areas for nematode analysis and evaluation of the efficiency of each treatment in terms of nematode control. The drip irrigation system was installed on November 5th and 1,120 seedlings of a parthenocarpic winter variety of cucumbers (Thiram treated Noble F1 produced by De Ruiter Seeds) were transplanted into the greenhouse in eight rows at an inter-row spacing of 30 cm, according to the experimental design previously developed.

Each of the four plantation rows in every treatment area contained twelve plants in length. Five plants were marked in the middle row of each treatment area for recording the resulting quantity of yield throughout the growing season. The first and last plants of rows in each treatment area were considered as buffers, and therefore yield data resulting from these plants was discarded. On November 19th, all plots except the control were treated with Vydate (Oxamyl) through the drip irrigation system, at the rate of 1 liter/1,000 m^2. Usual cultural practices were followed throughout the growing season in terms of irrigation, fertilization and pest control in all treatment areas identically. The first harvest was on December 23rd, and the season was completed on April 10th of the following year. Data collected included nematode count in the soil pre-and post-application of treatments, quantity of yield produced all over the growing season, and root gall index on a scale of 0-10 at the end of the season, as described in the previous chapter.

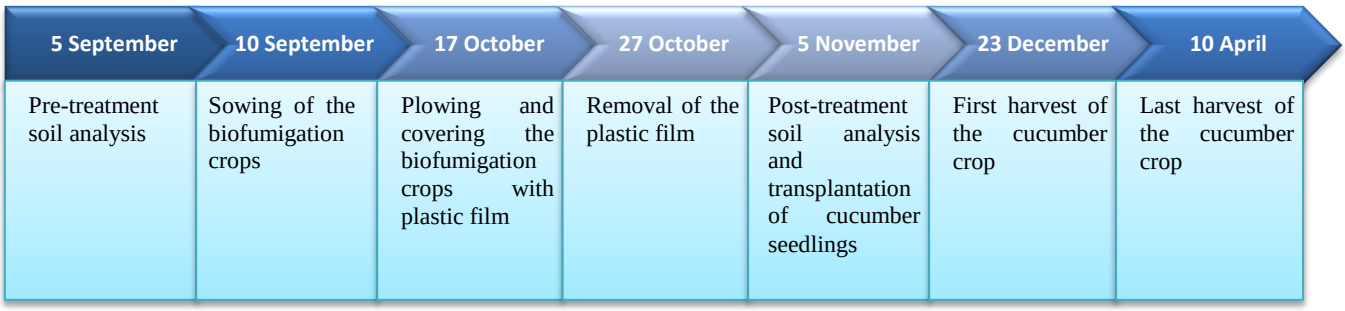
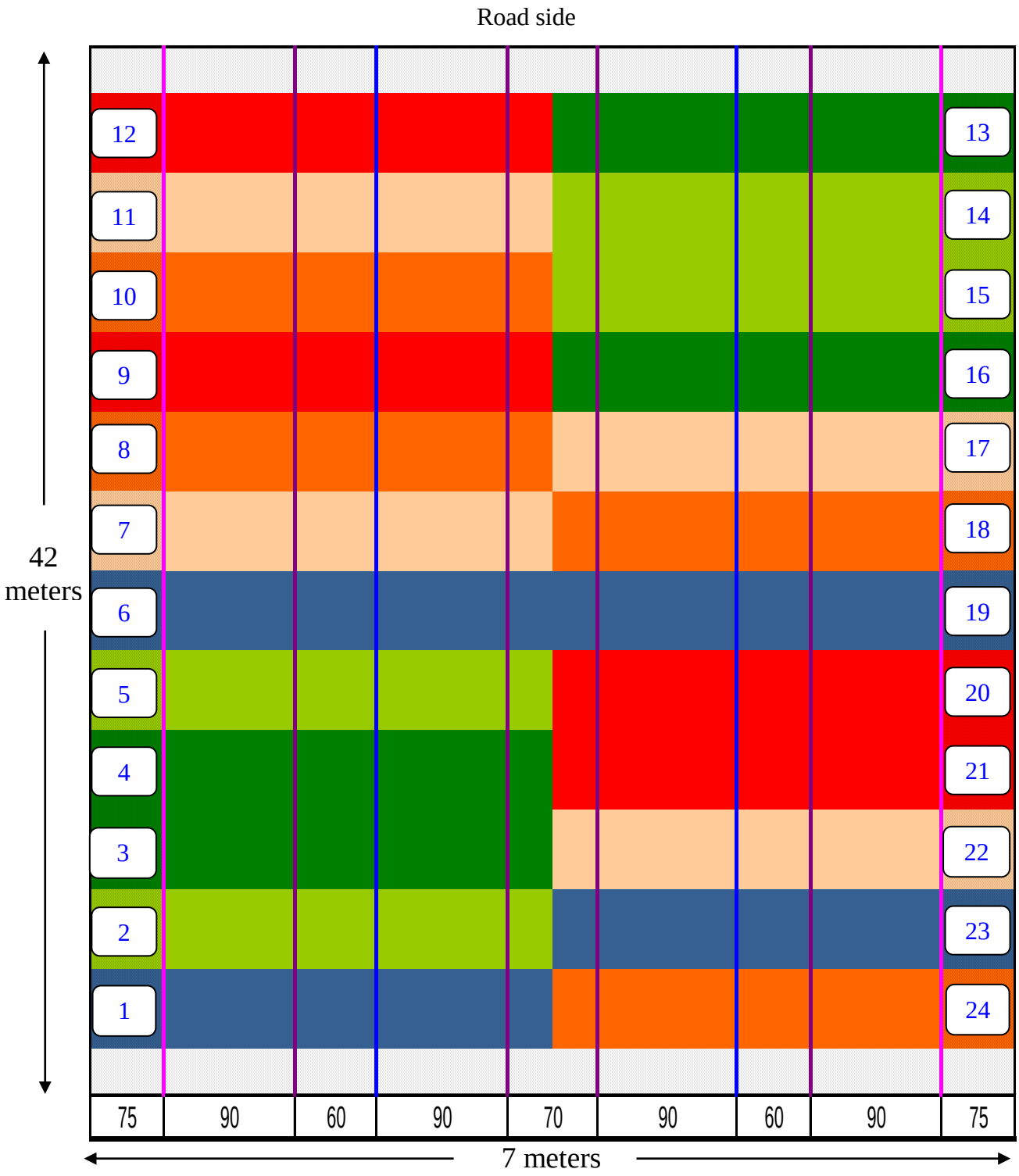


Figure # 19:
Timeline of operations (Experiment C)

Experimental design (Experiment C)



Legend

- Buffer zone
- Lines included in the buffer zone
- Lines of soil sampling
- Lines of harvest

Treatment areas

	Untreated Fallow (Control)
	Oxamyl (Vydate)
	Oil radish + Oxamyl
	Oil radish + Oxamyl + plastic cover
	Arugula + Oxamyl + plastic cover
	MeBr + Oxamyl

Figure # 20:
Experimental design (Experiment C)

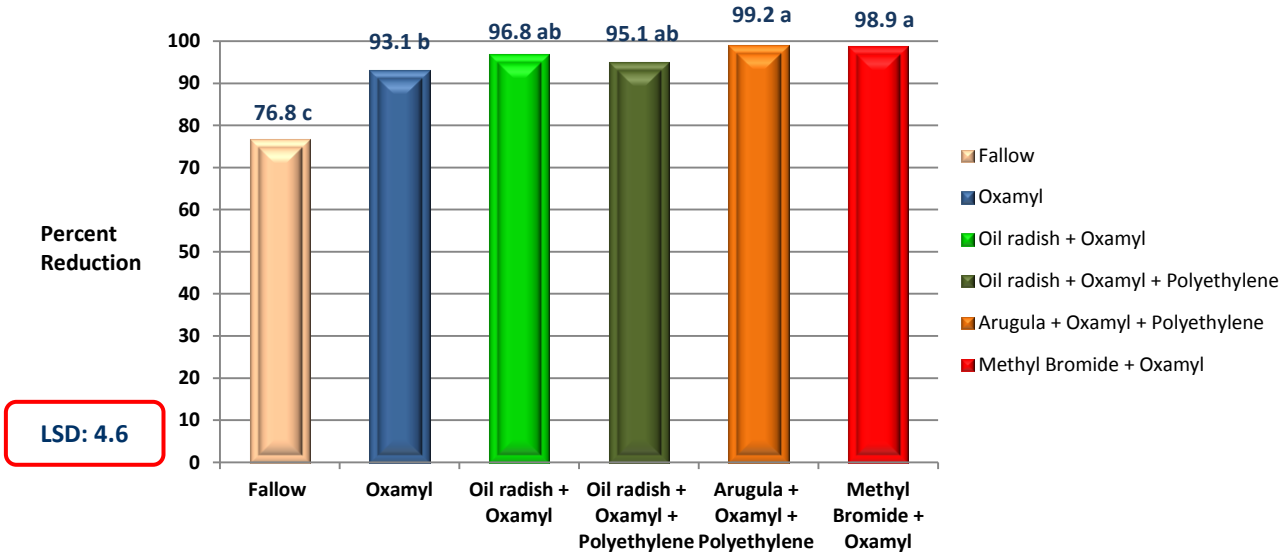
RESULTS AND DISCUSSION

The purpose of this experiment was to assess the effect of two green manure crops applied in combination with Oxamyl on the southern root-knot nematode in greenhouse cucumber crops. The three parameters evaluated were the effect of these applications on the nematode population in the soil, the root gall index at the end of the growing season and fruit yield.

Nematode Population

All treatments have significantly reduced nematode population as compared to the untreated fallow. The highest percent reduction was achieved by arugula with plastic cover + Oxamyl (99.2%) closely followed by Methyl Bromide + Oxamyl (98.9%). Percent reduction obtained with oil radish with plastic cover + Oxamyl was 95.1% and oil radish without plastic cover + Oxamyl (96.8%). There was no significant difference between any of these 4 treatments. Oxamyl applied alone reduced soil nematode population by 93.1%, being significantly different from untreated fallow, which was as low as 76.8% but did not reveal any significant difference compared to oil radish + Oxamyl with or without plastic cover. However, Oxamyl application was significantly less effective compared to arugula + Oxamyl with plastic cover and Methyl Bromide + Oxamyl (93.1% versus 99.2 and 98.9 respectively).

Chart # 2.1: Effect of different treatments on nematode population reduction in soil



Previous experiments A & B described in chapter I having been conducted under similar conditions (in terms of time of year, biofumigation crops used, cucumber cultivar grown and initial soil populations) have already revealed the efficiency of both biofumigant crops (either with or without plastic cover) used in the current experiment in reducing soil populations of the Southern root-knot nematode significantly as compared to the untreated fallow. In these experiments (A & B) percent reduction achieved has ranged from 94 to 98% with oil radish with plastic cover and reached 97.7% with arugula with plastic cover. These results have been attributed to the nematostatic effects of isothiocyanates released by these biofumigant crops into the soil. These assumptions are concomitant to the findings of several other studies on the efficacy of isothiocyanates in suppressing nematodes (Boydston and Vaughn, 2002; Brown *et al*., 1991; McFadden *et al*., 1992; Ploeg and Stapleton, 2001). They also match other studies having defined the nematostatic effect of isothiocyanates as the effect of certain volatile compounds released in the soil and acting as fumigants for the control of plant pathogens (Kirkegaard *et al*., 1993; Halbrendt, 1996; Kirkegaard and Sarwar, 1998; Garcia-Alvarez *et al*., 2004).

Some studies have found that in order for the isothiocyanates to produce the desired effects regarding the efficient management of the existing pathogens, they have to be

retained in the soil for at least two weeks (Garcia-Alvarez *et al*., 2004). However, our results indicate that covering the soil treated with the two biofumigant crops used in our research with transparent 50 microns thick polyethylene sheets for 10 days produce satisfactory results in terms of nematode control (both in experiment B described in Chapter I and the current experiment).

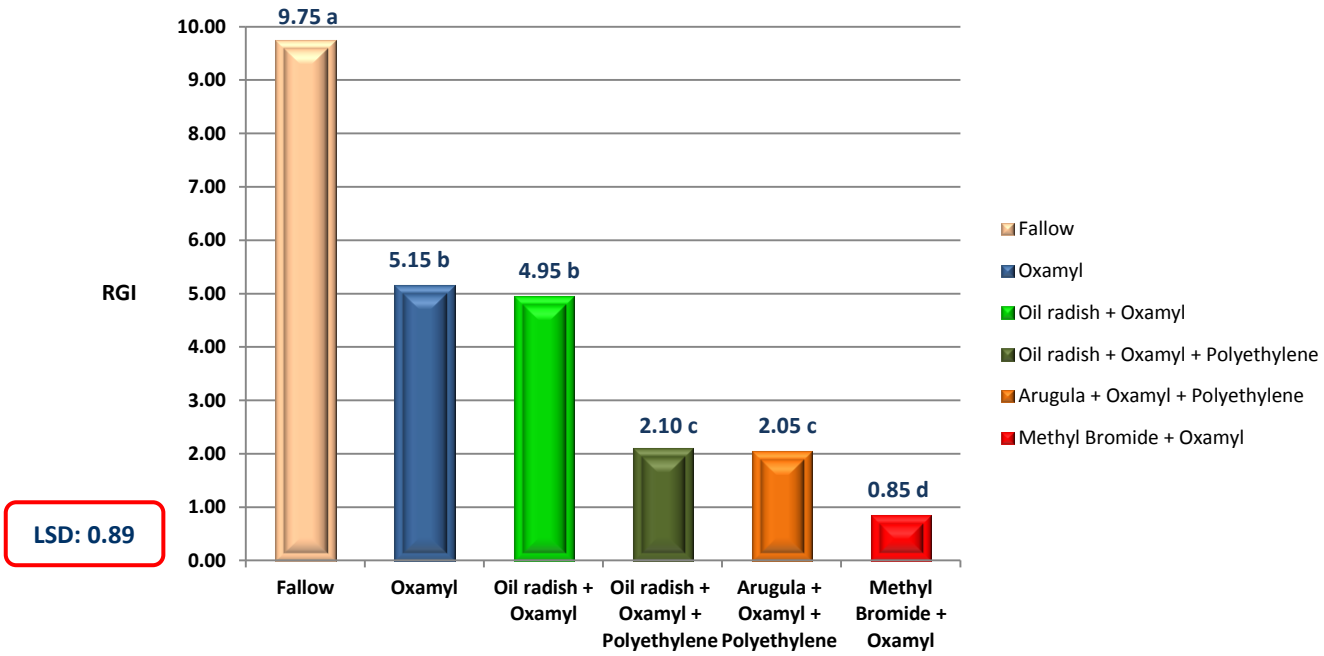
Unlike certain studies having found that Oxamyl when used alone through the drip irrigation is not efficient in reducing nematode infection (Ingham et al., 2007), Oxamyl used alone in our current experiment has reduced the nematode population significantly as compared the untreated fallow. This result coincides with the findings of Stephan (1995) and Desaeger and Csinos (2006) regarding the effect of Oxamyl on nematode populations. However, in our experiment adding Oxamyl to oil radish either with or without plastic cover has not significantly increased the efficiency of the oil radish in terms of nematode control, with no significant difference between those 3 treatments. Therefore, taking the results of the current experiment into consideration, it can be derived that addition of Oxamyl to oil radish is not necessary for adequate nematode control in the production of cucumbers in Lebanon. It can be similarly deducted that using oil radish in coordination with Oxamyl does not significantly increase the nematostatic effect of Oxamyl. Furthermore, results of both experiments A and B described in chapter I let us conclude that addition of Oxamyl to any of the biofumigation crops used (oil radish and arugula) is not necessary specifically when transparent plastic cover is used as tarp.

Root Gall Index

All treatments have reduced root galling significantly as compared to untreated fallow. The lowest root gall index was reached in the plots treated with Methyl Bromide + Oxamyl (0.85). There was a significant reduction in both oil radish and arugula planted plots covered with plastic + Oxamyl (2.10 and 2.05 respectively) as compared to fallow. Relatively heavier root galling was found in plots treated with oil radish + Oxamyl without plastic cover (5.15),

with no significant difference with plots treated with Oxamyl alone (4.95). The highest galling was found in the roots of untreated fallow plots (9.75).

Chart # 2.2: Effect of different treatments on the cucumber root gall index at end season



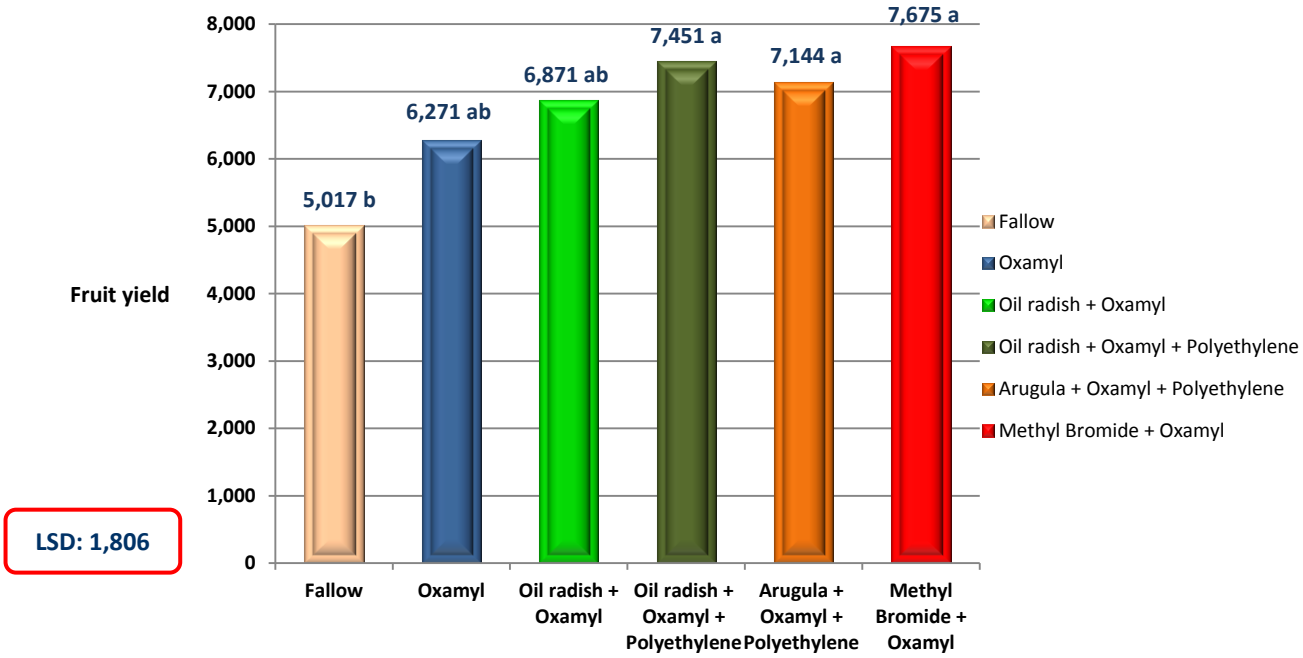
Oxamyl used alone in our current experiment has reduced root galling significantly as compared the untreated fallow. This result is conforming to the findings of research conducted by Desaeger and Csinos (2006) regarding the efficiency of Oxamyl in reducing root galling caused by the Southern root-knot nematode. However, the non-significant difference between Oxamyl and Oxamyl + oil radish indicates that once again Oxamyl did not add to the efficiency of oil radish used without plastic cover in the reduction of root galling. The addition of Oxamyl did not result into any further reduction of root galls. On the other hand, the significant difference existing between these two treatments and any of the two biofumigant crops used in addition to Oxamyl with plastic cover leads us to conclude that the additional reduction of root galls was more due to the plastic cover rather than Oxamyl. These findings further confirm the results of our previous experiment B described in Chapter I, where less root gall formation was encountered when oil radish was covered with

plastic as compared to the oil radish left without cover. Use of plastic cover with oil radish has resulted into higher reduction of root galls than adding Oxamyl to oil radish without plastic cover. Therefore, our study confirms that addition of Oxamyl to biofumigant crops is not as essential as plastic cover for the control of the Southern root-knot nematodes in greenhouse production of cucumber in Lebanon.

Fruit Yield

Fruit yield was assessed based on the harvest resulting from the five plants selected in each treatment plot for this purpose. No significant differences in fruit yield were observed among all treatments except untreated fallow. The significantly highest yield was obtained from plots treated with Methyl Bromide + Oxamyl (7,675 gram), and arugula or oil radish with Oxamyl and plastic cover as compared to untreated fallow (5,017 gram). No significant differences were obtained among Oxamyl, oil radish + Oxamyl and Untreated fallow. Yield produced by oil radish + Oxamyl and arugula + Oxamyl treatments both covered with plastic were only slightly less (7,451 gram and 7,144 gram respectively) than Methyl Bromide + Oxamyl (7,675 gram).

Chart # 2.3: Effect of different treatments on cucumber fruit yield



Results of our experiment confirm several previous studies having found that Oxamyl increases crop yields either when used alone (Stephan, 1995) or following fumigation with metam sodium (Desaeger and Csinos, 2006), which produces nematostatic gases in the soil similar to the ones released through the decomposition of the two biofumigant crops used in our experiment. Our previous experiments A and B described in chapter I, have revealed that oil radish consistently and significantly increases cucumber yields when covered with plastic. Results obtained in our current experiment have shown that Oxamyl does not increase yield significantly as compared to the untreated fallow neither when applied alone, nor in combination with oil radish without plastic cover. Significant difference with fallow is observed only with the addition of plastic cover to the oil radish + Oxamyl treatment. Furthermore, this treatment has shown no significant difference with the arugula + Oxamyl with plastic cover and specially the Methyl Bromide + Oxamyl treatment, which has produced the highest yield.

Therefore, based on the results of our previous and current experiments it can be concluded that addition of Oxamyl to the two biofumigant crops used in our study is not necessary for the proper management of the southern root-knot nematode in Lebanon, given that these crops are covered with plastic tarp for a period of 10 days following their incorporation into the soil. As such, both oil radish and arugula crops have shown consistent results and significantly decreased the soil population of the southern root-knot nematode and root galling, and increased cucumber yield. Consequently, it can be concluded that when covered with plastic, these biofumigation crops can be sound replacements of Methyl Bromide for the production of cucumbers in Lebanon.

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CHAPTER III

Cost-Benefit Analysis of Different Treatment Methods of Meloidogyne Control in Greenhouse Cucumber Crops.

INTRODUCTION

Nematodes cause serious economic losses to most agricultural crops (Araji and Hafez, 2001). Root-knot nematodes (*Meloidogyne* spp.) particularly are among the major soil-borne pests causing significant reduction of yields (Wang *et al.,* 2006). Surveys carried out in the United States of America (USA) have shown that total annual crop losses due to plant parasitic nematodes in 1994 reached 8-12 billion US Dollars in the United States (Barker *et al*., 1994). Estimation of worldwide total annual crop loss due to nematodes for the same year ranges from 78 billion (Barker *et al*., 1994) to exceeding 90 billion US Dollars (Koening et al., 1999). Root-knot nematodes cause severe yield losses particularly in vegetable crops, reaching 50% total yield loss (Darekar and Mhase, 1988) as a result of root galling, which not only affects general plant growth but also predisposes plants to other pathogens.

According to the statistics undertaken by the National Potato Council of the United States of America (www.nationalpotatocouncil.org) for the year 2003, the western states of the US produce 64% by weight and 61% by value of the total potato (*Solanum tuberosum* L.) grown in the country. The Columbia root-knot nematode (*Meloidogyne chitwoodi*) poses a serious threat to this US$1.5 billions worth annual potato production (Ingham et al., 2007). Although no yield reductions are attributed to *M. chitwoodi*, internal and external quality defects such as brown spots and surface galling make the yields unacceptable for fresh consumption. If 5-15% of the tubers display such symptoms the entire crop may be rejected. At an estimated crop value of US$ 9,900/hectare, rejection of the entire potato crop from an average size irrigated plot of 52.6 ha inflicts losses exceeding US$ 500,000 on the grower (Bosecker and Coba, 2006).

High populations of *Meloidogyne incognita* in the soil have caused 65% yield loss in melon (*Cucumis melo* L.) plantations in Southern California (Ploeg and Phillips, 2001). In a greenhouse experiment, the root-knot nematode *Meloidogyne hapla* has led to a decrease of marketable yields of carrots to 58% (Slinger and Bird, 1978).

It has been also found that the four identified species of root-knot nematodes in Cyprus, namely *M. incognita*, *M. javanica, M. arenaria and M. hapla* cause extremely serious crop losses in the production of vegetables, and potato, reaching up to 12% of the total production value (Philis J., 1995). Additionally, for every twenty (20) eggs of *Globodera rostochiensis* per gram of soil, a reduction of 2.2 tons per hectare in potato yield is recorded (Philis J., 1991). A study done under glasshouse conditions in Italy has shown a tolerance limit of 3.3 eggs and juveniles of *Meloidogyne incognita* per 1 cc of soil for different susceptible and resistant tomato cultivars, and minimum relative yields have been established as 0 and 0.77 for the susceptible and resistant tomato cultivars respectively (Di Vito and Ekanayake, 1983). In another experiment, Di Vito *et al*. (1991) have found that root-knot nematode infestation has negatively affected both fruit size and marketable yields of susceptible tomato cultivars (Di Vito *et al*., 1991).

Similarly, *M. incognita* and *M. javanica* are considered as being the most damaging species for vegetables, field crops, and fruit trees in the warm sandy soils of Pakistan. Damage caused to tomato production is estimated to range from 24–38% yield loss, to total crop failure in the absence of effective nematode management practices (Javed *et al*., 2007). The magnitude of damage is increased in the presence of certain soil-borne pathogens such as *Fusarium oxysporum* f. sp*. lycopersici* (Sikora and Fernandez, 2005).

The wide host range and high reproduction rates of *Meloidogyne* spp., make them difficult to control. In tropical soils the generation time ranges between twenty (20) and thirty (30) days. Females are usually capable to produce around 1,000 eggs (Natarajan et al., 2006). Serious yield losses of carrots were reported at initial population densities of *M. hapla* as low as 0.2 and 2.0 J2 juveniles/cm^3 of soil in Michigan (Slinger and Bird, 1978) and in Canada (Vrain *et al*., 1979), respectively. Similar effects of *M. hapla* on lettuce (Viaene and Abawi, 1996) and of *M. incognita* on tomato (Barker et al., 1976) have been also reported.

Extensive research for the evaluation of the different chemical and non-chemical control measures in the production of various commercial crops has been done.

Incorporation of organic amendments to soils for the management of plant parasitic nematodes has interested farmers and scientists since the beginning of the 20th century. Castor (*Ricinus communis* L.), collard (*Brassica oleracea* L.), sesame (*Sesame indicum* L.), sorghum-sudangrass (*Sorghum bicolor* x *Sorghum Sudanese*), velvet-bean (*Mucuna deeringiana*) and zinnia (*Zinnia elegans* L.) have all been used as organic amendments incorporated into the soil as mulch or in crop rotations for nematode suppression (Huang, 1984; Mankau, 1968; Mian and Rodriguez-Kabana, 1982; Rodriguez-Kabana *et al*., 1988, McSorley and Gallaher, 1993).

However, complete understanding of this technique as a control method was lacking at that time (Linford *et al*., 1938; Watson, 1945). Higher yields obtained by these organic amendments were initially attributed to many factors such as release of phenolic compounds, changes in soil pH and increased water holding capacity, or the C/N ratio of the organic material used (Brown, 1987; Mankau and Minteer, 1962; Rodriguez-Kabana, 1986). Warman (1998) reported that additions of organic amendments to soils have resulted into an increase of marketable carrot yields by 67-76%, as compared to application of conventional fertilizers (Warman, 1998). In a similar experiment extended over three years on tomato, Maynard (1995) has found that while the average fruit yields in the untreated plots ranged from 3.54 to 7.67 kg per plant, compost amended plots yielded an average of 4.89 to 8.85 kg per plant, thus providing an average yield increase of 18% (Maynard,1995).

Nonetheless, it was soon realized that yield increase due to the application of organic amendments may not be necessarily related to the dynamics of nematode population reduction, since organic amendments themselves result into healthy plants and subsequent yield increase due to the enhancement of soil fertility they promote (McSorley and Gallaher, 1995). Ritzinger and McScorley (1998) have found a significant relation between different types and forms (fresh or dry) of organic amendments used as mulch and both formation of egg masses by *M. arenaria* and number of juveniles hatched from these egg masses. Amendments of castor, collard, velvet-bean and zinnia have all been effective suppressants of *M*. *arenaria* (Ritzinger and McScorley, 1998).

In the recent years, the relatively high application cost of most chemical nematicides in addition to their potential detrimental effects on the ozone layer, the environment and human health, have led scientists to look for efficient and safe alternatives, and to study the possible use of certain plant materials for this purpose (Javed *et al*., 2007; Ijani and Mmbaga, 1988; Ploeg, 2002). A large variety of plant products have thus been tested for their effect on the management of plant parasitic nematodes. Several green manure and non-host crops have been identified to influence nematode populations in the soil (Johnson *et al*., 1992; Rodriguez-Kabana *et al*., 1992; Mojtahedi *et al*., 1993a; 1993b). In addition, it was perceived that these crops enhance soil fertility and water holding capacity, thus improving yields (Wang *et al*., 2002; Wang and Klassen, 2003; Wang *et al*., 2005). It was found that while their decomposition in the soil, many of these plants produce secondary metabolites which have the potential of suppressing nematodes. Additionally, organic residues of plant origin were proven to enhance the biological and physical properties of the soil and increase its water holding capacity (Chitwood, 2002).

Bhatti and Nandal (1994) have found that many plant parts and extracts suppress *Meloidogyne* species effectively (Bhatti and Nandal, 1994). In a pot experiment aiming to study the effects of castor (*Ricinus communis*), eucalyptus (*Eucalyptus citriodora*), and neem (*Azadirachta indica*) on *Meloidogyne incognita*, Walia *et al*. (1999) have found that fresh leaves of all three plants studied considerably reduce root galling in both okra and tomato (Walia *et al*., 1999). Among several other plant derivatives, walnut extracts have been used in California for nematode management in fruit trees (McKenry and Anwar, 2003).

Indian farmers have been using neem tree (*Azadirachta indica*) extracts for hundreds of years to control agricultural pests and diseases. Several formulations of neem have been recently demonstrated as being effective nematicides as well. It has been shown that neem extracts inhibit the ability of eggs of the root-knot nematode to hatch and mobility of juveniles, thus effectively reducing population and improving plant health (Javed, 2000, unpublished data). Through an experiment conducted in the UK, Javed *et al*. have found that different neem formulations used at a low concentration of 0.05% azadirachtin (a secondary metabolite present in the neem seeds) have protective and curative effect on the

invasion and development of J2 of *M. javanica* in the roots of tomato plants. At higher concentration of 0.1% azadirachtin neem cake have shown curative effect by restraining development of the root-knot nematode, and suppressing the numbers of eggs and egg masses on tomato roots (Javed *et al*., 2007). In a similar experiment conducted in Egypt, Butool *et al*. (1998) have found that neem, castor, mustard, groundnut and linseed cakes improve plant growth and effectively suppress *M. incognita* by reducing its population in the soil and the roots of Egyptian henbane (Hyoscyamus muticus L.) (Butool *et al*., 1998).

Throughout centuries plants of the mustard family (Brassicaceae) have been recognized for their breakdown products which were used as medicine. In the recent years extensive knowledge exists on the chemistry and mode of action of these products, some of which are found to be effective nematode suppressants (Fahey *et al*., 2001). All parts of Brassicaceae plants produce Glucosinolates, which react with myrosinase as a result of tissue damage to generate nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs) (Brown and Morra, 1997 and Fahey *et al*., 2001). The actual conversion rate of glucosinolates to isothiocyanates in the soil ranges from 1 to 25% (Borek *et al*., 1997). ITCs are volatile toxic compounds classified as general biocides (Brown and Morra, 1997). There is ample scientific evidence of their toxicity to fungi (Charron and Sams, 1999; Chung *et al.,* 2002) and bacteria (Delaquis and Mazza, 1995; Zasada and Ferris, 2004). Toxicity of ITCs to many plant parasitic nematode species has also been established through extensive research (Lazzeri *et al*., 1993; Buskov *et al*., 2002; Zasada and Ferris, 2003). In a laboratory experiment Zasada and Ferris (2004) have found that soil amendments of mustard (*Brassica juncea*) and broccoli (*Brassica oleracea var. botrytis*) have reduced *M. javanica* populations by 100% and 94%, respectively (Zasada and Ferris, 2004).

Hafez and Sundararaj have found that incorporation of different nematode-resistant green manure crops such as oil radish (*Raphanus sativus* L.) and white mustard (*Sinapis alba* L.) in sugar beet rotations prevent the completion of the life cycles of phytoparasitic nematodes, thus reducing nematode populations by 84-92% and increasing sugar beet yield by 22-25% as compared to fallow (Hafez and Sundararaj, 1998; Hafez, 1999; Hafez and Sundararaj, 1999). In a field experiment in *M. javanica* infested soils in Florida Fenamiphos has increased tobacco yields by 646 kg/ha raising net profits of US $1,983, while 1,3-D has

increased yields by 934 kg/ha with a net return of US$ 3,134/ha (Rich and Zimet, 1996). Rodriguez-Kabana and King (1985) have described the relationship between larval numbers of *M. arenaria* and peanut yield loss through a linear equation, which indicates a loss of 4.77 Kg/ha for each larva present in 100 cm^3 of soil. Highest yield responses and greatest profits were obtained with the lowest rates of several nematicides used, among which Oxamyl (Rodriguez-Kabana and King, 1985; Rodriguez-Kabana *et al*., 1982).

Gugino *et al*. (2006) have found that control of *Meloidogyne hapla* with the use of Oxamyl was economically beneficial in highly infested fields, but not at low infestation levels, and recommended to incorporate Oxamyl in an IPM system including crop rotations with non-host crops (Gugino *et al*., 2006). Oxamyl applied alone has increased tomato yields in Cyprus by 23.5% and reduced *M. javanica* eggs by 81.3%. A combination of a pre-plant application of Dazomet and Oxamyl was much more effective. This treatment increased yields by 47.1% and reduced *M. javanica* egg density by 96.1%. However, the incremental crop value over the cost of nematicide used was considerably higher with the use of Oxamyl alone (Philis, 1994).

A nematode suppressive green manure crop can be considered beneficial only if its suppressive effects last long enough to promote the desired level of development to the following crop. In this case economic and environmental benefits can be achieved and the high cost of soil fumigation with the use of chemicals might be avoided (Abdul-Baki *et al*., 2005; Wang *et al*., 2003, Wang *et al*., 2006). Therefore, nematode-suppressive green manure crops may be used as biological alternatives to Methyl Bromide in rotation with nematode-susceptible cash crops, having the potential to replace Methyl Bromide effectively in sustainable agricultural systems (Wang et al., 2007).

Accurate information about the commercial profitability of the use of green manure crops alone or in combination with some chemical nematicides for the control of *M. incognita* is limited. Therefore, comprehensive data was gathered throughout three field experiments, daily yield market values and application costs of nine different root-knot nematode management methods for the assessment of net profits resulting from each of these methods in comparison with Methyl Bromide and Methyl Bromide + Oxamyl in the production of greenhouse cucumber crops in Lebanon.

MATERIALS AND METHODS

In the three experiments described in chapters I and II, data on yield resulted from plants marked for harvest in each treatment area was recorded throughout the consecutive growing seasons. Day to day wholesale market values prevailing on each harvest date were also registered. The total revenue resulting from the sale of cucumbers harvested from each treatment area was summed up at the end of the season, and converted into total revenue in US Dollars per 1 dunum (1,000 m^2) for harmonization purposes, and ease of comparison. These calculations were done according to the following formula:

$$A = \frac{B \times 2{,}500}{C}$$

Where B is the total revenue resulting from yield obtained from plants harvested from the four replicated plots of the same treatment;

C is the total number of plants harvested from the four replicated plots of the same treatment;

A is the calculated total revenue which would result from yield that would be obtained from 2,500 plants under similar circumstances.

The figure of 2,500 has been considered as a constant, being the usual plant density in a greenhouse area of 1 dunum in Lebanon.

In parallel, the application cost of each treatment in US Dollars was calculated in detail, and converted into cost per dunum, for the assessment of the net profit resulting from each treatment as follows:

$$X = (A - Y)$$

Where X represents the net profit resulting from each treatment in US$ per dunum.

Y being the application cost of the treatment considered for 1 dunum.

Finally, the net profit percentage achieved by each treatment as compared to Methyl Bromide was assessed based on the following formula:

$$Y = \frac{X \times 100}{M}$$

Where M is the net profit resulting from the Methyl Bromide treatment.

Costs related to general cultural practices throughout the growing season such as soil tillage, seedlings, preparatory and seasonal fertilization, irrigation and control of pests and diseases not related to soil were calculated and summed up to a constant figure of 500 US$ per dunum. The cost of each treatment calculated separately was added to this constant figure for the estimation of the total production cost related to each treatment.

Table # 3.1 below summarizes the list of components considered in this study for the evaluation of the actual cost of each of the nine different treatments applied in the three experiments described:

Table # 3.1: Cost components of the different treatments

Treatment	Tractor	MeBr	Seeds	PE	Oxamyl	Labor
Fallow						
OR	**x**		**x**			**x**
OR + PE	**x**		**x**	**x**		**x**
AR + PE	**x**		**x**	**x**		**x**
MeBr		**x**		**x**		**x**
Oxamyl					**x**	**x**
OR + Oxamyl	**x**		**x**		**x**	**x**
OR + PE + Oxamyl	**x**		**x**	**x**	**x**	**x**
AR + PE + Oxamyl	**x**		**x**	**x**	**x**	**x**
MeBr + Oxamyl		**x**		**x**	**x**	**x**

Table # 3.2 illustrates the total cost of each treatment calculated based on the components described above, as well as the total production cost related to each treatment, including the seasonal production costs (constant figure of 616 US$ per dunum).

Table # 3.2: Total costs of the different treatments

Treatment	Cost of Treatment (US$/Dunum*)	Total Production Costs (Treatment + Seasonal) (US$/Dunum*)
Fallow	**0**	**616**
OR	**75**	**691**
OR + PE	**175**	**791**
AR + PE	**175**	**791**
MeBr	**600**	**1,216**
Oxamyl	**39**	**655**
OR + Oxamyl	**114**	**730**
OR + PE + Oxamyl	**214**	**830**
AR + PE + Oxamyl	**214**	**830**
MeBr + Oxamyl	**639**	**1,255**

* 1 dunum = 1,000 square meters

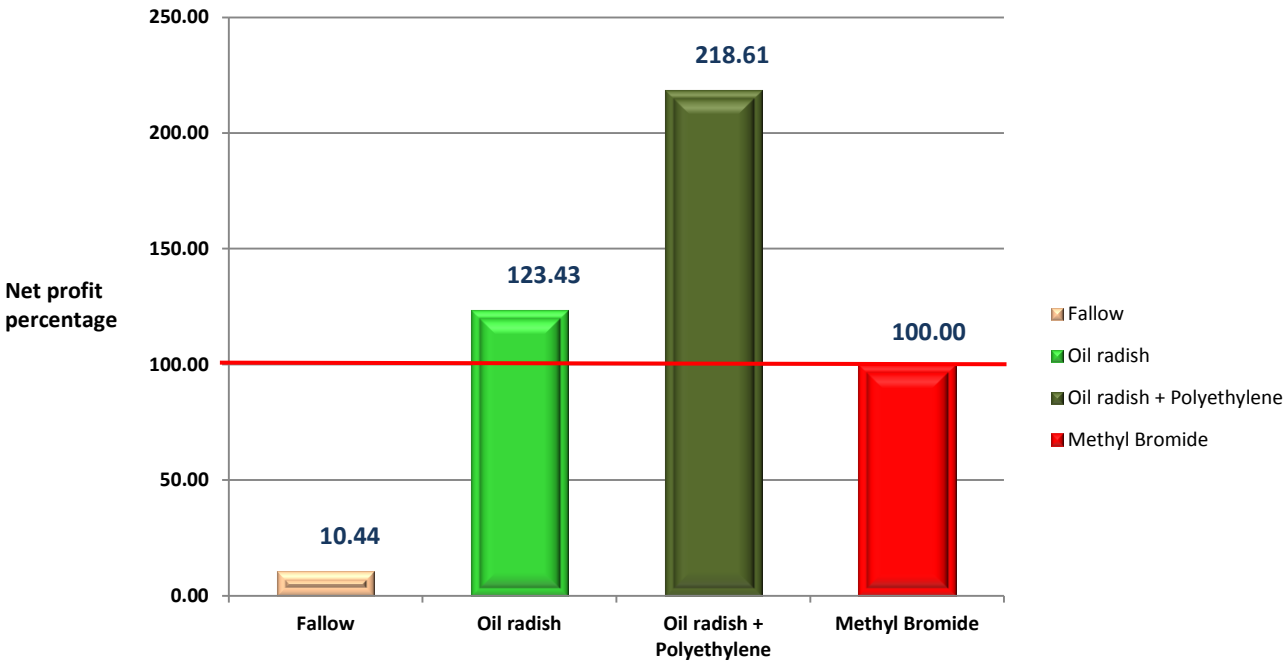
At the completion of harvest in each of the three experiments, cost to benefit analysis was calculated by deducting the total production cost related to each treatment from the total revenue resulted from the yield produced and marketed by the treatment being considered. Results of treatments considered as potential alternatives to Methyl Bromide thus obtained were compared to results of Methyl Bromide when applied alone (experiments A & B of chapter I) and Methyl Bromide + Oxamyl when applied in combination (experiment C of chapter II). Cost to benefit ratio (or the net profit percentage) of Methyl Bromide and Methyl Bromide + Oxamyl treatments has been estimated at 100% for comparison purposes.

RESULTS AND DISCUSSION

Experiment A (Chapter I)

In experiment A net profit percentages resulted from both treatments (oil radish and oil radish with plastic cover) have been 123.43% and 218.61% respectively as compared to Methyl Bromide (estimated at 100%), whereas net profit percentage of the untreated fallow has been as low as 10.44%.

Chart # 3.1: Effect of different treatments on net profit percentage (Experiment A)



Results of this experiment indicate that oil radish and oil radish with plastic cover have both increased nets profits by 23.43% and 118.61% respectively as compared to Methyl Bromide. Use of plastic cover with oil radish has led to a remarkable increase of net profit (95.18%) as compared to the uncovered oil radish. Planting without any soil treatment

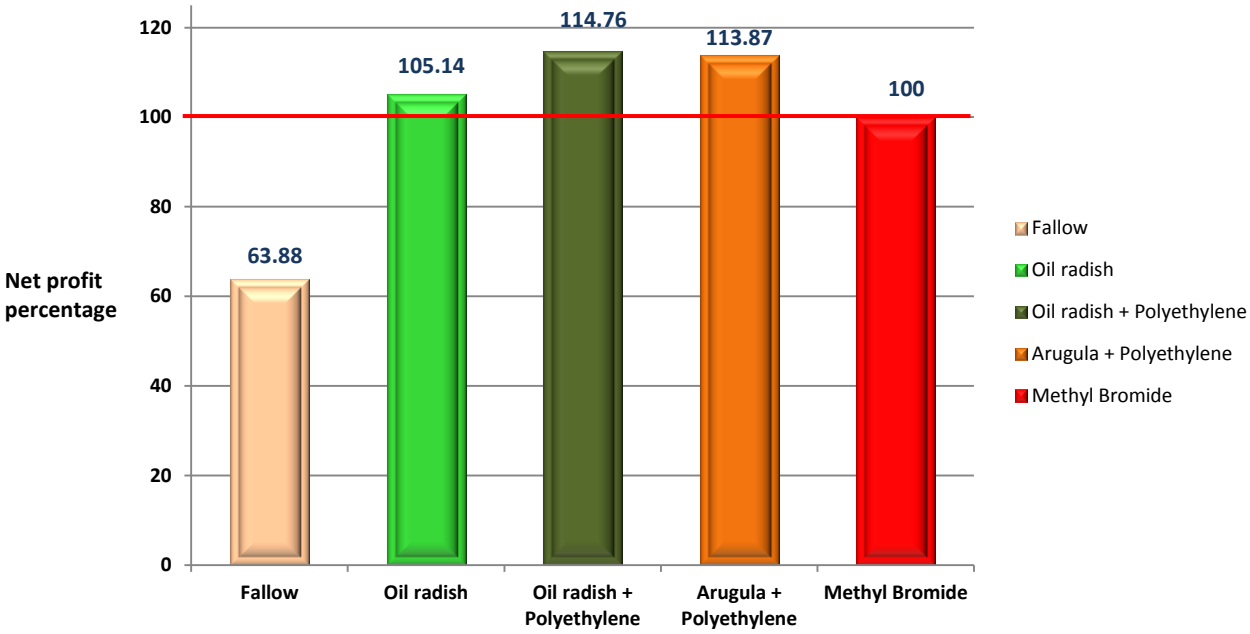
activity has led to a considerable decrease of 89.56% of net profits as compared to Methyl Bromide, and even more as compared to the remaining treatments.

It is to note that the highest yield of cucumber was obtained from the plants marked for harvest in plots treated with Methyl Bromide (11,054 grams), with significant difference between Methyl Bromide and each of oil radish (7,165 grams) and oil radish with plastic cover (8,780 grams), as described in chapter I (Chart # 1.5, page 47, Chapter I). Similarly, Methyl Bromide has led to significantly less root galling (0.9) than oil radish and oil radish with plastic cover (2.0 and 1.7 respectively) (Chart # 1.3, page 45, Chapter I). However, the higher net profits achieved by oil radish with and without plastic cover as compared to Methyl Bromide can be attributed to the relatively high cost of Methyl Bromide fumigation (600 US$) as compared to cost of oil radish (75 US$) and oil radish with plastic cover (175 US$), as described in Table # 3.2 above.

Experiment B (Chapter I)

The net profit percentages resulted from the alternative treatments (biofumigation crops) applied in experiment B either with or without polyethylene followed a similar pattern to the ones registered in experiment A. After deduction of the total production cost (including the treatment cost) from the total market value of yield produced by each treatment, it was noted that the net profit percentages obtained from plots treated with oil radish and oil radish with plastic cover were 105.14% and 114.76% respectively as compared to Methyl Bromide. Net profit percentage produced by arugula with polyethylene cover was 113.87%, and only 63.88% for untreated fallow.

Chart # 3.2: Effect of different treatments on net profit percentage (Experiment B)



Similar to the results obtained in experiment A, both biofumigation crops used in this experiment have increased nets profits as compared to Methyl Bromide. Oil radish and oil radish with plastic cover have increased nets profits by 5.14% and 14.76% respectively. Arugula with plastic cover has increased nets profits by 13.87%.

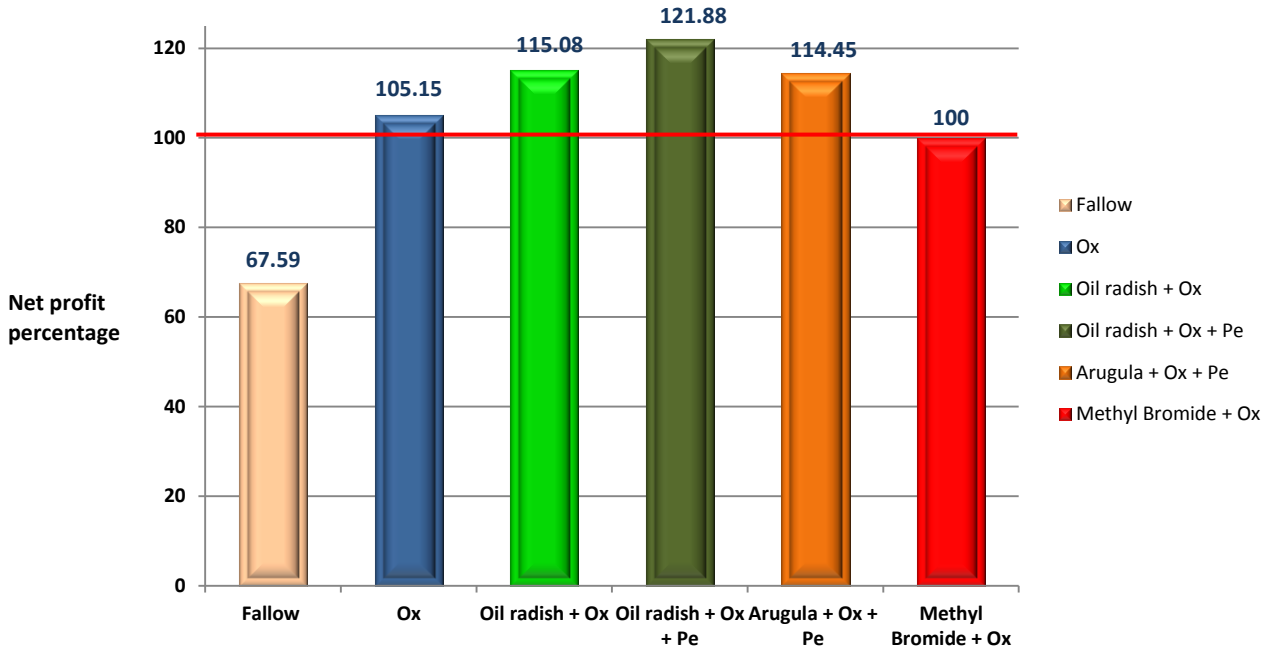
Yield records indicate that there has been no significant difference between quantities of yield achieved by the plants marked for harvest in plots treated with oil radish with plastic cover (29,863 grams), arugula with plastic cover (29,990 grams) and Methyl Bromide (29,903 grams). All of these three treatments have produced significantly higher yields than oil radish without plastic cover (26,910 grams) (Chart # 1.6, page 48, Chapter I). On the other hand, there has been significantly less galling in roots taken from plots treated with Methyl Bromide (0.7) than any of the remaining treatments (1.7 for arugula with plastic cover, 2.4 for oil radish with plastic cover and 4.7 for oil radish without plastic cover) (Chart # 1.4, page 46, Chapter I). As in the previous experiment A, higher net profits achieved by the biofumigation crops used in the current experiment (oil radish with and without plastic cover,

arugula with plastic cover) as compared to Methyl Bromide can once again be the consequence of the high cost of Methyl Bromide fumigation.

Experiment C (Chapter II)

Similar to the two previous experiments A and B, all treatments of this current experiment have increased net profits as compared to Methyl Bromide + Oxamyl. The least increase was achieved by Oxamyl applied alone (105.15%). Arugula with plastic cover + Oxamyl has increased net profits by 114.45%, while oil radish without plastic cover + Oxamyl increased net profits by 115.08%. The highest increase was reached by oil radish with plastic cover + Oxamyl (121.88%). Net profit of the untreated fallow has been only 67.59%.

Chart # 3.3: Effect of different treatments on net profit percentage (Experiment C)



In the current experiment as in the previous ones, all treatments implemented have increased nets profits as compared to the Methyl Bromide + Oxamyl treatment. Oxamyl used alone has caused an increase of 5.15%, while oil radish without plastic cover +

Oxamyl has produced an increase of 15.08%. Oil radish with plastic cover + Oxamyl has reached 21.88% of increase, and arugula with plastic cover + Oxamyl 14.45%. Net profit loss due to the absence of any treatment of the soil caused a decrease of 32.41% of net profits as compared to Methyl Bromide.

As described in chapter II, yield records show that there has been no significant difference between the quantities of yield produced by any of these treatments. The highest yield was achieved by the plants marked for harvest in plots treated with Methyl Bromide (7,675 grams), closely followed by oil radish with plastic cover + Oxamyl (7,451 grams), then arugula with plastic cover + Oxamyl (7,144 grams) and oil radish without plastic cover + Oxamyl (6,871 grams). Oxamyl alone has yielded 6,271 grams, while untreated follow has produced as little as 5,017 grams (Chart # 2.3, page 77, Chapter II). Root galling has shown a similar pattern, where significantly less galling was found in roots taken from plots treated with Methyl Bromide + Oxamyl (0.85) than any of the remaining treatments. There was also no significant difference between root galling found in arugula + Oxamyl + Polyethylene (2.05) and oil radish + Oxamyl + Polyethylene (2.10), neither between oil radish + Oxamyl (4.95) and Oxamyl alone (5.15) (Chart # 2.2, page 75, Chapter II). However, once again the higher net profits achieved by all treatments applied is due to their relatively low cost as compared to Methyl Bromide application. Table # 4.1 summarizes the net profits achieved by the different treatments applied in our 3 experiments.

Table # 4.1: Net profits achieved by the different treatments

Treatment	Experiment I	Experiment II	Experiment III
Fallow	- 89.56%	- 36.12%	-32.41%
OR	+ 23.43%	+ 5.14%	-
OR + PE	+ 118.61%	+ 14.76%	-
AR + PE	-	+ 13.87%	-
MeBr (Standard)	**100.00%**	**100.00%**	-
Oxamyl	-	-	+ 5.15%
OR + Oxamyl	-	-	+ 15.08%
OR + PE + Oxamyl	-	-	+ 21.88%
AR + PE + Oxamyl	-	-	+ 14.45%
MeBr + Oxamyl	-	-	**100.00%**

Based on the results of the cost-benefit studies of the current chapter, it can be deduced that the additional yield obtained through Methyl Bromide fumigation is not financially justified in terms of net profits achieved, even when there is significant difference between quantities of yield produced by Methyl Bromide and any of the treatments used in this study whether chemical, non-chemical or in combination. In contrast with these findings, use of plastic cover with oil radish provides the desired additional control of the root-knot nematode and lead to reasonable increases in net profits in the production of greenhouse cucumber crops in Lebanon.

Whereas only two biofumigation crops have been used in these experiments, there is a wide range of such crops either currently available or under development in the worldwide market. It is to note however, that there is a clear difference between green manure crops and biofumigation crops. Not all green manure crops are biofumigation crops. Green manure crops are primarily grown to add nutrients and organic matter to the soil, while biofumigation crops are used to control nematodes soil borne diseases in various crops. In other words, biofumigation is a natural approach of using plants containing specific biologically active compounds for the control of soil-borne pests and diseases in agricultural crops. It provides a promising sustainable alternative to the use of noxious chemicals and at the same time, delivers nutrients and organic matter to the soil. Both green manure and biofumigation crops are grown for a certain period, mainly between two growing seasons, and are ploughed under and incorporated to the soil while still green.

There is a large potential for the biofumigation technique to expand further and to be adopted more readily, specifically in the Middle East. Besides oil radish and arugula used in the current experiments, several other biofumigation crops such as white mustard, hybrid Sudan grass, sesame, Cahaba vetch, cabbage and wild Allium varieties are being successfully used in many European countries and the United States. It is worth evaluating these crops locally and testing their efficiency and feasibility under local conditions. It is probable that not all suit the climatic conditions prevailing in different regions, nor all can grow under greenhouses. It is also known that not all biofumigant crops release the same chemicals upon their decomposition in the soil. In fact, active ingredients released by the

most common biofumigant crops are related to four major chemical compounds, as described in the below table # 4.2:

Table # 4.2: Chemical compounds released by the most common biofumigant crops

Biofumigant crop	Chemical compound released
Brassica plants (Oil radish, arugula)	Glucosinolates
Crucifer plants (Cabbage family)	Carbon disulphide
Sudan grass	Cyanide
Wild Allium species (Leek, garlic, onion)	Dimethyl disulfide (DMDS)

These biofumigant crops contain high levels of naturally occurring compounds, which control soil-borne pests and diseases. However, the mechanism of breakdown of these naturally occurring compounds into different chemicals differ from one biofumigant crop to another. Brassica plants for example, release glucosinolates when incorporated into the soil. In parallel, the myrosinase enzyme produced by the decaying Brassica plants breaks down the glucosinolate, and hydrolyzes the glucosinolate into an active phytochemical called Methyl Isothiocyanate (MITC), which is detrimental to nematodes, fungi, and some weeds. It is to note that MITC is also the active ingredient found in some synthetic and widely used soil fumigants, such as metam sodium. This mechanism is different in Allium species (such as onion, leek, and garlic), which contain sulfur amino-acid precursors in their cytoplasms and an enzyme called allinase in their vacuoles. During the decomposition of these plants, these compounds come into contact and produce a new compound called dimethyl disulfide (DMDS), which has the characteristic smell of garlic and onion. DMDS is not the only compound produced through the decomposition process of Allium species. Garlic produces diallyl disulfide (DADS) as well, while onion and leek produce dipropyl disulfide (DPDS) in addition to DMDS.

Despite of the proven efficiency of DMDS in the adequate control of soil-borne pests and diseases, DMDS concentration levels are very low and insufficient in the species of onion, leek and garlic generally cultivated for commercial purposes. Therefore, the promising results obtained from the biofumigation technique in the past decade have promoted the development of several related industries, such as seed producing companies and breeding farms, for the development of elite species. There are already many such registered breeders and seed producing companies who develop efficient cultivars of biofugimation crops and produce huge amount of seeds and planting materials for the control of different nematode species in Europe and the USA. These industries have the potential of further development and expansion, especially in some regions where the biofumigation technique is not yet well known among farmer communities.

As the biofumigation technology is environmentally friendly, growers and governments are more supportive and tend to adopt it. However, not all farmers are yet well aware of the benefits of the biofumigation technique. Breeders and seed producing companies have therefore to further advertise their products and explain the long-term advantages and extra benefits of biofumigation to the farmers.

Biofumigation generally does not cause any harm to the cultivated crops, given that enough time is allocated for the dissipation of the chemical compounds released by the decomposition of the biofumigant crops before planting the main crop. Otherwise, these chemical compounds might have some phytotoxic effect on the following crop. This fact makes the period requested for the application of the biofumigation technique relatively longer than the time needed for the application of many synthetic chemicals and fumigants used for this purpose, such as Methyl Bromide. This is another hindering factor to the wide-scale adoption of the biofumigation technique by farmers. Research must be carried out on the development of new biofumigation crops which grow quickly and/or produce more abundant vegetative parts within a shorter period of time.

Finally, despite of the global phase out of Methyl Bromide, its either legal or illegal persistence in some markets, slows down the wide-scale adoption of the biofumigation technique. In Lebanon, most farmers having used biofumigation have expressed their satisfaction with the results obtained and stated their readiness in adopting biofumigation.

But this readiness will probably not be translated into actual action as long as Methyl Bromide is still available. However, in the light of the approaching deadline set for the complete and global ban of Methyl Bromide, in addition to its extremely high costs as compared to biofumigation, the biofumigation technique has a great potential of replacing Methyl Bromide in a great number of countries in a safe and sustainable manner.

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Conclusion and Future Areas of Study

Root-knot nematodes remain one of the most serious problems faced by greenhouse farmers of Lebanon and the Middle East region. The presence of these nematodes in the soil even in relatively small numbers causes serious damage to vegetable crops, specifically those lacking any resistance or tolerance to these pests, such as the cucurbits. In the past, soil fumigation with Methyl Bromide has been considered as the best control measure against root-knot nematodes. However, Methyl Bromide was completely banned worldwide for Ozone preservation concerns. Biofumigation with the use of green manure crops has been one of the potential alternatives suggested by the Methyl Bromide Alternatives Project Lebanon. Under these circumstances, scientific evaluation of the efficiency and cost effectiveness of this technique in Lebanon is an important area of research.

The objective of this study is to evaluate the efficacy of the two biofumigation crops oil Radish (*Raphanus sativus oleifera*) and arugula (*Eruca vesicaria sativa*) applied with and without plastic tarp separately and in combination with Oxamyl in the management of the root-knot nematodes in greenhouse cucumber crops. Additionally, this study aimed at assessing the financial feasibility of the biofumigation technique with the two crops used and the final profitability of these green manure crops in terms of cost to benefit ratio as compared to Methyl Bromide.

For this purpose, three field experiments were carried out in commercial greenhouses situated on the coastal zone of Lebanon. All experiments were designed based on a practical and applied research approach, in order to facilitate the adoption and application of biofumigation by farmers.

In all 3 experiments consistent results were recorded in terms of production of significantly higher yields and reduction of nematode population with the biofumigation crops used whether with or without plastic cover, as compared to untreated fallow.

As to the results achieved by the biofumigation crops with respect to Methyl Bromide, in experiment A yield produced by Methyl Bromide was significantly higher than oil radish with plastic cover. However, no significant difference was found between reduction of nematode population resulting from Methyl Bromide and oil radish with plastic cover.

Difference was significant between Methyl Bromide and oil radish without plastic cover in both yield and reduction of nematodes.

In experiment B no significant difference was observed in neither yield nor reduction of nematode population between any of the two biofumigation crops used with plastic cover and Methyl Bromide. These differences were significantly in favor of Methyl Bromide only when oil radish was used without plastic cover.

In experiment C where Vydate (Oxamyl) was incorporated at the rate of 1 liter per 1,000 m^2 to all treatments, there was no significant difference in neither yield nor reduction of nematode population between any of the two biofumigation crops used with or without plastic cover and Methyl Bromide.

Cost-benefit analysis made on all treatments applied in the 3 experiments showed that in all cases, all these treatments whether chemical, non-chemical (with or without plastic cover) or in combination have produced higher net profits than Methyl Bromide, even when yield produced by Methyl Bromide was significantly higher.

Use of plastic for covering oil radish has generally produced better results in terms of production of higher yields, better reduction of soil populations of root-knot nematodes and leading to reasonable increases of net profits.

Based on these findings and in the light of global phase out of Methyl Bromide, it can be concluded that the use of oil radish and arugula as biofumigation crops with plastic cover can be considered as an alternative management tool for the root-knot nematode in greenhouse cucumber production under Lebanese conditions.

Besides adequate control of a wide range of nematodes and fungal diseases in the soil, biofumigation with the use of green manure crops presents several other major advantages. It improves soil texture and structure and increases organic matter and water holding capacity, doesn’t harm most of the beneficial microorganisms of the soil and above all, it is one of the least expensive alternatives to Methyl Bromide. However, biofumigation has the major disadvantage of requesting a relatively a longer period of application time (45 days) as compared to Methyl Bromide (10 days). For most farmers this fact alone is a major handicap for the wide scale adoption of this technique as a viable alternative to Methyl Bromide.

Therefore, future research must focus on appropriate methods for the reduction of the application period of biofumigation crops. For this purpose, new fast growing varieties of oil radish (*Raphanus sativus oleifera*) and arugula (*Eruca vesicaria sativa*) producing more abundant vegetative parts within a shorter period of time, as well as other varieties of the Brassicaceae family must be developed and tested for their satisfactory performance under local conditions. The arugula cultivar tested in our research was a common variety purchased at the local market. It was till then used for rocket (salad) production purposes and its capacity as a biofumigant crop were unknown and not assessed. The results of our study indicate that arugula is worth of further development and additional testing is needed for the determination of its properties and eficacy as a biofumigant crop.

Finally, the combination of biofumigant crops with low doses of some chemical nematicides with limited impact on the environment remains a potential area of further investigation, having as purpose to evaluate new chemistry or to determine the minimal rates of the chemicals used in combination with the biofumigant crops and/or the maximum reduction of the application period of the biofumigation crops tested.