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EFFICIENCY OF SOME BIONEMATICIDES AGAINST ROOT-KNOT NEMATODE *Meloidogyne incognita* ON THREE TOMATO CULTIVARS UNDER GREENHOUSE CONDITIONS

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ABSTRACT: This study was carried out to determine the nematicidal efficiency of seven bionematicides (Nema end, Nema cont, Nema clean, Nema K, Bio-zeid, Bio-arc and Nemex) compared with one nematicide (Nemacur 10%) on three cultivars of tomato against the root-knot nematode (RKN) *Meloidogyne incognita* under greenhouse conditions. The screened tomato cultivars (endless summer, supermarmand and jueblle) were significantly different in their response to nematode infection. Detectable tolerance to nematode infection was recorded with cultivar Endless summer and Juebelle while the least tolerance was recorded with supermarmand cultivar. Number of galls and egg masses were significantly decreased in the investigated cultivars treated with Nemacur, and Nema k, Nema cont and Bio-zeid (82.49, 73.97; 68.48, 50.00; 30.74, 33.34; 61.09, 40.62%), respectively in endless summer, (78.90, 69.92; 73.70, 55.75; 68.51, 19.48; 72.31, 43.38%), respectively, in supermarmand and (71.15, 67.19; 61.90, 40.64; 59.38, 40.64; 41.18, 15.63%) respectively, in Juebelle. Fresh and dry weights of shoot had significantly increased with same components, (5.10, 8.77; 3.34, 7.21; 2.44, 3.24; 2.69, 4.80%) respectively, in Endless summer and (8.03, 5.09; 6.80, 3.96; 4.54, 3.75; 6.02, 2.44%) respectively, in Juebelle compared with untreated plants, while supermarmand was the most susceptible for infection with RKN. Random amplified polymorphic DNA (RAPD) marker using four primers detected polymorphism in DNA in percentage ranged between 33.33 – 75% with total polymorphism 58.33%. Primer OPC-09 gave the highest polymorphism (75%) while OPB-18 gave the lowest polymorphism (33.33%).

Key words: Tomato cultivars, Bionematicides, *Meloidygyne incognita,* Greenhouse, PCR.

INTRODUCTION

Vegetable crops are considered the most important crops all over the world. The production of vegetable crops in development countries increased 60% in last twenty years **(Anonymous, 2013).** Tomatoes (*Solanum lycopersicum* L.) are very important cultivated vegetable crop in Egypt, produced 6.07 million ton/year (**MALR, 2003-2005)** and are grown in three seasons (summer, autumn and winter seasons). The total cultivated areas in reclaimed sandy soil are 56432 hectare (**El-Nagar *et al.,* 1998)**. Plant parasitic nematodes especially root-knot nematodes, *Meloidogyne* spp. Are widely distributed all over Egypt and cause considerable losses in crops reach 30-40% of yields (**Bhatti and Jain, 1977; Sasser, 1980)**. Many of plant species especially vegetables are attacked by *Meloidogyne* spp. (**Trudgill and Blok, 2001**). The use of resistant varieties caused by one or more genes in tomato cultivars is good and cheap methods for controlling plant parasitic nematodes. **Taylor (1967) and Ammati *et al.* (1985)** reported the resistance of plants to parasitic nematode based on the ability of the

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parasite to reproduce. The plant resistance to *Meloidogyne* crushes in some plants when soil temperature raise above 28 C° due to increase of hot temperature (**Dropkin, 1969**). RAPD-DNA is used in tomato to detect genetic diversity. A lot of bands obtained in RAPD-PCR are good in solving "pattern recognition" problems, like the clustering of different vegetables varieties between species level (**Tedeschi *et al.*, 2011**). Genotypic differences between tomato cultivars detected by molecular markers can be used for identification cultivar. RAPD marker technique is simple, effective and significantly cheap. The objective of this study was to determine the efficiency of certain Bionematicides against *Meloidogyne incognita* and to confirm the genetic diversity among three tomato cultivars under laboratory conditions.

MATERIALS AND METHODS

Culturing of *Meloidogyne incognita*

The pure culture was collected from El Salhia district, Sharkia Governorate, Egypt, where susceptible tomato cultivar is used as source of inoculum. The identification of species was based on juvenile measurements and perineal pattern system examination of adult females (**Eisenback *et al.*, 1981; Jepson, 1987)**.

Tomato Cultures

Three tomato (*Solanum lycopersicum* L., Mill) cultivars namely Endless summer, supermarmand and Juebelle were chosen because they had different degree of tolerance to *Meloidogyne incognita* attack (**Khanzada *et al.*, 2012)**. Seeds of experimented tomato cultivars were steeped in petri dishes by sterile distilled water then put in incubator at 26±1°C. After 48 hr., seeds were cultivated in clay spots (25 cm diameter) containing sterilized sandy soil. Plants were transplanted at two-leaf stage to pots filled with sandy soil (95.7% sand, 1.2% silt and 3.1% clay). Tomato seedlings were inoculated with second stage juveniles (2000J/pot). The nematicide, Nemacur (10% fenamiphos Ec) was used at the rate of 0.2 ml per pot after *M. incognita* inoculation according to recommended dose based on formulated form.

Bionematicides Treatment

Nemacont (*Paecilomyces lilacinus* 10^9x), Nemaend (organic matter, saponin and cytokinine), Nema clean (*Serratia marcescens*, saponin and citric acid)

Nema K (garlic extract, nitrogen and cytokinine), Bio-arc (6% WP *Bacillus megaterium*), Bio- Zeid (2.5% WP *Trichoderma ablum*) and Nemix (*Serratia marcescens*) were used at the rate of 0.4 ml/plant. The experiment was terminated after 60 days from nematode inoculation. The fresh and dry weights of tomato plants were measured. Nematodes were extracted from soil by using combination of sieving and Baermann trays technique (**Hopper *et al.*, 2005**).

DNA extraction and PCR reaction

DNA was extracted from leaves of tomato by **Lodhi *et al.* (1994)** methods. Four random primers were used in PCR reaction; OPA- 04, OPA-05, OPB-18 and OPC-09 (Table 1). The reaction was prepared using 25 µl per tube containing 2 µl DNA of each sample (20 µg), 1 unit of Taq DNA polymerase, 2 µl 10X buffer, 2 µl MgCl2 (25 mM), 2µl dNTPs (2.5 mM of each), 2 µl primer (10 pmol) and 14.8µl dH_2O. The reaction mixture was durated for 1 min at 94°C then 40 cycles (94°C for 1 min, 35°C for 2 min., 72°C for 2 min) of PCR, followed by 5 min., at 72°C. Following PCR, products was electrophoresed on a 2% agarose gel.

Table 1. The four primers which enter RAPD-PCR reaction

No.	Code	Sequence
1	A-04	5-AATCGGGCTG-3
2	A-05	5-AGGGGTCTTG-3
3	B-18	5-CCACAGCAGT-3
4	C-09	5-CTCACCGTCC-3

Statistical Analysis

Means were compared by Duncan's multiple ranges test at 5% level of possibility according to **Duncan (1955)**.

RESULTS AND DISCUSSION

Efficacy of Bionematicides on Root-Knot Nematode *M. incognita*

Results in Table 2 show the effect of nematicide, Nemacur and 7 bionematicides (Nema end, Nemacont, Nema K, Nema clean, Bio arc, Bio zeid and Nemex) on root-knot nematode, *Meloidogyne incognita* infected tomato plants cv. Endless summer after two months of application. All treatments compared with control 2 (plants inoculated with RKN) significantly ($P \leq 0.05$) reduced soil and root parameters (number of galls, number of egg masses and number of IJs/100 g soil). The reduction percentage in number of galls was highly with Nemacur (82.49) followed by Nema k (68.48), Nema cont (63.03), Bio zied (61.09), Nemex (59.93), Nema end (53.70) and Bio arc (50.97) and less with Nema clean (30.74) compared with plants inoculated with RNK, while percentage of reduction in number of egg masses was highly recorded with Nemacur followed by Nema K, Nemex and Bio zeid with values 73.97, 50.00, 43.75 and 40.62%, respectively compared with treated plants with RKN. Number of IJs/100 g soil was significantly reduced with Nemacur, Nema K, Bio zeid; Nemex and Nema clean with values 90.77, 71.47, 66.77, 63.01 and 55.48%, respectively. On the other hand, plant parameters (fresh and dry weights) were significantly increased compared with untreated inoculated plants, with percentages 5.10 and 8.77% for Nemacur followed by Nema k 3.34 then 7.21%, Bio zeid 2.69 and 4.80%, as well as Nema clean 0.92 and 1.88% for fresh and dry shoot weight.

Results in Table 3 elucidate less resistance to *M. incognita* infection, where plant fresh weight of tomato cv. Supermarmand was reduced by 12.26%. Results showed significant reduction in number of galls, number of egg masses and number of IJs/100g soil by Nemacur, Nema K and bio zied by reduction percentage of 78.90, 69.92, 92.31 ; 73.70, 55.75, 82.42 and 72.31, 43.38, 78.02%, respectively. On the other hand each of fresh and dry shoot weight was significantly increased with same compounds by 3.37, 4.76; 3.35, 2.27; 2.42, 0.54g, respectively.

Results in Table 4 clarify the highly effect of *M. incognita* on roots of tomato plant cv. Juebelle in all parameters. Number of galls, egg masses and IJs/100 g soil were significantly decreased, reached to 71.15, 67.19, 83.78%; 67.97, 42.11, 67.07%; 61.90, 40.64, 74.40% for Nemacur, Nemex and Nema K, respectively. The percentage of increasing of fresh and dry shoot weight was 8.03, 5.09%; 6.80, 3.96% and 6.02, 2.44% by Nemacur, Nema K and Bio zeid, respectively.

Resistance of tomato cultivars to root- knot nematodes is considered as a useful method to decrease the yield loss **(Philis and Vakis, 1974)**. The tomato cultivars were tested for response to infection with *M. incognita* **(Alimeida and Santos, 2002)**. These results indicated that the 3 cultivars of tomato had different degrees of resistance or tolerance for infection by RKN; Endless summer and Juebelle cultivars had proximally same degree of tolerance for *M. incognita* with less degree for supermarmand cultivar. The use of Nemacur 10% in all cultivars gave good results in reducing number of galls, egg masses and IJs/100 g soil, and significantly increased fresh and dry shoot weight. In general the use of bionematicides were significantly decreased number of galls, egg masses and IJs/100 g soil, and significantly increase fresh and dry shoot weight of the three tomato cultivars. The effects of nematicides on the activity and survival of nematodes were studied by many workers **(Kaushal and Seshadri, 1989; Mohammad and Abdul Malik, 2000)**. The presence of cytokinine in structure of screened bionematicides acted as root activator and increased fresh and dry weight of tomatoes. **Lamberti, *et al.*, (1993)** documented some tomato cultivars *i.e.* "Brech, Bush, Piersol and VFN8" resistant to root-knot nematodes in Sri Lanka. The ability of plants for resistance by root-knot nematodes decreased when soil temperature becoming over 30°C **(Whitehead**

Table 2. Efficacy of some bionematicides on plant parameters of tomato, Endless summer cultivar and root-knot nematode, *Meloidogyne incognita* reproduction

Parameter		Treatments									
		Without RKN (Cont 1)	With RKN (Cont 2)	Nemacur 10%	Nema end	Nemacont	Nema clean	Nema K	Bio arc	Bio zeid	Nemex
Plant parameters increase	Fresh weight	24.69 a	22.71 E	23.88 a (5.10)	23.13 cd (1.80)	23.23 bcd (2.44)	22.93D (0.92)	23.4B (3.34)	23.03Cd (1.95)	23.33BC (2.69)	23.10CD (1.67)
	Dry weight	11.03 a	9.56 E	10.41 a (8.77)	9.98 bc (4.28)	9.88 bcd (3.24)	9.75 D (1.88)	10.26A (7.21)	9.84cd (2.81)	10.03B (4.80)	9.72DE (1.56)
Root and soil parameter reduction (%)	No. Galls	0.00 g	85.67 a	15.00 F (82.49)	39.67 c (53.70)	31.67 C (63.03)	59.33B (30.74)	27.00 E (68.48)	42.00C (50.97)	33.33D (61.09)	34.33D (59.93)
	No. egg masses	0.00 g	32.00 a	8.33 G (73.97)	22.00 c (31.25)	21.33 CD (33.34)	25.67B (19.81)	16.00 F (50.00)	22.67C (29.19)	19.00DE (40.62)	18.00EF (43.75)
	No. IJs/100g	0.00 g	106.33 a	8.00 G (90.77)	53.33 B (49.84)	43.33 CD (59.25)	47.33B (55.48)	30.33F (71.47)	51.00B (52.04)	35.33EF (66.77)	39.33DE (63.01)

Same letter (s) in each column indicate no significant difference (P ≤0.05) between treatments according to Duncan's multiple range test.

$$\text{Increase } (\%) = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100 \qquad \text{Reduction } (\%) = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Table 3. Efficacy of some bionematicides on plant parameters of tomato, Supermarmand cultivar and root-knot nematode *Meloidogyne incognita* reproduction

Parameter		Treatments									
		Without RKN (Cont 1)	With RKN (Cont 2)	Nemacur 10%	Nema end	Nemacont	Nema clean	Nema K	Bio- arc	Bio- zeid	Nemex
Plant parameter increase (%)	**Fresh weight**	23.98	21.04	22.06A (3.37)	21.34BCD (1.41)	21.62ABC (2.77)	21.39BCD (1.65)	21.75AB (3.35)	20.93D (1.04)	21.55BC (2.42)	21.25CD (1.00)
	Dry weight	10.50	9.24	9.68A (4.76)	9.26AB (0.21)	9.34AB (2.16)	9.25B (0.10)	9.45AB (2.27)	9.27AB (0.29)	9.29AB (0.54)	9.26AB (0.18)
Root and soil parameter reduction (%)	**No. Galls**	0.00	96.33	20.33F (78.90)	41.00C (57.44)	30.33D (68.51)	63.33B (34.26)	25.33E (73.70)	45.00C (58.48)	26.66DE (72.31)	30.33C (68.51)
	No. egg masses	0.00	37.67	11.33G (69.92)	23.33DE (38.07)	28.33CD (24.79)	25.67CDE (31.85)	16.67FG (55.75)	34.33AB (8.87)	21.33EF (43.38)	29.33BC (19.48)
	No. IJs/100g	0.00	121.33	9.33F (92.31)	65.67C (45.87)	51.00D (57.96)	73.33E (39.56)	32.33E (82.42)	50.00D (58.80)	26.67E (78.02)	52.33D (56.87)

Same letter (s) in each column indicate no significant difference (P ≤0.05) between treatments according to Duncan's multiple range test.

$$\text{Increase } (\%) = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100 \qquad \text{Reduction } (\%) = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Table 4. Efficacy of some bionematicides on plant parameters of tomato, Juebelle cultivar and root-knot nematode *Meloidogyne incognita* reproduction

Parameter		Treatments									
		Without RKN (Cont 1)	With RKN (Cont 2)	Nemacur 10%	Nema end	Nemacont	Nema clean	Nema K	Bio arc	Bio zied	Nemex
Plant parameter increase (%)	Fresh weight	21.08	19.10	20.63AB (8.03)	19.83BCD (3.84)	19.67BCD (4.54)	19.72BCD (3.23)	20.40ABC (6.80)	19.64CD (2.84)	20.25ABC (6.02)	21.00A (5.75)
	Dry weight	10.13	9.42	9.90A (5.09)	9.71BCD (3.15)	9.77BC (3.75)	9.60EF (1.87)	9.79B (3.96)	9.52F (1.03)	9.65DE (2.44)	9.70CD (2.97)
Root and soil parameter reduction (%)	No. Galls	0.00	119	34.33F (71.15)	55.33C (53.50)	48.33CD (59.38)	65.67B (44.81)	45.33DE (61.90)	70.00B (41.18)	49.00CD (58.82)	38.33EF (67.79)
	No. egg masses	0.00	42.67	14.00F (67.19)	28.33DE (33.60)	25.33DE (40.64)	34.00BC (20.32)	25.33DE (40.64)	36.00B (15.63)	30.33CD (28.92)	23.67E (42.11)
	No. IJs/100gm	0.00	135.67	22.00G (83.78)	64.67E (52.33)	57.67C (57.49)	61.00CD (55.04)	34.33F (74.70)	75.33B (44.47)	45.33E (66.59)	44.67E (67.07)

Same letter (s) in each column indicate no significant difference (P ≤0.05) between treatments according to Duncan's multiple range test.

$$\text{Increase (\%)} = \frac{\text{Treated - Control}}{\text{Control}} \times 100 \qquad \text{Reduction (\%)} = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

and Hemming, 1965; Zacheo *et al*., 1995). Finally this study reported that, use of resistant tomato cultivars with some bionematicides are effectively method for decreasing infection by root-knot nematode *M. incognita* (**Mohan and Subhashini, 2010)**, especially in spring, autumn and winter seasons, when soil temperature less than 30°C **(Singh and Sittaramiah, 1973)**. On the other hand, studied bionematicides are considered mostly cheap and non pollutant method for controlling root-knot nematodes comparing with chemical nematicides.

Genetic Diversity Among the three Tomato Cultivars

The random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate **(Fevzi, 2001)**.

Polymorphism percentage for all four primers was 58.33% (Table 5). Number of bands scored for all primers varied between 8 and 10 bands with total number 36 bands. From all this number 15 bands were monomorphic and 21 bands were polymorphic. The polymorphic percentage for first primer (OPA-04) was 70% and second primer (OPA-05) was 55.56% and third primer (OPB-18) was 33.33% and last primer (OPC-09) scored percentage 75%.

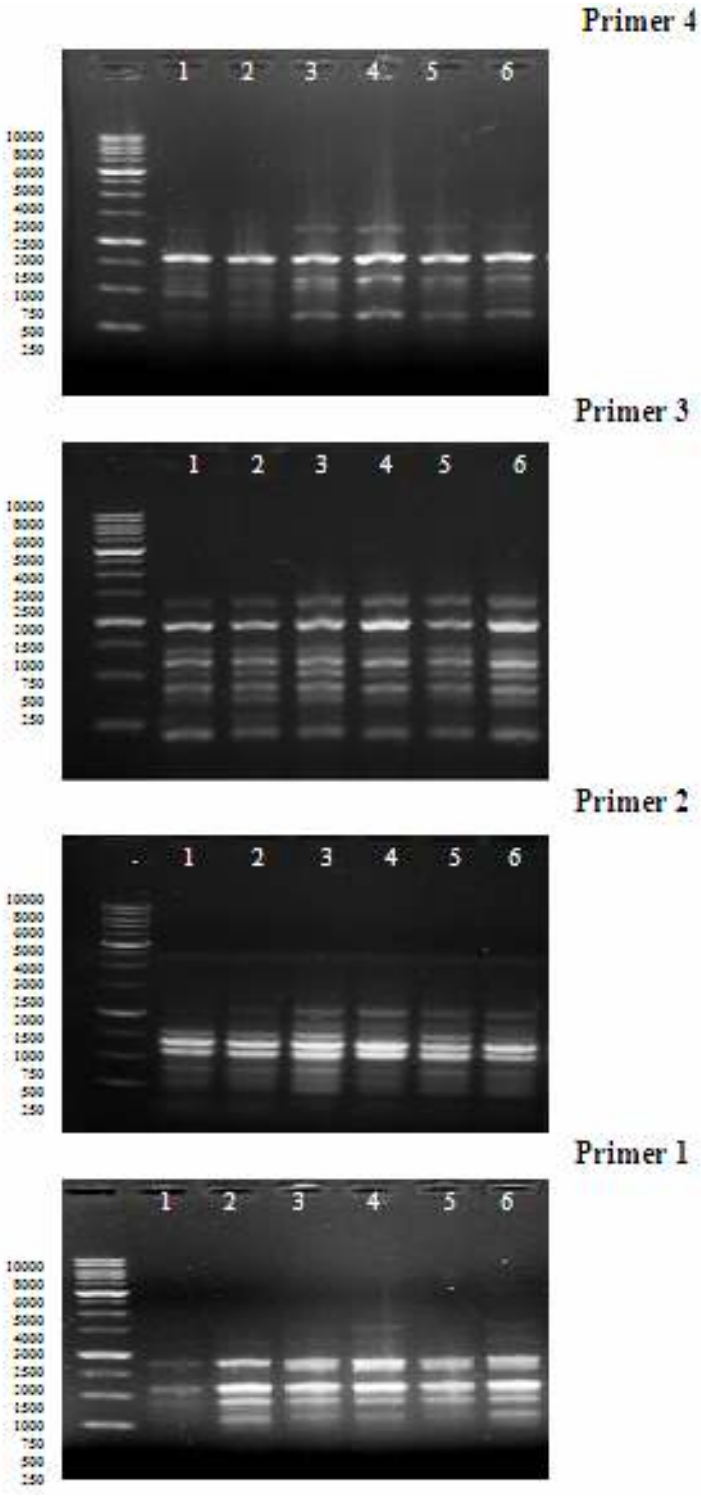
In first primer, the absence of 1500, 850 and 530 bp was detected in Juebelle sample. In second primer the absence of 1000, 900 and 200 bp bands was only recorded in Juebelle (Table 6 and Fig. 1). The result with OPB-18 primer revealed the presence of 250 bp only in Juebelle samples. With last primer the absence of 1250 and 550 bp bands was recorded only in Juebelle (Table 6 and Fig. 1).

Table 5. The Polymorphism in fragment size after RAPD-PCR reaction with the four primers

Primer	Range of fragment size	Juebelle		Supermarmand		Endless summer		Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism (%)
		Treated	Control	Treated	Control	Treated	Control				
A-04	250-1500 bp	3	7	8	9	7	8	10	3	7	70%
A-05	200-1000 bp	5	6	9	7	8	8	9	4	5	55.56%
B-18	200-1300 bp	9	9	8	8	6	6	9	6	3	33.33%
C-09	300-1250bp	5	4	6	7	5	6	8	2	6	75%
Total	200-1500 bp	22	26	31	31	26	28	36	15	21	58.33%

Table 6. RAPD-PCR bands of DNA in three tomato cultivars with four random primers

Primer name	FS	Juebelle		Supermarmand		Endless	
		Treated	Control	Treated	Control	Treated	Control
OPA-04	1500	-	-	+	+	+	+
	1300	-	+	+	+	+	-
	900	+	+	+	+	+	+
	850	-	-	+	+	+	+
	550	+	+	+	+	+	+
	530	-	-	+	+	+	+
	400	+	+	+	+	+	+
	350	-	+	-	-	-	-
	300	-	+	-	+	+	+
	250	-	+	+	+	-	+
OPA-05	1000	-	-	+	+	+	+
	900	-	-	+	+	-	+
	750	+	+	+	-	+	+
	700	+	+	+	+	+	+
	510	+	+	+	+	+	+
	400	+	+	+	+	+	+
	350	-	+	+	+	+	-
	250	+	+	+	+	+	+
	200	-	-	+	-	+	+
OPB-18	1300	+	+	+	+	+	+
	980	+	+	+	+	-	+
	700	+	+	+	+	+	+
	600	+	+	+	+	+	+
	500	+	+	+	+	+	+
	450	+	+	+	+	+	+
	350	+	+	+	+	-	+
	250	+	+	-	-	-	-
	200	+	+	+	+	+	+
OPC-09	1250	-	-	+	+	+	+
	800	+	+	+	+	+	+
	700	+	+	+	+	-	+
	550	-	-	+	+	+	+
	500	+	-	+	+	+	+
	450	+	-	-	-	-	-
	400	-	+	-	+	-	-
	300	+	+	+	+	+	+



Four primers for three cultivars of tomato

1 treated, 2 control Juebelle & 3 treated, 4 control supermarmand and 5 treated, 6 control Endless summer

Fig.1. RAPD-PCR

RAPD markers are good tools for detection polymorphism (**Fooland and Lin, 2011**). RAPD markers were used to identify polymorphism between three genotypes under study as it used by **Klein-Lankburst *et al.* (1992)**. The cultivars of tomato were screened by RAPD-PCR to examine resistance or tolerance to root-knot nematode *M. incognita* (**Fery and Thies, 1997; Fery *et al.*, 1998)**.

The presence of some bands in samples and absent in others this may be due to the resistance of screened cultivars to nematode infection this agree with **Trabelsi *et al.* (2007)** who detected by RAPD PCR the absence of bands in some phytophthora species in novel pathogenic behaviors. The possibility and application of the RAPD technique in varietal identification of tomato have been well explored **(Huh *et al.*, 2011).**

Ezekiel *et al*. (2011) reported 44.4- 83.3% and 12.5 - 85.7% polymorphism respectively in tomato genotypes by RAPD markers. RAPD was applied to assess genetic diversity in tomato varieties (**Saavedra *et al.*, 2001; Li Wang *et al.*, 2007**).

RAPD is a reliable and sensitive method for the environmental health risk **(Xiaolin *et al*., 2009)**. Amplified Polymorphic DNA (RAPD) has led to the development of a number of selective and sensitive assays for detecting DNA damage (**Aras *et al*., 2010**).

Atienzar *et al.* (1999) used the RAPD assay to determine the genotoxic effects of B[a]P in clonal *Daphnia magna.* Two RAPD primers revealed different values in RAPD band numbers, sizes and intensities between exposed and non-exposed individuals.

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فاعلية بعض مبيدات النيماتودا الحيوية ضد نيماتودا تعقد الجذور *Meloidogyne incognita* على ثلاث أصناف من الطماطم تحت ظروف الصوبة

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تعتمد هذة الدراسة على تحديد مدى فاعلية سبعة من المبيدات النيماتودية الحيوية وهي نيما اند، نيماكوت، نيماكين، نيماكي، بيوزيد، بيوأرك، نيمكس بالمقارنة مع مبيد نيماتودي كيماوي نيماكور ١٠% في مكافحة نيماتودا تعقد الجذور *M.incognita* ٣ أصناف من الطماطم تحت ظروف الصوبة، وأوضحت النتائج إن أصناف الطماطم لها درجات أستجابة مختلفة للأصابة بنيماتودا تعقد الجذور حيث وجد تحمل معنوي للإصابة بنيماتودا تعقد الجذور للصنفين Endless summer و Juebelle مقارنة بحساسية عالية للصنف Supermarmand، عدد العقد وكتل البيض انخفض معنويا في الثلاث أصناف مع مبيدات النيماكور، نيماكي، نيما كوت، بيوزيد (15, 8.33 ; 27, 16 ; 31.67, 21.33 and 33.33, 19) على التوالي في الصنف Endless summer بينما في الصنف Supermermand (20.33, 11.33; 25.33, 16.67 ; 30.33, 28.33 and 26.66, 21.33). الوزن الجاف والوزن الرطب للأجزاء النبات الخضرية زادت زيادة معنوية مع نفس المركبات (23.88, 10.41 ; 23.48, 10.26 ;23.23, 9.88 and 23.33, 10.03) مع الصنف Endless summer بينما كانت (71.15, 67.19;61.90, 40.64; 59.38, 40.64 and 41.18 ;15.63%) مع الصنف Juebelle وكان الصنف Supermarmand هو الأكثر حساسية، أظهر معلم RAPD بأستخدام أربعة بوادئ اختلافات على مستوى الدنا بنسبة تتراوح ما بين ٣٣,٣ -٧٥% بمعدل اختلافات كلى قيمته ٥٨,٣٣%، حيث أعطى البادئ OPC-09 أعلى أختلافات بنسبة ٧٥% بينما أعطى البادئ OPB-18 أقل نسبة للإختلافات بقيمة ٣٣,٣٣%.

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