Iraqi Journal of Market Research and Consumer Protection
(2026) 18(1): 37-47.
DOI: http://dx.doi.org/10.28936/jmracpc18.1.2026.(4)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

THE EFFECT OF AQUATIC AND ALCOHOLIC EXTRACTS OF *Brassica juicea* ON THE EGGS HATCHING AND VITALITY OF SECOND-STAGE JUVENILES (J2) OF *Meloidogyne* SPP ROOT-KNOT NEMATODES.

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Received 1/ 4/ 2024, Accepted 5/ 8/ 2024, Published 31/ 3/ 2026

ABSTRACT

The research aimed to evalauate the effectiveness of aquatic extract and Alcoholic extracts of seed and roots mustard under laboratory conditions at concentrations of(0.5, 1, 1.5) %to inhibiting the eggs' hatching and viability of second-stage juveniles (J2). The results indicated that the Alcoholic extract of mustard seeds at a concentration of 1.5% showed effectiveness in reducing egg hatching after (24 ,48, 72) (h) with percentages of (32, 67.67, 88) compared to the control treatment, which had (16.33, 11.67, 9) eggs successively. On the other hand, the aquatic extract of mustard seeds showed effectiveness after (24 ،48، 72) (h) with egg hatching percentages of (68, 58.67, 25) compared to the control treatment, which had (16.33, 11.67, 9) eggs successively. Additionally, the Alcoholic extract of root at 1.5% concentration demonstrated efficacy in inhibiting egg hatching after (24 ,48, 72)(h) with counts of (76, 49.67, 30)eggs successively compared to the control treatment, which had (16.33, 11.67, 9) eggs. The alcoholic extract of mustard seeds at 1.5% concentration also exhibited effectiveness against the viability of second-stage juveniles (J2) after (24 ,48, 72) (h), with counts of (92.33, 76.67, 40) juveniles successively compared to the control treatment, which had (23, 17, 9.67) juveniles. Similarly, the aquatic extract of mustard seeds at 1.5% concentration showed efficacy against the viability of (J2) after (24 ,48, 72) (h), with counts of (68, 72, 31.70) juveniles successively compared to the control treatment, which had (23, 17, 9.67) juveniles. The alcoholic extract of roots at 1.5% concentration also demonstrated effectiveness against the viability of (J2) after (72, 48, 24) (h), with counts of (72, 76.67, 30) juveniles successively compared to the control treatment, which had (23, 17, 9.67) juveniles.

Keywords: Alcohol, Aquatic, Extracts, Mastard.

[*] **This article is taken from the first researcher's master's thesis.**

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تأثير المستخلص المائي والكحولي لنبات الخردل *Brassica juncea* على فقس البيوض وحيوية يافعات الطور الثاني (J2) لنيماتودا تعقد الجذور *Meloidgyne* spp

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الخلاصة

اجري البحث لتقييم فعالية المستخلص المائي والكحولي للخردل تحت الظروف المختبرية وبالتراكيز(1.5,1,0.5)% في تثبيط بيوض وحيوية يافعات الطور الثاني Juveniles (J2) اذ أظهرت نتائج تأثير المستخلص الكحولي لبذور الخردل بتركيز 1.5% بعد (72 ,48, 24) ساعة فعالية في خفض نسبة فقس البيض والتي بلغت(88, 67.67,32) على التتابع مقارنة مع معاملة المقارنة والتي بلغت (9, 11.67 ,16.33) بيضة، على التتابع. في حين اظهر المستخلص المائي للبذور فعالية بعد (72, 48 , 24) ساعة اذ بلغت نسبة فقس البيض (30 , 58.67 ,68) بيضة على التتابع مقارنة مع معاملة المقارنة والتي بلغت (9, 11.67 ,16.33) بيضة، في حين اظهرت نتائج المستخلص الكحولي للجذور بتركيز 1.5% فعالية في تثبيط فقس البيض بعد (72, 48 ,24) ساعة اذ بلغت (30, 49.67 , 76) بيضة على التتابع مقارنة مع معاملة المقارنة والتي بلغت (9, 11.67 ,16.33) اظهر المستخلص الكحولي لبذور الخردل بتركيز 1.5% فعالية ضد حيوية يافعات الطور الثاني بعد (72,48,24) ساعة والتي بلغت (40 ,76.67, 92.33) يافعة على التتابع مقارنة مع معاملة المقارنة والتي بلغت(17, 9.67 ,23) يافعة،في حين اظهرت نتائج المستخلص المائي للبذور بتركيز 1.5% فعالية ضد حيوية ويافعات الطور الثاني بعد (72,48,24) ساعة والتي بلغت (68,72,31.70) يافعة على التتابع مقارنة مع معاملة المقارنة والتي بلغت (17, 9.67 ,23) يافعة، في حين اضهر المستخلص الكحولي للجذور بتركيز1.5 % فعالية ضد حيوية ويافعات الطور الثاني بعد (72,48,24) ساعة اذ اظهرت النتائج (30,76.67, 72) يافعة على التتابع مع معاملة المقارنة والتي بلغت ((17, 9.67 ,23) يافعة.

الكلمات المفتاحية: المستخلصات،الكحولية،المائية،خردل،نيماتودا تعقد الجذور.

INTRODUCTION

The root-knot nematodes, *Meloidogyne* spp, are characterized by large, extensive feeding structures and a wide host range **(Martinuz, 2012**).

The biocontrol fungus Hirsutella is used to inhibit egg hatching and kill second-stage juveniles (J2), resulting in reduced root galling and increased activity of essential enzymes. This leads to an improvement in the nutritional content of fig seedlings (**Al-Awabid &Yass, 2023)**Biological control was employed to combat root-knot nematodes in the soil surrounding the roots of infected plants، Antagonistic biological factors were selected from the soil surrounding roots of various plants and their effectiveness against early nematode stages was evaluated. Some of these biological factors exhibited strong effects in killing early nematode stages and preventing egg hatching, indicating their potential for nematode control in farms **(Dawood& Yass, 2023)**

Humic acid was applied to tomato plant roots infected with root-knot nematodes, showing high effectiveness against the nematodes. The effectiveness increased with higher concentrations of the acid, resulting in improved plant growth (**Yass *et al.*, 2020**).

Explore an effective and safe approach to manage root-knot nematodes (*Meloidogyne javanica*) and evaluate its impact on tomato growth. Providencia vermicola bacteria were isolated، revealing their effective capacity to suppress the hatching of root-knot nematode eggs and control infections efficiently in greenhouse environments. Additionally، these bacteria exhibited lethal activity against root-knot nematodes and produced a heat-stable active substance (**Aish, *et al.*, 2019**). The study demonstrates the effectiveness of rhizosphere bacteria *Pseudomonas* spp. and *Serratia* spp. in combating nematodes،These bacteria have the ability to inhibit egg hatching and effectively control infection, making them a promising alternative to oxamyl**(Abd El-Aal, *et al.*, *2021*).**

Fulvic acid (FA) and magnesium oxide (MgO) were used to study their effects on egg hatching and viability of second-stage juveniles on tomato plants. This led to a noticeable increase in both dry and wet weights of the infected plants (**Mohammed& Yass 2020).**

contain various compounds such as flavonoids, amino acids, carboxylic acids, phenols, proteins, and ketones (**Attia & Elsherry, 2020**).

The ethanolic extract of *Dodonaea viscosa* showed the highest inhibition percentage against the growth of both fungal hyphae and spores, surpassing the methanolic, chloroform, and ethyl acetate extracts at the same concentrations against the plant pathogens Fusarium oxysporum and Aspergillus niger (**Al-Salami & Jalil, 2015**).

Mustard is considered an important crop with a long history in China (**Chen *et al.*, 2013**). Mustard is used as oilseed and vegetable crops in China, with the cultivated area reaching 7.03 million hectares (**Jannat *et al.*, 2022**). The essential oils of mustard have inhibitory effects on many fungi **(Goi., 1985**).

Some of these compounds possess medicinal properties such as antioxidant, antimicrobial, and anti-inflammatory effects، Some also exhibit cytotoxic properties against cancer cells، While some are toxic، they play crucial roles in drug manufacturing and pesticide production**(Al-Mousuli, 2018**)

The total production for the year 2021 reached 14.70 million metric tons (**WAP, 2022**). Crop rotations have shown acceptable efficiency in reducing nematode populations, as studies in China have demonstrated that mustard plants attract nematodes when intercropped with rice (**Lu *et al.*, 2022**). In the study, an attempt was made to use mustard seed oil as an environmentally friendly alternative to petroleum oils in lubrication processes. It was found that blending mustard seed oil with commercial engine oil improves lubrication properties and reduces wear and friction. A specific ratio of this mixture showed excellent performance, indicating the potential use of mustard seed oil as an effective and environmentally friendly alternative in lubrication industries. (**Jabl & Khalifa, 2018**)

The recent years have not witnessed the development of safe and effective pesticides for root-knot nematodes, prompting researchers to explore alternative methods of control (**Selim, 2010**).

The aim of the study is to use mustard extract as an eco friendly method in two pathways first as a attractant plant for nematodes away from the main crop and second as inhibits their reproduction

MATERIALS AND METHODS

Meloidogyne inoculum

Samples were collected from the roots of Dodonaea infected with root-knot nematodes from nurseries located in Baghdad province. inoculum against root-knot nematodes from diferenet field located in Baghdad governorate eggs and J2 was extracteding to the (**Hussey & Bakker 1973**).

The infected roots were gathered and cut into small pieces 1-2 cm.

Prepared Sodum Hypochlorade with a concentration of 10 ml solm 50 ml distilled water, placed the pieces in the Sodum Hypochlorade for two minutes with gentle stirring, emptied the contents into sieves arranged in sequence of 300, 150, and 25 mesh sizes, and exposed the pieces to a flowing water stream for 5 minutes.

To eliminate any Sodum Hypochlorade traces, the eggs were collected on a 25-micron sieve under the influence of gentle water currents into a 125 ml glass cylinder (Beaker). The sample was examined under a microscope at a magnification of 40x, and the numbers of eggs and (J2) were counted per 1 ml of the total solution volume. inoculum was used for the laboratory experiment.

Mustard extracts

Preparations were made at three different concentrations (0.5%, 1%, and 1.5%). To create the aquatic extract, 25 g of the sample were weighed and transferred into a 500 ml beaker equipped with a magnetic stirrer. Subsequently, the mixture was blended for thirty minutes. Following this, it was filtered through a lint-free cloth, poured into a petri dish, and then placed in an oven at 65°C overnight until completely dried. As for the alcoholic extract, 100 grams of the sample were weighed and placed in a 500 ml beaker. Then, 250 ml of water and 250 ml of alcohol were added to it. The mixture was left in the refrigerator overnight. The next day, it was filtered, poured into a petri dish, dried at a temperature of 65°C, and scraped off.

To prepare the required concentrations of the extract:

Dissolve 0.25 grams of the extract in 49.75 mL of sterile water in a 50 mL container to achieve a concentration of 0.5%. Dissolve 0.5 grams of the extract in 49.5 mL of sterile water in a 50 mL container to achieve a concentration of 1%. Dissolve 0.75 grams of the extract in 49.25 mL of sterile water in a 50 mL container to achieve a concentration of 1.5%. To assess the effectiveness of the extract concentrations in hatching eggs and second-stage juveniles (J2) of *Meloidogyne* spp., the experiment was conducted by placing 1 mL of the vaccine containing 200 $\pm$5 eggs in a Petri dish with a diameter of 6 cm. In other dishes, 1 mL of the extract containing 200$\pm$5 freshly hatched juveniles from the roots of infected Dodonaea was added. The above-mentioned concentrations (0.5%, 1%, 1.5%) were added to the dishes, with 5 mL from each concentration added. Each treatment was repeated three times. In other dishes, the extract was added with sterile distilled water and placed with the rest of the treatments for

comparison. The dishes were then incubated at a temperature of 25 ± 2C, and the percentage of egg and juvenile mortality was caunted after 24, 48, and 72 (h) using the following (**Sharma & Sharma, 2017**).

$$\text{The percentage of juvenile mortality} \frac{\text{The number of dead J2 juvenile}}{\text{The total number of J2 juveniles}} \times 100$$

The plates were distributed according to a Completely Randomized Design (CRD)

RESULTS

The results of Table (1) illustrated the effectiveness of the three concentrations on the four extracts (alcoholic seed, aquatic seed, alcoholic root, aquatic root) in inhibiting egg hatching, with significant differences observed among the treatments compared to the control treatment. Concentration of 1.5% outperformed the other concentrations (0.5%, 1%), with the alcoholic seed extract showing the most efficient inhibition of egg hatching compared to the aquatic seed, alcoholic root, and aquatic root extracts after 24 h. Egg mortality reached 32, 25, 30, and 15% successively.

Table (1): Effect of Different Concentrations of Mustard Extract on Egg Hatching of Root-Knot Nematodes after 24 h.

inhibition of eggs after 24 h				
The extract	**Number of eggs%**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	20	15	32	22.33
Aquatic seed	15	15	25	18.33
Alcoholic roots	15	20	30	21.67
Aquatic roots	10	10	15	11.67
lsd5%	ns			7.67*
Average concentration	15	15	25.5	
lsd%	6.65**			
con	9			
lsd5%	12.68**			

Each number in the table represents the mean of three replicates.

As for the results from Table (2), the percentage of egg hatch inhibition after 72 h of treatment reached 67%, 58%, 49%, and 45% respectively.

Table (2): Effect of Different Concentrations of Mustard Extract on Egg Hatching of Root-Knot Nematodes after 48 h.

Inhibition of eggs after 48 h				
The extract	**Number of eggs%**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	45	45	67.67	52.56
Aquatic seed	40.67	45	58.67	48.11
Alcoholic roots	27	40.67	49.67	39.11
Aquatic roots	22.67	27	45	31.56
lsd5%	5.63*			3.25**
Average concentration	33.84	39.42	55.25	
lsd%	2.82**			
con	11.67			
lsd5%	5.37**			

Each number in the table represents the mean of three replicates.

Table (3) showed very high percentage of inhibition for the four extracts (alcoholic seeds, aquatic seeds, alcoholic roots,aquatic roots), reaching (88%, 68%, 76%, 44%) respectively.

Table (3): Effect of Different Concentrations of Mustard Extract on Egg Hatching of Root- Knot Nematodes after 72 h.

Inhibition of eggs after 72 h				
The extract	**Number of eggs%**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	64	76	88	76
Aquatic seed	44	48	68	53.33
Alcoholic roots	63	76	76	71.67
Aquatic roots	36	40	44	40
lsd5%	7.08**			4.09**
Average concentration	51.75	60	69	
lsd%	3.54**			
con	16.33			
lsd5%	6.76**			

Each number in the table represents the mean of three replicates**.**

The results described by **(Handiseni *et al.*, 2017**) confirm the effectiveness of using Brassica juncea and Sinapis albah seed extracts to control root-knot nematodes in Bermudagrass under laboratory conditions. These extracts significantly reduced the number of second-stage juveniles (J2) compared to the control treatments. *Brassica juncea* extracts were found to be significantly more effective in controlling root-knot nematodes, followed by

Sinapis albah extracts, with no observed toxicity. Extracts from *B. juncea* seeds are considered as nematicides for various crops. Several types of mustard have been studied, containing different chemical compounds such as fatty acids and isothiocyanates. Moreover, essential oils extracted from them exhibit antibacterial activity, especially against some Gram-negative bacteria such as Escherichia coli, Pseudomonas aeruginosa, and Klebsiella (**Jabbar *et al.*, 2021**).

Evaluation of the impact of three concentrations of extracts from mustard on the vitality of second-stage juveniles (J2).

Table (4) illustrates the effectiveness of the three concentrations of the four plant extracts (alcoholic seeds, aquatic seeds, alcoholic roots, aquatic roots) to inhibiting second-stage juveniles (J2), with significant differences observed among the treatments compared to the control treatment. Concentration of 1.5% showed superiority over the other concentrations (0.5%, 1%), as the alcoholic seed extract treatment exhibited greater efficiency inhibiting second-stage juveniles compared to the aquati**c** seed extract, as well as the alcoholic and aquatic root extracts after 24 hours. The mortality rate of juveniles reached 40%, 31%, 30%, and 15% respectively.

Table (4): Effect of different concentrations of mustard extract on the viability of second-stage juveniles (J2) of root-knot nematodes after 24 h.

Stage viability of J2 after 24 h				
The extract	**Number of Juvenile_2**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	15	25	40	26.67
Aquatic seed	10	15	31.7	18.9
Alcoholic roots	10	15	30	18.33
Aquatic roots	10	10	15	11.67
lsd5%	ns			6.12**
Average concentration	11.25	16.25	29.18	
lsd%	5.30**			
con	9.67			
lsd5%	10.13**			

Each number in the table represents the mean of three replicates.

Results from Table (5) demonstrate the effectiveness of the four extracts, showing significant differences among the treatments compared to the control treatment. The alcoholic seed extract treatment exhibited greater efficiency in the vitality of second-stage juveniles compared to the aquati**c** seed extract, as well as the alcoholic and aquatic root extracts after 48 h. The mortality rate of juveniles reached (76%, 72%, 76%, 72%) respectively.

Table (5): Effect of different concentrations of mustard extract on the viability of second-stage juveniles (J2) of root-knot nematodes after 48 h.

Stage viability of J2 after 48 h				
The extract	**Number of Juvenile_2**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	54	67.33	76.67	66
Aquatic seed	40.67	49.67	72	54.11
Alcoholic roots	40.33	54	76.67	57
Aquatic roots	31.67	49.67	72	51.11
lsd5%	ns			4.79**
Average concentration	41.67	55.17	74.34	
lsd%	4.15**			
con	17			
lsd5%	8.02**			

Each number in the table represents the mean of three replicates.

Table (6) showed a high mortality rate for juveniles from the four extracts (alcoholic seeds, aquatic seeds, alcoholic roots, aquati**c** roots), reaching (92%, 68%, 72%, 72%) respectively for the concentration of 1.5%. Some parts of Brassicaceae were used as volatile compounds released after enzymatic hydrolysis responsible for the bioactive volatile compounds' effectiveness, as the glucosinolate-myrosinase- isothiocyanates system controls many pests and pathogens, including root-knot nematodes transmitted through soil. The biological role of Brassicaceae green manure amendments was controlled (**Aires *et al.*, 2009**).

Table (6): Effect of different concentrations of mustard extract on the viability of second-stage juveniles (J2) of root-knot nematodes after 72 h.

Stage viability of J2 after 72h				
The extract	**"Number of Juvenile_2**			**Average extract**
Concentration	0.5	1	1.5	
Alcoholic seed	68	84	92.33	81.44
Aquatic seed	44	64	68	58.67
Alcoholic roots	44	55	72	57
Aquatic roots	40	44	72	52
lsd5%	7.42**			4.28**
Average concentration	49	61.75	76.08	
lsd%	3.71			
con	**23.00****			
lsd5%	**7.15****			

Each number in the table represents the mean of three replicates.

DISCUSSION

These results are consistent with those reported by (**Handiseni *et al*., 2017**), who used extracts from *Brassica juncea* and Sinapis albah seeds to control root-knot nematodes in Bermudagrass experimentally. They led to a significant reduction in the numbers of second-stage juveniles (J2) in comparison treatments, with *Brassica juncea* showing significantly greater efficacy in controlling root-knot nematodes compared to Sinapis albah, without plant toxicity.

extracts from the *Brassica juncea* genus have shown high efficacy against two types of nematodes, *Meloidogyne chitwood* and Pratylenchus penetrans. Compounds of glucosinolates from 16 species of Brassica, seven species of Lolium, and one species of Lupinus were used (**Cóndor Golec, 2019)** These chemical compounds, found in the Brassicaceae family such as mustard and broccoli, contain sulfur and nitrogen and are part of the defense system against pests and root-knot nematodes.

Certain parts of Brassicaceae have been used as volatile compounds released after enzymatic hydrolysis responsible for the efficacy of bioactive volatile substances. The glucosinolate- myrosinase- isothiocyanates system controls many pests and diseases, including root-knot nematodes transmitted through soil. The biological role of Brassicaceae green manure amendments has been controlled. (**Aires *et al*,. 2009**). Mustard seed extracts were utilized to inhibit the activity of root-knot nematodes (**Henderson *et al*., 2009**)

CONCLUSION

Studies have showed that alcoholic and aquatic extracts from the roots and seeds of the mustard naturally effect on hatching of nematode eggs and the vitality of second-stage juveniles (J2) of nematodes that infest the roots of green crops. The study indicates that extracts represent promising and environmentally friendly methods for providing inhibitors to root-knot nematodes, thereby contributing to environmental pollution reduction by reducing reliance on insecticides.

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