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Nematicidal effect of powder extractions of different coloured radish seeds against *Meloidogyne incognita* on tomato

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Abstract

The study aimed to evaluate the nematicidal effect of powder extracts of different coloured radish seeds against *Meloidogyne incognita* on tomato (Gülizar F1, susceptibile to root-knot nematode) under controlled conditions. Extractions were obtained from radish seeds of different colours (white, black, red, yellow) by using ethanol and acetone solvents. The experiment was carried out using 2, 4 and 6 g powder/plant application of the extracts in the pot. The experiment was set up in a random plot design with 5 replication for each radish seed extract and concentration. Radish seed powder was applied one week after nematode inoculation (1000 *M. incognita* eggs). After 50 days, the number of galls and egg masses on the roots were counted. It was determined that radish colour, extraction solvents and concentrations of extracts differed significantly for their nematicidal effects. The mean number of galls and egg masses was found to be 56 units in the negative control. Compared to the negative control, all treatments and concentrations decreased the number of galls and egg masses. The number of galls and egg masses was lower in acetone extract than in ethanole extract. The nematicidal effect was higher in yellow and red radish seeds powder application. The highest nematicidal effect was determined at 6 g powder/plant application. While the mean number of galls was 1.4 unit in the yellow seed powder application at a concentration of 6 g/plant of the extract prepared with acetone solvent, it was found to be 3.0 units in the red seed powder application at 6 g/plant of the acetone extract. The number of egg masses was 1.0 unit in the yellow seed powder application, while it was 2.8 units in the red seed powder application at 6 g/plant of the acetone extract. The acetone extract of radish seed powder can be used as an alternative to chemicals in the root-knot nematodes control.

Keywords: Radish, Seed powder, Acetone extract, Ethanol extract, Nematicidal effect, Root-knot nematode

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INTRODUCTION

Root-knot nematodes are among the most destructive nematode groups. The galls formed as a result of feeding on the roots of the root-knot nematodes, prevent the plant roots from absorbing nutrients and water from the soil. As a result of this, yellowing, wilting and stunted growth occur in the plant. In addition, root-knot nematodes suppress the host plant's defences, making the plant more susceptible to attacks by other plant pathogens (Goverse and Smant, 2014). *Meloidogyne incognita*, the most common root-knot nematode species in vegetable fields worldwide, can attack the roots of more than 3000 agricultural crops (Sahebani et al., 2011). Synthetic nematicides have been used to protect moderate-to-high-value crops in intensive production systems throughout most of the twentieth century. In the last decades, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides (Nyczepir and Thomas 2009; Sorribas and Ornat, 2011). Recently, plant extracts have become an environmentally friendly alternative to chemical pesticides in pest control (Aji, 2024). Most plants produce various secondary metabolites during their development. These compounds are generally antioxidant phenolic compounds with redox properties (El-Abbassi et al., 2012). Brassicaceae plants have been used to reduce plant parasitic nematode population levels for biological fumigation, cover and/or poor host characteristics (Fourie et al., 2016). When Brassicaceae seeds and green parts were compared together, they were found to have higher glucosinolate levels and were reported to be more advantageous due to lower loss of glucosinolate degradation products (Lazzeri et al., 2004). Brassicaceae seed treatments with a 100% herbal product that provides significant environmental benefits as an alternative to chemicals, as well as higher renewability, biodegradability, positive impact on atmospheric CO2 levels and overall potential on nematodes, could open a new perspective in the control of plant parasitic nematodes (Lazzeri et al., 2009).

Radish is a member of the Brassicaceae (Cruciferae) family and its scientific name is *Raphanus sativus* L. Radish plant contains glycocinolate compounds in cells as a result of decomposition of plant cells and hydrolysis of glycocinolates, toxic isothiocyanates are formed (Vallejo et al., 2004; Sandler et al., 2015). Isothiocyanates have a lethal effect on nematodes (Zasada and Ferris 2004). Radish is reported to be resistant to nematode reproduction and the formation of root galls (Pattison et al., 2006). It is also reported to be a very good trap plant for root-knot nematodes and a biofumigant when applied to soil as a green manure (Melakeberhan et al. 2008). However, the consistent suppressive effect of allelopathic extracts or ground material on nematode populations has also been attributed to the nematostatic effect of released ammonia, hidrolysis products of glycosinolate content (Mazzola et al., 2007, 2009). Aydınlı and Mennan (2018) found that in biofumigation plots treated with radish and arugula, *M. arenaria* infections in tomatoes were significantly reduced. Radwan et al. (2012) reported that the application of powdered *R. sativus* seeds suppressed root galling in tomatoes by 78.3%. Shalaby et al. (2021) determined that the application of *R. sativus* seeds significantly suppressed nematode development in *M. incognita*-infected pepper compared to the control. In addition, Zasada et al. (2009) reported that seed particle size altered the nematoxic effect. They found that ground *Sinapis alba* seeds had a higher suppressive effect on *Pratylenchus penetrans* compared to larger particles. This indicates that smaller particles are evenly distributed in the soil profile, whereas larger particles create pockets of toxicity where not all nematodes are exposed, in comparison, *Brassica juncea* seeds were found to have greater nematode toxicity than *S. alba*. The 2.5% and 10% *S. alba* were required for 100% suppression of *M. incognita* and *P. penetrans*, respectively, whereas 0.5% would be sufficient for *B. juncea*.

In Türkiye, there is a limited number of studies on seed applications of nematicidal plants on soil. However, it is known that different radish varieties are cultivated in Türkiye. It has been determined that these varieties are named according to their seed colours. Whether this colour difference will make a difference in the nematicidal effect has been the subject of research. In this study, it was aimed to determine the nematicidal effect of acetone and ethanole extracts obtained from the powder of different coloured radish seeds (yellow, black, white and red) on the root knot nematode, *M. incognita* under controlled conditions.

MATERIALS AND METHODS

Material

In this study, 4 different radish seeds of local varieties with white, yellow, red and black colours were used as material. Within the scope of this study, seeds were purchased commercially from Intfa Agricultural Shopping Center (Konya, Türkiye). The ISP isolate, which continues mass production under climate chamber conditions (24±1 °C, 60%±5% RH), was used as the population of the root-knot nematode, *M. incognita* (Göze et al., 2022). As tomato material, nematode susceptible Gülizar F1 variety was used which was purchased commercially from Olympos Seedling (Kumluca, Antalya, Türkiye).

Methods

Preparation of *Meloidogyne incognita* inoculum

Eggs of *M. incognita* were extracted from infected tomato roots using 1.5% sodium hypochlorite (NaOCl) as described by Hussey and Barker (1973). Eggs were poured into a 75 µm sieve and collected on in a 5 µm sieve. At this stage, they were washed with tap water to remove the sodium hypochlorite and the number of eggs was counted under a binocular microscope at 40x magnification, appropriate dilution was made with distilled water and nematode suspensions were prepared according to the study. They were kept in the refrigerator (+4ºC) until the experiment was established.

Preparation of seed extracts from radish seeds

Ethanol and acetone solvents were used in the study. Twenty g of each radish seed was separated and crushed in a spice grinder until it was powdered. Then 200 ml of different solvents (acetone (99%) or ethanol (96%) were added to the powder samples and kept at room temperature for 24 hours. At the end of this period, the samples were filtered using filter papers. The solvents in the filtrate were evaporated by drying in an evaporator (Kabil and Adam, 2020).

In pot experiment

The study was carried out under controlled conditions (24±1°C, 60%±5% RH) in the climate chamber of Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection. The tomato seedlings were transplanted into 250 ml plastic pots containing 300 g of sterile soil mixture (68% sand, 21% silt and 11% clay). The study was established in a randomized plots experimental design with 5 replicates for each radish seed extracts (acetone and ethanol) and concentrations (2, 4 and 6 g/plant). One week after transplanted, 1000 *M. incognita* eggs with 1 ml of water were inoculated into holes drilled near the root. One week after nematode inoculation, radish seed powder extracts according to the experiment were applied at doses of 2, 4 and 6 g/plant in pot (Shalaby et al., 2021). It was then allowed to mix thoroughly with the soil. While only nematode-inoculated plants were used as a negative control, nematicide with the active ingredient Fosthiazate (Nemathorin, Sygenta) was used as a positive control at a dose of 0.3 ml/plant. The experiment was terminated 50 days after inoculation. Afterwards, the plants were uprooted and washed with clean water to remove the soil from the roots and the number of gall and egg mass in the roots were counted.

The data obtained as a result of the study were analyzed in the standard analysis of variance technique (ANOVA) using the GLM procedure in the SAS (2009) statistical package program, and the differences between the means were determined according to the LSD multiple comparison test.

RESULTS AND DISCUSSION

In this study, it was determined that radish seed variety (V), extracts (E) and concentrations (C) differed significantly ($p<0.01$) on the galls caused by *M.incognita* on tomato roots. It was also found that E X V, E X C, V X C and E X V X C interactions were significant ($p<0.01$) (Table 1).

Table 1. The variance analysis of interaction on galling of tomato roots

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Extraction(E)	1	42.320	42.320	22.39**
Seed variety (V)	3	499.220	166.40667	88.05**
Concentration (C)	4	78139.680	19534.920	10335.9**
E X V	3	174.280	58.09333	30.74**
V X C	4	118.280	29.570	15.65**
V X C	12	578.08	48.17333	25.49**
E X V X C	12	872.52	72.71	38.47**
Error	160	302.40	1.89	
General	199	80726.780		

In the present study, compared to the negative control, all treatments and concentrations decreased the number of galls. The difference between acetone and ethanol extracts was found to be significant in terms of gall number. The number of galls was lower in acetone extract than in ethanol. The suppressiveness of gall number increased with high concentrations. The highest suppressiveness on the number of galls was found in 6 g/plant in a soil application. When the general mean was evaluated, when 4 g/plant was applied to the soil in terms of gall number, a similar effect was observed as the positive control. However, the mean number of galls in the 6 g/plant in soil application (2.4 galls/ plant) was found less than the positive control (6.2 galls/ plant). The lowest number of galls was determined in the yellow radish seed powder extractions. The highest number of galls was found in white and black seed powder extractions (Table 2).

It was determined that radish seed variety (V), extracts (E) and concentrations (C) differed significantly ($p<0.01$) on the number of egg masses in tomato. It was also found that E X V, E X C, V X K and E X V X C interactions were significant ($p<0.01$) (Table 3).

The mean number of egg masses was lower significantly in acetone extract (16.17 units) than in ethanol (17.42). It was determined that the suppressiveness on the number of egg masses was higher in 6 g/plant in a soil application. When compared with the negative control, all treatments and concentrations decreased the number of egg masses. When the general mean was evaluated, the egg mass number in 6 g/plant application (1.8 unit) was found less than the positive control (4.4 units). The lowest mean number of egg masses in tomatoes was obtained from yellow and red radish seed powder extraction which had similar suppressive effects. The highest number of egg mass was found in black radish powder extraction (Table 4).

The nematicidal effect of different coloured radish seed powder ethanol and acetone extracts on root-knot nematode were evaluated and significant suppression was determined in all extracts compared to the negative control. The nematicidal properties of radish have been reported in different previous studies. *Raphanus sativus* has a high ability to control nematodes present in the soil, such as *Meloidogyne hapla*, it is a biosynthesis plant for toxic compounds of nematodes (Jaafar et al., 2020). Shalaby et al. (2021) reported that *Brassica rapa, Eruca sativa, Juniperus communis, Lepidium sativum, R. sativus* and *Sinapis alba* seed powders caused a significant reduction in nematode population in pepper infected with *M. incognita* under greenhouse conditions, but *S. alba* was the most effective. El-Shaefeey et al. (2023) reported that mixing radish seed extract into the soil before nematode inoculation in eggplant reduced the number of *M. javanica* galls on the roots.

Table 2. Effect of different coloured radish seed powder extractions on the number of galls in tomato roots

Application	Seed variety	Number of galls						Mean
		Concentration g/plant in soil						
		2	4	6	Negative Control	Positive Control	ExV	
Acetone	White	18.2	7.4	3.8	56.0	6.2	18.32	17.23 b
	Black	25.4	10.2	1.8	56.0	6.2	19.92	
	Yellow	4.2	1.0	1.4	56.0	6.2	13.76	
	Red	12.8	6.6	3.0	56.0	6.2	16.92	
Ethanol	White	23.4	11.8	1.2	56.0	6.2	19.70	18.15 a
	Black	16.2	10.6	5.6	56.0	6.2	18.92	
	Yellow	22.0	2.2	1.2	56.0	6.2	17.52	
	Red	14.6	4.2	1.2	56.0	6.2	16.44	
Mean		17.1 B	6.75 C	2.40 D	56.0 A	6.2 C		

Lowercase letters indicate differences between extractions in the same column, and uppercase letters indicate differences between concentrations in the same row.

Table 3. The variance analysis of interaction on egg masses in tomato roots

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value
Extraction (E)	1	78.125	78.125	27.80**
Variety (V)	3	305.775	101.925	36.27**
Concentration (C)	4	80889.27	20222.3175	7196.55**
E X V	3	191.815	63.93833	22.75**
E X C	4	198.15	49.5375	17.63**
V X C	12	425.25	35.4375	12.61**
E X V X C	12	668.61	55.7175	19.83**
Error	160	449.6	2.81	
General	199	83206.595		

Table 4. Effect of different coloured radish seed powder extractions on the number of egg masses in tomato roots

Application	Seed variety	Concentration g/plant in soil						Mean
		2	4	6	Negative Control	Positive Control	ExV	
Acetone	White	13.2	5.2	2.0	56.0	4.4	16.16	16.17 b
	Black	20.8	11.4	1.8	56.0	4.4	18.88	
	Yellow	4.2	2.0	1.0	56.0	4.4	13.56	
	Red	12.6	4.6	2.8	56.0	4.4	16.08	
Ethanol	White	22.2	11.1	1.2	56.0	4.4	18.96	17.42 a
	Black	14.2	11.0	4.0	56.0	4.4	17.92	
	Yellow	21.0	2.8	1.4	56.0	4.4	17.12	
	Red	14.0	2.6	1.4	56.0	4.4	15.68	
Mean		15.28 B	6.33 C	1.98 E	56.0 A	4.4 D		

Lowercase letters indicate differences between extractions in the same column, and uppercase letters indicate differences between concentrations in the same row.

The study revealed that extraction method and concentration were important depending on seed colour. The nematicidal effect of acetone extract was higher than ethanol. In addition, The nematicidal effect of yellow and red radish seed extracts was higher than white and black. To determine the differences in these nematicidal properties, their compounds need to be identified. Flavonoids, saponins, and tannins present in radish have also demonstrated antioxidant, antimicrobial and antibacterial activity (Ahmad et al., 2012; Lim et al., 2019; Muthusamy & Shanmugam, 2020). Goyeneche et al. (2015) reported that the most abundant free and bound phenolic compounds in the roots and leaves of red radish are pyrogallol and vanillic acid; and epicatechin and coumaric acid, respectively. Radish produces isothiocyanate that is break down product of glicosinolates and this eliminates pathogens in the soil including fungi (Melakeberhan et al., 2008). The α-amylase inhibition activity as well as antibacterial activity of radish seed and rapeseed were also significantly high (Khatiwada et al., 2018).

In our study, although the nematicidal effect of ethanol extract was found to be lower than acetone, it was observed that significantly suppressed galls and egg masses compared to the negative control. Ahmad et al. (2012) reported that ethanolic and methanolic extracts of *R. sativus* seeds were effective against the bacterial species they used. Zaidat et al. (2020) reported that *Peganum harmala* L., *Raphanus raphanistrum* L., *Taxus baccata* L., *Sinapis arvensis* L., and *Ricinus communis* had high nematicidal potential on *M. incognita* when applied in a methanolic solvent. Aissani and Sebai (2022) found that radish methanol extract was rich in 4-methylthio-3-butenyl isothiocyanate and had high nematicidal activity on *M. incognita*. Törün et al. (2017) determined that the antimicrobial activity of methanol extract of *Echinophora tenuifolia* L. and boiled water extract of *R. sativus* was more effective than ethyl acetate extract. Göze Özdemir (2024) investigated the nematicidal effect of milk thistle leaves and seeds prepared with different solvents on *M. incognita*. No statistical difference could be determined between the solvents (acetone, ethanol, distilled water) in the number of galls and egg masses in seed extraction. On the contrary, a difference was found between acetone and ethanole extract in this study.

It was observed that the nematicidal effect increased as the concentration increased and it was determined that 6 g/soil concentration was more effective and a suppression above 60% was determined. Radwan et al. (2012) reported that 5 g/kg radish seed powder treatment reduced root galling in tomatoes by 78%. Göze Özdemir (2022) found that the control effect of 6 g/plant radish seed powder alone on *M. incognita* gall and egg masses in tomato and cucumber roots was similar to the control effect of arugula (2 g/plant) + radish (2 g/plant) and cress (2 g/plant) + radish (2 g/plant). Ibrahim et al. (2007) reported that fenugreek and lupin seed powder caused reduction (92.2–98.6%) in root galls and egg masses of *M. incognita*, while treatments of acacia seed powder and camphor dried leaves induced 54.6–66.3% reduction in root galls and egg masses on infected sunflower plants. Incorporated powder seeds of pig bean (*Canavalia ensiformis*) into the soil reduced galls and egg mass of *M. incognita* on tomato plants by 48% and 64%, respectively, with the application of 10 g/kg soil (Silva et al. 2002).

CONCLUSION

From this study, it is concluded that the extracts of radish shows promising nematicidal activity and offer possibilities as non-chemical alternatives for the management of *M. incognita*. It is envisaged that yellow and red seed extracts of radish can be used as an alternative to chemicals in the control of root-knot nematodes. Yellow and red radish nematicidal compound(s) are unknown. Therefore, We should be determined and purified them. Once identified, they or their derivatives can be artificially synthesized which as a source of nematicidal agents in future pesticide design and development. Additionally, microplots and field studies are required.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

Conceived & designed the experiment, Fatma Gül Göze Özdemir & Fadimana Maril; Performed experiment, Fadimana Maril & Harun Çimenkaya; Formal data analysis & Visualization of the data Bekir Tosun, Writing-original draft and data curation, Fatma Gül Göze Özdemir, Fadimana Maril, Bekir Tosun & Harun Çimenkaya.

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