Biological Forum – An International Journal **10(2): 06-09(2018)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Nematicidal Effects of *Datura innoxia* and *Sophora alopecuroides* from Balochistan for Environmental-friendly Control of Plant Parasitic Nematodes

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(Received 02 May 2018, Accepted 15 July, 2018)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The plants extracts are becoming more popular for ecofriendly control of plant parasitic nematodes because of increasing environmental pollution arising due to persistent pesticides especially including POPs (persistent organic pollutants). These are the major threat to the environment, human lives, beneficial microbes in soil and to the underground body water. There is a need for adopting environmentally-friendly remedies such as plant extractsas an alternative for controlling plant parasitic nematodes. Balochistan is a rich region of Pakistan in floral variety with a broad range of biological value, though no encouraging effort has been made yet in this region, to evaluate botanical-nematocide. Therefore *Datura innoxia, Sophora alopecuroides* collected from Balochistan were screened for their nematicidal activities against *Meloidogyne incognita*. The ethanol extracts of the said plants were tested for egg hatchability and nematicidal effects on the second stage juveniles of *M. incognita*. The ethanol extract of leaves of *Datura innoxia* and *Sophora alopecuroide* exhibited highly promising mortality after 72 hours of exposure.

Keywords: *Meloidogyne incognita*, *Datura innoxia*, *Sophora alopecuroides*, juveniles, Balochistan, environmentally-friendly

How to cite this article: Noreen Khan, Bibi Shagufta, Shahina Fayyaz, Salma Javed, Saima Maher, Shazia Iqbal and Nelofer Jamil (2018). Nematicidal Effects of *Datura innoxia* and *Sophora alopecuroides* from Balochistan for Environmental-friendly Control of Plant Parasitic Nematodes. *Biological Forum – An International Journal*, **10**(2): 06-09(2018).

INTRODUCTION

Nematodes are widely distributed on our earth; about 28,000 species have been identified all over the world out of which over 16,000 are reliant on plant and animalsas parasites (Hugot *et al*, 2001). Several nematodes are known as parasites causing diseases in plants and animalsthus can cause enormous economic destruction (Javad *et al.*, 2006).

There are number of plant-parasitic nematodes identified as ectoparasites or endoparasites. The ectoparasites get their food by injecting the stylet into the roots of plants whereasendoparasitic nematodes enter into the body of plant (Lambert & Bekal, 2002). Root-knot nematodes are among most criticizing endoparasitesthat cause massive damage by injecting their hormones into the roots to produce root knot gall which ingest the plant's nutrient cause nutrient deficiency, predominantly nitrogen deficiency (Eisenback and Triantaphyllous, 1991) (Siddiqui *et al*., 2001).

The plant parasitic nematodes can cause about 125 Billion US dollars'(12.3% yearly yield) misfortunes to plant edits annually (Chit Wood, 2003). Pakistan have ideal conditionsfor the growth a range of nematodes, therefore nematodes cause relatively higher yield misfortunes in this region than other well developed countries (Zia UlHa *et al.*, 2012). Saveral root-knot nematodes are reported including *Meloidogyne incognita*, *M. hapla*, *M. javanica*, *M. arenaria*, *M. Graminicola* and *M. Pakistanica* (Shahina *et al*., 2009; 2016).

In the past 50 years, various synthetic molecules have been successfully used to control harmful nematodesfor plants (Lambert and Bekal, 2002). The applications of chemical nematicides is notcostly and very harmful to environment.

Mostly organic compounds are utilized as nematocides which may accumulate in the most parts of plants (vegetables/crops, fruits *etc*.) thus can enter in food chain when reach human body can cause several disorders and even death (Zia UlHaq *et al*., 2012). Thereforemost of them were identified as persistent organic pollutants (POPs).

For more acceptable substitutes of these hazardous chemicals, various botanical remedies are being investigated by researchers to obtain nematode-combative plants as an alternative and safer source for controlling plant parasitic nematodes (Chitwood, 2002). The nematicides from natural origin particularly plant can deals with the ecological issues created by synthetic nematicides therefore the discovery of plants based nematicide become a focused area of research (Ntallie *et al*., 2011; Andres *et al*., 2012). Numerous plants and different parts of the plants has been identified as a potential source of management of plant parasitic nematodes.(Dawar*et al*., 2008, Muniasamy *et al*., 2010; Pavaraj *et al*., 2010). These nematocides can be considered as less polluted items resulting no buildups are released in the ecosystem.

Pakistan is considered a treasure dynasty of various diverse medicinal plants in term of composition and endemism. Balochistan is the biggest province of Pakistan blessed with floral biodiversity due to various climatic conditions. Due to diverse agro-ecological environments make the region more appropriate for the growth several types of medicinal and other beneficial plants (Khan, 2011). Therefore we targeted to find the plants based remedies for the management of root-knot nematodes using *Datura innoxia* and *Sophora alopecuroides* collected from Quetta and Hazar GanjiChiltan National Park situated in the province Balochistan respectively.

MATERIALS AND METHODS

A. Plant material

Fresh and healthy plant of *Datura innoxia*, a species from family Solanaceae collected from Quetta, Pakistan and *Sophora alopecuroide*, a species from family Fabaceae collected from Hazarganji, Pakistan. These plants generally used in folk medicines. Common name of *Sophora alopecuroide* is pea-flowered treeand local name is Musunduk, *Datura innoxia*is Downy thorn apple and local name is Daturag.Collected plants were washed with tap water and spread over polythene sheets for air dry. The dried material of different parts of the plants are changed over into fine powder using electric blender.

B. Preparation of crude extract

Datura innoxia (whole plant), *Sophora alopecuroides* (flowers and leaves) were extracted with ethanol and acetone by soaking these fine powders in solvents for seven days each the process was repeated thrice.The thick masses of crude extracts were obtained by filtration and evaporation of these extracts. All these extracts were tested for their nematicidal potential.

C. Nematicidal activity

The second stage infective juveniles of *M. incogniata* collected from pure culture grownin the microplot of a green house on *Lycopersicon esculentum* (tomato) in NNRC, University of Karachi, Pakistan. The Infected roots were cut from plant and egg masses were extracted with waterin a small cavity block. The cavity block was then incubated at 28˚C for three daysto get freshly hatched juveniles (J2). In glass cavity block (3 × 3 cm) 100 larvae were counted in a counting chamber for application of each dose and replicate. The stock solutions were prepared by dissolving 10 mg of plant extracts in 5% DMSO (dimethyl sulfoxide). Three dilutions were 1%, 0.5% and 0.125% were prepared from each stock solution and applied to each cavity block at a rate of 1 ml. Furadan, a synthetic nematicide, was used as standard and water as a control. The percent of mortality was observed after 24, 48 and 72 hours by using stereoscopic microscope at magnification 4 ×. Nematodes were assumed to be dead when they did not move even after mechanical prod; the immobility of nematodes was further checked by transferring them to water.

D. Statistical analysis

Treatment difference was found using ANOVA (a multifactor analysis of variance); after getting significant ANOVA, the DMRT($P \leq 0.05$) (the Duncans' multiple ranges test)was used to separate the difference using SPSS software.

RESULTS AND DISCUSSION

Plants play a vital role for control of destruction instigated by nematodes. Synthetic nematicides are usually expensive and exert hazardous effects towards the ecosystem which have assumed alarming range. Ecofriendly, safe, effective and cheap phytochemical extracts may appear as effective and safer alternatives for plant parasitic nematodes (Pavaraj *et al*., 2012). As Balochistan has a wealth of medicinal flora, therefore search of traditional nematocidesarerequired for utilization of these unexplored indigenous resources. The current study was focused to find phyto-based nematocides using medicinal flora of Balochistan. During this study two medicinal plants *Datura innoxia* and *Sophora alopecuroides* were evaluated for nematicidal potential against *M. incognita*.

The ethanolic extract of leaves of *D. innoxia* (DL-E) have showed highest mortality rate as compared to other extracts. Mortality ranges between 50–75 % after 72 hrs of exposure.

Table 1: Nematicidalpotential of *Datura innoxia* against *M. incognita.*

Mortality (%), Intervals (hours)				
Sample code	**Con. mg/mL**	**24 hrs**	**48 hrs**	**72 hrs**
DL-E	**1**	59 ± 1.0 aA	70 ± 2.0 aB	79 ± 1.0 aC
	0.5	58 ± 1.0 aA	68 ± 1.0 aB	78 ± 2.0 bC
	0.125	54 ± 1.5 bA	65 ± 1.2 bB	77 ± 2.0 bC
DL-A	**1**	40 ± 1.5 aA	55 ± 2.0 aB	67 ± 1.5 aC
	0.5	38 ± 1.0 bA	53 ± 1.5 abB	63 ± 1.5 bC
	0.125	20 ± 1.0 cA	40 ± 1.0 cB	50 ± 1.2 cC

Values in columns having same upper case are not significantly different (P < 0.001).
Values in rows having same lower case letters are not significantly different (P < 0.001).

Tables 2: Nematicidalpotential of *Sophora alopecuroides* against *M. incognita.*

Mortality (%), Intervals (hours)				
Sample code	**Con. mg/mL**	**24 hrs**	**48 hrs**	**72 hrs**
SL-A	**1**	27 ± 1.5 aA	42 ± 1.5 aB	52 ± 1.0 aC
	0.5	19 ± 1.0 bA	35 ± 2.0 bB	45 ± 2.0 bC
	0.125	15 ± 1.0 cA	34 ± 2.0 bB	44 ± 1.2 cC
SL-E	**1**	52 ± 2.0 aA	62 ± 1.5 aB	70 ± 1.5 aC
	0.5	48 ± 2.0 bA	58 ± 1.0 bB	66 ± 1.0 bC
	0.125	47 ± 1.5 bA	57 ± 1.5 bB	65 ± 2.0 bC
SF-A	**1**	39 ± 1.2 aA	54 ± 1.2 aB	66 ± 2.0 aC
	0.5	32 ± 1.0 bA	48 ± 1.0 bB	60 ± 1.5 bC
	0.125	30 ± 1.0 bA	42 ± 1.5 bB	54 ± 1.2 cC
SF-E	**1**	60 ± 1.0 aA	70 ± 2.0 aB	75 ± 1.0 aC
	0.5	58 ± 1.0 aA	68 ± 2.0 bB	73 ± 1.0 bC
	0.125	56 ± 1.0 abA	65 ± 1.2 cB	71 ± 1.0 cC

Values in columns having same upper case are not significantly different (P < 0.001).
Values in rows having same lower case letters are not significantly different (P < 0.001).

Whereas DL-A extract showed lowest mortality rate 20 40% after 24 hrs of treatment. (Table 1). The crude ethanolic extracts of flowers of *S.alopecuroides* (SF-E) exhibited mortality range 71-75 % after 72 hours of exposure which is highest as compare ethanolic extract of leaves of *S. alopecuroides* (SF-E). On the other hand the acetone extract of flowers of the plant (SF-A) showed moderate activity with mortality rates ranges 54-66% after 72 hours.

The different concentrations of crude ethanolic extract of leaves of *S. alopecuroides* (SL-E) possess moderate to good nematocidal activity with mortality rate 65-71 in 72 hours. Whereas the acetone extract SL-A showed lowest mortality rate 15- 27 % after 24 hrs and 44- 52 % after 72 hours in all three concentration (Table 2).

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