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PROFESSIONAL UNIT FOR PLANT PROTICTION DEPARTEMENT OF PLANT PROTECTION FACULTY OF AGRICULTURE- DAMASCUS UNIVERSITY



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DEMONSTRATION PROJECT ON ALTERNATIVES TO THE USE OF METHYL BROMIDE IN AGRICULTURE

(Second Report)

Introduction

Since the Montreal Protocol was established to protect the earth's fragile zone layer. Phase-out schedules have been established for chemical that destroys ozone molecules as CFCs, halone and, more recently, methyl bromide.

Methyl bromide is an agricultural fumigant, which is used to control pests in soil, commodities and structures. Under the Montreal Protocol it is due to be phased out by industrialized countries by 2005 and in developing countries by 2015.

Syrian government banned the use of Methyl bromide as a soil fumigant few years ago, since then many farmers are looking for alternatives for this material, even though many are using it illegally.

This project, which have been established as a cooperation between the UNIDO, Ozone unite (Syrian Ministry of Environment), and PUPP (Faculty of Agriculture, Damascus university), aimed to test and demonstrate different alternative method for Methyl Bromide. The farmers in the areas of these demonstrations will also learn about the new techniques to be adapted for the new methods.

Two locations were picked up to carry out this project. The first one in Zabadani 45 km west of Damascus, an area of cut flower production (Carnation). The second one in Baniyas one the Mediterranean cost 300-km northwest of Damascus, an area of tomato and cucumber production under tunnels.

This report will deal with the final results of the Zabadani Experiments and the first part of the results in the Baniyas area experiment, which have been collected by the time of writing this report as agreed on in the contract signed by the different parties.

1-The Zabadani Experiment

The design of this experiment and the finding of the first set of samples were discussed in detail in the first report. This report will deal only with the result of the final samples and the plant vigor data.

Results

A complete set of samples were collected from the Zabadani site on Jan. 1st 2000 for laboratory study to determine the population and presence of weed, bacteria, fungi, nematodes and arthropod in the samples at the end of the experiment. Plant vigor through out the experiments was measured in addition to the number of buds and flowers opened; comparisons were made between the different treatments.

A-Weed study result:

The results from the germination of weed seeds in soil samples showed no significant differences between the four different treatments plots at the end of the experiment even though the steam treatment had the lowest observed number of germinating seeds. In the other hand there was no differences between the three different depths (Tables 1-1&1-2). The weed species were the same as mentioned in the first report.

Treatments	Mean number of germin	ating weed seeds/m ²
O- control	46.33	a
A- Metam- Na	31	a
B- Methyl bromide	38.17	а
C- Steam	16.5	a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-1- Mean number of germinating weed seeds in the four different treatments.

Depth	Mean number of germinating weed seeds/m ²
0-20 cm	32.44 a
20-40 cm	32.69 a -
40-60 cm	33.88 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-2- Mean number of germinating weed seeds at the three different depths.

B- Nematodes study result

Data showed that the steam treatment have the lowest number of plant parasitic nematodes at the end of the experiment while the Metam-Na had the highest number of free living nematodes which suggest that there is some enhancements to this kind of nematode in the treatment (Table 1-3). The top layer of soil have the highest number of nematodes except the plant parasitic which present equally in all layers (Table 1-4).

Treatments	Fungi Feeding Nematodes	Plant Parasitic Nematodes	Free living Nematodes	Total Number of nematodes
O- control	11.42 a	15.92 a	35.83 b	63.17 ab
A- Metam- Na	12.42 a	15.58 a	64.08 a	92.25 a
B- Methyl bromide	12.33 a	16.75 a	34.83 b	63.92 ab
C- Steam	11.42 a	5.33 b	36 b	52.75 b

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-3- Mean number of nematodes at four different soil treatments.

Depth	Fungi Feeding Nematodes	Plant Parasitic Nematodes	Free living Nematodes	Total Number of nematodes
0-20 cm	19.56 a	14.25 a	64.5 a	98.31 a
20-40 cm	8.44 b	15.81 a	26.63 b	50.13 b
40-60 cm	7.69 b	10.13 a	36.94 b	55.63 b

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-4- Mean number of nematodes at the three different soil depths.

The genera of nematode present at the end of the experiment are listed in Table 1-5. Ten genera of plant parasitic nematode were present in addition to three fungi feeding and eleven free-living genera.

Plant Parasitic Nematodes	Plant & Fungi Feeding Nematodes	Free Living Nematodes
Trichodorus Pratylenchus Tylencorhynchus Helincotylenchus Tylenchus Ditylenchus Meloidogyne Xiphenima Macroposthia	Aphelenchus Aphelenchoides Paraseinura	Chiloplacus Mononchus Pelodera Panagrolainus Eudorylaimus Eucephalobus Plectus Rhabditis Acrobeles Cephalobus Dorylaimus

Table 1-5- Nematode genera found in the carnation root zone at the Zabadani experiment site.

C- Bacteria study results

Bacterial count study showed the same genera as mentioned in the first report. The MB treatment has significantly lower number of bacterial count compared to the control, while there was no significant differences between the treatments (Table 1-6). The bacterial count between the three depths showed no significant differences at the level of %5 (Table 1-7).

Treatments	Mean of bacterial count/1 gram dry soil
O- control	5.13 X 10 ⁷ a
A- Metam- Na	3.91×10^7 ab
B- Methyl bromide	3.13×10^7 b
C- Steam	4.04×10^7 ab

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-6 Mean of bacterial count /1 gram dry soil at the four different treatments

Depth Me	an of bacterial count/1 gram dry soil
0-20 cm	4.19×10^7 a
20-40 cm	4.31×10^7 a
40-60 cm	3.65×10^7 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-6 Mean of bacterial count /1 gram dry soil at the three different depths.

D- Fungi study results

Table 1-7 shows that the MB treatment has lower number of spore count at the end of the experiment compared to the steam and control treatments. In addition there was no significant differences between the MB and Metam-Na in one hand and the metam and steam in the other hand.

Treatments	Mean of fungi spores /1	gram dry soil
O- control	34413.42	a
A- Metam- Na	23331.58	bc
B- Methyl bromide	15103.67	с
C- Steam	25914.92	ab

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-7 Mean number of fungi spores / 1 gram of soil at the four different treatments

The top layer of soil has the highest number of fungi spores compared to the other layers (table 1-8). The genera of the fungi were the same as mentioned in the first report.

Depth	Mean of fungi spores/1 gram dry soil
0-20 cm	36618.38 a
20-40 cm	22497.5 b
40-60 cm	14956.81 b

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-8 Mean number of fungi spores /1 gram dry soil at the three different depths.

E- Arthropods study results

Results show that there are no significant differences between the treatments or the depths concerning the number of Arthropods (Tables 1-9 &1-10).

Treatments	Mean number of Arthropods in 500 g. of
O- control	3.167 a
A- Metam- Na	1.5 a
B- Methyl bromide	1.92 a
C- Steam	1.25 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-9 Mean number of Arthropods in 500 gram of soil at the four different treatments

Depth	Mean number of Arthropods in 500 g. of soil
0-20 cm	2.81 a
20-40 cm	1.75 a
40-60 cm	1.31 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 1-10 Mean number of Arthropods in 500 gram of soil at three different depths.

F- Plant vigor

Twenty-five plants were chosen from each plot to take measurements through out the experiment. The means from these data were calculated and plotted in the following graphs. Statistical analysis using LSD at level of %5 showed no significant differences between the four different treatments for all vigor measurements.

Figure 1-1 shows the maximum highest reached for the carnation plants before the harvest. The MB and the Metam-Na were superior compared to the other treatments. Even though there was no statistical differences observed.



Figure 1-1. Mean of maximum highest reached before harvest for carnation plants before harvest.

The average of plant height was also calculated and figures 1-2 shows the mean at the four different treatments. The steam treatment was observed to be the best even though there was no statistical differences between the four treatments.



Figure 1-2. Average height of carnation plants at four different soil treatments.

The mean number of buds and opened flower were observed to be the highest at the Metam-Na treatment (Figures 1-3 & 1-4). In spite of the fact that there is no statistical differences between the treatments.



Figure 1-3. Average number of buds on carnation plants at four different soil treatments.



Figure 1-4. Total opened flower in carnation plants at four different soil treatments.

2- The Baniyas Experiment

Experimental design

The experiment conducted in four plastic tunnels of 400 m² (a total 0f 1.6 Syrian Dunums), each tunnel with five growing plots each one had two rows with100 plants per row. The experimental design consisted of four completely randomized blocks, With 5 treatments and one replicate per tunnel. Each plot had an area of 60 m² (1.2 x 50 m). The tunnels planted with one variety of tomatoes (Karam) on the second week of September 1999 (Sept. 9th 1999 for the 1st and 2nd tunnels and Sept. 20th 1999 for the 3ed and 4th tunnels). Samples of soils were taken randomly from the four tunnels and analyzed to get the physical and chemical properties of the soil before treating it.

Treatments

The treatments were applied during the third week of August after the preparation of the soil. Samples were taken on July 7th 1999 for analysis. The treatments were as follows:

1

O. Control

A- MB (57 g/m^2)

One can of 680g MB for each 12 m^2 of soil surfaces under plastic covers.

B-Dazomet 98% GR + Solarization

Application ratio of 2.4 kg/plot then covered with plastic and solarized.

C-Organic matter + Solarization

5 kg of half fermented cow manure per m^2

D-Soilless cultivation

Plants were placed in plastic bags (30 cm x 2 m) filled with volcanic stones and compost (1:1). Irrigated with liquid nutrient solution.

Experiment layout

The experiment layouts in the tunnels are shown in the following figure.







Figure 2-1- Layout of the experiment.

Soil analysis before the trial

Soil samples were taken from experiment plots to determine the soil fertility on July 19th 1999. The soil was collected from 25 randomly selected spots per tunnel from three different depths (0-20 cm, 20-40 cm and 40-60 cm). Soil from each depth was mixed and an over all sample from each depth in each tunnel was collected. The samples were filled in plastic bags and transported to the lab where they were air dried and analyzed. The results of soil analysis showed that the soil is vary according to the depth and tunnel with high percentage of organic matter compared to the soils in the area (due to the addition of organic matter by the farmer). The percentage of calcium carbonate was low (2-10%). pH of the soil was neutral. Electrical conductivity was normal. The detailed results listed in the following table:

Sample	Depth	pH	EC 1:5	Active Lime	CaCo ³ O.M. T.			% G.A.		
	cm	1;2.5	µmho/cm	%	%	%	%		0.00	C1
								Sand	Silt %	Clay <u>%</u>
	0-20	7.1	375	1.10	5.2	3.9	0.156	37.5	37.5	25.0
	20-40	7.4	300	1.00	5.2	3.1	0.124	42.5	35.0	22.5
	40-60	7.4	280	1.85	7.8	3.2	0.127	32.5	20.0	47.5
	0-20	7.2	290	0.93	5.2	3.8	0.152	40.0	22.0	37.5
	20-40	6.9	300	1.51	6.5	3.3	0.132	37.5	20.0	42.5
	40-60	7.3	350	1.92	7.8	3.5	0.140	50.0	12.5	37.5
	0-20	7.3	240	0.50	2.6	5.5	0.220	47.5	17.5	35.0
	20-40	7.2	255	0.45	2.6	4.9	0.146	47.5	18.75	33.7
	40-60	7.1	210	1.87	7.8	3.9	0.156	37.5	17.5	45.0
	0-20	7.0	300	2.07	10.4	5.9	0.236	65.0	12.5	22.5
	20-40	7.2	250	1.80	7.8	4.3	0.172	50.0	20.0	30.0
	40-60	7.1	240	2.13	9.1	4.1	0.164	52.5	17.5	30.0

Table 2-1- Results of soil analysis in the Baniyas experiment's site.

RESULTS

In addition to the sample collected in July 19th 1999 for the soil analysis another set was also collected for laboratory study to determine the presence and population of Weed, Bacteria, Fungi, Nematodes and Arthropods in the samples before the treatments. Another set of samples was taken after 17 day from the day of treatment in early September. One total sample was taken from each plot and each one consisted of 10 randomized sub samples. The samples were also analyzed in the lab for the same categories as mentioned earlier. Results for each category are shown in the following paragraphs.

A-Weed study result:

Four broad leaf weed seeds and one grass species were found in the soil from the four tunnels, which were collected before the treatments and form the seed germination trails in the lab. These species are listed in the following table:

Broad leaves	Grasses
Amaranthus retroflexus L. Chenopodium album L. Oxalis sp. Sonchus oleraceus L.	Setaria glauca L.

Table 2-2- Weed species found in the Baniyas experiment site.

The results from the laboratory germination of weed seeds in soil samples showed high weed control in the Dazomet treatment, compared to the control (Table 2-3 & Figure 2-2). While there were no significant differences shown between the Dazomet treatment and the MB & Solarization treatments. This suggests that the Dazomet treatment is the best one on weed seeds. The detailed results are shown in Table 2-3 & Figure 2-2.

Treatment	Means of germinating weed seeds/m ²	
Before Treatments	68.16	
O – Control	109.83 a	
A – MB	35.25 ab	
B-Dazomet + Solarization	23.58 b	1
C – Solarization + organic matter	77.5 ab	

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-3- Mean number of germinating weed seeds in the four different treatments.





The total number of weed seeds in the upper layer of soil in the different treatments were significantly different at the level of 5% (table 2-4 and Figure 2-3).

Depth	Mean number of weed seeds/m2
0-20	137.125 a
20-40	28.75 b
40-60	18.75 b

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-4- Mean number of germinating weed seeds in the three different depths.





B-Nematodes study result

Twenty-one genera of nematodes were found in the soil surrounding the tomato roots. Samples were taken from the field and transported to the lab in a cooler. 50 gram of soil from each sample (one total sample from each plot) placed in a Berman funnel for 24 hours. Nematodes collected were fixed in TAF solution, then identified using light microscope. The nematode genera were divided to

three groups according to their feeding habitat. The following table shows the genera collected during the experiment.

Plant Parasitic Nematodes	Plant & Fungi Feeding Nematodes	Free Living Nematodes
		Chiloplacus
Pratylenchus	Aphelenchus	Mononchus
Tylencorhynchus	Aphelenchoides	Pelodera
Helincotylenchus	Paraseinura	Panagrolainus
Tylenchus		Eudorylaimus
Ditylenchus		Eucephalobus
Meloidogyne		Plectus
		Rhábditis
		Acrobeles
		Cephalobus 🕔
		Dorylaimus

Table 2-4- Nematode genera found in the Tomato root zone at the Baniyas experiment site.

Treatments	Fungi Feeding Nematodes	Plant Parasitic Nematodes	Free living Nematodes	Total Number of Nematodes
Before Treatments	3.83	18.91	40.17	72.83
O – Control	77.75 a	172.33 a	603 a	878.08 a
A – MB	3.5 b	7.75 b	16 a	22.25 b
B- Dazomet	15.5 b	16 b	261.75 a	297.5 ab
C – Solarization +	17.83 ab	123.67 a	472 a	625.08 ab
organic matter				

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-5- Mean number of plant parasitic, fungi feeding free living and total number of nematode in the Baniyas site.

Treatments	Fungi Feeding	Plant Parasitic	Free living	Total Number	
0-20 cm depth	54.06 a	93.44 a	761.75 a	940.63 a	200 ja 19
20-40 cm depth	29.68 a	97.56 a	194.56 b	321.5 ab	,
40-60 cm depth	2.18 a	48.81 a	58.25 b	108.81 b	

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-6- Mean number of plant parasitic, fungi feeding, free living and total number of nematode at three different depths in the Baniyas site.

Results showed that there is no significant difference between the treatments at the level of 5% (LSD) comparing the means of free living nematodes (Table 2-5 & Figure 2-5). The plant parasitic nematodes were significantly higher in the control and the Solarization treatments compared to the other treatments but there was no significant difference between the MB and Dazomet treatments.

While there were significant differences between the control and the MB treatment for the fungi feeding nematodes but no differences between the MB and the other treatments which was the case for the total number of nematodes (Table 2-5 & Figure 2-4). The means number of free living nematode in all treatments were significantly higher at the top soil compared to the other depths while for the total number of nematodes the only significant number was between the top soil and the 40-60 cm depth (Table 2-5 & Figure 2-3). These results suggest that the MB procedure was equal in effectiveness to the Dazomet treatment for the plant parasitic nematodes. While the Solarization treatment was less effective and did not damage the free living nematode which give a very beneficial effect on the soil fauna and keeping down the disease incidents.



Figure 2-4- Total number of nematodes/50 gram soil after treatments



Figure 2-5- Numbers of nematodes/50 gram soil after treatments at three different depths.

C-Bacteria study results:

1- Bacterial counts:

Total bacterial counts were performed using LPGA media. Three Petri dishes were used for each dilution. Table 2-7 & Figure 2-6 shows the mean of the four replicates for each of the four treatments and the control:

Treatment	Means of bacteria count/1 gram dry soil
Before Treatments	3133333.33
O – Control	23583333.33 ab
A – MB	21000000 b
B- Dazomet	38666666.67 a
C-Solarization +organic matter	29400000 ab

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).





Figure 2-6- Mean soil bacterial count /1 gram of soil in the four different treatments.

Depth	Means of bacteria count/1 gram dry soil
0-20	45906250 a
20-40	24125000 b
40-60	14456250 b

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-8- Bacterial counts for the Baniyas experiment at the three soil depths.



Figure 2-7- Mean soil bacterial count /1 gram of soil at three different depths.

The results of the bacterial count showed significant differences between the MB and the Dazomet treatment, but there was no significant difference with the control and the solarization treatments. Probably due to the high deviation of the data, large numbers and the need for a data transformation in order to get any significant results. The mean bacterial count on the upper soil layer showed a significant difference compared to the other two layers which is normal for agriculture soils.

2-Bacteria identification:

31 isolates were chosen from the colonies grown in the total count experiment. The isolates were chosen according to its color, shape, diameter, growth rate, and other characters. These isolate were picked up for the following tests:

1-Gram pigment test: 12 of the isolate were gram-positive and 19 were gram-negative.

A-The gram positive isolates belongs to the following genera: *Bacillus*

Nocardia

Streptomyces

b- The gram negative isolates belongs to the following genera: *Enterobacterie*

Pautoea

2-Respiration test: this test was performed using Heigh & Leifson media, to identify Entrobacter and Non Entrobacter bacterial groups.

3-Oxidation test: Fifteen isolates were found to be positive to this test.

4-Identification of bacteria: The method of API 20 was used utilizing API ZONE test kit, to identify Non Entrobacter bacteria.

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The following table shows the results of these tests:

	Bacterial species	Number of isolate
1	Flavimonas oryzihabitans	2
2	Pasteurella haemolytica	1
3	Chrysomonas indologens	2
4	Aeromonas salmonicida	1
5	Pseudomonas alcaligens	1
6	Burkholdecia apacia	2
7	Chrysomonas luteola	4
8	Isolates to be identified later	9

D-Fungi study results:

1-Fungi spores count in soil:

The soil samples were air-dried and mixed thoroughly. 5-gram soil sub-sample from each replicate was mixed with 50-ml sterile water. The solution was diluted three time taking 5 ml from the original dilution and mixes it with 45 ml of sterile water. One ml of the final solution was placed on a PDA+ antibiotic media in petri dish. Three replicates were performed for each soil sample. The fungi colonies on the media were counted and identified. Table 2-7 & Figure 2-8 shows the results of colonies count which represented as fungi spores in 1 gram of soil.

Treatment	Means of Fungi spores in 1 gram of soil
Before Treatments	· · · ·
O – Control	52858.33 a
A – MB	4433.33 b
B- Dazomet	13900 b
C- Solarization +organic matter	61375 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-9- Mean number of fungi spores in the different treatments at the Baniyas site.





Depth	Means of Fungi spores soil	s in 1 gram of
0-20 cm	37543.75	a
20-40 cm	33043.75	a
40-60cm	28837	a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-10- Mean number of fungi spores at the three different depths at the Baniyas site.



Figure 2-9- Mean number of fungi spores /1 gram of soil in the three different depths.

The results pointed out that MB and Dazomet treatments were equally effective in reducing the number of fungi spores compared to the solarization and the control treatments (Table 2-9 & Figure 2-8). In the other hand there was no significant differences between the mean number of spores at the three different soil depths (Table 2-10 & Figure 2-9).

2- Fungi identification:

All the fungi colonies from the spore count experiment were identified. Five of these fungi found out to be saprophyte (*Rhizopus, Aspergillus, Penicillium, Gliocladium*, and *Cladosporium*). Three of them wee found to be plant parasitic nematode (*Rhizoctonia, Alternaria, and Fusarium*). The effectiveness of treatment on each of these fungi could not be determined at this point of the experiment.

E-Arthropods study results:

A 500-gram of soil sub-sample was obtained from each replicate. The samples were placed in Berlese funnels for 48 hours under 40 watt electrical lamp. Arthropods collected in small jars filled with alcohol and a drop of glycerin. The total number of mites and insects in each sample was recorded and the Arthropods were identified to the order. Few insects were found in the soil (Coleoptera, Diptera, Collembola, and Himeptera) but most of the Arthropods found in the samples were Mites (Astigmata, Cryptostigmata, and Mesostigmata). Table 2-11 & Figure 2-9 shows the results of the Arthropods count.

Treatment	Means number of Arthropods / 500 gram soil
Before Treatments	0.4
O – Control	5.25 a
A – MB	1,167 a
B- Dazomet	1.167 a
C-Solarization+organic matter	3.417 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test).

Table 2-11- number of Arthropods found in 500-gram samples at the Baniyas site.



Figure 2-9- Number of Arthropods/500 gram of soil after one month of four different treatments.

Depths	Means number of Arthropods / 500 gram soil
0-20 cm	6.65 a
20-40 cm	0.81 a
40-60 cm	0.875 a

*Numbers followed by the same letter in the same column are not significantly different at level of 5% (LSD test). Table 2-12- number of Arthropods found in 500-gram samples at the three depths.

The result shows that there were no significant differences between the treatments and also between the three depths (Table 2-12). These results are probably due to the low numbers of arthropods in the different blocks.

Damascus, February 25, 2000

This report have been written by Dr. Majd JAMAL In behalf of PUPP Faculty of Agriculture Damascus University Damascus, Syria Fax. 963 11 511 6352