SHORT COMMUNICATION

EFFECT OF SELECTED INSECTICIDES ON SF9 INSECT CELL LINE

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ABSTRACT

The toxic effect of three insecticides: dimethoate (organophosphate insecticide), acetamiprid (neonicotinoid insecticide) and deltamethrin (pyrethroid insecticide) were evaluated in vitro on cultured Sf9 cell line. Cell growth inhibition was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Regression Analysis was used to estimate the 20% inhibition of cells growth (IC_{20}). The IC_{20} values obtained for deltamethrin, acetamiprid and dimethoate were: 46.8, 61.6 and 68.9 µM, respectively. The proportion of phagocytic cells was positively correlated with the applied concentrations of the insecticides.

Keywords: Sf9 insect cell line, insecticide, MTT assay, cell toxicity

INTRODUCTION

The world market for insecticides is still dominated by compounds inhibiting acetylcholine esterase (AChE). These AChE inhibitors (organophosphates and carbamates) and the insecticides acting on the voltage-gated sodium channel, in particular the pyrethroids (Cox, 2002), together account for approximately 70% of the world insecticide market (Smagghe, 2007). However, neonicotinoids, the most important novel class of synthetic insecticides in the past three decades, act as an antagonist at the nicotinic acetylcholine receptor (Ware & Whitacre, 2004).

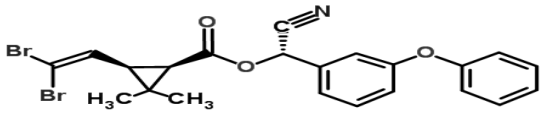
Over the past decades different industries have demonstrated an increasing interest in the development of *in vitro* methods for the studying of insecticides effect. Such methods are considered recently potential alternatives to conventional animal toxicity tests (Smagghe, 2007). They further reduce the need for time consuming and costly tests performed using animals or isolated organs (Decombel *et al.*, 2004; Watts *et al.*, 2003a). The commercially available Sf9 insect cell line derived from pupal ovarian tissue of *Spodoptera frugiperda* was used for *in vitro* assays to estimate the effect of different insecticides such as pyridalyl (Saito *et al.*, 2005; 2006) and to test the effect of fungal metabolites (Fornelli *et al*., 2004) or the effect of some insect fungi that could be developed as biopesticides (Watts *et al.*, 2003a; 2003b). Sf9 cell line was further used to study *Bacillus thuringiensis* toxins and to explore their mode of action (Rang *et al*., 1999; Agrawal *et al*., 2002).

The objective of this study was to test the effect of three categorically different but widely used insecticides, namely, dimethoate, acetamiprid and deltamethrin using a cultured Sf9 insect cell line and MTT assay to quantitatively assess cell proliferation during exposure to the selected insecticides.

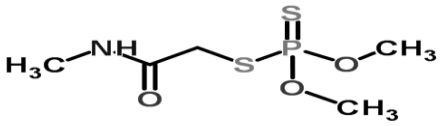
MATERIALS AND METHODS

Chemicals

The chemical compounds including dimethoate (purity 99.9%, lot: SZE5334X), deltamethrin (purity 99.7%, lot: SZE8297X), and acetamiprid (purity 99.4%, lot: SZE8067X), (Fig.1) dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were all purchased from Sigma-Aldrich (USA).



(A)



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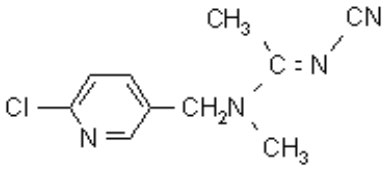

(C)

Figure 1. Chemical structure of: (A) deltamethrin , (B) dimethoate and (C) acetamiprid.

Sf9 cells

The Sf9 cells derived from pupal ovarian tissue of *Spodoptera frugiperda* (Vaughn *et al.*, 1977) were purchased from Gentaur-Belgium. The culture was routinely maintained at 27°C using an incubator (Selecta, Spain) in 25-cm^2 culture flasks (TPP, Switzerland) using the serum-free medium 5 ml of SF-900 II SFM culture media, purchased from Invitrogen (GIBCO). Cells formed a monolayer and were sub-cultured every 3–4 days after detaching using a scraper (TPP, Switzerland).

Cell bioassay

Cells were collected six days after subculturing and diluted with fresh medium to a density of 7.5x104 cells/ml. Each well of a 96-well microtiter culture plate (TPP, Switzerland) was loaded with 100 µl of cell solution containing 2 µl of the insecticide compound solution, prepared in ethanol. The final concentration range of insecticides was 0.5 to 500 µM. Each concentration tested consisted of four replicates and the test was repeated two times. After 72h of exposure, the MTT assay was performed based on the procedure described by Borenfreund *et al* (1988). The test medium was replaced with 20 µl of 2 mg/ml MTT dissolved in cell culture medium SF-900 II SFM. Following overnight staining at 27 ºC, the staining solution was carefully removed and 150 µl/well DMSO was added to solubilize the purple formazan crystals produced within the cell. The absorbance of each well was measured at 540 nm using a microplate reader (SCO, Germany). The cell growth was expressed as a percentage of absorbance ratio; absorbance in wells with insecticide treatment to control well (representing cells treated with 0 concentration of insecticide). The inhibition rate (**IR**) was calculated as follows (Liu *et al.*, 2010):

$$(1-At / Ac) \times 100 = IR.$$

At: absorbance value of tested wells.
Ac: absorbance value of control wells.

Statistical analysis

Analysis of regression was applied to estimate the 20% inhibition of cell growth (IC_{20}) and the theoretical IC_{50} was calculated using Microsoft excel 2010. Linear regression analysis of dose-response data was performed to obtain the mathematical curve.

RESULTS

Effect of insecticides on Sf9 cells

The tested insecticides, significantly inhibited cell growth at concentrations between 0.5 and 75 µM (Fig. 2). The lowest concentration with an effect was 25 µM. IC_{20} values, were calculated because the maximal cell growth inhibition was below 50% .The concentrations of insecticides resulting in 20% -inhibition of cell growth were: 68.9 µM, 61.6 µM and 46.7 µM for dimethoate, acetamiprid and deltamethrin, respectively, and $R^2 > 0.8$. The calculated theoretical IC_{50} for tested insecticides was 260, 232.9 , 132.5 µM for dimethoate, acetamiprid and deltamethrin, respectively.

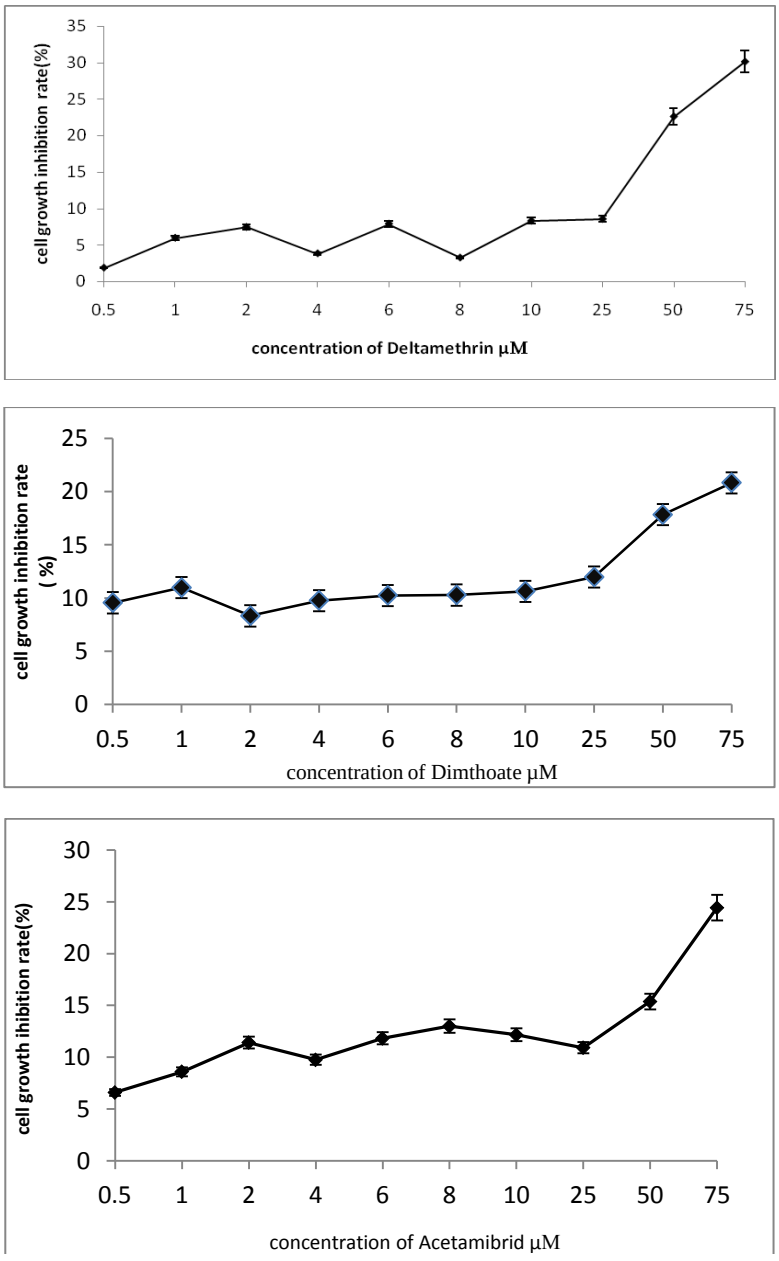

Figure 2. Quantitative effects of insecticides: deltamethrin ,dimethoate and acetamiprid on growth of Sf9 cells determined by MTT assay. The individual data points are expressed as the arithmetic mean percentage of control ± S.D.

Sf9 cell morphologies

The effect of tested insecticides was clear on Sf9 cell line. Dying cells (Fig. 3) showed cell shrinkage, loss of cell sphericity, increase of cell granularity and presence of multiple phagosomes which were absent in cells not exposed to the insecticides. The presence of phagocytic cells was also observed in exposed Sf9 cell culture, their proportion was positively correlated with concentration level of insecticide (Fig. 3).

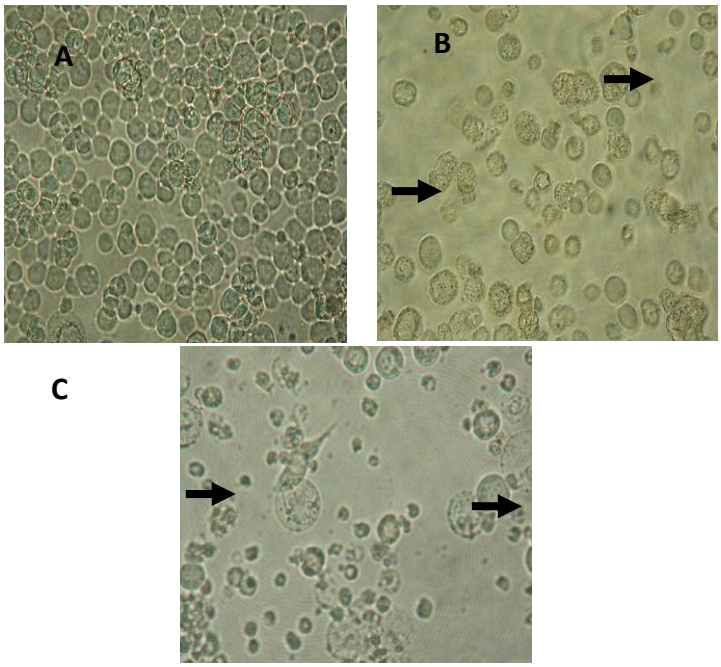

Figure 3. Sf9 cell morphologies: (A) cells before treatment by three insecticides (B), (C) cells after treatment by insecticide. The arrow in (B) indicates presence of phenotypicaly apoptotic cells; the arrow in (C) indicates presence of phagocytic cells (200X).

DISCUSSION

Three neurotoxic insecticides known to interfere with specific receptors were tested in the present strudy. The IC_{20} values obtained in this study for the three tested insecticides were relatively high (Decombel *et al.*, 2004) so the selected insecticides are judged to be of low toxicity. Dimethoate is an organophosphate insecticide that inhibits acetyl cholinesterase (AChE). The IC_{20} value obtained in this study for dimethoate was: 68.9 µM. McCarthy *et al*. (1987) studied the effect of dimethoate on the growth of two cell lines: TN368 (*Trichoplusia ni*) and IPLB-HZ1075 (*Heliothis zea*), the ID_{50} obtained was: 740 µM and 330 µM, respectively. The difference in values may be due to differences in cell cycle, cell metabolism (McCarthy *et al.*, 1987) and tissue origin of cells (Yanagimoto *et al.*, 1990). In addition, there are other factors that may affect toxicity values: the amount and type of serum additive, and

the type of solvent used to dissolve the insecticides. DMSO for example (not used in this study) could alter cell membrane permeability which may lead to easier access of insecticides to cell interior (McCarthy *et al.*, 1987).

Duangkaew *et al.* (2011) studied the effect of deltamethrin and other pyrethroid insecticides on Sf9 insect cells . The controlled expression of *CYP6P7* and *CYP6AA3* P450 genes in *CYP6P7* and *CYP6AA3*- transformed cells resulted in a higher LC_{50} values in (379.5 µM, 285 µM) than parental Sf9 cells (27.5 µM).

There appears to be no publication available yet that tested the effect of acetamiprid on insect cell culture. However, there are few studies on the effect of another member of the neonicotinoid insecticides: imidacloprid. The cytotoxic effect of this insecticide was tested on proliferation of Se cell line derived from *Spodoptera exiqua* (Decombel *et al*., 2004) and the IC_{50} value was 20 ppm on the latter cell line, but on the proliferation of gill cell line derived from Flounder *paralichthys olivaceus* (Feng *et al.*, 2007) IC_{50} value was 38.46 µg/ml.

A proportionate increase of phagocytic cells depending on increased concentrations of insecticides was observed in this study. The presence of these cells has been reported by Meneses-Acosta *et al.* (2001) who suggested that these cells eliminate cell debris from Sf9 cell culture.

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