Nematicidial Activity of Five Cruciferous Seed Extracts in Controlling the Root-Knot Nematode Infecting Cucumber Plants

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Abstract

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A greenhouse experiment was conducted to evaluate the effectiveness of five plant aqueous extracts of watercress, radish, turnip, cauliflower, and cabbage, either as seed extract or geminated seed extract, compared with oxamyl nematicide to protect cucumber plants from infection with the root knot nematode *Meloidogyne incognita.* In general, all the tested aqueous plant extracts and the chemical nematicide led to a significant decrease in the number of nematode root galls, egg masses, and eggs per gram of root as well as the number of larvae per 250 g of soil compared to the untreated inoculated control. The aqueous extracts of the germinated cauliflower seed and watercress seed had the highest nematicidal effect against root gall formation, egg-masses, and the number of eggs per gram of root. The cauliflower seed extract had the maximum nematicidal effect against nematode juveniles in soil, whereas the radish seed extract has turned the least effective. The tested aqueous plant extracts significantly increased the shoot's fresh weight compared to the untreated inoculated control. Soil drenching with the germinated cruciferous seed extracts exhibited similar activity against *M. incognita* in terms of a significant increase in ascorbic acid, salicylic acid, and phenol content.

Keywords: Plant extracts, nematicidal activity, *Meloidogyne incognita*, cucumber.

Introduction

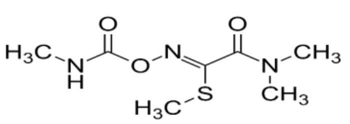
Plant pathogenic nematodes (PPN) are serious pests that invade vegetable crops grown in greenhouses around the world (Almohithef, 2020; El-Sherbiny, 2011; Sivaprakash *et al*., 2008). The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood, is one of the very important root-knot nematode species, which affects vegetable production (Almohithef, 2020; Sharma & Rich, 2005; Zarina & Shahina, 2010). Several strategies were used to manage plant-parasitic nematodes in agricultural areas, with a strong dependence on chemical nematicides, which lead to several problems, including pest resistance, and damage to beneficial organisms, in addition to its adverse effect on public health (Yudelman *et al*., 1998). Accordingly, dependence on synthetic chemical nematicides started to decline (Rich *et al*., 2004), and the search for more environment friendly pest control products started to increase (Giannakou, 2011; Khalil, 2013; Ntalli *et al*., 2009; Taniwiryono *et al*., 2009).

The botanical brassicaceae fumigant effect is a result of production of biologically active compounds following fermentation in the soil (Underhill, 1980). Arugula is able to act as a trap crop for the root-knot nematode species in addition to its properties as a green manure (Melakeberhan *et al*., 2006). This study aimed to estimate the effect of five cruciferous seed extracts with two methods of preparation on *M. incognita* which infects cucumber under greenhouse conditions.

Materials and Methods

Preparation of nematode stock cultures and inocula:
Uncontaminated *Meloidogyne incognita* (Kofoid &White) Chitwood (1919) culture raised from only one egg mass of recognized female (Taylor *et al.,* 1955), collected from coleus roots (*Coleus blumei* L.) and grown in sterilized soil under greenhouse conditions. The root-knot nematode second-stage juveniles (J2) were isolated by a modified Baermann technique after egg hatching at room temperature.

Chemical nematicide
Vydate®10% G (Oxamyl): [N,N-dimethyl-2methylcarbamoyloxyimino-2-(methylthio) acetamide] was applied to soil at the rate of 0.003 ml/plant.



Source of seeds
Cucumber seeds *Cucumis sativus*, as well as watercress *Eruca vesicaria*, radish *Raphanus sativus*, turnip *Brassica rapa*, cauliflower *Brassica oleracea botrytis*, and cabbage *Brassica oleracea capitata* seeds were obtained from the Seed Production Station, Ministry of Agriculture, Egypt.

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Preparation of aqueous extracts

Aqueous extracts were prepared according to the procedure of Claudius-Cole *et al*. (2010). Two types of seed extracts were prepared, a seed extract and a germinated seed extract. Seeds of watercress, radish, turnip, cauliflower, and cabbage were washed under running tap water, air-dried at room temperature, and then in an oven at 50°C. Plant seeds were ground in an electric blender to a fine powder. To prepare extract of germinated seeds, five seeds were washed underwater, then placed in a Petri plate with -distilled water added and then covered. 48 hours later, the germinated seeds for each plant were ground in a blender separately. One hundred grams of each germinated plant seeds extract was added to 1000 ml of distilled water, and then left for 72 h, then warmed for one hour over a water bath. The extract was allowed to cool and filtered through Whatman filter paper No.1. Each extract was considered a standard solution, at a concentration of 100 g/L.

Experimental design

The experiment was conducted under greenhouse conditions (25±3°C) using the Randomized Complete Block Design with six replications and eight treatments, included watercress, radish, turnip, cauliflower, and cabbage + oxamyl (0.03 mL/pot); nematode check (nematode alone); and plant without nematode (plant free-nematode). Seventy-eight plastic pots (10 cm in diameter) filled individually with one kg of autoclaved soil (clay: sand; 1:1, v/v) were planted with three cucumber seeds cv. F1/pot and irrigated with water. One week later, 2000 eggs of *M. incognita* were added for 7 treatments, which consisted of 42 pots. One week later, two types of seed extracts, as well as nematicide (oxamyl), were applied by drenching them to the soil. Plant pots were set in a wholly randomized strategy and wetted. 45 days after nematode inoculation, plants were collected. Measurements on the fresh shoot and root weights, dry shoot weight, shoot and root lengths were made. Nematodes were extracted from 250 g of soil by sieving and the modified Baermann technique (Goodey, 1963). Roots were stained with acid fuchsine in lactic acid (Byrd *et al*., 1983) and counted for galls, females, egg masses, and eggs/single egg mass were counted. Galls (GI) and egg masses indices (EI) were measured based on 0–5 scale according to Taylor and Sasser (1978).

Total phenols determination

Total phenols were estimated in fresh cucumber leaves using the Folin-Ciocalteau reagent (Kaur & Kapoor, 2002).

Salicylic acid and Ascorbic acid determination

Ascorbic and salicylic acids in fresh cucumber leaves were estimated by titration with 2.6 diclorophenol indophenol blue dye (A.O.A.C., 1980). The percentage of increase was calculated using the equation:

$$\text{Increase } \% = \frac{\text{Treated - Nematode only (control)}}{\text{Nematode only (control)}} \times 100$$

Statistical analysis

Results obtained were statistically analyzed by the analysis of variance (ANOVA), and the significance of means was determined by the Duncan multiple range test (DMRT) (Duncan, 1955).

Results

The efficacy of five aqueous extracts (watercress, radish, turnip, cauliflower, and cabbage) either as seed extract or geminated seed extract compared with oxamyl as a standard nematicide was evaluated against *M. incognita* on cucumber plants. Each of the tested extracts was applied as a soil drench under greenhouse conditions. Results obtained (Table 1) indicated that all of the tested aqueous cruciferous seed extracts (two extracts types) and the synthetic nematicide (oxamyl) significantly ($P \leq 0.05$) reduced the number of galls as compared with untreated inoculated control. All aqueous extracts were significantly less effective than oxamyl. In addition, the aqueous extract of the germinated seeds was superior to the traditional seed extract for all tested treatments. The highest reduction rate (%) was obtained from aqueous extract of the germinated watercress seed extract (69.08%), followed by cauliflower (68.66%), turnip (63.02%), and cabbage (57.39%). On the other hand, radish exhibited the lowest reduction rate of number of galls (39.55 %). Concerning the effects of different aqueous extracts on the reduction of egg-masses, all of the tested extracts and the synthetic nematicide; oxamyl significantly ($P \leq 0.05$) reduced the number of egg-masses as compared with untreated inoculated control. All aqueous extracts were significantly less effective than oxamyl (93.14%). The highest reduction was obtained by aqueous extract of the germinated watercress seeds (71.81%), followed by cauliflower (68.66%), turnip (63.07%), and cabbage (55.59%). On the contrary, radish exhibited the lowest reduction effect on the number of egg-masses (37 %).

Data concerning the effects of different aqueous extracts on the reduction of eggs/g root are summarized in Table 1. All of the tested aqueous extracts significantly ($P \leq 0.05$) reduced eggs/g root as compared with untreated inoculated control. All aqueous extracts were less significant than the synthetic nematicide oxamyl (93.11%). The highest reduction rate was obtained by the aqueous extract of the germinated cauliflower seeds extract (77.99%), followed by watercress (71.72%), cabbage (70.44%), and turnip (70.44%). On the other hand, radish extract exhibited the lowest reduction effect of number of eggs/g root (64.53%).

A similar trend was observed for all of the tested aqueous extracts which significantly ($P \leq 0.05$) reduced the number of J2 in the soil as compared with untreated inoculated control. All aqueous extracts were significantly less effective than oxamyl (93.11%). The highest reduction was obtained by the aqueous extract of the germinated cauliflower seeds extract (75%), followed by watercress (73.33%) and cabbage (68.66%). On the other hand, radish extract exhibited the lowest reduction effect on the number of J2 in the soil (43.33 %).

Results obtained (Table 2) revealed that all of the tested aqueous plant extracts and oxamyl significantly ($P \leq 0.05$) increased the shoot fresh weight as compared with untreated inoculated control (34.82 g/shoot). The treatment with germinated seed extracts gave similar result to non-inoculated control (56.84 g/ shoot). All the tested aqueous plant extracts and oxamyl significantly ($P \leq 0.05$) increased the shoot dry weight compared to untreated inoculated control (10.67 g/plant). All treatments of the two extraction methods gave similar results to the non-inoculated control (13.67 g/shoot). All the tested aqueous plant extracts and oxamyl significantly ($P \leq 0.05$) increased the plant height as compared with untreated inoculated control. All tested aqueous plant extracts significantly increased the root fresh weight compared to the untreated inoculated control, and the germinated seed extracts were similar to the non-inoculated control (Table 3). All of the tested aqueous plant extracts and oxamyl significantly ($p \leq 0.05$) increased the root dry weight as compared with untreated inoculated control.

Ascorbic acid content

Results obtained (Table 3) showed a significant decrease in ascorbic acid (0.341 mg/100g) due to nematode infestation. Utilization of five aqueous extracts (watercress, radish, turnip, cauliflower, and cabbage) either as seed extract or geminated seed extract enhanced the ascorbic acid content in cucumber leaves. The aqueous extract of the germinated seeds was superior to the traditional seed extract in all the tested treatments. The highest increase was obtained from the germinated turnip seed extract (94.4%), followed by cabbage (82.9%).

Table 1. Effect of five cruciferous seed extracts with two methods of preparation on galls number and egg-mass formation on cucumber plants infected with *M. incognita* under greenhouse conditions.

Treatments	Method of extraction	Mean of galls/root system	Reduction rate (%)	Egg-mass/root system	Reduction rate (%)	No. of eggs/g root	Reduction rate (%)	Mean No. of J2/250 g of soil	Reduction rate (%)
Cabbage	Seeds extract	250.00 c	47.77	266.67 b	37.00	150.00 cd	55.66	135.66 d	54.78
	Germinated seeds extract	201.33 d	57.39	188.00 de	55.59	100.00 ef	70.44	94.00 ef	68.66
Cauliflower	Seeds extract	185.00 d	61.35	168.33 ef	60.23	165.67 c	51.03	145.33 d	48.55
	Germinated seeds extract	150.00 ef	68.66	132.67 gh	68.66	100.00 ef	70.44	140.33 d	53.22
Radish	Seeds extract	300.33 b	37.25	276.00 b	34.8	200.00 b	40.88	200.0 b	33.33
	Germinated seeds extract	289.33 b	39.55	266.67 b	37.0	120.00 de	64.53	170.0 c	43.33
Turnip	Seeds extract	230.00 c	51.95	202.00 d	52.28	165.67 c	51.03	145.33 d	48.55
	Germinated seeds extract	177.00 de	63.02	156.33 fg	63.07	100.00 ef	70.44	140.33 d	53.22
Watercress	Seeds extract	200.00 d	58.21	182.67 de	56.88	154.67 c	54.28	100.66 e	66.44
	Germinated seeds extract	148.00 f	69.08	119.33 h	71.81	95.67 ef	71.72	80.00 gf	73.33
Oxamyl	0.03 ml/plant	34.00 g	92.89	29.00 i	93.14	22.33 g	93.39	20.66 h	93.11
Control	-	478.67 a	-	423.33 a	-	338.33 a	-	300.00 a	-

Each value is the mean of six replicates. Means followed by the same letter (s) in the same column are not significantly different based on Dncan's multiple range test at P= 0.05%.

Table 2. Effect of aqueous plant extracts on some plant growth parameters of cucumber plants infected with *M. incognita* under greenhouse conditions.

Treatments	Method of extraction	Fresh shoot weight (g)	Dry shoot weight (g)	Shoot length (cm)	Root fresh weight (g)	Root dry weight (g)
Cabbage	Seeds extract	45.33 e	12.23 d	48.33 c	20.59 cde	4.24 bc
	Germinated seeds extract	55.67 ab	14.60 abc	57.33 ab	23.66 abcd	4.75 abc
Cauliflower	Seeds extract	50.52 cd	14.00 abcd	46.00 c	20.46 de	4.07 bc
	Germinated seeds extract	54.26 abc	15.30 a	56.83 ab	23.95 ab	4.68 abc
Radish	Seeds extract	47.86 de	13.08 bcd	43.56 c	19.91 e	4.28 abc
	Germinated seeds extract	54.44 abc	14.50 abc	55.50 ab	23.33 abcd	4.60 abc
Turnip	Seeds extract	49.04 de	13.94 abcd	46.60 c	21.06 bcde	4.20 bc
	Germinated seeds extract	54.80 abc	14.40 abc	54.83 b	23.79 abc	4.69 abc
Watercress	Seeds extract	46.81 de	12.73 cd	46.60 c	19.66 e	3.87 cd
	Germinated seeds extract	51.38 bcd	13.42 abcd	56.20 ab	24.71 a	4.80 ab
Oxamyl	0.03 ml/ plant	50.01 abc	14.89 ab	55.50 ab	23.66 abcd	4.79 ab
Control	-	34.82 f	10.67 e	35.64 d	15.93 f	2.16 d
Healthy plants	-	56.84 a	13.67 abcd	60.86 a	25.66 a	5.14 a

Each value is the mean of six replicates. Means followed by the same letter(s) in the same column are not significantly different according at P= 0.05%.

Salicylic acid and phenol content

Salicylic acid and phenol levels were significantly affected by root-knot nematode, *M. incognita* infection of cucumber roots, and soil drenching with turnip or watercress geminated seed extracts increased the content of these components by 66.0 and 61.3% (salicylic acid), and 110.0 and 105.8% (phenol), respectively.

Discussion

Control of nematodes is vital to minimize crop yield losses and secure food. Among the various ecologically-based methods of Meloidogyne incognita control are pesticides of plant origin (Adesiyan *et al.*, 1990; Mangala & Mauria, 2006; Olowe, 1992). In this study, the five brassica germinated seed extracts inhibited *M. incognita* which attacked cucumber roots. The highest nematode reduction was obtained from the aqueous extract of watercress germinated seeds (69.08%), followed by cauliflower (68.66%), turnips (63.02%), and cabbage (57.39%). On the other hand, radish exhibited the lowest effect on gall formation. These data confirmed that brassica seed germinated extracts have higher toxic action than traditional seed extracts, but not as high as chemical nematicides. Numerous previous reports emphasized the role of cabbage in the control of plant pathogenic nematodes, and this nematicidal activity is due to the presence of myrosinase and glucosinolates which undergo enzymatic hydrolysis and are transformed into bioactive, volatile isothiocyanates (Cole, 1976; Fenwick *et al*., 1983; Kirkegaard & Sarwar, 1998; Poulton & Moler, 1993).

Isothiocyanates possess an inhibitory effect on fungi, bacteria, insects, and nematodes (Bello *et al*., 2004; Brown & Morra, 1997). Salicylic acid (SA) originates in plants as a phenolic phytohormone that plays a significant role in plant development and growth, and it can interfere with nematode juveniles attack and nematode multiplication (Molinari & Loffredo, 2006).

In this study, phenol content in treated plants decreased as root galling formation decreased. This result contradicts what has been reported by Nguyen *et al*. (2011), who suggested that the activity of the antioxidative enzymes in cucumber leaves treated with different concentrations of cinnamon extract increased as the relative galling formation of *M. incognita* decreased in cucumber roots.

Ascorbic acid synthesizes mitochondrial hydroxyproline proteins, which regulate the expansion of cyanide-resistant respiration and may play a role in plant defense to nematode infections (Bakr & Hewedy, 2018). The current results are in line with those of Anita (2012), who found that ethanol extracts of Chinese cabbage, radish, cabbage, and cauliflower leaves decreased the population of *M. hapla* and enhanced the growth of celery plants. Of these, radish leaf residue proved to be the most effective, leading to a significant decrease in the number of nematodes in the soil and an increase in the yield of celery green leaves and stalks. El-Sherbiny & Awd Allah (2014) observed a similar pattern, stating that addition of air-dried powder of some plants before planting decreased *M. incognita* on tomato plants and enhanced plant growth. The current findings concur with previous workers (Asif *et al*., 2017; Radwan *et al.,* 2012; Roberts *et al*., 1988; Runia & Molendijk, 2007) who reported that aqueous extracts were less effective than the synthetic nematicide oxamyl.

This study confirms that the use of germinated seed extracts is a practical tool to manage *M. incognita* infecting cucumber, which reduces the dependence on synthetic nematicides and mitigate environmental contamination generated by the intensive use of chemicals. Accordingly, germinated seed extracts should be considered as a component of nematodes management, and more large-scale field research is needed to support its general use.

Table 3 Ascorbic acid, salicylic acid and phenol content in cucumber leaves infected with *Meloidogyne incognita* as influenced by five cruciferous seed extracts with two methods of preparation under greenhouse conditions.

Treatments	Method of extraction	Ascorbic acid (mg/100g)	Increase %	Salicylic acid (mg/100g)	Increase %	Total phenol (mg/100g)	Increase %
Cabbage	Seeds extract	0.617 abc	80.9	17.3 abc	47.8	24.60 ab	80.9
	Germinated seeds extract	0.587 bc	82.9	16.0 bc	50.9	23.30 ab	94.2
Cauliflower	Seeds extract	0.587 bc	72.1	16.9 abc	44.4	24.40 ab	79.4
	Germinated seeds extract	0.577 c	79.8	16.5 abc	55.7	21.90 b	82.5
Radish	Seeds extract	0.615 abc	80.4	16.7 abc	42.7	24.30 ab	78.7
	Germinated seeds extract	0.584 bc	81.9	15.5 c	46.2	22.90 ab	90.8
Turnip	Seeds extract	0.593 bc	84.7	17.8 ab	52.1	0.66 ab	94.4
	Germinated seeds extract	0.663 ab	94.4	17.6 abc	66.0	25.20 ab	110.0
Watercress	Seeds extract	0.595 bc	74.5	17.2 abc	47.0	24.50 ab	80.1
	Germinated seeds extract	0.582 bc	81.3	17.1 abc	61.3	24.60 ab	105.0
Oxamyl	-	0.671 a	109.0	17.4 abc	64.2	25.30 ab	110.8
Control	-	0.341 d	-	11.7 d	-	13.60 c	-
Healthy	-	0.688 a	114.3	18.4 a	73.6	25.70 ab	114.2

N= 2000 J_2 of *M. incognita*. Each value is the mean of six replicates. Means in each column followed by the same letters(s) did not differ significantly at P< 0.05 according to Duncan's multiple range tests.

الملخص

سعدون، مراد سعدون، سمير جاد، محمد عثمان ومحمد السرجاني. 2025. النشاط الإبادي لخمسة مستخلصات من بذور العائلة الصليبية في مكافحة نيماتودا تعقد الجذور التي تصيب نباتات الخيار. مجلة وقاية النبات العربية، 43(2): 241–246.

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أجريت دراسة لتقييم فعالية خمسة مستخلصات مائية لنباتات الجرجير والفجل واللفت والقرنبيط والملفوف، إمّا كمستخلص بذور أو مستخلص بذور منبّتة، مقارنة بالمبيد النيماتودي (أوكساميل) في حماية نباتات الخيار من الإصابة بنيماتودا تعقّد الجذور (*Meloidogyne incognita*). عموماً، سبّبت جميع المستخلصات النباتية المائية المختبرة ومبيد النيماتودا الكيميائي انخفاضاً معنوياً في عدد العقد الجذرية وأكياس البيض وعدد البيض، وكذلك عدد يرقات النيماتودا في كل 250 غ تربة بالمقارنة مع مبيد الأوكساميل ومعاملة الشاهد غير المعاملة بالنيماتودا. سجلت معاملة المستخلص المائي لبذور القرنبيط المنبتة وبذور الجرجير أعلى تأثير إبادي للنيماتودا، وقلّلت من العقد النيماتودية وأكياس البيض وعدد البيض على الجذور. أظهر مستخلص بذور القرنبيط أعلى تأثير إبادي للنيماتودا في التربة، بينما كان مستخلص بذور الفجل أقلّها فعالية. أدت جميع المستخلصات النباتية المائية المختبرة إلى زيادة الوزن الرطب للمجموع الخضري بشكل ملحوظ مقارنة بمعاملة الشاهد المعامل بالنيماتودا فقط. أظهرت معاملة التربة بمستخلصات بذور النباتات الصليبية المنبتة نشاطاً مشابهاً ضدّ *M. incognita* وزيادةً ملحوظة في محتوى حمض الأسكوربيك وحمض الساليسيليك والفينول.

كلمات مفتاحية: مستخلصات نباتية، مبيد نيماتودي، نيماتودا تعقد الجذور، خيار، *Meloidogyne incognita*.

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