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Original article (Orijinal araştırma)

Screening of the nematicidal potential of some spice extracts against root-knot nematode, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae)[1]

Bazı baharat ekstraktlarının *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae)'ya karşı nematisidal potansiyellerinin araştırılması

Hissein Mahamad HAROUN[2] Gökhan AYDINLI[3] Sevilhan MENNAN[2*]

Abstract

Experiments were conducted in the laboratories and greenhouses of Plant Protection Department, Agricultural Faculty, Ondokuz Mayıs University in 2018 and 2019 to investigate the nematicidal effects of aqueous extracts of 13 spices on *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae). Spice extract concentrations of 0.5, 1 and 2% were tested in laboratory experiments for inhibition of egg hatching, mortality and immobility of second-stage juveniles (J2s). When used at a concentration of 2%, clove, *Syzygium aromaticum* L. (Myrtales: Myrtaceae) caused the greatest immobility and mortality of J2s. The extracts had a lesser effect on J2s than the egg hatching. For the pot experiment, five effective spices extracts were selected based on the laboratory experiments. These extracts were applied at 2% to 200 g of soil inoculated with 3,000 nematode eggs then susceptible tomato seedlings were transplanted into the soil. Forty-five days after inoculation, the gall index and the quantity of nematode eggs on roots were determined and reproduction factor of nematode calculated. All extracts, except cumin, *Cuminum cyminum* L. (Apiales: Apiaceae), reduced root gall index and the reproduction factor when compared to control. Basil, *Ocimum basilicum* L. (Lamiales: Lamiaceae) extract reduced nematode reproduction the greatest degree, followed by turmeric, *Curcuma longa* L. (Zingiberales: Zingiberaceae) and clove extracts.

Keywords: Egg hatching inhibition, J2 mobility, J2 mortality, nematicidal effect, spice extract

Öz

On üç baharattan elde edilen sulu ekstraktların *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine nematicidal etkilerini belirlemek amacıyla Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü Nematoloji Laboratuvarı ve seralarında, 2018 ve 2019 yıllarında denemeler yürütülmüştür. Laboratuvar çalışmalarında ekstraktların 3 farklı konsantrasyonunun (%0.5, 1, 2) yumurta açılımı, ikinci dönem larvaların (J2) hareketi ve canlılığına etkileri araştırılmıştır. J2'lerin hareketi ve canlılığına en fazla etkiyi %2'lik konsantrasyonda karanfil, *Syzygium aromaticum* L. (Myrtales: Myrtaceae) sağlamıştır. Genel olarak ekstraktların yumurta açılıma etkisi, larvalara olandan fazladır. Laboratuvar denemeleri sonucunda etkili bulunan 5 baharat ekstraktı saksı denemeleri için seçilmiştir. Ekstraktların %2 konsantrasyonları 3000 nematod yumurtası bulaştırılmış 200 g toprağa uygulanmış, sonrasında hassas domates fideleri şaşırtılmıştır. Nematod bulaştırılmasından 45 gün sonra, kök başına yumurta sayısı ve ur skalası bulunmuş, üreme faktörü hesaplanmıştır. Kimyon, *Cuminum cyminum* L. (Apiales: Apiaceae) hariç ekstraktların tamamı, ur skalası ve üreme faktöründe kontrole kıyasla azalmaya neden olmuştur. Nematodun üremesini en fazla azaltan baharat fesleğen, *Ocimium basilium* L. (Lamiales: Lamiaceae) olmuş onu zerdeçal, *Curcuma longa* L. (Zingiberales: Zingiberaceae) ve karanfil izlemiştir.

Anahtar sözcükler: Yumurta açılımı engelleme, J2 hareket, J2 ölüm, nematisidal etki, baharat ekstraktı

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[2] Ondokuz Mayıs University, Agricultural Faculty, Department of Plant Protection, 55100, Samsun, Türkiye
[3] Ondokuz Mayıs University, Vocational High School of Bafra, 55400, Bafra, Samsun, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: smennan@omu.edu.tr
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Introduction

Root-knot nematodes (*Meloidogyne* spp.) (RKNs) are sedentary endoparasites of over 3,000 plant species, cause significant economic losses and can be found in almost all agricultural soils and climates. RKNs are one of the most important nematode taxa that reduce the yield and quality of agricultural products in tropical and subtropical regions (Trudgill & Blok, 2001; Abad et al., 2003; Kiewnick & Sikora, 2006). The unusual root gall formation that alters water and nutrient uptake is the most obvious morphological response of susceptible plants to infection with RKNs and the name of the genus comes from this symptom (Jones et al., 2013). Like other plant-parasitic nematodes, RKNs reduce plant productivity while predisposing plants to fungal and bacterial infections (Zhou et al., 2016). RKNs infect a wide range of horticultural and field crops, especially vegetables, causing an estimated $157x10^9$ USD in annual damage worldwide (Abad et al., 2008). RKNs cause a 10% decline in annual vegetable yields (Koenning et al., 1999). However, yield loss in susceptible plants to this pest, such as tomatoes can reach 68% (Padilla-Hurtado et al., 2022).

Given their economic importance, there is a growing need for long-term management strategies to control RKNs. Cultural methods are widely used but have major limitations due to their broad host range and the presence of mixed populations of different RKN species in the field (Trudgill & Blok, 2001; Xiang et al., 2018). RKN-resistant cultivars have proven to be a useful management tool, but there are few commercially available resistant cultivars and the existence of resistance-breaking virulent populations has also been documented in many countries (Roberts, 1995; Devran & Söğüt, 2010; Xiang et al., 2018; Hajihassani et al., 2020). Given there are few effective chemicals that can be used on a large scale against plant-parasitic nematodes, and because resistant plant cultivars are not available for many species, they are among the most difficult pests to control (Jones et al., 2013). High molecular weight soil fumigants, carbamates and organic phosphorus compounds are commonly used for control, but several of these chemicals have been banned or restricted because of their broad spectrum of activity. Most of the nematicides are highly toxic, carcinogenic and leave residues in harvested products. They also have significant adverse effects on the environment, natural life, humans and animals (Dutta et al., 2019; Ebone et al., 2019). Given the negative effects of nematicides and the lack of supply of resistant plant cultivars, studies on alternative management methods have attracted considerable attention in recent years. The use of plant extracts as an alternative to synthetic pesticides for the management of RKNs has gained importance. Numerous plant species from 57 families, including Asteraceae, Lamiaceae, Lauraceae, Myrtaceae and Rutaceae, may contain nematicidal compounds (Andrés et al., 2012). The use of plant extracts against RKN has shown their efficacy in previous studies (Javed et al., 2007; Hassan et al., 2013; Curto et al., 2015; Xia et al., 2019). Some of the plant extracts are already used commercially for RKN management, especially in organic farming (Zaidat et al., 2020). Spice plants are also known to contain components that have a negative impact on nematodes (Oka, 2001; Abbas et al., 2009; Ntalli & Caboni, 2012; Nile et al., 2017; Zaidat et al., 2020).

Spices have been used for many years as medicinal materials, in religious rituals, in cosmetics and perfumery, or as food. They have also been tested for their potential use as pesticides. Spices obtained by drying various plant parts such as roots, leaves and seeds, may be toxic to nematodes. Many studies show that extracts and oils derived from spice plants have negative effects on nematodes by inhibiting egg hatching, causing second-stage juvenile (J2) immobility, or being lethal (Oka et al., 2000; Ibrahim et al., 2006; Abbas et al., 2009; Aydınlı & Mennan, 2014; Youssef et al., 2015; El-Nagdi Wafaa et al., 2017). Therefore, in this study, the nematicidal potential of aqueous extracts from 13 spices plants for management of the root-knot nematode *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) was investigated in the laboratory and in pot experiments. The effects of spice extracts on egg hatching, J2 mobility and mortality of *M. arenaria* were studied in the laboratory. Then, five most effective extracts were selected and used to study the effects of *M. arenaria* damage on tomato plants in pot experiments in a greenhouse.

Materials and Methods

Nematode inoculum

Meloidogyne arenaria was used in the study because it is the most abundant nematode species in greenhouses in the Black Sea Region of Türkiye (Aydınlı & Mennan, 2016). The population of the nematode species required for the study was obtained from nematode-susceptible tomato cultivars of Rio Grande (May Seed Company, Bursa, Türkiye) grown continuously as a mass culture in the greenhouses of the Nematology Laboratory of the Department of Plant Protection of Ondokuz Mayıs University (Aydınlı & Mennan, 2016).

The species of the root-knot nematode population was confirmed using morphological and biochemical methods. Female perineal patterns were used for morphological diagnosis (Taylor & Netscher, 1974), and the esterase enzyme phenotype was used for biochemical diagnosis (Esbenshade & Triantaphyllou, 1985). Females for both methods were collected from infested tomato plant roots using a stereomicroscope (Nikon SMZ1500). After evaluation of the perineal patterns of the females and the esterase enzyme phenotypes, it was confirmed that the root-knot nematode population used in the study was *M. arenaria*. The eggs and J2s of *M. arenaria* were obtained from this mass culture. For this purpose, tomato plants in mass culture pots were removed; the roots were washed with water, cut into 1-2 cm long pieces, and shaken for 3-5 min in a glass flask containing 0.5% NaOCl. This solution with the roots was sieved through a 200 mesh (75 µm) and 500 mesh (25 µm) sieve and the eggs in the lower sieve (500 mesh) were collected in a glass beaker (Hussey & Barker, 1973) and then counted under the stereo microscope. The J2s were collected daily from the eggs and stored at 15°C. The juveniles used for the experiments were less than 5 days old.

Preparation of the aqueous spice extract

For the laboratory experiments, 13 spice species were used (Table 1). The spices were purchased (Kaan Baharat A.Ş., Rize, Türkiye) and a 10% (w/v) stock solution of each spice was prepared. In a shaker, 10 g of spice were mixed with 90 ml of distilled water and shaken at 100 rpm in the refrigerator (4°C) (Heidolph, Unimax 2010). After 24 h in the shaker, the spice-water mixture was passed through a muslin cloth, then a 38-µm sieve, and lastly into a beaker. The supernatants were collected using Whatman No. 1 filter paper, transferred to dark plastic bottles, and kept refrigerated until as a stock solution (Oka et al., 2006). Stock solutions were used to make three concentrations (0.5, 1 and 2%) for each spice.

Laboratory experiments

Laboratory experiments were conducted to investigate the nematicidal effects of 0.5, 1 and 2% aqueous extracts of spice on egg hatching, J2 mobility, and mortality of *M. arenaria*.

Effect of spice extracts on egg hatching

The spice extract stock solution was immediately passed through a sterile 0.2 m syringe filter before use. All in vitro experiments were performed in 48-well cell culture plates (Sigma SIAL0548). Using a micropipette, 100 eggs, extracts and water were added to each well. As a result, the final volume of the prepared concentration was adjusted 100 µl. Each treatment was repeated four times. For 7 days, the plates were kept in a dark environment in an incubator at 24°C. To determine the effect of the treatments on egg hatching, the J2 and eggs in the wells of the plates were counted under a stereomicroscope at each day. The experiment was repeated once more under the same conditions (experiments 1 and 2). The inhibition rate of egg hatching was calculated at the end of the experiment by evaluating the unhatched eggs (Oka et al., 2000; Nile et al., 2017).

Table 1. Species, family, common name and plant part(s) from which the spice was made

Species	Family	Common name	Plant parts
*Anethum graveolens**	Apiacae	Dill	Fruit and leaves
Capsicum annium	Solanaceae	Chili pepper	Fruit
Cuminum cyminum	Apiaceae	Cumin	Fruit
Coriandrum sativum	Apiaceae	Coriander	Fruit and leaves
Curcuma longa	Zingiberaceae	Turmeric	Rhizomes
Helichrysium italicum	Asteraceae	Italian helichrysum, immortelle	Young shoots and leaves
Ocimium basilicum	Lamiaceae	Basil	Leaves
Piper nigrum	Piperaceae	Black pepper	Fruit
Prunus mahaleb	Rosaceae	Mahaleb cherry	Fruit
Rhus coriaria	Anacardiaceae	Sicilian sumac, tanner's sumac	Fruit
Syzygium aromaticum	Myrtaceae	Clove	Flower buds
Thymus vulgaris	Lamiaceae	Thyme	Young shoots and leaves
Zingiber officinale	Zingiberaceae	Ginger	Rhizomes

* Spice extracts were purchased from Kaan Baharat A.Ş., Rize, Türkiye.

Effect of spice extracts on J2 immobility and mortality

Tomato plants infested with *M. arenaria* were removed from mass culture and their roots were washed. Under a stereomicroscope, egg masses were collected from the roots with forceps to sterile water. The eggs were placed in an incubator at 24°C and checked every 2 days. Hatched J2s were collected and stored at 5°C until needed. J2s as young as 2 days old were used for extract applications (Ferris & Zheng, 1999; Oka et al., 2000). In 48-well plates, the effects of a 0.5, 1 and 2% aqueous spice extract on J2 immobility were studied using 100 J2 instead of eggs, as mentioned above. As controls, only water and J2s were used, with no extract application. After 48 h, the plates were examined under a stereomicroscope, and mobile and immobile J2s were counted and recorded (Zaidat et al., 2020). Treatments were applied to four replicates in an incubator at 24°C, and all experiments were repeated under the same conditions (experiments 1 and 2). To assess the effect of the aqueous spice extracts on J2 mortality, the extracts were removed with a micropipette and replaced with sterile water in the wells where J2s were counted. A second assessment was performed after 24 h, and they were classified as immobile and/or dead if the J2 was straight or slightly curved. The number of J2s in the sample was confirmed using small touches with a needle under a stereomicroscope, and the inactive J2s were considered dead. The treatment percentage mortality rate was calculated and compared to distilled water (Ferris & Zheng, 1999; Oka et al., 2000; Coltro-Roncato et al., 2018).

Pot experiment

The soil used in the pot experiments was heated for 150 min at 165°C for sterilization. For the experiments, the tomato cultivar Falcon (May Seed Company, Türkiye), which is known to be susceptible to root-knot nematodes, was used. Tomato seeds were sown and grown to the seedling stage with two to four leaves at a controlled temperature (25 ± 3°C). *Meloidogyne arenaria* eggs were obtained in the way described in the laboratory studies. After the laboratory studies, the five effective spices basil, clove, cumin, coriander, and turmeric were chosen for the pot experiments. The pot experiments consisted of seven applications with 5 spice extracts and negative and positive controls. Negative and positive controls were distilled water and nematicide (200 g/l ethoprophos), respectively. The stock solution of spice extracts (10% in 4 ml), 3000 nematode eggs (1,500 eggs/ml), and water (14 ml) were applied to 200 g of sterile sandy soil, resulting in a final concentration of spice extracts in the soil of 2%. For 1 week, soils were kept at room temperature (22-26°C). The soil was transferred to pots at the end of this period, and the susceptible tomato

seedlings were transplanted (Oka et al., 2006). The plants were grown in greenhouses at 25 ± 3°C applying daily routine requirements. The experiment was conducted using a randomized block design with eight replicates. Tomato plants were taken from pots 45 days after nematode inoculation and their roots were carefully cleansed. The gall index was determined using a 0-5 gall scale: 0, no galls; 1, traces of infestation with a few minor galls; 2, 25%; 3, 26-50%; 4 51-75%; and 5, >75% of the roots galled (Hussey & Janssen, 2002). The number of eggs in each root was counted under a stereomicroscope as reported before (Hussey & Barker, 1973). Subsequently, the reproduction factor (R_f) was calculated by the division of the final population of egg (P_f) by the initial population (3000 egg, P_i) (Oostenbrink, 1966).

Data analysis

The rates of egg hatching inhibition, the immobility and the mortality of J2s were expressed as a percent of total treatments. The percent inhibition in egg hatching was calculated by using the formula:

$$\% \text{ inhibition egg hatching} = ((C_0 - T_1) / C_0 \times 100)$$

where, C_0 is the number of juveniles hatched in control and T_1 is the number of juveniles hatched in each concentration of spice extract. In case of mortality, C_0 is the number of live nematodes in control and T_1 is the number of live nematodes after 24 and 72 h exposure (Khan et al., 2019). The raw data were $\log_{10}(x+1)$ transformed first to improve homogeneity for statistical analysis. The data obtained from the trials were evaluated in the SAS statistical program and Tukey's comparison test was applied to determine the means of different groups when variances were homogeneous ($P \leq 0.05$).

Results

Effect of spice extracts on egg hatching, J2 immobility and J2 mortality

Given there was no statistical difference between the values from the experiments 1 and 2, the results were combined and reported over eight replicates. The aqueous extracts of the 13 spices tested showed highly significant effects on egg hatching. It was found that all spice extracts inhibited egg hatching by 19.1-93.1% (Table 2). With increasing concentration, the rate of inhibition of egg hatching increased significantly. Two percent was used as the highest concentration; the inhibition rate of egg hatching was ranged from 33.3-93.1%. The lowest egg hatching inhibition rate was observed with immortelle extract (19.1%), followed by dill (19.4%) at concentrations of 0.5%. Pepper extract caused the highest inhibition of egg hatching at all concentrations, followed by basil and clove.

The effect of aqueous spice extract applications on larval immobility was evaluated with counts conducted 48 h after extract application; at the lowest concentration (0.5%) of spice extracts, the highest rate of immobile J2s was recorded for cloves at 16.8%, and the lowest rate was recorded for dill extract at 2.6% (Table 3). Coriander had the greatest effect after cloves (16.0%). Cloves were followed by sumac (12.5%), cumin (12.5%), black pepper (12.0%), thyme (11.6%), basil (9.8%), and hot pepper (8.0%), with no statistically difference ($P \leq 0.05$). At the 1% concentration of spice extract applications, the highest rate of J2 immobility was found in cloves at 27.9%, while the lowest rate of immobility was found in dill extract at 2.5%. Coriander (21.1%), thyme (20.1%), sumac (18.3%), cumin (18.3%), and basil (17.6%) had the highest immobility rates after cloves. Spice extracts, for which the highest and lowest J2 immobility rates were determined at concentrations of 0.5 and 1%, showed the same effect at a concentration of 2%. In general, as the concentration increased with each application of spice extracts, the J2 immobility rate also increased. Except for the applications of pepper, coriander, immortelle, basil and clove, the change in these increases was not statistically significant at all concentrations ($P \leq 0.05$).

Table 2. Inhibition rate of spice aqueous extracts at three concentrations on egg hatching of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	19.4	C de*	28.4	B c-e	35.8	A c-e
Capsium annium	Pepper	82.3	C a	86.1	B a	93.1	A a
Cuminum cyminum	Cumin	39.5	B b-d	51.9	AB a-d	59.3	A a-d
Coriandrum sativum	Coriander	64.0	A a-c	68.3	A ab	73.6	A a-c
Curcuma longa	Turmeric	49.5	A a-d	55.8	A a-d	59.6	A a-d
Helichrysium italicum	Immortelle	19.1	C de	29.1	B c-e	33.3	A c-e
Ocimum basilium	Basil	70.4	B ab	78.4	AB ab	83.4	A ab
Piper nigrum	Black pepper	61.9	A a-c	67.1	A ab	70.6	A a-d
Prunus mahlep	Mahaleb	51.3	A a-d	55.9	A a-d	60.9	A a-d
Rhus coriaria	Sumach	37.0	A b-e	46.4	A bc	52.1	A bc
Syzygium aromaticum	Clove	69.1	B ab	78.4	A ab	82.0	A ab
Thumus vulgaris	Thyme	51.1	A a-d	58.5	A a-c	65.5	A a-d
Zingiber officinale	Ginger	50.9	A a-d	53.1	A a-d	54.6	A a-d
Control	D. W.	0.00	A f	0.00	A f	0.00	A f

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at P ≤ 0.05. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

Table 3. Immobilization rate of spice aqueous extracts at three concentrations on J2s of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	2.6	B cd*	2.5	B de	3.8	A cd
Capsium annium	Pepper	8.9	C a-d	16.9	B a-d	22.9	A a-c
Cuminum cyminum	Cumin	12.5	A ab	18.3	A a-d	22.9	A a-c
Coriandrum sativum	Coriander	16.0	C a	21.1	B ab	32.9	A ab
Curcuma longa	Turmeric	6.1	B b-d	8.9	A b-e	10.5	A cd
Helichrysium italicum	Immortelle	2.8	C cd	4.3	B c-e	9.1	A cd
Ocimum basilium	Basil	9.8	C a-c	17.6	B a-d	22.8	A a-c
Piper nigrum	Black pepper	12.0	B ab	15.5	B a-e	23.6	A a-c
Prunus mahlep	Mahaleb	4.9	B b-d	14.1	A a-e	19.6	A b-d
Rhus coriaria	Sumach	12.5	A ab	18.3	A ad	20.5	A a-c
Syzygium aromaticum	Clove	16.8	C a	27.9	B a	39.5	A a
Thumus vulgaris	Thyme	11.6	C a-c	20.1	AB a-c	23.6	A bc
Zingiber officinale	Ginger	3.5	BC b-d	10.6	AB b-e	16.4	A b-d
Control	D. W.	0.0	A e	0.0	A f	0.0	A e

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at P ≤ 0.05. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

J2 mortality was more affected by all spices and concentrations than J2 immobility. Also, the extracts that were found to be effective in J2 immobility were effective in J2 mortality. The extracts with the greatest effect in each of the different concentrations of spice extracts were clove, coriander, thyme, cumin, and sumac (Table 4). Aside from these extracts, basil and hot pepper extracts showed statistically the same level of J2 mortality at 1 and 2% (P ≤ 0.05). J2 mortality increased with increasing concentration of spice extracts but was not statistically significant when the effects of each application at different concentrations

were considered. When the concentrations of the extracts of pepper (5.0-14.5%), coriander (10.5-19.4%), immortelle (0.37-4.25%), basil (5.3-14.6%), and clove (13.6-27.8%) were increased, the mortality rate increased significantly. Also, higher concentrations of dill, turmeric, black pepper, and mahaleb extracts resulted in a statistically significant mortality rate when compared to lower concentrations.

Table 4. Mortality rate of spice aqueous extracts at three concentrations on J2s of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	0.8*	BC c	1.1	B de	2.1	A cd
Capsium annium	Pepper	5.0	C bc	11.0	B a-e	14.5	A a-d
Cuminum cyminum	Cumin	8.5	B ab	12.4	AB a-c	16.4	A a-c
Coriandrum sativum	Coriander	10.5	C ab	13.8	B a-c	19.4	A ab
Curcuma longa	Turmeric	2.8	B b	3.5	B b-e	5.9	A b-d
Helichrysium italicum	Immortelle	0.4	C c	2.4	B cd	4.3	A b-d
Ocimum basilium	Basil	5.3	C bc	10.5	B a-e	14.6	A a-d
Piper nigrum	Black pepper	6.1	B bc	8.9	B a-e	13.0	A a-d
Prunus mahlep	Mahaleb	3.0	BC bc	5.3	B b-e	10.3	A b-d
Rhus coriaria	Sumach	8.6	A ab	12.4	A a-c	15.0	A a-d
Syzygium aromaticum	Clove	13.6	C a	20.1	B a	27.8	A a
Thumus vulgaris	Thyme	8.5	BC ab	14.8	AB ab	19.0	A ab
Zingiber officinale	Ginger	2.9	BC bc	5.4	AB b-e	8.6	A bc
Control	D. W.	0.0	A c	0.0	A ef	0.0	A e

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at $P \leq 0.05$. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

Pot experiments

Although the spice extract applications were not as effective as nematicides, they caused a significant decrease in galling index and egg count in tomato roots compared to the negative control ($P \leq 0.05$) (Table 5). No signs of phytotoxicity were observed on tomato plants during the growing season. Among the spice extracts, the lowest value of gall index was in the plants growing in the soils where basil and turmeric extracts were applied (1.12). In addition, the application of coriander and clove resulted in a significant decrease (2.0) in the gall index compared to the negative control (3.25). Plants treated with basil and turmeric extracts had the lowest number eggs in their roots, followed by clove, cumin and coriander. The R_f of the nematode was ranked similarly, and the plants with the least reproduction were those treated with basil, followed by turmeric and clove extracts. With the same statistical group, basil extract reduced *M. arenaria* reproduction by 84.0%, turmeric by 79.0%, and clove by 76.0%. Even coriander had the lowest R_f reduction, but it was still nearly 50% (49.6%). As a result, the R_f in all treated spice extracts is about half the R_f in the negative control (Figure 1). When compared to the controls, the application of the extracts resulted in a reduction in the R_f of 49.6 to 84.0%.

Table 5. Effect of the spice extracts on the gall index, eggs per root, and reproduction factor (R_f) of *Meloidogyne arenaria* on the roots of susceptible tomato plants in the greenhouse (25 ± 3°C)*

Spices	Gall index (0-5)[3]	Eggs x 10^3/root	R_f
Coriander (*Coriandrum sativum)*	2.0 bc	37.5 b	12.5 b
Cumin (*Cuminum cyminum*)	3.0 ab	23.1 c	7.7 c
Turmeric (*Curcuma longa*)	1.1 c	15.7 e	5.2 e
Basil (*Ocimium basilicum*)	1.1 c	11.9 f	4.0 f
Clove (*Syzygium aromaticum*)	2.0 bc	18.1 d	6.0 d
+ Control[1]	0.0 d	0.0 g	0.0 g
- Control[2]	3.3 a	74.4 a	24.8 a

***** The data are the averages of 8 replicates, and the values with the same letters in the column according to the Tukey test are not statistically different according to P ≤ 0.05.

[1] The positive control consisted of commercial nematicide with the active ingredient ethoprophos (200 g/l). [2]The negative control consisted of water without extracts. [3]0-5 gall scale: where 0 =no galling; 1 = trace infection with a few small galls; 2 =25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75% roots galled (Hussey & Jenssen, 2002).

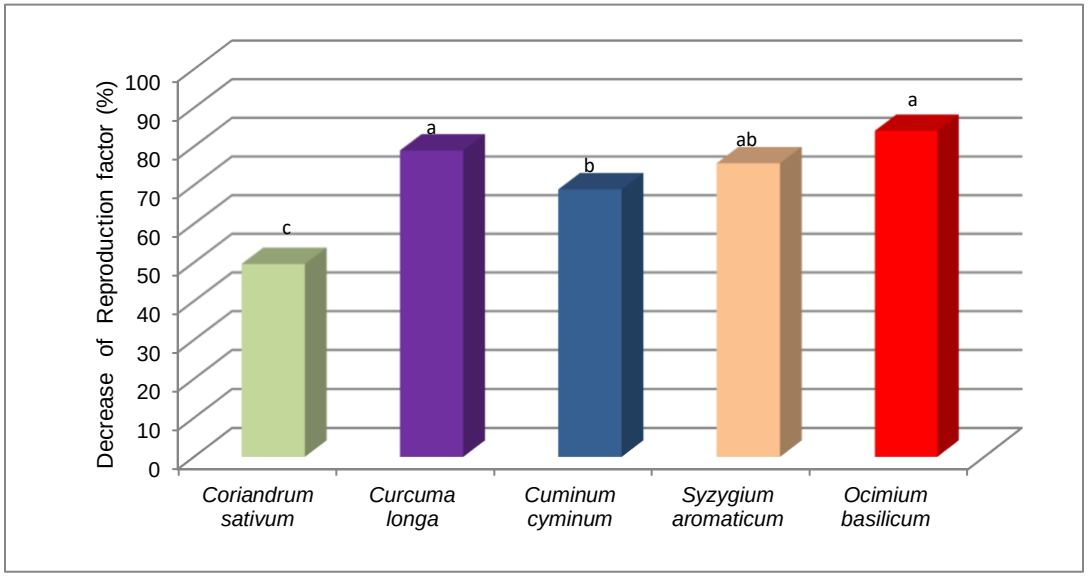

Figure 1. The effect of spice extracts on the reproduction factor of *Meloidogyne arenaria* in tomato plant roots.

Discussion

Spices are used as food additives, colorants, flavorings, and preservatives, as well as anthelmintic, antiseptic, antidiabetic and antipathogenic agents. The antimicrobial activity of spices was first described in 1880 and also nematicidal effects have been known (Rahman et al., 2011). In this study, the nematicidal potentials of 13 spice extracts were investigated in laboratory and pot experiments. Egg hatching tests are useful for screening nematicidal activity of extracts, because counting hatched juveniles is more accurate than counting juveniles in a particular J2 population (Oka et al., 2000). The highest inhibition rate of egg hatching was found to be 93.1% at a 2% concentration of pepper extract. When Abbas et al. (2009) investigated the effects of 50% and 100% aqueous concentrations of pepper spice extract on the hatching of *Meloidogyne javanica* (Treub, 1885) eggs, they found similar results. In the same study, the effects of cumin, coriander, turmeric, black pepper and ginger on egg hatching and larval mortality were investigated, and it was discovered that, contrary to the current study, the other spice extracts inhibited egg hatching more than black pepper. In our study, J2 mortality was also higher when these extracts applied. At all concentrations, black pepper extracts reduced egg hatching significantly (61, 67 and 70%, respectively).

Nile et al. (2017) found that black pepper extracts significantly suppressed galls in tomatoes and reduced RKN population in roots. Black pepper is a very important spice due to its valuable medicinal and aromatic properties. Piperamides, the primary component of *P. nigrum*, have a wide range of biological activities, including antimicrobial, antioxidant, and insecticidal properties (Scott et al., 2005). Özdemir (2014), in a similar study, investigated the effect of basil, black pepper, and ginger essential oils on J2 mortality of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1919 at three different concentrations (1, 3 and 5%) under laboratory conditions and found that black pepper treatments had the highest toxic effect (82%, 86-91%) with the highest mortality rate as a result of laboratory experiments.

All aqueous spice extracts had a greater effect on egg hatching than J2 immobilization and J2 mortality. At a 2% concentration of clove extract, the highest J2 mortality was found to be 27.75%, making clove the most successful extract in terms of J2 mortality. Salgado & Campos (2003) investigated the effects of aqueous clove extracts on J2 mortality of *Meloidogyne exigua* Goeldi, 1887, and it was discovered that clove extract killed more than 50% of the J2s compared to the control. Meyer et al. (2007) demonstrated in microwell tests that clove oil reduced *M. incognita* egg hatch and J2 viability. Clove oil has also been shown to have nematicidal effects on plant-parasitic nematodes (Sangwan et al., 1990; Pandey & Dwivedi, 2000). Previous research on the effects of clove oil on nematodes, mostly on taxa other than RKN has been conducted. Clove oil was nematotoxic to J2s of *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935 (Tylenchida: Anguinidae), *Tylenchulus semipenetrans* Cobb, 1913 (Tylenchida: Tylenchulidae), *M. javanica*, and *Heterodera cajani* Koshy, 1967 (Tylenchida: Heteroderidae) (Sangwan et al., 1990). A commercial standard of eugenol was also toxic to *M. incognita* J2s (Chatterjee et al., 1982). Meyer et al. (2007) also reported that the volatiles in 5% clove oil reduced nematode egg hatching by 30% and the viability of hatched J2s of *M. incognita* by up to 100%. El Badri et al. (2008) used clove extracts to kill the larvae of *Bursaphelenchus xylophilus* (Steiner & Bührer, 1934) Nickle, 1970 (Tylenchida: Parasitaphelenchidae). Clove oil extract has been shown to inhibit egg embryogenesis and to have complete nematicidal activity against J2s both free and in egg masses. In a separate study, extracts from clove were found more effective in killing *M. incognita*, with an effective concentration EC50 which was 5-10 times lower than the EC50 of the synthetic pesticides, chlorpyrifos, carbosulfan, and deltamethrin according to Taniwiryono et al. (2009). Among plant essential oils, eugenol, the main component of clove oil extracted from clove buds and basil leaves, was found to be active against pathogenic organisms including plant-parasitic nematodes (Pandey & Dwivedi, 2000; Park et al., 2005; Meyer et al., 2007; Huang & Lakshman, 2010). So, the application of clove buds as a plant pesticide for future use against nematodes is promising. Clove has a high nematicidal activity for future use against RKN (Taniwiryono et al., 2009). These characteristics make this product an intriguing tool for a novel nematode management strategy (Carlotti et al., 2011).

In our study, cumin extracts inhibited hatching in 39.5-59.3% of eggs, immobilized 12.5-22.9% of J2s, and killed 8.5-16.4% of J2s. The effects of essential oil and hydrosol isolated from cumin seeds on the mobility, hatching, and survival of J2s of *M. incognita* and *M. javanica* were studied by Pardavella et al. (2020). Lower hatching of RKN eggs was observed with an increasing concentration of extracts, which is consistent with the current study. In general, the nematicidal effect increased with increasing extract concentration in laboratory experiments.

In our pot experiments, the most effective extracts were basil and turmeric. The gall index of basil and turmeric applied to tomato was 1.1, and these extracts reduced the reproductive factor by 84.0 and 78.9%, respectively. Oka et al. (2000) found that when they studied the influence of essential oils from 27 spice and aromatic plants, basil extracts reduced egg hatching (68%) and the immobile J2 rate was 18%, which is similar to our findings. Basil extracts also reduced *M. arenaria* egg hatching by 70-83% and reduced immobilization by 9-23%, which agrees with Oka et al. (2000). In trials conducted by Douda et al. (2010), commercially available basil plant essences reduced the gall index of *M. hapla* in carrots (*Daucus carota* L.) (Apiales: Apiaceae). These results also confirmed the findings of the present study.

Turmeric also resulted in a significant reduction in the gall index (65.5%) and eggs per root of tomato plants in the pot experiments compared to the nematicide-treated control. The nematicidal activity of turmeric against RKN has been known for a long time (Pillai & Desai, 1978). Pandey et al. (2001), also stated that the extract of turmeric, a very well-known medicinal plant, had strong nematicidal and nematode hatching inhibitory activity against *M. incognita*. These findings supported the conclusions of the current study. Under in vitro conditions, Neeraj et al. (2020) used methanolic and hexane extracts of turmeric and discovered different levels of mortality of *M. incognita* at different concentrations. The percent mortality of J2s and the suppression of egg hatching, as well as our experimental results, were shown to be directly proportional to the concentration of the extracts and the time of exposure. Turmeric ethanolic extracts have been found to be more effective than all other plant extracts in increasing mortality and inhibiting egg hatching (Mioranza et al., 2016; Neeraj et al., 2017). Aqueous extract, fresh juice, and essential oil of turmeric have also been shown to have biopesticidal properties (Saju et al., 1998). Constituents of turmeric have been shown to be effective, in both in vitro and in vivo studies, against also various plant pathogens. According to Nair et al. (2015), turmeric suppressed the number of *M. hapla* in the roots of tomato cv. Rutgers while increasing the number of beneficial nematodes in the soil with minimal negative effects on plant health and growth, and the components of turmeric leaf macerates and extracts suppressed the ability of *M. hapla* to infect plant hosts without affecting plant growth. According to Babu et al. (2012), curcumin, the main component of turmeric, has a high nematicidal potential, with 92.5% inhibition of the activity of the enzyme glutathione S-transferase of *M. incognita*, an enzyme responsible for nematode survival in host plants. Mioranza et al. (2016) found that an aqueous extract of turmeric at four concentrations (1, 5, 10 and 15%) reduced *M. incognita* J2 mobility in an in vitro assay. Borges et al. (2013) investigated the toxicity of a 10% aqueous extract of turmeric against J2s of *M. incognita* and found that it was completely lethal. According to Ulfa et al. (2021), turmeric extract in various solvents significantly inhibited RKN egg hatching and root penetration but had no effect on RKN development or reproduction. Rashid et al. (2021) used turmeric against *M. incognita* and found that while maximum mortality was achieved up to 20%, root gall severity and final nematode population were significantly suppressed, which is consistent with our findings. It was discovered that the use of turmeric is crucial for RKN management.

Spice extracts have a nematicidal effect because of their ability to penetrate cell walls, which are characterized by high levels of certain oxygenated compounds (Knobloch et al., 1989). The mechanisms of action of spice extracts are also explained by the fact that they cause ADP phosphorylation, protein denaturation and degradation, enzyme inhibition, and interference with electron flow in the respiratory chain (Konstantopoulou et al., 1994). The ability of spice extracts to penetrate cell walls, which are characterized by a high content of certain oxygenated compounds, accounts for their nematicidal action (Knobloch et al., 1989). Clove contains eugenol and eugenol acetate compounds, cumin contains aldehyde, thymol, carvacrol, menthol, and menthone compounds, coriander contains carbohydrate and geranyl acetate compounds, black pepper contains capsaicin, phellandrene, dipentene, and sesquiterpene compounds, pepper contains capsaicin compounds, ginger contains sesquiterpenoid hydrocarbons, turmeric contains curcumin, thiamine, riboflavin, niacin and ascorbic acid. These compounds have been found to be effective against pests and diseases (Peter, 2001). There is a clear need for extract component fractionation to test each compound individually. However, it is possible to generalize that the nematicidal activity of each extract against nematodes follows a multisite mode of action. This is since each extract contains a large number of compounds, each with a distinct functional group and mode of action (Kesba et al., 2021). As a result, future research will focus on these natural active compounds isolated from plants as new compounds with nematicidal properties (Ferraz & De Freitas, 2004). Plant extracts may have a stronger nematicidal effect than synthetic nematicides (Kesba et al., 2021). In the future, all active and effective components of spices particularly basil, clove, and turmeric, could be isolated and analyzed for use as environmentally-friendly biopesticides against RKN.

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