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Short Communication

NEMATICIDAL PROPERTIES OF SOME MEDICINAL PLANTS FROM SELECTED FLORA OF GILGIT-BALTISTAN

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ABSTRACT

Man is interested in plants for the treatment of diseases from prehistoric times. Medicinal plants have great importance in treatment of different diseases. Pesticides are beneficial for crop protection, food preservation, disease control as well as it is risk for human health, ill impact on environment and ecosystem. Different plant species are considered to possess the toxicity against various nematodes and used as green alternatives to conventional harmful organophosphorus and carbamates. The main objective of the current study is to evaluate the nematicidal properties of *Peganum harmala, Clematis orientalis*, *Mentha longifolia,* and *Capparis spinosa* from Gilgit-Baltistan region of Pakistan against root-knot nematodes. The polar and non-polar extracts of these plant species were assessed to study the nematicidal effects against *Meloidogyne incognita*. The effect of 0.125, 0.5, and 1% methanolic, ethyl acetate, aqueous, and *n*-hexane extracts of these plants exhibited highly promising nematicidal activity (47-75% mortality) after 72 h of treatment. The 1% solution of methanolic extracts of *C. orientalis*, *P. harmala,* and *C. spinosa* were found to be the most active fractions that exhibited 75, 71, and 75% mortality, respectively after 72 h. At this concentration conventional nematicide furadan showed 100% mortality. It was concluded that the tested plants furnished a good option to control nematodes as green alternatives to conventional practices.

Keywords: *Capparis spinosa*, *Clematis orientalis*, *Mentha longifolia*, *Peganum harmala*, root-knot nematode, green pesticides.

INTRODUCTION

Meloidogyne incognita is a plant parasite and highly damage causing nematode. It is wide spread and found in all continents. Plants which are attacked by *M. incognita* are characteristically retarded and their performance is badly affected in terms of quality and quantity. To control these nematodes usually synthetic nematicides are used but the synthetic nematicides may also affect non-target organisms, cause environmental pollution and not safe to farmers and consumers (Siddiqui *et al.*, 2001). The importance of botanical pesticides can be gauged by the rapidly increasing growth of this discipline roughly by 10-15% per year. Despite these efforts, there remains much to be learned about the secondary chemicals of plants. It is estimated that only 14% (0.37 million) of total plant species have been identified and recorded while 86% of plant species on earth and 91% species in the ocean are yet to be explored. Out of this small proportion of known plants most of the research on natural products has been done on just a few plant species and only 31,128 known plant species have a documented use (Mora, *et al.*, 2011; Corlett, 2016; Christenhusz and Byng, 2016; Kew, 2016; Villaverde *et al.*, 2016). It has been estimated that each year 2.5 million tons of pesticides are used on crops worldwide which costs 100 billion dollars annually (Koul *et al*., 2009). The Royal botanical Garden Kew calculated 540 billion/year cost of agriculture if invasive pests and pathogens are not controlled (Kew, 2017). The disruption of natural control by the application of conventional chemical pesticides can even create new pest problems. Minor pests that are usually kept at low numbers by their natural enemies will multiply rapidly in the absence of their enemies and cause outbreaks. Therefore, it has become an important issue to find alternative controlling strategies, which are as effective as conventional pesticides, cheaper, easily available, more selective to target pests and safer to farmers, consumers and the environment (Ntalli and Caboni, 2012). Currently plants are used as effective and alternative green pesticides generally known as botanical pesticides (Zeng, *et al*., 2010) The plant extracts are easily degraded, pollution free, leave no harmful residues, cheaper and not toxic to host plants and humans (Isman, *et al*., 2011; Zoubiri and Baaliouamer, 2014).

In this paper, we have presented the nematicidal effects of 4 plant species including; *P. harmala, C. orientalis*, *M. longifolia* and *C. spinosa* collected from the diverse flora of Gilgit-Baltistan. The polar extracts (methanolic and aqueous) obtained from different parts of these plants showed up to 75% mortality against the common root-knot nematode, *Meloidogyne incognita* at 1% concentration after 72 h treatment.

MATERIALS AND METHODS

A randomized complete block experimental design in a factorial arrangement with 3 concentrations (1%, 0.5%, and 0.125%) for each and three exposure times of nematodes at each concentration, plus an absolute control, for a total of 16 treatments was used.

Collection of Plants and Preparation of Sample Material: The plant samples, 2 kg of each *Peganum harmala, Clematis orientalis*, *Mentha longifolia* and *Capparis spinosa* in the flowering time were collected from Skardu (Yultar- Kharpito) and Gilgit (Danyore) during Jun-Aug, 2016 and they were identified by the plant taxonomist Dr. Sujjad Hayder, a resident Botanist at the Department of Biological Sciences Karakoram International University, Gilgit-Baltistan. The plant samples of *P. harmala, C. orientalis*, *M. longifolia,* and *C. spinosa* were dried in laboratory under shade at room temperature, the direct exposure to sunlight was avoided as it might damage the secondary metabolites in plants. Each part of dried plant materials [roots (R), stems (S), Leaves (L) and flowers (F)] were grounded by using mortar and pestle.

Preparation of Extracts

***Methanol extracts*:** The powdered material of all plants was soaked in 200 mL methanol for four days then filtered and the filtrate was dried through rotary evaporator. After evaporation, the samples were dried and 2.5 g of each plant sample (methanolic extract) was kept in vials. Furthermore, *n*-hexane, ethyl acetate and aqueous extracts were also prepared.

***n-Hexane extracts*:** The dried extract of each sample was taken in a beaker and 100 mL of methanol was added in each, shaken well and transferred to 250 ml separating funnel. 100 mL of *n*-hexane was added to the separating funnel and mixture was left for 10 minutes stand-by. Two distinct layers were formed and the methanolic layers were drained out first and *n*-hexane later. The process was repeated three times and *n*-hexane fractions were combined together. The filtrate was dried through rotary evaporator and the solid residue (extract) of *n*-hexane of each sample was employed for further study. The remaining methanolic residue was evaporated to dryness then suspended in water and fractionated with ethyl acetate.

***Ethyl acetate and aqueous extracts*:** The methanol extract of each plant sample was taken in different beaker, dissolved in 100 mL of water and 100 mL of ethyl acetate was added. The mixture was placed in the separating funnel, shaken well and was allowed to stand for 10 minutes. Two distinct layers of ethyl acetate and water were observed. This process was repeated three times and all the extracts of ethyl acetate were combined together before drying by using rotary evaporator. The dried extracts of ethyl acetate and aqueous fractions were kept in different vials. All the extracts of *P. harmala, C. orientalis*, *M. longifolia* and *C. spinosa* were further analysed for their activity against root-knot nematode, *Meloidogyne incognita* through standard assay protocol (Sabira, *et. al.,* 2015).

Nematicidal activity: The effect of sixteen extracts was evaluated for larval mortality of root-knot nematode. Population of J2 infective stage juveniles of *M. incogniata* was collected from pure culture maintained on tomato plants in microplot of a screen house in National Nematological Research Centre, University of Karachi, Pakistan. Egg-masses were extracted from the roots of infected tomato plant and transferred to small cavity block contained water. The cavity block was incubated for egg hatching at 28 ˚C for 3 days. For nematicidal activity 100 larvae were counted in a counting chamber for each dose and replicated thrice to introduce in a 3 × 3 glass cavity block. The stock solutions (10 mg/ mL) from plant extracts were prepared in 5 % dimethyl sulfoxide (DMSO). Three concentrations 1%, 0.5% and 0.125% were applied at a rate of 1 mL at each cavity block. The synthetic nematicide furadan was taken as standard and 5 % DMSO used as a control treatment for the comparison of results. Stereoscopic microscope was used after 24, 48 and 72 h of intervals at magnification 4 x to study the percent mortality. Nematodes were considered dead when no movement was observed after mechanical nudge, their irreversible mobility was confirmed by transferring them to distilled water.

Statistical analysis: Treatments differences were analyzed by multifactor analysis of variance (ANOVA) and then the data sheet was subjected to Duncan's multiple range test (DMRT) ($P \leq 0.05$) using SPSS statistical software. Probit analysis was performed under survival analysis for LC_{50} values by SAS, 2000. With the average mortality data in each of the concentrations and by simple regression technique, the median lethal concentration was calculated.

RESULTS AND DISCUSSION

During current study, four medicinal plants, *P. harmala, C. orientalis*, *M. longifolia* and *C. spinosa* were examined for the evaluation of their nematicidal effect against *M. incognita*. Almost all sample extracts of the plants showed nematicidal effect except those which were insoluble in assay solution. The extracts of all tested plant species didn't show any mortality during initial 2 hours exposure whereas maximum mortality rate was observed after 24 hours. The polar organic extracts (methanolic and aqueous) of all plants under this investigation showed excellent results as compared to medium and less polar soluble fractions. The nematicidal

effect of tested extract indicates that some polar oxygenated secondary metabolites with lipophilic properties may be involved to damage the cytoplasmic membrane of nematode cells by interfering with the enzyme protein structure through their functional groups (Knoblock *et al.,* 1989). Plants have been considered as good source of nematicidal substances. During last few decades, the potential of using plant extracts in integrated pest management has been studied by many authors (Abid, *et al*. 1997; Pavaraj, *et al*., 2010, Tordable, *et al*., 2010, Nguyen, *et al*., 2014, Sabira *et al*., 2015).

In the current investigation, the highest mortality was found for methanolic extract of roots and stem (R, S) while for water extracts no mortality was found after 24 h of treatment. Significant differences were found in mortality at different time intervals. After 48 h, at concentration of 1 and 0.5%, the effect was maximum as compared to 0.125% as shown in Table-1. Similarly, the activity trend of L and F extract of *P. harmala* after 72 h was found to be maximum at higher concentration (Table-1). The ethyl acetate of L and F of the same plant also showed a good activity (62%) at the concentration of 1 and 0.5% with non-significant difference. However, the ethyl acetate and hexane extracts of roots and stem (R, S) and hexane extract of L and F were insoluble in reaction medium and precipitates were observed. The total % mortality presented by aqueous extracts of L and F, and R and S were 62 and 57%, respectively. The root-knot nematodes mortality increased with the increase in concentration and time of exposure. LC_{50} values are given in table-2.

Table-1. Nematicidal effects of different extracts of *P. harmala*

Plant part	Fractions	[%] in 5 % DMSO	Mortality (%) ± SD		
		Concentrations	24 h	48 h	72 h
R, S	H_2O	1	25 ± 0.0Aa	45 ± 1.5Ab	57 ± 1.0Ac
		0.5	20 ± 1.5Ba	40 ± 1.2Bb	52 ± 2.0Bc
		0.125	15 ± 1.2Ca	17 ± 0.5Cb	42 ± 2.0Ca
	MeOH	1	33 ± 1.5Aa	61 ± 1.5Ab	71 ± 1.0Ac
		0.5	30 ± 0.5Aa	59 ± 0.5Ab	70 ± 1.5Ac
		0.125	20 ± 0.5Ba	44 ± 2.0Bb	54 ± 0.5Bc
L, F	H_2O	1	0 ± 0Aa	35 ± 1.1Ab	62 ± 0.5Ac
		0.5	0 ± 0Aa	34 ± 1.5Ab	62 ± 0.5Ac
		0.125	0 ± 0Aa	31 ± 1.5 Bb	41± 1.5Bc
	MeOH	1	10 ± 1.0Aa	29 ± 1.5Ab	50 ± 1.5Ac
		0.5	09 ± 1.5Aa	28 ± 1.1Ab	50 ± 1.0Ac
		0.125	02 ± 1.0Ba	18 ± 1.1Bb	32 ± 2.0Bc
	EtOAc	1	35 ± 1.0Aa	50 ± 1.2Ab	62 ± 1.0Ac
		0.5	33 ± 1.1Aa	50 ± 2.0Ab	62 ± 0.5Ac
		0.125	16 ± 1.5Ba	40 ± 2.0Bb	50 ± 1.5Cc

Values in a column having same upper case/capital letters are not significantly different ($P \leq 0.01$).
Values in rows having same lower-case letters are not significantly different ($P \leq 0.01$).

Table-2. Median lethal concentration $LC_{50}$of different extracts of *P. harmala*

Fractions	LC_{50} (95 % CL)		
	24 h	48 h	72 h
R, S-H_2O	0.2008 (3.3003-1.9656)	0.0945 (1.3553-0.7273)	0.1938 (1.1648-0.123)
R, S-MeOH	0.1886 (2.5517-1.2984)	0.173 (0.866-0.2839)	0.1625 (0.3055-0.774)
L, F-H_2O	-	0.6686 (6.2908-1.8467)	0.1414 (0.7916- 0.0483)
L, F-MeOH	0.1518 (3.3354- 2.3214)	0.2141 (3.0361-1.6129)	0.1652 (1.3991- 0.3009)
L, F- EtOAc	0.1296 (1.9187- 1.0572)	0.5689 (2.9518- 0.1946)	0.1165 (0.6631- 0.0193)

The nematicidal activity of methanolic and aqueous extract of *C. orientalis* ranges between 51-75% at 1% and 0.5% extract concentrations, after 72 h (Table-3) and in methanolic extract at same concentration no significant difference was found. The ethyl acetate and hexane extracts of roots, stem and leaf were insoluble in assay mixture. The LC_{50} values are given in table 4.

Table-3. Nematicidal effects of different extracts of *C. orientalis*

Plant part	Fractions	[%] in 5 % DMSO	Mortality (%) ± SD		
		Concentrations	24 h	48 h	72 h
R, S	H_2O	1	18 ± 1.5Aa	19 ± 1.0Aa	48 ± 1.0Ab
		0.5	13 ± 1.0Ba	18 ± 1.5Ab	40 ± 1.5Bc
		0.125	10 ± 1.2Ca	13 ± 1.5Bab	36 ± 2.0Cc
	MeOH	1	20 ± 1.0Aa	50 ± 1.0Ab	75 ± 2.0Ac
		0.5	20 ± 2.0Aa	50 ± 1.0Ab	73 ± 1.5ABc
		0.125	15 ± 0.5Ba	30 ± 1.2Bb	60 ± 1.0Cc
L	H_2O	1	12 ± 1.0Aa	32 ± 1.7Ab	51± 1.0Ac
		0.5	11 ± 1.1Aa	30 ± 1.5Bb	50 ± 1.5Ac
		0.125	06 ± 1.5Ba	17 ± 1.0Cb	45 ± 1.2Bc
	MeOH	1	19 ± 1.1 Aa	35 ± 1.0Ab	51 ± 2.0Ac
		0.5	18 ± 0.5Aa	30 ± 1.5Bb	44 ± 1.0Bc
		0.125	08 ± 1.5Ba	20 ± 0.5Cb	41 ± 1.0Cc

Values in a column having same upper case/capital letters are not significantly different ($P \leq 0.01$).
Values in rows having same lower-case letters are not significantly different ($P \leq 0.01$).

Table-4. Median lethal concentration LC_{50} of different extracts of *C. orientalis*

Fractions	LC_{50} (95 % CL)		
	24 h	48 h	72 h
R, S-H_2O	0.1948 (3.8315- 2.537)	0.3082 (5.2308- 3.1826)	0.2297 (1.9245- 0.3981)
R, S-MeOH	0.3979 (6.2729-3.6282)	0.148 (1.3418-0.3582)	0.1732 (0.0323-0.001)
L-H_2O	0.2608 (4.83-3.0879)	0.1599 (2.3317-1.2686)	0.4956 (2.3906-0.9037)
L-MeOH	0.1586 (3.1059-2.0517)	0.1636 (2.2498-1.1627)	0.2796 (1.8734-0.0152)

The *Mentha longifolia* showed a mild mortality (41-50%) after 72 h exposure (Table 5). The rate of mortality decreased with theincrease dilution of all the extracts. The aqueous extract of leaf and flowers exhibited greater mortality effect as compared to its root and stem extracts. The general trend of increased motility for polar fractions (aqueous and methanolic) was same as rest of the tested plant extracts. The methanolic and hexane extracts of roots and stem andethyl acetate and hexane extracts of leaf were insoluble in DMSO solution of bioassay experiment. The LC_{50} values are given in table-6. The methanolic extract of L, F showed no mortality after 24 h of exposure in all concentration.

Table-5. Nematicidal effects of different extracts of *M. longifolia*

Plant part	Fractions	[%] in 5 % DMSO	Mortality (%) ± SD		
		Concentrations	24 h	48 h	72 h
R, S	H_2O	1	08 ± 1.0Aa	20 ± 1.5Ab	47 ± 1.0Ac
		0.5	05 ± 1.0Ba	10 ± 0.5Bb	36 ± 2.0Bc
		0.125	0 ± 0Ca	06 ± 0.5Cb	18 ± 2.0Cc
	EtOAc	1	11 ± 1.5Aa	22 ± 2.0Ab	43 ± 1.0Ac
		0.5	10 ± 2.0Aa	22 ± 1.0Ab	41 ± 0.5Ac
		0.125	09 ± 1.0Aa	20 ± 1.0Ab	38 ± 0.5Bc
L, F	H_2O	1	20 ± 1.0Aa	30 ± 0.5Ab	50 ± 0.0Ac
		0.5	20 ± 1.0Aa	29 ± 1.1Ab	49 ± 1.0Ac
		0.125	20 ± 1.0Aa	27 ± 0.5Ab	48 ± 1.5Ac
	MeOH	1	0 ± 0Aa	10 ± 1.0Ab	32 ± 2.0Ac
		0.5	0 ± 0Aa	05 ± 0.5Ab	12 ± 1.0Ac
		0.125	0 ± 0Aa	0 ± 0Aa	07 ± 0.5Ab

Values in a column having same upper case/capital letters are not significantly different ($P \leq 0.01$).
Values in rows having same lower-case letters are not significantly different ($P \leq 0.01$).

Table-6. Median lethal concentration $LC_{50}$of different extracts of *M. longifolia*

Fractions	LC 50 (95 % CL)		
	24 h	48 h	72 h
R, S-H_2O	0.1044 (2.3136- 1.6052)	0.1209 (2.4281-1.6207)	0.0892 (1.3238-0.7306)
R, S-EtOAc	0.6328 (12.5896- 8.3833)	1.0955 (14.848-7.5667)	0.564 (4.0465-0.2978)
L-H_2O	0.0892 (1.3238- 0.7306)	0.8295 (9.0189- 3.5056)	0.2485 (1.699- 0.0476)
L-MeOH	-	0.0942 (2.1179- 1.4787)	0.0678 (1.6329- 1.1821)

The roots and aerial parts of *C. spinosa* were appeared to be more active for nematicidal activity against larvae of *M. incognita*, as it caused 45-75 % mortality of the nematode larvae after 72 h exposure. The aqueous extracts of aerial parts showed the best activity as compared to its root extract whereas rest of the organic extracts of different extracts of *C. spinosa* were precipitated in assay solution. The aqueous extracts of root and stem showed no significant difference in all concentrations. LC_{50} values are given in table-7.

Table-7. Nematicidal effects of different extracts of *C. spinose*

Plant part	Fractions	[%] in 5 % DMSO	Mortality (%) ± SD		
		Concentrations	24 h	48 h	72 h
R, S	H_2O	1	22 ± 1.0Aa	40 ± 1.5Ab	47 ± 1.1Ac
		0.5	20 ± 1.0Aa	40 ± 1.5Ab	45 ± 2.0Ac
		0.125	19 ± 0.5ABa	38 ± 1.0Ab	44 ± 1.0ABc
Aerial	H_2O	1	18 ± 0.0Aa	28 ± 1.0Ab	48 ± 1.5Ac
		0.5	15 ± 1.0Ba	25 ± 1.5Bb	45 ± 1.0Bc
		0.125	10 ± 2.0Ca	15 ± 2.0Cb	30 ± 1.0Cc
	MeOH	1	20 ± 0Aa	70 ±1.5Ab	75 ±1.0Ac
		0.5	20 ± 0Aa	65 ±2.0Bb	70 ±1.0Bc
		0.125	19 ± 0Aa	35 ±1.0Cb	45 ±2.0Cc

Values in a column having same upper case/capital letters are not significantly different ($P \leq 0.01$).
Values in rows having same lower-case letters are not significantly different ($P \leq 0.01$).

Table-8. Median lethal concentration LC_{50}ofdifferent extracts of *C. spinose*

Fractions	LC 50 (95 % CL)		
	24 h	48 h	72 h
R, S-H_2O	0.0678 (1.6329-1.1821)	1.4528 (10.1786- 0.5223)	0.9366 (4.9969- 1.2285)
Aerial-H_2O	0.2054 (3.929- 2.5641)	0.1688 (2.6751- 1.553)	0.1594 (1.5228- 0.4631)
Aerial –MeOH	1.0322 (14.917-8.0572)	0.0712 (0.6632-0.19)	0.0914 (0.3989-0.0088)

In standard treatment of furadan 100 % mortality was observed while 0 % mortality was found in control treatments. Despite differences among investigated plants, all plants indicated time and concentration dependent activity in addition to tendency of greater mortality rate towards polar extracts. Since the methanolic extracts of the tested plant species showed the promising activity against a common root-knot nematode *M. incognita* therefore, additional phytochemical work is needed to isolate and characterize the possible compounds responsible for the nematicidal activity.

Conclusions: God has gifted Gilgit-Baltistan with a great floral diversity. Plants of Gilgit-Baltistan are medicinally important due to special geographic origin and climatic conditions. In this study, it was revealed that different extracts of all tested plants presented different larval mortality effect against the root-knot nematode. Although, all the samples exhibited nematicidal activity but polar fractions of *Peganum harmala, Clematis orientalis*, and *Capparis spinosa* were found to be significant. Therefore, it is concluded that the polar fractions of different parts of *P. harmala, C. orientalis*, and *C. spinosa* can be used against the nematode *M. incognita.* It is yet to be investigated whether the nematicidal activity demonstrated by the plant species is due to single compound or combination of compounds present in a mixture. Therefore, further investigations are required to isolate and identify the active ingredients. This will help to provide green alternatives to replace the conventional nematicides already available in the market.

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