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Effects of synthesized silver and chitosan nanoparticles using *Nerium oleander* and *Aloe vera* on antioxidant enzymes in *Musca domestica*

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ABSTRACT

Green synthesis of silver and chitosan nanoparticles have been achieved using *Nerium.oleander* and *Aloe vera* leaf extract as reducing agents. The formation of nanoparticles was quantified by UV-vis spectroscopy of wave length from 200-700. TEM, XRD and FTIR were used for characterization. The present investigation was carried out to assess the activity of antioxidant enzymes, SOD, CAT, GPx and GST in third instar larvae of *Musca domestica* under the effect of methanol leaf extract, synthesized silver and chitosan nanoparticles and the LC_{50} was used for biochemical assay. The activity of antioxidant enzymes in *M.* domestica larvae were varied with the time where an increase in SOD activity was recorded after 24hrs in methanol extract and AgNPs treated larvae, then decreased again after 48hrs. In CsNPs treated larvae, SOD induced a continuously decreased activity at all times of investigation. CAT activity followed the same trend of SOD. After 48hrs of treatment the activity of GPs was significantly decreased in all treated groups, at 1% and 5% level of significance in larvae treated with chitosan *Nerium* and chitosan *A. vera*, respectively. After an increase in GST activity at 24hrs, a decrease was noticed after 48hrs. From the present study, the use of *N. oleander* and *A. vera* as methanol crude extracts to synthesize silver and chitosan nanoparticles could be a new approach for the control of house flies.

Keywords: *Musca domestica*, AgNPs, CsNPs, *Nerium oleander*, *Aloe vera*

INTRODUCTION

Musca domestica causes a serious threat to human health by transmission of infectious diseases. The chemical insecticides widely used to control house flies are often harmful to human and environment (Abd El-Hamid *et al*., 2018). Therefore, it is imperative that control of house fly must be improved through the use of natural pesticides (Mohammed, 2018). Plant extracts have been the major weapon used, because they are believed to contain many phytochemicals that have insecticidal activity (Oni *et al*., 2019).

In the recent years, nanotechnology has become one of the most promising new approaches for pest control because; nanoparticles exhibit novel characteristics such as extraordinary strength (Rai *et al*., 2018; Tunçsoy, 2018; Ahmed *et al*., 2019; Khoshraftar *et al*., 2019 and Shahzad & Manzoor, 2019). Biosynthetic methods using plant extracts has received a great attention than chemical methods because nanoparticles synthesized by using plants is ecofriendly and safe for human use (Fouad *et al*., 2018; Shehata and Mahmoud, 2019; Sutthanont *et al*., 2019). The phenolic compounds, present in most plants, are prooxidants that often transformed to semi-Quinone radicals which in turn react with oxygen to generate superoxide radicals, hydrogen peroxide and hydroxyl radicals. These radicals may react with essential biomolecules such as proteins, lipids, causing alterations in their structures. Lipid peroxidation is harmful in insects because it changes the permeability of the cell membrane, thus disturbs the specific function of pheromones and juvenile hormone (Benelli, 2016). In response to the nanoparticles and allele-chemicals prooxidant properties, insects have developed various defense mechanisms towards the free oxygen radicals (Kaur *et al*., 2014). Among these mechanisms, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase were performed. Although there have been numerous studies on the effects of nanoparticles on bacteria, fungi, and animal pathogens (Tuncsoy *et al*., 2019), few research has been carried out to investigate the mode of action of the nanoparticles on insects. Since it is now an important field for biopesticides research, it is necessary to develop potent enzyme inhibitors. *Aloe vera* leaf extract against *M. domestica*, and aqueous leaf extract and synthesized nanoparticles using *N. oleander* against other insects have proven their insecticidal effect (Jesikha, 2012; Roni *et al*., 2013 and Anand *et al*., 2018). However, their inhibitory effects on different enzymes have not been evaluated yet. Therefore, the aim of the present work is to evaluate, for the first time, the inhibitory effects of methanol-leaf extract as well as the synthesized silver and chitosan nanoparticles, using *N. oleander* and *A. vera*, on the antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase of *Musca domestica.*

MATERIALS AND METHOD

The present study was carried out in the laboratory of Entomology Department, Faculty of Science, Benha University.

Maintenance and rearing of *Musca domestica*

The initial culture of *M. domestic* used in the present investigation was obtained from a susceptible strain colony in laboratory of the Medical Insect Research Center, Dokki, Giza. For raising the larvae of house fly the method of Elkattan *et al*., (2011) was followed.

Plant materials, extraction and biosynthesis of nanoparticles

The used plants are *Nerium oleander* (Apocynaceae) and *Aloe vera* (Asphodelaceae). Fresh leaves of *N. oleander* obtained from the plant nursery in Benha

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city, while *A. vera* leaves were collected from graves of El Ramla village, Qalyobia Governorate. The taxonomical identification of the collected plants was confirmed by Botany Department, Faculty of Science, Benha University.

The methanol-leaf extract of each plant was prepared following to the method of Fartyal and Kumar (2014). While, the techniques used for sliver nanoparticles (AgNPs) and chitosan-based nanoparticle production were carried out according to Fatima and Mohammed (2018) and Othman *et al*., (2018), respectively. The formation of nanoparticles was quantified by UV-vis spectroscopy with wave length of 200-700 nm. Transmission electron microscopy (TEM), Fourier transforms infrared (FTIR) and X-ray diffraction (XRD) were also used for characterization.

Biochemical assays

Acute toxicity of the used compounds against early third instar larvae of *M. domestica* was evaluated and the values of LC_{50} were determined. The resultant LC_{50} was used for biochemical assays. Meanwhile, the antioxidant enzymes were also measured. Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi *et al*., (1972), Catalase (CAT) activity was estimated according to the method of Aebi (1984). The method of Paglia and Valentine (1967) for extraction and estimation of Glutathione peroxidase (GPx) activity was used. However, Glutathione S-transferase (GST) activity was determined by the method of Habig *et al*., (I974).

Statistical analysis

Mortality data were subjected to probit analysis, the EPA Probit analysis program (version 1.5) was used for calculating the LC_{50} values. All data obtained from biochemical assays were subjected to one-way analysis of variance and means were separated with Tukey honestly significant test. SPSS version 21 was used for the analysis.

RESULTS

In the present study, the early third instar larvae of *M. domestica* treated in their artificial diet with different concentrations of methanol leaf extract, green synthesized silver and chitosan nanoparticles using *N. oleander* and *A. vera*. The LC_{50} values which calculated with probit analysis were 73.02 and 117.37 ppm for methanol leaf extract, 1.52 and 2.62 ppm for silver nanoparticles, and 0.65 and 0.79 ppm for chitosan nanoparticles of *N. oleander* and *A. vera*, respectively. The biochemical assays were performed with these concentrations.

Effect of methanol leaf extract on the activity of antioxidant enzymes

The effects of methanol leaf extract of *N. oleander* and *A. vera* on the activity of SOD, CAT, GPx and GST of 3rd instar larvae of *M. domestica* are presented in the table (1). As indicated from the table, the SOD activity during the normal course of development of larvae showed that, it increased by 1.1 fold from 0.70 U/ml at 0hr to 0.77 U/ml at 48hr. In the *N. oleander* treated larvae SOD showed a significant increase in activity after 24 hrs ($p \leq 0.05$), but decreased after 48 hrs, while, in *A. vera* treated larvae, a non-significant increase after 24hrs and non-significant decrease after 48hrs were observed. Under the normal developmental conditions, estimation of CAT activity showed a decrease from 0.46 U/L at 0hr to 0.43 U/L at 48hrs. In treated larvae, the catalase activity followed the same trend of SOD, but the values for *N. oleander* treated groups were highly significant ($p \leq 0.01$) after 24hrs and significant for *A. vera* treated groups. The GPx activity showed an increase by 1.53 fold from 0hr to 48hrs in the control group, while in all treated groups, a highly significant increase ($p \leq 0.01$) was observed. The investigation of GST activity showed some fluctuations during normal development from 0hr to 48hrs, but showed insignificant effect in most treated groups.

Table (1): Effect of methanol-leaf extract of *N. oleander* and *A. vera*, on the activity of antioxidant enzymes in *M. domestica* 3rd instar larvae.

Measured Enzymes	Activity of the enzyme						
	0hr	24hr			48hr		
	Control	Control	*N. oleander*	*A. vera*	Control	*N. oleander*	*A.* vera
SOD(U/ml)	0.70±0.01	0.71±0.02	0.91±0.05*	0.74±0.04^{NS}	0.77±0.07	0.69±0.02*	0.73±0.12^{NS}
CAT (U/L)	0.46±0.02	0.45±0.06	1.32±0.02**	1.06±0.06*	0.43±0.06	0.41±0.02^{NS}	0.42±0.06 ^{NS}
GPx (U/L)	63.6±0.00	63.7±0.08	102±20.7**	124±11.5**	97.1±8.9	150.0±12.3**	170±15.2**
GST (mmole/min/mg protein)	1.28±0.00	1.22±0.08	2.0±0.07*	1.30±0.07^{NS}	1.25±0.07	1.23±0.12^{NS}	1.23±0.04^{NS}

Data are presented in mean ± SD
** = Significant at 1% level, * = Significant at 5% level, NS = Non Significant

Effect of silver nanoparticles on the activity of antioxidant enzymes

Dada presented in the table (2) showed the activity of antioxidant enzymes after treatment with synthesized silver nanoparticles using *N. oleander* and *Aloe vera* leaf extract against *M domestica* 3rd instar larvae. SOD showed non-significant increase in activity after 24hrs of treatment, but showed decreased activity after 48hrs ($p \leq 0.05$) in larvae treated with of *N. oleander*-AgNPs, whereas significant increase was recorded in

A. vera-AgNPs treated insects after 24hrs and insignificant decrease after 48hrs of treatment was observed. The CAT activity followed the same trend of SOD, a significant increase was observed after 24hrs, 2.07 and 2.33 fold increase for *N oleander* and *A. vera* treated larvae respectively, then, activity decreased signific-antly after 48hrs in all treated groups. The GPx activity showed an increase in the control groups when assessed at different developmental times. While the treatment with AgNPs induced an increase in the activity of the enzyme which was significant in *N. oleander*-AgNPs treated and highly significant in *A. vera*-AgNPs treated larvae after 48hrs. After showing an induction, in the GST activity after 24hrs of treatment, insignificant decrease was observed after 48hrs of treatment.

Effect of chitosan nanoparticles on activity of antioxidant enzymes

The antioxidant enzyme activities of *M. domestica* 3rd instar larvae treated with synthesized chitosan nanoparticles using *N. oleander* and *A. vera* leaf extract is given in table (3). The SOD induced a continuously decreased enzyme activity at all times of investigation, the activity decreased significantly by 1.16 and 2.09 fold decrease after 24 and 48hrs of treatment with *N. oleander*-CsNPs. Whereas, insignificant decrease after 24hrs and significant decrease after 48hrs were observed in *A. vera*-CsNPs treated larvae.

The same trend was observed in the CAT activity, but non-significantly decreased at 24hrs post treatment. After 24hrs of treatment, the GPx activity nonsignificantly decreased in chitosan *Nerium* treated but significantly increased in chitosan *A. vera* treated larvae, whereas, after 48hrs of treatment the activity was significantly decreased in all treated groups, at 1% and 5% level of significance in larvae treated with chitosan *Nerium* and chitosan *A. vera*, respectively. The GST activity showed insignificant decrease in most treatments, except for, the chitosan *A. vera* treated group, the enzyme activity showed highly significant increase at 24hrs of treatment and decreased again at 48hrs.

Table (2): Effect of silver nanoparticles synthesized using *N. oleander* and *A. vera* on the activity of antioxidant enzymes in *M. domestica* 3rd instar larvae.

Measured Enzymes	Activity of the enzyme						
	0hr	24hr			48hr		
	Control	Control	*No*-AgNPs	*Av*-AgNPs	Control	*No*-AgNPs	*Av*-AgNPs
SOD (U/ml)	0.70±0.01	0.71±0.02	0.73±0.02 ^{NS}	0.83±0.03*	0.77±0.07	0.62±0.05*	0.72±0.03 ^{NS}
CAT (U/L)	0.46±0.02	0.45±0.06	0.95±0.02*	1.07±0.06*	0.43±0.06	0.38±0.01*	0.39±0.07*
GPx (U/L)	63.6±0.00	63.7±0.08	116.3±7.2**	166±7.10**	97.1±8.9	112.0±4.5*	181.2±17.8**
GST (m mole/min/mg protein)	1.28±0.00	1.22±0.08	1.37±0.01 ^{NS}	2.21±0.08*	1.25±0.07	1.19± 0.07 ^{NS}	1.21±0.07 ^{NS}

Data are presented in mean ± SD
** = Significant at 1% level, * = Significant at 5% level, NS = Non Significant
No-AgNPs = *Nerium oleander*-silver nanoparticles. *Av*-AgNPs = *Aloe vera*-silver nanoparticles

Table (3): Effect of chitosan nanoparticles on the activity of antioxidant enzymes in *M. domestica* 3rd instar larvae.

Enzymes	Activity of the enzyme					
	0hr	24hr		48hr		
	Control	*No*-CsNPs	*Av*-CsNPs	Control	*No*-CsNPs	*Av*-CsNPs
SOD (U/ml)	0.71±0.02	0.61±0.03	0.70±0.02 ^{NS}	0.77±0.07	0.34±0.02 **	0.68±0.02 *
CAT (U/L)	0.45±0.06	0.42±0.02 ^{NS}	0.44±0.02 ^{NS}	0.43±0.06	0.31±0.12 **	0.35±0.01 *
GPx (U/L)	63.7±0.08	62.7±11.4 ^{NS}	74.7±7.8*	97.1±8.9	32.0±12.9**	89.2.±4.8*
GST (m mole/min/mg protein)	1.22±0.08	1.18±0.07 ^{NS}	1.78±0.02 **	1.25±0.07	1.12±0.06 *	1.20±0.05 ^{NS}

Data are presented in ± SD
** = Significant at 1% level, * = Significant at 5% level, NS = Non Significant
No-AgNPs = *Nerium oleander*-silver nanoparticles. *Av*-AgNPs = *Aloe vera*-silver nanoparticles

DISCUSSION

Inhibition of the activities of enzymes is considered a well-known instrument to stop a large number of important physiological and biochemical processes (Zorlu *et al*., 2018). The dismutation ability of the SOD enzyme enables it to transform superoxide radicals (O_2-) to hydrogen peroxide (H_2O_2) and oxygen, thus, prevents the accumulation of oxygen free radicals. Therefore, it is the first line of defence against toxicity from superoxide radicals generated during metabolism helping in resistance development in insects (Kolawole *et al*., 2014). Results revealed an increase in the SOD activity in the larvae treated with methanol leaf extracts and silver nanoparticles after 24hrs, then decreased again after 48hrs. The role played by SOD as a first line of defence against toxicity with the generation of superoxide radical could be responsible for the observed increase in SOD activity. On the other hand, chitosan nanoparticles induced continuously decreased

in SOD activity at all times. This decrease is an indicator that more reactive oxygen species (ROS) had accumulated in the insect cells due to the enzyme inability to scavenge them, causing some levels of oxidative damages to the treated-stressed insects.

CAT activity followed the same trend of SOD. In antioxidant enzymes system of insects, the SOD and CAT act as complementary enzymes (Benelli, 2016), SOD removes the superoxide radical to hydrogen peroxide and a greater concentration of H_2O_2 in the cell induces CAT activity. Thus, the increase in CAT activity indicate that, this enzyme may play an important role in metabolism of toxic substances, and the reduction in CAT activity suggest that, H_2O_2 concentration in the cell was not sufficient to induce CAT activity. Results are in agreement with Kaur *et al*., (2014) who noticed an increase in SOD and CAT activities after 24 hrs of *Bactrocera cucurbitae* larvae treatment with Acacia plant derived fractions, whereas, a decrease after 48 hrs was observed. Studies made with, *S.litura* and *A. janata* (Yasur and Pathipati, 2015), and Culex pipiens and Aedes albopictus (Fouad *et al*., 2018) also showed that, silver nanoparticle treatments altered the antioxidant enzyme activity of insects. Oni et al. (2019) had also observed an increase in SOD and CAT activities in *Callosobrachus maculatus* when exposed to low concentrations of *Acalypha*-leaf extract and reduced drastically at higher dosages.

The GPx enzyme belongs to selenoproteins family. Its activity is associated to CAT activity, when CAT is saturated, GPx enzyme could be the second line of defence is activated (Ali, 2012). This enzyme plays an important role in defence mechanisms of insects against oxidative damage by catalysing, the reduction of H_2O_2 and lipid peroxides into less reactive species at the membrane level, thus, protects the cell from lipid peroxidation and oxidative stress (Sankar *et al*., 2012). The result of the present investigation revealed that, the GPx activity increased in most treatments. This induction in activity could be associated with the involvement of GPx in the reduction of the oxidative stress caused by the tested compounds in treated larvae. This finding agreed with the results of Asadpour *et al*., (2014), and Tuncsoy *et al*., (2019).

The GST enzyme is a multifunctional enzyme, it plays an important role in protecting insects from oxidative damage, as well as in antioxidant process , the detoxification of toxic substances by catalysing the conjugation of reduced glutathione , thus, rendering them less toxic (Tarigan *et al*., 2016). In this study, GST showing an increase in the activity at 24hrs post treatment. Milivojevic *et al*., (2015) also demonstrated an elevation of GST activities in Apis mellifera after the exposure to nano-materials for 10 days, and Yasur and Pathipati (2015) reported that, GST activities were increased in the larvae *S. litura* and *A. janata* larvae due to silver nanoparticles treatment. Then a reduction was noticed after 48hrs. GST enzyme made of 85% protein, thus, the decrease in its activity by the effect of plant extract could be due to a reduction in protein content of the insect. This study was supported by the report of Ebadollahi, *et al*., (2013), that stated that, the botanical extract cause low protein content in *Tribolium castaneum* and in turn causes GST inhibition, and Meng *et al*., (2017) suggest that AgNPs lowered the resistance to oxidative stress, affected cell apoptosis, and induced cell necrosis by regulating related protein metabolism and metabolic pathways in B. mori.

Conclusion

The hypothesis regarding the vital role which played by antioxidant enzyme system in the removal of free radicals in insects was strengthened in the present investigation. At biochemical level, it may be concluded that, the methanol leaf extract, green synthesized silver and chitosan nanoparticles using *N. oleander* and *Aloe vera* leaf extract showed an alterations in all antioxidant enzymes under investigations. The inhibitory effect was more pronounced in green synthesized CsNPs treated insects, it almost inhibits the activity of most enzymes. Based on these findings, green synthe-sized CsNPs using *N. oleander* and *A. vera* leaf extract could be suggested as a potential biopesticide for the control of *M. domestica*. Further studies relating to other suported antioxidant enzymes and detoxification enzymes are needed to explain the role of these enzymes in the metabolism of xenobiotics.

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تأثير جسيمات الفضة والكيتوزان النانوية المحضرة باستخدام مستخلص اوراق نباتي الدفلة والصبار علي نشاط الانزيمات المضادة للاكسدة في الذبابة المنزلية

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كلية العلوم، جامعة بنها، قسم علم الحشرات

الملخص العربي

تعد التقنية النانوية من اسرع التقنيات انتشارا علي المستوي العالمي وترجع سمية الجسيمات النانوية الي قدرة هذه الجسيمات علي اختراق جدار جسم الحشرة ثم اختراق الخلايا والارتباط بجزيئات البروتين وال (DNA) مما يؤدي الي تغيير طبيعة هذه العضيات والانزيمات. في هذا البحث تم تصنيع جسيمات الفضة والكيتوزان المتناهة الصغر من خلال الطرق البيولوجية باستخدام المستخلص الميثانولي لاوراق كل من نباتي الدفلة والصبار كعوامل اختزال وتوصيفها عن طريق أجهزة التحليل الطيفي للأشعة فوق البنفسجية (طول الوجة من 200-700) والمجهر الالكتروني النافذ (TEM)، وحيود الاشعة السينية (XRD) وتحويل فورييه للطيف بالاشعة تحت الحمراء (FTIR) . وتم اختبار النشاط الابادي لهذه الجسيمات علي يرقات العمر الثالث للذبابة المنزلية حديثة الانسلاخ واستنتاج التركيز القاتل ل 50% من اليرقات (LC_{50}) ثم تطبيق هذا التركيز عن طريق خلط الجسيمات مع الغذاء لتقييم تاثير هذه المواد علي نشاط الانزيمات المضادة للاكسدة مقارنة بالمستخلص الخام للاوراق لكل نبات. وقد أظهرت النتائج زيادة نشاط انزيم ال (SOD) بعد 24 ساعة وهذه تعتبر محاولة من الحشرات للتخلص من الجذور النشطة (free radicals) ثم انخفاضه بشكل ملحوظ بعد 48 ساعة في الحشرات المعاملة بالمستخلص الخام وجسيمات الفضة, بينما انخفض نشاط هذ الانزيم في الحشرات المعاملة بجسيمات الكيتوزان في كل الاوقات. وحيث ان انزيم (CAT) هو خط الدفاع الثاني في الحشرات الذي يقوم بإزالة ال (H_2O_2) الناتج من تحويل (O^{2-}) بواسطة ال (SOD) فقد اتبع نشاطه نشاط ال (SOD) . كما اظهرت النتائج ان جسيمات الكيتوزان المحضرة باستخدام مستخلص اوراق نبات الدفلة قد أدت الي تثبيط نشاط انزيم ال (GPx) بعد 48 ساعة. أما بالنسبة لانزيم ال (GST) فقد زاد نشاطه بعد 24 ساعة ثم انخفض بعد 48 ساعة . وقد خلصت الدراسة الي ان جسيمات الكيتوزان والفضة المحضرة باستخدام نباتي الدفلة والصبار كانت اكثر تأثيرا من المستخلص الخام للاوراق في تثبيط نشاط الانزيمات المضادة للاكسدة ويمكن استخدام هذه الجسيمات كوسيلة جديدة لمكافحة الذبابة المنزلية.