Discover Sustainability

Discover

Brief Communication

Evaluation of various seed extracts for their nematicidal efficacies against root nematode, *Meloidogyne incognita*

Zubair Rehman Nengroo[1] · Zeshan Umer Shah[2] · Adil Shafi Ganie[3] · Mohammad Danish[4]

Received: 9 January 2021 / Accepted: 25 February 2021

Abstract
The current study was assumed to identify the novel nematicidal activity of chloroform and methanol (50:50, *v/v*) 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. using the root-knot nematode *Meloidogyne incognita* in in vitro and greenhouse experiments. At 72 h exposure, the extracts were found to be highly nematostatic, where nematodes were completely paralyzed at 12 and 48 h of exposure. However, dominant mortality was observed by *T. officinale* 93.67% and *B. persicum* 89.66% seed extracts at 72 h. In greenhouse method, extracts of *T. officinale* and *B. persicum* extracts were found to be most potent in reducing number of galls (1.76 and 2.37) and number of egg masses (0.06 and 2.18) respectively as compared to inoculated control. The root knot index of all extracts 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 upon further isolation and purification of bioactive substance responsible for nematicidal activity.

Keywords Soxhlet extraction · Yield percentage · In vitro nematicidal activity · Greenhouse nematicidal activity

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 react with other disease causing organisms which lead to development of more complex diseases and loss of resistance to plants against pests and nematodes [2]. In last decades, the synthetic nematicides were the only available drugs used against these parasites, because of their indiscriminate use which leads to harmful side effects and consequently problems to the environment [3, 4]. Furthermore, the development of resistance of microbes to these drugs is a major setback to their continued use in livestock and humans [5, 6]. The development of new nematicides is very difficult task, because the living place of most nematodes is soil and plant roots. The target of any chemical substance is very away from its application. Moreover, cuticle and surrounding surface

✉ Zubair Rehman Nengroo, zubairchem33@gmail.com | [1]Section of Oils and Fats, Department of Chemistry, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India. [2]Department of Zoology, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India. [3]Environmental Chemistry Section, Department of Chemistry Aligarh, Muslim University, Aligarh 202002, India. [4]Department of Botany, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India.

of nematode is impermeable to chemical substances, leads to toxicity, environmental pollution and because of their high cost an average farmer cannot afford them easily [7, 8]. Hence, keep these things in consideration, the latest development is search for novel antimicrobial, antioxidant and nematicidal agents from plant sources which are less expensive, eco-friendly, biodegradable, safer and natural [9–13]. The report of WHO medicines obtained from plant sources serve about 80% of world population [14]. Hence, it is important to screen medicinally important plants which are used in ethanopharmacolgy or ethnomedicine. These plants may provide new, novel, inexpensive and above all locally available drugs especially for underdeveloped countries.

Hence, to address the above mentioned issues chloroform and methanol (50:50, *v/v*) seed extracts 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 were analyzed for nematicidal activity against *Meloidogyne incognita*.

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 h. 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* juvenile (J_2) mortality under different exposure time (24, 48 and 72 h). Briefly, 5 mL of nematode suspension containing 100 freshly second stage juveniles (J_2) of *M. incognita*, were put in vial with a final concentration of 100 ppm. These nematode vials were taken in eight petri dishes at 25 ^{0}C. The seed extracts at a concentration of 1000 ppm of the test extract were prepared by dissolving different extracts in 0.5 mL dimethyl sulphoxide (DMSO), and to it add distill water to make final volume 5 mL. The extracts were then transferred in each eight sterilized petri dishes so as to cover whole surface. The Petri dishes were then kept for exposure at 24, 48 and 72 h. The volume of 0.5 mL DMSO with 9.5 mL distilled water served as control. 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 h. 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 [15]. 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 [16].

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 which could play an important role in relation to their inexpensive cost nature in drug formulation.

3.2 Nematicidal activity

The worked plant extracts from Kashmir were used in vitro for the first time against plant parasitic nematode. 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. Data presented in the (Table 2) showed that all the seed extracts showed suppressive activity against *M. incognita* which increases in increasing exposure time. The highest mortality rate was observed at 72 h. The extract of *T. officinale* gave up to 93.67% immobilization activity when exposed for 72 h while (J_2) were innobilized up to 61.44% when exposed to 48 h in same extract. Laquale et al. [17] 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*. The extract of *B. persicum* showed inhibition of 89.66%

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 *Mincognita*.

Seed extracts	% Nematode mortality		
	24 h	48 h	72 h
P. hexandrum	$34.53 \pm 1.16^{b,c,d,e}$	$40.33 \pm 1.70^{g,h}$	60.31 ± 0.35^{fg}
B.persicum	$35.06 \pm 0.10^{b,c,d,e}$	56.55 ± 2.18^{b}	89.66 ± 1.26^{b}
A. precatorius	$34.33 \pm 2.08^{c,d,e,f}$	$41.19 \pm 1.11^{g,h}$	67.62 ± 1.37^{e}
T. officinale	38.71 ± 1.61^{a}	61.44 ± 1.17^{a}	93.67 ± 2.00^{a}
A.viridis	$32.24 \pm 1.00^{c,e,f,g}$	45.32 ± 1.04^{f}	62.46 ± 1.92^{f}
D. deltoidea	$31.47 \pm 0.97^{c,g}$	42.22 ± 1.19^{g}	$62.37 \pm 1.17^{f,g}$
M.neglecta	$36.67 \pm 1.53^{a,b}$	$55.73 \pm 0.35^{b,c}$	75.28 ± 0.51^{c}
R. pseudoacacia	$36.43 \pm 0.51^{a,b,c}$	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

juveniles at 72 h while the same extract showed 56.55% inhibition of *M. incognita* at 48 h exposure. Khan et al. [18] has performed the same study and they found that various plants from from apiaceae family such as *Coccinia grandis*, *Commelina benghalensis*, *Leucas cephalotes*, *Phyllanthus amarusand*, *Trianthema portulacastrum* displayed good nematicidal activity against *M. incognita* which validates the good nematicidal activity of *B. persicum* extracts. 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 h respectively. The results were confirmed by seed extracts of *Ricinus communis* and *Peganum harmala* used by Hasan et al. [19], Curto et al. [20] and Rich et al. [21] against Meloidogyne juveniles, and found them effective. Significant nematicidal activity of water extract of the Italian *M. azedarach* fruit pulp (IPWE) (EC50/48 h = 955 µg/mL) was obtained by Aoudia et al. [22] and among its active ingredient components were p-coumaric acid and p-hydroxybenzoic acid (EC50/48 h = 840 and 871 µg/mL, respectively).

In case of greenhouse method as presented in (Table 3), it has been observed that all seed extracts exhibit excellent nematicidal activity. Among the worked extracts, the *T. officinale* and *B. persicum* extracts were found to be more potent in reducing number of galls (1.76 and 2.37) and number of egg masses (0.06 and 2.18) respectively in comparison to all other seed extracts at ($p < 0.05$). The observed results are in complete agreement with the previous studies on roots and leaves [17]. The activity of other seed extracts showed good reduction of number of galls and egg masses as *M. neglecta* (5.04 and 4.06), *A. virdis* (4.25 and 8.08), *A. precatorius* (7.27 and 5.08) and *R. pseudoacacia* (8.09 and 6.17) respectively. However, the lowest reduction was noticed in case of *P. hexandrum* and *D. deltoidea* as compared to control (Table 3).

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)	32.21 ± 0.23^{a}	21.27 ± 0.22^{a}	4.12 ± 0.43^{a}	3.14 ± 0.22^{a}
P. hexandrum	15.26 ± 0.26^{b}	12.24 ± 0.27^{b}	3.23 ± 0.28^{b}	3.03 ± 0.37^{a}
B. persicum	2.37 ± 0.17^{h}	2.18 ± 0.37^{h}	$1.09 \pm 0.27^{h,i}$	1.14 ± 0.28^{g}
A. precatorius	$7.27 \pm 0.14^{d,e}$	$5.08 \pm 0.51^{e,f}$	2.35 ± 1.73^{d}	$2.17 \pm 0.08^{b,c,d,e}$
T. officinale	1.76 ± 0.33^{h}	0.06 ± 0.11^{i}	1.12 ± 0.09^{h}	0.57 ± 0.25^{h}
A. viridis	$4.25 \pm 0.09^{f,g}$	8.08 ± 0.23^{d}	$2.16 \pm 0.38^{d,e,f}$	$2.09 \pm 0.26^{b,c,d,e,f}$
D. deltoidea	12.04 ± 0.18^{c}	10.11 ± 0.25^{c}	$3.21 \pm 0.25^{b,c}$	2.43 ± 0.36^{b}
M. neglecta	$5.04 \pm 0.32^{e,f}$	$4.54 \pm 0.17^{f,g}$	$2.34 \pm 0.52^{d,e}$	$2.21 \pm 0.24^{b,c,d}$
R. pseudoacacia	8.09 ± 0.28^{d}	6.17 ± 0.19^{e}	$2.03 \pm 0.37^{d,e,f,g}$	$2.28 \pm 0.37^{b,c}$

RKI root-knot index, *EMI* egg mass index

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

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 particularly 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.

Acknowledgements 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.

Authors' contributions ZRN: Conceptualization, methodology, software, writing and supervision; ZUS: software validation and in vitro nematicidal activity; ASG: Material collection and identification; MD: Green house nematicidal activity. All authors read and approved the final manuscript.

Declarations

Competing interests The authors declare that they have no conflict of interest.

References

1. James WC. Estimated losses of crops from plant pathogens. In: Pimentel D, editor. Handbook of pest management in agriculture. Boca Raton, FL: CRC Press; 1991. p. 579.
2. Mukhtar T, Kayani MZ, Hussain MA. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against Meloidogyne incognita. Ind Crops Prod. 2013;42:447–53.
3. Qin S, Gan J, Liu W, Becker JO. Degradation and adsorption of fosthiazate in soil. J Agri Food Chem. 2004;52:6239–42.
4. Karpouzas DG, Karanasios E, Menkissoglu-Spiroudi U. Enhanced microbial degradation of cadusafos in soils from potato monoculture: demonstration and characterization. Chemospher. 2004;56:549–59.
5. Geerts S, Gryseels B. Drug resistance in human helminths: current situation and lessons from livestock. Clin Microbio Rev. 2000;13:207–22.
6. McGaw LJ, Jäger AK, Van Staden J. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J Ethnopharmacol. 2000;72:247–63.
7. Anonymous. *Protection of stratospheric ozone*: incorporation of clean Air Act Amendments for reduction in class 1, group V1 controled substances. Fed Reg. 2000;65:795–804.
8. Thomas WB. Methyl bromide: effective pest management tool and environmental threat. J Nematol. 1996;28:586–90.
9. Nengroo ZR, Rauf A. Fatty acid composition and antioxidant activities of five medicinal plants from Kashmir. Ind Crops Prod. 2019;140:111596.
10. Nengroo ZR, Rauf A, Danish M, Dar MS. Evaluation of fatty acid composition and antimicrobial activity of eight medicinal plants from Kashmir. Orient J Chem. 2020;36:44–53.
11. Nengroo ZR, Rauf A. *Inula racemosa* and *Digitalis purpurea* from Kashmir: fatty acid composition, antioxidant, antibacterial activities, and functional group evaluation. Flavour Fragr J. 2020. https://doi.org/10.1002/ffj.3604.
12. Valle DL Jr, Andrade JI, Puzon JJM, Cabrera EC, Rivera WL. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian Pacific J Trop Biomed. 2015;5:532–40.
13. Grainge M, Ahmed S. Handbook of plants with pest-control properties. Hoboken: John Wiley & Sons Limited; 1988.
14. Geneva World Health Organization. 2000. World Health Organization general guidelines for methodologies on research and evaluation of traditional medicine. Geneva: World Health Organization; 2001. p. 1.
15. Holbrook CC, Knauft DA, Dickson DW. A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Dis. 1983;67:957–8.
16. Taylor AL, Sasser JN. Biology, identification and control of some root-knot nematodes (*Meloidogyne* spp.). Raleigh, NC: Department of Plant Pathology and Genetics, North Carolina State University and USAID; 1978. p. 111.
17. Laquale S, Avato P, Argentieri MP, Candido V, Perniola M, D'Addabbo T. Nematicidal potential of *Taraxacum officinale*. Env Sci Pol Res. 2018;25:30056–65.
18. Khan F, Asif M, Khan A, Tariq M, Ansari T, Shariq M, Siddiqui MA. Evaluation of the nematicidal potential of some botanicals against root-knot nematode, *Meloidogyne incognita* infected carrot: in vitro and greenhouse study. Curr Plant Bio. 2019;20:100115.
19. El-Hassan M, Zahra F, Mina IHL. Anti-nematode effect assessment of *Peganum harmala* based-products against *Meloidogyne javanica* on melon. J Bio Agri Healthcare. 2013;3:5–10.
20. Curto G, Dallavalle E, Santi R, Casadei N, D'Avino L, Lazzeri L. The potential of *Crotalaria juncea* L. as a summer green manure crop in comparison to Brassicaceae catch crops for management of *Meloidogyne incognita* in the Mediterranean area. Eur J plant Pathol. 2015;142:829–41.
21. Rich JR, Rahi GS, Opperman CH, Davis EL. Influence of the castor bean (*Ricinus communis*) lectin (ricin) on motility of *Meloidogyne incognita*. Nematropica. 1989;19:99–103.

22. Aoudia H, Ntalli N, Aissani N, Yahiaoui-Zaidi R, Caboni P. Nematotoxic phenolic compounds from *Melia azedarach* against *Meloidogyne incognita*. J Agri Food Chem. 2012;60:11675–80.