Research Article

In Vitro Nematicidal Activity of an Endemic Plant *Pulicaria boissieri* Hook. F. Against Root-Knot Nematodes *Antinematodal* Activity of *Pulicaria boissieri*

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Abstract | In this current study, the *in vitro* nematicidal activity of *Pulicaria boissieri* against root-knot nematodes has been investigated. The results revealed that all the solvent extracts contained anti-nematodal properties; however, variations in mortality rates were observed. Specifically, the chloroform extract of the leaves of *P. boissieri* exhibited 84% inhibitory activity against nematodes after 72 hours of exposure. The results indicated that exposure of nematodes for 72 hours resulted in the maximum nematicidal activity compared to exposures of 24 and 48 hours. It was concluded that leaf and stem extracts in chloroform and methanol solvents exhibited the highest activity against nematodes. The sequence of nematicidal activity from high to low was as follows: chloroform > methanol > acetone > ethanol > water. Hence, Pulicaria boissieri demonstrates potential nematicidal activity with various organic solvents.

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Introduction

Nematoda is extremely diverse in relationships of species productivity and one of the most abundant metazoan groups on earth (Hugot *et al.*, 2001). Plant-parasitic nematodes (PPNs) are extremely specified parasites that cause diseases in plants using a variety of approaches (Williamson and Hussey, 1996). In terms of nourishing, they might be known as ectoparasitic or endoparasitic (Jasmer *et al.*, 2003). Ectoparasites do not go in the root nevertheless feed by introducing a stylet interested either in epidermal cells or in the root (Wyss, 1997). Plant parasitic nematodes are the greatest damaging group of plant pathogens all over the world and their control is extremely challenging (Sasser, 1998). Plant-parasitic nematodes are the main pests in several countries, especially in the tropical and subtropical regions, where they are renowned for causing severe damages to the yield on a wider variety of crops (Luc *et al.*, 2005). Up till now more

than 4,100 nematode species are discovered which have potential to being toxic for plants (Decraemer and Hunt, 2006). Many parasitic nematode species effect leaves and stems but most of the nematodes damage roots of the Plant (Sasser and Freckman, 1987). Plant-parasitic nematodes are the main pests that are affecting economic important crops and loss of billions of dollars. Numerous species of plant-parasitic nematodes belong from the Longidorus and Trichodorus serve as virus vector and carry several viruses from infected plants to healthy plants (Ehlers, 2001; Gaugler, 2002). Entomopathogenic nematodes have been commercialized as environment-friendly agents for numerous insect pests (Brown *et al*., 1994). Infected plants have different symptoms such as root galling, inhibiting height and nutrient deficiency, predominantly insufficiency of nitrogen (Siddiqui and Mahmood, 1996). They induce the formation of root galls, root system reduction, chlorosis, dwarfism, and wilting in their host plants (Manzanilla *et al*., 2002). In galled roots, the uptake and transport of water and nutrients are severely altered and therefore, the crop yields are reduced (Sikora and Fernandez, 2005).

It is problematic to control because nematodes have an extensive host variety and a high rate of reproduction, the female nematodes can able to reproduce above a thousand eggs (Natarajan *et al*., 2006). Currently, several medicinal plants having insecticidal properties are identified (Banerji *et al*., 1985). Nematicides of plant origin consist of phenolic compounds, isothiocyanates, thiophenics, fatty acids, alkaloids, and glucosides (Andres and Coupland, 2012; Chitwood, 2002). These compounds possibly contain the same compounds that are produced synthetically as respiratory poisons, insecticides, deterrents and repellents (Sukul, 1992). In several plant species belonging to 57 families, antinematicidal compounds have been identified (Banerji *et al*., 1985). Many plants including Brassicaceae, Asteraceae, Myrtaceae and Rutaceae family member plants contain nematicidal compounds (Sukul, 1992; Gommers, 1973).

Pulicaria boissieri Hook. F. is an endemic plant of Pakistan. *P. boissieri* belongs to the tribe Inulae from the family Asteraceae. It is a small shrub having villose leaves. There is no research work regarding the nematicidal activity on this plant that has been done yet. In this research, we are going to evaluate antinematodal activity of this plant in different solvent extracts.

Materials and Methods

Extraction of egg masses from galled roots

Egg masses were separated from root knot galls by needle and added into the Petri dishes which contained some amount of distilled water. For hatching egg, masses of nematodes were suspended in water. The Petri dishes were kept at room temperature for 24 hrs. Egg suspension was diluted, and the number of 260/20ml was maintained at about 15-25 nematodes in each block.

Cavity block studies

1ml of extract of each solvent were poured in blocks and left for evaporation for 24 hrs. After complete hatching of egg masses and evaporation of solvents, 1ml of nematodes suspension containing 15-25 living nematodes were added into the cavity blocks, each treatment replicated 3 times. The effect of different extracts on the motility of nematodes was determined at a three-observation time i.e., 24, 48 and 72 hours. Data were recorded, and comparisons of dead percentage were made on basis of the average of dead nematodes.

Plant collection

The plant was collected from the Kerthar range, Thana Bulla khan and adjoint areas. Healthy Stem and leaves were collected and brought to the lab and cleaned with running tap water to remove dust. The collected leaves and stems shade dried for one week. The dried ground to powder form in uniform size with the help of a grinding mill. In total 5 types of plant, solvents were used to extract compounds from plants: Methanol, Ethanol, Acetone, Chloroform and water making the final volume of 1000ppm.

Extraction process through Soxhlet

The first step in qualitative and quantitative analysis of plant constituent is plant extraction. In this process, plant compounds are extracted from crude plant material (Abubakar and Haque, 2020). By this process, phytochemicals from crude plant material with minimum material and solvent usage were extracted. So, we preferred to use the Soxhlet apparatus for purpose of extraction.

Solvent extraction

The powder form of 10g plant material in 150 mL of different solvents chloroform, methanol, ethanol, water and acetone was used. Extraction was done in

the Soxhlet apparatus. The extract was air-dried and stored in the refrigerator.

Nematodes culture

For a finding of egg masses and larvae, pure culture of nematodes maintained on eggplants in sterilized soil. Root-knot infected brinjal plants from the culture plate were up-rooted and washed gently under running tap water (Hooper *et al.*, 2005).

Statistical analysis

Statistical analysis was performed by using the software IBM SPSS Statistics 23. One way analysis of variance (ANOVA) followed by Bonferroni post hoc test and student's t-test were performed to compare the groups with level of confidence P <0.05; (where * = P < 0.05, ** = P < 0.01, *** = P < 0.001). Data are presented as mean ± SE.

Results and Discussions

This study was designed to evaluate the effect of *Pulicaria* sp. extracts (in different solvents) on living nematodes (Figure 1). The inhibition of nematode mortality was attributed to the exposure period and the extract concentration. However, the mortality rate significantly differed among tested plant extracts in methanol, ethanol, acetone, chloroform and water. All the extracts of leaf and stem showed variation against nematodes as shown in Tables 1-5 and Figure 2. Nematode survival was significantly affected by all types of plant extracts. The length of exposure affected nematode mortality remarkably. The results indicate that leaf and stem extracts of *Pulicaria* showed significant activity against plant parasitic nematodes.

At 24 hour the highest mortality rate observed in chloroform and methanol extracts as compared to control and other extracts of leaf and stems in different solvents. They showed a significant (p<0.05) nematicidal activity (Tables 1 and 2).

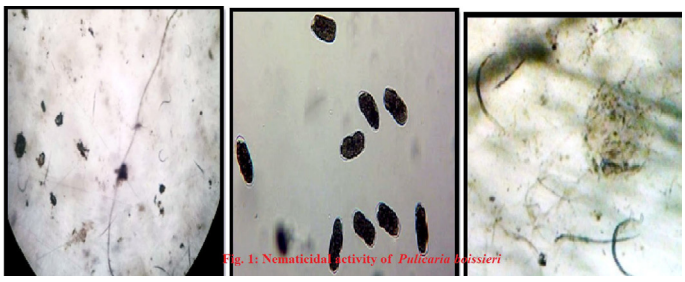

Figure 1: *Nematicidal activity of Pulicaria boissieri.*

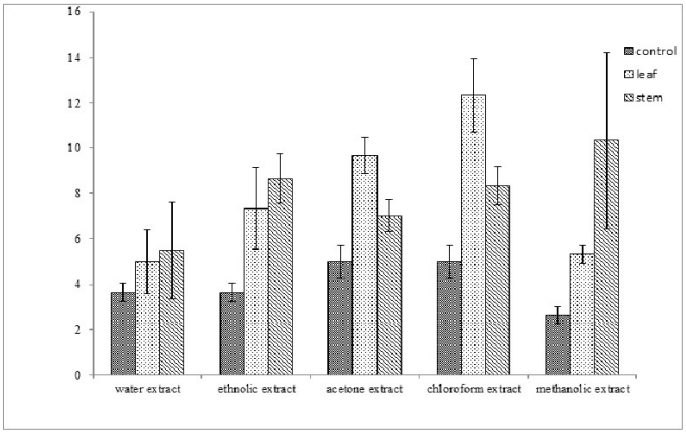

Figure 2: *Nemacidal effect of different extracts of Pulicaria boissieri.*

However, in the next 48 hours time period, a more significant and pronounced mortality rate observed in all solvent extracts of leaf and stem, but stem extract of chloroform showed the highest rate *i.e.,* 51% and leaf extract *i.e.,* 40% as compared to rest of the solvent extracts (p<0.01) (Tables 1-5).

Table 1: *Nematicidal activity of methanolic extract of Pulicaria boissieri and its statistical analysis.*

Methanolic extract	Mean and Standard Error	% of dead Nematodes in 24 hrs	% of Dead Nematodes in 48 hrs	% of Dead Nematodes in 72hrs	Degree of freedom	Means square	Fre-quency	Signifi-cance	Least significant difference
control	14±2.54	7.14	14.28	19	2	2.1	2.71	0.145	0.778
Leaf	15.66 ± 1.47	19.15	21.26	34.03	2	4.77	5.37	0.046	0.889
Stem	15.66 ± 3.55	19.15	31.92	65.96	2	40.11	3.28	0.109	12.22

Table 2: *Nematicidal activity of Chloroform extract of Pulicaria boissieri and its statistical analysis.*

Chloroform extract	Mean and Standard Error	% of Dead Nematodes in 24hrs	% of Dead Nematodes in 48 hrs	% of Dead Nematodes in 72 hrs	Degree of freedom	Means square	Frequen-cy	Signifi-cance	Least significant difference
Control	13.33 ± 1.77	22.5	24.98	37.5	2	3.4	2.38	0.173	1.44
Leaf	14.66 ± 2.16	27.28	40.92	84.1	2	52.11	21.31	0.002	2.44
Stem	13.66 ± 1.47	29.28	51.24	60.98	2	15.44	9.92	0.012	1.55

Table 3: *Nematicidal activity of acetone extract of Pulicaria boissieri and its statistical analysis.*

Acetone Extracts	Mean and Standard Error	% of Dead Nematodes in 24 hrs	% 0f Dead Nematodes in 48 hrs	% of Dead Nematodes in 72 hrs	Degree of freedom	Means square	Frequency	Significance	Least significant difference
Control	20.66 ± 1.77	14.51	16.11	24.2	2	0.778	0.875	0.464	0.889
Leaf	20.33 ± 1.77	21.29	22.99	47.51	2	26.77	26.778	0	0.667
stem	21.33 ± 2.85	12.87	17.15	32.8	2	15.44	27.8	0.001	0.556

Table 4: *Nematicidal activity of ethanolic extract of Pulicaria boissieri and its statistical analysis.*

Ethanolic Extracts	Mean and Standard Error	% of Dead Nematodes in 24 hrs	% of Dead Nematodes in 48 hrs	% of Dead Nematodes in 72 hrs	Degree of freedom	Means square	Frequency	Significance	Least significant difference
Control	22.33 ± 3.48	13.43	14.91	16.39	2	0.333	0.6	0.579	0.556
Leaf	22.33 ± 2.67	8.99	16.39	32.82	2	22.33	6.931	0.028	3.22
Stem	23.66 ± 3.61	11.24	16.9	36.6	2	27.44	24.76	0.001	1.11

Table 5: *Nematicidal activity of water extract of Pulicaria boissieri and its statistical analysis.*

Water	Mean and Standard Error	% of Dead Nematodes in 24 hrs	% of Dead Nematodes in 48 hrs	% of Dead Nematodes in 72 hrs	Degree of freedom (DF)	Means square	Frequency	Significance	Least significant difference
Control	22.33 ± 3.48	13.43	14.91	16.39	2	0.667	1.33	0.385	0.5
Leaf	17.5 ± 2.12	14.28	17.14	28.57	2	3.5	0.95	0.47	3.66
Stem	16 ± 1.41	18.75	21.87	34.37	2	3.5	2.3	0.245	1.5

At 72 hours, all the solvent extracts showed highly significant nematicidal activity ($p<0.001$). The highest mortality rate observed in leaf extract of chloroform solvent *i.e.,* 84% and stem extract of methanol solvent which is 65% (Tables 1-5).

The above result evaluated that all the solvent extracts contained anti-nematodal properties, however, the variation in mortality rate was observed. It was concluded that leaf and stem extract in chloroform and methanol solvent showed the highest activity against nematodes. The sequence of high nematicidal activity to low is as: Chloroform > methanol > acetone > ethanol > water.

In Plants, phytoconstituents are available that are utilized to fix the illness of humanity. Many plants have nematicidal properties in their foundations, shoot, leaves, blossoms, seeds and their concentrates, natural balm, oilseed cakes and items have been successfully tried against an assortment of nematodes (Tsay *et al*., 2004). In our review, the outcomes demonstrated that the leaf and stem concentrate of Pulicaria plant showed nematicitadal action against plant parasitic nematodes. It has been seen that nematicidal action could be connected with various kinds of compound present in plant. Plant can be the best hotspot for such bioactive mixtures (Bharadwaj and Sharma, 2007).

The quantity of plants contains intensifies that are poisonous to specific nematodes. Neem (*Azardirachta indica*) has such an assortment of organically dynamic fixings, which have various methods of activity, that it is workable for neem to oppose in excess of 200 types of bugs, parasites and nematodes (Akhtar and Mahmood, 1996). The concentrate fluctuated in their viability in various solvents. In our review, results showed that the plant extract in chloroform showed more viability against nematodes than other solvent's extract.

The current review has shown that chloroform concentrate of the plant is valuable for nematode control, which will be a prudent and ecofriendly choice for control of nematodes In our review the water concentrate of leaf and stem showed slight adequacy against plant parasitic nematodes with regards to killing and decreased the mortality of nematodes, Klimpel *et al.* (2011) likewise depicts various concentrates movement against nematodes.

This study exhibits that the plant extract contains gigantic anti-oxidant agent action. Anti-oxidant agents normally produce in plants kills the receptive

oxygen species (ROS) created by any injury or stress (Lee *et al*., 2003). Flavonoid presence demonstrated by the phytochemical examination of plant concentrate might be responsible for this anti-oxidant agent action (Karimi *et al*., 2010; Prochazkova *et al*., 2011). Plants produce a wide variety of auxiliary metabolites, some of them of the drug interest and others with antimicrobial properties (e.g., phenylpropanoids, flavonoids, terpenoids, alkaloids, and others), and a significant number of these accumulates assume an indispensable part in the association of plants with their current circumstance (Khalaf *et al*., 2008).

Conclusions and Recommendations

The results obtained from the testing of leaves and stem extract of *P. boissieri* against nematodes showed that methanolic extract of leaves of *P. boissieri* displayed significant inhibition of harmful microbial agents causing damage to plants and humans. It is also reported that the extract showed 84% activity against plant parasitic nematodes. Chloroform extract of leaf and stem showed maximum nematicidal activity as compared to other plant extracts. Thus, this study proved that *Pulicaria* sp. can be used for biocontrol of plant parasitic nematodes and this plant extract is environment friendly and can control of parasites easily. Antioxidant activity showed 35.35% activity against control from methanolic extract of stem of *P. boissieri*.

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Novelty Statement

This study is first of its kind in which an Endemic Plant P. boissieri extracts are used for nematicidal activity.

Author's Contribution

Rauf is the main student who performed main aspect of the research work under the supervision of Dr. Shazia Mansuri.

Conflict of interest

The authors have declared no conflict of interest.

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