Inhibitory Effect of Extracts of *Alpinia officinarum*, *Laurus nobilis* and *Solenostemma argel* on Egg Hatching of The Root-knot Nematode, *Meloidogyne incognita* and Their Possible Application in Nematode Control on Tomato

Amr A. El-Sherbiny[1] and Fahad A. Al-Yahya[2]

ABSTRACT

Crude ethanolic extracts of dried rhizomes of *Alpinia officinarum* and leaves of *Laurus nobilis* and *Solenostemma argel* at the concentrations 100, 250, 500 and 1000 ppm significantly inhibited egg hatching of the root-knot nematode, *Meloidogyne incognita* under laboratory conditions. The maximum inhibition (82.06%) was achieved by *S. argel* (1000 ppm), while the minimum one (29.11%) was recorded for *L. nobilis* (100 ppm). The probit analysis of all tested plant extracts revealed that the inhibitory concentration of egg hatching by 50% (IC_{50}) was 238.4, 346.2 and 141.8 ppm for *A. officinarum*, *L. nobilis* and *S. argel*, respectively. Thus, extract of *S. argel* was the best one in inhibiting egg hatching, followed by extracts of *A. officinarum* and *L. nobilis*, respectively.

The possible application of all tested plant extracts at the concentrations 500 and 1000 ppm as soil drenching following nematode inoculation, comparing to the nematicide Carbofuran 10% in controlling *M. incognita* on tomatoes cv. Marmande was studied under greenhouse conditions. All plant extracts and Carbofuran significantly reduced nematode infection as compared to the nematode check plants. Extract of *S. argel* (1000 ppm) achieved the highest reduction in numbers of root galls (80.6%), egg masses (91.0%) and reproduction factor of nematode, Rf (91.4%), while extract of *L. nobilis* (500 ppm) gave the lowest ones (59.1, 79.6 and 77.8% in galls, egg masses and Rf, respectively). Plant growth criteria of tomatoes were significantly increased as influenced by the application of plant extracts as compared to the nematode check plants, where extract of *L. nobilis* provided the highest increase (90.1 - 100%), followed by extracts of *A. officinarum* (37.5 - 65.8%) and *S. argel* (39.0 - 46.1%).

Extract of *S. argel* recorded a high relative nematicidal efficacy to Carbofuran 10% ranged from 81.6 - 112.7%, followed by extracts of *A. officinarum* (68.4 - 84.8%) and *L. nobilis* (48.2 - 62.7%).

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the major vegetable crops cultivated and consumed in Saudi Arabia and worldwide. The estimated area of tomato cultivation in the kingdom of Saudi Arabia was 15127 ha producing 542589 tons of tomato fruits in the year 2009 (Anonymous, 2010).

Root knot nematodes (RKN), *Meloidogyne incognita* and *M. javanica* are of great economic importance as damaging pests of tomato production in Saudi Arabia and throughout the warmer regions of the world (Al-Hazmi, *et al*., 1995).

Nematode control is still depending on the application of synthetic nematicides in fields and greenhouses. Despite their great efficacy in killing nematodes, they are costly and very toxic compounds which may cause real hazards to the human and environment. Thus, several trials have been created to develop new safe alternatives for nematode management. Extracts from many higher plants having a nematicidal activity towards plant parasitic nematodes become the major interest of many researchers worldwide. They almost delivered from natural sources, cheap, biodegradable easily and have a wide range of targeted plant pathogens and parasites in the soil (Oka *et al*., 2000; Chitwood, 2002; Ibrahim *et al*., 2006; Barbosa *et al*., 2010).

The herbal shops throughout the world are crowded by hundreds of medicinal plants and herbs having diverse therapeutic properties commonly used in the folkloric medicine and phytotherapy. Among them, the smaller galangal, *Alpinia officinarum* Hance (family Zingiberaceae) is a pungent and aromatic plant native to south east China, and cultivated in China, Indonesia, Thailand and Japan. Its rhizomes are widely used as a spice and in traditional medicine (Lawless, 1992). It has

[1]Nematology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
e-mail: amr_elsherbiny_1968@yahoo.com
[2]Plant Protection Department, College of Food and Agricultural Sciences, King Saud University, P.O. 2460, Riyadh 11451, Saudi Arabia
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strong antibacterial and antifungal (Srividya *et al*., 2010), antiviral (Hussein *et al*., 2000) and insecticidal activities (Samart *et al*., 2009).

Bay laurel (Sweet bay), *Laurus nobilis* L. (family Lauraceae) is an evergreen tree native to the Mediterranean region and extensively cultivated in France, Spain, Italy, Morocco, Yugoslavia, China, Israel, Turkey and Russia. The dried leaves are used extensively in cooking and in folk medicine (Lawless, 1992; Kenner and Requena, 1996). The essential oil of its leaves has a potential antifungal (Simić *et al*., 2004), antibacterial (Shan *et al*., 2007), antiviral (Loizzo *et al*., 2008) and insecticidal activities (Rizi, 2009).

Argel, *Solenostemma argel* (Del.) Hayne (family Asclepiadaceae) is a perennial wild herb of African origin, and has long been used by Africans in the folk medicine (Al-Doghairi *et al*., 2004; Ahmed *et al*., 2010). It has great antimicrobial activity towards 8 species of bacteria and 14 species of fungi and *Candida* (Abd El Hady *et al*., 1994), antiplasmodial (Ahmed *et al*., 2010) and insecticidal activities (Al-Doghairi *et al*., 2004).

Little reports have described the nematicidal activity of all above mentioned plants. Essential oils of the leaves of *L. nobilis* showed an *in vitro* nematicidal activity towards *Meloidogyne javanica* (Oka *et al*., 2000), *M. incognita* (Ibrahim *et al*., 2006) and the pinewood nematode, *Bursaphelenchus xylophilus* (Barbosa *et al*., 2010). Moreover, methanol or hexane extracts of the leaves of *S. argel* observed a nematicidal activity against eggs and juveniles of *M. incognita* (Elbadri *et al*., 2008).

Indeed, there is a lack of studies regarding possible use of the above mentioned plants in the management of RKN on susceptible host plants. Therefore, the aim of current study is to confirm the nematicidal activity of their crude ethanolic extracts on egg hatching of *M. incognita* under laboratory conditions, and to examine their possible application in the nematode control on tomato under greenhouse conditions.

MATERIALS AND METHODS

Plant materials and extraction:

Dried rhizomes of the smaller galangal (*Alpinia officinarum*) and leaves of bay laurel (*Laurus nobilis*) and argel (*Solenostemma argel*) were purchased from an herbal shop at Riyadh city, Saudi Arabia. Plant parts were finely ground in a coffee grinder, sieved through a 100 mesh stainless screen (pore aperture=150 μm), and a hundred grams of each plant powder was soaked (three times) in 300 ml ethanol 95% in 500-ml Erlenmeyer glass flasks sealed with parafilm and exposed to overnight shaking using an electric shaker at 150 hub/min at laboratory temperature (≈25°C) for three days. Crude plant extracts were filtered under vacuum and the solvent was evaporated at 40˚C under pressure using a Buchi Vacobox rotary evaporator (model B-177 with Buchi 461 water bath, Brinkman Instruments Inc., Westbury, NY) in order to concentrate extracts (up to 50 ml each). All concentrated extracts were poured into 50 ml amber glass bottles with glass stoppers and kept in the refrigerator (4-5ºC) till the bioassay.

Nematode inoculum and the bioassay (*in vitro* test):

Severely galled roots of the ornamental tree, *Albizia lebbeck* growing in a public garden at Riyadh city naturally infected with the root-knot nematode, *Meloidogyne incognita* [Kofoid & White] (El-Sherbiny, 2011) were selected as a stock inoculum source of the present study. Nematode eggs for the *in vitro* test and greenhouse experiment were extracted from the infected roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973).

All plant extracts were tested for their nematicidal activity towards egg hatching of *M. incognita* at the concentrations 100, 250, 500 and 1000 ppm under laboratory conditions. Prior to the bioassay, the tested plant extracts (standards) were emulsified with Triton X-100 at a concentration of 0.01% according to Elbadri *et al*., (2008). The tested concentrations were prepared from standards by adding the appropriate amounts of distilled water. Two ml of nematode egg suspension containing approximately 2000 eggs were poured into 20 ml capped glass vials over two ml of double tested concentrations in order to optimize the studied concentrations in test vials (Pandey *et al*., 2000). An extract-free treatment (distilled water) and a solvent blank (ethanol 95%) at the concentration 1000 ppm were served as checks. All treated vials were replicated three times. Eight days after treatments, hatched juveniles were examined under a stereo microscope and counted using Peter's 1 ml eelworm counting slide, and the inhibition (%) of egg hatching was assessed (Al-Rajhi *et al*., 1997).

Greenhouse experiment:

All plant extracts at the concentrations 500 and 1000 ppm were further examined for their efficacy in controlling *M. incognita* on tomato plants under greenhouse conditions. Seeds of tomato cv. Marmande were sown in a plastic transplanting tray (12 x 6 holes) filled with a mixture of peat moss and sand (2:1 v/v) and received their needs of water and nutrients. Uniform tomato seedlings (one month age) with 3-4 true leaves were transplanted (one/pot) in 12 cm diam plastic pots filled with 1 kg steam sterilized mixture of sand, silt and ground peat moss (4:2:1 v/v/v). One week

later, each seedling was inoculated with 5000 nematode eggs by pipetting the inoculum suspension into several holes around the seedling base (5-10 cm deep). Following nematode inoculation, soil was immediately drenched with 100 ml of the tested concentrations and thereafter every 3 days for 12 days period (Massa, 2010). The nematicide Carbofuran 10% (0.1g/pot) was applied to the soil following nematode inoculation in a comparative treatment. Nematode-free (healthy) and the other plants inoculated with nematode only were used as checks. All treatments were replicated five times and arranged in a completely randomized design in the greenhouse (air temperature 35±5ºC). Six weeks after nematode inoculation, plants were carefully uprooted and their roots were harvested, gently washed free of soil particles using tap water and stained in an aqueous solution of Phloxine B (0.15g/l. tap water) to emphasize nematode egg masses for counting (Holbrook *et al*., 1983). Fresh weights of shoots and roots were recorded. Final nematode egg populations (P_f) were extracted from roots using 1% NaOCl (Hussey and Barker, 1973) and the nematode reproduction factor (Rf) was calculated according to the formula $Rf = P_f / P_i$, where P_i = initial egg population (Oostenbrink, 1966).

Data analysis:

Inhibition percentages of nematode egg hatching given by all tested plant extracts were corrected using Abbott's formula (Abbott, 1925) and subjected to the probit analysis in order to estimate the inhibitory concentration of egg hatching by 50% (IC_{50}) according to Finney, 1971. Linear regression models were used to determine the relationship between tested concentrations of plant extracts and inhibition percents of egg hatching. Analysis of variance (ANOVA) concerning numbers of root galls, nematode egg masses, final nematode egg populations and Rf values were statistically analyzed using SAS computer software program and means of all treatments were compared according to Fisher's protected LSD (SAS, 1997). On the other hand, the relative nematicidal efficacy (%) of all studied plant extracts to Carbofuran 10% was calculated.

RERSULTS AND DISCUSSION

Data presented in Table 1 indicate that all tested concentrations of crude plant extracts significantly (P = 0.05) inhibited egg hatching of *M. incognita* under laboratory conditions. The maximum inhibition (82.06%), was achieved by *S. argel* (1000 ppm), while the minimum one (29.11%) was provided by *L. nobilis* (100 ppm). Other concentrations gave a satisfied inhibition of egg hatching ranged from 35.91 – 74.17%. These results are consistent with those previously given by Oka *et al*., 2000; Ibrahim *et al*., 2006 and Barbosa *et al*., 2010 on *L. nobilis*, and Elbadri *et al*., 2008 on *S. argel*.

Table 1. Effect of different concentrations of crude ethanolic extracts of *Alpinia officinarum*, *Laurus nobilis* and *Solenostemma argel* on egg hatching of *Meloidogyne incognita* (8 days of exposure) under laboratory conditions

Treatment		No. hatched J_2/ml ± SD	% Inhibition* ± SD	IC_{50}
Distilled water (Extract-free)		251.0 a ± 13.07	–	
Ethanol 95% (1000 ppm) (Blank)		232.3 b ± 10.26	–	
Alpinia officinarum	**100 ppm**	148.7 d ± 5.51	36.00 ± 2.37	238.4
	250 ppm	122.0 f ± 7.94	47.48 ± 3.41	
	500 ppm	80.3 hi ± 8.33	65.42 ± 3.58	
	1000 ppm	60.0 k ± 3.61	74.17 ± 1.55	
Laurus nobilis	**100 ppm**	164.7 c ± 4.04	29.12 ± 1.74	346.2
	250 ppm	137.0 de ± 4.58	41.00 ± 2.08	
	500 ppm	102.0 g ± 7.21	56.09 ± 3.11	
	1000 ppm	66.3 jk ± 11.72	71.44 ± 5.04	
Solenostemma argel	**100 ppm**	127.7 ef ± 8.51	45.04 ± 3.66	141.8
	250 ppm	93.3 gh ± 4.16	59.82 ± 1.79	
	500 ppm	77.0 ij ± 7.55	66.85 ± 3.25	
	1000 ppm	41.7 l ± 6.11	82.06 ± 2.64	

-Data are averages of 3 replicates.

-Values within column followed by the same letter(s) are not significantly different according to Fisher's protected LSD at P = 0.05.

* Inhibition (%) after correction using Abbott's formula (Abbott, 1925), where distilled water and ethanol 95% are served as checks.

IC_{50} = Inhibitory concentration of egg hatching by 50% after probit analysis (Finney, 1971).

The probit analysis of all tested plant extracts revealed that the inhibitory concentration of egg hatching by 50% (IC_{50}) was 238.4, 346.2 and 141.8 ppm for *A. officinarum*, *L. nobilis* and *S. argel*, respectively. Thus, extract of *S. argel* was the best one in inhibiting egg hatching, followed by extracts of *A. officinarum* and *L. nobilis*, respectively (Table 1).

Inhibition of egg hatching of *M. incognita* was significantly (P=0.0001) increased linearly with increasing concentration of the plant extract, recording R^2 = 0.86, 0.93 and 0.90 for *A. officinarum*, *L. nobilis* and *S. argel*, respectively (Fig. 1).

Results of the greenhouse experiment show that soil application with all plant extracts at the concentrations 500 and 1000 ppm, significantly (P = 0.05) reduced nematode infection on tomatoes cv. Marmande as compared to the nematode check plants (Table 2). Extract of *S. argel* (1000 ppm) provided the highest reduction in numbers of root galls (80.6%), egg masses (91.0%) and Rf (91.4%), while extract of *L. nobilis* (500 ppm) gave the lowest ones (59.1%, 79.6% and 77.8% in numbers of root galls, egg masses and Rf, respectively). Moreover, Carbofuran 10% recorded a high reduction in numbers of root galls (84.5%), egg masses (88.7%) and Rf (88.7%). Almost, no significant differences were found between the tested concentrations of studied plant extracts (except for *L. nobilis*) in reducing nematode infection on tomatoes (Table 2).

Regarding to the relative efficacy of all tested plant extracts to Carbofuran 10% (Table 2), it was found that extract of *S. argel* recorded a high efficacy (81.6 - 112.7%), followed by extracts of *A. officinarum* (68.4-84.8%) and *L. nobilis* (48.2 - 62.7%).

On the other hand, plant growth criteria of tomatoes were significantly increased as influenced by treatments of all plant extracts as compared to plants infected by nematode only and sometimes to the healthy ones too (Table 3). Almost, no significant differences were found between the tested concentrations in increasing growth of tomato plants. Only 47.8% increase was recorded by Carbofuran 10%, while extract of *L. nobilis* achieved the highest increase of total plant growth (90.1 - 100%), followed by extract of *A. officinarum* (37.5 - 65.8%) and *S. argel* (39.0 - 46.1%). Despite extract of *L. nobili*s gave the lowest nematode reduction (Table 2), it provided the highest growth of tomato plants (Table 3). It seems that *L. nobilis* extract may have an activating effect on the growth of tomato plants. Results of Bekhiet (2004) supported this finding. He found that extract of mint (*Mentha microphylla*) gave the lowest reduction of the root-knot nematode, *Meloidogyne javanica* and highly increased growth of potato plants cv. Nicola.. Efficacy of some plant extracts applied as soil drenching comparing to some nematicides such as Carbofuran and Oxamyl in the control of RKN and reniform nematode on some host plants, were reported by other authors (Eldeeb and Mansour, 2002; Khalil, 2002; Alshalaby and Noweer, 2003; Bekhiet, 2004).

The chemical constituents of all studied plants have been reported by many authors (Table 4). One or more of these constituents may have a nematicidal activity against *M. incognita* in the present study. Many glycosides, alkaloids, essential (volatile) oils, terpenoids, sesquiterpenoids, steroids, triterpenoids and phenolics found in some higher plants were described as nematicidal constituents (Al-Rajhi *et al*., 1997, Oka *et al*., 2000; Chitwood, 2002 and Barbosa *et al*., 2010). Further investigations are needed to study mode of action of the studied plant extracts on RKN. It was documented that the mode of action of most synthetic nematicides is attributed to the inhibition of acetylcholinesterase (AChE) activity of nematode (Opperman and Chang, 1990). Fortunately, Ferreira *et al*., (2006) in an *in vitro* study found that ethanolic extract of *L. nobilis* leaves showed a high AChE inhibition reached to 64%. Thus, it was suggested that the nematicidal activity of *L. nobilis* extract towards *M. incognita* in the current study may due to the inhibition of the AChE activity of nematode. Previous study of Korayem *et al*., (1993) confirmed that extracts of *Punica granatum*, *Thymus vulgaris* and *Artemisia absinthium* showed a significant inhibition of AChE activity of *M. incognita* and *Helicotylenchus dihystera* more than that by the nematicide Oxamyl. Furthermore, many alkaloids, glycosides and flavonoids found in other higher plants were described as AChE inhibitors (Mukherjee *et al*., 2007).

Generally, our results suggest that extracts of *A. officinarum, L. nobilis* and *S. argel* have the potential for use in controlling *M. incognita* on tomato plants under greenhouse conditions. However, we have to carry out further experiments to evaluate their economic aspects and nematicidal activity under field conditions with other nematode species affecting other high-value crops.

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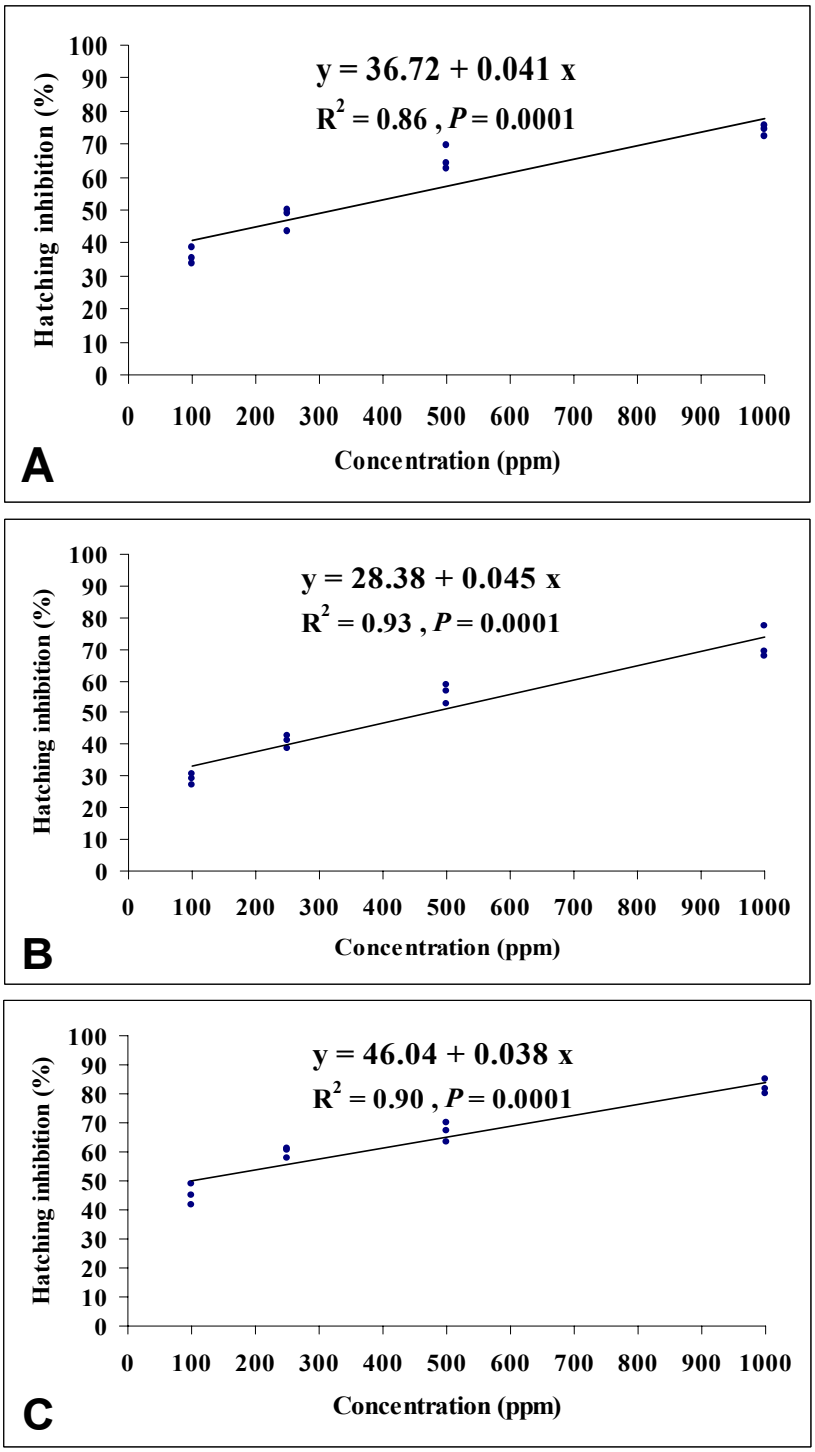

Fig. 1. Inhibition percentages of hatching of *Meloidogyne incognita* eggs exposed for 8 days to different concentrations of crude ethanolic extracts of dried rhizomes of *Alpinia officinarum* (A), and leaves of *Laurus nobilis* (B) and *Solenostemma argel* (C) under laboratory conditions

Table 2. Effect of soil drenching with extracts of *Alpinia officinarum, Laurus nobilis* and *Solenostemma argel* at the concentrations 500 and 1000 ppm, comparing to application of the nematicide Carbofuran 10% (0.1g/pot) on *Meloidogyne incognita* (*Mi*) infecting tomato cv. Marmande, 6 weeks after nematode inoculation under greenhouse conditions (35±5°C).

Treatment	No. of Root galls	Reduction (%)	No. of Egg masses	Reduction (%)	P_f	Rf	Reduction (%)	Relative efficacy (%)* to Carbofuran 10%			
								No. of Root galls	No. of Egg masses	Rf	Total mean
Nematode check (*Mi* only)	177.6 a	–	148.2 a	–	39026 a	7.804 a	–	–	–	–	–
Carbofuran 10% + *Mi*	27.6 f	84.5	16.8 de	88.7	4430 ef	0.886 ef	88.7	–	–	–	–
A. officinarum* (500 ppm) + *Mi	49.0 cd	72.4	22.2 cd	85.0	6038 cd	1.208 cd	84.5	56.3	75.7	73.3	68.4
A. officinarum* (1000 ppm) + *Mi	38.4 def	78.4	18.2 cde	87.7	4900 de	0.982 de	87.4	71.9	92.3	90.2	84.8
L. nobilis* (500 ppm) + *Mi	72.6 b	59.1	30.2 b	79.6	8626 b	1.736 b	77.8	38.0	55.6	51.0	48.2
L. nobilis* (1000 ppm) + *Mi	55.8 c	68.6	24.4 bc	83.5	6348 c	1.270 c	83.7	49.5	68.9	69.8	62.7
S. argel* (500 ppm) + *Mi	42.4 de	76.1	18.6 cde	87.5	4946 de	0.990 de	87.3	65.1	90.3	89.5	81.6
S. argel* (1000 ppm) + *Mi	34.4 ef	80.6	13.4 e	91.0	3338 f	0.668 f	91.4	80.2	125.4	132.6	112.7

- Data are averages of five replicates (one plant each).
- Values within each column followed by the same alphabetical letter(s) are not significantly different according to Fisher's protected LSD at $P = 0.05$.

P_f = final egg population.

Rf = reproduction factor = P_f / P_i, where P_i = initial egg population.

* Relative efficacy (%) = 1-[(Treatment – Nematicide ÷ Treatment)] X 100

Table 3. Effect of soil drenching with extracts of *Alpinia officinarum, Laurus nobilis* and *Solenostemma argel* at the concentrations 500 and 1000 ppm, and application of Carbofuran 10% (0.1g/pot) on growth criteria of tomatoes cv. Marmande infected by *Meloidogyne incognita* (*Mi*), 6 weeks after nematode inoculation under greenhouse conditions (35±5˚C)

Treatment	Fresh weight (g)					
	Shoot	Increase (%)*	Root	Increase (%)	Total	Increase (%)
Nematode check (*Mi* only)	5.88 d	–	2.02 d	–	7.90 d	–
Healthy plants	9.96 ab	69.4	3.06 bc	51.5	13.02 bc	64.8
Carbofuran 10% + *Mi*	8.86 bc	50.7	2.82 bc	39.6	11.68 c	47.8
A. officinarum (500 ppm) + *Mi*	8.22 bc	39.8	2.64 c	30.7	10.86 c	37.5
A. officinarum (1000 ppm) + *Mi*	9.86 abc	67.7	3.24 b	60.4	13.10 bc	65.8
L. nobilis (500 ppm) + *Mi*	10.92 a	85.7	4.10 a	103.0	15.02 ab	90.1
L. nobilis (1000 ppm) + *Mi*	11.32 a	92.5	4.48 a	121.8	15.80 a	100.0
S. argel (500 ppm) + *Mi*	8.04 c	36.7	2.94 bc	45.5	10.98 c	39.0
S. argel (1000 ppm) + *Mi*	8.42 bc	43.2	3.12 bc	54.5	11.54 c	46.1

- Data are averages of five replicates (one plant each).
- Values within each column followed by the same alphabetical letter(s) are not significantly different according to Fisher's protected LSD at $P = 0.05$.

* Increase (%) of growth over the treatment with nematode only.

Table 4. Phytochemical constituents of the rhizomes of *Alpinia officinarum* and leaves of *Laurus nobilis* and *Solenostemma argel*

Plant	Constituents	References
Alpinia officinarum	Tannins, alkaloids, flavonoids, saponins, terpenoids, steroids, volatile oil, diarylheptanoids, glycosides, and total phenol content (called gallic acid). The first four constituents are majors.	Lu and Jiang, 2006 An *et al*., 2010 Sirividya *et al*., 2010
Laurus nobilis	Essential oil, alkaloids, sesquiterpenes, glycosides, phenols, proanthocyanidins, and flavonoids. The essential oil (called 1,8-cineole) is the main active constituent.	Fiorini *et al*., 1998 Simić *et al*., 2004 Škerget *et al*., 2005 Barla *et al*., 2007
Solenostemma argel	Alkaloids, sterols, flavonoids, tannins, saponins, and acylated phenolic glycosides (namely argelin and argelosid).	Kamel, 2003 Ahmed *et al*., 2010

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الملخص العربي

التأثير المثبط لمستخلصات نباتات الخولنجان، الغار والحرجل لفقس بيض نيماتودا تعقد الجذور *Meloidogyne incognita* وإمكانية تطبيقهم في مكافحة النيماتودا على الطماطم

عمرو علي الشربيني، فهد بن عبد الله اليحيى

أدت مستخلصات الإيثانول الخام لكل من ريزومات نبات الخولنجان الجافة وأوراق نباتات الغار والحرجل الجافة عند تركيزات ١٠٠، ٢٥٠، ٥٠٠، ١٠٠٠ جزء في المليون، إلى تثبيط فقس بيض نيماتودا تعقد الجذور *Meloidogyne incognita* معنوياً تحت ظروف المختبر (المعمل). وقد حقق مستخلص الحرجل (١٠٠٠ جزء في المليون) أعلى نسبة تثبيط (٨٢,٠٦٪)، بينما سجل مستخلص الغار (١٠٠ جزء في المليون) أقل نسبة تثبيط (٢٩,١١٪). أظهرت نتائج تحليل البروبيت للمستخلصات الثلاثة أن التركيز المثبط لفقس البيض بنسبة ٥٠٪ (IC_{50}) كان ٢٣٨,٤، ٣٤٦,٢، ١٤١,٨ جزء في المليون لكل من مستخلصات الخولنجان والغار والحرجل، على التوالي. وعلى ذلك فإن مستخلص الحرجل هو الأفضل في تثبيط فقس البيض، يليه مستخلص كل من الخولنجان والغار، على الترتيب.

تمت دراسة إمكانية تطبيق المستخلصات الثلاثة عند تركيزي ٥٠٠، ١٠٠٠ جزء في المليون (كمعاملة غمر التربة عقب التلقيح بالنيماتودا)، مقارنة مع المبيد النيماتودي كاربوفيوران ١٠٪، لمكافحة تعقد الجذور على نباتات الطماطم صنف مارماند تحت ظروف الصوبة الزجاجية.

أدت جميع المعاملات إلى خفض معنوي في إصابة الطماطم بالنيماتودا مقارنة بالنباتات المصابة بالنيماتودا فقط. وقد حقق مستخلص الحرجل (١٠٠٠جزء في المليون) أعلى نسبة خفض في كل من أعداد العقد الجذرية (٨٠,٦٪)، أكياس بيض النيماتودا (٩١,٠٪) وقيمة عامل تكاثر النيماتودا (٩١,٤٪)، بينما أعطى مستخلص الغار (٥٠٠ جزء في المليون) أقل نسبة خفض للإصابة (٥٩,١٪، ٧٩,٦٪ ، ٧٧,٨٪ في كل من أعداد العقد، أكياس البيض وقيمة عامل التكاثر، على التوالي). كما أدت المعاملات بجميع المستخلصات إلى زيادة معنوية في مقاييس نمو نباتات الطماطم، مقارنة بالنباتات المصابة بالنيماتودا فقط. وقد أعطى مستخلص الغار أعلى نسبة زيادة في النمو (٩٠,١ - ١٠٠٪)، تبعه مستخلص الخولنجان (٣٧,٥٪ - ٦٥,٨٪)، ثم مستخلص الحرجل (٣٩,٠٪ - ٤٦,١٪).

سجل مستخلص الحرجل كفاءة إبادية للنيماتودا مرتفعة بالنسبة لمبيد الكاربوفيوران ١٠٪ تراوحت بين ٨١,٦ – ١١٢,٧٪، تبعه مستخلص الخولنجان (٦٨,٤ – ٨٤,٨٪) ثم مستخلص الغار (٤٨,٢ – ٦٢,٧٪).