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Anti-nematode Effect Assessment of *Peganum harmala* Based-Products Against *Meloidogyne javanica* on Melon

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Abstract
Natural products from plant origin have been tested for their potential to develop viable components of plant parasitic nematode management strategies especially against *Meloidogyne javanica*. Different products based on *P. harmala* seed evaluated in pot experiments against *M. javanica* associated to *Cucumis melo* L. crop showed that when they are used as soil amendment in pre-planting, not only reduces densities of *M. javanica* in soil and crop damage to melon crops, but also improves significantly growth parameters except for the emulsified oil of *P. harmala* which proved a phytotoxic effect although it gave a complete suppression of *M. javanica*. The aqueous extract and the seeds powder are among the less phytotoxic substances, which are efficient against these pathogens. *P. harmala*-seed-based-products have a potential antinematodes effect against *Meloidogyne javanica* and can be used as component of an integrated management system to control this root knot nematode.
Keywords: nematodes management, *Peganum harmala*; extracts, organic amendment, *Cucumis melo*

1. Introduction
Melon (*Cucumis melo* L.) is a very sensitive crop to root-knot nematodes (*Meloidogyne* spp.). Depending on the age of transplants and initial population density of nematodes in the soil, the threshold of tolerance may be located between 0.48 and 3.53 J2/100g soil (Ploeg 2001). The damage caused by *Meloidogyne* on melon crop are considerable (Sasser 1979 Di Vito et al., 1983; Netscher and Sikora, 1990). The yield losses can reach 65% depending on the level of infestation (Ploeg and Phillips, 2001). To manage these plant parasite, producers do not have efficient means though control by chemicals is the most common. Chemical nematicides, although they are often effective, they are very expensive and sometimes in reexamination or not registered on melon crop. For economic and environmental reasons, development of new management tools that respect the agro-ecosystem has become a necessity nowadays. The application of substances from plant origin is a promising option in crops protection against plant parasitic nematodes (Chitwood, 2002; Kokali-Burelle and Rodriguez-Kabana, 2006). *Peganum harmala* is a reputed medicinal plant for its high toxicity against several microorganisms and animals (Bellakhdar, 1997; Idrissi Hassani, 2000 and Mahmoudian, 2002). Substances from its different organs have various biological activities: antispasmodic, antimitotic, antibacterial, insecticidal and anthelmintic (Ross et al., 1980; Al-Shamma, 1981; Al Yahya, 1986; Sijilmassi, 1996; Bellakhdar, 1997; Jbilou et al., 2006; Arshad, 2008 and Edziri et al., 2010). In most cases, the biological activity observed was attributed to the alkaloids (Ross et al. 1980; Gupta et al. 1978; Duke, 1985 and Ayoub et al., 1994). This study aims to evaluate different products based on *P. harmala* seed. Dried powder, emulsified oil, aqueous, methanolic and hexanic extract for their nématotoxic potential against the most damaging root knot nematodes *Meloidogyne javanica* for melon (*Cucumis melo vars.* Leonardo) crop protection and its impact on this crop growth.

2. Material and Methods
2.1 Botanicals products preparation
The hexanic and methanolic extracts of *Peganum harmala* seeds have been prepared using a continous extraction by soxhlet apparatus and then evaporated on a rotatory evaporator under vacuum at a temperature of 45°C to a small volume. The residual syrup was used for the preparation of different concentrations used for bioassays. The residue obtained by hexane has been dissolved in distilled water using Tween 20 was used at 2% while the dry methanol extract was dissolved in distilled water using ultrasound. The solids residue have been dissolved and stored at +4°C

in a refrigerator.

The aqueous extract of *P. harmala* was performed by macerating plant powder distilled water under ultrasonication for 10 min and then filtered (Whatman No. 1) after shaking for about 24 hours in the dark and stored at 4°C.

Aqueous solution has been performed by macerating 250 g of seed powder in 2 liters of distilled water. After exposing the mixture to ultrasonication for 10 min and agitation for 24 hours, the mixture was centrifuged at 4500 rpm for 20 min and the supernatant was filtered. The resulted solution was stored until use at 4°C.

The oil of *P. harmala* was obtained by cold pressing extraction of seed. This oil was used to formulate an oil emulsion as "oil in water" 2% and stabilized by Tween 20 used at 0,2%.

2.2 Preparation of inoculum and inoculation

Infested Melon Roots with *Meloidogyne javanica* showing typical galls were washed thoroughly under tap water. Then, roots were cut and ground in a blender in 1% of sodium hypochlorite (NaOCl) solution for 2 min to release the eggs from the gelatinous mass. The mixture is filtered through a column of sieves (meshes ranging from 250 microns to 40 microns). The refusal of the 40µm sieve containing eggs of *Meloidogyne* spp. is deposited on a screen covered with a cellulose tissue and placed in a basin filled with water. Neonates (second stage juvenile J2) migrate through the cellulose. After 48hours, the suspension containing the J2 is recovered and the larvae's were collected and counted under a microscope (x40).

The inoculation was performed by a suspension of second stage juveniles of *Meloidogyne spp*. by soaking the soil surface in a circle around the center of jars with a pipette having an initial density of 80 J2/100 g soil. Three days after inoculation, the emulsified oil, hexane, methanolic and aqueous- extract based seed powder of *P. harmala* were applied by simply spraying with a corresponding volum to field capacity in three concentration 2%, 0.2% and 0.02% (w/w). The seeds powder was applied by mixing with the soil. Three controls were considered for this study. A positive control consists in inoculated pot, which received no further treatment. The negative control was represented by only sterilized soil and an adjacent control where the pots were inoculated and treated with Tween 20 used 2% applied in the same volume as the extracts. The treatments were performed in four replicates and arranged in a completely randomized design. Observations are made three months after transplantation to assess treatments effect on *M. javanica* population and melon plants growth. Watering was performed according to the needs of the crop by keeping the soil moist.

The collected data were statistically analyzed using the statistical software "SPSS 11.5". Treatments means were tested using the Newman and Keuls test ($P \leq 5\%$).

3. Results

Seedlings transplanted in soil treated with the seed oil of *P. harmala* at 2% showed phytotoxicity symptoms since the first weeks of the experiment. This increased phytotoxicity was expressed by a very slow growth and yellowing leaves. The amendment of soil by different products of *Peganum harmala* seeds, reduced significantly the population of *M. javanica* and the gall index ($p < 0,05$). The rate of reduction compared to control increases significantly with increasing product concentration. The aqueous extract and powder applied at 2% and oil used at 0.2 and 2% are most active substance from *P. harmala* seeds on soil population of *M. javanica* and do not show significant differences with the negative control. With the exception of the aqueous extract, the emulsified oil and methanol extract at 0.02%, all other treatments show a significant difference with the positive control (Table 1). The emulsified oil is the most effective product against *M. javanica*. However, the aqueous extract is the product that had an interesting potential of activity and has not show any phytototoxicity effect on melon crop. This constitutes an advantage compared to the emulsified oil. The hexane extract remained the least effective.

3.1 Impacts of treatments on root knot nematodes population

With the exception of the hexane extract, the majority of the tested products significantly reduce galling on root of melon. Powder, aqueous extracts and oil seeds of *P. harmala* applied at 2% allow better protection of plant roots of *C. melo* attacks against *M. javanica*. The number and the index of galls on the roots of plants grown on soil treated with these extracts is very low compared to the control treatment. The 2% oil completely inhibited the development of galls on roots and is the only product that does not reveal any significant difference with the negative control. The ground material, the aqueous extract and methanolic 2% have a relatively lower inhibition potential than the oil (Table 2)

3.2 Impact of treatments on crop growth

The vegetative growth (stem length and dry weight of root) increases very significantly when the soil is amended by

the homogenate of *P. harmala* or treated with aqueous and methanolic extract particularly at the dose 2% except where the soil is treated with hexane extract and emulsified oil. This treatment causes a strong inhibition of vegetative growth as result of a pronounced phytotoxicity when the dose increases. Improved growth was recorded when seed powder was applied at 0.2 and 2% as rate of amendment; as well as in the case when the soil is treated with aqueous or methanolic extracts at 2% (Table 3).

4. Discussion

Most products made from seeds of *P. harmala* at the high concentration (2%) reduce the population of *Meloidogyne javanica* associated to the melon crop and limit galling on roots significantly. At the highest used concentration, seeds aqueous extract and emulsified oil of *P. harmala* are the most effective on *M. javanica*. The emulsified oil provided a complete suppression of root knot nematodes and total protection of roots. Unfortunately this product has induced a pronounced phytotoxicity on melon plants expressed by partial inhibition of vegetative growth. But probably an increase of separating period (needed for the bio-decomposition to release the toxic products) between the transplantation date and the moment of product application would overcome this problem. The lack of difference between the treated soil with 2% of Tween 20 and the positive control indicates that it has no effect on the population density of *Meloidogyne javanica* in soil or growth of melon plants. On the other hand, the nematicidal effects induced by emulsified oil from *P. harmala* seeds could be attributed to the oil alone. Concerning the powder and the aqueous extract, which showed a similar effect, the population of *M. javanica* in soil and galling have been significantly reduced and vegetative growth was significantly improved. Various plants are known to produce and release antibiotic components via two major processes: (1) root exudation and (2) release of components during plant decomposition after incorporation in the soil. The antimicrobial activity of *P. harmala* seeds was demonstrated in many studies and was related to the abundance of the alkaloids in this organ. These alkaloids include β-carboline as: harmine, harmaline, harmalol, harmol and Harman and quinazolines as vascine and vasicinone. Bais and his colleagues reported that harmaline and harmine are endowed with antimicrobial activity against terrestrial pathogenic microorganisms and these two molecules are formed and released from the plant *Oxalis tuberosa* in the rhizosphere to meet the microbial invasion (Bais *et al*., 2003). Therefore, the effectiveness of *P. harmala* on the population of root-knot nematodes may also be attributed to the direct action of these alkaloids on J2. In addition, these alkaloids are nitrogen-containing molecules and very abundant in *P. harmala* seeds. Probably, soil amendment by powders from seeds increases the rate of nitrogen availability to crop, which could cause a reduction in nematode population and improves crop growth and vigor. Mian and Rodriguez-Kabana (1982) reported that the potential for management of nematodes with organic amendment is directly dependent on the nitrogen content and inversely related to C/N. Ammonium derived from microbial decomposition of the amendments play an important role in control of nematodes and fungi for a short period (days to weeks) after application (Oka et al., 1993; Tenuta and Lazarovits, 2002). The mode of biocidal action of ammonia is not yet clear, but several mechanisms may be involved: alteration of the cell membrane (Rush and Lyda, 1982), depletion of the chemical energy of the cells carrying the cytosolic ammonia against the concentration gradient (Britto et al., 2001). The limit of using organic amendment that release nitrogen is the necessary amount for an effective control because it is frequently phytotoxic or not economic (Stirling, 1991). Oka (2010) reported that the nematicidal activity of this type of amendment can be improved by manipulating the soil environment to reduce the amount needed for a control practice.

5. Conclusion

It is possible to conclude that powder, oil, aqueous and methanolic extract based from *P. harmala* seeds have a potential antinematode effect against the population of *Meloidogyne javanica* on melon crop, can limit their damage and improve its growth. To enhance the performance of these botanicals for nematode control, further investigation is needed to determine how *P. harmala* seed products act on nematodes (lethal, nematostatic, repellent or attractant) their mode of action, identify the active compound and appropriate conditions and methods to use as a component in a multifaceted approach in a sustainable management of agro ecosystem.

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Table 1. Effect of different products from *P. harmala* seeds on the *Meloidogyne javanica* population density in soil

Treatments	Concentration (%)	final Density J2/100 cm3
Dryed powder	0,02	29,25b,c,d*
	0,20	34,00b,c,d
	2,00	17,00a,b,c
Aquous extract	0,02	38,25d,e
	0,20	23,25b,c,d
	2,00	17,00a,b,c
Hexane extract	0,02	32,75 b,c,d
	0,20	32,25 b,c,d
	2,00	21,25b,c,d
Emulsified oil	0,02	36,67c,d,e
	0,20	**15,00a,b**
	2,00	**0,00a**
Methanolic extract	0,02	40,50d,e
	0,20	24,75b,c,d
	2,00	29,50b,c,d
Positif control		52,25e
Tween 20 (0,2%)		63,50e

* Numbers followed by the same letter in the same column are not significantly different according to Newman and Keuls test (P ≤ 5%).

Table 2. Effect of different products from *P. harmala* seeds on root galling induced by *Meloidogyne javanica* on *Cucumus melo* vars. Leonardo

Treatments	Concentration (%)	Galls number	Gall index
Dryed powder	0,02	14,75c,d*	02,75c,d
	0,20	15,67c,d	03,00c,d,e
	2,00	**01,50a**	**01,00b**
Aquous extract	0,02	29,25e,f	03,50c,d,e
	0,20	11,50b,c	02,50c
	2,00	**04,75a,b**	**01,75b**
Hexane extract	0,02	29,25e,f	03,75d,e
	0,20	19,75c,d,e	03,00c,d,e
	2,00	18,00c,d,e	03,00c,d,e
Emulsified oil	0,02	22,33c,d,e	03,00c,d,e
	0,20	23,00e	03,33c,d,e
	2,00	**0,00a**	**0,00a**
Methanolic extract	0,02	26,25e	03,25c,d,e
	0,20	24,75e	03,25c,d,e
	2,00	**13,00b,c**	**02,33b**
Positif control		37,00f	04,00e
Tween 20 (0,2%)		37,25f	04,00e

* Numbers followed by the same letter in the same column are not significantly different according to Newman and Keuls test (P ≤ 5%).

Table 3: Effect of different products from *P. harmala* seeds on growth parameters of *Cucumus melo* vars. Leonardo.

Treatments	**Concentration (%)**	**Stem dry weight (g)**	**Stem length (cm)**	**Root dry weight (g)**
Dryed powder	0,02	4,971b,c,d*	41,50b,c,d	**00,26e,f**
	0,20	6,429a,b	53,67a,b	00,33d,e
	2,00	7,936a	**62,00a**	**00,71a**
Aquous extract	0,02	5,599b,c,d	51,00a,b,c	00,50b,c
	0,20	5,031b,c,d	44,00a,b,c,d	00,48b,c,d
	2,00	**7,637a**	**61,25a**	**00,69a**
Hexane extract	0,02	5,352b,c,d	48,75a,b,c,d	00,49b,c,d
	0,20	5,343b,c,d	48,67a,b,c,d	00,49b,c,d
	2,00	4,941b,c,d	45,00a,b,c,d,	00,45b,c,d
Emulsified oil	0,02	3,586d	32,66d	00,33d,e
	0,20	3,769c,d	34,33c,d	00,34d,e
	2,00	1,949e	**17,75e**	**00,18f**
Methanolic extract	0,02	5,243b,c,d	47,75a,b,c,d	00,48b,c,d
	0,20	5,737b,c,d	52,25a,b	00,52b,c,
	2,00	6,631a,b	**58,25a,b**	**00,58b**
Negatif control		5,929b,c	**54,00a,b**	00,38c,d,e
Positif control		4,314c,d	52,25b	00,41c,d,e
Tween 20 (0,2%)		4,045c,d	53,01b	00,40c,d,e

* Numbers followed by the same letter in the same column are not significantly different according to Newman and Keuls test ($P \leq 5\%$).

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