

## ***In vitro* Nematicidal Activity of Ten Plant Extracts Against Juveniles of *Meloidogyne incognita***

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### **Abstract**

The nematicidal potential of aqueous leaf extracts of ten plant species distributed in agricultural lands in Yemen were assessed against second stage juveniles of *Meloidogyne incognita* in laboratory. The juveniles were exposed to 6, 12, 24 and 48 hrs in three concentrations (12.5, 25 and 50%) of leaf extracts. Leaf extracts of *Datura stramonium*, *Peganum harmala*, *Datura innoxia*, *Argemone mexicana* and *Nicotiana glauca* were effective in causing juvenile mortality, while leaf extracts of the other five plant species, *Azadirachta indica*, *Catha edulis*, *Solanum incanum*, *Tagetes minuta* and *Withania somnifera* showed no or little nematode mortality (< 30 %) even at 50 % concentration and 48 hrs exposure time. The juveniles mortality was increased with increase of concentration and exposure time. Hundred percent juveniles mortality was observed at the modest concentration (25%) of leaf extracts of *D. stramonium* and *D. innoxia* after 24 and 48hrs, respectively. Whereas 100 % mortality of juveniles was found at highest concentration (50%) in leaf extract of *D. stramonium* after 12 hrs and in leaf extracts of *D. innoxia*, *P. harmala* and *N. glauca* after 24 hrs or after 48 hrs in case of leaf extracts of *A. mexicana*. Leaf extract of *D. stramonium* achieved the highest mortality percentage at different concentrations and was significantly ( $p \leq 0.05$ ) superior over the rest of the extracts applied over time.

**Keywords:** *Meloidogyne incognita*, Nematicidal activity, Juveniles mortality, Leaf extracts.

### **Introduction**

Plant parasitic nematodes are serious threats to crop production, causing significant yield losses (**Sasser and Freckman, 1987**). Among the plant-parasitic nematodes, are root-knot nematodes particularly *Meloidogyne incognita* Kofoid and White (Chitwood) causing crop losses of around 15% alone in tropical countries (**Sasser, 1979**). Moreover, yield losses of 50-80% caused by these nematodes in vegetable crops are common (**Siddiqi, 2000**). In Yemen, it is one of the main pests in vegetables and fruit orchards, causing severe and significant yield losses, especially in polyhouses cultivations and nurseries (**Saeed and Shawkat, 2014**). The use of synthetic nematicides has been the most effective method of controlling

plant-parasitic nematodes, but their cost and hazardous effect on human health, environment, ground water contamination and non-target organisms create a necessity to search new, cheap, eco-friendly and harmless methods of nematode control (**Chitwood, 2003**). In recent years, studies have shown the importance of natural nematicidal compounds in the plants themselves that have potential to suppress nematode populations (**Sharma and Trivedi, 2002; Zasada et al., 2002; Banna et al., 2003; Neves et al., 2005; Awadh et al., 2008; Muniasamy et al., 2010; Pavaraj et al., 2010; Abdelnabby and Abdelrahman, 2012; Moosavi, 2012; Pavaraj et al., 2012 and Nelaballe and Mukkara, 2013**). However, little attention has been drawn in Yemen towards the use of plant extracts as nematicides. **Awadh et al., (2008)** investigated *in vitro* the nematicidal effects of some plant extracts against *Ditylenchus dipsaci*. Also, **Saeed and Shawkat, (2014)** studied the nematicidal effect of some botanical powders and poultry manure against the root-knot nematode *M. incognita* in tomato plants under greenhouse conditions. Therefore, the present study was carried out to evaluate efficacy of some plant extracts *in vitro* against J2 of *M. incognita*.

## Materials and Methods

### Plant collection:

Fresh mature healthy leaves of *Argemone mexicana*, *Datura innoxia*, *Datura stramonium*, *Nicotiana glauca*, *Peganum harmala*, *Solanum incanum*, *Tagetes minuta* and *Withania somnifera* were collected from Sanaa University campus, while leaves of *Azadirachta indica* and *Catha edulis* were collected from Tehama and Al Hada regions, respectively. The collected leaves were washed under running tap water and spread for two weeks on polythene sheets on laboratory benches for air drying. The dried leaves were ground separately to coarse particles using DE motore and further grinding to fine powder was done using a Warring® electric blender and stored at room temperature in closed dark containers until use.

### Preparation of Plant Extracts:

Aqueous extracts (10% concentration) were prepared by soaking 15 gm of powdered leaves in 150 ml of sterilized distilled water (SDW) and kept on a rotary shaker at 190-200 rpm for 24 hrs. Then, the mixture was filtered using Whatman® No. 1 filter paper and the residues were soaked and shaken by hand in 20 ml distilled water for 5 minutes. The solution was filtered again and the first and second filtrates were mixed and freeze dried using freeze dryer system/lyph lock 4.5. All residues so obtained were kept in dark glass bottles in a freezer until use.

### Culture preparation:

Adequate second stage juveniles (J2) of *M. incognita* were obtained from stock culture maintained on eggplant cv. Long purple in a glasshouse at the Faculty

of Agriculture, Sana'a University. Only freshly hatched second stage juveniles collected within 48 hrs were used in the study.

### Nematicidal Assay:

Residue of each plant was dissolved in 50 ml distilled water and out of which only 1 ml was poured into 25 ml beaker containing 4 ml distilled water. This solution was considered as a stock solution (S/S) or 100% concentration. Dilutions of S/2 (50%), S/4 (25%) and S/8 (12.5%) were freshly prepared by dilution with distilled water. Freshly hatched second stage juveniles of *M. incognita* were suspended in sterile distilled water. This suspension contained about 25 juveniles /1/2 ml. For mortality assay, 1/2 ml of *M. incognita* suspension was poured into autoclaved 5 cm Petri dishes and 2 ml of each diluted plant extract concentration (12.5%, 25% or 50%) was added separately to Petri dishes. For control treatment, 2 ml of distilled water was added to each Petri dish containing the nematode suspension. All the treatments were replicated thrice. Dead and survived juveniles were counted under a stereoscopic microscope after 6, 12, 24 and 48 hrs of extract exposure. Percentage mortality was calculated from the number of J<sup>2s</sup> that did not move even after pricking the tail.

Data were subjected to ANOVA SPSS 21 software (SPSS Inc., Chicago, IL, USA) and significant differences among the treatments were portioned by **Duncan's (1955)** multiple range test at probability levels of P=0.05.

## Results

Results in Table (1) show the effect of aqueous leaf extracts of ten plant species at different concentrations on mortality of *M. incognita* juveniles over time. The leaf extracts of five plant species, out of the ten tested, showed remarkable nematicidal effects against *M. incognita*. Percentage of juveniles mortality varied, according to the type of plant extract, concentration level and the exposure time. The juvenile mortality was increased with increase of concentration and exposure time. Leaf extracts of the five plant species viz., *D. stramonium*, *P. harmala*, *D. innoxia*, *A. mexicana* and *N. glauca* were effective in causing juvenile mortality, while leaf extracts of the other five plant species viz., *A. indica*, *C. edulis*, *S. incanum*, *T. minuta* and *W. somnifera* showed no or little nematode mortality (< 30 %) even at 50 % concentration and 48 hrs exposure time. With plant species that showed remarkable nematicidal effect it could be noticed that the percentage of juveniles mortality was significantly increased with increase of the concentration over exposure time, except in case of *N. glauca* which caused no or little juveniles mortality when the concentration was increased from 12.5 to 25%, while it achieved the highest mortality increase with increasing concentration to 50%.

Table (1): Effect of aqueous leaves extracts of ten plant species on mortality % of second-stage juveniles (J2) of *Meloidogyne incognita* at different exposure time.

Plant	Concentration (%)	Mortality (%)			
		6h	12h	24h	48h
<i>Argemone mexicana</i>	12.5	*0 ± 0 g	*0 ± 0 g	*5 ± 0 ijk	*10 ± 7 ghijk
	25	21 ± 4 f	35 ± 2 e	63 ± 1 c	77 ± 6 c
	50	42 ± 11 d	88 ± 2 b	98 ± 4 a	100 ± 0 a
<i>Azadirachta indica</i>	12.5	0±0 g	0±0 g	0±0 k	6±3 jk
	25	0±0 g	0±0 g	0±0 k	8±4 ijk
	50	0±0 g	0±0 g	2±3 k	17±9 fghi
<i>Catha edulis</i>	12.5	0±0 g	0±0 g	0±0 k	0±0 k
	25	0±0 g	0±0 g	0±0 k	0±0 k
	50	0±0 g	0±0 g	5±0 hijk	24±5 ef
<i>Datura innoxia</i>	12.5	0±0 g	2±3 g	15±2 ef	36±3 d
	25	8±3 g	52± 11 d	73±9 b	100±0 a
	50	58±4 c	86±4 b	100±0 a	100±0 a
<i>Datura stramonium</i>	12.5	0±0 g	8±4 f	28±0 d	36±12 d
	25	58±1 c	86±3 b	100±0 a	100±0 a
	50	82±6 a	100±0 a	100±0 a	100±0 a
<i>Nicotiana glauca</i>	12.5	0±0 g	5±0 fg	16±1 ef	24±2 ef
	25	0±0 g	9±4 f	17±1 e	34±7 d
	50	32±4 e	85±4 b	100±0 a	100±0 a
<i>Peganum harmala</i>	12.5	3±4 g	5±0 fg	11±1 fgh	16±1 fghi
	25	43±7 d	60±10 c	76±7 b	91±7 b
	50	72±6 b	90±3 b	100±0 a	100±0 a
<i>Solanum incanum</i>	12.5	0±0 g	0±0 g	0±0 k	16±2 fghi
	25	0±0 g	0±0 g	3±4 jk	19±7 fg
	50	0±0 g	2±3 fg	9±0 ghij	30±1 de
<i>Tagetes minuta</i>	12.5	0±0 g	4±0 fg	6±3hijk	8±0 hijk
	25	0±0 g	6±2 fg	10±2 ghi	14±2 ghij
	50	2±3 g	10±2 f	13±1 efg	17±1 fgh
<i>Withania somnifera</i>	12.5	0 ± 0 g	0 ± 0 g	0 ± 0 k	0 ± 0 k
	25	0 ± 0 g	0 ± 0 g	0 ± 0 k	0 ± 0 k
	50	0 ± 0 g	0 ± 0 g	5±0 hijk	17±4 fgh
<b>Control</b>	0	0 ± 0 g	0 ± 0 g	0 ± 0 k	0 ± 0 k

Means in each column followed by the same letter (s) are not significant at  $P \leq 0.05$  according to Duncan's multiple range test.

\* Mean ± standard deviation

Leaf extract of *D. stramonium* achieved the highest mortality percentage at different concentrations and was significantly superior over the rest of the extracts applied over time. Significant mortality was found in leaf extract of the five plant species that showed nematicidal effect, except in case of lowest concentration of *D. stramonium* after 6 hrs; *D. innoxia*, *N. glauca* and *P. harmala* after 6 and 12 hrs, and after all exposure time for *A. mexicana*, and after 6hrs at modest concentration of *D. innoxia* and *N. glauca*. On the other hand, insignificant mortality percentages were found in leaf extracts of the other 5 plant species, except in case of *A. indica*, *C. edulis* and *W. somnifera* at the highest concentration after 48hrs; *S. incanum* at highest concentration after 24 hrs and at the other concentrations after 48 hrs; and *T. minuta* at highest concentration after 12 hrs and after 24 and 48 hrs at modest or highest concentrations.

Hundred percent juveniles mortality was observed at the modest concentration (25%) of leaf extract of *D. stramonium* and *D. innoxia* after 24 and 48hrs, respectively. Whereas 100 % mortality of juveniles was found at highest concentration (50%) in leaf extract of *D. stramonium* after 12hrs and in leaf extracts of *D. innoxia*, *P. harmala* and *N. glauca* after 24 hrs or after 48hrs in case of leaf extract of *A. mexicana*.

At lowest concentration (12.5%) leaf extracts of the tested plants found to be no or less active in nematicidal activity causing only 37 % as a highest mortality after 48 hrs for *D. stramonium* and *D. innoxia*. At modest concentration (25%) leaf extracts of *D. stramonium* exhibited the highest mortality (58%) after 6 hrs followed by *P. harmala* and *A. mexicana* with 43 and 21% mortality, respectively. Lowest mortality 8% was obtained by *D. innoxia*, while the rest extracts showed no mortality at the same exposure time. After 12 and 24 hrs the highest values mortality (86 and 100 %) were achieved by *D. stramonium* followed by *P. harmala*, *D. innoxia* and *A. mexicana* with (60 & 76%), (53 & 73%) and (35 & 63%), respectively. *D. stramonium* and *D. innoxia* gave 100% mortality after 48 hrs followed by *P. harmala* and *A. mexicana* with 91 and 77%, mortality, respectively. At highest concentration (50%) leaf extracts of *D. stramonium* achieved the highest mortality (82%) after 6 hrs followed by *P. harmala*, *D. innoxia* and *A. mexicana* with 72, 58 and 42% mortality, respectively. After 12 hrs 100% juveniles mortality was observed only in leaf extract of *D. stramonium* followed by leaf extracts of *P. harmala* (90%), *A. mexicana* (88%), *D. innoxia* (86%) and *N. glauca* (85%). Whereas 100 % juveniles mortality was observed in leaf extracts of the five effective plants after 24 and 48hrs, except in case of leaf extract of *A. mexicana* after 24hrs which caused 98% mortality.

## Discussion

The obtained results indicated that the aqueous leaf extracts of *D. stramonium*, *P. harmala*, *D. innoxia*, *A. mexicana* and *N. glauca* had nematicidal

activity against *M. incognita*. Leaf extracts of the other tested plant species (*A. indica*, *C. edulis*, *S. incanum*, *T. minuta* and *W. somnifera*) showed no or low juveniles mortality. A positive correlation was found between the juveniles mortality and each of the extract concentration and the exposure time. Extracts of *Datura* species were more effective by increasing the exposure time being the most effective extract. Aqueous leaf extract of *D. stramonium* significantly showed the highest percentage of juvenile mortality, as it caused 100 % mortality after only 12 hrs of exposure time at highest concentration level and after 24 hrs at modest one. These results are in agreement with those obtained by **Nandal and Bhati (1986)**; **Sellami and Mouffarah, (1994)**; **Akhtar and Farzana (1996)**; **Prasad *et al.* (2002)**; **Elbadria (2008)** and **Chaudhary *et al.* (2013)** who reported that *D. stramonium* caused the highest mortality percentage in comparison with other tested plants. Phytochemical analysis revealed that this plant is rich in alkaloids atropine, meteloidine, nicotine, scopolamine, hyoscyamine, terpenoids and flavonoids which have high rate of nematicidal activity (**Shahwar *et al.*, 1995** and **Pavela, 2004**). The alkaloids killed 90 to 100% of *Hoplolaimus indicus*, *Helicotylenchus multicinctus*, and *M. incognita* (**Qamar *et al.*, 1995**). Our results could prove that aqueous leaf extracts of *P. harmala*, *A. mexicana* and *N. glauca* contain active ingredients that exhibit strong nematicidal properties. Nematicidal activity of *P. harmala* and *A. mexicana* was noticed at modest and highest concentrations, while that of *N. glauca* was only noticed at highest concentration. These findings are similar to other results in previous reports (**Mojumdar and Mishra, 1991**; **Shaukat *et al.*, 2002**; **Patel *et al.*, 2004**; **El Allagui *et al.*, 2007**; **Abdelnabby and Abdelrahman 2012**; **Parihar *et al.*, 2012**; **Rizvi *et al.*, 2012**; **El-Hassan *et al.*, 2013**; **Sholevarfard and Moosavi, 2015**).

The bioactivity of *P. harmala* against nematodes may be attributed to the presence of the alkaloids in its leaves including  $\beta$ -carboline, harmine, harmaline, harmalol, harmol and harman and quinazolines as vascine and vasicinone (**El-Hassan *et al.*, 2013**; and **Moloudizargari *et al.*, 2013**). Nematicidal activity of *A. mexicana* might be attributed to its contents of alkaloids (berberine, protopine, sarguinarine, coptisine, chelerytherine), amino acids, phenolics, fatty acids (myristic, palmitic, oleic, linoleic acids) and triglyceride (sn-glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate) (**Saleh *et al.*, 1987**; **Mojumdar and Mishra, 1991**; **Facchini, 2001** and **Shaukat *et al.*, 2002**). In case of *N. glauca*, the biological activity can be attributed to presence of nicotine, and piperidine alkaloid, which can be toxic in high doses to both animals and human. Because of its ability in water it could explain the high affectivity of its water extracts (Webb and Dalzell, 1997). Also, it contains anabasine, as a highly toxic piperidine like alkaloid constituting about 70% of the plant as a whole (**Mizrachi *et al.*, 2000** and **Mhinana *et al.*, 2010**).

Neem (*A. indica*) and marigold (*T. minuta*) have been certified as biological

nematicides by various researchers and used extensively all over the world (**Good et al., 1965; Pluke et al., 1999; Akhtar and Malik, 2000; Chitwood, 2002; Ferraz and de Freitas, 2004; Javed et al., 2007a,b; Adegbite, 2011; Nelaballe and Mukkara, 2013**), but in our results leaf extracts of these plants exhibited no or very little juveniles mortality even at the highest concentration level. A possible explanation for this may be that most studies on the use of neem and miragold leaf extracts in nematode control used high concentrations (**Agbenin et al., 2005, Akpheokhai et al., 2012 and Chaudhary et al., 2013**). These concentrations were many times higher than concentrations of neem and miragold extracts used in this study. **Zongo et al. (1993)** reported that the concentration of active ingredients present in leaf extracts may differ according to environmental conditions, geographical area and the year of collection. **Tibugari, et al. (2012)** postulated that active ingredients may be lost during air drying and preparing process of powder extracts.

The nematicidal potential of leaf extracts of *W. somnifera* and *S. incanum* were very little, but some previous studies found that extracts of *W. somnifera* (**Goel et al., 2005; Khan et al., 2008; Parihar et al., 2012**) and *S. incanum* (**Akhtar and Farzana, 1996**) were effective in causing juvenile mortality. *C. edulis* is one of the most cultivated plant in Yemen. It is a good resistant plant to many pests and diseases. The present study is the first report on the evaluation of its leaf extract for their nematicidal activity against the root knot nematode, *M. incognita*. Only at the highest concentration after 48hrs it significantly caused low mortality percentage (24%).

In this study, leaf extracts of five plants, namely; *D. stramonium*, *P. harmala*, *D. innoxia*, *A. mexicana* and *N. glauca* have the potential in controlling the root- knot nematodes and can be efficiently used as soil amendments. Future research should be focused on micro plot and field experiments, along with assessment the combination use of these amendments with other controlling methods.

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## الملخص العربي

### التأثير الإباضي لمستخلص عشر أنواع نباتية ضد يرقات نيماتودا تعقد الجذور (ميلويد وجين انكوجنيتا) تحت ظروف المعمل

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تحت ظروف المعمل تم دراسة الكفاءة الإبادية للمستخلصات المائية لأوراق عشرة أنواع نباتية منتشرة في الأراضي الزراعية اليمنية ضد يرقات الطور الثاني لنيماتودا تعقد الجذور *Meloidogyne incognita*. عرضت اليرقات لثلاث تراكيز (١٢.٥ و ٢٥ و ٥٠ %) من مستخلصات الأوراق وسجلت نسبة الموت بعد ٦ و ١٢ و ٢٤ و ٤٨ ساعة من التعرض. ولقد أظهرت النتائج كفاءة خمس أنواع نباتية هي الداتورا نوع استرامونيوم *Datura stramonium* والحرمل *Peganum harmala* والداتورا نوع اينوكسيا *Datura innoxia* والسنف (الارغمون المكسيكي) *Argemone mexicana* والتبغ البري *Nicotiana glauca* في قتل النيماتودا، بينما كانت نسبة القتل معدومة أو منخفضة (أقل من ٣٠%) في مستخلص أوراق النباتات الأخرى (الليم - القات - العرصم - النرجس البري - العيب) حتى عند التركيز العالي ٥٠% وبعد ٤٨ ساعة من التعرض. زادت معنوياً نسبة الموت بزيادة التركيز وكذا فترة التعرض. سجلت نسبة موت كاملة ١٠٠% عند التركيز الأوسط ٢٥% لمستخلص أوراق الداتورا نوعي استرامونيوم واينوكسيا بعد فترة ٢٤ و ٤٨ ساعة على التوالي، بينما سجلت عند التركيز الثالث لمستخلص أوراق الداتورا نوع استرامونيوم بعد ١٢ ساعة وبعد ٢٤ ساعة لمستخلص أوراق الداتورا نوع اينوكسيا والحرمل والتبغ البري وبعد ٤٨ ساعة لمستخلص أوراق السنف (الارغمون المكسيكي). سجل مستخلص أوراق الداتورا نوع استرامونيوم أعلى نسبة موت عند كل التركيزات وبفارق معنوي عن مستخلصات الأنواع النباتية المختبرة وعند فترات التعرض المختلفة.