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ORIGINAL ARTICLE/CONTROL

Inhibition of *Meloidogyne incognita* Egg Hatching by Herbal Extracts

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Abstract

The inhibitory effect of herbal extracts using methanol and hexane collected from Sudan and Korea was evaluated on egg hatching of *Meloidogyne incognita*. The concentration of herbal extracts were 10,000, 1,000 and 100 ppm, respectively. They were treated to *Meloidogyne incognita* eggs after 3, 7, and 21 days of hatching. All herbal extracts inhibited egg hatching significantly compared to the control. The degree of inhibition was directly related to the concentration of the extracts. At 10,000 ppm, very fewer individuals were hatched at all the periods of exposure with the exception of the extract of fruits of *Quisqalis indica* which gave 84.4 and 54.5% at 7 and 21 days, respectively. Egg hatching was completely ceased, especially after 7 days for most of the extracts. While at 1,000 ppm relatively high numbers of eggs were hatched compared to the higher concentration, as well the extracts *Q. indica* reported many number of hatched eggs compared to others, especially at 21 days. On the other hand, the extract from pods of *Cucumis mello* var *agrestis* gave the least hatching number of eggs at all periods of exposure which gave 2, 8, and 3% at 3, 7, and 21 days, respectively in comparison to the control. Whereas at 100 ppm, inhibition increased with exposure time. However, the extract leaf of *Desmodium caudatum* represented the fewer hatching eggs at 3 and 7 days.

Key words Herbal extracts, egg hatching, *Meloidogyne incognita*, root-knot nematodes

Introduction

Traditional chemical control using nematicides available for the last few decades for insects, weeds, and pathogens is declining internationally in the last decades. Health and hazards concerns which have elicited close security by regulatory agencies have resulted in increasing restriction or prohibition of use (Osman and Viglierchio, 1988). Natural products seem to resolve environmental problems caused by synthetic pesticides and many researchers are trying to find out effective natural products to replace synthetics ones (Kim *et al.*, 2005). Considering the probability of plant secondary products being involved in plant pest's interactions, the strategy of randomly isolating, identifying and bioassaying these compounds may also be an effective method of pesticide discovery (Duke, 1990).

Many plant species are known to be highly resistant to plant parasitic nematodes, the well documented of these includes marigolds (*Tagetes* spp.), rattlebox (*Crotalaria spectabilis*), chrysanthemum (*Chrysanthemum* spp.), and neem (*Azardiracta indica*) (Duke, 1990). Natural products are selective to pests and have no or little harmful effect

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on non-target organisms. The uses of herbal extracts against plant parasitic nematodes had been studied by many authors, for example, Amer-Zareen *et al.* (2003), Coxa *et al.* (2006), Husan-Bano *et al.* (1999), Mennan and Melakeberhan (2006), Onfade (2007), Pandey (2000), Pandey and Kalra (2005), Shaukat and Siddiqui (2001), for plant pathogens (Lee *et al.*, 1998, 2000, 2001a; Park *et al.*, 2003), and for insect pests (Lee *et al.*, 2001b).

Root-knot nematodes are the most widely distributed group of plant parasitic nematodes (Castagone-Sereno *et al.*, 1994) and the genus is one of the most wide spread pests limiting agricultural productivity causing average loss of yield of about 5% worldwide (Nickle, 1991). Root-knot nematodes attack many plant species including monocotyledons and dicotyledons and cause loss of millions of dollars worldwide (Piotte *et al.*, 1992). They are the pests of major food crops, vegetables, fruits and ornamental plants grown in tropical, sub-tropical and temperate regions (Jepson, 1987; Eisenback and Hirschmann, 1991).

The damage caused by plant parasitic nematodes especially the root-knot nematodes represents one of the major obstacles for the production of adequate food supply (Carter and Sasser, 1982). In Sudan the recent intensification and diversification of cropping, to meet the ever increasing of food consumption and export, have led to more and more nematodes problem being evident (Yassin *et al.*, 1992; Yassin, 1984). In the Republic of Korea, as well root-knot nematodes especially *M. incognita* causing servere damage to crops especially in greenhouses (Choi and Choi, 1982; Cho *et al.*, 2000).

Our objectives of this work was to test the inhibition of root knot nematode, *M. incognita* eggs hatching using 12 herbal extracts under laboratory conditions.

Materials and Methods

Twelve different herbal plants species (trees and weeds) were collected from Gezira locality in Sudan and Korea. Leaves and stems of *Desmodium caudatum* were collected from Seogwipo, Jeju and fruits of *Quisqalis indica*, rhizomes of *Zingiber officinale*, flower buds of *Daphane genkwa* and seeds of *Areca catechu* were purchased from the herbal shop at Gyeongdong Market, Seoul. Some of them were separated to different portions (leaves and seeds), then were dried under shade in the laboratory, after being dried they were crushed and finely blended using home blender and labeled. Methanol extracts of the 8 herbal plants and hexane extracts of the 4 herbal plants (Table 1) were screened against *M. incognita* egg hatching.

The *Meloidogyne* egg masses were brought from an infected cucumber grown in the greenhouse. The eggs were separated from egg masses by cutting the roots containing egg masses into small portions of 0.5-1 cm long and agitated for 2-3 minutes in 0.5% sodium hypochloride (Orion, 2001). Then rinsing for three times in tap water over 60 and 30 um sieve. The separated eggs were collected in 250 mL beaker and homogenized using automatic stirrer. Then, 3 mL were taken separately for counting the number of eggs and the average of three readings was calculated and was found to be 230 eggs.

The extracts were diluted to give 5,000, 500 and 50 ppm. Test samples were suspended in distilled water with Triton X-100 added at the rate of 0.1 mL/L. Each dilution was put in a Petri dish then nematodes eggs were added. The dishes were kept on the bench in the laboratory

Table 1. Herbal extracts used in this test and their yields in grams

Herbal extracts	Portions used	Yield (%)[a]
Quisqalis indica	Fruit	12.1
Desmodium caudatum	Leaf	17.2
Zingiber officinale	Rhizome	7.0
Daphane genkwa	Bud of flower	2.9
Areca catechu	Seed	3.7
Dinbera retroflexa	Leaf	7.6
Cucumis mello var *agrestis*	Fruit	8.7
Ziziphus spina-christi	Leaf	5.1
Acacia nilotica	Pod	33.7
Chenopodium album	Leaf	6.2
Azadirachta indica	Seed	34.7
Calotropis procera	Leaf	9.3

[a](Dried weight of methanol or hexane extract/dried weight of the same sample plant)X100.

under normal room temperature. Each extract dilution was replicated 4 times. Control was set with only distilled water with Triton X-100 added at the rate of 0.1 mL/L.

Nematode eggs hatching were counted after 3, 7, and 21 days after treatment using the stereo-microscope.

Data were analysed by using SAS programme (2004), then ANOVA and means separation were set.

Results and Discussion

Generally speaking, all herbal extracts at all concentrations levels and time of exposures inhibited eggs hatching in varying degrees. As well under all herbal extracts including the control eggs hatching started with very low numbers of eggs hatching after three days and then increased slowly after 7 and 21 days of exposure (Table 2, 3, and 4). The degree of inhibition of egg hatching depends upon the concentrations.

At 10,000 ppm all the herbal extracts inhibited nematode egg hatching at all levels of exposure in varying degrees (Table 2), and significantly different from the control with the exception of the extract of *Quisqalis indica* although gave significantly different after 3 days compared to control (df=12, 39, F=16.7, $P<0.0001$). In most of the extracts there was no increase or very little increment in egg hatching after 7 days of exposure (Table 2).

Pandey (2000) found that neem cake at higher dose of 38 g cake/ kg soil gave maximum reduction of *Meloidogyne incognita* was found at high dose of neem cake followed by *Adhatoda vasica* leaf powder. On the other hand Husan-Bano *et al.* (1999), as well used crude extracts of *Tagetes patula* and *Commicarppus boissieri* on egg hatching of *M. javanica* and they found that *T. patula* flowers have more nematicidal components than leaves and roots. The inhibitory of water extracts of seed, leaf, and bark of five plants viz *Tamarindus indica*, *Cassia siamea*, *Isoberilinia doka*, *Dolnix regia* and *Cassia sieberiana* were evaluated on larval hatch of *M. incognita*, in which they found all plant parts inhibited larval hatch of this nematode. The highest inhibition rate was from seeds followed by leaves and bark (Bello *et al.*, 2006).

At 1000 ppm dilution we found that the herbal extracts of *Cucumis mello* var agrestis, *Zizphus spina-christ*, *Acacia nilotica*, *Dinbera retroflexa* and *Areca catechu*, they gave the best result of inhibition of hatching at all levels of exposure. Whereas, the herbal extracts of *Quisqalis indica* and *Zingiber officinale* they were not significantly different from the control after 3 days of exposure (df=12, 39,

Table 2. Inhibition of egg hatching of *Meloidogyne incognita* with 10.000 ppm at 3, 7 and 21 days

Treatment	Mean number of egg hatching ± SE		
	3 days after treatment	7 days after treatment	21 days after treatment
Quisqalis indica	2.0 ± 0.7 XY[a]	32.5 ± 4.7 Z	96.8 ± 7.2 Y
Desmodium caudatum	1.0 ± 0.6 X	5.8 ± 1.9 Y	11.3 ± 5.1 VW
Zingiber officinale	0.0 ± 0.0 X	10.0 ± 0.7 Y	48.8 ± 7.2 X
Daphane genkwa	0.0 ± 0.0 X	1.8 ± 0.9 Y	21.8 ± 4.4 W
Areca catechu	0.0 ± 0.0 X	5.5 ± 0.9 Y	5.3 ± 0.6 VW
Dinbera retroflexa	0.0 ± 0.0 X	1.3 ± 0.9 Y	1.5 ± 1.2 W
Cucumis mello var *agrestis*	3.3 ± 1.2 XY	1.8 ± 0.9 Y	1.8 ± 0.9 W
Ziziphus spina-christi	0.0 ± 0.0 X	0.5 ± 0.3 Y	0.5 ± 0.3 W
Acacia nilotica	3.3 ± 1.2 XY	9.3 ± 1.4 Y	9.3 ± 1.4 VW
Chenopodium album	2.0 ± 0.7 XY	2.5 ± 0.5 Y	2.5 ± 0.5 W
Azadirachta indica	3.3 ± 1.2 XY	5.8 ± 1.1 Y	5.8 ± 1.1 VW
Calotropis procera	2.0 ± 0.7 XY	2.3 ± 1.0 Y	4.8 ± 1.0 VW
Control	15.0 ± 1.1 Z	38.5 ± 5.6 Z	178.0 ± 7.3 Z

[a]Means with the same letter (s) in the column are not significantly different according to Tukey's Studentized Range Test at $P=0.0001$.

F=22.24, P<0.0001). However, the extracts of *Quisqalis indica*, *Zingiber officinale*, *Desmodium caudatum*, *Azadirachta indica*, *Calotropis procera* and *Daphane genkwa* their activity towards egg inhibition was not significantly different compared to control after 7 days of exposure (df=12, 39, F=16.21, P<0.0001). However all the herbal extracts were found to be significantly different from the control after 21 days of exposure (df=12, 39, F=77.88, P<0.0001). Amer *et al*., (1999) had higher concentration of ginger (100%) reduced root-knot egg hatching and caused juvenile mortality.

At 100 ppm all the herbal extracts inhibited eggs hatching at 3 (df=12, 39, F=8.33, P<0.0001) and 21 days (df=12, 39, F=31.76, P<0.0001) of exposures which were significantly different compared to the control. Whereas, after 7 days of exposure all the herbal extracts were not significantly different from control except the extracts of *Desmodium caudatum* and *Acacia nilotica* which gave results that were significantly different compared to the control (df=12, 39, F=5.05, P<0.0001).

In banana crop soil applications of powdered neem seed or neem cake at 100 g/plant and subsequently at 3-months intervals reduced the populations of *Pratylenchus goodeyi* Sher and Allen and *Meloidogyne* spp. (Musabyimana and Saxena, 1999). Meyer *et al*. (2006) had tested *Plantago lanceolata* and *P. rugelii* extracts against *M. incognita*, they found that all the extracts were toxic to eggs and J2, with the *P. lanceolata* shoot extract tending to have the most activity against *M. incognita*. However, at lower concentrations J2 were found to be more sensitive than eggs. While at higher concentrations (75% and 100%) were found to be equally toxic to both life stages. In other study, Zasada *et al*. (2006) found that velvet bean (*Mucuna* spp.) crude aqueous extracts (1: 15 dry weight/ volume water were made from velvet bean parts, they found that *M. incognita* eggs were less sensitive to extracts than the juveniles (J2) stage. Also they found that for root-knot nematode management, extracts from the above ground portions of the plants are much more toxic than the roots. Leaf blades and vines have similar toxicities to the root-knot nematodes. However, recently Kong *et al*. (2007) have used cassia and cinnamon oil and related compounds to control *Bursaphylenchus xylophilus* and they came up with promising results.

Plant extracts and generally phytochemicals have potential as products for plant parasitic nematode control, because many of them are selective, and they may be biodegradable to non-toxic products, and can applied to control plant

Table 3. Inhibition of egg hatching of *Meloidogyne incognita* with 1000 ppm at 3, 7 and 21 days

Treatment	Mean number of egg hatching ± SE		
	3 days after treatment	7 days after treatment	21 days after treatment
Quisqalis indica	22.0 ± 3.1 Z[a]	47.5 ± 6.0 Z	86.0 ± 4.6 Y
Desmodium caudatum	6.5 ± 0.3 VWX	38.5 ± 5.6 YZ	36.0 ± 2.7 UV
Zingiber officinale	20.8 ± 1.9 Z	49.3 ± 7.4 Z	59.3 ± 8.3 VWX
Daphane genkwa	9.3 ± 1.7 WXY	40.0 ± 6.6 YZ	45.5 ± 6.3 VW
Areca catechu	12.3 ± 1.0 XY	25.8 ± 3.2 WXY	51.8 ± 3.4 VWX
Dinbera retroflexa	2.0 ± 1.1 VW	5.3 ± 1.7 VW	10.3 ± 2.4 UV
Cucumis mello var *agrestis*	0.3 ± 0.3 V	3.5 ± 1.3 V	5.5 ± 2.2 T
Ziziphus spina-christi	1.8 ± 1.1 VW	10.0 ± 0.9 VWX	12.0 ± 1.4 TU
Acacia nilotica	4.3 ± 1.3 VW	6.8 ± 1.9 VW	8.5 ± 2.3 T
Chenopodium album	3.0 ± 0.7 VW	29.5 ± 1.8 XYZ	56.3 ± 6.6 VWX
Azadirachta indica	9.0 ± 1.9 WXY	46.0 ± 3.9 YZ	65.0 ± 7.6 WXY
Calotropis procera	5.5 ± 1.6 VWX	30.8 ± 4.9 XYZ	73.5 ± 3.6 XY
Control	15.0 ± 1.2 YZ	43.5 ± 2.9 YZ	178.0 ± 7.3 Z

[a]Means with the same letter (s) in the column are not significantly different according to Tukey's Studentized Range Test at P=0.0001.

Table 4. Inhibition of egg hatching of *Meloidogyne incognita* with 100 ppm at 3, 7 and 21 days

Treatment	Mean number of egg hatching ± SE		
	3 days after treatment	7 days after treatment	21 days after treatment
Quisqalis indica	8.0 ± 0.8 XY[a]	26.3 ± 3.2 WXY	47.3 ± 2.2 WXY
Desmodium caudatum	2.8 ± 1.1 X	21.0 ± 3.2 W	57.5 ± 5.4 WXY
Zingiber officinale	7.0 ± 1.7 XY	33.8 ± 3.4 WXYZ	48.8 ± 5.6 WX
Daphane genkwa	6.0 ± 1.1 XY	35.8 ± 3.2 WXYZ	51.8 ± 4.0 WXY
Areca catechu	4.0 ± 0.4 XY	26.0 ± 3.3 WX	49.5 ± 6.9 WX
Dinbera retroflexa	5.5 ± 1.6 XY	24.3 ± 2.6 WX	59.0 ± 8.5 WXY
Cucumis mello var agrestis	6.5 ± 1.0 XY	29.3 ± 1.4 WXYZ	39.0 ± 3.1 W
Ziziphus spina-christi	6.5 ± 1.1 XY	31.5 ± 2.1 WXYZ	62.0 ± 3.2 WXY
Acacia nilotica	4.3 ± 0.5 XY	22.5 ± 1.9 Z	34.3 ± 3.2 W
Chenopodium album	9.3 ± 0.9 Y	45.8 ± 3.5 YZ	65.8 ± 6.7 WXY
Azadirachta indica	4.8 ± 0.9 XY	47.8 ± 7.5 Z	83.3 ± 10.6 X
Calotropis procera	4.5 ± 1.0 XY	35.3 ± 7.4 WXYZ	78.3 ± 10.0 XY
Control	15.0 ± 1.1 Z	43.5 ± 2.9 XYZ	178.0 ± 7.3 Z

[a]Means with the same letter (s) in the column are not significantly different according to Tukey's Studentized Range Test at P=0.0001.

parasitic nematodes as conventional nematicides. Hence we save environmental pollutions and control our crops in cheap way because many of the plant materials used in this study are available naturally and found freely in many fields.

Data from the present experiment indicate that herbal extracts can be effective in controlling root-knot nematodes. This experiment needs to be further executed in the glass-house and/or field level to obtain the optimal goal in controlling this pest under field conditions.

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식물체 추출물이 뿌리혹선충(*Meloidogyne incognita*)의 부화에 미치는 영향

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요 약 수단과 우리나라에서 수집된 12 가지 식물체 추출물(메탄올 추출 9종, 헥산추출 3종)을 이용하여 실내에서 뿌리혹선충(*Meloidogyne incognita*)의 부화에 미치는 영향을 조사하였다. 10,000 ppm, 1,000 ppm, 100 ppm농도의 식물체 추출물에 뿌리혹선충 알을 넣고, 3일과 7일, 21일 후 부화 된 알의 수를 조사하였다. 모든 식물체 추출물 처리에서 무처리에 비해 뿌리혹선충 난의 부화억제 활성이 있었다. 농도가 높을수록 부화억제 활성이 높았는데, 10,000 ppm 농도에서는 *Quisqalis indica* 처리를 제외하고 소수의 개체만이 부화하였다. 그리고 *Q. indica* 처리에서만 7일과 21일째 84.4%와 54.4%가 부화하였고, 대부분의 처리에서는 7일째 이후에 부화되는 알이 없었다. 1,000 ppm농도에서는 10,000 ppm농도에 비해 부화율이 높았는데, *Cucumis mello* var *agrestis* 처리에서 부화율이 가장 낮아 3, 7, 21일 후에 각각 2%와 8%, 3% 만이 부화하였다. 100 ppm 처리에서는 *Desmodium caudatum* 처리에서 처리 3일과 7일째 부화되는 알의 수가 가장 적었다.

색인어 식물체 추출물, 부화, *Meloidogyne incognita*, 뿌리혹선충