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EFFECT OF APPLICATION OF CAPSAICIN AND PYRETHROID ON METABOLIC RATE IN MEALWORM *Tenebrio molitor*

WPŁYW ZASTOSOWANIA KAPSAICYNY I PYRETROIDU NA TEMPO METABOLIZMU U LARW MĄCZNIKA MŁYNARKA *Tenebrio molitor*

Abstract: In the research presented the effect of Bulldock insecticide and capsaicin on CO_2 release in mealworm *Tenebrio molitor* was assessed. Metabolic rates of insects intoxicated with tested substances were measured using flow-through respirometry. The results obtained showed considerable increase in CO_2 release after application both pyrethroid and capsaicin. The highest metabolic rates were observed after simultaneous intoxication with capsaicin and insecticide. These results suggest that capsaicin enhances the toxic effect of Bulldock and may be used as this insecticide's synergist against mealworms.

Keywords: capsaicin, mealworms, metabolic rate, pyrethroids.

Environment contamination caused by excessive use of insecticides, as well as growing problems connected with resistance force us to look for new directions in crop protection. One of the ideas is to use synergists that will increase insecticidal properties of pesticides. Synergist is safe to environment and people chemical substance, which added to insecticides will increase their toxicity. Our research focuses on the use of capsaicin, an alkaloid responsible for the spicy taste of pepper, as a synergist to pyrethroids.

Pyrethroids are a class of insecticides derived from *Chrysantenum* plant extracts. This natural insecticide served as a base for synthetic pyrethroids production [1]. For almost forty years these insecticides have been commonly used in crop protection, and by the mid-1990's they amounted to 23 % of the world insecticide market and occupied the second position just after organophosphate pesticides [2]. The mode of action of pyrethroids is connected with action of these substances on insects' nervous system. They open the sodium channels, cause membrane depolarization and disturb nerve

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impulse conduction, which leads to insect's paralysis [3]. Unfortunately the extensive use of pyrethroids caused resistance occurence in many species, which is especially disadvantageous in the case of such species as *Anopheles gambiae*, main malaria vector in Africa [4]. Resistance may come into existence as a result of many different molecular mechanisms, mainly due to the increased concentration of detoxification enzymes or by reduction of sensitivity of insects sodium channels to pyrethroids, called 'knockdown' resistance [5]. Despite resistance pyrethroids are profitable to use because of low toxicity to mammals and impermanence in the environment. For that reasons pyrethroids are extensively used all the time, so it is very important to find substances which will increase their toxicity without any harm to living organisms other than pests.

The aim of the presented studies was to assess the effect of capsaicin and pyrethroid insecticide on metabolic rate of *Tenebrio molitor* larvae.

Materials and methods

The experiments were performed on mealworms (*Tenebrio molitor*) reared in 25 ^{o}C and under 12 h/12 h light-dark cycle.

Mealworms were intoxicated with the following substances:

No.	Quantity	Substance
1	10 mm^3	Water (control group)
2	10 mm^3	Insecticide Bulldock 025 EC solution (182 mm^3 dissolved in 1 l of water) – this pesticide belongs to pyrethroids, the active substance is β-cyfluthrin
3	10 mm^3	Capsaicin dissolved in alcohol solution (10^{-4} M)
4	10 mm^3	Ethyl alcohol in concentration 0.1 % – capsaicin does not dissolve in water, but in alcohol. (To make sure that the observed results are real effects of action of capsaicin)
5	10 mm^3	Mixture of alcohol and insecticide in the same concentrations
6	10 mm^3	Mixture of capsaicin (dissolved in alcohol) and Bulldock in the same concentrations

The metabolic rates for each mealworm were measured using flow-through respirometry. After intoxication with the tested substances the mealworms were placed in 2 cm diameter glass-aluminium chambers in 25 ^{o}C. Dry, CO_2 – free air was pumped through the chambers at 50 cm^3/min to a infrared CO_2 analyzer (Qubit Systems Inc., Kingston, Canada). CO_2 release of insects was measured for four hours from intoxication.

Mean metabolic rate of 6–8 mealworms intoxicated with particular substance was subjected to statistical analysis. t-Student test was used to compare the difference between control and intoxicated groups. Significant difference was achieved when $p < 0.05$.

Results and discussion

Many toxic compounds may have an influence on physiological processes in insects, which results very often in organisms dysfunction. Under stress conditions, such as toxicant application, insect may increase their metabolism to cope with changed energy demands [6].

The study presented clearly showed that both capsaicin, as well as pyrethroid increase CO_2 release in *Tenebrio molitor* larvae (results are shown in Fig. 1 and Fig. 2). In study presented, application of a natural alkaloid, capsaicin, resulted in a shift in carbon dioxide release (on average 1.33 times higher in comparison with control group) during all four hours of the experiment. The experiments performed on mice and rats revealed that capsaicin promotes secretion of adrenaline and increase in oxygen consumption after single administration [7, 8].

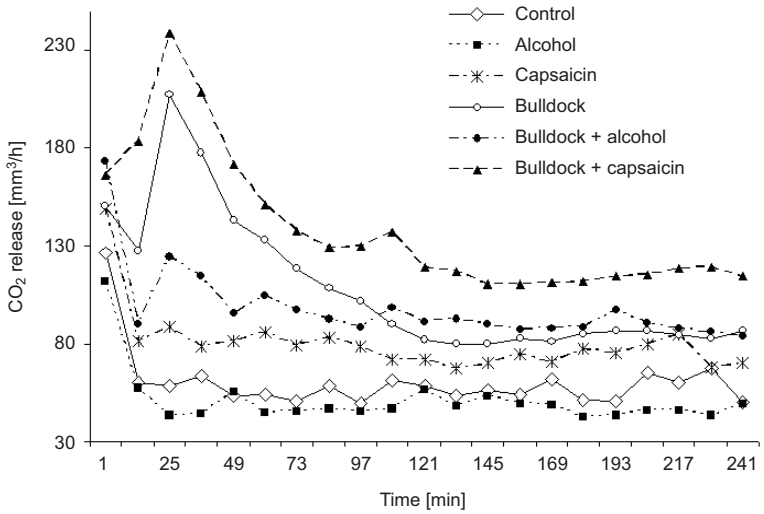

Fig. 1. Changes in CO_2 release in *Tenebrio molitor* larvae after intoxication with the tested substances during four hours of experiment

Mealworms intoxicated with Bulldock demonstrated significant increase in CO_2 release (the intoxicated insects released about 1.8 of CO_2 times more than control ones). Similar results were obtained by Zafeiridou and Theophilidis [9], where application of 50 and 100 ng of deltamethrin (pyrethroid) per mealworm beetle caused concentration-dependent increase of respiratory rate. Enhanced metabolic rate in insects may be a result of detoxication processes initiation, convulsion caused by poisoning or excitation of respiratory motoneurons. Pyrethroids increase the probability of the sodium channels' opening and cause gradual membrane depolarization, which triggers excitation of the cells. This leads to increased work of respiratory motoneurons and enhanced CO_2 release in insects. Another study showed that application of insecticides has a great influence on glycogen metabolism in intoxicated insects body. Increased levels of trehalose and glucose in the haemolymph are suggested to be a result of raised transportation of these compounds from fat body to haemolymph to supply energy for enhanced detoxification processes [6]. Similar processes may be observed after application of other toxins, such as capsaicin.

Combination of insecticide and alcohol did not change significantly the metabolic rate of the examined insects in comparison with Bulldock alone. In that case we can

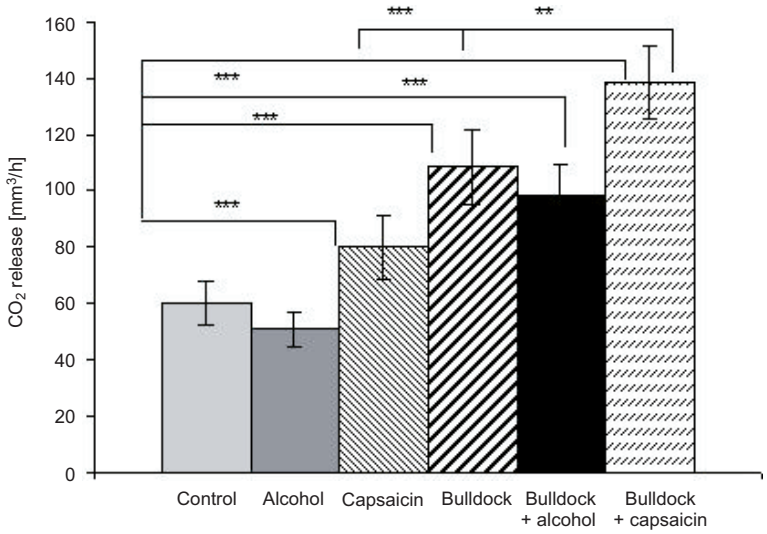

Fig. 2. CO_2 release in mealworms after intoxication with the tested substances (mean ± SE). Statistical significant difference is shown as * (** – $p < 0.01$;*** – $p < 0.001$)

exclude the effect of alcohol on the metabolism of both capsaicin (because alcohol alone did not have any significant influence either), as well as insecticide.

Simultaneous application of capsaicin and pyrethroid resulted in the highest growth of CO_2 release. We can observe synergism between capsaicin and insecticide, appearing as increased metabolic rate in intoxicated mealworms. Simultaneous effect of these two compounds probably resulted in activation of intensive detoxification processes. What is more, recent researches showed that pyrethroids may also act on voltage-dependent calcium channels and increase calcium influx [10]. The main target for capsaicin in mammals is vanilloid receptor subtype 1 (TRPV1), which is calcium-permeable cation channel. Capsaicin binding to this receptor results in channel opening and calcium influx. It may be possible that capsaicin exerts an influence on calcium homeostasis in insects as well. Simultaneous action of capsaicin (possible calcium influx) and pyrethroids (sodium and calcium influx) would result in strong response of insects. This would explain why one hour after application the intoxicated insects were paralysed. Capsaicin intensified the effect of pyrethroid action. This result suggest that capsaicin may be used as pyrethroid insecticides synergist against mealworms. Because of increased metabolic rate insects have higher energy demands. Combination of capsaicin and pyrethroid may not cause death of pests, but after intoxication insects will have higher metabolic rate and energy consumption, so it may not be enough energy supplies to reproduce.

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References

[1] Seńczuk W.: Toksykologia. Wydawnictwo Lekarskie PZWL, Warszawa 2002.
[2] Soderlund D.M., Clark J.M., Sheets L.P., Mullin L.S., Piccirillo V.J., Sargent D., Stevens J.T. and Weiner M.L.: Toxicology 2002, **171**, 3–59.
[3] Węgorek P., Obrępalska-Stęplowska A., Nowaczyk K. and Zamojska J.: Post. Ochr. Rośl. 2007, **47**, 383–388.
[4] Corbel V., Stankiewicz M., Bonnet J., Grolleau F., Hougard J.M. and Lapied B.: Neurotoxicology 2006, **27**, 508–519.
[5] Soderlund D.M. and Knipple D.C.: Insect Biochem. Molecular Biol. 2003, **33**, 563–577.
[6] Nath B.S.: Pesticide Biochem. Physiol. 2003, **74**, 73–84.
[7] Ohnuki K., Haramizu S., Oki K., Watanabe T., Yazawa S. and Fushiki T.: Biosci. Biotechnol. Biochem. 2001, **65**, 2735–2740.
[8] Kobayashi A., Osaka T., Namba Y., Inoue S., Lee T.H. and Kimura S.: Amer. J. Physiol. Regulatory Integrative Comp. Physiol. 1998, **275**, 92–98.
[9] Zafeiridou G. and Theophilidis G.: Pesticide Biochem. Physiol. 2006, **86**, 211–217.
[10] Clark J.M. and Symington S.B.: Invert. Neurosci., 2007, **7**, 3–16.

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Abstrakt: W prezentowanych badaniach określano wpływ insektycydu Bulldock i kapsaicyny na tempo uwalniania CO_2u larw mącznika młynarka *Tenebrio molitor*. Tempo metabolizmu owadów intoksykowanych badanymi substancjami mierzono przy użyciu układu respirometrycznego, służącego do pomiarów w układzie przepływowym. Uzyskane wyniki wykazały znaczący wzrost ilości uwalnianego CO_2 po aplikacji zarówno pyretroidu, jak i kapsaicyny. Najwyższe tempo metabolizmu odnotowano po jednoczesnej intoksykacji kapsaicyną i insektycydem. Przedstawione wyniki sugerują, że kapsaicyna zwiększa toksyczny wpływ pestycydu Bulldock i prawdopodobnie może być używana jako synergetyk dla tego insektycydu przeciwko larwom mącznika młynarka.

Słowa kluczowe: kapsaicyna, larwy mącznika młynarka, tempo metabolizmu, pyretroidy