ALLELOPATHIC ACTIVITY OF SOME DESERT PLANTS AGAINST PLANT PATHOGENIC BACTERIA AND NEMATODES

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ABSTRACT

The extensive use of chemicals in plant protection has given rise to concerns about pesticide residues in the environment and to the development of pesticide-resistance by any organisms. Allelopathic plants offer better alternative for this purpose due to being cost-saving, eco-friendly, easy to use, efficient and safe. So, the present work was carried out to evaluate the allelopathic effect of three desert plants (*Artemisia judaica, Asphodelus microcarpus, Solanum nigrum*) on root knot nematode (*Meloidogyne incognita*) and three gram negative pathogenic bacterial strains (*Erwinia carotovora*, *Xanthomonas campestris* and *Ralestonia solanacearum*) using root exudates and leaf extracts of each plant with different concentrations. Under laboratory conditions, the data indicated that the three plants contain nematicidal compounds; the inhibitory effect was proportional to the concentrations used. *Artemisia judaica* extract showed the highest level (100%) of nematicidal activity and indicated good antibacterial activities against all the tested bacterial strains also recorded the best result of minimum inhibitory concentration followed by *A.microcarpus* root exudate. While the root exudate of *A.judaica*, *S. nigrum* had no antibacterial activity, leaf extracts of *S. nigrum* had weak antibacterial activity. Aqueous leaf extracts of *A.judaica* appears to be attractive for the development of nematicidal and bactericidal bio- pesticides.

Key words: Allelopathy, desert plants, leaf extracts, root exudates, root knot nematode, bacteria.

INTRODUCTION

Biological control of plant diseases and plant pathogens is highly significant in agricultural field. There is great stimulant to discover biologically active natural products from higher plants to act as herbicides, fungicides, bactericides, nematicides and insecticides that are better than synthetic agrochemicals and are much safer, from a health and environmental point-of view. Allelopathy thus offers an attractive environmentally friendly alternative to pesticides in agricultural pest management (Inderjit and Mukerji, 2006). In 1996, the International Allelopathy Society presented its definition of allelopathy to refer to any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (Cheng and Cheng, 2015).Incorporating allelopathy into agricultural management systems may reduce the use of pesticides, decrease environment/soil pollution, and diminish auto toxicity dangers, increase crop growth and productivity enhancement (Sodaeizadeh and Hosseini, 2012).

Root knot nematode is plant-parasitic nematodes which attack plants and cause crop losses annually in fruit crops and vegetable production. *Meloidogyne* spp. being the most common and widespread group of root knot nematodes in the world (Jones *et al*.,2013) which cause weak growth, poor yield, and low quality and can break the resistance of host plant and expose them to other pathogens and diseases. (Aissani *et al*., 2013)Among the huge diversity of plant pathogens, which include viruses, bacteria, fungi, nematodes, and insects, roughly 150 are bacterial species that cause diseases

to plants which restrict the crop production and record annual crop losses in all countries. Use of agrochemicals to control bacterial diseases is not promising because of the high risk of its high toxic residues (Kannan and Bastas, 2015).The future looks bright for identifying new classes of pesticides (cheap and safe) from natural plant extracts and root exudates to replace the synthetic harmful and expensive chemicals used in the control of these pests at present (Danahap and Wonang 2016).

The objective of the present work was to evaluate the allelopathic potential of compounds contained in leaf extracts and root exudates of three desert plants against root-knot nematode *M. incognita* and three plant pathogenic gram negative bacteria.

MATERIALS AND METHODS

1. Collection of plant materials

Plant samples were collected as follow:

1. Wild onion, *Asphodelus microcarpus* (family, Liliaceae) and black night shade, *Solanum nigrum* (family, Solanaceae) from the North western coast of Egypt.
2. Worm wood, *Artemisia judaica* (family, Asteraceae) was collected from Saint Catherine, Sinai, Egypt.

Samples from each plant were then classified to roots and vegetative parts to obtain the root exudates, the aqueous leaf extract and the decayed residue of each plant.

2. Extraction of root exudates and plant leaves of the tested plants

According to Lu *et al*., (2015), the collection of allelopathic chemicals from the undisturbed plant root system was difficult because of their low concentrations and the high level of contaminants in the soil. *Asphodelus microcarpus* was collected and transported to the laboratory at the Plant Protection Department, Desert Research Center, for sterilization and collection of root exudates (Tang and Young, 1982; Balah, 2015) Onion bulbs were used for germination as it was very difficult to use its seeds in germination under laboratory conditions. *Solanum nigrum* and *Artemisia judaica* seeds were germinated using tissue culture technique which proved to be the best way to obtain the secondary metabolites using MS basal hormone free media (Murashige and Skoog, 1962; Wu *et al*., 2000).Root exudates were then collected from 400 seedlings grown on MS liquid media and centrifuged at 5000 rpm for 10 min to remove any cellular debris, pH was adjusted at pH≤4, root exudates supernatant was extracted by adding ethyl acetate (added 3 times), exudates were concentrated to dryness by aeration then dissolved in ethanol (constant volume) to prepare 25, 50, 75 and 100 μg ml^{-1} concentrations (Balah 2015; Dangash *et al*., 2015). Aqueous extraction from leaves of the three plants was carried out according to (Balah and Nassar, 2011.)

3. Evaluation of allelochemicals pesticidal activity:

In this experiment, pure culture of juveniles 2(J2) root knot nematode *Meloidogyne incognita* were used. In addition, three species of bacteria namely (*Erwinia carotovora*, *Xanthomonas campestris*and *Ralestonia solanacearum*) were used.

3.1. Nematicidal activity

A. Hatchability assay:

Eggs of *M. incognita* were collected from heavily infected tomato roots, three egg-masses of almost equal size (each mass contains 350 eggs approximately) Eggs were washed by rinsing with tap water through a 200 mesh (75 pm) sieve, collected on a 450 meshsieve and transferred into distilled water forming egg suspension (Hussey and Barker, 1973). One ml of egg suspension was placed in the petri plate and eggs were counted under the stereoscope. (40-55 eggs/ml) and 1 ml of each of the root exudates or leaves extracts were transferred in 5-cm-diameter petri dishes and were maintained at 25^{o}C. 1 ml egg suspension and 1ml distilled water served as control. After four days of exposure, the number of hatched eggs was counted under a low power stereomicroscope. Each treatment was replicated three times. The toxicity of allelochemicals was assessed as the mean percentage of the hatched eggs (Kayani *et al*., 2001). Hatchability inhibition (HI)was calculated from the formula

HI% = [(C-T)/C] × 100 where C is the number of hatching control and T is number of hatching after treatment. Lethal concentrations were calculated using probit analysis according to Finney (1971).

B. Larvicidal assay:

Pure culture of J2 root knot nematode *M. incognita* was obtained from tomato plants. Different concentrations of allelochemicals were prepared. Two- ml of each concentration was transferred to 5 cm diameter petri dishes into which 0.1 ml of freshly hatched larval suspension containing 50 Juveniles was added. Juveniles kept in sterilized distilled water were used as controls. Each treatment was replicated three times and the dishes were kept in an incubator (27±2°C). Mortality of juveniles was recorded after 24 h of exposure. The nematodes were considered dead if they did not move when probed with a fine needle (Akyazi, 2014). The percentages of mortality were corrected according to Abbott, (1925). Lethal concentration (LC_{50}) was calculated through probit analysis (Finney, 1971).

3.2. Bactericidal activity

The estimation of bactericidal activity of plant allelochemicals was carried out according to agar well method (Cooper, 1963) based on the observation of inhibition clearing zone of microbial growth on an agar medium.

The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile petri dish nutrient agar .The seeded plates with target test organisms were cut by sterile cork borer to make holes (9 mm in diameter). Only 0.1 ml of each allelochemicals was transferred into each hole under aseptic conditions. The plates were kept then in a refrigerator for 1–2h before incubation of each organism. The antimicrobial activity was detected as a result of clear inhibition zone around holes. Inhibition of the bacterial growth

was measured in mm (SEN and Batra, 2012).The minimal inhibitory concentration was determined according to the broth macro-dilution method (concentrations ranged from 0.1 to 5mg/mL of each allelochemicals) as recommended by (NCCLS, 1985; Ruangpan and Tendencia, 2004).

4. Data analysis

The experiment was arranged in factorial randomized complete block design (RCBD) with three replications where the first factor was tested substances and the second factor was the concentrations. Analysis of variance (ANOVA) was calculated using MSTAT-C computer statistical software according to Russel (1991)and Freed *et al*., (1991).Duncan's multiple range test (DMRT) was used as a posteriori test for multiple comparison of means (Duncans, 1955). P values of ≤ 0.05 were considered indicative of statistical significance.

RESULTS

1. Nematoda: Data presented in Table 1 showed that the highest hatchability inhibition of nematode eggs was recorded at concentration of 100µg/ml of root exudates of the three tested plants. Mean percent hatchability inhibition ranged between 87.78% in case of *A. microcarpus* to 81.15% in case of *A. judaica*. Minimal inhibition was recorded in case of *S. nigrum* 64.73%; similar results were recorded for the mean larval mortality. Highest larval mortality percent was recorded at concentration of 100µg/ml for *A. microcarpus* 87.97% and the least mortality was recorded in case of *S. nigrum* 64.13%.Results of LC_{50} values ranged between (40.02µg/ml and 59.97µg/ml) in case of hatchability inhibition and between (52.46µg/ml and

88.50µg/ml) in case of larval mortality which confirms the degree of toxicity of each allelochemicals against egg hatching and second stage juveniles.

Data analysis revealed that the percent hatching inhibition of root knot nematode significantly increased as the concentration of root exudates of the three tested plants increased ($F_{5, 34}$= 5843169.13; P< 0.0001). Duncan's multiple range tests among the tested concentrations revealed highly significant difference for each paired comparison (P< 0.0001).Results of factorial ANOVA revealed a significant interaction between the type of root exudates extracted from the three tested plants and the concentrations used on the hatchability inhibition of nematodes eggs ($F_{10, 34}$= 73297.39; P< 0.0001).

Highest inhibition was observed when a concentration 100 µg/ml of root exudates of *A. microcarpus* was used followed by a 100µg/ml root exudates of *A. judaica*. Least hatchability inhibition was observed when *S. nigrum* was used with concentration of 12.5 µg/ml. Statistical analysis also revealed that a concentration of 100µg/ml of *A. microcarpus* and *A. judaica* caused similar larval mortality. A concentration of 12.5µg/ml of *S. nigrum* caused least larval mortality.

Table 1: Hatchability inhibition and larval mortality of *M. incognita* second stage juveniles as affected by the tested plant root exudates and LC_{50} values

Root exudates	Hatching inhibition (Mean±SD) (%) Concentrations (µg/ml)						LC_{50} (µg/ml)
	Control	12.5	25	50	75	100	
A. judaica	0.05^{o}	174±0.21^{m} (13.05)	306±0.25^{l} (26.94)	607±0.15^{g} (58.63)	791±0.40^{d} (78.00)	821±0.35^{b} (81.15)	40.02
A. microcarpus	0.05^{o}	308±0.21^{k} (27.15)	493±0.19^{j} (46.63)	704±0.45^{e} (68.84)	802±0.31^{c} (79.15)	884±0.17^{a} (87.78)	26.87
S. nigrum	0.05^{o}	153±0.13^{n} (10.84)	308±0.47^{k} (27.15)	496±0.14^{i} (46.94)	567±0.16^{h} (54.42)	665±0.11^{f} (64.73)	59.97
Larval mortality Mean±SD (%)							
A. judaica	2.6^{h}	4.7±0.20^{fg} (4.43)	11.7±0.17^{d} (19.19)	21±0.25^{c} (38.81)	32±0.35^{b} (62.02)	43±0.26^{a} (85.23)	52.46
A. microcarpus	2.6^{h}	6.3±0.14^{ef} (7.81)	13±0.31^{d} (21.94)	20±0.21^{c} (36.71)	31.3±0.65^{b} (60.54)	44.3±0.24^{a} (87.97)	49.89
S. nigrum	2.6^{h}	3±0.36^{gh} (2.95)	8.2±0.07^{e} (9.28)	12.2±0.10^{d} (19.83)	22.1±1.37^{c} (40.92)	33.6±0.90^{b} (64.13)	88.50

Means followed by the same letter are not significantly different, according to Duncan Multiple Range Test

Data presented in Table 2 showed that highest hatchability inhibition of nematode eggs was recorded at concentration of 100µg/ml of aqueous leaf extracts of the three tested plants. Mean percent hatchability inhibition ranged between 98.84% in case of *A. judaica* to 91.68% in case of *A. microcarpus.* Minimal inhibition was recorded in case of *S. nigrum*78.63%. Similarly, the aqueous leaf extracts caused the highest larval mortality when a concentration of 100µg/ml for *A. judaica* (100.00) was used. Least hatchability inhibition was recorded in case of *S. nigrum* (81.01).Results of LC_{50} values ranged between (19.28µg/ml and 38.58µg/ml) in case of hatchability inhibition and

between (22.92µg/ml and 57.88µg/ml) in case of larval mortality which confirms the degree of toxicity of each leaf extract against egg hatching and second stage juveniles.

The percent hatching inhibition of root knot nematode significantly increases as the concentration of aqueous leaf extracts of the three tested plants increased ($F_{5, 34}$ =4309132.30; P< 0.0001). Duncan's multiple range tests among the tested concentrations revealed highly significant difference for each paired comparison (P< 0.0001).Results of factorial ANOVA revealed a significant interaction between the aqueous leaf extracts from the three tested plants and the concentrations used on the hatchability inhibition of nematodes eggs ($F_{10, 34}$ = 33770.92; P< 0.0001).

Highest inhibition was observed when a concentration of 100µg/ml of aqueous leaf extract of *A. judaica* was used followed by a 100µg/ml aqueous leaf extracts of *A. microcarpus*. Least hatchability inhibition was observed when *S. nigrum* was used with concentration of 12.5 µg/ml. Statistical analysis also revealed that a concentration of 100µg/ml of *A. judaica* caused the highest larval mortality. In addition, the concentration of 12.5µg/ml of *S. nigrum* caused the least larval mortality

Table 2: Hatchability inhibition and larval mortality of *M. incognita* second stage juveniles as affected by the tested plant leaf extracts and LC_{50} values.

Leaf extract	Hatching inhibition Mean±SD (%) Concentrations (µg/ml)						LC_{50} (µg/ml)
	Control	12.5	25	50	75	100	
A. judaica	0.05^{p}	429 ± 0.70^{m} (39.89)	564 ± 1.33^{j} (54.10)	826 ± 1.35^{d} (81.68)	876 ± 1.78^{c} (86.94)	989 ± 1.30^{a} (98.84)	19.28
A. microcarpus	0.05^{p}	266 ± 1.47^{n} (22.73)	532 ± 1.93^{k} (50.73)	688 ± 1.72^{g} (67.15)	806 ± 1.67^{e} (79.57)	921 ± 1.31^{b} (91.68)	27.33
S. nigrum	0.05^{p}	248 ± 1.19^{o} (20.84)	434 ± 0.59^{l} (40.42)	589 ± 1.05^{i} (56.73)	642 ± 1.25^{h} (62.31)	797 ± 1.34^{f} (78.63)	38.58
Larval mortality Mean±SD (%)							
A. judaica	2.6^{l}	14.4 ± 1.18^{h} (24.89)	26.8 ± 0.62^{f} (51.05)	37.6 ± 1.12^{d} (73.83)	48.3 ± 1.52^{ab} (96.41)	50 ± 1.49^{a} (100.00)	22.92
A. microcarpus	2.6^{l}	4.6 ± 0.61^{k} (5.06)	11.4 ± 1.66^{i} (19.83)	20 ± 1.94^{g} (36.70)	40.2 ± 1.17^{c} (78.90)	47.2 ± 1.60^{b} (93.67)	43.99
S. nigrum	2.6^{l}	3.3 ± 0.55^{k} (1.50)	9.2 ± 0.80^{j} (13.50)	19.3 ± 1.81^{g} (34.59)	33.2 ± 1.38^{e} (64.13)	41.6 ± 1.51^{c} (81.01)	57.88

Means followed by the same letter are not significantly different according to Duncan Multiple Range Test.

2- Bacteria: The antibacterial activity of crude root exudates (Table 3) showed that *A. microcarpus* inhibited the bacterial growth of the three tested bacterial species. The inhibition zone diameter of *A. microcarpus* root exudates ranged between 23 and 27 mm and its minimum inhibitory concentration (MIC) values ranged between 1.2 and 1.5 mg/ml. The root exudates of *A. judaica* and *S.nigrum* showed no bactericidal effects on the gram- negative bacteria studied.

Data given in Table 4 demonstrated that the crude leaf extract of *A. judaica* showed the maximum bactericidal activity against the three studied bacterial species. *A. judaica* leaf extract inhibits the growth of bacteria with

inhibition zone diameter ranging between 26 and 29 mm. Also, *A. microcarpus* extract showed good bactericidal activity with mean inhibition zone diameter ranging between 15 and19 mm, while the lowest antibacterial activity was observed when *S. nigrum* extract was used (inhibition zone ranged between 6 and 10 mm).

The high antibacterial activity had low MIC values while low antibacterial activity gave high MIC values. Results showed that the highest MIC value was detected for *A. judaica* leaf extract (0.25 to 0.45 mg/ml), while the minimum MIC value was detected for *S. nigrum* leaf extract (3.5 to 4.5mg/ml) which represented the weakest bactericidal effect.

Table 3: Antibacterial effect and minimum inhibitory concentration (MIC) of crude root exudates against the three tested bacteria species.

Root exudates	**Mean diameter of inhibition zone (mm±SD)**		
	Bacteria		
	E. carotovora	**X. campestris**	**R. solanacearum**
A. judaica	-	-	-
A. microcarpus	27±0.10	25±0.15	23±0.25
S. nigrum	-	-	-
MIC(mg/ml)			
A. judaica	-	-	-
A. microcarpus	1.5	1.4	1.2
S. nigrum	-	-	-

Table 4: Antibacterial effect and minimum inhibitory concentrations (MIC) of crude leaf extracts against the three tested bacteria species.

Leaf extracts	Mean diameter of inhibition zone (mm±SD)		
	Bacteria		
	E. carotovora	***X. campestris***	***R. solanacearum***
A. judaica	29±0.26	27±0.20	26±0.47
A. microcarpus	19±0.22	20±0.21	15±0.25
S. nigrum	10±0.15	7±0.17	6±0.23
MIC(mg/ml)			
A. judaica	0.45	0.35	0.25
A. microcarpus	2.5	1.7	2.0
S. nigrum	4	3.5	4.5

DISCUSSION

Our results indicated that the aqueous leaf extracts of the three studied plants was more potent than the root exudates of the same plants against the root knot nematodes and plant pathogenic gram-negative bacteria.

In the present study a 100 µg/ml aqueous leaf extract of *A. judaica* revealed it was the most effective against M. *incognita* egg hatchability inhibition and second stage juvenile (J2) mortality. Korayem *et al.*, (1993) proved that the exposure to standard extract of *Artemisia absinthium* for 72 h caused reduction in the number of active *Meloidogyne incognita* by 100% and reduction in the egg hatching by 98.7%. In addition to Costa *et al*., 2003 showed that the ethanolic rhizome extract of *Artemisia vulgaris* at concentration 50 mg/ml inhibited 100% *Meloidogyne megadora* egg hatching and at concentration 55.67 mg/ml caused 50% second stage juvenile mortality, both in a dose-dependent manner, D'Addabbo *et al*., 2013 reported that aqueous extract of *Artemisia annua* was toxic to *M. incognita* juveniles

where it caused more than 90% mortality and significant reduction of egg hatchability at 500µg/ml after 24 h. The differences in lethal concentration and doses reported in the present study might be attributed to differences in the plant and nematode species used as well as differences in extraction method.

A 100 µg/ml of aqueous leaf extract of *Asphodelus microcarpus* was less potent than *Artemisia judaica* as it significantly reduced egg hatchability of *Meloidogyne incognita* by 92% and significantly increased larval mortality by 95%. *A. sativum*was reported to possess nematicidal effect against *Meloidogyne* sp. (Gupta and Sharmaj, 1993; Zasada *et al*., 2002) In addition, (Ahmed *et al*., 2016) reported that the aqueous whole plant extracts of *Asphodelus tenuifolius* caused *Meloidogyne javanica* mortality and egg masses reduction.

Aqueous leaf extracts of *S. nigrum* was the least effective extract against *M. incognita* egg hatchability inhibition and J2 mortality. Different species of *Solanum* plant showed similar effects on nematodes. (Dias *et al*., 2012; Correia, 2014)

A. judaica leaf extract was the most effective extract against the three bacterial species investigated as it caused the highest zone inhibition and MIC. These results corroborate with those recorded by Badawy and Abdelgalil, (2014), where they reported that the MIC of *Artemisia judaica* oil against *Erwinia carotovora* was 550mg/l. Also, Massiha *et al*., (2013) proved that *Artemisia annua* leaf oil showed antibacterial activity with inhibition zone diameter ranging between (18-28 mm).Present studies indicated that

Asphodelus microcarpus leaf extracts demonstrated moderate bactericidal activity. In the same trend, other reports have addressed the antibacterial effect of *Asphodelus* sp. on the gram negative bacteria and recommended their use as a bio-control agents of plant bacterial diseases(Dangi *et al*., 2013;Eddine *et al*., 2015)Our results showed the weak bactericidal activity of *S. nigrum* aqueous leaf extracts and these are quite similar to findings of Doss *et al*., (2009) who reported that MIC of the tannins isolated from *Solanum trilobatum* against tested bacteria ranged between 1-4 mg/ml and showed bacterial growth inhibition with zone diameter ranged from 7 to 13 mm

CONCLUSION

In conclusion, the leaf extracts of *A. judaica* has an effective nematicidal activity against root knot nematode by exhibiting some toxic compounds which cause reduction in egg hatching and death of second stage juveniles. Moreover, the leaf extracts of the *A. judaica* showed the highest antibacterial activity against gram negative bacteria. Therefore, the leaf extracts of the *A. judaica* is strongly recommended for root knot nematode and plant pathogenic gram negative bacteria management. These plant extracts should also be tested against other parasitic nematodes. Allelopathy offers safe solutions in pest management; in the long run it would be a luminous direction to proceed in order to develop a bioactive pesticide using the allelochemicals.

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النشاط الاليلوباثي لبعض النباتات الصحراوية ضد البكتريا والنيماتودا الممرضة للنبات

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المستخلص

الاستخدام المفرط للكيماويات في وقاية النبات اثارت القلق حول بقايا المبيدات في البيئة وتطور مقاومة اي كائن حي لها. النباتات الاليلوباثية تقدم بديل افضل لهذا الغرض نظرا لكونها موفرة في التكلفة، صديقة للبيئة، سهلة الاستخدام، امنة وفعالة.و قد اجريت الدراسة الحالية لتقييم التأثير الاليلوباثي لثلاث نباتات صحراوية (الشيح الجبلي، بصل العنصل، وعنب الديب) علي نيماتودا تعقد الجذور *Meloidogyne incognita* وثلاث سلالات بكتريا ممرضة للنبات (*Erwinia carotovora*, *Xanthomonas campestris* and *Ralestonia solanacearum*) باستخدام راشح الجذور ومستخلص اوراق كل نبات علي حده بتركيزات مختلفة. اوضحت النتائج تحت الظروف المعملية ان الثلاث نباتات تحتوي علي مركبات مبيدة للنيماتودا،وان التأثير المثبط يتناسب تناسب طرديا مع التركيزات المستخدمة . وقد سجلت اعلي النتائج عند استخدام مستخلص اوراق الشيح الجبلي في ابادة النيماتودا بنسبة ١٠٠ % واعطت افضل تاثير مضاد للبكتريا المختبرة حيث سجلت افضل نتيجة في اقل تركيز مثبط للبكتريا(MIC) يليه راشح جذور بصل العنصل في حين ان راشح جذور الشيح الجبلي وعنب الديب ليس لهما تاثير مضاد علي البكتريا المختبرة اما مستخلص اوراق عنب الديب فان له تاثير مضاد محدود علي البكتريا. النتائج المسجلة للمستخلص المائي لاوراق الشيح الجبلي تسلط الضوء علي تطوير المبيدات الحيوية المضادة للنيماتودا والبكتريا الممرضة للنبات