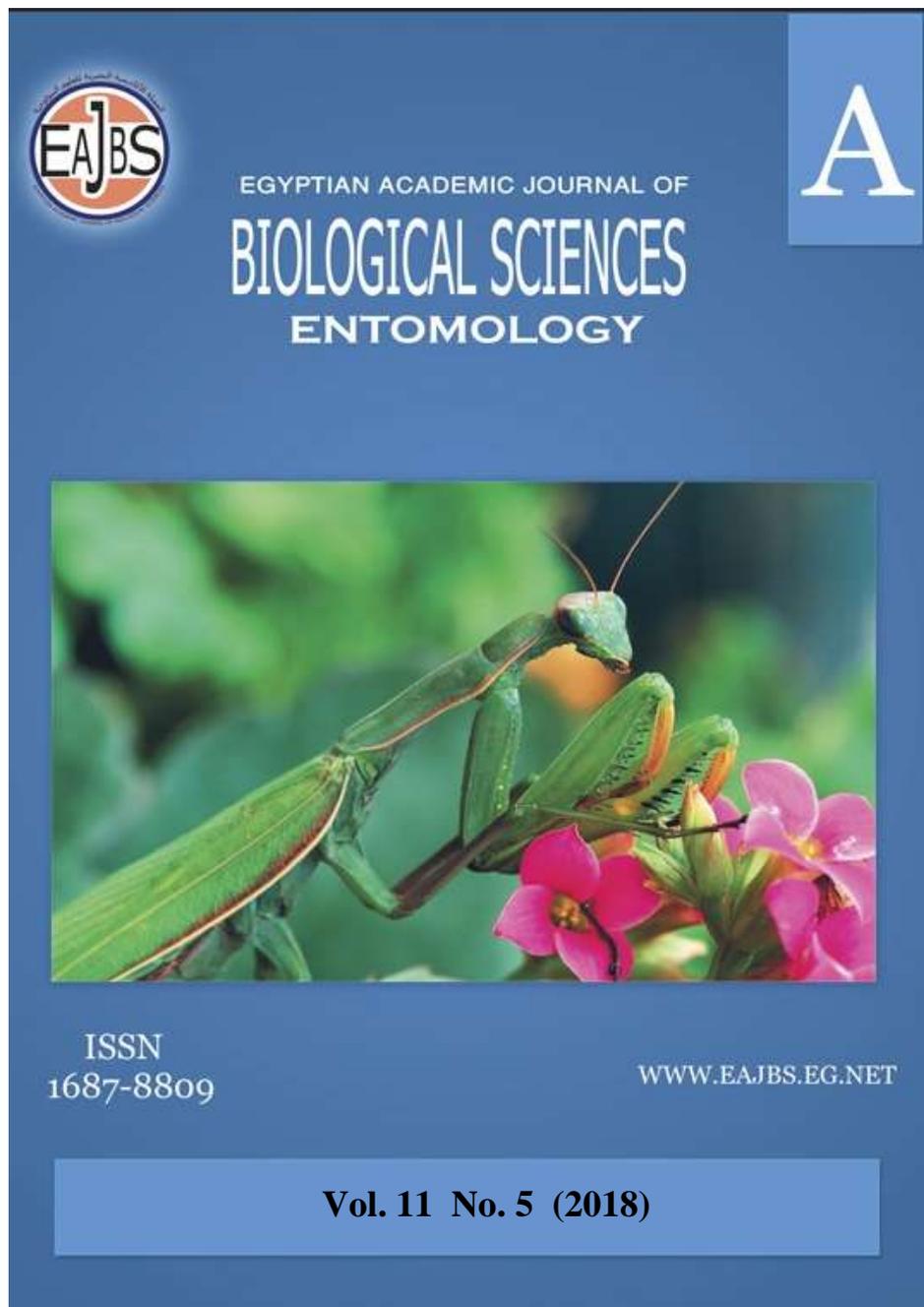


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**Nano Bioinsecticides based on Essential oils Against *Phenacoccus solenopsis***

Sawsan, M. A. <sup>1</sup>; Afaf, A. Abasse<sup>2</sup>; Horeia, A. H. <sup>3</sup>; Naema, A. A. <sup>1</sup> and Enayat, Mohammed, M. <sup>1</sup>

1-Microbiology Department Atomic Energy Authority- Egypt.

2-Zoology Department- Faculty of Science (Girls) - Al-Azhar University.

3- Plant Protection Research Institute Dokki- Giza, Egypt

E.Mail: [drenayat2017@gmail.com](mailto:drenayat2017@gmail.com)

**ARTICLE INFO**

**Article History**

Received:1/7/2018

Accepted:27/7/2018

**Keywords:**

*Phenacoccus solenopsis*, lemon grass *Cymbopogon citratus*, thyme *Thymus vulgaris* and geranium *Pelargonium graveolens*, Phyto-Synthesized Silver Nanoparticles AgNPs.

**ABSTRACT**

In the present study, insecticidal Activity of essential oils (lemon grass *Cymbopogon citratus*, thyme *Thymus vulgaris* and geranium *Pelargonium graveolens*) and silver nanoparticles (AgNPs) synthesized by using tested essential oils were evaluated against female of *P. solenopsis* Tinsley. At high concentration (40µl/l), the percentages of *p. solenopsis* mortality recorded 100, 100 & 86.70%, for thyme, lemon grass and geranium EOs, while it recorded 100, 90 and 83.3 % at (400ppm) of AgNPs-thyme, AgNPs-lemon grass, and AgNPs-geranium after 72hrs of treatment. All essential oils and AgNPs were proved to be very toxic to female of *p. solenopsis*. However, thyme essential oil and AgNPs-thyme recorded the highest mortality percentage with LC<sub>50</sub> = 8.094 µl/l & 86.645 ppm, respectively, followed by lemon grass (bulk & AgNPs- lemon grass oil) and geranium oil (bulk & with AgNPs-geranium oil).

**INTRODUCTION**

*Phenacoccus solenopsis* Tinsley; (1898) (Hemiptera: Pseudococcidae) is a polyphagous insect feeding on a large variety of plants species including fruits, vegetables, other crops and a few ornamentals belonging to Malvaceae, Solanaceae and Leguminaceae families (Arif *et al.*, 2009). In addition to the direct losses that the insects can cause by sucking the phloem sap, its feeding secretions (honeydew) cause additional losses to the plants by disturbing the photosynthesis activity and inducing fungal contaminations (Arif *et al.*, 2012). The chemical pesticides are simple and cost-effective, but their massive use has caused problems such as resistant behavior and environmental pollution with negative side effects on human health and on arthropods, disrupting biological control (Islam *et al.*, 2010; Ali *et al.*, 2012). In order to reduce the effects of conventional synthetic pesticides, biopesticides based on essential oils (EOs) appeared to be a complementary or alternative method in crop production and integrated pest management (Werdin Gonzalez *et al.*, 2011, 2013). Despite these promising properties, problems related with the EO volatility, poor water solubility, and aptitude for oxidation have to be resolved before they are used as an alternative pest control system (Moretti *et al.*, 2002). Nano formulation of the EOs could solve these problems protecting them from degradation and losses by

evaporation, achieving a controlled release of these products and facilitating their handling (Martin *et al.*, 2010). Furthermore, this kind of formulation is expected to be more effective than the bulk substances (Anjali *et al.*, 2010, 2012). On the other hand, it has been found that nano formulation of pesticide showed less toxicity towards non-target organisms compared with bulk or commercial formulations and therefore a higher specificity was observed (Frederiksen *et al.*, 2003). Plant-mediated synthesis of metal nanoparticles has more advantages compared to micro-organism mediated synthesis (Ibrahim, 2015).

## MATERIALS AND METHODS

### **The Culture of *phenacoccus solenopsis*:**

The laboratory culture of *P.solenopsis* was established from individuals collected from tomato plants in my home garden those do not have any previous exposure to pesticides. *P.solenopsis* was reared in on potato sprouts as per the method of Amarasekare *et al.* (2008) and potato plants under laboratory conditions. The culture was left for several generations until reach too much mass rearing to begin the experiment.  $23 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  R.H. and 13:11 L: D h photoperiod.

### **Essential Oils:**

The essential oils of lemon grass *Cymbopogon citratus*, thyme *Thymus vulgaris* and geranium *Pelargonium graveolens* EOs were obtained from Unite of Extraction and press Oils, National Research Centre, Giza.

### **Synthesis of Silver Nanoparticles By Essential Oils:**

For the biosynthesis of silver nanoparticles, 1.5 ml of plant essential oil was mixed with 34 ml of  $\text{AgNO}_3$  solution (1 M/ml) and incubated at  $29^\circ\text{C}$  for 24 h (Thanighaiarassu *et al.*, 2014). The small aliquot of solution was used for the Characterization of AgNPs was performed by Dynamic light scattering (DLS) and Transmission electron microscopy (TEM). Five different concentrations (50, 100, 200, 300 & 400 ppm.) for AgNPs-EOs and (5, 10, 20, 30 & 40 ppm.) for bulk EOs of each of the tested oils were prepared from the stock solution by diluting with SiSi-6 (Potassium alkyl sulphonate) as emulsifier (3ml/1liter of distilled water) in volumetric flasks to give the necessary concentrations.

### **Statistical Analysis:**

The data were statistically analyzed and graphing employed IBM by using program, StatPlus: Mac Pro; AnalystSoft Inc 2017.

### **Insecticidal Assay:**

#### **Direct Spray Method:**

- 1-The fresh unsprayed leaves of potato were collected from the garden, washed under tap water and left to dry.
- 2-The dried leaves were kept in glass Petri dishes (6 cm diameter), and in each Petri dish placed a tissue paper for dehydration of acceded fluid in petri dish. Twenty of 2<sup>nd</sup> nymphal instars and adult females were placed on each leaf. Each treatment was replicated three times for each concentration 3 replicate/ conc. /bio-insecticide.
- 3- A particular concentration from each tested eco-friendly AgNPs-EOs was sprayed directly on pre-adult and adult female cotton mealybugs using a hand sprayer (20 ml.) and the Petri dish was covered with nylon net. The sprayed by 3 ml of potassium alkaline sulphate in 100 ml. of distilled water as a control. Cotton Mealy bug *p. solenopsis* mortality was counted at 24, 48 and 72 hours, respectively, following initial application. The mortality records for all treatments were obtained in percentage values.

## RESULTS AND DISCUSSION

In the present study, the insecticide activity of tested bulk essential oils and AgNPs-EOs was studied against the adult *P. solenopsis* Tinsley after 24, 48 & 72hr. from treatment.

### Effect of Bulk Essential Oils against The Adult Female of *Phenacoccus solenopsis*:

The data indicated that, the mortality % of 24hr. 48hr. and 72hr. from treatment was [(30, 36.7, 63.3, 83.3 & 96.7%) (9.3, 25.3, 44, 86.7 & 93.3%) & (0, 15.30, 43.3, 60 & 73.3%)], [(36.7, 43.3, 86, 93.3 & 100%), (20.3, 33.3, 63.3, 93.3 & 100%) & (13.3, 21.3, 50, 73.3 & 83.3%)] and [(36.7, 46.7, 86.7, 93.3 & 100%), (27.7, 46.7, 73.3, 93.3 & 100%) & (20, 33, 56.7, 73.3 & 86.70%)] for thyme, lemon grass and geranium bulk essential oils at concentration 5, 10, 20, 30 & 40 µl/L., respectively. Table, (1)

Table, (1): Corrected mortality % of *P. solenopsis* female treated with bulk essential oils.

Essential oil	Concentration (µl/L)	Mortality %		
		24hr	48hr	72hr
Thyme <i>Thymus vulgaris</i>	5	30	36.7	36.7
	10	36.7	43.3	46.7
	20	63.3	86	86.7
	30	83.3	93.3	93.3
	40	96.7	100	100
Lemon grass <i>Cymbopogon citratus</i>	5	9.3	20.3	27.7
	10	25.3	33.3	46.7
	20	44	63.3	73.3
	30	86.7	93.3	93.3
	40	93.3	100	100
Geranium <i>Pelargonium graveolens</i>	5	0.00	13.3	20
	10	15.30	21.3	33.00
	20	43.3	50.00	56.70
	30	60.0	73.3	73.30
	40	73.3	83.30	86.70
Control	-----	nil	nil	nil

### Determination of LC<sub>50</sub> of Bulk EOs against the Adult Female of *Phenacoccus solenopsis* after 72hr. of Treatment:

Data in table, (2) revealed that thyme was the most effective oils to the female of *p. solenopsis* with LC<sub>50</sub>: 8.094 and LC<sub>90</sub>: 23.97, while LC<sub>50</sub> of lemon grass and geranium oils were 9.546 and 14.619, respectively and LC<sub>90</sub>: 27.699 and 59.719, respectively, (Fig.1a, b&c )

In agreement with the present results, Mohamed *et al.* (2018) tested ten plant essential oils for their toxicity against the adult females of cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) under laboratory conditions. Mortality varied according to the essential oil type and the delivered dose (ppm). The most remarkable toxic essential oils after 24h and 72h of treatment were *Thymus vulgaris* followed by *Mentha longifolia*, and *Cyperus articulatus* essential oils. The LC<sub>50</sub> values were 29.03, 34.32 and 54.69 ppm, respectively after 24h while after 72h of treatments were 15.04, 24.93 and 29.21 ppm, respectively. Hayat *et al.* (2015) indicated that the neem seed extracts (acetone and n-hexane extracts) caused 100 % mortality of *P. solenopsis* after 48 hrs. Mamoon *et al.* (2011) revealed that neem oil at 2.0% registered 69.63% mortality of the pest 72 hours after exposure

under laboratory conditions. Also coincidence with Banu *et al.* (2010) who exposed both nymphs and adults of *P. solenopsis* to pesticides and bio-pesticides and reported that neem oil at 2.5 l/ha +Nirma Evaluation of different chemicals for the management of powder 0.1% provided more than 50% mortality of mealybug nymphs 48 hours after treatment. Muhammad *et al.* (2017) cleared that lesser concentrations of botanicals can be used to manage cotton mealybug, *Phenacoccus solenopsis* Tinsley, and have non-toxic effects on natural enemies.

Table, (2): LC<sub>50</sub> and LC<sub>90</sub> values after 72 hr. of treatment with bulk essential oils

Essential oils	LC <sub>50</sub> and LC <sub>90</sub> values (µl/L)				
	LC <sub>50</sub>	LC <sub>90</sub>	Slope ±S.E	Index	LC <sub>90</sub> /LC <sub>50</sub>
Thyme	8.094	23.97	2.718±0.732	87.283	2.961
Lemon grass	9.546	27.699	2.77±0.712	56.994	2.901
Geranium	14.619	59.719	2.097±0.62	34.760	4.085

The values are statistically significant at  $p < 0.05\%$

### Biosynthesis and Characterization of AgNPs-Eos:

In the present study, silver nanoparticles were synthesized using essential oils, within 30 min of incubation. It was observed that upon addition of the essential oil into the flask containing the aqueous silver nitrate solution, the color of the medium changed to yellowish brown within 30 min which was due to the excitation of surface plasmon vibrations within the synthesized silver nanoparticles. This indicates the formation of silver nanoparticles (Ahmad *et al.*, 2003). (Shankar *et al.*, 2004; Ankamwar *et al.*, 2005). The intensity of color increases with the increase of incubation period. Kasture *et al.* (2008) reported the synthesis of silver nanoparticles from the seed of *Cuminum cyminum* and reported that the color of solution changed from pale yellow to reddish brown indicating formation of silver nanoparticles.

Synthesized silver nanoparticles were characterized by different techniques. The main important techniques implemented were Dynamic Light Scattering (DLS) analysis and Transmission Electron Microscopy (TEM) analysis.

### Dynamic Light Scattering (DLS):

To determine the particle size we performed dynamic light scattering technique analysis. Here the radius of the particle is responsible for light scattering. Particle with greater radius diffract the light less than that of smaller radius particles. The particle size determined by DLS as found to be {32.67-190.1 d.nm more density size 43.82 d.nm (21.4%)}, {13.54-91.28 d.nm more density size 21.04 d. nm (28.8%)} and {43.82-295.3d. nm more density size 58.77d. nm (21.1%)} for AgNPs synthesized by lemon grass, thyme and geranium oils, respectively. The results indicated that there is different reduction rate of silver nitrate by tested essential oils and thyme has the most acceleration reduction between other tested oils which showed from the average size of AgNPs in the case of thyme oils. Fig., (2a, b &c).

### Transmission electron microscopy (TEM) analysis:

The transmission electron microscope image of AgNPs synthesized by tested essential oils was noted in Fig., (3a, b &c). The pictures concluded that the synthesized AgNPs are of spherical shape and coating with the essential oil.

The results coincidence with Shiv Shankar *et al.* (2003) revealed that the rate of

reduction of the silver ions by the geranium leaf extract is faster than that observed by us in an earlier study using a fungus, *Fusarium oxysporum*, thus highlighting the possibility that nanoparticle biosynthesis methodologies will achieve rates of synthesis comparable to those of chemical methods. This study also represents an important advance in the use of plants over microorganisms in the biosynthesis of metal nanoparticles. Also in agreement with, Thanighaiarassu *et al.* (2013) synthesized gold nanoparticles from plant essential oil *menthapiperita* and these nanoparticles were characterized and Similarly Vyom *et al.* (2009) described the synthesis of silver nanoparticles using Parthenium leaf extract. Transmission Electron Microscopy analysis of these particles shows that they are 50 nm in range and assembled in irregular shape of variable morphology.

**Effect of AgNPs-EOs against Female of *Phenacoccus solenopsis*:**

Data in table, (3) and fig. ( 3 ), revealed that the reduction rates of *P. solenopsis* after 24hr from treatment were (10, 26.7, 46.7, 86.7&100%), , (3.3, 23.3, 50 & 60) and (0, 0, 6.7, 30& 53.3%), 48hr. (16.7, 40, 63.3, 100 & 100%), (6.7, 30, 63.3, 70 & 86.7 % ) and (0, 16.7, 46.7, 56.7 & 76.7 %) and 72hr. (23.3, 56.7, 83.3, 100 & 100 %), (13.3, 40, 66.7, 73.33 & 90%) and (6.7, 23.3, 53.3, 66.7 & 83.3%) for AgNPs-thyme, AgNPs-lemon grass and AgNPs-geranium oils at concentration 50,100, 200, 300 &400 ppm., respectively. Previous results proved that AgNPs-thyme oil was the most effective followed by AgNPs-lemon grass.

Table, (3): Corrected mortality % of *P. solenopsis* female treated with tested AgNPs- EOs.

Treatment	Conc. ppm	After treated (Mortality %)		
		24hr.	48hr.	72hr.
AgNPs-Thyme <i>Thymus vulgaris</i>	50	10	16.7	23.3
	100	26.7	40	56.7
	200	46.7	63.3	83.3
	300	86.7	100	100
	400	100	100	100
AgNPs-Lemon grass <i>Cymbopogon citratus</i>	50	3.3	6.7	13.3
	100	23.3	30	40
	200	50	63.3	66.7
	300	60	70	73.33
	400	83.33	86.7	90
AgNPs-Geranium <i>Pelargonium graveolens</i>	50	0	0	6.7
	100	0	16.7	23.3
	200	6.7	46.7	53.3
	300	30	56.7	66.7
	400	53.3	76.7	83.3
Control	20	nil	nil	nil

**Determination of LC<sub>50</sub> of Tested AgNPs-EOs against Female of *Phenacoccus Solenopsis* after 72hr. from Treatment:**

Results indicated that, all tested essential oils very effective against *P. solenopsis* and thyme was the most effective oil of insecticidal activity to the female of *P. solenopsis* with LC<sub>50</sub>: 86.645 and LC<sub>90</sub>: 203.234 followed by lemon grass with LC<sub>50</sub>: 114.169 and LC<sub>90</sub>: 269.013, while LC<sub>50</sub> of geranium oil was 188.318 and LC<sub>90</sub>: 575.128, respectively. (Table 4 and Fig., 4a,b&c )

The present data proved that the AgNPs-EOs were more effective than the bulk phase against *p. solenopsis* and thyme (bulk and nano phases) based on LC<sub>50</sub>

was strong toxic against *p. solenopsis* followed by lemon grass oil while, geranium oil was the least effective under study.

**Table, (4):** LC<sub>50</sub> and LC<sub>90</sub> values for AgNPs-EOs after 72 hr. of treatment

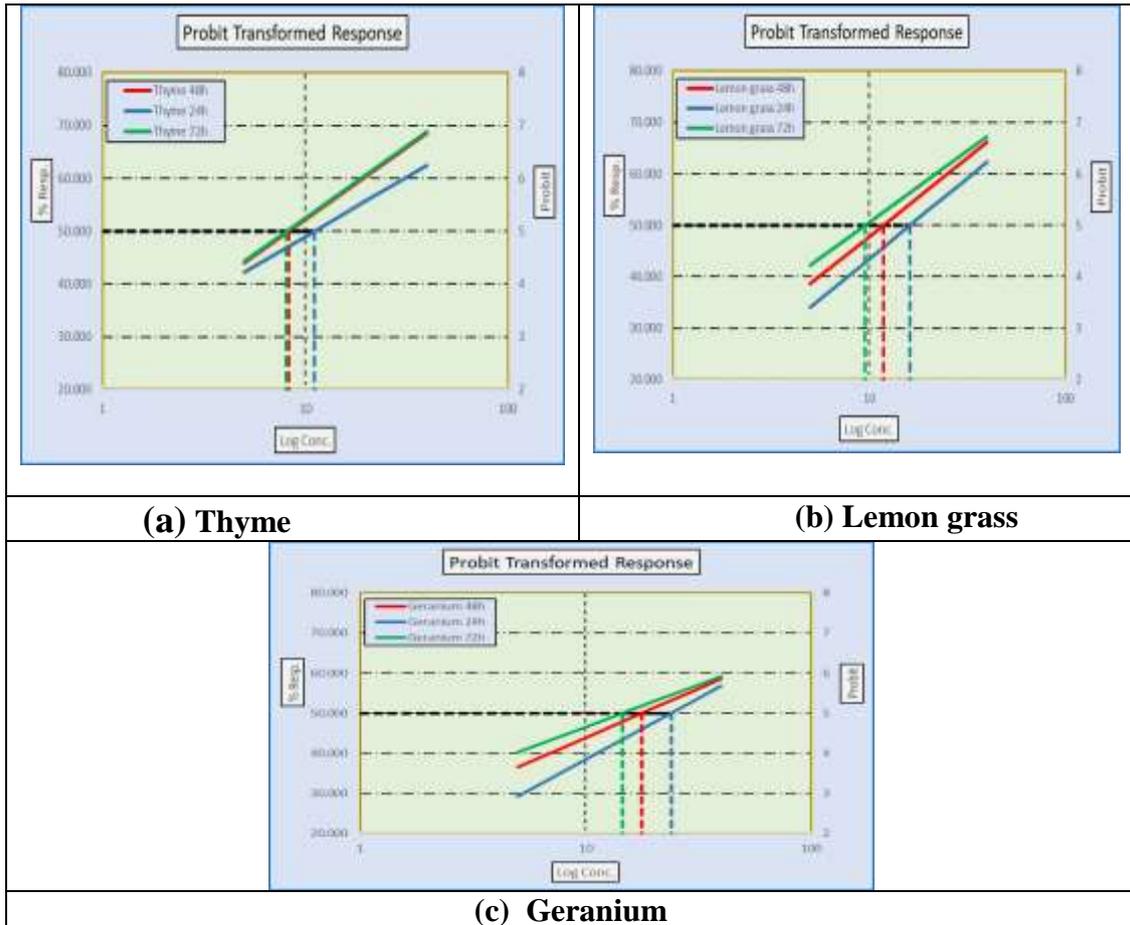
AgNPs-EOs	Lc <sub>50</sub> (ppm.)				
	LC <sub>50</sub>	LC <sub>90</sub>	Slope ±S.E	Index	LC <sub>90</sub> / LC <sub>50</sub>
Thyme	86.645	203.234	3.461±0.865	100	2.345
Lemon grass	114.169	269.013	1.837±0.6	75.892	2.356
Geranium	188.318	575.128	2.643±0.704	46.010	3.054

The values are statistically significant at  $p < 0.05\%$  (LSD, Tukey's test).

These results are in agreement with Yang *et al.* (2009) who indicated that polyethylene glycol-coated nanoparticles loaded with garlic essential oil were efficacious against adult *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) found in stored products. They observed that the control efficacy was about 80 % mortality. Similarly Magda *et al.* (2016) tested the essential oils (bulk & nano phase) of Purslane, Mustard and Castor oil for their toxicity against larvae of *E. cautella*. Their results reported that the most effective oil was Purslane oil (bulk and nano) followed by Mustard and the least one was Castor against larvae of *E. cautella* and the nano oils were more effective than the bulk phase. Ganesh *et al.* (2016) obtained results infer that green synthesized Ni NPs are in cubical shape with an average particle size of 47 nm. Synthesized Ni NPs were subjected to pesticidal activity against agricultural pest *Callasobruchus maculatus* which resulted in 97.31% mortality. The results not coincidence with Carvalho *et al.* (2012) who tested perimented neem (*Azadirachta indica*) oil in nano formulations containing beta -ciclodextrin and poli- epsilon -caprolactone (PCL) against eggs and nymphs of *Bemisia tabaci* (Genn.) and indicated that the nano formulations were less efficient to control the *B. tabaci* biotype B nymphs than the commercial neem oil.

### Conclusion

Essential oils were successfully utilized for the consistent and quick synthesis of silver nanoparticles. The biosynthesized silver nanoparticles using oils of lemon grass, thyme and geranium were characterized by DLS and TEM; they are circular, uniform and monodispersed nanoparticles. From the aforementioned results, it obvious that the size particles of AgNPs which synthesized by essential oils based on DLS analysis arranged in descending order as follows: AgPNs-Thyme>Lemon grass>Geranium. Bulk EOs and biosynthesized silver nanoparticles proved to be of the insecticidal agent against *p. solenopsis*. This green synthesis approach appears to be a cost-effective, non-toxic, eco-friendly alternative to the conventional physical and chemical methods, and would be suitable for developing a biological process for large-scale production. Further studies are needed to assess the long-term toxic effects of AgNPs in the system, and to extend this knowledge to other experimental models.



**Fig. 1 (a, b & c):** Regression lines representing toxicity of bulk thyme, lemon grass and geranium EOs against *P. solenopsis* female after 24, 48 & 72hr. of treatment.

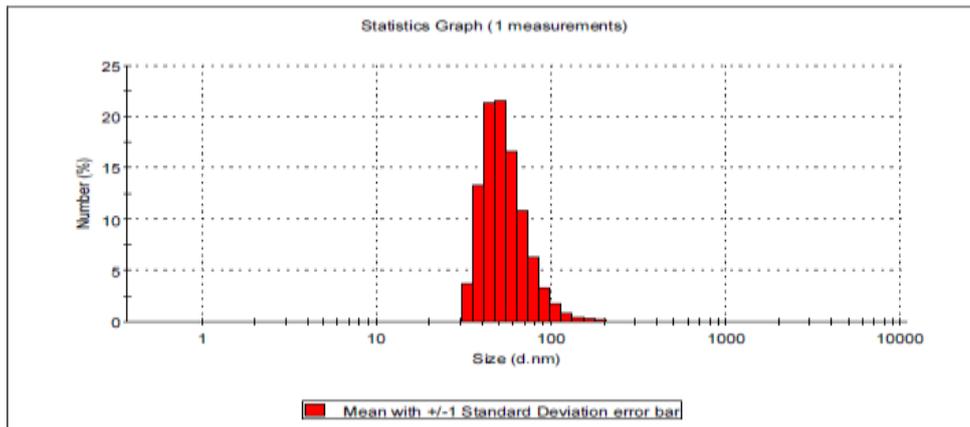


Fig., (2a) : Dynamic light scattering (DLS) of AgNPs synthesized by lemon grass *C. citratus*

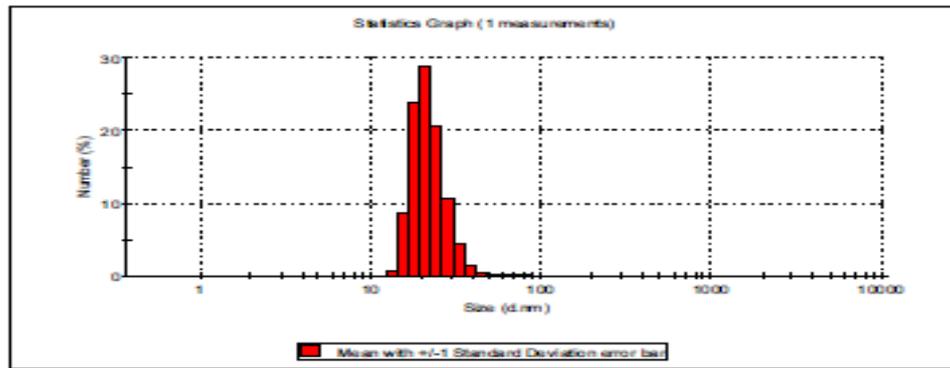


Fig., (2b) : Dynamic light scattering (DLS) of AgNPs synthesized by thyme *T. vulgaris*

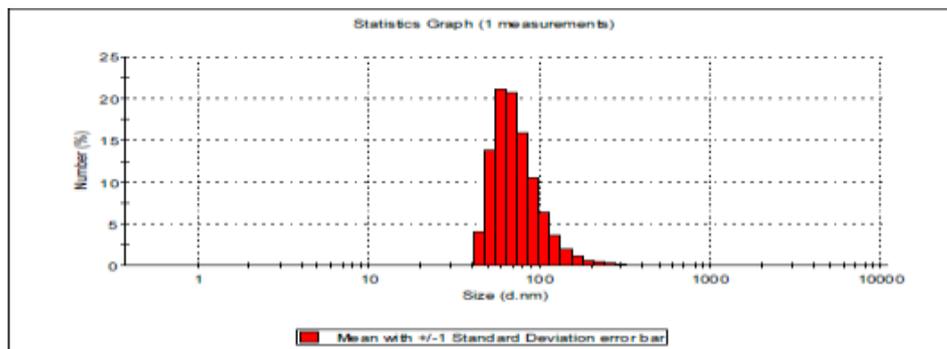


Fig.,( 2c): Dynamic light scattering (DLS) of AgNPs synthesized by geranium *P. graveolens*.

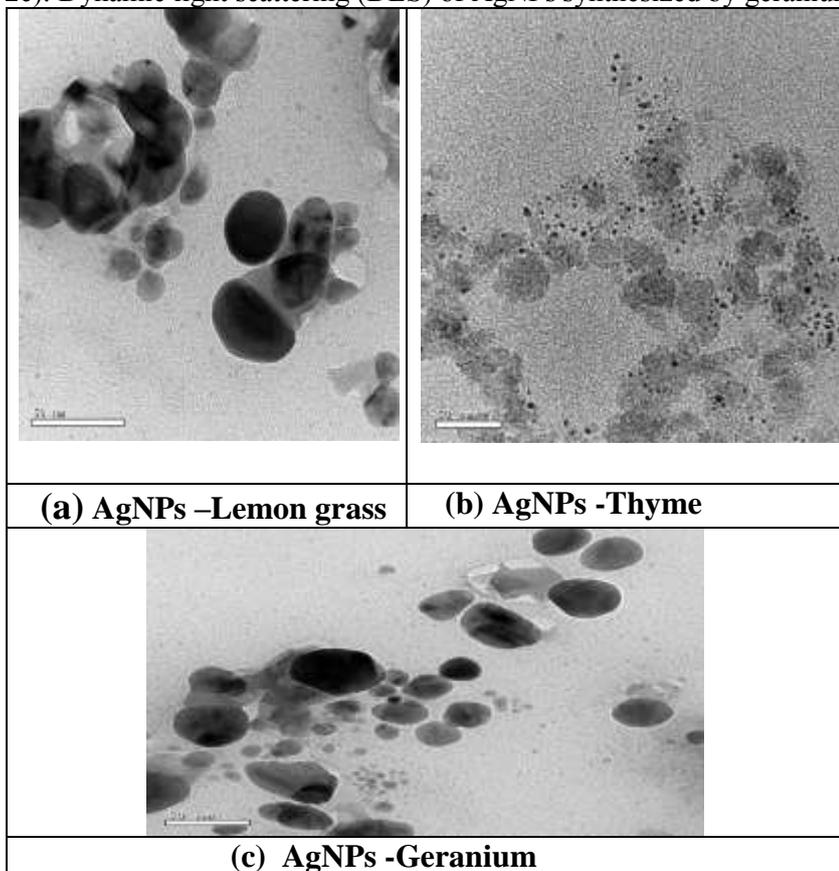
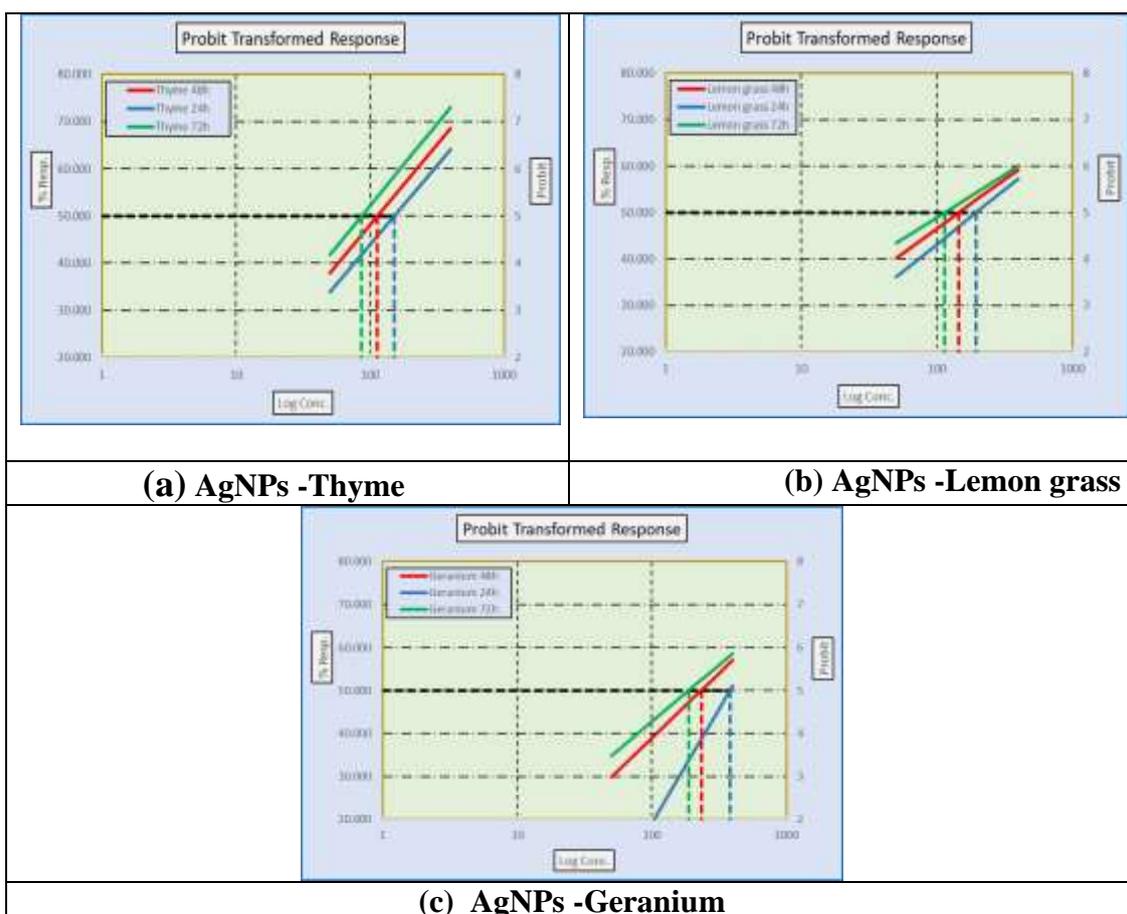


Fig., (3a, b&c) : Transmission electron microscope (TEM) image of AgNPs synthesized by (a) Lemon grass, (b) thyme & (c) geranium.



**Fig., (4 a,b&c) :** Regression lines representing toxicity of AgNPs- thyme, lemon grass and geranium oil, respectively against *P. solenopsis* female after 24, 48 & 72hr. from the treatment.

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## ARABIC SUMMERY

مبيدات حشرية حيوية في صورة النانو يعتمد تكوينها على الزيوت العطرية لمكافحة بق القطن الدقيقي  
فييناكوكس سولينوبس

سوسن محمد احمد<sup>1</sup> ، عفاف عبد الوهاب عباس<sup>2</sup> ، حورية علي عبد الوهاب<sup>3</sup> ، نعمة احمد عبد الحميد<sup>2</sup> ،  
عنايات محمد محمد احمد<sup>2</sup>

- ١- المركز القومي لتكنولوجيا الإشعاع بهيئة الطاقة الذرية
- ٢- كلية العلوم (بنات)- جامعة الأزهر
- ٣- معهد بحوث وقاية النبات بالدقى

تم إختبار ثلاث زيوت عطرية (الزعتر، حشيشة الليمون والعتري) بتركيزات ٥، ١٠، ٢٠، ٣٠ و٤٠ مللي لتر وكذلك جزيئات النانو للفضة المكونة فزيائياً بإستخدام الزيوت العطرية الثلاثة المختبرة بتركيزات ٥٠، ١٠٠، ٢٠٠، ٣٠٠ و٤٠٠ لكل جزء من المليون ومعاملتهم لإينات حشرة بق القطن الدقيقي فييناكوكس سولينوبس. وأظهرت النتائج أن الزيوت العطرية الثلاثة المختبرة لها تأثير سمي قوى على إينات حشرة بق القطن الدقيقي، و أن زيت الزعتر هو الأكثر سمية على إينات حشرة بق القطن الدقيقي فييناكوكس سولينوبس بإستخدامه في حالة النقية وكذلك حالته مع جزيئات الفضة، كما أعطى الزعتر أعلى نسبة إختزال وأقل حجم لجزيئات الفضة بين الزيوت العطرية الأخرى المختبرة الذى بينه تحليل DLS .