Nematicidal Properties of Medicinal Plants against Root-Knot Nematode - A Systematic Review

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Abstract:
Plant parasitic nematodes are small roundworms that feed on plants and damage them. The most serious among them was root-knot nematodes that found in cultivated crops throughout the world. Root-knot nematode infection in plants has become the biggest issue nowadays due to the significant agricultural losses. The greatest barrier to environment-friendly crop production in modern agriculture is the reliance on chemical nematicides for crop protection. Instead of using hazardous chemical nematicides, it should be encouraged to apply plant-based nematicides. The mechanism of root-knot nematode infection and eco-friendly management strategies were covered in this review. This information should be done in this area so that eco-friendly and cost-effective controlling measures against root-knot nematodes.

Keywords: Root-knot nematode, Phytochemical, Medicinal plant, Management strategies, eco-friendly.

Introduction
Root-knot nematode, Meloidogyne genus contains 90 species but the most dangerous ones are M. arenaria, M. hapla, M. javanica and M. incognita. Meloidogyne is a greek word that means "apple-shaped female" (Sharma et al., 2018). Their ability to reproduce rapidly and possess unique characteristics contributes to their effectiveness as parasites (Cetintas et al., 2018). The majority of temperate and tropical crops have yield losses due to root-knot nematodes, which are the most significant plant parasitic worms worldwide which cause nutritional deficiencies as well as galling, stunting, and yellowing (Crow, 2019).

Various strategies, including improved cultural practices, the utilization of resistant crop varieties, the application of allelochemicals found in cover crops, organic amendments, through sanitation measures, solarization, and the implementation of widely-used synthetic nematicides, are employed to effectively control root-knot nematodes in agriculture fields. Nematicides may cause environmental and health issues, as well as the emergence of
nematode resistance if they are used frequently. Hence, it is preferable to utilize alternative management techniques that are effective, inexpensive, and safe for consumers, farmers, and the environment (Abbassy et al., 2017).

Root-knot nematode control practices such as crop rotation; intercropping, and chemical control by soil fumigants such as methyl bromide. Certain phytochemicals are antagonistic to other nematodes including plant parasitic nematodes (Regaieg et al., 2017). Higher plants have produced a wide range of bioactive substances that are effective against parasite infestation and are less expensive and biodegradable than toxic pesticides (Mukhtar et al., 2017; Ismail et al., 2020).

Life Cycle of Root-Knot Nematode

Female lays eggs up to 400-500 in the gelatinous egg mass which gives rise to first-stage juveniles (J1) within the egg. Gelatinous egg mass gives protection from adverse environmental conditions. Once favorable conditions such as adequate soil moisture, temperature and pH levels are met, the first-stage juveniles (J1) emerge from the eggs and subsequently develop into second-stage juveniles (J2) (Lima et al., 2018). The second stage juvenile (J2) possesses stylet which pierces the root tissue and also takes a role in the secretion of the esophagus and nutrient up taking. They also have a sedentary movement to penetrate the root. Root-knot nematodes after that show the 4 successive moltings they become adult males or adult females (Kumar & Yadav, 2020). The adult male also contains piercing stylet with knob and spicule at the tail region. They also have a slow sedentary movement. Males are thought to play little involvement in the reproduction of root-knot Nematodes, which reproduce mostly through mitotic parthenogenesis. Life-cycle of root-knot nematode is influenced by the temperature. Root-knot nematode adult females become visible approximately 13-15 days after penetration in roots, while the initial instances of egg laying females within the roots tissue can be observed around 19-21 days after penetration. So the life span of the root-knot nematodes females extend up to 2 to 3 months but in the case of male it might be shorter (Figure 1) (Eisenback et al., 2020).

Figure 1. The Life Cycle of Root-Knot Nematode and Gall Formation in Host Plant
Source: Bui et al. (2021)
Root-Knot Nematode Infection in Plant

A cell wall covers the root tissue of plants, providing defense against intracellular pressure. Because of its chemistry and structure, the cell wall also prevents the nematode. Invading plant tissue as a result, the root-knot nematode breaks down the cell wall with digestive enzymes (Bhowmik et al., 2021).

Similarly, the life cycle of the root-knot nematode comprises 6 distinct stages: eggs, first-stage juvenile (J1), second-stage juvenile (J2), third-stage juvenile (J3), fourth-stage juvenile (J4), adult male and an adult female. Only the second stage juvenile (J2) and adult males invade the root tissue. Here in the case of newly hatched second-stage juvenile (J2) moves freely in the rhizome of the root tissue. After migration into the root the second stage juvenile (J2) formed the giant cells and from there they feed for almost 10-12 days and molt 3 times (J2, J3 & J4) (Narasimhamurthy et al., 2018).

Symptoms of Root-Knot Nematode Infection

Plants infested with root-knot nematode include symptoms of galling, stunting, wilting, and leaf curling (Charei, 2017). Eventually, the induced gall formation in roots caused by nematode infection resumes normal water and nutrient intake. These root-knot nematodes initiate the growth of giant cells that cause gall to accumulate in roots; these giant cells provide the nutrition up to reproduction (Yigezu, 2021). Chlorosis is also seen in foliage which leads to crop loss and the quality of the crop (Makhubu et al., 2021; Bhowmik et al., 2021). Soil infested with root-knot nematode may result in the death of young plants. Generally, root-knot nematode females can conceal and lay about 400-500 eggs in particular root tissue and form a gall (Mani, 2022).

Control Methods for Root-Knot Nematodes

Chemical Control

In contrast, chemical nematicides that control the root-knot nematodes are categorized as fumigant & non-fumigant nematicides. In fumigants nematicides such as EDB (Ethylene dibromide), DD (dichloropropane), and methyl bromide were extensively employed. However, the use of methyl bromide has been prohibited in certain countries owing to its detrimental impact on the stratospheric ozone layer, contributing to ozone depletion (Ravichandra, 2018). As a substitute for methyl bromide, several alternatives have been introduced, including chloropicrin (CP), dazomet (DZ), metham sodium (MS), 1, 3-dichloropropene (1, 3-D), allyl isothiocyanate (AITC) and other related products. These alternatives serve as replacements for methyl bromide in the context of fumigant nematicides (Huang et al., 2019). Non-fumigant nematicides are generally considered to be less potent in suppressing nematode populations compared to fumigant nematicides. This is primarily due to their limited or specific spectrum of activity. Unlike fumigants, non-fumigant nematicides may not exhibit broad-spectrum effectiveness against a wide range of nematode species (Desaeger et al., 2020). Carbofuran also has a standard synthetic nematicide which is widely used towards the root-knot nematodes (Izuogu et al., 2019). Velum Prime 400 SC (active ingredient fluopyram, Bayer) is also used as a potential nematicide to control root-knot nematodes (Dahlin et al., 2019).

Mode of Action

The mode of action of nematicides refers to its specific lethal impact on certain nematode processes. Nematicides such as organophosphates and carbamates operate by inhibiting the enzyme acetylcholinesterase (AChE), resulting in the cessation of nerve impulses and eventual death of the nematode. Similarly, abamectin acts as a neurotoxin by affecting acetylcholinesterase and impairing...
ATPase activity, leading to respiratory failure and nematode mortality (Ebene et al., 2019).

**Limitations to Using Chemical Nematicides**

1) It also affects the environment and soil sterility.
2) A risk in consuming such chemicals through the root-knot nematode infected plant products.
3) Small farmers could not purchase it due to its high-cost value (Ji et al., 2019).
4) Improper utilization of chemical nematicides, including excessive doses, frequent application, expiry date, and illiteracy of applicators can have detrimental effects on crop production (Makhubu et al., 2021).

**Biological Control of Root-Knot Nematode**

The biological control of root-knot nematode in field conditions can be achieved through inoculation with effective antagonists. While chemical nematicides are known for their effectiveness, ease of application, and quick results, they have started to be phased out in certain developed countries due to concerns related to public health and environmental safety (Naz et al., 2021). The search for novel, environmentally friendly alternatives for controlling root-knot nematode populations has become increasingly critical. Generally, commercially utilized biological antagonists that have demonstrated efficacy against root-knot nematodes include fungi, bacteria, predatory nematodes, and plant extracts (Tapia-vazquez et al., 2022).

**Other Management Approaches for Root-Knot Nematode**

Cultural methods, such as summer ploughing, crop rotations, antagonistic crops, trap crops, destructions of crop residues, applications of organic amendments, and utilization of resistant varieties/hybrids/genotypes (host plant resistance), have proven to be highly effective in managing root-knot nematodes management (Mani, 2022). Stream sterilization is a physical method that can be employed to address the high occurrence of root-knot nematodes in protected cultivation. Additionally, the combined application of nitrogen-rich organic amendments and soil solarization has been found to be effective in controlling root-knot nematodes in organic greenhouse farming systems. These practices serve as valuable strategies in the management of root-knot nematodes within protected cultivation and organic farming contexts (Rosskopf et al., 2020).

**Use of Medicinal Plants Against Root-Knot Nematodes**

Eleven plants have been examined in this review to demonstrate their efficiency against the four species of root-knot nematodes that are most frequently found: *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla*, and *Meloidogyne arenaria*. According to (Table 1) these plants contain a variety of secondary metabolites that prevent second-stage juveniles (J2) from hatching from eggs, cause larval mortality, and impede nematode proliferation.

Distinct water leaf extract of Neem (*Azadirachta indica*) and Bael (*Aegle marmelos*) shows 86.6% and 11.9% mortality of the second-stage juveniles of *Meloidogyne javanica* in 100% concentration in the In-vitro experimental study (Shakya & Yadav, 2020). Similarly, leaf extract from five different plants viz., margosa, marigold, datura, hemp, and tobacco plant repressed the growth of *Meloidogyne incognita*, reduced root galling and enhanced the plant growth (Shah et al., 2018). *Azadirachta indica* seed powder shows a maximum reduction in egg masses on tomato and brinjal plants infected with *Meloidogyne hapla* (Askary, 2020).

Under greenhouse conditions, the use of *Tagetes erecta* shows the treatment at concentrations of 500 and 2,500 ppm has been observed significantly inhibit the growth and reproduction of *Meloidogyne javanica*. This is evident in terms of reduced eggs/plant root, decreased formation of egg-masses and galls, lower nematode populations in the soil and enhanced plant growth (Gholaminezhad, 2022). The standard aqueous extract of *Tagetes erecta* and *Datura stramonium* demonstrated effective inhibition of egg hatching of *Meloidogyne incognita* in vitro. At a concentration of 5% (w/v), the hatching inhibition rates were 91.43% for *Tagetes erecta* and
59.78% for *Datura stramonium*. With a higher concentration of 10% (w/v), the mortality rates increased to 75.60% and 54.20% for *Tagetes erecta* and *Datura stramonium*, respectively after 5 days post-treatment. The larvicidal effect was found to be directly proportional to the concentration and duration of exposure in the in-vitro study. In an in-vivo study, it was observed that these plant extracts significantly increased the biomass of infected pepper plants while reducing the reproduction of *M.incognita* (Ali et al., 2018).

<table>
<thead>
<tr>
<th>Name of the Plant</th>
<th>Family</th>
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<th>Solvent of extract</th>
<th>Root-knot nematode Species</th>
<th>Study Type</th>
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<td>Distill Water</td>
<td><em>M.incognita, M.javanica, M.hapla</em></td>
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<td>suppressing nematode multiplication, Inhibit J2 hatching from eggs, inducing larval mortality</td>
<td>Shah et al., 2018; Shakya &amp; Yadav, 2020; Askary, 2020</td>
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<td>Ali et al., 2018; Gholamnezhad, 2022; Shah et al., 2018</td>
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<td>Tobacco (<em>Nicotiana tabacum</em>)</td>
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<td>Okechalu et al., 2020; Haroun et al., 2022</td>
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Datura stramonium exhibits high efficacy in hatching juvenile (J2s) mortality of *M. javanica* under laboratory conditions. They conduct an experiment in a greenhouse using 4000 eggs and J2s of *M. javanica*, as well as soil that has been saturated with 100 ml/pot of three different concentrations of *Datura stramonium* (Moazezikho et al., 2020). Leaf extract at higher concentrations, 20ml/plant, was found to be more successful at suppressing *Meloidogyne incognita*. In tomatoes infested with *Meloidogyne javanica*, aqueous crude extract of *Calotropis procera* Linn. has the maximum effectiveness at 50% concentration (Agatha & God, 2018). The root gall index and nematode reproductive factor of *Meloidogyne incognita* were affected, as were the development and yield of root-knot nematode-infected tomatoes in the field, by leaf and seed extracts of *Ricinus communis* and *Jatropha curcas* (Oluwatayo et al., 2019). Aqueous extract of *Tinospora cordifolia* shows (97.75%) at the concentrations of 2500 µg/ml, respectively, after 5 days of exposure in inhibition of hatching second-stage juveniles (J2s) of *Meloidogyne incognita* (Tariq et al., 2022). *Aegle marmelos* leaf aqueous extract induces 70.16% inhibition on the juveniles of *Meloidogyne incognita*. In this study, the mean of three replication and the number of larval mortality were quite reduced (Jamir et al., 2018). Aqueous extract of *Ocimum basilicum* also reduced *M. arenaria* egg hatching by 70-83% and reduced immobilization by 9-23% (Haroun et al., 2022). *Ocimum basilicum* at the concentration of 60 mg/ml shows the highest efficacy on *Meloidogyne javanica* and *Meloidogyne incognita* in field conditions (Okechalu et al., 2020).

**Conclusion**

Nematicides derived from plant material are effective tools against root-knot nematodes. In vivo study is used to investigate plant based formulations for nematode control. In-vitro methods serve as valuable tools to assess the efficacy of nematode control and the inhibition of egg hatching. Implementing plants in the form of powdered or extracts for field application poses challenges due to seasonal growth patterns of specific plants, which may vary based on environmental conditions. The creation of novel plant extract-based nematicides as a substitute for synthetic nematicides requires research on bioactive compounds. Compared to chemical nematicides, all plant extracts are more affordable and accessible to farmers. The development of innovative nematicides based on plant extracts requires research on bioactive compounds. Compared to chemical nematicides, all plant extracts are more affordable and accessible to farmers.

With the findings of this study, it may be improved to benefit both farmers and the environment. In this instance, a lot of work is still required in different aspects like life-cycle, control strategies and mechanism of action of the other root-knot nematodes in the host plant. However the mechanism of action of different medicinal plant extract towards the root-knot nematode is still unexplored.

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**Conflicts of interest**

Authors declared no potential conflict of interest.

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