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Nematicidal activity of aqueous and organic extracts of local plants against *Meloidogyne incognita* (Kofoid and White) Chitwood in Algeria under laboratory and greenhouse conditions

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Abstract

The nematicidal efficiency of two types of extracts (aqueous and methanolic) of 5 plants *Peganum harmala* L., *Raphanus raphanistrum* L., *Taxus baccata* L., *Sinapis arvensis* L., and *Ricinus communis* L. on second stage juveniles (J2s) of *Meloidogyne incognita* was evaluated at 4 doses in vitro (40, 50, 60 and 80%) at 4 exposure times (12, 16, 24, and 32 h). In a greenhouse, methanol extracts obtained from 0.75, 1.5, or 3 g of the dry matter of *T. baccata*, *S. arvensis*, and *P. harmala* were added in pots of tomato plants (Moneymaker cultivar) to test the effect of the extracts on the nematode, its reproduction, and the plant's growth. After 32 h of exposure, the in vitro results on J2s showed that all the aqueous and methanolic extracts of the 5 plants had positive effects on J2 mortality compared to controls; water and DMSO (2%) (dimethyl sulfoxide). In general, methanolic extracts were significantly more effective than the aqueous ones. Methanolic extracts of *T. baccata*, *P. harmala*, and *S. arvensis* had the highest mortality rates (100, 89.2, 86.6%), respectively, followed by the aqueous extract of *T. baccata* (73.8%). After 12 days, the hatching inhibition varies between (61.4%) for the aqueous extract of *R. communis* and (84.2%) for the methanolic extract of *T. baccata*. The average was significantly different from controls (DMSO 23.8 and water 21.8%) for all extracts. Methanolic extracts were not always significantly different than the aqueous ones. In vivo methanolic extracts of *S. arvensis*, *P. harmala*, and *T. baccata* reduced infestation than the controls. The gall index varied between 3.5 and 5.5 for the extracts than the positive control 6.5. The present study revealed the effectiveness of all tested plant extracts to root-knot nematodes without any chemical inputs.

Keywords: *Meloidogyne incognita*, Plant extracts, Hatch suppression, Juvenile mortality

Background

Cecidogenic nematodes are pests that cause serious damage to several crops, mainly horticulture (Adegbite et al. 2005). Crop production problems induced by nematodes therefore generally occur as a result of root

dysfunction, reducing rooting volume and foraging and utilization efficiency of water and nutrients (Hemlata and Jyoti 2018).

In Algeria, the distribution of *Meloidogyne* nematodes affects areas of high vegetable crop productivity (Alger, Constantine, Biskra, Ouargla, Adrar) (Babaali et al. 2016). Several methods are available to control the plant parasitic nematodes. Synthetic nematicides are used most often compared to other known nematode control

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strategies because they are effective and quick in action (Odeyemi et al. 2013). The indiscriminate use of synthetic pesticides to control nematodes was likely to give rise to phytotoxicity, environmental pollution, and resistance to nematodes, in addition to its very high cost (Adegbite et al. 2005). The growing concern for the environment and the recent ban on many nematicides has required a reduction in the amount of chemical nematicides and the development of non-chemical options (Odeyemi et al. 2013). In addition, the search for effective, environmentally sound and safe alternative control methods has been intensified.

One of the possible alternatives is the utilization of biopesticides from plant origin, known as botanical pesticides (Javed et al. 2007). These biopesticides are generally considered to be non-persistent under field conditions as they are readily transformed by light, oxygen, and microorganisms into less toxic products (Wiratno et al. 2009). Numerous plant species, representing 57 families including *Lamiaceae*, *Asteraceae*, *Myrtaceae*, *Rutaceae*, and *Lauraceae*, can contain nematicidal compounds (Andrés et al. 2012). The use of plant extracts as an alternative tool to synthetic pesticides for control of root-knot nematode has become important (Kepenekçi et al. 2016). The use of plant extracts against *Meloidogyne* spp. has shown their effectiveness in several previous studies (Hassan et al. 2013; Curto et al. 2015; Xia et al. 2019).

The objective of the present work was evaluation of juvenicidal and ovicidal potential of aqueous and organic extracts of 5 local plants on *Meloidogyne incognita* under controlled conditions.

Materials and methods

Plant extracts

Five plants (*Peganum harmala*, *Raphanus raphanistrum*, *Taxus baccata*, *Sinapis arvensis*, and *Ricinus communis*), belong to 4 different botanical families, were collected at their vegetative stage from different regions of Algeria at different bioclimatic levels in 2017. The collected plants were identified using voucher specimens deposited in the herbarium of the Botany Department of Ecole Nationale Supérieure d'Agronomie (ENSA, ex. INA, Algiers, Algeria); identification was further confirmed by B. FARSSI, Doctor of Ecology in the same Department. Plant leaves were dried in shade for 15 days under laboratory conditions and then turned to fine powders using a commercial grinder.

The species used (wild rue, wild radish, field mustard, and castor bean) were chosen because of their nematicidal effects as shown in several previous studies (Hassan et al. 2013; Curto et al. 2015; Xia et al. 2019). *T. baccata* was chosen for its medicinal properties and it has not been tested previously on nematodes (Guenard et al.

1993). In order to prepare the inoculum and/or in plant test, tomato plants were used. The seeds of tomato (*Solanum lycopersicum* L. cv. Moneymaker, Solanaceae) were sown in alveolate plates and raised in a greenhouse for 1 month, and then the seedlings were transplanted into 2.5 L plastic pots (17.5 × 14.5 cm: diameter × height) containing 1 L of a sand mold mixture (1/2:1/2).

Preparation of aqueous and organic extracts

Two types of extracts (organic and aqueous) were used in this study. A quantity of 25 g of powder of each plant species (*P. harmala*, *R. raphanistrum*, *T. baccata*, *S. arvensis*, and *R. communis*) was placed in 500 ml glass flasks containing 250 ml of solvent (distilled water or methanol at 80% for aqueous or organic extraction, respectively) (Dane et al. 2015). The flasks were placed under an orbital shaker (Ika-Werke-GMBH & CO.KG D-79219 Staufen, Germany) for 4 h at 500 rpm. The mixture was filtered through a funnel equipped with filter paper (N°1; 100 µm) and centrifuged (Horizontal centrifuge, Swing-3000, Apogee, Germany) for 15 min at 1500 rpm to remove debris. The solvent (methanol) of the organic extract was evaporated using the Rotavapor (Laborgerate, GmbH, ISOLAB) at 60 °C, while the water of aqueous extract was evaporated by water bath (LWB-111D, LabTech, Korea) at 60 °C. All quantities obtained from aqueous extracts after evaporation were diluted by 25 ml of distilled water, while the methanolic extracts were diluted by 25 ml of DMSO (2%) (dimethyl sulfoxide). The solution was considered as a stock solution and stored at 4 °C for up to 24 h. (Babaali et al. 2017).

Nematode inoculum

Tomato roots infected with *Meloidogyne incognita* were sampled from the Staoueli region (25 km west of Algiers, Algeria) and transferred to the laboratory. The egg masses were carefully detached from the roots and placed in hatchers containing sterile distilled water (SDW). After 48 h, the juveniles were recovered in graduated beakers. One milliliter of the suspension was collected and observed under an optical microscope to determine the number of existing J2s in the solution (the recommended concentration of J2s of inoculum was always adjusted by adding sterile distilled water for juveniles). This step was repeated at least 10 times. The multiplication of the number of juveniles (J2s) was carried out on tomato seedlings (Moneymaker cultivar) on greenhouse at experimental station of ENSA. The tomato seedlings were inoculated 1 month after transplanting by 2500 J2s (tomato seedlings were irrigated regularly for 60 days). After 2 months, fresh egg masses were collected and hatched.

Table 1 Percentages of the different volumes of the stock solution in the juvenile mortality test

Juvenile suspension (1 ml)				
Volumes of stock solution (ml)	0.8	0.6	0.5	0.4
Percentages (%)	80	60	50	40

Biological assays

In vitro test

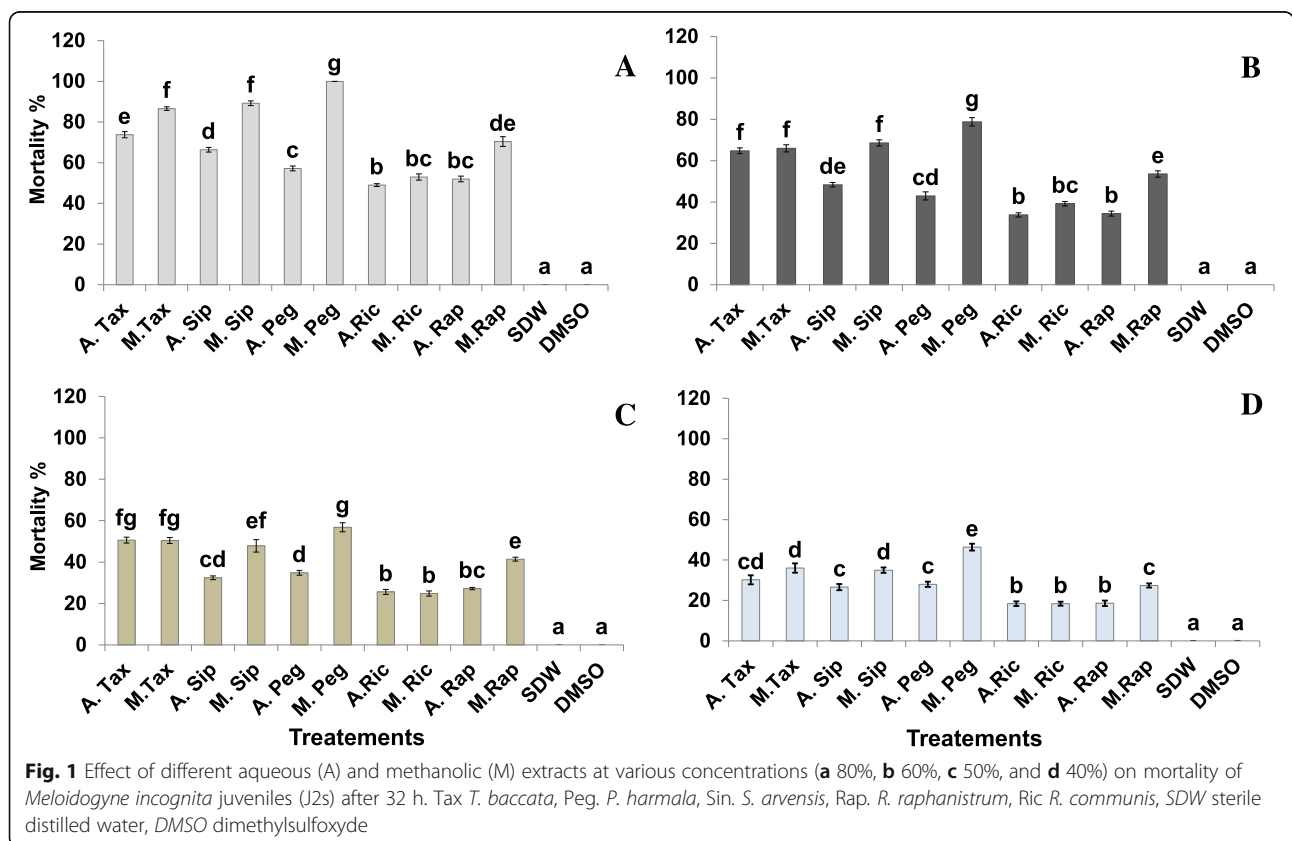
Juveniles mortality bioassay The effect of aqueous and organic (methanolic) extracts at various concentrations (Table 1), obtained from the stock solution (considering that 1 ml of stock solution with 1 ml of J2s suspension was the 100% concentration) on the juveniles of 48 h was evaluated. One milliliter nematode suspension > 100 J2s in a 24-well plate maintained in an incubator, in the dark, at 24 °C. Each treatment was repeated 5 times. Using a double counter, 100 J2s between dead and live juveniles were counted after 12, 16, 24, and 32 h of exposure considering a young straight or motionless juvenile as dead, which was confirmed by touching them with a fine needle. To compare the results, natural mortality was counted in water and in DMSO at 2% and they were considered as controls. The lethal concentration (LC₅₀)

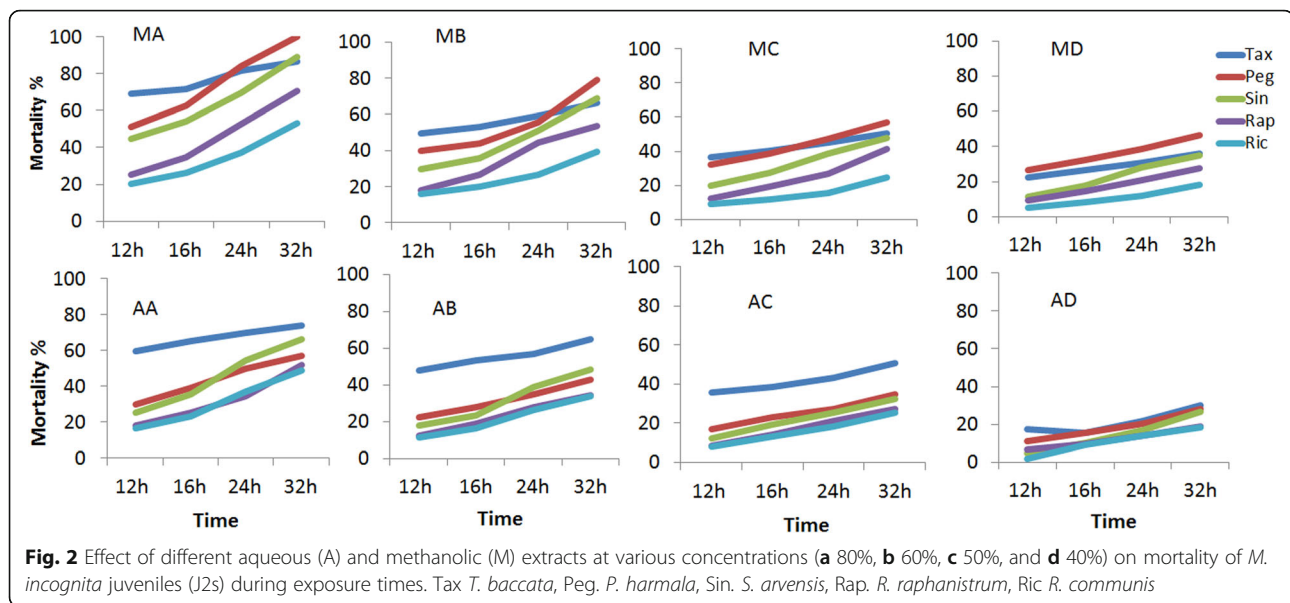
to exterminate 50% of juveniles was calculated using probit analysis (Bliss 1934).

Hatching bioassay For the evaluation of the effect of the 2 types of extracts (aqueous and organic) at the same concentrations, used in the previous test on the hatching of *M. incognita* eggs, a suspension was prepared from the eggs extracted from the tomato roots with a 0.5% NaOCl (sodium hypochlorite) solution, knowing that the *M. incognita* eggs were obtained by passing the solution through a 250-µm sieve and rinsing the eggs with sterile water in a 25-µm sieve (Hussey and Barker 1973). Each treatment consisted of 5 replicates of 100 eggs in 1 ml of each extract concentration. The experiment was conducted in 24-well plate maintained in an incubator, in the dark, at 24 °C. Hatched J2s were counted after 12 days in treatments. The controls used were eggs treated with DMSO (2%) and eggs treated with sterile distilled water (Babaali et al. 2017). The hatching inhibition rate R (HI) was calculated using the following formula:

$$R (HI) = [(Nie - Neh)/Nie]*100$$

where Nie is the initial number of eggs and Neh is the number of eggs hatched.





In vivo infestation of nematodes into roots of the tomato bioassay To confirm the results obtained in vitro, plant test was performed. In this test, the methanolic extracts used were *P. harmala*, *T. baccata*, and *S. arvensis* (their doses were 3, 1.5, and 0.75 ml for each dose, and water was added to reach 20 ml). This experiment was conducted in pots using tomato seedlings of the Moneymaker cultivar, which is very sensitive to *M. incognita* under greenhouses. The treatments were applied 1 day before transplanting the tomato seedlings. After 1 day of transplantation, 1500 J2s (48 h old) were deposited in 5 holes around the stem at a depth of 3 cm. Unlike the positive control seedlings, which were treated by the nematodes juveniles and by sterile distilled water, the negative control seedlings were treated by sterile distilled water only. Each treatment was repeated 6 times. At the end of the test, the infestation parameters (galls index, number of juveniles per root, number of galls, and number of egg masses) and growth parameters (dry weight of the aerial part and of the root) were estimated. The gall index was estimated according to Zeck's rating of 1 to 10 (Zeck 1971).

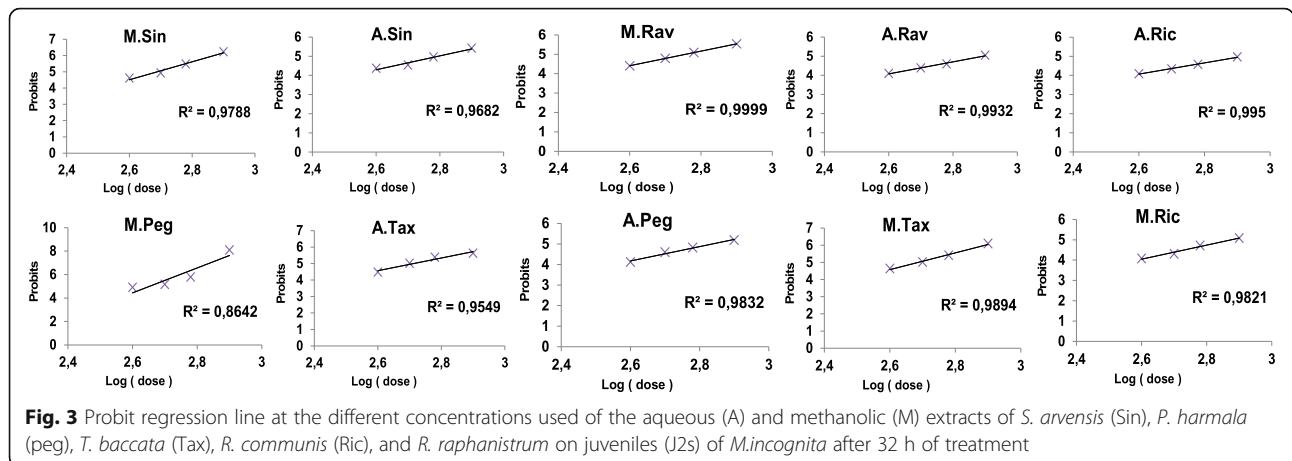
Data processing Experiments were conducted in a complete randomized design. The data were performed by the analysis of variance (two-way ANOVA) for juvenile mortality and for in vivo test parameters, three-way ANOVA for egg hatching, and their means were compared, using HSD of Tukey at $P < 0.05$, using the STATISTICA software (Statistica version 8.5 year 2014).

Results and discussion

The two types of extracts of the 5 plant species at different concentrations and at different times of exposure showed different effects on the J2s mortality rate, on the hatching rate, and on the degree of infestation of juveniles in the roots of tomato plants. To the best of our knowledge, this is the first time that the leaves of local species; *P. harmala*, *T. baccata*, *R. raphanistrum*, and *R. communis* have been tested. Various plant species have been studied as a potential biocontrol tool against *M. incognita* and were therefore proposed as nematicidal. This was the case for *Artemisia vulgaris* L., *Azadirachta indica* A. Jass, roots of *Ricinu scommunis* L., *Nicotiana tabacum* L., *Syzygium aromaticum* L., *Piper betle* L., *Capsicum*

Table 2 Lethal concentration 50 (LC₅₀) of the effect of extracts on *M. incognita* J2s mortality after 32 h of treatment

Species	Methanolic extract		Aqueous extract	
	LC ₅₀ (ml/mlwate)	Slope	LC ₅₀ (ml/mlwate)	Slope
<i>T. baccata</i>	0.43	$y = 4.9306x - 8.2421$	0.51	$y = 3.8489x - 5.4401$
<i>P. harmala</i>	0.44	$y = 10.64x - 23.221$	0.69	$y = 3.4938x - 4.908$
<i>S. arvensis</i>	0.48	$y = 5.5311x - 9.8702$	0.62	$y = 3.6387x - 5.1658$
<i>R. raphanistrum</i>	0.57	$y = 3.7723x - 5.4048$	0.78	$y = 3.1408x - 4.0865$
<i>R. communis</i>	0.75	$y = 3.4586x - 4.9463$	0.83	$y = 2.9389x - 3.5748$



annuum L., *Zingiber officinale* Roscoe, *Parkiabiglobosa* (Jacq.), *Datura stramonium* L., and *Datura innoxia* Mill. (Costa et al. 2003; Adegbite and Adesiyani 2005; Wiratno et al. 2009; Bawa et al. 2014; Babaali et al. 2017).

In vitro test

Effect of plants extract on the juveniles mortality

The two types (methanolic or aqueous) of extracts from the 5 tested plants (*P. harmala*, *R. raphanistrum*, *T. baccata*, *S. arvensis*, and *R. communis*) showed efficacy on *M.incognita* J2s (Fig. 1). The results are confirmed by Rich et al. (1989), Hassan et al. (2013), and Curto et al. (2015), when they used the seed extracts of *Ricinus communis* and *Peganum harmala* and the extracts of the 3 brassicaceae species of (*Eruca sativa*, *Raphanu ssativus* sp. *oleiformis*, *Reseda luteola*) on *Meloidogyne* juveniles, and found them effective.

This effect was consistent with the increase in concentration and the exposure period (Fig. 2), which is consistent with that reported by (Adegbite and Adesiyani 2005; Chaudhary et al. 2013; Kepenekçi et al. 2016).

The species, *T. baccata* and *P. harmala*, showed the highest nematicidal effect as they acted quickly with a shock effect from the first hours of immersion (12 h) J2s. At the highest concentration (80%), the mortality rate of J2s treated with methanolic extracts of *T.baccata* and *P.harmala* reached 69 and 51%, respectively, while the mortality rate of J2s treated with methanolic extracts of *S.arvensis* and *R.raphanistrum* was lower. It reached 44 and 25%, respectively. Moreover, the lowest mortality rate of methanol extracts was recorded at *R.communis* with 20.2%. Mortality rates of J2s treated with aqueous extracts of *T.baccata*, *P.harmala*, *S. arvensis*, *R.*

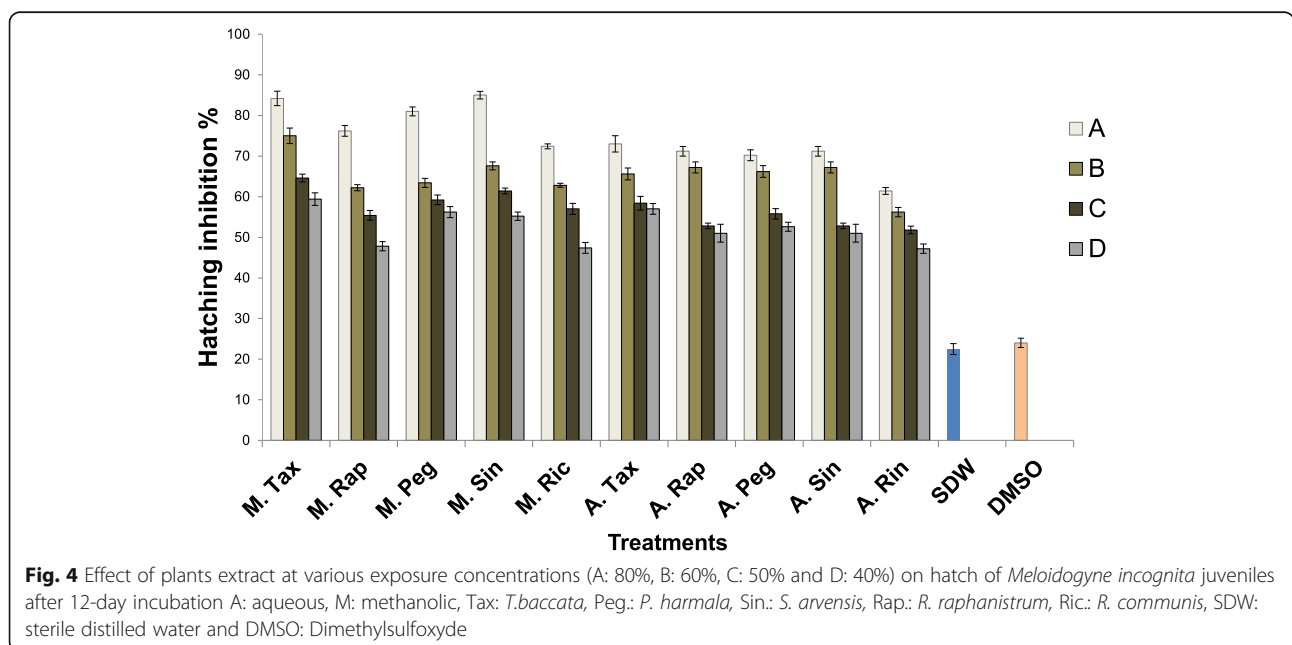


Table 3 Effect of the methanolic extracts of *S.arvensis*, *P.harmala*, and *T.baccata* applied in the soil on the reproduction of *M.incognita* and the growth of tomato plants under greenhouse

Plant extract	Extract dosage (ml/pot)	Gall index	Number of J2s	Number of egg masses	Number of galls	Dry weight of the aerial part	Dry weight of roots
Methanolic <i>S.arvensis</i>	3	4.5 ± 0.22 bcd	3500.33 ± 231.45bc	72.50 ± 5.65b	121.66 ± 5.23bc	7.10 ± 0.46cde	2.09 ± 0.26a
	1.5	5.0 ± 0.25 cde	3687.33 ± 238.61cd	97.00 ± 11.30bce	128.33 ± 4.93c	6.01 ± 0.47bcd	2.12 ± 0.20a
	0.75	5.66 ± 0.21 de	4416.83 ± 263.12d	104.5 ± 4.89cef	146.83 ± 12.07cd	4.76 ± 0.60b	1.24 ± 0.15a
Methanolic <i>T. baccata</i>	3	4.00 ± 0.25 bc	2682.50 ± 151.36b	87.33 ± 3.30bc	125.33 ± 8.08bc	7.54 ± 0.51de	1.53 ± 0.31a
	1.5	4.500 ± 0.22 bcd	3723.50 ± 205.96cd	129.83 ± 12.12f	176.00 ± 5.70de	6.55 ± 0.54bcde	1.21 ± 0.08a
Methanolic <i>P. harmala</i>	0.75	4.83 ± 0.30 cd	4390.00 ± 205.47d	164.00 ± 11.93g	190.33 ± 9.85e	5.23 ± 0.34bc	1.07 ± 0.15a
	3	3.50 ± 0.22 b	3140.50 ± 73.78bc	68.16 ± 5.28b	86.83 ± 4.60.b	6.87 ± 0.58bcde	1.38 ± 0.10a
	1.5	4.83 ± 0.30 cd	4017.0 ± 0177.32cd	121.00 ± 5.5ef	144.66 ± 5.94cd	6.54 ± 0.56bcde	1.37 ± 0.19a
Positif control (inoculum)	0.75	5.50 ± 0.22 de	4437.16 ± 178.38d	164.16 ± 3.09g	160.33 ± 12.78cde	5.45 ± 0.64bcd	1.76 ± 0.11a
Negatif control	/	6.16 ± 0.40 e	7319.66 ± 197.74e	178.16 ± 4.25g	244.66 ± 10.84f	1.24 ± 0.15a	1.32 ± 0.25a
<i>P</i> value	/	00 ± 00a <i>P</i> = 0.01	00 ± 00a <i>P</i> = 4.4.10 ⁻⁵	00 ± 00a <i>P</i> = 3, 6.10 ⁻⁷	00 ± 00a <i>P</i> = 9.10 ⁻⁶	8.21 ± 0.16e <i>P</i> = 0.13	4.28 ± 0.25b <i>P</i> = 0.15

Means with the different letters are significantly different according to the Tukey's test ($P \leq 0.05$). Values with ± represent the standard errors

raphanistrum, and *R. communis* at the same exposure time and the same concentration attained 59, 30, 25, 18, and 16.2% respectively. After 32 h, the percentage of mortality reached 100% at 80% by the methanolic extract of *P.harmala*. The same extract of *S.arvensis* recorded a considerable increase up to 89.2% unlike the extract of *T.baccata*, which showed an irregular increase to reach 86.6%. The best aqueous extracts were of *T. baccata*, which caused a mortality rate of 73.8 at 80% after 32 h of exposure, exceeding the recorded rate of 70.4 and 53% for methanolic extracts (80%, 32 h) of *R. raphanistrum* and *R. communis*, respectively. In this test, the lowest mortality rate (1.8%) was recorded in juveniles treated with aqueous extract of *R.communis* at a concentration of 40% and exposure time of 12 h, followed by mortality rates of 4.6, 6.4, 5.4, and 9.2% and recorded in juveniles treated with aqueous extract of *S. arvensis* and *R. raphanistrum* and with methanolic extract of *R. communis* and *R. raphanistrum*, respectively. Obtained results showed that the organic extract had better efficacy than the aqueous one on J2s at the 4 concentrations and all exposures times.

The results presented in (Table 2 and Fig. 3) showed that the LC₅₀ values calculated for the 32 h of exposure time ranged from 0.43 ml/ml (methanolic extract of *T.baccata*) to 0.83 ml/ml (aqueous extract of *R. communis*). The highest LC₅₀ of all methanolic extracts expressed by 0.75 ml/ml (extract of *R.communis*) and the lowest LC₅₀ of all aqueous extracts by 0.51 ml/ml (extract of *T. baccata*).

Effect of plants extracts on egg hatching of *M.incognita*

Different extracts (methanolic and aqueous) of the tested 5 plants showed very highly significant effects ($P = 5, 3.10^{-5}$) on egg hatching. After 12 days of exposure of *M. incognita* eggs to the various extracts, significant differences with the DMSO 2% and water controls recorded egg hatch inhibition of 23.8 and 21.8%, respectively (Fig. 4). The inhibition rate varied between 47.2 at 40% for the aqueous extract of *R. communis* and 85.2 at 80% for the methanolic extract of *S. arvensis*, followed by the methanolic extracts of *T. baccata* and *P. harmala* at the same dose with 84.2 and 81%, respectively. Concerning the juveniles, the methanolic extract of *P. harmala* gave (100%) mortality rate; the methanolic and aqueous extracts of *R. communis* had a better yield than its juvenicidal effect. The percentage of inhibition in hatching increased with the increase of the concentration of the extracts from 40 to 80%. In the present study, the efficacy of methanolic extracts compared to aqueous extracts was also verified for the hatch inhibition of *M.incognita* eggs. Similarly, in previous studies, they reported that the effect of organic extracts was better than the effect of aqueous extracts, and the rate of egg inhibition increased by increasing the concentrations

of extracts (Chaudhary et al. 2013; Kepenekçi et al. 2016; Babaali et al. 2017).

In vivo test

Effect of plant extracts on root infestation of nematodes

At the end of the test, the roots of the tomato plants inoculated with the suspension of juveniles of *M.incognita* and treated with the 3 selected plant extracts (*S. arvensis*, *P. harmala* and *T. baccata*) that showed symptoms of disease (galls) with different degrees of infestation. The variance analysis at two factors (plants extracts and doses) of the methanolic extracts of the 3 selected plants showed 2 effects on the index of *M.incognita* infestation: one significant and the other highly significant on the roots of tomato seedlings, and 2 effects on growth parameters (dry weights of the aerial part and the roots): one significant and the other non-insignificant (Table 3). *P. harmala* extract showed a significant difference than other extracts to reduce *M.incognita* infestation for the highest concentration, while it ranked 2nd in reducing the number of juveniles after *T. baccata* extract. The efficacy of the methanol extracts of the 3 plants increased at the high concentration but it was different from one parameter to another. The reduction of the infestation rate and number of galls on the roots after the application of the 3 methanolic extracts of the 3 plants was at the same line with other researches like that Costa et al. (2003), Wiratno et al. (2009), and Bawa et al. (2014).

These results can be attributed to the presence of toxic substances released by the extracts such as alkaloids, flavonoids, saponins, and amides including benzamide and ketones, which have juvenicidal and ovocidal potentials and can effect singly or combination (Adegbite and Adesiyun 2005). *Peganum harmala*, *Taxus baccata*, and *Ricinus communis* have always been known for their biological activities (medicinal, antibacterial, antifungal, antioxidant, insecticide) (Nenaah 2010; Behidj-Benyounes et al. 2014; Bhakta and Das 2015; Abbassi et al. 2015 and Wilson and Hooser 2018).

Conclusion

Obtained results suggest that *P. harmala*, *R. raphanistrum*, *T. baccata*, *S. arvensis*, and *R. communis* have potentials as nematocidal on *Meloidogyne incognita*, especially when applied in a methanolic solvent. A study of the biochemical composition of these 5 plants and detection of molecules with nematocidal potential is worth for further researches. These species are well adapted to humid, arid, and semi-arid conditions, where wild tomato is cultivated, and *M.incognita* is its common threat.

Abbreviations

A: Aqueous; DMSO: Dimethyl sulfoxide; E: East; ENSA: Ecole Nationale Supérieure d'Agronomie; J2s: Juvenile of second stage juveniles; LC

50: Lethal concentration 50; M: Methanolic; N: North; NaOCl: Sodium hypochlorite; Nhe: Number of eggs hatched; Nie: Initial number of eggs; Peg: *Peganum harmala*; R (hi): Rate of hatching inhibition; Rap: *Raphanus raphanistrum*; Ric: *Ricinus communis*; RKNs: Root-knot nematode; SDW: Sterile distilled water; Sin: *Sinapis arvensis*; Tax: *Taxus baccata*

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All authors read and approved the final manuscript

Authors' contributions

SAZ carried out the experiment and was a major contributor in writing the manuscript. FM conceived and planned the experiments. NA carried out the experiment and revised the manuscript and corrected references. DJB followed the work in the laboratory and in greenhouses. DM assistance in statistical analysis and correction of the manuscript. MH supervised the work. The author(s) read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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