EFFECT OF SOME PLANT EXTRACTS ON ROOT-KNOT NEMATODE (*MELIODOGYNE* SPP) ASSOCIATED WITH CUCMBER

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ABSTRACT

An experiment was conducted to test the nematicidal activity of different concentrations of the aqueous extracts of marigold (*Tagetes s*pp.) and garlic (*Allium sativum*). Cucumber plants were grown in pots in 2018 in a greenhouse at the University of Sulaimani–College of Agricultural Sciences Engineering. Plant extract concentrations of 0, 10%, 20%, and 30% were prepared in1 liter of hot water. The extracts were applied to soil infested with nematodes which was brought fromYaranbag. The results showed that garlic recorded maximum shoot length (8.18 cm and 7.80 cm) when concentrations of 30% and 20% were used. The maximum root length (3.67 cm) was recorded with concentration 30 of garlic. Moreover, maximum number of leaves (7.33 cm) was recorded when 30% of garlic extract was applied. The significantly minimum numbers of galls/ plant (0.33 cm) were recorded when 30% of garlic was used. These results suggest that the application of marigold and garlic extracts would be a good alternative to manage root-knot nematode populations.

Keywords: Botanicals, *Meloidogyne*, Root-knot nematodes, Cucumber.

دراسة تاثير بعض المستخلصات النباتية في نيماتودا تعقد جذور (*Meilodogyne spp*) في نبات الخيار

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الملخص

تم اجراء التجربة لدراسة تأثير تراكيز مختلفة من مستخلص مائي نبات القطيفة والثوم في الفعالية المضادة للنيماتودا. تم زراعة نبات الخيار في أصص في داخل البيت الزجاجي في عام 2018 في كلية علوم الهندسة الزراعية /جامعة السليمانية . تم تحضير اربع تراكيز من كل مستخلص نباتى وهي صفر و10٪ و20٪ و30٪ وتم اضافتها الى تربة الا صيص المصابة بالنيماتود التي تم جلبها من يارانبك. اظهرت النتائج ان النباتات المعاملة بمستخلص الثوم بتركيز 30٪ اعطى اعلى معدل لطول الساق (8.18 سم) واعلى معدل الطول الجذر (3.67 سم) واعلي معدل لعدد الاوراق (7.33 سم) كما ادت هذه المعاملة الى تقليل عدد العقد على الجذور بشكل معنوي (0.33 سم). تشير من هذه النتائج ان اضافة مستخلص القطيفة والثوم بديل مناسب للسيطرة على تجمعات نيماتودا التعقد الجذرية.

كلمات مفاتحية : مستخلصات،نيماتودا، تعقد جذور،خيار .

INTRODUCTION

Cucumber (*Cucumis sativus*) is an economic crop that is considered as one of the most popular vegetables and is ranked the fourth most important vegetable after tomato, cabbage and onion in the world. Cucumber belongs to the family of Cucurbitaceae (Weng and Sun, 2011). It is one of the most grown crops under protected greenhouses throughout the world. Cucumbers grown in plastic house are prone to many diseases caused by bacteria, fungi, viruses, insects, and nematodes, which are the most important and result in yield losses (Koch, 1942). Most of the plant parasitic nematodes that attack the roots of plants are Root-Knot Nematode (RKN). The most economically important genus, which parasitzes the plant roots, is the worldwide-distributed genus *Meloidogyne*. The most well-known species *M. incognita*, *M. Javanica*, *M. arenaria*, and *M. hapla* (Agrios, 2005). Nematode control is largely based on synthetic nematicides which are one of the main causes of environmental pollution, nematode resistance, and

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phytotoxicity.Unsafe use of nematicides may result in poisoning of humans is a problem, especially in the developing countries (Yudelman*et al*., 1998). The use of plant extracts is one of the methods for nematode control. They are cheap, easy to apply, produce no pollution hazards, and have the capacity to improve the soil health (Sultana *et al*., 2010). Some studies have shown that marigolds were capable of suppressing a wide range (up to 14 genera) of nematode pests (Suatmadji 1969). Evaluated nematicidal effect of five plant essential oils on the root-knot nematode *Meloidogyne incognita*, which was one of major pest in many vegetables plants including tomato which showed that garlic and thyme essential oils were more effective in reducing the nematode infestation(Cetintas & Yarba 2010). The current study was designed to extract nematodes from soil and root of cucumber, determine the intensity of the disease, compare the antinematodal activity of the aqueous extracts between marigold and garlic.

1. MATERIAL AND METHODS

Collection of samples

Soil sample and root used for the study were obtained from eight greenhouses in Yaranbag that cultivated two cultivars of cucumber (Alamdar, saef). We have taken soil samples from a depth of 15–20 cm and five cucumber roots from each greenhouse. Soil and root were brought to the laboratory.The roots were washed with tap water. The number of Gall index (GI) was counted. Gall index (GI) was determined using a scale that is shown in Table 1 (Anonymous 1993).

Table 1: Root-knot index for *Meilodogyne spp*

Number of galls	Scale (based on number of root-knot galls/root)	Reaction
0	1	HR (Highly Resistant)
1-10	2	R (Resistant)
11-30	3	MR (Moderately Resistant)
31-100	4	S (Susceptible)
101 and above	5	HS (Highly Susceptible)

Extraction of nematodes from plant and soil samples

Soil sample and root wereused for extraction by three extraction methods, namely root method, Cobb's sieving, and Baermann funnel technique. Illustrated procedures followed for each method of extraction are given below.

Root method. Roots were cut into small pieces (1–2 cm) and put into a container of 200 ml of 0.5% NaOCI (sodium hypochlorite) solution and left for 48 hours. Later, the suspension observed for nematodes using digital microscope (Hussey and Baker, 1973).

Cob's sieving. 200cc of soil was taken and put in a 1000-ml capacity beaker and sufficient quantity of water was added to make a soil suspension. Soil and water were stirred for 60 seconds, and then the suspension was passed through a cascade of several sieves with decreasing sizes (710 µm, 500 µm, 355 µm, 250 µm,180 µm, 125 µm, 90 µm, 75 µm, 45 µm, and 5 µm).The remaining sediment on the 5 µm sieve was rinsed with tap water and transferred into a beaker (Cobb, 1918). Then,the sediment was examined under a digital microscope.

Baermann funnel technique. The Baermann funnel consists of a glass funnel to which a piece of rubber tubing is attached, with a clamp placed on the tubing. The funnel (12 to 15 centimeters in diameter) is placed on a stand. 100 cc of soil sample was put on a piece of cheese cloth.The cloth was placed in a funnel and filled with water, and then left for 24 hours. The live nematodes move actively and migrate through the rubber tubing. More than 90% of the live nematodes are recovered in the first 5 to 8 milliliters of water drawn from the rubber tubing, and this sample is placed in aPetridish (Agrios,

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2005).Then, it was observed by a digital microscope.

Identification of root-knot nematode

The roots infested with root-knot nematode were washed. Then the roots were cut from well-developed galls of the root transferred to petri plate containing water transferred to a clean microscopic slide. A cover slip was placed on it, and observed under digital microscope. Main Morphological Characters that it Body elongated in larvae and male, spheroid with a distinct neck in female and female swollen as described by Chitwood (1949).

Preparation of plant extract

Mature plant marigold was collected in October, 2018 from different areas of Sulaimani. Root marigold were washed from soil particles and put on a carton in the shade to air-dry. The roots were cut into very fine pieces. Powde garlic bulb was used for the study were obtained from a local market. Extractcon centrations of 0%, 10%, 20%, 30% were prepared by dissolving 0g, 100g, 200g, 300g of each sample in1 liter of distilled water at 90°C for an hour and allowed to cool for 30-45 minutes. the cooled extract was filtered using filter paper (Aydinli and Mennan,2014).

Potted Experiment

The soil infested of nematode was collected from the green houses in Yaranbag, and put into 21 pots (3kg per pot).Then, two seedlings had sowing of cucumber of each pot. One week after transplanting, 25 ml of each extract was poured into the soil in the pot around the base of the cucumber stem weekly for six weeks with the exception of concentration 0, which was only supplemented with water. All treatments were replicated three times. Observations were taken on the following plant growth parameters:shoot length (cm), root length, numbers of leaves and numbers of root gall. Statistical Analysis for this experiment was conducted designed by Factorial CRD, and Least significant difference Test was used to separate means at the level of significance $P<0.05$.

2. RESULTS AND DISCUSSION

Data shown in Table (1) indicate the number of galls per root system in eight greenhouses in Yaranbag between two cultivars. The results showed that all greenhouses were either susceptible or highly susceptible. However, maximum susceptibility was recorded in greenhouses three and six (250 and140, respectively) of cultivar Alamdar with gall index of 5.0, indicating its high susceptibility. This result was previously reported by Al-Ghonaimy and Zawam(2016). The authors evaluated six cucumber cultivars were evaluated for their resistance against *M. incognita* and *M.javanica* under greenhouse conditions. None of the tested cultivars was found completely resistant to the infection root-rot nematode.

Table 1: Root-knot nematode index for *Meloidogyne* spp

Green house	Cultivars	Numberof galls	Scale	Reaction
1	Alamdar	103	5	Highly Susceptible
2	Saef	52	4	Susceptible
3	Alamdar	250	5	Highly Susceptible
4	Saef	74	4	Susceptible
5	Saef	42	4	Susceptible
6	Alamdar	140	5	Highly Susceptible
7	Saef	60	4	Susceptible
8	Alamdar	80	4	Susceptible

The life cycle of *Meloidogyne* spp. Was clearly seen under microscope that they extracted from root and soil of cucumber. Generally, the life cycle of RKN *Meloidogyne* spp. consists of six stages, egg stage with four juvenile stages and finally adult, which is shown in Figure 1(Coyne*et al*., 2014).

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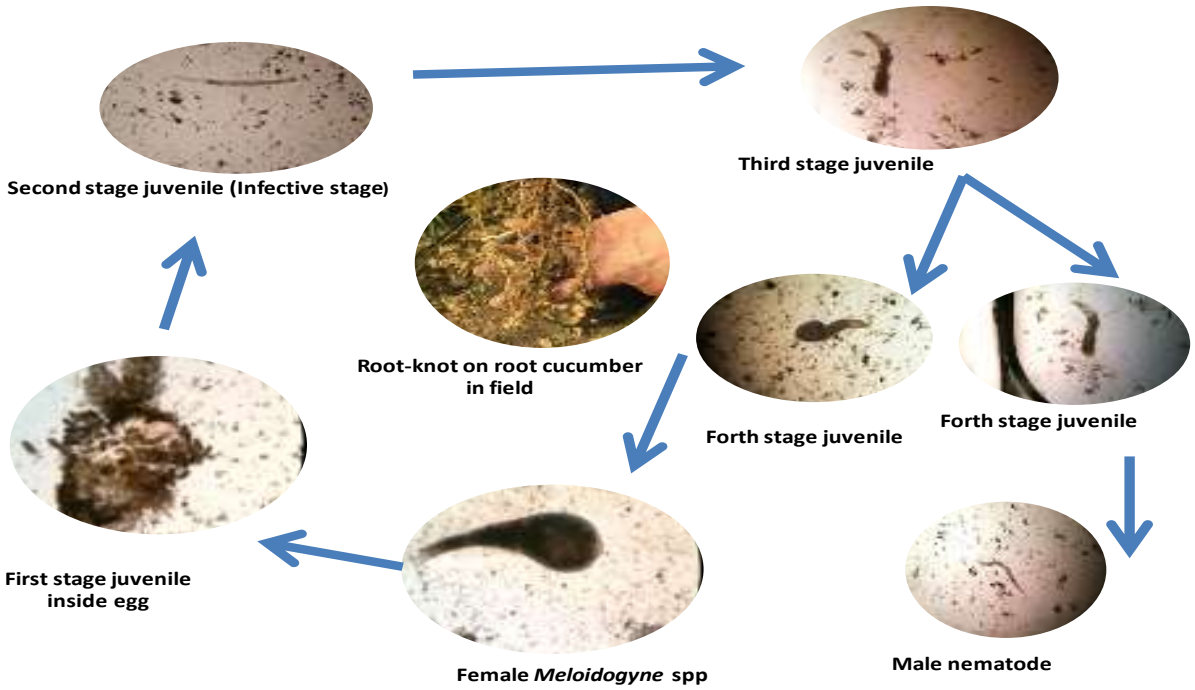

Figure 1: The life cycle of nematodes (*Meloidogyne* spp)

Data presented in the Table 2 show the effect of plant extracts of marigold and garlic on the growth of shoot length (cm) of cucumber grown on nematode-infested soil. There were no significant differences in plant extracts marigold and garlic. Tagetes (marigold) is a nematode allelopathic crop and act as a non-host or a poor host and a trap crop produce allelopathic compounds α ternithyl, that are fatal to Plant Parasitic Nematodes (PPNs) development and creates a favorable microenvironment for nematode antagonistic flora (PudasainiMP*et al*., 2008). There were no significant differences between the effects of concentrations 20 and 30. However, the effect of the two concentrations was more confound than concentration 0. A considerable increase of the plant parameter in shoot length at 30 concentration shows that the extracts have a toxic effect on the activity of the nematodes in comparison with the concentration 0. The significantly maximum shoot length (8.33 cm) was recorded for concentration 30 followed by 8.17cm and 8.03 cm in concentration 20 garlic and 30 marigold. However, minimum shoot length (6.00 cm) was recorded in concentration 0. The results are in agreement with those of Fayzia *et al*.(2018) who studied seven treatments *Paecilomyceslilacinus*, *Arthrobotrysoligospora*, *Glomus faciculatum*, *Eucalyptus globules*, *Tageteserecta*, *Allium sativum*, and *Fenamiphos* to control *M. incognita* on cucumber . All the treatments gave increase in shoot length of cucumber.

Table 2: Effects ofextractsof marigold and garlic on the growth of shoot length (cm) cucumber grown on nematode infested soil.

Conc. / Plant extracts	0	10	20	30	Effect of plant extracts
Marigold	6.00 b	6.67 ab	7.43 ab	8.03 ab	7.03 a
Garlic	6.00 b	7.67 ab	8.17 a	8.33 a	7.54 a
LSD 0.05=	2.15				LSD 0.05= 1.07
Effect of extract concentration	6.00 b	7.17 ab	7.80 a	8.18 a	
LSD 0.05=	**1.52**				

Means followed by the same letter in the same column are not significantly different by least significant difference (P≤0.05)

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The effect of plant extracts marigold and garlic on the growth of root length (cm) cucumber grown on nematode-infested soil is illustrated in Table 2. There is no significant difference between each other while significant difference was found with concentration 0. The significantly maximum root length (3.67 cm) was recorded with concentration 30 from garlic followed by 2.67cm and 2.67 cm in concentration 20 garlic and 30 marigold. Smilar result with bakr (2018). The highest increasing in root length recorded with plants treated with garlic 3% followed by onion 3% and lemongrass 3%.The lowest plant growth parameters observed in plants with nematode alone. Dried, powdered garlic contain approximately 1%allin (S-allyl cysteine sulfoxide). One of the most biologically active compounds, allicin (diallythiosulfinate) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes allin to allicin (Londhe*et al*., 2012).

Table 3: Effects ofplant extracts marigold and garlic on the growth of root length (cm) cucumber grown on nematode infested soil.

Conc. / Plant extracts	0	10	20	30	Effect of plant extracts
Marigold	2.67 b	2.67 b	2.37 b	2.67 b	2.59 a
Garlic	2.67 b	2.33 b	2.67 b	3.67 a	2.83 a
LSD = 0.05	0.99				LSD 0.05= 0.50
Effect of extract concentration	2.67 a	2.50 a	2.52 a	3.17 a	
LSD 0.05=	**0.70**				

Means followed by the same letter in the same column are not significantly different by least significant difference (P≤0.05)

Data presented in Table 4 show the effects of marigold and garlic extracts on the number of leaves of cucumber grown on nematode-infested soil. There is no significance difference between the effects of the plant extracts.There were no significant differences between concentrations 10, 20, and 30 while significant difference was found with concentration 0. In the present study, garlic extract showed no phytotoxicity in contrast to some earlier reports (Sukul*et al*., 1974), as they used 250 ml of 50% garlic extract concentration. High concentrations may lead to osmotic loss of water from the root tissues resulting in wilting.The significantly high number of leaves (7.33) was recorded with concentration 30 of garlic followed by (6.67 and 6.67) in concentration 20 garlic and 30 marigold.The lowest number of leaves was observed in plants with nematode alone. Marigold plants suppress plant parasitic nematodes through the process of biochemical interaction known as allelopathy, which is defined as the release of compounds from plants thatare toxic to other plants and nematodes. Marigold plants produce a number of potentially bioactive compounds among which alpha terthienyl is recognized as one of the most toxic chemical. The sulfur-containing bioactive compounds are abundant in marigold tissues, including roots. Mainak(2017) mentioned that garlic possesses biochemical substances, and the allelopathic substances are toxic to nematodes (Nigh E.L., 1985).

Table 4: Effects of marigold and garlic extracts on the number of leaves cucumber grown on nematode infested soil

Conc. / Plant extracts	0	10	20	30	Effect of plant extracts
Marigold	4.67 c	6.33 ab	6.33 ab	6.67 ab	6.00 a
Garlic	4.67 c	5.67 bc	6.67 ab	7.33 a	6.08 a
LSD 0.05	1.58				LSD0.05= 0.79
Effect of extract concentration	4.67 b	6.00 a	6.50 a	7.00 a	
LSD 0.05=	**1.12**				

Means followed by the same letter in the same column are not significantly different by least significant difference (P ≥ 0.05)

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Data presented in Table 5 reveal the effects of marigold and garlic extracts on the number of root-gall grown on nematode-infested soil. There were no significant differences between marigold and garlic but there were significant differences between concentrations 0, 10, 20, and 30. Applications of botanicals at all tested concentrations significantly inhibited nematodes. Plant parameters showed an effective role of the extracts in controlling the infestation of nematodes into cucumber roots compared to concentration 0. Efficacy of plant extracts, however, depends on the concentration and duration of exposure of the nematode to the extract (Mahmood *et al*., 1979; Kali and Gupta, 1980).The lowest numbers of galls/ plant (0.33) were recorded in concentration 30 of garlic followed by 1.33 and l.66 in 30 marigold and 20 garlic. However, highest galls/ plant (4.33) were recorded in concentration 0. The results were similar to the findings of Sukul*et al*.(1974) on tomato, thatgarlic extract was highly effective in reducing root-knot infection.

Table 5: Effects of plant extracts marigold and garlic on the number of root -gall grown on nematode infested soil

Conc. / Plant extracts	0	10	20	30	Effect of plant extracts
Marigold	4.33 a	3.33 ab	2.33 bc	1.33 cd	2.833a
Garlic	4.33 a	3.00 b	1.66 c	0.33 d	2.333a
LSD 0.05	1.27				LSD 0.05= 0.64
Effect of extract concentration	4.333 a	3.167 b	2.000 c	0.83 d	
LSD 0.05=	**0.90**				

Means followed by the same letter in the same column are not significantly different by least significant difference (P≥ 0.05)

Conclusion

The present study revealed that out of different botanicals tested, garlic bulb extract at 30% concentration reduced root-knot nematode infestation and increased shoot length and number ofleaves and can be used effectively for the management of root-knot nematode in cucumber. Also, the authors suggest testing the activity of marigold and garlic bulb with other solvents, different durations of exposure, and different method of application as further research work.

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