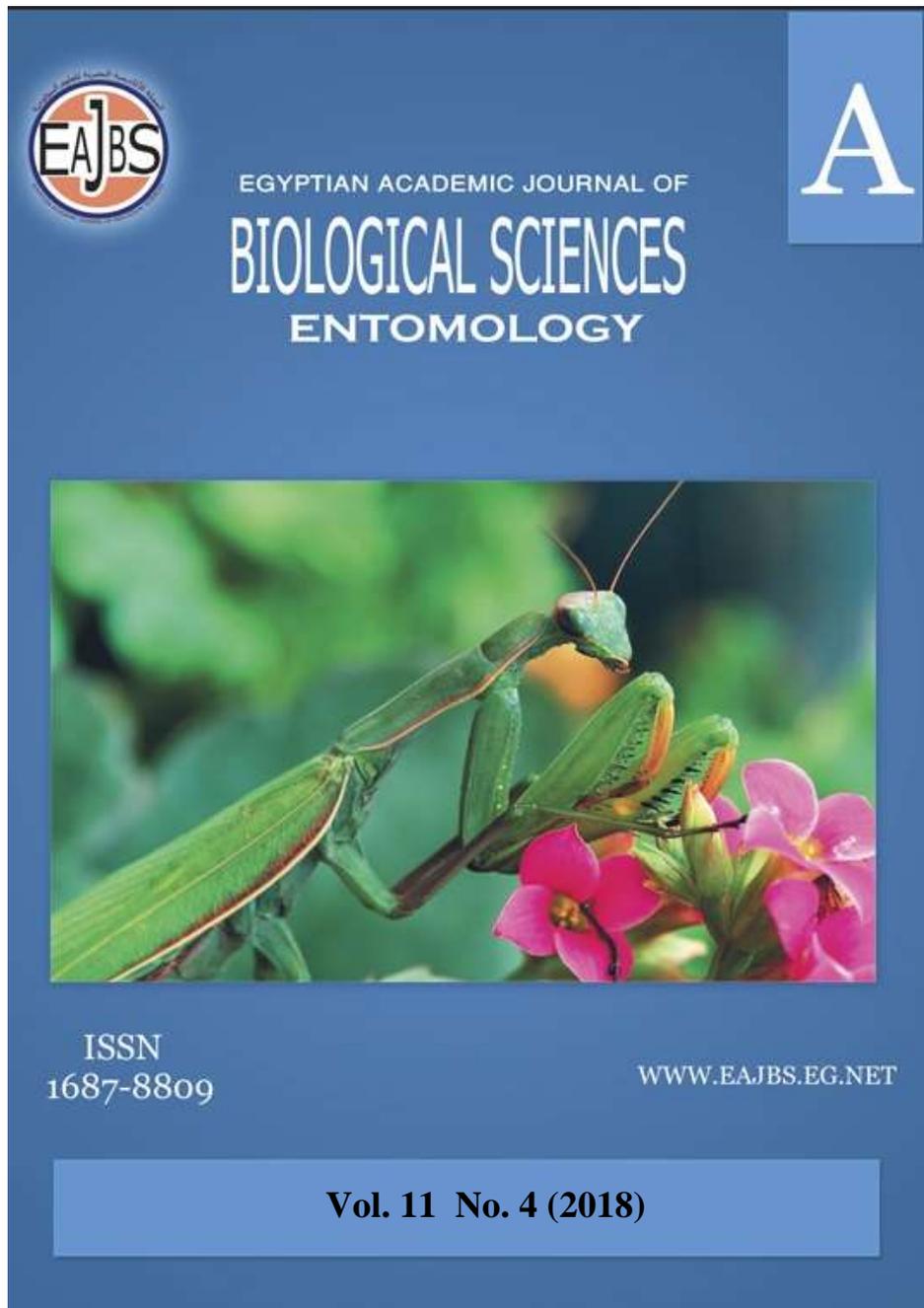


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Insecticidal Activity of *Moringa oleifera* Synthesized Silver and Zinc Nanoparticles against the House Fly, *Musca domestica* L.

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ABSTRACT

Green synthesized nanoparticles have been studied as new and more effective biopesticides against insects of medical importance and this for their eco-friendly aspects. In the current study *Moringa oleifera* leaf extract (Mo-LE) was used to synthesis silver nanoparticles (Mo-AgNPs) and zinc oxide nanoparticles (Mo-ZnONPs). Low concentrations of Mo-LE, Mo-AgNPs and Mo-ZnONPs showed the larvicidal and pupicidal toxicity against *Musca domestica*. In larvicidal bioassay, the values of LC₅₀ were 16.50, 2.03 and 6.41 mg/ml for Mo-LE, Mo-AgNPs and Mo-ZnONPs, respectively. In addition, the LC₅₀ was determined against pupae, which were 129.77, 9.604 and 17.10 mg/ml for Mo-LE, Mo-AgNPs and Mo-ZnONPs, respectively. Moreover, treatment larvae with LC₅₀'s of Mo-LE, Mo-AgNPs and Mo-ZnONPs produced a significant reduction ($P < 0.05$) in the fecundity of female and a highly significant reduction ($P < 0.01$) in the egg hatchability. The total protein content and activities of nonspecific esterases, acetylcholine esterase, and glutathione S-transferase enzymes were significantly declined after larvae had fed on LC₅₀ Mo-AgNPs and Mo-ZnONPs treated diets as compared with the control. This study indicates that it is feasible to use *M. oleifera* synthesized nanoparticles as effective candidates to develop newer and cheap control tools for immature stages of *M. domestica*.

INTRODUCTION

Housefly *Musca domestica* L. (Diptera: Muscidae) is a vector and resource insect (Abbas *et al.* 2014; Bahrndorff *et al.* 2014; Barin *et al.* 2010). This insect is distributed worldwide and highly adaptable to various environments. The control of houseflies largely depends on chemical insecticides. The imprudent application of chemical insecticides resulted in many problems such as insecticide resistance in the insect and negative impact on non-target organisms including man (Acevedo *et al.* 2009; Hemingway & Ranson 2000; Naqqash *et al.* 2016). So, there is an increasing demand to search for alternative control materials that are highly effective and safe for humans.

In recent years, nanomaterials have been receiving great attention because of their antimicrobial and pesticidal activities (Bhattacharyya *et al.* 2010; Bodaiah *et al.* 2016; Rai & Ingle 2012). A number of plants with various reductive groups can

act as reducing and protective agents for the preparation of nanoparticles. The application of botanicals for the synthesis of nanostructured materials offers numerous benefits as the process do not use toxic chemicals in the synthesis (Borase *et al.* 2014). Furthermore, these green synthesized nanoparticles are reported to be more effective insecticides, less expensive, biodegradable and safe for mankind and environment than synthetic counterparts (Murugan *et al.* 2016; Sabbour & Abd El-Aziz 2015). The bioinsecticidal effects of green synthesized nanoparticles on the housefly, *M. domestica* have been reported (Gul *et al.* 2016; Kamaraj *et al.* 2012; Kumar *et al.* 2014).

The biosynthesis of silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) by using extracts from *Moringa oleifera* (F: Moringaceae) have been recently described (Elumalai *et al.* 2015; Matinise *et al.* 2017; Prasad & Elumalai 2011). The *M. oleifera* synthesized AgNPs (Mo-AgNPs) had insecticidal activity against *Aedes aegypti* (Sujitha *et al.* 2015) and *Culex quinquefasciatus* (Murugan *et al.* 2016). While, *M. oleifera* synthesized ZnONPs (Mo-ZnONPs) had antimicrobial activities (Elumalai *et al.* 2015). In course of this study, the larvicidal and pupicidal activity of *M. oleifera* leaf extract (Mo-LE), Mo-AgNPs and Mo-ZnONPs were tested against *M. domestica*. Also, the study assessed the latent effects of these materials on reproductive function, total protein and detoxification enzymes.

MATERIALS AND METHODS

Insect Rearing:

Different stages of *M. domestica* were obtained from stock culture reared in the animal research laboratory, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams Univ., Egypt. Larvae were reared on the modified artificial diet according to Pavela (2008) containing sterilized bran (38 g), milk powder (2 g) and distilled water (60 ml). Adults were fed with 10% sugar solution and provided with cotton pads saturated by milk 10% (10g milk powder dissolved in 100 ml water) as a surface to lay eggs. The culture was maintained at 27°C in 60–70% relative humidity, with a 16 L:8 D h photoperiod.

Plant Material:

Fresh green leaves of *M. oleifera* were collected from the garden of Department of Biological and Geological Sciences, Faculty of Education, Ain Shams Univ., Egypt. Leaves were washed with running tap water then with distilled water and air dried at room temperature. The dried leaves were ground using an electric mill. *M. oleifera* leaf extract (Mo-LE) was prepared by mixing 10 g of dried leaf powder with 100 ml deionized water in 500 ml of Erlenmeyer flask and boiled for 20 min and then filtered (Prasad & Elumalai 2011).

Green Synthesis of Silver and Zinc Oxide Nanoparticles:

The green synthesis of AgNPs through the reduction of AgNO₃ (1 mM aqueous) by Mo-LE was carried out by the method of Prasad & Elumalai (2011). The formation of Mo-AgNPs was observed and it is perceived by the color change from brown to reddish color.

Mo-ZnONPs was synthesized as previously described by Elumalai *et al.* (2015) and Matinise *et al.* (2017) by using Mo-LE with some modifications. To obtain Mo-ZnONPs, 5 g of zinc nitrate hexahydrate was dissolved in 50 ml Mo-LE at 80 °C under constant stirring. Then the mixture was boiled until it becomes deep yellow colored paste. Then, it dried at 400 °C for 2 h. Finally, Mo-ZnONPs was obtained as light yellow colored powder. This powdered product was stored at 4 °C until use.

Larvicidal Assay:

Insecticidal efficacy of Mo-LE, Mo-AgNPs and Mo-ZnONPs were evaluated against newly hatched 1st instar larvae of *M. domestica* by using diet incorporation method. The larval artificial diets (100g) were prepared by replacement the water with 60 ml of each of the following concentrations of Mo-LE (0.5, 1, 5, 10 and 50 mg/ml), Mo-AgNPs (0.5, 1, 2, 4 and 8 mg/ml) and Mo-ZnONPs (0.5, 1, 2, 4 and 8 mg/ml). Twenty-five 1st instar larvae were introduced to each concentration and the experiment was repeated four times. Simultaneously, controls with deionized water only were prepared. Bioassays were conducted under the laboratory conditions. The number of surviving larvae was counted after 48 h.

Pupicidal Assay:

Dipping method was used to evaluate the insecticidal efficacy of Mo-LE, Mo-AgNPs and Mo-ZnONPs against newly formed pupae of *M. domestica*. Twenty five pupae were submerged in one of the following concentrations of Mo-LE (0.5, 1, 5, 10 and 50 mg/ml), Mo-AgNPs and Mo-ZnONPs (0.05, 1, 2, 4 and 8 mg/ml) for 2 minutes. Controls prepared by dipping pupae in deionized water only. The experiment was repeated four times. Pupae were transferred into new vials and kept in the previously mentioned laboratory condition. The number of emerged adults was recorded hereafter.

Latent Effect of LC₅₀ on Fecundity:

First instar larvae were exposed to LC₅₀ of Mo-LE, Mo-AgNPs and Mo-ZnONPs. After adult emerged from each treatment, ten males and females adults were transferred to oviposition cages, containing sugar and cotton pads served for feeding and oviposition, the cotton pads were renewed daily. The total number of eggs was recorded and the number of eggs laid per female (fecundity) was calculated. Percent fecundity was determined according to Crystal (1964). The oviposition deterrent index (ODI) was calculated according to Lundgren (1975). The eggs were moved to Petri dishes containing filter paper moisten by water. Control and treated eggs were incubated under the same laboratory conditions. One day later, the emerged larvae were counted and the percent of egg hatch was determined. The sterility was calculated according to Topozada *et al.* (1966).

Assay of Total Protein and Detoxification Enzymes:

First instar larvae were exposed to LC₅₀ of Mo-LE, Mo-AgNPs and Mo-ZnONPs. After 48 h post treatment, live treated larvae from each treatment were collected, weighed then homogenized in 2 times volumes (w/v) of 0.7 % saline solution. Control specimens were obtained by homogenizing control larvae through the same technique. Homogenates were centrifuged at 10000g for 10 min. The supernatant was used directly or frozen as a source of enzymes. Total protein content was estimated according to Bradford (1976). Total esterase activity was determined according to Prabhakaran & Kamble (1993). The activity of acetylcholine esterase (AChE) was assayed according to the method of Ellman *et al.* (1961). The activity of Glutathione-S transferase (GST) was assayed according to the method of Habig *et al.* (1974).

Statistical Analyses:

Mortality for Mo-LE, Mo-AgNPs and Mo-ZnONPs were corrected for control mortality using Abbott's formula (Abbott 1925) and subjected to probit analysis (Finney 1971) for calculating LC₅₀ using SPSS software (V. 20.0). For testing the significance of the data obtained, statistical analyses was carried out using Tukey's HSD test using SPSS software.

RESULTS

In the present study, the larvicidal and pupicidal activities of Mo-LE, Mo-AgNPs and Mo-ZnONPs against 1st instar larvae and newly formed pupae of *M. domestica* are represented in terms of their medium lethal concentrations (LC₅₀) along with upper and lower confidence limit values and regression equation (Table 1). In all treatments, the mortality rates increase by increasing concentrations. Mo-AgNPs was more effective than Mo-ZnONPs and Mo-LE against 1st instar larvae with LC₅₀ values were 2.03, 6.41 and 16.50 mg/ml respectively. In addition, the LC₅₀ was determined against pupae, which were 129.77, 9.604 and 17.10 mg/ml for Mo-LE, Mo-AgNPs and Mo-ZnONPs respectively.

Table 1: Larval and pupal toxicity of Mo-LE, Mo-AgNPs and Mo-ZnONPs against *M. domestica*

Stage	Treatment	LC ₅₀	95% Confidence limits (UL-LL)		Regression equations	χ ² (df=3)
Larva	Mo-LE	16.50	10.71	29.54	Y= 0.6*x + (-0.9)	1.42
	Mo-AgNPs	2.03	1.33	3.15	Y= 1.78*x + 0.28	5.92
	Mo-ZnONPs	6.41	4.46	11.49	Y= 1*x + (-0.8)	1.23
Pupae	Mo-LE	129.77	63.72	415.48	Y= 0.8*x + (-1.6)	2.67
	Mo-AgNPs	9.604	2.79	340.89	Y= 0.35*x + (- 0.37)	0.66
	Mo-ZnONPs	17.10	6.49	107.96	Y= 0.50*x + (- 0.75)	2.09

LC₅₀=lethal concentration (mg/g for larvae and mg/ml for pupae) that kills 50 % of the exposed larvae. χ² =chi-square value, df=degrees of freedom, (α=0.05)

The data presented in Table 2 showed that the fecundity of untreated female was 514.6±64.6 eggs/female and the percentages hatch was 93.57%. It was noticed that Mo-LE has a negative impact on the number of eggs laid per female. LC₅₀-treatment produced a significant reduction ($P<0.05$) in the fecundity of female, it was 262.3 ± 57.17 eggs/female. Fecundity value was reduced by about 50.97 % from that of control and the ODI value was 32.47 32.475 %, as compared with controls. Also, a highly significant reduction ($P<0.01$) in the egg hatchability, it was 89.64%. As a result of that reduction, the sterility value reached 19.44%. Both Mo-AgNPs and Mo-ZnONPs treatment caused a highly significant reduction ($P<0.01$) in the fecundity of female, treated females with LC₅₀ of Mo-AgNPs and Mo-ZnONPs produced 86.5 ± 16.18 and 115.3 ± 25.05 eggs/female, respectively. Fecundity values were reduced by about 16.81 and 22.41 % from that of control and the ODI values were 71.22 and 63.39%, as compared with controls. Also, a highly significant reduction ($P<0.01$) in the egg hatchability, they were 89.29 and 75.77 %. The sterility values reached 56.69 and 79.29 %, respectively.

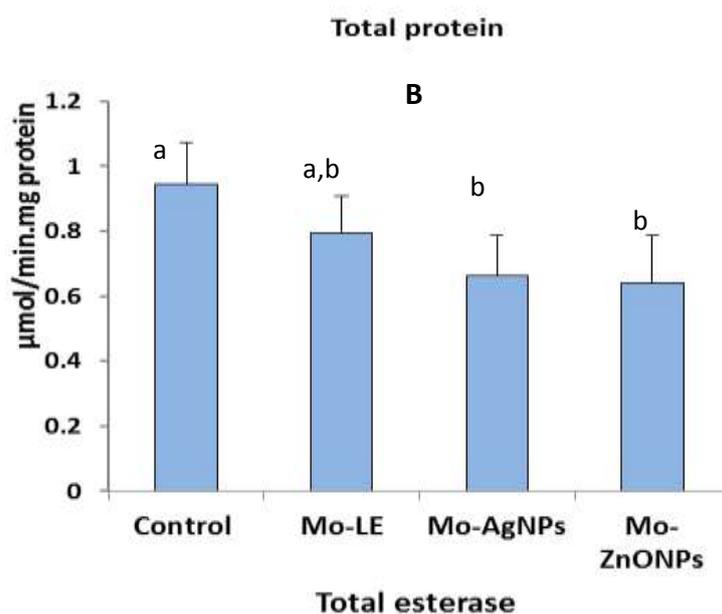
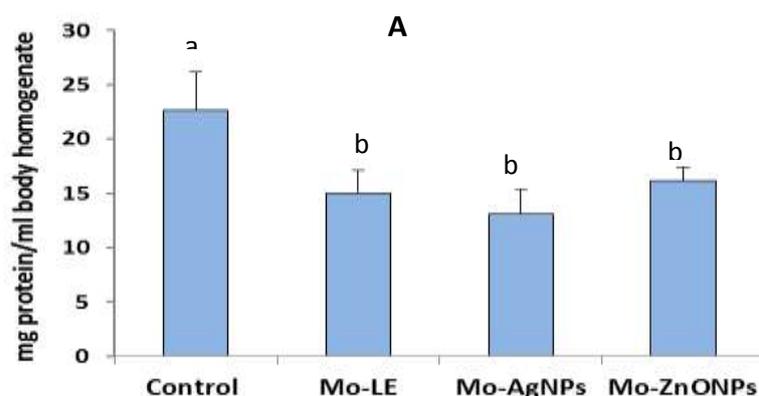
Table 2: Latent effects of Mo-LE, Mo-AgNPs and Mo-ZnONPs on reproduction of *M. domestica* following treatment of larval media.

Treatment	Fecundity	% Fecundity	Fertility	% hatch	% ODI	% Sterility
Control	514.6± 64.6 a	100.00	480.9 ± 61.58 a	93.57	0.00	-
Mo-LE	262.3 ± 57.17 b	50.97	210.3 ± 53.12 b	80.61	32.47	77.71
Mo-AgNPs	86.5 ± 16.18 c	16.81	45.9 ± 16.61 c	53.09	71.22	98.39
Mo-ZnONPs	115.3 ± 25.05c	22.41	79 ± 19.94 c	69.18	63.39	96.32

Means within columns followed by different letters indicate statistically significant differences. Fecundity and fertility refer to eggs and larvae produced per female, respectively.

The total protein concentration of larval body homogenate treated with Mo-LE, Mo-AgNPs and Mo-ZnONPs were analyzed and compared to the untreated larval homogenate (Fig. 1A). The treatment of the larvae with Mo-LE exhibited the total protein content from 22.62 mg/ml in control to 14.96 mg/ml. While, treatment with Mo-AgNPs and Mo-ZnONPs were found to be highly significant reduced the total protein amounted to be 13.09 and 16.15 mg/ml, respectively, ($F_{3,19} = 14.42$, $P = 0.000$). Application of LC_{50} of Mo-LE produced non-significant ($P > 0.05$) difference on the total esterase on treated larvae. Conversely, LC_{50} of Mo-AgNPs and Mo-ZnONPs treatments produced highly significant decrease ($P < 0.01$) on the total esterase of the treated larvae from that of the control, amounted to be -30.03 and -32.14 % from that of the control (Fig. 1B).

In the present study, AchE and GST activities were significantly decreased in larvae fed diets treated with LC_{50} of Mo-LE, Mo-AgNPs and Mo-ZnONPs compared with the controls (Fig. 1C & 1D). After treatment with Mo-LE, Mo-AgNPs and Mo-ZnONPs, the activity of AchE in the larvae declined by -33.63, -62.50 and -58.30 %, respectively ($F_{3,19} = 31.21$, $P = 0.000$). The GST activity was approximately 1.3, 1.6, and 1.8 times lower in larvae treated with Mo-LE, Mo-AgNPs and Mo-ZnONPs than in control larvae, respectively, ($F_{3,19} = 51.8$, $P = 0.000$). However, there was **no** significant difference between the Mo-AgNPs and Mo-ZnONPs treatment but both produced a significant inhibition in AchE and GST from that of the control and Mo-LE.



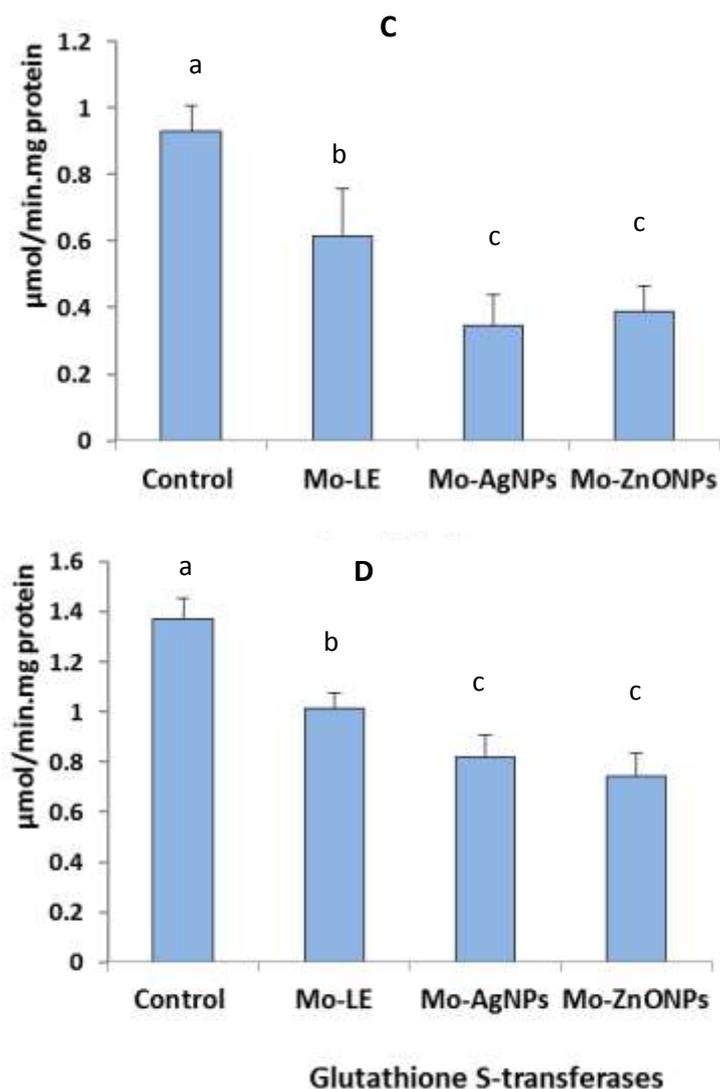


Fig. 1. Effect of LC_{50} of Mo-LE, Mo-AgNPs and Mo-ZnONPs on the enzyme activities and total proteins in larval homogenates of *M. domestica* (A) total protein concentration, (B) Acetylcholine esterase, (C) total esterase, (D) Glutathione S-transferases enzyme. Each column represents the mean \pm SE of five replicates. Errors bars followed by different letters indicate statistically significant differences.

DISCUSSION

An attempt was made in the present study to test the efficacy of *M. oleifera* leaf extract synthesized silver and zinc oxide nanoparticles to control housefly. It was observed from the results that both Mo-AgNPs and Mo-ZnONPs were highly toxic against larvae and pupae of *M. domestica*. The Mo-LE was comparatively less active against immature stages of housefly in the present investigation, reflecting the role of nanostructured materials in insecticidal activity determination. These results are in agreement with the findings of Sujitha *et al.* (2015) and Murugan *et al.* (2016) reported that the *M. oleifera* seed extract -synthesized AgNPs is toxic than *M. oleifera* seed extract against larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus*. The insecticidal potential of green silver nanoparticles against *M.*

domestica has been investigated recently (Kamaraj *et al.* 2012) evaluated the toxicity of *Manilkara zapota* leaf extract and its synthesized silver nanoparticles against the adult of *M. domestica*. The most efficient activity was observed in synthesized AgNPs (LD₅₀=3.64 mg/mL). While, the aqueous extract of *M. zapota* had reported LD₅₀=28.35mg/mL. In addition, nanoparticles of *Mentha piperita* essential oil showed considerable mortality against housefly larvae in laboratory as well as in field application (Kumar *et al.* 2014). Furthermore, Gul *et al.* (2016) found that melon aqueous extract synthesized AgNPs showed significantly high mortality against housefly adults.

Vani and Brindhaa (2013) reported that nanoparticles are more reactive than their bulk counterpart because of their increased surface to volume ratio. The toxicity of nanoparticles may be due to partial lysis of the midgut epithelial cells; vesicles and damaged membranes at the apical side of epithelial cells (Foldbjerg *et al.* 2015; Sultana *et al.* 2018). Moreover, nanoparticles caused death in insects by being absorbed into the cuticular lipids causing physical damage (Barik *et al.* 2008). Also, the exposure to silver nanoparticles has a major impact on the induction of oxidative stress and detoxification genes if compared over the exposure to free silver ions (Nair *et al.* 2013). Furthermore, the application of ZnONPs led to several morphological, histological abnormalities and accumulation of ZnONPs in the thorax and abdomen of the insect (Abinaya *et al.* 2018; Banumathi *et al.* 2017; Ishwarya *et al.* 2018). Therefore, detailed studies on how the exposure to ZnONPs can affect insect physiology and genetics are lacking (Benelli 2018).

In the present study, the fecundity was significantly decreased after treatment with Mo-LE, Mo-AgNPS and Mo-ZnONPs. Our results are further supported by the observations of Malaikozhundan and Vinodhini (2018) reported the reduction effect of *Pongamia pinnata*-ZnONPs on the fecundity and hatchability of *Callosobruchus maculatus*. Also, the pungam oil based gold nanoparticles significantly reduced the fecundity of *Pericalia ricini* (Sahayaraj *et al.* 2016). Silica nanoparticles reduced egg laying by *C. maculatus* (Arumugam *et al.* 2015). Roni *et al.* (2015) reported that *Hypnea musciformis* extract and AgNP strongly reduced longevity and fecundity of *A. aegypti* and *Plutella xylostella* adults.

In the current study, the total protein level of larvae treated with LC₅₀ of Mo-LE, Mo-AgNPS and Mo-ZnONPs was found to be declined. Also, the total protein levels reduced after application of various nanoparticles was also shown in 1st instar larvae *A. aegypti* (Ghramh *et al.* 2018) and *Aedes albopictus* (Ga'al *et al.* 2018). Also, AgNPs prepared using *Cassia fistula* extract showed a decrease in total protein levels of 4th instar larvae of *Aedes albopictus* and *Culex pipiens* (Fouad *et al.* 2018). The inhibitory effect of nanoparticles might be due to the direct toxic effect on the protein synthetic pathways of the insect larvae (Ga'al *et al.* 2018).

According to the results of enzymes assays, the activity of the tested enzymes was more sensitive to Mo-nanoparticles than Mo-LE. However, there was not any significant difference between Mo-AgNPs and Mo-ZnONPs. AChE is a key enzyme in the nervous system that rapidly terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine (Solairaj & Rameshthangam 2016). Glutathione S-transferases are major detoxification enzymes that are found mainly in the cytosol. In addition to their role in catalyzing the conjugation of electrophile substrates to glutathione, these enzymes also exhibit peroxidase and isomerase activities (Krishnan & Kodrik 2006; Weinhold *et al.* 1990). These enzymes efficiently catalyze the hydrolysis of ester- and amide- containing chemicals and responsible for the detoxification or metabolic activation of various xenobiotics and

also play an important physiological role in lipid metabolism. Nonspecific esterases and GSTs have been shown to play a role in the dietary tolerance of allelochemicals (Bass & Field 2011; Li *et al.* 2007). In the present investigation, total protein and metabolic enzymes were inhibited in housefly larvae after treatment with Mo-AgNPs and Mo-ZnONPs, which might suggest a poor defense mechanism in the detoxification of Mo-AgNPs and Mo-ZnONPs and this may be the reason for larvicidal property of Mo-AgNPs and Mo-ZnONPs. These results are similar to that reports by Malaikozhundan and Vinodhini (2018) who reported the reduction effect of *P. pinnata* synthesized ZnONPs on the mid-gut α -amylase, cysteine protease, α -glucosidase, β -glucosidase, glutathione S-transferase and lipase activity in *C. maculatus*. Fouad *et al.* (2018) reported that 4th instar larvae of *A. albopictus* and *C. pipiens* treated with LC₅₀ *Cassia fistula*-AgNPs showed a decrease of acetylcholinesterase and α - and β -carboxylesterase activities. AgNPs inhibit the activity of acetylcholine esterase and ultimately kill the mosquito larvae (Solairaj & Rameshthangam 2016). The results confirmed that inhibition in the metabolic enzymes and protein may be the reason explained why Mo-AgNPs showed higher activity than Mo-ZnONPs and Mo-LE

CONCLUSIONS

It was concluded that Mo-AgNPS and Mo-ZnONPs are effective against *M. domestica*. Results suggest that the use of Mo-AgNPS and Mo-ZnONPs cause death of treated larvae and pupae at low concentration and reduced the fecundity of adults. The activity of acetylcholine esterase, esterases, GST and total protein were decreased by the action of Mo-AgNPS and Mo-ZnONPs and were comparatively lower than pure Mo-LE. Hence, green synthesized nanoparticles are recommended in housefly control, because of its unique biological mode of action, more effective, less expensive, and safe to environment.

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ARABIC SUMMARY

كفاءة جسيمات الفضة والزنك النانوية المصنعة بواسطة نبات المورينجا كمبيد حشري لمكافحة الذبابة المنزلية، مسكا نوميسكا ل.

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تمت دراسة الجسيمات النانوية المخلفة عن طريق النباتات الخضراء كمبيدات بيولوجية جديدة أكثر فعالية ضد الحشرات ذات الأهمية الطبية وهذا من أجل جوانبها الصديقة للبيئة. في الدراسة الحالية ، تم استخدام مستخلص أوراق نبات المورينجا (Mo-LE) لتخليق جسيمات الفضة النانوية (Mo-AgNPs) وجسيمات أكسيد الزنك النانوية (Mo-ZnONPs). أظهرت التركيزات المنخفضة من Mo-LE و Mo-AgNPs و AgNPs سمية مميّنة ضد يرقات و عذارى الذبابة المنزلية. في الاختبار الأحيائي لليرقات ، كانت قيم التركيز نصف المميت هي 16,50 و 2,03 و 6,41 ملجم/مل لكل من Mo-LE و Mo-AgNPs و Mo-ZnONPs على التوالي. بالإضافة إلى ذلك ، تم تحديد التركيز نصف المميت ضد العذارى ، والتي كانت 17,10 و 9,604 ، 129,77 ملجم/مل لكل من Mo-LE و Mo-AgNPs و Mo-ZnONPs، على التوالي. وعلاوة على ذلك ، أدت معاملة اليرقات بالتركيز نصف المميت من Mo-LE و Mo-AgNPs و Mo-ZnONPs الى انخفاضاً معنوياً ($p < 0.05$) في خصوبة الأنثى و انخفاضاً معنوياً ملحوظاً ($p < 0.01$) في معدل فقس البيض. كما انخفض محتوى البروتين الكلى وأنشطة الاسترات غير النوعية ، أستيل كولين استيريز ، وإنزيمات الجلوتاثيون S-transferase بشكل ملحوظ بعد تغذية اليرقات على التركيزات نصف المميّنة لكل من Mo-ZnONPs و AgNPs مقارنة مع اليرقات غير المعاملة. تشير هذه الدراسة إلى أنه من المجدي استخدام الجزيئات النانوية المخلفة باستخدام المورينجا كبديل فعال لتطوير أدوات مكافحة جديدة ورخيصة للاطوار غير الناضجة للذبابة المنزلية.