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Biological Control of Root-knot Nematode *Meloidogyne incognita*: 2- Evaluation of the Nematicidal Effects of *Tagetes erecta* Tissue Culture under Laboratory and Greenhouse Conditions

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The effects of extracts of *Tagetes erecta* plants cultured on modified Murashige and Skoog medium (MS+0.1mg/l NAA), using ethyl or methyl alcohol or petroleum ether or chloroform or hexane extracts on *Meloidogyne incognita* second stage juveniles (J_2) were measured *in vitro*. Their mortality at standard (100%) concentration of the extracts was 100,74,87,34 and 49%, respectively. Ethanolic extracts of *T. erecta* calli were prepared from seed, leaf, stem or root grown on Ms- medium supplemented with three combinations of the two growth regulators namely; naphthalene acetic acid (NAA) and 6-benzylamine purine (BAP) and cultured for 8 weeks under dark or light conditions. The percentage mortality of *M. incognita* J_2 treated with extracts from these tissues was studied under laboratory conditions. Generally, the net mortality was positively correlated with the concentrations of all callus extracts. Under greenhouse conditions, the application of *T. erecta* (root + modified Ms-medium) extract as a seed soaking or soil applications have significantly ($P \leq 0.01$) reduced the population of *M. incognita* in cowpea root and soil and this reduction was not significant in soil treated at sowing time. Plant growth parameters, in this study, were enhanced by all the tested treatments.

Keywords: Biological control, cowpea, *Meloidogyne incognita* and tissue culture.

Tagetes species (marigolds) belong to family Asteraceae, were used by ancient civilizations such as the Aztecs for various purposes (Neher, 1968). Uhlenbroek and Bijloo (1958 and 1959) isolated and described some active principles from Tagetes plants. These chemicals belong to a group of heterocyclic sulphur-containing compounds, the thiophenes. *Tagetes* species contain biocidal compounds of the thiophene group as non polar products of secondary metabolites (Bohlmann *et al.*, 1973 and Gommers, 1981). Thiophenes were frequently found in *Tagetes* species where their accumulation changes according to the physiological state of plants and during organogenesis (Sutfeld, 1982). Many investigators identified several strong nematicidal compounds such as α-terthienyl and some unstable derivatives in marigold plants and these compounds seemed to be activated by illumination. The photo-toxins, α-terthienyl and other naturally occurring acetylenes seem to have

potential pesticidal activity (Marchant, 1987). Kyo *et al.* (1990) reported that marigold (*T. patula*) hairy roots induced by infection with *Agrobacterium rhizogenes* produced α-terthienyl when grown in darkness, and an n-hexane extract of the roots had a nematicidal activity. They added that, depending on the hairy root line used, the level of α-terthienyl varied from 15 to 1268 µg/g dry weight, a level that corresponded to 0:15 to 12.7 fold that in intact roots. Poli *et al.* (1991) evaluated thiophene accumulation in leaf calli and callus suspension of *T. patula.* They reported that the secondary calli showed 4 to 6 fold greater fresh weight in 2 weeks, while the tertiary ones only doubled their weight in the same period. Moreover, they investigated the influence of light/dark succession on the production of thiophene. A considerable increase of thiophene production (about 16.5 fold) was obtained when growing calli under successive light/dark periods. Repeated sub-culturing of callus tissues resulted in a slow decline of the thiophene yield. Ekes-Kretovics *et al.* (1993) reported that various species of the genus *Tagetes* are well known for their insecticidal properties. *T. minuta* contains large amounts of thiophenes. Recently, El-Gengaihi *et al.* (2001) reported that, the roots of *Tagetes erecta, T. patula* and *T. minuta,* extracted by petroleum ether and chloroform were highly potent against the reniform nematode, *Rotylenchulus reniformis.* The chloroform extract of *Tagetes erecta* roots produced a higher mortality rate than the individual component isolated by column and purified on preparative thin layer plates. Generally, the nematicidal activity of the isolated materials from *T. erecta* was concentration dependent. Spectral analysis of the three potent substances revealed that they are: 5 (pent-1-ol)-2, 2-bithienyl, stigma-4, 22-dien-3-beta-ol, and 5-(4-acetoxy-1-butenyl)-2, 2-bithienyl. The aim of this study is to evaluate the nematicidal effects of *T. erecta* plants cultured on basal-MS-medium and calli produced by tissue culture on *M. incognita* under laboratory and greenhouse conditions.

Materials and Methods

1. In vitro studies:

a- Extraction of Tagetes erecta plants grown on modified MS-medium using different solvents and evaluation of their nematicidal effects:

Twelve jars each contained three plants of *T. erecta* previously grown from seeds on modified MS-medium (MS+0.1mg/l NAA) for four months, to enhancement of mass value of root production from *Tagetes erecta*, were collected. Each two jars containing roots plus residual medium were soaked for 24 hrs in the following solvents: ethanol or methanol or petroleum ether or chloroform or hexane or distilled water, then filtered through Whatman No.1 filter paper. Each extract was evaporated under reduced pressure using Rotavapour Equipment. Residues of the extracts were separately weighed (1mg), collected, and then each residue was emulsified by one drop of tween 20 and diluted to a final volume of 100 ml. using distilled water (El-Gengaihi *et al.*, 2001). Each extract was considered as a standard solution, *i.e.* S (100%) concentration. Dilutions of S/2(50%) and S/10(10%) were freshly prepared by dilution with distilled water and bio-assayed for their nematicidal activity.

b- Bioassay tests:

Ten ml of the above solutions previously described (S (100%), S/2(50%) and S/10 (10%)) for each extract was added to one ml of nematode suspensions containing 250 *M. incognita* second stage juveniles in 50 ml plastic capsule. A control treatment was made by adding 10 ml of distilled water plus 1 ml of the nematode suspension. Each treatment was replicated five times. The number of viable and dead nematodes was counted with the aid of a light microscope after 24, 48 and 72 hrs at 25±1°C and the nematode mortality was calculated for each treatment. The nematodes in each concentration after 72 hrs exposure period were transferred to another plastic capsule containing distilled water and they were observed for 48 hrs to make sure that they did not regain activity. Only the nematodes which did not regain motility were considered "dead". The mortality percentage was calculated according to the Abbott's formula:

$$\text{Mortality (\%)} = \frac{m - n}{100 - n} X\ 100$$

Whereas: m and n stand for the percentages mortality in treated sample and control, respectively.

c- Extraction of Tagetes erecta calli derived from seed, leaf, stem and root explants grown on MS-medium supplemented with combinations of naphthalene acetic acid (NAA) & 6-benzylamine purine (BAP) and evaluation of their nematicidal effects:

Ten grams of fresh calli derived from seed, leaf, stem and root explants of Tagetes, were tested in the following media: **1**-MS+5mg/l NAA+5mg/l BAP, **2**-MS+7mg/l NAA+10mg/l BAPand **3**-MS+12mg/l NAA+12mg/L BAP. Then, they were collected after 8 weeks and soaked in 50 ml ethanol 70 % in conical flasks overnight. Then, filtered through Whatman No.1 filter paper, re-soaked again in 70% alcohol and then, the filtrates of each explants were combined separately and evaporated under vacuum using Rotavapour Equipment and under temperature not exceeding 40° C. The weighed residue of each callus extract was emulsified and diluted with distilled water according to El-Gengaihi *et al.* (2001), *i.e.* (100%), S/2 (50%) and S/10 (10%) and bio-assayed for its nematicidal activity as mentioned before. The comparative studies were done under dark or light conditions.

2. Greenhouse studies:

Evaluation of the nematicidal effect of Tagetes erecta cultured on modified MS-medium against Meloidogyne incognita infecting cowpea, Vigna sinensis:

a- Extraction procedures:

Forty jars each contained three plants of *T. erecta* cultured from seeds on modified MS-medium (MS+0.1mg/l NAA) for four months, for enhancement of root mass production, were collected. Then, the plants were uprooted. The root plus the medium were soaked in 50 ml ethanol 70% for 24 hrs then, filtered. The extracts of all jars were combined and evaporated under reduced pressure. Two and half grams of the residue were dissolved in 100ml distilled water (*i.e.* 2.5% concentration).

b- Nematicidal experiment:

Thirty, 10 cm diameter pots were filled with one kg solarised sandy clay soil (1:1 w/w) and arranged into five groups, each consisted of five replicates. Cowpea seeds were soaked in 9 ml (2.5%) of the previously mentioned Tagetes extract solution for 30 and 60 min, separately, then three seeds of each period were sown in each pot (Group 1 and 2, respectively). The soil of (Group 3) received 9 ml of the same extract at sowing time of three cowpea seeds in each pot. While each pot of the (Group 4) was sown with three cowpea seeds, then, 9 ml of the same extract was added at time of nematode inoculation. The (group 5) was used as inoculated-untreated control Ten days later, only two healthy plants were kept in each pot. All groups were inoculated with 1600 newly hatched J_2 of *M. incognita* / pot. The pots were maintained in a greenhouse bench and watered as needed. Two months later, the number of root galls, nematode egg masses, females and developmental stages in roots were recorded. The root gall index was categorized according to Sharma *et al.* (1994). The fresh and dry weights and lengths of shoot and root and number of bacterial nodules were recorded. Data were statistically analyzed using Fisher's Least Significant Differences (Carmer and Swanson, 1973).

Results

1. Evaluation of the nematicidal effects of different extracts of Tagetes erecta tissue-cultures on Meloidogyne incognita juveniles under laboratory conditions:

The nematicidal effects of the extracts of *T. erecta* plants cultured on modified MS-medium are presented in Table (1). The estimated percentage net mortality of *Meloidogyne incognita* juveniles at standard concentration(S) was 100, 74, 87, 34 and 49% for ethyl and methyl alcohol, petroleum ether, chloroform and hexane, respectively. *Tagetes erecta* extracted by chloroform or hexane was found to be the least toxic to *M. incognita* J_2 at S/10 concentration after each exposure period as the percentage net mortality for each of chloroform and hexane extracts was 4% (Table 1).

2. Screening of the nematicidal effects of the ethanolic extract of calli derived from different parts of T. erecta plant on M. incognita larvae under laboratory conditions:

Data in Tables (2, 3, 4 and 5) showed the nematicidal effects of ethanolic extracts of different *T. erecta* calli-derived from seed, leaf, stem and root, respectively grown on MS-medium, supplemented by the three combinations of the two growth regulators, and cultured for eight weeks under dark or light conditions on the percentage mortality of *M. incognita* (J_2). Generally, it could be noticed that the percentage nematode net mortality slightly increased with increasing of the concentration of all callus extracts. The net mortality increased at "S" followed by S/2 and S/10 concentrations. Callus tissue cultured under dark produced more residues after solvent evaporation in the most tissues. However, this was not absolutely correlated with the nematicidal effect of the extract. For example, in Table (3) the weight of the residue of leaf explants cultured on 5mg/l NAA + 5mg/l BAP under dark conditions was 0.25g which led to net mortality of 57% at (S)

Table 1. Evaluation of the nematicidal effects of different extracts of *Tagetes erecta* root plus modified medium produced by tissue culture on *Meloidogyne incognita* 2nd stage larvae under laboratory conditions

Solvent	Dilution	Mortality (%)			Recovery (%)	Net mortality (%)
		24 hrs	48 hrs	72 hrs		
Ethyl alcohol	S	100*	100	100	0	100
	S/2	98	98	96	21	75
	S/10	90	92	70	15	55
Methyl alcohol	S	98	93	74	0	74
	S/2	80	75	70	0	70
	S/10	76	63	65	0	65
Petroleum ether	S	99	94	87	0	87
	S/2	81	86	75	0	75
	S/10	19	30	45	0	45
Chloroform	S	97	82	70	36	34
	S/2	35	15	13	0	13
	S/10	25	12	4	0	4
Hexane	S	90	76	58	9	49
	S/2	40	27	19	8	44
	S/10	27	12	11	7	4
Distilled water	-	21	19	26	-	-

* Each figure represents the mean of 5 replicates.

concentration, however, the weight of the residue from similarly treated tissue under light conditions was 0.18 g which led to net mortality of 71%. Data also indicated that, the stem explant cultured on 5 mg / l NAA + 5 mg/l BAP under dark conditions and leaf explant cultured on 7 mg / l NAA + 10 mg / l BAP under dark condition led to the highest net mortality percentages of 92 and 89 %, respectively at "S" concentrations (Table 3 and 4).

3. Evaluation of the nematicidal effects of the ethanolic extract of Tagetes erecta tissue- culture on M. incognita infecting cowpea under greenhouse conditions:

The application of *T. erecta* (root plus modified MS-medium) extract as seed soaking or soil application significantly ($P \leq 0.01$) reduced the population of the root-knot nematode *M. incognita* in roots and soil and insignificantly reduced the number of juveniles in soil treated at the sowing time of cowpea compared to inoculated-untreated control plants. When the seeds were soaked for 60 min, the percentages reduction were 61, 66, 50, 80 and 81 for galls, egg-masses, females, developmental stages and number of 2nd stage juveniles / pot, respectively, while the percentage reduction in nematode numbers were 56, 53, 40, 77 and 79%, respectively, for the above mentioned parameters when the seeds were soaked for 30 min. (Table 6).

Table 2. Nematicidal effects of the ethanolic extracts of calli derived from seed explants of *T. erecta* cultured for 8 weeks on different media and incubated at 26±1°C under light or dark conditions on *M. incognita* 2nd stage juveniles under laboratory conditions

Culture medium	Dilution	Weight of residues (g)		Mortality (%)						Recovery (%)		Net mortality (%)	
				24 hrs		48 hrs		72 hrs					
		L*	D	L	D	L	D	L	D	L	D	L	D
NAA(5mg/l) + BAP(5mg/l)	S	0.36	0.50	100	100	92	93	88	88	30	26	58	62
	S/2			100	98	68	89	69	76	24	20	45	56
	S/10			69	57	33	43	15	35	0	9	15	24
NAA(7mg/l) + BAP(10mg/l)	S	0.12	0.16	98	100	95	96	88	89	32	26	56	63
	S/2			68	99	57	91	69	88	39	32	30	56
	S/10			34	46	27	25	21	15	4	0	17	15
NAA(12mg/l) + BAP(12mg/l)	S	0.28	0.30	98	100	93	95	88	89	33	29	55	60
	S/2			96	97	87	90	78	84	34	31	44	53
	S/10			63	59	38	43	28	35	0	3	28	32
Distilled water	-	-		18		5		7		-		-	

* L: light conditions, D: dark conditions. Each figure represents the mean of 5 replicates.

Table 3. Nematicidal effects of the ethanolic extracts of calli derived from leaf explants of *T. erecta* cultured for 8 weeks on different media and incubated at 26±1°C under light or dark conditions on *M. incognita* 2nd stage juveniles under laboratory conditions

Culture medium	Dilution	Weight of residues (g)		Mortality (%)						Recovery (%)		Net mortality (%)	
				24 hrs		48 hrs		72 hrs					
		L*	D	L	D	L	D	L	D	L	D	L	D
NAA(5mg/l) + BAP(5mg/l)	S	0.18	0.25	100	100	96	98	95	81	24	24	71	57
	S/2			91	100	78	89	76	51	28	27	48	24
	S/10			43	45	38	38	22	18	0	0	22	18
NAA(7mg/l) + BAP(10mg/l)	S	0.28	0.38	100	100	97	100	99	96	27	7	72	89
	S/2			100	100	96	100	83	94	5	64	28	30
	S/10			49	48	17	11	18	11	3	0	15	11
NAA(12mg/l) + BAP(12mg/l)	S	0.25	0.29	100	100	99	100	85	94	70	66	15	30
	S/2			98	100	83	85	57	73	14	43	43	28
	S/10			87	92	19	51	21	24	0	0	0	24
Distilled water	-	-		12		4		7		-		-	

* As described in footnote of Table (2).

Table 4. Nematicidal effects of the ethanolic extracts of calli derived from stem explants of *T. erecta* cultured for 8 weeks on different media and incubated at 26±1°C under light or dark conditions on *M. incognita* 2nd stage juveniles under laboratory conditions

Culture medium	Dilution	Weight of residues (g)		Percentages mortality						Recovery (%)		Net mortality (%)	
				24 hrs		48 hrs		72 hrs					
		L*	D	L	D	L	D	L	D	L	D	L	D
NAA(5mg/l)	S	0.19	0.40	100	100	100	100	97	100	9	8	88	92
+	S/2			85	100	88	99	89	89	19	43	50	46
BAP(5mg/l)	S/10			38	91	33	39	23	13	3	0	20	13
NAA(7mg/l)	S	0.32	0.24	100	100	100	100	100	91	30	48	70	43
+	S/2			100	99	100	84	92	38	55	18	37	20
BAP(10mg/l)	S/10			77	28	7	7	6	7	1	0	5	7
NAA(12mg/l)	S	0.22	0.21	100	100	100	100	81	85	32	55	49	30
+	S/2			90	97	73	78	27	65	17	55	10	10
BAP(12mg/l)	S/10			71	87	28	29	5	-	0	100	5	0
Distilled water	-	-		12		4		7		-		-	

* As described in footnote of Table (2).

Table 5. Nematicidal effects of the ethanolic extracts of calli derived from root explants of *T. erecta* cultured for 8 weeks on different media and incubated at 26±1°C under light or dark conditions on *M. incognita* 2nd stage juveniles under laboratory conditions

Culture medium	Dilution	Weight of residues (g)		Percentages mortality						Recovery (%)		Net mortality (%)	
				24 hrs		48 hrs		72 hrs					
		L*	D	L	D	L	D	L	D	L	D	L	D
NAA(5mg/L)	S	0.27	0.29	90	98	94	100	87	97	5	33	82	64
+	S/2			93	100	88	92	79	65	19	10	60	55
BAP(5mg/L)	S/10			75	80	42	27	38	14	19	1	19	13
NAA(7mg/L)	S	0.37	0.27	100	100	100	100	98	82	35	40	63	42
+	S/2			99	74	96	73	82	44	45	19	37	25
BAP(10mg/L)	S/10			21	23	14	14	12	9	0	0	12	9
NAA(12mg/L)	S	0.17	0.12	98	100	75	80	72	76	41	0	31	76
+	S/2			80	73	45	31	30	57	18	24	12	33
BAP(12mg/L)	S/10			60	71	2	13	-	-	100	100	0	0
Distilled water	-	-		14		11		4		-		-	

* As described in footnote of Table (2).

Table 6. Evaluation of the nematicidal effects of the ethanolic extract of *T. erecta* cultured on modified MS-medium on *M. incognita* population injecting cowpea plants grown under greenhouse conditions

Treatment	RGI	No. of galls	Red. (%)	No. of Egg mass	Red. (%)	No. of females	Red. (%)	No. of RDS g/root	Red. (%)	No. of J^2 soil/pot	Red. (%)
Seed soaking period											
30 min	6	46*	56	48	53	61	40	5.4	77	250	79
60 min	6	41	61	35	66	51		4.6	80	219	81
Soil application											
At sowing time	6	46	56	46	55	59	50	3.4	85	451	62
At inoculation time	6	32	69	31	70	46	42	1.2	95	236	80
Control	9	104	-	102	-	101	-	23	-	1181	-
LSD 0.05		27.8		18.5		23.89		1.55		879.32	
LSD 0.01		25.6		16.97		21.95		1.43		808.18	

* Each figure represents the mean of 5 replicates. RGI= Root gall index, Red.= Reduction, RDS=Root developmental stages.

For soil application, the effect of extracts added at inoculation time of nematodes was greater than its effect at seed sowing. The percentages reduction for the treatment at nematode inoculation time were 69, 70, 54, 95 and 80 % for galls, egg masses, females, developmental stages and 2ndstage juveniles, respectively. While, soil application at sowing time reduced nematode counts by 56, 55, 42, 85 and 62%, respectively, for the above mentioned parameters. Plant growth parameters were enhanced by all treatments, (Table 7). A statistically significant increase in shoot length and root dry weight (seed soaked for 60 min. and at nematode inoculation time) over inoculated-untreated control was observed. From Tables (6 & 7), it is clear that the treatment at nematode-inoculation time gave the highest percentage reduction in nematode counts and the greatest improvement in plant growth.

Table 7. Influence of the ethanolic extract of *T. erecta* extract cultured on modified MS-medium on growth parameters of cowpea infected by *M. incognita* under greenhouse conditions

Treatment	Length (cm)				Fresh weight (g)				Dry weight (g)					
	Root	Increase (%)	Shoot	Increase (%)	Root	Increase (%)	Shoot	Increase (%)	Root	Increase (%)	Shoot	Increase (%)	No. of nodules	Increase (%)
Seed soaking period														
30 min	26	32	57	44	1.05	11	5.84	24	0.18	20	1.05	28	14	17
60 min	28	42	65	64	1.22	28	7.12	51	0.21	40	1.15	40	40	33
Soil application														
At Sowing time	26.5	36	44	11	1.30	37	5.3	14	0.19	27	0.94	15	19	60
At inoculation time	26.7	37	50	27	1.38	45	6.2	33	0.23	53	1.10	34	21	71
Control	19.5	-	39.8	-	0.95	-	4.7	-	0.15	-	0.82	-	12	-
LSD. 005	NS	-	199	-	NS	-	NS	-	NS	-	NS	-	NS	-
LSD. 001	NS	-	183	-	NS	-	NS	-	NS	-	NS	-	NS	-

NS = Not significant.

Discussion

The nematicidal effects of the extraction of *T. erecta* plants cultured on modified MS-medium using five different solvents indicated that the nematicidal activity of *T. erecta* root medium extracts differed according to the solvents used. These differences may be attributed to the differences in the chemical nature, composition and concentration of the toxic compounds extracted, which were separated from the root medium by different solvents and presented in the form of aqueous solutions.

Although the nutrition and the light or dark conditions have great effects on the callus weight and callus growth rate, it seems that, the dry weight of the residue of alcoholic extract of the callus did not correlate well with its nematicidal activity on *M. incognita*. The same trend was obtained for the different nutritional supplements of the media. This could be attributed to the differences in the quantity of the active principles produced. The present data are in line with the observation of Ketel (1986), who found that the type of nutrient solution exerted no effect on the number of thiophene like compounds. It seems that the type of explant affects the thiophene content. In the present data, it was noticed that the leaf explant at 7.0 mg / l NAA + 10.0 mg / l BAP under dark conditions and stem explant at 5mg / l NAA + 5 mg /l BAP under the same conditions led to the highest net mortality percentage (89% & 92%), respectively. Ketel (1986) reported that, the number of thiophenes in primary calli of *T. minuta* decreased when compared with the leaves, but in *T. patula* or *T. erecta*, the number of thiophenes in calli was higher than in the corresponding

leaves. Ketel (1985) reported that callus of *Tagetes erecta* rapidly formed roots and shoots in culture and contained a number of hexane-soluble secondary metabolites, some of which corresponded with thiophenes in their high performance liquid chromatography (HPLC) retention times and ultra violet (UV) spectra. However, *T. minuta* callus did not differentiate and lacked secondary metabolites. Callus of *Tagetes patula* showed intermediate behaviour. Lately, the studies on thiophene production *in vitro* cultures were performed with the aim to select the explants having high thiophene production (Ketel, 1986 and 1987).

The present results revealed a powerful nematicidal effect of the alcoholic extract of *Tagetes erecta* tissue culture grown on modified MS-medium. The effect was observed *in vitro* (Table 1) as well as under greenhouse conditions (Table 6). These results agree with Benavides and Caso (1993) who found four thiophene metabolites in Tagetes seedlings grown *in vitro*. The results obtained from applying Tagetes roots plus MS-medium extracts as a seed soaking or soil application proved that Tagetes extract provided an early protection to cowpea plants against *M. incognita*. The low multiplication of the root-knot nematode may be due to low penetration and later retardation in different activation of the nematode such as feeding and reproduction as suggested by Bunt (1975).Also, in case of seed soaking, it is possible that certain chemicals were absorbed by cowpea seeds or they might have initiated some chain reactions triggered by some activating factors present in the seeds (Bell, 1981).

From a biotechnological point of view, this study revealed that callus is a promising starting material for the production of thiophenes. However, many efforts are still necessary needed.

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المكافحة البيولوجية لنيماتودا تعقد الجذور *ميلودوجينا إنكوجنيتا*: ٢- التقييم البيولوجى لتأثير زراعة الأنسجة لنبات القطيفة تحت ظروف المعمل والصوبة

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تم تنمية نبات القطيفة (*Tagetes erecta*) على بيئة MS المحسنةالمضاف اليها هرمون حمض النفثالين أسيتك ثم أستخلصت هذه البيئة بواسطة كل من كحول الإيثايل ،كحول الميثايل ،ايثير البترول الكلوروفورم والهكسان . وعند دراسة تأثير هذه المستخلصات على الطور اليرقى الثانى لنيماتودا تعقد الجذور ميلودوجين انكوجنيتا تحت ظروف المعمل وجد أن نسب موت الطور اليرقى الثانى فى التركيز الاعلى هم ٨٧،٣٤،٤٩،٧٤،١٠٠% فى المذيبات المختلفة على الترتيب. وعند دراسة تأثيرالمستخلص الايثانولى للكالوسات المشتقة من البذرة، الورقة، الساق والجذور النامية على بيئة MS المزودة بهرمونات النمو (BAP+NAA) والنامية لمدة ٨ أسابيع فى الظلام او الضوء وجد أن النسبة المئوية لموت اليرقات ازدادت مع زيادة تركيز الكالس. وتحت ظروف الصوبة تم اختبار مستخلص نبات القطيفة (جذور+بيئةMSالمحسنة) كنقع للبذرة او معاملة للتربة فقد أدت المعاملات المستخدمة إلى خفض معنوى (١%) للنوع *Meloidogyne incognita* فى تربة وجذور اللوبيا صنف بلدى ، بينما لم يحدث نقص معنوى فى حالة معاملة التربة فى وقت زراعة البذور. كما تحسنت الصفات الخضرية تحت الدراسة لنباتات اللوبيا.