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<Short Communication>

Resistance and control of cypermethrin and chlorpyrifos as acaricide for control of hard tick *Haemaphysalis longicornis* (acari: ixodidae)

Myung-Jo You*

Laboratory of Veterinary Parasitology, College of Veterinary Medicine and Bio-safety Research Centre, Chonbuk National University, Jeonju 561-756, Korea

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Abstract : Chemotherapeutic treatment is still the foundation of tick control programs. This study investigated the acaricidal efficacy of cypermethrin alone and in combination with chlorpyrifos against *Haemaphysalis* (*H.*) *longicornis.* Unfed larval ticks were exposed to 0.1, 1.0, and 10 mg/mL cypermethrin for 60 min, after which the acaricidal efficacy was examined based on tick mortality. All compounds showed similar suppression curves, with the best control being achieved by cypermethrin and chlorpyrifos (1 : 1 ratio) at 10 mg/mL. Effective cypermethrin concentrations for tick control were two to seven times higher than the recommended doses, indicating resistance by *H. longicornis.*

Keywords : acaricide, *Haemaphysalis longicornis*, tick control

Tick-borne diseases are among the most important vector-borne diseases that cause significant losses to humans as a result of damage to domestic and wild animals. A number of different tick control strategies have been used by livestock farmers and animal practitioners, including grooming [10], genetic manipulation through increasing *Bos indicus* content in the progeny [8], biological control in poultry [13], entomopathogenic fungi [15], immunological control through production of vaccines against tick species [7], and ethnoveterinary practices. Ticks transmit several disease pathogens, including *Babesia* and *Theileria* parasites, *Borrelia* bacteria, and the encephalomyelitis virus. To reduce or prevent these diseases in domestic animals, control of the tick vector is necessary. Tick monitoring is also important to detect its early stage. It helps to reduce the spread resistance and to obtain knowledge of distribution of acaricide resistance. Standard methods are needed to assess resistance evolution and allow the comparison of resistance data between laboratories. It is also important to study specific effects of selected acaricide on specific species. There are a variety of methods that have been used to suppress tick vector populations [14]; however, chemotherapeutic control remains the foundation of tick control programs for the eradication of livestock infestations in the developing world [2]. A progressive decrease in the efficiency of acaricidal drugs through the development of resistance [1] would undermine this method. Epidemiological investigations have suggested that a reduction in acaricide-treatment frequency that permits high tick attachment rates allows for the development of endemic stability [9]. To this end, a regular screening of compounds is required to determination their efficacy. Thus far, various groups of acaricides have been found to have significant efficacy for tick control, including pyrethroids [20], avermectins, organophosphates, organochlorines, carbamates, and insect growth regulators.

Pyrethroid acaricides have been used for more than 40 years and account for 25% of the worldwide acaricide market [3]. The pyrethroid class of acaricides was derived from natural compounds isolated from the *Chrysanthemum* genus of plants. The primary mode of pyrethroid action in both insects and mammals is disruption of voltage-sensitive sodium channel (VSSC) function, and any potential disruptor of VSSC function has potent acaricidal or toxicological activity. Often, pyrethroids are sold and/or used as mixtures containing a combination of two or more compounds. Generally, the pyrethroid acaricide cypermethrin is used in combination with chlorpyrifos. Chlorpyrifos is a toxic crystalline organophosphate acaricide that inhibits acetylcholinesterase and is used to control insect pests.

The main objectives of this study were to detect the levels of tick resistance to acaricides, to compare *in vitro* test methods, and to investigate the lowest concentrations of cypermethrin alone and cypermethrin in combination with chlorpyrifos for maximum tick suppression. There is no available data regarding the effects of these chemical compounds on the hard tick *Haemaphysalis* (*H.*) *longicornis*. We examined effi-

*Corresponding author
Tel: +82-63-270-3887, Fax: +82-63-270-3780
E-mail: tick@chonbuk.ac.kr

cacy of cypermethrin alone and cypermethrin in combination with chlorpyrifos at 1 : 1 and 1 : 2 ratios as a tick acaricide using unfed tick larvae. The *H. longicornis* hard ticks that were collected from the bodies of cattle native to Jeju island of South Korea were morphologically and genetically identified. *H. longicornis* ticks have been maintained on rabbits and mice for several generations since 2003 in this laboratory.

The test cypermethrin compounds used were the commercially available products Cyperkiller (ChoongAng Biotech, South Korea), cypermethrin and chlorpyrifos at a 1 : 1 ratio from Cymax (Dow AgroScience, USA) and cypermethrin and chlorpyrifos at a ratio of 1 : 2 from Newgoldenmil (Dongbuchem, Korea). All three chemical compounds were dissolved in 1.0 mL DW at 0.1 mg, 1.0 mg, and 10.0 mg concentrations, respectively. Test compound A (cypermethrin) has a recommended label dose concentration of 0.15 mg/mL, test compound B (cypermethrin : chlorpyrifos, 1 : 1) is recommended at 0.13 ~ 0.5 mg/mL, and test compound C (cypermethrin : chlorpyrifos, 1 : 2) is recommended at 0.15 mg/mL. The compounds were swept into 9-cm-diameter petri dishes and allowed to dry. Approximately 100 unfed larva ticks were placed into the petri dishes, which were closed with a plastic cover. After 5 min, the ticks were first evaluated to count the living and dead tick numbers. We measured tick mortality at different time points from 5 min to 1 h. The experiment repeated three times. Fifty percent suppression rates were calculated from the numbers of dead ticks recorded at each time point over the different doses of each treatment using probit analysis on dose (95% fiducial limits) using SAS software (ver. 8.x; SAS Institute, USA).

The effects of 0.1, 1.0, and 10.0 mg/mL cypermethrin on tick mortality were relatively reproducible between experiments. The results of the three different cypermethrin test groups had suppression curves that were similar in appearance. Compounds A, B, and C at 10.0 mg/mL concentrations killed all unfed larva ticks within 15, 12, and 14 min, respectively. At the 1.0 mg/mL and 0.1 mg/mL concentrations, all unfed larva ticks were killed within 15, 22, and 18 min and 56, 39, and 41 min, respectively. The observed effects of compounds A, B, and C were similar at the 10.0 mg/mL concentration with 50% mortality by 3.8, 5.8, and 5.4 min, respectively. The 50% mortality rates at the 1.0 mg/mL and 0.1 mg/mL concentrations of compounds A, B, and C were observed at 3.6, 10.7, and 11.7 min and 25.2, 24.0, and 18.9 min, respectively. The effect of the 0.1 mg/mL concentration was clearly less than that of the 10.0 mg/mL and 1.0 mg/mL concentrations for compounds A, B, and C (Fig. 1). Fifty percent suppression was identified within 10 min and 100% suppression within 30 min when larvae were treated with 1.0 mg/mL or 10.0 mg/mL of cypermethrin, and 50% suppression were seen within 30 min and 100% suppression within 1 h with 0.1 mg/mL cypermethrin (Fig. 2). At the lowest doses of 0.1 mg/mL and 1.0 mg/mL, the compounds still had effects as acaricides. Thus, we observed that a concentration of greater than 1.0 mg/mL cypermethrin was sufficient for tick suppression. Compounds A, B, and C at 10.0 mg/mL

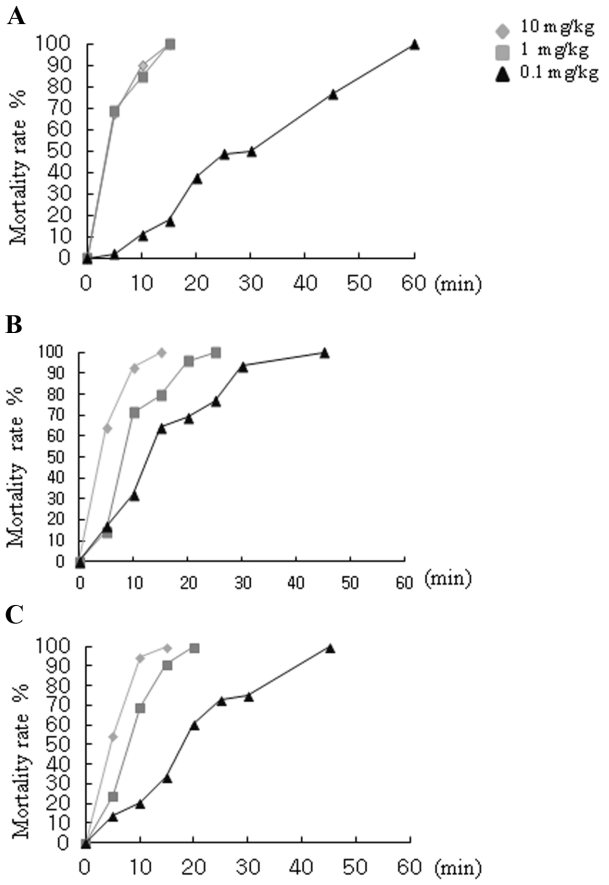

Fig. 1. Mortality curve of *Haemaphysalis longicornis* after treatment with chemical compounds A, B, and C. (A) Cypermethrin at 0.1 mg/mL, 1.0 mg/mL, and 10.0 mg/mL concentrations. (B) Cypermethrin and chlorpyrifos at a 1 : 1 ratio at 0.1 mg/mL, 1.0 mg/mL, and 10.0 mg/mL concentrations. (C) Cypermethrin and chlorpyrifos at a 1 : 2 ratio at 0.1 mg/mL, 1.0 mg/mL, and 10.0 mg/mL concentrations.

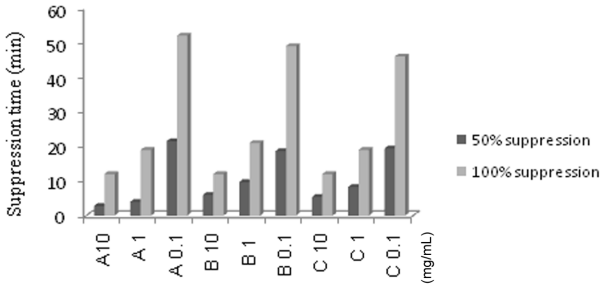

Fig. 2. The results of three cypermethrin treatments were similar in appearance. A 50% suppression before 10 min and 100% suppression before 30 min was observed in the case of stimulation with 1.0 mg/mL or 10.0 mg/mL cypermethrin and 50% suppression was observed before 30 min and 100% suppression before 1 h with 0.1 mg/mL cypermethrin.

and 1.0 mg/mL displayed 50% suppression within 12 min and 100% suppression within 25 min, but the 0.1 mg/mL concentration showed 50% suppression within 25 min and 100% suppression within 1 h.

The survival curves for the 10.0 mg/mL and 1.0 mg/mL concentrations were similar for compounds A, B, and C, but the survival curves showed a distinct difference between the 1.0 mg/mL and 0.1 mg/mL concentrations. Therefore, we concluded that a concentration of greater than 1.0 mg/mL cypermethrin was sufficient for tick suppression. The results of cypermethrin compound concentrations identified for efficient tick control were two- to seven-times higher than the recommended dose. Ticks that acquired resistance to cypermethrin compounds may have been the cause of this increase. The application of an acaricide to the ticks permits the assessment of the capacity of the product to affect tick attachment.

The application of cypermethrin alone at a 0.1 mg/mL concentration, in comparison to cypermethrin and chlorpyrifos at a 1 : 1 ratio and cypermethrin and chlorpyrifos at a 1 : 2 ratio, showed the greatest amount of time to reach 100% mortality in the tested ticks. The compared two compounds may have a synergistic effect, which may shorten mortality time. It was established that the compounds are potential pyrethroids [12] when used in combination mixtures for the control of cattle ticks. However, cypermethrin alone showed the shortest time to achieve 50% mortality, at 3.6 min at a 1.0 mg/mL concentration. A similar result was found where cypermethrin was applied to a resistant strain of cattle tick [11] and was found to acquire 99% mortality with the use of 0.33% concentrate cypermethrin w/v DDT-resistant cattle ticks. The cypermethrin and chlorpyrifos acaricides have long residual activity [17] and are very effective compared with amitraz or chlorfenvinphos. Cypermethrin and chlorpyrifos in combination rapidly eliminated all susceptible members of the target tick population and this resulted in higher incidence of interbreeding between resistant members [16].

da Silveira Novelino *et al.* [16] observed that thymol, menthol, methyl salicylate, and salicylic acid were potential tick inhibitors. Of the four products tested, only thymol caused significant (up to 100%) mortality in *Boophilus microplus* larvae. At concentrations of 0.25%, 0.5%, and 1%, the mortality was 22.73, 73.59, and 100%, respectively. Kröber *et al.* [6] verified that products containing fipronil and ivermectin were potential tick inhibitors since fipronil at 10.0 μg/mL killed all female ticks (*Ixodes ricinus*) within 2 days, and at 1.0 μg/mL and 0.1 μg/mL concentrations, no females survived longer than 4 and 7 days, respectively. The effect of ivermectin was clearly less than that of fipronil as 10 μg/mL ivermectin killed all female *I. ricinus* only after 9 days compared with 100% mortality of all females feeding on fipronil-treated blood at this dose by day 2.

Jonsson *et al.* [5] verified the efficacy of amitraz, cypermethrin, coumaphos and moxidectin as tick inhibitors. Engorged adult female ticks (*Boophilus microplus*) were immersed in one of a series of dilutions of the commercial acaricide in water. For amitraz, the discriminating concentration recommended was 0.25%, but the estimates ranged from 0.46% to 9000%. For cypermethrin, the recommended DD was 0.0050%, with estimates ranging from 0.00022% to 0.74%. For coumaphos, the recommended DD was 0.50% but estimates ranged from 0.66% to 130%. Finally, for moxidectin, the recommended DD was 0.10%, while estimates ranged from < 0.0001% to 5.9%. Analyses of cypermethrin and cypermethrin in combination were expected to result in a valuable increase in the period of protection against *H. longicornis* as well as to decrease farm load in environmental samples.

There are some technique recommanded and provided protocols to evaluate bioassay and tick resistance as the larval pack test (LPT) describe by Stone and Haydock [18], adult immersion test (AIT) developed by Drummond *et al.* [4], larval immersion microassay (LIM) describe by White *et al.* [19]. It needs further study covering full range of chemical grous use againg *H. longicornis* its effectivess and resistance identification.

The results of cypermethrin compounds in tick control were demonstrated at two- to seven-times higher than the recommended dose. Acquired cypermethrin resistance in the tested ticks may have caused this increase in dose. The choice of cypermethrin compound concentration should be considered for suppression *H. longicornis*. The dose effects did not clarify the distinction of their efficacy; therefore, these results suggest the necessity for new standards.

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