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INFLUENCE OF SOME PLANT EXTRACTS ON VITALITY OF EGGS AND SECOND STAGE JUVENILES OF ROOT – KNOT NEMATODE *Meloidogyne javanica**

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ABSTRACT

Ethanol and aqueous extracts of leaves of oleander, river red gum and fruit of chili pepper were used against egg hatching and vitality of 2nd juveniles of root – knot nematode *Meloidogyne javanica* in in-vitro study. Results revealed that the maximum inhibition in egg hatching (70.65%) was caused by ethanol extract of chili pepper fruit at concentration 1.2% with significant difference compared to aqueous extract of the same plant, as well as with ethanol and aqueous extracts of the other two plants at all concentrations, while minimum inhibition (10.36%) was recorded by the aqueous extract of oleander leaves but the difference was not significant with the other extracts of the same plant and aqueous extract of river red gum at all concentration and ethanol extract at concentration 0.3%. Higher mortality of J2s (62.93%) was recorded by ethanol extract of chili pepper fruit at concentration 1.2% with no significant difference with ethanol extract of the same plant at concentration 0.6%, however minimum J2s mortality (7.45%) achieved by aqueous extract of river red gum at concentration 0.3% but the difference was not significant with aqueous extracts of the same plant and aqueous extract of oleander at concentration 0.3 and 0.6%.

KEYWORDS: Eggs, 2nd stage juveniles, *M.javanica,* plant extracts.
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INTRODUCTION

The most important species of root-knot nematodes are *M.javanica, M.arenaria, M.incognita* and *M.hapla* which include 98% populations in the worldwide (Hussain , 2011) and cause high economic damages in different crops (Khalil, 2013).The use of

plant extracts as an alternative to synthetic pesticides for controlling of *Meloidogyne* species is becoming important. In recent years, studies on this subject have increased quickly in the Mediterranean coast (Ntallie *et al.,* 2011 and Andres *et al.,* 2012).The use of plant extract with nematicidal properties is cheaper, safer and effective control measure than pesticides (Elzawahry *et al.,* years, studies on this subject have increased quickly in the Mediterranean coast (Ntallie *et al.,* 2011 and Andres *et al.,* 2012).The use of plant extract with nematicidal properties is cheaper, safer and effective control measure than pesticides (Elzawahry *et al.,* 2014).Various plant extracts and decomposed products of numerous medicinal plants and their parts such as fruit, leaves, seeds, roots and stems are known to have anthelminthics activities. These plant parts have been recorded to be toxic to many nematodes including *Meloidogyne* species (Montasser *et al.,* 2012).

In laboratory conditions (In-vitro test) the extracts of some plants including chili pepper (*Capsicum frutescens*) were used against *M.incognita* at different concentrations and it was shown that those plant extract caused mortality of J2s and inhibited of egg hatching (Kepenekci *et al.,* 2016). Ethanol extract of *Capsicum annuum* fruit at 1000ppm caused mortality of J2s of *M.incognita* by 100% before attacking tomato plants (Bawa *et al.,* 2014).Different parts of *Eucalyptus* sp., including fruits, leaves, barks and stems increased J2s mortality and decreased egg hatching of *M.javanica* when used as ethanol and aqueous extracts (Dawar *et al.,* 2007 and Hassan *et al.,* 2015).The aim of this study is to test the effect of aqueous and ethanol extract of

some plants on egg hatching and mortality of 2nd stage juveniles of root – knot nematode *M. javanica* in in-vitro assay in order to test the most efficient one as anti-nematodes in the field or greenhouses.

MATERIALS AND METHODS

1-Preparation of plant extracts:

Leaves of oleander (*Nerium oleander* L., Apocyanaceae), river red gum (*Eucalyptus camadulensis* Dehnh., Myrtaceae) and fruit of chili pepper (*Capsicum frutescens* L., Solanaceae), were washed, air dried in the laboratory and milled into powder by a coffee mill.Water and ethanol extracts of these plant materials were prepared by soaking 100gm powder of each one individually in 1000 ml of distilled water and ethanol for 48 hrs., in the dark on a shaker at 150 rpm and then filtrated through filter paper. The filtrated ethanol extracts were evaporated in oven at 70 ºC, and the final stock extracts became 100ml then diluted with distilled water at ratio 1:9, after that 3 concentrations (0.3, 0.6 and 1.2%) for both solvents and for each plant material prepared**.**

2- Extraction and preparation of eggs of *M.javanica*:

Nematode eggs were extracted by a method described by Coyne *et al.*(2007) and counted with the aid of counting dish under stereomicroscope then it was found that the mean number of eggs and for 6 replications was 50± 5eggs mL^{-1}

3-Egg hatching test (*In-vitro* bioassay):

One ml of nematode egg suspension consisted of 50±5eggs of *M.javanica* with 4ml of distilled water and 5ml of plant extract at ratio1:1, were placed in labeled sterilized Petri dishes (7cm).Four concentrations (0.0, 0.3, 0.6, 1.2%) for each of water and ethanol extract of mentioned plant materials were used, while 0.0 concentration was considered as control treatment consisted of 1ml of nematode egg suspension with 9 ml of distilled water. Each treatment was replicated 3 times. Petri dishes were placed in an incubator at 30±2ºC. After 10 days number of hatched eggs were calculated under stereomicroscope then corrected inhibition of egg hatching was calculated according to the equation mentioned by Zukreman and Rohde (1981) as follow :100 – 100 (number of hatched eggs in treatment ÷ number of hatched eggs in control treatment).

4- Extraction and preparation of J2s of *M.javanica* :

2ndstage juveniles were prepared from freshly hatched eggs of *M.javanica* where egg suspension were incubated at 30±2ºC and after 3 days suspension volume of emerging juveniles were adjusted for counting number of juveniles that reached 50±5 J2s/2ml as an Average of 6 replications.

5-Influence of some plant extracts on vitality of J2s of *M.javanica* (In-vitro bioassay):

This experiment was conducted by the same method used in egg hatching test but 2ml of freshly hatched J2s suspension (50±5 J2s) was used and incubated at 27±2ºC. Numbers of dead and living juveniles were calculated after 10 days under stereomicroscope. The dead juveniles either have curly (∞), bent, sigmoid (Σ), and straight (I) shape depending on Hassan *et al.*(2015).Juvenile's mortality percentage was corrected according to an equation mentioned by Ami(1998) as follows:

100-100 (Number of living juveniles in treatment ÷ Number of living juveniles in control).

6- Experimental design:

Both experiments (egg hatching and J2s vitality) were consisted of 24 treatments (3 plants × 2 solvents × 4 concentrations) with 3 replications performed as factorial experiment in CRD. Data were analyzed using SAS program to compare the mean of egg inhibition and corrected mortality of J2s for the tested treatments depending on Duncan's multiple range test, $p \leq 0.05$ (SAS, 2001).

ERESULTS AND DISCUSSION

1-Effect of plants extracts on inhibition of egg hatching of root – knot nematode *M.javanica*:

Results of statistical analysis (Table, 1) showed that the highest inhibition of egg hatching (63.62%) was caused by fruit of chili pepper with significant difference with extracts of leaves of oleander and river red gum which caused an inhibition percentage by 15.02 and 15.58% respectively. Highest concentration (1.2%) was also more effective, and caused an inhibition in egg hatching by 33.32% but the

difference was significant only with the lower concentration (0.3%) which caused an inhibition by 29.68%.

Results also demonstrated that the ethanol extract was more effective in reducing egg hatching with an inhibition percentage reached 35% regardless of plant extracts. It is obvious from the results of interaction between plant species, solvents and concentrations that the highest inhibition in egg hatching (70.65%) was caused by ethanol extract of chili pepper fruit at concentration 1.2% but the difference was not significant with the ethanol extract of the same plant at concentrations 0.3 and 0.6%, while the difference was significant with aqueous extract of the same plant, as well as with ethanol and aqueous extracts of oleander and river red gum at all concentrations.

Table (1): Egg hatching inhibition of root-knot nematode *M. javanica* as affected by plant extracts.

Plants	Corrected inhibition percentage of egg hatching						Effect of concentrations
	Chili pepper		Oleander		River red gum		
Conc.%	Ethanol	Water	Ethanol	Water	Ethanol	Water	
0.3	65.39 ab	55.56 c	17.81 def	10.36 f	17.83 def	11.12 ef	29 .68 b
0.6	66.87 ab	60.87 bc	17.83 def	14.1 ef	18.6 de	11.13 ef	31.57 ab
1.2	70.65 a	62.39 bc	17.85 def	14.22 ef	22.17 d	12.62 ef	33.32 a
Effect of solvents	35 a	27.82 b					
Effect of plant extracts	63.62 a		15.02 b		15.58 b		

*means with different letter(s) are significantly differ for each of interaction or for the independent factors based on Duncan's Multiple Range test (P≤0.05).
*interaction = each value is a mean of 3 replications.
*plant effect = each value is a mean of 18 replications (2 solventx3concentrations x3 replications).
*solvent effect =each value is a mean of 27 replications (3plants x 3concentrations x 3replications).
*concentration effect = each value is a mean of 18 replications (3 plants x 2 solvents x 3 replications) * Conc. = concentrations.

On the other hand less inhibition in egg hatching (10.36%) was recorded by the aqueous extract of oleander leaves which was not significant with the other extracts of the same plant and aqueous extract of river red gum at all concentration, in addition to its ethanol extract at concentration 0.3%.

2-**Effect of plant extracts on vitality of J2s of *M.javanica*:**

Results of statistical analysis (Table,2) showed that extracts of chili pepper caused a significant increase in mortality of J2s of *M.javanica* by 56.88%, followed by oleander (15.86%) which significantly differed also with the less effect (12.95%) caused by river red gum. On the other hand, the mortality percentage of J2s of *M.javanica* went up with increasing of plant extracts concentrations started by 25.64% as less mortality percentage with the lower concentration (0.3%) and ended by 31.72% with the higher concentration (1.2%).The solvents were also significant in their effects on J2 mortality and it was found that the highest J2 mortality (32.71%) was recorded by ethanol extracts while the lowest (24.42%) by aqueous extracts.

Table (2): Effect of plant extracts on vitality of J2s of *M.javanica*

Plants	Corrected mortality percentage of 2nd juveniles						Effect of concentration
	Chili pepper		Oleander		Eucalyptus		
Conc. %	Ethanol	Water	Ethanol	Water	Ethanol	Water	
0.3	58.21 b	49.76 c	16.61 fg	8.87 hi	12.91 gh	7.45 i	25.64 c
0.6	59.92 ab	52.85 c	19.84 ef	11.98 ghi	16.53 fg	8.89 hi	28.33 b
1.2	62.93 a	57.59 b	24.47 d	13.41gh	22.97 de	8.97 hi	31.72 a
Effect of solvent	32.71 a	24.42 b					
Effect of plant extracts	56.88 a		15.86 b		12.95 c		

*interaction = each value is a mean of 3 replications.
*plant effect = each value is a mean of 18 replications (2 solvents x3concentrationsx3 replications).
*solvent effect=each value is a mean of 27 replications (3 plants x 3 concentrations x 3 replications) .
*concentration effect= each value is a mean of 18 replications (3 plants x 2 solvents x 3 replications) .
*means with different letter(s) are significantly differ for each of interaction or from the independent factors based on Duncan's Multiple Range test ($P \leq 0.05$).

In general, the interaction between plant species, solvents and concentrations were significant on their effect on the percentage of J2 mortality. Maximum mortality (62.93%) was caused by ethanol extract of chili pepper at concentration 1.2%, and the superiority was not significant only with the ethanol extract of the same plant at concentration 0.6%. Regarding plant extracts of oleander and eucalyptus, the highest mortality were seen with ethanol extract by 24.47 and 22.97 % respectively at concentration 1.2% and the lowest with aqueous extract by 8.87 and 7.45% correspondingly for the same plants at their low concentration (0.3%).

The effect caused by these plant extracts in reducing egg hatching and increasing J2 mortality of root – knot nematode *M.javanica* is consistent with the results of each of Meira *et al.* (2006);Dawar *et al.*(2007) and Kepenekci *et al.*(2016) for extract of chili pepper, oleander and eucalyptus respectively as well as with Bakr *et al.* (2015) for extract of chili pepper, thus it seems that these plants possess nematicidal properties and as confirmed by previous studies, regarding extracts of chili pepper (Ayazpour *et al.*, 2010), oleander (Radwan *et al.*, 2007 and Nour El-Deen *et al.*, 2011) and river red gum (Youssef and Lashein, 2013 and Centintas and Qadir, 2014).The nematicidal activity of these plants may be attributed to the presence of active or toxic materials such as allyl isothiocyanate, capsainoids, capsaicinin in chili pepper (Mackeen *et al.*, 1997 and Abbas *et al.*, 2009) and flavonoids, alkaloids,tannins,saponins and sterolsin in oleander (Mousa*et al.*,2011) in addition to alkylhalids, aromatics aliphatic amines, amides and alkanes in the same plant (oleander) (Bakr *et al.*, 2015) while for river red gum it has been recorded that it contains also some active substances against nematodes such as ketones, benzamid, amides, saponins, flavonoides and alkaloids (Goswami and Vijayalakshmi, 1986) as well as some acids included hydroxyl benzoic, chlorogenic, vanelic, benzoic, coumaric, ferulic and caffeic (Hassan *et al.*, 2015).The mechanism effect of nematicidal activities of these plant extracts may return back to their ability in breaking up of cytoplasmic membrane of nematode cells and interfere of this functional group with structure of protein enzyme (Knobloch *et al.*, 1989), through degrading and denaturing of protein and enzyme inhibiting (Konstantopoulou *et al.*, 1994).

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كارتيكرنا ده رخسنه رين هنده ك رووه كان لسه ر زينده يا هيك و زر كورين كرمى ده زو له يى (نيماتودا Meloidogyne javanica)

بوختە

كارتيكرنا ده ر خسته رى كحولى و ئافى يين هه ر ئيك ز به لكين ريلى و كارفورى (يوكاليبتوس) وبه رين فافلين سوور و تيز هاته جه رباندن لسه ر زينده يا هێك و زركورێن كرمێ دەزوولەی (نيماتودا .M.javanica (هاته ديار كرن ز ئه نجامين با يو تيستى كو ئه ف ده ر خسته ره بين ئه كرين نه ده ركه تنا تيزكين فى نيماتودى ز هيكان وهه ر وه سا ن بين ئه كرى مرنا زركورێن فى نبماتودى بريزه يه كا جياوازى بشتى 10 روزان ز نوقيكرنا وان د ناف فان ده رخسته راندا ودیار بو كو دەرخستنەرێ كحولى يێ بەرێن فلفلين سوور ب تيراتيا 1.2% بو ئەگەرێ نەدەركەفتنا تيزكان ز هێكان و مرنا زركوران ب رێژا 70.65 و 62.93% ل ديڤ ئێك. ژ بەر هندێ ئەڤى دەرخستنەری پتر كاريگەری هەبوو ژ ناڤ دەرخستنەرێن تاقيگەهێ. دەرخستنەرێن ئاڤێ يێ بەلگێن يوكاليبتوسى بو ئەگەرێ نەدەركەفتنا تيزكان زهێكان و مرنا زركوران ب رێژا 12.62% و8.97% و ژبەر هندێ ئەڤ دەرخستنەرە كێمترين كاريگەری هەبو.

تأثير مستخلصات بعض النباتات في حيوية البيوض ويافعات الطور الثاني لنيماتودا تعقد الجذور *Meloidogyne javaniac*

الخلاصة

اختبر تاثير المستخلص الكحولي والمائي لكل من اوراق الدفلة والكافور (اليوكاليبتوس) وثمار الفلفل الحار في فقس البيوض وحيوية يافعات الطور الثاني لنيماتودا تعقد الجذور *M.javanica*.اتضح من نتائج الاختبار الحيوي ان هذه المستخلصات سببت تثبيطا في فقس البيض وموتا ليافعات الطور الثاني لهذه النيماتودا وبنسب مختلفة بعد 10 ايام من غمرها في هده المستخلصات وقد لوحظ ان المستخلص الكحولي لثمار الفلفل الاحمر بتركيز 1.2% سبب تثبيطا في فقس بيض النيماتودا و موتا ليافعاتها بنسبة 70.65و 62.93% على التوالي فكانت من اكثر المستخلصات كفاءة فيما اثر المستخلص المائي لاوراق اليوكاليبتوس بنسبة 12.62و 8.97% لكل من تثبيط فقس البوض وموت اليافعات على التوالي لتكون بدلك من اقل المستخلصات كفاءة.