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EFFECT OF THREE DIFFERENT CONTROL METHODS ON ROOT KNOT NEMATODES UNDER GREENHOUSE CONDITIONS

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ABSTRACT

Experiments were conducted in a greenhouse, located in Awkar, heavily infected with Meloidogyne incognita to study the effect of three different soil control methods on root knot nematodes. The greenhouse was divided into four plots: control plot where no treatment was applied, a plot treated with methyl bromide, a plot covered with thermal polyethylene and a plot covered with regular polyethylene designed as T1, T2, T3 and T4 respectively. Temperatures, measured using thermometers put in plots where treatments T1, T3 and T4 were applied, revealed that thermal polyethylene preserve heat more than regular polyethylene. Nematodes were extracted using the sieving and Baermann funnel methods (1) before application of treatments in order to assess initial nematode population; (2) immediately after removal of polyethylene for evaluating of post-treatment nematode population; (3) and by the end of the experiment for determining final nematode population. The initial nematode population was high (328 nematodes/500 cc of soil) and similar in the four plots with no significant differences. The three applied treatments were effective as they decreased post treatment nematode population counted just after the removal of plastic polyethylene with sometimes 0 nematode in 500 cc of soil. One hundred fourteen days later, final populations were counted showing an increase in *nematode population if compared with post treatment nematode population but a decrease if compared with initial nematode population. Yield of 20 plants per plot was weighed starting from November 9th 2003 to December 28th. Yield in T1 was very low (5,2 Kg) while yield for T2, T3 and T4 were respectively 13,2, 13,6 and 11,85. A cost effective analysis was discussed. The study revealed that solarisation is the most easy, environmental friendly and the most economic method for elimination of root knot nematodes.*

Keywords: *Meloidogyne incognita, soil solarisation, thermal polyethylene, regular polyethylene, temperature, methyl bromide.*

RÉSUMÉ

Des expériences ont été réalisées dans une serre dans la région de Awkar infestée par les nématodes. Les expériences ont visé à démontrer l'effet de trois moyens de lutte sur le développement de la maladie des racines noueuses des concombres due à Meloidogyne incognita. Ces trois moyens ont été fondés sur la fumigation et la solarisation à l'aide de deux types de polyéthylène. A cet effet, la serre a été divisée en 4 blocs : le premier comme bloc témoin où aucun traitement n'a été effectué, le second a été traité par du bromure de méthyle, le troisième a été couvert avec du polyéthylène thermique et le quatrième avec du polyéthylène normal. Les quatre blocs sont désignés respectivement par T1, T2, T3 et T4. Les températures du sol ont été mesurées à l'aide de thermomètres dans les blocs T1, T3 et T4. Les résultats obtenus ont montré que le polyéthylène thermique préserve la chaleur pour une durée plus longue que le polyéthylène normal. Les nématodes ont été extraites et après tamisage par la méthode Baermann à trois moments différents : avant l'application des traitements, 39 jours plus tard après l'enlèvement du polyéthylène et 114 jours plus tard à la fin de l'expérience. Dix échantillons sont prélevés de chaque bloc. La population initiale de nématodes, de l'ordre de 328 nématodes / 500 cc de sol, a été similaire dans les quatre blocs. Les trois traitements effectués ont entraîné une baisse considérable du nombre des nématodes. A la fin de l'expérience, le nombre de nématodes comptés dans les 4 blocs a augmenté par rapport à celui effectué juste après les traitements sans toutefois dépasser le nombre de nématodes initial. Le rendement de 20 plantes par bloc a été également pesé à partir du 9 novembre 2003 jusqu'au 28 decembre 2003. Le rendement dans T1 était très faible (5,2 Kg) tandis que le rendement des plantes dans T2, T3 et T4 était plus élevé et de l'ordre respectivement 13,2, 13,6 et 11,85. Une analyse du coût d'investissement a été effectuée. En se basant sur les résultats du rendement et *du coût d'investissement, il a été démontré que la solarisation à l'aide du polyéthylène thermique est un moyen de lutte efficace contre les nématodes, peu cher et non nuisible à l'environnement.*

Mots-clés : *Meloidogyne incognita, solarisation, polyéthylène thermique, polyéthylène normal, température, bromure de méthyle.*

INTRODUCTION

New production systems for fresh market vegetables evolved to take advantage of technological developments in plasticulture, fertilizer, pesticide application methods and cultivars (Geraldson, 1975). To compensate for the lack of land suitable for these new production systems and for the corresponding increases in production costs, growing seasons were extended and continuous monocultures were practiced. With intensive cultivation practices, came a dramatic increase in the incidence and severity of soilborne pests, commonly referred to as "old land syndrome" (Overman *et al.*, 1965; Gowen, 2003). Cultivated areas with vegetables and mainly cucumbers, grown in greenhouses, occupy a wide surface of the Lebanese coastline (Ministry of Agriculture, 2001). However, this vegetables production is economically constrained by several pests and diseases of which nematodes.

Non-chemical management of soilborne pests has been practiced for centuries. Host resistance, organic amendments, crop rotation, soil solarisation, and cultural practices have been used to control soilborne pests in fresh market vegetable production systems (Callies, 2002 ; Chellemi, 2002). Only in the last 40 years, agricultural producers come to rely on synthetic chemicals for the control of soilborne pests. During this period, agricultural production systems were modified or redesigned to use these chemicals such as methyl bromide (Chellemi, 2002).

In 1985, it was revealed that methyl bromide released from human activities is responsible for 5 to 10% of total ozone depletion in the earth's stratosphere. In 1987, to phase out the use of methyl bromide and manage the consumption of Ozone Depleting Substances, the Montreal Protocol was achieved (UNDP, 2001).

The Methyl Bromide Alternatives Project of Lebanon is being executed by the Ministry of Environment, having as objective to phase out methyl bromide in Lebanon. Many alternatives to methyl bromide were used; some are chemical alternatives (Cadusafos, Fenamiphos, etc.) and some non-chemical alternatives (biofumigation, solarisation, etc.). The three main criteria taken into consideration were: efficiency, economical cost relatively to methyl bromide fumigation, and safety on the environment and public health (UNDP, 2001).

Thus, the critical question facing vegetable producers who have relied exclusively on methyl bromide fumigation are whether soilborne pests could be managed without chemicals in production systems designed or based on the use of broad-spectrum biocides to control them.

Regarding the importance of soil solarisation as the most used alternative to methyl bromide by Lebanese farmers (Haroutunian, 2002) and according to McBride (1999), soil solarisation can be a viable alternative to methyl bromide for vegetable farmers.

Our interest is to compare the efficiency of three different control methods on root knot nematodes under greenhouse conditions, especially between solarisation, using thermal and normal polyethylene as alternatives to methyl bromide, on one side, and methyl bromide fumigation on the other side.

MATERIALS AND METHODS

The main objective of soil solarisation was to increase soil temperature to levels which would be lethal for phytopathogenic nematodes. For this reason, solarisation was conducted in July / August when solar radiation levels were high enough (Robin *et al.*, 1988).

The site where experiments were conducted was in Awkar region. The choice of this site was made according to the density of nematodes in this region which is the major problem for vegetable growers who, until now, rely exclusively on methyl bromide fumigation to reduce nematode population (Haroutunian, 2003).

The greenhouse with an area of 350 m^2 (50 m long x 7 m wide) was divided, using appropriate zoning bans, into four plots designed as P1, P2, P3 and P4. The surface area of P1 was 7 x 5 m, P2, P3 and P4 had the same surface area and that of 7 x 15 m.

A sampling was conducted on 15th of July after tomato plants were removed, in order to establish a base population density for each zone and before treatment application. After a deep plow, the greenhouse was irrigated until saturation point. Seven days later, on 22nd of July 2003, a 10 cm plow was conducted and the greenhouse was treated with 3 different control methods. The zone P1 represented the control area where no treatment was conducted. The zone P2 was treated with methyl bromide, zones P3 and P4 were solarized for 39 days using two types of polyethylene, thermal (P3) and regular (P4). Soil thermometers were placed at 25 cm beneath the soil surface for daily temperature monitoring at 2 pm. In addition to the normal thermometers, under the regular plastic polyethylene, a temperature logger (COX tracer recorder) was provided by the MASTERPAK Company on 19th of August. This device was programmed to take temperature readings every hour, 24 hours a day at 35 cm depth, 0 cm and outside, under the regular plastic polyethylene. Under the same environmental conditions, another temperature logger with the same characteristics was put under the thermal polyethylene 50 meters away from the experiment site.

After soil treatment and 39 days later, samples, from each zone, were taken for post treatment nematode extraction and identification.

Sampling method was done using the W method (Geagea, 2003). Ten samples were taken from the intended plant root zone, because nematode population may vary greatly from one core to another specially that of *Meloidogyne incognita* which occur in aggregates. The upper 5 cm of soil were first removed, and then samples were taken from a depth up to 35 cm using soil augers and shovels (Barker *et al.,* 1986; Hafez, 1997).

Sampling was done three times:

a) Before the application of the three treatments and in order to determine the initial nematode population (pop_i).

b) Just after removing the plastic polyethylene and before cucumber plantation. The goal of this sampling was to evaluate the post-treatment nematode population (pop_p).

c) By the end of the season and after the removal of cucumber plants, a third and final sampling was undertaken and has aimed to determine the final nematode population (pop_f).

Nematodes were extracted using the sieving method and Baermann funnel. This combined method is the most appropriate, rapid and simplest method for extracting stage male *Meloidogyne incognita* from different kinds of soil (Kotcon *et al.*, 1987; Najm, 2002).

After 65 days (November 9th), an area of 4,5 m^2 (1,5 x 3) in each zone with 20 cucumber plants was selected for yield inspection in the middle of each zone in order to avoid border effect. According to this layout, yield was monitored every day for 49 days (December 28th). After 114 days from plantation, cucumber plants were harvested and final sampling was conducted. In addition, root samples were taken for galls indexing. Root galls were counted individually and were indexed on a 0 to 6 scale (0= no galls; 1 = 1 to 10%, mild; 2 = 11 to 20%, moderate; 3 = 21 to 50%, severe; 4 = 51 to 80%, severe; 5 = 81 to 90%, highly severe and 6 = 91 to 100%, highly severe) (Barker *et al.,* 1986).

Data generated from the different analysis methods were treated by SPSS® (version 10) for windows. Data from yield, nematode population and temperature were analyzed using the one way ANOVA, post hoc, Bonnferroni method. Data obtained from the temperature logger were tested for correlation and regression. One sample T-Test was used to show the homogeneity between soil textures, pH, EC.

RESULTS AND DISCUSSION

The soil characteristics, revealed in the analysis undertaken, are favorable for nematode infestation as density and damage of *Meloidogyne incognita* are great in coarse-textured sandy soils as stated by Van Gundy (1985) and Starr *et al.* (1993).

The temperature logger, placed under the regular polyethylene from 19 to 28 of August, was programmed to take temperature readings each hour from the surface of the soil (0 cm), 35 cm deep and the outside temperature, during 24 hours a day. This was done to study the variability of temperature under the regular plastic polyethylene. Similarities between curves of measured temperatures were noticed. Therefore, data collected on 23rd of August was chosen to show variability of measured temperatures at different levels under regular polyethylene as presented in figure 1.

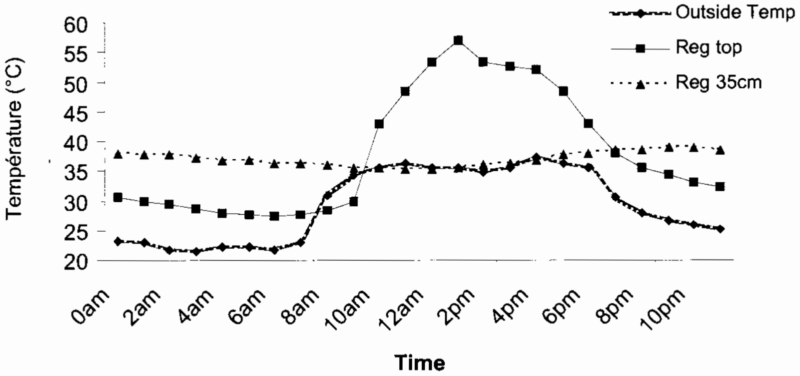

Figure 1. Temperatures recorded by the temperature logger on 23rd of August for 24 hours under the regular polyethylene at 0 cm, 35 cm and outside temperature.

According to Pearson's correlation, there was a correlation between the outside and 35 cm temperatures measured at p = 0,01 and p = 0,05. However at 35 cm, the values were negatively correlated. In fact at 12 pm, outside and at 0 cm temperatures were at their maximum (35,6 °C and 53,4 °C respectively), however at 35 cm, temperatures were at their minimum (35,2 °C), while maximum temperature recorded at 35 cm was at 10 pm (39 °C). This is certainly due to the conductivity of the soil and the characteristics of the polyethylene to preserve heat and assure its conductivity as deep as possible.

As for the soil thermometers placed at 25 cm beneath the non covered soil (T1), the thermal plastic polyethylene (T3) and the regular plastic polyethylene (T4) showed that temperatures measured at 25 cm beneath the non covered soil was normal during the 39 days of recording and did not reach even 31 °C.

Temperature under regular polyethylene reached 41 °C in some days especially by the end of the solarisation period and was 6 to 10 °C higher than temperature under non covered soil.

Data obtained from the temperature logger placed under the thermal polyethylene showed that, at 35 cm, preservation of heat was better and more stable under the thermal polyethylene (Fig. 2). A temperature of 39 °C was recorded for many successive hours extending from 2 pm and up to 6 pm. After 6 pm, temperature started decreasing and just for a short period and then it stabilized and stayed high (around 39°C for 6 hours) which is not the case under regular polyethylene where temperatures remained around 39°C for only 2 hours. This preservation of heat is due to the composition of the thermal polyethylene and to the fact that thermal polyethylene treatment efficiency varies according to the ambient condition during solarisation period (Chellemi, 2000).

Thermal polyethylene did not entail an increasing in temperature higher than the one under regular polyethylene. In fact, maximum temperature recorded was 40°C.

Though the temperature under regular polyethylene was 0,5-1°C higher than thermal polyethylene, but thermal polyethylene preserves heat for longer period and this preservation compensates the higher temperature recorded under the regular polyethylene.

On the other hand and in both cases, nematodes could not survive for long periods at temperature above 40°C (Heald and Robinson, 1987) or at temperatures around 40°C for less than 1 hour to 25 hours (Gowen, 2003).

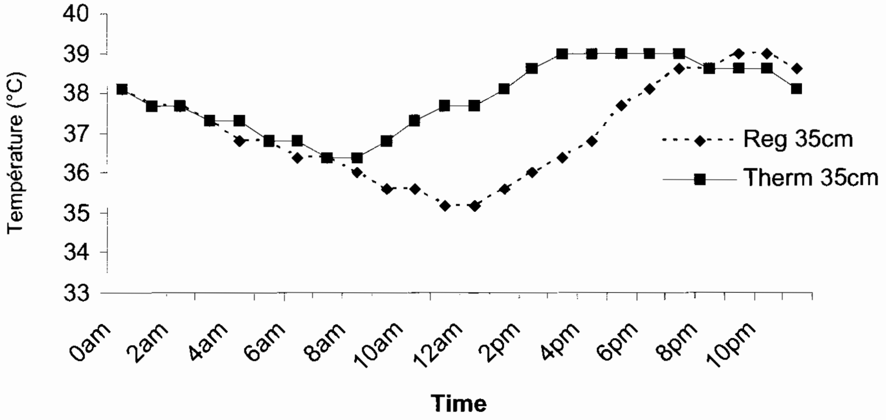

Figure 2. Variability of temperature at 35 cm depth under the regular and thermal polyethylene.

The pretreatment identification revealed that the basic population distribution pattern, in each area, was random, with no significant difference (p = 0,05) between the basic populations in the four plots.

After removal of polyethylene, a second sampling (pop_P) was done. Results showed that all phytopathogenic nematodes were suppressed and no significant difference was observed between the four treatments or between the ten samples of each treatment at p = 0,01. Although no treatment was applied in T1, number of nematodes was very low as the soil was not irrigated for almost 40 days leading to a decrease in humidity. This decrease in humidity might explain the low number of nematodes, as nematode numbers decrease with decreasing humidity according to Savin *et al.* (2001).

T2 led to the suppress of nematodes as methyl bromide has killed all nematodes and microorganisms (Lembright, 1990).

Regarding T3 and T4 which consisted in ocntrolling soil by using regular and thermal polyethylene, the suppression of nematodes could be explained by the increase of soil temperature, where temperature around 40°C is considered lethal for nematodes as mentioned by Gowen (2003). Similar results were found by Mcsorley and Mcgovern (2000) who stated that immediately after solarisation, numbers of all plant parasitic nematodes can be suppressed.

The increase of *Meloidogyne incognita* population, in the four plots, from post treatment population until the time of harvest could be explained by the vertical migration of the nematodes from deeper to upper soil layers in the presence of the host plant (Sorribas and Verdejo-Lucas, 1994), especially that cucumber plants were not resistant varieties and cucumber is known to be a host plant for nematodes (Agrios, 1997).

Comparison between pop_i, pop_P and pop_f of the four treatments was made to evaluate their efficiency on *Meloidogyne incognita* (Fig. 3, 4, 5, 6).

In T1, final nematode population increased because no treatment was applied in this area.

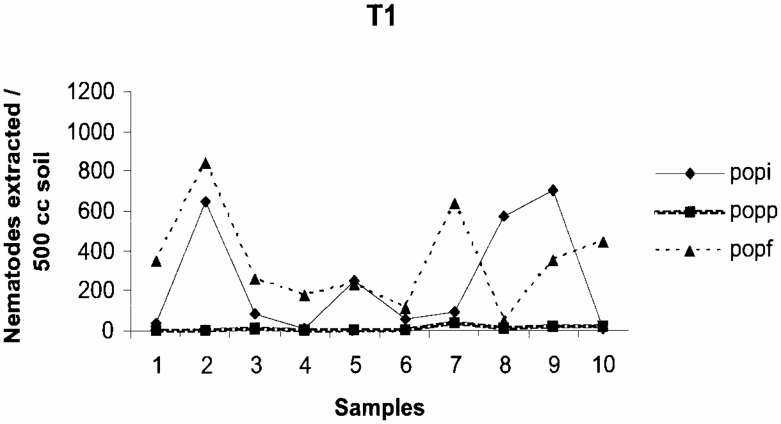

Figure 3. Comparison between the basic population, post treatment population and final population of the control plot.

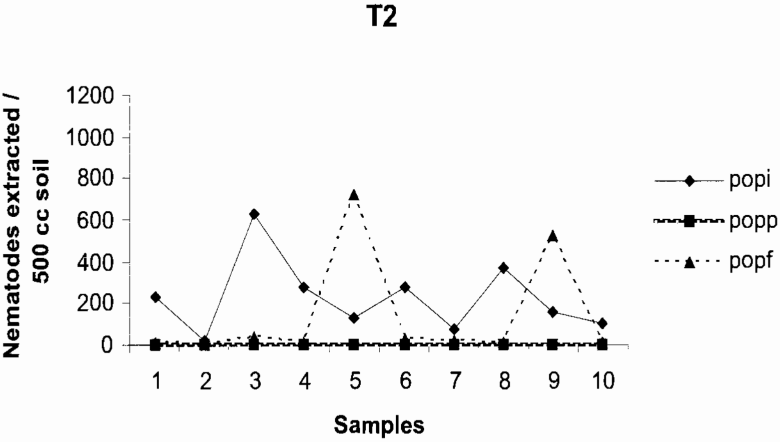

Figure 4. Comparison between the basic population, post treatment population and final population of the methyl bromide treated plot.

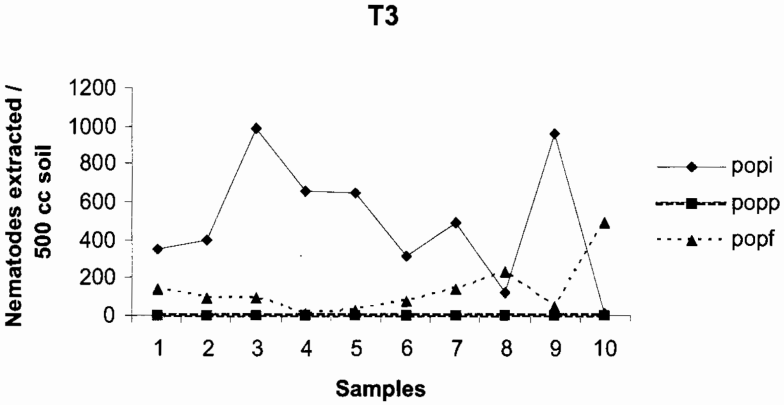

Figure 5. Comparison between the basic population, post treatment population and final population of the regular polyethylene treated plot.

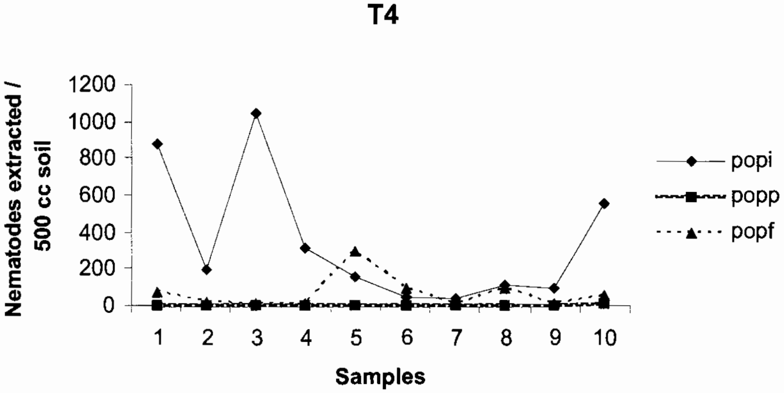

Figure 6. Comparison between the basic population, post treatment population and final population of the thermal polyethylene treated plot.

In T2, final nematode population has decreased, but there was no homogeneity between the final ten population samples (p>0,05), and some final popu-

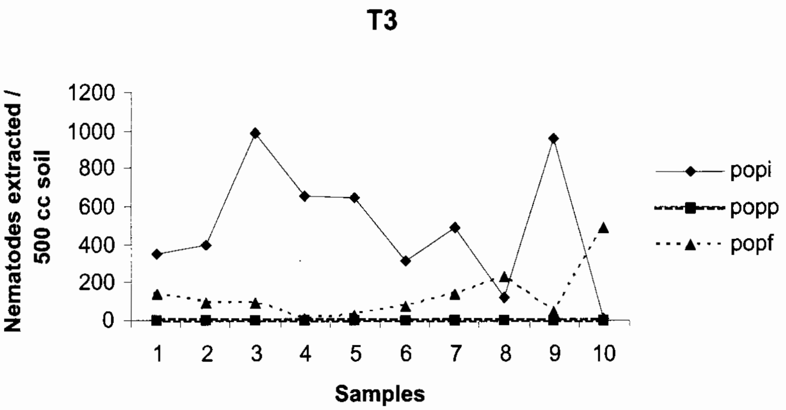

Figure 5. Comparison between the basic population, post treatment population and final population of the regular polyethylene treated plot.

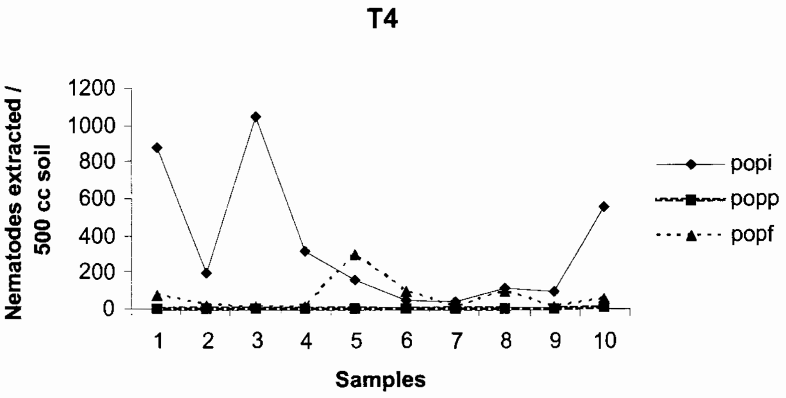

Figure 6. Comparison between the basic population, post treatment population and final population of the thermal polyethylene treated plot.

In T2, final nematode population has decreased, but there was no homogeneity between the final ten population samples (p>0,05), and some final population samples taken from T2 which contained more *Meloidogyne incognita* than the initial population.

In T3, number of *Meloidogyne incognita* has decreased with a significant difference between the initial and the final population ($p<0,05$) and a homogenous decrease of nematodes between the ten samples of the final population ($p<0,05$).

In T4, there was a significant difference between the initial and the final population ($p = 0,05$). In fact, a decrease in final population was noticed when compared with initial nematode populations, these results indicate the influence of high temperature on nematode population.

When comparing the final nematode population between T2, T3 and T4, the plot the lowest population of *Meloidogyne incognita* at harvest was the one treated with methyl bromide. However, there was no homogeneity between populations of *Meloidogyne incognita*, this is due to the bad distribution of soil aggregates, air spaces as well as the difficult way of application reducing thus, the efficiency of methyl bromide (Lembright, 1990).

Yield in T1 or the control area was relatively low (5,2 Kg) (Fig. 7) when compared with other treatments. This significant difference in visual observation was confirmed by One way-ANOVA statistical analysis at $p=0,05$. While comparing total yields between T2, T3 and T4, no significant difference was noted ($p=0,05$).

Total yield for T2, T3 and T4 were respectively 13,2, 13,6 and 11,85 Kg. This positive effect on yield could be due to decreasing in nematode population as in the case of methyl bromide (UNDP, 2001), but also to the revival of beneficial microorganisms as in the case of soil solarisation, with regular or thermal polyethylene (Mcbride, 1999). The increase in yield due to soil solarisation was also observed by Heald and Robinson (1987) while evaluating the effect of soil solarisation on lettuce and cowpeas. In parallel and despite having the lowest population of *Meloidogyne incognita*, yield in the methyl bromide treated plot was slightly lower than the thermal polyethylene treated area because the elimination of soil beneficial microorganisms led to a less balanced soil (Mcbride, 1999).

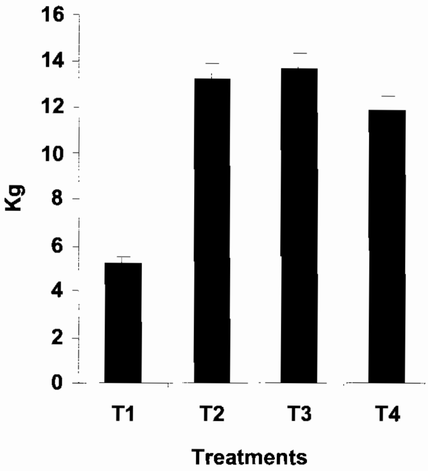

Figure 7. Total yield (Kg) of cucumber obtained in the four treatments.
Each histogram represents the sum of 20 tagged plants yield per treatment for 49 days.

A cost effective analysis was made between cost, yield and profit of each treatment, and that in order to reveal the treatment with the highest income to the farmer. To do this, the harvested yield generated from the 20 plants within 4,5 m^2 was generalized to represent the approximate yield value in 100 m^2. Labor, water, electricity and other fertilization costs were not included in the cost analysis; these parameters are considered equal for all treatments.

Figure 8 shows that income of thermal polyethylene (same for the regular polyethylene) was approximately two times higher than the income concerning control area. However, income of methyl bromide treated area was approximately 1,5 times lower than the control area and 3 times lower then the polyethylene treated area because of the product price.

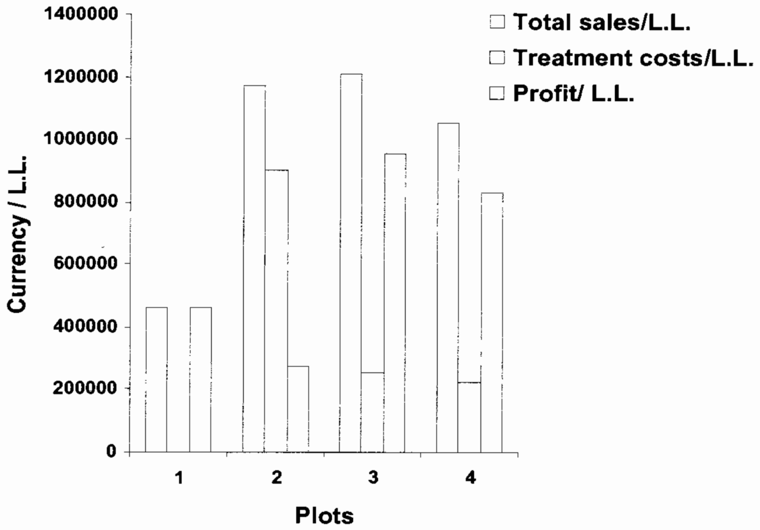

Figure 8. Cost effective analysis of the three treatments and the control plot.

CONCLUSIONS

The experiment was completed in order to help farmers choose new control methods used as alternatives to methyl bromide and to understand the effect of these treatment methods by comparing with the methyl bromide treatment on root-knot nematodes which is the major soil borne pest in coastal areas in Lebanon.

Sampling was done three times: before the application of the three treatments and in order to determine the initial nematode population, just after removing the plastic polyethylene, before cucumber plantation for evaluating post treatment nematode and by the end of the season, after the removal of cucumber plants, a third and final sampling was undertaken and has aimed to assess the final nematode population.

Initial nematode population was similar in the 4 plots and nematode counted were around 328 nematodes per 500 cc of soil. Thirty nine days after application of T2, T3 and T4, nematodes were suppressed (5-15 nematodes per 500 cc of soil). The decrease of nematode population in the control plot T1 is due to the decrease of humidity and to the migration of nematodes to deeper soil layers where humidity is higher (Savin *et al.*, 2001). After 114 days, an increase of *Meloidogyne incognita* final population was noticed in all plots. The increase of *Meloidogyne incognita* population, in the four plots, from post treatment population until the time of harvest could be explained by the vertical migration of the nematodes from deeper (deeper than 35 cm) to upper soil layers in the presence of the host plant and the recovery of nematodes who survived the treatments (Sorribas and Verdejo-Lucas, 1994).

In parallel, methyl bromide led also to the decrease of nematode population but this decrease was not uniform where some samples from the final identification contained more nematodes than the initial identification, this indicated that methyl bromide was not uniformly distributed through soil particles.

Soil solarisation using thermal polyethylene had the highest yield, where maximum reached temperatures were maintained longer and more stable then the regular polyethylene. This increase in yield by solarisation is due to the decomposition of the organic matter by beneficial microorganisms present in the soil which are eliminated by the use of methyl bromide.

The study being a part of the broader project undertaken by UNDP "Alternatives to Methyl Bromide" has proven that soil solarisation deserves serious consideration as a fundamental component of nematode management and that it could be recommended to farmers. It allowed us to understand the efficiency of soil solarisation on root knot nematodes, and because nematodes can not be suppressed totally, it is important that soil solarisation should be contributed with other IPM programs to maintain the nematode population as low as possible. Other studies should be done as continuity to this research to know lethal temperatures for *Meloidogyne incognita* in the laboratory, as well as more root samples has to be taken for galls indexing.

On the other hand, data collected during this research from plantation to harvest time as well as yield weighed and cost analysis showed satisfactory results concerning the area treated with the thermal polyethylene. The thermal polyethylene solarised plot showed an increase in yield, less management cost, easy to apply and it is non toxic.

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