Minufiya J. Agric. Res. Vol.39 No. 2(2): 737 - 745 (2014) "http://www.mujar.net"

EFFECT OF SOIL TYPES AND NEMATICIDES ON THE VEGETATIVE GROWTH AND SOME CHEMICAL CONTENT OF STEVIA PLANTS INFECTED WITH *MELOIDOGYNE INCOGNITA* NEMATODE

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(Received : Jan 12 , 2014)

ABSTRACT : *A pot experiment was conducted to determine the effect of three soil types, (sandy – loamy sand (1:1) – loamy) and nematode inoculum levels (0 - 500 - 1000 - 1500) second stage juveniles of Meloidogyne incognita on vegetative growth of Stevia plants (Stevia rebaudiana) and nematode development. In addition, to evaluate the efficacy of the nematicides, (Mocap – Nemathorin – Vydate – Nemacur – Rugby - Cropguard) in the control of M. incognita infecting Stevia plants and its effects on the total soluble carbohydrates and steviosides of Stevia plants under green house conditions. Results indicated that the highest numbers of M. incognita in Stevia roots (galls and females) were recorded in sandy soil, while the lowest was observed in loamy soil. Application of nematicides Vydate, Nemathorin, Nemacur and Rugby recorded 80, 75, 75, 70% reduction in root knot nematode galls, respectively, and recorded 80, 70, 60, 50% reduction in numbers of nematode females, respectively. As for the contents of Stevia plants of total soluble carbohydrates and steviosides , results indicated that there were no effects and no significant differences between the content of treated plants with nematicides and control treatment . The content of total soluble carbohydrates were ranged between 45.23 – 47.81 mg/1g dry leaves in nematicides treatments compared with 44.73 mg/1g dry leaves in control , in addition the content of Stevia plants of steviosides were ranged between 39.24 – 41.93 mg/1g dry leaves at nematicides treatments compared with 38.72 mg/1g dry leaves in control treatment.*

Key words: *Meloidogyne, nematicides, Stevia, soil type , total soluble carbohydrates, steviosides .*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is an important medicinal plant, recently introduced to Egypt. *Stevia* is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions from western North America to South America. The species *Stevia rebaudiana*, commonly known as sweet leaf, sugar leaf, or simply stevia, is widely grown for its sweet leaves. As a sweetener and sugar substitute, stevia's taste has a slower onset and longer duration than that of sugar and some of its extracts may have a bitter or licorice-like after taste at high concentrations.

With its steviol glycoside extracts having up to 300 times the sweetness of sugar, stevia has attracted attention with the rise in demand for low-carbohydrate, low-sugar sweeteners. Because stevia has a negligible effect on blood glucose it is attractive to people on carbohydrate-controlled diets.

The availability of stevia varies from country to country. In a few countries, it has been available as a sweetener for decades or centuries; for example, it has been widely used for decades as a sweetener in Japan. In some countries health concerns and political controversies have limited its availability; for example, the United States banned stevia in the early 1990 unless labeled as a dietary supplement, but in 2008 it approved rebaudioside. A extract as a food additive. Over the years, the number of countries in which stevia is available as a sweetener has been increasing. In 2011, stevia was approved for use in the EU. Stones (2011) and Wikipedia, (2014)

The leaves of Stevia contain steviosides, which used an alternative to artificially produced sweeten. Stevia was considered hypoglycemia, hypostasis, diuretic, cordial and tonic (Baiuomy, 2007). The leaves are used against diabetes, obesity (Darise *et al.,* 1983; Martelli *et al.,* 1985; Crammer and Ikan, 1986; Lewis, 1992; Hanson and Oliveira, 1993 and Megeji *et al.,* 2005) Root knot nematode infection was observed in the roots of stevia (Sultan *et al.,* 2010) .

The aim of this experiment was to study the effect of different soil types on the vegetative growth and nematode development of Stevia plants infected with *Meloidogyne incognita*, in comparison with control, in addition to study the efficacy of six nematicides against nematode, in relation to the Stevia plant contents of total soluble carbohydrates and steviosides , under greenhouse conditions.

MATERIALS AND METHODS

Source of nematodes:

The nematode population used in this study originated from greenhouse culture maintained at the Plant Pathology Research Institute, Agric. Res. Centre, where *Meloidogyne incognita* (Treub) Chitwood was reared in a greenhouse on tomato plants. Eggs of *M. incognita* were extracted from roots in 0.5% sodium hypochlorite (Hussey and Barker, 1973) and caught on 25 µ sieve. Second stage juveniles (J_2) were hatched from these eggs on Baermann funnels and only (J_2) less than 2 days old was used for inoculation.

Soil types:

Three soil type textures i.e., sandy soil, loamy sand soil (1 : 1) and loamy soil were separately steam-sterilized and used in this experiment.

Greenhouse experiments:

A- Twenty Stevia seedlings three weeks old were transplanted into plastic pots (25 cm diam.) filled with autoclaved sandy soil, twenty Stevia seedlings three weeks old were transplanted into plastic pots (25 cm diam.) filled with autoclaved loamy sand (1 : 1) and twenty Stevia seedlings three weeks old were transplanted into plastic pots (25 cm in diam.) filled with autoclaved loamy soil. Each pot contained one Stevia seedling. After seven days each soil type pots were assigned to four groups:

1) Five Stevia pots inoculated with 500 second stage juveniles of *M. incognita* / pot.
2) Five Stevia pots inoculated with 1000 second stage juveniles of *M. incognita* per pot.
3) Five Stevia pots inoculated with 1500 second stage juveniles of *M. incognita* / pot.
4) Five Stevia pots was free of *M. incognita* inoculum and served as control treatment.

Each treatment was replicated five times and arranged as randomly block design. Pots were kept in the greenhouse at 30 ± 2°C receiving water and ordinary nutrient solution as required. Sixty days after the beginning of the experiment Stevia plants were uprooted and washed with tap water and the number of root galls, eggmasses, females and number of egg in eggmasses was counted and recorded. Juveniles of *M. incognita* in 250 g soil were extracted by sieves and Baermann funnels and counted. Plant growth criteria recorded i.e., root length, root weight, shoot length and shoot weight.

B- Thirty-five Stevia seedlings three weeks old were transplanted to plastic pots (25 cm diam.) filled with autoclaved loamy sand (1 : 1) after seven days each Stevia pot was inoculated with 1500 second stage juveniles of *M. incognita*. After thirty days pots were assigned to seven groups and nematicides were added as follows:

1) The first group (five pots) were received 0.5 g / pot of Mocap 10% G
2) the second group (five pots) were received 0.2 g/ pot of Nemathorin 10% G.
3) the third group (five pots) were received 0.1 ml / pot Vydate 24% sL.
4) the fourth group (five pots) were

received 0.1 ml / pot Nemacur 40% Ec.

5) the fifth group (five pots) were received 0.1 ml / pot of Rugby 20 % Cs.
6) the sixth group (five pots) were received 0.5 ml each pot Cropguard 90 % Ec.
7) the seventh group (five pots) without nematicides and served as control treatment.

Forty-five days after the application of nematicides Stevia plants were uprooted and washed with tap water. Numbers of root galls, and females were counted and recorded. Juveniles of *M. incognita* in 250 g soil from each pot were extracted by sieves and Baermann funnels and counted.

Total soluble carbohydrates and steviosides were recorded as mg/one g dry leaves. Total soluble carbohydrates were determined in Sugar Crops Res. Inst. ARC, Egypt, according to AOAC (1995) method.

Moreover, stevioside was calculated according to the formula suggested by Nishiyama *et al.* (1991) and applied by Allam *et al.* (2001) and Nassar *et al.* (2006) as the following formula:

$$\text{Stevioside} = \frac{\text{total carbohydrates} - 7.56}{0.96}$$

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan's multiple range tests to compare means (Duncan, 1955) using L.S.D. at 5%.

Reduction percentages were counted according to of Abbott's formula (1925).

RESULTS AND DISCUSSION

Data presented in Table (1) revealed that treatments of sandy , loamy sand (1 :1) and loamy soil types were significantly encouraged the number of *M. incognita* population in Stevia plants. Results showed that the soil type loamy sand (1:1) registered the first rank in the high number of *M. incognita* juveniles in soil at all inoculum levels of the root knot nematode, *Meloidogyne incognita* 500, 1000 and 1500 juveniles per pot. Numbers of galls and females in Stevia roots were the highest in the Stevia plants in sandy soil alone at the level of 1500 second stage juveniles per pot. While the loamy soil recorded the lowest numbers of *M. incognita* in soil and root (number of galls or number of females). Statistical analysis of the obtained data (Table 1) indicated that there were significant differences in the population numbers of nematode stages among the three soil types , as well as among the three inoculum levels of nematodes.

Table (1). Effect of three soil types and three inoculum levels of *Meloidogyne incognita* on their developing stages in Stevia plants under greenhouse conditions.

Soil types	Nematode inoculum	juveniles /250 g soil	Average no of nematode stages / 1 g roots			
			galls	egg masses	eggs/egg mass	females
Sandy	500	120.0 ^{f}	12.0 ^{cd}	0.0 ^{b}	0.0 ^{c}	5.0 ^{bc}
Sandy	1000	360.0 ^{d}	12.0 ^{cd}	1.0 ^{b}	226.0 ^{b}	6.0 ^{bc}
Sandy	1500	720.0 ^{c}	38.0 ^{a}	13.0 ^{a}	235.0 ^{a}	24.0 ^{a}
Loamy sand	500	900.0 ^{b}	10.0 ^{cde}	0.0 ^{b}	0.0 ^{c}	6.0 ^{bc}
Loamy sand	1000	960.0 ^{b}	17.0 ^{bc}	0.0 ^{b}	0.0 ^{c}	9.0 ^{b}
Loamy sand	1500	1560.0 ^{a}	22.0 ^{b}	0.0 ^{b}	0.0 ^{c}	9.0 ^{b}
Loamy	500	100.0 ^{f}	2.0 ^{e}	0.0 ^{b}	0.0 ^{c}	0.0 ^{c}
Loamy	1000	120.0 ^{f}	7.0 ^{de}	0.0 ^{b}	0.0 ^{c}	0.0 ^{c}
Loamy	1500	240.0 ^{e}	17.0 ^{bc}	0.0 ^{b}	0.0 ^{c}	5.0 ^{bc}

L.S.D 5%	-	103.60	8.55	1.48	3.49	5.62

Means in each row followed by the same letter (s) are not significantly different at 5%

The obtained data on the vegetative growth of Stevia plants in Table (2) show that there were significant differences in the values of shoot and root weights and heights, except length of shoots, among all treatments as well as the values of the weights of whole plants , while no significant differences in the values of shoot lengths were recorded. Results indicated that the treatments of loamy sand (1:1) occupied the first rank in the fresh weight of whole Stevia plant, while the loamy soil recorded the lowest numbers in fresh weight of whole plant.

Results Table (3) showed that all nematicides greatly suppressed the nematode juveniles in the soil and reduced number of root galls and number of females in roots.

Statistical analysis of the data in Table (3) indicated that there were significant differences in the numbers of M. incognita stages in both of soil and roots between all used nematicides and control treatment.

The obtained results (Table 3) revealed that the use of nematicides Vydate, Nemathorin, Nemacur and Rugby recorded 94.8, 93.1, 93.1 and 91.8 % reduction in the number of *M. incognita* juveniles in the soil, respectively. Whereas the same nematicides recorded 80, 75, 75 and 70 % reduction in root knot nematode galls, respectively. While reduction in numbers of females has reached 80, 70, 60 and 50% at the same treatments, respectively.

Table (2). Effect of soil types and inoculum level of *Meloidogyne incognita* on the vegetative growth of Stevia plants under greenhouse conditions

Soil types	Nematode inoculum	Shoot		Root		weight of whole plant
		Length (cm)	Weight (g.)	Length (cm)	Weight (g.)	
Sandy	0	61.7 ^{a}	4.3 ^{a}	19.0 ^{abc}	3.0 ^{bcd}	7.3 ^{bc}
Sandy	500	53.7 ^{a}	2.3 ^{bc}	19.0 ^{abc}	2.1 ^{cd}	4.4 ^{def}
Sandy	1000	55.3 ^{a}	2.9 ^{b}	19.3 ^{abc}	1.9 ^{d}	4.8 ^{de}
Sandy	1500	39.7 ^{a}	1.5 ^{bc}	24.0 ^{a}	6.8 ^{a}	8.3 ^{ab}
Loamy sand	0	56.7 ^{a}	5.1 ^{a}	14.0 ^{c}	3.1 ^{bcd}	8.2 ^{ab}
Loamy sand	500	41.3 ^{a}	4.4 ^{a}	19.7 ^{abc}	3.0 ^{bcd}	7.4 ^{abc}
Loamy sand	1000	51.7 ^{a}	4.7 ^{a}	17.0 ^{bc}	4.3 ^{b}	9.0 ^{a}
Loamy sand	1500	53.0 ^{a}	2.3 ^{bc}	22.7 ^{ab}	3.7 ^{bc}	6.0 ^{cd}
Loamy	0	61.7 ^{a}	2.6 ^{b}	18.3 ^{abc}	2.7 ^{bcd}	5.3 ^{d}
Loamy	500	55.7 ^{a}	2.1 ^{bc}	16.7 ^{bc}	2.6 ^{bcd}	4.7 ^{def}
Loamy	1000	49.0 ^{a}	1.8 ^{bc}	18.0 ^{abc}	1.7 ^{d}	3.5 ^{ef}
Loamy	1500	49.3 ^{a}	1.1 ^{c}	16.3 ^{c}	2.0 ^{cd}	3.1 ^{f}
L.S.D 5%	-	ns	1.22	5.5	1.45	1.7

Means in each row followed by the same letter (s) are not significantly different at 5%

Table (3). Efficacy of nematicides on number of juveniles, galls and females of *Meloidogyne incognita* infecting Stevia plants under greenhouse conditions.

Treatments	Ave. no. juveniles / 250 g soil	Juveniles reduction (%)	no galls/1 g root	Galls reduction (%)	no females/1 g root	Females reduction (%)
Mocap 10% G	120.0 [b]	89.7	8.0 [b]	60.0	4.0 [b]	60.0
Nemathorin 10 % G	80.0 [bc]	93.1	5.0 [b]	75.0	3.0 [b]	70.0
Vydate 24 % SL.	60.0 [c]	94.8	4.0 [b]	80.0	2.0 [b]	80.0
Nemacur 40 % Ec	80.0 [bc]	93.1	5.0 [b]	75.0	4.0 [b]	60.0
Rugby 20 % Cs.	95.0 [bc]	91.8	6.0 [b]	70.0	5.0 [b]	50.0
Cropguard 90 % Es.	100.0 [bc]	91.4	6.0 [b]	70.0	4.0 [b]	60.0
Control	1160.0 [a]	–	20.0 [a]	–	10.0 [a]	–
LSD 5%	45.3	–	5.52	–	4.40	–

Means in each row followed by the same letter (s) are not significantly different at 5%

Statistical analysis of data in Table (4) revealed that there were no significant differences in the total soluble carbohydrates and steviosides contents of Stevia plants between all used nematicides and control treatment , where the total soluble carbohydrates in the nematicides treatments were ranged between 45.23 – 47.81 mg/1 g dry leaves, in comparison with 44.73 mg/1 g dry leaves at control treatment. In addition steviosides content was ranged between 39.24 – 41.93 mg/1 g dry leaves in used nematicides , in comparison with 38.72 mg/1 g dry leaves at control treatment.

Data illustrated in Fig. (1) reveal that the application of the used nematicides against *M. incognita* infecting Stevia plants slightly increased the total soluble carbohydrates and steviosides at all treatment in comparison to control treatment. Meanwhile, Vydate showed a considerable increase percentages in carbohydrates and steviosides as 6.9 and 8.3 % , respectively, while Rugby recorded only 1.1 and 1.3 % .

Finally, the obtained results indicated that the use of nematicides to control root knot nematode , *Meloidogyne incognita* infecting Stevia plants didn't significantly increased the plant content of both total soluble carbohydrates and steviosides, this due to that Stevia plants are considered poor host for this species of nematodes , thus it could be recommend that there aren't need to apply any nematicides to Stevia cultivation in the pest control programs.

Table (4). Effect of nematicides application on contents of total carbohydrate and steviosides in Stevia plant leaves, grown under greenhouse conditions.

Treatments	Total soluble carbohydrates mg/1 g dry leaves	Increase (%)	Steviosides mg/1 g dry leaves	Increase (%)
Mocap 10% G	46.81[a]	4.7	40.89 [a]	5.6
Nemathorin 10 % G	47.73 [a]	6.7	41.84 [a]	8.1
Vydate 24 % SL.	47.81[a]	6.9	41.93 [a]	8.3
Nemacur 40 % Ec	46.86 [a]	4.8	40.94 [a]	5.7
Rugby 20 % Cs.	45.23 [a]	1.1	39.24 [a]	1.3
Cropguard 90 % Es.	45.91 [a]	2.6	39.95 [a]	3.2
Control	44.73 [a]	-	38.72 [a]	-
LSD 5%	ns		ns	

Means in each row followed by the same letter (s) are not significantly different at 5%

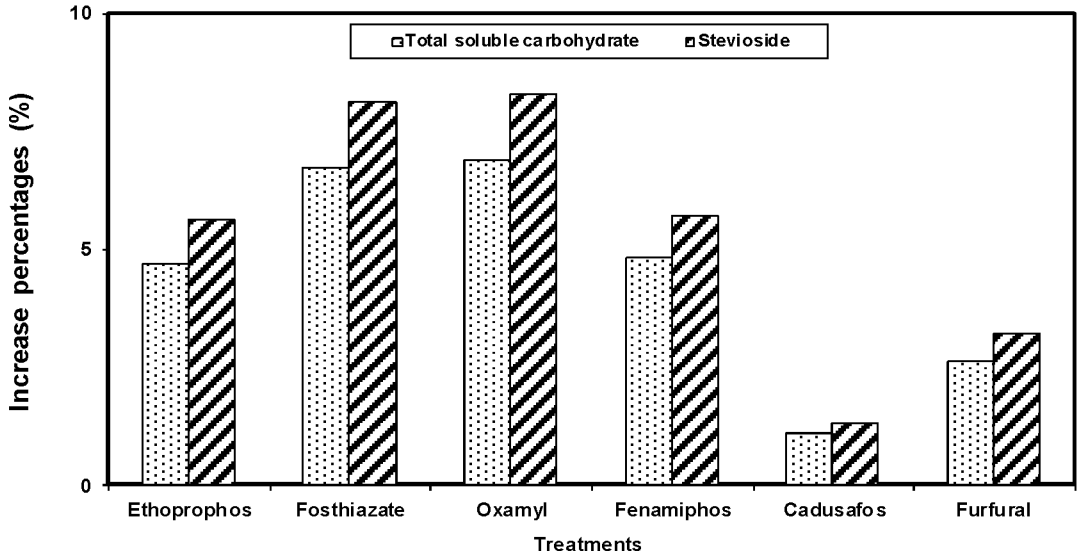

Fig. (1). Effect of nematecides in the increase percentages of total soluble carbohydrates and steviosides of stevia plants.

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تأثير نوع التربة والمبيدات النيماتودية على النمو الخضرى والمحتوى الكيميائى لنباتات الإستيفيا المصابة بنيماتودا تعقد الجذور

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الملخص العربى

يُعتبر نبات الإستيفيا من النباتات التى تتميز بأنها من النباتات الطبية ، فإن بضعة وريقات من نبات الإستيفيا كافية لتحلية كوب من الشاى وقد سجل جورج برادى عدم سُمية نباتات الإستيفيا وإمكانية استخدامها على حالتها الطبيعية بمجرد تجفيفها وطحنها ، وأنها سكر مثالى وآمن للمصابين بمرض السكر . وقد سُجلت مادة الإستيفيوسيد بمنظمة الأغذية والعقاقير الأمريكية FDA عام ١٩٩٥ ، وقد تم التصريح باستخدام مسحوق خلاصة الإستيفيا فى الصناعات الغذائية . الإستيفيوسيد هى عبارة عن مركبات جليكوسيدية ومن المعروف أن الجليكوسيد عبارة عن جزيء جلوكوز مرتبط بشق غير سكرى يُعرف بالجليكون . وقد وجد أن هذه الجليكوسيدات لا تتكسر تحت ظروف الطهى ودرجات الحرارة العالية وأن الشق السكرى للجليكوسيد يظل مرتبط ويخرج على نفس حالته بعد عملية الهضم وهذه تُعتبر ميزة فى الإستيفيا حيث أنها تحتفظ بالطعم السكرى للشق السكرى فى الجليكوسيد وفى نفس الوقت لا يستطيع الجسم استخلاص سكر الجلوكوز منها وإدخاله فى الدم وبالتالى عدم الحصول على السعرات الغير مرغوب فيها وخاصةً لمرضى السكر وراغبى خفض الوزن .

وقد أجريت هذه الدراسة بهدف التعرف على العلاقة بين نيماتودا تعقد الجذور ونباتات الإستيفيا تحت ظروف ثلاث أنواع من التربة وهى الرملية والطينية الرملية والطينية وأثر ذلك على النمو الخضرى للنباتات . وكذلك تم دراسة أثر استخدام المبيدات النيماتودية (موكاب – نيماثورين – فايدت – نيماكور – راجبى – كروب جارد) في المكافحة الكيماوية لنيماتودا تعقد الجذور المتطفلة على نباتات الإستيفيا تحت ظروف التربة الرملية الطينية وعلاقة ذلك بمحتوى النباتات من الكربوهيدرات والإستيفيوسيد .

وقد أوضحت النتائج أن أعداد النيماتودا فى التربة الرملية الطينية كان أعلى من الأعداد الموجودة فى التربة الرملية أو التربة الطينية حيث سجلت ١٥٦٠ ، ٧٢٠ ، ٢٤٠ طور يرقى / ٢٥٠ جرام تربة على الترتيب وذلك عند مستوى الإصابة الأعلى . أما بالنسبة للإصابة فى جذور نباتات الإستيفيا فقد أحدثت النيماتودا عُقد على المجموع الجذرى ولكن لم تتمكن الإناث من وضع أكياس بيض وذلك فى حالة التربة الطينية الرملية وأيضاً فى التربة الطينية أما بالنسبة للتربة الرملية فقد تم وضع أكياس بيض ولكن بنسبة منخفضة مقارنةً بعدد العقد الجذرية . لم تُسجل النتائج فروق معنوية فى أطوال المجموع الخضرى أما بالنسبة للوزن فقد سجلت النباتات النامية فى التربة الطينية الرملية أعلى وزن خضرى مقارنةً بالنباتات النامية فى التربة الطينية أو التربة الرملية.

أما بالنسبة لاستخدام المبيدات النيماتودية فقد حققت كل المعاملات خفض فى أعداد النيماتودا مقارنةً بالكنترول ولكن لم يُوجد فروق معنوية بين المركبات فى التأثير على العقد الجذرية أو أعداد الإناث . أما فى حالة اليرقات الموجودة بالتربة فكان من أفضل المركبات الفايدت ثم تلاه كل من النيماكور والنيماثورين.

وأظهرت النتائج أن محتوى النباتات من الكربوهيدرات وكذلك من الإستيفيوسيد لم تُسجل فروق معنوية بين المركبات المستخدمة والكنترول ، ولذا يتم التوصية بعدم استخدام المكافحة لنيماتودا تعقد الجذور المتطفلة على نبات الإستيفيا حيث أن هذه النباتات غير حساسة للإصابة بنيماتودا تعقد الجذور ولذلك لا يتأثر محتوى النبات من الكربوهيدرات والإستيفيوسيد نتيجة الإصابة وذلك تحت الظروف التى أُجرى فيها البحث .

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