Green Silver Nanoparticles Effects on The Susceptible and Field Strains of The Housefly, *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT: The housefly is considered a major household pest, as it carries many pathogens that threaten human health. The increase in awareness of the dangers of chemical insecticides on the environment and the increase in insect resistance to various pesticides, led to the continuation of the search for safe and alternative sources of insecticides. The present study was carried out using crude rice starch paste as a natural bio reducing agent to produce green synthesis of AgNPs due to its cheapest and safety on environment. The larvicidal effects of (AgNPs) were tested against the third larval stage of both strains (the susceptible laboratory strain). (SS) and field strain (Gol-RR) of the house fly. Larvae were fed on contaminated medium with AgNPs concentrations at 0, 10, 20, 30, 40 and 50 µg /ml. The numbers of dead larvae were recorded in both strains, at intervals 24, 48 and 72 h. Then, larvae were tracked for survival to adulthood and observed different biological aspects. The results showed that the larval mortality rate of both strains of the house fly, increased with increasing concentrations and increased gradually with increasing the exposure period, while it was found that the comparison treatment did not show any mortality. However, silver nanoparticles (AgNPs) at a concentration of 50µg/ml recorded maximum larval death (%) in both strains of *Musca domestica*, followed by 40 µg /ml. But the susceptible strain was affected more often than the field strain, followed by a significantly higher number of abortive fledgling adults, which show morphological abnormalities.

Keywords: *House fly, Musca domestica, green synthesized, nanoparticles, silver nanoparticles (Ag NPs), larvicidal effects.*

INTRODUCTION

Musca domestica , commonly known as the house fly, is critical because it is a major vector for several infectious pathogens, including those that cause cholera, typhoid fever, dysentery and some skin diseases, in addition to trachoma and trachoma considered a household pest and a public health hazard (**Greenberg, 2019**). House flies thrive in unsanitary environments such as slaughterhouses, dairies, and ranches that encourage the spread of disease. Pathogen transmission can occur when insects come into contact with human habitats (**Khan *et al*., 2012**).

Although the systemic application of chemicals has brought progress, the dangers posed by chemicals, such as the loss of beneficial arthropods, environmental imbalances, contamination of manure, the environment, freshwater, food, and employees, are quickly becoming apparent. These factors have supported pest resurgence and contributed to the rapid emergence of resistant individuals, leading to irreversible environmental and health hazards such as cancer, genetic aberrations, poisoning and death (**Prabhaker *et al*., 1998**). House flies are known for their ability to evolve resistance strategies to evade and detoxify chemical pesticides. Resistance to organophosphates, carbamates, and pyrethroid insecticides has been documented **(Boxster and Campbell, 1983; Kaufman *et al*., 2001; Butler *et al*., 2007).**

Recently, pesticide formulations have been developed using nanotechnology techniques **(Abd-Elnabi *et al.* 2021**). As nanoparticles exhibit new properties, nanotechnology has emerged as one of the most promising new approaches to pest control in modern agriculture and is expected to revolutionize the field of pest control in the near future. (**Bhattacharya *et al*., 2010, Rai *et al*., 2018, Tunçsoy, 2018, Ahmed *et al*., 2019, Khoshraftar *et al*., 2019, Shahzad and Manzoor, 2019)**

Metallic nanoparticles produced by various chemical processes are of interest applications of biomedical worldwide. These methods reveal a wide range of toxicities in non-target organisms. The biosynthesis of NPs is an environmental safety manner without the use of toxic chemicals, and increasing the safety of environment in the future. Therefore, the development of green synthesis of Ag NPs has emerged as an important area of nanotechnology, and the use of biological entities such as plant extracts and biomass, as well as microorganisms for the production of NPs, can be used both chemically and physically. In addition, conventional methods were safety to the environment **(Reddy *et al.* 2012**). Advances in green synthesis beyond physical and chemical methods are environmentally friendly, inexpensive, and easily scaled up for large scale synthesis of NPs, but green synthesis involves high temperature, energy, pressure and hazardous no chemicals are required (**Ahmed *et al*. 2016).**

El Monery *et al*. (2021) found that its components such as lignin, pectin and hemicellulose can be used for the reduction of silver ions to produce silver nanoparticles Ag NPs. Therefore, the main aim of this study is evaluate the efficacy of green synthesized from silver nanoparticles as alternative insecticides on the two experimental houseflies strains (Diptera: Muscidae) susceptible and resistant in the laboratory conditions.

MATERIALS AND METHODS

Insect rearing

The house fly, *Musca domestica* used during this study was reared in the laboratory of Economic Entomology in Plant Protection Department, Faculty of Agricultural (Saba Basha), Alexandria University at 28$\pm$1°C, 60% $\pm$10 R.H. and 18 hrs illumination. Adults of a susceptible and field strains of *M. domestica* were maintain cages in $40\times 30 \times 30$ cm, with a plywood floor and wooden frame. Larval, adult, oviposition media were prepared according to **Singh and Jerram (1976).**

The preparation of starch paste, silver nitrate solution and silver nanoparticles

Prepared amount of Na OH 0.3 g then dissolve it in water, and lye is added gradually to a known amount of crude rice starch 2 g while stirring. This prepared mixture was stirred continuously until the starch was completely dissolved. The pH of the prepared solution reached to 11 and its temperature reached to 60 °C. After that, the previous solution was used for preparing different concentrates of silver nitrate 0.25 and 0.5 g then all concentration was dissolved in 100 ml of distilled water. After that, the different concentrations of the silver nitrate solution which prepared was dropped to the starch solution which stirring at 60 ºC and pH 11. After 15 minutes, the silver color was changed gradually from dark white to transparent yellow. Then the silver nanoparticles Ag NPs were formed and the color intensity of Ag NP changed due to the amount of silver nitrate added. So, the darker color means the higher concentration of Ag NPs formed. Based on (**El-Rafiea *et al* 2013 and Abdelsalam *et al* 2019**) they have two concentrations of Ag NP denoted Ag NPs-2000 and Ag NPs-4000 ppm

The characterization description of silver nanoparticles Ag NPs

In this experimental work the characterization of silver nanoparticles were used according to (**Abdelsalam *et al.* 2019**) description.

Bioassay of housefly:

In the laboratory 1 ml of AgNPs was taken from different concentrate (10, 20, 30, 40 and 50 $\mu g/ml$) and mixed with 20 ml of larval media into plastic cup, each concentration included three replicates each of them had 10 larvae in third stage of age. In addition, the compared larvae in control (C) were feed with 20 ml of media mixed only with 1 ml of distilled water. The experiments were carried out in third generation at field strain and eighth in sensitive strain. The ratio of mortality calculated by counting the number of dead larvae after 24, 48, 72 hrs and trace the pupal weight, inhibition of pupation, adult emergence, sex ratio and other biological aspects were appeared. **(Abd El-Hamid *et al.* 2018)**.

Statistical analysis:

The statistical analysis were used to analysis the obtained results by SPSS 26 program to analysis of variance (ANOVA) and all means compared using L.S.D at 0.05 (**Wagner and William 2015**). Some results analyzed by M. Excel (**Schmuller, 2013**). In addition, statistical treatments were analyzed with probit analysis curve (**Finney, 1952**)

RESULTS AND DISCUSSION

Mortality tests by green silver nanoparticles on *Musca domestica*

The presented results in Tables (1 & 2) showed the comparison between sensitive strain and field strains concerning the mortality rate of *Musca domestica* larvae when they were treated by feeding on the mixed diet with different tested concentrate of Ag NPs 10, 20, 30, 40, and 50 $\mu g/ml$. The mortality % were 47, 57, 73, 90 and 97% susceptibly in sensitive strain after 72 h (Fig., 1) while the field strain recorded a lower percentages than that of the susceptible one 43, 50, 67, 83 and 93% respectively (Fig. 2). The control treatment showed no incidence of larval mortality.

The average larval mortality data were submitted to probit analysis as shown in (Fig. 3 & 4) to determine the LC_{50} value which calculated by 21.18$\mu g/ml$, 18.014 $\mu g/ml$ in felid and sensitive strains. From this result, the percentage of larval mortality of house fly, *M. domestica* increased as the concentrations of Ag NPs increased and as the exposure period increased in both strains. There was a significant difference in LC_{50} values between field strain and sensitive strain represented by a linear relationship. The observed minimal selection effects were caused by developed insecticides resistance in field strain than sensitive one. **Scott *et al.* (1991**) suggested that resistance to certain active ingredients occurs among field populations. In addition, synthesized nanoparticles are toxic to the nervous system which more sensitive in susceptible strain than field one **Fouad *et al.*** (**2018**).

Marimuthu *et al.* (2011) found that, biosynthesized Ag NPs were effective against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. In addition, Ag NPs can cause toxicity through a variety of mechanisms, including surface attachment and changes in membrane properties, affect cell permeability **(Morones *et al*., 2005).** The physical and chemical properties of Ag NPs can be altered by interactions with biochemical and physiological structures and processes **(Nel *et al*., 2006 and Kim *et al*., 2009).** Insect death can be due to gastrointestinal dysfunction, changes in the skin surface due to dehydration, or obstruction of the stomata and trachea **(Abo-Arab *et al*., 2014)**.

Table (1) Effects of silver nanoparticles Ag NPs on mortality of susceptible strain 3rd larval instar larvae after 72h.

Conc.	Number of treated larvae	Number of dead larvae	Larval Mortality (%) after 72h ± S.E	LC50 $\mu g/ml$ (LCL-UCL)	χ^2
Control	30	1	3±0.33		
10	30	14	47±0.67		
20	30	17	57±1.33	18.014(13.90-21.67)	6.307
30	30	22	73±0.33		(df=4)
40	30	27	90±1		
50	30	29	97±0.33		

Significant at P<0.05 level LC_{50} lethal concentration that kills 50% of the exposed larvae UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ chi-square, df degree of freedom. A Mean calculated from three replicate.

Table (2) Effects of silver nanoparticles Ag NPs on mortality of field strain 3rd larval instar of after 72 h.

Conc.	Number of treated larvae	Number of dead larvae	Larval Mortality (%) after 72h ± S.E	LC50 µg/ml (LCL-UCL)	χ^2
Control	30	0	0±0		
10	30	13	43±0.88		
20	30	15	50±1	21.18(16.9-25.05)	8.601
30	30	20	67±0.88		(df=4)
40	30	25	83±0.33		
50	30	28	93±0.33		

Significant at P<0.05 level LC_{50} lethal concentration that kills 50% of the exposed larvae UCL upper confidence limit, LCL lower confidence limit, $\chi 2$ chi-square, df degree of freedom. A Mean calculated from three replicate.

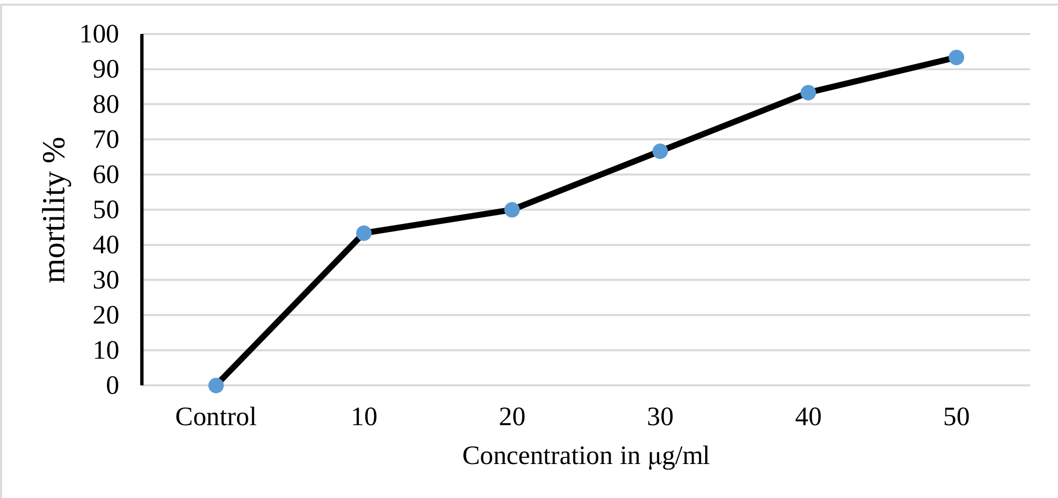

Fig (1): effects of silver nanoparticles Ag NPs on the mortality of sensitive 3rd larval instar after 72h.

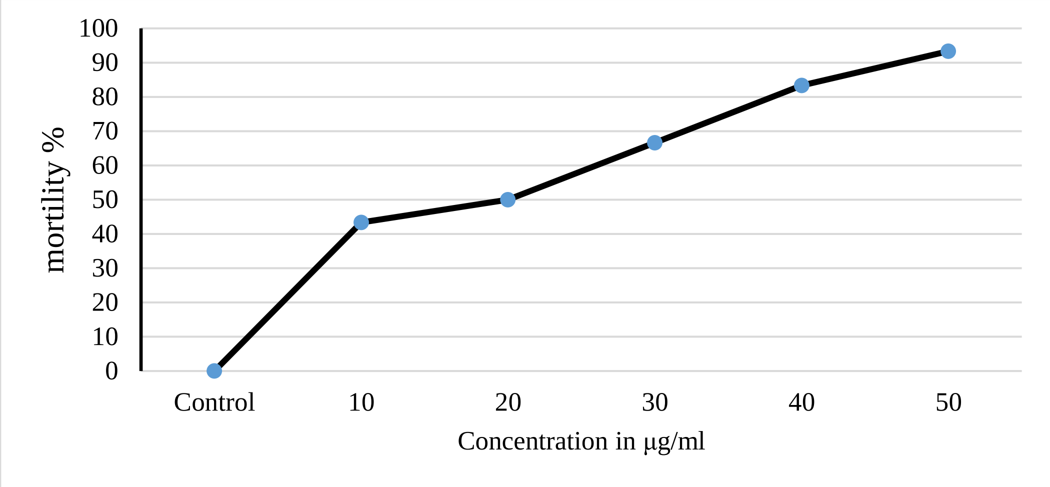

Fig. (2): effects of silver nanoparticles Ag NPs on the mortality of field strain 3rd larval instar after 72h

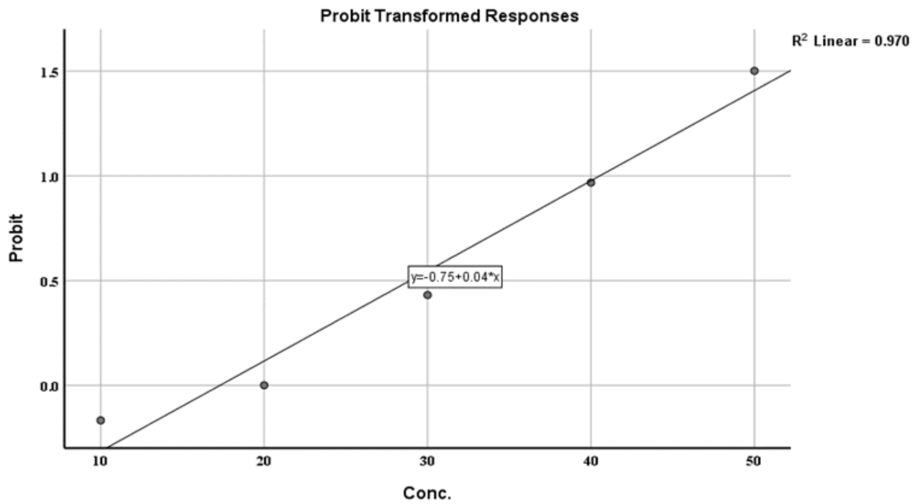

Fig. (3): The probit analysis of the silver nanoparticles Ag NPs on field strain illustrate the liner formula of LC_{50}

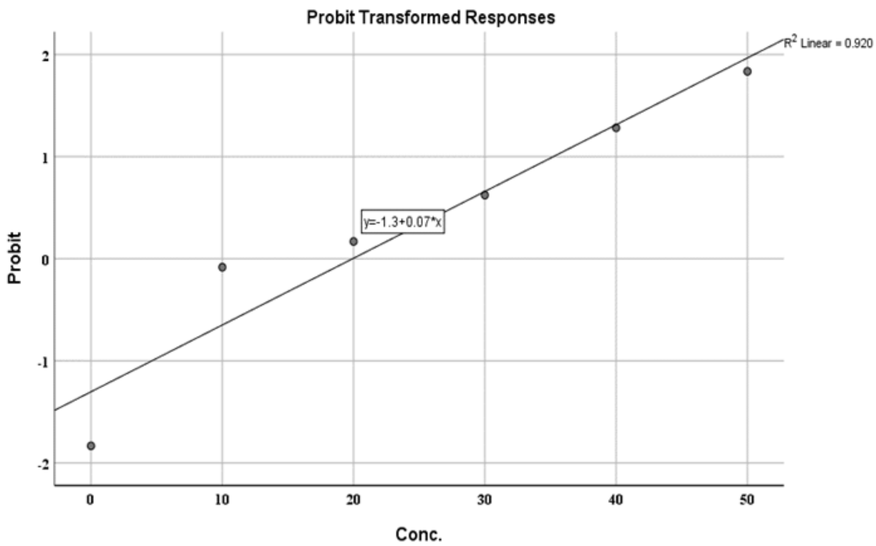

Fig. (4): The probit analysis of the silver nanoparticles Ag NPs on sensitive strain illustrate the liner formula of LC_{50}

The pupae weights in the two strains after treatments

The results of the effect of Ag NPs on pupal weight of those larvae survived treatment and formed pupa in table (3) and the results showed that treatments had a significant effect on pupal weights of the two tested strain as they were compared with the control (Fig., 5). It was found the weights of the pupae decreased throughout the pupal stage. The treatment with Ag NPs had a noticeable effect showing the presence of abnormalities in pupae inhibiting adult emergence. Advanced physiological will be test and clear in the next research.

Table (3) the mean of the pupa weight (mg) in field and sensitive strain of *M.domestica* under effect of silver nanoparticles Ag NPs concentration

Concentration in µ/ml	Pupa_F	Pupa_S
0	0.0176	0.0173
10	0.0164	0.0159
20	0.0162	0.0154
30	0.0158	0.0150
40	0.0145	0.0135
50	0.0122	0.0120

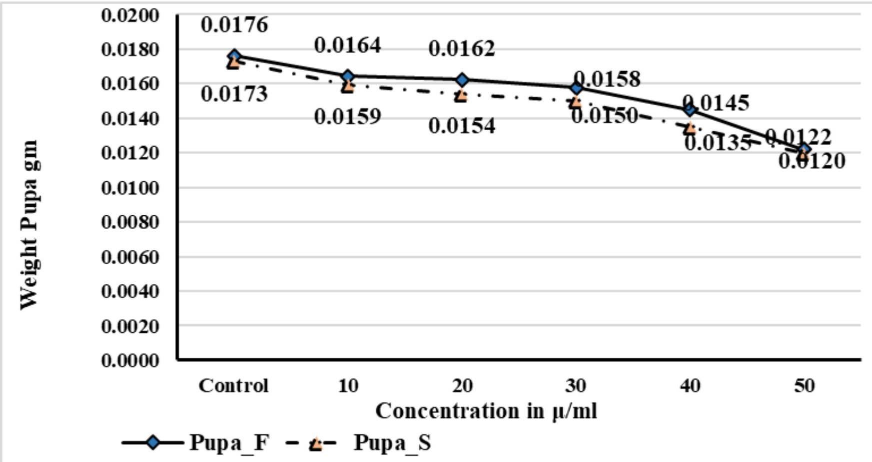

Fig (3) the mean of the pupa weight (mg) in field and sensitive strain of *M.domestica* under effect of silver nanoparticles Ag NPs concentration

Effects of Silver nanoparticles on the two strains adults and their sex ratio

The results in table (4) described the reduction in adult emergence of *M. domestica* as a consequence effect of the treatment with the green synthesized AgNPs The results showed that the adults insects emergence from pupae was affected in field and sensitive strains, and most effective concentration was 50 $\mu g/ml$ where the inhibition rate reached 100% , On the other hand, it was noted effective of Ag NPs on the sex ratio of adults emergence produced from treated larvae and the efficiency of females to laying egg ,this was confirmed by the recording of a number of females with deformed ovipositor. These results agree with **Abd El-Hamid *et al*. (2018)** where they notice the abnormalities appearance in pupae, and adult stages. In pupae, morphological changes included elongation, dwarfism, and swelling, whereas in adults, some individuals have shown partial emergence and others popped up with wrinkled wings.

Table (4): The effect of silver nanoparticles Ag NPs concentration on adults, their sex ratio and eggs laid in the two strains F & S *M domestica*

C.	R.	Adult (F)	Adult (S)	Inhibition Adult (F)	Inhibition adult (S)	sex _F		Sex_S		F Egg No./f	S Egg No./f
						m.	f.	m.	f.		
0	30	29	28	1	2	15	14	14	14	550	420
10	30	8	6	7	8	5	3	4	2	70	50
20	30	9	7	4	4	6	3	5	2	65	50
30	30	4	3	5	4	3	1	3	0	0	0
40	30	2	1	2	1	0	2	1	0	0	0
50	30	0	0	2	1	0	0	0	0	0	0

F: field strain S: sensitive strain R.: replicates m.: male f.: female

The decrease in adult longevity (especially oviposition period) of *M. domestica* obtained by the treatment with the AgNPs might be due to their accumulation in the different developmental stages. The obtained results indicated that the AgNPs affected fecundity and fertility of *M. domestica*. Similar results were tested using AgNPs on *D. melanogaster* (Armstrong *et al*., 2013), *Heliothis sp*., and *Trichoplasia ni* (Afrasiabi *et al*., 2016), some of other researchers opinioned that the NPs toxicity may be effect on midgut epithelial cells and disruption to the cells of epithelial apical membrane (Sultana *et al*., 2018)

Comparison the experimental results with other investigations **(Debnath *et al.*, 2011)** demonstrated that used application of SNP increasing significantly the mortality due to the effect of NPs by increasing the exposure time. They reveal that SNP has a high potential as pesticide. This may be one of the possible reasons why there is a centuries old tradition of using silica dust as a seed protectant stored by various ethnic races around the world **(Ebeling, 1971**).

The presence of a dose response gradient is one of the main criteria for determining whether a toxic effect is causal (**Robertson and Rappaport, 1979**). Ag NPs can induce toxicity through multiple mechanisms including surface binding, alteration of membrane properties that can affect cell permeability **(Morones *et al.*, 2005).** AgNPs can alter physical and chemical properties due to their interactions with biochemical and physiological structures and processes (**Nel *et al.*, 2006 and Kim *et al.*, 2009).**

The reduction in adult emergence of *M. domestica* as a consequence of the treatment with the tested NPs was in consistent with previous data on other insect species, as reported against *S. littoralis* using ZnONPs **(Osman *et al.*, 2015)**. **(Sabbour, 2013)** noted that the higher surface to volume ratio of NPs makes them more reactive than their bulk counterpart **(Vani and Brindhaa 2013)**. NP toxicity may also be due to partial lysis of midgut epithelial cells and disruption of the apical membrane of epithelial cells (**Sultana *et al*., 2018**). They kill insects by absorbing the cuticle lipids and causing physical damage (**Barik *et al.*, 2008)**.By penetrating the exoskeleton and entering the intracellular region, NPs usually cause damage to the insect **(Rai *et al.*, 2014)**. The efficiency of NPs varies depending on their sizes, coatings, concentrations, and exposure period **(Jiang *et al.*, 2015)**.

Konenda *et al.* (2018) record the same result.

The results showed that nano-silver were moderately toxic to the 3rd generation larvae of the housefly in both treatments. **(Kamaraj *et al*., 2012**) determined the food blocking activity of biosynthesized Ag NPs against adult house flies (L.D_{50} = 3.64 mg/mL). While **(Gul *et al.*, 2016)** demonstrated that synthetic Ag NPs were effective against adult *M. domestica* at lower doses to suppress feeding, other authors noted high mortality rates. The most from nanoparticles of biosynthesized silver nanoparticles (**Marimuthu *et al*., 2011**) were found that synthetic silver nanoparticles of Mai Duong pudica had the highest killing index compared with larvae of *Anopheles subpictus* (L.C_{50} = 8.89ppm) and compared with larvae *Culex quinquefasciatus* (L.C_{50} = 9.51ppm).

Based on the result, it can be concluded that the present research investigated the entomotoxic effects of green synthesis of silver nanoparticles on two strains (susceptible and field) of the housefly, *Musca domestica* (Diptera: Muscidae) by feeding the larval and counting the mortality. Meanwhile, the effect also extended to the stage of pupae, where the weights and shapes of pupae were severely affected and that has been reflected on adult emergence using biosynthesizes silver particles as a new approach in insects control specially houseflies, is successful and they are safer, environmental friend, non-toxic and cheaper, so that biosynthesized Ag NPs can be used in practice for insect control.

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الملخص العربي

الجزيئات المتناهة الصغر للفضة الخضراءعلى سلالاتي الذبابة المنزلية المقاومة والحساسة

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في البحث تم دراسة التأثير السام لجزيئات القضة المتناهية الصغر المخلقة حيويا على سلالتين من الذبابة المنزلية: سلالة معملية حساسة، وسلالة حقلية تم جمعهما من البيئة المحيطة، تم تربية كلا السلالتين في الظروف الملائمة للتجربة فى معمل وقاية النبات بكلية الزراعة (سابا باشا) جامعة الإسكندرية.استندت اختبارات السمية. على تغذية العمر اليرقي الثالث للذباب على بيئة تغذية مضاف اليها التركيزات المختلفة 10، 20، 30، 40، 50مجم/ مل من جزيئات الفضة النانوية. وقد أظهرت نتائج المقارنة بين السلالة الحساسة والسلالة الحقلية نسبا أعلى للسلالة الحساسة عن المقاومة وبالاخص في النسبة المئوية لموت اليرقات بعد مرور (24 ، 48 ، 72 ساعة). كما أظهرت النتائج أن هنالك تزيدا مطردا بين نسب موت اليرقات وزيادة التركيز . سجلت جزيئات الفضة النانوية (AgNPs) بتركيز 50مجم/ مل الحد الأعلى لموت اليرقات (97% و93 %) في كلا من السلالتين الحساسة والحقلية على التوالي. وامتد التأثير السام ليشمل العذارى الناتجة والتي تناقصت أوزانها وظهرت عليها تشوهات مورفولوجية عديدة مقارنة بالكنترول. وقد سجلت نسبة تثبيط خروج الحشرات الكاملة بنحو 100% عند التركيز 50مجم/ مل لكلا السلالتين مما يدفعنا الى الحث على بدء الاعتماد على استخدام جزيئات الفضة النانوية المخلقة بيولوجيا كأحد الاتجاهات الحديثة في مكافحة الأفات وذلك لقدرتها وكفاءتها بالأضافة لكونها آمنة ورخيصة الثمن وصديقة للبيئة.