Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Myco- silver nanoparticles synthesized using *Beauveria bassiana* and *Metarhizium brunneum* as a smart pest control

Atef M. M. Sayed[1] and Sanghoon Kim[2]

[1]*Biological Control Research Department, Plant Protection Research Institute, Agricultural Research Center, Ismailia Agricultural Research Station, Egypt.*
[2]*United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, Illinois, 61604, USA.*

ARTICLE INFO
Article History
Received:15/5/2018
Accepted: 20/7/2018

Keywords
Nanotechnology,
silver nanoparticles,
Beauveria bassiana ,
Metarhizium brunneum and
Tetranychus urticae.

Abstract:

Nanotechnology has recently been considered as a modern potential tool for crop protection at the nanoscale level. Biosynthesis of silver nanoparticles by using the entomopathogenic fungi, *Beauveria bassiana* (*Bb*) and *Metarhizium brunneum* (*Mb*) is ~~as~~ an eco-friendly and cost effective production system. The produced nanoparticles revealed a brownish color that is characteristic for silver nanoparticles. *Bb*-synthesized AgNPs and *Mb*-synthesized AgNPs were produced at various concentrations and characterized by UV-Vis spectrophotometer and Dynamic Light Scattering (DLS). The variation of hydrodynamic diameter (D_h) of silver particles at various concentration of culture in conjunction with UV-Vis spectra showed that production of AgNPs was maximized when using 15% of culture for both fungi and the size of particles was around 87 nm for both. The efficacy of *Bb*-synthesized AgNPs and *Mb*-synthesized AgNPs against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) were tested. Results demonstrated that the treatments of either *Bb*- or *Mb*-synthesized AgNPs were found to be highly significantly virulent toward newly emerged adult female of *T. urticae*.

Introduction

Nanotechnology has increasingly developed as an effective, novel tool, generating various promising applications in different active fields at the nanoscale level. Such areas include: electrochemical sensors, biosensors, pharmacology, medicines, agriculture, food industry and pest management (Kim *et al*., 2007; Bhattacharyya *et al*., 2010; Rai and Ingle, 2012; Tarafdar *et al*., 2013; Bengalli, 2016 and Sayed *et al*., 2017a). Nanoparticles are materials at nanoscale levels that usually range in dimension from 1-100 nanometers (nm). They possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications (ISO, 2010). There is a growing interest in the synthesis of metallic

nanoparticles such as silver that are involved in several applications (Wei *et al*., 2015). Silver is known to have been used by the Persians, by ancient Phoenicians, Greeks, Romans and Egyptians for treatment related to bacterial infections (21-22) (Alexander, 2009).

Recently, the use of silver in nano-particulate form (AgNPs) is one of the most vital nanomaterials among several metallic nanoparticles. It has gained more importance due to their potential properties, as well as the increase in the development of products that contain nano-silver in the fields of biology, biotechnology, medicine, chemistry and agriculture (Sharma *et al.*, 2009; Wei *et al*., 2015 and Sandhu *et al*., 2017).

Various chemical and physical methods have been used for synthesis of metal nanoparticles. However, these methods are more expensive, highly energy-consuming and potentially toxic to the environment. It is necessary to develop alternative, eco-friendly methods. Therefore, biological synthesis method that are based on bacteria, fungi, yeast, actinomycetes, algae, viruses, bioderived chemicals and plant extracts have the ability to synthesis various types of nanoparticles and could be more advanced than other methods. Biological synthesis methods are cost effective, biocompatible, environmentally friendly approaches for nanoparticles synthesis without using hazardous materials, easily scaled up for large scale synthesis and there is no need to use high pressure, energy, temperature and toxic chemicals (Thakkar *et al*., 2010; Castro-Longoria *et al*., 2011; Sathya and Ambikapathy, 2012 and Sunkar and Nachiyar, 2013).

Microbial synthesis using fungi provide wide advantages over other methods using plants or bacteria. Fungi are easy to handle in the laboratory, require simple nutrients and possess high wall-binding capacity. In addition, they could be used as a source for the production of large amounts of nanoparticles. Various fungal species are gaining more attention in synthesizing different kinds of metal nanoparticles. These species show great potential, since they secrete large amounts of enzymes and are easy to use in the laboratory, which lead to higher yields of nanoparticles (Ahmed *et al*., 2003; Bhainsa and D'Souza, 2006; Mohanpuria *et al*., 2008; Dhillon *et al*., 2012; Banu and Balasubramanian, 2014; Soni and Prakash, 2012; Roy *et al*.,2014; Wei *et al*., 2014 and Amersan *et al*., 2016).The mechanism of synthesis of metal nanoparticles by microbes is not clearly explored.

Among these fungi are the anamorphic entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium brunneum* (Metschnikoff) Sorokin. Both fungi belong to the order Hypocreales (Ascomycota), infecting a wide range of pests and having cosmopolitan distributions (Roberts and St. Leger, 2004 and Rehner, 2005). Much effort has been put into research on the development of *B. bassiana* and *M. brunneum* as biological control agents to be applied in agriculture and forestry.

Indeed, nanotechnology has offered potential solutions for many problems to revolutionize a wide array of applications in the fields of biomedicine and pest management (Benelli and Lukehart, 2017 and Athanassiou *et al*., 2018). Rai and Ingle (2012) concluded that nanotechnology can provide green and eco-friendly alternatives for pest management without harming nature. Nano-pesticides, nanofungicides and nanoherbicides are being used in agriculture (Owolade *et al*., 2008 and Athanassiou *et al*., 2018). The mycosynthesis of metal NPs has also revealed interesting prospects for the management of certain insect pest species (Amerasan *et al*., 2016).

However, this technology has been studied particularly in the research of larvicides with potential focus on the protection against mosquito vectors or ticks of veterinary importance (Salunkhe *et al.*, 2011; Amerasan *et al.*, 2016; Prabakaran *et*

al., 2017 and Benelli, 2016). Little information is available about the toxicity of nanoparticles against other pests such as phytophagous mites.

In this research, we have made an attempt to present a rapid and eco-friendly approach of silver nanopartes production using two fungal species: free cell filtrates of *B. bassian*a and *M. brunneum*. In addition, we are submitting the preliminary results of toxicity of biosynthesized silver nanoparticles against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).

Materials and methods

1. Materials:

Beauveria bassiana strain GHA (ARSEF6444) was originally isolated from *Diabrotica undeciumpunctata* Barber (Coleoptera: Chrysomellidae) in Corvallis, Oregon, USA, and was obtained from the ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) in Ithaca, NY. The GHA strain is the active ingredient of a commercial product marketed by LAM International (BotaniGard® ES, Butte, MT, USA). *Metarhizium brunneum* (Petch) strain F52 was first cultivated from the codling moth *Cydia pomonella* in Austria. F52 has been incorporated into a commercial product Met52® (ATCC 90448, Novozyme Biologicals, Salem, VA, USA).

Silver nitrate ($AgNO_3$, 99%) was obtained from Fisher Scientific Co., Fair Lawn, NJ, USA. Commercial silver nanoparticles were purchased from Sigma-Aldrich as silver, dispersion nanoparticles, 10 nm particle size, 0.02 mg mL^{-1} mass concentration, in aqueous buffer containing sodium citrate as stabilizer. All chemicals used were purchased from Sigma-Aldrich unless otherwise stated. All suspensions were prepared in either sterilized deionized water or Milli-Q water.

2. *Beauveria bassiana* and *Metarhizium brunneum* cultures:

Fungal growth and media preparations were carried out based on the method previously described by Sayed and Behle (2017b) and Behle and Jackson (2014). Briefly, stock cultures of both fungi were grown on potato dextrose agar (PDA) media (Difco, Detroit, MI, USA) in Petri dishes for 3 weeks at 25 ± 2 ^{o}C with a 12:12 h (L:D) photoperiod until sporulation. Each fungal conidia were harvested by scraping plates with 10 mL of sterile aqueous solution of 0.04% polyoxyethylene sorbitan mono-oleate (Tween 80, Sigma, St. Louis, US) using a fine sterile loop (Fisherbrand™ Disposable Inoculating Loops, Fischer Scientific, Pittsburg, PA, USA). Stock culture each was used to inoculate the culture medium at an initial concentration of 1.4×10^5 conidia mL^{-1} for *B. bassiana* and 1.1×10^5 conidia mL^{-1} for *M. brunneum*. Conidia concentrations were measured light microscopically (400 magnification) with Nomarski optics (BH2, Nikon America, Center Valley, PA, USA) using a hemacytometer (Bright-line, Hausser Scientific, Horsham, PA). Each whole culture fungi (1 L) was grown in 5 L baffled Erlenmeyer flasks (Bellco Glass, Vineland, NJ, USA) with 200 mL of the liquid media. This liquid media was incubated in a rotary shaker incubator (INNOVA 4000, NewBrunswick Scientific, Edison,NJ) for 4 days at 28^{o} C and 350 rpm.

The liquid media used for *B. bassiana* and *M. brunneum* cultures contained basal salts as described by Jackson *et al.* (1997) which were supplemented with glucose (Fisher Scientific) at 80 g L^{-1} (40%) carbon (C) and acid hydrolyzed casein (derived from bovine milk, Hy-case™ MSF, Kerry Bioscience, New York, NY, USA) at 25 g L^{-1} (8.5% nitrogen (N) and 53% C), which produced a medium with a carbon-to-nitrogen ratio (C:N) of 23:1 and ~~had~~ an initial pH of 5.3. The basal salts per liter were as follows: KH_2PO_4, 2.0 g; $CaCl_2 \cdot 2H_2O$, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $CoCl_2 \cdot 6H_2O$, 37 mg; $FeSO_4 \cdot 7H_2O$, 50 mg; $MnSO4 \cdot H_2O$, 16 mg; $ZnSO_4 \cdot 7H_2O$, 14 mg; thiamin, riboflavin, pantothenate, niacin, pyridoxamine, thioctic acid, 500 mg each; folic acid, biotin, vitamin B12, 50 mg each. Glucose stock solutions (20% w/v) were

autoclaved separately and added prior to inoculation. Sterilization of liquid cultures and glucose stock solutions were performed at 121°C for 20 min.

Both cultures were inoculated with conidial suspensions in the liquid culture medium. Flasks were hand-shaken frequently during the fermentation process to minimize mycelial growth and sporulation on the flask walls. For quality assurance, the fermentation broth was streaked onto nutrient agar plates, incubated for 48 hours at 30 ºC, and visually evaluated for bacterial contamination. In all experiments, pH was uncontrolled during culture growth. The final 4-d-old fermentation product of 1 L was expected to contain 35.3×10^8 conidia mL^{-1} for *B. bassiana*, 6.3×10^8 conidia mL^{-1} for *M. brunneum* with 20-25 g solids L^{-1} each. The whole fungal cultures were each stored at 4°C until used.

3. Biosynthesis of silver nanoparticles:

After growing *B. bassiana* and *M. brunneum* for 4 days, the whole fungal products were blended in a blender (Kitchen-Aid, St. Joseph, MI, USA) at high speed for one minute and filtered through Whatman #1 filter paper (GE Healthcare UK Limited, Buckinghamshire, UK). Then, they were washed thrice in sterile distilled deionized water to remove any nutrient media that might interact with the silver ions. The resulting filter cake from each cultural fungal product was spread over baking sheets and allowed to air dry under an air drying chamber with lateral air inflow and controlled RH atmosphere (RH 50–60%) for 24 h to achieve moisture less than 4% (w/w) (Jackson and Payne, 2007). Water activity of theses dried conidia were measured with a water activity analyzer (Aqua Lab Model Series 3, 4TEV, Decagon Devices, Inc., Pullman, WA, USA). Dried conidia preparations were stored in 50 mL conical tubes at 4 °C until used. Approximately 10 g of each fresh fungal biomass was transferred to a 500-mL baffled Erlenmeyer shaker flasks containing 100 mL milli-Q water or sterilized deionized water. These flasks were incubated in a rotary shaker incubator for 3 days at 25° C and 200 rpm. The whole fungal products were filtered through Whatman #1 filter paper to obtain cell-free filtrates.

In a typical procedure, stock solution of silver nitrate ($AgNO_3$) was dissolved in sterilized deionized water at a concentration of 0.01 *M* (1.69 g L^{-1}). Various desired volume concentrations of the cell-free filtrates of each fungus were added to 250 mL baffled Erlenmeyer shaker flasks (each containing 5 mL of 0.01 *M* $AgNO_3$ solution) to provide 50 mL total volume of the whole mixture suspension. The final twelve treatment concentrations of 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, and 80% of each fungal cell-free filtrate were obtained with the final constant concentration of silver nitrate at 1 m*M* (0.169 g L^{-1}). Flasks containing the whole mixture suspensions of both fungi were incubated at 25 °C in a rotary shaker incubator (INNOVA 4000, New Brunswick Scientific Co., Enfield, CT, USA) at 200 rpm for 5 d in ~~a~~ the dark to avoid any photochemical reactions during the experiment and until complete bioreduction of silver ions was achieved. Simultaneously, two 250 mL baffled Erlenmeyer shaker flasks, one containing only broth medium (50 mL) without silver nitrate solution, and the other consisting of only silver nitrate solution (50 mL), were maintained under similar experimental conditions as a control. These experiments were done with both fungi in triplicates.

According to visual observation, the cell-free filtrates of each fungus maintained in the presence of silver nitrate showed a color change from yellow to brown, whereas no color change could be observed in broth culture of fungi without silver nitrate and silver nitrate solution without the cultures. These control experiments indicate that the Ag^+ ions' reduction is not just a thermal process. The bioreduction of the Ag^+ ions in the samples and color change of the resulting solution were monitored, thus indicating the formation of silver nanoparticles. The

filtrates of *B. bassiana* and *M. brunneum* derived silver nanoparticles were named as *Bb*-AgNPs and *Mb*-AgNPs, respectively. The presence of *Bb*-synthesized silver nanoparticles (AgNPs) and *Mb*-synthesized silver nanoparticles was confirmed by UV–vis spectra, at the wavelength of 300–800 nm in the UV–vis spectrophotometer. The difference in color depends on size and shape of the nanoparticles formed as reported previously by Wiley *et al.*, 2006; Soni and Prakash, 2012 and Prabakaran *et al.*, 2017) .

After incubation, the silver nanoparticles obtained by these treatments were purified by centrifugation at 10,000 × g for 10 min, and then freeze-dried. The obtained AgNPs were weighed and stored at 4 ^{o}C until used as described by Ingle *et al.*, 2008 and Soni and Prakash, 2012). Sterilized deionized water or Milli-Q water was used to suspend the *Bb*-AgNP or Mb-AgNP powders for the characterization and bioassay of prepared silver nanoparticle. Dynamic Light Scattering (DLS) experiment was performed for the measurement of particle size of *Bb*- and *Mb*-synthesized AgNPs. The concentration of silver nanoparticles was calculated according to the equation of Liu *et al.* (2007).

4. Characterization of AgNPs:

The nanoparticles were characterized using UV-Vis, Dynamic Light Scattering (DLS), and Fourier-transform infrared (FT-IR) spectroscopy. The synthesized silver nanoparticle suspensions at different percent concentrations were recorded via UV-Vis spectrum analysis. The change in spectra of these solutions was monitored in the range of 300-800 nm. UV-Vis spectra were obtained using a spectrophotometer (UV-2600, Shimadzu Scientific Instruments, Kyoto, Japan). Since each of *Bb* or *Mb* showed very strong absorption in the range of 200-500 nm, the UV absorption peaks of silver nanoparticles were isolated through deconvolution of the obtained spectra. Otherwise, the color changes of reaction mixtures were used as evidence for AgNP formation. The size distribution and average size of the synthesized AgNPs was measured by DLS (NanoBrook Omni Particle Size Analyzer, Brookhaven Instruments Corp., Holtsville, NY, USA).

The characterization of functional groups on the surface of AgNPs was performed by Fourier-Transform Infra-Red (FT-IR spectrum) spectroscopy. Measurements of the samples were monitored using a FT-IR spectrometer (Varian Escalibur 3100, Varian Inc., Randolph, MA) with diffuse reflectance mode (DRS-800) attachment. The bio-transformed products were diluted with potassium bromide in the ratio of 1:100. All measurements were carried out in the range of 400–4,000 cm-1 at a spectral resolution of 4 cm-1 (Prabakaran *et al.*, 2016 and Amerasan *et al.*, 2016). Unless otherwise stated, the synthesized silver nanoparticle was measured for more than three independent runs for all experiments using the cultures of *B. bassiana* and *M. brunneum* with the same procedures.

5. Mite colonies:

Mitcidial activities of the treatments were determined. Mite mortality was determined using adult female of mite *T. urticae* from laboratory colonies maintained at the Agricultural Research Center, Ismailia Agricultural Research Station, Ismailia, Egypt. The batches of mite colony were reared in the laboratory under constant conditions at 25 ± 2 °C, 60 ± 5% RH, and a photoperiod of 16: 8 (L:D) h away from any pesticide contaminations according to the method adapted by El-Esnawy *et al.* , 2012. Sweet potato cuttings (*Ipomoea batatas* (L.) Lam, family convolvulaceae, cv. 195A) with about 7 leaves each were used as a source of food. These cuttings were kept in 250 mL glass jars filled with tap water; four cuttings were placed in each jar. These cuttings were changed twice weekly in summer and once every week in winter. The old infested cuttings were placed on top of the new cuttings for a couple of days to facilitate mite transfer onto the new plant and avoid losing

selected mites in the process. The colony was established with five jars, and kept in special cages (60 X 60 X 60 cm) covered with cheese cloth (muslin) and provided with fluorescent light tubes of 40 watts to give constant illumination (16 hours/day).

6. Bioassay:

Leaf disk bioassays were performed to estimate the median lethal concentrations (LC_{50}) for the obtained *Bb*-AgNPs and *Mb*-AgNPs against newly emerged adult females of *T. urticae*. To obtain fixed-age females for the bioassay, quiescent deutonymphs were collected from the mite colony and isolated on fresh leaf discs. Sterilized deionized water or Milli-Q water was used to dissolve the samples of *Bb*-AgNPs or *Mb*-AgNPs to desired concentrations. For the freshly made *Bb*-AgNPs and *Mb*-AgNPs, six suspensions of 150, 125, 100, 75, 50 and ~~50~~ 25 ppm were prepared. The untreated control consisted of four solutions of 20, 10, 5, and 2.5 ppm of commercial silver nanoparticles (Sigma-Aldrich); four dosage dilutions of 170, 17, 1.7 and 0.17 ppm of $AgNO_3$ solution; and five concentrations of conidial serial suspensions of each *Bb* and *Mb* only ranging from 10^8 to 10^4 conidia mL^{-1}. Only liquid broth media of *Bb* and *Mb* and sterilized deionized water served to assess mortality. Leaf disks (3 cm diameter) were excised with a cork borer from 8-10 week old field-grown sweet potato. Disks were placed top-side up on water-saturated cotton pads in a Petri dish (90 mm diameter). Each dish contained 2 disks and each dish represented a replicate. Each leaf disk was immersed individually for 5 seconds with gentile agitation and allowed to air dry. Once treatments were dry, each disk was infested with 10 newly emerged adult females using a fine brush (Pelikn brush No. 000) and incubated (WTC binder, 7200 Tuttlingen, Germany) in the dark at 25^o C and 60-70% relative humidity for the desired exposure period. After incubation, the numbers of live and dead adult female mites were counted to calculate the activity of each treatment. For both exposure nanoparticles, adult females were considered dead if they did not respond when touched with a fine brush. The entire experiment was repeated three times on different dates using different mite cohorts.

7. Data analysis:

Data of bioassay mortalities was log transformed prior to analysis of variance to meet the normality assumptions and provided the best fit due to its lowest deviance. Dose response results obtained in bioassays were subjected to probit analysis using Probit Analysis-MSChart 2009 software (Chi, 2009) to calculate the median lethal concentrations of LC_{50} with its corresponding fiducial limits (95% FL) and slope for each. Mortalities due to *Bb*-AgNP and *Mb*-AgNP treatments were based on the mean of four replicates of dishes with 80 newly emerged adult females each, which were carried out three times on different dates using different mite cohorts with a total of 240 females for each treatment. Controls were included with each assay to indicate handling mortality, and thus data were not corrected for control mortality. Analysis of variance was used to determine the efficacy of each *Bb*-AgNP and *Bb*-AgNP treatments on the newly adult female stage of *T. urtiacea.* Treatment means were separated using the least significant difference (LSD) test at *p-value* 0.05 with the statistical software SPC for Excel (Knoware International, Inc., Denver, CO, USA).

Results and Discussion

1. Synthesis of *Beauveria bassiana and Metarhizium brunneum*-silver nano-particles (*Bb* and *Mb*-AgNPs):

Based on visual observation, when the free-cell fungal filtrates of *Bb* and *Mb* were mixed in the aqueous solution of silver nitrate $AgNO_3$, the color of the reaction mixture solutions changed from pale yellow to brownish due to reduction of silver ion, which indicated formation of silver nanoparticle *B. bassiana* - synthesized silver nanoparticles (*Bb*- AgNPs) and *M. brunneum* -synthesized silver nanoparticles

(*Mb*- AgNPs) as illustrated in Figure (1). Figure (1) highlights the change in color intensity of synthesized *Bb* or *Mb* –AgNPs, which increased with duration of incubation. The color of the solution

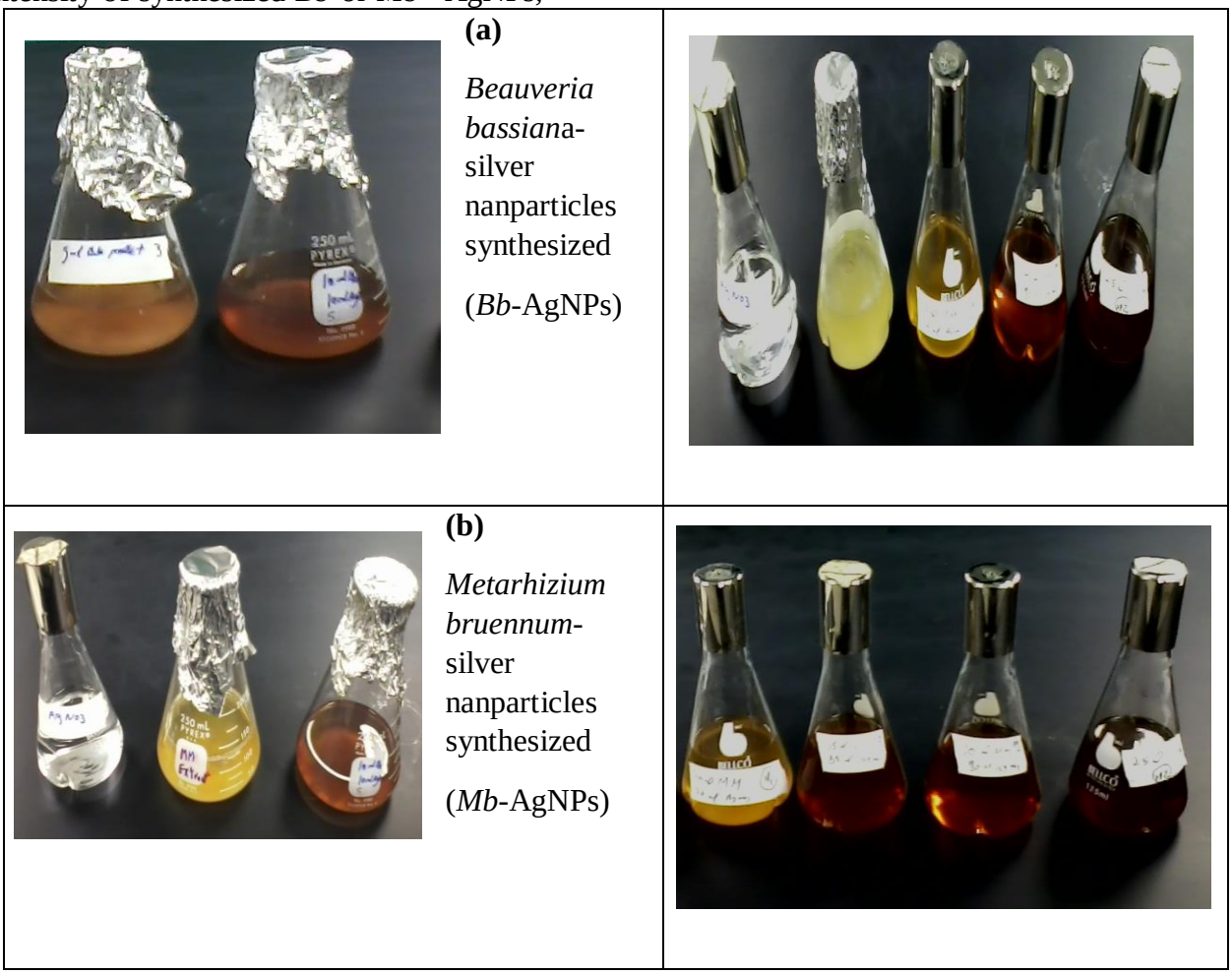


Figure (1): a, b, The change in color intensity in cell free filtrate of *Beauveria bassiana* and *Metarhizium bruennum* after exposure to silver nitrate of synthesized a=*Bb* - AgNPs or b=*Mb* -AgNPs increased with duration of incubation.

changed to dark brown after 5 d of incubation for the synthesis of nanoparticles as the concentration of *Bb* or *Mb* filtrates increased to 10% in the solution, then faded as the concentration of *Bb or Mb* exceeded 15%. In the case of the samples of fungal cell free filtrates without $AgNO_3$ or broth media of Bb and Mb or $AgNO_3$ solution alone, no color development was observed. Silver nanoparticle concentrations were characterized by UV spectrophotometry. The measurement of UV-Vis spectra for the silver nanoparticles was performed in the range of 300-700 nm, as illustrated in Figures 2 and 3. The analysis of UV-Vis spectra showed an appearance of a surface plasmon resonance peak (SPR) at 300-700 nm wavelength range, which corresponds to silver nanoparticles formation for both *Bb*-AgNPs and *Mb*- AgNPs. As the amounts of fungal cell free filtrates of *Bb* and *Mb* increased in the solution, higher absorption peaks were observed, indicating that there were increases in the reduction of silver precursor to silver nanoparticles. However, further increase in the amounts of fungal filtrates of *Bb* and *Mb* lowered the production of silver nanoparticles. In order to see the overview of this behavior, the signal intensity of UV-Vis spectra for each sample was integrated for the 300-700 nm range and plotted against the amount of Bb

and Mb. These data indicate that the development of color is maximized when the concentration of Bb or Mb was 10-15%. The size of the silver particles in each sample was analyzed by DLS measurement (Figures 4 and 5). This result illustrates the relationship between the hydrodynamic diameter (Dh) of produced silver nanoparticles and amount of Bb or Mb. As shown in Figures (4 and 5), the amount of silver nanoparticles increased until the concentration of fungal filtrates increased to around 15%. Therefore, combining the UV spectrophotometer and DLS data sets shows that the production of silver nanoparticles increased until the amount of Bb or Mb increased to around 15% without significant change in the size of silver nanoparticles. Further increase in the concentration of fungal filtrates caused a rapid increase in the size of particles in the solution. Since UV data showed that the concentration of silver nanoparticle decreased with higher fungal filtrate concentrations, the increased size of particles indicated that the produced silver nanoparticles were bound together, yielding aggregated nanoparticle product.

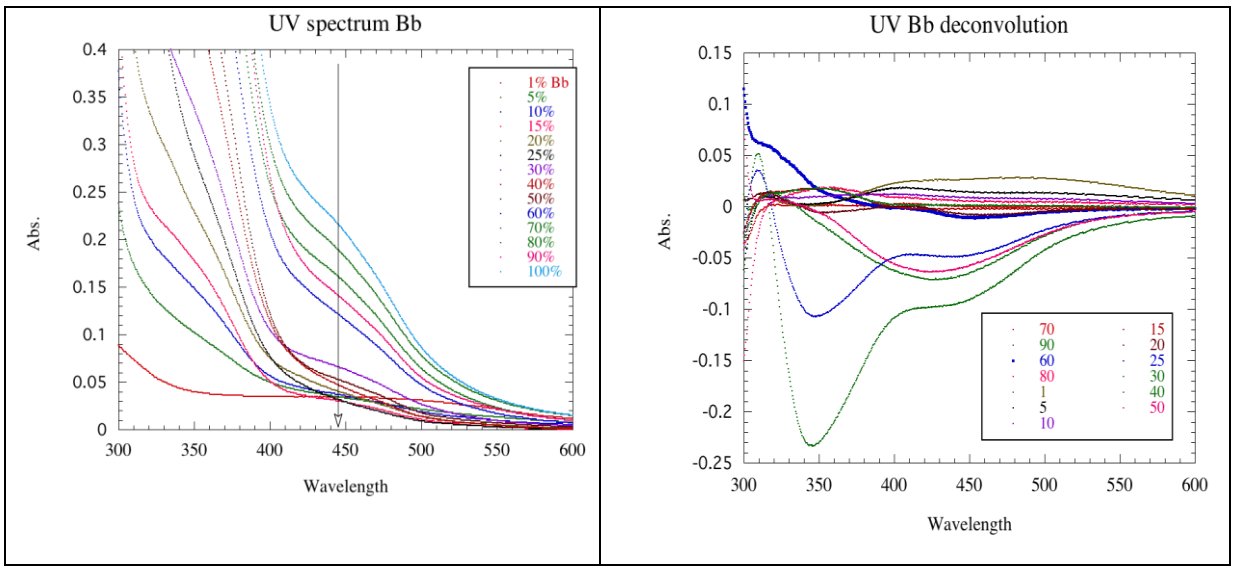


Figure (2): UV-Vis spectra response indicating the development of silver nanoparticles by *Beauveria bassiana* .

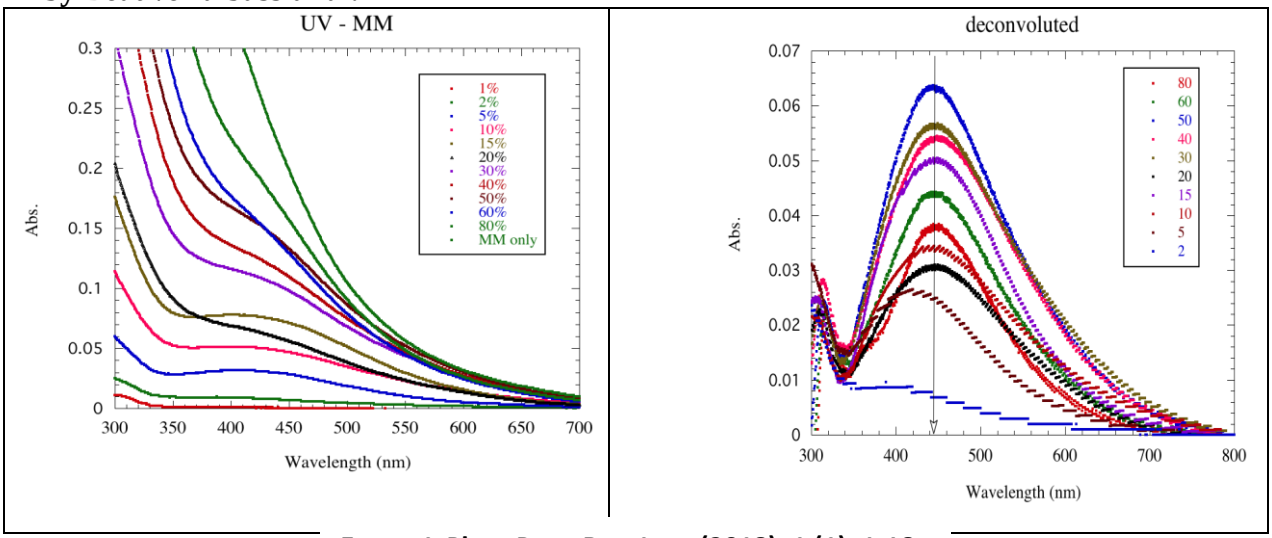


Figure (3): UV-Vis s **of silver nanoparticles by *Metarhizium brunneum***

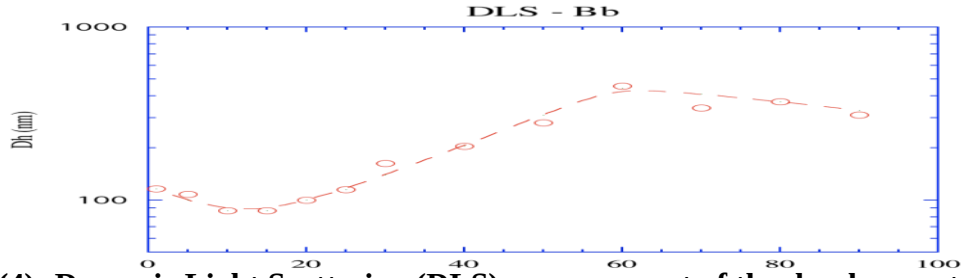


Figure (4): Dynamic Light Scattering (DLS) measurement of the development of silver nanoparticles by *Beauveria bassiana.*

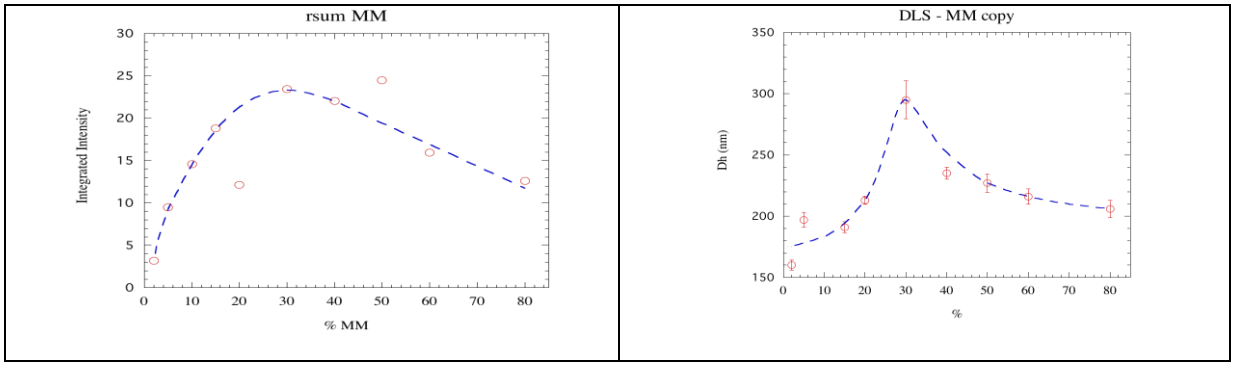


Figure (5): Dynamic Light Scattering (DLS) measurement of the development of silver nanoparticles by *Metarhizium brunneum* .

The presence of *Bb*-AgNPs or *M*b-AgNPs was confirmed and measured by UV–vis and DLS. As the concentration of fungal free cell filtrates increased, the observed particle size decreased and the UV absorption increased. These findings reveal that the average particle size of sample decreased as more silver nanoparticles were produced. The trend continued until 15% of *Bb* or *Mb* filtrates was added to the sample. The size of particles in silver nitrate/*Bb* filtrate was similar to that in silver nitrate/*Mb* filtrate sample. At higher concentrations of *Bb* filtrates, the overall behavior of *Mb* filtrates was similar to that of silver nitrate/*Bb* filtrate, i.e., the produced nanoparticles were bound together to form larger particles (Figure, 6).

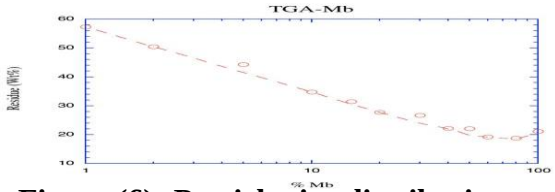


Figure (6): Particle size distribution curve of silver nanoparticles obtained by *Metarhizium brunneum.*

Although the DLS data for Bb and Mb systems looked quite different, both data showed the inflection point at the same concentration of 15% and the size of particle was around 87 nm for both. FT-IR spectroscopy analysis was carried out to identify the potential functional groups present in the bioactive molecules which may play a role in the formation and stability of AgNPs synthesized using the fungi Bb and Mb. The absorption spectra peaks of the Bb-AgNPs and Mb-AgNPs synthesized were elucidated in Figures (7 and 8). Figure (7) shows the absorbance bands analysis in bio-reduction of AgNPs synthesized by fungal cell filtrate of B. bassiana were mainly located at 3304.05 cm−1 for O–H hydroxy group, 2358.49 cm−1 for methylene C–H stretch, 1641.42 cm−1 for stretching carbonyl modes of –C–O and –C–O–C, 1396.46 cm−1 for –CH3 nitro compounds, and 1076.28 cm−1 for C-N stretching vibrations of aromatic and aliphatic amines. The band values at 729.24, 680.87, 594.01, and 534.81 cm−1 (aliphatic iodo-compounds, –C–H alkynes, and C–I stretch, respectively) were related to fungi-borne functional groups. On the other hand, the FT-IR spectrum of Mb-AgNPs indicated

absorption peaks located at positions 3437, 1635, 1376, and 1053 cm−1 (Figure, 8). Absorption bands are due to vibration of chemical bonds, and can be assigned to hydroxyl, carbonyl, aliphatic amine, or carboxylic acid groups involved in the reduction of AgNO3 to Ag+. These bending peaks were entirely different from corresponding control samples and may describe the presence of protein molecules involved in the reduction of AgNO3

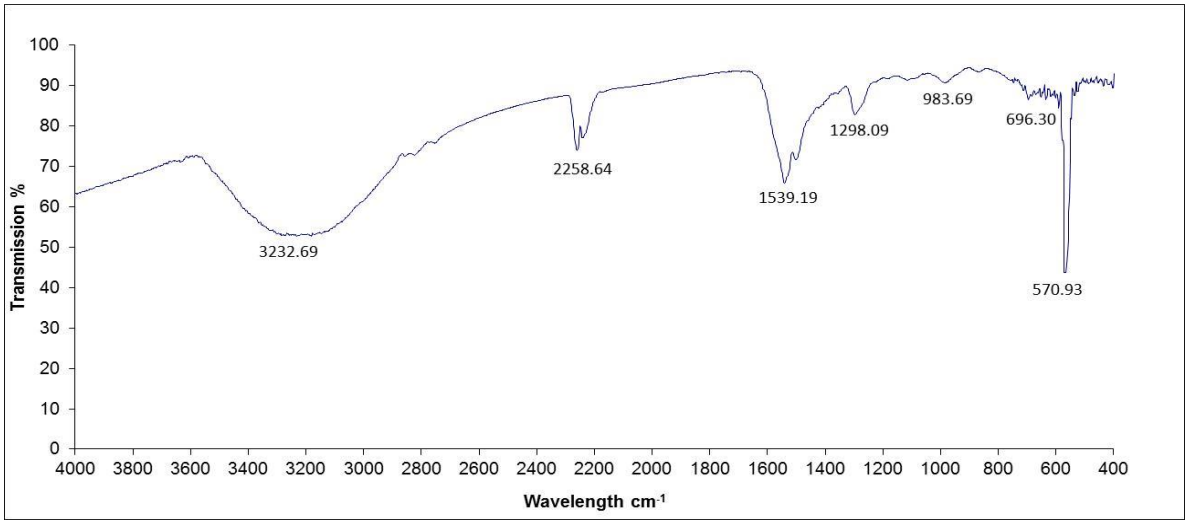


Figure (7): Fourier transform infrared spectrum (FT-IR) of vacuum-dried powder of myco-synthesized silver nanoparticles using *Beauveria bassiana* filtrate solution.

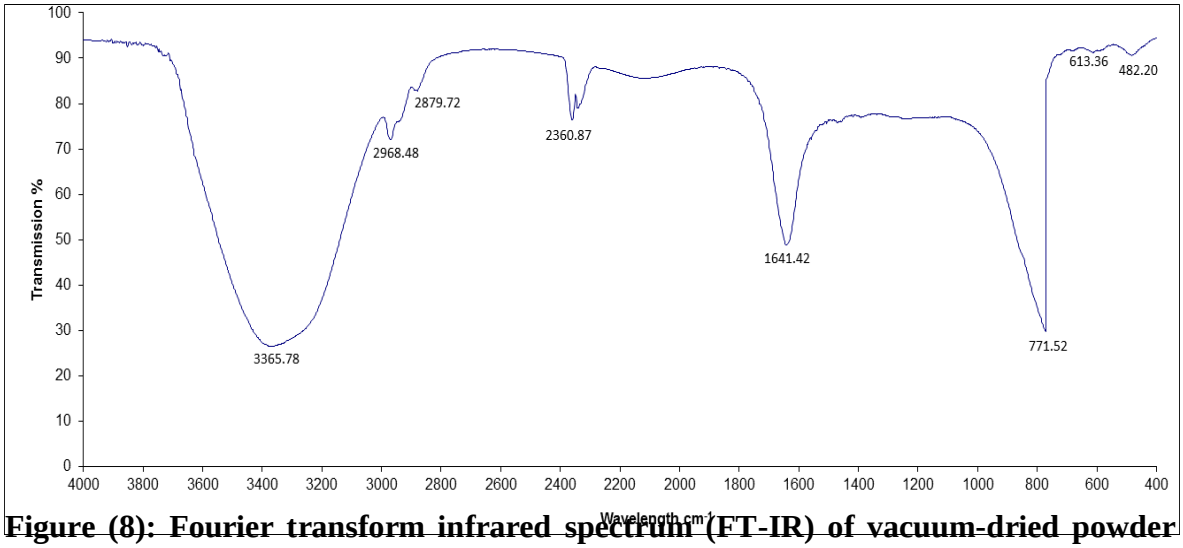


Figure (8): Fourier transform infrared spectrum (FT-IR) of vacuum-dried powder of myco-synthesized silver nanoparticles using *Metarhizium bruennun* filtrate solution.

2. Mitcidal activity of *Bb*-AgNPs *and Mb*-AgNPs:

The efficacy of the myco-synthesized *Bt*-AgNPs and *Bt*-AgNPs treatments against newly adult females of the two-spotted spider mite, (*T. urticae*) were tested based on dosage response data to assess relative LC_{50} values. Experimental conditions and LC_{50} values are noted in Table (1). The LC_{50} values of *Bb*-AgNPs and *Mb*-AgNPs compared after 24 h were estimated to be 35.45 and 41.22 ppm for *T. urticae*, respectively. There were greatly significant differences between adult female mortalities of *T. urticae* at various concentrations of *Bb*-AgNPs produced using *B. bassiana* ($F_{5,71}$ = 16.46, P < 0.0001*)* and *Mb*-AgNPs produced with *M. brunneum* ($F_{5,71}$ = 16.04, P < 0.0001*)*. There were no significant differences between *Bb*-AgNPs and *Mb*-AgNPs ($F_{1,10}$ = 0.16, P < 0.7*)*. However, it is noted that silver nanoparticle treatments using *M. brunneum* were more toxic to the adult female of *T. urticae* than in the case of *Bb*-AgNPs made using *B. bassiana*. The commercially purchased silver nanoparticles (AgNPs) did not kill the tested insects at the applied dosages up to 20 ppm. Furthermore, the highest concentration of 170 ppm of $AgNO_3$ had little or no effect on *T. urticae*. Therefore, it obvious that the efficacy of *Bb*-AgNPs or *Mb*-AgNP*s* against new emerged adult females of *T. urticae* are much more toxic than treatment with *Bb* or *Mb* only.

We desired to create biosynthesis silver nanoparticles with entomopathogenic fungi to investigate the potential use to enhance pest control. As was shown during visual observation, cell free fungal filtrates of Bb and Mb incubated with silver nitrate (AgNO3) showed a color change from yellow to reddish/dark brown. It is noted that the addition of silver nitrate (AgNO3) caused precipitation both inside and outside the microbial cells. We observed reduction of silver ion to form silver particles when silver nitrate was exposed to both fungal species as indicated by the color change of mixtures. Silver nanoparticles exhibit dark brownish color in aqueous suspensions due to the surface plasmon resonance phenomenon, and this color variation confirms formation of the silver nanoparticles. Several studies using different fungal species reported similar trends (Wiley *et al*., 2006; Riddin *et al*., 2006; Ingle *et al*., 2008; Birla *et al*.,2009; Soni and Prakash, 2012; Banu and Balasubramanian, 2014; Amerasan *et al*., 2016 and Kamil *et al*., 2017).

In this finding, formation of silver nanoparticles was investigated by treating silver nitrate with various concentrations of the cell free filtrates of *Bb* and *Mb* to find optimum conditions for the preparation of silver nanoparticles. The relative amount of silver nanoparticles formed in each culture concentration was quantified by using UV-Vis spectroscopy. The optimal production of nanoparticles could be estimated from the UV-Vis spectra that showed the highest integrated intensity (Figures 2 and 3). However, it should be noted that these data contain errors associated with the deconvolution process of raw spectra from the sample solutions. Each spectrum shown in Figures (4 and 5) was obtained by subtracting the spectrum for the culture solution from that for the mixture of culture and silver nitrate solutions. Since the absorbance of the former is much larger than that of the latter, the individual spectrum, especially the spectrum for silver nitrate/*Bt* pellet, was not smooth. As a result, the plot for integrated intensity of spectra showed roughness. DLS data clearly revealed the inflection point, and the physical meaning of this point could be explained by comparing DLS data with UV-Vis data.

The present study emphasizes the use of entomopathogenic fungi for the synthesis of silver nanoparticles with potent biological effect. Several species of fungi have been widely used for the biosynthesis of silver nanoparticle (AgNPs) production, including *Aspergillus fumigates* (Bhainsa and D'Souza, 2006), *Aspergillus niger* (Gade *et al.*, 2008), *Aspergillus oryzae* (Binupriya *et al*., 2010),

Table (1): The insecticidal activity of synthesized of *Metarhizium brunneum* and *Beauveria bassiana* –silver nanoparticles (*Mb*-AgNPs and *Bb*-AgNPs), ideal (standard) silver nanoparticles (AgNPs), and silver nitrate ($AgNO_3$) against newly adult female stage of *Tetranychus urticae*.

Material used	Concentration Ppm	Mortality[a] ±SD	LC_{50}[b] ppm	Fiducial limits		Slope[c]± SD	Chi-square
				Upper	Lower		
Mb-AgNP	150	81.3 ± 3.5	35.45	24.51	51.04	1.20 ± 0.13	6.60
	125	76.3 ± 3.4					
	100	67.1 ± 3.7					
	75	61.7 ± 2.2					
	50	55.4 ± 3.3					
	25	46.3 ± 3.1					
Mb-AgNP	150	77.9 ± 3.7	41.22	31.01	54.65	1.17 ± 0.13	4.85
	125	71.3 ± 2.8					
	100	67.5 ± 3.8					
	75	56.7 ± 2.9					
	50	54.6 ± 2.7					
	25	42.1 ± 3.8					
Mb only	spores mL^{-1}		1.49 E+04	1.82 E+01	9.43 E+06	0.252 ± 0.91	1.03
Bb only	spores mL^{-1}		3.17 E+04	1.01 E+01	8.00 E+06	0.254 ± 0.07	1.03
AgNPs Standard	20	2.9 ± 0.7					
	10	1.3 ± 0.4					
	5	00					
	2.5	00					
Silver nitrate ($AgNO_3$)	170	7.1 ± 1.4					
	17.0	5.0 ± 1.7					
	1.70	3.8 ± 1.1					
	0.17	2.1 ± 0.7					
Water		0.4 ± 0.4					

[a]Total number of adult female *Tetranychus urticae* tested 240 (three repetitions / four replicates per concentration).

[b]Delivered median lethal concentration (LC_{50}) expressed by *Mb*- or *Bb*-AgNPs ppm and estimated by the logistic model. Mortality censored after one-day application.

[c]Slope for mortality represents regression of proportion of adult female mortality versus $\log_{10}$ of *Mb*- *Bb* -AgNPs or *Bb* -AgNPs ppm.

*Chrysosporium tropicum (*Soni and Prakash, 2012*), Cladosporium cladosporioides (Balaji et al*., 2009), *Coriolus versicolor* (Sanghi and Verma, 2009), *Fusarium solani* (Ingle *et al.* , 2009), *Fusarium oxysporum* (Ahmad *et al*., 2003), *Penicillium* species (Sadowski *et al*., 2008), *Phaenerochaete chrysoporium* (Vigneshwaran *et al.*, 2006), *Phoma glomerata* (Birla *et al*., 2009), *Phytophthora infestans, Pleurotus sajor caju* (Nithya and Ragunathan, 2009), *Streptomyces hygroscopicus, Trichosporon beigelii* (Ghodake *et al*., 2011), *Trichoderma reesei* (Vahabi *et al.*, 2011), *Trichoderma viride* (Thakkar *et al*., 2010) and *Verticillium* species (Mukherjee *et al*., 2001).

However, the mechanism of myco-synthesis of the intercellular or extracellular synthesis of silver nanoparticles is represented by two main steps: trapping of Ag^+ ions on the surface of fungal cells and subsequent reduction of silver ions by the enzymes present in the fungal biomass (Mukherjee *et al*., 2001). Promising synthesis of nanoparticles appears by the use of specific enzymes or proteins secreted by fungi, *F. moniliformae* (Duran *et al*., 2005) and *F. oxysporum* (Kumar *et al.*, 2015? and Mohanpuria *et al*., 2008). Balaji *et al*. (2009) hypothesized that proteins, polysaccharides and organic acids released by the fungus *C. cladosporioides* were responsible for formation of spherical crystalline silver nanoparticles. The bioreduction of silver ions occurring on the surface of the cells and proteins might have a critical role in formation and stabilization of the synthesized nanoparticles. FT-IR spectra is an important tool for identifying types of chemical bonds in a molecule by making an infrared absorption spectrum that is like a molecular "fingerprint" (Senapat *et al*., 2004), which are responsible for the reduction of the ag+ ions and capping of the bio-reduced silver nanoparticles using the fungal filtrates. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in Figures (7 and 8). The results confirmed the presence of OH/COO– , -C-O-C- and -C=C- functional groups, which may indicate the presence of possible proteins. The proteins could most possibly form a coating which covers the metal nanoparticles, acting as a capping of silver nanoparticles to prevent agglomeration of the particles and providing stability in the medium.

Overall, our FT-IR spectrum indicates that the biomolecules (possibly proteins or enzymes) present in the fungal filtrates are responsible fr synthesis and stabilization of silver nanparticles (Ganesh Babu and Gunasekaran, 2009 and Dhanasekaran and Thangaraj, 2013).

The lethal activity of *Mb*-AgNPs was significantly greater than *Bb*-AgNPs for *T. urticae*, and the mortality rate was dosage dependent at higher concentrations. The functionality of nanoparticles produced by *Mb* is slightly better than that of nanoparticles produced by *Bb* filtrates. Other studies found that acute toxicity using *B. bassiana* and *M. anisopliae* synthesized silver nanoparticles were effective as mosquitocides (Banu and Balasubramanian, 2014; Amerasan *et al*., 2016 and Prabakaran *et al.*, 2016). Therefore, previous studies confirmed the metal nanoparticles are effective against plant pathogens and insect pests. Thus, nanoparticles could be included in the preparation of new formulations of biopesticides (Goswami *et al*., 2010; Bhattacharyya *et al*., 2010; Rai and Ingle, 2012; Kah and Hofmann, 2014; Roni *et al*., 2015; Benelli, 2017; Sayed *et al*., 2017a and Athanassiou *et al*., 2018).

Our research points out that use of *B. bassiana* and *M. brennum* to synthesize silver nanoparticles is a rapid, eco-friendly and easy approach and the produced myco-synthesized nanoparticles can be useful agents to enhance efficacy of pest control programs. Nanotechnology has potential to provide efficient alternatives for the management of pests in agriculture without harming the environment. However, in-depth studies on toxicological impacts of AgNPs to the environment and assorted life forms need to be performed to verify AgNPs as sustainable

biocontrol agents. There is now increased interest to consider potential issues relating to the use of nanotechnology for crop protection by developing nanopesticides that are less harmful to the environment than conventional formulations, in terms of both cost and performance (Kah and Hofmann, 2014). These novel products may present far more effective control of pests with lower quantities of pesticides, reducing the spray application times and promising improved human and environmental safety. Consequently, they could contribute to enhancement of agricultural productivity involving integrated pest management.

Acknowledgment

The authors thank Dr. Robert Behle for his valuable assistance in this research. This cooperative research was made possible by the Egyptian Ministry for Scientific Research, Science & Technology Development Fund, Agricultural Research Center (ARC), Plant Protection Research Institute (PPRI), Egypt and United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Center for Agricultural Utilization Research (NCAUR), USA.

Disclaimer

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

Ahmad, A.; Mukherjee, P.; Senapat, S.; Mandal, D.; Khan, M.I.; Kumar, R. and Sastry, M. (2003): Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Coll Surf B Biointerface., 28:313–318.

Alexander, J. (2009): History of the Medical Use of silver. Surgical infections,10(3):289-92.

Amerasan, D.; Nataraj, T; Murugan, K.; Panneerselvam, C.; Madhiyazhagan, P.; Nicoletti, M. and Benelli, G. (2016): Myco-synthesis of silver nanoparticles using *Metarhizium anisopliae* against the rural malaria vector Anopheles culicifacies Giles (Diptera: Culicidae. J Pest Sci., 89:249–256.

Athanassiou, C. G.; Kavallieratos, N. G.; Benelli, G.; Losic, D.; Usha Rani, P.and Desneux, N. (2018): Nanoparticles for pest control: current status and future Perspectives. J Pest Sci., 91:1–15.

Balaji, D.S.; Basavaraja, S.; Deshpande, R.; Mahesh, D.B.; Prabhakar, B.K.; Venkataraman, A. (2009): Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Coll Surf B Biointerface, 68:88–92.

Banu, A.N. and Balasubramanian, C. (2014): Myco-synthesis of silver nanoparticles using *Beauveria bassiana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae). Parasitol Res., 113: 2869-2877.

Banu, A.N., and Balasubramanian, C. (2014): Myco-synthesis of silver nanoparticles using *Beauveria bassiana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae). Parasitology Research, 113:2869–2877.

Benelli, G. and Lukehart, C.M. (2017): Special issue: applications of green synthesized nanoparticles in pharmacology, parasitology and entomology. J Clust Sci , 28(1):1–2.

Bhainsa, C.K. and D'Souza, F.S.(2006) : Extracellular biosynthesis of silver nanoparticles using the fungus, *Aspergillus funigatus*. Colloids Surf B,47:160-164.

Bhainsa, C.K., and D'Souza, F.S. (2006): Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids and Surfaces B., 47:160–164.

Bhattacharyya, A.; Bhaumik, A.; Usha Rani, P.; Mandal, S. and Timothy, T.E. (2010): Nano-particles - A recent approach to insect pest control. African Journal Biotechnology, 9(24): 3489–3493. Retrieved from www.ajol.info/index.php/ajb/article/download/82345/72501.

Binupriya, A.R. ; Sathishkumar, M. and Yun, S.I. (2010): Myco-crystallization of silver ions to nanosized particles by live and dead cell filtrates of *Aspergillus oryzae* var. *viridis* and its bactericidal activity toward *Staphylococcus aureus* KCCM12256. Ind Eng Chem Res., 49: 852-858.

Birla, S.; Tiwari, V.V.; Gade, A.K.; Ingle, A.P.; Yadav, A.P. and Rai, M.K. (2009): Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lett Appl Microbiol 48:173–179.

Castro-Longoria, E.; Vilchis-Nestor, A.R. and Avalos-Borja, M. (2011): Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neuropora crassa. Colloids and Surfaces B: Biointerfaces, 83(1):42–48. DOI 10.1016/j.colsurfb.2010.10.035.

Chi, H. (2009): Probit-MSChart: a computer program for probit analysis. http://140.120.197.173/Ecology/ (accessed January 2012).

Dhanasekaran, D. and Thangaraj, R. (2013): Evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector. Asian Pacific Journal of Tropical Disease, 3(3):174–179.

Dhillon, G.S.; Brar, S.K.; Kaur, S. and Verma, M. (2012): Green approach for nanoparticles biosynthesis by fungi current trends and applications. Critical Reviewer in Biotechnology, 32(1), 49-73. doi:10.3109/07388551.2010.550568.

Duran, N.; Priscyla, D.; Marcato, P.D.; Alves, O.; De Souza, G. and Esposito, E. (2005): Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. Journal of Nanobiotechnology, *3,1–7.*

Gade, A.K.; Bonde, P.; Ingle, A.P.; Marcato, P.D. and Durän, N., *et al.* (2008): Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. Journal of Biobased Materials and Bioenergy, 2: 243-247.

Ganesh Babu, M.M. and Gunasekaran, P.(2009): Production and structural characterization of crystalline silver nanoparticles from Bacillus cereus isolate, Colloids and Surfaces B: Biointerface, 74: 191-195.

Ghodake, V.P.; Kininge, P.T.; Magdum, S.P.; Dive, A.S. and Pillai, M.M. (2011): Biosynthesis of silver nanoparticles using *Trichosporon beigelii* NCIM 3326 and evaluation of their antimicrobial activity. J Eng Res Studies, 2:32–36.

Goswami, A.; Roy, I.; Sengupta, S. and Debnath, N. (2010): Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin

Solid Films, 519:1252–1257. doi:10.1016/j.tsf.2010.08.079.

Ingle, A.; Gade, A.; Pierrat, S.; Sonnichsen, C. and Rai, M. (2008): Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Curr Nanosci, 4:141–144.

Ingle, A.; Rai, M.; Gade, A. and Bawaskar, M. (2009): *Fusarium solani:* A novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanopart Res., 11: 2079-2085.

ISO (2010): ISO/TS 80004-1:2010 Nanotechnologies-Vocabulary– Part1: Core terms. International Organization for Standardization, Geneva, Switzerland.

Kah, K. and Hofmann, T. (2014): Nanopesticide research: Current trends and future priorities. Environment International, 63: 224–235.

Kamil, D.; Pramela Devi, T; Ganesh, S. ;Narayanasamy, P.; Kumar, R. N. and Thomas, S. (2017): Green synthesis of silver nanoparticles by entomopathogenic fungus *Beauveria bassiana* and their bioefficacy against mustard aphid (*Lipaphis erysimi* Kalt.). Indian journal of experimental biology, 55(8):555-561.

Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.S.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; Kim, Y.K; Lee, Y.S.; Jeong, D.H. and Cho, M.H. (2007): Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med., 3:95–101.

Kumar, S.; Viney, L. and Deepti, P. (2015): Green synthesis of therapeutic nanoparticles: An expanding horizon. Nanomedicine, 10(15), 2451–2471. doi:10.2217/nnm.15.112

Liu, X.; Atwater, M.; Wang, Q. and Huo, J. (2007): Colloid Surf. B: Biointerfaces, 58: 3–7.

Mohanpuria, P.; Rana, K.N. and Yadav, S.K. (2008): Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res., 10:507–517.

Mukherjee, P.; Ahmad, A.; Mandal, D.; Senapati, S.; Sainkar, S.R.; Khan, M.I.; Ramani, R.; Parischa, R.; Kumar, P.A.V.; Alam, M.; Sastry, M. and Kumar, R. (2001): Bioreduction of AuCl by the fungus, *Verticillium* sp. and surface trapping of the gold nanoparticles formed. Angew Chem Int Ed., 40:3585–3588.

Nithya, R. and Ragunathan, R. (2009): Synthesis of silver nanoparticles using *Pleurotus sajor caju* and its antimicrobial study. Digest Journal of Nanomaterials and Biostructures, 4: 623-629.

Nithya, R., and Ragunathan, R. (2009): Synthesis of silver nanoparticles using *Pleurotus sajor caju* and its antimicrobial study. Digest Journal of Nanomaterials and Biostructures, 4: 623-629.

Owolade, O.F.; Ogunleti, D.O. and Adenekan, M.O. (2008): Titanium dioxide affects disease development and yield of edible cowpea. Electrical Journal Environmental Agricultural Food Chemstry, 7(50): 2942–2947.

Prabakaran, K.; Ragavendran, C. and Natarajan, D. (2017): Mycosynthesis of silver nanoparticles from Beauveria bassiana and its larvicidal, antibacterial, and cytotoxic effect on human cervical cancer (HeLa) cells. Royal Society of Chemistry Advances, 6: 44972-44986.

Rai, M., and Ingle, A. (2012): Role of nanotechnology in agriculture with

special reference to management of insect pests. Applied Microbiology and Biotechnology, 94(2): 287–293. doi:10.1007/s00253-012-3969-4.

Rehner, S.A. and Buckley, E.P. (2005): A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps teleomorphs*. Mycologia, 97: 84–98.

Riddin, T.L.; Gericke, M. and Whiteley, C.G. (2006): Analysis of the inter- and extracellular formation of platinum nanoparticles by *Fusarium oxysporum* f. sp. *lycopersici* using response surface methodology. Nanotechnol, 17:3482–3489.

Roberts, D.W. and St. Leger, R.J. (2004): *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. Advances Applied Microbiology, 54: 1–70.

Roni, M.; Murugan, K.; Panneerselvam, C.; Subramaniam, J.; Nicoletti, M.; Madhiyazhagan, P.; Dinesh, D.; Suresh, U.; Khater, H.F.; Wei, H.; Canale, A.; Alarfaj, A.A. Munusamy, M.A.; Higuchi, A. and Benelli, G. (2015): Characterization and biotoxicity of *Hypnea musciformis*-synthesized silver nanoparticles as potential eco-friendly control tool against *Aedes aegypti* and *Plutella xylostella*. Ecotoxicol Environ Saf, 121:31–38.

Roy, S.; Mukherjee, T.; Chakraborty, S. and Das, T.K. (2014): Biosynthesis, characterization and antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* MTCC8876. Digest J Nanomats Biost., 8:197–205.

Sadowski, Z.; Maliszewska, I.H. ; Grochowalska, B. ; Polowczyk, I. and Kozlecki, T. (2008): Synthesis of silver nanoparticles using microorganisms**.** Materials Science - Poland., 26: 419-424.

Salunkhe, R.B.; Patil, S.V.; Patil, C.D. and Salunke, B.K. (2011): Larvicidal potential of silver nanoparticles synthesized using fungus Cochliobolus lunatus against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). Parasitol Res. doi:10.1007/s00436-011-2328-1

Sandhu, S.S.; Shukla, H. and Shukla, S. (2017): Biosynthesis of silver nanoparticles by endophytic fungi: Its mechanism, characterization techniques and antimicrobial potential. African Journal of Biotechnology, 16(14): 683-698

Sanghi, R. and Verma, P. (2009): Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresource Technology, 100:501–504.

Sayed, A.M.M. and Behle, R.W. (2017b): Evaluating a dual microbial agent biopesticide with *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana* blastospores. Biocontrol Science and Technology, 27 (4) :461-474. DOI: 10.1080/09583157.2017.1303662.

Sayed, A.M.M.; Sanghoon, K. and Behle, R.W. (2017a): Characterization of silver nanoparticles synthesized by *Bacillus thuringiensis* as a nanobiopesticide for insect pest control. Biocontrol Science and Technology, 27: 11, 1308-1326. DOI: /10.1080/09583157.2017.1397597.

Senapati , S.; Mandal, D. and Ahmad, A. (2004): Fungus mediated synthesis of silver nanoparticles: a novel biological approach. Indian J. Physics A., 78A:101–105.

Sharma, V.K.; Yngard. R.A. and Lin, Y. (2009): Silver nanoparticles: Green

synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145:83-96.

Soni, N. and Prakash, S. (2012): Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. Parasitol Res.,110:175–184. DOI 10.1007/s00436-011-2467-4.

Sunkar, S. and Nachiyar, C.V. (2013): Endophytic fungi mediated extracellular silver nanoparticles as effective antibacterial agents. Int. J. Pharm. Pharm. Sci. 5:95-100.

Tarafdar, J.C.; Sharma, S. and Raliya, R. (2013): Nanotechnology: Interdisciplinary science of applications. Afr J Biotechnol, 12:219–226.

Thakkar, K.N.; Mhatre, S.S. and Parikh, R.Y. (2010): Biological synthesis of metallic nanoparticles. Nanomedicine, 6: 257-262.

Vahabi, K.; Mansoori, G.A. and Karimi, S. (2011): Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei* **(**a route for large-scale production of AgNPs). Insci J., 1:65–79.

Vigneshwaran, N.; Kathe, A.A.; Varadarajan, P.V.; Nachane, R.P. and Balasubramanya, R.H. (2006): Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. Coll Surf B Interface., 53:55–59.

Wei, L.; Lu, J.; Xu, H.; Patel, A.; Chen, Z.S. and Chen, G. (2015): Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug Discov Today., 20:595–601.

Wei, L.; Lu, J.; Xu, H.; Patel, A.; Chen, Z.S. and Chen, G. (2015): Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug Discovery Today, 20:595–601.

Wiley, B.J.; Im, S.H.; Li, Z.Y.; McLellan, J.; Siekkinen, A., and Xia, Y. (2006): Manoeuvring the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. J Phys Chem B, 110:15666–15675.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 19-31

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Toxicity of certain essential oils loaded on silica nanoparticles against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults

Abdel-Halim, K.Y. [1] **and Manal A. Attia**[2]

[1]*Mammalian and Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Egypt*

[2]*Insecticide Bioassay Department, Central Agricultural Pesticides Laboratory Agricultural Research Center, Egypt*

ARTICLE INFO

Article History

Received: 20/4/2018

Accepted: 1/6/2018

Keywords

Essential oils,
solid lipid nanoparticles,
alternative pesticides and
stored product insects.

Abstract:

Nano silica was chemically prepared from rice husk and characterized using X-Ray Diffractometer and Electron Dispersive Analysis instruments. The data indicated an amorphous structure with 96.9% of silica. In addition, the synthesized nanoparticles were coated with essential oils (EOs); either clove or peppermint (20%) to form solid lipid nanoparticles (SLNs/EO). Scanning Electron Microscopy (SEM) investigation indicated that, SLNs/EO was almost spherical.

Furthermore, the Fourier Transform Infrared Spectroscopy (FTIR) analysis of these formulations showed asymmetric vibration for Si-O, Si-OH as well as bands of alkyl, hydroxyl and carbonyl groups of EOs. Subsequently, toxicity of EOs and their SLNs/EO formulations were assessed against adults of red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). The mortality was recorded after 5, 7, 10 and 14 days of exposure. The data showed that, SLNs/clove was more toxic than clove oil alone (4.86-folds) after 7 days. On the other hand, no significant differences in the total protein, carbohydrate contents and percentages of germination were observed between treatments and control group. These findings suggest that, SLNs/EO could serve as alternative potent insecticides for controlling stored product insects. However, further researches are required to improve SLNs/EO efficacy and investigate their environmental impact.

Introduction

Post-harvest grain crops are exposed to many insects that decrease their quantity and quality. Despite the fact that, there is no accessible accurate estimation of the amount of grain loss during storage in Egypt, it is believable to range between (10 -20%) (FAO, 2015). Among the stored product insects, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a cosmopolitan and a serious pest of cereal grains and their products. Adult beetles and larvae feed on stored food stuff viz. dry fruits, pulses, bran, coat, germ, grain dust and prepared cereal foods (Khattak and Khatoon, 1999 and Dars *et al*., 2001).

Regarding the insecticide resistance, pesticide residues in food, and health/environment concerns it is obvious that, chemical control is not the appropriate strategy for controlling stored grain pest populations (Debnath *et al*., 2011). As an alternative approached Diatomaceous Earths (DEs) which composed mainly of amorphous silica and derived from fossilized phytoplankton are used against stored product insects (Subramanyam and Roesli, 2000 and Mewis and Ulrichs, 2001). DEs could be more effective against insects if they possessed high silica content, uniform size distribution and number of physical properties which could achieved by alter their size approach to nanoscale (Debnath *et al*., 2011).

Nanotechnology has been offered a powerful tool in modern agricultural practices throughout the past few years (Scott and Chen, 2013). Despite, nanoformulations are widely engaged in pharmaceutical and personal care industry, using nanomaterials in agriculture is still at a rudimentary stage (Anton and Vandamme, 2011 and Ptorchilin, 2006). Lately, nanoparticles have received great attention for controlling pathogens in agriculture (Guan *et al*., 2008; Kim *et al*., 2009 and Elek, 2010). Another alternative is the employing of essential oils (EOs) as potential toxic agents against stored product insects. They showed toxic, repellent and antifeedent effects against stored product insects (Regnault-Roger, 1997; Isman, 2006 and Regnault-Roger *et al*., 2012). Despite these promising properties, problems related to EO volatility, poor water solubility and aptitude for oxidation have to be resolved before application (Moretti *et al*., 2002).

Fortunately, nanoformulation of EOs could conquer these problems protecting them against degradation and evaporation achieving a controlled release of these products and facilitating their handling (Martín *et al*., 2014). Hence, nanoparticles (NPs) represent a new generation of promising technologies that could provide a cost-effective solution for the most challenges and could help to produce new pesticides, insecticides and pest repellents (Owolade *et al*., 2008 and Athanassiou, 2018). Nanopesticides are defined as any formulation that intentionally includes elements in nm range and/or claims novel properties associated with small size ranges (Kah *et al*., 2013). Solid Lipid Nanoparticles (SLNs) are typically spherical with an average diameter (10–100 nm). Since, they possess a solid lipid core matrix that can solubilize lipophilic molecules, while the lipid layers on solid particles is stabilized by surfactants. SLNs were demonstrated as modern approaches in drug delivery and pest control (Scheffel *et al*., 1972). In addition, nanomaterials hold promising properties for application in plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties (Puoci *et al*., 2008). For instance, Wan and Nain (2005) demonstrated that, mixtures of two NPs with insecticides were effective against mites, *Epitrimerus pyri* (Nalepa) (Acari: Eriophyidae). Also, Yng *et al.* (2009) stated that, nanoparticles loaded with garlic essential oil were effective against *T. castaneum*. As well, Stadler *et al.* (2010) showed that, nanoalumina could be successfully used to control stored grain pests and Khoobdel *et al.* (2017) suggested that formulated nano encapsulated essential oils from *Rosmarinus officinalis* were significantly more toxic against *T. castaneum* than the non-formulated oil.

However, few studies had been carried out to investigate the toxic effects of NPs against stored product insects.

The aim of this work is to prepare practical formulas of SLNs/EOs to control *T. castaneum* in comparison with either unaccompanied essential oils or silica salts.

Materials and methods

1. Essential oils:

Peppermint oil was obtained from Elgmhoria Company for Drug and Chemicals, while clove oil was supplied by Al-Kapten Company for Medical Products, Egypt.

2. Rearing insects:

A laboratory-susceptible strain of *T. castaneum* has been continuously reared in the laboratory for more than eight years at the Faculty of Agriculture, Alexandria University. The strain was maintained as described by Beeman *et al.* (2017) on whole wheat flour contains 5% (w/w) brewer's yeast at constant condition (28 °C±1, relative humidity 70±5 and photoperiod L/D 12:12 hr).

3. Nanosilica extraction:

Rice husk was burnt to white ash and the remaining ash was boiled in 2.5M NaOH solution for 3 hr. Then, a solution of 5M H_2SO_4 was added to produce highly purified silica. Subsequently, the continuous refluxing process with 3M HCl was proceeded for 6 hr to obtain nanosilica (SiO_2NPs) powder. Finally, the precipitated NPs were washed and dried at 50 °C for 24 hr (Awizar *et al*., 2013).

4. Solid lipid nanoparticles preparation:

SLNs/EO formulations were prepared using ultrasonic–solvent emulsification technique at 45-50 °C. Twenty percentage (w/w) of EOs were mixed with nanosilicate in diethyl ether (analytical grade) and sonicated for 1 hr. Then, 0.5 to 1% of emulsifier agent was dispersed into the mixture with continuous mixing. Finally, the sample was evaporated to dryness and stored in dissector at 4 °C until used.

5. Solid lipid nanoparticles characterization:

The samples of silica crystals and NPs were subjected to X-Ray Diffractometer (XRD) (APD 2000 PRO, GNR Co., Bonn, Germany), wavelength 1.54 A and scanning time 0.52 per sec, while, Electron Dispersive Analysis (EDA) was performed by X-ray Oxford (model 6647, England), at 5.9 Kev. On the other hand, the prepared formulations were scanned using Scanning Electron Microscopy (SEM) (JEOL, JSM-5300) at the Faculty of Science, Alexandria University. Regarding the active groups of the products, the formulated SLNs/EO was examined by Fourier Transform Infrared Spectroscopy (FTIR) (Tensor 27 Bruker) in comparison with silicate salt absorbance. The samples were ground and mixed with KBr to make pellets. FTIR spectra were obtained by the transmission mode (400 – 4000 cm^{-1}).

6. Susceptibly test:

In 500 ml glass Jars with tight glass cap, 50 g of sterilized wheat grains (Seeds 12) was well mixed with the appreciate weight of each silica salt, NPs and the SLNs/EO formulations by shaking and overturning for 1 min to give the desirable concentration as mg/kg. A series of 6-8 concentrations were tested for each material. The treated jars were left to set for 10 min, and adults of *T. castaneum* (25 beetles) were introduced to each jar and incubated at 28 °C±1, RH 70±5 and L/D 12:12 hr. Each concentration was replicated three times and mortality was recorded after 5, 7, 10 and 14 days of treatment. The LC_{50} values and the regression equations were calculated according to Finney (1971) using LdP Line ® software.

The same procedure mentioned above was followed except that, the appreciate weight of the tested EO was dissolved in 1ml acetone, then applied to 50 g of sterilized whole wheat grains to give the desirable concentration and mixed well as previously mentioned. Afterward, the cap was opened to allow acetone evaporate. After 15 min the procedure was completed as stated previously.

7. Grain quality:

7.1. Humidity determination:

A bulk of 100 g wheat grains treated with LC_{50} either NPs, SLNs/EO formulations or EOs as well as silica salt were stored for two weeks. Samples (2-3 g) from each treatment were weighed, grounded and heated at 130 °C for 1 hr. After that they were kept at room temperature to cool, and weighed again. The moisture content was calculated as percentages (ISTA, 1985).

7.2. Water absorption:

The effect of SLNs/EO formulations at LC_{50} level on the water absorption capacity of the treated grains was determined at the initial time of storage and after 2 weeks (Liu *et al*., 2006). Twenty five g of the treated grains (three replicates each) were immersed in 100 ml of water. The water swelling capacity was expressed as the difference in the weight of seeds before and after the chosen periods post submergence in water.

7.3 .Grain germination:

Sterilized whole wheat grains (50 g) were treated with LC_{50} concentration of each tested material as previously mentioned. The treated grains were stored for 14 days at the same rearing conditions. After the storage period, a number of 100 grain seeds were randomly collected treatment and divided by four. Afterward, each 25 seeds were placed on a moistened slight sheet of cotton in a Petri dish and incubated at 25±1 °C in a dark place. The seeds were sprayed if required with water to keep the moisture. The numbers of germinated seeds were counted after 4 and 7 days and the percentages of germination were calculated (Horwitz, 1980).

7.4. Total protein:

The treated grains and untreated (0.1 g) were extracted by 10 ml of borate buffer (28.63 boric acid+ 29.8 g KCl + 3.5 g NaOH in one litter of distilled water), kept overnight, then centrifuged at 3000 rpm for 10 min, filtered and completed to 10 ml. Protein concentration was determined according to Lowry *et al.* (1951) and expressed as mg/g dry weight.

7.5. Total carbohydrates:

The powder (0.1 g) of the treated and untreated grains was mixed with 4% of NaOH and boiled in water bath for 2 hr. After cooling, the samples were centrifuged at 3000 rpm for 10 min. An aliquot (0.5 ml) was mixed with the same volume of phenol reagent 5% and 2.5 ml of concentrated H_2SO_4 for 30 min at room temperature. The optical density was measured at wavelength 490 nm against blank on Spectronic 21D (Milton Roy Co. USA). Glucose was used as a standard to calculate the extension coefficient and expressed as % of total weight (Dubois *et al*., 1965).

7.6. Lipid peroxidation:

Lipid peroxidation was estimated as malondialdehyde (MDA) content following the method of De Vos *et al.* (1991). Half g of wheat grains from each treatment was homogenized with 5 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 min. Two ml of the supernatant were mixed with 3 ml of thiobarbituric acid (TBA) (0.65% w/v TBA in 100 mM HCl). After cooling, the sample was centrifuged as described before and the absorbance was recorded at 535 nm. MDA was quantified using an extinction coefficient of 156 $mM^{-1}$and its concentration was expressed as mmol/g dry mass.

8. Statistical analysis:

The LC_{50} values and the regression equations were calculated according to Finney (1971) using LdPLine®software. All data were presented as means ±SE and subjected to analysis of variance (ANOVA). The statistical analysis was performed using COSTAT , Costat User Manual, version 3. Cohort Tucson, Arizona, USA (1985).

Results and Discussion

1. Characterization of solid lipid nanoparticles:

The SEM images (Figure 1a) exhibit characteristic sharp edges for SiO_2NPs or agglomerates composed by NPs. Figure 1 (b and c) shows EOs layers loaded on NPs.

Additionally, XRD patterns of the formulated SiO_2NPs and SiO_2 crystals are plotted in Figure (2) showing board peaks in the range of 15-35 with Laser beam 2θ which indicate an amorphous structure. On the other hand, EDA pattern for elemental analysis is plotted in Figure (3) displaying the dominance of Si (96.9%) of the total contents. The FTIR spectra of SLNs/EOs are plotted in Figure (4) where, (a) and (b) show absorption bands at 1045 cm^{-1} and 964 cm^{-1} which may be attributed to the asymmetric vibration of Si-O and Si-OH, respectively. However, the band at 793 cm^{-1} can be referred to the symmetric vibration of Si-O (Beganskienė *et al*., 2004). As well as, the absorption bands of alkyl group are at 2396 and 2873 cm^{-1} (Figure 4, c and d) are attributed to bands of *cis* olefin group or carbonyl group from the EOs. The board absorption bands between 3200 and 3600 cm^{-1} can be attributed to the hydroxyl groups of the EOs and SiO_2. However, the presence of residual silanol (Si-OH) group is frequently observed in many derived materials reflecting the incomplete polycondensation (Lee *et al*., 2009).

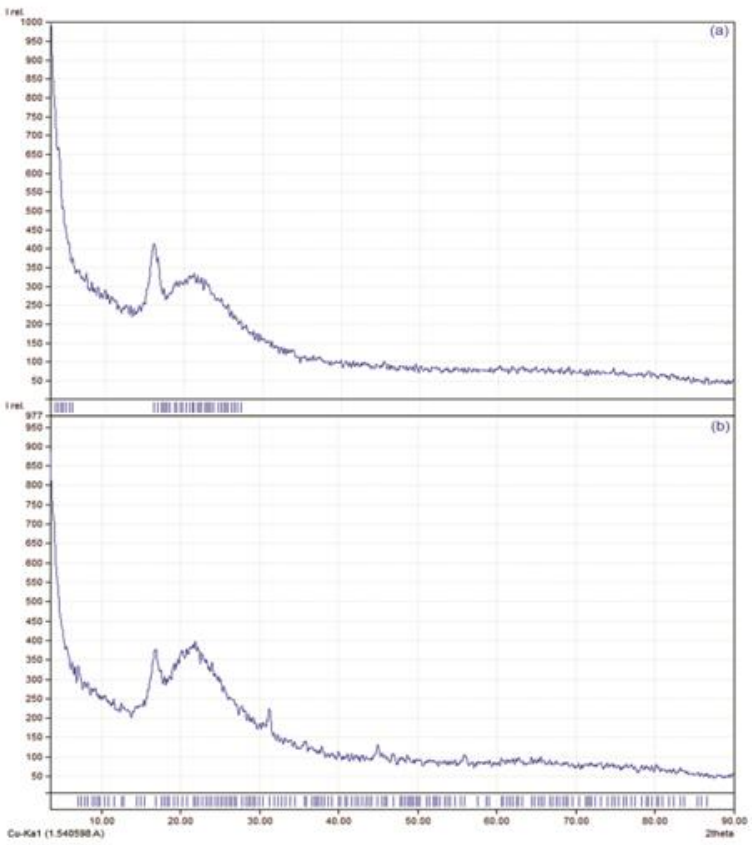
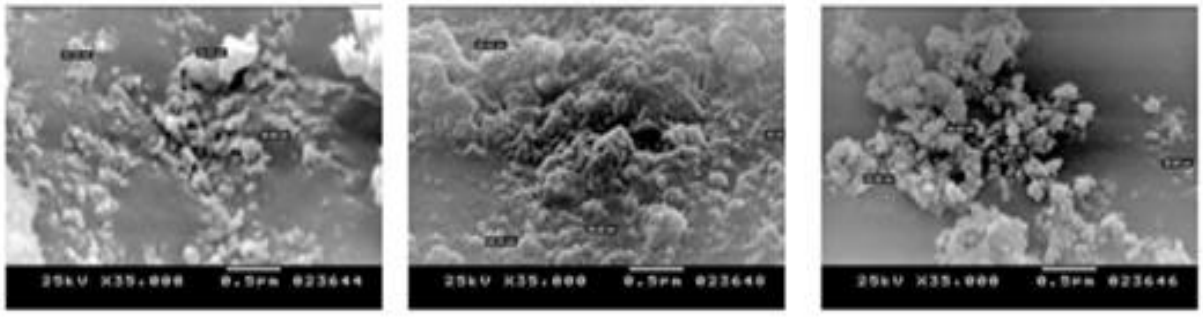


Figure (2): X-ray diffraction pattern graphics of SiO_2 crystals (a) and SiO_2nanaoparticles (b).



(a) (b) (c)

Figure (1): SEM photograph of Silica nanoparticles, NPs (a) and agglomerated nanosilica loaded essential oils peppermint, SNL/Peppermint(b) or clove, SNL/Colve(c).

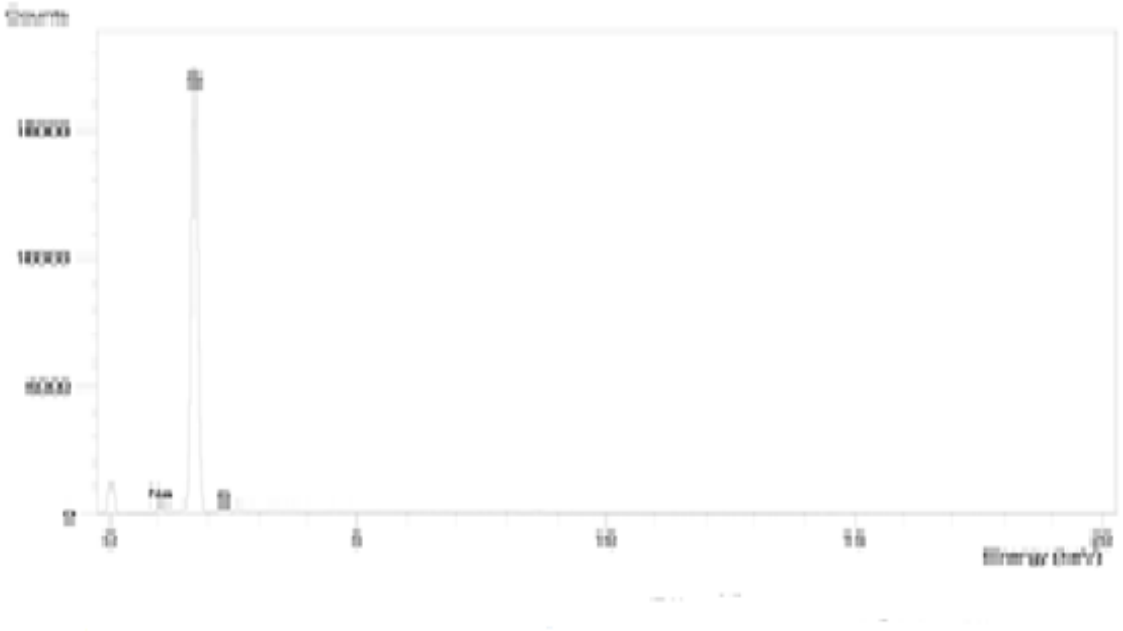


Figure (3): EDA of SiO_2 nanoparticles on X-ray elemental analysis instrument

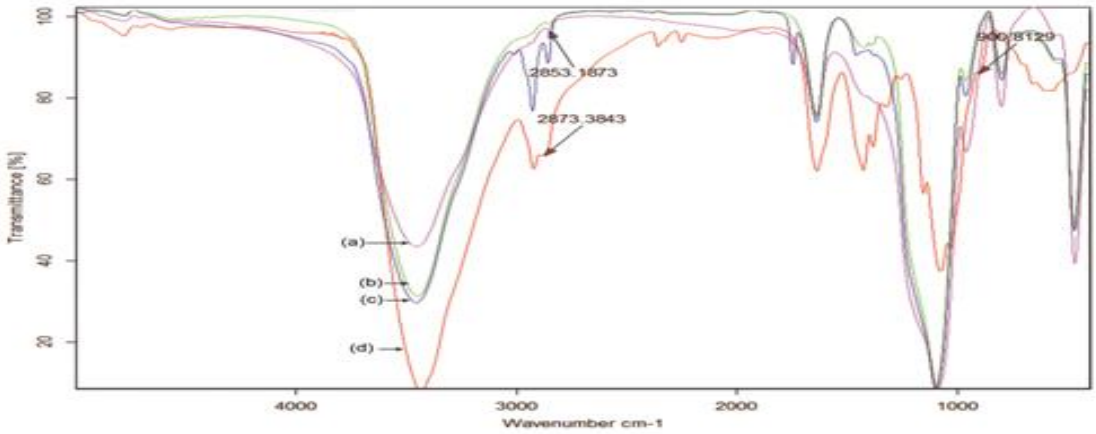


Figure (4): FTIR spectra of SiO_2 crystals (a), SiO_2 nanoparticles, NPs (b), SiO_2 nanaopaticles contained clove oil, SLN/clove of peppermint.

2. Insect susceptibility:

The toxicity values of SLNs formulations and EOs are presented in Table (1). The prepared SLNs/Clove formulation was more toxic to adults of *T. castaneum* compared with clove oil treatment, since LC_{50} values were 1156.26 and 5619.03 ppm after 14 days post treatment, respectively. Despite SLNs/Clove formulation was not effective up to five days after the treatment, its potential employed 4.86 folds after 7 days against clove oil treatment. Moreover, this difference was observed up to 14 days (the end of the experiment). On the other hand, no significant differences were observed between the SLNs/Peppermint formulation and peppermint oil alone. Since the LC_{50} values were 486.63 and 494.35 ppm, after 7 days of treatment, respectively and their confidence limits were overlapped. However, the silica salts and SiO_2NPs were obviously none effective against *T. castaneum* adults up to 50.000 ppm.

Table (1): Acute toxicity of some essential oils and their nanoformulations against *Tribolium castanuem* after different time intervals

Days after Treatment	LC_{50} (ppm)	Confidence limits		Slope	variance	Chi^2
		lower	upper			
5 day						
Peppermint oil	454.05	309.74	541.58	3.67	0.76	0.09
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	500.14	490.23	511.06	13.71	1.33	1.84
SLNs/Clove	None	None	None	None	None	None
7 day						
Peppermint oil	494.35	484.49	505.53	12.71	1.10	4.70
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	486.63	477.79	495.68	15.38	1.37	2.59
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024
10 day						
Peppermint oil	461.78	443.57	480.03	15.28	1.18	9.6
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	464.82	457.85	471.16	16.45	1.18	7.64
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024
14 day						
Peppermint oil	448.72	423.89	470.12	14.94	1.20	14.34
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	464.14	454.09	472.44	20.57	2.09	2.35
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024

.

3. Biochemical quantifications:

The data of moisture contents, water absorption capacity and germination percentages of wheat grains are listed in Table (2) no significant differences of moisture contents were detected neither between all treatments nor the untreated wheat, where the percentages ranged between 10.88 and 11.58%. However, results of water absorption percentages indicated that, all treatments significantly enhanced the water absorption capacity after 5 and 24 hr compared with the control group. SLNs/clove treatment showed the maximum significant improvement of the water absorption capacity (253.53% and 392.40%) compared with the control (237.60% and 3 29.33%) after 5 and 24 hr, respectively. While no significant differences between the entire treatments and control were recorded after 1 hr. SiO_2 and SiO_2NPs showed high germination percentages after 4 days (80.67 and 83.50%, respectively), but no significant differences were observed between them and other treatments after 7 days. Meanwhile, the peppermint oil treatment showed the least germination percentage (67.33%) followed by SLNs/clove treatment (71.33%) and control (92.67%).

On the other hand, the data of total protein, total carbohydrates and lipid peroxidation of the treated grains are listed in Table (3). SLNs/clove formulation caused the highest significant enhancement of protein content (32.55%) but clove oil showed the least significant decreasing (10.16%). The other treatments were in the following order: peppermint oil (25.08%)> SiO_2NPs (23.67%) > SLNs/peppermint (22.28 %)> control (16.61)> and SiO_2 (15.89), respectively. Although the entire treatments showed increase in total carbohydrate contents compared, no significant differences were observed between them and the control group. However, significant increase in total carbohydrate contents was observed as

following SiO_2 (82.23%), clove oil (70.00%), peppermint oil (64.85%) and SiO_2NPs (62.10%), treatments, respectively. In case of peroxidation, malnodialdhyde (MAD) contents, there were no significant differences between SLNs/clove treatment and control (0.0007 mmol/g tissue). However, the highest MAD contents (0.0011 mmol/g tissue), was recorded for SLNs/peppermint treatment, while the least value was recorded for SiO_2 treatment (0.0002 mmol/g tissue).

Obviously, the challenges associated with applying synthetic insecticides such as insecticide

Table (2): Effect of nanoformulations of certain essential oils on moisture, water absorption and germination percentage of wheat grains after different storage periods.

Treatment	Moisture%	Water Absorption%			Germination%	
		After 1 h	After 5 h	After 24 h	after 4 day	after 7 day
Control	$11.32^{a} \pm 0.07$	$120.20^{a} \pm 1.74$	$237.60^{e} \pm 4.00$	$329.33^{f} \pm 10.21$	$78.56^{a} \pm 5.49$	$92.67^{c} \pm 1.66$
SiO_2	$10.88^{a} \pm 0.15$	$113.67^{c} \pm 2.13$	$247.43^{b} \pm 4.01$	$380.00^{d} \pm 9.64$	$80.67^{a} \pm 4.53$	$92.60^{c} \pm 1.48$
SiO_2NP_s	$11.13^{a} \pm 0.03$	$100.47^{e} \pm 3.00$	$240.13^{c} \pm 4.87$	$392.80^{c} \pm 36.34$	$83.50^{a} \pm 1.26$	$87.00^{bc} \pm 1.68$
Clove oil	$11.58^{a} \pm 0.32$	$116.10^{b} \pm 3.27$	$238.20^{d} \pm 1.23$	$394.63^{b} \pm 2.47$	$76.67^{a} \pm 0.95$	$90.50^{c} \pm 0.33$
Peppermint oil	$11.08^{a} \pm 0.04$	$112.43^{c} \pm 1.98$	$240.47^{c} \pm 0.73$	$346.23^{e} \pm 9.23$	$64.17^{a} \pm 0.95$	$67.33^{a} \pm 1.26$
SLNs/ Clove	$11.29^{a} \pm 0.03$	$115.00^{b} \pm 1.51$	$253.53^{a} \pm 3.20$	$392.40^{c} \pm 6.14$	$60.17^{a} \pm 3.50$	$71.33^{a} \pm 0.63$
SLNs/peppermint	$11.04^{a} \pm 0.21$	$105.10^{d} \pm 4.10$	$232.87^{f} \pm 4.03$	$403.30^{a} \pm 29.22$	$76.67^{a} \pm 3.82$	$83.75^{b} \pm 0.96$

Each value represents the mean of three replicates ± SE.
No significant difference obtained of the same letters at 0.05 levels.

Table (3): Biochemical alterations in treated wheat grains

Treatment	Total protein (%)	Total carbohydrates (%)	Lipid peroxidation (mmole/g tissue)
Peppermint oil	$25.05^{b} \pm 1.73$	$64.85^{b} \pm 0.31$	$0.0010^{c} \pm 0.00005$
Clove oil	$10.16^{d} \pm 0.83$	$70.00^{e} \pm 1.80$	$0.0007^{d} \pm 0.00001$
SLNs/peppermint	$22.28^{a} \pm 6.63$	$56.45^{c} \pm 1.31$	$0.0011^{b} \pm 0.00010$
SLNs/clove	$32.55^{a} \pm 3.66$	$66.75^{c} \pm 3.84$	$0.0010^{c} \pm 0.00019$
SiO_2NP_s	$23.67^{b} \pm 2.83$	$62.10^{e} \pm 0.31$	$0.0006^{d} \pm 0.00003$
SiO_2	$15.89^{b} \pm 2.37$	$82.23^{a} \pm 10.01$	$0.0002^{a} \pm 0.00004$
Control	$16.61^{c} \pm 0.27$	$53.60^{c} \pm 2.20$	$0.0007^{d} \pm 0.00002$

Each value is the mean of three replicates ± SE.
The same letters indicate no significant different at 0.05 levels.

resistance, residues in stored products and environment as well as health problems have been necessitated the seeking for more effective and environmentally friendly controlling agents such as Eos (Lorinia and Galleya, 1999 and Zettler and Arthur, 2000). EOs has been known as a natural source of insecticides (Gbolade, 2006). Their lipophilic nature gives them possibility to interfere with an assortment of vital functions of insects (Nishimura, 2001). Nevertheless, their high volatility and poor water solubility are difficulties, which confine their advancement as commercial pesticides. Besides, a principal disadvantage of using EOs as pesticides is their lack of persistence, which required two or more applications to exert a satisfactory management of the pests (Isman *et al*., 2011). Nanoformulation of pesticides aims toward measure releases of the necessary and sufficient amounts of the active ingredients for a period of time to obtain the fullest biological efficacy (Ghormade *et al*., 2011). As well, NPs have chemical activity higher than the bulk material (González and Alicia, 2014). Likewise, several investigators reported that, NPs could be applied to facilitate the management of stored product insects (Goswami *et al*., 2010; Zahir *et al*., 2012 and Rouhani *et al*., 2012). Moreover, it has been demonstrated that, application of SiO_2NPs could significantly increase the mortality of *Sitophilus oryzae* (L.) as a result of increasing the time of exposure (Debnath *et al*., 2011). Therefore, depending on the present results and previous reports the developed SLNs/EO could prove a possible solving for such problems. The prepared SLNs/EO

formulations in this study could protect the EOs through slowing down their rapid evaporation and degradation and successfully improve their stability. This is in agreement with what stated by Lai *et al.* (2006), where they reported that, a formulation of SLNs of *Artemisia arborescence* extract showed controlled release of the EO and decreased its rapid evaporation. Meanwhile, the presented formulations could enhance EOs toxicity *via* increasing their bioavailability as a consequence of the high mobility of the NPs.

The activity of EOs mainly relies on the synergistic effects of their major constituents, where 97.77% of peppermint oil contents are terpenes (Bazargania and Rohloff, 2016), but clove oil contains 85.2% eugenol (Rajkowska, 2016). Hence, it had been demonstrated that, terpenoids have a biological action against several post-harvest Coleopteran insects (Regnault-Roger *et al*., 2012; Sahaf *et al*., 2007 and Tripathi *et al*., 2009). The efficacy of the prepared SLNs/EO formulations against *T. castaneum* adults may be referred to terpenes, which represent the major components of the peppermint and clove oils loaded on NPs (Abdelgaleil *et al*., 2009; Ukeh and Umoetok, 2011 and Zhang *et al.*, 2011). These terpenes may act against insects throughout the interference with nervous system, including γ-aminobutyric acid (GABA)-gated chloride channels, acetylcholine esterase, sodium channels, octopamine receptors, tyramine receptors, nicotinic acetylcholine receptors (nAChR) and others (Tong , 2010). On the other hand, the diffusion and transport processes of the amorphous materials are considerably faster than the crystals (Hancock and Zografi, 1997). Therefore, the toxicity of the developed SLNs/EO formulations could be referred to the high mobility of NPs, which enable to penetrate into insect tissues. The penetration can be achieved either by means of faster penetration through the direct contact with the insect's cuticle, or by ingestion and diffusion through the digestive tract (Margulis and Magdassi, 2012). Despite the fact that, the unusual physicochemical and toxicological properties of NPs are attributed to their small size, chemical composition, and aggregation, yet the high surface area creates the opportunity for increasing the uptake and interaction with a biological target (Nel *et al*., 2006 and 2009).. For example, efficacy of SiO_2NPs against *T. castenum* adults could be attributed to impairment of the digestive tract (Smith, 1969) or to surface enlargement of the integument a consequence of dehydration or blockage of spiracles and tracheas. Also, their enormously increased exposed surfaces could allow more interaction with the insect cuticle resulting damage to insects' protective wax which coat on the cuticle, both by sorption and abrasion (Rouhani *et al*., 2011). NPs display large specific surface, resulting in higher adhesiveness of EO-NPs to insect's body, and increasing the exposure time. Furthermore, the detoxifying oxidation enzymes role had been reported in EOs detoxication process (Rossi *et al*., 2012).

Thus, we can assume that SLNs/EO formulations in particularly the SLNs/clove may decrease the detoxication rate compared with EOs alone, since NPs reserved the oil into the extracellular tissues and allowed it to reach its site of action (Sahaf *et al*., 2007 and Isman, 2000). Hence, the grain quality is an important concern in the stored product insect management. These findings revealed that the prepared formulations enhanced the efficacy of the EOs meanwhile they positively affected the grain quality with the exception of slight effect on the lipid peroxidation. So, further studies are required to determine the mode of action, enhance of the formulation efficacy and improve grain quality.

It is concluded that the benefits of SLNs/EO formulations evaluated in this work are: the efficacy enhancement due to the higher surface area, the lower expected detoxication rate and sustained controlled release. Also, the induction of systemic activity attributable to their smaller particle size, higher mobility and possible lower ecotoxicity are considerable advantages of SLNs/EO. These designed formulations may be useful to promote the massive use of the

EO in stored product insect controlling systems and develop sustainable environmentally friendly controlling agents.

References

FAO (2015): Food and Agriculture Organization of the United Nations Rome, Investment Centre, and Egypt wheat sector review. [Accessed 14 Nov. 2017] http://www.fao.org/3/a-i4898e.pdf .

Abdelgaleil, S.A.M.; Mohamed, M.I.E.; Badawy, M.E.I. and El-Arami, S.A.A. (2009): Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. J. Chem. Ecol., 35: 518–525.

Anton, N. and Vandamme, T.F. (2011): Nano-emulsions and Micro-emulsions: Clarifications of the Critical Differences. Pharm. Res., 28: 978–985.

Athanassiou, C.G.; Kavallieratos, N.G.; Benelli, G.; Losic, D.; Usha, P. and Desneux, R. N. (2018): Nanoparticles for pest control: current status and future perspectives. J Pest Sci, 91:1–15.

Awizar, D.A.; Othman, N.K.; Jalar, A. and Daud, A.R. (2013): Nanosilicate extraction from rice husk ash as green corrosion inhibitor. Int. J. Electrochem. Sci., 8: 1759–1769.

Bazargania, M.M. and Rohloff, J. (2016): Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 61: 156–164.

Beeman, R.W.; Haas, S. and Friesen, K. (2017): Beetle Wrangling Tips. (An Introduction to the Care and Handling of *Tribolium castaneum*). (Accessed 14 February 2017) https://www.ars.usda.gov/plainsarea/mhk/cgahr/spieru/docs/itribolium i-stock-maintenance/. 2009.

Beganskienė, A.; Sirutkaitis, V.; Kurtinaitienė, M.; Juškėnas, R. and Kareiva, A. (2004): FTIR, TEM and NMR Iinvestigations of Stober Silica Nanoparticles. Mater. Sci., 10: 1392–1320.

Costat (1985): Cohort Software Inc. Costat User Manual, version 3. Cohort Tucson, Arizona, USA.

Dars, F.; Rustamani, M.A; Khuhro, R.D. and Baloch, H. (2001): Effect of wheat grain moisture on infestation of red flour beetle, *Tribolium castaneum* (Herbst.). Pakistan J. Zool., 33: 189–200.

De Vos, C.H.R.; Schat, H.; De Waal, M.A.M; Vooijs, R. and Ernst, W.H.O. (1991): Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. Physiol. Plant., 82: 523–528.

Debnath, N.; Das, S.; Seth, D.; Chandra, R.; Bhattacharya, S.C. and Goswami, A. (2011): Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). J. Pest. Sci., 84: 99–105.

Dubois, M.; Gilles, K.; Hamilton, J.; Rebers, P. and Smith, F. (1965): Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350–356.

Elek, N.; Hoffman, R.; Raviv, U.; Resh, R.; Ishaaya, I. and Magdassi, S. (2010): Novaluron nanoparticles: Formation and potential use in controlling agricultural insect pests. Colloids Surf. Physicochem. Eng. Asp., 372: 66–72.

Finney, D.J. (1971): Probit analysis. Third. Cambridge, UK: Cambridge University press.

Gbolade, A.A.; Oyedele, A.O.; Sosan, M.B.; Adewoyin, F.B. and

Soyelu, O.L. (2000): Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. J. Trop. Med. Plants., 1: 146–148.

Ghormade,V.; Deshpande, M.V. and Paknikar, K.M. (2011): Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechn. Advan., 29 : 792-803.

González, J.O.W. and Alicia, A.A.F. (2014): Essential oils nanoformulations for stored-product pest control – Characterization and biological properties. Chemosphere, 100: 130–138.

Goswami, A.; Roy, I. ; Sengupta, S. and Debnath, N. (2010): Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin. Solid Films, 519: 1252–1257.

Guan, H.; Chi, D.; Yu, J. and Li, X. (2008): A novel photodegradable insecticide: Preparation, characterization and properties evaluation of nano-imidacloprid. Pestic. Biochem. Physiol., 92: 83–91.

Hancock, B.C. and Zografi, G. (1997): Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci., 86: 1–12.

Horwitz, W. (1980): Official Methods of Analysis, 13th ed. The Association of Official Analytical Chemists, Washington, DC. 211, 1980.

Isman, M.B. (2000): Plant essential oils for pest and disease management. Crop Prot., 19: 603–608.

Isman, M.B. (2006): Botanical Insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol., 51: 45–66.

Isman, M.B.; Miresmailli, S. and Machial, C. (2011): Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem. Rev., 10: 197–204.

ISTA (1985): International Seed Testing Association. International rules for seed testing. Seed Sci. Technol., 13: 299–513.

Kah, M.; Beulke, S.; Tiede, K. and Hofmann, T. (2013): Nanopesticides: State of Knowledge, Environmental Fate, and Exposure Modeling. Crit. Rev. Environ. Sci. Technol., 43: 1823–1867.

Khattak, S.U. and Khatoon, R. (1999): Insect attack nutritional losses in stored dried fruits. Nucleus., 36: 133–166.

Khoobdel, M.; Ahsaei, S.M. and Farzane, M. (2017): Insecticidal activity of polycaprolactone nanocapsules loaded with Rosmarinus officinalis essential oil in *Tribolium castaneum* (Herbst). Entomol. Res., 47: 175-184.

Kim, S.W.; Kim, K.S. and Lamsal, K. *et al*. (2009): An in vitro study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea sp.* J. Microbiol. Biotechnol., 19: 760–764.

Lai, F.; Wissing, S.A.; Müller, R.H. and Fadda, M.F. (2006): *Artemisia arborescens* L essential oil-loaded solid lipid nanoparticles for potential agricultural application: Preparation and characterization. AAPS Pharm. Sci. Tech., 7: 1–9.

Lee, E.J.; Shin, D.S. and Kim, H.E. *et al.* (2009): Membrane of hybrid chitosan–silica xerogel for guided bone regeneration. Biomaterials, 30: 743-750.

Liu, C.H.; Mishra, A.K. and Tan, R.X. *et al.*, (2006): Repellent and

insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Biores. Tech., 97: 1969–1973.

Lorinia, I. and Galleya, D.J. (1999): Deltamethrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), A pest of stored grain in Brazil. J. Stored Prod. Res., 35: 37–45.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265–75.

Margulis, K.G. and Magdassi, S. (2012): Nanotechnology: An Advanced Approach to the Development of Potent Insecticides. In: Ishaaya I, Palli SR, Horowitz AR. (editors) Advanced Technologies for Managing Insect Pests. New York London. Springer, p. 295-314.

Martín, Á.; Varona, S.; Navarrete, A. and Cocero, M.J. (2014): Encapsulation and co-precipitation processes with supercritical fluids: applications with essential oils. Open. Chem. Eng. J., 5: 31–41.

Mewis, I. and Ulrichs, C. (2001): Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum, Tenebrio molitor*, *Sitophilus granarius* and *Plodia interpunctella*. J. Stored Prod. Res., 37: 153–164.

Moretti, M.D.L.; Sanna-Passino, G.; Demontis, S. and Bazzoni, E. (2002): Essential oil formulations useful as a new tool for insect pest control. AAPS Pharm. Sci.Tech., 3: 64-74.

Nel, A.; Xia, T.; Madler, L. and Li, N. (2006): Toxic Potential of Materials at the Nanolevel. Science, 311: 622–627.

Nel, A.E.; Mädler, L.; Velegol, D.; Xia, T. ; Hoek, E.M.V.; Somasundaran, P.; Klaessig, F. ; Castranova, V. and Thompson, M. (2009): Understanding biophysicochemical interactions at the nano–bio interface. Nat. Mater., 8: 543–557.

Nishimura, H. (2001): Aroma constituents in plants and their repellent activities against mosquitoes. Aroma Res., 2:25–32.

Owolade, O.F.; Ogunleti, D.O. and Adenekan, M.O. (2008): Titanium dioxide affects diseases, development and yield of edible cowpea. Electron. J. Environ. Agric. Food Chem., 7: 2942–2947.

Ptorchilin, V. (2006): Nanoparticulates as drug carriers. In: Vladimir P Torchilin, editor. Nanoparticulates as drug carriers. London: Imperial College Press.

Puoci, F.; Iemma, F.; Spizzirri, U.G.; Cirrillo, G.; Curcio, M. and Picci, N. (2008): Polymer in Agriculture: a Review. Am. J. Agric. Biol. Sci., 3: 299–314.

Rajkowska, K.; Nowak, A.; Kunicka-Styczyńska, A. and Siadura, A. (2016): Biological effects of various chemically characterized essential oils: investigation of the mode of action against *Candida albicans* and HeLa cells. RSC Adv., 6: 97199–97207.

Regnault-Roger, C. (1997): The potential of botanical essential oils for insect pest control. Integr. Pest. Manag. Rev., 2: 25–34.

Regnault-Roger, C.; Vincent, C. and Arnason, J.T. (2012): Essential oils in insect control: Low-risk products in a high-stakes world. Ann. Rev. Entomol., 57: 405–424.

Rossi, Y.E.; Canavoso, L. and Palacios, S.M. (2012): Molecular response of *Musca domestica* L. to *Mintostachys verticillata* essential

oil, (4R) (+)-pulegone and menthone. Fitoterapia., 83: 336–342.

Rouhani, M.; Samih, M.A. ; Aslani, A. and Beiki, K. (2011): Side effect of non-ZnO-TiO_2-Ag mix-oxide nanoparticles on *Frankliniella occidentalis Pergande* (Thy: Thripidae). In: Proceedings, Internat. Symp. Insect Physiol. Biochem. Mol. Biol., Shanghai, China, p. 51.

Rouhani, M.; Samih, M. and Kalantari, S. (2012): Insecticidal effect of silica and silver nanoparticles on the cowpea seed beetle, *Callosobruchus maculatus* F. (Col.: Bruchidae). J. Entomol. Res., 4: 297–305.

Sahaf, B.Z.; Moharrmipour, S. and Meshkatalsadat, M.H. (2007): Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetles. Insect Sci., 14 : 213–218.

Scheffel, U.; Rhodes, B.A.; Natarajan, T.K. and Wagner, H.N. (1972): Albumin microspheres for study of the reticuloendothelial system. J. Nucl. Med., 13: 498–503.

Scott, N. and Chen, H. (2013): Nanoscale Science and Engineering for Agriculture and Food Systems. Ind. J. Biotech., 9: 17–18.

Smith, B.C. (1969): Effects of silica on the survival of *Coleomegilla maculatelengi* (Coleoptera: Coccinellidae) and *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Canad. J. Entomol., 101 : 460–462.

Stadler, T.; Buteler, M. and Weaver, D.K. (2010): Novel use of nanostructured alumina as an insecticide. Pest Manag. Sci., 66: 577-579.

Subramanyam, B. and Roesli, R. (2000): Inert Dusts. In: Subramanyam B, Hagstrum DW. (Editors). Alternatives to Pesticides in Stored-Product IPM. Springer, Boston, MA., p. 321–380.

Tong, F. (2010): Investigation of mechanisms of action of monoterpenoid insecticides on insect gamma-aminobutyric acid receptors and nicotinic acetylcholine receptors. [dissertation] Iowa State University.

Tripathi, A.K.; Upadhyay, S.; Bhuiyan, M. and Bhattacharya, P.R. (2009): A review on prospects of essential oils as biopesticide in insect-pest management. J. Pharmacogn. Phyther., 1: 52–63.

Ukeh, D.A. and Umoetok, S.B.A. (2011): Repellent effects of five monoterpenoid odours against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) in Calabar, Nigeria. Crop Prot., 30 : 1351–1355.

Wan, S.Q. and Nain, G.Z. (2005): Effect of action of mixture of two nano-particles with two insecticides to pest mite (Epitrimerus pyri). Chinese J. Pestic., 44: 570–572.

Yng, F.L.; Li, X.G. and Lei, C.L. (2009): Structural Characterization of Nanoparticles Loaded with Garlic Essential Oil and Their Insecticidal Activity against *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae). Agric. Food Chem., 57: 10156-10162.

Zahir, A.A.; Bagavan, A.; Kamaraj, C.; Elango, G. and Abdul, R. (2012): Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*. J. Biopestic., 288 : 95- 102.

Zettler, J.L. and Arthur, F.H. (2000): Chemical control of stored product insects with fumigants and residual treatments. Crop Prot., 19 : 577–582.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 32-42

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Seasonal activity of fresh water crayfish, *Procambarus clarkii* (Decapoda: Cambaridae) in irrigation canal of Abou-Kabir, Sharkia Governorate, Egypt

Samah M. Abdel-Kader

Agricultural Animal Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Egypt

ARTICLE INFO

Article History
Received: 10/3/2018
Accepted: 15/6/2018

Keywords
Procambarus clarkii,
crayfish,
seasonal activity and
water temperature.

Abstract:

The red swamp crayfish, *Procambarus clarkii* (Girard) (Decapoda: Cambaridae) which was introduced into the Egyptian fresh water system, became widely distributed all over the country. The seasonal activity of *P. clarkii*, was conducted using baited trap in irrigation canal of Abou-Kabir (Gynabeit Bahr Fakous), Sharkia Governorate during the year 2016, through direct counts of active animals caught throughout 24 hours period. The results revealed that the number of caught crayfish fluctuated during the year, the highest catchability (activity) synchronized with high temperature prevailing in summer seasons as compared with those prevailing in spring and autumn seasons. Large crayfish (> 60 mm TL) were the most dominant in catches during the whole search period, whereas medium size (35-60mm TL) were more common in summer than spring and autumn, both sizes were mostly active at night. Small size crayfish (<35mmTL) were more abundant in spring, autumn but absent in summer and were mostly active at daylight. Females were dominant over males during spring, while males were dominant over females during summer and autumn.

Introduction

The red swamp crayfish, *Procambarus clarkii* (Girard) (Decapoda: Cambaridae), native to the south central United States (Louisiana) and north-eastern Mexico. But now, due to introductions by human (Machino *et al*., 2004), it has been transplanted world-wide (Torres and Álvarez, 2012). *P. clarkii* is considered one of the 100 worst alien invasive species (Savini *et al*., 2010), as consequence of deleterious impacts where introduced on native ecosystems (Reynolds and Souty-Grosset, 2012). After the introduction of *P. clarkii* to Egypt in the early 1980's for aquaculture (Ibrahim *et al*., 1995), the crayfish populations have rapidly increased without control, invading the whole area of freshwater ecosystem, i.e., streams, marshes, ponds of fish farms, irrigation canals and ditches, causing complex changes on aquatic communities and the whole ecosystem function (Cruz *et al*., 2008). Its invasive

potential being related to its high adaptability to the new habitats (burrow environments), early maturity, rapid growth rate, high fecundity, aerial exposure, disease resistance, plastic life history traits, polytrophism, and active dispersal capability (Gherardi *et al*., 2011), features that favor its establishment in new available habitats, when other environmental conditions are favorable (Correia, 2002). Soil nature, location and permanence of the water table and food supply, enough cover vegetation, low predators, temperature and light are considered the most important factors for distribution and activity of crayfish within their habitat (Souty-Grosset *et al*., 2014). In natural conditions photoperiod, temperature and water are the most critical factors controlling the most vital activity of aquatic animals (Farhadi and Jensen, 2015). Knowledge of the activity of this invasive *P. clarkii* is of fundamental importance for successful control understanding their invasions and in attempt to mitigate their occurrence and detrimental impacts (Simberloff, 2003). The aim of this research is to study the seasonal activity of *P.clarkii* (size and sex activity) in irrigation canal of Abou-Kabir and to investigate the factors inducing its acclimatization and rapid spread as well as if this species is more active during night (nocturnal) or during daylight hours (diurnal).

Materials and Methods

1. Description of the study areas:

The field experiment was carried out in irrigation canal of Abou-Kabir (Gynabeit Bahr Fakous) district at Sharkia Governorate during 2016 year. Abou-Kabir canal is approximately about 4 Km in length, its width and depth range from 2 to 4 m and 2–3 m, respectively. The bottom is varied from sand and clayey to loam in sediment nature. Due to the permanence of the water table during the whole year (Genabiet Bahr Fakous), the studied canal is surrounded by an extensive bank cover vegetation includes, *Salix tetrasperma, Pulchea dioscroidis,* as well as, *Cynodon dactylon* and *Vossia cuspidate* which create suitable refuges for crayfish. For each sampling date, water temperature was recorded by mercury thermometer at a depth of 50cm (Tolba, 1981). The variances in the seasonal activity of *P. clarkii* during the year were analyzed in accordance with temperature values. Some physicochemical parameters of the water were measurement by spectro-photometric measurements of multi-lab. P5 (WTW) in the laboratory (Table, 1).

Table (1): Some physico-chemical parameters of water at Abou-Kabir district during seasons of 2016.

Chemical analysis	Seasons			
	Winter	Spring	Summer	Autumn
PH	7.1	7.7	7.8	7.6
Temperature (^{o}C)	6	20	27	17
Salinity (ppm)	200	100	100	100
Iron (ml/l)	0.1	0.1	0.1	0.02
Copper (ml/l)	0.1	0.1	0.1	0.01
Nitrite (ml/l)	0.08	0.03	0.4	0.6
Phosphate (ml/l)	0.5	0.4	0.5	0.5
Ammonium (ml/l)	0.1	0.1	0.6	0.1
Dissolved 02 (ml/l)	0.4	0.9	1.4	1.4

2.Crayfish capture:

To determine the daily activity of *P.clarkii*, sampling of the crayfish population was done bimonthly from March to November 2016, using small-mesh (10 mm in diameter), cylindrical trap (Gobbia), that baited with dead fish were placed directly into the bottom at approximately 1.5−2.0 m intervals at sunrise (0600 hr) across the irrigation channel. This trap was emptied at

sunset (1800 hr) and left again in the water overnight till sunrise (0600 hr) and the number of crayfish per-trap were separately and recorded through 24-hr period. Trap was placed on the same position during the whole research period. The crayfish were transferred alive to the laboratory where subsequent data were recorded for each specimen as following: a. the number of specimens for each trap and the total length as in all of the previous studies, (from the anterior tip of the rostrum to the posterior point of the telson) using Vernier caliper. As an index of size; the collected specimens were divided into three groups on the basis of total length (TL), small crayfish (juvenile) if TL < 35mm, medium; immature crayfish if TL 35-60mm and large mature crayfish if TL> 60 mm as reported in previous studies (Cheese *et al.*, 2006).

b. Wet weight was determined using an electronic balance (accuracy 10^{-4} g).

c. The specimens were sexed by the presence or absence of developed gonopodia

Results and Discussion

The seasonal variation in daily activity of *P. clarkii* expressed by the number of crayfish entering trap per 24 hours (daylights and night) in irrigation canal of Abou-Kabir from March to November 2016, was shown in Tables 2,3,4 and 5 and Figures 1,2 and 3. Water quality parameters in the irrigation canal of Abou-Kabir remained within an acceptable ranges throughout the experiment as recorded in Table (1). A total of 744 red swamp crayfish specimens were caught from irrigation canal of Abou-Kabir in one year 2016. All catches constitute as number of specimens, total length and weight, were obtained. During spring 241specimens were caught (daylights 97and night144), in summer 320 specimens (daylights 120 and night 200), whereas catches were relatively decreased in autumn being 183 specimens (daylights 68 and night 115) and no specimen was caught in the winter seasons (Tables, 2, 3 and 4 and Figures, 2 and3). Of the total crayfish counts made during this study: 45small, 108 medium and 591 large (Figure, 1).

In early spring (March) at the average water temperature was 12^0C, a few number of length classes was represented in the trap, with predominance of large individuals (90 to 120 mm TL) with an the body weight (BW) varied between 225 and 630 mg for both sexes.

As the water temperature gradually increased from 16 to 20 ^{0}C in the mid and late spring (April and May) some fairly activity of small size, greenish-gray juvenile (18n. in daylights and 8n. in night, ranged from16 to35mm in TL and BW from 10-34.8mg) and medium size, immature stage (15n. in daylights and 17n. in night, ranged from 40-55mm in TL with BW ranged from 34.8 to 52mg) were found along with the large size, mature (64n. in daylights and 119n. in night, ranged from 80-120mm TL and BW 74-120 mg) (Table, 2). The trap catches during spring months showed a predominance of females (113) compared to males (102) that remained this way until the end of season (Table, 5). Female were slightly more active (74) than males (62) during night while, both sex were similar in its activity at daylights (males 40 and females 39). Juveniles were more active at daylights (18) than nighttime (8) (Table, 5). It can be seen that when the averaged of water temperature was 20^0C in spring, the total catch of *P. clarkii* was consist of individuals with mean TL varying between 16-35, 40-55 and 80-120 mm and BW varying between 10-34.8,34.8-52 and74-120 mg for small, medium and large individuals, respectively (Table, 2).

Table (2): Seasonal variation in daily activity of *Procambarus clarkii* represented by catch per trap at 0600 hr and 1800 hr in irrigation canal of Abou-Kabir during Spring months of 2016 year.

Time of capture	Sex	Number of different size capture/ Gobbia			Total
		Small 16-35mm(TL) 10-34.8mg(BW)	Medium 40-55mm(TL) 34.8-52mg(BW)	Large 80-120mm(TL) 74-120mg(BW)	
Daylights	Juvenile	18	-	-	18
	Male	-	10	30	40
	Female	-	5	34	39
	Total	18	15	64	97
Night	Juvenile	8	-	-	8
	Male	-	8	54	62
	Female	-	9	65	74
	Total	8	17	119	144
Grand total		26	32	183	241

TL: Total length BW: Body weight N:Number of individuals

Table (3): Seasonal variation in daily activity of *Procambarus clarkii* represented by catch per trap at 0600 hr and 1800 hr in irrigation canal of Abou-Kabir during summer months of 2016 year.

Time of capture	Sex	Number of different size capture/ Gobbia			Total
		Small	Medium 40-55mm(TL) 40.6-56.2mg(BW)	Large 60-120mm(TL) 65.6-530mg(BW)	
Daylights	Juvenile	-	-	-	-
	Male	-	8	48	56
	Female	-	14	50	64
	Total	-	22	98	120
Night	Juvenile	-	-	-	-
	Male	-	17	90	107
	Female	-	11	82	93
	Total	-	28	172	200
Grand total		-	50	270	320

Table (4): Seasonal variation in daily activity of *Procambarus clarkii* represented by catch per trap at 0600 hr and 1800 hr in irrigation canal of Abou-Kabir during autumn months of 2016 year.

Time of capture	Sex	Number of different size capture/ Gobbia			Total
		Small 25-35mm 30-45mg	Medium 45-55mm 47.4-56.3mg	Large 60-120mm 75.4-630mg	
Daylights	Juvenile	17	-	-	17
	Male	-	4	28	32
	Female	-	1	18	19
	Total	17	5	46	68
Night	Juvenile	2	-	-	2
	Male	-	12	55	67
	Female	-	9	37	46
	Total	2	21	92	115
Grand total		19	26	138	183

Table (5): Activity of juveniles, males and females *Procambarus clarkii* in daylight and night throughout different seasons of 2016 year

Time of capture	Total catch numbers of active crayfish/Gobbia												Total
	Spring				Summer				Autumn				
	J.	♂	♀	Total	J.	♂	♀	Total	J.	♂	♀	Total	
Daylights	18	40	39	97	-	56	64	120	17	32	19	68	285
Night	8	62	74	144	-	107	93	200	2	67	46	115	459
Total	26	102	113	241	-	163	157	320	19	99	65	183	744

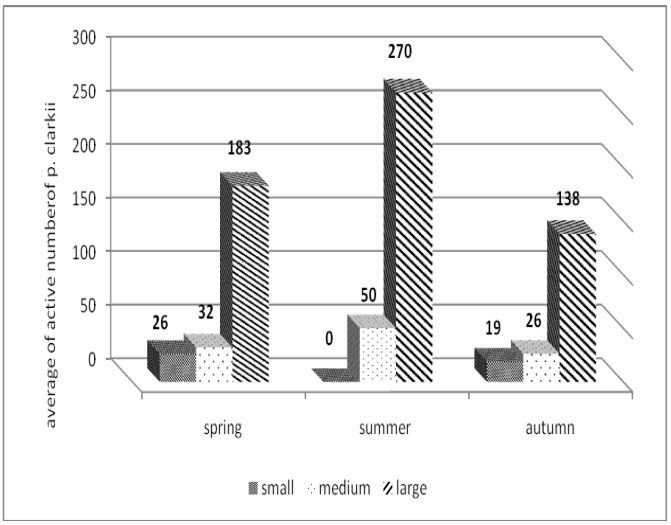


Figure (1): Catchability (activity) of small, medium and large Procambarus clarkii per seasonally during 2016 .

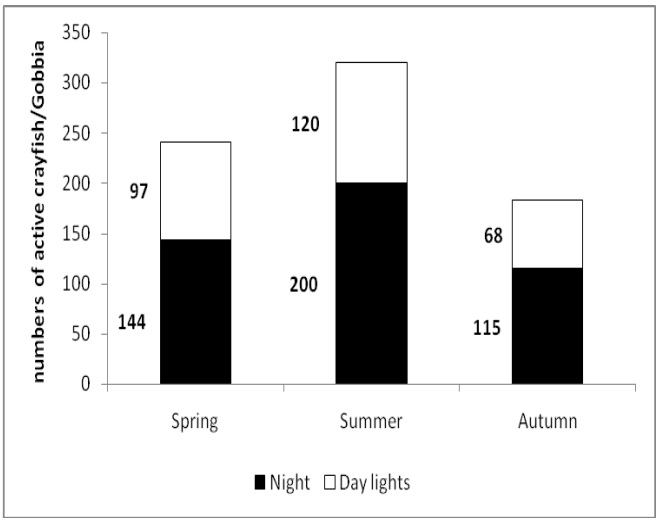


Figure (2): Daily activity of Procambarus clarkii individuals in night and daylights during seasons of 2016.

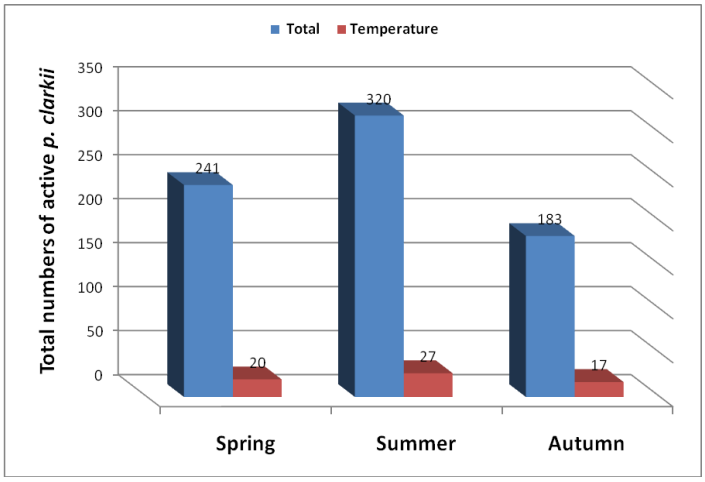


Figure (3): Total catch numbers of active *Procambarus clarkii* in relation to watertemperature°C

In summer, when the average water temperature was increased above 20°C, there was a higher activity for all crayfish individuals of medium and large sizes of both males and females, indicated by increased in the caught number of *P. clarkii* by the traps (n.320) through June to August at the average of water temperature 27°C. Whereas the caught number for medium sizes of males and females were 22 n. in daylights and 28 n. in night. Their TL ranged from 40-55mm and their BW ranged from 40.6 to 52.5 mg and from 41.3 to 56.2 mg in females and males, respectively. On the other hand, the caught number for large sizes of both males and females were 98n. in daylights and 172 n. in night, with TL measuring from 60 to 120mm in males, in females from 60 to 110mm with body weight varied between 69.4 and 530 mg and between 65.6 and 430 mg, for males and females, respectively Table (3). Furthermore, two sexes were more active in night (200n.) than daylights (120n.) as show in Tables (3and5). It can be seen that the trap catches during summer months showed a predominance of males (163n.) that remained this way until the end of autumn (99n.) (Table, 5).

It can be seen that when the averaged of water temperature was 27^0C in summer, total catches of *P. clarkii* was consist of individuals with mean TL varying between 40-55 and 60-120 mm and with BW ranges of 40.6-56.2 and 65.6-530mg for medium and large individuals, respectively (Table, 3).

After a high activity in summer months which extended to early autumn (September), a remarkable decrease in activity occurred when the averaged water temperature gradually decreased from 22 in September to 12 ^{0}C in November, indicated by decreased taken in the traps. At the water temperature 22^0C in mid September, green–gray colored of both juveniles (17n. in daylights and 2 n. in night with 25 -35 TL mm, BW 30-45mg) and medium (5n. in daylights and 21 n. in night with 45-55 TL mm, BW 47.4-56.3mg) were found with the majority were always large males and females,46n. in daylights and 92 n. in night with the total length varied from 60 to 120 mm and BW ranged from75.4-630mg in catches during this time (Table, 4). Adults males are dominant (99n.) over females (65n.) during autumn months (Table, 5). It can be seen that when the average of water temperature was 17^0C in autumn, total catches of *P. clarkii* was consist of individuals with mean TL varying between 25-35, 45-55 and 60-120 mm and BW varying between 30-45,47.4-56.3 and75.4-630 mg for small, medium and large individuals, respectively (Table, 4).

It can be seen that adults (large) dominated all trapping during the different seasons of 2016 year (spring n.183, summer, n.270 and autumn, n.138 individuals/Gobbia) followed by medium (spring n.32, summer, n.50 and autumn,n.26 individuals/ Gobbia), while the lowest small size recorded in spring n.26, autumn,n.19 (individuals/ Gobbia) and absent in summer) Figure (1). With the drop in temperatures of water during the winter months (December to February) no specimen was captured in theses months whereas the mean water temperature was 6°C (the coldest period in the year). During early morning hours in the early of winter (December) two males were observed with very sluggish weak

movement over the substrate of irrigation canal around their burrow.

These results on *P. clarkii* activity in irrigation canal of Abou-Kabir were based on the number of captured crayfish, showed that all of crayfish individuals were active during the whole year except in the winter season and the changes in observed numbers represent changes in activity levels (Tables 2, 3, 4 and 5 and Figures 1, 2 and 3). Temperature has been recognized as one of the main environmental variable influencing the abundance and distribution of many aquatic ectothermal organisms (Lagerspetz and Vainio, 2006).

Data revealed that the increased or decreased of *P.clarkii* activity seem to be directly related with the change in water temperature of irrigation canal during the different seasons of the year, whereas the relatively high water temperature (12-27°C) in irrigation canal throughout most of the year was the suitable range for the activity of *P.clarkii* which has been previously reported to prefer warm water (Espina *et al.*, 1993). Our record for starting the activity of *P.clarkii* at the mean water temperature in spring (12-20°C) is similar to finding of Oluoch (1990) in Lake Naivasha, Kenya recording the mean monthly temperature range for Louisiana *P.clarkii* as being between 15.9 and 20.6°C. Similar results were presented by Gherardi *et al*. (2000) who showed that, at least in spring, more than 50% of *P. clarkii* collected by baited traps in an irrigation canal in Tuscany were active at daytime. The higher trapebility (activity) of *P. clarkii* in summer compared with other seasons is directly related to rising values of water temperature which ranged between 20-27^{0}C (Ackefors, 1999) and due to the fact that the summer is a period of food abundance (Noblitt *et al*., 1995). This finding is similar to what was found by other authors. Trimble and Gaude (1988) reported that the optimum temperature for growth, population structure and the abundance of harvestable crayfish in pond system ranges from 20 to 25°C. Provenzano and Handwerker (1995) reported that temperature should be kept below 30°C for survival of *P. clarkii.* Gherardi *et al.* (2000) in a laboratory reported that the rise of water temperature within the range 5-25°C was associated with the increase of *P. clarkii* locomotion activity. Furthermore, Payette and McGaw (2003) assured that *P. clarkii* were significantly more active at 22°C and avoided water temperature above 30°C and below 12°C. Aquiloni *et al*. (2005) reported that temperature (in some cases also water level) is the most influential factor in the movement pattern of *P.clarkii* and in determining the distribution of crayfish (Bohman *et al.,* 2013). Also similar to those reported for another crayfish species, Maguire (2002) found a positive effect between the water temperature and the number of animals caught for *Austropotamobius torrentium* as well as for *Astacus astacus* (Lucic, 2004). Kalder *et al. (*2016) recorded that the best temperature for culture of Marble crayfish (*Procambarus fallax*) was at 18-25°C, but could withstand temperature below 8°C and above 30°C for many weeks. The gradual reduction in capture of crayfish (low activity in autumn months probably caused by a lowering of the water temperature and the recession of water level (Huner, 2002) or may be a result of the inactive phase during moulting period (Dorr *et al*., 2006) and or mating period which occurred in autumn (Gherardi and Barbaresi, 2000). On the other hand, catchability reach to zero with the drop in temperature of the water during winter seasons (less than 10°C). These low temperatures seem to inhibit the activity of *P. clarkii* (Freitas *et al*., 2010), may also be the result of a suppression of temperature dependent metabolic processes such as heart rate and oxygen consumption rates (Chung *et al.,* 2012). Similar observations were recorded by Gherardi *et al*. (2002) who reported that individuals of *P.clarkii* stop their movement in winter. Crayfish in different size categories were seen throughout the whole year, except in the winter season, large crayfish were predominant in all seasons throughout the year of the study (Gherardi and Barbaresi,

2000), whereas medium size were more abundant in summer than spring and autumn. While small crayfish were abundant in spring and autumn and absent during summer as exactly formerly reported by Correia (2001).

As regard daylights and night individual activities, bimonthly total collection indicated that the night activity was more intense (Gherardi, 2002) for both large and medium size (males and females individuals), possibly as a result of an increase in feeding intensity and burrowing activity that occurred mainly at night (Barbaresi *et al*., 2004) or to avoid daytime predators such as birds and fishes (Aquiloni *et al*., 2005). The noticeable high activity of small crayfish (Juveniles) during daylights may be to avoid nocturnal predators of large ones (Furse *et al*., 2006), mammals (Beja,1996) or the maturation and survivorship require not only a hormonal induction by the longer daylight (Liu *et al*., 2013) but also a hydroperiod longer than four months, a temperature above 18 ^{0}C and pH ranged between 7 and 8 (Gutie´rrez-Yurrita, 1997). The two sexes were nearly equally found in nocturnal and diurnal hours during spring and summer months, similar results were represented by Barbaresi *et al.* (2004).

The finding that adult females were dominant over males during May and June, similarly as Ligas (2008). The dominance of male, compared to females in the trap specimens collected during summer and autumn was due to searching for the reproductive opportunities with females (Furse *et al*., 2004) or the most of females became ovigerous and need suitable shelters to protect their broods (Gherardi, 2002).

References

Ackefors, H. (1999): The positive effects of established crayfish. In: Gherardi F, Holdich DM, editors. Crayfish in Europe as alien species. How to make the best of a bad situation? Rotterdam: A.A. Balkema., pp 49–61.

Aquiloni, L. ; Ilheu, M. and Gherardi, F. (2005): Habitat use and dispersal of the invasive crayfish *Procambarus clarkii* in ephemeral water bodies of Portugal. Mar Freshw. Behav. Physiol., 38:225–236.

Barbaresi, S.; Tricarico, E. and Gherardi, F. (2004): Factors inducing the intense burrowing activity by the red-swamp crayfish, *Procambarus clarkii*, an invasive species. Naturwissenschaften 91: 342–345.

Barbaresi, S. ; Tricarico, E. ; Santini, G. and Gherardi, F. (2004): Ranging behaviour of the invasive crayfish, *Procambarus clarkii*. J. Nat. Hist., 38:2821–2832.

Beja, P. R. (1996): An analysis of otter Lutra lutra predation on introduced American crayfish *Procambarus clarkii* in Iberian streams. Journal of Applied Ecology 33:1156–1170.

Bohman, P.; Edsman, L.; Martin, P. and Scholtz, G. (2013): The first Marmorkrebs (Decapoda: Astacida:Cambaridae) in Scandinavia. BioInvasions Rec., 2: 227–232.

Chiesa, S. ; Scalici, M. and Gibertini, G. (2006): Occurrence of allo-chthonous freshwater crayfishes in Latium (Central Italy). Bull. Fr. Pêche. Piscic., 380-381, 883–902.

Chung, Y.S.; Cooper, R.M. ; Graff, J. and Cooper, R.L. (2012): The acute and chronic effect of low temperature on survival, heart rate and neural function in crayfish (*Procambarus clarkii*) and prawn (*Macrobrachium rosenbergii*) species. J. Mole. Integrat. Physiol., 2: 12.

Correia, A. M . (2001): Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish, *Procambarus clarkii*

(Crustacea, Cambaridae) in a freshwater marsh (Portugal). J. Zool. (Lond.), 255: 533–541.

Cruz, M.J.; Segurado, P.; Sousa, M. and Rebelo, R. (2008): Collapse of the amphibian community of the Paul do Boquilobo Natural Reserve (central Portugal) after the arrival of the exotic American crayfish *Procambarus clarkii*. Journal of Herpetology, 18: 197-204.

Dorr, A.J.M.; La Porta, G.; Pedicillo, G. and Lorenzoni, M. (2006): Biology of *Procambarus clarkii* (Girard, 1852) in Lake Trasimeno. Bulletin Francais De La Peche Et De La Pisciculture, 380(81):1155-1167.

Espina, S.; Diaz-Herrera, F. and Bucle, L.F. (1993): Preferred and avoided temperature in the crayfish *Procambarus clarkii* (Decapoda, Cambaridae). J. Thermal Biol., 18: 35–39.

Farhadi, A. and Jensen, M.A. (2015): Effects of photoperiod and stocking density on survival, growth and physiological responses of narrow clawed crayfish (*Astacus leptodactylus*). Aquaculture Research, 47:2158–2527.

Freitas, V.; Cardoso, J.F.M.; Lika, K.; Peck, M.A. ; Campos, J.; Kooijman, S.A.L.M. and van derVeer, H.W. (2010): Temperature tolerance and energetics: a dynamic energy budget-based comparison of North Atlantic marine species. Phil. Trans. R. Soc. B, 365: 3553–3565.

Furse, J. M.; Wild, C. H., and Villamar, N. N. (2004): In-stream andterrestrial movements of *Euastacus sulcatus* in the Gold Coast hinterland:developing and testing a method of accessing freshwater crayfish movements. Freshwater Crayfish, 14: 213–220.

Furse, J. M.; Wild, C. H.; Sirottii, S. and Pethybridge, H. (2006): The daily activity patterns of *Euastacus sulcatus* (Decapoda: Parastacidae) in southeast Queensland. Freshwater Crayfish, 15: 139–147.

Gherardi, F. (2002): Behaviour. In: Holdich DM (ed) Biology of freshwater crayfish. Blackwell, London, 258–290.

Gherardi, F.; Acquistapace, P.; Tricarico, E. and Barbaresi, S. (2002): Ranging and burrowing behaviour of the red swamp crayfish in an invaded habitat: the onset of hibernation. Freshwater Crayfish, 13: 330–337.

Gherardi, F.; Aquiloni, L.; Diéguez-Uribeondo, J. and Tricarico, E. (2011): Managing invasive crayfish: is there a hope? Aquatic Sciences, 73: 185-200.

Gherardi, F. and Barbaresi, S. (2000): Invasive crayfish:activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). Archiv fur Hydrobiologie, 150: 153–168.

Gherardi, F.; Barbaresi, S. and Salvi, G. (2000): Spatial and temporal patterns in the movement of the red swamp crayfish, *Procambarus clarkii*, an invasive crayfish. Aquatic Sci., 62:179–193.

Girard, C. (1852): Revision of the North American Astaci with observation on their and geographical distribution. Proc. Acad.Nat. Sci. Phila., 6: 87–91.

Gutie´rrez-Yurrita, P.J. (1997): El papel ecolo gico del cangrejo rojo de la marisma, *Procambarus clarkii* (Crustacea: Decapoda: Cambaridae),en el Parque Nacional de Don˜ ana. Una perspectiva bioenerge´ tica con implicaciones en la gestio´ n (Ph.D. thesis). Facultad de Ciencias, Universidad Auto´noma de Madrid, Spain, 380

(InSpanish, with English summary).

Huner, J.V. (2002): *Procambarus*. In: Holdich D.M. (ed.), Biology of Freshwater Crayfish, Blackwell Scientific Press, Oxford, 541–574.

Ibrahim, M.A.; Khalil ,M.T. and Mubarak, M.F. (1995): On the feeding behavior of the exotic crayfish *P. clarkii* in Egypt and its prospects in the bio-control of local vector snails. J.Union. Arab Biol. Cairo, 4(A):321-340.

Kalder,K.; Mezenin,A.; Paaver,T. and Kawai,T.(2016): A preliminary study on the tolerance of Marble crayfish *Procambarus fallax f. virginalis* to low temperature in Nordic climate. Freshwater Crayfish, 4:55–62.

Lagerspetz, K. Y. H. and Vainio, L. A. (2006): Thermal behavior of crustaceans. Biological Reviews, 81: 237–258.

Ligas, A. (2008): Population dynamics of *Procambarus clarkii* (Girard, 1852) (Decapoda, Astacidea, Cambaridae) from southern Tuscany (Italy). Crustaceana, 81: 601–609.

Liu, S.; Gong, S.; Li, J. and Huang, W. (2013): Effects of water temperature, photoperiod, eyestalk ablation, and nonhormonal treatments on spawning of ovary-mature red swamp crayfish. North American Journal of Aquaculture, 75: 228–234.

Lucic, A. (2004): Physiological characteristics of three species of the freshwater crayfish from the Astacidae family. Ph.D Thesis, pp. 122 ,University of Zagreb, Croatia.

Machino, Y. ; Füreder, L.; Laurant, P.J. and Petuschnig, J. (2004): Introduction of the white-clawed crayfish *Austropotamobius pallipes* in Europe. Ber. Naturwiss.-medizin. Ver. Innsbruck, 91: 187–212.

Maguire, I. (2002): Family Astacidae in north-west Croatia. PhD thesis (in Croatian with English abstract),pp.128, University of Zagreb, Croatia.

Noblitt, S.B.; Payne, J.F. and Delong, M. (1995): A comparative study of selected physical aspects of the eggs of the crayfish *Procambarus clarkii* (Girard, 1852) and *Procambarus zonangulus* Hobbs & Hobbs, 1990 (Decapoda,Cambaridae). Crustaceana, 68(5): 575-582.

Oluoch, A.O. (1990): Breeding biology of the Louisiana red swamp crayfish *Procambarus clarkii* (Girard, 1852) in Lake Naivasha, Kenya. Hydrobiologia, 208: 85–92.

Payette, A.L. and McGaw, I.J. (2003): Thermoregulatory behavior of the crayfish *Procambarus clarkii* in a burrow environment. Comp. Biochem. Physiol., 136: 539–556.

Provenzano, A. J. Jr. and Handwerker, T. S. (1995): Effects of photoperiod on spawning of red swamp crayfish, *Procambarus clarkii*, at elevated temperature. Freshwater Crayfish, 8:311–320.

Reynolds J. and Souty-Grosset, C. (2012) : Management of freshwater biodiversity: crayfish as bioindicators. Cambridge: Cambridge University Press, 384 pp.

Savini, D.; Occhipinti-Ambrogi, A.; Marchini, A.; Tricarico, E.; Gherardi, F.; Olenin, S. and Gollasch, S. (2010): The top 27 animal alien species introduced into Europe for aquaculture and related activities. Journal of Applied Ichthyology, 26 (2): 1–7.

Simberloff, D. (2003): How much information on population biology

is needed to manage introduced species? Conserv Biol., 17:83–92

Souty-Grosset, C.; Reynolds, J.; Gherardi, F.; Aquilon, L.; Coignet, A.; Pinet, F. and Del, M.M.M. (2014): Burrowing activity of the invasive red swamp crayfish, *Procambarus clarkii*, in fishponds of La Brenne (France). Ethology Ecology and Evolution, 26: 263–276.

Tolba, M.R. (1981): The effect of water quality on the biology of *Asellus aquatcus* (L)(Crustacea, Isopoda) PH.D.Thesis, Nottingham Uni.England.

Torres, E. and Álvarez, F. (2012): Genetic variation in native and introduced populations of the red swamp crayfish *Procambarus clarkii* (Girard,1852) (Crustacea, Decapoda, Cambaridae) in Mexico and Costa Rica.Aquatic Invasions, 7: 235-241.

Trimble, W. C. and Gaude A. P.(1988): Production of red swamp crawfish in a low-maintenance hatchery. Progressive Fish-Culturist, 50:170–173.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 43-50

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Joint action of certain insecticides by sub lethal dose effect on the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae

Seham M. Ismail

Insect Ppulation Toxicology Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Egypt

ARTICLE INFO

Article History
Received: 5/4/2018
Accepted: 10/7/2018

Keywords
Joint action, indoxicarb, imidacloprid, pyridalyl, lufenuron, cotton leafworm, polyphenol oxidase and chitinase.

Abstract :

Toxicity of the four compounds belong to different groups of insecticides: indoxicarb, imidacloprid, pyridalyl and lufenuron against the 2nd and 4th larval instars of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) . The joint toxic action of lufenuron with tested compounds against 2nd instar larvae *S. littoralis* was studied. The activities of polyphenol oxidase (PPO) and chitinase were also studied. Indoxicarb against 2nd and 4th larval instars (LC_{50} = 0.087 and 0.31 ppm, respectively) was more toxic than lufenuron (LC_{50} = 0.23 and 0.62 ppm, respectively), followed by imidacloprid (LC_{50} = 0.93 and 1.23 ppm, respectively) and pyridalyl (LC_{50} = 1.28 and 3.13 ppm, respectively) after 96 hours of treatment. Lufenuron/ indoxicarb mixtures resulted in potentiation effect more than the lufenuron/imidacloprid mixtures and lufenuron/pyridalyl mixtures respectively, the co-toxicity factor (CTFs) gave potentiation effect with three mixtures tested, the CTF ranged from +62.6 to +20. The *in vivo* interaction of LC_{25} values of each tested compounds, with polyphenol oxidase (PPO) and chitinase caused significant increase in the activities in all treatment. Therefore, mixtures of lufenuron with these tested insecticides can be used for cotton leafworm control. The results generally indicate that indoxicarb, imidacloprid and pyridalyl are a successful insecticides at sub lethal dose, which may be used to prevent or delay appearance of resistance to conventional pesticides and save the environment.

In respect with the joint toxic action, the combination of lufenuron with tested compounds results in a synergistic effect; this will shed some lights in the possible joint toxic action and the sequence of alternative spraying programs. So, indoxicarb, imidacloprid and pyridalyl are a promising compounds in integrated pest control programmes.

Introduction

The extensive use of insecticides to control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae, the major lepidopterous cotton leafworm **(**CLW) infesting more than 150 crop in Egypt, the rate of development to multiple insecticide resistance toward the majority of compounds (Johnson and Gnanadhas 2016). Therefore, the determination of the most effective safe methods and practices for decreasing the level of infestation with CLW on economically important crops, by used a great need to develop noval alternative control agents with new mode of action where they disrupt the development of target pest, or functional combinations of pest control techniques is emphatically a product of this decade to reduce resistance to conventional insecticides (Yongqiang *et al*., 2016). Attention was therefore paid to control insect using different non-traditional insecticides, e.g., insect growth regulators (lufenuron) (El-Helaly and El–Bendary 2015), and **s**elective insecticides with modes of action differed from older classes of insecticides, these insecticides are: indoxicarb, imidacloprid, pyridalyl and lufenuron. If this trend continues, new compounds will be required to replace these insecticides (Haijing *et al*., 2017).

Insecticides mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests attack simultaneously. Mixtures are available as pre-mixes from pesticides companies or they are tank-mixed by farmers. Ideally, the insecticides with different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pests**.** When two compounds are mixed, they can be potentiating, additive or antagonistic in an insect species. These effects can be varied on different insect species or strains depending upon their physiology and the mechanisms of resistance developed. Nowadays, the scientists of pest control and environmental protection oriented their activities to limit the environmental pollution. The efficiency of broad-spectrum neurotoxic insecticides and their mixture with insect growth regulators (IGRs) against the CLW affected several investigators (Bushra *et al*., 2017).

The present work aimed to investigate the effect of pre-treatment of sub lethal dose of lufenuron on the efficacy of indoxicarb, imidacloprid and pyridalyl against 2nd and 4th instars larvae of *S. littoralis.* polyphenol oxidase and chitinase activities as biochemical parameter were studied.

Materials and Methods

1. Test insects:

Larvae of *S.littoralis* were obtained from Central Lab. of Pesticides, Agricultural Research Center, Cairo, Egypt and reared on castor oil leaves under laboratory conditions (27 ± 2 ^{o}C and RH 65 % ± 5) for several years, according to Eldefrawi *et al*. (1964).

2. Test insecticides:

Commercial formulations of indoxicarb (Steward, 15% EC); imidacloprid (Sinodor, 70% W.G) were supplied by Du Pont Co.; pyridalyl (Pleo, 50% EC) was supplied by Sumitomo Chem., Co., and lufenuron (Match, 5% EC) was supplied by Syngenta Co.

3. Toxicity tests:

3.1. Toxicity of insecticides against *Spodoptera littoralis* larvae:

Toxicity of the four-mentioned insecticides against the 2nd and 4th larval instars of *S. littoralis* was evaluated. To assess the insecticidal activity of the tested compound, series of aqueous concentrations for each compounds were prepared using the commercial formulations. The leaf dipping technique was adopted according to Eldefrawi *et al*. (1964) where fresh castor oil leaves were cut into discs (2 cm^2) each disc was dipped for 30 seconds in one of the prepared concentrations. The treated leaves had dried under laboratory conditions before being offer to *S. littoralis* larvae. Ten larvae in three replicated, were used for each concentration. Larvae were fed on leaves immersed in only water as a control. Newly moulted 2nd and 4th larval instars were fed on the treated leaves in

a glass jar covered with muslin for 24 hrs for the tested compound. The treated leaves were replaced by another untreated ones. Mortality percentages were recorded after 24, 48, 72 and 96 hrs of treatment, percent mortality was corrected according to Abbott equation (Abbott, 1925). The LC_{25}, LC_{50} and slope values of the tested compounds where calculated using Finney's equation (1971), through software computer program.

3.2. Joint toxic action of lufenuron with tested insecticides against *Spodoptera littoralis* larvae:

Joint toxic action of lufenuron with tested insecticides (indoxicarb, imidacloprid and pyridalyl) against the 2nd larvae of *S. littoralis* was investigated. LC_{25} of lufenuron was mixed with the LC_{25} of the other insecticides after 96 hrs of treatment. The percent mortality of each mixture was recorded after 24 hrs. The combined effect of the different mixtures was expressed as the co-toxicity factor (CTF) which was estimated according to the equation given by Mansour *et al*. (1966).

4. Biochemical studies:

4.1. Polyphenol oxidase activity assay:

Surviving larvae treated with LC_{25} value of each tested insecticide, after 24 hrs of treatment. The larvae were homogenized in 10 ml of 0.1 M potassium phosphate buffer (pH 7.0) using polytron Kinemetica on ice. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10,000 rpm for 10 min at 4 ^{o}C using Beckman J2-21 hotor centrifuge. The supernatant was used as the crude enzyme extract. The activity of PPO (EC 1.10.3.2) was determined according to Zhi-qing *et al*. (2008) by mixing of 1.5 ml of 0.2 mol/L pyrocatechol, 1.4 mL enzyme extract, respectively. The mixture was incubated at 25 ^{o}C for 25 min and the absorbance was measured λ420 nm by spectrophotometer (Unico 1200- Spectrophotometer, USA). The specific activity of PPO was calculated and expressed as OD_{420}. 30 min^{-1} .mg $protein^{-1}$.

4.2. Chitinase activity assay:

Chitinase (EC 3.2.1.14) is specific hydrolyze enzyme which hydrolyse chitin (chitobiose polymer) to N-acetyl-D-glucosamine (reduced sugar monomer). The specific activity was determined in the surviving larvae treated with LC_{25} value of each tested insecticide, after 24 hrs of treatment. The larvae were homogenized in 0.1 M phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenates were then centrifuged at 5000 rpm for 20 min at 0 ^{o}C. The supernatants were used as enzyme source for chitinase activity assay. Enzyme activity was measured according to Monreal and Reese (1969**)** method. One ml of colloidal chitin, as a substrate, in 5 M citrate phosphate buffer (pH 6.6) was mixed with 1 ml of enzyme extract. Colloidal chitin was prepared by Shimahara and Takiguchi (1988). A suspension containing 1% (w/v) of moist colloidal chitin is prepared in appropriate buffer and pH. The vials were placed sufficient to keep the chitin in suspension. Subsecquently the vials were placed into a boiling water bath for 5 min then were cooled to room temperature by placing the vials in a cold water bath. The reaction mixtures were centrifuged at 5000 rpm for 10 min at 0 ^{o}C. The supernatants was retained. Enzyme activity was assayed by measuring the amount of reducing sugar that produced by enzyme reaction (Miller, 1959). Reducing sugar was determined by mixing of 1 ml of the supernatant with 2 ml phosphate buffer (pH 6.8) and 1.5 ml of 3,5-dinitrosalicylic acid (96 mM, 438 mg of 3,5-dinitrosalicylic acid in 20 mL of deionized water and heat in a boiling water bath to dissolve). The tubes were boiling for 5 min. After cooling the tubes, the optical density (OD) was measured at λ450 nm using a Unico 1200- Spectrophotometer, USA. The specific activity of chitinase was calculated as OD_{450}. mg $^{-1}$ protein. $1h^{-1}$. Blank sample was determined in the manner described above without enzyme solution.

4.3. Determination of total protein:

The Lowry *et al*. (1951) method was used to determine protein content in the supernatant comparing to the standard curve of Bovine Serum Albumin (BSA).

5. Statistical analysis:

All the quantitative estimation of toxicity and biochemical parameters were based on three replicated and the values are expressed as mean ± standard error. The data were statistically analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Mean values were compared using Duncan's multiple rang test (1955).

Results and Discussion

1. Toxicity of tested insecticides against *Spodoptera littoralis* larvae:

Toxicity of the indoxicarb, imidacloprid, pyridalyl and lufenuron against the larval instars were recorded. Tables (1 and 2) shown that the toxicity of indoxicarb was the most effective at LC_{50} level after 96 hours of treatment whereas pyridalyl was the least active with LC_{50} level 0.087; 1.28 ppm for 2nd instar larvae, and 0.31; 3.13 ppm for 4th instar larvae respectively. The lufenuron and imidacloprid gave LC_{50} 0.23; 0.93 ppm for 2nd instar larvae, and 0.62; 1.23 ppm for 4th instar larvae respectively. From these data, it was clear that the toxicity of the tested compounds against two larval instars of *S. littoralis* were increased with the increasing of the exposure time and decreased with the advancement of larval instar**,** also, Von Keyserlink (1988) stated that sublethal concentration of pesticides can provide useful information concerning the basic physiological and behavioral responses of the target insect pest and this could be of high important value when new compounds are evaluated for potential application in pest management program. These results are in agreement with that obtained by many investigators, Cox, 2001; Sakamoto *et al.*, 2005; Lisa, 2015; Silva *et al.*, 2016 and Mushtaq and Sanobar, 2017. They reported that emamectin benzoate is the most potent compound followed by indoxacarb, imidacloprid, pyridalyl and chlorantraniliprole of *S. littoralis*.

Table (1): Toxicity of some insecticides against 2nd larval instar of *Spodoptera littoralis* at different exposure times.

Insecticides	Time (hrs)	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits		Slope values ± SE
				Lower	Upper	
Indoxicarb	24	4.42	19.35	2.91	7.68	1.05 ± 0.22
	48	0.55	2.22	0.25	0.86	1.11 ± 0.18
	72	0.13	0.562	0.027	0.28	1.06 ± 0.20
	96	0.010	0.087	0.001	0.07	0.71 ± 0.21
Imidacloprid	24	7.99	77.55	4.03	56.7	0.68 ± 0.22
	48	1.88	14.42	0.92	3.18	0.76 ± 0.19
	72	0.4704	2.365	0.17	0.80	0.96 ± 0.18
	96	0.19	0.93	0.04	0.39	0.97 ± 0.19
Pyridalyl	24	4.73	49.18	2.51	16.54	0.66 ± 0.20
	48	3.05	19.03	1.81	5.40	0.85 ± 0.20
	72	0.98	8.17	0.32	1.68	0.73 ± 0.18
	96	0.32	1.28	0.12	0.55	1.13 ± 0.19
Lufenuron	24	10.2	88.25	4.98	93.5	0.72 ± 0.23
	48	5.20	56.31	2.72	21.6	0.65 ± 0.20
	72	1.61	11.32	0.77	2.62	0.79 ± 0.19
	96	0.07	0.23	0.010	0.18	1.31 ± 0.27

Table (2): Toxicity of some insecticides against 4th larval instar of *Spodoptera littoralis* at different exposure times.

Insecticides	Time (hrs)	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits		Slope values ± SE
				Lower	Upper	
Indoxicarb	24	3.50	26.54	1.99	7.26	0.77 ± 0.20
	48	1.44	10.83	1.44	0.63	0.77 ± 0.19
	72	0.31	1.21	0.12	0.53	1.14 ± 0.19
	96	0.076	0.31	0.007	0.20	1.09 ± 0.26
Imidacloprid	24	9.66	84.78	4.78	81.9	0.72 ± 0.22
	48	2.32	21.21	1.13	4.33	0.70 ± 0.19
	72	1.42	6.42	0.81	2.09	1.03 ± 0.19
	96	0.33	1.23	0.14	0.55	1.19 ± 0.19
Pyridalyl	24	5.37	55.57	2.84	22.1	0.66 ± 0.21
	48	3.53	26.11	2.03	7.28	0.78 ± 0.20
	72	1.65	12.96	0.75	2.76	0.75 ± 0.19
	96	0.77	3.13	0.39	1.16	1.11 ± 0.18
Lufenuron	24	8.26	98.5	8.55	95.86	0.63 ± 0.21
	48	7.80	75.7	3.96	52.96	0.68 ± 0.22
	72	0.99	9.93	0.28	1.78	0.68 ± 0.18
	96	0.13	0.62	0.023	0.29	0.99 ± 0.19

2. Joint toxic action of lufenuron with tested insecticides against *Spodoptera littoralis* 2nd larvae:

The joint toxic action of lufenuron with the tested insecticides at LC_{25} against *S. littoralis* 2nd larvae is shown in Table (3). It is clear that, all mixtures of lufenuron (at LC_{25}) with the tested insecticides (at LC_{25}) resulted in potentiation effect with co-toxicity factors (CTFs) ranged between +62.6 to +20. The highest potentiation effect was observed, when lufenuron mixed with indoxicarb CTF value was +62.6 while CTFs were +40.4 and +20 when lufenuron was mixed with the imidacloprid and pyridalyl respectively. So, it could be concluded that all tested combinations positive effect, these effect depending upon their different modes of action for these insecticides are mixed on the assumption that they would complement the action of each other for killing the target pest. Also, these mixtures are potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance, in this case, there may be potential for reducing the application rate of one or both components of the mixture, so, favorable to mix lufenuron with tested insecticides. These results were compatible with the results obtained by El-Helaly and El–Bendary 2015 and Bushra *et al*., 2017. They reported that insect growth regulator mixtures with the insecticides resulted in additive effect, these positive effect may due to the insecticides from different chemical groups with different mod of action to act influenced on *S. littoralis*.

Table (3): Joint action for three mixed tested insecticides against 2nd instar *Spodoptera littoralis*.

Combination	Concentration levels[1]	Observed (%)Mortality	CTFs	Type of interaction
Lufenuron + Indoxicarb	$LC_{25} + LC_{25}$	81.3	62.6	Potentiatio
Lufenuron + Imidacloprid	$LC_{25} + LC_{25}$	70.2	40.4	Potentiatio
Lufenuron + Pyridalyl	$LC_{25} + LC_{25}$	60	20	Potentiatio

[1]Concentration level of each insecticide in the paired combination was calculated from its corresponding LC-p lines at 96 hrs of exposure.

3. Influence of tested insecticides on the polyphenol oxidase and chitinase activity in the *Spodoptera littoralis* larvae:

The *in vivo* effects of tested insecticides when applied to the 2nd instar larvae of *S. littoralis* on the PPO and chitinase activities after 4 days of treatment the results are shown in Tables (4 and 5). In general, the PPO activity and chitinase with all treatments significantly decreased compared to the control. The inhibition was recorded with LC_{25} of lufenuron followed by indoxicarb; imidacloprid and pyridalyl with inhibition percentages of 58.97, 43.83, 30.87 and 23.43% of PPO activity respectively. On the other hand, the inhibition percentages of 64.13, 38.63, 27.67 and 21.33% of chitinase activity respectively. Ishaaya and Casida (1974) reported that house fly larvae showed an increase of both the cuticle chitinase and phenoloxidse activities up to about 180 and 155% respectively, when treated with one ppm of the compound TH-6040. Moreover, insect excluded exotic substances by means of melanin. As the key enzyme to compose melanin, PPO was important for insect immunoreaction. Usually, PPO was located in insect blood lymph in the form of hydroxybenzene oxidase, which was activated by specific cascade reaction of serine protease and hydrolysis (Jing *et al*., 1998). Luo *et al*. (2005) reported that, the inhibition of PPO and chitinase by insecticides were concentration dependent. The present results are confirmed by the results of investigators, McKinley, 2002; Merzendorfer and Zimoch, 2003; Liu *et al*., 2014; Xing-Liang *et al*., 2015 and Shi, 2017, where they reported that PPO was important for insect immunoreaction and chitinase plays an essential role during ecdysis chitin, therefore, the PPO and chitinase had become one of the targets of pesticides studies,

Table (4): *In vivo* effect of tested insecticides at their LC_{25} values on polyphenol oxidase activity in larvae of *Spodoptera littoralis* after 4 days of exposure.

Insecticides	LC_{25} (mg./L^{-1})	Specific activity (OD_{420}. min^{-1} .mg $protein^{-1}$)± SE	Inhibition %± SE
Control	--------	17.33±0.612 a	----------
Indoxicarb	0.010	9.17±0.606 b	43.83±0.353
Imidacloprid	0.19	11.67±0.617 cd	30.87±0.809
Pyridalyl	0.32	12.93±0.636 c	23.43±0.740
Lufenuron	0.07	6.53±0.491 b	58.97±0.851

Means in the same column followed by the same letter are not significantly at P = 0.05.

Table (5): *In vivo* effect of tested insecticides at their LC_{25} values on chitinase activity in larvae of *Spodoptera littoralis* after 4 days of exposure.

Insecticides	LC_{25} (mg./L^{-1})	Specific activity (OD_{450}. mg $protein^{-1}$. $1h^{-1}$)± SE	Inhibition %± SE
Control	--------	14.83±0.524 d	----------
Indoxicarb	0.010	7.43±0.996 ab	38.63±0.769
Imidacloprid	0.19	10.43±0.348 ab	27.67±1.39
Pyridalyl	0.32	11.07±0.491 cd	21.33±0.674
Lufenuron	0.07	7.0±0.173 a	64.13±0.120

Means in the same column followed by the same letter are not significantly at P = 0.05.

It is concluded that the results presented, indoxicarb, imidacloprid, pyridalyl and lufenuron are potentially potent insecticides for controlling *S. littoralis*. Their high activity all mixtures of lufenuron with the tested insecticides, so, it is preferred to use these mixtures for controlling *S. littoralis*, which can lead to increase the efficacy of insecticides. The use of these insecticides with low doses may introduce good control results. Such treatments will reduce the used doses of this groups to not effect on non-target organisms and to save the environment, on the other hand, the

alternation between these insecticides can reduce resistance and avoid increasing selection pressure of *S. littoralis* populations to these insecticides.

References

Abbott, W. S. (1925): A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Bushra, S.; Masood, E.; Sarfraz, A. S. and Muhammad, A. (2017): Assessing combined toxicity of conventional and newer insecticides on the cotton mealybug *Phenacoccus solenopsis*. Ecotoxicologym, 26: 9-15.

Cox, C. (2001) : Imidacloprid. J. of Pestic. Reform., 21: 15-21.

Duncan, D. B. (1955) : Multiple rang and multiple F test. Biometerics, 11: 1-42.

Eldefrawi, M. E.; Toppozada, A.; Mansour, N., and Zeid, M. (1964) : Toxicological studies on the Egyption cotton leafworm, *Prodenia litura*. I. Susceptibility to different larval instars of *Prodenia* to insecticides. J. Econ. Entomol., 57: 591-593.

El-Helaly, A. A., and El–Bendary, H. M. (2015) : Field study to evaluate the joint action of certain insecticides, IGR's and baculoviruses against *Spodoptera littoralis* (Bosid.). J. Entomol. Zoology Studies, 3: 289-293.

Finney, D. J. (1971) : Probit analysis, 3rd edn, Cambridge Univ. Press, Cambridge, England. pp: 318.

Haijing, C.; Wenjing, L.; Yuansen, C.; Hui, C.; Yang, Z.; Wenping, X. and Limong, T. (2017): 5′- *Epi* - SPA-6952A, a new insecticidal 24-membered macrolide produced by *treptomyces diastatochromogenes* SSPRC-11339. Natural Product Research, 16: 1-6.

Ishaaya, I., A.; and Casida, J. E. (1974) : Dietary TH6040 alters composition and enzyme activity of housefly larval cuticle. Pestic. Biochem. Physiol., 4: 484-490.

Jing, H. B.; Wang, Y., and Kanost, M. R. (1998) : Pro-phenol oxidase activating proteinase from an insect, *Manduca sexta*, a bacteria-infducible protin similar to *Drosophila easter*. In Proceedings of the National Academy of Sciences of the USA, 95: 12220-12225.

Johnson, S., and Gnanadhas, P. (2016) : Pesticide toxicity to parasitoids: exposure,toxicity and risk assessment methodologies. Pesticide Toxicity to Non-Target Organisms, 99-152.

Lisa, J. B. (2015) : Baseline susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to indoxacarb, emamectin benzoate, and chlorantraniliprole in Australia. J. Economi. Entomology, 108: 294-300. **Liu, X. L.; Gulijimila, M. J. T.; Wang, Z. Y. and Wu, H. Q. (2014) :** Effects of several pesticides on the activities of *in vitro* enzymes in *Clostera anastomosis*. J. Northeast For. Univ., 42: 128–133.

Luo, W. C.; Gao, X. X.; Yu, T. U. and Wang, S. D. (2005) : Inhibitory effect of quercetin on the activity of phenoloxidase in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Acta Entomologica Sinica, 48: 36-41.

Lowry, O. H.; Rosebrough, N. J.; Farry, A. L. and Randall, R. J. (1951) : Protein measurements with folin phenol reagent. J. Bio. Chem., 193: 265-271.

Mansour, N. A.; Eldefrawi, M. E.; Toppozada, A. and Zeid, M. (1966) : Toxicological studies in the Egyption cotton leafworm, *Prodenia litura*. VI. Potentiation and antagonism of

organophosphorus and carbamate insecticides. J. Econ. Entomol., 59: 307-311.

McKinley, N.; Kijima, S.; Cook, G. and Sherrod, D. (2002) : Avaunt (Indoxacarb): a new mode of action insecticide for control of several key orchard pests. Proc. 76th Ann.

Merzendorfer, H. and Zimoch, L. (2003) : Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinase. J. Experimental Biol., 206: 4393-4412.

Miller, G. L. (1959) : Use of dinitrosalicyclic acid reagent for the measurement of reducing sugar. Analytical Chem., 31: 426-428.

Monreal, J. and Reese, E. (1969) : The chitinase of *Serratia marcescens*. Canadian J. Microbiol., 15: 689-696.

Mushtaq, A. and Sanobar, G. (2017) : Susceptibility of armyworm *Spodoptera litura* (Lepidoptera: Noctuidae) to novel insecticides in Pakistan. The Canadian Entomologist, 149: 649-661.

Sakamoto, N.; Ueda, N.; Umeda, K.; Matsuot, S.; Hagat, T.; Fujisawa, T. and Tomigahara, Y. (2005) : Research and development of a novel insecticide "pyridalyl" "Sumitomo Kagaku" Chem. Abst., 1: 133-144.

Shi, X.; Tang, F.; Zhou, X. and Bu, X. (2017) : *In vitro* inhibition of polyphenol oxidase activity by insecticides and allelochemicals in *Clostera anastomosis* (Lepidoptera: Notodontidae) larvae and poplar trees. J. Entomol Sci., 52: 239-247.

Shimahara, K., and Takiguchi, Y. (1988) : Preparation of crustacean chitin. Methods in Enzymol., 161: 417-423.

Silva, T. B. M. ; Silva, W. M. ; Campos, M. R. ; Silva, J. E. ; Ribeiro, L. M. S. and Siqueira, H. A. A. (2016) : Susceptibility levels of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to minor classes of insecticides in Brazil. Crop Protection, 79: 80-86.

Von Keyserlink, H. C. (1988) : The significance of behavior studies in insecticides Research. Pestic. Sci., 16: 558-559.

Xing-Liang, W.; Wen, S.; Jian-Heng, Z.; Yi-Hua, Y.; Ke, D. and Yi-Dong, W. (2015) : Two novel sodium channel mutations associated with resistance to indoxacarb and metaflumizone in the diamondback moth, *Plutella xylostella*. Insect Sci., 19: 537-545.

Yongqiang, L.; Xiangying, L.; Chao, Z.; Feng, L. and Wei, M. (2016) : Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. Sci. Reports., 6: 1-10.

Zhi-qing, M. A.; Xiu-ling, H.; Jun-tao, F.; Guang-ze, L. and Xing, Z., (2008) : Effects of Terpinen-4-ol on four metabolic enzymes and polyphenol oxidase (PPO) in *Mythimna separta* Walker. Agric. Sci. China., 7: 726-730

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 51-57

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Biocontrol of the pomegranate whitefly, *Siphoninus phillyreae* (Hemiptera: Aleyrodidae) by augmentation, releasing and evaluation of *Eretmocerus parasiphonini* (Hymenoptera: Aphelinidae) in Egypt

Shaaban Abd-Rabou

Scale insects and mealybugs Research Department , Plant Protection Research Institute, Agricultural Research Center , Egypt

ARTICLE INFO

Article History

Received: 15/3/2018

Accepted: 1/7/2018

Keywords

Parasitic hymenoptera, pomegranate whitefly, biological control and *Eretmocerus parasiphonini*.

Abstract:

The pomegranate whitefly, *Siphoninus phillyreae* (Haliday) (Hemiptera : Aleyrodidae) is one of the most important pests infested pomegranate in Egypt. The aim of this work was to evaluate the biological control potential of the parasitoid, *Eretmocerus parasiphonini* Evans and Abd-Rabou (Hymenoptera: Aphelinidae) against the pomegranate whitefly, *Siphoninus phillyreae* (Haliday) (Hemiptera: Aleyrodidae) on pomegranate (*Punica granatum* L.) by mass rearing and augmentative releases of this parasitoid during 2011-2014 in Egypt. This parasitoid species were mass reared and monthly releases were made in the fields of pomegranate during each of three consecutive years (2011-2014). About 142578 *E. parasiphonini* individuals were released in fields in Assuit, Daqahylia and Giza governorates in Egypt on pomegranate which were naturally infested by *S. phillyreae.* Populations of the parasitoid and parasitism were much higher in field plots where releases were made as compared with where no releases were made. The maximum rate of parasitism reached 48.9, 42.1 and 46.7 % in Assuit, Daqahylia and Giza governorates, respectively in the field treatment where releases were made, while parasitism peaked at 2.6, 4.6 and 2.5% in Assuit, Daqahylia and Giza governorates, respectively where no releases were made. These observations indicated that *E. parasiphonini* is a promising bioagent in controlling *S. phillyreae* in Egypt.

Introduction

Recently, the pomegranate whitefly. *Siphoninus phillyreae* (Haliday) (Hemiptera : Aleyrodidae) is the most important pest of pomegranate in Egypt. Pomegranate leaves infested with *S. phillyreae* have a demand for fluid transport substantially increased beyond the tree's normal capacity to respond. The loss of phloem fluids certainly represents a loss of potential productivity and heavy infestation caused leaf wilt, early leaf drop and smaller fruit (Abd-Rabou, 1998 and 2001b). This pest attacking 60 host economic

plant species including, apple, pear, citrus and olive. It distributed in Palearctic region (Bellows *et al.,* 1990). *Eretmocerus parasiphonini* Evans and Abd-Rabou (Hymenoptera: Aphelinidae) is recorded associated with *S. phillyreae* in Egypt for the first time, 2002 and corrected to be a valid name, 2004 (Abd-Rabou and Evans, 2002 and 2004). Abd-Rabou and Abou-Setta (1998) recorded seven parasitoids attacking *S. phillyreae* these are *Encarsia davidi* Viggiani and Mazzone, *E. galilea* Rivany, *E. inaron* (Walker), *E.lutea* (Masi), *Eretmocerus corni* Haldeman, *E. diversicilatus* Silvestri and *E. mundus* Mercet. They stated that *E. inaron* is the effective parasitoid attacking this pest with maximum parasitism percent of 78%. Biological control of the pomegranate whitefly has been attracted many scientists of the world ex. McDonald *et al.*(1996), Hackney *et al.* (1997), Abd-Rabou (1998 and 2006) and Abd-Rabou and Simmons (2010).

The present work deals with the biocontrol of *S. phillyreae* by using augmentation, releasing and evaluation of *E. parasiphonini* in different localities in Egypt.

Materials and methods

Mass rearing of the parasitoid: In the laboratory, the parasitoid *E. parasiphonini* was successfully mass reared on the infestation of *S. phillyreae* that were feeding on pomegranate (*Punica granatum*) ((According to the method of Abd-Rabou, 1998). Approximately 142578 adults of this parasitoid were released (Tables 1- 3) in Assuit, Daqahylia and Giza governorates in fields of pomegranate which were naturally infested with *S. phillyreae*. Releases were made during each of 3 consecutive years (2011-2014). From August to July parasitoids were released each year. Within a given year, similar numbers of parasitoids were released each month. The parasitoids were released as adults from containers (vials or cups) which were attached to pomegranate trees about 0.21 hectares. One container of 20-30 parasitoids was released per tree by allowing parasitoids to fly or walk from the containers. Half of the field (0.21 hectares) was used as a control and no release was made in this field plot.

Assessments of released parasitoid were estimate through dissection of recovered samples. Cardboard containers, 0.5-liter with ventilated tops, were used to hold samples for two weeks at 25-29˚C. The samples were 600 pomegranate leaves (4replications) each replicate was 150 leaves. This was achieved by holding 150 pomegranate leaves in each container. All materials found at the bottom of the rearing containers were examined for dead stages of pomegranate whitefly and the parasitoid, *E. parasiphonini*. The parasitoid was identified by comparison with voucher specimens. Leaf samples were collected at the beginning of every month from Sep. to Aug. in 2011-2014. The samples were taken after each monthly release. For each month of sampling, 50 trees were sampled in the parasitoid release plot and 50 trees were sampled in the control plot.

Percent parasitism was defined as: Percent parasitism = [number of prepupae, pupae, and adult parasitoids / (number of *S. phillyreae*, excluding eggs and first larval instars + number of prepupae, pupae, and adult parasitoids)] x 100. Some time was expected to elapse before the maximal level of impact from this parasitoid could be observed on the target pest.

Results and Discussion

The release of approximately 142578 adult *E. parasiphonini* in the fields on pomegranate resulted in elevated parasitism by this species for each year from 2011 to 2014 as compared with the control fields plots wherein no releases were made (Figures 1-9). The maximum rate of parasitism by *E. parasiphonini* (48.9, 42.1 and 46.7%) was attained in September and July 2013-2014 in the release plot in Assuit, Daqahylia and Giza governorates, respectively. Parasitism gradually increased in May and peaked during September or July of each year, but was also high in June and August of each

year. The peak in parasitism was due to higher populations of *E. parasiphonini* in the field **.** Overall seasonal populations of *S. phillyreae* (including parasitized and non-parasitized individuals) were higher in August - September followed by a decrease over the February - April of the study. For example, the percent parasitism during the last year was high the percent parasitism observed during the first year. This trend occurred for both the control plots and the insect release plots. The statistical analysis between the differences of increase after releasing the parasitiod during the three years under consideration SE. and SD were 62.81 and 108.8, respectively, in Assuit, While in Daqahylia and Giza were 25.36, 43.9 and 48.65, 84.3, respectively. These results showed that the incearse of realeasing parasitids individuals followed by increasing of persent parasitism in the three regions under considerations.

Viggiani and Battaglia (1983), Bellows *et al.* (1990) and Gould *et al.* (1992) studied the population dynamics , parasitoids and predators of *S. phillyreae* in California and Italy , respectively. Biological control of pomegranate whitefly, *S. phillyreae* studied by Viggiani and Mazzone (1980), McDonald *et al.* (1996), Hackney *et al.* (1997) and Abd-Rabou and Simmons (2010). Abd-Rabou (1998) studied the indigenous parasitoids of *S. phillyreae*, from different localities in Egypt were manipulated, reared and mass produced for classical biological control in Upper Egypt, more than 82,019 parasitoids were released. Several releases were made between July to October in both 1995 and 1996. Releases of the following indigenous parasitoids of the pomegranate whitefly in Upper Egypt*: Encarsia inaron* (Walker), *Eretmocerus mundus* (Mercet), *Encarsia lutea* Masi, *Eretmocerus corni* (Haldeman), *Encarisa davidi* Viggiani, *Encarsia galilea* Rivnay and Gerling and *Eretmocerus diversicilatus* Silvestri (Hymenoptera: Aphelinidae). Increases of the rate of parasitism from 6 to 67% indicate that En. inaron is the most effective parasitoid in controlling *S. phillyreae* in Egypt. Other parasitoids found associated with *S. phillyreae* in other localities in Egypt were manipulated and released in Upper Egypt. Some of these parasitoids became established in the release areas. Here recorded new parasitoid *E. parasiphonini*, also increased after more releasing and established in new are in Egypt. The host plants, distribution, parasitoids, predators and biological control studies were carried out in Egypt by Abd-Rabou, 1997, 1999, 2001a, 2002 2003, 2006, Abd-Rabou and Abou-Setta, 1998 and Abd-Rabou and Ahmed, 2006 and 2007.

The role of parasitoids in controlling *S. phillyreae* with augmentation releases was conducted in different parts of the world, India and USA (Mani and Krishnamoorthy, 1995; Hackney *et al.* ,1997; Bellows *et al.* ,2007). Pickett and Pitcairn (1999) stated that the released of the parasitiod, *E. inaron* rapidly established populations and spread throughout areas occupied by ash whitefly. The dispersal and overwintering ability could play a role in the extraordinary success of this parasitoid and we measured the impact of released parasitoids using a new method at a single location in northern California. This result agree with the data recorded here which showed that the increase of releasing parasitiods individuals followed by increasing of percent parasitism in the three regions, Assuit, Daqahylia and Giza.

Table (1): Total numbers of the adult parasitoid, *Eretmocerus parasiphonini* released in different fields of pomegranate in Assuit in Egypt during each year from 2011 to 2014.

Year	Number of released *Eretmocerus parasiphonini* individuals by *Siphoninus phillyreae*												
	Months												
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Total
2011-12	1120	1555	1230	1390	1540	1700	1415	1380	1400	1365	1250	1125	16470
2012-13	1240	1212	1105	1530	1650	1510	1504	1430	1220	1310	1210	1350	16271
2013-14	1324	1025	1005	1690	1555	1620	1320	1510	1410	1565	1310	1210	16544

Table (2): Total numbers of the adult parasitoid, *Eretmocerus parasiphonini* released indifferent fields of pomegranate in Daqahylia in Egypt during each year from 2011 to 2014.

Year	Number of released *Eretmocerus parasiphonini* individuals by *Siphoninus phillyreae*												
	Months												
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Total
2011-12	1210	1321	1410	1114	1324	1321	1302	1014	1335	1300	1411	1100	15162
2012-13	1150	1001	1113	1320	1441	1422	1116	1212	1078	1240	1310	1321	14724
2013-14	1104	1012	1124	1421	1341	1521	1246	1720	1312	1500	1410	1256	15967

Table (3): Total numbers of the adult parasitoid , *Eretmocerus parasiphonini* released in different fields of pomegranate in Giza in Egypt during each year from 2011 to 2014.

Year	Number of released *Eretmocerus parasiphonini* individuals by *Siphoninus phillyreae*												
	Months												
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apri	May	June	July	Total
2011-	1115	1500	1120	1240	1340	1501	1324	1240	1365	1300	1242	1360	15647
2012-	1223	1245	1135	1450	1450	1420	1421	1450	1265	1335	1255	1342	15991
2013-	1300	1009	1145	1501	1235	1510	1229	1325	1478	1500	1325	1245	15802

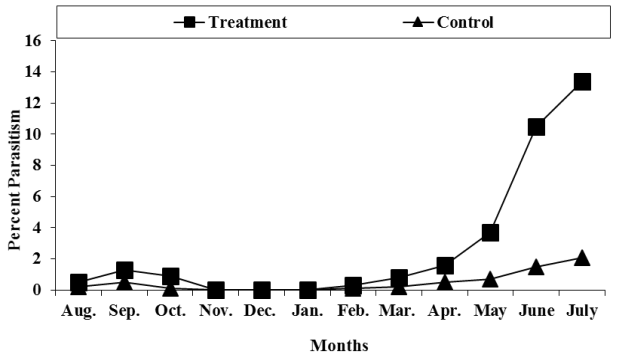


Figure (1): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Assuit during 2011-2012.

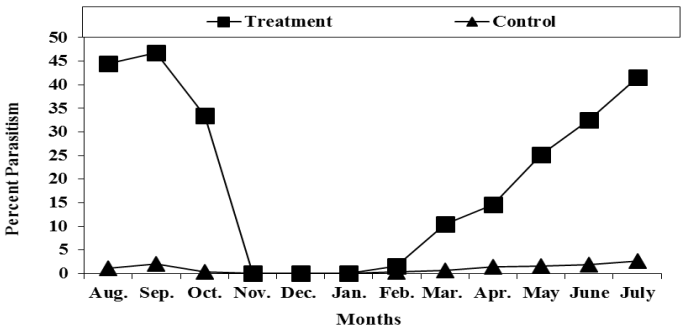


Figure (2): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Assuit during 2012-2013.

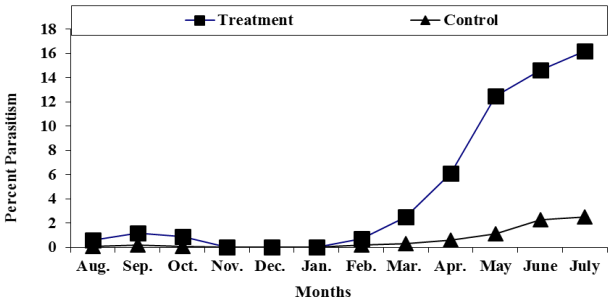


Figure (4): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Daqahylia during 2011-2012.

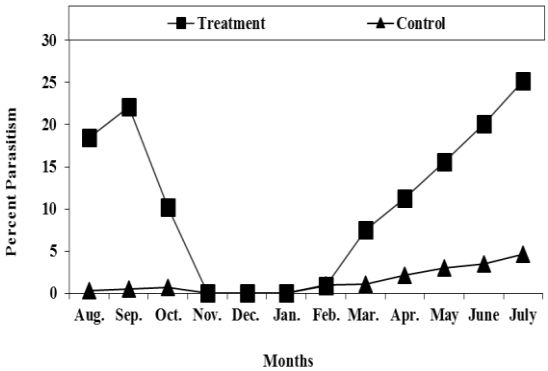


Figure (5): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Daqahylia during 2012-2013.

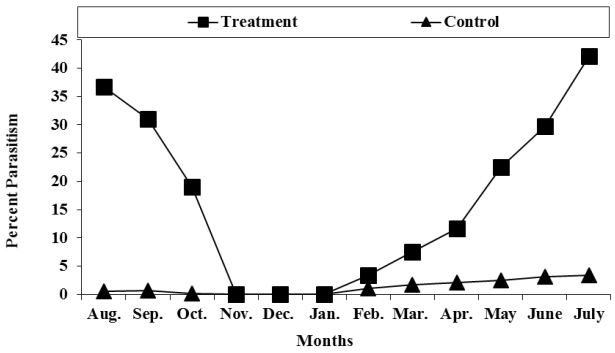


Figure (6): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Daqahylia during 2013-2014.

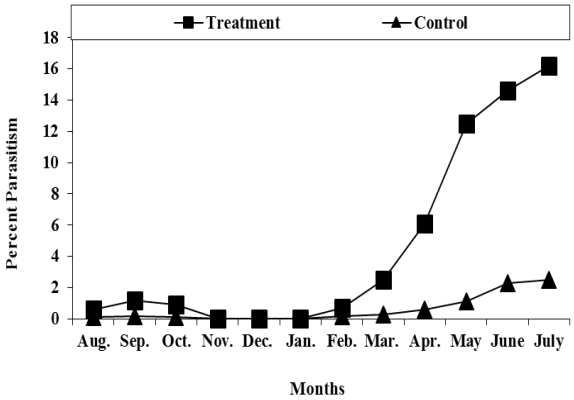


Figure (7): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Giza during 2011-2012.

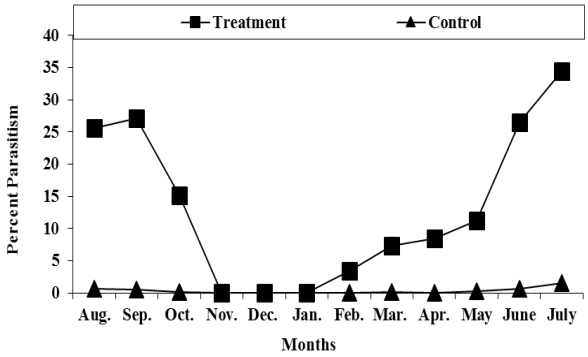


Figure (8): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Giza during 2012-2013.

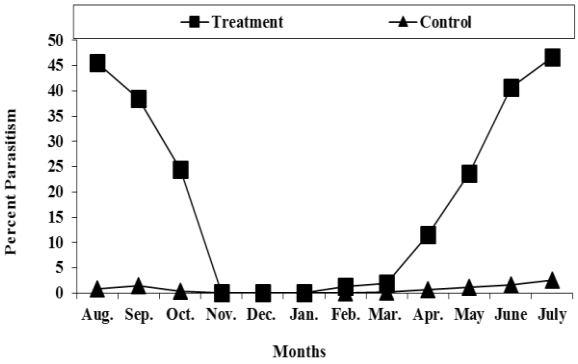


Figure (9): Percent parasitism of *Eretmocerus parasiphonini* associated with *Siphoninus phillyreae* infested pomegranate before and after releasing in Giza during 2013-2014.

References

Abd-Rabou, S. (1997): Hosts, distribution and vernacular names of whiteflies (Homoptera : Aleyrodidae) in Egypt. Annals of Agric. Sci. Moshtohor, 35 (2): 1029-1048.

Abd-Rabou, S. (1998): The efficacy of indigenous parasitoids in the biological control of *Siphoninus phillyreae* (Homoptera: Aleyrodidae) on pomegranate in Egypt. Pan-Pacific Entomolgists, 74 (3): 169-173.

Abd-Rabou, S. (1999): Parasitism of *Eretmocerus siphonini* (Hymenoptera : Aphelinidae) in Egypt. Boll. Lab. Ent. Agr. Filippo-Silvestri , 55: 27-31.

Abd-Rabou, S. (2001a): Role of *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae) in biological control of some whitefly species (Homoptera: Aleyrodidae) in Egypt. Shashpa,7(2):187-188.

Abd-Rabou, S. (2001b): Whiteflies of Egypt : Taxonomy, biology, ecology and means of their control. Adv. Agric. Res. In Egypt, 3 (1): 1-74.

Abd-Rabou, S. (2002): Biological control of two species of whiteflies by *Eretmocerus siphonini* (Hymenoptera: Aphelinidae) in Egypt. Acta Phytopathologica et Entomologica Hungerica, 37(1-3):257-260.

Abd-Rabou, S. (2003): First record of pomegranate whitefly, *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae) on olive in North Sinai, Egypt. Egypt. J. of Agric. Res. 81(4): 1577-1579.

Abd-Rabou, S. (2006): Biological control of the pomegranate whitefly, *Siphoninus phillyreae* (Homoptera: Aleyrodidae: Aleyrodinae) by using the bioagent, Clitostethus *arcuatus* (Rossi) (Coleoptera: Coccinellidae). Journal of Entomology, 3(4): 331-335.

Abd-Rabou, S. and Abou-Setta, M. (1998): Parasitism of *Siphoninus phillyreae* (Homoptera : Aleyrodidae) by aphelinid parasitoids at different locations in Egypt. J. of Hym. Res., 7 (1): 57-61.

Abd-Rabou, S. and Evans, G.A. (2002): The *Eretmocerus* Haldeman of Egypt (Hymenoptera: Aphelinidae). Mitt.internat. entomol. Ver.27(3/4):115-123.

Abd-Rabou, S. and Evans, G.A. (2004): Corrections to the *Eretmocerus* (Hymenoptera: Aphelinidae) of Egypt and the validation of two new species. Insecta Mundi, 17(1-2):68.

Abd-Rabou, S. and Ahmed, N. (2006): Seasonal abundance of the pomegranate whitefly, *Siphoninus phillyreae* (Homoptera: Aleyrodidae) and its natural enemies on olive trees in Egypt. J.Agric. Sci. Mansoura Univ.,31(9): 6029-6035.

Abd-Rabou, S. and Ahmed, N. (2007): Survey on host plants, distribution and natural enemies of the pomegranate whitefly, *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae). J. Agric. Res., 85 (5): 1695-1701.

Abd-Rabou, S. and Simmons, A. (2010): Augmentation and evaluation of a parasitoid, *Encarsia inaron*, and a predator, *Clitostethus arcuatus*, for biological control of the pomegranate whitefly, *Siphoninus phillyreae*. Archives of Phytopathology and Plant Protection, 34(13): 1318-1334.

Bellows, T.S.; Paine, T.D.; Arakawa, K.Y.; Meisenbacher, C.; Leddy, P. and Kabashimo, J. (1990): Biological control sought for ash whitefly. California Agriculture ,44: 4-6.

Bellows, T.S.; Paine, T.D.; Bezark, L.G. and Ball, J. (2007): Optimizing natural enemy release rates, and associated pest population decline rates, for *Encarsia* inaron walker (Hymenoptera: Aphelinidae) and *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae). Biological Control, 37:25–31.

Gould, J.R.; Bellows, Jr. T.S. and Paine, T.D. (1992): Population dynamics of *Siphoninus phillyreae* in California in the presence and absence of a parasitoid, *Encarsia partenopia*. Ecol. Entomol., 17:127-134.

Hackney, G.D.; Kidd, K.A.; McDonald, R.C. and Robbins, N.S. (1997): Ash whitefly biological control in North Carolina. NCDA Benef. Insect lab. Ann. Rep. of Activities, 33-35.

Mani, M. and Krishnamoorthy, A. (1995): Natural enemies of *Siphoninus phillyreae* (Homoptera: Aleurodidae) and Aphis punicae (Homoptera: Aphididae) on pomegranate. Entomon. 20(1):31-34.

McDonald, R.C.; Robbins, N.S.; Baker, J.R. and Bellows, T.S. (1996): Release and colonization of *Encarsia inaron* (Walker) (Homoptera: Aleyrodidae), in North Carolina. NCDA Benef. Insect lab Ann. Rep. of Activities. 37-45.

Pickett, C.H. and Pitcairn, M.J. (1999): Classical biological control of ash whitefly: factors contributing to its success in California. BioControl, 44: 143–158.

Viggiani, G. and Mazzone, P. (1980): *Encarsia pseudopartenopea* n.sp.,

parassita di *Siphoninus phillyreae* (Haliday) (Hom. Aleyrodidae). Bollettino el Laboratorio di Entomologia Agraria 'Filippo Silvestri', 37: 9-12.

Viggiani, G. and Battaglia, D. (1983): Le specie italiane del genere *Eretmocerus* Hald. (Hymenoptera: Aphelinidae). Bolletino del Laboratorio di Entomologia Agraria 'Filippo Silvestri', 40: 97-101.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 58-68

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Reducing infestions of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in cantaloupe using intercropping with non-host aromatic plants

Yomna, N.M. Abd-Allah[1] ; Samia A.G.Metwally[1]; Barka, M.Refaai[2] and Bahira , M.El-Sawaf[2]

[1] *Vegetable, Aromatic , Medicinal and Ornamental Plant Pests Research Department, Plant Protection Research Institute, Agriculture Research Center, Egypt.*

[2]*Entomology Department, Faculty of Science, Ain Shams University*.

ARTICLE INFO

Article History

Received: 5/3/2018

Accepted: 10/6/2018

Keywords

Cucumis melo, intercropping, *Allium sativum*, *Anethum graveolense*, *Coriandrum sativum*, biological management and *Bemisia tabaci*

Abstract:

To manage and reduce the severity of damage caused by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in the field, there is an enormous demand for safe and potential alternative strategiesto the use of chemical control. Trials were performed to determine the impact that the intercropping three aromatic plants garlic, *Allium sativum* (Amaryllidaceae), dill, *Anethum graveolense* (Umbelliferaeae) and coriander, *Coriandrum sativum* (Apiaceae) with cantaloupes has on the eggs and nymphs of *B. tabaci.* . The study was conducted during nili of the two successive growing seasons of 2015 and 2016 by intercropping these aromatic plants with cantaloupe in alternating rows. It was found that, intercropping cantaloupe with garlic, dill and coriander reduced infestations of *B. tabaci* and recorded highly significant differences between the intercropped and the cantaloupe monoculture. The mean numbers of *B. tabaci* eggs were 4.1, 4.4 and 4 eggs /leaf, respectively, compared to 13.7 eggs /leaf in cantaloupe monoculture during 2015 and 0.1, 0.07 and 0.02 eggs/leaf, respectively, compared to 14.6 eggs/leaf in cantaloupe monoculture during 2016]. Intercropping also markedly reduced the number of*B. tabaci* nymphs in 2015 and 2016 with garlic, dill and coriander to 20.7, 26.3 and 21.7 and 16.98, 15.5 and 16.8 nymphs /leaf, respectively compared to 61.8 and 55.6

nymphs /leaf, respectively in cantaloupe monoculture plots. We suggest that, intercropping non - hosts garlic, dill and coriander promotes the reduction of *B. tabaci* infestations in cantaloupe by insect repellency due to volatiles which may affect preference of *B. tabaci* as compared with cantaloupe. These plants appear to be good candidates for intercropping schemes and may be recommended in Integrated Pest Management programs as a safe and effective method in controlling *B. tabaci*. The tested aromatic plants have short life cycles, and are easy to be established and removed from the field

Introduction

Cantaloupe, *Cucumis melo* L. (Cucurbitaceae) is among the most important tasty, nutritional and exportable cucurbitaceous vegetable crops, that are cultivated in Egypt. Cantaloupe adapted to be cultivated in different types of soils and climates (FAO, 2006). In Egypt, cultivated areas of cantaloupe in old and new land reaches about 42,037 feddans and produces 475,817 tons in summer plantations (Qalyubia Governorate produced 26 tons of cantaloupe from the total production) while winter plantation resulted in about 24,254 feddans producing 370,115 tons (Anonymous**,** 2015). Azab *et al.* (1971) reported that whitefly damage generally includes a reduction of plant vigor due to adults and nymphs feeding on the plant phloem, the development of disorders including silver leaf, irregular ripening of fruit and excretion of honey-dew which promotes sooty mold. The most destructive and widely distributed *B. tabac*i biotypes are B and Q. Demichelis *et al.* (2000) reported that biotype Q is distributed in the Mediterranean region. *B. tabaci* is an extremely polyphagous pest that causes significant damage and can act as a vector of plant viral diseases. Jones (2003) found that *B. tabaci* transmits more than 100 species of viruses. Azevedo and Bleicher (2003) reported that *B. tabaci* has become the most important pest for melon crop in several countries, causing losses of millions of dollars /years. The worldwide spread of *B. tabaci* continues to cause severe crop losses which forces producers to use pesticides on many crops (such as tomato, cucurbits, beans, cotton, potatoes and sweet potatoes). In conjunction to this the biological and behavioral characteristics of the whitefly fast development, high fecundity and capacity for wide dispersion increase the probability of selecting individuals resistant to the most used insecticides (Byrne *et al.*, 2003).The strategy most commonly used to control B. tabaci continue to be the use of chemical pesticides, but this practice is becoming problematic and is considered high risk for beneficial arthropods and the environment. Metwally *et al*. (2013) demonstrated that cantaloupe is liable to be infested by numerous pests throughout seedling, flowering and fruiting stages of plant development. Among these are the cotton whitefly (also called tomato whitefly) *B. tabaci*. Many investigations have been carried out to estimate biocontrol agents as biological and safe control methods to manage the population of *B. tabaci* (Castle *et al*. 1996 and Faria and Wraight 2001). Li *et al*. (2001) and Hata *et al.* (2016) suggested that, mixed cropping or intercropping plays an important role in agriculture. This is due to the effective utilization of resources and improve crop productivity compared to that of monocultured crop. Letourneau *et al*. (2011) found extensive support for models with intercropping schemes in 552 experiments published in scientific papers concerning plant diversity in agroecosystems. Togni *et al*. (2010) suggested that intercropping prevent *B. tabaci* from becoming established in the tomato crop. In Egypt, garlic, (Allium *sativum*, Amaryllidaceae), dill (*Anethum graveolense*, Umbelliferaeae) and coriander, (*Coriandrum sativum*, Apiaceae) haven't been recorded as hosts of *B. tabaci*, in addition, these plants are easy to be cultivated, lost cost and each has short crop cycle.

This current investigation was aimed to testing the effect of intercropping these aromatic plants as a safe control method in reducing populations of *B. tabaci* attacking cantaloupe plants in the field.

Materials and Methods

1. Intercropping cantaloupe with aromatic plants:

1.1. Study site and aromatic plants used:

A field trial was carried out on the Experimental Farm of Plant Protection Research Institute at Qaha region, southeast of Qalyubia Governorate (30° 17'00 "N, 31° 12'00 "E) in Egypt during two successive growing seasons, 2015 and 2016 of cantaloupe. Aromatic plants used in the experiment garlic, dill and coriander.

1.2. Seedlings preparation:

Seeds of cantaloupe (cv. Darvina) were obtained from the Horticultural Research Institute (Agricultural Research Center). Cantaloupe were seeded in a greenhouse on August 30 in 2015 and on August 28 in 2016. The seedlings were watered and maintained for growth but plants didn't receive any insecticidal treatments. The cantaloupe seedlings were transplanted in the field on September 29 during both years.

2. Experimental design and cultivation method:

An experimental area of 1026 m^2 (27 x 38m) was divided into four equal plots (254.75$m^2$of each) that were 2.5 m apart and each plot was arranged longitudinally to the others. Three of the plots were intercropped with the experimental aromatic plants. Each treatment plot was established with one aromatic plant interplanted with cantaloupe to determine the effect of each aromatic plant alone without interfering their volatiles or any other effect. One plot as control (monoculture or monocrop) of only cantaloupe. Each experimental plot was divided equally to represent three parts. Eeach part was represented as a replicate was 84.91 m^2 and consisted of six planting rows. The rows were designed as 7 plants 0.30 m apart spacing between rows. Intercrop of aromatic plants was established in alternating rows in the southern edges of single rows facing cantaloupe plants which were planted in the eastern edges. A space of 20 cm was provided between aromatic plants and cantaloupe (Figure,1). The total number of plants per replicate was 42 plants (21 aromatic plants and 21 as cantaloupe plants). A total of 126 plants were established per plot [(n = 7 plants) x 6 rows x 3 replicates]. The same plot design was established in the cantaloupe monoculture plot. Seeds for the intercrop aromatic plants were sown in the field two weeks before the cantaloupe was seeded because these aromatic plants require more time to grow than cantaloupe.All the standard agricultural practices for cantaloupe cultivation in Egypt were adopted, and the entire experimental area remained free from any chemical control of pests and diseases.

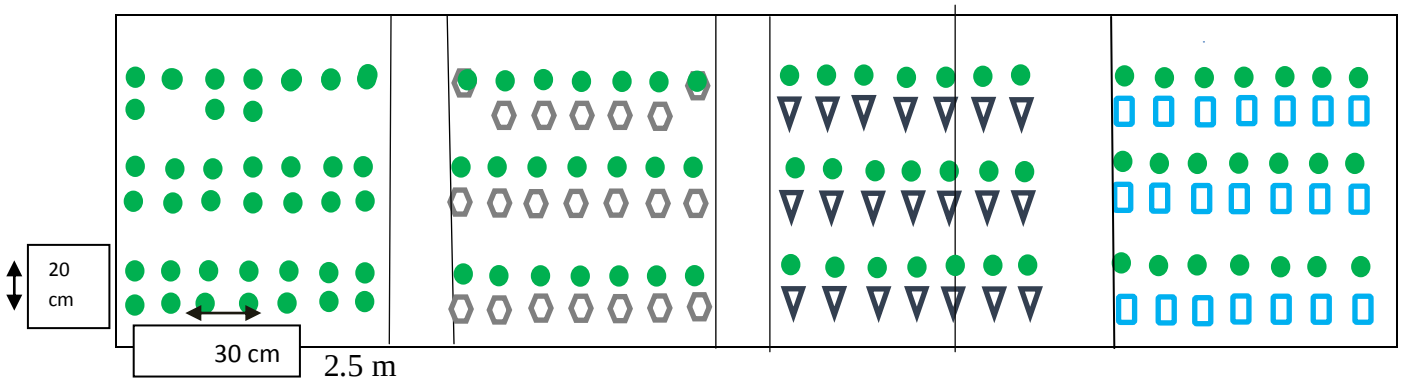


Figure (1): Design of one replicate of cantaloupe intercropping with garlic, dill and coriander or cantaloupe monoculture in th e field (cantaloupe, garlic, dill, coriander).

3. Sampling technique:

During the two growing seasons (2015 and 2016), samples were taken 15 days after crops were seeded and sampling continued weekly for 10 weeks. A sample of 120 leaves of canatloupe (10 leaves x 3 replicates x 4 plots) were randomly picked from three levels of plants (upper, middle and lower levels) during the morning of each sample date. Leaves that were sampled form each plot were kept separately in tightly closed polyethylene bags and they were then transferred to the laboratory. The presence of whitefly (*B. tabaci*) eggs and nymphs were examined by aid of a binocular stereomicroscope. The number of eggs and nymphs were assessed on the abaxial leaf surface. The number of eggs and nymphs per the three replicates in each treatment and the monoculture were estimated.

4. Statistical analysis:

Data for the intercropped cantaloupe and cantaloupe monoculture were subjected to analysis of variance. Mean separation was conducted according to the Duncan's multiple range test (Snedecor and Cochran,1971) to arrange any treatments in groups according to their infestation levels by *B. tabaci* population. The data were statistically analyzed by using SAS (SAS Institute Inc., 1988) program.

Results and discussion

1 .Effects of intercropping cantaloupe on *Bemisia tabaci* eggs:

Generally, B. tabaci showed oviposition preference to cantaloupe in monoculture as compared with cantaloupe intercropped with either garlic, dill or coriander. The females deposited eggs on cantaloupe leaves in all plots (intercropped or monoculture), during the study from 13 October through 14 December during 2015 (Table, 1). The data showed that, the number of B. tabaci eggs in cantaloupe plots intercropped with garlic, dill or coriander ranged from 0 to 24.7, 0 to 25.3 and 0 to 19.3 eggs/30 leaves (10 leaves in each replicate), respectively (Figure, 2). However, the egg counts in cantaloupe monoculture ranged from 0.3 to 55 eggs/30 leaves. Our study revealed that, all intercropped plots had lower seasonal mean number of whitefly eggs than cantaloupe monoculture. This pattern was observed in plots of cantaloupe intercropped with garlic (4.1±1.5), dill (4.4±1.5) and coriander (4±1.1) (mean ± SD) compared to 13.7±3.8 in the plot of monoculture cantaloupe. After the first three weeks of sampling, the counts of whitefly eggs were very low in all plots. Although there were no significant differences among the aromatic plant treatments in reducing *B. tabaci* laying eggs ($P< 0.05$), there was a significant difference in whitefly oviposition between intercropped cantaloupe and monoculture cantaloupe ($P=0.0001$). Infestation with *B. tabaci* eggs was observed on the first sample date of 12 October and eggs were observed through the last sample date of 14 December (Table, 2). The number of eggs ranged from 0 to 0.3, 0 to 0.3, 0 to 0.1 eggs /30 leaves in cantaloupe + garlic, cantaloupe + dill and cantaloupe + coriander, respectively. However, the number of eggs in the monoculture plot. of cantaloupe ranged between 0 to 142.5 eggs/30 leaves. The presence of whitefly eggs on leaves was low (a mean of less than 3 eggs per 30 leaves) on cantaloupe in all plots regardless of intercrop or monoculture). Cantaloupe intercropped with garlic, dill or coriander had mean numbers of eggs of 0.1±0.03, 0.07 ±0.02 and 0.02±0.01eggs, respectively versus a mean of 14.6±7.9 eggs in cantaloupe monoculture across the season. The obtained data are in accordance with the data of the previous season without significant different in reduction of B. *tabaci* eggs.

Table (1): Mean number of *Bemisia tabaci* eggs on cantaloupe plants intercropped with garlic, dill or coriander versus monoculture of cantaloupe during the nili plantation of 2015.

Sample Date	Mean number of *B. tabaci* eggs /30 leaves			
	Garlic	Dill	Coriander	Cantaloupe monoculture
October,13	7.9	4	5.9	51.4
21	24.7	25.3	10.3	55
27	7.8	14.3	19.3	12.4
November,2	0.3	0.3	0.6	1.1
9	0.1	0	0.5	0.6
16	0.2	0.1	2.4	5.9
23	0	0.1	0.8	3.1
30	0	0.03	0.03	0.3
December,7	0	0	0.1	5.2
14	0	0	0	2.2
Seasonal mean ±SE	4.1 ±1.5b	4.4±1.5b	4 ±1.1b	13.7 ± 3.8a
F value	16.4			
P	0.0001			
LSD	4.1			

Means within a row followed by the same letter do not differ significantly (Duncan's test: *P*<.0.05). LSD= Least significant difference.

Table (2): Mean number of *Bemisia tabaci* eggs on cantaloupe plants intercropped with garlic, dill or coriander versus monoculture of cantaloupe during the nili plantation of 2016.

Sample date	Mean number of *B. tabaci* eggs/ 30 leaves			
	Garlic	Dill	Coriander	Cantaloupe monoculture
October,12	0.2	0.03	0	142.5
19	0.03	0.2	0.1	0
26	0	0	0	0
November,2	0	0.3	0.1	2.5
9	0	0	0	0.03
16	0.2	0.2	0.05	0.63
23	0.3	0	0	0.1
30	0	0	0	0
December,7	0.3	0	0	0
14	0	0	0	0
Seasonal mean±SE	0.1 ± 0.03 b	0.07 ± 0.02b	0.02 ± 0.01b	14.6 ± 7.9a
F value	3.8			
P	0.0001			
LSD	10.1			

Means within a row followed by the same letter do not differ significantly (Duncan's test:P<.0.05)
LS D = Least significant difference.

2 .Effects of intercropping cantaloupe on Bemisia tabaci nymphs:

The data in Table (3) from October 13 through 14 December in 2015 indicated that, the counts of B. *tabaci* nymphs ranged between 0 to 66.7, 0 to 68.3 and 0.8 to 50.4 nymphs per 30 leaves in plots intercropped with garlic, dill or coriander, respectively (Table, 3). However, the count ranged between 5.3 to 146.4 nymphs per 30 leaves in cantaloupe monoculture (Figure, 4). Additionally, the lower incidence of *B. tabaci* adults in intercropped plots with garlic, dill or

coriander was reflected by lower number of nymphs per leaf (20.7 ±4.5, 26.3±4.8 and 21.7±3.4), respectively) across the sampling period. The colonization of cantaloupe monoculture by B. tabaci adults was apparently reflected by higher mean number of B. tabaci nymphs (61.8±9.4) than all intercropped cantaloupe plots. There were no significant differences in counts of nymphs among the treatments with aromatic plants. However, there was a significant reduction in number of nymphs between the treatment of aromatic plants and cantaloupe monoculture.

A similar pattern was observed in 2016 (Table, 4) as was observed in 2015. The mean number of *B. tabaci* nymphs ranged from 0.4 to 56.2, 0 to 55.3 and 0.1 to 53.6 nymphs/30 leaves in plots intercropped with garlic, dill or coriander, respectively (from 12 October through 14 December) in 2016. On the other hand, the mean number of nymphs in cantaloupe monoculture ranged between 5.2 to 127 nymphs/30 leaves. The populations of whitefly nymphs were clearly reduced in intercropped plots with garlic (16.98±2.9), dill (15.5±3.2) or coriander (16.8±2.9) as compared the plot of cantaloupe monoculture which harboured higher number of B. tabaci nymphs (55.6 ±7.98). There was a highly significant reduction reduction in the number of nymphs in all intercropped plots as compared to the plot monoculture cantaloupe which suffered from highest infestation level (P=0.0001).

Table (3): Mean number of *Bemisia tabaci* nymphs on cantaloupe plants intercropped with garlic, dill or coriander versus monoculture of cantaloupe during the nili plantation of 2015

Sample date	Mean number of *B. tabaci* nymphs / 30 leaves			
	Garlic	Dill	Coriander	Cantaloupe
October,13	5.9	4.6	0.8	5.3
2 1	14.6	27.2	8	146.4
27	66.7	68.3	27.3	122.1
November,2	22.6	62.4	46.5	106.3
9	63.5	52.9	43.2	107.6
16	26.9	29.2	50.4	38.8
23	6.3	16.2	18.9	27.2
30	0	0.1	12.1	24.1
December,7	0	2.3	2.9	32.5
14	0	0	6.5	7.7
Seasonal mean ±SE	20.7 ± 4.5b	26.3 ±4.8b	21.7 ± 3.4b	61.8 ± 9.4a
F value	22.8			
P	0.0001			
LSD	10.5			

Means within a row followed by the same letter do not differ significantly (Duncan's test : $P<.0.05$). LSD= Least significant difference.

Table (4): Mean number of *Bemisia tabaci* nymphs on cantaloupe plants intercropped with garlic, dill or coriander versus monoculture of cantaloupe during the nili plantation of 2016

Sample date	Garlic	Dill	Coriander	Cantaloupe monoculture
October,12	18.5	0	0.1	41.6
19	10.2	15.9	23.3	120
26	30.7	8.9	14.5	62.8
November,2	11.5	24	25	88.3
9	10.8	35.5	22.5	62.9
16	56.2	55.3	53.6	127
23	15.3	9.6	17.9	29.4
30	8.6	1.7	5	9
December,7	7.4	1.3	2.5	9.8
14	0.4	2.7	3.2	5.2
Seasonal mean ±SE	16.98 ±	15.5 ±	16.8 ±	55.6 ± 7.98a
F value	26.8			
P	0.0001			
LSD	8.3			

Means within a row followed by the same letter do not differ significantly (Duncan's test: $P<.0.05$). LSD= Least significant difference

Our findings demonstrated that intercropping garlic, dill or coriander plants of cantaloupes can result r in reduction in B. *tabaci* infestation during the growing season. This work supports the hypothesis that *B. tabaci* has strong repellent behavior to intercropped areas with each of the tested aromatic plants. This was apparent when cantaloupes intercropped with the aromatic plants under investigation had lower number of eggs and nymphs than cantaloupe monoculture. It is known that, *B. tabaci* avoids plant species which contain aromatic oils, such as ginger oil but repellence or deterrence of plant volatiles to *B. tabaci* is not well established.

It is quite evident from the obtained results that, the infestation levels of both eggs and nymphs were significantly reduced in cantaloupe that was intercropped with either garlic, dill or coriander as compared with cantaloupe monoculture. It is noteworthly that in some instances, only records for nymphs were observed on cantaloupe monoculture and no records of eggs. Hongtao and Yuchuan (2017) stated that, intercropping cucumber with different celery (Apium *graveolens)* varieties showed repellent effects and oviposition deterrent effects in *B. tabaci* and thus acted as a good repellent against *B. tabaci*. The volatiles of celery, asparagus (*Asparagus officinalis*), lettuce, Malabar spinach (Basella *alpa*), and edible amaranth (Amaranthus *tricolor*) have also been shown to strongly repel *B. tabaci* biotype B in a Y-tube assay, however, the repellency was different among plants (Zhao *et al*., 2014). In addition, Ying *et al*. (2003) demonstrated that, *B. tabaci* biotype B is attracted by host plant volatiles.

The same pattern of reduction in number of nymphs of *B. tabaci* was observed when garlic, dill or coriander was added. The population of nymphs significantly declined for garlic, dill or coriander intercropping during 2015. The same trend was evident in 2016 and the population was significantly treatments in garlic, dill and coriander compared to the cantaloupe monoculture. Although the number of whitefly eggs tended to be more depressed in the 2016 experiment than in the 2015, the difference was statistically insignificant. However, the population of *B. tabaci* nymphs was lower in 2016 than 2015. El-Khayat *et al.* (2010) reported different *B. tabaci* infestations during two years of their study. Naranjo *et al*. (2004) attributed the mortality of *B. tabaci* in

spring and fall cantaloupes as well as other crops to predation, parasitism, desiccation, weather factors and other factors besides control methods. It was apparent here that garlic, dill and coriander show strong and positive effect of similar force in helping to keep *B. tabaci* away from intercropped plots. The reduction in the number of nymphs *B. tabaci* was observed by Costa and Bleicher (2006) when melon (*C. melo* L). or watermelon (*Citrullus lanatus* Thumb), plants were intercropped with coriander in a greenhouse.

The suppression of *B. tabaci* populations in coriander plots may be explained here by aromatic volatiles which may have affected the preference of the insect to cantaloupe. Suppression could be due to different reasons, eg., Togni *et al*. (2010) found that, tomato intercropped with coriander reduced the population of *B. tabaci* attacking tomato plants as compared with tomato monoculture plots. They suggested that coriander produces a high amount of volatile secondary metabolites that had an odour that mask the odor tomato volatiles, and interfered in the host plant selection of *B. tabaci.* Also, coriander plant can be attractive to some natural enemies such as Coccinellidae, especially under organic management (Togni *et al*., 2009). Bickerton (2011) found that, intercropping dill, coriander or buckwheat with bell pepper (Capsicum annuum) attracted natural enemies of *Ostrinia nubilalis*. The same approach was previously reported when the South American tomato pinworm (*Tuta absoluta* Meyrick) (eggs and adults) were found in lower numbers in intercropped coriander and Gallant soldier (Galinsoga *parviflora*) with tomato compared to tomato alone in an organic cropping system. However, there were higher population of predators such as spiders and ladybeetles. Ladybeetles appeared at the flowering season of coriander (Medeiros *et al*., 2009). Hilje and Stansly (2008) stated that coriander *C. sativum* reduced the number of incoming whitefly adults, delayed the onset of *Tomato yellow mottle virus* (ToYMoV) and decreased disease severity, resulting in higher yields and profits in a field experiment in Costa Rica.

More recently, Sujayanand *et al.* (2015) reported that intercropping eggplant (*Solanum melongena* L.) with coriander, marigold or mint resulted in lower numbers of *B. tabaci* on eggplant versus sole eggplant. In addition, they found highest mean number of coccinellids in treatment had coriander as intercrop. They found 21 volatile compounds in coriander plants. Carvalho *et al*. (2017) assayed the behavior and population development of *B. tabaci* biotype B in the field (adults) and in the greenhouse (nymphs) on tomato plants alone and in tomato intercropped with aromatic plants. In Y-tube olfactometer assays coriander, Greek basil and citronella show strong repellency of similar magnitude to whiteflies characterizing their odors as repellents. In field tomato intercropped with coriander and basil adult populations of whiteflies were reduced by the same magnitude.

The reduction of *B. tabaci* in cantaloupe due to intercropping with garlic was less than that was previously reported. However, a recent study concluded that, garlic can be used effectively as a repellent crop under intercropping (Karavina *et al*., 2014). In that study, no significant difference in larval populations of the diamondback moth (Plutella *xylostella*) was observed between cabbage plots treated chemically versus cabbage intercropped with garlic. According to Hata *et al.* (2016), strawberry (*Fragaira ananassa*) intercropped with garlic plants reduced *Tetranychus urticae* Koch (two spotted spider mite) populations in the field. They suggested that interplanting garlic between rows may be a promising strategy to reduce T. *urticae* and attributed the mortality in mite population to the release of strong plant odor. Volatiles released from plants can act as toxins or repellents (Kant *et al*., 2009). Such volatiles could be responsible for reducing B. *tabaci* by garlic plants in the current investigation due to volatile bioactive

compounds which can enter the insect body through the tracheal system (Isman, 2000). Intercropped with garlic has also been shown to reduce mites in the tomato field in Malawi (Mtambo and Zeledon, 2000). Garlic oil has oviposition deterrent and repellent effects on *B. tabaci* adults as well as affects the survival of larvae (Hussein, 2017). In cantaloupe intercropped with garlic the reduction effect on *B. tabaci* population may specifically be due to vinyl dithiin, diallyl disulfide and diallyl trisulfide, that have been identified in garlic essential oil (Attia *et al.* 2012). We suggested that releasing sulfur volatile compounds could play a role in keeping *B. tabaci* away from the cantaloupe fields intercropped with garlic according to Attia *et al.* 2012.

It is concluded that the developing whiteflies in plots intercropped with garlic, dill or coriander were apparently decreased because of repellency of plant volatiles to the adult whiteflies. Consequently, intercropping cantaloupe with aromatic plants reduced the incidence of *B. tabaci* in the open field. Particularly, intercropping with dill or coriander kept B. *tabaci* nymphs lower than cantaloupe monoculture during 10 weeks. This approach emphasize that garlic or dill and coriander could be good candidates in an integrated pest management programs due to their promising results in reducing *B. tabaci* populations in cantaloupe plants as safe alternative control methods to chemicals. Moreover, these plants have short life cycle, provide additional economic returns when sold and was easier to establish and remove.

References

Anonymous (2015): Cultivated area in old and new land. Agriculture directorates of governorates. Egyptian Ministry of Agriculture.

Attia, S.; Griss, K.L.; Mailleux, A.C.; Lognay, G.; Heuskin, S.; Mayoufi, S. and Hance, T. (2012): Effective concentrations of garlic distillate (*Allium sativum*) for the control of *Tetranychus urticae* (Tetranychidae). Journal of Applied Entomology, 136:302–312.

Azab, A.K.; Megahed, M.M. and El-Mirsawi, D.H. (1971): On the biology of *B. tabaci* (Genn.)[Hemiptera: Homoptera: Aleyrodidae]. Bulletin of the Entomological Society of Egypt, 55: 305-315.

Azevedo, F.R. and Bleicher, E. (2003): Distribuicao vertical e setorial das ninfas de mosca-brancanasfolhas do meloeiro. Horticultural Brasileira, 21: 464-467.

Bickerton, M.W. (2011): Intercropping method for conservation biocontrol of European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) in bell peppers. Thesis. Graduate School New Brunswick Rutgers, the State University of New Jersey. U.S.A.

Byrne, F.J.; Castle, S.; Prabhaker, N. and Toscano, N.C. (2003): Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. Pest Management Science, 59:347-352.

Carvalho, M.G.; Bortolotto, O.C.and Ventura, M.U. (2017): Aromatic plants affect the selection of host tomato plants by *Bemisia tabaci* biotype B. Entomologia Experimentalis et Applicata, 162:86-92.

Castle, S.J.; Henneberry, T. J.and Toscano, N.C. (1996): Suppression of *Bemisia tabaci* (Homoptera: Aleyrodidae) infestations in cantaloupe and cotton with sprinkler irrigation. Crop Protection, 15:657-663.

Costa, R.S. and Bleicher, E. (2006): Comportamento da mosca-branca (*Bemisia tabaci* biotipo B) emsistemas de plantio de coentro, melancia e melao. Revista Ciencia Agronomica, 37:195-199.

Demichelis, S.; Bosco, D.; Manino, A.; Marian, D. and Caciagli, P. (2000): Distribution of *Bemisia tabaci* (Hemiptera, Aleyrodidae) biotypes in Italy. The Canadian Entomologist, 132(4):519-527.

El-Khayat, E.F.; El-Lakwah, F.A.; Rady, G.H.H.; Ghallab, M.A.and Wahba, B.S. (2010): Impact of planting dates on infestation of cowpea plants with some pests. Egyptian Journal of Agricultural Research, 88(4): 1107-1119.

FAO - Food and Agriculture Organization of the United Nations (2006): 30 de novembro. Agricultural production, primary crops.

Faria, M. and Wraight, S.P. (2001): Biological control of *Bemisia tabaci* with fungi. Crop Protection, 20:767-778.

Hata, F.T.; Ventura, M.U.; Carvalho, M.G.; Miquel, A.L.; Souza M.S.; Pauala, M.T. and Zawadneak, M.K. (2016): Intercropping garlic plants reduces *Tetranychus urticae* in strawberry crop. Experimental Applied Acarology, 69 (3):311-321.

Hilje, L.and Stansly, P.A. (2008): Living ground covers for management of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and tomato yellow mottle virus in Costa Rica. Crop Protection, 27:10-16.

Hongtao, T. and Yuchuan, Q. (2017): Repellent effects of different celery varieties in *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotype Q. Journal of Economic Entomology, 110: (3)1307-1316.

Hussein, H.S. (2017): Behavioral and Insecticidal Effects of Three Plant Oils on *Bemisia tabaci*. Journal of Entomology, 14: 87-95.

Isman, M.B. (2000): Plant essential oils for pest and disease management. Crop Protection, 19:603–608.

Jones, D. (2003): Plant viruses transmitted by whiteflies. European Journal of Plant Protection Pathology, 109:197-221.

Kant, M.R.; Bleeker, P.M.; van Wijk, M.; Schuurink, R.C. and Haring, M.A. (2009): Plant volatiles in defence. In: van Loon LC (ed) Advances in botanical research. Academic Press, London, pp 613–666.

Karavina, C.; Mandumbu, R.; Zivenge, E.and Munetsi, T. (2014): Use of garlic (*Allium sativum*) as a repellent crop to control diamondback moth (*Plutella xylosttella*) in cabbage (*Brassica oleraceae* var. Capitata) Journal of Agricultural Research, 52(4): 615-621.

Letourneau, D.K.; Armbrecht, I.; Rivera, B.S.R.; Lerma, J.M.; Carmona E.J.; Daza, M.C.; Escobar, S.; Galindo, V.; Gutie´rrez, C.; Lo´pez, S.D.; Mejı´a, J.L.; Rangel, A.M.A.; Rangel, J.H.; Rivera, L.; Saavedra, C.A.; Torres, A.M. and Trujillo, A.R. (2011): Does plant diversity benefit agroecosystems? A synthetic review. Ecological Applications, 21:9–21.

Li, L.; Sun, J. H.; Zhang, F.S.; Li, X.L.; Yang, S. C. and Rengel, Z. (2001): Wheat /maize or wheat /soybean strip intercropping. Yield advantage and interspecific interactions on nutrients. Field Crops Research, 71: 123-137.

Medeiros, M.A.I.; Sujii, E.R. and Morais, H.C. (2009): Effect of plant diversification on abundance of South American tomato pinworm and predators in two cropping systems. Horticultura Brasileira, 27: 300–306.

Metwally, S.A.G.; Shoukery, I.F.; Younes, M.W.F. and AbdAllah, **Y.N.M. (2013):** The susceptibility of certain cantaloupe cultivars to different

three pests' infestation in Qualyobia Governorate, Egypt. Egyptian Journal of Agricultural Research, 91(1):151-167.

Mtambo, C.C. and Zeledon, I.H. (2000): The development of integrated control methods for the tomato red spider mite (*Tetranychus evansi*) in Malawi. In: Agricultural technologies for sustainable development in Malawi. Proceedings of the first annual scientific conference held at the Malawi Institute of Management, pp 139–147.

Naranjo, S.E.; Canas,L. and Ellsworth, P.C. (2004): Mortality factors affecting populations of sweetpotato whitefly, *Bemisia tabaci* in a multi-crop system . Horticultura Internacional, pp14-21.

SAS Institute (1988): SAS / capital state user's guide, 6.03 ed. SAS Institute, Cary, N.C.

Snedecor, G.W. and Cochran, W.G. (1971): Statistical Methods. Iowa State University Press, Ames, Iowa, USA.

Sujayanand, G.K.; Sharma, R.K.; Shankarganesh, K.; Saha, S .and Tomar, R.S. (2015): Crop diversification for sustainable insect pest management in eggplant (Solanales: Solanaceae). Florida Entomologist, 98: 305–314.

Togni, P.H.B.; Frizzas, M.R.; Medeiros, M.A.; Nakasu, E.Y.T.; Pires, C.S.S. and Sujii, E.R. (2009): Din^amica populacional de *Bemisia tabaci* biotipo B em tomate monocultivo e consorciado com coentro sob cultivo org^anico e convencional. Horticultura Brasileira, 27:179-184.

Togni, P.H.B.; Laumann, R.A.; Medeiros, M. A. and Sujii, E. R. (2010): Odour masking of tomato volatiles by coriander volatiles in host plant selection of *Bemisia tabaci* biotype B. Entomologia Experimentalis et Applicata, 136:164-173.

Ying, J.; Huang, J.; Rui-yan, M. and Ju-cai, H. (2003): Host plant preferences of *Bemisia tabaci* Gennadius. Insect Science, 10:109-114.

Zhang, W.; McAuslane, H.J. and Schuster, D.J. (2004): Repellency of ginger oil to *Bemisia argenifolii* (Homoptera: Aleyrodidae) on tomato. Ecotoxicology, 97:1310-1318.

Zhao, Q.; Zhu, J. J.; Qin, Y.; Pan, P. and Tu, H. (2014): Reducing whiteflies on cucumber using intercropping with less preferred vegetables. Entomologia Experimentalis et Applicata, 150:19-27.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 69-73

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Effect of using Pritchardia dates honey as a nutritive source on the quality of *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)

Amany, N. Siam

Agricultural Animal Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Egypt

ARTICLE INFO

Article History

Received: 13/3/2018

Accepted: 15/7/2018

Keywords

Pritchardia dates honey, *Trichogramma evanescens*, nutrition and general productivity.

Abstract:

Experiments were carried out to determine the effects of date's honey of Pritchardia spp. palm as a new nutritive source for adults of *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae). Certain nutritive solutions were tested (pure bee honey, pure sugar cane honey, 10% sucrose solution and water compared with this new source. Fecundity, longevity, percentage of parasitoid emergence, percentage of females in progeny and general productivity were investigated. Obtained results revealed that, parasitoids fed on Pritchardia date honey parasitized the highest number of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs (65.2 egg/female), lived longest period (6.05 days) and recorded the highest adult emergence (97.45%) comprising the highest ratio of females in progeny (78.45%). Control females parasitized the lowest number of *S. cerealella* eggs (30.7 eggs/female), they lived the shortest life span (1.75days), and resulting in the lowest adult emergence (77.1%) comprising the lowest percentage of females in progeny (61.95%). Generally, feeding trichogrammatids on Pritchardia date honey resulted in the highest productivity (49.85 females/female, while the lowest productivity (14.66 females/female) was calculated from the unfed ones.

Introduction

Feeding of adults is an important factor in *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) mass rearing in laboratory to ensure the production of high quality parasitoids. The choice of the nutritive source is usually based on trials and errors (Wackers, 2005). Many authors relied on sugar or bees honey (Abd Elhafez *et al.*,1999; Gurr and Nicol, 2000 and Karimi and Hatami, 2010), others relied on sugar cane honey as a diet for adults of *T.*

evanescens (Siam *et al*., 2014). Pritchardia palms draw my attention with its ripe fallen fruits (dates) in huge amounts, their dates are locally edible as they are delicious and sweet. From the point of view that aiming to exploit the surrounding environmental sources and saving expenses of mass rearing, the date's honey of those palms was used to nitrify adult parasitoids, however no data regarding the nutritional composition of that kind of dates are available. This work was carried out to investigate the effect of introducing Pritchardia dates honey as a new nutritive source for adults on the fitness components of *T. evanescens* in order to high light its potential.

Materials and Methods

Experiments were conducted at Fayoum Laboratory, Plant Protection Research Institute, Agricultural Research Center. Experiments were conducted at 25±2°C and 70±5% R.H. *T. evanescens* was reared on *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs.

1. *Sitotroga cerealella* rearing:

S. cerealella rearing method was a modification of that reported by Hassan (1995) where soft wheat was chosen as the rearing medium.

2. *Trichogramma evanescens* rearing:

Fresh *S. cerealella* eggs < 24hrs old were put on self-adhesive paper cards (21×15) and exposed to *T. evanescens* adults in transparent jars (2 liters capacity) provided with the nutritive source and covered with cloth wrapped cotton kept in position by rubber band. Parasitized eggs cards were kept in clean jars.

3. Preparation of Pritchardia dates honey:

Ripe fruits were collected, washed with water, and then the outer cover of the fruit is pulled off the stone seed, and then weighed. About one kilogram of the dates is covered with one liter of water in a wide pot, boiled on low heat with stirring with a wooden spoon until the mixture thickened. The thick, boiled mixture poured through two mesh cheese cloth. The cloth squeezed well to retrieve more juice as possible from the cooked date mixture. Then it was returned to heat till be thicker cooled and kept in a refrigerator.

4. Experimental technique:

This experiment was conducted to investigate the effect of Pritchardia date honey on the fitness components of *T. evanescens* as an alternative nutritive source. The nutritive sources were, water, bee honey, sugar cane honey, sugar solution and Pritchardia date honey. Unfed females were the control. For each tested nutritive source, twenty newly emerged females were released individually in rearing glass vials (4×8.5cm) containing about 70 *S. cerealella* eggs. Each vial was provided with a droplet of the tested diet (about 2ml of each nutritive source) put on a piece of filter paper with a thin dissecting needle. Unfed females were left as control. All vials were checked daily to observe mortality and longevity of females. Parasitized eggs were counted as fecundity. The percentage of emerged adults and produced females in progeny were determined. General productivity was calculated according to Tshernyshev and Afonina (1995).

GP= rate of emergence ×rate of produced females in progeny × fecundity

Analysis of variance (ANOVA) was used to process data and means were separated by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

1.Effect of nutritive sources on fecundity:

Tested nutritive sources affected significantly the fecundity of *T. evanescens* females ($P<0.05$). The lowest fecundity was 30.7±0.41 parasitized eggs with unfed females, while droplets of water increased slightly the fecundity to 33.35±0.54 parasitized eggs. Sugar solution, honey bee and sugar cane honey raised female's fecundity to 44.1±0.66, 55.25±0.69 and 59.95±0.41 eggs respectively. The highest fecundity was recorded with females fed on Pritchardia date honey 65.2±0.74 eggs (Table,1).

2. Effect of nutritive sources on longevity:

Longevity of females was significantly affected with the tested nutritive sources (P<0.05). The shortest life span was recorded with unfed females 1.75±0.14 days. While, water and sugar solution fed females lived 2.55±0.11 and 3.05±0.88 days resp. This gradually increased by feeding females with bee honey and sugar cane honey reaching 4.65±0.19, 5.35±0.17 days respectively. While, Pritchardia dates honey caused the longest life span 6.05±0.11 days (Table,1).

3.Effect of nutritive sources on percentage of adults' emergence:

Percentage of off spring emergence was significantly different with all the tested nutritive sources (P<0.05). The highest percent of adult emergence was recorded from parasitoid females fed on Pritchardia dates honey 97.45±0.19% followed by those from cane honey diet (95.1±0.35%), meanwhile, bee honey or sugar solution diets showed close rates of off spring emergence 91.1±0.46% and 92.65±0.29% respectively. Unfed females gave the lowest adult emergence 77.1±1.13%, but access of water increased the percentage of off spring emergence 81.45±0.82%. (Table,1)

Table (1): Effect of different nutrition sources on fitness components of *Trichogramma evanescens* reared on *Sitotroga cerealella* eggs

Nutrition source	Fecundity Mean±SE	Longevity Mean±SE	Emergence% Mean±SE	Female % Mean±SE
Unfed	30.7±0.41^{f}	1.75±0.14^{f}	77.1±1.13^{f}	61.95±0.78^{e}
Water	33.35±0.54^{e}	2.55±0.11^{e}	81.45±0.82^{e}	71.85±0.51^{d}
Honey bee	55.25±0.69^{c}	4.65±0.19^{c}	91.1±0.46^{d}	73.85±0.17^{c}
Sugar cane honey	59.95±0.41^{b}	5.35±0.17^{b}	95.1±0.35^{b}	77.75±0.34^{b}
Sugar solution 10%	44.1±0.66^{d}	3.05±0.88^{d}	92.65±0.29^{c}	71.3±0.29^{d}
Pritchardia dates honey	65.2±0.74^{a}	6.05±0.11^{a}	97.45±0.19^{a}	78.45±0.28^{a}

Means within columns followed by different letters are significantly different at the p < 0.05 level by Duncan's multiple range test

4. Effect of nutritive sources on female percentage:

Produced female percentage in progeny was significantly different among the tested nutritive sources (P<0.05). The highest females ratio 78.45±0.28 was recorded in progeny from Pritchardia dates honey fed females, followed by those resulted from cane honey fed females 77.75±0.34%. Water and sugar solution produced nearly the same percentages of produced females in progeny 71.85±0.51% and 71.30±0.29% resp. Among the treatments, parent females fed on bee honey produced 73.85±0.17% females in progeny and the lowest produced females' ratio 61.95±0.78% was produced from unfed females (Table,1).

5. Effect of nutritive sources on general productivity (GP):

The highest general productivity of *T. evanescens* 49.85 females/female was recorded with the nutrition of Pritchardia date honey followed by those fed on sugar cane honey 44.33 females/female. Low GP 19.52 females/female was recorded from females fed on water. Adding sugar solution increased females GP to 29.13females/female, while bees honey raised GP to 37.17females/female. On the other hand, the general productivity was drastically reduced to 14.66 females/female when parent mothers were unfed, Figure (1).

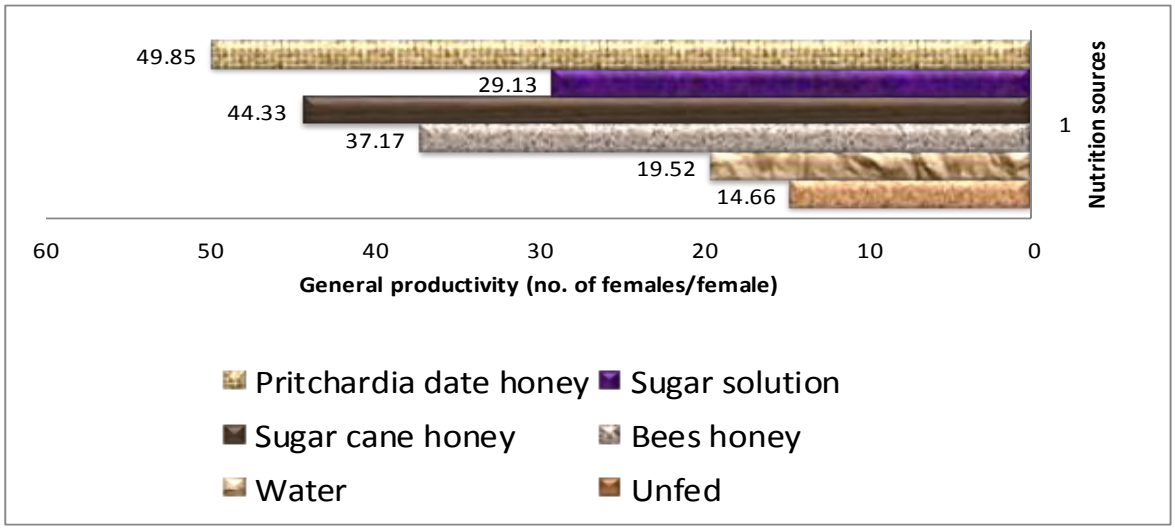


Figure (1): General productivity of *Trichogramma evanescens* females fed on different nutrition sources

In this study, and from the point is that, one must exploit the surrounding environmental resources which are represented in Pritchardia palms in the gardens with its fallen ripe dams in huge amounts; those dams are rounded, small and black, with delicious and sweet taste. Pritchardia dams is commonly edible by human being, so, thinking about the using it to serve as an alternative or supplemental nutrition source for *T. evanescens* parasitoids in the laboratory, as it is available and cheap source of carbohydrates to improve their fitness components. Pritchardia palm belongs to the family: Arecaceae which is a botanical family of perennial climbers, shrubs and trees commonly known as palm trees, it is of enormous economic importance for human beings. Some authors work on the nutritional characterizations of fruits obtained from that family, they reported that they had a relevant concentration of nutrients and bioactive compounds with importance for human health. (Lescano *et al*., 2018). This could be coincided with our results which was represented in the highest fecundity of *T. evanescens* females fed on Pritchardia dates honey, survival the longest life days, the highest emergence percentage comprising the highest ratio of produced females in progeny. In addition, results of this work revealed that, cane honey fed females survived long days with large number of parasitized *S. cerealella* eggs resulting in high percent of off spring emergence with high rate of females ratio in progeny, those results are in consistence with those of (Siam *et al.*, 2014) who conducted experiments on feeding *T. evanescens* females on different nutritive sources for high quality parasitoids production. They demonstrated that, feeding females on cane honey recorded high percentage of off spring emergence over 96% from the highest number of parasitized host eggs with the highest sex ratio of females in progeny over 78.18%. In addition, (Abd Elhafez *et al*., 1999) and (Salijoqi and Khajjak, 2007) supported our results, they confirmed that bees honey fed females parasitized high number of host eggs with the high number of off spring emergence and high rate of produced females in progeny.

It is concluded that the Pritchardia date honey could serve as a very cheap nutritive source, followed by cane honey and bees honey as a favorable nutritive source to adult parasitoid in laboratory mass rearing for a sustainable and efficient *T. evanescens*.

References

Abd Elhafez, A.; EL-Khayat, E. F.; Fawzy, F. S. and EL-Sharkawy, M. (1999): Effects of imaginal food on general productivity of two trichogrammatids (Hymenoptera: Trichogrammatidae), cultured on eggs of *Pectinophora gossypiella*

Saunders. (Lepidoptera: Gelechiidae). Proceedings of the 1st Regional Symposium for Applied Biological Control in Mediterranean Countries. Cairo Egypt. October 25-29, 1998. Canard M. and Bessat. Arnaouty V (Eds). Toulouse. France.

Duncan, D. B. (1955): Multiple range and multiple F tests. Biometrics, 11: 1-41.

Gurr, G. and Nicol, H. I. (2000): Effect of food on longevity of adults of *Trichogramma carverae* Oatman and Pinto and *Trichogramma* nr *brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). Australian J. of Entomology, 39:185-187.

Hassan, S. A. (1995): Improved method for the production of the Angoumois grain moth *Sitotroga cerealella* (Oliv.). *Trichogramma* and other egg parasitoids, Cairo, Egypt. 4-7 October 1994, 157-160.

Karimi, A. M. and Hatami, B. (2010): Effect of feeding and male presence on some biological characteristics of female *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). J. Entomol. Soci. Iran, 29(2):1-11.

Lescano, C. H.; Oliveira, I.; de Lima, F.; Baldivia, D.; Justi, P.; Cardoso, C.; Junior, J. and Argandona, E. (2018): Nutritional and chemical characterizations of fruits obtained from *Syagrus romanzoffiana, Attalea dubia, Attalea phalerata* and *Mauritia flexuosa*. Journal of food Measurements and Characterization, available at https://doi.org/10.1007/s11694-018-9742-3

Salijoqi, A. U. R. and Khajjak, A. S. K. (2007): Effect of different artificial diets on the efficiency and development of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). J. Econ. Entomol., 97:789-792.

Siam, A. N.; Abd EL Hafez, A.; Zohdy, N.; ELShrief, H. and Moursy, L. (2014): Influence of adult nutrition on the fitness of the egg parasitoid *Trichogramma evanescens* (Westwood). Egypt. J. Agric. Res., 92(2):477-484.

Tshernyshev, W. B. and Afonina, V. M. A. (1995): Optimal light and temperature conditions for *Trichogramma evanescens* (Westwood) rearing. In: *Trichogramma* and other parasitoids. Fourth International Symposium. Cairo, Egypt, 4-7 October 1994. 173-175. INRA Publ., Paris, France.

Wackers, F. L. (2005): Suitability of extra floral nectar, pollen and honey dew as insect food. Food sources. pp.17-75. In: Wacker F. L., VanRijn PC and Bruin J (Eds.) Plant Provided Food for Carnivorous Insects: A protective Mutualism and its Applications, Cambridge University Press.

Egypt. J. Plant Prot. Res. Inst. (2018), 1(1): 74-83

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Seasonal fluctuation of the cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) and its natural enemies on mulberry trees in Egypt

Maha I. Abd El-Razzik

Scale insects and mealybugs Research Department , Plant Protection Research Institute, Agricultural Research Center , Egypt

ARTICLE INFO

Article History
Received: 5/4/2018
Accepted: 15/7/2018

Keywords
Phenacoccus solenopsis ,cotton mealybug, seasonal fluctuation, natural enemies and mulberry trees.

Abstract:

The seasonal fluctuation of the cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) was studied for two years (January 2014 until mid of December, 2015) on mulberry trees at Giza Governorate. The obtained results showed that, P. *solenopsis* has two peaks and presence of three overlapping generations in both years under field conditions. The 1st generation started from mid April to early August /mid of July with duration of 3-3.5 months at field condition during two years respectively. The 2nd generation occurred from early of August to mid of October /mid of July to mid of October with duration of 2.5-3 months in both years respectively. The 3rd generation occurred between mid of October to mid of December and they lasted 2 months in both years respectively. The favorable time for its abundance on mulberry branches occurred in early and late summer season due to the high temperature, whereas decrease until disappear during in winter season referred to the cold weather. On the other hand, the relationship between the meaybug fluctuation and abiotic factors (minimum, maximum temperatures and relative humidity) were studied where the simple correlation of the maximum and minimum temperatures was positive and high significant but R.H. % gave negative and insignificant effect. The natural enemies (predators and parasitoids) were surveyed and identified.

The results indicated that three species of predators, these are *Hyperaspis vinciguerrae* Capra (Coleoptera: Coccinelledae), Dicrodiplosis *manihoti* Harris (Diptera: Cecidomiidae), *Scymnus syriacus* Mars. (Coleoptera: Coccinellidae). Also, there were two different primary parasitoids associated with the mealybug, *Acerophagus gutierreziae* Timberlake (Hymenoptera: Encyrtidea) and *Chartocerus dactylopii* (Ashmead) (Hymenoptera: Signiphoridae). The results also observed, *H. vinciguerra* and *D. manihoti* had two and three peaks during the two years of study, respectively.

Introduction

The newly world species of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has emerged as a serious pest of cotton in Pakistan and India, and is now being as a serious threat to cotton in China. It has been infested from 175 host plant species in 45 families, and from 26 countries in different ecological zones (Abbas *et al*., 2010). *P. solenopsis* cause crinkling, twist and condense flower, bud, bolls growth and finally it cause yield loss (Sahito *et al*., 2009).In Egypt, this pest was recorded for the first time infesting *Hibiscus* sp. In September, 2009 by Abd-Rabou *et al*. (2010). This pest spread rapidly on different host plants to the extent that recorded it on 29 host plant species belonging to 16 plant families including field crops (3), vegetables (3), ornamentals (7), weeds (13) and fruits (3) (Abdel-Razzik *et al*., 2015).

Mulberry (*Morus* spp.) is cultivated throughout the world wherever silkworms are raised where their leaves were used as food for silkworms. Mulberry fruit may be eaten raw or cooked (mad into Jam, syrup or juice) (Duke, 1983). Many countries cultivate mulberry trees in field such as China, Korea and India. Others cultivate them as wild trees around the field of different crops or at the side of roads and streams. Egypt is one of the second categories in mulberry cultivations (Hosny *et al*., 1995).

The present work is to study the activity period of *P. solenopsis* on mulberry trees, annual generations and effect of biotic and abiotic factors on the populations of this mealybug.

Materials and methods

1. Seasonal fluctuation of *Phenacoccus solenopsis:*

These experiments were carried out in a free insecticides private farm in El-Saff district, Giza Governorate, Egypt during two successive seasons from January, 2014 to December, 2015 on 15 years old mulberry trees. Twelve infested trees, nearly of the same age and size were used for sampling. Samples of sixty branches (15cm length) were picked from the four cardinal directions and center core of each tree with rate of five branches every two weeks throughout two years. Branches were preserved in labeled paper bags and transferred to the laboratory and carefully inspected using a binocular microscope and the insect population was counted and stored into:

1.1. Alive unparasitized individuals:

Numphs, adult females and ovipositing females that were counted on the mulberry branches using a binocular microscope . The total number of live individuals in each sample was taken as the population index.

1.2. Parasitized individuals:

To calculate age structure per sample, the mean number of each stage was divided by total and multiplied by 100. This way gave each stage a percent proportion of the total per sample regardless the total number of presented insects (*i.e* population density). The number of generations was determined using the obtained data throughout the two successive years using the age-structure technique per sample over the year. Generation was defined as the time required for an insect to complete its life cycle (*i.e.* egg to egg). In the case of Monophelibid,

eggs were oviposited under the female in ovisac until they hatch and crawl out. Ovipositing females were defined as female with eggs. The presence of ovipositing females (*i.e*. the transformation of adult females to ovipositing females) was considered in this study as presence of the egg stage. This phenomenon was used to determine the end of each generation and the beginning of the next one (El-Amir, 2009).

Weather factors data assumed to effect studied insects (i.e. maximum, minimum daily temperatures and mean percentage of daily relative humidity) were obtained for the Giza area from the Egypt-Weather Underground https:/www.wunderground.com/global/EG.html.

Obtained data was summarized for each fourteen days previous to the sampling date. Considered weather factors means over each determined generation was calculated and presented. To investigate the relationship between the climatic factors and the population density of *P. solenopsis* were tested using simple correlation, and multiple regression analysis. All statistical analyses were done using the software package (Costat, 2005).

2. Survey of natural enemies of *Phenacoccus solenopsis:*

Mulberry branches were examined on different months in Giza Governorate. Samples of mulberry branches infested with *P. solenopsis* were collected. The specimens were confined in glass jar kept in laboratory for securing any emerging parasitoids or predators. The immature and mature stags of the predators were counted on the branches mulberry using a binocular microscope every two weeks. The total number of the alive individuals in each sample was taken as the population index.

Results and Discussion

1. Seasonal fluctuation of *Phenacoccus solenopsis*:

Figures (1 and 2) showed that the half-monthly means of nymph, adult female and ovipositing female population density of *P. solenopsis* infesting mulberry trees throughout the two successive years of investigation (2014-2015). The figures also showed the half monthly (maximum &minimum of temperature) and relative humidity recorded during the same two years. In 2014, population density of *P. solenopsis* nymphs on mulberry branches was lowest during January and February (winter season), according to prevailing

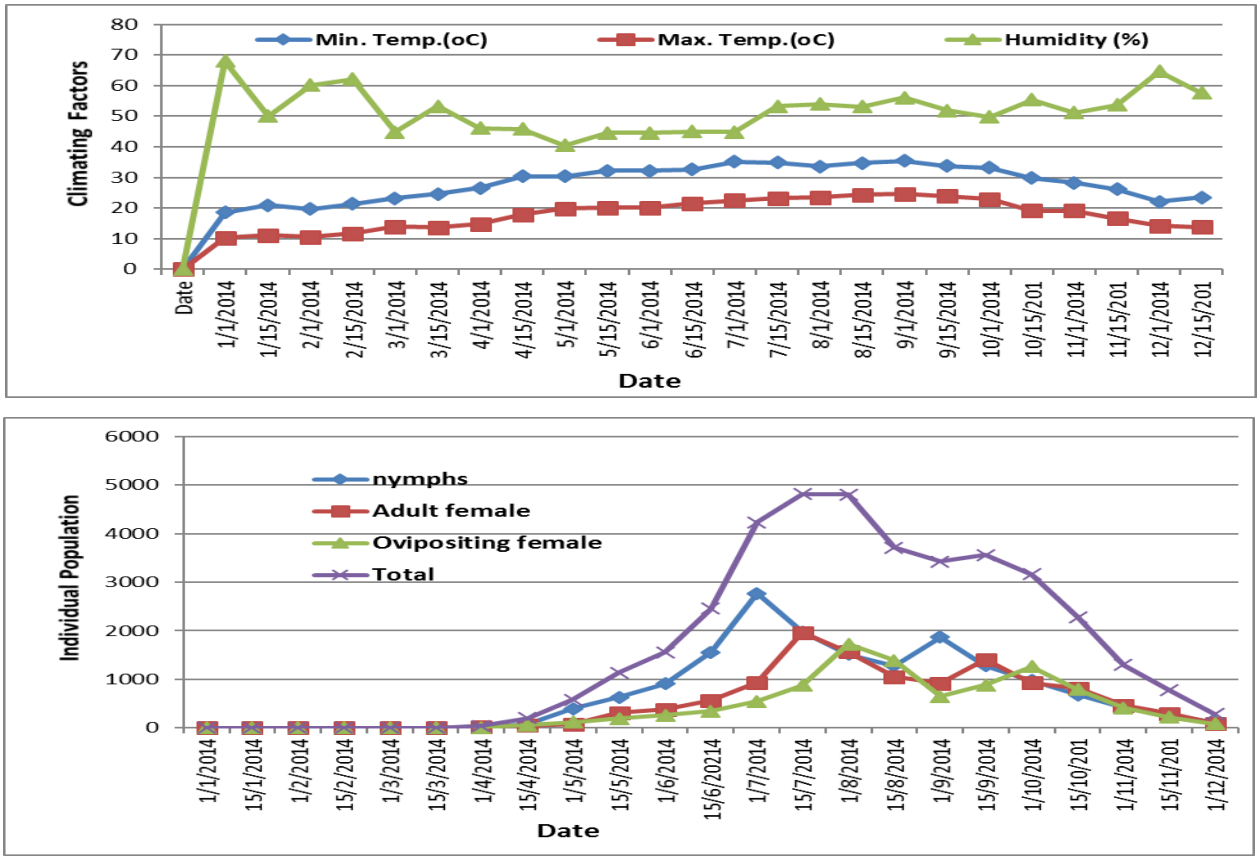


Figure (1): Seasonal fluctuation of *Phenacoccus solenopsis* population in response to max., min. temperature and relative humidity on mulberry trees at El-Saff, Giza Governorate during 2014.

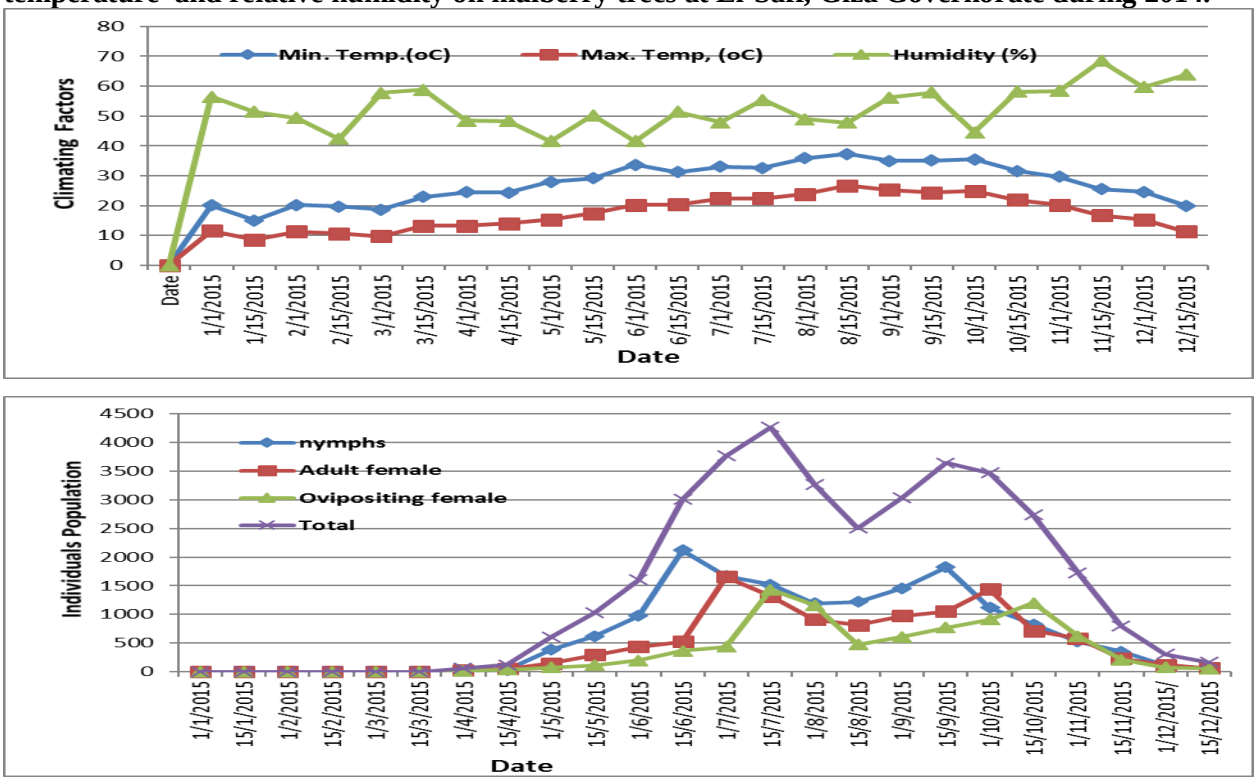


Figure (2): Seasonal fluctuation of *Phenacoccus solenopsis* population in response to max., min. temperature and relative humidity on mulberry trees at El-Saff, Giza Governorate during 2015.

environmental conditions. In April, the population starts to increase gradually, the population high increased to reach large first peak by first July (2765 nymphs/60brumches). Intermediate peak of nymphs was recorded on the first September (1866 nymphs/60 branches). Also, two peaks of adult females were observed on mid July (1958 females/60 branches) and mid September (1405 females /60 branches). Ovipsiting female also had two peaks, the first peak showed in first August (1724 ovipositing female/60 branches) and second peak in mid September (1263 ovipositing females). Over all combined numbers of total population (Nymphs, adult females and ovipositing females) on mulberry branches indicated that activity of *P. solenopsis* extended from April to November with large activity peak on mid July (4815 individuals/60 branches) and intermediate peak of total population occurred during September (3561 individuals/60branches).

The results of the second year of investigation (2015) as represented in Figure (2) showed that population trends and number of peaks of nymphs, adult females, ovipositing female and total population of *P. solenopsis* were nearly similar to those recorded in the previous season (2014). The population of *P. solenopsis* was not finding during January, February and March according to prevailing environmental conditions. In April, the population of nymphs starts to increase gradually and highly increased during June recording the 1st peak on mid June (2117 nymphs/60 branches) then the population decreased again. In July gradually increased and reached to 2nd peak on mid September (1822 nymphs/60 branches). The adult female population has the same trend as the nymphal population, the population gradually decreased from January until April after that it gradually increased in June, the population highly increased and reached to 1st peak by early July with (1654 adult female/60 branches) then decreased again from mid-July until early September Gradual increase was observed in the adult population during mid-September, the population highly increased recording 2nd peak on first October (1437 adult female /60branches). The ovipositing female stage also had two peaks in this year. The population greatly increased in mid July recording the 1st peak (1431 ovipositiog female/60 branches). The ovipositing females gradually decreased during August and September in early October, it increased and reached to high number in mid October recording the 2nd peak (1198 ovipositing female/60branches). On the other hand, the total population (nymphs, adult females and ovipositing females) on mulberry branches indicated that activity of *P. solenopsis* extended to increase from April to October with 1st activity peak on mid July (4268 individuals/60 branches). 2nd peak of total population recorded during mid September (3641 individuals/60 branches). Large activity peak on mid July (4815 individuals/60 branches) and intermediate peak of total population occurred during September (3561 individuals/60branches).

The obtained results showed that, P. *solenopsis* have two peaks during July and September While the results of Arve *et al.* (2011) and Singh and Kumar (2012) indicated that the peaks during October and December of the year in India. Also, they result observed mealybug population decreased from January to March with agreement with the results conducted here. The results here also indicated that the favorable time for its abundance on mulberry branches occurred in early and late summer season due to the high temperature, whereas decrease until disappear during in winter season referred to the cold weather. The data of Arif *et al.* (2012) indicated the same trend. They result showed that, after winter, population build-up of

mealybug took place and its number was high in March on weeds and in April on crops, being 328 and 434 per sample respectively. Its number remained low in mid cotton season followed by a second peak of 320 mealybugs per sample on weeds and 474 on crops in November. Population declined in December. On ornamental plants, incidence of mealybug started increasing in May. Its highest number of 432 mealybugs per sample was recorded in July followed by a decline. Second peak of 304 mealybugs per sample was observed in November followed by a gradual fall. Cotton mealybug was active throughout the year on various crops, ornamental plants and weeds with two population peaks in Multan and nearby districts. Population peaks of mealybug on vegetables (okra, brinjal etc.) in March/April and other crops (cotton etc.) in October/November were observed near their maturity/termination.

2. Duration and number of generations:

Number of annual field generations was estimated from the graphical representation of age structure technique to the seasonal abundance data of *P. solenopsis* obtained over the two years on mulberry trees and illustrated on Figures (3 and 4). The first generation started at the beginning of mid April in both years and extended until early August in the 1st year and mid July in the 2nd one. The duration of the 1st generation lasted 3.5-3 months in both years respectively. The 1st generation peaked in first July in 1st year and mid June in 2nd year. The second generation

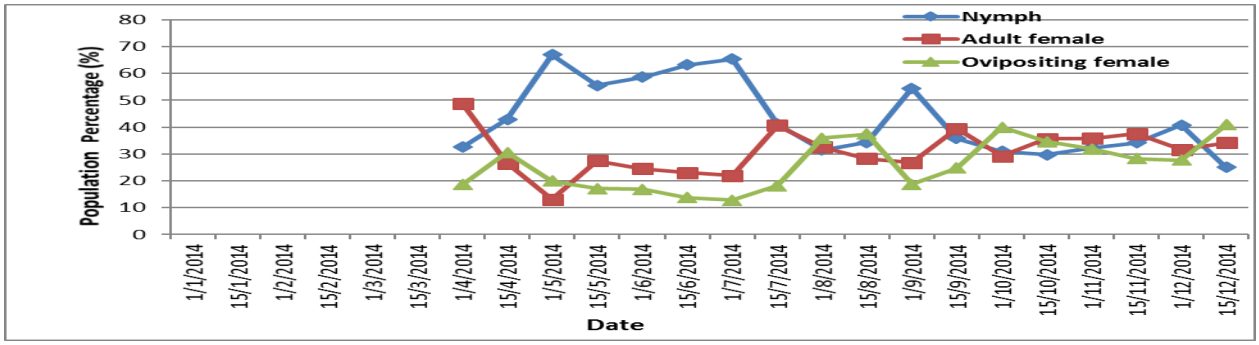


Figure (3): Age structure of *phenacoccus solenopisis* on mulberry trees 2014.

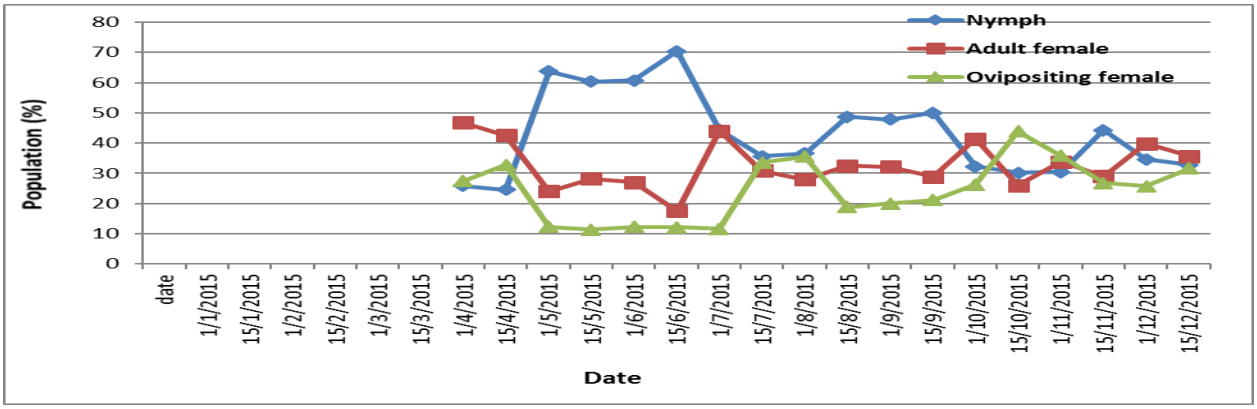


Figure (4): Age structure of *Phenacoccus solenopisis* on mulberry trees during 2015 .

started from first Aug. until mid October 2014 with duration of 2.5 months and mid July until mid October 2015 with duration of 2.5-3 months, it peaked early/mid September in the two years respectively. The third generation which started from early October until mid December with duration 2 months in both years respectively. The obtained results showed that, P. *solenopsis* had three overlapping generations in both

years While, Anonymous (2013) in Australia stated that depending on temperature, mealybug can produce 6-8 generations per year.

3. Effect of ecological factors on *Phenacoccus solenopsis* population fluctuations:

The effectiveness abiotic factors on *P. solenopsis* total population were studied during two successive years (2014-2015) in Giza Governorate as in Table (1).

3.1. Effect of daily maximum temperature:

Results of the statistical analysis of simple correlation on *P. solenopsis* total population during the two studied years showed that, the simple correlation gave positive highly significant correlation with (r) value = (.842±0.1and 0.875±0.10) in both years (2014-2015). Also, the partial regression value (P.reg=24.155±1.8 & 21.9±1.9) showed highly positive significant in the two years respectively.

3.2. Effect of the night minimum temperature:

The effect of night minimum temperature on the total population during two studied years indicated highly positive significant correlation, (r) value= (0.880±0.1&0.912±0.09) respectively. On the other hand, the partial regression showed positive significant relation with (P.reg=21.9±1.9 and12.451±1.9) in the two years respectively.

3.3. Effect of daily mean relative humidity:

The daily mean relative humidity had negative relation, insignificant on the total population (r=-0.1488±0.211 and -0.362±0.214) in the two years respectively. The single effect of this factor on the total population activity appeared from the partial regression coefficient value was insignificant effect with (P.reg=53.1±7.31 and 53.5±3.88) in both years respectively.

3.4. The combined effect of daily mean temperature and humidity:

The combined effect of climatic factors on the cotton mealybug *P. solenopsis* during the two studies years was significant (F=36.516 and 35.651) and the explained variance (E.V) presented (50.2% and 58%) during the two years of study, respectively. The results conducted here indicated that the simple correlation of the maximum and minimum temperatures was positive and high significant but relative humidity % gave negative and insignificant effect. The same results conducted by Dhawan *et al.* (2009). They stated that there was positive correlation among the mealybug population with temperature, whereas negative correlation was observed with relative humidity.

4. Survey of natural enemies associated with *Phenacoccus solenopsis*:

4.1. Predators:

The present data showed the presence of three species of insect predators identified as *Hyperaspis vinciguerrae* Capra (Coleoptera: Coccinelledae), *Dicrodiplosis manihoti* Harris (Diptera: Cecidomiidae), *Scymnus syriacus* Mars. (Coleoptera: Coccinellidae) associated with *P. solenopsis.*

4.2. Parasitoids:

Also, the present data indicated that two primary parasitoids associated with *P. solenopsis*. These parasitoids are as following:

a. *Acerophagus gutierreziae* Timberlake (Hymenoptera: Encyrtidea)

b. *Chartocerus dactylopii* (Ashmead) (Hymenoptera: Signiphoridae)

Two species of encyrtid and signiphorid parasitoids were recorded from samples of *P. solenopsis*, these species are *A. gutierreziae* and *C. dactylopii* (Attia and Kamal, 2016).

5. Population fluctuations of the cotton mealybug, *Phenacoccus solenopsis* predators:

5.1. *Hyperaspis vinciguerra* Capra:

Data in Figures (5 and 6) revealed that the population density of *H. vinciguerra*, had two peaks during the two years of study. The1stpeak recorded in first August /first July in the two years, while the second peak was recorded in first October /early September in

the two years under considerations, respectively. Laila *et al*. (2015) reported that *H. vinciguerra* when associated with *P. solenopsis* had four peaks on lantana plants during two years of study. Kedar *et al.* (2011) recorded that, the coccinellid predators play a good role in reducing the infestation of *P. solenopsis*

Statistical	First year (2014)			Second year (2015)		
Parameters	Temperature		R.H.%	Temperature		R.H.%
	Tmax.	Tmin.		Tmax.	Tmin.	
Simple correlation						
Corr.Coef.(r)	0.842 ±0.115	0.880±0.101	-0.148±0.211	0.875 ±0.103	0.912 ± 0.17	-0.362±0.214
Probability(p)	< 0.0001	< 0.0001	0.4908	0.0001	0.0001	0.1070
Correlation significant	Yes	Yes	No	Yes	Yes	No
Partial Regression						
Partial Regres. Coef (b)	24.155±1.770	7.733 ± 3.20	58.855 ±	21.90±1.946	12.451±1.946	53.466± 3.883
Regression Coefficient r^2	0.710	0.774	0.022	0.766	0.832	0.131
F-value	53.762	75.427	0.491	72.190	108.705	2.863
Probability (p)	< 0.0001	< 0.0001	0.4908	0.0001	0.0001	0.1070
Regression significant	Yes	Yes	No	Yes	Yes	No
Combined factors						
E.V (Explained variance)	50.1			58		
F-value	36.516			35.651		

Table (1): Effect of both temperature and relative humidity on *Phenacoccus solenopsis* population on mulberry trees at El-Saff, Giza Governorate, Egypt during the studied years.

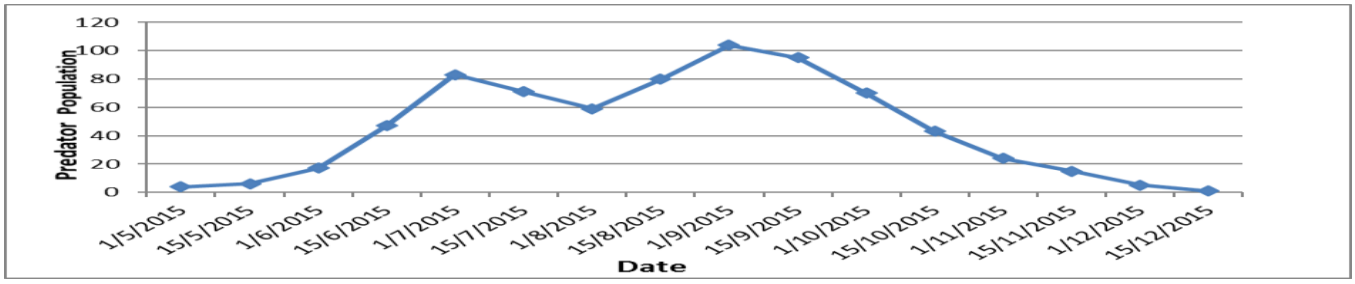


Figure (5):Population density of *Hyperaspis vinciguerrae* on mulberry branches at El-Saff, Giza Governorate. during 2014.

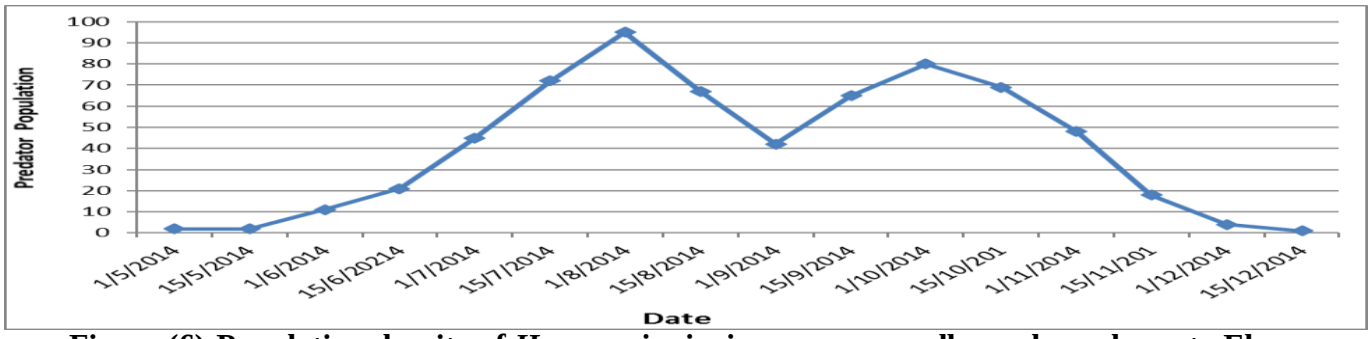


Figure (6):Population density of *Hyperaspis vinciguerrae* on mulberry branches at El-Saff, Giza governorate. during 2015.

5.2. *Dicrodiplosis manihoti* Harris:

The obtained results in Figures (7 and 8) revealed that *D. manihoti* had three peaks in 2014 and 2015 years. The1st peak recorded in first July in the two years, while the second peak was recorded in mid August /mid September in two years respectively. The third peak occurred in mid October /first November in the two years under considratios, respectively. *D. manihoti* was found to associate with the long-tailed mealybug, *P. longispinus* and the citrus mealybug, *P. citri*. *D. manihoti* was obtained during almost months whenever its prey, *P. longispinus* or *P. citri* occurred. The highest number of the predator, *D. manihoti* collected from the sample of mealybug was 93 individuals in November 1994 in Sultanate of Oman (Abbas, 1999). It is concluded that from the results here, in general, the population fluctuation of insect pests in the agricultural field is helpful for assessing the pesticide productivity and timing of pesticide application.

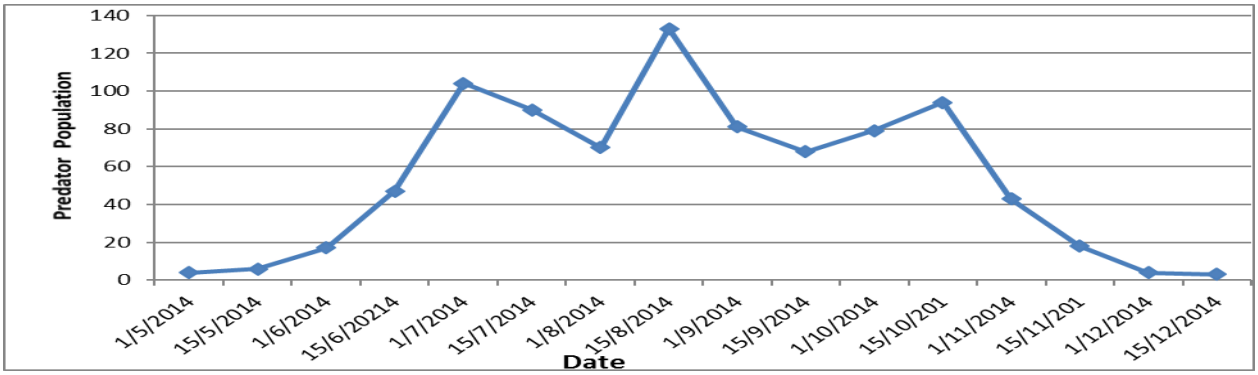


Figure.(7):Population density of *Dicrodiplosis manihoti* Harris on mulberry branches at El-Saff, Giza Governorate during 2014

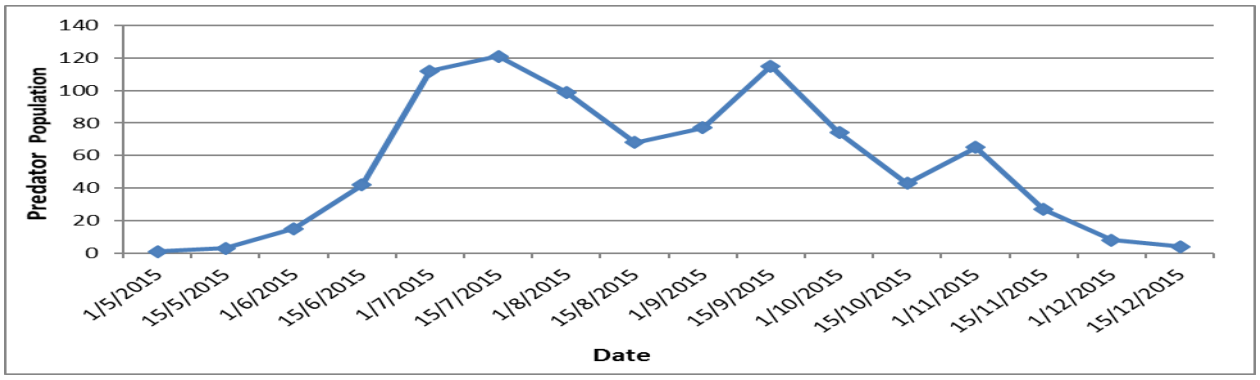


Figure (8):Population density of *Dicrodiplosis manihoti* Harris on mulberry branches at El-Saff, Giza Governorate during 2015 .

References

Abbas, G.; Arif, M. J.; Aslam, M. and Saeed, S. (2010): Host plant distribution and overwintering of cotton mealybug (*Phenacoccus solenopsis)* Himemptera: Pseudococcidae). Int. J. Agric. Biol., 12: 421-425.

Abbas, M.S.T. (1999): Studies on *Dicrodiplosis manihoti* Harris (Diptera: Cecidomyiidae), a common predator of mealybug. Anz. Schä dlingskunde /J. Pest Science, 72: 133-134.

Abdel-Razzik, M. I.; Attia, A. R. and Abdel-Aziz, M. (2015): Newly host plants of cotton mealybug

Phenacoccus solenopsis Tinsley (Hemiptera: Psedococcusdae) in Egypt. Egypt.Acad. J. Biolog., 8(3): 31-33.

Abd-Rabou, S.; Germain, J. F. and Malausa, T. (2010): *Phenacoccus parvus* Morrison and *P. solenopsis* Tinsley, two new scale insects for Egypt. Bulletin de la societec Entomologique de France, 115(4): 509-510.

Anonymous (2013): Solenopsis mealybug in Australia – an overview . State of Queensland, Department of Agriculture, Fisheries and Forestry, 1-2.

Arif, M. I.; Rafiq, M.; Wazir, S.H.; Mehmood, N. and Abdul Ghaffar , M. (2012): Studies on cotton mealybug *Phenacoccus solenopsis* (Hemiptera :Psedococcidea) and its natural enemies in Punjab, Pakistan. Int. J. Agric. Biol., 14(4): 557-562.

Arve, S. S.; Patel, K. G.; Chavan, S,M. and Vidhate, P.K. (2011): Investigation on population dynamics of hibiscus mealybug, *Phenacoccus solenopsis* Tinsley in relation to biotic factors under south Gujarat. J. Biopesticides, 4(2): 211-213.

Attia, A. R. and Kamal, T. A. (2016): Predators, parasitoids and hyperparasitoids associated with cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) infesting different host plants at Giza region. Egyptian Academic Journal of Biological Sciences A. Entomology, 9(4):97-103.

Costat (2005): Version 6.311,Copyright (c), CoHort Software, 798 Lighthouse Ave.

Dhawan, A.K.; Singh, K.; Aneja, A. and Saini, S. (2009): Distribution of mealybug, *Phenacoccus solenopsis* Tinsley in cotton with relation to weather factors in South-Western districts of Punjab. Journal of Enotomological Research, 33(1):59-63.

Duke, J. A. (1983): Handbook of energy crops. P. 1-61.In: ASA special symposium 32, crop tolerance to suboptimaml land conditions. Am. Soc. Agron. Madison, Wi.

El-Amir, S.M.(2009): Studies on some scale insects infesting citrus treesand their control. Ph. D. Thesis Fac. Agric. Al-Azhar Univ. 102 PP.

El-Batran, L.A.; Ghanim, A. A.; Shanab, L.M. and Ramadan, M. M. (2015): Population density of some insects infesting *Lantana camara* L. shrubs ,their predatory insects at Mansoura district. J. Plant Prot. and Path., Mansoura Univ., 6 (5): 813 – 823.

Hosny, A. ; El-Karasky, L.A. and El-Sayed, M.E. (1995): Effect of defoliant materials on mulberry trees and silk production of silkworm, *Bombyx mori* L. Zeitschrift FÜr Angewandte Zoologie, 81(2):279-284.

Kedar, S.C.; Saini, R.K. and Pala, R. (2011): Relative abudance of coccinellid predators associated with *Phenacoccus solenopsis* on cotton. Annals of Plant Protection Sci., 19 (2):475-477.

Sahito, H.,A.; Abro, G. H.; Khuhro, R. D. and Buriro, A.S. (2009): Varietal Resistance of Cotton Crop against Mealybug, *Phenacoccus solenopsis* Tinsley. Pak. Jr., Agri., Agri. Engg., Vet. Sci., 25(1):34-38.

Singh, A. and Kumar, D. (2012): Population dynamics, biology of mealybug *Phenacoccus solenopsis* (Tinsley) and its natural enemies in Vadodara, Gujarat. Recent Res. In Scie. and Tech., 4 (11): 22-27.