FURTHER STUDIES ON INSECTICIDES RESISTANCE IN THE COWPEA APHID

BY

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B.Sc. Agric. Sci. (Pesticides), Fac. Agric., Zagazig Univ., 1991
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APPROVAL SHEET

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ABSTRACT

The cowpea aphid, *Aphis craccivora* (Koch) is an important pest of legumes crops in Egypt. Aphid has developed resistance to many insecticides. Organophosphates are considered important group of insecticides being used against piercing insects, in addition to neonicotinoids which introduced to the market science 1990.This study was carried out to explore resistance characteristic of cowpea aphid to chloropyrifos-methyl and acetamiprid and studying some biochemical and genetic differences. Selection pressure was used to build up resistance within 24- generations with both insecticides. Resistance to acetamiprid increased to 38.6 -fold compared with susceptible strain(S-strain), while chloropyrifos-methyl reached to about 82 fold in the same period. Cross resistance patterns, in acetamiprid resistant strain (R-acetamiprid) showed that, all tested members of neonicotinoids, as well as the synthetic pyrethroid lambda-cyhalothrin and s-fenvalerate possess obvious resistant level. However, the rest of tested insecticides didn't show obvious cross resistance. On the other hand, Cross resistance patterns, in chloropyrifos-methyl showed that, the organophosphate compound, malathion as well as the synthetic pyrethroid, lambda-cyhalothrin possess obvious resistant level. While the other tested insecticides except for acetamiprid and thiamethoxam exhibited various levels of tolerance. Selection pressure produced some differences in total protein content and SDS-protein banding patterns. The analysis of esterase patterns and esterase bands classification by inhibitors obtained by the native-PAGE and by using two substrates revealed differences as a result for selection. Acetylcholinesterase (AChE), total esterase activity and glutathione-s-transferase (GST) activity was also measured. Total esterase and (GST) activity demonstrate a significant role for these enzymes in chloropyrifos-methyl resistance. Synergistic studies showed that, esterase play the key role of resistance in chloropyrifos-methyl selected strain and the monooxygenase also play a potent role. In acetamiprid selected strain, P450 mediated detoxification plays a substantial role in acetamiprid resistance but other secondary mechanism(s) may be involved. DNA polymerase chain reaction (PCR) is a less expensive and rapid molecular method. The size and number of PCR products obtained using multiprimer sets, the multiplex PCR method, can be used for distinguishing several strains. Then the amount of genetic variation within and among strains, using a molecular assay that rapidly distinguishes the species. The results confirmed that RAPD profiling is a powerful method for identification and distinguishing for pesticides resistance between different strains.

Keywords: *Aphis craccivora*, acetamiprid, chloropyrifos-methyl, resistance, synergists, esterases, P 450- monooxygenase, SDS protein, polymerase chain reaction (PCR).

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LIST OF ABBREVIATIONS

Abbreviation	
A-St.	Acetamiprid resistant strain
AChE	Acetylcholinesterase
ATC	Acetylthiocholine
ATChI	Acetylthiocholine iodide
BLRV	Bean leaf roll virus
BPH	Brown plant hopper
CbE	Carboxylesterase
CDNB	1-chloro-2-4-dinitrobenzene
Ch-m St.	Chloropyrifos-methyl strain
CL	Confidence limit
DBM	Diamond back moth
DEF	Tribufos (S, S, S-tributyl phosphorotrithioate)
DEM	Diethyl malate
DNA	Deoxyribonucleic acid
FBNYV	Faba bean necrotic yellows virus
F-St.	Field strain
GABA	γ- amino butyric acid
MFO	Mixed function oxidase
MACE	Modification of acetylcholine esterase
ng	Nano gram
1-NA	α-naphthyl acetate
№	Number
PBO	Piperonyl butoxide
PAGE	Polyacrylamide gel electrophoresis
R H	Relative humidity
Rm	Relative mobility
SE	Standard error
S-St.	Susceptible strain
SR	Synergistic ratio
TPP	Triphenyl phosphate

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INTRODUCTION

Cowpea aphid (*Aphis craccivora* Koch) is a major insect pest on leguminous food crops. When aphid infestation level is large; it may stunt or kill plants. As a result of aphid feeding, a considerable amount of honeydew is produced upon which sooty mold grows. Such black sooty mold reduces photosynthesis and may render leaves unpalatable to livestock. Honeydew also makes the plants sticky, and causes problems at harvest. Cowpea aphid causes major yield losses, due to transmission of two major viruses; faba bean necrotic yellows virus (FBNYV) and bean leaf roll virus (BLRV) (Laamari *et al.,* 2009). It has become a serious pest on a variety of legumes such as faba bean, cow pea and pea in Egypt (El-Ghareeb *et al.,* 2002).

Insecticide resistance is one of the major obstacles to the control of agricultural pests. This worldwide problem has been documented for over 500 arthropod species which include over 20 resistant aphid species (Georghiou, 1990). The continuous use of insecticides to control insect infestations and the ability of aphids to travel over long distances could lead to widely distributed insecticide resistant populations. Resistance results in increased pesticide application frequencies and dosages, decreased yields, together with likely risks of more residues in food and environmental damages due to side effects on beneficial organisms, as well as to the increased release of xenobiotics in air, soil and water. Due to the great difficulty and large investments associated with the development of new insecticides; there is always a need to preserve the efficacy of current and future developed active ingredients. It is important, therefore to monitor the presence of insensitive specimens and to understand which resistance mechanisms are co-selected, so that strategies to delay their diffusion can be designed (Criniti *et al.,* 2008).

The use of organophosphorus (Ops) insecticides in the agricultural and urban settings is still high and is expected to remain so, at least in the near future, because of the efficacy of OPs, their relatively low cost and their lack of bioaccumulation in the ecosystems. Meanwhile, other classes of insecticides are gaining a considerable market share (*e.g.,* pyrethroids) and new classes have been developed such as neonicotinoids (Costa, 2006). Neonicotinoids insecticides represent a relatively new group of chemicals with novel mode of action that includes imidacloprid, thiametrhoxam, clothianidin, acetamprid and dinotefuran. The increasing popularity of these compounds reflects their rapid action, high systemicity, generally long residual activity, potency against pests at low concentration active ingredients, and effectiveness against species already resistant to other insecticide classes (Ambrose, 2003).

Rotating insecticides with novel mode of action is one of the most commonly recommended approaches to delay the occurrence of insecticide resistance. However successful implementation of this technique hinges on a good understanding of resistance and cross-resistance patterns in the populations of target pests. The idea behind the rotation of insecticides is that, when an insecticide is withdrawn, the susceptibility of resistant insects will be restored within several generations allowing the insecticide to be reincorporated again into pest management programs. However, in certain cases, resistance persists over many generations after the withdrawal of selection pressure and such stable resistance prevents the successful reuse of an insecticide for pest management (Ninsin and Tanaka, 2005).

Insecticide resistance can be ascribed to three general mechanisms. One mechanism is modifying behavior to reduce exposure to toxic compounds. A second physiologically based mechanism involves alteration of absorption, excretion, transport, or sequestration of insecticides. A third biochemically based mechanism entails change in target-site binding activity and enhanced detoxification by several types of metabolic enzymes (Wilson and Ashok, 1998). Understanding the mechanisms by which insects develop resistance to insecticides is crucial for designing a successful resistance management program. As a preliminary investigation to elucidating the mechanisms of resistance, we determined the involvement of metabolic enzymes in the resistance to pesticides by using the synergist piperonyl butoxide (PBO), an inhibitor of cytochrome p_{-450} monooxygenase, and s,s,s-tributyl phosphorotrithioate (EDF), an inhibitor of esterases.

Random amplified polymorphic DNA-Polymerase chain reaction technique (RAPD-PCR) has been previously used for population genetics studies of a number of insects including aphids. RAPDs are viewed as having several advantages over other molecular markers and DNA fingerprints as the technique randomly samples the genome and hence multiple amplifiable fragments are present for each primer. Amplification of genomic DNA by the RAPD-PCR was used to differentiate between deltamethrin resistant and susceptible *Culex pipiens pallens* (Zhu *et al.,*