

Efficacy of metam sodium and metam potassium on root-knot nematode and *Fusarium* root rot fungus in cucumber plants in controlled agricultural systems

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Abstract: Effects of metam sodium (Solasan 51% SL, a.i., w/v) [MS] and metam potassium (Tamifume 69% SL, a.i., w/v) [MP], in addition soil treated with essential plant oils such as *Aloe-vera barbadensis* (Aole-vera), *Eucalyptus globules* (Eucalyptus), *Lupinus luteus* (Lupinus) and *Sesamum indicum* (Sesame) on populations of root-knot nematode (*Meloidogyne incognita*), disease incidence (%) of root rot (*Fusarium solani*), total microbial counts of fungi, aerobic bacteria and spore forming bacteria, frequency (%) of common fungi and yield of cucumber plants (*Cucumis sativus* L.) were studied, in controlled agricultural systems, under commercial plastic house conditions after 45 and 90 days of treatment. Two experiments were conducted. An experiment I was designed to estimate the effect of MS and MP, separately. A second experiment was conducted to study the effect of MP only or combined with essential plant oils. Results revealed that all treatments highly reduced the populations of *M. incognita* parameters, than untreated control. In experiment I, MP highly reduced the total final population of nematode, than MS as well as untreated control, where rates of built up reached to 0.035 & 0.065 with MP, 0.068 & 0.080 with MS, compared to 1.130 & 2.620 in untreated control, respectively. In experiment II, soil fumigated with MP plus treated essential plant oils of Aole-vera, Eucalyptus and Sesame highly reduced the nematode population, than other treatments. Our results also revealed that all treatments highly reduced the incidence (%) of root rot disease, than untreated control. MP highly reduced the disease incidence, than MS in experiment I. In experiment II, MP only, MP + Aole-vera and MP + Eucalyptus highly reduced the disease incidence, than other treatments. Effects of treatments on microbial counts as well as on frequency % of common fungi in rhizospheres of treated cucumber plants as well as untreated control were determined. All treatments increased the yield parameters of cucumber fruits. i.e. averages of fruits per plant and fruit weight per plant in one harvest as well as fruit yield weight per plant in one season (about 12 harvests).

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1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops in Egypt. Increasing productivity and improving fruit quality of cucumber are mainly dependent upon using healthy plants (Sawan *et al.*, 1998). Plant parasitic nematodes and soilborne pathogens also attack a wide range of vegetables reducing its yield quality and quantity (Nchore *et al.*, 2010 and 2011). *Meloidogyne* spp. form disease complex with root rot pathogens causing major losses in vegetable production. Such problem is widely spread in controlled agricultural systems (Bashour *et al.*, 2010 and Kago *et al.*, 2013). Therefore, the most important problem in vegetable tunnel production is managing soilborne pests.

Fumigant has been used against plant-parasitic nematodes with good results since the 1950. They have been used extensively for root-knot nematode and soil borne pathogens control in the production of

many fruit, vegetable and nursery crops (Noling and Becker, 1994). Methyl bromide (MBr) has been the main tool to reduce the incidence of soilborne pests in tunnel production (Santos *et al.*, 2008). MBr as soil fumigant has been banned completely since 1992 in many countries because it is an atmosphere ozone depleting agent (Watson *et al.*, 1992) and several alternatives can be applied to control soilborne pathogens and pests (Runia & Molendijk, 2007). Metam sodium (MS), metam potassium (MP) and dazomet are broad spectrum soil fumigants that can be used to control pathogens and pests affecting a wide array of economically important fruit and vegetable crops.

MS, at rates of 63-702 L/ha in form 2.5 to 14.2 cm water, significantly reduced nematodes in soil before planting as well as root gall ratings at midseason and harvest and increased yield in most cases (Roberts *et al.*, 1988). For *Pythium ultimum* and

Fusarium sp., MS at rates of 187 – 702 L/ha reduced the number of sporangia and propagules per gram of dry soil, respectively (Roberts *et al.*, 1988). MS treatments suppressed different parasitic nematode populations in “Braeharn” apple (*Malus domestica* Borkh) [Fallahi *et al.*, 1998].

A possible alternative replacement to MBr is metam sodium (MS, sodium N- methyl dithiocarbamate) studied by Nelson *et al.* (2004). They reported that the total yield of tomato from MS fumigation was not significantly different than that of MBr. Effect of MBr plus chloropicin (MBr+ Pic 98:2 w/w) at a rate of 500 kg ha⁻¹, emulsifiable concentrate of 1,3 – dichloropropene (1,3-D) plus Pic at 275 L ha⁻¹, emulsifiable concentrate of metam sodium (metam-Na) at 275 L ha⁻¹ and non-treated control on soil borne pest control and Campana hot pepper (*Capsicum frutescens* L.). Among the fumigants, MBr + Pic and 1, 3 –D + Pic had the best performance controlling nematode (Santos *et al.*, 2008). The highest reduction of the second juvenile stage of root-knot nematode of between 42% and 81% and up to 88% reduction were observed in plots treated with metham sodium at 012g/L in rose plants grown under polyethylene covered tunnels (Oloo *et al.*, 2009).

The objective of this study was to; (i) investigate the effect metam sodium and metam potassium in controlling of root-knot nematode and *Fusarium* root rot fungus, (ii) study their effect on total counts of fungi, aerobic bacteria and spore forming bacteria as well as frequency (%) of common and (iii) evaluate the fruits yield response for soil application, in cucumber plants in plastic house conditions.

2. Materials and Methods

I. Fumigants:

Metam sodium [sodium methyl dithiocarbamate (Solasan 51% SL) a.i., w / v (MS)] and metam potassium [potassium methyl dithiocarbamate (Tamifume 69% SL) a.i., w/v (MP)] were applied at recommended dose in seed beds. Fumigant was thoroughly incorporated into the soil at the recommended rate 100 Cm³/m² to a depth of 30 cm. Then, soil was irrigated with water. The plots were covered with clear polyethylene of 0.14 mm thickness. The polyethylene edges were buried 15 cm into the soil to ensure airtight conditions for 15 days.

II. Essential plant oils:

Essential plant oils (EPO) of Aloe- vera (*Aloe-vera barbadensis*), Eucalyptus (*Eucalyptus globulus*), Lupinus (*Lupinus luteus*) and Sesame (*Sesamum indicum*) were applied. EPO-water mixture (2%) was carried out by mixing of 2 ml of EPO to 98 ml of water and then 0.01 ml of Twine 20 % as triton emulsifier was add. One liter of EPO-water mixture

(2%) was added to m² of soil after cucumber seedlings transplanting.

III. Pathological studies:

1. Samples:

In previous season, roots samples of cucumber plants, showing yellowish and wilting symptoms, were obtained from commercial plastic house of Gazzart El-Dahab, Giza Governorate. The samples were kept in polyethylene bags and immediately sent to the Plant Pathology Department, National Research Center (NRC) for pathological studies.

2. Detection of *Meloidogyne incognita*:

Adult females were isolated from galled roots of cucumber plants and identified as *M. incognita* by examination of their cuticular perineal patterns and morphological characteristics according to the methods described by Eisenback (1985).

3. Isolation of *Fusarium solani*:

Isolation of pathogenic fungi from cucumber roots was carried out according to the method described by Raviv *et al.* (2005). Firstly, the roots were washed by tap water and surface sterilized for two min by dipping in 2% sodium hypochlorite solution. Then, the roots were washed several times in sterile distilled water and then it's dried between two filter papers. The dried roots were cut into small pieces (2 cm). The root pieces were placed on the surface of the PDA medium in sterile Petri dishes. The inoculated plates were incubated for 7 days at 28 °C. The growing fungi were identified based on the pathological, morphological and cultural characteristics (Barnett & Hunter, 1972 and Nelson *et al.*, 1983).

IV. Plastic house experiments:

Each plastic house was about 360 m² (9 m width x 40 m length). All treatments were replicated three times in a randomized complete design. Each commercial plastic house consists of 5 rows, each 0.5m width and 40m length. The spaced between seedling bed and other was 0.5 m. The experiment I consists of three plastic houses. The first one was fumigated with MS; the second was fumigated with MP and third was untreated as control. Each experiment divided into three experimental units as replicates. Cucumber seedlings cv. Paracoda were sown at above distances after 15 days of treatment. The experiment II consists of one plastic house. The plastic house was fumigated with metam potassium and then it was divided into five trials. The first one treated with metam potassium only. In experimental trials of 2, 3, 4 and 5 the soil fumigated with MP and treated with EPO of Eucalyptus, Aole-vera, Sesame and Lupinus, separately.

Effects of all applied treatments on the nematode parameters, incidence % of root-rot disease, total counts of fungi, aerobic bacteria and

spore forming bacteria, frequency (%) of common fungi and cucumber fruit yield parameters were studied in experiments I and II as follows:

1-Samples:

1.1. Primary soil samples (before fumigation):

Five samples of soil rhizosphere were separately collected from each commercial plastic house of Gazert El-Dahab, Giza Governorate before fumigants treatment. Soil samples (about 250 g of each sample) were collected from cores 2 cm in diameter X 20 – 30 cm deep (Roberts *et al.*, 1988). The samples were kept in polyethylene bags and immediately sent to the Plant Pathology Department, National Research Center (NRC). The initial counts of nematode juveniles (J_2) in soil and total counts of fungi, aerobic bacteria and spore forming bacteria were determined.

1.2. Samples after sowing:

Ten samples of rhizosphere soil as well as feeder roots were separately collected from cucumber plants from each treated plastic house as well as untreated control. Each soil sample (about 250 g of each sample) was composite of five cores 2 cm in diameter and at the depth of 20-30 cm. Feeder root samples also were collected from the same place of soil samples. Samples were obtained after 45 and 90 days of treatment. The samples were kept in polyethylene bags and immediately sent to the Plant Pathology Department, National Research Center (NRC) for following studies.

2-Effect on *M. incognita*

Observations of *M. incognita* parameters as numbers of juveniles (J_2) in soil and both the developing larva stages (Third stage, L_3 and Fourth stage, L_4), females, egg-masses and galls in roots of treated cucumber plants as well as untreated control were recorded after 45 and 90 days of treatment. The juveniles of nematode were extracted in soil samples of cucumber plants by sieving and decanting methods (Barker 1985). In cucumber roots samples, roots were gently washed free of adhering soil and an aliquot of 5 g per plant was cut into 2-cm- long pieces and put in Petri dishes with clean fresh water and stained by acid fuchsin lactophenol (Franklin and Goodey, 1949). Numbers of L_3 , L_4 , females, egg-masses and galls were estimated in roots 5 g per plant using a binocular microscope (Mai and Lyon, 1975). Rate of build-up was calculated according to the following formulae (Oostenbrink, 1966):

Rate of build up = Total nematode populations in soil and roots (Pf) / Initial population of J_2 at sowing time (Pi).

Where: Pf is number of J_2 in soil and numbers of L_3 , L_4 , females and egg-masses in roots.

3-Effect on *Fusarium root rot incidence %*:

The cucumber roots samples were firstly washed thorough tap water to remove any adhering soil particles. Then, the roots were cut into small pieces (2-cm length) and disinfested by immersing in sodium hypochlorite (2%) for 5 min. The disinfested root pieces were washed serial times with sterilized water and then dried between two sterilized layers of filter paper. The root pieces were cultured onto the surface of sterilized Petri dishes containing freshly preparing Potato Dextrose Agar (PDA) medium. After 5 days of incubation at $25 \pm 1^\circ\text{C}$, the occurrence of *F solani* infection was recorded.

4-Effect on total microbial counts:

Serial dilutions of each soil sample were used for determining the number of fungi, aerobic bacteria and spore forming bacteria by the plate count technique using suitable media (Ghini *et al.*, 2007). Ten gram of each soil sample, separately, was shaken in 90 ml of sterilized distilled water in a 250 ml flask for 20 minutes on a shaker to give a dilution of 10^{-1} . Then, serial dilutions up to 10^{-7} of fresh suspension of each sample were prepared in a similar fashion by transferring 1 ml of sample suspension to 9 ml sterilized distilled water in test tube under sterile conditions. About of 1 ml soil of each of the dilutions 10^{-3} to 10^{-7} were transferred to each Petri dish and sterile four Petri dishes were used as replicates. Martin medium (g/L): glucose 10g, peptone 5g, KH_2PO_4 1g, MgSO_4 0.5g, Rose Bengal 30 μg , streptomycin 0.03g, agar 20.0g was used for count the common fungi after 7 days of incubation at $25 \pm 1^\circ\text{C}$. Nutrient agar medium (g/L): peptone 5 g, beef extract 3 g, sodium chloride 5 g, agar 20 g, pH 7; was used for count of both total aerobic bacteria and spore forming bacteria after 2 days of incubation at 28°C (Bridson, 1995). The results are presented as a number of colony-forming units (CFU) per gram of soil sample.

5-Effect on frequency % of common fungi:

The growing fungi were identified to genus and species level according to the morphological and cultural characters (Barnett & Hunter, 1972 and Nelson *et al.*, 1983). The frequency percentages of isolated fungi were counted and calculated according to the equation:

Fungal frequency percentage (%) = (Fungus no. / Total fungi no.) x 100

V-Statistical analysis:

Statistical analyses of all the previously designed experiments have been carried out according to ANOVA procedures which reported by Snedecor and Cochran (1999). Treatment means were compared by the least significant difference test "LSD" at 5% level of probability using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co.

3.Results

1-Effect on nematode parameters:

A. Experiment I:

The soil fumigated with MS and MP significantly reduced number of juveniles (J_2) of *M. incognita* in rhizosphere soil, as well as, the numbers of L_3 , L_4 , females, egg-masses and galls in roots of cucumber plants, compared to their numbers in untreated control. Results showed that the total final population (Pf) of J_2 in soil and numbers of L_3 , L_4 , females and egg-masses of *M. incognita* in roots of cucumber plants reduced to 7 & 14 and 13 & 17 with MP and MS, compared to 267 and 621 in untreated control after 45 and 90 days of treatment,

respectively. Results also showed that the MS and MP reduced the rates of build-up (Pf/Pi) of *M. incognita*, compared to untreated control after 45 and 90 days of treatment (Table, 1). MP gave highly reduced the nematode build-up about 0.035 and 0.065, compared to 0.068 & 0.080 with MS and 1.130 & 2.620 in untreated control, after 45 and 90 days, respectively. Soil fumigated with MP significantly reduced the number of galls of *M. incognita* in roots of cucumber to 5 & 9 galls, compared to 8 & 12 galls with MS and 40 & 52 galls in untreated control after 45 and 90 days of treatment, respectively (Table, 1).

Table (1): Effects of metam sodium and metam potassium on *Meloidogyne incognita* parameters in the rhizosphere and roots of cucumber plants under commercial plastic house conditions (Experiment I).

Treatments	<i>Meloidogyne incognita</i> parameters								
	Initial J_2 in soil (Pi)	J_2 in soil	Egg-masses in roots	Third stage (L_3) in roots	Fourth stage (L_4) in roots	Females in roots	Total final population (Pf)	Pf/Pi	Gall no. in roots
45 days									
Metam sodium	207 a	0 c	3 c	4 cd	4 c	3 c	14 cd	0.068	8 cd
Metam potassium	200 a	0 c	1 c	2 d	2 c	2 c	7 d	0.035	5 d
Untreated control	237 a	211 a	15 b	20 a	11 b	10 b	267 b	1.130	40 b
90 days									
Metam sodium	207 a	0 c	2 c	7 b	3 c	5 c	17 c	0.080	12 c
Metam potassium	200 a	0 c	1 c	5 bc	3 c	4 c	13 cd	0.065	9 cd
Untreated control	237 a	574a	21 a	18 a	15 a	20 a	621 a	2.620	52 a

Means followed by same small letter, in each column, are no significantly differ using L.S.D. test ($P = 0.05$).

B. Experiment 11:

The total final population (Pf) of J_2 in soil and numbers of L_3 , L_4 , females and egg-masses of *M. incognita* in cucumber roots was in the range of 5 to 12 and 9 to 17 in soil fumigated with MP plus EPO treatment, compared to 12 & 20 with MP only and 311 & 447 in untreated control after 45 and 90 days of treatment, respectively. After 45 days of application, MP + Aole-vera significantly reduced Pf of *M. incognita* to 5, followed by MP + Eucalyptus (6), MP + Sesame (8), MP + Lupinus (12) and MP only (12), compared to 311 in the untreated control. After 90 days of application, MP + Eucalyptus significantly reduced the Pf of *M. incognita* to 9, followed by MP + Aole-vera (13), MP + Sesame (13), MP + Lupinus (17) and MP only (20), compared to 447 in the untreated control (Table, 2).

Results showed that the MP only or combined with EPO reduced the rates of build up of *M. incognita*, compared to untreated control after 45 and 90 days of treatment. MP + Aole-vera significantly reduced the build-up rate of *M. incognita* to 0.026 after 45 days, followed by MP + Eucalyptus (0.030),

MP + Sesame (0.039), MP + Lupinus (0.057) and MP only (0.056), compared to 1.410 in the untreated control. After 90 days of application, MP + Eucalyptus gave highly reduced the nematode build-up rate to 0.045, followed by MP + Sesame (0.063), MP + Aole-vera (0.068), MP + Lupinus (0.081) and MP only (0.093), compared to 2.030 in the untreated control (Table, 2).

The number of galls of *M. incognita* in cucumber roots in soil fumigated with MP plus treated four EPO, separately, was in the range of 4 to 5 galls and 6 to 15 galls, compared to 5 & 12 with MP only and 34 & 54 galls in untreated control after 45 and 90 days of treatment, respectively. The soil fumigated MP and treated with Eucalyptus or Aole-vera or Sesame, separately, significantly reduced the number of galls to 4 galls, followed by 5 galls in both MP only and MP + Lupinus, compared to 34 galls in untreated control after 45 days. MP + Eucalyptus significantly reduced the number of galls to 6 galls, followed by MP + Sesame (10 galls), MP+ Aole-vera (11 galls), MP only (12 galls) and MP + Lupinus (15

galls), respectively, compared to 54 galls in untreated control after 90 days (Table, 2).

2- Effect on *Fusarium root rot incidence (%)*:

A-Experiment I:

Results revealed that MP fumigant highly reduced the percentages of root rot incidence caused by *F. solani* in cucumber plants, followed by MS and untreated control. The percentages of cucumber root

pieces that infected with root rot disease was < 20% from total assayed root pieces in soil fumigated with MP, while it was $\geq 20\%$ - < 40% and ≥ 40 - < 60% of total assayed root pieces in soil fumigated with MS after 45 and 90 days of treatment, respectively, compared to ≥ 60 % of root pieces in the treated control (Table,3).

Table (2): Effects of metam potassium only and combined with essential oils of some plants on *Meloidogyne incognita* parameters in the rhizosphere and roots of cucumber plants, under commercial plastic house conditions (Experiment II).

Treatments	<i>Meloidogyne incognita</i> parameters								
	Initial J ₂ in soil (Pi)	J ₂ in soil	Egg-masses in roots	Third stage (L ₃) in roots	Fourth stage (L ₄) in roots	Females in roots	Total final population (Pf)	Pf/pi	Gall no. in roots
45 days									
Metam potassium (MP) only	215 a	0c	1 c	6 c	3 c	2 c	12 de	0.056	5 d
MP + Eucalyptus	200 a	0c	1 c	3 c	1 c	1 c	6 de	0.030	4 d
MP + Aole-vera	190 a	0c	0 c	3 c	1 c	1 c	5 e	0.026	4 d
MP + Sesame	205 a	0c	1 c	2 c	3 c	2 c	8 de	0.039	4 d
MP + Lupinus	210 a	0c	1 c	6 c	2 c	3 bc	12 de	0.057	5 d
Untreated control	220 a	167b	14 b	55 a	25 a	25 a	311 b	1.410	34 b
90 days									
Metam potassium (MP) only	215 a	0c	2 c	9 c	3 c	6 b	20 c	0.093	12 cd
MP + Eucalyptus	200 a	0c	1 c	4 c	2 c	2 c	9 d	0.045	6 d
MP + Aole-vera	190 a	0c	1 c	4 c	2 c	6 b	13 cd	0.068	11 cd
MP + Sesame	205 a	0c	3 c	5 c	2 c	3 bc	13 cd	0.063	10 cd
MP + Lupinus	210 a	0c	2 c	7 c	2 c	6 b	17 c	0.081	15 c
Untreated control	220 a	357a	32 a	21 b	14 b	23 a	447 a	2.030	54 a

Means followed by same small letter, in each column, are no significantly differ using L.S.D. test ($P = 0.05$).

Table (3): Effects of metam sodium, metam potassium and essential oils some plants on root-rot disease incidence % (caused by *Fusarium solani*) in cucumber plants, under commercial plastic house conditions (Experiments I and II).

Treatments	Root-rot disease incidence % (days)	
	45	90
Experiment I		
Metam sodium	++	+++
Metam potassium	+	+
Untreated control	++++	++++
Experiment II		
Metam potassium (MP) only	+	+
MP + Eucalyptus	+	+
MP + Aole-vera	+	+
MP + Sesame	++	+++
MP + Lupinus	++	+++
Untreated	++++	++++

Root rot infection % of root pieces: + < 20%, ++ ≥ 20 - < 40%, +++ ≥ 40 - < 60% and ++++ ≥ 60 %.

B-Experiment II:

Results revealed that MP only or combined with EPO of Eucalyptus and Aole-vera, separately, were more effective against root rot disease (*F. solani*), than other treatments as well as untreated control. Where the infected cucumber root pieces %

was < 20% of total assayed root pieces after 45 and 90 days of treatment. In soil fumigated with MP plus Sesame and/or Lupinus oil, the infected root pieces % was ≥ 20 - < 40% after 45 days of treatment, while it was ≥ 40 - < 60% after 90 days of treatment, compared to ≥ 60 % in untreated control (Table, 3).

3-Effect on total microbial counts:**A-Experiment I:**

MP fumigant significantly reduced the total fungal count in the rhizosphere of cucumber plants, than MS as well as untreated control. The fungal count was 10.7×10^4 ($5.2 \log_{10}$) CFU/g soil, 16.7×10^4 ($5.0 \log_{10}$) CFU/g soil and 23.0×10^4 ($5.4 \log_{10}$)

CFU/g soil with above treatments after 45 days of treatment, while it was 2.7×10^4 ($4.4 \log_{10}$) CFU/g soil, 6.7×10^4 ($4.8 \log_{10}$) CFU/g soil and 16.0×10^4 ($5.2 \log_{10}$) CFU/g soil with MP, MS and untreated control after 90 days of treatment, respectively (Table,4).

Table (4): Effects of metam sodium and metam potassium on total counts of fungi, aerobic bacteria and spore-forming bacteria in the rhizosphere of cucumber plants under commercial plastic house conditions (Experiment I).

Treatments	Periods (days)	Total microbial count					
		Fungi at dilution of 10^4		Aerobic bacteria at dilution of 10^6		Spore-forming bacteria at dilution of 10^4	
		CFU/g ¹	Log ₁₀ ²	CFU/g	Log ₁₀	CFU/g	Log ₁₀
Metam sodium	Initial	18.3	5.3ab ³	19.0	7.3b	7.3	4.9bc
	45	16.7	5.2b	10.0	7.0c	5.7	4.8c
	90	6.7	4.8d	30.0	7.5a	12.7	5.1a
Metam potassium	Initial	23.0	5.4a	17.3	7.2b	9.1	5.0ab
	45	10.7	5.0c	2.7	6.4e	3.0	4.5d
	90	2.7	4.4e	4.7	6.7d	5.7	4.8c
Untreated	Initial	25.2	5.4a	18.3	7.3b	9.1	5.0ab
	45	23.0	5.4a	35.7	7.6a	10.7	5.0ab
	90	16.0	5.2b	39.0	7.6a	12.7	5.1a

- 1) CFU/g = Colony forming unit per gram soil.
- 2) log₁₀ of counts of fungal, aerobic bacteria and spore forming bacteria.
- 3) Means followed by same small letter, in each column, are no significantly differ using L.S.D. test ($P=0.05$).

Results also revealed that the count of total aerobic bacteria was 2.7×10^6 ($6.4 \log_{10}$) CFU/g soil and 4.7×10^6 ($6.7 \log_{10}$) CFU/g soil with MP, while it was 10.0×10^6 ($7.6 \log_{10}$) CFU/g soil and 30.0×10^6 ($7.5 \log_{10}$) CFU/g soil with MS, compared to 35.7×10^6 ($7.6 \log_{10}$) and 39.0×10^6 ($7.6 \log_{10}$) CFU/g soil in untreated control after 45 and 90 days of treatment, respectively (Table, 4).

MP also significantly reduced the count of spore forming bacteria to 3.0×10^4 ($4.5 \log_{10}$) CFU/g soil and 5.7×10^4 ($4.8 \log_{10}$) CFU/g soil, compared to count of 5.7×10^4 ($5.7 \log_{10}$) CFU/g soil and 12.7×10^4 ($5.1 \log_{10}$) CFU/g soil with MS and 10.7×10^4 ($5.0 \log_{10}$) and 12.7×10^4 ($5.1 \log_{10}$) in untreated control after 45 and 90 days, respectively. Significant differences were recorded between recorded periods as well as among treatments with counts of tested microbes (Table, 4).

B-Experiment II:

Effects of MP only and/or combined with EPO on the total counts of tested microbes are listed in Table (5). The fungal count was in the range of 11.0×10^4 ($5.0 \log_{10}$) to 20.0×10^4 ($5.3 \log_{10}$) CFU/g soil and 3.7×10^4 ($4.6 \log_{10}$) to 10.0×10^4 ($5.0 \log_{10}$) CFU/g soil with MP + EPO, compared to 11.6×10^4 ($5.1 \log_{10}$) CFU/g soil and 2.9×10^4 ($5.3 \log_{10}$) CFU/g

soil with MP only and 21.0×10^4 ($5.3 \log_{10}$) CFU/g soil and 18.0×10^4 ($5.3 \log_{10}$) CFU/g soil with untreated control after 45 and 90 days of treatment, respectively. Significant differences were recorded in total fungal count between recorded periods (Table, 5).

The total count of aerobic bacteria was in the range of 9.7×10^6 ($7.0 \log_{10}$) to 15.7×10^6 ($7.2 \log_{10}$) CFU/g soil and 14.0×10^6 to 19.7×10^6 ($7.3 \log_{10}$) CFU/g soil in the rhizosphere of MP + EPO treated soil, compared to the count of 3.0×10^6 ($6.5 \log_{10}$) CFU/g soil and 5.0×10^6 ($6.7 \log_{10}$) CFU/g soil with MP only and 33.7×10^6 ($7.5 \log_{10}$) and 35.0×10^6 ($7.5 \log_{10}$) in untreated control after 45 and 90 days of treatment, respectively (Table, 5).

The spore forming bacteria counts in soil fumigated with MP and treated with EPO were in the range of 3.8×10^4 ($4.6 \log_{10}$) to 17.7×10^4 ($5.2 \log_{10}$) and 5.0×10^4 ($4.7 \log_{10}$) to 14.7×10^4 ($5.2 \log_{10}$), compared to 4.0×10^4 ($4.6 \log_{10}$) & 6.0×10^4 ($4.8 \log_{10}$) CFU/g soil with MP only and 10.0×10^4 ($5.0 \log_{10}$) & 13.0×10^4 ($5.1 \log_{10}$) CFU/g soil in untreated control after 45 and 90 days of treatment, respectively. No significant differences were recorded periods in counts of aerobic bacteria as well as spore forming bacteria (Table 5).

Table (5): Effects of metam potassium only and combined with essential oils some plants on total counts of fungi, aerobic bacteria and spore-forming bacteria in the rhizosphere of cucumber plants, under commercial plastic house conditions (Experiment II).

Treatments	Periods (days)	Total microbial count					
		Fungi at dilution of 10 ⁴		Aerobic bacteria at dilution of 10 ⁶		Spore-forming bacteria At dilution of 10 ⁴	
		CFU/g ¹	Log ₁₀ ²	CFU/g	Log ₁₀	CFU/g	Log ₁₀
Metam potassium (MP) only	45	11.6	5.1bc ³	3.0	6.5g	4.0	4.6f
	90	2.9	4.5f	5.0	6.7f	6.0	4.8de
MP + Eucalyptus	45	11.0	5.0cd	12.0	7.1d	3.8	4.6f
	90	3.7	4.6f	14.0	7.2c	7.7	4.9cd
MP + Aole -vera	45	16.7	5.2ab	15.7	7.2c	6.7	4.8de
	90	8.0	4.9dc	19.7	7.3b	5.0	4.7ef
MP + Sesame	45	13.0	5.1bc	9.7	7.0e	10.7	5.0bc
	90	6.0	4.8e	15.7	7.2c	14.7	5.2a
MP + Lupinus	45	22.0	5.3a	13.7	7.1d	17.7	5.2a
	90	10.0	5.0cd	15.7	7.2c	10.7	5.0bc
Untreated control	45	21.0	5.3a	33.7	7.5a	10.0	5.0bc
	90	18.0	5.3a	35.0	7.5a	13.0	5.1ab

1) CFU/g = Colony forming unit per gram soil.

2) log₁₀ of counts of fungal, aerobic bacteria and spore forming bacteria.

3) Means followed by same small letter, in each column, are no significantly differ using L.S.D. test ($P=0.05$).

4-Effect on frequency % of common fungi:

A-Experiment I:

Effect of both MS and MP on the frequency (%) of common fungi in the treated rhizosphere of cucumber plants, after 45 and 90 days of treatment, are listed in Table (6). Results revealed that *Penicillium* spp., *Aspergillus* spp., *Aspergillus niger*, *Fusarium* spp., *Rhizoctonia* sp., *Rhizopus* spp. and others were the common isolated fungi in fumigated cucumber rhizospheres as well as untreated control. The fumigated soil significantly increased the

frequency (%) of saprophytic fungi such as *Penicillium* spp. and *Aspergillus* spp., while the same soil significantly decreased the frequency (%) of pathogenic fungi such as *Fusarium* spp. and *Rhizoctonia* spp. MP was more effective on the frequency of common fungi, than MP as well as untreated control. Significant differences were recorded among the frequency (%) of isolated fungi, between recorded periods as well as between fumigant treatments and the untreated control (Table, 6).

Table (6): Effects of metam sodium and metam potassium on the fungal frequency % in the rhizosphere of cucumber plants, under commercial plastic house conditions (Experiment I).

Treatments	Periods (days)	Common fungal frequency %						
		<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.	<i>Aspergillus niger</i>	<i>Fusarium</i> spp.	<i>Rhizoctonia</i> spp.	<i>Rhizopus</i> spp.	Others
Metam sodium	45	32.1b	35.9a	9.4c	7.6d	7.6d	3.7e	3.7e
	90	38.5a	30.8b	0.0e	7.7c	7.7c	0.0e	5.3d
Metam potassium	45	43.9a	22.0b	0.0f	9.8d	4.9e	14.6c	4.8e
	90	30.0b	36.0a	12.9c	6.0e	4.0f	4.0f	7.1d
Untreated control	45	19.6b	15.2c	10.9e	23.9a	13.0d	13.0d	4.4f
	90	18.8b	25.0a	9.4c	18.8b	9.4c	9.4c	9.2c

Means followed by same small letter, in each row, are no significantly differ using L.S.D. test ($P = 0.05$).

B-Experiment II:

Results showed that the isolated fungi that mentioned before were the common fungi in the rhizosphere of cucumber plants fumigated with MP plus EPO- treated soil as well as MP only and untreated control. The frequency % of isolated fungi is listed in Table (7). Results also showed that the MP plus EPO- treated soil significantly increased the frequency (%) saprophytic fungi of *Penicillium* spp. and *Aspergillus* spp., than other fungi. The MP plus

EPO- treated soil also decreased the frequency of *Fusarium* spp. and *Rhizoctonia* spp., than untreated control. After 90 days of treatment, the frequency of *Fusarium* spp. was in the range of 8.3 to 18.8% with MP + essential plant oils, compared to the frequency of 6.2 and 19.8 % with MP only and untreated control, while the frequency of *Rhizoctonia* spp. was in the range of 0.0 to 8.3% with MP + EPO, compared to 4.8 and 11.4% with MP only and the untreated control, respectively (Table, 7).

Table (7): Effects of metam potassium only and combined with essential oils some plants on fungal frequency % in the rhizosphere of cucumber plants under commercial plastic house conditions.

Treatments	Periods (days)	Common fungal frequency %						
		<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.	<i>Aspergillus niger</i>	<i>Fusarium</i> spp.	<i>Rhizoctonia</i> spp.	<i>Rhizopus</i> spp.	Others
Metam potassium (MP) only	45	38.9a	23.0b	0.0g	11.8d	7.9e	13.6c	4.9f
	90	32.0b	32.3a	13.0c	6.2e	4.5g	5.0f	7.0d
MP + Eucalyptus	45	31.8b	40.9a	4.6e	13.6c	0.0f	0.0f	9.1d
	90	28.6b	42.9a	0.0e	14.7c	0.0e	0.0e	13.8d
MP + Aole-vera	45	27.4b	45.2a	8.2c	5.5d	2.7e	5.5d	5.5d
	90	31.3a	25.0b	0.0f	18.8c	6.3e	6.3e	12.3d
MP + Sesame	45	34.6b	39.6a	0.0e	11.5c	3.9d	0.0e	10.4c
	90	33.3b	41.7a	0.0d	8.3c	8.3c	0.0c	8.4c
MP + Lupins	45	22.7b	31.8a	6.8e	15.9c	6.8e	11.7d	4.6f
	90	40.0a	25.0b	0.0e	10.0c	5.0d	0.0e	10.0c
Untreated control	45	21.0b	18.0c	11.0e	24.0a	14.5d	7.1f	4.4g
	90	19.8b	23.0a	10.4e	19.8b	11.4c	11.4c	4.2f

Means followed by same small letter, in each row, are no significantly differ using L.S.D. test ($P = 0.05$).

5- Effect on cucumber yield:

A-Experiment I:

Results revealed that the MP highly increased the average of cucumber fruits number per plant to 20 fruits in one harvest, followed by MS and untreated control, where the cucumber fruits average was 14 and 10 fruits, respectively (Table, 8). MP increased the average of fruits weight/plant in one harvest to

2.25 Kg, followed by MS (1.60 Kg) and untreated control (1.60 Kg). Average of fruits yield / plant in one season (About 12 harvests) reached to 27.0 Kg/plant with MP, while it reached to 19.2 and 16.8 Kg/plant with MS and treated control, respectively. Significant differences in plant yield in season were recorded between MP and MS treatments, comparing with untreated control (Table, 8).

Table (8): Effects of metam sodium, metam potassium and essential oils some plants on fruits yield parameters of cucumber plants, under commercial plants house conditions (Experiments I and II).

Treatments	Yield parameters		
	Av. fruits no./plant ¹	Av. fruit weight/ plant ² (kg)	Av. fruit yield /plant ³ (kg)
Experiment I			
Metam sodium	14	1.60	19.2b ⁴
Metam potassium	20	2.25	27.0a
Untreated control	10	1.40	16.8c
Experiment II			
Metam potassium (MP) only	16	2.15	25.8A
MP + Eucalyptus	18	2.15	25.8A
MP + Aole-vera	17	2.10	25.2A
MP + Sesame	16	2.15	25.8A
MP + Lupinus	16	2.00	24.0B
Untreated	12	1.65	19.9C

1-Average of cucumber fruits number/plant in one harvest,

2-Average of cucumber fruits weight /plant in one harvest,

3-Average of cucumber fruits yield/plant in season (12 harvests) and 4- Means followed by same small or capital letter, in each column, are no significantly differ using L.S.D. test ($P = 0.05$).

B-Experiment II:

Results revealed that average of cucumber fruits number was 16 fruits/plant with MP, while it was in the range of 16-18 fruits/plant with MP + EPO, compared to 12 fruits/plant in untreated control (Table, 8). Average of fruits weight / plant was 2.15 Kg, compared to the range of 2.00-2.15 Kg in mixed treatment (MP + EPO) and 1.65 Kg in untreated

control. Average of fruits yield/plant was 25.8 Kg, in the range of 24.0 – 25.8 Kg and 19.9 Kg with MP only, mixed treatment and untreated control, respectively (Table, 8). Significant differences in fruits yield / plant in one season were recorded among MP only or combined with EPO and untreated control (Table, 8).

4. Discussion

Our results revealed that the cucumber plants were infected with root-knot nematode (*M. incognita*) and root rot fungus (*F. solani*) under plastic house conditions. These results are agreement with those recorded by Boshour *et al.* (2010). They reported that the plant parasitic nematodes are among the most important pathogen that adversely influence tomato production in Lebanon and its neighboring countries. *Fusarium* and *Verticillium* wilts also cause severe constraints on agricultural production in near east (Boshour *et al.*, 2010). Plant parasitic nematodes cause losses of up 80% on vegetables (Kaskvalci, 2007 and Nchore *et al.*, 2011). The root knot nematodes (*Meloidogyne* spp.) also severely attack tomato resulting in yield loss (Hemeng, 1980).

The fumigants of MS and MP highly effective in reducing the *M. incognita* parameters such as J_2 in soil and numbers of L_3 , L_4 , females and egg-masses in roots, where the results indicated that MP was more effective than MS (Experiment I). Soil treated with essential oil of Aole-vera, Eucalyptus and sesame, separately, incased the efficacy of MP in reducing the above nematode parameters (Experiment II). The fumigant of MP has highly affected against incidence of root rot disease, than MS, where MP significantly reduced the numbers of root pieces that infected with *F. solani* pathogen (Experiment I). In experiment II, no clear differences were recorded among MP only, MP + Aole-vera and MP + Eucalyptus in their effect against fungal disease incidence. On the other hand, soil treated with EPO of Sesame and Lupinus reduced the efficacy of MP against root rot disease. It is clear that MP only is highly effective against disease and may be playing an important role it controls.

Metam sodium, metam potassium and dazomet are broad spectrum soil fumigants that can be used to control pathogens and pests affecting a wide array of economically important fruit and vegetable crops. Especially all three fumigants are registered for use in controlling nematodes (e. g. root-knot, cysts, etc) and several species of soil fungi (e.g. *Rhizoctonia*, *Fusarium*, *Pythium*, *Phytophthora*, *Verticillium* and *Sclerotinia*). Products also containing these active substances can be applied within Integrated Pest Management systems, as they can be used in conjunction with resistant / rootstocks, improved sanitation techniques, biological control agents and soil pasteurization (by solarization, hot water or steam injection) [Roberts *et al.*, 1988]. Santo & Qualls (1984) and Ben-Yephet *et al.* (1986) also reported that several methods of applying metam sodium to soil to control plant-parasitic nematodes and pathogenic fungi have been evaluated over the last 30 yr with variable results. Some investigators

controlled this pest by using oils of some essential and medicinal plants (Yuji *et al.*, 2000 and El-Nagdi & Said-Al Ahl, 2010). The monoterpenes, of which D-carvone, D-limonene and α -phellandrene are impact compounds of many essential oils responsible for biological effects (Delaquis *et al.*, 2002 and Leopold *et al.*, 2003).

Our results revealed that the soil fumigated with MS and MP, separately, reduced the total of fungi, while the treatments reduced total counts of aerobic bacteria and spore forming bacteria were recorded after 45 days of treatment and then it was increased after 90 days of treatment (Experiment I). These notes also were recorded in soil fumigated with MP only and/or plus soil treated with essential plant oils (Experiment II). The fumigants of MS and MP reduced the frequency % of pathogenic fungi (*Fusarium* spp. and *Rhizoctonia* spp.), while it's were increased the frequency % the saprophytic fungi such as *Penicillium* spp., *Aspergillus* spp., *Aspergillus niger* and *Rhizopus* spp. (Experiment I and II). These results are agreement with those recorded by (James, 1989). He reported that soil fumigation with broad-spectrum biocides is a non-selective means of killing soil borne pathogens in forest seedling nurseries. Beneficial microorganisms are also killed by most fumigants. Dormant structures of microorganisms are usually more resistant to fumigant action. Specific fumigants are more effective against certain microorganisms, i.e. *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctinia*, *Macrophomina* and *Phoma*.

All applied treatments increased the cucumber fruits yield parameters such as average of fruits number per plant and average of fruit weight per plant in one harvest as well as increased the fruit yield per plant in one season. These results are agreement with those recorded by McMillan and Bryan (2001). In the forest-savanna transitional zone, the highest yield increase of 95.2% was recorded when tomato cultivar "Asewewa" was grown in 1, 3 D soil (Hemeng, 1980)

Conclusions

Fumigants, metam potassium and metam sodium at the rate of 100 Cm^3/m^2 controlled of root-knot nematode and *Fusarium* root rot fungus. Metam potassium was highly effective in reducing the nematode parameters such as numbers of J_2 in soil and the numbers of L_3 , L_4 , females, egg-masses and galls in roots of cucumber plants as well as root rot disease incidence, than metam sodium. Soil treated with essential oils (Aloe-vera, Eucalyptus and Sesame) was increased the efficacy of metam potassium against root-knot nematode, while it was not ineffective against root rot fungus. The tested

fumigants reduced the frequency % of pathogenic fungi (*Fusarium* spp. and *Rhizoctonia* spp.), while it's were increased the frequency % the saprophytic fungi such as *Penicillium* spp., *Aspergillus* spp., *Aspergillus niger* and *Rhizopus* spp. The tested fumigants increased the tested yield parameters of cucumber fruits per plant. Our results recommended that can be used metam potassium and metam sodium in controlling the root – knot nematode and *Fusarium* root rot fungus in cucumber plants in controlled agricultural systems.

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