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In-vitro Nematicidal Activity of Different Solvent Extracts of *Solanum torvum* Fruit against *Meloidogyne incognita*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Root-knot nematodes are harmful plant parasites that significantly reduce agricultural productivity, affecting about 2,000 plant species and causing 5% of global crop losses. Due to concerns about the environmental impact of chemical nematicides, plant-based alternatives are gaining attention. This study, conducted in September-October 2023 at the University of Mysore, tested four solvent extracts (aqueous, petroleum ether, ethanol, and methanol) from *Solanum torvum* fruit at varying concentrations (10-100%) for their effects on egg hatchability and juvenile mortality of *Meloidogyne*

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incognita. Results showed that the methanolic extract was most effective, achieving 99% inhibition of egg hatching and 100% juvenile mortality at 100% concentration. This suggests that *Solanum torvum* extract could be an eco-friendly and economical method for managing root-knot nematodes. Further research is needed to evaluate its field efficacy and to identify the active compounds responsible for its nematicidal properties.

Keywords: Root-knot nematode; Meloidogyne incognita; Solanum torvum; egg hatching; juvenile mortality.

1. INTRODUCTION

Plant parasitic nematodes, particularly Root-Knot Nematodes (*Meloidogyne* species), pose a significant threat to global crop plants, causing an estimated annual loss of 125 billion dollars (Chitwood, 2003). These nematodes, obligate root parasites, are responsible for substantial agricultural productivity reduction, affecting over 3,000 plant species worldwide and resulting in estimated crop losses of nearly USD 100 billion annually (Abad et al., 2003; Dejene, 2014; Hunt and Handoo, 2009). Their impact is pervasive, affecting most cultivated crop plants (Sasser and Carter, 1985), leading to root dysfunction, reduced root volume, and compromised nutrient and water uptake efficiency (Noling, 2002). In response to the environmental and health hazards associated with chemical nematicides, there is a growing interest in botanical alternatives. However, in India, efforts to utilize botanicals for Root-Knot Nematode control on crops are limited. Botanical nematicides are seen as safer alternatives with biodegradability, selective toxicity to pests, and minimal impact on non-target organisms. This study focuses on *Solanum torvum* Swartz, commonly known as Turkey berry, as a potential botanical nematicide. Distributed widely in Asia and Tropical America, *S. torvum* has been traditionally used for medicinal purposes. Rich in alkaloids, flavonoids, saponins, tannins, and glycosides, its extracts have shown antimicrobial properties (Fui, 2012; Biney *et al.*, 2019). The objective of this research is to evaluate the nematicidal activity of various solvent extracts (Aqueous, Methanol, Ethanol, and Petroleum ether) of *S. torvum* fruit against root-knot nematodes under in-vitro conditions.

2. MATERIALS AND METHODS

Collection of Plant material: The experiments were carried out during September-October 2023 at the Department of Studies in Botany, Manasagangotri, University of Mysore, Mysuru, Karnataka – 570006. Fruits of *Solanum torvun* were collected during the field survey around the Mysore district of Karnataka state. Collected samples were kept in polythene bags, tagged, and brought to the laboratory, then the fruits were cut and shade dried for up to 10 days inside the laboratory.

Preparation of *Solanum torvun* fruit extracts: Shade-dried fruits of S. *torvum* are finely ground to powder with the help of a grinder and passed through a sieve. Weighed 10gm of powder in 100ml of methanol, ethanol, petroleum ether, and distilled water in conical flasks and kept in an incubation shaker for two days to prepare crude extracts treated as stock. From this stock, required concentrations *viz*., 10%, 25%, 50%,75%, and 100% were prepared.

Preparation of Nematode inoculum: The culture of *M*. *incognita* was maintained on tomato plants (*Solanum lycopersicum* L.). The infected plants were uprooted, roots were thoroughly washed in a running tap, and then cut into 2-3 cm in length. The roots were then placed in a jar with about 300 ml of 1% Sodium hypochlorite (NaOCl) and agitated vigorously for about 3-5 minutes. The agitation in NaOCl solution dissolved the egg masses and eggs were released in the solution. The egg mass of *M. incognita* was gently picked using forceps under a stereo zoom-microscope. Egg masses were incubated for 48 hours by the modified Baermann funnel method to obtain infective second-stage juveniles. Population density of J_2 was calculated from 5 replications of one ml aliquots of inoculum suspension.

***In-vitro* analysis for Juvenile Mortality:** Freshly hatched 150 second-stage juveniles (48 hours old) were taken in 2.5 cm diameter petri plates containing 5 ml of each treatment solution. J2s kept in distilled water treated as control. Plates were covered with a lid and incubated at room temperature (25±2 0 C) during the experiment period. Each treatment was replicated three times. Data on mortality was recorded after 12, 24, 48, and 72 hours after incubation. Mortality of

the J2 was assessed by observing the mobility of the J_2 under a stereo zoom-microscope at 60 X magnification and expressed as the percentage of the total population. The mobile and non-mobile J2 were prodded using a fishing needle to check for mobile responses (Das et al., 2011). The percentage of mortality was calculated using the formula;

$$\%\ Juvenile\ mortality = \frac{Number\ of\ dead\ juveniles}{Total\ number\ of\ juveniles\ taken} \times 100$$

In-vitro **analysis for Egg Hatching:** A single egg mass of M. incognita was kept in 2.5 cm diameter petri plates containing 5 ml solution of each treatment. Egg mass kept in distilled water was treated as a control. Each treatment was replicated three times. Petri plates were kept at room temperature (25±20 ^{0}C). The number of hatched J_2 was counted under a microscope and the solution of each treatment was replaced every counting. Data was recorded at every 24-hour interval and continued up to 72 hours. Percent egg hatching inhibition over control was calculated using the formula (Mahesha et al., 2017);

$$\%\ Egg\ hatching = \frac{Number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ taken} \times 100$$

3. RESULTS AND DISCUSSION

In-vitro **analysis for Egg Hatching:** Four solvent extracts *viz*., Aqueous, Petroleum ether, Ethanol, and Methanol of *S. torvum* fruit were selected at different concentrations were tested for egg hatchability of root-knot nematode M. *incognita* (Table 1). The different concentrations (10, 25, 50, 75, and 100%) were tested among the four solvents. The results showed a decrease in egg hatchability as increasing concentrations of the extracts. The increase in exposure period and increasing concentration also decreases the egg hatchability. The methanolic extract is the most toxic when compared to other solvent extracts. The egg hatchability has been found to decrease with increasing concentration from 10 to 100% after 72 hours of exposure time. The inhibition of egg hatching was observed in the decreasing order of Aqueous > Ethanol > Petroleum ether. Significant results were found in Methanolic extract at 75 and 100% concentration rates.

Table 1. Percentage of egg hatching in different solvent extracts of *Solanum torvum* fruit

Treatment	Concentration	Percentage of egg hatched at different exposure time		
		24 hrs	48 hrs	72 hrs
Control	**-**	28.66	52.33	97.33
Aqueous extract	**10%**	23.33	44.66	68
	25%	21	38.66	53.66
	50%	17.66	31.33	50.33
	75%	17	25.66	37.66
	100%	7.66	15	21.33
Petroleum ether extract	**10%**	12.33	28.66	58.33
	25%	12	18.33	28.33
	50%	8.66	14.33	17.66
	75%	7.33	12.33	12.33
	100%	3.66	6.33	8
Ethanol extract	**10%**	22.66	39.66	57.66
	25%	17.66	30	45.33
	50%	14.33	24.33	37.33
	75%	10.66	16	25.33
	100%	6	8.33	10.33
Methanol extract	**10%**	6.33	10.33	14
	25%	4	6.66	7.66
	50%	2.66	4.66	6.33
	75%	0.66	1	1
	100%	0.33	1	1

Table 2. Percentage of juvenile mortality in different solvent extracts of *Solanum torvum* fruit

Treatment	Concentration	Percentage of juvenile mortality at different exposure time		
		24 hrs	48 hrs	72 hrs
Control	**-**	00	0.33	2.33
Aqueous extract	**10%**	2.66	5.66	8
	25%	9	15	21
	50%	16.66	28.33	41.66
	75%	24.33	39.66	53
	100%	31.33	57.66	78
Petroleum ether extract	**10%**	4	8	14
	25%	9.33	15.33	21.33
	50%	13.33	21.33	30.66
	75%	17.66	29.33	45.66
	100%	29.33	53.66	86
Ethanol extract	**10%**	7.33	15.33	20.66
	25%	11	19.33	29
	50%	16.33	26.66	37
	75%	19.66	28	42.66
	100%	27.66	48.66	88.33
Methanol extract	**10%**	12	21.33	37.66
	25%	25.33	38.33	54.66
	50%	44.33	64	71.66
	75%	81.66	92.33	98
	100%	98	100	100

Fig. 1. *Solanum torvum* plant

Similarly, petroleum ether extracts and ethanolic extracts were found to be more effective than aqueous extracts (Table 1). A similar result was reported by Irdani *et al.* (2023) and Azhagumurugan and Rajan (2015) in different plant leaf extracts. They also reported that decreased egg hatchability and increased juvenile mortality were found in higher concentrations of plant extracts. 99% of egg hatching was inhibited after 48 hours of exposure time was found in methanolic extract followed by petroleum ether extract (92%) and ethanolic extract (89.66%) at their 100% concentration rate after 72 hours of exposure time respectively. Aqueous extract at 50, 75, and 100% concentration rates also showed inhibitory action of egg hatching and it resulted in 49.66, 63.33, and 79.66% inhibition of egg hatching respectively (Table 1 and Figs 3 to 5).

Fig. 2. Dried fruits of *Solanum torvum*

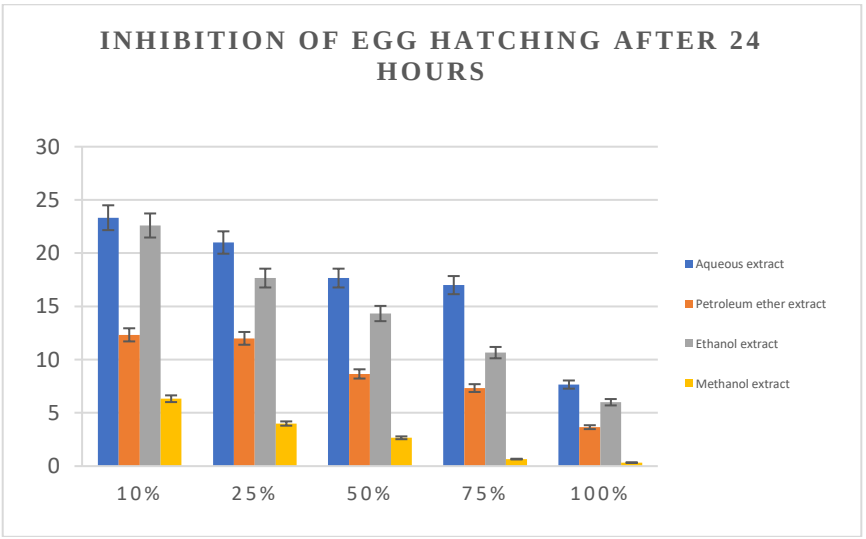

Fig. 3. Inhibition of egg hatching after 24 hours treated in different solvents

***In-vitro* analysis for Juvenile Mortality:** The second-stage juveniles were exposed for 24-, 48- and 72-hours exposure time in different concentrations (10,25,50,75 and 100%) and the juvenile mortality was found to increase with increasing concentrations of different solvent extracts of which aqueous (78%), petroleum ether (86%), ethanol (88.33%) and methanol (100%) were found effective after 72 hours of exposure period at their highest concentration rate of 100% (Table 2 and Figs 6 to 8). The increase in juvenile mortality was found associated with an increase in concentrations of the extracts was also reported by Irdani *et al.* (2023) and Azhagumurugan and Rajan (2015), where they found the same in leaf extracts of different plants. The characteristic shape of nematodes killed by extracts of the fruits of S. *torvum* was found with straight shapes. The highest percentage of inhibition of egg hatching and juvenile mortality (99% and 100% respectively) was obtained at a higher concentration of methanolic extract at a 100% concentration rate. Similarly, applying different solvent extracts at all concentrations in different time intervals significantly inhibited egg hatching and caused the juvenile mortality of *M. incognita* compared to the control (Tables 1 and 2). However, there were some variations among treatments in reducing egg hatching and juvenile mortality. Treatments applied at lower concentrations were less effective than higher concentrations in all tested treatments, a similar result was found by Terefe, (2015).

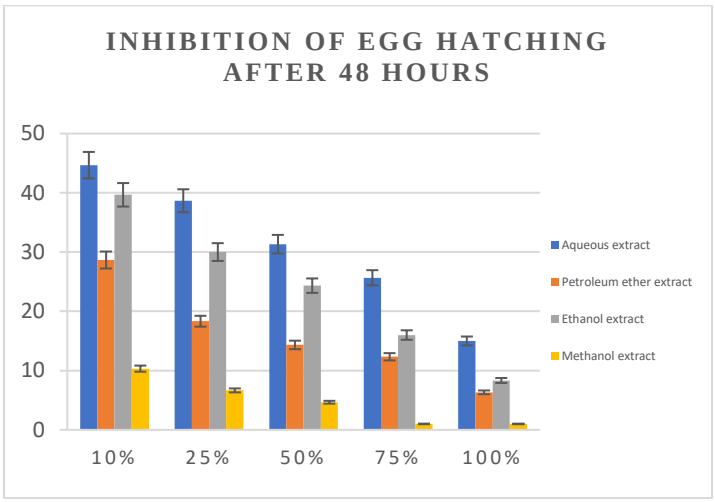

Fig. 4. Inhibition of egg hatching after 48 hours treated in different solvents

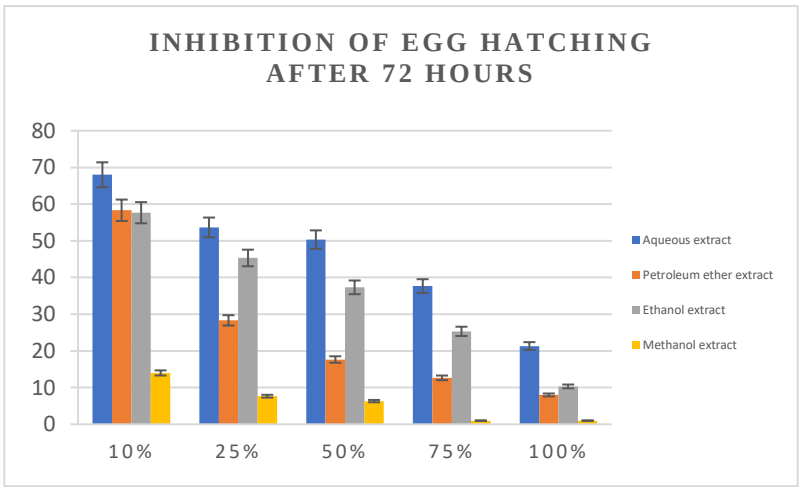

Fig. 5. Inhibition of egg hatching after 72 hours treated in different solvents

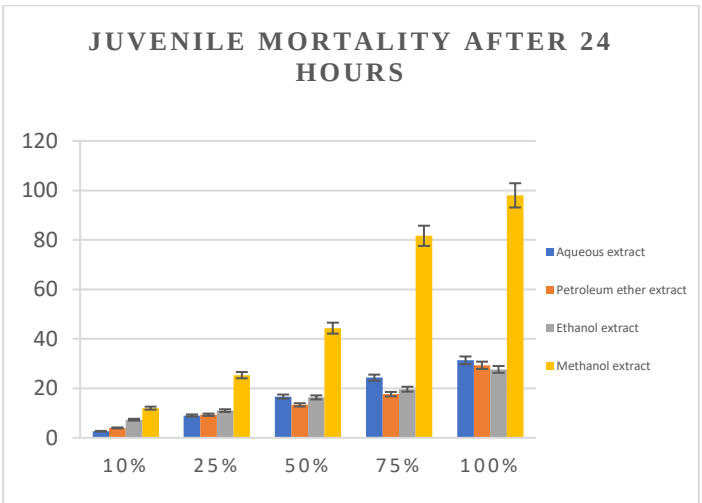

Fig. 6. Juvenile mortality after 24 hours treated in different solvents

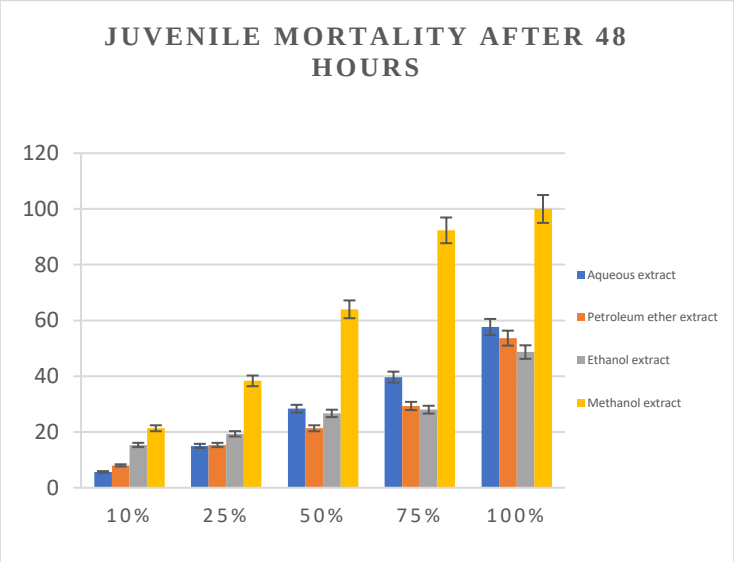

Fig. 7. Juvenile mortality after 48 hours treated in different solvents

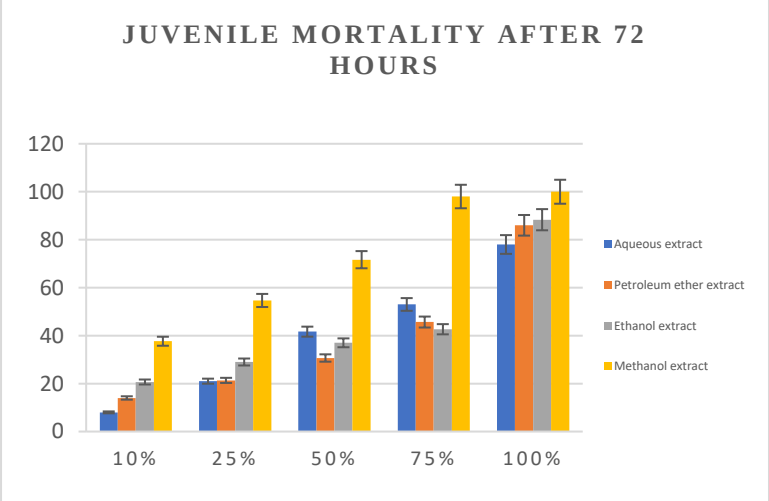

Fig. 8. Juvenile mortality after 72 hours treated in different solvents

4. CONCLUSION

The present study showed the fruit extracts of *Solanum torvum* in different solvents at different concentrations gave significant results of inhibition of egg hatching and juvenile mortality and may be useful for nematode management, which will be an environmentally and economically safe option and also recommended for the promotion of organic agriculture. However, further study on field efficacy trials and identification of the active chemical compounds of these extracts are needed.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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