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Short Communication

The mutagenic testing of different brands of commonly used insecticides

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Insecticides are chemical agents used to control insects, but humans are usually exposed to insecticides and this may have a long term toxicological effects on their health. Different brands of insecticides including Baygon^R, Mobile^R, Mortein^R and Total^R were subjected to Ames spot Forward Mutation Assay, using *Escherichia coli*. The assay was examined for the presence of revertant strains of the organism in the presence and absence of white albino rat liver metabolizing enzymes (S9). The results showed that these insecticides produced no mutant strain of the organism and no alteration in the phenotypic characteristics of the organism as compared with the standard mutagen (ethidium bromide), which produced revertant strains of the organism and altered the phenotypic characteristics of the organism. Finally, this result showed that these insecticides can be considered not mutagenic in bacteria and may not be mutagenic or carcinogenic in human.

Key words: Insecticides, mutant strains, ames spot assay, bacteria.

INTRODUCTION

Insecticides are natural or man made preparations that are used to kill or otherwise control insects or may work to prevent them from reproducing (Ware and Whitcre, 2004). They belong to a group of pesticides that are usually chemical substances, which could be of plant or synthetic origin. The use of insecticides has become very important because of the increasing menace of insects especially in the tropics, posing a threat to health or competes for food or other materials with man (Bloomquist, 1993). In addition to their intended effects, they are sometimes found to affect non-target organism, including humans (Chantelli-Forti et al., 1993; Chaudhuri et al., 1999). Thus, the genotoxicity of these environmental chemicals/toxicants and their influence on ecosystems are of worldwide concern (Karabay and Oguz, 2005).

Studies have demonstrated that some pesticides have mutagenic and clastogenic activities in several biological test systems (Shirasu et al., 1976; Celik et al., 2003) and certain types of cancer have been associated with long term exposure to synthetic chemicals that are used in industry, household and agriculture. Thus these chemicals can cause changes in the genetic material in the nucleus of cells in ways that allow the changes to be transmitted during cell division.

Insecticides are made up of different composition. For example, Baygon^R insecticides are composed of 1% propoxur (carbamate), 1% dichlorvos (organophosphate) and 0.04% cyfluthrine (pyrethroid). Mobil^R insecticide is composed of 0.3% tetramethrin (pyrethroid), 0.2% phenothrin (pyrethroid) and 0.44% Allethrin (pyrethroid). Mortein^R is composed of 0.1% D-phenothrin (pyrethroid) and 0.04% Imiprothrin (pyrethroid), while Total^R is composed of 0.12% D-tetramethrin (pyrethroid), 0.035% prallethrin (pyrethroid), 0.0063% deltamethrin (pyrethroid and D-D-transcyphenothrin (pyrethroid).

Generally, organophosphate insecticides act by inhibiting acetylcholinesterase enzyme resulting in the accumulation of acetylcholine at the neuromuscular junctions (Schnellman and Manning, 1990). While, pyrethroids are natural insecticides which are considered to be axonic poisons and act by keeping open the sodium channels in neuronal membranes, thus causing paralysis.

As the use of insecticides have become increasingly widespread throughout the world and particularly in Nigeria, additional studies are needed to evaluate the potential toxic risk of insecticides for non-target organisms. Therefore, this research intends to assess the

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Table 1. Biochemical characteristics of *E. coli* [0157:H7 (1 and 7) obtained with the insecticides Baygon^R, Mobile^R, Mortein^R and Total^R and its revertant strains obtained with ethidium bromide (positive control).

Strain	Kliger iron agar (KIA)				Motility indole urea			Simmon's citrate
	Butt	Slant	H_2S	Gas	Motility	Indole	Urease	
***E. coli* [0157:H7) obtained with the insecticides Baygon^R, Mobile^R, Mortein^R and Total^R**								
0157: H7(1)	GF	LF	-	+	+	+	-	-
01`57;H7(7)	GF	LF	-	+	+	+	-	-
Revertant strains of *E. coli* [0157:H7) obtained with ethidium bromide (positive control)								
0157: H7(1)	NGF	NLF	-	-	+	-	-	+
01`57:H7(7)	NGF	NLF	-	-	-	-	-	+

GF = Glucose fermenting, NGF = Not glucose fermenting, LF = Lactose fermenting, NLF = Not Lactose Fermenting, - = Negative, + = Positive.

mutagenic potentials of commonly used insecticides in Nigeria.

MATERIALS AND METHODS

Chemicals

Baygon^R, Mobil^R, Mortein^R and Total^R insecticides were obtained from Oke-Arin Market, Idumota, Lagos, Nigeria.

Study design

The study was carried out using modified Ames spot forward mutation assay as described by Ames et al. (1975), Maron and Ames (1983) and modified Ames test in wikipedia, the free encyclopedia. The study was performed in the toxicology laboratory of the Pharmacology department, College of Medicine, University of Lagos, Nigeria.

Media preparations

The agar used were MacConkey agar, Kliger iron agar (KIA), Brain heart infusion agar, Simmon's citrate agar, Microbiological agar and peptone water. The media were prepared as described by LAB M^{TM}, Topley House, 52 Washlane, Bury, Lancashire, BL9 6AU, UK.

Inoculation

The MacConkey agar plate was inoculated with two strains of *Escherichia coli* [0157:H7 (1 and 7)] obtained from Nigeria Institute for Medical Research, Yaba, Lagos, which were lactose and glucose fermenting, motile, urease negative, indole positive and citrate negative, using a wire loop and incubated for 24 h.

Bacterial mutation assay

The assay was performed using *E. coli* [0157:H7 (1 and 7)] which has been grown on MacConkey media to obtain discrete colonies. The bacterial mutation assay was done according to the methods of Maron and Ames (1983) Ames et al. (1975) and modified Ames test in wikipedia, the free encyclopedia.

The experiment was performed in the presence and absence of metabolic activation by phenobarbitone 10 mg/kg/day for 3 days which induced whilstar albino rat liver enzyme (S9). The fraction of the liver enzyme was used at a concentration of 10% $^v/_v$ in the S9 mix. The S9 mix was freshly prepared for the experiment according to the methods of Maron and Ames, 1983. Test agents and positive controls were tested in all strains of the experiment. Ethidium bromide which is a known mutagen was used as the positive control.

Fresh cultures of tester strains obtained from MacConkey plates were grown on LAB M^{TM} nutrient broth. The cultures were incubated for 10 - 12 h at 37ºC in order to ensure adequate aeration. A portion of the nutrient broth that contains the organism was mixed with S9 (mix) and was seeded into the nutrient-agar plates using a swab stick, while the other portion without S9 was also seeded on other nutrient agar plates.

Whatman paper discs were separately impregnated in each of the insecticides and ethidium bromide. The discs were then placed on the nutrient agar plates and incubated for 48 h. This was done for the two strains of organism and a discrete colony of the organism in the nutrient agar plate was re-inoculated into freshly prepared KIA, MIU and Simmon's citrate media. The results obtained were recorded.

RESULTS AND DISCUSSION

The results obtained showed all the tested insecticides on the two organisms to be negative to Ames test. Table 1 shows the biochemical characteristic of *E. coli* [0157:H7 (1 and 7)] before exposure to standard mutagen and insecticides. It shows the organisms to be glucose and lactose fermenting, motile, urease negative, indole positive citrate negative and gas producing. The results on All the tested insecticides produced no revertant strains, while ethidium bromide produced some noticeable revertant strains in the presence and absence of S9 mix. The alteration in the normal biochemical characteristic of the organism when inoculated with the revertant strains produced by ethidium bromide is also shown in Table 1.

The forward mutation assay which is an *in vitro* test with rat liver microsomal fraction S9 is one of the most frequently used tests for assessing the mutagenic potential of both pure compounds and complex mixtures (Karabay and Oguz, 2005). Short term test series including combination of the microorganism/microsome assay together with the chromosome aberration test in mammalian cells, were found to significantly improve the sensitivity for detection of potential human carcinogens (Sierra-Tores et al., 1998). Moreso, WHO (1985) reported that the bacteria mutation test has proved to be effective in screening chemicals for potential mutagenic and carcinogenic activity. The result obtained from this study

showed that insecticides containing either pyrethroids or combination of pyrethroids, organophosphate and carbamate are not mutagenic on bacteria. This could be due to the percentage of the constituents in each of the insecticides studied. However, this result is consistent with the studies carried out by Herrera and Larboda (1998) on resmethrin and cypermethrin that showed non-mutagenicity on bacteria. Hallenbeck and Cunningham-Bums (1985) also showed propoxur not to be mutagenic on six different types of bacteria. Furthermore, the work of Batiste-Alentorn et al. (1986) demonstrated using *Drosophilia* that no significant increase in the frequency of sex chromosome loss or non-disjunction after exposure of male flies to cypermethrin at concentration up to 20 ppm were observed.In view of the above facts, it has been shown that individual agents are not mutagenic and their combinations have been demonstrated in this study not to be mutagenic. However, more *in vivo* studies should be carried out on these agents to elucidate their toxicological profile as this will go a long way to make a conclusive report on their genotoxic potentials.

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