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Eco-friendly root-knot nematode management: Potential of *Adhatoda vasica* leaf extract in protecting *Abelmoschus esculentus* from *Meloidogyne incognita*

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Abstract
The impact of nematode pathogenesis is reflected at tissue level with reduced sugar and an increased lipid and protein levels. The redox enzyme activities showed a specific increase of dehydrogenase activities in the infected host plant. Varied concentrations of acetone leaf extract treatment of *Adhatoda vasica* improved the biochemical profiles in nematode infected plants. The infected treated plants showed an increased activities of all the parameters studied. The impact of pathogenesis was reflected at tissue level with the depletion of sugar, and an elevated lipids and protein levels in the host plant. The increment of protein observed in inoculated and untreated plant tissue might be due to the action of proteolytic enzymes from plant and nematode source. The total lipid content was found to be reduced in the shoot system, whereas in the root system, it was found to be increased. The resultant of these catalytic and synthetic enzyme is quite evidenced in the increments of sugar, protein and lipid when leaf extract of *Adhatoda vasica* was introduced as nematicide to the infected host plant. Further works are needed to provide this fact.

Keywords: *Meloidogyne incognita*, *Adhatoda vasica*, Root-Knot Nematode, Total dehydrogenase, Total endogenous reductase

Introduction

Root-knot nematodes (Meloidogyne spp.) are among the most destructive plant-parasitic nematodes, causing significant yield losses in a wide variety of crops worldwide. These microscopic pests invade plant roots, inducing the formation of characteristic galls or "knots," which interfere with the plant's ability to uptake water and nutrients. This not only results in stunted growth but also predisposes plants to secondary infections, compounding the agricultural and economic impact. Plant-parasitic nematodes have the ability to reproduce on more than 2,000 plant species (Sasser and Freckman, 1987) and account for approximately 50% of total agricultural damage (Abbasi *et al*., 2008). Among these, the root knot nematode (Meloidogyne incognita) induces galls on the roots of various vegetable crops, pulses, certain fruit crops, tobacco, and ornamental plants, leading to significant economic losses. This nematode is known to infest over 3,000 distinct host plants. In Bihar, M. incognita is responsible for a 33 percent reduction in vegetable yields, while in New Delhi, the loss escalates to 60 percent. Root-knot nematodes threaten agricultural productivity, affecting around 2,000 plant species and causing an estimated 5% of global crop losses. With concerns over chemical nematicides, interest in plant-based alternatives is rising. This research at the University of Mysore (September-October 2023) tested four solvent extracts (aqueous, petroleum ether, ethanol, and methanol) from Solanum torvum fruit at concentrations of 10-100% on Meloidogyne incognita. The methanolic extract was the most effective, achieving 99% inhibition of egg hatching and 100% juvenile mortality at 100% concentration (Basavaraj *et. al.*, 2025) [3].

Organic additives derived from plants can offer a safe and environmentally friendly method for managing plant-parasitic nematodes. Numerous studies have evaluated various plant-based organic additives for their effectiveness in controlling nematodes (Muller and Gooch, 1982 [14]; Vaitheeswaran *et al*., 2007). Research has identified nematicidal properties in decomposing plant materials (Sayre *et al*., 1964; Patrick *et al*., 1965) [15]. The observed beneficial effects of these organic additives in combating infections are attributed to specific nematicidal compounds that are released during the decomposition of organic matter in the soil. Taylor (1936) and Steiner (1941) documented the resistance of *Tagetes* spp. to *Meloidogyne* sp. Additionally, it has been noted that leaf extracts from various plants can effectively reduce the population of root-knot nematodes. The present work was undertaken to study the nematicidal effect of leaf extract of *Adhatoda vasica* on a selected host plant.

Materials and Methods

Source of inoculum: Naturally infected *Acalypha indica* (Plate.1)

The pot culture study was carried out with totally 75 earthen pots which contains autoclaved sand soil mixture (4:1). Among 75 pots, 15 pots were maintained for control uninoculated and another 15 pots for infected untreated. The remaining 45 pots were meant for varied concentration of leaf extract treatments viz 1000, 1500 and 2000ppm. The acetone leaf extract was prepared using soxhlet apparatus. Among 45 pots, each 15 pots were maintained for different concentration treatment.

Varied concentration of acetone leaf extract of *Adhatoda vasica* were treated to the respective pots contained about 1.5 Kg autoclaved soil. The pots were kept 10 days for proper decomposition. After 10 days the seedlings of the host plant were raised in the treated and untreated autoclaved soil. (Plate .3)

The *Abelmoschus esculentus* was selected as host plant. The seeds were surface sterilized by treating them with 1% mercuric chloride solution, washed well and raised in the experimental plants. After 10 days the plants were inoculated with 2000 larvae around the root of the host plant, the plants were watered regularly (Plate.2). The acetone leaf extract treatment was given on alternative days. The pot study was maintained for a period of 30 days. After 30 days the plants were uprooted and the shoots and roots were then separately dried in a hot air oven at (50°C±5°C) and ground to 60m mesh powder. These dried samples were used for analyzing various biochemical profiles viz., Sugar (Seifter *et al.,* 1950) [18], Protein (Lowry *et al.*, 1951) [13], Lipid (Bragdon, 1951) [4]. Tripharyl tetrazolicine chloride was used to determine the enzyme activities (Kun and Abood, 1949) [12]. All the data were subjected to statistical analysis.

Results and Discussion

The impact of pathogenesis was reflected at tissue level with the depletion of sugar, and an elevated lipids and protein levels in the host plant (Table.1). The reduced level of sugar in the inoculated-untreated plant than in uninoculated control might be due to the possible consumption by the obligate endoparasite to complete its life cycle and also for its sustenance and part mobilization in the metabolic pool for the synthesis of protein as suggested by Kannan (1977) [6]. Similar report were observed by many workers (Das Gupta *et al.*, 1977) [5]. The increment of protein observed in inoculated and untreated plant tissue might be due to the action of proteolytic enzymes from plant and nematode source.

The total lipid content was found to be reduced in the shoot system, whereas in the root system, it was found to be increased. A general reduction in the activities of catabolic enzymes i.e. total dehydrogenase was discernible in the host plant under infection system (Table 2 and 3). The reduced activities of glucose and alcohol dehydrogenase both in root and shoot system might be due to the reduced availability of sugar. The redox enzyme activities show a specific increase of dehydrogenase activities (Table 2 and 3) in the infected plant under extract treatment. However, this seem to be offset by increased velocities of endogenous reductases involved in synthesis.

Similar research was carried out to assess the nematicidal efficacy of jojoba oil, potassium silicate, and Bio-Nematon (a commercial formulation of *Paecilomyces lilacinus*), both individually and in combination, against the root-knot nematode, *Meloidogyne incognita*, under in vitro and in vivo conditions. All treatment groups demonstrated a statistically significant increase ($P \leq 0.05$) in the mortality rate of *M. incognita* juveniles, which varied according to the specific treatment applied and the duration of exposure (Basavaraj *et al*,, 2025.) [3]

Recent research areas like phytochemicals, natural compounds in plants, can effectively control nematodes as deterrents or lethal agents, offering an environmentally sustainable alternative to chemical nematicides. Classes such as terpenes, phenols, alkaloids, and flavonoids have shown efficacy against nematodes (Krishnamoorthy *et al.*, 2014[a] [9]; 2014[b]; Krishnamoorthy and Kamatchi, 2022). They can be applied directly to soil, developed into biopesticides, or used in crop rotation to manage nematode populations. These compounds disrupt nematode molting, hinder root invasion, and induce stress, leading to their death (Puvaneswari *et al.*, 2021 [16]; Ajith *et al.*, 2024 [1]; Amulu *et al.*, 2023 [2]; Khairy 20025). The resultant of these catalytic and synthetic enzyme is quite evidenced in the increments of sugar, protein and lipid when leaf extract of *Adhatoda vasica* was introduced as nematicide to the infected host plant. Further works are needed to provide this fact.

Conclusion

Recent advancements in biological and chemical screening have opened new avenues for identifying natural and synthetic compounds with nematicidal properties. Plant-derived metabolites, microbial bioagents, and novel synthetic formulations have demonstrated promising potential in controlling *Meloidogyne* spp. These alternatives aim to minimize environmental risks while effectively managing nematode infestations.

Table 1: Influence of varied concentration of acetone leaf extract of *Adhatoda vasica* on biochemical profiles in the root and shoot system of *Abelmoschus esculentus* infected by *M. incognita*. (Each value mean ± SD represents an average performance of 3 observations. Readings are expressed in mg/g dry weight)

Treatment	Sugar		Protein		Lipid	
	Root	Shoot	Root	Shoot	Root	Shoot
CUI	7.34±0.08	7.04±0.02	11.73±0.06	11.54±0.16	1.26±0.12	2.00±0.08
IUT	6.02±0.26	5.77±0.31	15.44±0.16	14.45±0.18	2.80±0.32	1.13±0.21
IT_1	6.16±0.15	7.34±0.09	19.33±0.28	17.51±0.36	3.40±0.07	1.73±0.08
IT_2	7.51±0.20	7.54±0.28	20.13±0.06	19.38±0.17	4.16±0.30	1.91±0.05
IT_3	7.70±0.02	7.76±0.09	21.10±0.17	20.43±0.22	5.13±0.18	2.33±0.14
CD 5%	0.1214	0.0394	1.1950	0.2563	0.0501	0.2011
CD 1%	0.1891	0.0613	1.8606	0.3990	0.0779	0.3130

IUT- Infected untreated; IT_1 –Infected+Treatment1000ppm; IT_2 –Infected +Treatment 1500ppm; IT_3-Infected+Treatment 2000ppm

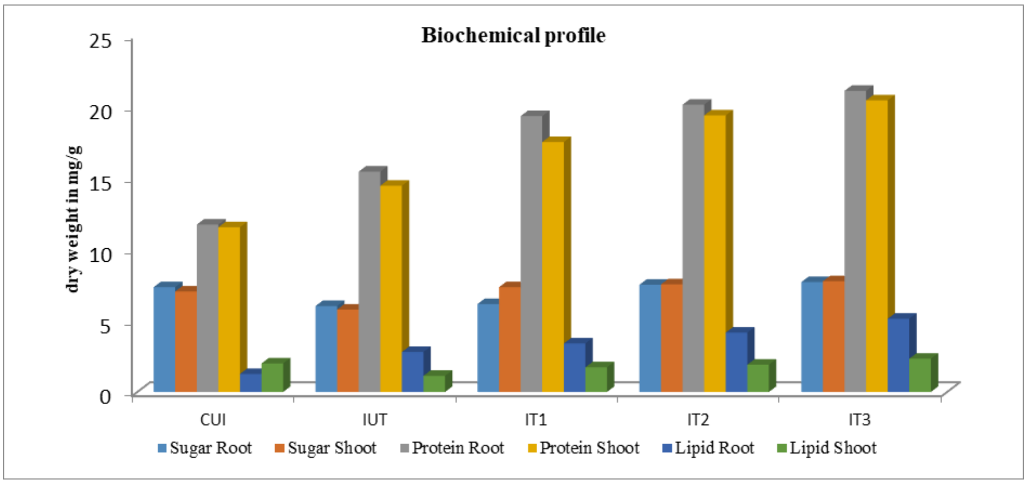

Fig 1: Shows varied concentrations of acetone leaf extract of *Adhatoda vasica* treatment on the biochemical profile in root and shoot system of host plant infected by *M. incognita*.

Table 2: Influence of varied concentration of acetone leaf extract of *Adhatoda vasica* treatment on the activities of various enzymes in the root system of *Abelmoschus esculentus* infected by *M. incognita*. (Each value mean ± SD represents an average performance of 3 observations. Readings are expressed in mg) TTC reduced /gm dry weight

Treatment	GL.D	A.D	F.D	S.D	G.D	A.O
CUI	1.54±.0.02	1.97±0.06	2.13±0.08	1.37±0.03	1.51±0.05	2.35±0.16
IUT	2.18±0.18	2.52±0.20	2.81±0.03	2.68±0.13	2.10±0.19	2.45±0.07
IT_1	2.28±0.05	3.81±0.06	3.86±0.08	3.30±0.09	2.72±0.12	3.27±0.23
IT_2	3.07±0.10	4.37±0.16	4.90±0.02	4.76±0.06	3.12±0.17	4.24±0.08
IT_3	4.05±0.02	5.04±0.06	5.22±0.18	5.39±0.10	4.06±0.16	5.41±0.05
CD 5%	0.0303	0.0226	0.0166	0.0241	0.0075	0.0347
CD 1%	0.0472	0.0353	0.0259	0.0376	0.0117	0.0541

IUT- Infected untreated; IT_1 –Infected+Treatment1000ppm; IT_2 –Infected +Treatment 1500ppm; IT $_3$-Infected+Treatment 2000ppm

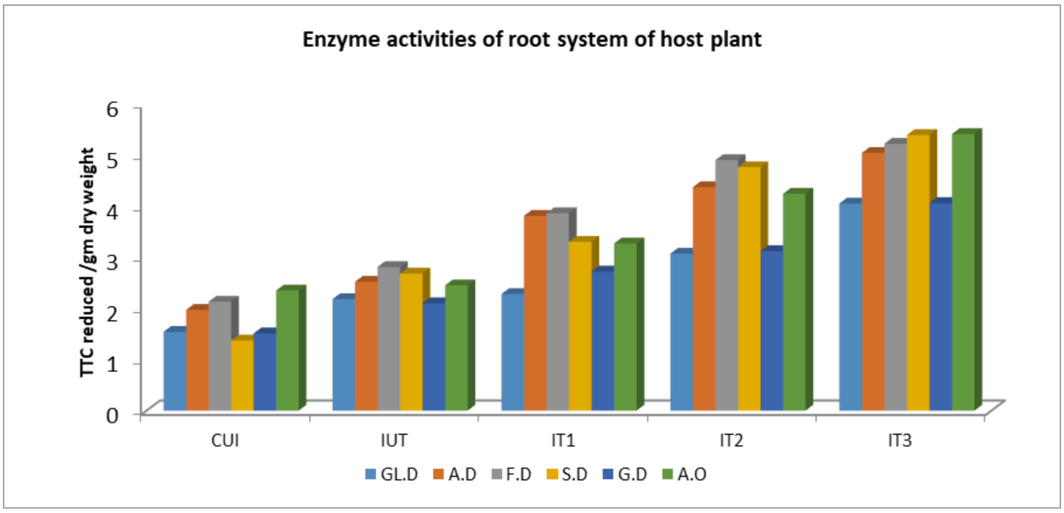

Fig 2: Shows varied concentrations of acetone leaf extract of *Adhatoda vasica* treatment on the activities of various enzymes in the root system host plant infected by *M. incognita*.

Table 3: Influence of varied concentration of acetone leaf extract of *Adhatoda vasica* treatment on the activities of various enzymes in the shoot system of *Abelmoschus esculentus* infected by *M. incognita*. (Each value mean ± SD represents an average performance of 3 observations. Readings are expressed in mg) TTC reduced /gm dry weight

Treatment	GL.D	A.D	F.D	S.D	G.D	A.O
CUI	1.43±0.07	2.69±0.02	2.42±0.08	2.72±0.10	2.03±0.19	1.84±0.26
IUT	2.64±0.04	2.93±0.51	3.30±0.02	3.16±0.10	2.56±0.14	2.24±0.17
IT_1	2.97±0.03	4.83±0.06	4.29±0.11	3.52±0.07	2.77±0.03	3.13±0.09
IT_2	4.26±0.08	5.99±0.14	6.79±0.08	4.45±0.05	4.07±0.12	3.99±0.17
IT_3	6.31±0.11	6.34±0.18	8.66±0.02	4.93±0.21	5.52±0.06	4.75±0.04
CD 5%	0.0303	0.0226	0.0166	0.0241	0.0075	0.0347
CD 1%	0.0472	0.0353	0.0259	0.0376	0.0117	0.0541

IUT- Infected untreated; IT_1 –Infected+Treatment1000ppm; IT_2 –Infected +Treatment 1500ppm; IT $_3$-Infected+Treatment 2000ppm

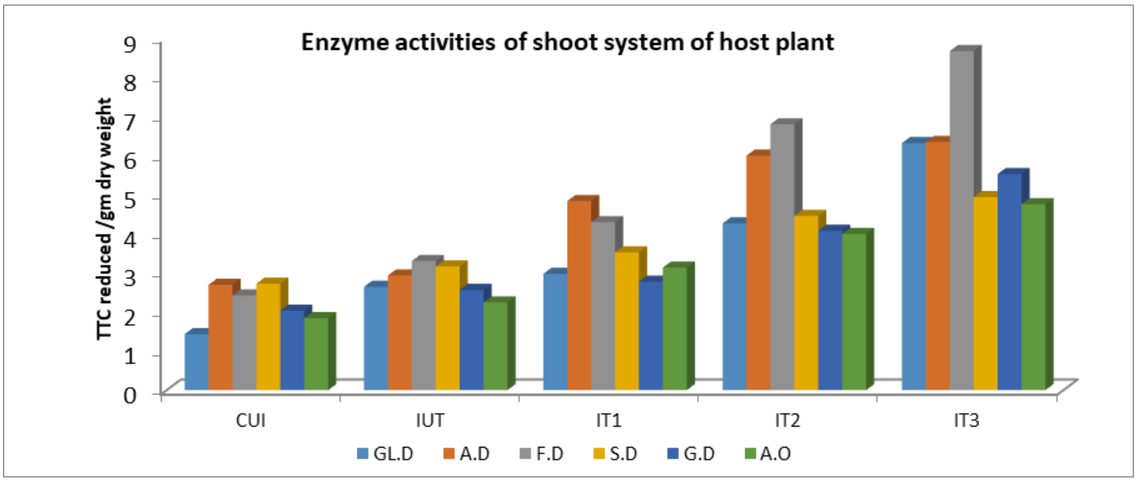

Fig 3: Shows varied concentrations of acetone leaf extract of *Adhatoda vasica* treatment on the activities of various enzymes in the shoot system host plant infected by *M. incognita*.

Plate 1: Infected *Acalypa indica* - a source of inoculum.

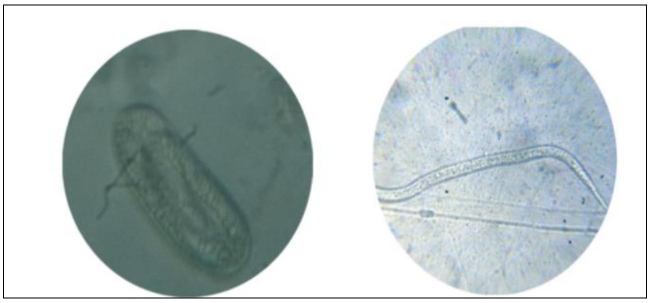

Plate 2: Microscopic view of J2 larvae and Adult of *Meloidogyne incognita*

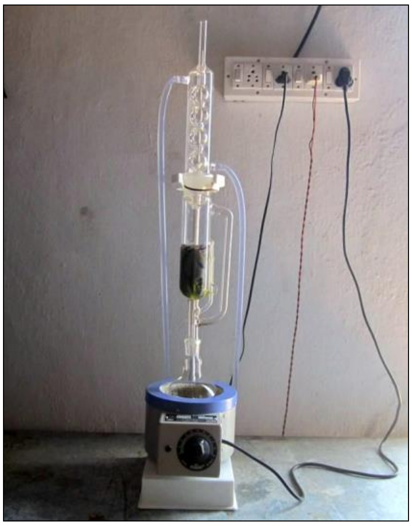

Plate 3: Extraction of *Adathoda vasica* using Soxhelt apparatus unit

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