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Study of Inhibition of Root-Knot Nematode (Meloidogyne incognita) in Tomato Plants by the Effect of Extracts from Certain Wild Plants

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Abstract
This study aims to determine the effectiveness of extracts from some wild plants commonly found in the desert regions of the south against the root-knot nematode Meloidogyne incognita, which infects tomato crops. The fruits of the colocynth plant, as well as the leaves and flowers of the sunflower plant and the leaves and fruits of the Calotropis procera plant were collected to prepare the plant extracts. The second-stage larvae (2 (J) of the root-knot nematode that infects tomato were obtained. The perineal model morphologically identified them. Laboratory results showed that second instar larvae were affected when treated with concentrations of (30, 20 and 29)% of wild plant extracts (colocynth, sunflower, Calotropis procera) for 3 days at laboratory temperature. This led to their movement stopping. The highest level of dead nematodes reached 100% at high concentrations for both colocynth, sunflower and Calotropis procera plant extracts, with an average of (30, 20 and 29)%. The results showed that all high concentrations of plant extracts had significant positive effects compared to the control on the number of root-knot nematodes (250 g/soil). There were differences in the highest number of root nodules in plants treated only with nematodes (326) nodes per plant root compared to the other concentrations of treatments. The best treatment was the 100% and 150% colocynth extracts, as no nodules formed on the roots. The greenhouse results showed that the use of these wild extracts, especially at high concentrations of 100%, resulted in a clear improvement in the vegetative growth of tomato plants treated with extracts of colocynth, sunflower, and Calotropis procera compared to the control.

Keywords: Plant extracts, Colocynth, Calotropis procera, Sunflower, Root-knot nematode, Meloidogyne incognita, Tomato plant.

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Introduction
Tomatoes are one of the most widely used and popular vegetables globally. They belong to the nightshade family. They are characterized by their attractive red colour and delicious taste, which combines acidity and sweetness. Tomatoes are used in a wide variety of dishes, such as salads, soups, and sauces. They are a basic ingredient in many international cuisines. They contain a high water content and are a rich source of vitamins, such as C and A, as well as antioxidants like lycopene, which contribute to the prevention of certain chronic diseases. Tomatoes are cultivated worldwide. Tomatoes vary in shape, size, and taste (FAO, 2018). They are not too hard to cultivate. Depending on the weather, they can be cultivated in greenhouses or open fields. They are used a lot in the food business, mainly to make paste, juices, and canned meals. Many individuals eat tomatoes every day since they are good for their health and taste good. Tomatoes are also thought to be one of the plants with a lot of nutritional value since they provide a lot of vitamins and amino acids that people require (Sikora and Fernandes, 2005).

Root-knot nematodes (Meloidogyne spp.) are one of the worst pests that may attack tomato plants. They hurt crop yield a lot and are a big danger to farming, especially in tropical areas. This pest may infect more than 2,000 different types of plants (Abdullah et al., 2025), including the Solanaceae family, which includes tomatoes, one of the most important crops for the economy (Al-Jubouri et al., 2024). Also, root-knot nematodes are among of the worst pests that may hurt tomatoes, worse than fungal and bacterial illnesses.

The developing tips of the roots let second-instar nematode larvae in. They settle into the tissues and release substances that cause the growth of enormous feeder cells. This causes root nodules, which are typical swellings (Abdullah and Hasan, 2020). These nodules make it harder for plants to take in water and nutrients, which slows down their growth (Humada et al., 2025). Plants that are sick show signs like yellowing leaves, wilting, and slow development, especially in warm, sunny soils. Plants that are infected are also more likely to get sick from various illnesses and environmental stress (Alatawi et al., 2024).

People commonly get nematodes via dirty soil, irrigation water, or agricultural implements that haven't been cleaned. They are a big reason why tomato yields and quality go down since they directly affect the roots (Younis et al., 2025). This pest costs the world economy over $100 billion a year (Oka et al., 2000). Chemical nematicides were the main way to control them in the past. Research has moved toward safer and more effective alternative options (Hasan et al., 2023) because of the negative impact these chemicals have on the environment and human health (Hasan et al., 2023). Plant extracts have been a potential strategy for controlling nematodes (Abdullah et al., 2023). They have active ingredients like alkaloids, organic and aromatic acids, and things that directly effect soil organisms (Putnam, 1987).

The efficiency of plant extracts depends on the type of plant, the place where it grows, and the section of the plant that is utilized for extraction. This study looked at how well wild plant extracts that grow in desert areas can control root-knot nematodes that infect tomatoes. This was done because tomatoes are important for both nutrition and the economy, and to reduce the use of chemical pesticides (Younis et al., 2022a).

Research Materials and Methods

To make the nematode inoculum, we got second-instar larvae, which are the infective stage, and used the perineal model to identify them morphologically (Hartman and Sasser, 1985; Jepson, 1987). We took egg sacs from the roots of plants that were sick. Then, they were put on Petri plates that had been wet so that the eggs may hatch. After the eggs hatched, second-instar larvae were collected and then utilized in infection tests to learn more about how nematodes affect plants.

Collecting samples of wild plants for use: We took samples from wild plants that grow naturally in some places. The samples had the fruits of the colocynth plant, the leaves and flowers of the sunflower plant, and the leaves and fruits of the Calotropis procera plant. We cleaned the plant components well and then squeezed them to get them ready for extraction with 70% ethyl alcohol. Following these processes, 20 grams of plant material and 400 ml of ethyl alcohol were mixed together to extract the desired substance:

The first step was to transfer the mixture to a 1-liter beaker and let it sit in a water bath set at 40°C for one day.
Second, after the incubation period, the extract was filtered using filter paper. This was followed by drying in an incubator at 37°C for 24 hours. After the drying process was complete, the final extract weight was recorded, and the percentage yield of the extracted material was calculated.

Plant Extract Preparation: After the extraction process was completed, three different concentrations of each plant extract (colocynth fruit, sunflower leaves and flowers, and Calotropis procera leaves and fruits) were prepared: 50%, 100%, and 150%. The extracts were diluted with distilled water. These concentrations were placed in opaque, sterile, and airtight bottles. They were then clearly labelled and refrigerated at 5°C until they were used in the experiments. In addition, a commercial nematicide (24% oxamyl) was used as a control. It was obtained from the Agricultural Offices and produced by a manufacturer in Thailand. The pesticide was used to compare the effectiveness and concentration of different plant extracts in controlling root-knot nematodes and their potential impact on tomato plants.

In vitro study of the effectiveness of plant extracts:
The effect of plant extracts on root-knot nematodes (Meloidogyne spp.) was studied using in vitro experiments. 10 mL of each plant extract (colocynth fruits, sunflower leaves and flowers, and Calotropis procera leaves and fruits) was placed in Petri dishes with a diameter of 10 cm and a height of 1.5 cm, at each concentration (50%, 75%, and 100%), with four replicates for each concentration. 100 second-instar nematode larvae were added to each dish. The percentage of immobile (dead) larvae was recorded daily for 3 days. The results were compared with a control group containing only distilled water and 100 nematode larvae. After treatment, dead larvae were transferred to Petri dishes containing 2 ml of distilled water to confirm their death using Vital's blue stain. Larvae stained black are considered dead. While the transparent, unpigmented ones are considered alive (Ogiga and Estey, 1974).

Study of the effectiveness of extracts in a greenhouse:
The effect of 12 treatments on the growth and reproduction of root-knot nematodes on tomato seedlings of the "Amira" cultivar was tested. The treatments were as follows:
1. 50% Colocynth fruit extract
2. 100% colocynth fruit extract
3. 150% colocynth fruit extract
4. 50% sunflower leaf and flower extract
5. 100% sunflower leaf and flower extract

6. 150% sunflower leaf and flower extract
7. 50% Calotropis procera leaf and fruit extract
8. 100% Calotropis procera leaf and fruit extract
9. 150% Calotropis procera leaf and fruit extract
10. 24% oxamyl pesticide (according to the company's concentration) (Artificial)
11. Tomato plant with nematodes only (no treatment)
12. Negative control (tomato plant without nematodes or treatment)

Seedlings were planted in 48 pots, each 10 cm in diameter and filled with 250 grams of sterile sandy loam soil. Approximately 30 days after planting, when the fourth leaf appeared, each pot was inoculated with 500 second-instar nematode larvae, except for three pots that served as negative controls. Treatments were added one week after infection, and the experiment continued for 40 days. Data were then collected regarding:
• Plant fresh weight
• Root weight
• Number of root nodules
• Number of egg sacs
• Number of females
• Number of second-instar larvae per 250 grams of soil.

Larvae were extracted from the soil using the Berman filtration method. They were counted using a Hawksley slide under a microscope at 10×10 magnification. Infected roots were washed with plain water. They were then fixed in 4% formalin for 24 hours and stained with acid fuchsin (Byrd et al., 1983) to facilitate counting of nodules, egg sacs, and females.

A completely randomized design (CRD) was employed in the experiment. Data were statistically analyzed using the least significant difference (L.S.D.) test at a 0.05 probability level to assess the significance of differences between treatments (Humada et al., 2024).

Results

Laboratory Study:
The results of Table (1) showed that second-instar larvae of root-knot nematodes were significantly affected when treated with three different concentrations (50%, 100%, and 150%) of wild plant extracts (colocynth, sunflower, and Calotropis procera). This was achieved after exposure for three days at room temperature. The highest rate of immobile (dead) larvae was observed at the highest concentration (150%) on the third day. The average mortality rates for the colocynth, sunflower, and Calotropis procera extracts were 30%, 28%, and 29%, respectively. This was followed by concentrations of 100% for both colocynth and sunflower, with average mortality rates of 24% and 23%, respectively. Meanwhile, the 100% Calotropis procera extract showed a lesser effect. The average mortality rate reached 12% on the second day. The lowest concentrations (50%) recorded the lowest rates of larval movement inhibition, with the rates being (5%, 8%, and 9%), respectively, for the three extracts, compared to the control treatment, which recorded no significant change.

Greenhouse Experiment:
The results of the experiment showed that high concentrations of plant extracts had a clear positive effect in reducing the number of female root-knot nematodes compared to the control treatment. Sunflower and Calotropis procera extracts were less effective in controlling nematodes than oxamyl and the two high concentrations (100% and 150%) of colocynth extract. Table (2) also showed significant differences between the different treatments. Plants treated with nematodes alone recorded the highest number of root nodules (326). In contrast, plants treated with colocynth extract at concentrations of 100% and 150% showed the best results, as no nodules were observed on their roots. Meanwhile, plants treated with sunflower and Calotropis procera extracts recorded a small number of nodules, with the effectiveness of the extract clearly decreasing as the concentration decreased.

The same applies to the number of egg sacs. The highest percentage was recorded in plants infected with nematodes only (276 sacs). However, in treatments with low concentrations, a slight variation in the number of sacs was observed. The data in Table (3) showed that the use of plant extracts, especially at a concentration of 150%, led to a significant improvement in the vegetative growth of tomato plants compared to the control. As for the weight of the fresh root system, it varied between treatments. The highest weight was recorded in plants treated with Calotropis procera extract, while the lowest weight was in the control treatment.

Table (1) The percentage of dead nematodes in the three days for different concentrations of wild plant extracts			
Percentage (%) of larval death after			Treatment
Third day	Second day	First day	
9	5	1	Colocynth Extract 50%
24	13	3	Colocynth Extract 100%
30	15	5	Colocynth Extract 150%
8	6	3	Sunflower leaf and flower extract 50%
23	7	2	Sunflower Leaf and Flower Extract 100%
28	13	6	Sunflower leaf and flower extract 150%
5	1	1	Calotropis procera leaf and fruit extract 50%
16	12	6	Calotropis procera leaf and fruit extract 100%
29	5	5	Calotropis procera leaf and fruit extract 150%
0	0	0	Comparison treatment

Table (2) The effect of extracts of some wild plants on root-knot nematodes that infect tomato plants under greenhouse conditions.						
Number of larvae		Number of egg sacs		Number of nodes on the roots		treatment
shortage %	root/soil plant	shortage %	plant soil%	shortage %	250 soil/gm	
95	5	95	9	96	6	Colocynth Extract 50%
100	0	100	0	100	0	Colocynth Extract 100%
100	0	100	0	100	0	Colocynth Extract 150%
93	7	95	8	94	25	Sunflower leaf and flower extract 50%
95	5	97	23	95	7	Sunflower Leaf and Flower Extract 100%
97	3	99	28	98	13	Sunflower leaf and flower extract 150%
92	8	94	5	93	14	Calotropis procera leaf and fruit extract 50%
96	4	97	16	94	12	Calotropis procera leaf and fruit

						extract 100%
100	0	98	29	95	5	Calotropis procera leaf and fruit extract 150%
100	0	100	0	100	0	nematicide
0	295	0	276	326	0	Comparison treatment
	61.64		42.76		66.43	L.S.D

Table (3) The effect of extracts of some wild plant species on the growth of tomato plants infected with root-knot nematodes.

Fresh weight of the vegetative group		Fresh weight of the root system		treatment
increase %	Weight gm	increase%	Weight gm	
40.80	16.52	38.69	11.45	Colocynth Extract 50%
51.48	20.46	62.78	13.60	Colocynth Extract 100%
54.89	22.12	56.16	13.03	Colocynth Extract% 150
42.76	16.81	56.13	13.06	Sunflower leaf and flower extract 50%
48.12	19.07	53.12	12.80	Sunflower Leaf and Flower Extract100%
53.70	21.02	69.32	14.19	Sunflower leaf and flower extract 150%
42.57	18.91	60.42	13.39	Calotropis procera leaf and fruit extract 50%
48.19	17.07	75.22	14.71	Calotropis procera leaf and fruit extract 100%
48.80	19.07	72.76	14.49	Calotropis procera leaf and fruit extract 150%
39.17	16.04	56.61	13.05	Nematicide
0	9.19	0	7.91	Comparison treatment
2.99			1.18	L.S.D

Figure A

Figure B

Figure C

Figure D

Figures (A, B, C, and D) show the presence of root nodules and egg sacs on tomato roots infected with root-knot nematodes.

Discussion

Meloidogyne incognita can be treated using plant extracts because these extracts include natural chemical components that have poisonous or nematode-inhibiting characteristics. These chemicals include alkaloids, flavonoids, and volatile oils. These chemicals kill or severely limit the nematode's ability to reproduce by interfering with its critical processes or damaging its cell walls (Jaber et al., 2025). According to Hasan and Abdullah (2021), some of the extracts can help boost the plant's immune and make it more resistant to infections. Compared to chemical pesticides, this technique is more safer and better for the environment. Plant extract treatment of Meloidogyne incognita significantly reduced egg sac production and root nodule number (Younis et al., 2022a). Additionally, the nematode was killed by these natural extracts. The results of Begum et al. are in agreement with this discovery (2008). There are a number of natural plants that can inhibit the growth of nematodes. According to Gong et al. (2013), the use of garlic extracts positively contributes to the suppression of root-knot formation in M. incognita. Auger et al. (2004) demonstrated that botanical pesticides have a direct effect in controlling these nematodes. Otipa et al. (2003) confirmed that garlic extract prolongs the life cycle of nematodes and reduces their reproductive efficiency. Elgawad-Abd et al. (2009) reported that wild plant extracts have direct benefits in promoting tomato growth and reducing the reproduction of root-knot nematodes. It was also observed that the application of commercial preparations rich in garlic extract to tomato plants reduced the incidence of these nematodes and increased the vegetative mass compared to the untreated control. The results also showed that all high concentrations of wild plant extracts were effective in eliminating root-knot nematodes. This helps reduce reliance on environmentally harmful chemical pesticides. Begum et al. (2008) noted that many wild plants produce compounds that are lethal to M. incognita and contribute to their defense against the nematode.

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