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Seasonal fluctuation and biological control of root- knot nematodes *Meloidogyne incognita* on cucumber

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Abstract

The experiments were conducted under both greenhouse and field conditions to determine seasonal fluctuation and application of some bioagents to control root-knot nematode *Meloidogyne incognita* on cucumber. Seasonal fluctuation of the root-knot nematode *M. incognita* was studied on two cucumber cultivars (*Madia* and *Slaprty*) during the period from March to November in seasons 2015/2016 and 2016/2017. Soil population increased gradually on two cultivars during spring and throughout summer to reach peak in August began to decrease during autumn and reach the lowest in winter (January and February) in two seasons where soil temperature reached 15± 4ºC. Seven treatments *Paecilomyces lilacinus*, *Arthrobotrys oligospora*, *Glomus faciculatum*, *Eucalyptus globules*, *Tagetes erecta*, *Allium sativum* and Fenamiphos (10%G) were evaluated to control *M. incognita*. All the treatments decreased the nematode population of *M. incognita* in soil and on roots compared with check. Data indicated percent increase of fresh weight of the whole plant which was greatly improved in all treatments.

Keywords: *Meloidogyne incognita*, seasonal fluctuation, biological control, cucumber.

Cucumber (*Cucumis sativus* L.) is the most important tropical vegetable crop widely used throughout the world. In recent years, root-knot nematodes *Meloidogyne* spp. problem has become a threat to cucumber cultivation under Egyptian conditions (Ibrahim, 1985). Control of root-knot nematodes has been primarily accomplished through chemical nematicides. However, indiscriminate use of chemical pesticides causes great harm to human beings, animals, vegetation and to environment. Shawky *et al*., (2010) found out that nematode population of *M. incognita* increased during July, August and September in two cassava cultivars (*Brasilia* and *Endonisy*) and then nematode population decreased from November to December. Stephan *et al*., (1998) studied bio-control agents and nematicides for the control of *M. incognita* on tomato and eggplant. They revealed that ethoprophos was most effective followed by *Paecilomyces lilacinus* and *Acremonium butyric.* Gupta & Sharma (1993) tested three *Allium* sp. against root-knot nematode *M. incognita* for their nematicidal and vicinal action they found that larvae were killed with all plant extracts at 5% concentration; also after 12 days, it was noticed that garlic was less toxic to nematicide. Recently, numerous researchers has focused on biological control agents with the objective of controlling the parasitic nematodes and to overcome the nematode damage by using *mycorrhizal* fungus (Duponnois & Plenchette, 2003; Serfoji *et al*., 2010; Soliman *et al*., 2011). Management of root-knot nematode, *M. incognita* on tomato *cv.*

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Pusa Ruby by using compost, *Arbuscular mycorrhiza* fungus was studied. Both Vydate and *Arbuscular mycorrhiza* were the most effective treatments in decreasing the final nematode population and rate of build-up of root-knot nematode (Soliman *et al*., 2011). They found out that combination of *P. lilacinus + D. stramonium* showed the highest efficacy in reducing the nematode population in both soil and roots. Plant extracts of *Tagetes erecta* and *T. patula* have nematicidal effect on root-knot nematode (Kimpinski *et al*., 2000; Verma, 2006; Khalil & Shawky, 2008).

The aim of the present work was to study the control to *M. incognita* using different bio-agents and plant extracts. Furthermore, seasonal fluctuation was also the part of assessment targeting infected cucumber from *M. incognita*.

Material and Methods

1- Greenhouse experiments

Efficacy of bioagents and plant extracts to control *Meloidogyne incognita*

The treatments used for the nematicidal activity are:

1- *Paecilomyces lilacinus*, 2-*Arthrobotrys oligospora*, 3-*Glomus faciculatum*, 4-*Eucalyptus glolbules*, 5-*Tagetes erecta*, 6- *Allium sativum*, 7-Fenamiphos (10% G).

The concentrations of both *Paecilomyces lilacinus* and *Arthrobotrys oligospora* were (1×10^6, 3×10^8, 5×10^8cfu)/ml/plant while, *Glomus faciculatum* was (500 spores/g) at 5, 10, 15 g / plant and were obtained from Microbiology Department, Soils, Water and Environment Research Institute, Agriculture Research Center.

The nematicide Fenamiphos (10% G) was used as 0.4g/pot recommended concentration. Cucumber (*cv. Slaprty*) seedlings of two weeks old were transplanted individually in 25cm diameter clay pots. Each pot filled with steam sterilized sandy loam soil (18% clay, 10% silt and 72% sand). In the same day after transplantation of cucumber seedling five pots were amended with powdered leaves of *Tagetes erecta* and other five pots were with powdered garlic gloves.

Two weeks after cultivation each pot was inoculated with 3000 newly hatched larvae of *M. incognita* around the roots. All treatments received the same agricultural treatments. Each treatment replicated five times. Also, five cucumber plants were treated with newly hatched larvae of *M. incognita* alone and served as control. All pots were arranged in Randomized Complete Block Design (RCBD), and kept under greenhouse conditions at about 25-28^{o}C.

Sixty days after inoculation, all plants were carefully uprooted and fresh root and shoot systems were weighted. Nematode populations in soil per pot were determined according to Goodey (1957). Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*., (1983) and examined for counting number of developmental stages and females/root. Number of galls, egg-masses and eggs/egg-mass per plant were counted. *M. incognita* was extracted by using sodium hypochlorite (NaOCl) method as described by Hussey & Barker (1973). Also the final nematode population (Pf) and rate of buildup of *Meloidogyne incognita* (Pf/Pi) were calculated according to Oostenbrink (1966) as follows:

Final nematode population (Pf) = (No. of egg-masses x no. of eggs/egg-mass) + No. of females + No. of developmental stages + No. of juveniles in soil/pot.

Reproduction factor= Pf/Pi

RGI Root gall index (RGI) was rated on a scale of 0-5 where: 0= no galls or egg-masses, 1= 1-2, 2= 3-10, 3= 11- 30, 4= 31-100 and 5= more than 100 galls or egg-masses (Taylor & Sasser, 1978).

Rate of build up = Pf/Pi

Reduction % = Rate of build up in control - rate of build up in treatment x1 x100

Increase % = Weight plants in the treatment - Weight plants in the control x 100/weight plants

in the control.

2- Field experiment

Seasonal fluctuation of root-knot nematode *Meloidogyne incognita*

The seasonal fluctuation of the root-knot nematode *M. incognita* was studied on two cucumber cultivars (*Slaprty* and *Madia*) under two different field conditions in sandy and silt, naturally infested soil at El-Giza and El-Ismailia. Fifty samples were taken per month from each field.

The root-knot nematode *M. incognita* population was followed on the two cucumber cultivars during two seasons in the period from March to February in 2015/2016 and 2016/2017. Nematode population in soil (number of juveniles/250g soil) was determined each month. Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*., (1986) to record the number of developmental stages and egg laying females/g of root. Eggs/egg-mass of *M. incognita* was extracted by using sodium hypochlorite (NaOCl) method as described by Hussey & Baker (1973).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) (Gomez & Gomez, 1984) and means were compared by using L.S.D. at 5 % level of significance.

This work was undertaken in the greenhouse of Nematology Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, and in a naturally infested field with *M. incognita* on cucumber plants in two farms in Giza and in Ismailia, Egypt.

Results

1- Greenhouse experiments

Efficacy of bioagents and plant extracts to control *Meloidogyne incognita*

The population of root-knot nematode *M. incognita* was significantly influenced by the applied treatments bioagents as well as dry plant extracts. *Glomus faciculatum* was most effective bioagent at the rate of 15g, significant differences have been found as compared to control treatments.

In *G. faciculatum* minimum nematode population was traced in 250g of soil. The parameters of nematode population are directly proportional to RGI in all applied treatment whereas reduction percentage was inversely proportional to the concentration of treatments.

Least population reduction was obtained by applying *Arthrobotrys oligospora* at the concentration of $1x10^6$cfu/ml. Among plant extracts *Tegetes erecta* was found to be the most efficient plant extract as compared to control (Table 1).

All treatments induced remarkable improvement in plant growth parameters. Significant increased was observed in shoot and root length and fresh weight treated with *G. faciculatum* at concentration of 15 g and *T. erecta* at 20g applied concentration. Fresh weight and increased % were also obtained by applying *T. erecta* at the concentration of 20g. Conventional nematicide response corresponded more or less to the plant extract showing non-significant differences among them (Table 2).

Efficacy of combinations of bioagents and dry leaves plants on cucumber (*cv. Slaprty*) infected with *M. incognita*.

Data in Table (3 & 4) illustrated that all tested treatments in combination were effective in controlling root-knot nematode *M. incognita* under greenhouse conditions.

Data revealed that *Allium sativum* in combination with *Glomus faciculatum* has shown non-significant difference in nematode population as compared to Fenamiphos (nematicide). Minimum numbers of galls were obtained from *Eucalyptus globule*s + *Glomus faciculatum* as compared to control. Non-

significant (reduction percentage) was found for all treatment combinations. The combined effect of treatments has shown significant results on growth parameters of cucumber plants. Maximum shoot length was obtained after the treatment of *Allium sativum* + *Glomus faciculatum* i.e. 125cm whereas minimum was gained from *Eucalyptus globules* + *Paecilomyces lilacinus* (79). The plants treated with *Eucalyptus globules* + *Paecilomyces lilacinus* significantly reduced root length (9.4); maximum was obtained by treating *Tegetes erecta* + *Glomus faciculatum* (107). Weight of shoot was significantly high in treatments of plant extract. Root weight was found maximum after treating *Allium sativum* + *Glomus faciculatum.* Maximum weight increased (147) was calculated of *Allium sativum* + *Glomus faciculatum.*

2- Field experiment

Seasonal fluctuation of the root-knot nematode *Meloidogyne incognita*

Results showed that *Slaprty* cultivar was more susceptible than *Madia* cultivar in two seasons. The sandy soil favored nematode *M. incognita* reproduction. The nematode population in soil, number of galls, and egg-masses per root were remarkably higher on the cultivar *Slaprty* in season 2015/2016 than season 2016/2017 (Fig. 1-4). Nematode Population of *M. incognita* increased during spring to summer from March to September. The maximum population fluctuation of *M. incognita* multiplied in summer during July and August, on the two cultivars of cucumber and decreased in autumn from November to reach minimum in winter in January and February in two seasons. The numbers of galls and egg-masses were remarkably higher on cultivar *Slaprty* in sandy soil than silty soil.

Discussion

The present study has demonstrated the significant effectiveness of bio-agents alone and in combinations with plant extracts for controlling growth parameters of cucumber plants and nematode population in soil. On the other hand, seasonal fluctuations in relation to host type and nematode population were also evaluated and obtained positive correlation between these parameters. Widmer & Abawi (2000) found that the pathogenic fungus *Paecilomyces lilacinus* is the most widely tested biological control agent used for management of plant-parasitic nematodes. According to Ahmed *et al*., (1992) Marigold (*Tagetes* spp.), castor bean and chrysanthemum plants grown alone or along with nematode susceptible crops suppressed the population density build up of *M. incognita*. However, the treatment of *P. lilacinus* alone showed the least effect. Khalil & Shawky (2008) studied the combine effect of *P. lilacinus* + *D. stramonium* induced a remarkable reduction in the nematode build up and improved both plant growth and tuber weight of potato plants in relation to nematode alone. Khan *et al*., (1997) reported that *P. lilacinus* at1.0g/kg gave better control against *M. incognita* and increased growth of papaya plants. Nematicide Fenamiphos (10%G) caused significantly reduction in nematode infection on cucumber plants which reached 98.3% and reduction in egg-masses 98.3% compared with control (nematode alone). These results are in agreement with Siddiqui *et al*., (2009) who cleared that prorated Fenamiphos at 0.6 g a.i/m^2, and carbofuran at 0.3g. /m^2 were effective in improving plant growth and reducing *M. incognita* galling in cucumber. Khalil & Shawky (2008) added the information that *Arbuscular mycorrhiza* was the most effective treatment in decreasing the final nematode population of root- knot nematode. Further, the combination of *P. lilacinus*+ *D. stramonium* showed the highest efficacy in reducing the nematode population in both soil and roots Kellam & Schenck (1980). Soliman *et al*., (2011) worked on the combination of *P. lilacinus* + *D. stramonium* and showed the highest efficacy in reducing the nematode population in both soil and roots. Similar results found in the experiments of Khalil & Shawky (2008). The combing effect of *P. lilacinus* + *D. stramonium* induced a

remarkable reduction in the nematode build up and improved both plant

Table 1. Influence of some bioagents and dry leaves plants on population and reproduction of *M. incognita* infecting on cucumber under greenhouse conditions.

Treatments	Conc.	Nematode population				Red %	No. galls	RGI*	No. egg-masses	Red %
		250g soil	Count*	Total	Pf/Pi					
Paecilomyces lilacinus	1×10^6cfu/ml	144^{c}	110^{d}	254	0.127	72.7	98d	4.0	110^{f}	74
	3×10^8cfu/ml	125^{d}	107^{d-e}	232	0.116	75.1	88^{e}	4.0	95^{g}	78.26
	5×10^8cfu/ml	99^{e}	75^{f}	174	0.087	81.3	75^{g}	4.0	85^{h}	80.54
Arthrobotrys oligospora	1×10^6cfu/ml	165^{b}	130^{b}	295	0.147	68.3	112^{b}	5.0	155^{b}	53.31
	3×10^8cfu/ml	143^{c}	117^{cd}	260	0.130	72.1	106^{c}	5.0	138^{c}	
	5×10^8cfu/ml	120^{d}	$88e^{-f}$	200	0.100	78.5	100^{d}	4.0	120^{d}	63.85
Glomus faciculatum	5g	130^{b}	120^{c}	250	0.125	71.5	103^{cd}	5.0	123^{b}	62.95
	10g	110^{e}	86^{e}	196	0.098	78.9	96^{d-e}	4.0	114^{e}	65.66
	15g	80^{f}	64^{g}	154	0.077	83.4	81f	4,0	78^{h-i}	74.50
Eucalyptus globule	20g	64^{g}	34^{h}	98	0.044		54^{h}	4.0	51^{h}	84.63
Allium sativum	20g	45	30^{h}	75	0.375	92.9	35i	3.0	31^{j}	92.90
Tagetes erecta	20g	43h	27i	70	0.035	92.4	30i	3.0	35i	89.45
Fenamiphos	0.4g	3^{i}	6^{j}	9	0.003	98.8	3^{j-k}	2.0	$3j^{-k}$	99.31
Control		416^{a}	516^{a}	932	0.466	-	230^{a}	5.0	437^{a}	-

No. of nematode /250g soil; *No. of females+ Developmental stages; Each value is the mean of five replicates.
Mean in each column followed by the same letter (s) are not significantly different ($P\leq 0.05$) according to Duncan's multiple range test.

Table 2. Impact of bioagents and dry leaves plants on growth parameters of cucumber infected with *Meloidogyne incognita* under greenhouse conditions.

Treatments	Conc.	Growth parameters					
		Length (cm)		Weight (g)		Fresh wt.(g)	Increase%
		Shoot	Root	Shoot	Root		
Paecilomyces lilacinus	1×10^6cfu/ml	34^{e-f}	5.4^{d}	8.5^{e}	2.5^{c}	11.0	46.6
	1×10^8cfu/ml	37^{e}	5.6^{d}	9.0	2.7c	11.7	56.0
	5×10^8cfu/ml	40d-^{e}	6.4^{c}	9.5^{d}	3.4^{b-c}	12.9	72.0
Arthrobotrys oligospora	1×10^6cfu/ml	31^{f}	5.5^{d}	7.4^{f}	2.0^{d}	9.4	25.3
	1×10^8cfu/ml	34^{e-f}	6.6c	8.5	2.5^{c}	11.0	46.6
	5×10^8cfu/ml	37^{e}	8.0^{b}	9.4^{d}	3.4^{b-c}	12.8	70.6
Glomus faciculatum	5g	42^{d}	9.1^{a}	10.4^{e}	2.8^{c}	13.2	77.3
	10g	47^{c}	9.3^{a}	11.0^{b}	3.5b-^{c}	14.5	93.3
	15g	55^{b}	9.6a	12.7^{a}	4.4a	17.1	128.0
Eucalyptus globules	20 g	49^{c}	8.4^{b}	8.4^{e}	2.6^{e}	11.0	46.6
Allium sativum	20g	58^{b}	9.4^{a}	8.6^{e}	3.8^{h}	12.4	65.3
Tagetes erecta	20g	54^{b-c}	9.5^{a}	10.4^{c}	3.7^{b-c}	14.1	88.0
Fenamiphos	4g	62^{a}	9.7^{a}	11.0^{b}	3.9^{b}	14.9	98.6
Nematode alone (control)	-	21^{f}	3.1^{e}	7.3^{f}	0.2^{e}	7.5	-

Table 3. Influence of some combinations of bioagents and dry leaves plants on population and reproduction of *M. incognita* infecting on cucumber under greenhouse conditions.

Treatments	Nematode population				Red %	No. of gall	RGI	Egg-mass	Red %
	In soil*	No. of females**	Pf	Rf					
Tagetes erecta+Paecilomyces lilacinus	37[d]	32c[-d]	69	0.02	89.4	37b	4	24d[e]	93.6
Tagetes erecta+Arthrobotrys oligospora	32[c]	27[c]	59	0.01	90.0	31c	4	21[c]	94.4
Tagetes erceta+Glomus faciculatum	81[f]	17[c]	38	0.01	94.1	24d-e	3	17[e-f]	95.4
Allium sativum+Paecilomyces lilacinus	30[e]	25[d]	55	0.01	95.9	27[d]	3	19[e]	94.9
Allium sativum+Arthrobotrys oligospora	21[g]	13f	31	0.01	95.2	16[f]	3	14[f]	96.2
Allium sativum+Glomus faciculatum	14[g-h]	11[f]	24	0.00	96.3	14[f]	3	12[g]	96.8
Eucalyptus globules+Paecilomyces lilacinus	61[b]	43[b]	104	0.03	84.4	23[e]	3	39[b]	89.6
Eucalyptus globules+Arthrobotrys oligospora	46[c]	37[c]	83	0.02	87.2	16[e-d]	3	33[c]	91.2
Eucalyptus globules+Glomus faciculatum	32[e]	26[d]	58	0.02	91.1	4[g]	2	27[d]	92.2
Fenamiphos	7[h]	4[g]	11	3.66	98.3	7[g-h]	2	6[h]	99.3
Nematode alone (control)	321[a]	332[a]	653	0.21	-----	372[a]	5	377[a]	-----

*No. of nematodes - ** Count of females + developmental stages

Table 4. Impact of some combinations bioagents and dry leaves plants on growth parameters of cucumber infected with *M. incognita* under greenhouse conditions.

Treatments	Growth parameters				Fresh wt.	Increase%
	Length (cm)		Weight (g)			
	Shoot	Root	Shoot	Root		
Tagetes erecta + Paecilomyces lilacinus	87[e]	10.3[f]	21.4e	10.7[d]	31.1	80.8
Tageteserecta+Arthrobotrys oligospora	94[d]	11.1[e]	23.3[d]	12.9	36.2	110.4
Tageteserceta +Glomus faciculatum	107[b-c]	14.2[b]	24.4[b]	13.4[c]	37.8	119.7
Allium sativum +Pecilomyces lilacinus	95[d]	10.7[e]	22.4[c]	13.6[c]	35.8	108.1
Allium sativum +Arthrobotrysoligospora	110[b-c]	12.4[d]	24.5[a-b]	15.6[b]	40.1	133.1
Allium sativum + Glomus faciculatum	125[a]	15.0[a]	25.6[a]	16.9[a]	42.5	147.9
Eucalyptus globules+Pecilomyceslilacinus	79[f]	9.4[g]	15.2[g]	8.6[f]	23.8	38.3
Eucalyptus globules+ Arthrobotrysoligospora	85[e-f]	10.0[f]	17.3[f]	10.5[e]	27.8	61.6
Eucalyptus globules + Glomusfaciculatum	105[e]	13.1[e]	18.5[d]	10.9[d]-[e]	29.4	70.9
Fenamiphos	115[b]	13.3[e]	19.3[d]	14.0[b]	33.3	93.6
Nematode alone (control)	72[g]	4.6[h]	11.0[h]	6.2[f-g]	17.2	------

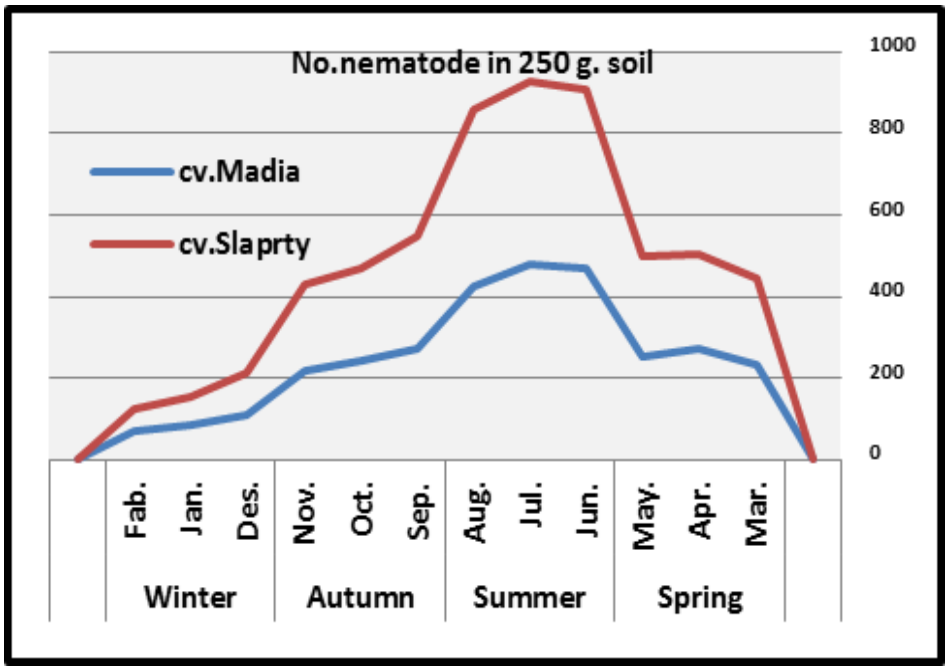

Fig. 1. Seasonal fluctuation on numbers of *M.incognita* on two cucumber cultivars in sandy soil in season 2015/2016.

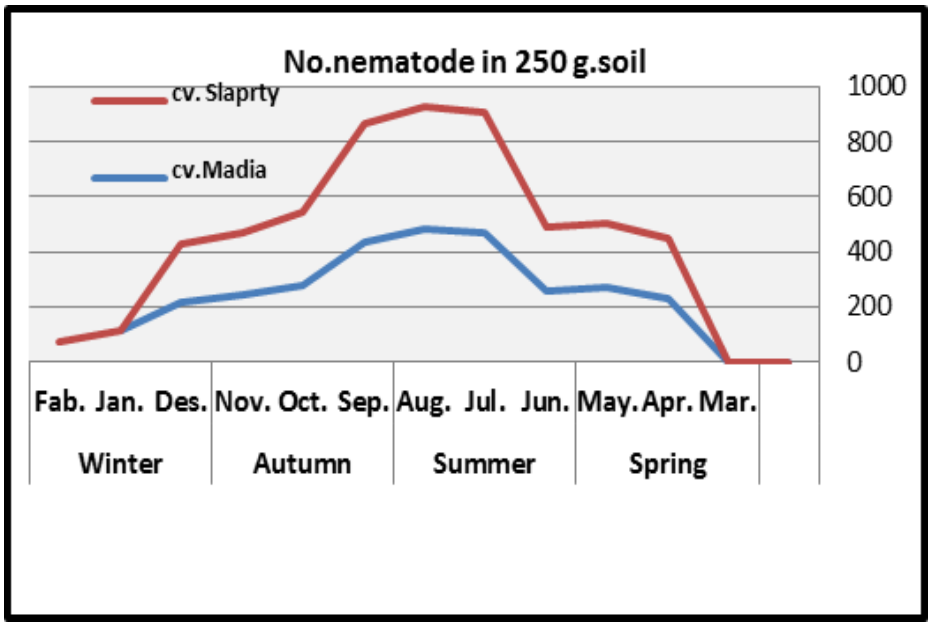

Fig. 2. Seasonal fluctuation on numbers of *M. incognita* on two cucumber cultivars in slit soil in season 2015/2016

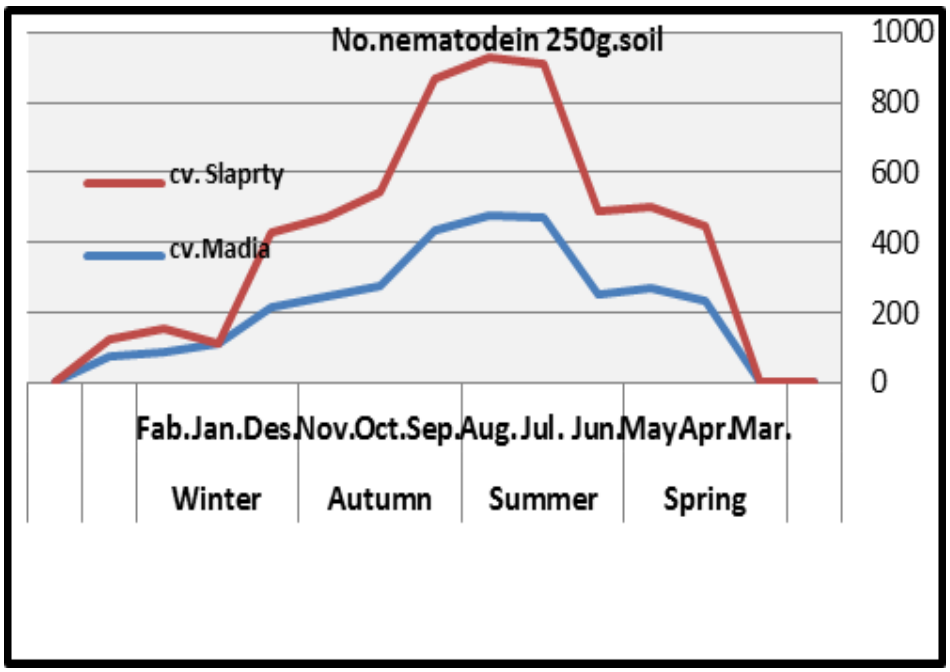

Fig. 3. Seasonal fluctuation effect on nematode population on two cucumber cultivars in slit soil in season 2016/2017.

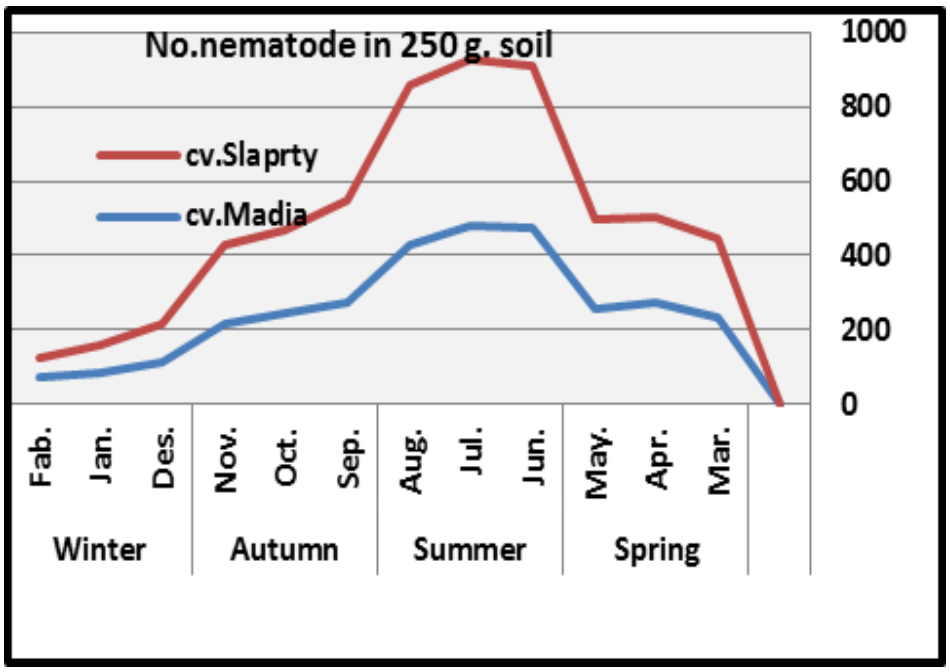

Fig.4. Seasonal fluctuation effect on nematode population on two cucumber cultivars in sandy soil in season 2016/2017.

growth and tuber weight of potato plants in relation to nematode alone. Further, they found that the presence of *Glomus faciculatum* reduced the number of galls produced by *M. incognita*. The present findings are also in agreement with those of Sivaprasad *et al*., (1990) who indicated that inoculation with *G. fasiculatum* significantly reduced galling and population of *M. incognita* infected *Piper unigram* plants and agree with those of Jothi & Sundarababu (2002) who observed that the root galling indices caused by *M. incognita* was reduced significantly with the mycorrhizae fungus (*Glomus mosseae*) on treated eggplants (*Solanum melongena*). Yossef & Aboul-Eid (1996) reported that population peaks of *M. incognita* were negatively correlated with moderate soil temperature of 17-19ºC.

However, the population of *M. incognita* in roots reached its peaks in July and August and positively correlated with the highest soil temperature of 26-30ºC. The results are in agreement with those of Mittal & Prasad (1998) who studied the seasonal population fluctuation of *M. incognita* during October, 1989 to September, 1990 in soil of soybean–wheat rotation and stated that the major population fluctuation with maximum seasonal fluctuation found in August with *M. incognita* multiplication during July, August and December.

Barker (1986) reported that yield losses were greatest in sandy and in clay soils. As well as Ahmed & Sayed (1991) reported that the lowest populations of *M. incognita* occurred in clay loam soil grown with cowpea while the highest nematode populations in sandy soil. In 1997, El-Shawadfy & Mousa stated that the distribution of *Meloidogyne* spp. was related to soil texture and the incidence of *M. incognita* was greatest in the sandy soil. The lowest occurrence of nematodes was recorded in the heavy soil of middle of the Delta region. Shawky *et al*., (2010) added that nematode population of *M. incognita* increased during July, August and September in two cassava cultivars (*Brasilia* and *Endonisy*) and then nematode population decreased from November to December. This study revealed that root-knot nematode was the common nematode disease on cucumber plants in the reclaimed area in Egypt. Seasonal fluctuation of *M. incognita* was increased in the months (June, July and August) on two cucumber cultivars (*Madia* and *Slaprty*). *Slaprty* cultivar was more susceptible than *Madia* to the root-knot nematodes. The distribution of *M. incognita* was greatest in the sandy soil. Biological control gave very promising results in this concern. All these treatments of fungi have shown potential to retain nematode population. The possibility of using this trend for integrated control of nematode clearly needs further investigation.

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