Evaluation of some Methyl bromide alternatives on management of cucumber root-rot

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ABSTRACT---Six chemicals alternative to Methyl bromide, *i.e.* Metam sodium, Metam sodium covered with polyethylene sheets, Dichloroprene + Carbendezim, Metam sodium +Oxamyle , Metam sodium +Oadusafs and Metam sodium + Carendazim, were used to control damping-off and root rot of cucumber plants (cv. Rocket). Isolation trials from rotted roots collected from north Gaza, Palestine yielded five fungal isolates. The isolated fungi were purified and identified as *Alternaria* sp., *Fusarium oxypsorum , F.solani, Pythium ultimum* and *Rhizoctonia solani* . All the tested fungi were pathogenic to cucumber plants and *F. oxysporum* was the most virulent one and of high frequency during isolation trial. The virulent pathogen has been identified as *F. oxysponwn* and symptomology and host range studies indicated that this is likely a new forma specialis , *i.e.* f. sp. *radicis-cucumerinum* (FORC). Among the six tested chemicals, Metam sodium +Cadusafs was the best alternative treatment to methyl bromide for reducing the number of the causal fungus spores. In addition,l the tested six treatments alternative to Methyl bromide significantly decreased the severity of root-rot with significant increase to the estimated crop parameters of cucumber plants, *i.e.* fresh weight of the foliage growth and the roots and fruit yield . In all cases, Methyl bromide , although , was the superior treatment in reducing the number of the viability of FORC spores at 20 and 40 cm. depth ,managing root-rot and increasing foliage growth and root weight as well as the produced fruit yield . Furthermore, the six chemicals alternative to Methyl bromide as well as Methyl bromide resulted in improving the growth vigor and the produced fruit yield of the grown cucumber plants and extended the fruiting period compared with the control.

Keywords: Cucumber, Carbendezim , Cadusafs , Dichloroprene, *Fusarium osysporum* f. sp. *radicis-cucumerinum,* Methyl bromide, Metam sodium, and Oxamyle

1 INTRODUCTION

Cucumber (*Cucumis sativus* L.) is considered as one of the important crops in Palestine. It has a valuable nutritional, where it has been its accepted by the consumer.

Cucumber is liable to infection by many bacterial fungal and viral diseases in addition to physiological disorder and nematode infection. However, fungal diseases especially damping-off and root-rot are among the most constrain diseases (Zitter *et al.*,1996). The fungus *Fusarium osysporum* f. sp. *radicis-cucumerinum* is responsible to cause damping off and root-rot that reduce plant stand and fruit yield and its quality (Kuwata *et al*. , 1994 ; Vakalounakis, 1996 ; Martínez *et al.,*2002 and Paker, 1999).

Methyl bromide has been used around the world since the 1930s as a quarantine treatment for plants and to control insects in buildings and commodities. It is also widely used as a pre plant soil fumigant to control nematodes, insects, pathogens and weeds. It is the method of choice of many pesticide applicators because of its rapid action and broad spectrum of activity. In 1992, it was listed as an ozone-depleting substance under the Montreal Protocol on substances that deplete the Ozone Layer, and all developed countries are scheduled to eliminate the bulk of their consumption of methyl bromide by 2005 . The loss of this important pesticide has forced pest control operators, plant managers in the food industry and entomologists to find alternatives to methyl bromide (Bekhradi *et al*. , 2006)).

The present study aimed to find alternative treatments to methyl bromide to control damping-off and root -rot diseases of cucumber plants.

2 MATERIALS AND METHODS

1. Isolation , purification and identification of the causal fungi

Samples from infected cucumber plants cv. Rocket showing root – rot symptoms were collected from fields at North Gaza, Palestine.

Roots of the infected plants were washed in running tap water for several times , then cut into small pieces and surface sterilized with 1% sodium hypochlorite for 2 minutes under aseptic condition, the plant pieces were rinsed in sterilized water for several times and dried between sterilized Whitman 1 filter papers . The pieces plated on potato dextrose agar (PDA) medium and incubated for 7 days at 25 °C.

The developing fungal colonies were purified using hyphal tip technique and identified depending on their morphological features and the description of Gilman (1957) and Booth (1971).

2. Pathogenicity test

The inoculum of *Fusarium osysporum* was grown in bottles contained autoclaved barley – sand – medium for two weeks . Formalin sterilized soil was infested with the inoculums of the isolated fungi at the rate of 2 % inoculums level the distributed in plastic pots (25 cm in diam.). Un-infested formalin sterilized soil was used a control treatment. All pots were sown with cucumber seeds . Five seeds were sown in each pot and three replicates were used for each treatment.

Pre- and post – emergence damping –off were recorded 15 and 30 days after sowing, respectively.

Cucumber (cv. Roket) , bean (cv. Pronco) ,cantaloupe (hybrid Premal) , melon (cv. Sahd Edfina) ,pea (cv. Master B) ,Squash (cv. Eskandarany), tomato (cv.GS) and watermelon (cv.Giza 1) seeds were sown in soil infested with the inoculum of *Fusarium osysporum* (2% inoculums level) to know the behavior of the infection on these hosts (4 seeds / pot) . The grown plants were pull-off gently two months after sowing and symptoms of the infection on the tested plants were observed .

3. Survival of *F. oxysporum* f. sp. *radicis-cucumerinum* in infected roots of cucumber plants

Samples of rotted –roots of cucumber plants infected by *F. oxysporum* (grown in soil infested with the tested fungus and showing root-rot symptoms) were incubated in an incubator (25º C) for three days to encourage spore formation and buried in the soil at two depths ,*i.e.* 20 and 40 cm. of soil surface .Soil was treated with the tested six treatments (Table 1) alternative to methyl bromide . Methyl bromide and chemical-free treatment were used as controls. One week and 60 days after soil treatment, samples were taken from both depths and *F. oxysporum* spores were counted in 1ml. of spore suspensions (x10 ^{3}) in the aforementioned treatments using haemocytometer slide (Abada, 1986).Also, the number FORC spores in the infected roots pre-treatment with the tested chemicals were counted and recorded.

Table (1). Chemical treatments alternative to methyl bromide.

No.	Treatment
1	Control (untreated soil).
2	Methyl bromide at 50g/m^2 .
3	Metam sodium (Metmor) covered with polyethylene sheets at 40cm/ m^2 .
4	Metam sodium at 40cm/ m^2 .
5	Dichloropropene (Kandor) at 20cm /m$^{2\ -1}$ + Carbendazim (Bavistin) at 0.3g/m^2 .
6	Metam sodium at 40 cm/ m^2 + Oxamyl (Vydate) at 15 cm/ m^2 .
7	Metam sodium at 40cm/ m^2 + Cadusafs (Ragby super) at 15 cm/ m^2 .
8	Metam sodium at 40cm/ m^2 + Carbendazim at 0.3g/m^2 .

4. Effect of the tested treatments on the severity of root-rots and some crop parameters of cucumber plants

The experiments were carried out during 2011 and 2012 growing seasons in a land at north Gaza- Palestine , has a back history of severe infection by cucumber root-rot , mainly with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) due to the frequent sowing cucumber and un-using crop rotation. The land was prepared for sowing cucumber in plots of (42 m^2 of 6 ridges) . The naturally infested soil with the pathogenic fungi , especially the tested pathogen was treated with the six treatments (Table, 1) alternative to Methyl bromide before sowing. Soil infested soil with the tested pathogen and treated with Methyl bromide as well as chemical – free soil, were used as controls . All treatments were sown with apparently healthy cucumber transplants of 21 days old (grown in foam trays) at 25 cm interval (one transplant in each hill) during the first week of August of both seasons . Four replicates were used for each treatment. All recommended agriculturaltur practices , hoeing, irrigation, fertilization and pest control were applied

Two months after sowing 10 randomly plants were gently pulled-off from each treatment and examined for root-rot severity.

Root-rot severity was then assessed using the devised scale (0-5) according to Salt (1982) as follows:

$$DS\ \% = \frac{\text{Sum of (nxv)}}{5N} \times 100$$

Where:

DS= Disease severity,

n= Number of roots in each category,

v= Numerical value of each category,

N= Total number of roots in samples.

Crop parameters of 10 randomly plants in each treatment , *i. e.* fresh weight of the foliage growth and roots as well as number of fruits and their weight / plant were estimated and recorded for the six treatments alternative to methyl bromide as well as methyl bromide and chemical – free treatments as controls.

5. Statistical analysis:

The complete randomized block design was adopted. The obtained data were tabulated and statistically analyzed according to Duncan's Multiple Range Test (1955).

3 RESULTS AND DISCUSSION

1. Isolation, purification and identification of the causal fungi

Isolation trials from the rotten roots of cucumber roots collected from north Gaza , Palestine yielded many fungal isolates . The isolated fungi were purified and identified as *Alternaria* sp., *Fusarium oxypsorum (* of high frequency during isolation trial) *, F.solani* , *Pythium ultimum* and *Rhizoctonia solani .*

The isolated fungi were previously isolated from rotted cucumber roots (Paternotte ,1987 ; Vakalounakis, 1996; Paker, 1999 ; Martínez *et al.,*2002 and Tuwaijri, 2015).

2. Pathogenicity test

Data presented in Table (2) show that the five isolated fungi caused pre-and post-emergence damping – off to cucumber. The fungus *Fusarium oxypsorum* caused the highest percentages of pre-and post damping-off , being 23.3 and 26.7 % followed by *F. solani*, being 20.0 and 20.3 % then *Rhizoctonia solani,* being 16.7and 20.0%, respectively *.* Meanwhile, *Alternaria* sp*.* caused the lowest figures of pre-and post damping-off, being 3.3 and 10.0 % followed by *P.ultimum*, being 13.3 and 16.7 %respectively.

Reisolation from the damped-off seedlings proved pathogenicity of the tested fungi.

Among the tested species of Cucurbitaceae, *i.e.* cucumber, cantaloupe , melon , squash and watermelon that were artificially inoculated with the inoculums of *F.oxysporum* developed root and stem rot symptoms similar to those developed on cucumber in the nature and low infection to bean, pea and tomato was occurred. Therefore, the pathogen has been identified as *F. oxysporum* and symptomology and host range studies indicate that this is likely a new forma specialis, f. sp. *radicis-cucumerinum* . Also, when cucumber plant grown in soil infested with the inoulum of *Fusarium osysporum* , the pathogen develops on the exterior of cucumber plants as well as within cortical tissues causing rot . Therefore, it named *Fusarium osysporum* f. sp. *radicis-cucumerinum* (FORC) (Vakalounakis, 1996).

Table(2). Pathogenicity test of the isolated fungi on cucumber plants (cv. Rocket) , pot experiment.

Treatments	%, Damping off	
	Pre - emergence	Post – emergence
Alternaria sp.	3.3 *	10.0
F.oxysporum	23.3	26.7
P.ultimum	13.3	16.7
F.solani	20.0	23.3
R.solani	16.7	20.0
Control	0.0	0.0

* each figure represents 3 replicates.

3. Survival of FORC in infected roots of cucumber plants

The total count of FORC spores was assessed at the beginning of the experiment. No alive spores were counted just after the tested treatments at 20 cm. depth and low numbers were counted at 40 cm. depth. In addition, the initial number of the spores at 20 and 40 cm. depths for the control treatment (un-treated soil) was 71.8 and 66.0 at 20 and 4 cm. depths, respectively Table (3). The obtained data show that all treatments decreased the number of FORC spores compared with the control . In general, no significant differences were detected due to the effect of the six alternative chemicals to methyl bromide. In addition, Methyl bromide was the best treatment in this regard , being 3.0 and 2.6 x10^3 spore / g. soil and Metam sodium + Cadusafs was the best alternative treatment in this respect, being 4.2 and 1.0 x10^3 spore / g. soil, respectively. In all cases, the number of the causal pathogen spores was greatly affected at 20 cm. depth than those at 40 cm. depth.

The obtained results are in harmony with those reported by Abada (1986) and Ibekwe *et al.*(2010).

4. Effect of the tested treatments on the severity of root-rots and some crop parameters of cucumber plants

Data in Table (4) reveal that the percentages of the severity of rotted-roots of cucumber plants infected with FORC were significantly decreased due to treating the soil with methyl bromide and the six alternatives during 2011 and 2012 growing seasons. In this respect,
methyl bromide was the superior treatment , where decreased root-rot severity to 30.9 and 32.6 % during 2011 and 2012 growing seasons, respectively. Meanwhile, treatment with Metam sodium + covered with polyethylene was the efficient alternative treatment , being 37.3 and 35.3 % during 2011 and 2012 growing seasons, respectively. The treatment with Metam sodium + Oxamyl was the lowest efficient alternative treatment , being 50.1 % during 2011 growing season and Metam sodium + Cadusafs , being 50.0% during 2012 growing season, respectively. Un-treated treatment (control) recorded 77.5 and 82.0 % during 2011 and 2012 growing seasons, respectively .

The obtained results showed , to somewhat, efficient management to cucumber root-rot due to making a combination between to chemicals alternative to Methyl bromide and these chemicals could be used as alternative to Methnyl bromide for managing such soil borne pathogens.

Duniway (2002) reported that Methyl isothiocyanate generators such as Metam sodium have broad biocidal activity in soil, but are more difficult to apply effectively. In most soil applications, the available alternatives are likely to be used in combinations, either as mixtures (e.g., 1,3-dichloropropene and chloropicrin) or sequentially (e.g., chloropicrin followed by Metam sodium). They may also be supplemented with other more specific pesticides and cultural controls. Among the alternatives currently under active development but not yet available, methyl iodide and Propargyl bromide probably have activity that most closely parallels that of methyl bromide in soil. However, all of the chemical alternatives to Methyl bromide will be subject to continuing review and more regulation. Furthermore, he added that we do not know the actual prospects for registration of the new fumigants currently under development and there is a risk that registered fumigants will not be available for large-scale use in soil indefinitely.

Vachev (2015) suggested the possible reinfestation of freshly steamed or fumigated soils by airborne propagules of the pathogen *Fusarium osysporum* f. sp. *radicis-cucumerinum* .He added that propagules of the pathogen that are trapped from the air or recovered from greenhouse interior structures display typical root and stem rot symptoms on inoculated cucumber plants. It is still not clear whether the airborne inoculum has the capacity of inducing disease on intact cucumber plants under natural production conditions.

Table (3). Effect of the tested six treatments, alternative to methyl bromide on the total count of *F.oxysporum* f. sp. *radicis-cucumerinum* spores in infected roots of cucumber plants (cv. Rocket) buried in the soil at 20 and 40 cm. depth, one week after and 60 days of the treatments.

Treatments	Initial number of spores (x10^{3}g / soil) one week after treatment at depth of (cm.)		Number of FORC spores (x10^{3}g / soil) at depth of (cm.) 60 days after treatment			
	20	40	20	%, Efficacy	40	%, Efficacy
Methyl bromide	0.0 ^{b}	0.02 ^{b}	1.0^{b} *	98.6	1.6 ^{b}	97.6
Metam sodium + covered with polyethylene	0.0 ^{b}	0.40 ^{b}	4.8 ^{c}	93.3	5.0 ^{c}	92.4
Uncovered Metam sodium	0.0 ^{b}	0.84 ^{c}	5.2 ^{c}	92.8	5.4 ^{c}	91.8
Dichloropropene + Carbendazim	0.0 ^{b}	0.22 ^{b}	4.8 ^{c}	93.3	5.2 ^{c}	92.1
Metam sodium + Oxamyl	0.0 ^{b}	0.24 ^{b}	5.0 ^{c}	93.0	5.4 ^{c}	91.8
Metam sodium + Cadusafs	0.0 ^{b}	0.18 ^{b}	4.2 ^{c}	94.2	5.0 ^{c}	92.4
Metam sodium + Carbendazim	0.0 ^{b}	0.48 ^{b}	4.4 ^{c}	93.9	6.0 ^{c}	90.9
Control	72.0 ^{a}	72.8 ^{a}	71.8 ^{a}	-----	66.0 ^{a}	----

* Average of four replicates.
Means followed by the same letters within columns are different significantly at P= 0.05 according to Duncan's multiple range test.

Table (4). Effect of the tested treatments on the percentages of disease severity of the rotted-roots of cucumber plants infected with FORC, field experiment at north Gaza, Palestine during 2011 and 2012 growing seasons .

Treatments	% Disease severity during	
	2011	2012
Methyl bromide	30.9 ^{d} *	32.6 ^{d}
Metam sodium + covered with polyethylene	37.3 ^{c}	35.3 ^{cd}
Uncovered Metam sodium	48.1 ^{b}	46.5 ^{b}
Dichloropropene + Carbendazim	45.5 ^{b}	49.7 ^{b}
Metam sodium + Oxamyl	50.1 ^{b}	48.5 ^{b}
Metam sodium + Cadusafs	49.1 ^{b}	51.0 ^{b}
Metam sodium + Carbendazim	45.0 ^{b}	44.1 ^{bc}
Control	77.5 ^{a}	82.0 ^{a}

* Average of four replicates.
Means followed by the same letters within columns are different significantly at P= 0.05 according to Duncan's multiple range test.

It has been found that the obtained results are in agreement with those obtained by Ingham *et al*. (2007) who stated that Metam sodium could be reduce propagates of soil borne pathogenic fungi, particularly *Verticillium dahliae* to prevent yield loss caused by potatoes early dying disease. These results was confirmed by Giannakou and Anastasiadis (2005) who found that all fumigant treatments reduced soil propaga-tions of *R. solanii* and *Pythuim* spp. They added that variability of *R. solani* was reduced by most treat-ments . In

The obtained results are in agreement with the obtained results by many scientists (Fahim *et al.,*1994 ; Emmanuel , 2003 ; Giannakou and Anastasiadis , 2005; Santos *et al*., 2005; Bielinski *et al*., 2007; Ingham *et al*., 2007; Lopez-Aranda *et al*., 2009; and Qiao *et al.*, 2010).

The effect of soil treatment with chemicals alternative to Methyl bromide on some crop parameters of cucumber plants is presented in Tables (5 and 6).

Table (5) shows that the six methyl bromide alternatives as well as methyl bromide significantly increased foliage fresh and roots weight compared with control treatment. In addition, the treatment with Methyl bromide sill the superior treatment in this respect, where foliage fresh and roots weight recorded 1207.1 and 310.4 g / plant, respectively . Also, the alternative Metam sodium and covered with polyethylene was efficient treatment in increasing foliage fresh and roots weight , being 1064.8 and 201.1 g / plant, respectively . Meanwhile, Metam sodium + Cadusafs was the lowest efficient treatment, being 814.1 and 177.7 g / plant, respectively . Un-treated (control) plants recorded 663.6 and 135.1 g / plant, respectively .

The produced fruit yield (number of fruits and their weight / plant) of cucumber plants (Table, 6) was significantly increased due to the six treatments alternative to methyl bromide as well as the treatment with Methyl bromide compared with un-

addition, all fumigant treatments, except chloropnozin , reduced soil population of *F. solani* and total *Fusarium* spp.

All treatments alternative to methyl bromide increased growth parameters of cucumber plants i.e. the shoot system was taller than that in non treated treatment, the leaf areas were increased fresh weight of shoot system was increased after 105 day; fresh – and dry – weight of root system ; reduction of flowering date and finally increased the yield.

treated (control) treatment during 2011 and 2012 growing seasons.

Plants treated with Methyl bromide produced the highest number of fruits , being 53.6 and 57.3 fruit/ plant and their weight, being 4.9 and 5.3 kg. / plant during both seasons ,respectively. In addition, plants treated with Metam sodium as alternative to Methyl bromide produced the highest number of fruits during both seasons , being 52.9 and 53.4 fruit/ plant and their weight, being 4.6 and 4.3 kg. / plant during both seasons , respectively. Untreated (control) plants produced poor number and weight of fruits, being 27.1 and 29.1 fruit / plant and their weight, being 2.5 and 2.8 kg. / plant , respectively .

The obtained results are logic , where these alternative chemicals resulted in great reduction to the severity of cucumber root-rot and this significantly reflected on the vigor of the foliage growth and the root system, hence increasing the produced fruit yield. The obtained data are in agreement with the obtained results by many authors who used soil fumigation in many crops (Fahim 1994 ;Ingham *et al*. ;2007 ; Qias *et al*. ,2010 and Lopez-Aranda *et al.,*3009 and 2016).

Generally, the six chemicals alternative to Methyl bromide as well as Methyl bromide resulted in improving the growth vigor and the produced fruit yield of the grown cucumber plants and extended the fruiting period compared with the control.

Table (5). Effect of soil treatment with chemicals alternative to methyl bromide on the foliage and roots fresh weight(g) 105 days after transplanting , field experiment at north Gaza, Palestine during season of 2012 growing season.

Treatments	Foliage and roots fresh weight (g) / plant 105 days after transplanting	
	Foliage growth	Roots
Methyl bromide	1207.1 * a	310.4 a
Metam sodium covered with polyethylene	1064.8 b	301.1 a
Uncovered Metam sodium	922.1 c	193.2 b
Dichloropropene + Carbendazim	1006.3 b	196.8 b
Metam sodium+ Oxamyl	890.8 d	179.3 c
Metam sodium + Cadusafs	814.1 d	177.7c
Metam sodium + Carbendazim	975.3 c	211.2 b
Control	663.6 e	135.1 d

* Average of 4 replicates.
Means followed by the same letters within columns are different significantly at P= 0.05 according to Duncan's multiple range test.

Table (6). Effect of the six treatments alternative to methyl bromide on fruit yield (kg.) of cucumber plants, , field experiment at north Gaza, Palestine during 2011 and 2012 growing seasons.

	Average fruit yield during			
	2011		2013	
	Number of fruits / plant	Total fruit yield (kg) /plant	Number of fruits / plant	Total fruit yield (kg.) /plant
Methyl bromide	53.6 * a	4.9 * a	57.3 a	5.3a
Metam sodium covered with polyethylene	52.9 a	4.6 ab	53.4 a	4.5 b
Uncovered Metam sodium	46.4 b	3.9 b	49.8 ab	4.0 b
Dichloropropene + Carbendazim	50.9 ab	4.1 bc	51.2 a	4.5 a
Metam sodium+ Oxamyl	46.5 b	3.9 b	47.4 b	4.0 b
Metam sodium + Cadusafs	47.1 b	4.0 bc	48.2 b	4.0 b
Metam sodium + Carbendazim	48.1 ab	4.1b	47.9 b	4.0 b
Control	27.1 c	2.5 d	29.1 b	2.8 c

* Average of 4 replicates.
Means followed by the same letters within columns are different significantly at P= 0.05 according to Duncan's multiple range test.

4 REFERENCES

Abada, K. A. (1986) . Pathological studies on root-rot of strawberry. Ph. D. Thesis, Fac. of Agric., Cairo Univ.

Al-Tuwaijri, Majdah M.Y. (2015).Studies on Fusarium wilt Disease of Cucumber. J. of App. Pharmace. Sci.,5 (2): 110-119,

Bekhradi, F.; A. Kashi and M. Delshad (2006). Evaluation the effects of different rootstocks of cucurbit on growth and yield of Charleston gray Watermelon and its influence on blossom end rot disease. Abstract of 5th Iranian Horticul. Sci. Cong., Shiraz, : 384-385 (In Persian).

Bielinski, M. S. ; J. P. Gilreath ; Jose M. Lopez-Aranda ; L. Miranda ; C. Soria and Juan J. Medina (2007). Comparing Methyl bromide alternatives for strawberry in Florida and Spain. J. of Agron,, 6: 225-227.

Booth,C. (1971). The genus *Fusarium*. Kew, UK: Commonwealth. Mycological

Duncan, D.B. (1955).Multiple range and multiple F-tests. Biometrics,11:1-42.

Duniway, J.M. (2002). Status of chemical alternatives to Methyl Bromide for pre-plant fumigation of soil. Phytopathology, 92 (12): 1337-1343 .

Emmanuel, A T. (2003). Investigation of alternatives to methyl bromide fo management of *Meloidogyne javanica* on green-house grown tomato . Pest Manag. Sci., 59, (12) : 1311–1320.

Fahim, M.M. ; M.F. Attia ; A.K. Okasha and K.A. Abada (1994). Control of strawberry root-rot disease by soil fumigation. Egypt .J. Phytopathol.,22 (1):1-15.

Giannakou, I. O. and I. Anastasiadis (2005). Evaluation of chemical strategies as alternatives to Methyl Bromide for the control of root-knot nematodes in greenhouse cultivated crops. 24 (6): 499-506.

Gilman, J.C. (1957). A Manual of Soil Fungi . The Iowa State College Press, Iowa, USA.450 pp.

Ingham, R. E; P. B. Hamm ; M. Baune ; N. L. David and N. M. Wade (2007): Control of *Meloidogyne chitwoodi* in potato with shank-injected Metam sodium and other nematicides. J. Nematol Vol. 39 (2): 161–168.

Kuwata , H.; H. Saithoh and K. Shinada (1994). Occurrence of Fusarium wilt of tomato caused by *Fusarium oxysporum f.sp.lycoprersici* race J2, in Aonori prefecture and its control by grafting to rootstocks. Aomor :- Ken Nogyo Shikenzo kenkya Hokoka = Bolletin of the Aomori Agricultural Experiment Station No. 34, 1-10 (c.f. Rev. Plant Pathol.,73 :647).

Ibekwe , A.M.; S. K. Papiernik and C. H. Yang (2010). Influence of soil fumigation by methyl bromide and methyl iodide on rhizosphere and phyllosphere microbial community structure. J.of Environ. Sci. and Health,45 (5): 427-436.

Lopez-Aranda, J. M.; M. Luis ; J. Medina ; Soria Carmen ; de los Santos' Berta Romero ; F. Perez-Jimenez; M. Rosa ; T. Miguel; F. A. Steve and Santos M. Biclinski (2009). Methyl bromide alternatives for high tunnel strawberry production in southern Spain. Hort .Technol., 19:187-192.

Lopez-Aranda, J. M.; F. Gómez; M. Puga ; R. Zamora; O. Daugovish and M. A. Cotero (2016). Chemical soil fumigation for strawberry nursery in Jalisco (Mexico). J. of Fruit Sci., 1-12.

Martínez. R.; M.I. Aguilar ; M.L. Guirado; A. Álvarez and J. Gómez (2002) . First report of Fusarium wilt of cucumber caused by *Fusarium oxysporum* in Spain. J. ISSN 2044-0588.

Paker, Monika (1999). Fusarium root and stem rot of greenhouse cucumbers in British Columbia - host range, epidemiology and disease control. M.S, Thesis., Dept. of Biol. Sci., Simon Fraser Univ.Canada.

Paternotte, S. J. (1987). Pathogenicity of *Fusarium solani* f. sp. *cucurbitae* to courgette . Nether .J . Plant. Pathol., 93 :245-252.

Qiao, K. ; H.Y. Wang ; X.B. Shi and K.Y. Wang (2010). Effects of 1,3-dichloropropene on nematode, weed seed viability and soil-borne pathogen. J. Crop Prote., 29 : 1305-1310.

Santos, B.M.; J.P. Gilreath and T.N. Motis (2005). Managing nutsedge and sting nematode with reduced methyl bromide plus chloropicrin rates under virtually impermeable films in pepper. Hort. Technol., 15,596–599.

Salt, G. A. (1982): Factors affecting resistance to root-rot and diseases (eds. G.Hawtin and C. Webb); faba bean improvement. ICARDA, Aleppo, Syria, 260-270.

Vakalounakis, D.J. (1996) . Root and stem rot of cucurnber caused by *Fusarium oxysporum* f. sp. *radicis*-cucumerjnum f. sp. nov. Plant Dis. ,80:313-316.

Vachev , D. (2015). Fusarium root and stem rot of greenhouse cucumber: Aerial dispersal of inoculums. Bulgarian J. of Agric. Sci., 21 (3): 650-654

Zitter T.A.; D.L.Hopkins and C.E. Thomas (1996). Compendium of Cucurbits Diseases. APS Press, St Paul, Minn.