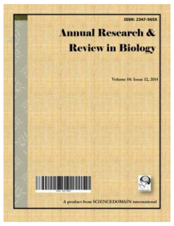

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Toxicity of Some Plant Extracts and Entomopathogenic Nematode against Pomegranate Aphid, *Aphis punicae* under Laboratory Condition

A. S. Alghamdi[1], A. H. Nour El-Deen[1,2*], A. F. Al-Barty[1] and M. M. Hassan[1,3]

[1]Department of Biology, Faculty of Science, Taif University, Saudi Arabia.
[2]Nematology Research Unit, Department of Agricultural Zoology, Faculty of Agriculture, Mansoura University, Egypt.
[3]Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ASA and AHNED designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AFAB managed the literature searches. Author MMH managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The *in vitro* aphicidal activity of five medicinal and aromatic plant extracts at concentrations of 10, 5 and 2%; and entomopathogenic nematode, *Steinernema feltiae* with levels of 2000, 1000, 500 and 250 IJs on the mortality of pomegranate aphid, *Aphis punicae* (Hemiptera: Aphididae) were evaluated after 12, 24, 48 and 72 hrs of exposure time. The plants used were *Azadiracta indica*, *Calotropis procera*, *Catharanthus roseus*, *Withania somnifera* and *Tagetes erecta*. Results revealed that individual mortality percentages generally increased with the increase of extract concentrations and nematode levels for every exposure times of four tests. Among treatments of the extracts, *A. indica* significantly gave the highest value (77.9%) for every concentrations and exposure times; followed by *C. procera* with value of 71.5%; whereas, treatment of *C. roseus* gave the lowest

**Corresponding author: E-mail: ahnoureldeen2003@yahoo.com;*

significant value of pomegranate aphid mortality that was amounted to be 23.4%. In respect to entomopathogenic nematode, 2000 IJs was the best level at all durations of exposure. On the other hand, level of 250 IJs gave the lowest mortality of *A. punicae* individuals after 24 and 48 h of exposure. The study indicates the possible use of such botanicals and *S. feltiae* as well against pomegranate aphid.

Keywords: Aphis punicae; botanicals; Steinernema feltiae; aphicidal activity; in vitro.

1. INTRODUCTION

There are many insects attack pomegranate. Worldwide, the pomegranate aphid, *Aphis punicae* (Hemiptera: Aphididae) is one of the key pests in pomegranate orchards. Both adults and nymphs feed on leaves, inflorescences and fruits. Infestation with aphids result in pale and curled leaves; retard development and fallen flowers; besides it transmit viral diseases and secret honey dew on which fungi grow and consequently reduce crop quality and yield [1]. In Al-Taif city, Saudi Arabia the highest infestation by aphids on pomegranate was recorded on the leaf more than the fruits [2]. Unfortunately, aphids have a high reproductive capacity and extensive usage of insecticide to control them for the development of resistance. The effective aphicides are highly toxic, very expensive, and have negative environmental impacts [3]. Recently, botanical insecticides have long been considered as acceptable as alternatives to synthetic chemical insecticides for pest management [4,5]. Over 2000 plant species belonging to about 170 natural families are known to have insecticidal properties and are considered comparatively safe for environment and public health [6]. For several years, entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae (Rhabditida) have received the most attention as a bio-agents against a wide spectrum of insect pests [7,8,9]. Entomopathogenic nematodes have been used against soil pests such as white grubs, root weevils, rootworms, sciarid flies, cutworms, armyworms and root-knot nematodes [10,11]. Entomopathogenic nematodes (EPNs), steinernematids and heterorhabditids hosting the mutualistic bacteria, are extraordinarily lethal to many insect pests. Infective juvenile stage (IJ) enters insects through the mouth, anus, spiracles, or areas of thin cuticle [8]. After penetrating into the haemocoel, the nematodes release their bacteria, which quickly multiply and kill the hosts, usually within 24 to 48 hrs. Developing nematodes feed upon the bacteria and liquefying host tissues, mate, and produce two or more generations before emerging as infective juveniles from the depleted insect cadaver in search of fresh hosts [9]. *Steinernema yirgalemense* and *Heterorhabditis zealandica* were identified as being the most effective against the subterranean adult females of woolly apple aphid, with infection rates of 39% and 28%, respectively [12]. Only a few literatures are investigated on the effects of native plant extracts and EPN species on aphids. Therefore, the aim of this work was to evaluate the toxicity of five plant extracts and entomopathogenic nematode against pomegranate aphid under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Insect Collection

Apterae pomegranate aphid, *Aphis punicae* adults of the same age (24-48 hrs) were collected from infested pomegranate trees located at the gardens of Taif University, Saudi Arabia, 30 min prior to start the experiment.

2.2 Plant Materials and Extraction

The plant parts of five medicinal and aromatic plants used in this study are leaves of Neem (*Azadiracta indica*), Sodom apple (*Calotropis procera*), and Periwinkle (*Catharanthus roseus*); leaves and fruits of ashwagandha (*Withania somnifera*); and leaves and flowers of marigold (*Tagetes erecta*). Plant materials were collected randomly from plants grown in the university experimental farm and gardens. Plant materials were taken and cut into small pieces, then shade-dried for 10 days until these pieces were fully dried. Dried plant materials were homogenized to a fine powder and stored in airtight bottles. Forty grams of each plant powder were extracted with 250 ml of distilled water for 24 hrs. The suspension was filtered with Whatman No. 1 filter paper, the residue re-extracted for an additional 24 hrs, and similarly filtered. Filtrates were combined, evaporated to dryness in a water bath for 60 min and the left over powder was considered 100%. The concentrations of 10, 5 and 2% were prepared by redissolving the extracted crude and a volume was made up to 100 ml with a distilled water.

2.3 Entomopathogenic Nematode

Entomopathogenic nematode (EPN) used in this study was isolated from soil of lemon trees grown in Taif University gardens using the *Galleria mellonella* L. baiting method described by [13]. White trap technique was used for re-culturing of EPN as described by [14]. This will be maintained in the dark at 20–25°C to allow for the emergence of infective juvenile nematodes (IJs). The isolated nematodes were morphologically identified as *Steinernema feltiae* by examining morphometrics for IJs and first-generation males [15]. The required IJ levels were prepared by serial dilution of the stock solution of 10,000 IJs/ml to be 2000, 1000, 500 and 250 IJs.

2.4 Toxicity Test

Discs measuring 1.5 cm in diameter were cut out from the center of Taify pomegranate leaves, from garden-grown plants. Each leaf disc was dipped into diluted aqueous extracts of previous plants or control suspension with distilled water for 30 seconds and then air dried. Other discs were sprayed with 5 ml of *S. feltiae* suspensions with different levels. Then discs were placed upside down in petri dish (10 cm diameter); ten healthy aphid adults were placed on the treated discs surface. A complete randomized design was used throughout the experiment. Five replicate batches of aphids (i.e. 50 insects) were used per each concentration of each plant extract or nematode level. Petri dishes containing aphids were carefully closed and kept at 25±1ºC to count died individuals and recorded mortality percentages after 12, 24, 48 and 72 hrs of application. Mortality was confirmed by touching the aphid with a fine brush. Aphids that appeared no realistic movement were considered as dead. The percentage mortality of the individuals was calculated and recorded. Mortality percentages were transformed to arcsin [16] values just before statistical analysis.

2.5 Statistical Analysis

Statistically, the obtained data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range to compare means [17] using the COSTATE software package.

3. RESULTS AND DISCUSSION

Data in Table (1) represent the impact of aqueous extracts of five medicinal and aromatic plants with three concentrations on mortality percentages of pomegranate aphid after four exposure times. In general, aphid mortality percentages increased with increase in plant extract concentrations tested at the different exposure duration. Results indicated that all treatments caused remarkable mortality of aphids with various degrees as compared to control. Among all tested materials, neem extract significantly gave the highest mortality percentage reaching to 77.9%. However, discs receiving sodom apple ranked second with mortality percentage that was amounted to 71.5%, followed by marigold extract treatment with value of 51.7%. On the other hand, periwinkle caused low mortality (23.4%). Neem extract at 10% gave the highest mortality percentage after 72 hrs of exposure that was amounted to 100%, followed by sodom apple (98%), marigold (94%), ashwagandha (60%) and periwinkle (38%) at the same exposure time. The same mortality percentage was obtained from neem extract at 10% after 24 hrs and 5% after 72 hrs with value averaged to 90%. Treatment of periwinkle extract at 2% gave the lowest value of aphid mortality after 12 hrs of exposure (Table 1). These results are in accordance with those recorded by [18] regarding neem extract which ameliorated aphicidal activity. Many compounds with insecticidal activities have been found in plants including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls [19]. Several phytochemicals extracted from various botanical sources, have been reported to have antibiotic effects on insects [20, 21,22]. Plant extracts are the recent trend which elicited effect against aphids in the field [23] and under laboratory conditions [24]. The efficacy of two plant extracts, namely glicolic extract of comfrey (*Symphytum officinale*) and fluid extract of pot marigold (*Calendula officinalis*) was tested against apple aphid (*A. pomi*) at 0.5, 1 and 5% concentrations [25]. Results on the third day at 25°C indicated that all of the tested substances showed aphicidal properties, and the highest mean corrected mortality rates. Marigold extract exceeded a 50% aphicidal efficacy at 15°C and at 5% concentration of the used suspension. Our results also on bar with [1] who recorded that the ethanolic extract of *Ruta chalepensis* at concentration of 0.015% showed the highest repellency (75) and mortality (79.5) of pomegranate aphid, *A. punicae*, followed by ethanol extract of *Aerva lanata* which gave high repellent effect and moderate mortality percentage at the same concentration under laboratory condition.

Data in Table (2) showed the efficacy of entomopathogenic nematode, *S. feltiae* with four levels in comparison with distilled water on *A. punicae* mortality percentages. Likewise, a similar trend was observed concerning aphid mortality percentages that were increased as the levels and exposure durations increased. The highest mortality percentage was achieved from 2000 IJs after 72 hrs of exposure with value reached to 67%, followed by treatment of the same level with value of 60% after 48 hrs. Level of 1000 IJs scored moderate percentages of mortality after 72 and 48 hrs of exposure with the same value each (50%).

Slight lethal effects were obtained after treatment with levels of 500 and 250 IJs, recording 37 and 17%, respectively after 72 hrs. Exposing aphids for 24 hrs to 250 IJs of *S. feltiae* showed lowest lethality with value of 5%. It was clear that no dead aphids were recorded after 12 hrs of exposure (Table 2). This finding is in line with the work of [12] who mentioned that EPNs offer an alternative biocontrol option for woolly apple aphid (WAA), *Eriosoma lanigerum*. EPN have been utilized as classical, conservational, and augmentative biological control agents. Steinernematids are symbiotically associated with entomopathogenic bacteria (EPBs) from the genus *Xenorhabdus*. This symbiotic bacterium multiplies and rapidly kills the insect within a day or two. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics [26] and anti-feedants that preserve the dead insect from putrefaction while the nematodes feed and reproduce in it. Our results disagree with those of [27] who reported the lowest efficacy of EPNs against insect pests in above ground habitats. The major limiting factor of foliar application of EPNs to leaf surfaces is the rapid desiccation of the IJs, although anti-desiccants have been shown to increase the effectiveness of EPNs against certain pest species.

Table 1. Mortality percentages of *Aphis punicae* Passerini exposed to aqueous extracts of five medicinal plants

Treatments	Conc. (%) / Time (hrs)	*Mortality % 10	5	2	Treat. mean
Neem	12	84 ef	75 hi	40 no	77.9 a
	24	90 cd	84 ef	50 l	
	48	94 bc	86 ed	70 j	
	72	100 a	90 cd	72 ij	
Marigold	12	72 ij	32 qr	16 u	51.7 c
	24	80 fg	38 nop	20 tu	
	48	86 ed	50 l	34 pqr	
	72	94 bc	53 l	45 m	
Sodom Apple	12	80 fg	64 k	34 pqr	71.5 b
	24	90 cd	72 ij	40 no	
	48	90 cd	78 gh	64 k	
	72	98 ab	78 gh	70 j	
Periwinkle	12	30 t	20 tu	5.2 vw	23.4 e
	24	38 nop	24 st	8 v	
	48	38 nop	25 s	10 uv	
	72	38 nop	25 s	20 tu	
Ashwagandha	12	45 m	30 t	24 st	42.0 d
	24	52 l	32 qr	35 pq	
	48	60 k	41.2 mn	36 opq	
	72	60 k	53 l	36 opq	
Distilled Water	12	0.0 w	0.0 w	0.0 w	0.0 f
	24	0.0 w	0.0 w	0.0 w	
	48	0.0 w	0.0 w	0.0 w	
	72	0.0 w	0.0 w	0.0 w	

**Each figure represents the mean of five replicates. Means in each column followed by the same letter did not differ at P= 0.05 according to Duncan's multiple range tests.*

Table 2. Effect of four levels of entomopathogenic nematode, *Steinernema feltiae* on *Aphis punicae* Passerini mortality

Time (hrs) / IJ levels	*Mortality %				Levels mean
	72	48	24	12	
2000	67 a	60 b	32 d	0.0 j	39.8 a
1000	50 c	50 c	20 f	0.0 j	30.0 b
500	34 d	25 e	16 g	0.0 j	18.8 c
250	17 fg	10 h	5 i	0.0 j	8.0 d
Distilled water	0.0 j	0.0 j	0.0 j	0.0 j	0.0 e

**Each figure represents the mean of five replicates. Means in each column followed by the same letter did not differ at P= 0.05 according to Duncan's multiple range tests.*

4. CONCLUSION

These findings could be important from the point of view of controlling the pomegranate aphid without the use of chemical pesticides avoiding the bad effects to the environment of these. Therefore, future studies are needed to characterize the active compounds in the tested plant extracts and EPNs symbiotic bacteria that are aphicidal and possessing complex modes of action before recommend it for IPM program against aphids.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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