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EFFECTS OF IVERMECTIN ON *Culex quinquefasciatus* LARVAE

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SUMMARY

The effects of ivermectin, a semi-synthetic drug widely used for treatment of livestock parasitic diseases, were observed on *Culex quinquefasciatus* larvae. Toxic effects and mortality evaluation were carried out after 5, 15, 30 and 60 minutes of exposure to 1, 5 or 10 ppm of ivermectin solutions. Observations were made 24 and 48 hours after the beginning of the experiment, and loss of mobility, progressive paralysis and high mortality of larvae were recorded. The observed effects of ivermectin on the mosquito larvae is probably correlated with chloride channel activation on cell membranes.

KEYWORDS: *Culex quinquefasciatus*; Ivermectin; Control; Larvae mortality.

INTRODUCTION

Culex quinquefasciatus Say, 1823 is a cosmopolitan mosquito, plaguing dwellers in most tropical areas of the world. It is known as the domestic tropical mosquito and its distribution is strongly influenced by human settlement. In Brazil, it occurs throughout the country, in urban areas and in rural villages[15]. This mosquito occurs throughout the year, but is most abundant in rainy and warm months. Females of *Cx. quinquefasciatus* oviposit in artificial water collections, either in recipients or in soil containing water rich in organic material. Adults have predominantly night behavior and, being highly anthropophylic, *Cx. quinquefasciatus* is the main vector of Bancroftian filariasis in Brazil.

Several insecticides has been tested to control mosquito populations, in order to reduce the transmission of many diseases by these insects. DDT is still the chemical insecticide most used for mosquito control in Brazil, although it remains in environment after application. Other such as organophosphates, carbamates and pyrethroids have been used against on adults and larvae[10].

Ivermectin, a semi-synthetic derivative of avermectins produced by an actinomycete, *Streptomyces avermitilis*[6], has been used as an antiparasitic drug in cattle[1,2], horses[7], sheep[2] and other domestic animals by its low toxicity in most of vertebrates, depending on the therapeutic doses[6]. The drug was also used in human therapeutics against a wide range of parasites[18], including the treatment of human filariasis[12,23]. In fact, the mass distribution of ivermectin to rural communities associated to a vector control strategy leads a strong impact on the population dynamics of the major vector species of the blinding onchocerciasis in West Africa[4]. The mode of action of this compound remains elusive. Some reports show the release of GABA (γ-amino butyric acid) on the

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post-junction of a muscle cell in arthropods, resulting in a flow of chloride ions and a consequent hyperpolarization which eliminates signal transmission[5,36]. The effects of ivermectin on adults of *Cx. quinquefasciatus, Aedes fluviatilis, Aedes aegypti, Anopheles quadrimaculatus* and other mosquito species have already been reported[11,14,16,21,35]. The aim of this present study was to observe some effects of ivermectin on larval instar of *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Sixteen groups of 3rd or 4th instar of *Culex quinquefasciatus* larvae raised and obtained from artificial tanks in the laboratory (100 larvae per group) were placed to plastic recipients containing a solution of ivermectin* 1% w/v in a final concentration of 1, 5 or 10 ppm during different times of exposition (5, 15, 30 and 60 minutes). Control groups were placed in recipients containing just dechlorinated water.

After the time of exposure, larvae were washed and transferred to other plastic recipients containing dechlorinated water. The experiments were conducted by adapting WHO methodology[37], used to test insecticides in mosquito larvae. *Cx. quinquefasciatus* larvae were examined in a stereomicroscope 24 and 48 hours after starting the experiment.

RESULTS

The mortality was assessed in different levels in all groups of larvae exposed to Ivermectin. None of the larvae in the control group died neither at 24 nor at 48 hours after exposure.

Data (in percentage) of *Cx. quinquefasciatus* larvae mortality are summarized in Figure 1.

The insects affected by the drug display ataxia and progressive paralysis – also observed in live larvae of each group. It was most obvious in groups exposed to 5 and 10 ppm, although the group exposed to 1 ppm concentration showed a lower – but expressive – percentage of larvae without movement (65% after 24 hours/5 minutes of exposure) than the groups exposed to 5 and 10 ppm. Figure 2 shows the percentage of larvae without movement during the observation periods.

DISCUSSION

Larvicide treatments have been carried out in order to control population of vector insects. Several works have reported the effects of insecticides on larval instar of mosquito population, as the Abate®, an organophosphorus insecticide considered toxic to the larvae of the *Aedes* mosquito[30]. Plant extracts have been considered alternative larvicides. The effect of black-pepper extracts on *Cx. quinquefasciatus* larvae was reported[8]. In the present study, ivermectin was found to have a lethal effect on 3rd or 4th instar of larvae *Cx. quinquefasciatus*. The lowest mortality level was found to occur after 5 minutes of exposure to 1 ppm for 24 hours and highest recorded mortality 24 hours after exposure occurred when larvae were exposed to 10 ppm for 60 minutes.

The lack of movements in some live larvae observed 24 and 48 hours after the beginning of the experiment suggests the action of ivermectin on the paralyzation of larvae, probably by the activation of both ligant and voltage gated chloride channels as reviewed by BLOOMQUIST (1996)[4]. In some reports, it has been discussed the ivermectin binding on specific receptors of motorneurons γ-aminobutyric acid (GABA) mediated. In this situation, the drug increases the probability of GABA binding on $GABA_a$ sites activating Cl^- channels of presynaptic membranes. It leads to an influx of chloride ions and irreversible hyperpolarization with consequent inhibition of signal transmission[5,36]. Ivermectin can also bind directly on specific sites of ionic channels following the disorder of hydroelectric balance. However, the action of ivermectin can be associated with the releasing of neurotransmitters in conjunction of GABA antagonism, probably contributing to excitatory signs of intoxication[4,36].

Development of species resistant to synthetic insecticides has been described[11]. Studies on some strains of the house fly and Colorado potato beetle have been suggested that resistance to organophosphates, pyrethroids and other insecticides are not cross-resistant to abamectin, an ivermectin-like chemical. Mechanisms of resistance to avermectins suggested to these insects are mainly the oxidative metabolism, altered target site and the esteratic metabolism/sequestration[9]. Resistance of some strains of mosquito larvae to insecticides has been proposed by the action of detoxifying enzymes[17,22]. In the present study, a percentage of live larvae were observed 48 hours after exposure to some concentrations of ivermectin.

Avermectins in soil and aquatic environments show a rapid degradation which is accelerated by exposure to sunlight, reducing half-lives to 0.5 days or less[20,38]. On the other hand, our results that solutions of ivermectin

*IVOMEC. M,S&D-22,23-dihydro avermectin B_1.

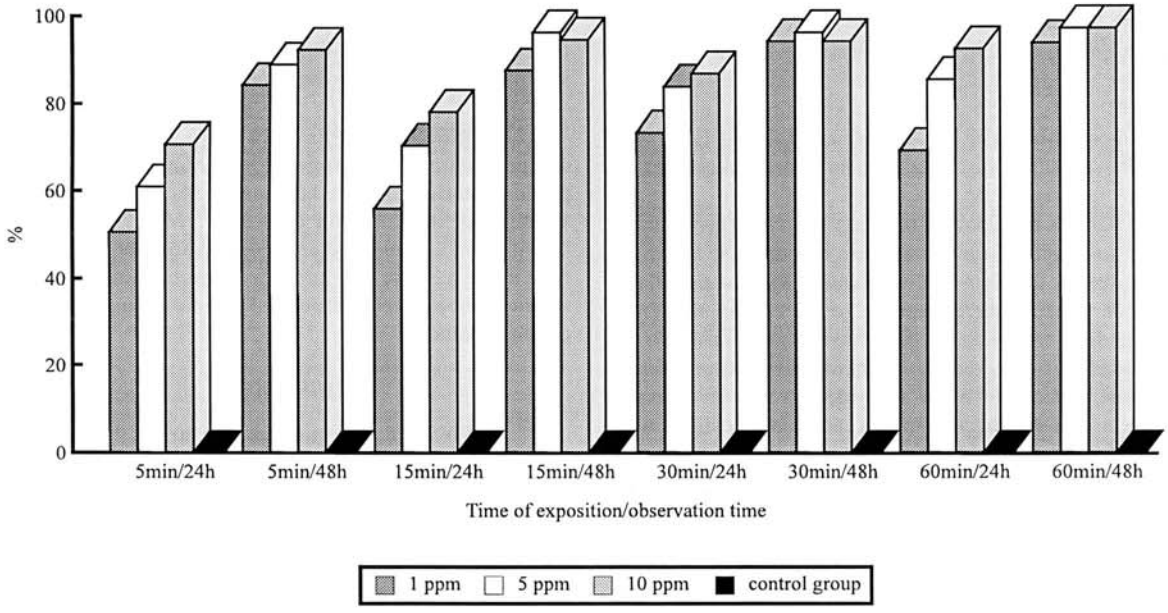

Fig. 1 - Percentage of mortality of *Culex quinquefasciatus* larvae exposed to 1, 5 and 10 ppm ivermectin concentration, in different times of exposure. 24 and 48 hours after start of the experiment.

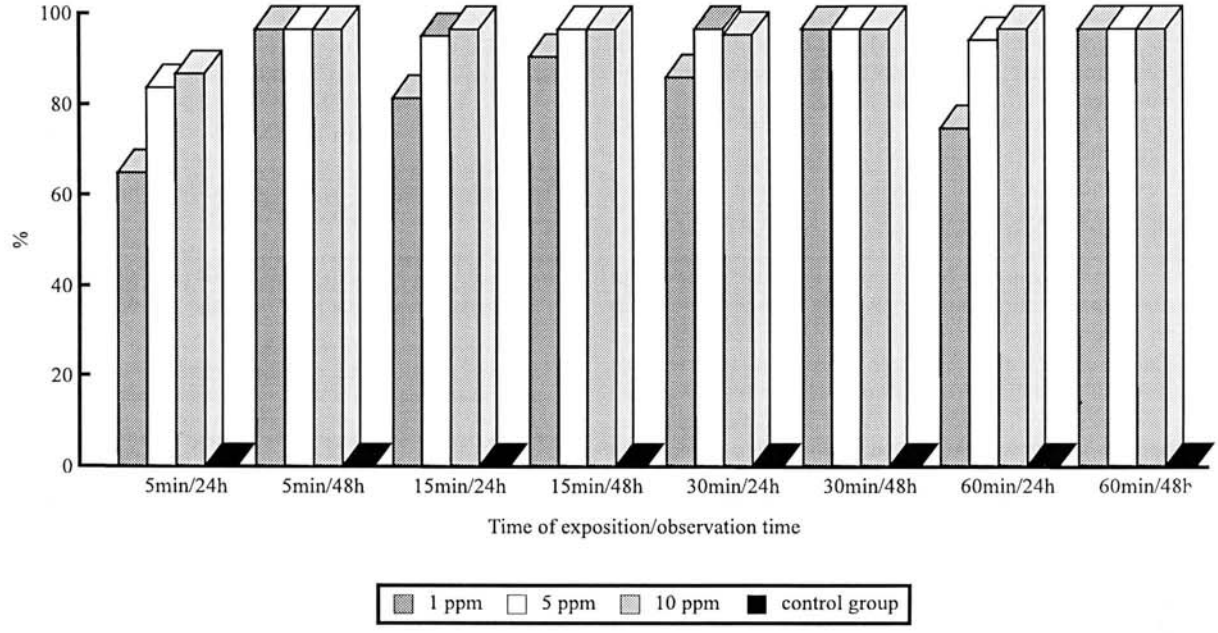

Fig. 2 - Percentage of *Culex quinquefasciatus* larvae found paralyzed after exposure to 1, 5 and 10 ppm ivermectin concentration at 24 or 48 hours after start of the experiment.

(1, 5, 10 ppm) kept in a glass jar for at least 1 month, at room temperature in absence of direct sunlight, remains active against mosquito larvae. Indeed, in laboratory conditions, when placed in soil-faeces mixtures, ivermectin showed a half-life of more than 90 days[20]. The same was shown for dung from bolus-treated cattle[23].

Periods of exposure presented in the experiment on *Cx. quinquefasciatus* larvae were less than the half-lives reported for the drug and were able to kill an appreciable number of specimens exposed to the compound. Thus, depending on the drug concentration and considering a reduced half-life degradation in other environment conditions, damage to water ecosystems could be expected after use of ivermectin. In fact, some publications have reported possible negative consequences of the effect of toxic substances on invertebrate populations as an increase of polluted areas[24,34]. This drug has been studied on non-parasite invertebrates such as cattle dung fauna[32,33]. A feature of ivermectin is that most of the given dose and similar compounds are eliminated in livestock faeces. At least 98% of the ivermectin dose is excreted in faeces of cattle and sheep, regardless the dosage route[20,31]. Invertebrate dung fauna did not survive when faeces contained a certain concentration of avermectin and its residues[28,29]. Although the avermectins are generally used in livestock, ivermectin and abamectin have been tested and used as a pesticide in horticulture and in agriculture[6,13,38]. Ivermectin has been considered profitable to control pest insects[26]. Otherwise, it has an effect on nontarget invertebrates, reducing their population[28,32]. Ivermectin usage can affect soil environments depending on its concentration in soil. Models of soil food web has been proposed to measure the levels of toxicity of pesticides residues (including the avermectins) on soil invertebrate fauna[27]. GUNN & SADD (1994)[19] suggest the side-effects of this compound on *Eisenia fetida* and possible increase of soil pollution, following loss of soil quality, and a repercussion on agricultural yield.

Apart from the finding of ivermectin residues in soil, consideration must be given to avermectin residues in running and ground-water. Avermectin residues in the field probably offers a damage to terrestrial invertebrates or freshwater organisms as the cladoceran *Daphnia magna*, probably one of the most sensitive freshwater species to ivermectin[20]. In our laboratories, some toxic effects of ivermectin were also verified in other freshwater organisms species, as the snail *Biomphalaria glabrata* and the planarian *Dugesia tigrina* (unpublished data). Extended use of ivermectin on cattle and plantation increases the probability of toxic effects on pastureland fauna, either in terrestrial or aquatic ecosystems. Contamination of aquatic environment by Ivermectin and similar compounds is an important matter to study the effects of this drug on all the freshwater adults specimens and their larval instar, besides the specimens that live near the running water.

Larvicide treatments have been changed species composition of insect population in some regions. Synthetic insecticide usage has been considered the main strategy for insect vector control[25,39]. Mosquito species distribution can also be altered. We attempt to inquire how many adverse effects the avermectins and their derivatives may offer to target and non-target organisms in the environment.

RESUMO

Efeitos da ivermectina em larvas de *Culex quinquefasciatus*

Os efeitos da ivermectina, uma droga semi-sintética amplamente utilizada para o tratamento de doenças parasitárias do gado, foram observados em larvas de *Culex quinquefasciatus*. Os efeitos tóxicos e a avaliação da mortalidade foram investigados após 5, 15, 30 e 60 minutos de exposição a 1, 5 ou 10 ppm de solução de ivermectina. As observações foram realizadas 24 e 48 horas após o início do experimento, e perda de mobilidade, paralisia progressiva e alta mortalidade de larvas foram registradas. Os efeitos da ivermectina observados nas larvas de mosquito estão provavelmente correlacionados com a ativação de canais de Cloro em membranas celulares.

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