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Eucalyptus (*Eucalyptus camaldulensis*) and chinaberry (*Melia azedarach*) extracts' potential for sustainable control of root-knot nematode, *Meloidogyne javanica*

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Abstract

Root-knot nematode has a widespread distribution and has caused significant damage, as in some cases, led to complete crop loss. Environment-friendly and safe methods are essential for the replacement of chemical nematicides. In the present study, the lethal effect of aqueous extracts from eucalyptus (*Eucalyptus camaldulensis*) and chinaberry (*Melia azedarach*), which are locally available plants, on second-stage juveniles (J_2) of the root-knot nematode, *Meloidogyne javanica* was investigated under laboratory conditions. For this purpose, concentrations of 1000 to 7000 ppm were evaluated on 100 numbers of J_2 at two different time intervals, two four days, using a factorial experiment (concentrations of 1000 to 7000 and times of two and four days after extract exposure) within a completely randomized design with three replications. The median lethal concentrations (LC_{50}) for each extract were calculated. The results of this study indicate a significant correlation between the increased mortality rate of juveniles and the concentration and exposure period. The highest concentration exhibited the best results after four days, with mortality rates of 73% and 68% for *M. azedarach* and *E. camaldulensis* compared to the control, respectively. The probit analysis revealed that LC_{50} values were 5044 and 5694 ppm for chinaberry and eucalyptus, respectively. No significant changes were recorded in the internal organs of the dead larvae, except for the contraction in parts of the intestine and their straight standing. In general, plant extracts present a promising method for managing *M. javanica*.

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Introduction

Plant-parasitic nematodes annually cause a loss of 12.6% of global crop production, equivalent to $157 billion per year (Nicol et al., 2011; Das et al., 2021; Marin-Bruzos et al., 2021). Root-knot nematodes (*Meloidogyne* spp.) have significantly increased damage to crops worldwide due to their broad host range and high reproduction rates under favorable conditions (Dutta et al., 2012; Mesa-valle et al., 2020). Farmers, in many regions, prefer the use of chemical nematicides as one of the effective options to achieve the acceptable yield of their agricultural products (Mesa-valle et al., 2020; Sharma et al., 2020). Unfortunately, this method is not only expensive but also poses risks to human health and the environment, as well as they can eliminate beneficial soil microorganisms. Therefore, it is crucial to adopt nature-friendly and cost-effective methods to overcome these issues (Rutter et al., 2022). We need a serious determination to replace chemical pesticides, so far, various methods have been proposed for managing this category of pests. As cultural management, biological control, nanoparticles derived of bio or chemical origin, encapsulation and resistant cultivars based on silencing of genes involved in the establishment of nematodes (Torabi & Zeynadini, 2024). Numerous studies have reported the importance of extracts from various plants, ranging from shrubs to trees, including those with medicinal and nutritional value, in managing plant diseases, which among them plant extracts hold a special position (Nolling & Becker, 1994; Shabana et al., 2017; Motlagh & Ebrahimi, 2024). The nematicidal potential of some plant extracts such as *Allium sativum*, *Azadirachta indica* has been proven due to the presence of compounds such as alkaloids, saponins, terpenoids and phenolic compounds (Imafidor et al., 2023). Many local and international studies have been well documented the toxicity or inhibitory effects of extracts and essential oils from various plants on root-knot nematodes (D'Addabbo et al., 2024; Asadi et al., 2024). These include extracts from medicinal plants such as thyme (*Thymus vulgaris*), chamomile (*Chamaemelum nobile*), fennel (*Ferula communis*), mallow (*Malva sylvestris*), sage (*Salvia officinalis*), and clove (*Syzygium aromaticum*), as well as extracts from tree species like neem (*Azadirachta indica*), chinaberry (*Melia azedarach*), walnut (*Juglans regia*), pomegranate (*Punica granatum*), and eucalyptus (*Eucalyptus camaldulensis*) (Javed et al., 2008; Asadi Sardari et al., 2015; Alikarami et al., 2017; Maleita et al., 2017; Shazdeh Ahmadi, 2024). The eucalyptus plant belongs to the *Myrtaceae* family and is native to Australia. Among more than 400 *Eucalyptus* species, only a few have been introduced to Iran, with *Eucalyptus camaldulensis* being the most prevalent species in the country. This plant contains compounds belonging to the categories of sterols, alkaloids, glycosides, flavonoids, tannins, and phenols, exhibiting anticancer, anti-inflammatory, blood sugar-lowering, antioxidant, and antiviral properties (Ansari, 2013; Sabo & Knezevic, 2019). A study conducted by El-Baha et al. (2017) analyzed the chemical compounds of eucalyptus and identified that Eucalyptol and α-pinene comprise the majority of its chemical composition, which may be related to their toxic effects on J_2 of the nematode *M. incognita*. In a recent study conducted by Sarri et al. (2024) it was observed that the essential oil from eucalyptus leaves (*E. globulus*) had significant effects against J_2 of root-knot nematodes (*Meloidogyne* spp.) under laboratory conditions. Dawar et al. (2007) also reported that aqueous and ethanolic extracts from the leaves, stems, and bark of eucalyptus (*Eucalyptus* spp.) significantly reduced the number of galls and egg masses on the roots of chickpea and mung bean plants. The chinaberry tree, known as Persian lilac in the *Meliaceae* family, is another plant of interest due to its medicinal properties and antioxidant, antibacterial, and antiviral effects (Krif et al., 2024; Sarri et al., 2024). Among the active compounds of the extract from *M. azedarach* are limonoids, with azadirachtin being the most well-known limonoid. These compounds are significant due to their insecticidal and antifeedant properties (Saraç et al., 2004). A study on the extract and dried powder of chinaberry revealed that extracts from various parts of this plant, including fruit and leaves, had suitable control effects against *M. incognita* (Ardakani, 2012). Furthermore, Fahmy et al. (2023) reported that the ethanolic extract of ripe chinaberry fruit had a significant control effect on root-knot nematode (*M. incognita*) and suppressing its growth under greenhouse conditions. With regard to the dominance of *M. javanica* species in the Rafsanjan region and the lack of investigation of native plant extracts from *E. camaldulensis* and chinaberry against second-stage juveniles (J_2) of this species present research seems necessary. This study also determined the LC_{50} of each plant extract and the morphological deformations of J_2 after exposure to the extracts.

Materials and Methods

Inoculum preparation of *M. javanica*

To prepare the inoculum of *M. javanica*, samples were collected from pistachio orchards located in the Rafsanjan region. Subsequently, the nematodes were purified and propagated using the single egg mass method (Hussey & Barker, 1973). Petri dishes containing eggs were incubated in a dark environment at a temperature of 32 °C for 48 to 72 hours. After this period, the number of juveniles was obtained by multiplying the mean of three counts with the counting slide by the volume of the collected suspension.

Preparation of aqueous leaf extracts

One kilogram of fresh leaves from eucalyptus and chinaberry plants were collected from the agricultural faculty of Vali-e Asr University of Rafsanjan. The leaves were dried at room temperature. The dried leaves were then ground using an electric grinder (Pars Khazar, ML-320P model, Iran). For the preparation of the aqueous leaf extract, 100 grams of powdered leaves from each plant were separately mixed with one liter of sterilized distilled water and placed on a magnetic stirrer for 24 hours at a speed of 110 rpm. The resulting solution was then filtered through Whatman filter paper (Whatman, No. 42, filter speed: slow), and the obtained solution was used for bioassay testing (Motlagh & Ebrahimi, 2024).

Bioassay testing

The effect of the aqueous extracts from the leaves of eucalyptus and chinaberry on the mortality rate of J_2 of

M. javanica was examined in Petri dish. Seven concentrations were prepared: 1000, 2000, 3000, 4000, 5000, 6000 and 7000 ppm of the aqueous leaf extract from each plant. Each plastic Petri dish (five cm diameter), contained one milliliter of nematode suspension containing 100 J_2 and nine milliliters of the respective plant extract. The control Petri dish received nine milliliters of sterilized distilled water. The Petri dishes were maintained in an incubator at a temperature of 27 °C, in the dark. The treatments were finally observed and examined with the aid of a stereomicroscope (Olympus SZH, Japan) at a magnification of 10X, after two, four, and six days and were grouped in to two distinct categories: motile or paralyzed. Moreover, at that point, nematodes were transferred to sterile distilled water after washing in tap water through a 20 µm pore screen to remove the excess of extracts. The larvae were considered paralyzed with straight body and absence of any mobility after touching a needle. Deformation of internal organs was surveyed after plant extracts exposure under an optical microscope.

Statistical analysis

This study was conducted as a factorial experiment (1000 to 7000 concentrations of extracts and times of two and four days after extract exposure) within a completely randomized design with three replications. The data were statistically analyzed using SAS software and mean comparisons were analyzed using Duncan's test. Based on the results obtained from the J_2 mortality rates, only the data from the fourth day were analyzed using the Polo (Probit and Logit Analysis) software to calculate the meaning lethal concentration (LC_{50}). The images were drawn using Excel software.

Results

The results obtained from the comparison of mean data using Duncan's multiple range test indicated a significant difference between the treatments of the aqueous leaf extracts of eucalyptus and chinaberry compared to the control. The extracts of these plants significantly increased ($P \leq 0.01$) the mortality rate of J_2 of the nematode *M. javanica*. The results of statistical analysis on the mortality of J_2 following treatment with eucalyptus extract showed that the concentration of 7000 ppm on the fourth day had the greatest effect with 68% mortality, although there was no significant difference with a concentration of 6000 ppm on the same day with 60% mortality. These two mentioned concentrations, with mortality rates of 38% and 45%, exhibited similar lethal effects on the second day. The mortality rate of J_2 in concentrations of 1000 to 3000 ppm on the second day showed no significant difference with the control. Although over time, by the fourth day, the concentrations of 2000 and 3000 ppm significantly demonstrated higher mortality rates, by 37.6% and 38.6%, respectively compared to the control (Fig. 1).

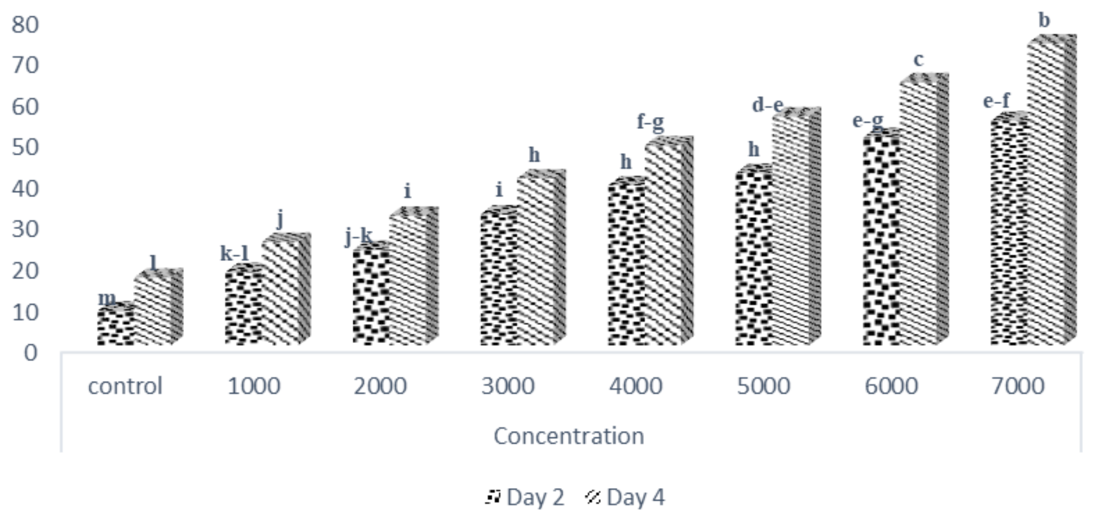

Fig. 1. Effect of different concentrations of aqueous leaf extract of *Melia azedarach* and exposure time on mean mortality rate of second stage juveniles (J_2) of root-knot nematode (*Meloidogyne javanica*). Means by the same letter are not significantly different at $P \leq 0.01$.

According to the statistical analysis results concerning the chinaberry extract against J_2 of *M. javanica*, the best inhibitory effect, by 73%, was observed at a concentration of 7000 ppm on the fourth day. Following that, the concentration of 6000 ppm on the fourth day demonstrated a mortality rate of approximately 64% (Fig. 2). The concentration of 1000 ppm on the second day did not show a significant difference compared to the control; however, this concentration on the fourth day exhibited a significant difference with a mortality rate of 25% compared to the control treatment. The concentration of 3000 ppm on the fourth day showed an equivalent mortality rate when compared to the concentrations of 4000 and 5000 ppm on the second day (Fig. 2). The results of probit analysis showed that the LC_{50} of the two studied extracts have significant differences with each other (ratio= 0.8860). The median lethal concentrations for *M. azedarach* determined 5044 ppm with a 95% confidence interval and 5694 ppm for *E. camaldulensis* with a 95% confidence interval (Table 1).

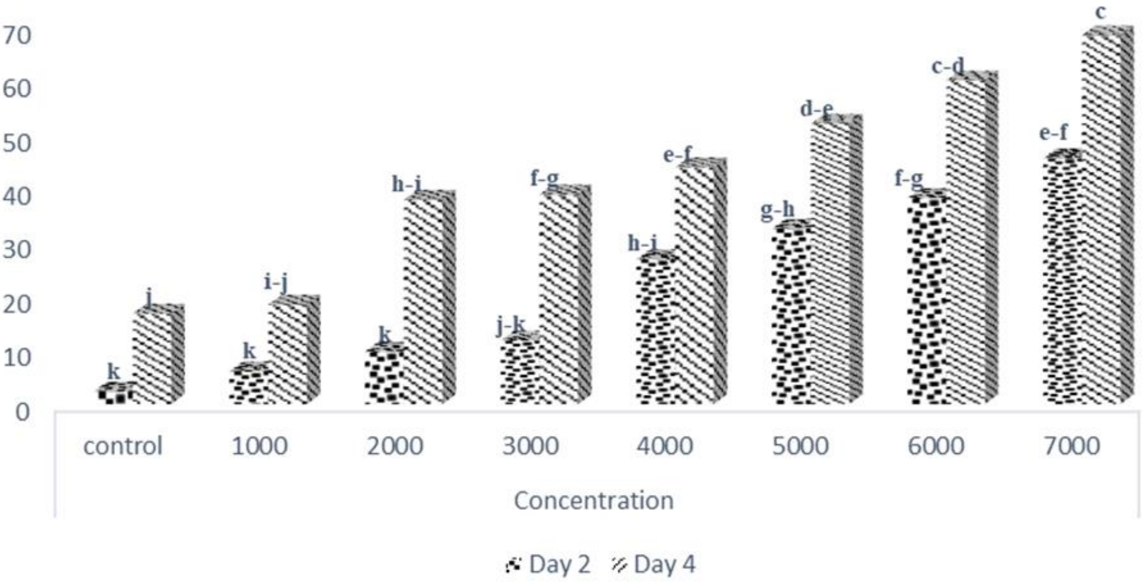

Fig. 2. Effect of different concentrations of aqueous leaf extract of *Eucalyptus camaldulensis* and exposure time on mean mortality rate of second stage juveniles (J_2) of root-knot nematode (*Meloidogyne javanica*). Means by the same letter are not significantly different at $P \leq 0.01$.

Table 1. Probit analysis results for LC_{50} after four days of exposure with leaf extracts.

Treatment	NT	LC50	CI 95%	Slope ± SE	Chi-square
Eucalyptus camaldulensis	240	5694	5292-6116	2.5 ± 0.2	2.354
Melia azedarach	240	5044	4494-5789	2.2 ± 0.2	7.142

LC_{50}: Median Lethal Concentration, NT: Number of Treatment, CI: Confidence Interval, SE: Standard Error.

Discussion

In the present study, the treated Petri dishes with plant extracts showed a significant difference of mortality on J_2 of *M. javanica* with compared to the control treatment, which indicating the parricidal activity of chemical compounds in eucalyptus and chinaberry extracts against this species. This subject has been previously reported for instance, it was observed that using a 20% concentration of aqueous eucalyptus extract against *M. incognita* resulted in a 100% mortality rate for J_2 and a 94.5% inhibition of egg hatching after 24 hours (Shazdeh Ahmadi, 2024). The eucalyptus extract was capable of reducing the gall index and egg mass count by 68% and 52%, respectively, compared to the control (Krif et al., 2024). In another study, extracts from various parts of the chinaberry plant, including leaves, fruit, and seeds, at concentrations of 2% and 4%, caused immobilization and 100% mortality of J_2 of *M. incognita* after 72 hours, and also significantly reduced the number of galls and egg mass (Ardakani, 2012). Based on the mortality results for the *M. javanica* juveniles after application of plant extracts it can be concluded that this effect is due to the presence of certain nemato-toxins compounds or phytochemicals in aqueous solvent. Previous studies indicated that eucalyptus leaves contain compounds such as 1,8-cineole (Eucalyptol), citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, α-pinene, aromadendrene, γ-terpinene, and α-terpineol. Among these, the highest concentrations are comprised of two compounds, Eucalyptol and α-pinene, which belong to the categories of terpenoids and terpenes, respectively. These chemical compounds exhibit antimicrobial properties and inhibitory activity on the enzyme acetylcholinesterase, like many chemically synthesized nematicide compounds that have an inhibitory effect on the activity of the acetylcholinesterase enzyme. (Dellacassa et al., 1990; Liu et al., 2008; El-Baha et al., 2017; Almas et al., 2021). In a study, it was found that extracts of some plants from the *Asteraceae*, *Polygonaceae* and *Rutaceae* families contain compounds with an inhibitory effect of over 80% on the activity of the acetylcholinesterase enzyme, and two species *Ruprechtia apetala* and *Trichocline reptans* completely inhibited the activity of this enzyme (Carpinella et al., 2010). Other previous reports have shown the anti-nematoid properties of chinaberry extract which contains compounds such as p-hydroxybenzoic acid and coumaric acid in significant amounts in the leaves and fruit. These phenolic compounds are important due to their extensive biological activities, including antioxidant, antifungal, antibacterial, antiviral, paralytic, and nematicidal properties (Manuja et al., 2013; Garg et al., 2020; Deng et al., 2023). In both the examined extracts, it was found that concentration and time are both key and determining factors, as higher concentrations demonstrate their parricidal effects in a shorter period. So that, concentration of 6000 ppm of chinaberry showed the same results as the 7000 ppm, but with a 48-hour delay. Specifically, eucalyptus extract at the concentrations of 4000 to 7000 ppm on day four, observed an approximately one and a half times increase in mortality compared to the second day. The mortality rate decreased from 90% to 50% at reducing the concentrations of 7000 to 5000 ppm. In previous studies on the extract of chinaberry, it has been demonstrated that the application of a 10% concentration of aqueous chinaberry leaf extract against the root-knot nematode *M. chitwoodi* resulted in 100% mortality of J_2 after a 24-hour exposure period. In contrast, a 5% concentration of this extract was only able to kill 49% of the J_2 within the same time frame (Uludamar, 2024). The median lethal concentrations of each extract, LC_{50} values, had the positive correlation with the time exposure periods. However, the lower concentrations showed significant parricidal results with increased exposure time to these extracts. Reducing the concentration from 10% to 5% yielded similar results regarding mortality and inhibition of J_2 and egg hatching over a 72-hour period (Shazdeh Ahmadi, 2024). The efficiency of the concentration of an extract is related to its chemical compounds, which must reach lethal levels after being absorbed or adsorbed by the nematode. As in the previous study the only Italian extract of the *M. azedarach* had notable nematicidal activity against *M. incognita* compared to the Algerian extract. Differences in chemical composition based on geographical distribution leads to different nematicide effects (Aoudia et al., 2012). The presence of chemical compounds with insecticidal properties, growth inhibition, and antifeedant effects in extract of *M. azedarach*, as well as the presence of polyphenols in extract of *E. camaldulensis*, suggests that the high parricidal activity of these extracts may be attributed to the presence of these compounds (Khan et al., 2019; Almas et al., 2021; Deng et al., 2023). Observations indicate that treated nematodes with eucalyptus and chinaberry were paralyzed in a straight shape, in a similar result as reported by Aissani et al. (2013) that treated nematodes with horseradish plant. Previous studies have shown that the reaction of a nucleophilic addition of a cuticle amino or thiol group to an α, β-unsaturated carbonyl leads to evident cuticle damage and leakage of the internal fluid nematode material (Aoudia et al., 2012).

Conclusions

The present results highlight the significance and special role of plant extracts in the sustainable management of plant-parasitic nematodes. As the findings showed, the native extracts of *E. camaldulensis* and *M. azedarach* had remarkable efficacy on J_2 mortality. In agreement with previous studies, it observed that the mortality rate was higher with increasing concentration and time of extract exposure. The results obtained in the present study suggest that the use of aqueous leaf extracts from eucalyptus and chinaberry plants could represent a suitable and reliable strategy for managing root-knot nematodes. Over time, there has been increasing acceptance and attention towards plant compounds and products, including extracts and essential oils, as factors for controlling and managing plant pests and diseases. The use of plant extracts not only addresses the issues arising from the application of chemical nematicides but also has the potential to enhance and improve the organic matter in the soil. Understanding the mode of action of the chemical compounds of plant extracts is of practical importance for developing new formulations and the compatibility of their combination with other biological agents for nematode control.

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Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

S. Torabi: Laboratory works & writing original draft.

A. Zeynadini Riseh: Supervision, methodology, writing, reviewing & editing.

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