Behavioural and Metabolic Reactions of Common Predatory Carabid Beetle to Thiamethoxam Intoxication

Lara Ivanković Tatalović
Department of Biology, Faculty of Science, University of Zagreb, 10000 Zagreb
Tomislav Mašek
Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb
Lucija Šerić Jelaska (slucija@biol.pmf.hr)
Department of Biology, Faculty of Science, University of Zagreb, 10000 Zagreb

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Abstract

Carabids (Coleoptera: Carabidae) are abundant predators in ecosystems that act as agents in biocontrol against pest species in agroecosystems and forestry. Studies have shown that exposure to pesticides, including neonicotinoids, can cause lethal and sub-lethal effects on their behaviour and physiology, which can impact predation efficiency. Here we test the impact of thiamethoxam, among the most used neonicotinoids on the feeding rate, locomotion, metabolomics, and oxidative stress level in a predatory carabid, *Abax parallelus*. Beetles were exposed to increasing concentrations of thiamethoxam by dipping method, and left to feed overnight. The mass of the consumed food per body weight was calculated for each beetle. The locomotor ability was observed for 48 hours after the treatment. Metabolomics profile and superoxide dismutase (SOD) levels in their tissue were scanned. The results showed that individuals treated with higher concentrations (20 and 40 mg/L) had significantly lower feeding rates and a higher share of intoxicated and moribund individuals. Feeding rate and locomotion did not differ significantly between control and groups treated with lower concentrations of thiamethoxam. There are statistically significant differences in concentrations of some metabolites between treated individuals and control group, primary in succinate and d-glucose, indicating a disruption in energy production. On the other hand, there is no statistically significant differences in SOD activity among the groups. To conclude, short-term exposure to thiamethoxam can result in negative sub-lethal effects in predatory activity, behaviour and energy budget, while the effects of long-term exposure to lower doses require further research.

Introduction

Neonicotinoids are the most widely used insecticides in the world (Sparks 2013). Their use affects non-target invertebrate and vertebrate species of agroecosystem communities (Hallmann et al. 2014; Douglas et al. 2015). Furthermore, they have been found to spread to surface waters and negatively affect the macroinvertebrate communities (Van Dijk et al. 2013). Neonicotinoids are nicotinic acetylcholine receptor agonists that bind to nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects. Lower concentrations of neonicotinoids cause nervous stimulation, while higher concentrations result in paralysis and death (Goulson, 2013). Abnormal clean food consumption rate (CFCR) and hypoactivity have been noted in other predatory carabids (Tooming et al. 2017) after being treated with thiamethoxam. Sublethal effects of pesticides on insects can be expressed in metabolism and at a cellular level in the form of oxidative stress (Plavšin et al. 2015; Cook 2019). Oxidative stress is defined as an imbalance between the organism's production of reactive oxygen species (ROS) and their elimination, which can result in damage of biomolecules that are necessary for organisms' normal function (Rahman et al. 2006). It is established that the efficacy of some insecticides is based on the oxidative imbalance that they cause in insects (Kolawole et al. 2014). Superoxide dismutase (SOD) is one of the enzymes in insects that serves as an antioxidant defense by transforming damaging superoxide anions (O2-) into two less damaging species: oxygen (O2) and hydrogen peroxide (H2O2) (Plavšin et al. 2015).

Carabid beetles are among the most important groups of beneficial arthropods in the agroecosystem food chain, acting as agents in natural pest and weed control (Lundgren, 2009, Bohan et al. 2011), by feeding on slugs (Symondson 1997; Hatteland et al. 2010), moths (Suenaga and Hamahura 1998), aphids (Bryan and Wratten 1984) dipteran larvae (Kromp 1999), and weed seed (Saska 2004; Bohan et al. 2011). They are species-rich, easy to identify, and sensitive to anthropogenic changes, which gives them bioindication value (Kromp 1999). Field and laboratory tests show that exposure to pesticides can cause lethal and sub-lethal effects in behaviour and physiology (Mauchline et al. 2004; Desneux et al. 2007; Tooming et al. 2017), and on the population level of non-target arthropods, including the Carabid beetles. They can be exposed to neonicotinoids through contact with residues via body surface (Yao et al. 2015), spray droplets (Kunkel et al. 2001), and also through feeding on a contaminated food source (Prasifka et al. 2008). Mullin et al. (2010) demonstrated that exposure to field doses of either imidacloprid, thiamethoxam, or clothianidin via corn seedlings results in nearly 100% mortality over four days.

Every organism has a limited energy budget (Hoffman and Parsons1989) that is distributed on biomass production, reproduction, and respiration. Additional detoxification that carabids have to conduct after being exposed to toxicants such as neonicotinoids is energetically costly (Calow 1991). Tooming et al. (2007) noted hypoactivity and reduction in food consumption and alteration in the behaviour of carabid *Platynus assimilis.* Several earlier studies (Elzen et al. 1990; Pisa et al. 2015; Yao et al. 2015) described how these sublethal effects can have a negative impact on the efficiency of predators in pest and weed control. However, the comprehensive research on behavioural and metabolic impacts of short-term neonicotinoid intoxication in carabid beetles is still lacking. Even though Thiamethoxam was banned for outdoor use in EU in 2018, 15 EU countries have granted emergency authorizations for products containing thiamethoxam since then, making further studies of its effect on natural predators necessary (HC Deb, 16 February 2022, cW).

In this study, we aim to assay the sublethal effects of short-term thiamethoxam exposure on the predatory Carabid species *Abax parallelus*. This includes (i) clean food consumption rate (CFCR), (ii) locomotor activity, (iii) metabolomics, and (iv) oxidative stress directly after exposure to thiamethoxam.

Materials And Methods

Beetle sampling and rearing prior to the treatment

Beetles were caught by hand and pitfall traps in late March and during May 2020, in the edge of deciduous forests in Zagreb County, in the area far from agricultural sites and pesticide use, ensuring that experimental beetles had not previously been under long- or short-term pesticide exposure. Plastic containers with a volume of 300 mL were placed in the ground and used as pitfall traps with vinegar serving as a lure. Traps were emptied every two days and specimens of *Abax parallelus* were transferred to the laboratory for rearing until a sufficient number of the specimens were collected. Before the start of the experiment, they were kept in plastic containers with 2 cm of humid soil (Mauchline et al. 2004; Douglas et al. 2015), and fed with moistened dog food (Kunkel et al. 2001; Mauchline et al. 2004;

Douglas et al. 2015). Water mist was applied to the towelling to provide humidity and drinking fluids (Young 2008). Pieces of wood and moss were offered as shelter (Hatteland et al. 2010). Containers were kept at room temperatures near the window, so beetles experienced local outside photoperiods.

Four days before the treatment, beetles were separated into the individual plastic containers (6 cm diameter, 1.5 cm deep) with wet paper at the bottom as a source of water and humidity, and transferred to the chamber with 12h light, 4h dimmed light, and 8h dark photoperiod and 20°C (± 2°C) temperature. They were offered food *ad libidum* for 24 hours to ensure that they all enter the treatment in the same condition. After that period the food was removed and beetles were starved for the next 72 hours. On the day of the treatment, every individual was weighed and had its sex observed.

Treatment with the thiamethoxam

Beetles were exposed to thiamethoxam (Thiamethoxam, 100 MGM, Sigma-Aldrich) by conventional dipping method, thus ensuring that every beetle was treated equally and all body parts were exposed to the pesticide. First, stock solution (100mg Ai/L) was prepared and then diluted with distilled water (dH_2O) to the following concentrations: 3.9 mg/L (C1), 9.1 mg/L (C2), 20.0 mg/L (C3), and 40.0 mg/L (C4). Control group (C0) was treated with the dH_2O. Fifteen beetles per concentration were submerged in the 15 mL of the solution for 15 seconds, then returned to the clean plastic container.

Feeding trial and testing of locomotor activity

Two hours after treatment each beetle was offered two fresh blowfly larvae (Calliphoridae) and left to feed on them for twelve hours. The larvae were notched to facilitate the feeding. Food was weighed before being offered to the beetles, and right after it was removed from the container twelve hours later. Wet filter paper ensured 100% humidity in the container so no weight was lost via evaporation.

The locomotor ability of each beetle was tested by turning it on its back and observing the reaction for 10 seconds. They were then classified in the four categories: (1) Normal - normal walk, energetic and fast movement of legs, 1 min or less to turn themselves over after being placed on their backs; (2) slightly intoxicated - beetles are still reacting to a stimulus, but their movements are slower and less coordinated. They may demonstrate excessive twitching and cleaning of antennae, needed more than 1 min to turn themselves over after being placed on their backs; (3) intoxicated - extremely uncoordinated movement, walking with legs fully extended, unable to return on their legs when put on their backs; (4) dead or moribund - completely paralyzed or had only minute nerve twitches. Those beetles whose behaviour fell between these categories were given the average value of two categories. Beetle's condition was observed 4, 12, 24, and 48 hours after the treatment. After 48 hours specimens were frozen at -80°C for the metabolomics profiling.

Non-target analysis for metabolomics profiling

Metabolites were extracted using the procedure described by Pan et al. (2010). Briefly, chloroform/methanol/water (1:2:1, v/v/v) was used for the first extraction and methanol for the second

extraction. Combined supernatants (after centrifugation at 12000 x g, 4°C) were transferred into analytical vials and evaporated to dryness. Methoximation was performed using 80 µL of methoxyamine hydrochloride (15 mg/mL) (Sigma-Aldrich, St. Louis, USA) in pyridine (BDH PROLABO, UK). The resultant mixture was incubated for 16 h at room temperature. Derivatization was performed using 80 µL of N-methyl-N-trimethylsilytrifluoroacetamide (MSTFA) with 1% of Trimethylsilyl chloride (TMCS) (Sigma-Aldrich, St. Louis, USA) at 70°C for 1h. The metabolic profiling analysis was carried out on a Shimadzu single quadrupole GCMS-QP2010 gas chromatograph-mass spectrometer (Shimadzu, Kyoto, Japan). Metabolites were separated on a 30 m × 0.25 mm × 0.25 µm BPX-5 capillary column (SGE, Austin, TX, USA). The injector temperature was 250°C and high-purity helium was used as the carrier gas. The GC temperature was programmed with initial temperature at 60°C for 2 minutes, then increased to 330°C at 15°C/minute and maintained for 10 minutes. Ion source temperature was 200°C and interface temperature was 280°C. The selected mass range was set to 45–600 m/z. The identification of low molecular weight metabolites was carried out using a commercially available GC-MS Metabolite Mass Spectral Database (NIST and Wiley). The metabolites concentrations are expressed as total ion current (TIC) for each individual beetle.

Measuring SOD activity

The beetles were fed *ad libidum* and then starved for 24h to ensure they all enter the experiment in the same condition. They were treated with thiamethoxam (0, 3.9, 9.1, and 20.0 mg/L) as described in section *Treatment with the thiamethoxam* and allowed to rest for 30 minutes. Then, they were killed by quick freezing in a liquid nitrogen and preserved at -80°C. The SOD activity in beetle tissue was measured using Superoxide Dismutase Activity Assay Kit (Colorimetric) by Abcam and calculated as the inhibition activity of xanthine oxidase (XO) by SOD.

Statistical analysis

The mass of consumed food 12 hours after the treatment was divides with the mass of the beetle, to standardize the numbers. A Shapiro-Wilks test was used to test the normality of the data ($p < 0.05$), after which a nonparametric Kruskal-Wallis test was performed to analyse differences in the mass of consumed dipteran larvae between the different treatments and sexes. The same test was performed to check the difference in metabolomics and SOD activity in tissue between the groups. The changes in metabolites were calculated as a deviation of treated groups from the control group.

Results

Feeding trial

The Kruskal-Wallis tests ($H(4.71) = 42.1531$, $p < 0.0001$) showed significant differences for the mass of the consumed food per body mass based on the thiamethoxam concentration used in the treatment. The food consumption decreased with the increasing concentrations of thiamethoxam in the treatment (Fig. 1). Multiple Comparisons *p* values (2-tailed) revealed that group C3 statistically differed from groups

C1 (p = 0.0154) and C2 (p = 0.0491), and group C4 was statistically different from groups C0 (p = 0.000016), C1 (p = 0.000001), and C2 (p = 0.000006). The mean food consumption per body mass was higher in females, but not significantly different compared to male beetles (H (1.67) = 0.1615, p = 0.6878). The mean food consumption per body mass within each treatment was higher in females, but not significantly different compared to male beetles (H (1.67) = 0.1615, p = 0.6878).

Locomotor activity and mortality following thiamethoxam exposure

The signs of intoxication were visible only in groups C3 and C4, with the shortest time period for signs to appear being 12 hours (Figure). All beetles in C3 survived a 48-hour long period after treatment, while one individual was found dead in C4 (Fig. 2). Overall, 20% of C3 beetles showed signs of intoxication at one point during the observation period, while for C4 beetles that share was 46.7%. In the period of 48h after the treatment, there was one dead individual, belonging to the C4 group.

Untargeted metabolic profiling

Kruskal-Wallis test revealed that there are statistically significant differences in concentrations of metabolites between treated groups (C3 and C4) and the control group (Table 1).

Table 1
Results of Kruskal-Wallis with multiple comparison *p* values (two-tailed). Column Separated treatments depicts results when C3 and C4 are observed as separated groups and each is compared to C0, while column merged treatments depicts results when C3 and C4 are observed as one group (treated) and compared to C0 (non-treated). Statistically significant values are bolded.

	Separated treatments				**Merged treatments**		
Metabolite	H	df	*p* (C3)	*p* (C4)	H	df	*p* (C3&C4)
Aminoisobutryc acid	7.0403	2	**0.0393**	1	1.8933	1	0.1688
Cholesterole	7.2221	2	**0.0222**	0.5121	5.0402	1	**0.0247**
d-Glucose	11.508	2	**0.0021**	0.5918	6.4248	1	**0.0113**
Glutamine	15.397	2	0.0661	**0.0003**	14.182	1	**0.0001**
2-hydroxyglutaric acid	11.227	2	**0.0195**	1	0.8863	1	0.3465
L-isoleucine	7.4396	2	**0.0371**	1	1.6993	1	0.1923
L-ornithine	9.6318	2	**0.0076**	0.1451	7.9773	1	**0.0047**
L-proline	8.9987	2	**0.0378**	1	0.8863	1	0.3465
Pyroglutamic acid	8.8623	2	**0.0183**	1	2.0979	1	0.1475
Succinate	13.253	2	**0.0008**	0.3773	7.9773	1	**0.0047**
Uric acid	5.9851	2	**0.0434**	0.9414	3.5455	1	0.0597

An increase of TIC was detected in L-isoleucine, succinate, pyroglutamic acid, d-Glucose, uric acid, and cholesterol, while glutamine and aminoisobutryc acid showed a decrease in TIC in both groups. The largest difference was in succinate, whose concentration was 439% higher in the C3 group and 47% higher in the C4 group (Fig. 3) than in the control group. Several metabolites showed opposite changes between the groups. L-proline and L-ornithine decreased in C3 but increased in C4 (overall decrease in treated groups), and the opposite occurred in 2-hydroxyglutaric acid concentration (Fig. 3). The full list of scanned metabolites and their TICs can be found in Supplementary Table S1.

Superoxide Dismutase activity after thiamethoxam exposure

Kruskall-wallis test (H (3, N = 51) = 3.601957) revealed that there is no statistically significant difference ($p = 0.3078$) in SOD activity between groups C0, C1, C2, and C3. SOD activity was higher in C0 groups.

Discussion

The effect of thiamethoxam exposure on CFCR

The CFCR decreased significantly in C3 and C4 treatment groups, shortly following thiamethoxam application, while lower doses C1 and C2 did not cause this effect. However, all beetles fed, proving that thiamethoxam did not render them incapable to recognise and consume the food. Furthermore, reduced feeding occurred in the first 14h after the treatment. This behavioural change following thiamethoxam intoxication was noted by Tooming et al. (2017) in predatory carabid *Platynus assimilis*, where significant reduction in feeding occurred in beetles treated at high doses on the first day, and even at doses ten to a hundred-fold lower on the next day. Systemic exposure to thiamethoxam caused biological agent *Serangium japonicum* to reduce feeding on the pest eggs during 24h exposure period (Yao et al. 2015). Martinou et al. (2014) reported that sublethal effects of thiacloprid on a common generalist predator in Mediterranean agro-ecosystems, *Macrolophus pygmaeus* Rambur, included an increase in resting and preening time and, decreased plant-feeding, and a significantly reduced predation rate. In nature, adequate feeding is an important factor in survival, growth, and fecundity (Knapp and Uhnavá 2014), and toxic stress resulting in decreased feeding could lead to a reduced abundance of beneficial insects.

Locomotor activity and mortality following thiamethoxam exposure

Between 12- and 48-hours post-treatment, beetles were showing visible signs of intoxication in their locomotion, but only one individual was dead after 48h. Thiamethoxam is a neurotoxin that bind to nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects (Goulson 2013), causing loss of coordination and orientation, paralysis and death (Jensen et al. 1997; Desneux et al. 2007; Moser and Obrycki 2009). In our study, beetles demonstrated adverse effects in locomotion at doses 20 and 40 mg/L, after 12h. Tooming et al. (2017) noted that when higher doses of thiamethoxam are administered orally, carabids display hyperactivity shortly after the treatment, and day after the treatment, all beetles were in the state of hypoactivity, regardless of the dose. Similar results on insect predators were observed for other neurotoxic pesticides such as pyrethroids (Prasifka et al. 2008) and organophosphates (Singh et al. 2001). In our study, thiamethoxam caused neurotoxic sublethal effects including impaired walking, inability to turn on the legs after being flipped on the back, and excessive grooming. Carabid beetle *Harpalus pennsylvanicus* showed the same effects after being exposed to neonicotinoid imidacloprid, through consumption of contaminated food or direct contact with spray, which left them vulnerable to other predators (Kunkel et al. 2001). Field application rate of dimethoate caused a 2.5% mortality rate of *Pterostichus melas italicus* adults and in 7.5% of specimens a reduction of normal activity (knocked out) after 48h exposure via substrate in containers (Giglio et al. 2011). Predatory carabid beetles are active hunters, meaning that neonicotinoid intoxication can easily disable them both in capturing prey and avoiding predators. Hypoactivity as a result to neonicotinoid poisoning could explain lower carabid activity density in field where neonicotinoid seed treatment is used (Douglas et al. 2014).

Untargeted metabolic profiling

The full list of scanned metabolites and their concentrations can be found in Supplementary table 1. Here we discuss metabolites with statistical changes in treated groups compared to the control and their connection to thiamethoxam intoxication. The highest change was in succinate levels (198% increase), following d-glucose (112% increase). Succinate is involved in the formation and elimination of reactive oxygen species. Leakage from the mitochondria requires succinate overproduction or underconsumption. Mutations in SDH, hypoxia or energetic misbalance are all linked to succinate accumulation (Tretter et al. 2016, Pekny et al. 2018). This may indicate the increase in oxidative stress, as neonicotinoids are proven to cause it (Yan et al. 2021). Furthermore, increased glutamine metabolism also leads to the accumulation of succinate, and we detected decreased glutamine concentrations in C3 and C4 groups. In the cytosol, glutamate is produced when glutamine donates its γ (amide) nitrogen for the synthesis of nucleotides and hexosamines. Cytosolic glutamate is critical for maintaining redox homeostasis and protecting cells against oxidative stress through the production of glutathione (GSH) (Yu 2008; Zhang et al. 2017). Neonicotinoids clothianidin and imidacloprid altered important aspects of nutritional and metabolic physiology in honey bees, where high-dose imidacloprid exposure resulted in bees having depressed metabolic rate (Derecka et al. 2013; Cook 2019). The lowered metabolic rate could explain both the decrease in food consumption and higher glucose concentrations. Succinate dehydrogenase, an enzyme that catalyses the oxidation of succinate into fumarate in the Krebs cycle, was significantly lowered in silkworms following treatment with organofosfate, suggesting a decrease in respiration rate at the tissue level in silkworms due to toxicity induced by these insecticides (Nath 2002). Another metabolite whose concentrations increased in treated beetles is uric acid. Protein depletion in tissues following insecticide exposure was noted in the earlier studies (Srinivas 1986; Jeschke et al. 2016). This may provide intermediates to the Krebs cycle, by retaining free amino acid content in hemolymph and compensating for osmoregulatory problems during insecticide intoxication (Srinivas 1986). Jeschke et al. (2016) concluded that the amino acids derived from protein breakdown were largely deaminated producing ammonia that was detoxified by conversion to uric acid. Furthermore, uric acid has a positive role in resistance against oxidative stress as demonstrated in the research conducted on thermites (Tasaki et al. 2017), where the accumulation of uric acid, as well as externally administered uric acid, considerably aided termite survival under highly oxidative conditions. Lastly, Etebari et al. (2007) noted an association between decreased protein levels in silkworm larvae after pesticide (pyriproxyfen) application and increased glucose levels in the hemolymph. It was hypothesised that it may be due to the enhancement of trehalase activity in silkworm haemolymph because it was reported that trehalase activity was enhanced in the midgut of silkworms treated with insecticides. Cholesterol, whose concentrations in tissue also increased in post-treatment, is the dominant sterol found in most insects (Behmer and Nes 2003). Etebari et al. (2007) found that changes in cholesterol were similar to uric acid and glucose, whereas it showed a significant increase in treatments after 120 h. In this study, the increase in cholesterol, uric acid, and glucose levels was more prominent at the lower doses of pesticide application. The increase in cholesterol following insecticide intoxication was noted in mammals as well (Ozsahin et al. 2014).

The changes in metabolites differed between the C3 and C4 groups in our experiment, and contrary to the expectation, they were often more pronounced in C3. Cook (2019) demonstrated that the effect of neonicotinoids on bee metabolism is dose dependant. This arises from the extent to which the compounds were perceived and detoxified, from the impact that levels of affected compounds have on secondary molecular pathways, specifically those related to the stress response.

Superoxide Dismutase activity after thiamethoxam exposure

Kruskal-Wallis test revealed no significant differences in SOD activity in beetle tissue following thiamethoxam application. However, trend showed slightly higher activity in the control group compared to the three treated groups, contrary to our hypothesis. Plavšin et al. (2015) demonstrated that the application of neurotoxic pesticides pirimiphos-methyl and deltamethrin can in fact significantly suppress total antioxidative capacity in beetle Tribolium castaneum, as they had an inhibitory effect on SOD molecules. This was due to the pesticide's interference with adipokinetic hormone (AKH) which plays a role in insect defence responses against oxidative stress. Večeřa et al. (2007) measured SOD activity in *Spodoptera littoralis* larvae fed on an artificial diet containing tannic acid which induces the formation of ROS, but no change in SOD activity was recorded. Song et al. (2017) tested the impact of three commonly used pesticides (chlorpyrifos, trifluralin, and chlorothalonil) on SOD and glutathione S-transferase (GST) activities in *Daphnia magna*, and found that both SOD activity and GST activity were induced at low concentration, but inhibited at high concentration. Furthermore, GST activity was more sensitive to three commonly-used pesticides than SOD activity. Thus, it is possible that while thiamethoxam did induce oxidative stress in the beetles, their antioxidative response (SOD activity) was suppressed, which would explain the lower SOD activity in the treated beetles. The high intragroup variability could also indicate that some other factors, such as age, soundness, and/or environmental conditions (Simone-Finstrom et al. 2016) prior to the capture had a higher impact on SOD activity than short term thiamethoxam exposure.

To conclude, short-term exposure to higher doses of thiamethoxams negatively affected the CFCR and locomotor abilities of predatory carabid *Abax parallelus*, which could have detrimental impact on both their survival and predatory abilities in nature. However, mortality was low even 48h after the treatment. Intoxication with thiamethoxam appears to increase the protein catabolism in insects, indicated by various changes in free amino acids and significantly elevated uric acid concentrations. On the other hand, opposite was noted for carbohydrate metabolism, as the glucose and succinate increased in the tissue. Mild SOD activity in treated groups could be due to the potential inhibiting effect of the thiamethoxam, or the sensibility of beetles to other inner and/or outer stress agents. Changes in glutamine, succinate, uric acid (metabolites which play a role in anti-oxidative response), together with earlier studies on oxidative stress caused by insecticides, imply that thiamethoxam did cause oxidative damage in the beetle, but some other biomarker than SOD may be more indicative. All observed effects are dose-dependent.

Declarations

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Author contribution: Lara Ivanković Tatalović and Lucija Šerić Jelaska contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Lara Ivanković Tatalović and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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Figures

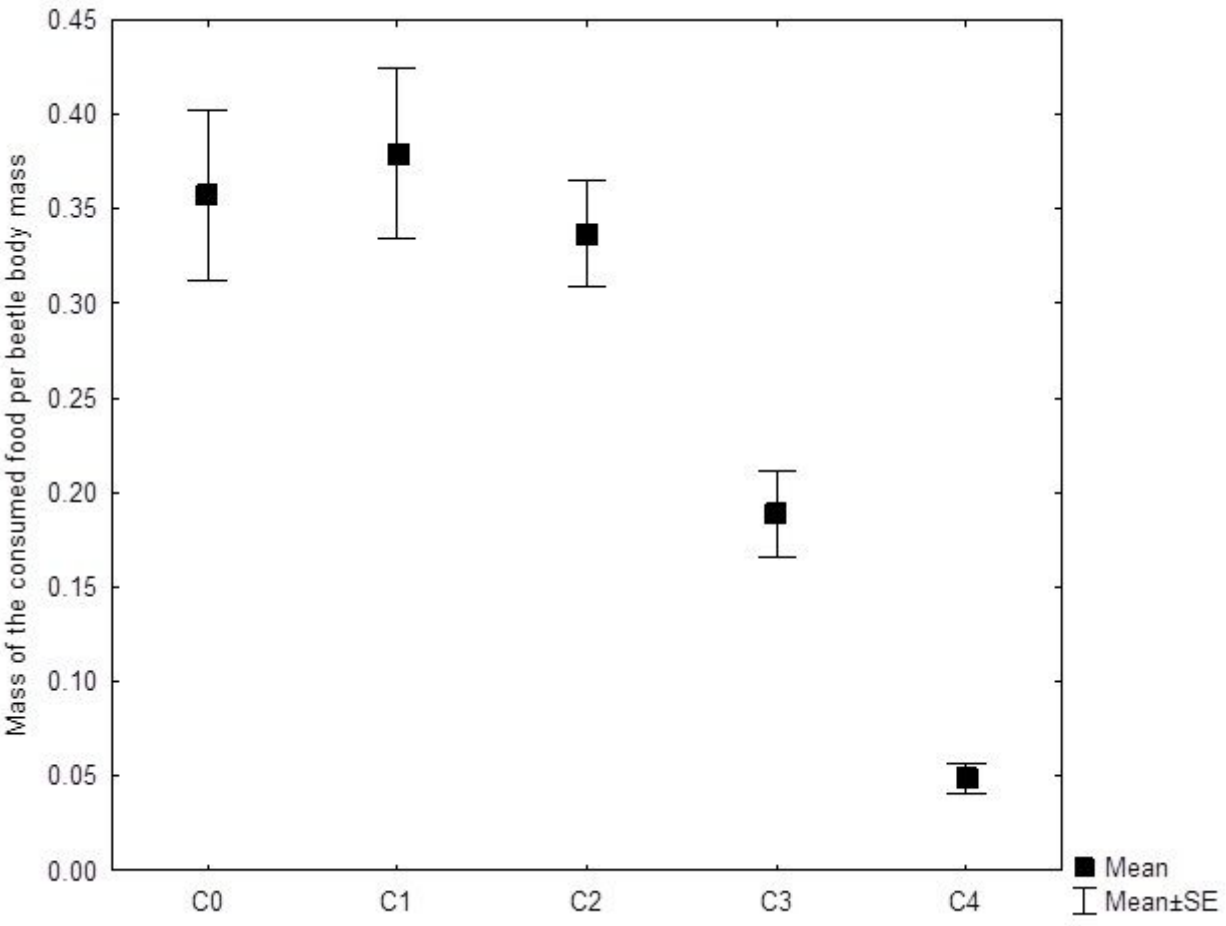

Figure 1

Mean of consumed food (g) per beetle body mass (g) for each treatment. Vertical bars denote standard errors.

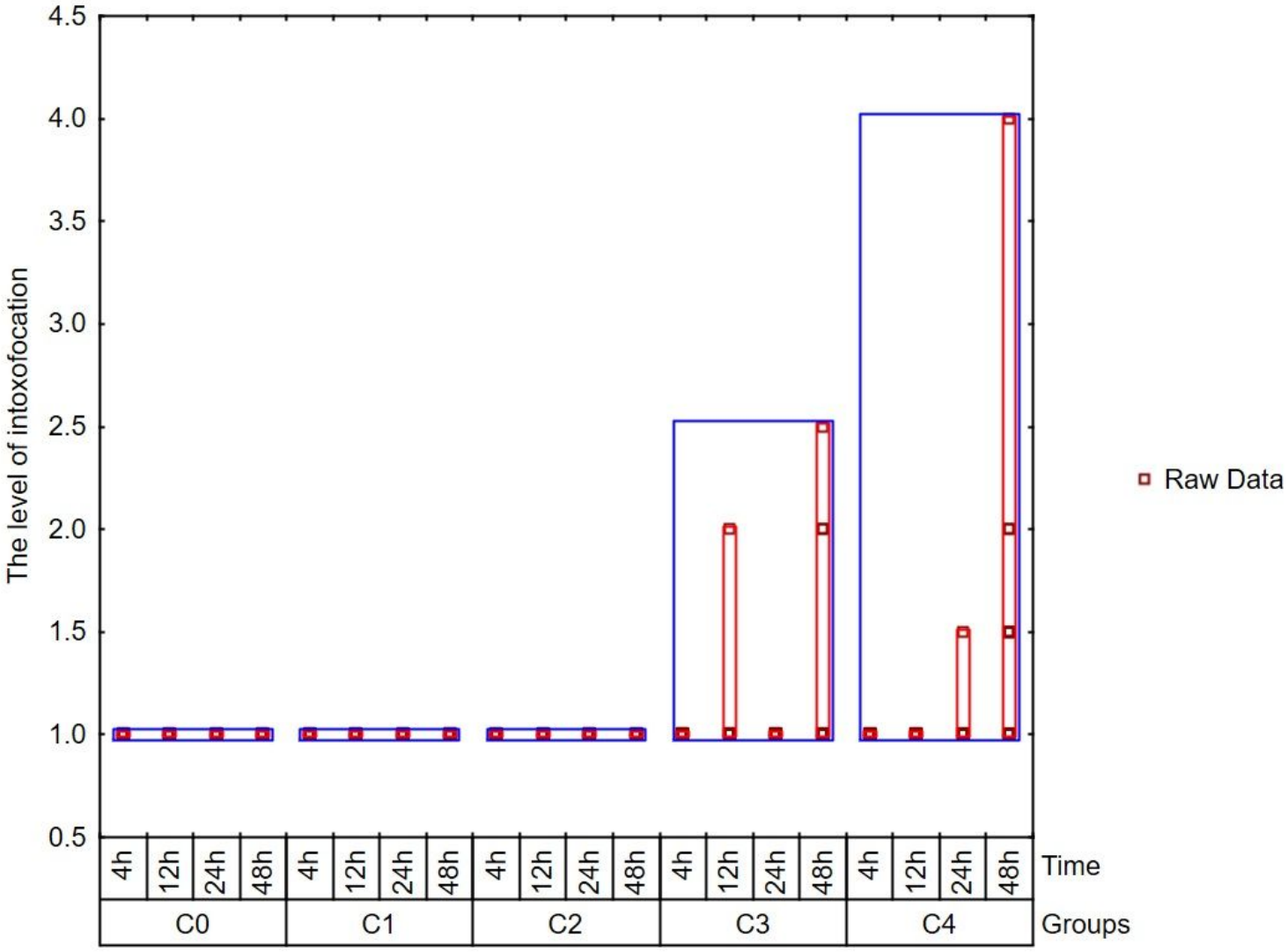

Figure 2

The number of beetles in each group expressing the signs of intoxication in their locomotor activity at some point during the 48h period after the end of the treatment.

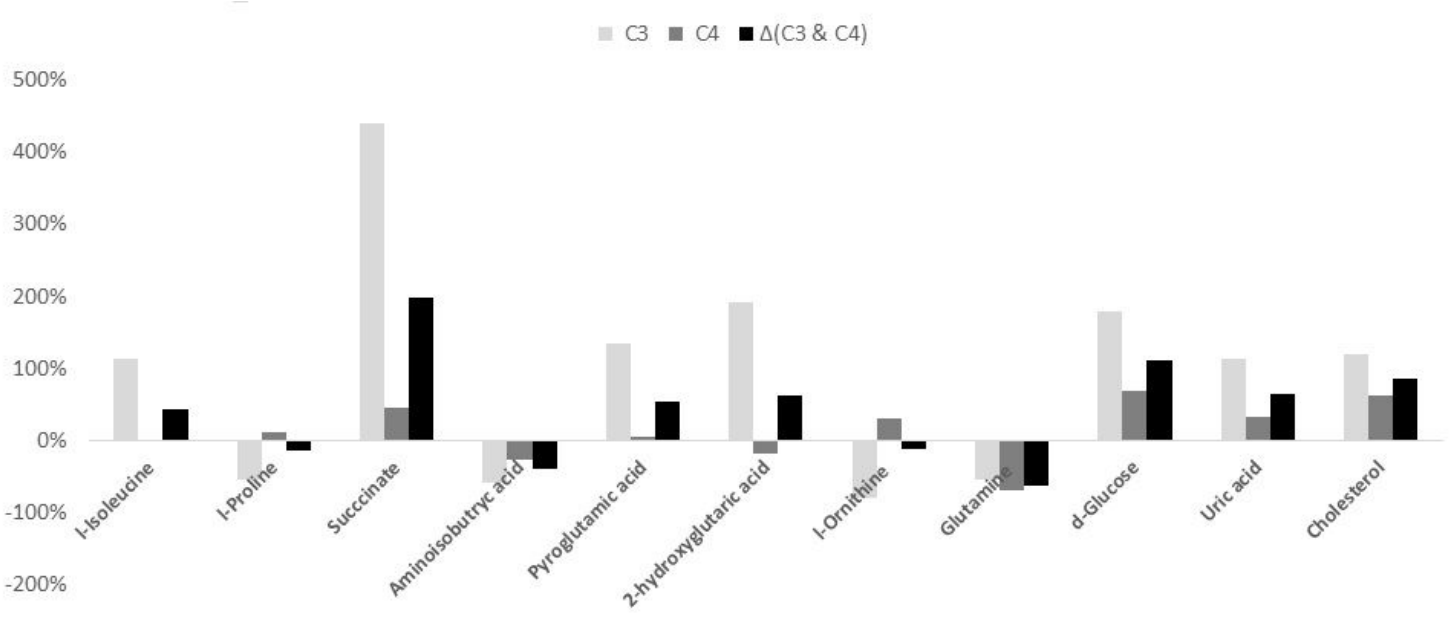

Figure 3

Deviation (%) of average TIC of different metabolites in C3 and C4 (individual group and combined) from C0. Only metabolites with statistically significant deviations are depicted.

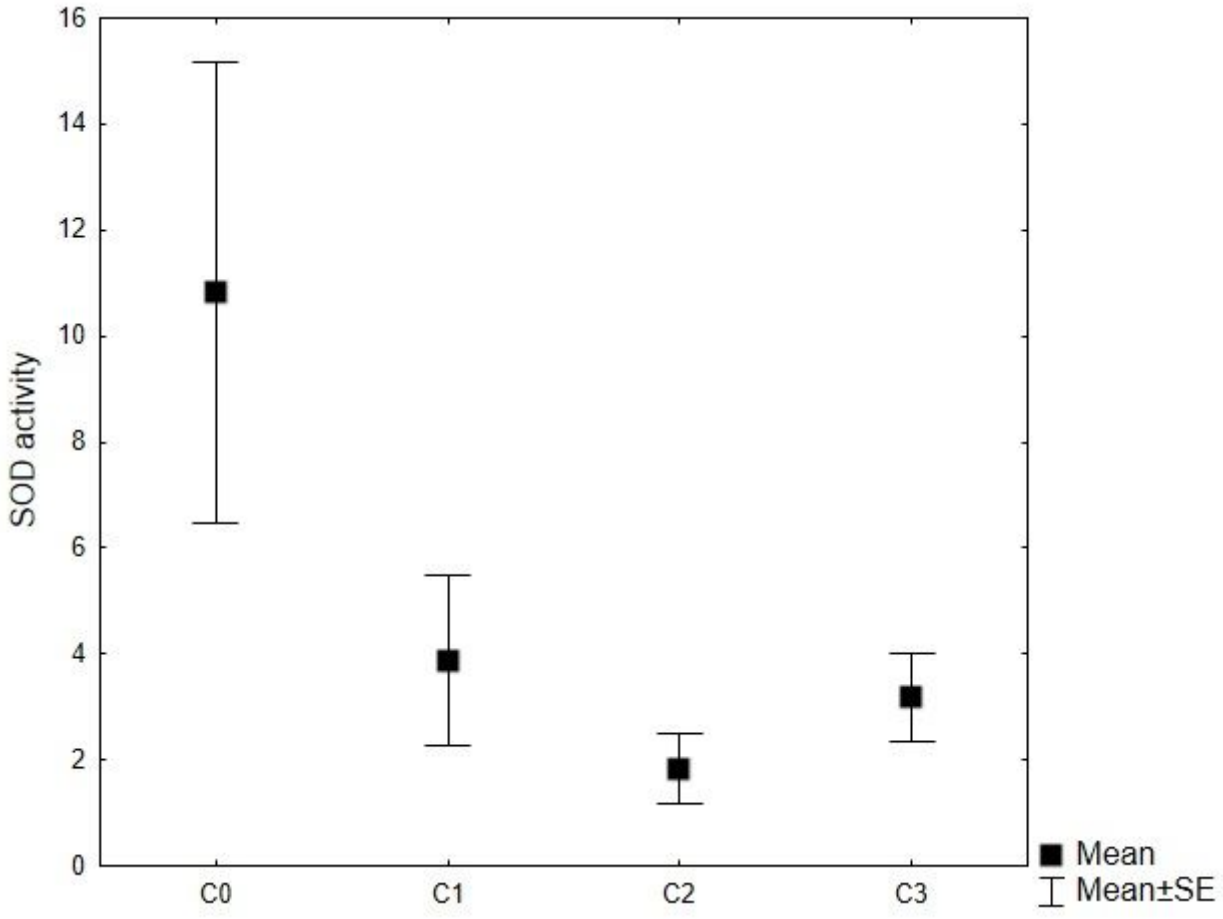

Figure 4

SOD activity in carabid beetle tissue depending on thiamethoxam treatment, calculated as the inhibition activity of xanthine oxidase (XO) by SOD. Vertical bars denote standard errors.

Supplementary Files

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- Supplementarytable1.docx