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## Antagonistic Effect of Azadirachtin on Cyfluthrin and Permethrin

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### ABSTRACT

The aim of this study was to assess the efficacy of some traditional pesticides either individually or in combination, against *Blattella germanica* (Linnaeus) (Dictyoptera: Blattellidae). Cockroaches are of great public health importance worldwide and chemical methods remain one of the most popular approaches to combat the problem. Using contact method German cockroaches were exposed to pesticides in glass jars containing pesticides. The results showed that maximum knockdown was achieved with 0.5 cyfluthrin with  $KT_{50}$  of 8.9 min. The  $KT_{50}$  values for 0.1% permethrin, 2.5% Primifos methyl, 2.5 and 5% malathion, 0.0 and 5% Diazinone, and also 0.25 and 0.5% Bendiocarb were 13.8, 88.03, 131.4, 75.16, 86.94, 41.08, 103.5 and 42.9 min, respectively. Surprisingly the combination of azadirachtin with permethrin or cyfluthrin showed the least effect which could be due to competition of azadirachtin and pyrethroids for same site or some conformational changes in target sites or receptors.

**Key words:** Cockroaches, Hamadan, Iran, chemical control

### INTRODUCTION

Because of their ubiquitous presence in human habitations and their filthy habits cockroaches are of great public health importance. Although, they live indiscriminately on any places from dirty conduits to unattended book shelf and cabinet, because of their necessity to humidity and moderate temperature cockroach are usually more abundant in warm and damp places like washrooms and around pipelines in basements. They are nocturnal and omnivorous so cockroaches can occupy every niche in buildings such as cookery and hospitals and in some cases even without being discovered by the residents of these places. Since they have a flattened body and are able runner cockroaches can move freely almost to any places and touch any thing in dwellings. Many pathogenic and nonpathogenic microorganisms have been found on or in their body (Saitou *et al.*, 2009) and implication of these insects have traced in some nosocomial epidemics (Fotedar and Banerjee, 1992). Allergy to the excretions or body parts of cockroaches is another problem which have been reported in many part of world (Arruda and Chapman, 2001). They discharge a nauseous secretion both from their mouths and from glands opening on the body which give a long-lasting, offensive cockroach smell to food stuff and other materials in infested areas (Agrawal *et al.*, 2005).

Over the years many pesticides have been used to control cockroaches but because of their superior adaptability power and high proliferation capacity the extinction of these creatures from

human habitats is far from over. Many experiments have been designed to screen suitable pesticides to resolve the problem of cockroaches and despite introduction of new pesticides because of resistant problem there have been plenty of reports of failure in chemical control (Pridgeon *et al.*, 2002).

To launch any chemical control program in infested places one of the key points is to find the susceptibility of them to proposed pesticides. At present study the efficacy of some insecticides alongside with azadirachtin (Fig. 1) a phytochemical extracted from neem seed kernel, were examined against *Blattella germanica* (L.) individually or in combination with permethrin and cyfluthrin (Fig. 2a, b).

The terpenoids of the neem tree (*Azadirachta indica* A Juss.) represent a group of plant metabolite which has become seriously considered for commercial use in the past few years in many parts of worlds. The most potent neem compound is azadirachtin which is most abundant and the

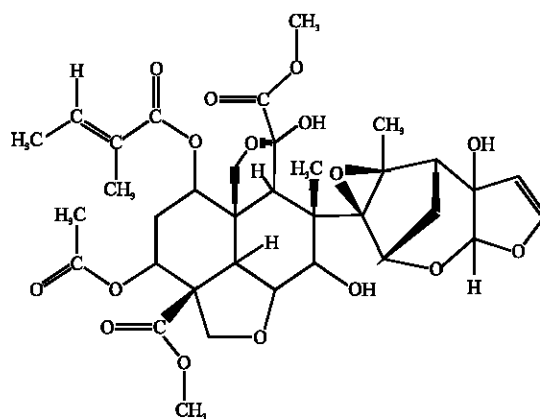


Fig. 1: The structure of Azadirachtin A

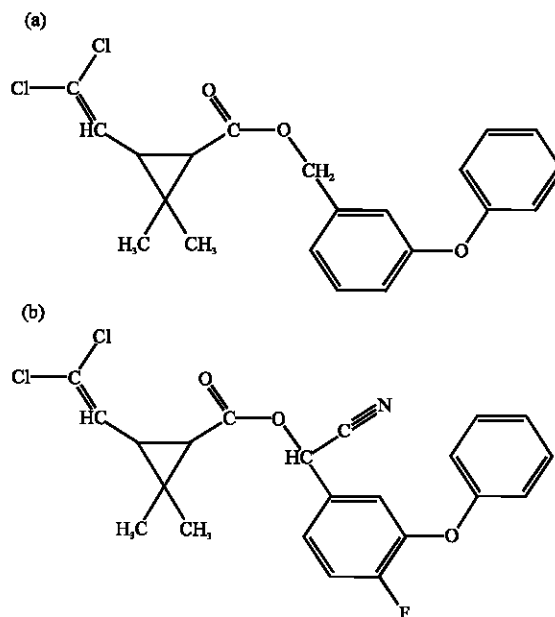


Fig. 2: The structure of (a) Permethrin and (b) Cyfluthrin

most biologically active component of neem tree (Salehzadeh *et al.*, 2003). Also, pyrethroid is an important family of synergistic insecticides because, in addition to low mammalian toxicity they rapidly paralyze insects and are less persistent in the environment.

## MATERIALS AND METHODS

**Test cockroaches:** German cockroaches *Blattella germanica* (L.) collected from some hospitals of city of Hamedan during Jul. 2005 to Sep. 2006, reared in laboratory under 65-75% humidity and at 28±2°C. With a photoperiod of 8:16 (L: D) h. Cockroaches were provided with food milk powder and water.

**Test chemicals:** The insecticides evaluated contained active ingredients in following concentrations: 0.1% Permethrin of 25 WP 25% Coopex, 2.5% Primifos methyl of 50% WP Actelic, 5% Malathion of 57% Emulsifiable Concentrate, 0.5 and 5% Diazinone of 60% Emulsifiable Concentrate, 0.25% and 0.5% of 80% WP Bendiocarb and 0.5% Cyfluthrine of 10% WP Sulfac.

All insecticides were commercially available formulations except azadirachtin which was kind gift of Dr. Strang University of Glasgow Scotland.

**Method:** For susceptibility test commercially available formulations of pesticides dissolved in HPLC grade acetone and diluted with distilled water. In each case 2.5 mL of solution was pipetted into a 0.51 L glass jar (surface area about 336 cm<sup>2</sup>). The jars were rolled horizontally over a flat surface until all of the acetone had evaporated, so that the insecticide was deposited evenly over the inner surface of the jar and allowed to dry overnight. The upper inside of jars walls were smeared with Vaseline to prevent escape cockroaches.

Both male and female cockroaches from a synchronously reared laboratory colony were exposed to different concentrations of pesticides.

Control batch was run simultaneously and each set of test was repeated 5-10 times (10 cockroaches for each replicate). After 24 h of exposure the alive cockroaches were transferred into a plastic container and checked for mortality. Treated insects monitored for mortality for further 24 h under the same temperature and photoperiod as the colony.

Another set of experiment carried out with 1.5% azadirachtin individually or in combination with Permethrin or Cyfluthrine. All experiments were performed under laboratory conditions.

The cumulative average knockdown/mortality was used to assess the susceptibility of cockroaches to each pesticide. If insects on their backs were unable to right themselves when prodded, they were considered dead. Mortality data from the replicates were pooled and the time exposure mortality and knockdown cockroaches were assessed by probit analysis as used in our previous work (Salehzadeh and Mahjub, 2007).

## RESULTS

Our evaluation of the susceptibility of german cockroach to Permethrin, Primifos methyl, Malathion, Diazinone Bendiocarb, Cyfluthrine and azadirachtin revealed that the maximum knockdown achieved with 0.5 cyfluthrin with  $KT_{50}$  of 8.9 min. Other Toxicological test indicated that  $KT_{50}$  values for 0.1% permehrin, 2.5% Primifos methyl, 2. 5 and 5% Malathion, 0.5 and 5% Diazinone and also 0.25 and 0.5% Bendiocarb were 13.8, 88.03, 131.4, 75.16, 86.94, 41.08, 103.5 and 42.9 min, respectively (Table 1).

Table 1: Susceptibility of german cockroaches to some pesticides in the laboratory test

Chemicals	KT <sub>50</sub>	KT <sub>90</sub>	Scaled deviance
0.1% permethrin	13.80	33.31	4.42
0.5% diazinone	86.94	121.10	5.99
5% diazinone	41.08	78.51	5.36
0.25% bendiocarb	103.50	320.20	23.89
0.5% bendiocarb	42.90	134.10	6.58
2.5% primifosmethyl	88.03	142.50	9.45
5% malathion	75.16	133.70	4.64
2.5% malathion	131.40	216.60	6.15
0.5% cyfluthrin	8.90	18.40	4.21

Table 2: Susceptibility of german cockroaches to combination of 1.5% azadirachtin with 0.1% permethrin or 0.5% cyfluthrin (knockdown after 24 h). There was not mortality or knockdown in control batches

Pesticide combination	Azadirachtin	Azadirachtin and permethrin	Azadirachtin and cyfluthrin
Knockdown	70%	2%	11%

Azadirachtin could not cause 50% knockdown even after 10 h and total mortality never exceed 70% of treated insect. Using combination of pyrethroids and azadirachtin interestingly there were less effect on cockroaches. In case of combination of 0.1% permethrin and 1.5% azadirachtin all samples were alive even after 24 h and combination of 1.5% azadirachtin and 0.5% Cyfluthrine showed similar results (Table 2).

## DISCUSSION

In spite of growing interest in the toxicological effects of pesticides compounds on pest and increasing studies on mode of action of pesticides, one of the remaining concern in filed of pest management is resistance of insect and other hazardous arthropods to pesticides.

Investigating the sensitivity of pests and application of compounds with higher selectivity is one approach to alleviate the problem. Using mixture of chemicals is another approach to overcome pesticide resistance which has been suggested by some authorities (Pennetier *et al.*, 2008; Boerboom, 2001; Mallet, 1989).

This in turn needs some more exact information related to simultaneous application of pesticides. In addition due to pesticide residues it is likely that pests expose to more than one pesticide simultaneously. So investigating the effects of probable combinations of pesticides can be very helpful. Although, there are lot of information about interaction of drug in mammals there is few data regarding the potential changes in effects of single pesticides by the presence of other compounds.

In present study the susceptibility of german cockroaches to some pesticides as well as possible potentiation or antagonism of mixture of pesticide was investigated. As our results shows there are great diversity in susceptibility of german cockroach to different pesticides in laboratory condition. Regarding estimated KT<sub>50</sub> and KT<sub>90s</sub> cyfluthrin and permehrin are the most effective pesticides which is in agreement with the study which carried out in Kashan, Iran (Doroudgar and Asadi, 2001) but there was huge difference between the results of this study and our eariler study which had been done in Tehran, Iran (Salehzadeh and Mahjub, 2007) which may reflect extensive application of pesticides for control of Blattidae in big cities such as Tehran. This is in agree with the works of some other researchers (Nasirian *et al.*, 2009; Strong *et al.*, 2008).

During these experiments it has been shown that despite sensitivity of german cockroaches to permethrin and cyfluthrin the combination of these pyrethroids with azadirachtin has little or no effect on them. There are few experiments regarding combination application of azadirachtin with other pesticides which have shown either synergistic or antagonistic effects (Mohan *et al.*, 2007).

Although, there is some analogy between these findings and some other research, there are other findings that show synergistic effect of azadirachtin on efficacy of other chemicals (US Patent 5352672).

It is not clear how azadirachtin interfere with different compounds. There are different opinions about mode of action of this terpenoid. Mordue *et al.* (1998) found that azadirachtin affect endocrine system. Shafeek *et al.* (2004) proposed that azadirachtin exerts excitatory action on the electrical activity in the nervous system of cockroach by interfering with the ion channels in the nerve membrane so lower toxicity of combination of azadirachtin and pyrethroids could be interpreted as competition for the same site on the sodium channels or represent a more indirect effect on pyrethroids binding. Due to the presence of a large number of functional groups in the azadirachtin molecule (Baldonia *et al.*, 1997). Conformational changes in target sites or receptors is another possibility and direct action on pyrethroids is another possibility which need further experiments.

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