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Evaluation of the ethanol liquid obtained from the important medicinal plants against *Meloidogyne incognita* infecting *Vicia faba L*

L Joymati Devi[1*], N Sanjoy Singh[2], M Suresh Singh[3], O Dwijamani Singh[3], O K Monoroma[4]
[1] Department of Zoology, Nematology Laboratory, DM College of Science, Imphal, Manipur, India
[2] Department of Botany, GP Women's College, Imphal, Manipur, India
[3] Department of Zoology, G.P Women's College, Imphal, Manipur, India
[4] Department of Zoology, Manipur College, Imphal, Manipur, India

Abstract
The effects of ethanol liquid extracts of *Allium hookerrii* (local name Maroinapakpi), *Brucea javanica* (local name heining*), Clerodondrum indicum (*local name Kuthap), *Citrus latipes (*local name Heiribop), and *Elsholtizia Communis* (local name Lomba) against infection caused by the *Meloidogyne incognita* in *Vicia faba* were determined. The seeds were soaked in 100 ppm stock solutions of the ethanol extracts of the above-mentioned medicinal plants for 24 h, dried in sunlight, and sowed in sterilized soil. After proper inoculation of juveniles of the *M. incognita,* the plants were allowed to grow. After 75 days of inoculation, the experimental plants were uprooted and observed. It was found that plants treated with the ethanol liquid from *Brucea javanica* showed much-improved growth rates and reduction in disease incidence when compared with the other treated plants. The ethanol extract of *Allium hookerrii* was found to be the least effective when compared with the extracts of other plants.

Keywords: infestation, ethanol extract, medicinal plants, control, *Meloidogyne incognita*, seed treatment

Introduction
Root-knot nematodes are a crucial cosmopolitan group of pests that are found in different crops throughout the country. The first incident of root-knot nematode injury to tea plants was reported by Barber (1901) [1]. The infestation sites for this pest occur in the root system, where it spends most of its life. The objective of nematode control is to improve plant growth and yield. The use of essential oils obtained from locally grown plants is an effective method for nematode control, considering the prohibitive cost of nematicides (Joymati, 2010) [8]. The state of Manipur is a hub of different varieties of medicinal plants (Sinha, 1996), which have been explored for their various functions by different researchers; however, studies on their role as eco-friendly nematicides are still lacking. To address this knowledge gap, the present investigation was undertaken at the P. G. Department of Zoology, D.M. College of Science, Imphal, from 3/10/2018 up to 20/5/2019 to evaluate the effects of ethanol extracts of different medicinal plants against infection caused by *M. incognita* in Vicia faba L.

Material and Methods
Plant extract preparation: Healthy leaves of *Allium hookerrii*, *Brucea javanica*, *Clerodondrum indicum*, *Citrus latipes*, and *Elsholtizia Communis* were collected. The plant parts were washed with water and oven-dried at 58 ± 2^0 C for 48 h. The material was powdered using a clean grinder. For oil extraction, 20 g (dry weight) of each plant material was taken, and the ethanol liquid was prepared with the help of a Clevenger apparatus (Carvalho *et al*., 1981). The solvent was distilled off, and the mixture was transferred into a separate beaker. The solvent was completely evaporated from the extract in an oven until it turned into a semi-solid material. A 1000 ppm stock solution of 1% Triton X100 was prepared in distilled water for use as an emulsifier. The seeds of the test plant were soaked in100 ppm stock solutions of the oil extracts of the above-mentioned plants for 24 h. The seeds were then spread and dried under sunlight before sowing and soaked in distilled water for treatment as control. Five seeds from each treatment were sown in a 15 cm clay pot containing 1 kg autoclaved soil. Two-week-old seedlings were thinned to one plant per pot. Three-week-old experimental plants were inoculated with freshly hatched 1000 second-stage juveniles (J_2) of *Meloidogyne incognita*. A control was also set up. All the sets were replicated five times. Seventy-five days after inoculation, the plants were carefully removed, their roots were washed with water, and plant growth parameters (shoot and root length and number of leaves) were recorded. Additionally, the number of galls, the root-knot index (0.5 scales), and final nematode population (both in soil and root) were counted using the sieving and decanting method described by Cobb (1918), followed by the modified Baermann's funnel technique. The above-mentioned experiment was undertaken on the lawn of the PG Department of Zoology, D.M. College of Science, Imphal, from October 3, 2018, to May 20, 2019.

Result and discussion

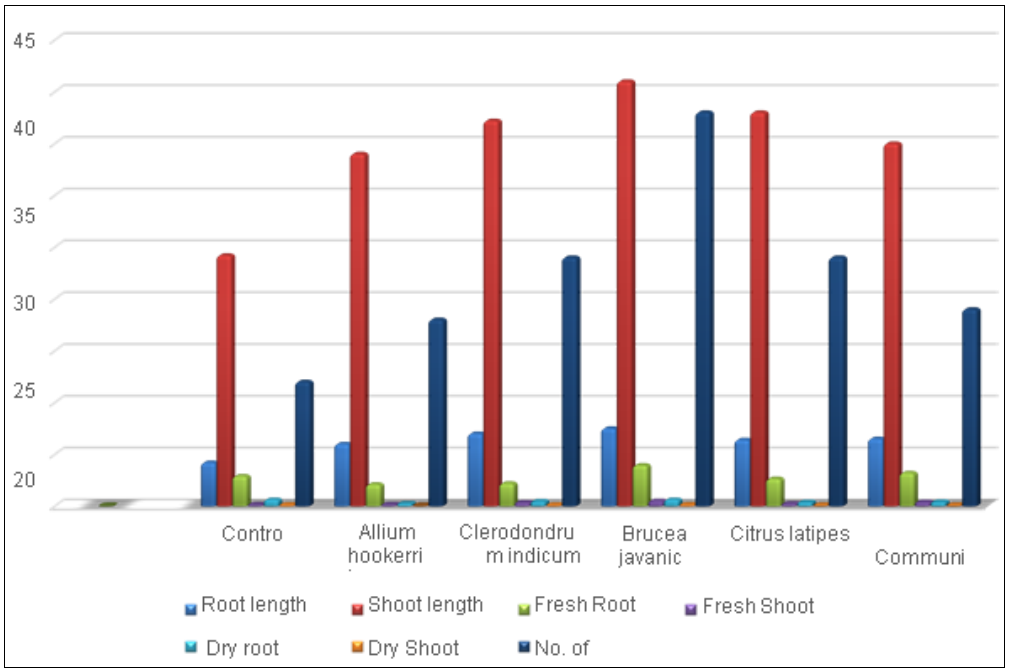
Tables 1 and 2 show the effects of ethanol liquid obtained from *Allium hookerrii*, *Brucea javanica*, *Clerodondrum indicum*, *Citrus latipes*, and *Elsholtizia Communis* against *Meloidogyne incognita* infection in *Vicia faba*. Treatment of seeds by soaking in different ethanol liquid extracts of the above-mentioned plants not only reduced the damage from root-knot infestation but also improved plant growth parameters. Treatment with the extract of *Brucea javanica* resulted in the maximum root length (7.5cm), shoot length (41cm) fresh shoot weight (0.5 g), fresh root weight (3.95 g), and a leaf number of 38. *Allium hookerrii* was the least effective among the five tested plants and resulted in a root length of 6 cm, shoot length of 34cm, fresh shoot weight of. 11 g, and fresh root weight of 2.1 g compared to those in the untreated plants, which showed 4.2 cm root length, 24.2 cm shoot length, 0.10 g fresh shoot weight and 2.9 g fresh root weight. Furthermore, in terms of disease incidence, *Brucea javanica* treatment resulted in a reduced infestation rate, with the presence of only 13 galls; additionally, the total population was reduced to 258, which was a 5 times reduction from that of the initial inoculum level. By contrast, in the untreated plants, 40 galls and 1.5 times higher nematode population (1299) than the initial population was observed. *Allium hookerrii*, although least effective, reduced nematode infestation rate to a greater extent than that observed in the untreated plants, which had 32 galls and a nematode population of 348 i.e., 3 times decrease than the initial population level. These observations suggested that the treated plants had low infestation rates and improved overall plant growth parameters.

According to Chopra *et al*. (1956) [4], all the plants investigated in this study contain alkaloids as their principal components and several compounds that act as inhibitory substances to nematodes, for example, the triterpene quassinoids in *Allium hookeri*i, a flavonoid in *Clerodendrum indicum***,** phytochemicals such as carotenoids in *Citrus latipes***,** phenylpropanoids in *Elsholtzia communis***,** and presence of sesquiterpenes with remarkable antitumor activity in *Brucea javanica*

The present investigation is in adjustable conformity of the results of Gokte *et al*. (1991), [6] who reported the effects of seven different oil extracts against the root-knot and cyst nematodes and described the oil extracts of Indian basil and sacred basil to be the most effective among other extracts.

The results of the present study are consistent with the findings of Papadoulou *et al*. (2016), Mendoza *et al*. (2008) [9], Nyaku *et al*. (2017) [10], Ozdemir and Gozel (2017) [13], and Taye and Sakhuza (2013). These results are also in agreement with those of Ononuju and Okoye (2003) [12] for *Tectona grandis*, *Mussendra globra*, *Xylosoma longifolia*, *Ocimum sanctum*, and *Melia azedarach*. Ononuju and Kalu (2004) [11] obtained results similar to those obtained with *Vernonia armygdaina* and *Rauwolfia vomitoria* in *Meloidogyne* spp. Furthermore, Joymati (2009) [7] reported the effects of the chloroform-methanol extracts of different medicinal plants on egg hatching and larval mortality of *Meloidogyne incognita*, which support the findings of the present study. The results of the present investigation support the work by Brito *et al*. (2020) [2] on the management of root-knot nematodes in tomatoes using agro-industrial wastes.

The significant increase in plant growth parameters and seed weight recorded in the treated plants, when compared with that in the untreated plants, could be attributed to the reduction of root-knot infestation by the essential oil extracts of the tested plant. From this investigation, it can be concluded that the essential oils from the plants tested in this study can be used for eco-friendly and effective nematode control practices. This work, as a native control program, provides a basis for future research on root-knot disease management.



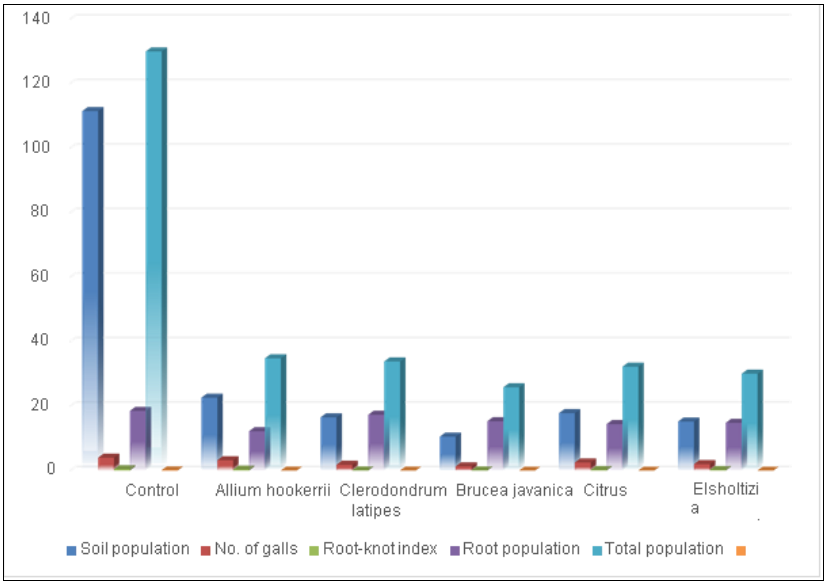
Graph 1: Effect of ethanol liquid extracts of medicinal plants showing plant growth parameter against *Meloidogyne incognita* infecting broad bean

Table 1: Effect of ethanol liquid extracts of medicinal plants showing plant growth parameter against *Meloidogyne incognita* infecting broad bean.

Plants	Root length(cm)	Shoot length (cm)	Fresh Root wt(g)	Fresh Shoot wt(g)	Dry root wt(g)	Dry Shoot wt(g)	No. of leaves
Control	4.2	24.2	2.90	0.10	0.61	0.06	12
Allium hookerrii	6.0	34.0	2.10	0.11	0.24	0.02	18
Clerodondrum indicum	7.0	37.2	2.20	0.35	0.47	0.04	24
Brucea javanica	7.5	41.0	3.95	0.5	0.61	0.09	38
Citrus latipes	6.4	38.0	2.65	0.21	0.39	0.03	24
Elsholtizia Communis	6.5	35.0	3.20	0.39	0.42	0.03	19

Table 2: Effect of ethanol extracts of some medicinal plants on nematodes multiplication against *Meloidogyne incognita* infecting broad bean.

Plants	Soil population	No. of galls	Root-knot index	Root population	Total population	Rf
Control	1114	40	4	185	1299	1.299
Allium hookerrii	226	32	3	122	348	0.348
Clerodondrum indicum	165	18	1	173	338	0.338
Brucea javanica	105	13	1	153	258	0.258
Citrus latipes	178	25	2	144	322	0.322
Elsholtizia Communis	152	20	2	148	300	0.3



Graph 2: Effect of ethanol extracts of some medicinal plants on nematodes multiplication against *Meloidogyne incognita* infecting broad bean.

Fig 1: Photograph showing the evaluation of ethanol liquid obtained from plants against root-knot nematode infecting Broad bean

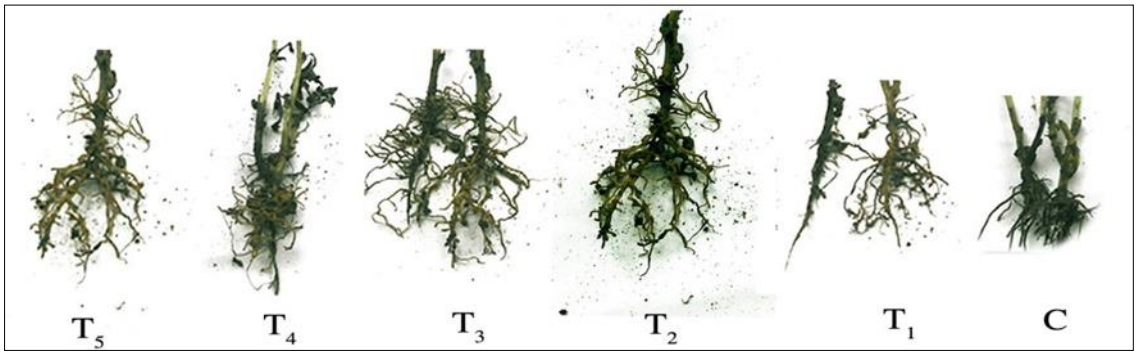


Fig 2: Photograph showing the infected root of broad bean with the root-knot nematode.

Conclusion

Thus, the significant increase in plants growth parameters recorded in treated plants as compared to the untreated one could be attributed to the beneficial effect of root-knot nematode infestation of the tested plants.

Acknowledgment

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