Persistence and residual activity of an organophosphate, pirimiphos-methyl, and three IGRs, hexaflumuron, teflubenzuron and pyriproxyfen, against the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract

Three insect growth regulators (IGR), the chitin synthesis inhibitors (CSI) teflubenzuron and hexaflumuron and the juvenile hormone mimic (JHM) pyriproxyfen, as well as the organophosphate (OP) pirimiphos-methyl, were evaluated for their activity against the cowpea weevil, *Callosobruchus maculatus* (F), in cowpea seeds stored for up to 8 months post-treatment. The initial activity data showed that, based on LC_{50} level, teflubenzuron had strong ovicidal activity (LC_{50} = 0.056 mg kg^{-1}) followed by pirimiphos-methyl (1.82 mg kg^{-1}) and pyriproxyfen (91.9 mg kg^{-1}). The residual activity data showed that none of the IGRs tested had strong activity when applied at 200 mg kg^{-1} in reducing the oviposition rates of *C maculatus* at various storage intervals up to 8 months post-treatment. However, teflubenzuron reduced adult emergence (F1 progeny), achieving control ranging from 96.2% at 1 month to 94.3% at 8 months. Hexaflumuron showed a similar trend in its residual activity, ranging between 93.8% control at 1 month to 88.2% control at 8 months post-treatment. However, pyriproxyfen was more active than the CSIs tested and caused complete suppression (100% control) of adult emergence at all storage intervals. Unlike the IGRs tested, pirimiphos-methyl applied at 25 mg kg^{-1} was more effective in reducing oviposition rates of *C maculatus*up to 8 months post-treatment. A strong reduction of adult emergence was also observed at various bimonthly intervals (98.6% control at 1 month to 91.6% control at 8 months post-treatment). The persistence of hexaflumuron and pirimiphos-methyl in cowpea seeds was also studied over a period of 8 months. The loss of hexaflumuron residue in treated cowpeas (200 mg kg^{-1}) was very slow during the first month post-treatment (4.43%). At the end of 8 months, the residue level had declined significantly to 46.4% of the initial applied rate. The loss of pirimiphos-methyl residue in treated cowpeas (25 mg kg^{-1}) was relatively high during the first month post-treatment (36.7%) and increased to 81.6% after 8 months.

Keywords:

Callosobruchus maculatus; insect growth regulators; OP; pirimiphos-methyl; ovicidal activity; residual activity; persistence; cowpea seeds

Monitoring and Characterization of Insecticide Resistance in the Cotton Leafworm, *Spodoptera littoral is* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract

Resistance to several classes of insecticides was diagnosed in the cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.), from cotton fields in the Nile Delta Egypt through 2002-2004 seasons. Two types of laboratory bioassays were used for the detection of insecticide resistance. Leaf-dip larval bioassay and discriminating concentration (LC_{99} for susceptible laboratory strain) technique revealed the presence of resistance to insecticides tested. Both larval and adult stages showed relatively similar response to most insecticides tested. However, the glass vial-discriminating concentration technique is particularly more useful when resistance is related to the target -site insensitivity rather than to increased metabolism. The pattern of reversion of resistance to five insecticides was determined in two field strains that have been released from continuous insecticide application and then reared under laboratory conditions for 6-8 generations in the absence of insecticide pressure. It was considered that losing of resistance to Cypermethrin in both resistant field strains seemed to be quicker than other insecticides tested, in which resistance ratios decreased from 47.7-fold (F_0- parents) to 17.8- fold (F_6 generation) in MNF- strain, and from 38.5- fold (F_0-parents) to 10.7-fold (F_6 generation) in KPR-strain. On the other hand, biochemical assays indicated that both MNF- and KFR-field strains, expressed higher levels of acetylcholinesterase (AChE) activity by 13.2- and 8.4-times, respectively, than that of the susceptible strain. AChE activity was sharply decreased to 1.01- and 1.92- times in MNF- and KFR-strains, respectively, compared to that of susceptible strain, following insect rearing for six successive generations without insecticide pressure. Esterases activity was also decreased 2.02-times (F_0) to 1.41 (F_6) in MNF-strain and from 2.0-times (F_0) to 1.21 -times (F_6) in KFR-strain, compared to that of susceptible strain. On the other hand, analysis of esterases by native Polyacrylamide gel electrophoresis (PAGE) confirmed the association between esterases activity and resistance to insecticides tested in both field strains.

Key Words: acetylcholinesterase; discriminating vial technique; esterase; insecticide resistance; resistance reversion; *Spodoptera littoralis*

Insecticidal Activity And Chemical Composition Of Extracts Dervied From Annona Squamosa Linn. (Annonaceae) Against Three Insect Species.

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Abstract

The insecticidal properties of methanol extracts derived from leaves and seeds of the Custard Apple, Annona squamosa (Annonaceae), were tested on three insect species, i.e. cotton leafworm, Spodoptera littoralis; cowpea weevil, Callosobruchus maculates; and house fly, Musca domestica. The bioassay tests for both crude extracts and purified fractions were examined for their toxicity and inhibitory growth effects, whereas phytochemical analysis was done by GC/MS analysis. Crude seed extracts showed high toxicity against C. maculatus adults, M. domestica larvae and S. littoralis larvae with 48 hour LC50's of 0.67, 1.29, and 1.67 %, respectively. Feeding assay showed that sublethal concentrations (LC25 and LC50) of both seed and leaf extracts showed deterrent effects, in a concentrationdependent manner, on larvae of S. littoralis and M. domestica. Pupation rate and fecundity of the adult females were significantly decreased, at LC50, compared to the control treatment. The insecticidal properties of the fractions eluted from methanol extracts of A. squamosa seeds and leaves were also evaluated against three insect species. The LC50 data of eluted fractions from A. squamosa extracts showed high toxicity against 4th S. littoralis instar, 2nd M. domestica instar, and C. maculatus adults. The biological activity of the most effective fractions was evaluated against the insect species tested. Pupation, adult emergence and fecundity were remarkably inhibited at LC50s of A. squamosa extracts in both S. littoralis and M. domestica. GC/MS analysis of A. squamosa extracts demonstrated the presence of some phytochemicals (phthalic acid esters, alkaloids, terpenes, and fatty acids) which may provide the insecticidal properties of these extracts against tested organisms. Phthalic acid esters were found to be the most bioactive components in the eluted fraction of methanolic extract from A. squamosa seeds and leaves.

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PHYTOCHEMISTRY AND ANTIMICROBIAL PROPERTIES OF METHANOL EXTRACTS OF SELECTED PLANT SPECIES

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ABSTRACT:

Methanolic extracts of three plant species, Custard apple, Annona squamosa L.; Madagascan periwinkle, Catharanthus roseus syn. Vinca rosus, and Conyza, Pluchea dioscoridis (L.) DC., were screened for their phytochmical and antimicrobial properties. Two Gram-positive bacteria (Bacillus subtilis and Streptomyces spp.) and three fungi strains (Fusarium oxysporum, Macrophomina phaseolina and Aspergillus niger) were used for evaluation of antimicrobial properties of selected plant extracts. The agar gel diffusion method was used to assay for the antimicrobial properties on the test isolate. The methanol extract from Conyza dioscoridis leaves was superior to other tested extracts showing an obvious inhibitory effect on the growth of bacterial isolates. The results of inhibitory activity of tested extracts against Streptomyces spp. indicated that C. dioscoridis extract showed the highest zone of growth inhibition occurred after 48 h of treatment with a zone diameter of 27.0 mm at a concentration of 1.0 % and of 20.0 mm at a concentration of 0.5%. However, the most antifungal activity was observed in methanol extract of Annona squamosa seeds, at concentration of 1.0%, against Fusarium oxysporum showing inhibition zone with a diameter of 44.0 mm, while at concentration of 0.5 %, the growth inhibition occurred with a zone of 19.0 mm. The extract from Catharanthus roseus leaves, at 1.0%, showed also an obvious inhibitory effect, where the zone diameter of growth inhibition was 22.0 mm. GC/MS analysis of tested plant extracts demonstrates the presence of some phytochemicals (phythalic acid esters, alkaloids, terpenes, and fatty acids) which may provide the antimicrobial properties of these extracts against tested organisms.

Key words: Antimicrobial activity, Annona squamosa, Cathranthus roseus, Pluchea dioscoridis, Phytochemical comp

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RISK ASSESSMENT OF CHLORPYRIFOS-METHYL USING 21-DAY SUBACUTE DIETARY STUDY IN THE JAPANESE QUAIL (Coturnix coturnix japonica)

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ABSTRACT:

The risk assessment of the organophosphate pesticide, chlorpyrifos-methyl (CPM), was conducted to determine the probability of adverse effects occurring from exposure to CPM in the Japanese quail (Coturnix japonica). The quail were exposed to sublethal levels, 1/10, 1/100 and 1/1000 LC50 of CPM, for 21-day dietary toxicity test, based on OECD workshop report. At 1/10 LC50, quail body weight was significantly decreased by 22.7% than that of the control. However, absolute weight of liver from quail exposed to 1/10 and 1/100 LC50 showed significant increases than that of the control. Kidney weight was also increased at the lower dose, 1/1000 LC50, while showed a significant decrease at the higher dose, 1/10 LC50. Data on hepatic function showed increased levels of ALT and ALP at concentrations of 1/10 and 1/100 LC50. Total protein levels were also increased at these doses. In kidney function parameters, data showed an obvious increase in concentrations of uric acid, at 1/10 and 1/100 LC50 without significant change in creatinine level due to pesticide exposure. The data obtained verified the toxic hazard of chlorpyrifos methyl, at concentration used, on Japanese quail that could be an excellent bird model for monitoring the toxicological risks of pesticides in Egypt.

Key words: Dietary toxicity test; Japanese quail; Chlorpyrifos-methyl

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Significance of the sulfonylurea receptor (SUR) as the target of diflubenzuron in chitin synthesis inhibition in Drosophila melanogaster and Blattella germanica

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Abstract
Diflubenzuron (DIMILIN®) is a powerful insecticidal chemical which has been known for many years to inhibit chitin synthesis in vivo in insects and related arthropod species. However, its action mechanism has remained unresolved partly because
of its inaction on any of the enzymes involved in chitin synthesis in vitro. Based on our previous work (Diflubenzuron affects
gamma-thioGTP stimulated Ca^{2+} transport in vitro in intracellular vesicles from the integument of the newly molted American
cockroach, Periplaneta americana L. Insect Biochem. Mol. Biol. 24 (1994) 1009) showing that diflubenzuron inhibits Ca^{2+} uptake
by vesicles obtained from the integument of American cockroach, Periplaneta americana (L.), in vitro, we tested the hypothesis
that the action site of diflubenzuron is an ABC (ATP binding cassette) transporter, probably a sulfonylurea-sensitive transporter.
Glibenclamide, one of the most commonly used sulfonylureas for type II diabetes treatment, was the positive control. When given
to immature insects, glibenclamide clearly caused toxicity, with symptoms indicating molting abnormality comparable to diflubenzuron.
Its LD_{50} (0.472 lg/nymph) was approximately 2.8 times the value obtained for diflubenzuron (0.17 lg/nymph, topical)
in German cockroach, Blattella germanica (L.). However, in terms of the inhibitory activities on chitin synthesis, in isolated integuments
glibenclamide showed an identical potency to diflubenzuron in B. germanica nymphs. A competitive binding assay with
[^{3}H]-glibenclamide and unlabeled diflubenzuron clearly established that the latter is capable of competitively displacing the former
radioligand. The K_D values observed for vesicles prepared from fruit fly larvae, Drosophila melanogaster M., were 44.9 nM for
glibenclamide and 65.0 nM for diflubenzuron, respectively. Furthermore, glibenclamide was found to affect Ca^{2+} uptake by
isolated cuticular vesicles from B. germanica in a manner very similar to diflubenzuron. These results support our conclusion that
the sulfonylurea receptor (SUR) is the target of diflubenzuron in inhibition of chitin synthesis in these two insect species.

Keywords: Diflubenzuron; Sulfonylurea receptor (SUR); Binding site; Glibenclamide; Chitin synthesis

Pyrethroid Pediculicide Resistance of Head Lice in Canada Evaluated by Serial Invasive Signal Amplification Reaction

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Background: Most people in the United States and Canada with pediculosis will be treated with neurotoxic pediculicides containing pyrethrins or pyrethroids. Their widespread use led to significant resistance reported from various countries. Although treatment failures are frequently observed in Canada, the resistance frequency to pyrethroid pediculicide of human head lice (*Pediculus humanus capitis*) has not been determined.

Objective: To determine the knockdown resistance (*kdr*) allele frequency in human head louse populations in Canada.

Methods: Patients infested with *Pediculus humanus capitis*, aged 4 to 65 years, residents of Ontario, Quebec, and British Columbia, were participants. Head lice were collected by combing and picking the enrolled subjects' hair. Lice were analyzed by serial invasive signal amplification reaction (SISAR) for genotyping the T917I mutation of lice indicating permethrin resistance. The permethrin-resistant *kdr* allele (R allele) frequency could then be evaluated in the head lice collected in Canada.

Results: Of the head louse populations analyzed, 133 of 137 (97.1%) had a resistant (R) allele frequency, whereas only 4 of 137 (2.9%) had a susceptible (S) allele frequency.

Conclusions: The 97.1% resistant (R) allele frequency in head lice from Canada could explain the treatment failures encountered with pyrethrin and pyrethroid pediculicide treatments in Canadian populations infested with *Pediculus humanus capitis* as the latter will not be eliminated by those pediculicides.

Antécédents: La plupart des personnes atteintes de pédiculose au Canada et aux États-Unis sont traitées au moyen de pédiculicides neurotoxiques à base de pyréthrines ou de pyréthroïdes. La résistance à ces produits, en raison de leur usage généralisé, a été rapportée dans plusieurs pays. Bien que l'échec du traitement soit fréquemment observé au Canada, la fréquence de la résistance aux pédiculicides à base de pyréthroïdes dans le traitement des poux (*Pediculus humanus capitis*) n'a pas été déterminée.

Objectif: Déterminer la fréquence allélique des gènes de résistance *kdr* dans la population des poux de tête au Canada.

Méthodes: Étude regroupant des patients infestés au *Pediculus humanus capitis*, âgés de 4 à 65 ans et résidant en Ontario, au Québec, et en Colombie-Britannique. Des poux de tête ont été recueillis chez les participants au moyen d'un peigne ou à la main. Les poux ont été analysés par SISAR en vue d'établir le génotypage de la mutation T917I chez les poux et d'indiquer la résistance à la perméthrine. La fréquence allélique du gène *kdr* résistant a été évaluée par la suite chez les poux de tête prélevés au Canada.

Résultats: Au sein de la population de poux analysée, 133/137 (97,1 %) présentait une fréquence allélique résistante (R) alors que seulement 4/137 (2,9 %) présentait une fréquence allélique susceptible (S).

Conclusions: La fréquence allélique résistante (R) de 97,1 % chez les poux au Canada pourra expliquer les échecs de traitement au moyen de pédiculicides neurotoxiques à base de pyréthrines ou de pyréthroïdes.

Biochemical characteristics of insecticide resistance in the fall armyworm, Spodoptera frugiperda (J.E. Smith)q

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Abstract
A strain of the fall armyworm, Spodoptera frugiperda (J.E. Smith), collected from corn in Citra, Florida, showed high resistance to carbaryl (562-fold) and methyl parathion (354-fold). Biochemical studies revealed that various detoxification enzyme activities were higher in the field strain than in the susceptible strain. In larval midguts, activities of microsomal oxidases (epoxidases, hydroxylase, sulfoxidase, N-demethylase, and O-demethylase) and hydrolases (general esterase, carboxylesterase, b-glucosidase) were 1.2- to 1.9-fold higher in the field strain than in the susceptible strain. In larval fat bodies, various activities of microsomal oxidases (epoxidases, hydroxylase, N-demethylase, O-demethylases, and S-demethylase), glutathione S-transferases (CDNB, DCNB, and p-nitrophenyl acetate conjugation), hydrolases (general esterase, carboxylesterase, b-glucosidase, and carboxylamidase) and reductases (juglone reductase and cytochrome c reductase) were 1.3- to 7.7-fold higher in the field strain than in the susceptible strain. Cytochrome P450 level was 2.5-fold higher in the field strain than in the susceptible strain. In adult abdomens, their detoxification enzyme activities were generally lower than those in larval midguts or fat bodies; this is especially true when microsomal oxidases are considered. However, activities of microsomal oxidases (S-demethylase), hydrolases (general esterase and permethrin esterase) and reductases (juglone reductase and cytochrome c reductase) were 1.5- to 3.0-fold higher in the field strain than in the susceptible strain. Levels of cytochrome P450 and cytochrome b_5 were 2.1 and 1.9-fold higher, respectively, in the field strain than in the susceptible strain. In addition, acetylcholinesterase from the field strain was 2- to 85-fold less sensitive than that from the susceptible strain to inhibition by carbamates (carbaryl, propoxur, carbofuran, bendiocarb, thiodicarb) and organophosphates (methyl paraoxon, paraoxon, dichlorvos), insensitivity being highest toward carbaryl. Kinetics studies showed that the apparent K_m value for acetylcholinesterase from the field strain was 56% of that from the susceptible strain. The results indicated that the insecticide resistance observed in the field strain was due to multiple resistance mechanisms, including increased detoxification of these insecticides by microsomal oxidases, glutathione S-transferases, hydrolases and reductases, and target site insensitivity such as insensitive acetylcholinesterase. Resistance appeared to be
correlated better with detoxification enzyme activities in larval fat bodies than in larval midguts, suggesting that the larval fat body is an ideal tissue source for comparing detoxification capability between insecticide-susceptible and -resistant insects.

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Determination of knockdown resistance allele frequencies in global human head louse populations using the serial invasive signal amplification reaction

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Abstract

BACKGROUND: Pediculosis is themost prevalent parasitic infestation of humans. Resistance to pyrethrin- and pyrethroid-based pediculicides is due to knockdown (*kdr*)-type point mutations in the voltage sensitive sodium channel α-subunit gene. Early detection of resistance is crucial for the selection of effective management strategies.

RESULTS: *Kdr* allele frequencies of lice from 14 countries were determined using the serial invasive signal amplification reaction. Lice collected from Uruguay, the United Kingdom and Australia had *kdr* allele frequencies of 100%, while lice from Ecuador, Papua New Guinea, South Korea and Thailand had *kdr* allele frequencies of 0%. The remaining seven countries investigated, including seven US populations, two Argentinian populations and populations from Brazil, Denmark, Czech Republic, Egypt and Israel, displayed variable *kdr* allele frequencies, ranging from 11 to 97%.

CONCLUSION: The newly developed and validated SISAR method is suitable for accurate monitoring of *kdr* allele frequencies in head lice. Proactive management is needed where *kdr*-type resistance is not yet saturated. Based on sodium channel insensitivity and its occurrence in louse populations resistant to pyrethrin- and pyrethroid-based pediculicides, the T917I mutation appears to be a key marker for resistance. Results from the Egyptian population, however, indicate that phenotypic resistance of lice with single or doublemutations (M815I and/or L920F) should also be determined.

Keywords: genotyping; human head lice; *kdr* allele frequency; *Pediculus humanus capitis*; pyrethroid resistance; SISAR

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Abstract: Three insect growth regulators (IGR), the chitin synthesis inhibitors (CSI) teflubenzuron and hexaflumuron and the juvenile hormone mimic (JHM) pyriproxyfen, as well as the organophosphate (OP) pirimiphos-methyl, were evaluated for their activity against the cowpea weevil, *Callosobruchus maculatus* (F), in cowpea seeds stored for up to 8 months post-treatment. The initial activity data showed that, based on LC_{50} level, teflubenzuron had strong ovicidal activity (*LC_{50} = 0.056mgkg^{-1}*) followed by pirimiphos-methyl (*1.82mgkg^{-1}*) and pyriproxyfen (*91.9mgkg^{-1}*). The residual activity data showed that none of the IGRs tested had strong activity when applied at 200mgkg^{-1} in reducing the oviposition rates of *C maculatus* at various storage intervals up to 8months post-treatment. However, teflubenzuron reduced adult emergence (F1 progeny), achieving control ranging from 96.2% at 1 month to 94.3% at 8months. Hexaflumuron showed a similar trend in its residual activity, ranging between 93.8% control at 1 month to 88.2% control at 8 months post-treatment. However, pyriproxyfen was more active than the CSIs tested and caused complete suppression (100% control) of adult emergence at all storage intervals. Unlike the IGRs tested, pirimiphos-methyl applied at 25mg kg^{-1} was more effective in reducing oviposition rates of *C maculatus* up to 8months post-treatment. A strong reduction of adult emergence was also observed at various bimonthly intervals (98.6% control at 1 month to 91.6% control at 8months post-treatment). The persistence of hexaflumuron and pirimiphos-methyl in cowpea seeds was also studied over a period of 8months. The loss of hexaflumuron residue in treated cowpeas (200mgkg^{-1}) was very slow during the first month post-treatment (4.43%). At the end of 8months, the residue level had declined significantly to 46.4% of the initial applied rate. The loss of pirimiphos-methyl residue in treated cowpeas (25mgkg^{-1}) was relatively high during the first month post-treatment (36.7%) and increased to 81.6% after 8 months.

Keywords: *Callosobruchus maculatus*; insect growth regulators; OP; pirimiphos-methyl; ovicidal activity; residual
activity; persistence; cowpea seeds