Is there a relationship between insect metabolic rate and mortality of mealworms *Tenebrio molitor* L. after insecticide exposure?

Czy istnieje zależność pomiędzy tempem metabolizmu a śmiertelnością mącznika młynarka *Tenebrio molitor* L. eksponowanego na insektycydy?

Justyna MALISZEWSKA* and Eugenia TĘGOWSKA

Nicolaus Copernicus University in Toruń, Department of Animal Toxicology, Poland;

*correspondence: ojustyna@umk.pl

Abstract

Pesticides are known to affect insects metabolic rate and CO_2 release patterns. In the presented paper metabolic rate and mortality of mealworms *Tenebrio molitor* L. exposed to four different insecticides was evaluated, to find out whether there is a relationship between mealworms sensitivity to pesticides and their metabolic rate. *Tenebrio molitor* mortality was determined after intoxication with pyrethroid, oxadiazine, neonicotinoid and organophosphate. Metabolic rate before and after intoxication with insecticides was also determined. The highest CO_2 production and mortality rate was observed after mealworms exposition to neonicotinoid insecticide. The results suggest that high CO_2 release after intoxication is adequate to the intensity of the non-specific action of the xenobiotic (e.g. hyperactivity of neuromuscular system), rather than the intensity of detoxification processes, and it is correlated with mealworms mortality.

Keywords: insecticides, metabolic rate, mortality, *Tenebrio molitor* L.

Streszczenie

Pestycydy mają wpływ na tempo metabolizmu owadów oraz wzorzec uwalniania przez nie dwutlenku węgla. W prezentowanej pracy oceniano tempo metabolizmu oraz śmiertelność mącznika młynarka *Tenebrio molitor* L. eksponowanego na cztery różne insektycydy, w celu określenia, czy istnieje zależność pomiędzy wrażliwością

mączników na pestycydy a ich tempem metabolizmu. Śmiertelność dorosłych osobników mącznika młynarka oceniano po intoksykacji insektycydami z grupy pyretroidów, oksadiazyn, neonikotynoidów oraz fosforoorganicznych. Dodatkowo, badano tempo metabolizmu mączników przed oraz po intoksykacji insektycydami. Najwyższa produkcja CO_2 oraz śmiertelność została odnotowana u mączników eksponowanych na insektycyd neonikotynowy. Uzyskane wyniki wskazują, że wysoki poziom produkcji i uwalniania CO_2 po intoksykacji jest adekwatny do intensywności niespecyficznego działania ksenobiotyku (np. wysokiej aktywności układu nerwowego i mięśniowego owada), natomiast w mniejszym stopniu jest adekwatny do intensywności procesów detoksykacyjnych, oraz jest powiązany z śmiertelnością mącznika młynarka.

Słowa kluczowe: insektycydy, śmiertelność, tempo metabolizmu, *Tenebrio molitor*

Streszczenie szczegółowe

Zadziałanie ksenobiotykiem wiąże się z wydatkiem energetycznym organizmu wykorzystanym na walkę z zatruciem. Po intoksykacji, owady muszą wydatkować dodatkową energię na procesy detoksykacyjne, w związku z czym zmniejszone zostają zasoby energetyczne na inne procesy życiowe, takie jak wzrost i reprodukcja.

Celem badań było określenie czy wrażliwość owadów na insektycyd jest powiązana z ich poekspozycyjnym tempem metabolizmu.

Doświadczenia przeprowadzono na dorosłych osobnikach mącznika młynarka, które eksponowane były na insektycydy o różnym mechanizmie działania owadobójczego. Zastosowano preparaty z grupy: pyretroidów (Bulldock 025 EC), oksadiazyn (Steward 30 WG), neonikotynoidów (Mospilan 20 SP) oraz fosforoorganicznych (Actellic 500 EC). Po ekspozycji na insektycydy oceniano śmiertelność oraz tempo metabolizmu badanych mączników. Śmiertelność obserwowano w 25 °C przez trzy doby. Produkcję CO_2 mączników badano przy użyciu respirometrii z otwartym przepływem powietrza, z wykorzystaniem analizatora CO_2 (Qubit Systems Inc., Kanada). Uzyskane wyniki opracowano statystycznie za pomocą programu SPSS z wykorzystaniem analizy kowariancji oraz testu Bonferroniego dla wielokrotnych porównań (tempo metabolizmu) oraz analizy wariancji i post hoc testu Tukeya (analiza śmiertelności).

Produkcja dwutlenku węgla u mączników eksponowanych na insektycydy istotnie wzrosła w porównaniu do grupy kontrolnej. Najsilniejszy efekt odnotowano po aplikacji insektycydu neonikotynowego (ponad 10-krotnie wyższe tempo metabolizmu niż w grupie kontrolnej) oraz pyretroidu (ponad 2-krotnie wyższe tempo metabolizmu niż w grupie kontrolnej). Śmiertelność badanych owadów była najwyższa po ekspozycji na insektycyd neonikotynowy (69,4±13,2% po 3 dobach) oraz insektycyd fosforoorganiczny (60,6±3,7% po 3 dobach).

Uzyskane wyniki wskazują na istnienie zależności pomiędzy wrażliwością mącznika młynarka na insektycydy, jego śmiertelnością a produkcją dwutlenku węgla. Wysoki poziom tempa metabolizmu nie jest jednak, w naszej opinii, wynikiem jedynie siły

procesów detoksykacyjnych. Jest on raczej odzwierciedleniem niespecyficznego działania ksenobiotyku, które objawiają się drgawkami, zaburzeniami behawioralnymi i konwulsjami, prowadzącymi do paraliżu. Powoduje to zużycie dużych ilości energii, które nie są przeznaczone np. na procesy detoksykacyjne, i w konsekwencji prowadzi do śmierci owada.

Introduction

Intoxication with a toxin is associated with organism's energy expenditure allocated to cope with poisoning. The theory of energetic trade-offs states that energetic budget of every organism is limited, which means that the energy available must be distributed in a way that maximize the organism's fitness (Maryański et al., 2002). This means that when intoxicated insect must expend additional energy for detoxification processes and then resources for fitness-related traits, such as reproduction or growth, are reduced (Spurgeon and Hopkin, 1996).

One of the method for having insight into energy budget of organisms under stressful conditions is respiration rate measurements. A general prediction is that metabolic rate should increase with increasing intoxication. Actually a significant increase in respiration rate of ground beetle *Pterostichus oblongopunctatus* (Fabricius, 1787) collected from metal polluted areas was observed (Bednarska and Stachowicz, 2013). This toxic effect seem to be long-lasting since the confused flour beetle was reported to have higher initial metabolic rate when originating from copper-exposed populations, even in the absence of heavy metal. This resulted in lower fecundity and shorter lifespan of these insects (Lukasik and Laskowski, 2007). Also after pesticide exposure such relationship was strongly implied (Karise and Mänd, 2015). However, not all published results was obtained by direct measurement of CO_2 production or O_2 consumption, basing only on changing in respiratory patterns as a measure of intensity of poisoning. Moreover, there is no general experimental protocol and experimental methods differ within published results, e.g. "closed system" metabolic chambers in *Blattella germanica* L. (Hostetler et al., 1994); calorimetry in *Pieris brassicae* L. (Harak et al., 1999); flow-through respirometry in *Amblyomma americanum* L. (Zheng et al., 2013), which makes it difficult to compare results.

Recently a general tendency was demonstrated showing that a high specific maintenance rate correlates with species sensitivity to toxicants. The most sensitive species to chemical pesticides had before exposition the highest metabolic rates (Baas and Kooijman, 2015). Piechowicz (2006) reported in his experiments on several insect species exposed to insecticides, that after poisoning, with an increase in insects metabolic rate, their survival decrease. Here, metabolic rate and mortality of mealworms exposed to four different insecticides was evaluated, to find out whether there is a relationship between mealworms sensitivity to pesticides and their after-exposure metabolic rate.

Materials and methods

Experiments were performed on adult individuals of mealworm *Tenebrio molitor* L., both sexes. Insects were kept at plastic containers (32×20×20 cm) filled with flour,

oat flakes and cottonwool. Containers were kept in 25 °C and 12:12 L:D. Insect were fed with slices of apple.

Four different commercially available preparations were used in this study:

- Bulldock 025 EC (Makteshim Agan Industries Ltd, Israel) with ß-cyfluthrin (2.75%) as an active substance. Dose used for mealworms in this study was 0.0004 μg · insect^{-1}.
- Steward 30 WG (Du Pont de Nemours, USA) with indoxacarb (30%) as an active substance. Dose used for mealworms in this study was 1 μg · insect^{-1}.
- Mospilan 20 SP (Nisso Chemical Europe, Germany) with acetamiprid (20%) as an active substance (dose used: 0.25 μg · mealworm^{-1}).
- Actellic 500 EC (Syngenta Crop Protection, Switzerland) with pirimiphos-methyl (49.02%) as an active substance (dose used: 0.0008 μg · mealworm^{-1})

These insecticides are represents of pyrethroids (Bulldock 025 EC), oxadiazines (Steward 30 WG), neonicotinoids (Mospilan 20 SP) and organophosphates (Actellic 500 EC) with different modes of toxic action in insects. All doses used were estimated to be LD_{50} (or as close as possible to LD_{50}) in a preliminary study at 25 °C (standard procedure for testing chemical toxicity).

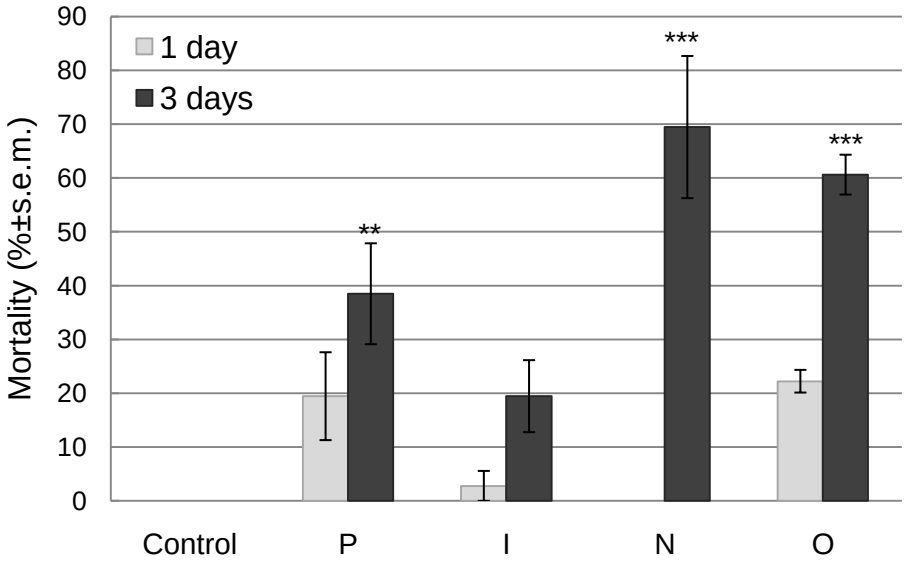
The mortality of the examined mealworms was monitored for 72 hours. Mealworms were exposed to 10μl of water (control group) or insecticide solutions, which were applied topically on the ventral part of prothorax. After intoxication, mealworms were placed in glass containers, which were kept in 25 °C and 12:12 L:D in laboratory incubator ILW 15 STD (POL-EKO APARATURA). The mealworms had free access to food (apple slices). Each experimental series was repeated six times, with six different individuals (total: 36 insect per each experimental series). The mortality rate was generally monitored for 72 hours, but to ascertain whether insects' paralysis is irreversible, observation was extended to 12 days.

The CO_2 production of each mealworm was measured using flow-through respirometry. After intoxication with tested substances, mealworms were placed into 2 cm diameter glass – aluminium chambers. Dry, CO_2-free air was pumped through the chambers at 150 ml · min^{-1} to a infrared CO_2 analyzer (Qubit Systems Inc., Kingston, Canada). The CO_2 release of examined mealworms was measured always during poisoning progress phase – that is until two hours from intoxication, as Goñalons and Farina (2015) showed that symptoms of poisoning with pesticides occur already after 15 minutes from exposition. All measurements were carried in 25 °C. In each experimental series, CO_2 production of six mealworms was measured, each mealworm individually. Before measurements, each mealworm was weighted on precise balance (Radwag WTB 200, USA).

Data concerning mealworms mortality was arcsine square root transformed and tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test). To test whether insecticide treatment affected mortality, one-way analysis of variance and post hoc Tukey test was performed. The CO_2 production of intoxicated mealworms was compared using ANCOVA, with body mass as covariate, and pairwise comparisons with Bonferroni correction for multiple comparisons. The level of significance for all test was 0.05. Analyses were performed in IBM SPSS Statistics 22 software. Data are presented as means±s.e.m.

Results

Mealworms mortality after intoxication with insecticides is presented in Figure 1. Significant differences form control group was observed after 72 hours from exposition (ANOVA: F=13.75; df=4.25; P = 0.0004). The highest mortality was observed after neonicotinoid – 69.4±13.2% and organophosphate treatment – 60.6±3.7%, whereas intoxication with indoxacarb resulted in low overall mortality – 19.4±6.7%.



* inidicates significant differences from insect not intoxicated (control) (ANOVA; post hoc Tukey: **- P < 0.01; ***- P < 0.001)

* wskazuje różnice istotne statystycznie w stosunku do grupy kontrolnej (ANOVA; post hoc test Tukey'a: **- P < 0.01; ***- P < 0.001)

Figure 1. Mortality (%±standard error of the mean) of mealworms exposed to pyrethroid (P), oxadiazine (I), neonicotinoid (N) and organophosphate (O) after first and three days from intoxication

Rysunek 1. Śmiertelność (%±s.e.m.) mącznika młynarka eksponowanego na insektycydy z grupy pyretroidów (P), oksadiazyn (I), neonikotynoidów (N) i fosforoorganicznych (O), po pierwszej i trzeciej dobie od intoksykacji

Mealworms that were not exposed to insecticides produced 530.8±6.1 µl · h^{-1}· g^{-1} CO_2. After insecticide treatment, their metabolic rate significantly increased (ANCOVA: F=5.4; df=1.4; P = 0.03). The CO_2 production of insects was 2.4 times higher after pyrethroid exposure (1286.5±26.9 µl · h^{-1}· g^{-1}) and 10.6 (5609.3±219.4 µl · h^{-1}· g^{-1}) times higher after intoxication with neonicotinoid. After oxadiazine and organophosphate treatment, mealworm CO_2 production was 1.8 and 1.5 times higher, respectively (Table 1a and 1b).

Table 1a. Results of ANCOVA for CO_2 production ($\mu l \cdot h^{-1} \cdot g^{-1}$) in mealworms after insecticides treatment, with body mass (g) as covariate

Tabela 1a. Wyniki analizy kowariancji dla produkcji CO_2 przez mączniki eksponowane na insektycydy, z masą ciała jako współzmienną

Factor	SS	d.f.	MS	F-ratio	P-value
INTERCEPT	8914.7	1	8914.7	4.5	0.046
BODY MASS	10682.2	1	10682.2	5.4	0.031
INSECTICIDE	2579990.3	4	644997.6	322.2	0.000
ERROR	44044.4	22	2002.1		

C - control, P - pyrethroid, I - oxadiazine, N - neonicotinoid, O – organophosphate

C – kontrola, P- pyretroid, I – oksadiazyna, N – neonikotynoid, O – insektycyd fosforoorganiczny

Table 1b. P-values for pairwise comparisons with Bonferroni correction of CO_2 production ($\mu l \cdot h^{-1} \cdot g^{-1}$) in mealworms intoxicated with insecticides

Tabela 1b. Wartości P porównań wielokrotnych z poprawką Bonferroniego dla produkcji CO_2 przez mączniki eksponowane na insektycydy

	C	P	I	N	O
C	-	0.006	0.137	0.000	0.965
P	0.006	-	1.000	0.000	0.357
I	0.137	1.000	-	0.000	1.000
N	0.000	0.000	0.000	-	0.000
O	0.965	0.357	1.000	0.000	-

C - control, P - pyrethroid, I - oxadiazine, N - neonicotinoid, O - organophosphate

C – kontrola, P- pyretroid, I – oksadiazyna, N – neonikotynoid, O – insektycyd fosforoorganiczny

Discussion

The CO_2 release in insects depends on many factors, both physiological status of an individual (starvation, activity, level of dehydration), as well as external factors (temperature, water availability) (Chown and Nicolson, 2004). One of the factor that may influence patterns of CO_2 release in insects is exposure to pesticides. The presented results demonstrate that mealworm CO_2 release is increased after exposure to insecticides. High CO_2 release in insecticide-treated beetles may stem from disturbances of spiracles normal functioning. The intoxicated insects became

paralysed and their spiracles were constantly open, which was observed as the transformation from discontinuous gas exchange cycles to continuous respiration, for example in *Platynus assimilis* (Paykull, 1790) (Kivimägi et al., 2013). However, high CO_2 release is a result of increased CO_2 production, which is a consequence of energy expenditure for detoxification processes or intensified activity. It seems that very high CO_2 production after intoxication with pyrethroid and neonicotinoid result from spontaneous contractions of somatic muscles due to hyperactivity of neuromuscular system. Both insecticides induce insects hyperactivity, neonicotinoids through activation of nicotinic acetylcholine receptors, while pyrethroids through increased permeability of the membrane to sodium and its depolarization. Similar results were observed for *Leptinotarsa decemlineata* L. exposed to neem extracts (Jõgar et al., 2006) and *Tenebrio molitor* exposed to deltamethrin (Zafeiridou and Theophilidis, 2006). Moreover, neuromuscular hyperactivity after pesticide exposure occur even when it is not noticeable. Kuusik et al. (2001) reported that nonlethal doses of neem induce irregular and externally invisible contractions of abdominal and thoracic muscles in diapausing adults of Colorado potato beetle. This irregular muscular contractions lasted for one-two days and had a strong ventilating effect on tracheae. In the first stage of poisoning, due to muscular activity, the metabolic rate increased about two times comparing to control individuals (Kuusik et al., 2001).

Insects that do not respire using discontinuous gas exchange after exposure to insecticides, are more susceptible to dessication, and therefore, easier to control (Appel et al., 1997). However, Muljar et al. (2012) claimed that oxidative damage (caused by freely entering oxygen through open spiracles) could be a factor that diminishes bees fitness after pesticide exposure.

The results obtained show that with an increase in metabolic rate of mealworms exposed to insecticides, the increase in their mortality is observed. Although the mealworm mortality was not significantly higher from control group in the first 24 hours of experiment, after 72 hours significant changes were observed. It is assumed that the increase in mealworms CO_2 release after insecticide exposure is not adequate to the intensity of detoxification processes, but it is rather adequate to the intensity of stressor that acts nonspecifically (behavior disorders, convulsion that lead to paralysis). The hyperactivity observed after neonicotinoid and pyrethroid exposure led to very high CO_2 production and metabolic rate increase in mealworms. A large amount of energy was used when mealworms were intoxicated, which could not be allocated for detoxification processes, and in consequence led to mortality of intoxicated mealworms.

Mealworms were slightly susceptible to indoxacarb. It is blocker of sodium-dependent action potentials (Lapied et al., 2001), bioactivated through metabolism to decarbomethoxyllated derivative (Wing et al., 1998). A high dose of Steward 30 WG (1 μg · mealworm^{-1}) resulted in only 20 percent mortality after three days. This dose is higher than reported LD_{50} for Colorado potato beetle (50 ng· insect^{-1}) or housefly (150 ng· insect^{-1}) (Wing et al., 2000). Bioactivation of indoxacarb occur at high rates in the target organisms (Lepidoptera species), while nontarget organisms break down indoxacarb via metabolites other than decarbomethoxyllated derivative (Wing et al., 1998). However, indoxacard intoxicated mealworms in presented experiments became paralysed after 24 hours from intoxication. The 50 percent mortality was observed after eight days, while after eleven days all exposed mealworms were

found dead. This corresponds with slower conversion rate of indoxacarb in nontarget insect, such as mealworm.

Conclusions

The results obtained suggest a relationship between mortality of mealworms exposed to insecticides and their metabolic rate. This imply that high CO_2 release is not adequate to the intensity of detoxification processes. In such case intensive detoxification processes should lead to recovery from the poisoning. However, the mortality of intoxicated insects was observed, and it was the highest in mealworms that showed the highest metabolic rate after intoxication.

Acknowledgements

This work was supported by statutory funds of Faculty of Biology and Environment Protection Nicolaus Copernicus University [no. 182/2016/1146].

References

Appel, A. G., Vogt, J. T., Shelton, T. G. (1997) Respiration patterns play key role in pest biology. Highlights of Agricultural Research, 44 (4), 20-21.

Baas, J., Kooijman, S. A. L. M. (2015) Sensitivity of animals to chemical compounds links to metabolic rate. Ecotoxicology, 24 (3), 657-663. DOI: 10.1007/s10646-014-1413-5

Bednarska, A. J., Stachowicz, I. (2013) Costs of living in metal polluted areas: respiration rate of the ground beetle *Pterostichus oblongopunctatus* from two gradients of metal pollution. Ecotoxicology, 22 (1), 118-124. DOI: 10.1007/s10646-012-1008-y

Chown, S. L., Nicholson, S. W. (2004) Insect physiological ecology. Mechanisms and patterns. New York: Oxford University Press.

Goñalons, C. M., Farina, W. M. (2015) Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. PLOS One, 10 (10), e0140814. DOI: 10.1371/journal.pone.0140814

Harak, M., Lamprecht, I., Kuusik, A., Hiiesaar, K., Metspalu, L., Tartes, U. (1999) Calorimetric investigations of insect metabolism and development under the influence of a toxic plant extract. Thermochimica Acta, 333 (1), 39-48.DOI: 10.1016/S0040-6031(99)00093-3

Hostetler, M. E., Anderson, J. F., Lanciani, C. A. (1994) Pesticide resistance and metabolic rate in German cockroaches (Dictyoptera: Blattellidae). Florida Entomologist, 77 (2), 288-290.

Jõgar, K., Kuusik, A., Metspalu, L., Hiiesaar, K., Luik, A., Grishakova, M. (2006) Results of treatment with natural insecticidal substances on the development and physiological state of insects. Agronomy Research, 4, 203-210.

Karise, R., Mänd, M. (2015) Recent insight into sublethal effects of pesticides on insect respiratory physiology. Open Acess Insect Physiology, 5, 31-39. DOI: https://dx.doi.org/10.2147/OAIP.S68870

Kivimägi, I, Kuusik, A., Ploomi, A., Metspalu, L., Jõgar, K., Williams, I., Sibul, I., Hiiesaar, K., Luik, A., Mänd, M. (2013) Gas exchange patterns in *Platynus assimilis* (Coleoptera: Carabidae): Respiratory failure induced by a pyrethroid. European Journal of Entomology, 110 (1), 47-54. DOI: 10.14411/eje.2013.006

Kuusik, A., Metspalu, L., Hiiesar, K., Hermann, P. (2001) Neuromuscular and respiratory response of Colorado potato beetle *Leptinotarsa decemlineata* (Say) adults on the treatments with neem preparation. In: Metspalu, L., Mitt, S., Proceedings of the international workshop: Practice oriented results on the use of plant extracts and pheromones in pest control, Tartu, Estonia, 24-25 January 2001, Tartu, Estonia: Institute of Plant Protection, Estonian Agricultural University.

Lapied, B., Grolleau, F., Sattelle, D. B. (2001) Indoxacarb, an oxadiazine insecticide, blocks insect neuronal sodium channels. British Journal of Pharmacology, 132 (2), 587-595. DOI: 10.1038/sj.bjp.0703853

Lukasik, P., Laskowski, J. (2007) Increased respiration rate as a result of adaptation to copper in confused flour beetle, *Tribolium confusum* Jacquelin du Val. Bulletin of Environmental Contamination and Toxicology, 79 (3), 311-314. DOI: 10.1007/s00128-007-9227-2

Maryański, M., Kramarz, P., Laskowski, R., Niklińska, M. (2002) Decreased energetic reserves, morphological changes and accumulation of metals in carabid beetles (*Poecilus cupreus* L.) exposed to zinc- or cadmium-contaminated food. Ecotoxicology, 11 (2), 127-139. DOI: 10.1023/A:1014425113481

Muljar, R., Karise, R., Viik, E., Kuusik, A., Wiliams, I., Metspalu, L., Hiiesar, K., Must, A., Luik, A., Mänd, M. (2012) Effects of Fastac 50 EC on bumble bee Bombus terrestris L. respiration: DGE disappearance does not lead to increasing water loss. Journal of Insect Physiology, 58 (11), 1469-1476. DOI: 10.1016/j.jinsphys.2012.08.014

Piechowicz, B. (2006) Wpływ insektycydów modyfikujących funkcjonowanie kanałów sodowych na owady o odmiennej genezie potencjału czynnościowego. Ph.D. dissertation. Nicolaus Copernicus University, Toruń.

Spurgeon, D. J., Hopkin, S. P. (1996) Effects of metal-contaminated soils on the growth, sexual development, and early cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. Ecotoxicology and Environmental Safety, 35 (1), 86-95. DOI: 10.1006/eesa.1996.0085

Wing, K. D., Sacher, M., Kagay, Y., Tsurubuchi, Y., Mulderig, L., Connair, M., Schnee, M. (2000) Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Protection, 19 (8-10), 537-545. DOI: 10.1016/S0261-2194(00)00070-3

Wing, K. D., Schnee, M. E., Sacher, M., Connair, M. (1998) A novel oxadiazine insecticide is bioactivated in Lepidopteran larvae. Archives of Insect Biochemistry and Physiology, 37 (1), 91-103. DOI: 10.1002/(SICI)1520-6327(1998)37:1<91::AID-ARCH11>3.0.CO;2-5

Zafeiridou, G., Theophilidis, G. (2006) A simple method for monitoring the respiratory rhythm in intact insects and assessing the neurotoxicity of insecticides. Pesticides Biochemistry and Physiology, 86 (3), 211-217. DOI: 10.1016/j.pestbp.2006.05.001

Zheng, H., Li, A. Y., Fielden, L. J., Liu, J., Seshu, J., Pérez de León, A. A. (2013) Effects of permethrin and amitraz on gas exchange and water loss in unfed adult females of *Amblyomma americanum* (Acari: Ixodidae). Pesticides Biochemistry and Physiology, 107 (2), 153-159. DOI: 10.1016/j.pestbp.2012.12.004