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Evaluation of vEvaluation of Various Seed Extracts for Their Nematicidal Efficacies Against Root Nematode, Meloidogyne Incognitaarious Seed Extracts for Their Nematicidal Efficacies Against Root Nematode, Meloidogyne Incognita

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Short Report

Keywords: Soxhlet extraction, Yield percentage, In vitro nematicidal activity, Greenhouse nematicidal activity

Posted Date: January 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-144055/v1

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Version of Record: A version of this preprint was published at Discover Sustainability on March 9th, 2021. See the published version at https://doi.org/10.1007/s43621-021-00023-0.

Abstract

Nematicidal activity of seed extracts of eight medicinally important plants *viz. Abrus precatorius* Linn., *Amaranthus virdis* Linn., *Bunium persicum* Boiss., *Dioscorea deltoidea* Wall. Ex Griseb., *Teraxacum officinale* Weber., *Malva neglecta* Wall., *Podophylum hexandrum* Royle and *Robina pseudoacacia* Linn. against *Meloidogyne incognita* was evaluated *in vitro* at (24, 48 and 72 h) and greenhouse method. After 48 h of exposure, the *in vitro* results of second stage juveniles (J2s) displayed almost all extracts possess positive effects on J2 mortality. However, dominant mortality was observed by *T. officinale* 93.67% and *B. persicum* 89.66% at 72 h exposure. In case of greenhouse effect extracts of *T. officinale* and *B. persicum* reduced infestation as compared to inoculated control. The root knot index varied between 1-3 and egg mass index 0-3 as compared to control. This study suggested that seed extracts of these plants can be used for the management of *M. incognita* and could be used in replacement of synthetic nematicides.

1 Introduction

Agriculture is one of the important backbones of food production in the world. Due to rapid increase in world population and damage caused by various microorganisms and nematodes on various plants and vegetables which leads pressure on agriculture and ultimately production of various foods. Nematodes are very much responsible for the damage of plants and industrial, edible crops. The various species of Meloidogyne causes 90% loss in agronomical damages caused by root-knot nematodes [1]. Additionally these nematodes reacts with other disease causing organisms which leads to development of more complex diseases and loss of resistance to plants against pests and nematodes [2]. The development of new nematicides is very difficult task, because the living place of most nematodes is soil or plant roots. The target of any chemical substance is very away from its application, more over cuticle and surrounding surface of nematode is impermeable to chemical substances, so it leads to toxicity, environmental pollution and an average farmer cannot afford them easily [3, 4]. There is recent trend in search for the discovery of various antimicrobial, antioxidant and nematicidal agents from plant sources which are less expensive, eco-friendly, biodegradable, safer and natural [5–9]. Hence taken in consideration of the vast abundance of with tremendous traditional and biological use of *Abrus precatorius* L., *Amaranthus virdis* L., *Bunium persicum* Boiss., *Dioscorea deltoidea* Wall., *Malva neglecta* Wall., *Podophylum hexandrum* Royle, *Teraxacum officinale* Weber., and *Robina pseudoacacia* L. from Kashmir [6] these plants were selected for present study.

2 Materials And Methods

2.1 Source of seeds and chemicals

The seeds of worked plants were collected from nurseries in Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKAUST-K) and various other parts of Kashmir in 2019. Dr. Sajad Gangoo authenticated the plants. The herbarium specimens were placed at Kashmir University Herbarium (KASH) under 823, 827, 891, 1374, 1357, 1391, 1398 and 1411 respectively for *A. precatorius*, *A. virdis*, *B.*

persicum, *D. deltoidea*, *M. neglecta*, *P. hexandrum*, *T. officinale* and *R. pseudoacacia* of Voucher Specimen Numbers [Ref. No. F1 (Specimen voucher, CBT) KU/2019] and nematode *Meloidogyne incognita* were obtained from the section of plant pathology, Department of Botany, Aligarh Muslim University, Aligarh (India). The solvents and chemicals used were purchased from Sigma-Aldrich, USA.

2.2 Soxhlet extraction of seeds

The seeds of all worked plants were air dried and then ground into powder. The coarsely powder (40 g) of each plant in the form of packets was transferred into a 250 ml Soxhlet apparatus. About 180 ml of solvent chloroform and methanol (50:50, v/v) was taken and the apparatus was placed in 80 °C water bath and extracted for 3 hr. After complete extraction the solvent is removed with rota vapor and extract of each plant was collected. The yield of each extract (%) was obtained by following formula:

$$\text{Yield of an extract (\%)} = \frac{\text{mass of extract obtained (g)}}{\text{mass of seed material (g)}} \times 100$$

2.3 Nematicidal activity

2.3.1 In vitro method

The seed extracts of *viz., P. hexandrum*, *B. persicum*, *A. precatorius*, *T. officinale*, *A. virdis*, *D. deltoidea*, *M. neglecta* and *R. pseudoacacia* were used for nematicidal activity to evaluate the effectiveness on *M. incognita* juveniles (J_2) mortality under different exposure time (24, 48 and 72 hours). In this experiment seed extracts were taken in sterilized petri dishes while tap water served as a control [10]. Hundred freshly second stage juveniles (J_2) of *M. incognita*, reared in the greenhouse in eggplant roots as pure culture, were added to each petri dishes by a pipette. Each treatment was triply replicated. All the dishes were kept randomly in laboratory at room temperature. Number of dead nematodes was observed at 24, 48 and 72 hours. Percentage mortality was calculated as: [mean of number of dead juveniles in treatment/total number of juveniles in treatment] × 100. After 72 h treatment the juveniles were transferred to distilled water for 24 h to ensure no recovery will occur.

2.3.2 Greenhouse method

This experiment is based in accordance to Holbrook et al. method [11]. Ten days old tomato seedlings were planted in clay pots with steam sterilized sandy loam soil. The plants were inoculated with counted number of infective juveniles (500 juveniles in 10 ml of water) and 100 ml of each plant extract separately by making holes of 3–5 cm deep near base of the plant. The holes were plugged with soil soon after inoculation. Watering of plants was done on regular basis. The plant inoculated with nematode without plant extracts serve as inoculated control. Each treatment had three replicate and experiment was repeated twice. After 30 days of inoculation, the plants were uprooted and washed gently with tap water. Number of galls per plants was counted visually. Number of egg masses per root system was counted by staining the infected root with phloxin-B (Holbrook). Disease intensity was determined by root-knot index and egg mass index on 0–5 rating scale according to [12].

2.4 Statistical analysis

Treatment results were statistically evaluated by using analysis of variance (ANOVA) by using the statistical software (IBM SPSS statistics software 20). Duncan's multiple range tests (DMRT) were performed to determine the differences between groups and means were considered significant at 5% significance level.

3 Results And Discussions

3.1 Yield of seed extracts

The Soxhlet extraction is one of the simple, useful and well known universal extraction technique. In seed extraction the polarity of solvent and time required for extraction process plays an important role in extraction efficiency. As displayed in (Table 1) the percentage yield obtained of all extracts were good however, highest yield was obtained in *P. hexandrum* 67.9% followed by *R. pseudoacacia* 57.5%, *A. virdis* 56.1%, *M. neglecta* 50.4 and *T. officinale* 40.3%. The lowest yield was observed in seed extracts of *D. deltoidea* 23.9% followed by *A. precatorius* 13.8% and *B. persicum* 12.4%. In this work the Soxhlet extraction gives comparatively good extraction efficiency of seed extracts that can play an important role in relation to their nematicidal nature.

3.2 Nematicidal activity

All the seed extracts tested were observed to exhibit good level of inhibition towards juveniles of *M. incognita in vitro* method**,** but the level of toxicity varied with increase in exposure time. It has been found that all extracts showed higher mortality rates following 72 h. Data presented in the (Table 2) showed that all the seed extracts showed suppressive activity against *M. incognita* which increases in increasing exposure time. However, dominant inhibition was shown by *T. officinale* (93.67%, 61.44% and 38.71%) and *B. persicum* (89.66%, 56.55% and 35.06%) mortality of *M. incognita* at 72, 48 and 24 hours respectively. The extracts of *M. neglecta* (75.28%, 55.73% and 36.67%) and *R. pseudoacacia* (70.39%, 48.66% and 36.43%) showed good inhibition of *M. incognita* while as normal effect was displayed by *A. precatorius* (67.62%, 41.19% and 34.33%), *A. virdis* (62.46%, 45.32% and 32.24%), *D. deltoidea* (62.37%, 42.22% and 31.47%) and *P. hexandrum* (60.31%, 40.33% and 34.53%) mortality of *M. incognita* at 72, 48 and 24 hours respectively. The results were confirmed by seed extracts of *Ricinus communis* and *Peganum harmala* which were used by Hasan et al. [13], Curto et al. [14] and Rich et al. [15] against Meloidogyne juveniles, and found them effective.

Laquale et al. [16] earlier reported that the leaf and root extracts of *T. officinale* showed 36 and 50% juvenile mortality and 14.8 and 23.8% egg hatchability reduction of *M. incognita*. Khan et al. [17] found that various extracts of plants from apiaceae family such as *Coccinia grandis*, *Commelina benghalensis*, *Leucas cephalotes*, *Phyllanthus amarusand*, *Trianthema portulacastrum* displayed good nematicidal activity against *M. incognita*.

In case of greenhouse method as presented in (Table 3) *T. officinale* and *B. persicum* extracts were found to be more potent than in reducing number of gall and number of egg masses in comparison to all other treatments. The seed extracts of *M. neglecta*, *A. precatorius* and *R. pseudoacacia* showed good inhibition of nematodes while as Lowest reduction was noticed in case of *P. hexandrum* and *D. deltoidea* as compared to control.

4 Conclusion

This study highlighted the nematicidal activity of eight seed extracts against root nematode, *M. incognita*. Both *in vitro* and green house method of nematicidal activity revealed that seed extracts especially those of *T. officinale* and *B. persicum* extracts were very potent in suppressing nematode infestation. From this study it is concluded that the extracts of these plants can be used as a source of nematicidal agents in future drug, design and development.

Declarations

Acknowledgement

The authors are thankful to UGC for the award of Non-Net fellowship and Department of Chemistry, Department of Botany and Instrumentation Facility (USIF), Aligarh Muslim University for providing the requisite facilities to complete this study. Moreover, Shazia Bhat is dully acknowledged for proper help in this work.

Conflict of interest

The authors declare that they have no conflict of interest.

Author credit statement

Zubair Rehman Nengroo: Conceptualization, methodology, software, writing and supervision **Zeeshan Umar Shah:** software validation and in vitro nematicidal activity **Adil Shafi Ganie:** Material collection **Mohammad Danish:** Green house nematicidal activity

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Tables

Table 1. List of plant species used for chloroform and methanol (50/50, v/v) extraction, with their yields obtained by Soxhlet extraction.

Botanical name	Family	% Yield (*w/w*)
A. precatorius Linn.	Fabaceae	13.8
A. virdis Linn.	Amaranthaceae	56.1
B. persicum (Bioss.) B.	Apiaceae	12.4
D. deltoidea Wall. Ex Griseb.	Dioscoreaceae	23.9
M. neglecta Wallr.	Malvaceae	50.4
P. hexandrum Royle	Berberidaceae	67.9
R. pseudoacacia Linn.	Fabaceae	40.3
T. officinale Weber.	Asteraceae	57.5

Table 2. *In vitro* nematicidal activity of seed extracts of *P. hexandrum*, *B. persicum*, *A. precatorius*, *T. officinale*, *A. virdis*, *D. deltoidea*, *M. neglecta* and *R. pseudoacacia* against *M. incognita*.

Seed extracts	% Nematode mortality		
	24 h	48 h	72 h
P. hexandrum	34.53 ± 1.16 bcde	40.33 ± 1.70 gh	60.31 ± 0.35 fg
B.persicum	35.06 ± 0.10 bcde	56.55 ± 2.18 b	89.66 ± 1.26 b
A. precatorius	34.33 ± 2.08 cdef	41.19 ± 1.11 gh	67.62 ± 1.37 e
T. officinale	38.71 ± 1.61 a	61.44 ± 1.17 a	93.67 ± 2.00 a
A.viridis	32.24 ± 1.00 cefg	45.32 ± 1.04 f	62.46 ± 1.92 f
D. deltoidea	31.47 ± 0.97 cg	42.22 ± 1.19 g	62.37 ± 1.17 fg
M.neglecta	36.67 ± 1.53 ab	55.73 ± 0.35 bc	75.28 ± 0.51 c
R. pseudoacacia	36.43 ± 0.51 abc	48.66 ± 0.57 e	70.39 ± 0.52 d

Each value represents the mean of three determinations (n=3). Different letters within each column are significantly different at ($p < 0.05$) by Duncan's test.

Table 3. Green house method for determination of nematicidal activity of seeds extracts of *P. hexandrum*, *B. persicum*, *A. precatorius*, *T. officinale*, *A. virdis*, *D. deltoidea*, *M. neglecta* and *R. pseudoacacia* against

M. incognita.

Treatment	Number of galls	Number of egg masses	Disease Intensity	
			RKI	EMI
Inoculated control (C)	32 a	21 a	4	3
P. hexandrum	15 b	12 b	3	3
B. persicum	2 h	2 h	1	1
A. precatorius	7 de	5 ef	2	2
T. officinale	1 h	0 i	1	0
A. viridis	4 fg	8 d	2	2
D. deltoidea	12 c	10 c	3	2
M. neglecta	5 ef	4 fg	2	2
R. pseudoacacia	8 d	6 e	2	2

RKI: root-knot index; EMI: egg mass index.

Each value represents the mean of three determinations (n=3). Different letters within each column are significantly different at ($p < 0.05$) by Duncan's test.

Each value represents the mean of three determinations (n=3) ± standard deviation. Different letters within each column are significantly different by the ANOVA ($p < 0.05$).