



THE ROLE OF SILVER NANOPARTICLES AND SOME EXTRACTS OF DAMAS *CONOCARPUS LANCIFOLIUS* IN CONTROLLING STORED DATES MOTH *EPHESTIA CAUTELLA* (WALKER)

Naseer Malaky Abbood* and Sadik Thajib Ali

Department of Biology, College of Science, University of Thi-Qar, Iraq.

Abstract

In this study the crude (ethanolic & hot aqueous) extract of leaves of the damas *C. lancifolius* were prepared. Two chemical compounds from ethanolic extract were separated and characterized by GC-MS-MS that they were Di(2-ethylhexyl) ester terephthalic acid and Dododecane. The effect of (hot aqueous & ethanolic extracts, Di(2-ethylhexyl) ester terephthalic acid, Dodecane and silver nanoparticles) was studied on many biological parameters of the insect *E. cautella* during larval and adult stages. The treatment with silver nanoparticles was the most efficient concerning antifeedant index (AFI) of the moth larvae with average (0.70%) with significant difference from other treatments, the concentration 1500 ppm was the most effective with average (0.65%) with significant difference from other concentrations and control. The average of percentage of sterility index (SI) achieved in treatment with silver nanoparticles (99.65%) was higher than others and the concentration 1500 ppm was the most efficient concerning this parameter with average (99.88%) that was significantly different from control and other concentrations.

Key words: Silver nanoparticles, ethanolic extract, *E. cautella*, Damas.

Introduction

Stored dates moth insect (*Ephestia cautella*) is one of the most common insect pests that spreads in agricultural crops, cocoa, corn, soybeans and rice (CABI, 2006) and it affects many stored food products such as dates (Hussein, 1974) Flour, cereals, pistachios, etc. (Sedlacek *et al.*, 1995), the larval stage is the harmful stage for nutrients (CABI, 2006) by feeding them (Navarro and Noyes, 2000). In addition to fecal residues, cocoons and uric acid (Singh and Moore, 1985) and being a major carrier of fungi, including the production of aflatoxin (Abbood *et al.*, 2014), this insect was registered as a pest in Europe, Africa, Asia and North, Southern, middle America and Caribbean countries (Haines, 1991), this insect is classified in the order of Lepidoptera and in the family of Pyralidae (Sasaki *et al.*, 1999). The entire life cycle of this insect takes 1-3 months in conditions of the field according to the climate and foodstuff conditions, but under laboratory conditions with a temperature of 24°C and a relative humidity of 75%, it takes about six weeks (Navarro *et al.*, 1991) Because of the multiple problems caused by the chemical control of insects in

*Author for correspondence : E-mail: jullanarfanar@gmail.com

general, which are the appearance of the resistance characteristic of insects and the many environmental and health problems, which made the researchers move towards eco-friendly and little harm to health and with low costs control and from these directions the use of nanoparticles and plant extracts, this research came to control stored dates moth *E. cautella* by some eco-friendly methods by the following points:

1. Preparation of the alcohol ethanol and hot aqueous extract of the leaves of the *Conocarpus lancifolius*.
2. Separation and characterization of some chemical compounds from the ethanol extract of leaves of *C. lancifolius*.
3. Biological effects of alcohol ethanol and hot aqueous extracts and some chemical compounds of alcohol extract for leaves of *C. lancifolius* and silver nanoparticles on stored dates moth insect of *E. cautella*.

Materials and Methods

Collection and breeding of *Ephestia cautella*

A pure culture of the insect was brought from the Center for Integrated Control / Ministry of Science and

Technology / Baghdad/ Iraq and it was bred on a medium consisting of wheat groats (semolina), molasses, yeast of dough and glycerin, placed in glass bottles, covered with pieces of gauze and tightly attached to a rubber band. It was placed at the laboratory temperature and continuously renewed for the duration of the research.

Silver nanoparticles

It was readily obtained through one of the specialized office and they are of American origin (US research nanomaterials, Inc) and they have the following characteristics

(CAS number = 7440-22-4, 50 nm, assay = 99.5%, MW = 107.87).

Concentrations used in the study

Three concentrations (500, 1000, 1500) ppm were prepared by dissolving (50, 100, 150) mg, respectively, of silver nanoparticles with 2 ml of solvent (Hexane 3: Ethyl acetate 7). Then the volume was completed to 100 ml deionized distilled water.

Preparation of hot aqueous and alcoholic ethanol extract of leaves of *C. lancifolius*

Hot aqueous and alcoholic ethanol extracts were prepared according to the method (Harborne, 1984).

Concentrations used in the study

As in the paragraph (materials: 2-1), the silver nanoparticles were replaced by the dry powder of the alcoholic ethanol and hot aqueous extract of the leaves of the *C. lancifolius* and the deionized distilled water with normal distilled water.

Separation and characterization of some chemical compounds of the alcoholic ethanol extract of leaves of *C. lancifolius*

After obtaining the dry alcoholic extract, many chemical solvents were tried to determine the most effective solvent for use in separating some of the chemical compounds from the alcoholic extract through method (Harborne, 2000) and using thin layer chromatography (TLC) and accordingly the solvent was approved (Hexane 3: Ethyl acetate 7). Some of the chemical compounds in the alcoholic ethanol extract were isolated by column chromatography method (Sarker *et al.*, 2006) and through this method two chemical compounds were isolated from the alcoholic ethanol extract and characterized by GC- MS / MS(MS Model= 5973 Network Mass Selective Detector, Ion source= Electron Impact (EI) 70ev, Ion source Temperature = 230°C, Analyzer = Quadrupole, Analyzer Temperature = 230°C) where one of two compounds was Alkane (Dodecane) and the other was ester Di(2-ethylhexyl) ester

terephthalic acid.

Concentrations used in the study

The same method was used in Paragraph (materials: 2-1) with the substitution of silver nanoparticles with Dodecane and Di(2-ethylhexyl) ester terephthalic acid and deionized distilled water with normal distilled water.

Biological effects of silver nanoparticles, hot aqueous and alcoholic ethanol extracts and some chemical compounds of leaves of *C. lancifolius* on stored dates moth *E. cautella*.

- The effect on larvae:

Five larvae were taken at the age of one or two days and were placed in special cans with artificial food mixed with it (1) ml from each of the previously mentioned concentrations of various treatments. As for the control treatment, it was done with distilled water added to it (2) ml of solvent (Hexane 3: Ethyl acetate 7) / 100 ml and in three replicates, all cans were incubated at a temperature (28 ± 2)°C and relative humidity (5 ± 70%) and examined after (7) days where Antifeedant Index (AFI) was calculated from :

$$AFI = \frac{C-T}{C+T} \times 100 \quad (\text{Isman } et al., 1990)$$

Where, C = food consumed in control,

T = food consumed in treatment.

The effect on adults

Two adult insects (1 female: 1 male) at the age of one or two days were placed in a lantern bottle on a black filter paper in a Petri dish and the opening of the top bottle was sealed with a piece of gauze placed in the center of it a cotton swab moistened with a 10% sugar solution added to it 1 ml of the concentrations of the previously mentioned treatments all separately. This bottle represents one replication for each of the aforementioned concentrations of various treatments. As for the control treatment, the cotton was moistened with 10% sugar solution added to it 2 ml of the solvent (Hexane 3: Ethyl acetate 7) / 100 ml and by three replications. After three days, the eggs were collected in relation to each concentration of the previous treatments and control separately, Sterility index (SI%) is calculated by the formula of Robb and Parrella, 1984.

Statistical analysis

All experiments were carried out according to the complete randomized design (C.R.D). The results were analyzed statistically by analysis of variance and by using the SPSS statistical program. The Least Significant Difference (LSD) was used to compare between the statistical differences and various interferences between

Table 1: Antifeedant index (AFI) of larvae of stored dates moth insect *E. cautella* control and treated with (alcoholic ethanol and hot aqueous extract of leaves of *C. lancifolius*, di (2-ethylhexyl) ester terephthalic acid, Dodecane and silver nanoparticles).

Type of substance concentr. (ppm)	Alcoholic ethanol extract	Hot aqueous extract	Di(2-ethylhexyl) ester terephthalic acid	Dodecane	Silver nano particles	Rate of concentration
500	0.28*	0.15	0.28	0.35	0.63	0.34
1000	0.29	0.10	0.29	0.37	0.57	0.32
1500	0.30	0.37	0.75	0.96	0.90	0.65
Rate of substance	0.29	0.21	0.44	0.56	0.70	

* The numbers represent an average of three replications
LSD 0.05 for materials = 0.10 LSD 0.05 for concentration = 0.10 LSD 0.05 for interference = 0.13.

the rates and the probability level is 0.05.

Results and Discussion

Biological effects of silver nanoparticles, hot aqueous and alcoholic ethanol extracts and some chemical compounds of leaves of *C. lancifolius* on stored dates moth *E. cautella*

• The effect on larvae:

Antifeedant index (AFI): Through table 1, with regard to the effect of the substance type, it can be observed that the highest rate of Antifeedant index (AFI) was in the treatment with silver nanoparticles, as it reached 0.70% and it differed significantly ($p \leq 0.05$) from each of the alcoholic ethanol extract and the hot aqueous extract and the ester compound Di (2-ethylhexyl) ester terephthalic acid and Dodecane, whose Antifeedant index was (0.29%, 0.21%, 0.44% and 0.56%) respectively and the current result is that the silver nanoparticles achieved the highest rate of Antifeedant index consistencies with what Kitherian, (2017) concluded that these particles have a significant Antifeedant effectiveness against larvae of cotton worm moth, *Helicoverpa armigera*, where these particles can adversely affect digestive enzymes in the insect stomach. Bharani and Namasivayam, (2017) found

that these particles, when mixed with food, affected enzymes (Lipase, Amylase and Protease) in the stomach of tobacco worm *Spodoptera litura*. Shockman and Barren, (1983); Raffi *et al.*, (2008) have demonstrated that, after the arrival of silver nanoparticles on the epithelial membrane of the insect stomach, they inhibit the enzymes and produce Peroxide, which leads to the death of stomach cells. As for the effect of concentration, The concentration (1500 ppm) was the most efficient, as it produced the largest Antifeedant index (0.65%) and it differed significantly ($p \leq 0.05$) from each of the two concentrations, 500 ppm (0.34%) and 1000 ppm (0.32%), while the last two concentrations did not differ significantly ($p \leq 0.05$).

It is clear from table 1 that the concentration (1500 ppm) within the treatment with alkane Dodecane achieved the largest Antifeedant Index (0.96%) and has differed significantly ($p \leq 0.05$) from the rest of the concentrations within the different treatments, followed by the same concentration within the treatment of silver nanoparticles (0.90%) and both did not differ significantly ($p \leq 0.05$) and this result was consistent with what Poonsri and *et al.*, (2015) observed that the isolated alkanes from the *Bauhinia scandens* plant had a

Table 2: AFI correlation matrix of stored *E. moth* insect *E. cautella* control and treated with (hot alcoholic ethanol and aqueous extract of leaves of *C. lancifolius*, ester compound Di (2-ethylhexyl) ester terephthalic acid, alkane Dodecane and silver nanoparticles).

Inter-Item Correlation Matrix						
	Alcoholic ethanol extract	Hot aqueous extract	Di(2-ethylhexyl) ester terephthalic acid	Dodecane	Silver nano particles	Mean
Alcoholic ethanol extract	1					0.28
Hot aqueous extract	-0.164	1				0.21
Di(2-ethylhexyl) ester terephthalic acid	-0.229	0.894**	1			0.44
Dodecane	0.495	0.648	0.697*	1		0.56
Silver nanopartic-les	-0.577	0.512	0.603	-0.029	1	0.7

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed).
Note : (r = 0 No correlation, 0.00 < r < 0.25 weak extreme, 0.25 < r < 0.75 moderate extreme, 0.75 < r < 1 strong extreme, r = 1 perfect, negative value inversely).

Table 3: Percentage of sterility index of larvae of stored dates moth insect *E. cautella* control and treated with (alcoholic ethanol and hot aqueous extract of leaves of *C. lancifolius*, di (2-ethylhexyl) ester terephthalic acid, alkane Dodecane and silver nanoparticles).

Type of substance conc. (ppm)	Alcoholic ethanol extract	Hot aqueous extract	Di(2-ethylhexyl) ester terephthalic acid	Dodecane	Silver nano particles	Rate of concentration
500	98.00	97.00	98.00	99.00	99.00	98.20
1000	99.13	99.27	99.66	99.86	99.98	99.58
1500	99.45	100.00	99.99	99.99	99.97	99.88
Rate of substance	98.86	98.75	99.22	99.61	99.65	
* The numbers represent an average of three replications LSD 0.05 for materials = 0.09 LSD 0.05 for concentration = 0.09 LSD 0.05 for interference = 0.13.						

antifeedant effect on the larvae of *Plutella xylostella*.

From table 2, it appears that there was a positive correlation ($p \leq 0.05$) between the ester di (2-ethylhexyl) ester terephthalic acid and the hot aqueous extract, in which the higher the effect of the first compound (the higher the Antifeedant Index) the greater the Antifeedant Index caused by the hot aqueous extract on the larvae of stored dates moth *E. cautella* and the opposite is true and in the case of a decrease in the effect of the first on the larvae of the insect, the effect of the second decreases and the opposite is also true. Likewise, with regard to the correlation between the ester compound Di (2-ethylhexyl) ester terephthalic acid and alkane Dodecane.

• The effect on the adults:

Percentage of sterility index: From table 3, it can be noted that with regard to the effect of the substance type, the treatment with silver nanoparticles achieved the highest average percentage of sterility index (99.65%), which did not differ significantly ($p \leq 0.05$) from the rate achieved in the treatment with the alkane Dodecane (99.61%), but it differed significantly ($p \leq 0.05$) from treatment with the ester Di(2-ethylhexyl) ester terephthalic acid (99.22%) and differed significantly with treatment with hot aqueous extract and ethanol alcoholic extract (98.75%,

98.86%) respectively, these results are consistent with what was recorded by Armstrong *et al.*, (2013) that confirmed that mixing silver nanoparticles with food of *Drosophila melanogaster* reduced its fertility by decreasing the hatching rate of eggs laid by females of this insect. and this result was supported by AL-Naami *et al.*, (2017) when they treated the second larval stage of Khapra beetle *Trogoderma granarium* with silver nanoparticles, which resulted in a significant decrease in the percentage of hatching eggs laid from the adults resultant from the larvae treated with these particles. Regarding the effect of the concentration, the concentration (1500 ppm) caused the highest rate of percentage of sterility index (99.88%) and differed significantly ($p \leq 0.05$) from concentration 500 ppm (98.20%) and concentration 1000 ppm (99.58%). Note that table 3 showed that the concentration (1500 ppm) and for various treatments has caused the highest percentage of sterility index, especially in the treatment with hot aqueous extract where the percentage was 100%, which differed significantly ($p \leq 0.05$) from the treatment with alcoholic extracts at the same concentration (99.45%), but it did not differ significantly ($p \leq 0.05$) from treatment with the ester di (2-ethylhexyl) ester terephthalic acid, the alkane compound Dodecane

Table 4: Correlation Matrix Percentage of Sterility index of Stored dates moth Insect *E. Cautella*, control and treated with (hot aqueous and ethanolic alcohol extract of Leaves of *C. lancifolius*, ester compound Di (2-ethylhexyl) ester terephthalic acid, alkane compound Dodecane and Silver Nanoparticles).

Inter-Item Correlation Matrix						
	Alcoholic ethanol extract	Hot aqueous extract	Di(2-ethylhexyl) ester terephthalic acid	Dodecane	Silver nano particles	Mean
Alcoholic ethanol extract	1					98.86
Hot aqueous extract	0.991**	1				98.75
Di(2-ethylhexyl) ester terephthalic acid	0.948**	0.969**	1			99.22
Dodecane	0.977**	0.968**	0.933**	1		99.61
Silver nanopartic-les	0.962**	0.969**	0.966**	0.955**	1	99.65
** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed). Note : (r = 0 No correlation, 0.00 < r < 0.25 weak extreme, 0.25 < r < 0.75 moderate extreme, 0.75 < r < 1 strong extreme, r = 1 perfect, negative value inversely).						

and silver nanoparticles within this concentration (99.99%, 99.99% and 99.97%) respectively and did not differ significantly ($P \leq 0.05$) from concentration (1000 ppm) within the treatment with silver nanoparticles (99.98%). These results are somewhat compatible with Shehu *et al.*, (2010) that found that treating adults of stored moth insects *E. cautella* with neem oil extract resulted in a high percentage of sterility index that was represented by a low percentage of egg hatching laid by the adults of this insect.

Table 4, through which a positive correlation ($p \leq 0.05$) can be observed between the ethanol alcoholic extract and the hot aqueous extract, that is, the higher effect of one of them on the adults of the stored dates moth *E. cautella* by increasing the percentage of Sterility index increases the effect of the other in relation to the same criterion and when the effect of one of them is reduced by the low percentage of sterility index of the adults, the effect of the other decreases according to the same criterion, Likewise, with regard to the correlation between the ester Di(2-ethylhexyl) ester terephthalic acid and both the hot aqueous extract and the ethanol alcoholic extract, as well as with the correlation between the Dodecane and all of the ester di (2-ethylhexyl) ester terephthalic acid and hot aqueous extract and ethanol alcoholic extracts, the same applies to the correlation between silver nanoparticles and all of the alcoholic and hot aqueous extracts and the ester di (2-ethylhexyl) ester terephthalic acid and the alkane compound Dodecane.

References

- Abbood, N.M., S.T. Ali and S.H. Mohammad (2014). Qualitative and quantitative contamination with fungi accompanying the infestation of some foods with fig moth insect *Ephesia cautella* (Walker) (Lepidoptera : Pyralidae) and test of ability of *Aspergillus flavus* isolates to produce aflatoxin. *Thi-Qar J. Agric. Res.*, **3(2)**: 2014.
- AL-Naami, M.T., A.M. Emad and E.A.M. Hussam (2017). The effect of silver nanoparticles on second larval Instar of *Trogoderma granarium* Everts (Insecta : Coleoptera: Dermestidae). *I.J.S.N.*, **8(2)**: 303-306.
- Armstrong, N., M. Ramamoorthy, D. Lyon, K. Jones and A. Duttaroy (2013). Mechanism of silver nanoparticles action on insect pigmentation reveals intervention of copper homeostasis. *PLoS One.*, **8(1)**: e53186.
- Bharani, R.A. and S.K.R. Namasivayam (2017). Biogenic silver nanoparticles mediated stress on developmental period and gut physiology of major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)-An eco-friendly approach of insect pest control. *Journal of environmental chemical engineering.*, **5(1)**: 453-467.
- CABI CAB International (2006). Data sheet for *Cadra cautella*. Crop Protection Compendium 2006 Edition.
- Devi, G.D., K. Murugan and C.P. Selvam (2014). Green synthesis of silver nanoparticles using *Euphorbia hirta* (Euphorbiaceae) leaf extract against crop pest of cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Biopesticides.*, **7**: 54.
- Haines, C.P. (1991). Insects and Arachnids of tropical stored products. Their biology and identification. 2nd edition Chatham UK: Natural Resources Institute, 246.
- Harborne, J.B. (1984). Methods of plant analysis. *In Phytochemical methods* (1-36). Springer, Dordrecht.
- Harborne, J.B. (2000). Phytochemical methods a guide to modern techniques of plant analysis., 2nd edition Chapman hall, London, New York, 240.
- Hussain, A.A. (1974). Date palms and Dates and their Pests in Iraq, Mosul University Press. 166.
- Isman, M.B., O. Koul, A. Luczynski and J. Kaminski (1990). Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *Journal of Agricultural and Food Chemistry.*, **38(6)**: 1406-1411.
- Kitherian, S. (2017). Nano and Bio-nanoparticles for insect control. *Research Journal of Nanoscience and Nanotechnology.*, **7**: 1-9.
- Navarro, S., E. Donohaye and R. Dias (1991). Insects of Stored products. Israel Agricultural Organization and Ministry of Health, 87.
- Navarro, S. and R.T. Noyes (2001). The mechanics and physics of modern grain aeration management. CRC press.
- Poonsri, W., W. Pluempanupat, P. Chitchirachan, V. Bullangpoti and O. Koul (2015). Insecticidal alkanes from *Bauhinia scandens* var. *horsfieldii* against *Plutella xylostella* L. (Lepidoptera: Plutellidae). *Industrial Crops and Products.*, **65**: 170-174.
- Raffi, M., F. Hussain, T.M. Bhatti, J.I. Akhter, A. Hameed and M.M. Hasan (2008). Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *Journal of materials science and technology.*, **24(2)**: 192-196.
- Robb, K.L. and M.P. Parrella (1984). Sublethal effects of two insect growth regulators applied to larvae of *Liriomyza trifolii* (Diptera: Agromyzidae). *Journal of economic entomology.*, **77(5)**: 1288-1292.
- Sarker, S.D., Z. Latif and A.I. Gray (2006). Natural product isolation. *In Natural products isolation* (1-25). Humana press.
- Sasaki, T. and H. Ishikawa (1999). Wolbachia infections and cytoplasmic incompatibility in the almond moth and the mediterranean flour moth. *Zoological Sci.*, **16(5)**: 739-745.
- Sedlacek, J.D., P.A. Weston and R.J. Barney (1996). Lepidoptera and psocoptera. *Integrated management of insects in stored products*, 41-70.
- Shehu, A., D. Obeng-Ofori and V.Y. Eziah (2010). Biological efficacy of Calneem TM oil against the tropical warehouse moth *Ephesia cautella* (Lepidoptera: Pyralidae) in stored maize. *International Journal of Tropical Insect Science.*, **30(4)**: 207-213.
- Shockman, G. D. and J.F. Barren (1983). Structure, function and assembly of cell walls of gram-positive bacteria. *Annual review of microbiology.*, **37(1)**: 501-527.
- Singh, P. and R.F. Moore (1985). Handbook of insect rearing. *Elsevier Science Publishers*, Amsterdam.