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Research Article

Effects of aqueous extract of walnut leaves against *Meloidogyne javanicaon* tomato plant

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ABSTRACT
Greenhouse and laboratory studies were conducted to test the efficacy of walnut aqueous extract against *Meloidogyne javanica*on tomato plant. Aqueous extract of walnut leaves prepared with various concentrations range (5%, 10%and 20%) that they diluted from stock solution and water were used as control. Tomato seedling roots were soaked in one hour in the mentioned concentrations then tomato plants were transplanted to the pots and 20 cc of walnut extract injected to the soil. Then, second stag juveniles (J2s) of nematode were incubated around the roots. According to the results, increasing in the concentrations of aqueous extract (20%) caused a significant reduction in final population of nematode and reproduction factor, but there is a reduction in root length and stem length of tomato with 20%concentration, as compare to control. However an increase in dry weight of roots and stem were observed. In laboratory test, to assess the nemtocidal effect of these extracts, J2s were exposed to the mentioned extracts for 24, 48 and 72 hours. The mortality of J2s, were affected by percentage of extract concentrations and time in petri dish environment. In case of any increase in concentration and time, there was a significant increase in mortality of nematodes. Totally the results showed the aqueous extracts of walnut leaf have nematocidal efficiency .
Key words : plant extract, Walnut, Nematode, Inhibition, Mortality

1. INTRODUCTION:
The common tomato, *Lycopersiconesculentum* Mill, belongs to solanaceae family. It is one of the most important products which are cultivated throughout the world.

Plant diseases are a limitation forcrop production. Plant parasitic nematodes are widespread through the world and among this Root-Knot Nematode, *Meloidogyne* spp., are one of the mostimportant nematodes, whichmakes some problems for growth and also serious crop losses. *Meloidogyne* spp. is an obligate root –parasite of more than 3000 plants species. More than 90 species of this nematode have been recognized at various parts on the world (Karssen and Moens, 2006). It may cause highest rates of damages to various garden and agricultural products. It may cause a reduction of 5% in agricultural products in the world (Agaryus, 2010). Also it is obvious in all parts of Iran as well. (Nasr Esfahani, 2009) This nematode is one of the most important pathogens of tomato plant. The magnitude of damage caused by nematodes in combination with other soil-borne pathogens increases. Tomato plants can be killed when *Fusariumoxysporum* f. sp. *lycopersici* is present with *Meloidogyne* spp. (Sikora and Fernandez, 2005).

It is impossible to have a complete removal of these nematodes due to their high rate of its reproduction, underground symptom of disease and wide host range of this pathogen. No specific control measures have yet been adopted in Iran to control of the root-knot nematode disease. Chemical control of root knot nematode is costly and hazardous to agro-ecosystem and

environment. So phytochemicals are safer to the environment andhumans than traditional chemicals. Many plant extracts have been reported to have nematicidal properties (Irshad*etal*., 1982,Stephan *etal*., 1989).Also plants are capable of producing secondary metabolites, which have an allelopathic effect (Chitwood, 2002) allopathic component are present in various parts of plants like the stem, root, leaves, rhizome, fruits and seeds. They may release some fallen leaves or dead parts of live plants and/or microbial/ chemical decompositions as well (Patrick*etal*, 1965).Other metabolites are effective in defense of plants against any insects and plant infection when exposed to mentioned component (Cowan, 1999). Therefore, they are effective in recognition of these metabolites and control of insects and diseases. For this purpose, today any replacement of natural preventive compositions with chemical ones is a modern guideline for nematode controls. Up to now, the anti-microbial effects of most of natural plant products have been studied as well. (Cowan, 1999). One of the oldest known and most noticeable allelopathic effects in trees occurs in walnut (*Juglans* spp.) associations. As early as 77 AD, the *Juglans* genera was cited by a Roman naturalist as having a poisonous effect on other plants and weeds.

Allelopathic materials in walnut, is juglone. All species in the genera of *Juglans* will generate measurable amounts of juglone potential. A number of these phenolic compounds in walnut can act as antioxidants and are antimicrobial. Juglone is released in different ways, leaves fall and decaying of them; root exudates, in the husks of abscised fruit; and by erosion from rain trickling over leaves and periderm. (kim, 2011).

There is little research about applying of walnut compositions for controlling of nematodes. The use of walnutextract has been explored in California for managingnematodes on fruit trees (McKenry and Anwar, 2003).In 2001, Wuyts*etal*. stated that *Juglan* is poisonous for various types of internal parasitic nematodes like *Pratylenchuspenetrans.* In 2002, in another research in a governmental project at California, it was reported that the extract of walnut out of watering of walnut branches is mortal for *Pratylenchuspenetrans*.

Therefore in order to find out any controlling effect of walnut extract on *Meloidogynejavanica*, aqueous extract of Iranian walnut leaves were used for controlling ofthis nematode on tomato for the first time in a petri dish and greenhouse environments. In addition, the phytotoxic effect of the mentioned concentration was studied on tomato as well.

Data analysis was made by SAS software, MSTAT-C and comparing the average data with Duncan test. By inserting data, Microsoft Excel has been used to make graphs.

2. MATERIALS AND METHODS

2.1 Culture of nematode

We received contaminated root with *Meloidogynjavanica* which was recognized in prior with molecular test from Plant protection Department of ShahidBahonar University. Then we could extract required juveniles from contaminated roots by Berman funnel method (Southy, 1970). Seedlings of tomato cultivar Early Urbana with two real leaves wereplanted in 1lpots. Tomato roots were infested with 1000fresh Juveniles and the pots were kept at greenhouse conditions and culture them. About 8 weeks after inoculation, the infected roots of thetomato plants were washed and the second stage juveniles were extracted with the mentioned method.

2.2 Preparation and inoculation of aqueous extract (In vivotest)

All healthy leaves of walnuts were washed and dried in shadeand grinded to powder after well dried. 20 gram of the leave powder was soaked in 100 ml water for twenty-four hours (24hrs). After that the soaked leaves were sieved. The filtrateswere taken as stocksolution; serial dilutions were taken as stock solution such that 5%, 10% and 20% concentrated. Water was served as the control.

The healthy and equal size of five weeks old tomato seedlings were uprooted. Theroots oftomato plant were dipped into each treatment (0, 5, 10 and 20%) for 1 hour then transplanted to pot and about 25cc of this solution were injected into the soil. One day later, one fresh

juvenile (J2s) pergram ofsoil wasinoculated around the tomato roots. The tomato plants maintained for 57 days at greenhouse condition. Then the following parameter was evaluated.

2.3 Evaluation of egg and larva at root system and Reproduction Factor (RF)

After 57 days from the incubation of tomato plant with nematode, each pot was hand harvested then the Population densities of *M. javanica* were estimated (Pf) including second-stage juveniles inside the pot soilsand eggs on the root system. Root knot nematode eggs were extracted from tomato root using a 5% sodium hypochlorite solution (Zaki *etal*. 1998). In addition, the numbers of second-stage juveniles were extracted from each pot soil by using Caveness and Jensen method(1955). Regarding the initial population density (Pi) at time of root tissue infection, we could obtain the reproduction factor of nematodes through the following formulation (Oostenbrink, 1966).

RF= Pf÷PiReproduction Factor= final Population÷ initial population

2.4 Effects of aqueous extract on mortality of larva (in vitrotest)

The effect of fresh walnut leave extract on larval mortality was assessed on Petri dish environment in a factorial experiment based on completely randomized design with fivereplications. Fresh second stage juveniles were exposed to concentrations of 5%, 10% and 20% of aqueous extract of walnut leaves (diluted from stock solution) and water as control for 24, 48 and 72 hours in petri dish and kept atroom temperature (Mahmood *etal*., 1979). With help of binocular thelive and dead juvenile recorded (Faheem *etal.* 2010, Osei*etal.* 2011). We considered those nematodes without any movements and with smooth and elongated body as dead ones.

2.5 Evaluation of plant growth

The length of aerial and roots were measured by a ruler. The weight of roots was measured by carefully removing the plants from the pots after complete washing of roots. The fresh roots and shoots were weighed separately.

To measure the dry weight, all aerial part of plant and roots were put in oven for 48 hours at 60° C and weighted with an accuracy balance (0.001 gr).

3. RESULTS

3.1 Evaluation of final population of nematodes and reproduction factor

There was a considerable difference in number of final population of nematodes in treated tomato with different concentration of aqueous extract after 57 days from incubation of plant. The aqueous extract of walnut leaves (20%) could make a significant reduction in average number of eggs and larva (65760),compere to control (182120). Although there was not a significant difference in number of final population density between treatments of 5%and 10%concentration of extract. But upon any increase of concentration, there was a reduction in number of larva and eggs. In addition, there was a significant difference in reproduction factor (Rf) andby increasing the concentration of extract a reduction in Rfobserved. So that20% concentration could reduce the reproduction factor of nematodes than the others (2.04)(As shown inTable 1).

In vitroexperiment

3.2 The effects of aqueous extract of walnut on mortality of J2s.

Second stag juveniles (J2s) of nematode exposed to different concentrations within 24, 48 and 72 hours. The results of variance analysis showed that mortality rate of larva were under the effect of percentage of extract concentration and the time of exposer (As shown in Table 2). The minimum mortality of larva observed in control (used water) within 24 hours (6%) and the maximum was in 20% of concentration at 72 hours which received 100% of mortality. Generally any increase in extract concentration may cause significant increase in mortality of nematodes. Also any increase in exposed times within equal concentration may cause a significant increase in number of dead larva. (As shown in Fig.1)

3.3 Evaluation of growth index

After 57 days from inoculation, plants were uprooted carefully to study the length of shoot, root, fresh weight of shoot and root, length of root and shoot. In comparison with control

treatment, shoot and root length with 20% concentration of extract was reduced. Although according to the dry weight and fresh shoots weight and roots, it was obvious that any increase in extract concentration may cause an increase in weight of dry and fresh shoots and roots (As shown in Table 3).

4. DISCUSSION

Today many researchers are searching to find suitable product to manage plant pathogens without any environmental pollution. Up to now, most of plants have been recognized with allopathic properties and great preventive or killing rates.

It has been concluded from present research that certain plant extracts are a source of cheap and effective nematicides of root knot nematodes. In the juvenile mortality test (in vitro) with the extracts of walnut leave, it was revealed that 100% mortality observed within 72 h at 20% concentration so the leave extracts of walnut were found to have nematicidal properties. In addition, any increase in time of exposition of nematodes may cause a significant increase in number of dead larva. Walnut is a great and tall tree among lots of weeds and controlling the surrounding area. This paper is a primary study about aqueous extract of walnut leaves for controlling of root-knot nematodes on tomato at greenhouse conditions. In comparison with previous researches about nematodes, this research is an acceptable one. For instance, Inga *etal*. reported in 2010 aqueous and alcohol extract of green peel of walnut, *Juglansnigra*, have effecton killing of *Pratylenchuspenetrans* on raspberry. Furthermore, in 2001, Wuyts *etal*. stated that juglonof walnut tree is poisonous and killing for *P.penetrans*. In 2002, it was reported at California that walnut extract out of soaking of walnut branches in water is the lethal effect for *P.penetrans*. In addition, more than 35 phenol compositions have been recognized in walnut along with 12 compositions just in green peel of this fruit. Most of the mentioned materials are recognized as allopathic compositions for plants and other micro-organisms (Cosmulescu, *etal*. 2010). Therefore they could kill or control *Meloidogynejavanica* nematode. According to the greenhouse tests of reproduction factor which are the most important index for evaluation of plant resistance and nematode controlling, it was revealed that dipping the seedling roots in all concentrations of aqueous walnut leaf extract reduced reproduction of nematodes, so they may active plant defence response against invasion of nematodes.

Increasing in the concentration of extract (more than 20%), could reduce reproduction of these nematodes but it may have a negative effect on the length of shoot and root of tomato. So that by increasing the concentration to 20%, a reduction in stem and root growth was observed. Increasing in dry and fresh weight of shoot and root was found by increasing of concentration. Perhaps, this is due to decline in number of nematode in the root system. The mentioned reduction in length mayrelate to allopathic property of compositions for tomato plant as well. Therefore walnut components are possible to be used against *Meloidognejavanica* before cultivation. According to the studies of Kim in 2011, Anwar &McKenry in 2003 and Shrestha in 2009, the allopathic property of *Juglon* in plant parts of black walnut proved for controlling the plant diseases but may have allelopaty property for some plants.

Although researchers made some studies on black walnut, therefore it is necessary to analysisexact materials of *Juglan regia*and their effecton controlling of other plant pathogens. Also it is necessary to make complementary tests like ethanol and aqueous extraction of green peel of walnut. Furthermore, it is necessary to study any effects of walnut residue as compost in soil to evaluate controlling of plant pathogen nematodes and allelopathy effect on other plants.

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Table 1: The mean number of eggs and larva and reproduction factor of nematodes with 5 replications.

Treatment	Avg no. of eggs & larva	Rf
Control (water)	[a] 182120	[a] 2.488
Walnut leave extract 5(%)	[ab] 166595	[b] 2.446
Walnut leave extract (10%)	[ab] 118010	[c] 2.297
Walnut leave extract (20%)	[b] 65760	[d] 2.046

Data are means of 5 replicated plants per treatment. Means followed by the same letter do not differ significantly ($P < 0.05$) according to Fisher's protected LSD test. Rf means Reproduction Factor. Data were transformed to log10 (X + 1) before analysis.

Table 2: Variance analysis of J2s when it is subject to various concentration of aqueous extract of walnut leaves within 24, 48 and 72 hours.

Resource of changes	Mortality
Walnut aqueous extract	0.87
Time	0.44
Extract & Time *	0.03
Error	0.002
Coefficient of changes	2.6

* Shows the significant treatment at $P < 0.05$

Table 3: The average of Stem Length (S.L), Root Length (L.R), wet weight of stem (W.W.S), Wet Weight of Root (W. W. R), Dried Weight of Stem (D. W.S), Dried Weight of Root (D.W.R) in treatment of plant with extract of walnut leaves of 5%, 10% and 20% concentration and water as control.

Treatment	S.L (cm)	R.L (cm)	W.W.S (gr)	W.W.R (gr)	D.W.S (gr)	D.W.R (gr)
Control (water)	32.37^{a}	20.1^{ab}	12.02^{ab}	5.15a	1.86^{b}	0.48^{b}
5% Concentration	32.3^{a}	21.1^{ab}	10.19^{a}	6.25^{ab}	1.64c	0.51c
10% Con.	32.6^{a}	26.4^{a}	11.65^{ab}	6.66^{b}	1.74^{d}	0.52a
20% Con.	$25/87^{b}$	17.12^{b}	13.04^{b}	7.52^{b}	2.09^{a}	0.54^{d}

Non-similar letters show the significant treatment at $P < 0.05$ with Duncan's test.

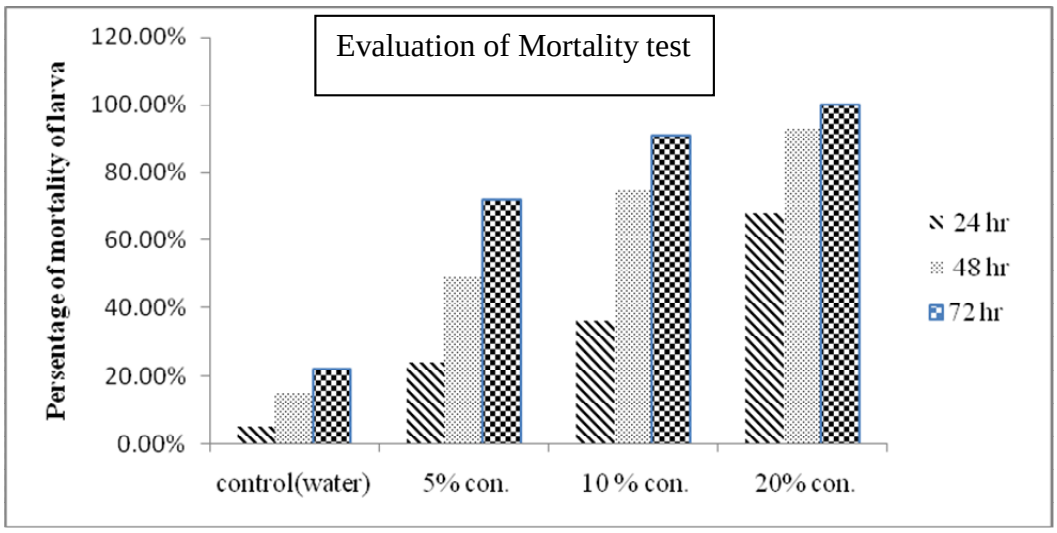

Figure 1: Mortality rate of nematode at various concentration of aqueous extract (5, 10, 20 and water as control) after 24, 48 and 72 hours. Different letters indicate that mortality is significantly different from each other at $P < 0.05$