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Biological and Biochemical Evaluation of Synthesized Green Nanoparticles and Ethanol Extract of Lemongrass (*Cymbopogon citratus* L.) against House fly, *Musca domestica* (Diptera: Muscidae)

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ABSTRACT

The house fly is one of the most important medical and veterinary insects for humans and farm animals. In the current study, the effect of two compounds of plant extract was evaluated against the larvae of the house fly, *Musca domestica* (Diptera: Muscidae), ethanolic extract of lemongrass plant *Cymbopogon citratus* L.), and the nanoparticles of the extract of the same plant. The effect of both compounds against housefly larvae and pupae resulting from larvae treated using the technique of feeding and dipping were compared. The results showed that the feeding technique is more effective against larvae when compared with the dipping technique. The results also indicated that the nanoparticles of the plant extract were more effective against larvae, which led to an increase in the period of larvae and pupae, and an increase in death rates in each of them, also led to deformities in the adult insects resulting from the treated larvae compared to the control. Treatment with the compounds caused some biochemical changes, where the treatment with nanoparticles of the plant extract caused significant increasing in the levels of total protein, total carbohydrates, total lipids and acetylcholinesterase activity (Ach E) compared to the control in the larval stage. As for the pupae resulted from treated larvae, the treatment resulted in a significant increase in the case of using nanoparticles of the plant extract in all the tested biochemical indicators. Therefore, nanoparticles can be used to control house fly larvae, with further study to know the effect of these particles.

Key words: *Cymbopogon citratus*, silver nanoparticles, *Musca demostica,* insecticidal activity, biochemical parameters

INTRODUCTION

The house fly, *Musca domestica* (Diptera: Muscidae) one of the most widespread species in the world, as it is present in many human daily activities, and as a result of these activities, the house fly multiplies in huge numbers. House flies can transmit many diseases, such as: infantile diarrhea, cholera, typhoid, bacillary dysentery and tuberculosis (Fotedar 2001; Malik *et al*., 2007 and Pavela 2008). Although the fact that the assemblies of flies do not

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bite, but their assemblage causes a lot of disgust and aversion in humans when standing on food and leaving fecal stains on it, as well as causing annoyance during its flight (Hinkle 2002; Steenberg and Jespersen 2002 and Winpisinger *et al*., 2005). Generally, control of house flies depends on the use of pyrethroids, organic chlorine, and organic phosphates. Despite this, the frequent use of chemical pesticides causes deterioration and pollution and the development of resistance in insects (Srinivasan, *et al*., 2008 and Kamaraj, *et al*., 2012) and therefore must switch to using new methods of insect control such as biological control and environmentally friendly compounds as well as using nanoformulation insecticides, which have been considered for agriculture and is one of health applications, which are the nanoformulations have new physical properties that have a good and effective effect on the control process (Wildenberg, 2005; Chinnamuthu and Murugesa-Boopathi 2009). Khot, *et al*., 2012, showed that nanoparticles have become as new plans in all applied fields, including the field of pest control, where nanomaterials acquire new characteristics that make them more effective because they provide a thermal stability, large specific surface area, biodegradability, and increased affinity for the target, and thus, quickly penetrate the body of the organism. (Bordes *et al*., 2009 and Margulis-Goshen and Magdassi, 2013). For example, Silver nanoparticles (AgNPs) have attracted interesting attention for the wide range of application in biomedical and agriculture applications. Silver nanoparticles are created in many ways, such as: mechanical milling (Arbain *et al*., 2011), electrochemical (Starowicz *et al*., 2006), microwave-assisted process (Sreeram *et al*., 2008), thermal decomposition (Navaladian *et al*., 2007), and Green Chemistry Methods (Begum *et al*., 2009). Therefore, this study was designed to evaluate the insecticidal activity ethanolic extract of lemongrass plant, *Cymbopogon citratus* L. and its nanoparticles against larvae of house fly and resulted pupae of via feeding and dipping methods.

MATERIALS AND METHOD

This search was done at Faculty of Agriculture (Economic Entomology & Agricultural Zoology Department) Menoufia University, Egypt. Leaves of Lemongrass (*Cymbopogon citratus* L.) were collected from the farm of the Faculty of Agriculture, Menoufia University, located in Shebin El-Kom. Lemongrass plants were identified by the specialist in horticulture department (Faculty of Agriculture, Menoufia University). An ethanolic extract of Lemongrass and the nanoparticles of the extract were prepared as follows:

Five hundred grams of dried leaves of plant were used for preparation of Lemon grass ethanol extract and placed in one liter of ethanol (absolute). The mixture was shaken and incubated for one day (24 h) at room temperature. Then filtered and centrifuged the mixture at 3000 rpm. The ethanol was evaporated (by using Freez-Drier). Finally, the dried extract was kept in the refrigerator until used (Dorman, *et al*., 2003).

Preparation of nanoparticles form of lemongrass ethanol extract:

Preparation of silver nitrate solution (1 ml): Sixteen milligrams of $AgNO_3$ were weighed and transferred into flask. The sold matter was dissolved slowly in the flask containing distilled deionized water (500 mL). After complete dissolving, more water was slowly added to obtain

a volume of one liter. The prepared 1 mL silver nitrate solution was stored in amber colored bottle at 4°C (Rajkumar and Malathi, 2015). Preparing of lemongrass ethanol extract AgNPs: One gram from the dry ethanol extract was put in flask and dissolved in 100 mL distilled water, pH was adjusted at 12 using 0.01N NaOH. This system was kept under magnetic stirring, when the temperature reaches 80 °C, 1mL 0.1N $AgNO_3$ was added, and keep under magnetic stirring for 30 min, (EL-Bisi *et al*., 2013). Characterization technique of AgNPs UV-vis spectra was used to the formation of silver colloids.

Transmission Electron Microscopy (TEM):

Shape and size Characterization of AgNPs were obtained using TEM (JEOLJEM-1200). Samples for TEM measurements were prepared by using a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and the solvent was evaporated in open air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of one hundred nanoparticles found in several chosen areas in enlarged microphotographs. Five serial concentrations of both ethanol extract and Ag NPs of Lemongrass extract were prepared in distilled water as follow; (200, 150, 100, 50 and 25 ppm) to evaluate theirs insecticidal activities against house fly larvae.

Insect rearing

Larvae of *M. domestica* were collected from manure piles. The insect samples were transferred to the insectary house in the Department of Economic Entomology and Agricultural zoology, Faculty of Agriculture, Menoufia University. Larvae were reared in special glass cages with dimensions 60 × 35 × 40 cm under laboratory conditions (27 ± 2°C and 60% ± 5% relative humidity). The cages were covered with a mesh screen with a cloth opening at the top and were equipped with 20-watt light lamps to control the temperature during the cold periods of the winter months. Housefly larvae were fed nutrient media in plastic cups, and the meal ingredients were as follows: 9 g of powdered milk and 5 g of yeast dissolved in 100 ml of water and combined with 100 g of fine bran (Wilkins and Khalequzzaman, 1993).

Bioassay tests:

1- Dipping method: one hundred of the first instar larvae were dipped into each tested concentrations (10 mL) for 30 sec and then transferred to a filter paper. Three replicates were prepared from each concentration, while control larvae were dipped in distilled water. Larval mortality was recorded by larval wasting and immobility after twenty four h of treatment. The LC_{50} values and 95% CL were calculated by using P.C.Probit analysis software program according to Finney's method (Finney, 1971); the test included a set control group by distilled water (Sinthusiri and Soonwera, 2010).

2- Feeding method was evaluated according to the standard method described by Wright (1971).

The biological effects were evaluated by monitoring the treated larvae (that have been treated by mixed food media technique) until they reached the pupal stage. In addition, the larval and pupal periods, percentages of larval and pupal mortality adult emergence were recorded. Also, percentages of adult

malformations were recorded in treated and untreated larvae (control).

Biochemical assays:

The house fly larvae were treated with LC_{50} of both extracts to study their effects on biochemical parameters (Total carbohydrates, total protein, Acetylcholinesterase (AchE) and total lipids,) of larvae and resulted pupae after twenty four h from treatment. Total carbohydrates were estimated by the phenol-sulphuric acid method (Dubois *et al*., 1956); total proteins were determined according to Bradford, (1976); acetylcholinesterase (AchE) activity was measured according to the method described by Simpson *et al*., (1964) and total lipids were estimated by the method of Knight *et al*., (1972). All these biochemical parameters were determined in Institute of Plant Protection Research, Physiological Department, ARC. Giza.

Statistical analysis

The bioassay data were statistically analyzed using statistical package for Social Science (SPSS, ver.20). All data were expressed as mean ± SE and subjected in the one-way analysis of variance (ANOVA) was applied to compare the significant at P≤ 0.05 differences between treated and control groups (Snedecor, 1952 and Duncan, 1957).

Results

Nanoparticle formation of AgNP Lemongrass ethanol extract:

Brown color appearance indicates the successful formation of silver nanoparticles AgNPs. Where an immediate appearance of the brown color occurred as a result of quick reaction between the silver nanoparticles and the ethanolic extract. This color is evidence of an oxidation-reduction reaction between the silver particles and the extract, in which the silver ions are reduced by the extract particles.

The successful synthesis of Ag nanoparticles was used to ensure by UV-Vis spectroscopy. Fig. (1) Showed the characteristic absorption peak of Ag-NPs in the spectra at 450 nm.

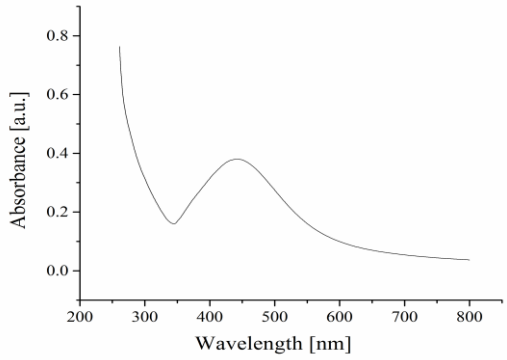

Fig 1. UV-Visible spectrum of the prepared Ag NPs lemon grass (ethanol extract)

Fig.2: TEM image of the prepared Ag NPs particles in the polymermatrix

The size and shape of AgNPs were examined by using transmission electron microscopy (TEM) (Ag, this investigation was made by using JEOL JEM l0xl0 (Electron microscope-Japan). Fig. (2) showed

TEM images which explained that they have an average size of 12 nm for the particle and spherical shape.

Insecticidal activity of lemongrass extract and its nanoformulation:

Toxicity levels for two tested compounds against *M. domestica* larvae were estimate in the presented study after 24 h of treatment. Data presented in Table (1) showed that the LC_{50} value of *C. citratus* extract nanoparticles was the most toxic for larvae in two ways of treatments than anther Ethanol extract of *C. citratus*. LC_{50} values were 121.25 and 117.32 ppm for dipping and feeding techniques, respectively where *C. citratus* extract nanoparticles were applied but LC_{50} values were 142.33 ppm and 124.11 ppm for dipping and feeding techniques, respectively when Ethanol extract of *C. citratus* was applied.

Table (1): LC_{50} data of ethanol extract and nanoparticles of *C. citratus* extract against *M. domestica* larvae after 24 h under laboratory conditions.

Plant extract	technique	LC_{50} (ppm) (95% cl)	Slope
Ethanol extract of *C. citratus*	dipping	142.33	1.63 ±0.41
	feeding	124.11	1.71 ±0.43
***C. citratus* extract Nanoparticles**	dipping	121.25	1.31 ±0.61
	feeding	117.32	1.51 ±0.29

cl = confidence limits

It is clear from the results presented in Table 2 that *C. citratus* Ethanol extract and *C. citratus* extract nanoparticles have an effect on the periods of the larval and pupal stage and the death rate, as well as the percentage of exit of adults and the percentage of deformities in adults.

Larval period and resulted pupa

The results in Table (2), show that the examined compounds have a significant effect on the larval period compared to the control, and it was *C. citratus* extract nanoparticles more effective on the larval period, followed by *C. citratus* Ethanol extract where the larval periods were 6.1, 5.4 and 4.5 days for the compounds *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively.

Also, the same compounds had a significant and clear effect on the death rates; as it were 64, 44 and 5% for the compounds *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and the control, respectively.

Pupal period and resulted adults

From the results in Table (2), it is obvious that the examined compounds have a significant effect on the pupal period compared to the control, and it was *C. citratus* extract nanoparticles more effective on the pupal periods, followed by *C. citratus* Ethanol extract Where the periods were 6.8, 6.6, and 5.3 days for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively.

Also, the same compounds had a significant and clear effect on the death rates; as it were 57.29, 41.67 and 6.49% for the compounds *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and the control, respectively.

Adult malformation

The results presented in Table (2) show the occurrence of deformities in the

adult insects resulting from the larvae treated with both *C. citratus* extract nanoparticles and *C. citratus* Ethanol extract and the results indicated that the incidence of deformities was higher in *C. citratus* extract nanoparticles than in *C. citratus* Ethanol extract compared to the control. The percentage of deformities in adults was 42.68 % & 14.29 % in each of *C. citratus* extract nanoparticles and *C. citratus* Ethanol extract, respectively.

Table 2: Biological parameters of larval and pupal stages after larval feeding on LC_{50} of *C. citratus* ethanol extract and nanoparticles of *C. citratus* extract

Tested plant extract	Initial number	larval stage Period (days) ±SE	Mortality %± SE	number of resulted pupae	Pupal stage period (days)± SE	Mortality %± SE	number of resulted adults	Malformed adults %± SE
***C. citratus* Ethanol extract**	300	5.4±1.33 b	44±1.6 b	168	6.6±1.5 a	41.67 ±1.32b	98	14.29±1.7 b
***C. citratus* extract Nanoparticles**	300	6.1±.921 a	64±1.5 a	108	6.8±1 a	57.29 ±0.64a	64	42.68±1.7 a
Control	300	4.5±.73 c	5±1.2 c	285	5.3±2.3 b	6.49 ±1.71c	266	_
F value		11.31	33.42		9.54	14.73		22.31
P value	_	0.0001***	0.00012***	_	0.002**	0.0005***	_	0.0003***

In each column, means followed by the same letter are not significant at the 5% level.

Biochemical studies

Results presented in Table (3) revealed that tested plant extracts caused significant increase in total protein, total carbohydrate, total lipids and AchE activity in treated larvae when feeding technique was used. The means of total protien were 11.51, 10.21 and 8.31(mg/g.b.wt) for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively. The mean values of total carbohydrate were 19.41, 18.12 and 17.7 (mg/g.b.wt) for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively. As shown in the table (3) the mean values of total lipids were 18.11, 17.33 and 16.14 (mg/g.b.wt) for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively. Finally, the mean values for AchE were 288.41, 265.12 and 204 (µg AchBr/min /g.b.wt) for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively.

As for the pupae resulted from treated larvae, the results recorded in table (3) show that significant increasing in total protein and total carbohydrate when pupae resulted from larvae which were treated with *C. citratus* extract nanoparticles compared with control, but in case of *C. citratus* Ethanol extract the

increasing was non-significant. The mean values of total protein were 9.8, 8.6 and 8.13(mg/g.b.wt) for *C. citratus* extract nanoparticles , *C. citratus* Ethanol extract and control ,respectively, while the mean values of total carbohydrate were 15.53, 14.51 and 14.3 (mg/g.b.wt) for *C. citratus* extract nanoparticles , *C. citratus* Ethanol extract and control ,respectively.

The results indicated a significant increase in both total lipids and AchE activity for two tested compounds compared with control. As shown in table (3), the means of total lipids were 12.5, 11.62 and 10.31 (mg/g.b.wt) for *C. citratus* extract nanoparticles , *C. citratus* Ethanol extract and control, respectively, while in case of AchE activity, the mean values were 223.18 , 211.51 and 170,31(µg AchBr/min/g.b.wt) for *C. citratus* extract nanoparticles, *C. citratus* Ethanol extract and control, respectively.

Table 3: Some biochemical parameters of treated larvae with LC_{50} concentrations of *C. citratus* Ethanol extract and *C. citratus* extract Nanoparticles using feeding technique and pupae resulted from treated larvae.

Biochemical parameters	Treated larvae (Mean±SE)				
	Control	*C. citratus* Ethanol extract	*C. citratus* extract Nanoparticles	F value	P value
Total protein (mg/g.b.wt)	8.31±0.51c	10.21±0.42b	11.51±0.31 a	19.11	0.001 **
Total carbohydrate (mg/g.b.wt)	17.71±1.61c	18.12±0.1.66 b	19.41±1.61 a	17. 21	0.0001 ***
Total lipids (mg/g.b.wt)	16.14±1.35a	17.33±2.73b	18.11±1.42 c	32.31	0.0005 ***
AchE activity (µg AchBr/min /g.b.wt)	204±22.26c	265.12±12.71b	288.41±22.11a	121.32	0.0001 ***
	Pupae resulted from larvae in feeding technique (Mean±SE)				
	Control	*C. citratus* Ethanol extract	*C. citratus* extract Nanoparticles	F value	P value
Total protein (mg/g.b.wt)	8.13±1.04b	8.63±2.31b	9.81±1.34a	11.14	0.0001 ***
Total carbohydrate (mg/g.b.wt)	14.3±2.21b	14.51±2.41b	15.53±2.12a	22.11	0.0002 **
Total lipids (mg/g.b.wt)	10.31±1.72c	11.62±1.52b	12.51±2.51a	32. 11	0.001 ***
AchE activity (µg AchBr/min /g.b.wt)	170.3±21.64 c	211.51±23.61b	223.18±21.31a	41.15	0.0004 ***

In each row, means followed by the same letter are not significant at the 5% level.

Discussion

Results revealed that the nanometric extract is more toxic against the larvae of the house fly, when applied by both methods of dipping and feeding, respectively. (Soni and Prakash, 2014;

Said, 2017; Said and Abdelaal, 2020 and Eldefrawy *et al*., 2022).

The previous results show the effective effect of nanoparticles against the house fly larvae than ethanol extract, where the feeding treatment led to a significant increase in the period of the larvae and death rates in both larvae and pupae resulting from the treated larvae, as well as a significant increase in the percentage of deformities in the adult insects resulting from the treated larvae. This may be due to the toxic effect and accumulation of nanoparticles which has a high penetration rate into the insect's body, causing cell destruction and their transfer into next stage of insect, which in turn may cause increase in death percentages and defects or deformities in the body of adult insects. (Gnanadesigan *et al*., 2011; Kamaraj *et al*., 2012; Marimuthu *et al*., 2011; Gul *et al*., 2016 and Abd El-Zaher, 2017).

Recorded results indicated that silver Nano particles has entomotoxic effective on larvae and pupae resulted from treated larvae, this reaction may be to toxixity mechanism by feeding such as oxidative stress, membrane disruption and protein unfolding. The silver nanoparticles which entre to the intracellular space can destroyed DNA leading to destroy some organs and enzymes (El-Bisi, *et al*.,2013; Veerakumar *et al*., 2013; Velayutham *et al*., 2013; Abd El-Raheem, and Eldafrawy,2016; Benelli, 2016 and Abd El-Zaher, 2017).

Conclusion

Our data demonstrated that nanoparticles of lemongrass extract has high insecticidal activity against the larvae of the house fly, where the treatment was done in two ways, immersion and feeding, and the treatment with feeding was more effective. The results also showed that larval treatment with nanoparticles caused significant increasing in the larval periods and death rates in both larvae and pupae resulting from the treated larvae. It also caused a significant increase in percentages of deformities in the adult insects resulting from the treated larvae. Nanoparticles could therefore be used to control house fly larvae, although further research is needed to determine what these particles do.

Author Contributions:

Conceptualization, MFE, BME and SMS; data curation, BME and SMS; formal analysis, MFE and SMS; investigation BME and SMS; methodology, MFE, BME and SMS; SMS, writing—original draft, and writing and editing, MFE, BME and SMS. All authors have read and agreed to the published version of the manuscript.

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