

SUPERVISION SHEET

**BIOCHEMICAL STUDIES OF SOME
PESTICIDES ON DATE PALM TO CONTROL
Ephestia cautella (WALKER)**

**Ph.D. Thesis
In
Agricultural Sci. (Biochemistry)**

By

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ABSTRACT

Dates are exposed to many insects pest in all its stages of maturity. *Ephestia cautella* is the major insect pest attacking dates at harvest time and storage particularly Siwy and Frihy cultivars. Five insecticides (Tracer, Radiant, Coragen, Pleo and Achook) from new groups were selected as safe alternatives to human and environment against *E. cautella* in storehouse of Siwy and Frihy dates. Three concentrations of each insecticide (single, half and double concentrations) were sprayed on clean and untreated fruits of each cultivar for storing. The single concentration was a prescribed concentration, based on the recommended concentration to be used on different crops. Half concentration did not appear to be effective in all insecticides used on both cultivars, the double concentration was achieved 100% mortality during larvae stage period and single concentration was effective particularly in Radiant while Tracer was the lowest influence on insects. Data exhibited no significant changes in the main chemical composition of these treated dates. The residual levels of tested insecticides in the fruit were studied after 28 days from application. The results showed a complete dissipation of the Coragen after 21 days for both cultivars, and the residue of the Achook pesticide was less than LOQ (limited of quantitation) the lowest concentration of the pesticide could be detected. The Risk assessment were studied based on chronic exposure of pesticides, the calculated EDI (estimated daily intake) was lower than ADI (acceptable daily intake) indicating no risk associated with the exposure via the consumption of treated date after storage period. Also, the toxicological, biochemical and molecular studies were carried out on 3rd instar larvae of *E. cautella* in laboratory conditions. Bioassay is done in order to determine the LC₂₅, LC₅₀ and LC₉₀ for each compound and the results showed that Coragen was the most potent insecticide with LC₅₀ followed by Radiant and Achook. Effects of the used insecticides on the vital enzymes activity were studied. The Data revealed that treatment with Radiant showed an elevation in AST, ACP, ALP and GST activities and reduction in AChE and ALT and protease activities. Insects treated with Coragen demonstrated an elevation in the activities of AChE, GST, ALP, ACP, ALT and protease. Finally, to screen the effect of the five insecticides on genome-wide DNA mutability, 15 RAPD primers where used against both control and treatments. The RAPD analysis results indicated that Radiant is a genotoxic insecticide which causes large changes in genomic DNA. While, Coragen and Achook had the lowest mutability effect against the insect DNA.

Keywords: *Ephestia cautella*, Date, Insecticides, Chemical composition, Pesticides residues, Protein, Protease, AChE, GST, ALP, ACP, AST, ALT, RAPD-PCR.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
A.O.A.C.	Association of Official Analytical Chemistry
ACP	Acid Phosphatase
ADI	Acceptable daily intake
ALP	Alkaline phosphatase
ALT (GPT)	Alanine aminotransferase
ARfD	Acute reference dose
AST (GOT)	Aspartate aminotransferase
ECD	Electron capture detector
EDI	Estimated daily intake
EPA	Environmental Protection Agency of the United States
FAO	Food and Agricultural Organization of the United Nations
GC	Gas chromatography
GEMS/FOOD	Global Environment Monitoring System - Food
GSTs	Glutathion-S-Transferases
HPLC	High performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC ₅₀	Median lethal concentration
LOQ	limit of quantitation
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MRL	Maximum residue limit
ND	Not detected
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
ppm	Part per million
PSA	Primary-Secondary amine
RAPD	Randomly Amplified Polymorphic DNA
S.E.	Stander error
SPE	Solid Phase Extraction
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
WHO	World Health Organization

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INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an economic crop in Egypt and many other countries all over the world with nourishing fruits. Egypt is the largest date producer in the world (FAO, 2016), level in terms of production by 18% of the world production of dates, and the first at the level of the Arab world by 23%. Nevertheless the exported amount of dates fruit is less than 3% (Worldatlas 2018), although the high quality of the main exported cultivar Siwy in addition to some other cultivars. Main reason is the fruit infestation with a number of pests. On the top of these pests *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) which infest the fruit on the tree before harvest, but the major infestation occurs during storage and cause a lot of losses, whereas the infestation starts from the field with about 4% and this percentage increases after storing up to 35%. (Howard *et al.*, 2001). The damage caused by the larvae by feeding on the fruits or by contaminating them with webbing and feces. It also infests flour, rice, maize, cereal grains, dried fruits such as fig, almond, raisins, dates, pears, ground nuts, walnuts, and confectionery items like biscuits and chocolates. (Hodges and Farrell, 2004; Rees, 2007 and Girish, 2017)

The conventional insecticides such as Malathion and Chlorpyrifos-methyl, and the fumigants such as Methyl-bromide (MB) and Phosphine were being used for control efforts with great danger on the human health and environment. Therefore, MB is

facing a phase-out worldwide since 2015 under the terms of the Montréal Protocol (UNEP, 1998), but still the most effective way for fumigating the dates illegally. Recently, many researchers have been devoted for seeking alternatives to MB against insect pests in warehouses (Fields and White, 2002 and Robin, 2013). Among these alternatives is using other new groups of insecticides with new mode of action, have low toxicity (Rat oral LD₅₀ > 5000 mg/kg of body weight) and with lower adverse impact on the environment. Among the modern proposed insecticides; groups/families such as Spinosyns, Diamide, Pyridalyl and Azadirachtin were investigated (Gavkare *et al.*, 2013). Many researchers have applied these insecticides to control *E. cautella* or other storages products insects such Shehu *et al.* (2010) used Azadirachtin agaist *E. cautella*, Athanassiou *et al.* (2005) studied Azadirachtin on stored grains, Vassilakos *et al.* (2012) tested Spinetoram on stored grains, Almasi *et al.* (2013) experimented Spinosad on wheat and stored peanuts, Kavallieratos *et al.* (2013) used Diamide, Chlorantraniliprole against *Ephestia* on stored-product and Abd El-Razik and Zayed (2014) studied the effect of Pyridalyl on cowpea.

Spinosyns are a large family of unprecedented compounds produced from fermentation of two species of *Saccharopolyspora*. Many reports are conforming their insecticidal potency in additional to their broad-spectrum activities against all stages of many lepidopterous pests were released (Rodriguez *et al.*, 2016.). Spinosad, Tracer® is the only one insecticide registered for

use against *E. cauttella* on date in Egypt. Meanwhile, another member of Spinosyns family which is Spinetoram, Radiant® showed greater potency and longer duration of control against certain insects when compared with Spinosad (Dripps *et al.*, 2008).

Among the Diamide insecticides, Coragen®, Chlorantraniliprole is one of those with a high controlling power, almost covering all economically important Lepidoptera and other species. It is active on chewing pests by ingestion and by contact, showing good larvicidal active (Su *et al.*, 2017).

Pyridalyl, Pleo®, is a novel insecticidal class that has a Phenoxy-pyridaloxo derivative structure that not related to any other existing insecticide showing extraordinary activities against various lepidopteran larvae (Isayama *et al.*, 2005)

One of the natural pesticides, Azadirachtin, Achook®, is derived from the Neem tree. It known as a repellent, a potent anti-feedant and disrupting growth, development of insect larvae and sterilizes adults (Chaudhary *et al.*, 2017).

Generally, the different groups of insecticides cause defect in the vital and enzymatic systems for insects through the interaction between xenobiotic and the enzymes or a transport protein and inhibit its normal function (McKinlay *et al.*, 2012). To evaluate the effectiveness of novel insecticides, many biochemical markers in insects can be employed, such as, the nerve conduction enzyme (Acetylcholinesterase; AChE), the detoxifying enzymes