

Evaluation of nematocidal effects of some medicinal plant extracts against root-knot nematodes (*Meloidogyne incognita*)

Muhammad Ismail,¹ Shahina Fayyaz,² Attia Kowsar,¹ Salma Javed,² Iftikhar Ali,¹ Sajjad Ali,¹ Farhad Hussain,¹ Haibat Ali³

¹Department of Chemistry, Karakoram International University, Gilgit; ²National Nematological Centre, University of Karachi, Karachi; ³Department of Environmental Sciences, Karakoram International University, Gilgit, Pakistan

Abstract

Hundreds of highly toxic chemical pesticides and their conventional unsafe formulations of varying toxicities are used extensively to control pests, diseases, and weeds to increase agricultural production. The use of bio or botanical pesticides, which have been found effective, safe, and eco-friendly, could possibly provide a viable solution. Thus, the development and production of environment-friendly botanical pesticides and their water-based formulations, to replace the highly toxic agro-chemicals and unsafe formulations, has gained significant importance towards developing appropriate strategies for crop protection. The present study was aimed to evaluate the nematocidal nature of the aqueous extracts of *Allium sativum*, *Urtica dioica*, *Sophora mollis*, *Ephedra intermedia*, and *Tanacetum baltistanicum*. For this pur-

pose, the plant material was dried in shade and mechanically ground into a powder form. The methanolic extracts of each plant sample were obtained and further extracted into different organic and aqueous fractions. The polar organic and aqueous fractions were further subjected to *in vitro* studies against *Meloidogyne incognita*, a common root-knot nematode. The results revealed that the polar organic and aqueous extracts of all the tested plants showed excellent results with total mortality of 75-95% at the concentrations of 0.125-1% after 72 h of the treatment. These results can be exploited further for their efficacy against *M. incognita* through on-field applications. The nematocidal effect of tested extracts indicates that some polar oxygenated secondary metabolites with lipophilic properties may be responsible to damage the cytoplasmic membrane of the nematode cells by interfering with the enzyme protein structure through their functional groups.

Correspondence: Muhammad Ismail, Department of Chemistry, Karakoram International University, University Road, Gilgit-15100, Pakistan.
Tel.: +92.345.3094385 – Fax: +92.5811.980039.
E-mail: dr.ismail@kiu.edu.pk

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Introduction

Gilgit-Baltistan is rich in floral diversity and contains 70% of wild plant species. It is reported that 70-80% people in Gilgit-Baltistan use wild and domestic plants for the treatment of different diseases as well as crop protection (Khan *et al.*, 2011). It is globally admired that pesticides are the best source of disease protection in plants (Hubert *et al.*, 2013). Pesticides are the chemical substances having significant potential to resist insect growth that spoil or interfere with the growth of crops, shrubs, trees, timber, and other vegetation desired by humans. Synthetic pesticides contain harmful agents that pose long term danger to the human health and environment through their persistence in nature or body tissue. Many pesticides are poisonous in nature and are threat for living things being non-friendly to the environment, hence are useless (Nabile and Wakeil, 2013). The main purpose of using pesticides in agriculture is to protect crops from insects and to get better productivity (Jacobsen *et al.*, 2015). Pesticides mainly dissolve in soil and undergo many chemical changes through a complex mechanism thus are used to treat plant diseases, destroy weeds, and inhibit insect-growth (Andrey and Pico, 2004). Previous study showed that before World War II people used both organic pesticides like pyrethrum, neem, nicotine, *etc.* and inorganic pesticides like sulphur, lead, copper, arsenic and lime (Kaberia, 2007). Some fifty years ago, synthetic pesticides provided a very safe means for crop protection worldwide but soon people realized their adverse effects on environment as well as on human health (Zadoks and Waibel, 2000). In 1940 when DDT, BHC and chlorinated cyclodienes were introduced, a great revolution came about in the world of agriculture as these chemical substances provided great agricultural productivity and protection. Over the time, the negative side

effects of these synthetic chemicals like their residues on food etc. also came into account (Varma and Dubey, 1999). The use of organochlorine pesticides also causes ecological problems and are dangerous to human health due to their non-degradable properties (Martins *et al.*, 2013). Since synthetic pesticides become a serious issue due to their toxic effects. Many other problems like effects on non-target organisms, non-biodegradability, and denatured-active components also weigh their importance (George *et al.*, 2014). It is estimated that about three million people working in different field of agriculture around the world are affected by the toxicity of synthetic pesticides every year and around 20,000 people died due to direct use of agrochemicals. From the experiments, it is concluded that very small amount (less than 1%) of pesticides used for crop protection affect the target organisms and the remaining can pollute soil, air, water, and food (Amoabeng *et al.*, 2014).

Study of the human history showed that in every era human being depended on plants to fulfil their needs. Human used plants as a source of food, medicine, household tools, fuel, etc (Jabeen *et al.*, 2015; Kumar and Simon, 2016). Globally medicinal plants received significant importance and provide financial support to the nations (Gilani *et al.*, 2006). Plants contain vast number of biologically active compounds (Ujvary, 2000; Uy and Villazorda, 2015). A survey of World Health Organization reported that about 80% peoples in developing countries use drugs obtained from medicinal plants (Priya and Gopalan, 2015). Historically plants and their phytochemicals are the best source to control insects, nematodes, *etc.* (Dang *et al.*, 2005). Medicinal plants take attention of researchers worldwide to use them as botanical pesticides and drugs due to their eco-friendly nature and nontoxic behaviour (Adebayo *et al.*, 2014). Pesticides derived from plant sources are easily available, economical, harmless, and biodegradable (Tapwal *et al.*, 2011). Plants contain repulsive aromatic substances which provide defence and work as repellents against insects (Kaberia 2007). It is reported that there are many plant species which have great potential in pesticide formulation used in integrated pest management (Sola *et al.*, 2014).

The use of botanical pesticides has been introduced for hundreds of years for food preservation. Many kinds of plant derived products like essential oils, powder and plant are used as pesticides and to control insect's growth (Kedia *et al.*, 2015). The active chemical compounds like proteins, oxalates, glycosides, terpenes, phenolic, alkaloids, anthocyanins *etc.* present in plants play major role in their defence mechanism (Priya and Gopalan, 2015). Bio-pesticides based on plants provide great methodology for crop protection. Secondary metabolites pay significant contribution to resist insect growth as they contain several phytochemicals such as flavonoid, monoterpenes, and organosulphur compounds (Zubari, 2006; Hubert *et al.*, 2013). The secondary metabolites inhibit insect's growth by affecting their locomotion, feeding behaviour, oviposition, development, and physiological processes (Odeyemi, *et al.*, 2013). Many plant species of the families like Rutaceae and Myrtaceae possess pesticidal activity and are used for insect control and are considered as botanical insecticides (Khan *et al.*, 2014).

A variety of the management strategies for botanical based nematodes control has been adopted effectively to reduce the hazards of chemical nematicides to human and the environment. Identification of plant based nematicides has been considered a green approach, where many plants extracts, and its active compounds have been already discovered (Ahmad *et al.*, 2010; Echeverrigaray *et al.*, 2010; Chaudhary *et al.*, 2013). In the present study, it is aimed to achieve the target of pest-control by using the plant extracts with nematocidal properties including *Sophora mol-*

lis, *Ephedra intermedia*, *Urtica dioica*, *Allium sativum* and *Tanacetum baltistanicum* against *Meloidogyne incognita*, a root-knot nematode.

Materials and methods

Collection of plant material

Whole plants, each weighing 1-2 kg of *S. mollis* (1.3 kg), *U. dioica* (1.1 kg), *E. intermedia* (1.4 kg), *A. sativum* (1.0 kg), and *T. baltistanicum* (1.9 kg), were collected from different areas of Gilgit region namely Sonicot, Napora, Nomal, Danyor, and Sumayar respectively, in June 2016. The plants were identified by Dr. Sujjad Hyder, resident botanist, Department of Environmental Sciences, Karakoram International University, Gilgit.

Preparation of the sample extracts

The whole plant materials were dried in shade and ground separately. Each dried and ground plant material was soaked in enough methanol (95%) for one week at room temperature with occasional shaking and stirring. The whole mixture was then filtered by using sheets of commercial grade filter paper. The filtrate obtained was evaporated by using rotatory evaporator to obtain solidified crude methanolic extract (ME) of each plant sample. The soaking and extraction process were repeated three times.

Fractionation process

The crude methanolic extracts were then successively partitioned by solvent-solvent fractionation into four major fractions; methanol (ME), *n*-hexane (HE), ethyl acetate (EE), and aqueous extracts (AE). The ethyl acetate fraction of *T. baltistanicum* was further fractionated by using liquid column chromatography into 11 fractions with gradient increase of ethyl acetate starting with *n*-hexane (100%) as mobile phase. All the plant extracts of *S. mollis* (roots and stems), *U. dioica*, *E. intermedia*, *A. sativum*, and ethyl acetate fractions of *T. baltistanicum* were analysed further for their nematocidal activity against *M. incognita* by following standard protocol.

Nematocidal activity

The effect of 16 extracts was evaluated for larval mortality of root-knot nematode. Population of J2 infective stage juveniles of *M. incognita* was collected from pure culture maintained on tomato plants in microplot of a screen house in National Nematological Research Center, University of Karachi, 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 nematocidal activity 100 larvae were counted in a counting chamber for each dose and replicated thrice to introduce in 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 4x to study the percent of 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

Treatment differences were analysed by multifactor analysis of variance (ANOVA) and then the data sheet was subjected to Duncans' 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.

Results

In present study, the methanolic and aqueous extracts of roots, stems, and leaves of *Sophora mollis* and *Urtica dioica* whereas, roots and aerial parts of *Ephedra intermedia* and only bulbs of *Allium sativum* were evaluated as nematocidal agents. In addition to these, the hexanes, methanolic and ethyl acetate extracts of *T. baltistanicum* were also screened for its potential against root knot nematodes.

The aqueous bulbs extract of *A. sativum* showed highest mor-

tality of *M. incognita* at the concentration (conc.) of 1% after 78 h. The leaf (L) extract of *Urtica dioica* showed maximum mortality response (40-47 %) after 24 h of exposure as compared to the rest of the extracts studied during this study. The roots and stems (R, S) showed no mortality response after 24 h exposure (Table 1), while the leaf extract of *U. dioica* exhibited the surprising activity of 90, 85, and 82% after 72 h at 1, 0.5, and 0.125% conc., respectively. The AE of roots and stems of *Sophora mollis* showed significant nematocidal activity against *M. incognita* at different concentrations. In case of *S. mollis*, the most active extract was that of the leaf parts after 72 h treatment with the activity of 88% larval mortality of *M. incognita* at 1% conc. (Table 1). The lethal dose concentrations (LC_{50}) values of tested aqueous extracts are given in Table 2. The tested extracts were found to exhibit varied nematocidal effects ranging from weak, moderate and strong as determined by LC_{50} . The lowest LC_{50} observed for roots and stems extracts of *S. mollis* (0.039) and bulbs of *A. sativum* (0.032) against the common nematodes (*M. incognita*). A low LC_{50} value is indicative of greater antinematode activity. The leaf extract of *S. mollis* had a LC_{50} value of 0.043.

Table 1. Nematocidal effects of aqueous extracts (AE) of different plant species.

Plant species	Fractions	[%] in 5% DMSO Concentrations	Mortality (%) \pm SD		
			24 h	48 h	72 h
<i>Sophora mollis</i>	R, S	1	22 \pm 1.0 ^{Aa}	52 \pm 0.5 ^{Ab}	78 \pm 1.0 ^{Ac}
		0.5	20 \pm 2.0 ^{Aa}	50 \pm 1.0 ^{Ab}	72 \pm 1.5 ^{Bc}
		0.125	17 \pm 1.5 ^{Ba}	45 \pm 1.0 ^{Bb}	67 \pm 1.1 ^{Cc}
<i>Sophora mollis</i>	L	1	30 \pm 1.0 ^{Aa}	67 \pm 1.0 ^{Ab}	88 \pm 0.5 ^{Ac}
		0.5	28 \pm 1.5 ^{Aa}	60 \pm 1.0 ^{Bb}	82 \pm 1.5 ^{Bc}
		0.125	22 \pm 1.0 ^{Ba}	50 \pm 1.5 ^{Cb}	70 \pm 1.5 ^{Cc}
<i>Ephedra intermedia</i>	R	1	33 \pm 1.5 ^{Aa}	40 \pm 1.0 ^{Ab}	80 \pm 0.5 ^{Ac}
		0.5	22 \pm 1.1 ^{Ba}	36 \pm 1.5 ^{Bb}	78 \pm 0.5 ^{Ac}
		0.125	20 \pm 1.0 ^{Ba}	30 \pm 1.2 ^{Cb}	62 \pm 2.0 ^{Bc}
<i>Ephedra intermedia</i>	A	1	32 \pm 2.0 ^{Aa}	40 \pm 1.1 ^{Ab}	80 \pm 1.0 ^{Ac}
		0.5	30 \pm 2.0 ^{ABa}	35 \pm 0.5 ^{Bb}	78 \pm 1.0 ^{Ac}
		0.125	28 \pm 1.0 ^{Ba}	32 \pm 1.0 ^{Cb}	65 \pm 1.0 ^{Bc}
<i>Urtica dioica</i>	R, S	1	0 \pm 0 ^{Aa}	25 \pm 2.0 ^{Ab}	30 \pm 1.5 ^{Ac}
		0.5	0 \pm 0 ^{Aa}	18 \pm 2.0 ^{Bb}	25 \pm 0.5 ^{Bc}
		0.125	0 \pm 0 ^{Aa}	16 \pm 1.0 ^{Bb}	20 \pm 1.0 ^{Cc}
<i>Urtica dioica</i>	L	1	47 \pm 1.5 ^{Aa}	75 \pm 1.0 ^{Ab}	90 \pm 2.0 ^{Ac}
		0.5	45 \pm 1.5 ^{Aa}	72 \pm 1.0 ^{Bb}	85 \pm 2.0 ^{Bc}
		0.125	40 \pm 1.0 ^{Ba}	65 \pm 2.0 ^{Cb}	82 \pm 0.5 ^{Cc}
<i>Allium sativum</i>	B	1	20 \pm 1.1 ^{Aa}	55 \pm 1.0 ^{Ab}	92 \pm 2.0 ^{Ac}
		0.5	20 \pm 0.5 ^{Aa}	55 \pm 1.0 ^{Ab}	90 \pm 2.0 ^{Ac}
		0.125	08 \pm 1.0 ^{Ba}	40 \pm 1.5 ^{Bb}	77 \pm 1.0 ^{Bc}

R, roots; S, stems; L, leaves; A, aerial parts; B, bulbs. ^{A-C}Values in columns having same upper-case letters are not significantly different ($P < 0.001$); ^{a-c}Values in rows having same lower-case letters are not significantly different ($P < 0.001$).

Table 2. Median lethal concentration (LC_{50}) of aqueous extracts of plant species.

Plant species	LC_{50} (95 %CL)		
	24 h	48 h	72 h
<i>Sophora mollis</i> (R, S)	0.402 (6.0903-3.4185)	0.4194 (2.0635-0.0002)	0.0399 (0.7963-0.0001)
<i>Sophora mollis</i> (L)	0.3019 (3.8571-1.8506)	0.1641 (0.6235-0.0003)	0.0435 (0.0701-0.0003)
<i>Ephedra intermedia</i> (R)	0.1713 (2.5447-1.4062)	0.2693 (2.684-0.8939)	0.0672 (0.1024-0.0005)
<i>Ephedra intermedia</i> (A)	0.6256 (6.6397-2.4817)	0.3317 (3.1406-0.9358)	0.1711 (0.0827-00.00)
<i>Urtica dioica</i> (R, S)	-	0.2174 (3.5562-2.1114)	0.2283 (3.2012-1.6836)
<i>Urtica dioica</i> (L)	0.4145 (2.6859-0.0003)	0.0479 (1.0699-0.0001)	0.3745 (0.0373-0.000)
<i>Allium sativum</i> (B)	0.1534 (2.9404-1.921)	0.3992 (1.2093-0.1002)	0.0329 (0.0482-0.0001)

This result suggested that the effectiveness of aqueous extracts of *S. mollis*, *A. sativum* and roots extract of *E. intermedia* were closely similar to each other. The LC₅₀ values of 0.17, 0.22, and 0.37 were recorded for aerial extract of *E. intermedia* and roots and stems and leaf extracts of *U. dioica*, respectively.

The observed nematocidal activity of the methanolic extract was 40 and 55% at 1% conc. after 24 and 48 h, respectively with the total mortality of 95% and it is very close to the standard used during these experiments. Whereas, the larval mortality at the conc. of 0.5% was 40 and 54% after 24 and 48 h, respectively with total activity of 92% mortality. The results remain very precise at all concentrations from 0.125-1% with minor increase in rate of mortality with the increasing concentrations. The aerial parts of *Ephedra intermedia* showed 45-74 % mortality after 72 h of the treatment for lowest to highest dose (Table. 3). The LC₅₀ values of the methanolic extracts are given in Table 4.

The nematocidal activities of the hexanes, methanolic and the aqueous extracts of *T. baltistanicum* were more consistent at the conc. of 1, 0.5 and 0.125% with the total larval mortality of 70-80% after 72 h of the exposure time. The EE of *T. baltistanicum* was further fractionated by using silica gel column chromatography into 8 sub-fractions by using 10% gradient increase of ethyl acetate with 100% hexane as a mobile phase. Among these fractions, 3-sub-fractions eluted through silica gel column at 30, 50, and 80% of ethyl acetate in hexane showed excellent activity (80%) after 72 h exposure at the conc. of 1% (Table 5). The LC₅₀ values of different extracts and ethyl acetate fractions of *T. baltistanicum* are given in Table 6. The hexanes extract showed the lowest LC₅₀ (0.034) followed by 100% ethyl acetate extract and the aqueous extract (0.077). The highest LC₅₀ values were exhibited by 50% ethyl acetate extract and considered the least active fraction of *T. baltistanicum* against the nematodes of the *M. incognita*.

Discussion

The *Meloidogyne incognita* is a parasite, plant-damage causing nematodes. It is a widespread nematode found in all continents. Plants attacked by *M. incognita* are characteristically retarded and show slow performance in quality and quantity. Usually, synthetic

nematicides are used for controlling these harmful nematodes. But the synthetic forms also affect non-target organisms. These synthetic nematicides may cause the problems of environmental pollution due to the degradation issues associated with the synthetic organic compounds. The botanical nematicides are more selective, eco-friendly, and locally produced as compared to synthetic ones. In current study, the nematocidal effects of different extracts of *Sophora mollis*, *Ephedra intermedia*, *Urtica dioica*, *Allium sativum* and *Tanacetum baltistanicum* against *Meloidogyne incognita* were discussed.

The effects of different extracts obtained from *A. sativum*, *U. dioica*, *S. mollis*, *E. intermedia*, and *T. baltistanicum* on larval mortality of root-knot nematode was determined after 20 min., 1, 2, 24, 48, and 72 h at different concentrations. According to the literature, *Allium sativum* has been used to control different insects. The ethyl acetate extract of the bulbs (B) shown effective resistance to *Colletotrichum lindemuthianum* (Masangwa *et al.*, 2013). Nath and Singh, 2015 reported that aqueous extract of the bulbs of *A. sativum* has been used to control harmful insects in the fields. The aqueous and the ethanol extracts of *A. sativum* effectively controlled *M. javania* (Abbas, *et al.*, 2009). Ferris and Zheng (1999) also reported the nematocidal activity of aqueous extract of *A. sativum* against *M. javania*. It has been revealed that *A. sativum* has great potential as an active pesticide due to the presence of an active chemical compound called diallyl sulphide (Tijjani *et al.*, 2014). The chemical products obtained from *A. sativum* shows effective resistance against the different insect populations. In the field, *T. urticae* population was controlled by extract of *A. sativum* (Mackeen *et al.*, 1997). In addition, *A. sativum* possesses anti-nematocidal (Attia *et al.*, 2013), and antibacterial properties (Amonrar and Reeves, 1970). The present study is also in-line with the reported studies that *A. sativum* can be used as a potential pesticide to control different insect species whereas, its excellent activity against *M. incognita* is reported for the first time. In the present study, the ME, and AE obtained from the bulbs of *A. sativum* showed the remarkable nematocidal activity. The methanolic extract of *A. sativum* showed more pronounced activity as compared to its aqueous extract. The results revealed that among all the tested plant extracts, *A. sativum* has maximum nematocidal activity against *M. incognita* (Tables 1 and 2).

Table 3. Nematocidal effects of methanolic extracts (ME).

Plant species	[%] in 5% DMSO Concentrations	Mortality (%) ±SD		
		24 h	48 h	72 h
<i>Allium sativum</i> (B)	1	40±1.1 ^{Aa}	55±0.5 ^{Ab}	95±1.5 ^{Ac}
	0.5	40±2.0 ^{Aa}	54±2.0 ^{Bb}	92±1.0 ^{Bc}
	0.125	30±1.0 ^{Ba}	50±2.0 ^{Cb}	90±1.0 ^{Bc}
<i>Ephedra intermedia</i> (A)	1	12±2.0 ^{Aa}	32±1.5 ^{Ab}	74±1.0 ^{Ac}
	0.5	05±1.5 ^{Ba}	25±1.5 ^{Bb}	50±1.0 ^{Bc}
	0.125	0±0 ^{Ca}	10±0.5 ^{Cb}	45±1.0 ^{Cc}

^{A-C}Values in columns having same upper-case letters are not significantly different (P<0.001); ^{a-c}Values in rows having same lower-case letters are not significantly different (P<0.001).

Table 4. Median lethal concentration (LC₅₀) of methanolic extracts (ME).

Plant species	LC ₅₀ (95 %CL)		
	24 h	48 h	72 h
<i>Allium sativum</i> B	0.1383 (1.312-0.3878)	0.3414 (3.7109-0.0042)	0.3034 (2.7505-0.000)
<i>Ephedra intermedia</i> A	3.4947 (28.6301-0.1166)	0.0617 (1.1223-0.71)	0.9336 (7.5147-0.0333)

Stinging nettle, *Urtica dioica* has been used as an active pesticide against *Aphids*. Previous study shows that aqueous extract of *U. dioica* was mostly used to control *Aphids* in preference to the synthetic pesticides (Kaberia 2007). Previous studies indicate that the aqueous extract of *Urtica dioica* shows antifungal activity

against *Alternaria solani*, *A. zinnia*, *Curvularia lunata*, *Rhizoctonia solani* and *Fusarium oxysporum* (Tapwal *et al.*, 2011). Moreover, the antifeedant property of *U. dioica* towards *A. bipunctata* has also been reported (Roy *et al.*, 2016). Nassar, 2016 reported that *U. urens* showed prominent resistance to nematodes such as

Table 5. Nematocidal effects different extracts and ethyl acetate fractions of *T. baltistanicum*.

Sample code	Conc. %	24 h	48 h	72 h
HE	1	18±0.5 ^{Aa}	42±2.0 ^{Ab}	80±0.5 ^{Ac}
	0.5	15±1.0 ^{Ba}	40±1.5 ^{Ab}	75±1.5 ^{Bb}
	0.125	12±1.0 ^{Ca}	35±1.5 ^{Bb}	70±1.1 ^{Cc}
AE	1	22±1.0 ^{Aa}	50±1.5 ^{Ab}	82±1.0 ^{Ac}
	0.5	18±1.0 ^{Ba}	45±1.1 ^{Bb}	78±1.1 ^{Bc}
	0.125	15±2.0 ^{Ca}	42±1.0 ^{Cb}	65±1.0 ^{Cc}
ME	1	20±1.1 ^{Aa}	48±2.0 ^{Ab}	80±1.5 ^{Ac}
	0.5	10±1.0 ^{Ba}	42±1.5 ^{Bb}	70±1.0 ^{Bc}
	0.125	08±1.5 ^{Ba}	40±1.0 ^{Bb}	57±1.0 ^{Cc}
20% EE	1	30±1.0 ^{Aa}	45±1.5 ^{Ab}	58±1.5 ^{Ac}
	0.5	27±1.0 ^{Ba}	40±2.0 ^{Bb}	55±0.5 ^{Bc}
	0.125	25±0.5 ^{Ba}	35±1.0 ^{Cb}	47±1.0 ^{Cc}
30% EE	1	32±1.0 ^{Aa}	52±1.0 ^{Ab}	80±0.5 ^{Ac}
	0.5	30±2.0 ^{Aa}	50±1.5 ^{Ab}	70±1.1 ^{Bc}
	0.125	25±1.0 ^{Ba}	47±1.0 ^{Bb}	60±1.0 ^{Cc}
40% EE	1	0±0 ^{Aa}	30±1.0 ^{Ab}	42±1.0 ^{Ac}
	0.5	0±0 ^{Aa}	20±2.0 ^{Bb}	31±2.0 ^{Bc}
	0.125	0±0 ^{Aa}	10±2.0 ^{Cb}	22±0.5 ^{Cc}
50% EE	1	10±0 ^{Aa}	38±1.1 ^{Ab}	80±1.0 ^{Ac}
	0.5	0±0 ^{Ba}	27±1.0 ^{Bb}	69±2.0 ^{Bc}
	0.125	0±0 ^{Ba}	25±0.5 ^{Bb}	60±1.5 ^{Cc}
60% EE	1	16±1.5 ^{Aa}	40±1.0 ^{Ab}	62±1.1 ^{Ac}
	0.5	10±1.0 ^{Ba}	30±1.0 ^{Bb}	55±0.5 ^{Bc}
	0.125	07±2.0 ^{Ca}	20±1.0 ^{Cb}	40±1.5 ^{Cc}
70% EE	1	18±2.0 ^{Aa}	40±2.0 ^{Ab}	52±1.0 ^{Ac}
	0.5	15±0.5 ^{Ba}	32±1.5 ^{Bb}	48±1.0 ^{Bc}
	0.125	12±2.0 ^{Ca}	27±1.0 ^{Cb}	35±1.5 ^{Cc}
80% EE	1	40±1.5 ^{Aa}	65±0.5 ^{Ab}	80±2.0 ^{Ac}
	0.5	35±1.0 ^{Ba}	60±2.0 ^{Bb}	72±1.1 ^{Bc}
	0.125	30±1.0 ^{Ca}	52±2.0 ^{Cb}	65±1.0 ^{Cc}
100% EE	1	18±0.5 ^{Aa}	42±2.0 ^{Ab}	80±0.5 ^{Ac}
	0.5	15±1.0 ^{Ba}	40±1.5 ^{Ab}	75±1.5 ^{Bb}
	0.125	12±1.0 ^{Ca}	35±1.5 ^{Bb}	70±1.1 ^{Cc}

HE, hexanes extract; AE, aqueous extract; ME, methanolic extract; EE, ethyl acetate extract. ^{A-C}Values in columns having same upper-case letters are not significantly different ($P < 0.001$); ^{a-c}Values in rows having same lower-case letters are not significantly different ($P < 0.001$).

Table 6. Median lethal concentration (LC₅₀) of different extracts and ethyl acetate fractions of *T. baltistanicum*.

Fractions	LC ₅₀ (95 %CL)		
	24 h	48 h	72 h
HE	0.2798 (5.0428-3.1828)	0.4023 (3.2563-0.5825)	0.0346 (1.1785-0.0000)
AE	0.2713 (4.46-2.6567)	0.1591 (1.4534-0.3959)	0.0774 (0.0871-0.0001)
ME	0.1206 (2.6777-1.8763)	0.3456 (2.4016-0.1047)	0.3456 (2.4016-0.1047)
20% EE	0.6076 (6.2505-2.1918)	0.6076 (6.2505-2.1918)	0.1871 (0.8903-0.0094)
30 % EE	0.2497 (2.6424-0.9744)	0.2497 (2.6424-0.9744)	0.0772 (0.1281-0.0013)
40 % EE	-	0.2497 (2.6424-0.9744)	0.1356 (1.6248-0.7189)
50% EE	0.1356 (1.6248-0.7189)	0.6076 (6.2505-2.1918)	0.6076 (6.2505-2.1918)
60% EE	0.6076 (6.2505-2.1918)	0.6076 (6.2505-2.1918)	0.1179 (0.5665-0.1369)
70% EE	0.1474 (3.289-2.3095)	0.1981 (2.2777-0.9611)	0.1713 (1.3773-0.2387)
80% EE	0.5045 (8.6175-5.264)	0.2161 (0.6534-0.0029)	0.1159 (0.113-0.0001)
100% EE	0.2554 (5.0762-3.3704)	0.2389 (3.2301-1.6423)	0.0598 (0.1504-0.0085)

M. incognita, *Pratylenchus*, *Aphelenchoides* and *Helicotylenchus*. The present finding agrees with those studies carried out earlier that *U. dioica* actively inhibited insect growth but in literature we could not find nematocidal activity of *U. dioica* against *M. incognita*. In this study, the aqueous extract of the leaves, roots, and stems of *U. dioica* effectively controlled the larvae of *M. incognita* whereas, the leaves extract showed more pronounced activity than that of the roots and stems. *Sophora mollis* is widely used as a pesticide and insecticide (Khan *et al.*, 2011; Bazmi *et al.*, 2014; Jabeen *et al.*, 2015). *Sophora flavescens* possesses varied percentage of pesticidal activity against red spider mites. This study provides the first report about the nematocidal activity of *S. mollis* against *M. incognita*.

The present study showed that the aqueous and the methanolic extracts of *E. intermedia* (aerial parts) and the aqueous extracts of the roots showed the best resistance to *M. incognita* at different conc. whereas aqueous extracts was found more effective than the methanolic extracts. This is the baseline study for the first time about the nematocidal activity of *E. intermedia* against the larvae of *M. incognita*. However, Iqbal *et al.* (2010) reported the pesticidal activity of *E. intermedia* against *Tribolium castaneum*. Moreover, *E. sinica* has also exhibited potential antifungal activity against *Alternaria panax*, *Phytophthora cactorum*, *Rhizictonia solani*, *Fusarium solani* and *Ustilago coicis*, due to the presence of certain essential oils (Shu-tong *et al.*, 2001). Literature study shows that pyrethrum, rotenone, neem, and essential oils are the most commonly used insecticides obtained from plants sources. Pyrethrum is obtained from flower of *Tanacetum cinerariaefolium* which contains 20-25% pyrethrins as an active component having pesticidal property. Pyrethrum acts in the similar fashion as synthetic pesticide DDT, blocking sodium gates and acts as a neurotoxin. Pyrethrum as an active pesticide was introduced in 17th century. The organic extracts of *Tanacetum baltistanicum* has been reported to possess insecticidal properties against few common insect pests (Ismail *et al.*, 2014). The results presented in Table 3 indicate that *ME*, *HE*, *EE*, and *AE* of *T. baltistanicum* have 80% inhibitory activity on the growth of *M. incognita*. The active fractions of *T. baltistanicum* were comparable to each other showing the excellent activity against root-knot nematodes. We are not sure about the exact mechanism and the mode of the action of the certain plant extracts on root-knot nematodes, but it was found that the aqueous polar extracts showed the best activity as compared to lest polar fractions. It means that some lipophilic, polar oxygenated secondary metabolites from the plant may involve damaging the cytoplasm of the nematode cells causing toxicity to enzyme protein structure by the functional group interaction. There may be multiple factors for the nematocidal activity of the plant extracts which can be revealed through structure activity relationship of compounds present in these extracts. Korayem *et al.* associated the nematocidal activity of the plant extracts with its AChE inhibition activity (Korayem *et al.*, 1993).

We recommend *in-vivo* testing of the active extracts, which have been never reported yet to promote the green practices for sustainable development in agriculture and the protection of the environment.

Conclusions

The investigation on green pesticides from natural sources is fundamentally important for the development of new botanical pesticides, especially in view of the vast worldwide flora. Based on

the results presented in this paper, the *EE* of *T. baltistanicum*, the *AE* and *ME* of the bulb of *A. sativum*, *AE* of *S. mollis*, *AE* of aerial parts of *E. intermedia* and the leaves, roots, and stems of *U. dioica* offers an opportunity for new botanical nematicides. Based on earlier findings on some other plant species and the present screening on selected plant species, we reached to the recommendations that medicinal plants extracts can be the best alternatives to the conventional nematicides. These plants may offer an alternative source for the control of certain nematodes without any negative impact on consumer health and the environment. However, more detailed studies are needed to identify and evaluate the active components and mechanism of action of these plant extracts to replace some of the existing toxic chemicals available in the market.

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