Short communication

In-vitro Nematicidal Activity of Different Solvent Extracts of *Solanum torvum* Fruit against Root-Knot Nematode (*Meloidogyne incognita*)

ABSTRACT

Introduction: 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. **Methodology:** 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 incognita*.

Results: Results showed that the methanolic extract was most effective, achieving 99% inhibition of egg hatching and 100% juvenile mortality at 100% concentration.

Conclusion: 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.

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 around 2000 susceptible plant species and causing approximately 5% of global crop loss (Hussey and Jenssen, 2002). 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 (Chah *et al*., 2000; David *et al*., 1998). The objective of

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Comment [PR3]: Add Recent Data on crop loss by nematode

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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.

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 root-knot nematodes (*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. Then the eggs were passed 50 µm sieve after passing through a 150 µm sieve to trap root fragments. The eggs on a 50 µm sieve were gently washed to remove any of the excess bleach and collected in a beaker (Hussey and Barker, 1973). The eggs were then processed on extraction trays for the emergence of second-stage juveniles (Whitehead and Hemming, 1965). The freshly hatched juveniles were used for the mortality tests.

Comment [PR7]: How do you identify second-stage juveniles? Give description

***In-vitro* analysis for Juvenile Mortality:** 5ml of suspension of freshly hatched juveniles (approximately 100-150 juveniles) of M. *incognita* was diluted with 5ml of different concentrations of the fruit of S. *torvum* extract, covering it by aluminium foil and kept in BOD incubator at room temperature for 24, 48 and 72 hours. Each experiment was replicated thrice. Control was maintained by distilled water simultaneously. After the desired period of incubation, the mortal juveniles were counted. The percent mortality had been calculated from the average of three replication in each case and data were analyzed and converted to natural mortality. Percent of mortality was calculated using the formula;

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

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***In-vitro* analysis for Egg Hatching:** For egg hatching experiments the protocols of Saravanapriya *et al.* (2004) was followed. 5ml of each concentration of fruit extracts of S. *torvum* were taken into a sterile Petridis. Two uniform egg masses with an average of 200 eggs were kept on Petridis. Observation on the number of juveniles hatched out in each Petridis was made at every 24-hour

interval, up to 3 days, control was maintained by distilled water simultaneously and Each treatment was replicated thrice in a completely randomized design. Percent of Egg hatching was calculated using the formula:

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

RESULT 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.

Similarly, petroleum ether extracts and ethanolic extracts were found to be more effective than aqueous extracts (Table-1). A similar result was reported by 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 Graph 1 to 3).

***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 Graph- 4 to 6). The increase in juvenile mortality was found associated with an increase in concentrations of the extracts was also reported by 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.

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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 (Table 1 and 02). 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 Metasebia (2015).

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Figure - 1: *Solanum torvum* plant

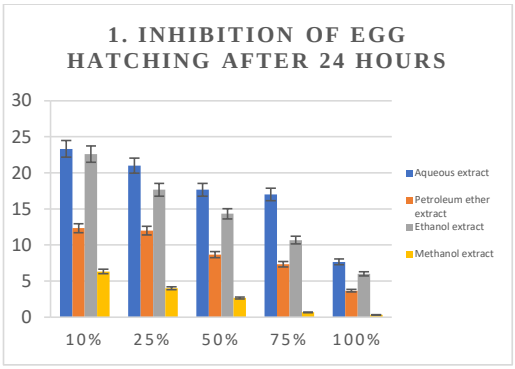
Figure –2: Dried fruits of *Solanum torvum*

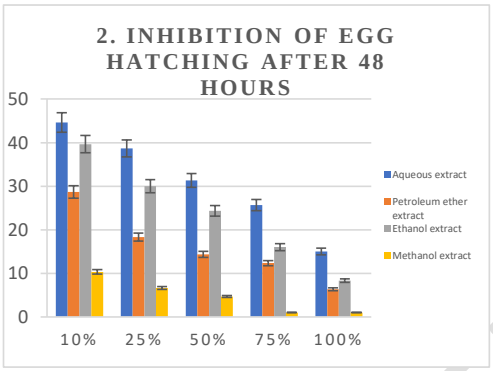
Treatment	Concentration	Percentage of Egg Hatched at different Exposure time		
		24 hrs	48 hrs	72 hrs
Control	**-**	28.66	52.33	97.33
Aqueous Extract	**10%**	23.33	44.66	68
	25%	21	38.66	53.66
	50%	17.66	31.33	50.33
	75%	17	25.66	37.66
	100%	7.66	15	21.33
Petroleum ether extract	**10%**	12.33	28.66	58.33
	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

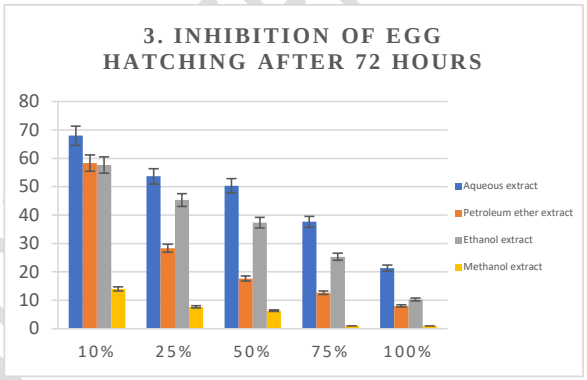
Table 1: Percentage of Egg hatching in different solvent extracts of *Solanum torvum* fruit.

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

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







Graph 1-3: Inhibition of egg hatching at different time intervals treated in different solvents.

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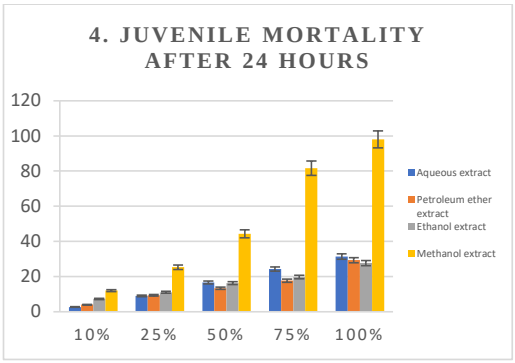

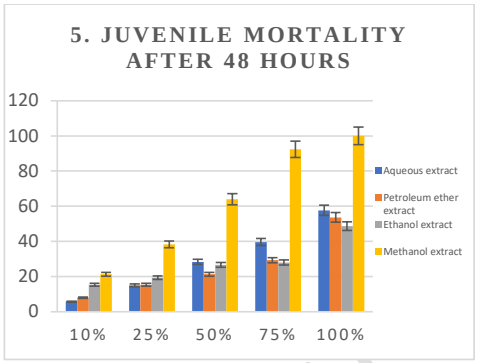

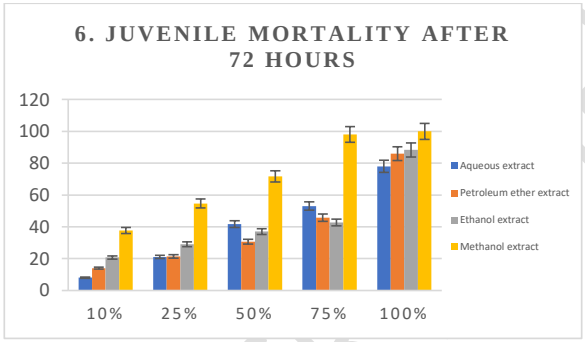

Graph 4-6: Juvenile mortality at different time intervals treated in different solvents.

CONCLUSION

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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.

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