Ebeid *Bull Natl Res Cent (2020) 44:202*
https://doi.org/10.1186/s42269-020-00460-3

Bulletin of the National Research Centre

RESEARCH

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Impact of neem-nanoemulsion and neem petroleum ether extract on some biological parameters of the internal parasitoid, *Meteorus gyrator* (Thub.) (Hymenoptera: Braconidae)

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Abstract

Background: The neem tree, *Azadirachta indica*, is very promising for the control of many insect pests, where it acting as feeding deterrents, oviposition obstruction and as a growth regulator.

Results: In this study, both neem extract and neem nanoemulsion significantly decreased the adult emergence of *Meteorus gyrator*. It was found that all concentrations of neem nanoemulsion especially high concentrations (800, 400, and 200 ppm) have adversely affected some biological parameters of this parasitoid. Also, they have a harmful effect as reducing fecundity, oviposition, and shortened the longevity of *M. gyrator*.

Conclusion: It has been proved that neem nanoemulsion is more harmful than neem oil extract for the hymenopteran parasitoid *M. gyrator*.

Keywords: *Azadirachta indica*, *Meteorus gyrator*, Biological parameter, Neem formulations

Background

Although insecticides are synthetic chemicals used in the agricultural field to improve the yield production by protecting it against most pests, but the permanent use of these products caused harmful environmental residues and also harm nontarget insects (natural enemies) that are responsible for insect pest control (Gesraha 2001; Yadav et al. 2015; Ebeid 2020). Consequently, concentrates as an afterthought impact of different plant extracts that are responsible for the suppression of some pest populations are pre-requests for successful integrated pest management (IPM). Neem (*Azadirachta indica*) is one among plant extracts that considered a profoundly intense biopesticide (Mallick et al. 2013).

Neem seeds oil contains ≈ 40% of the active ingredient 63 (azadirachtin), which is responsible for the toxic action for most insect pests and affects their fertility (Paul et al. 2011; Raut et al. 2014; Kumar et al. 2015). The utilization of nanotechnology in the agriculture field has been studied, including the development of insecticides formulations by polymeric encapsulation (Das et al. 2014). It has been proved that nano-neem formulation is more toxic than neem oil on most insect pests, where they affected cell renovation, nutrient absorption, and interfering with food conversion, but they have a negative impact on most parasitoid (Preetha 2018). Therefore, the effect of these products on predators, parasitoids, and some beneficial arthropods enables an assessment of their compatibility with the ecosystem (Gesraha 2001). *Meteorus gyrator* is one of the most efficient parasitoids recorded as solitary internal parasitoid of many lepidopterus larvae, e.g., *Spodoptera littoralis, S. exigua, Heliothis armigera, and*

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Sesamia cretica, *Autographa* spp.) under laboratory conditions (Gesraha and Ebeid 2020).

The purpose of this work is to investigate the influence of the two different neem formulations (neem-nanoemulsion and petroleum ether extract of neem) on some biological parameters of *Meteorus gyrator.*

Methods

The effects of neem-nanoemulsion and petroleum ether extract of neem oil, were evaluated toward longevity, fecundity, percent parasitism, and adult emergence of the internal larval parasitoid wasp of the, *M. gyrator*.

Insecticidal materials used

Neem-nanoemulsion and neem oil extraction were obtained as commercial formulations from Sigma-Aldrich, India, and was applied in five concentrations (800, 400, 200, 100, and 50 ppm).

Parasitoid treatment

Direct treatment

Four groups (five pairs of wasps/each) of newly emerged *Meteorus gyrator* parasitoid adults (male and female) were allowed to feed on the concentrations (800, 400, 200, 100, and 50 ppm) of each tested formulation mixed with honey droplets as the food supply for 24 h. The control treatment group was fed on honey droplets only (without treatment).

Twenty-five of newly molted fourth instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) per female wasp (replicated five times) were introduced daily for each tested parasitoid's female wasp along with their longevity. The exposed larvae were then transferred to rearing plastic cups (100 ml volume) till the emergence of the internal parasitoid's third instar larvae for pupation. The longevity of parasitoid's adult female, the number of deposited eggs/female, and oviposition period were calculated in each case.

Indirect treatment

Another group of twenty-five of newly molted fourth instar larvae of the cotton leafworn, *S. littorals* (that were previously fed as third larval instar on castor bean leaves treated with the same above mentioned concentrations, i.e., 800, 400, 200, 100, and 50 ppm of each tested material), were offered to the newly emerged female wasp. This step replicated five times and introduced daily for each tested groups of the parasitoid's female wasp along their longevity.

Statistical analysis

All data were subjected to statistical analyses using ANOVA F-test throughout the "SPSS" Computer program for comparison between treatments. Duncan multiple range test (Duncan 1955) was applied to differentiate between means. Henderson and Tilton equation (Henderson and Tilton 1955) was applied to compute the percentage of the reduction. The "Student *t*-test" was applied to compare the difference between the two neem formulations.

Results

The First experiment (direct treatment)

Effect of neem oil emulsion and neem nanoemulsion on Meteorus gyrator Longevity

Statistical analyses showed that both formulations of neem at all tested concentrations (800, 400, 200, 100, and 50 ppl), significantly shortened the longevity of *M. gyrator* female wasp if compared with the control treatment ($F_{2,12}=113.138^{**}$, $P=0.000$). With respect to 100 and 50 ppm concentrations, statistically, there was no significant variation between both neem products ($F_{2,12}=27.889^{**}$ and $F_{2,12}=7.658^{**}$, for 100 and 50 ppm, respectively, and $P=0.000$ for both). On the other hand, significant variation was recorded at 800, 400, and 200 ppm concentrations between both formulations and the control treatment (Table 1).

These figures indicated a negative relation between the concentration of both tested formulations and the longevity of wasps. This negative relation reflecting the percentage of reduction ranged between 21.38 and 79.62% for neem nanoemulsion and between 15.09 and 69.81% for neem oil treatments (Table 1).

Effect of neem oil emulsion and neem nanoemulsion on Meteorus gyrator Fecundity

Female wasp exhibited a reduced number of eggs that laid when exposed to the neem-treated host larvae feed on treated diet at all tested concentration levels. The total number of deposited eggs/female was significantly decreased at 800, 400, and 200 ppm treatments of both neem formulations compared with the check ($F_{2,12}=55.192^{**}$, 99.801^{**}, 18.196^{**}), respectively (Table 1). Also, both neem formulations at 800 ppm concentration reflected a drastic reduction (92.99 and 92.21%, respectively) in a number of the laid eggs as compared with the control treatment.

Concerning to the concentrations 800 and 200 ppm, there was insignificant variation between both neem formulations on the mean number of deposited eggs, but they were significantly varied with the control treatment ($F_{2,12}=55.192^{**}$, 18.196^{**}), respectively. The opposite effect was shown in the case of 100 ppm, where there was no significant effect between both formulations and the control treatment ($F_{2,12}=1.494^{NS}$) (Table 1).

Table 1 Effect of two neem formulations on longevity and fecundity of *Meteorus gyrator*

Tested materials	Concentration (ppm) Mean ± SE						*F*-value (1) (*df* = 2,12)
	800	400	200	100	50	Control	
	Adult female longevity (in days)						
Neem nanoemulsion (% reduction)	3.40 ± 0.47 c F (79.62%)	5.90 ± 0.37 c E (62.89%)	9.30 ± 0.41 c D (41.51%)	10.80 ± 0.39 b C (32.08%)	12.50 ± 0.57 b B (21.38%)	15.90 ± 0.29 A	113.138**
Neem (% reduction)	4.80 ± 0.26 b E (69.81%)	8.10 ± 0.49 b D (49.06%)	11.50 ± 0.88 b C (27.67%)	12.25 ± 0.71 b BC (22.96%)	13.50 ± 0.89 b B (15.09%)	15.90 ± 0.29 A	38.417**
Check	15.90 ± 0.29 a	15.90 ± 0.29 a	15.90 ± 0.29 a	15.90 ± 0.29 a	15.90 ± 0.29 a	15.90 ± 0.29	–
F-value(*df* = 2,12)	28.364**	17.930**	33.151**	27.889**	7.658**	–	
	Total number of eggs/female						
Neem nanoemulsion (% reduction)	1.09 ± 0.14 b E (92.99%)	5.20 ± 0.45 c D (62.83.14%)	9.50 ± 0.16 b C (32.09%)	12.60 ± 0.26 a B (9.94%)	12.99 ± 0.43 b B (7.15%)	13.99 ± 0.50 A	23.193**
Neem(% reduction)	0.98 ± 0.18 b E (92.21%)	8.30 ± 0.38 b D (40.67%)	10.99 ± 0.77 b C (21.44%)	12.00 ± 1.33 a BC (14.22%)	13.00 ± 0.25 a A (7.08%)	13.99 ± 0.50 AB	53.421**
Check	13.99 ± 0.50 a	13.99 ± 0.50 a	13.99 ± 0.50 a	13.99 ± 0.50 a	13.99 ± 0.50 ab	13.99 ± 0.50	
F value(*df* = 2,12)	55.192**	99.801**	18.196**	1.494^{NS}	4.387*	–	
	Oviposition period						
Neem nanoemulsion (% reduction)	0.95 ± 0.14 b E (92.4%)	4.60 ± 0.37 c D (63.2%)	6.90 ± 0.49 c C (44.8%)	10.75 ± 0.43 b B (14.00%)	11.70 ± 0.59 a AB (6.40%)	12.50 ± 0.50 A	105.480**
Neem (% reduction)	1.20 ± 0.91 b D (90.4%)	7.30 ± 0.75 b C (41.6%)	8.80 ± 0.60 b C (29.6%)	11.40 ± 0.39 ab B (8.8%)	12.80 ± 0.79 a A (2.4%)	12.50 ± 0.50 AB	64.072**
Check	12.50 ± 0.50 a	12.50 ± 0.50 a	12.50 ± 0.50 a	12.50 ± 0.50 a	12.50 ± 0.50 a	12.50 ± 0.50	
F value (2) (*df* = 2,12)	46.240**	51.009**	28.638**	3.961*	2.897^{NS}	–	

Means in a single column followed with the same small letters are not significantly different ($P = 5\%$)

Means in a single row followed with the same capital letters are not significantly different ($P = 5\%$)

NS not significant

**Highly significant

*Significant

Effect of neem oil emulsion and neem nanoemulsion on Meteorus gyrator oviposition period

Both tested neem formulations exhibited a pronounced shortening effect on the oviposition period of *M. gyrator.* The reduction percentage for both formulations ranged between 92.40 and 14.00% and between 90.40 and 8.80% for neem nanoemulsion and neem treatments, respectively (Table 1). Furthermore, all tested concentrations showed significant differences as compared with the check except at 50 ppm concentration ($F_{2,12} = 3.961^{NS}$) (Table 1). The obtained results showed that the aforementioned neem formulations especially neem nanoemulsion exhibited harmful effects to the *M. gyrator* parasitoid female.

The second experiment (indirect treatment)

This experiment was carried out to study the effect of both neem formulations against *M. gyrator* parasitized on the treated larvae of *S. littoralis.*

The results presented in (Table 2) showed the effect of different concentrations of neem nanoemulsion compared with neem oil on the parasitism percentage, adult emergence, and mortality of the tested parasitoid, *Meteorus gyrator*. The obtained data showed that parasitism was significantly decreased by increasing the two neem formulation concentrations (50–800 ppm), i.e., concentration dependent. All treatments were significantly different compared with the control treatment, which reflect a fully parasitism percentage (100%).

Despite what might be expected, the parasitism percentage was not significantly varied between all concentrations of both tested neem formulations with an exception in the case of 50 ppm concentration ($T_8 = 2.477^{NS}$), where nanoemulsion treatment was more effective than neem treatment (Table 2).

The lowest percent of adult emergence (58.00 and 60.10%) was recorded in the highest concentration (800 ppm) of neem-nanoemulsion and neem oil extract,

Table 2 Effect of neem oil and neem nanoemulsion on the parasitism, adult emergence, and mortality of the internal parasitoid larvae of *Meteorus gyrator*

Concentration (ppm)	Treatments Mean ± SE								
	Parasitism			Adult emergence			% Mortality		
	Neem nanoemulsion	Neem	T-value ($df=8$)	Neem nanoemulsion	Neem	T value ($df=8$)	Neem nanoemulsion	Neem	T value ($df=8$)
50	88.70 ± 0.78 b B	92.00 ± 1.08 b A	2.477*	74.30 ± 1.22 b B	81.90 ± 0.72 b A	5.373**	9.20 ± 0.36 e A	6.00 ± 0.86 d B	4.130**
100	88.40 ± 0.69 b A	90.00 ± 2.05 bc A	0.473^{NS}	71.40 ± 0.55 c A	75.60 ± 1.94 c A	2.080^{NS}	14.10 ± 0.42 d A	7.00 ± 0.48 d B	11.047**
200	83.90 ± 2.65 bc A	87.00 ± 1.92 cd A	0.947^{NS}	65.16 ± 1.14 d B	70.50 ± 0.50 d A	4.281**	18.30 ± 1.04 c A	12.00 ± 0.49 c B	5.473**
400	78.90 ± 1.79 cd A	85.00 ± 1.83 d A	2.268^{NS}	60.90 ± 0.35 e B	64.70 ± 0.75 e A	4.609**	23.70 ± 1.05 b A	15.00 ± 0.46 b B	7.619**
800	73.30 ± 1.93 d A	84.00 ± 1.10 d A	2.120^{NS}	58.00 ± 0.52 f A	60.10 ± 1.36 f A	1.440^{NS}	29.00 ± 1.26 a A	25.50 ± 0.30 a B	2.703*
Control	100 a	100 a	–	100 a	100 a	–	0 f	0 e	–
F value($df=5$, 24)	11.816**	15.308**	–	39.711**	17.611**	–	15.872**	37.523**	–
P value	0.001	0.001		0.000	0.000		0.000	0.000	

Tabulated T at 5% = 2.306 T at 1% = 3.355

Means in a single column followed with the same small letters are not significantly different ($P=5\%$)

Means in a single row followed with the same capital letters are not significantly different ($P=5\%$)

NS not significant

**Highly significant

*Significant

respectively, being statistically not significantly varied ($T_8=1.440^{NS}$), followed by 100 ppm concentration ($T_8=2.080^{NS}$), while the highest percentage of adult emergence (100%) was recorded in the control treatment, followed by the 50 ppm concentration that being significantly varied ($T_8=5.373^{**}$), then 200 and 400 ppm (Table 2). In this way, it was seen that the adult emergence expanded by diminishing the concentrations of both neem formulations.

Various concentrations of both neem formulations were proved to be unsafe to *M. gyrator* adult females when they utilized in high concentrations, particularly in the case of neem nanoemulsion that reached to 29.00% mortality (Table 2).

Discussion

First of all, very little literatures were found to be deals with the application of commercial neem oil or nanoformulations against the beneficial insects (natural enemies and pollinators). It is well known that the neem tree, *Azadirachta indica* A. Juss, is very promising for controlling many insect species (Lima et al. 2010). Therefore, neem assumes a part as a growth regulator and as feeding and/or oviposition deterrent agent (Isman 2006). Using nanotechnology in the agriculture field has been studied, including the development of insecticides formulations by polymeric encapsulation (Das et al. 2014; Oliveira et al. 2014).

The parameters usually measured to quantify the effect of neem products on parasitoids are: level of parasitization, the survival of adults (mortality), parasitoid development, adult emergence, longevity, and antifeedant.

In this study, all tested doses of neem nanoformulation especially high doses have adverse effects on the previously mentioned parameters of the internal parasitoid, *Meteorus gyrator,* and hence, these results matched with that of Lowery and Isman (1995). The results of the present study clarify that neem formulations especially that of nanoform have a very harmful effect as reducing fecundity, oviposition, and shortened the longevity of treated natural enemy *M. gyrator*.

A deterrence effect caused by neem products toward parasitoid *M. gyrator*, these results are in a harmony with those findings of Srivastava et al. (1997), but he mentioned that there were no obvious side effects of these products on the longevity of natural enemies especially at low concentrations.

Generally, neem products should not be applied in the abundance seasons of the target parasitoid, *M. gyrator,* and/or any other natural enemies in the field. It was observed that the adult emergence was significantly inhibited by these products especially neem nanoformulation at concentrations of 50 ppm or more, and these results were matched with that of Silva et al. (2019).

Also, our results indicated that a high concentration of azadirachtin (800 ppm) induced a reduction in the mean number of larval parasitism due to a decrease in the survival of tested parasitoid females and also their developmental success, these findings were matching with that reported by Lyons et al. (2003). There were many cases where neem treatments did not affect the parasitoids and can be normally compatible with IPM programs. This may be due to either the concentration is low or other operational concentration (common field concentration); these findings were coordinated with Tang et al. (2002). It has been proved that neem nanoemulsion is more harmful than neem oil on the tested parasitoid, *M. gyrator;* this result was in accordance with Correia et al. (2009).

Under laboratory conditions, it was obvious that the emergence of the parasitoid, *M. gyrator* was not harmed when neem formulation was applied at 50 and 100 ppm concentrations. However, the same concentrations of neem affected the host *S. littoralis* larvae by reducing its feeding. On the other hand, the parasitoid may be killed when the host larvae are treated with a higher concentration in the field so we must delay the application of neem formulation in the period of the parasitoid abundance. Therefore, at the low application of different neem formulations, the parasitoid could survive and complete its life cycle, so these compounds were effective for biological control. These results were harmonized with that mentioned by Schmutterer (1995), Stark et al. (1992), and Lakshmi et al. (2004).

Although there are many authors applied commercial neem formulations on many insect pests, but little of them applied neem formulations against natural enemies. Amin et al. (2019) examined the capability of nano-details of neem and peppermint oils on the bionomics and enzymatic potancy of *Agrotis ipsilon* hatchlings. They concluded that these formulations exhibited critical stretching of the larval duration, rate of mortality, and extended larval malformations. These formulations (neem and pepper mint oil) were significantly affected the enzymatic potency. Essential increase in the activities of cuticle phenoloxidase and chitinase was recorded, in any case, noteworthy restraints were recorded for amylase, invertase, trehalase, protease, and alkaline phosphatase. Dimetry et al. (2019) assessed the harmfulness of neem and peppermint oil extracts against *Agrotis ipsilon* hatching. They revealed that the estimated LC_{50} of neem or pepper mint were lower than neem or pepper mint oil nanoemulsion for the second larval instar. Dimetry (2020) reported that neem oil extract and/or its formulations (either regular or nanoemulsion formulation) was applied safely to many nontarget insects (natural enemies, bees, pollinators, and mammals). She added that neem nanoemulsion was more toxic than the regular neem oil emulsion.

Conclusion

The application of two neem formulations adversely affected the adult emergence, parasitization, and mortality, of the tested parasitoid, *Meteorus gyrator*; these are the most important parameters when assessing the effects of different neem emulsions on parasitoids. There was a minimal impact of neem on the parasitoids if applied in low concentrations. In addition, neem oil emulsions are less toxic than neem nanoemulsion. The timing of the application (host stage, i.e., the female parasitoid prefer the fourth and fifth larval instar of the host, but not attack prepupae or pupa) plays an important role in order to preserve the parasitoids (self communication).

Acknowledgements
The authors are very grateful to all colleges, Pests and Plant Protection Department, National Research Centre, for their valuable advises and help provided.

Authors' contributions
The author read andapproved the final manuscript..

Funding
This work was personally funded by the author.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declare that they have no competing interests.

Received: 27 October 2020 Accepted: 1 December 2020
Published online: 10 December 2020

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