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HOW CAPSAICIN CHANGES THE TOXICITY OF PYRETHROIDS IN AMERICAN COCKROACH
(Periplaneta americana)

W JAKI SPOSÓB KAPSAICYNA ZMIENIA TOKSYCZNOŚĆ PYRETROIDÓW W STOSUNKU DO KARACZANA AMERYKAŃSKIEGO
(Periplaneta americana)

Abstract: Intensive use of insecticides has led not only to enhanced environment pollution, but most of all, to an increase in insects’ resistance to the chemicals in use. That is why presently the scientists focus on searching for new, alternative means to aid crop protection. One of the ways to achieve it is finding a compound which, when added to an insecticide, magnifies significantly its toxicity against the pests and in the same time it is safe for people. In the presented study we have attempted to analyse whether the active compound of pepper, capsaicin, increases toxicity of a pyrethroid (Bulldock 025 EC) against American cockroach, a main insect factor causing allergies and asthma in people. The effect of the tested substances was measured by means of changes observed in hemolymph pH and acute toxicity test, performed in various ambient temperatures (15 °C, 25 °C and 35 °C). The results of the experiments suggest that the tested substances affect significantly acid-base homeostasis. Moreover, it was demonstrated that different concentrations of capsaicin have different influence on insecticidal properties of the tested pesticide in higher ambient temperatures. It was established that simultaneous application of capsaicin (in 10⁻⁸ M concentration) and pyrethroid (LD₅₀) causes an increase in death rate among the intoxicated insects. These results give hopes as for applicability of capsaicin as a synergist of pyrethroid insecticides against Periplaneta americana, especially in conditions in which the toxicity of the pesticide itself is lower (i.e., in higher ambient temperatures).

Keywords: capsaicin, pyrethroids, cockroach, death rate, changes in pH

There are many organisms, both vertebrates and invertebrates, associated with human environment. Among them, the cockroaches found their niche in blocks of flats, warehouses, public toilets, hot spots and so on. One of the most popular synanthropic cockroach species is American cockroach (Periplaneta americana). It is widely

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recognisable as a pantry pest, but the same cannot be said about its role as an allergic factor. So far the following cockroach allergens have been isolated: Bla g2 (inactive aspartic proteinase), Bla g4 (calycin), Bla g5 (glutatione S-transferase), Bla g6 (troponin) and group I of cross allergens including Bla g1 and Per a (arylophorin), as well as Per a 7 (tropomyosin) [1].

The research conducted on children in poor city districts in the United States showed that the exposition to cockroach-derived allergens plays a significant role in asthma pathogenesis. The most common allergens in children’s bedrooms were Bla g1 and Bla g2 [2, 3]. Among the methods of preventing asthma the most important is to clear the place of cockroaches using non-toxic traps and safe insecticides.

In our study we have been trying to pick up a substance which is safe for mammals, but when added to an insecticide, it raises its activity. We have decided to use a neurotoxin – capsaicin. It is an organic compound of amide structure responsible for spicy taste of red pepper. Capsaicin activates TRPV1 receptor, which is localised on endings of Aδ and C nerve fibres. Activation of this receptor results in burning sensation and affects mammalian and insect thermoregulation [4–6]. Long history of this spice use proves that it is safe for human, so it meets the pivotal condition laid down for synergists of insecticides. The aim of the study was to investigate whether capsaicin meets the second key provision – raising the insecticide’s toxicity. Since the tested pyrethroid shows a negative temperature coefficient [7], the most important was to determine how capsaicin affects the pyrethroid’s toxicity in high ambient temperatures.

Thus, the objective of the presented research was to test whether capsaicin may cause an increase in toxicity of pyrethroid insecticides in different ambient temperatures and whether capsaicin can be used as a synergist of these insecticides in wide spectra of temperature.

Materials and methods

In the experiments adult Periplaneta americana of both sexes were used. The animals were bred in insectariums of Department of Animal Toxicology and Department of Biophysics at the Nicolaus Copernicus University in Torun, at constant temperature of 22–25 °C and under a natural photoperiod. During the whole time of stay in the breeding premises the insects had unlimited access to water and food.

The substances in use

In the research a natural neurotoxin, capsaicin (product of Sigma) was used. In regards to its poor solubility in water, two kinds of solvents were used: alcohol (product of POCH – Polish Chemical Reagents) and a mixture of alcohol and Tween 80 (product of Sigma). In order to obtain proper solutions, a 1 M solution of capsaicin in alcohol 96 % or in a mixture of alcohol 96 % and Tween 80 in 1:1 relation was prepared. This initial solution was subsequently used for preparing a 10⁻⁸ M solution of capsaicin (the alcohol and Tween concentration was 0.1 %).
In the experiments a pyrethroid insecticide, Bulldock 025EC in \( \text{LD}_{50} \) dose was used. Its active compound is beta-cyfluthrin.

**Method of application**

The substances were administered in volume of 10 mm\(^3\) (\(\mu\)l) on the insects’ dorsum, under the wings, near the thoracic ganglia.

**Abbreviations used in the paper**

- C – the insects administered saline; A – alcohol 0.1 %; AT – a mixture of alcohol and Tween; k8 – capsaicin solved in alcohol in \(10^{-8}\) M concentration; P – pyrethroid.

**Temperature-dependant survival rate**

The intoxicated animals (\(n = 20\)) were placed for three days in glass cubical chambers (13 × 13 × 13 cm). A shelter and isolation for the insects was made of cardboard containers. The glass cubes were placed in rooms of defined temperatures: 15 °C, 25 °C or 35 °C. During the experiments water and food for the animals were provided. In 12-hour intervals the survival rate was noted, dead individuals removed and water and food changed.

**Determining of the insects’ hemolymph pH**

The hemolymph pH assessment was performed after three days since application. In order to measure the hemolymph pH, the insects’ body fluids were collected using thin capillary tubes, which were placed between the body segments right under the second pair of legs. The hemolymph was placed in ependorph tubes thermostated in temperatures equal to the ones in the experiments (15 °C, 25 °C or 35 °C). The pH value was measured using Lazar Research Laboratories PHR (146 Micro Combination pH electrode) and Hanna Instrument microprocessor pH meter. Calibration was performed on standard pH buffers (4.01 and 7.01). The measurements were taken at the temperatures relevant to the experimental ones, after 10 s since putting the electrodes in. After each measurement pH value was checked in the buffers.

**Results and discussion**

An influence of joint and separate application of pyrethroid and capsaicin in three different ambient temperatures (15 °C, 25 °C or 35 °C) on the insects’ death rate was studied. Since it is known that every change in the organism’s homeostasis may lead to disturbances in its functioning, a decision to investigate whether the xenobiotics administration affects acid-base homeostasis and whether the changes are correlated with the toxicity of the substances in use was made. As applying both the solvents
themselves and capsaicin in various configurations may cause changes in insects’ body fluids pH as well as in their death rate, the results are presented as differences observed in relation to the control group (the insects administered only saline) (Fig. 1).

The insects’ death rate assessment

The assessment of the capsaicin effect on insecticidal activity of the pyrethroid was conducted after 72 hours since the intoxication on the basis of acute toxicity test.

Table 1

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<tr>
<th>The substance tested</th>
<th>Changes in the insects’ death rate [%]</th>
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<tr>
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<td>Pk8</td>
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Application of pyrethroid caused the highest death rate at temperature of 15 °C – 95 %, while at 25 °C – 35 % and at 35 °C – only 5 % (Table 1). It was established that co-application of A and pyrethroid at temperatures of 15 °C and 25 °C caused a decrease in the insecticide’s activity (death rate fell by 20 % and 35 %, respectively). At temperature of 35 °C their co-operation led to an increase in the death rate by 20 %. However, unexpectedly, adding AT to pyrethroid only gave the highest results in the toxicity test. The intoxicated insects’ death rate was almost equal at various temperatures (15 °C – 80 %, 25 °C – 80 % and 35 °C – 85 %), thus while in colder environment the toxicity fell by 15 %, at higher temperatures it rose by 35 % and 45 %, respectively.

The results of the performed experiments suggest that the tested concentration of capsaicin affects insecticidal activity of the pyrethroid in various ways. While analysing co-operation of capsaicin with the insecticide it was noted that co-application of the pesticide and k8 caused an increase in the insects’ death rate by 35 % and 45 % at 25 °C and 35 °C, respectively, however, application of kT8 raised the toxicity by as much as 70 %, but only at the temperature of 35 °C. At temperature of 15 °C every combination of capsaicin and pyrethroid caused lowering of the death rate by 20 % in the case of Pk8 and 35 % in the case of PkT8 in comparison with the toxicity of the pyrethroid itself.

Analysing the insects’ hemolymph pH

On the basis of bibliographical data [8] and unpublished own studies it was concluded that in control insects pH value slightly lowers along with the rise in
temperature. Application of the pyrethroid reversed this relation: the lowest pH was noted at the temperature of 15 °C – 6.70 ± 0.18 and the highest at 35 °C – 7.31 ± 0.02. At 25 °C it was 7.27 ± 0.02 and was higher than in the insects administered saline (C) by 0.31 ± 0.01 (p < 0.001). After co-application of alcohol 0.1 % and insecticide at 15 °C a shift by 0.20 ± 0.01 (p < 0.001) towards more acid values of the insects’ hemolymph pH was noted. At 25 °C a slight increase in the pH value – by 0.08 ± 0.01 (p < 0.05) was observed (Fig. 1). PAT administration caused a decrease in pH value by 0.58 ± 0.01 (p < 0.001) at 15 °C. At 25 °C and 35 °C a minor increase in hemolymph pH was statistically insignificant. While analysing the effects of combinations of pyrethroid and capsaicin it can be noted that in both cases only changes at 15 °C were statistically significant (a decrease by 0.11 ± 0.01, p < 0.05 and by 0.19 ± 0.01, p < 0.001 for Pk8 and PkT8, respectively). In every case at 25 °C and 35 °C the insects’ hemolymph pH was similar to this noted in a control group and it was 6.96 ± 0.01 and 6.97 ± 0.01 for Pk8, respectively and 6.94 ± 0.04 and 7.00 ± 0.01 for PkT8, respectively.

![Figure 1](attachment:figure1.png)

Fig. 1. Changes in the insects’ hemolymph pH triggered by co-application of pyrethroid (P) with substances: alcohol 0.1 % (A), a mixture of alcohol and Tween 80 (AT), capsaicin in 10⁻⁸ M concentration, solved in alcohol 0.1 % (k8) and a mixture of alcohol and Tween 80 (kT8) at temperatures of 15 °C, 25 °C and 35 °C (* – p < 0.05, ** – p < 0.01, *** – p < 0.001)

When comparing the toxicity of the insecticide itself at the three experimental ambient temperatures it can be noted that it is 19-fold lower at 35 °C than at 15 °C, which is consistent with the fact that the tested insecticide is characterised by a negative temperature coefficient [7]. Our earlier studies showed that capsaicin cannot be used separately against cockroaches [9]. However, in the presented study we have demonstrated that when added to a pyrethroid pesticide, it affects markedly its toxicity. Average death rate at all ambient temperatures was 45 %; after co-application of pyrethroid and alcohol – 33 %; after co-application of pyrethroid with a mixture of Alcohol and Tween – 81 %; after co-application of pyrethroid and capsaicin – 65 % and after co-application
of pyrethroid and capsaicin dissolved in a mixture of alcohol and Tween – 58 %. Although a synergistic effect of capsaicin is poor, reaching only 20 %, an addition of kT8 to pyrethroid renders its toxic properties less susceptible to ambient temperatures (at 35 °C the toxicity of PkT8 was higher than of the pyretroid itself by as much as 70 %). Thus, a potential separate and joint use of the substances, depending on the season or ambient temperature (eg in hot spots) should be considered. However, a more attention should be paid to the results of acute toxicity test in the case of joint insecticide and a mixture of alcohol and Tween 80 application, where at every tested temperature the cockroaches’ death rate was almost the same, ~80 %. Tween 80 (polysorbate 80) is a nonionic surfactant used as a food ingredient (E433), applied along with substances limiting absorption of cholesterol [10] and also as a vector for antineoplastic drugs [11]. Thus, Tween 80 as a synergist might be considered as safe and the results obtained as highly advantageous. Nevertheless, it must be taken into account that studies on microorganisms proved that it changes markedly membrane fluidity and it affects many enzymes’ synthesis [12], so as far as pyrethroid and a mixture of alcohol and Tween 80 may be used at homes, the case is not that simple for crops, since its potential effect on soil microorganisms should be considered.

Every tested combination of the insecticide and capsaicin caused also temperature-dependent changes in the hemolymph pH. Noteworthy is the fact that in the case of application of the pyrethroid itself a certain dependency between ambient temperature, hemolymph pH and death rate was observed: an increase in ambient temperature was accompanied by a decrease in death rate and a rise in pH values. However, addition of any of the tested substances triggered such various changes that all attempts to find a uniform pattern for them failed. For instance, although joint application of pyrethroid with capsaicin and pyrethroid with capsaicin dissolved in a mixture of alcohol and Tween 80 reduced the effect of pyrethroid itself on acid-base homeostasis (total difference in pH is 0.52 and 0.40, respectively), this change was not accompanied by a change in these substances’ toxicity. However, the fact that the measurements were conducted 72 hours after intoxication and that the results obtained come from the individuals which survived the experiment should be taken into account. Thus, in order to investigate more thoroughly the relation between death rate and changes in hemolymph pH, it is necessary to measure time-dependent changes in pH in relation to progressing paralysis caused by the applied substances. Nevertheless, the tolerance of cockroaches to changes in their hemolymph pH (the range of changes covered one whole pH unit, from 6.4 to 7.4) is noteworthy.

The results obtained allow to assume that capsaicin may be used as a toxicity magnifier for pyrethroid insecticides against Periplaneta americana, although only in specific environmental conditions (ie high ambient temperature). The death rate observed in the experiments was independent of the changes in pH caused by application of the xenobiotics, as joint intoxication with pyrethroid and a mixture of alcohol and Tween 80 resulted in 80 % death rate and in the same time – lowering the value of pH by 0.57 at 15 °C, while in higher temperatures death rate remained the same
(80 %), but the changes in pH were significantly lower, reaching only 0.04 and 0.05 in 25 °C and 35 °C, respectively.

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References

Wykazano także, że testowane stężenia kapsaicyny w różny sposób wpływają na aktywność owadobójczą zastosowanego insektycydu w wyższych temperaturach otoczenia. Stwierdzono, że jednoczesna aplikacja kapsaicyny (w stężeniu \(10^{-8}\) M) i pyretroidu (LD\(_{50}\)) powoduje wzrost śmiertelności intoksykowanych owadów. Na podstawie otrzymanych wyników można przypuszczać, że kapsaicynę będzie można stosować jako synergetyk dla insektycydów z grupy pyretroidów w stosunku do Periplaneta americana, zwłaszcza w tych warunkach środowiska, w których toksyczność pyretroidu jest mniejsza (duże temperatury otoczenia).

**Słowa kluczowe:** kapsaicyna, pyretroidy, karaczan, śmiertelność, zmiany pH