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Research Article

**EVOLUTION OF NEMATOCIDAL POTENTIAL OF CRUDE
EXTRACT OF MEDICINAL PLANTS FROM PAKISTAN FLORA****Dr. Nadra Naheed¹, Dr Shahina Fayyaz², Sajida Sultana¹, Suad Naheed³, Iqra Anam¹,
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Pakistan, ²National Nematological Research Center University of Karachi, Pakistan³Department of Biotechnology Jinnah University for Women Karachi, Pakistan**Abstract:**

Root knot nematodes are harming various plants which is economically harmful and can lead towards serious food and economics problem. The research was carried out with aim to point out nematocidal potential of Methanolic extract of five selected medicinal plants, *Chinopodium album*, *Withania Coagulans*, *Saphora mollis*, *Peganum harmala* and *Rosamarinus officinalis*. Methanolic extract were used for extract preparation. 80% Methanolic solvent was used. All extracts were prepared in SBKWU lab and nematocidal analysis of crude extract of medicinal plants was carried out in NNRC (national nematocidal research center) Karachi. Culture for incognita species obtained from *Lycopersicon esculentum* (tomato) plant were prepared at 28 °C incubation temperature. For nematocidal assay, second jvelined stage was utilized. The analysis was carried out with different dose including 1%, 0.5%, 0.25% and 0.125%. Analysis was made with duration of 0.5, 1, 2, 3, 4, 42, 48 and 72 hours. Significant response was observed in *Chinopodium album* whose response was 100% reduction in nematodes population at 48 and 72 hours of exposure. All dose of this plant extract also showed positive response but among five selected herbal plants, lowest response was found in *Saphora mollis* Methanolic extract with 21-51.5 % response at even maximum exposure with highest dose.

Key Words: Root-Knot Nematodes, Nematocidal potential, Methanolic Extract, Medicinal Plants, *Meloiogyne* Species.

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INTRODUCTION:

Root-Knot nematodes which are member of *Meloidogyne* genus, are said to be plant parasite nematodes [1]. The member of this species are pathogenic for wide range of crop ranging from fiber, horticulture till food and vegetable crops [2]. Almost all plants get infected by these plants parasite nematodes [3]. These member may be responsible for major loss of crop yield. A study shows loss estimation of \$ 100 billion dollar per year around the world. Most specie which are subjected to face rot-knot damage are found to show growth in warm environment. The presence of these pants is evident by many symptoms involve reduction in yield, fading colour and presence of galls.

Four species named *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria*, are common in Pakistan [4]. These species are responsible for significance damage of different types of crops. So need is to protect this loss of crop as this beloved country is agriculture sector based country since birth [5]. Number of scientists have been reported damage of crop in Pakistan due to nematodes such as loss in rice crop in Punjab [6] in Soybean [7-8] damage to okaracrop. Lose in yield of pomegranate in Balochistan and Sindh [9]. Damage to peanut field [10] and potato crop [11] have also been documented. Present study was conducted in Jaffarabad, south east to Quetta city [12]. The damage to crop is mainly due to root-knot nematodes as it possess warm climate which is suitable for growth of *Meloidogyne*.

Eradication of root-knots nematodes is possible by chemical nematicidal agents but such methods are cost effective and possess harmful impact on human and environment as well. These factor have harmful impact which limits the use of chemical nematicides. So natural way to kill these species are necessary to find out. One of effective approach is to find out nematicidal potential in medicinal plants. Plants possess many hidden potentials which are to be explored. One of these hidden potential is ability to kill parasitic nematodes. Potential of different plant extract against nematodes have been reported [13-16]. One of research shows that 57 families of plant kingdom contain natural product which is responsible for nematicidal potential of those plants [17]. The use of

plant to kill nematodes is cheap, environmental friendly and economically beneficial method with no hazardous impact [17].

Aim of present research is to point out hidden potential of selected medicinal plants against nematodes. The plants selected for present research were *Chinopodium album*, *Withania Coagulans*, *Saphora mollis*, *Peganum harmala* and *Rosamarinus officinalis*. Ability of these plant to access as nematicidal activity were observed with different dose with variable duration of exposure period by in-vitro analysis.

MATERIAL AND METHOD:**Collection and identification of plant**

Plant samples of selected plants (*C. album*, *W. Coagulans*, *S. mollis*, *P. harmala* and *R. officinalis*) were collected in summer season from Usta Mohammad, Gandakha, Jhatpat and Suhbat Pur, different areas of Jaffarabad. The collected plants samples were preserved in polythene bag till access of lab.

Dr. Rasool Bakhsh Tareen who is prof. in university of Balochistan and also chairperson of botany department, Identified all the plants.

Extract preparation

All plants were washed with tap water and then with distil water to remove unwanted objects. After washing all sample, all collected plants were spread on clean sheet in shade and left for drying for 15 days. After duration of 15 days, the shade dried plants were put in mortal and roughly grinded with pestle. After it electrical grinder was used to get fine powder of each sample. Fine powder of each plant was preserve in glass bottles. All dried samples were placed in 80% methanol solvent for 10 days with purpose to attain Methanolic extract. This was soaking period which was followed by filtration through whatmann filter paper No 4. The filtrate was place in rotatory evaporator to separate solvent. The operation was carried out at 50-500 °C. After that thick gummy viscus extracts obtained were collected on petri-dish. All procedure till here was performed in SBKWU, chemistry lab. The extract obtained were analyzed for nematicidal activity at NNRC (National Nematological Research Center).

Table 1. Plant information with part used and quantity obtained.

Plant name	Soaked part of plant	Solvent system 80% methanol
<i>C. album</i>	Whole plant	40 g/ 400ml
<i>W. Coagulans</i>	Whole plant	60 g/ 500 ml
<i>S. mollis</i>	Stem and leave	40 g/ 400ml
<i>P. harmala</i>	Seeds, leaves	40 g/ 400ml
<i>R. officinalis</i>	Leaves	35 g/ 300 ml

Analysis of Nematicidal activity

Culture of juvenile's stage for *M. incognita* spp. were cultured in NNRC. The root-knot collected for culture initiation were obtained from *Lycopersicon esculentum* (tomato) plant.

Egg mass of this nematodes were isolated from plant and then incubated for some time at 28 °C. The period was hatching periods which result in production of larvae. These cultured larvae were utilized for studying nematicidal activity of extracts of selected plants. For each analysis 100 larva were placed in each petri-dish. Different doses (1%, 0.5%, 0.125% and 0.25%) of each plant extract were analyzed with intervals. Stock solution of plant extracts were made in 5% dimethyl sulfoxide. Analysis were carried out at interval of 0.5, 1, 2, 3, 4, 24, 48 and 72 hours.

RESULT AND DISCUSSION:

The observation gave direct time and dose relation against nematodes. As concentration increase death rate of nematodes also increases. Similarly exposure for longer time increase chance for reduction and population. Five plants were analyzed in this

research named *Chinopodium album*, *Withania Coagulans*, *Saphora mollis*, *Peganum harmala* and *Rosamarinus officinalis*. Among five under-studied plant least activity was observed in *Saphora mollis*, 21.8-51.5 % motility rate of nematodes even at largest exposure of time viz 72 hours. The most effective plant against root-knot nematodes was *Chinopodium album*. The plant extract was able to kill all nematodes resulting 100 % expressing positive response with all dose analysis at 72 hours.

The plant was also able to cause 91-100 % death of nematodes at 48 hours. In case of *Withania coagulans*, positive response was observed. Plant extract was able to kill nematodes after 24 hour while till 72 hours it's activity was significant. The nematicidal potential of *Peganum harmala* was found to be with positive response after 3 hours of exposure. Potential of *Rosamarinus officinalis* was less as compare to *Rosamarinus officinalis*. The observed nematicidal potential is of order *Chinopodium album* > *Peganum harmala* > *Rosamarinus officinalis* > *Withania Coagulans* > *Saphora mollis*. Our result shows that all plant exhibit nematicidal activity.

Table 2. Nematicidal activity

	Mortality percentage at different intervals (hrs)								
	Dose	½	1	2	3	4	24	48	72
<i>Peganum harmala</i>	1	31	41	61	71	86	91	100	100
	.5	30.5	43.5	57.5	66	83	90.5	100	100
	.25	0	27	29	36	50.5	68	78	83
	.125	0	23	26	33	46	63	68	75.5

<i>Withania coagulans</i>	1	0	22.5	38	56	62.5	76	86	98
	.5	0	21	31	48	51	74	83	91
	.25	0	16.5	28	43.5	52	66	73	88
	.125	0	16	23.5	36.5	48	52	66	78
<i>Chenopodium album</i>	1	34	46	68	74	83	96	100	100
	.5	26	41	61	61	76	91	97	100
	.25	24	38	56	59	76	88	97	100
	.125	24	33	54	61	71	81	91	100
<i>Rosmarinus officinalis</i>	1	0	39	63	78	86	93	97	100
	.5	0	34	56	73	81	88	90	93
	.25	0	31	48	68	76	83	86	91
	.125	0	0	33	56	70	76	81	88
<i>Saphora mollis</i>	1	0	0	23	26	31	36	41	51.5
	.5	0	0	0	0	18	21	26	31
	.25	0	0	0	0	0	26	26	21.8
	.125	0	0	0	0	0	0	11	31

CONCLUSION:

Root-knot nematodes are responsible for heavy loss of crop yield. In any agriculture based country this loss should prevent to maintain good yield for getting economic benefits. Chemical used to control this damage offer negative impact to our environment so need is to find out natural ways to protect and prevent this lose. One of such source are medicinal plants.

Plants found in Pakistan such as *Chinopodium album*, *Withania Coagulans*, *Saphora mollis*, *Peganum harmala* and *Rosamarinus officinalis* are also among those plants which exhibit potential against Root-knot nematodes. Among these plant *C.album* have strongest potential while lowest was in *Saphoramorous*.

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