Influence of Neonicotinoids on Selected Characteristics of the Earthworm *Dendrobaena Veneta* (Rosa) in Laboratory Conditions

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**ABSTRACT**

In the laboratory experiment, the effect of neonicotinoid Nuprid 200SC at the dose recommended by the manufacturer on the dynamics of population of earthworms *Dendrobaena veneta* was assessed. The studies were conducted on mature *D. veneta* specimens, in 4 replications of beddings (control, insecticide) in the climatic chamber. Condition of the population was checked five times, using a method of manual segregation of beddings. Insecticide limited the increase in number and biomass of *D. veneta* population (both the whole population, mature specimens and immature earthworms), but also restricted its reproduction (it decreased the number of cocoons laid by earthworms (p<0.05)).

**Keywords:** ecotoxicology, *Dendrobena veneta* Rosa, imidacloprid Nuprid 200 SC

**INTRODUCTION**

Nowadays, neonicotinoids (NEO) are the most well-known group of insecticides in the world and they constitute approximately 25% of the global insecticide market, while they are mainly applied as an additive to seed treatments (Renaud et al. 2018). They are used in 120 countries to control agricultural pests, mainly due to a wide spectrum of their application. Most of these insecticides were marketed in 1995–2002 (Hladik et al. 2018). They affect the central nervous system of target organisms (whiteflies, aphids, some microlepidoptera), but also the so-called nontarget organisms (e.g. pollinators) (Bonmatin et al. 2014; Hladik et al. 2018; Rayman et al. 2018). Up to now, many papers have been focused on the effect of neonicotinoids on bees, however, there has been more evidence indicating that low neonicotinoid level persisting in soil environment has a negative effect on other organisms (Wang et al. 2015 a,b; Eng et al. 2017; Wood & Goulson 2017).

Botías et al. (2016) demonstrated that the remnants of these compounds in soil were discovered more than three years after the application of seed treatments in agricultural crops. Thus, they should be applied carefully and in the situations when there are no other possibilities (Hladik et al. 2018).

The influence of xenobiotics on earthworm populations, even at low doses recommended by the manufacturers as safe, can be very different (Alves et al. 2013; Uhl et al. 2015; Garczyńska et al. 2018). In relation to this, there is a need for a broader assessment of the effect of NEO on soil fauna under varying environmental conditions.

The aim of the present study was to assess the effect of the neonicotinoid Nuprid 200 SC (at the dose described by the manufacturer as safe) on the characteristics of the population of *Dendrobaena veneta* earthworms Rosa 1893.

**MATERIAL AND METHODS**

**Earthworms- test organisms**

Earthworms (Oligochaeta; Lumbricidae) *Dendrobaena veneta* (Rosa 1893) (Figs 1, 2) originating from the conservative breeding in the Department of Biological Basis of
Agriculture and Environmental Education of the University of Rzeszów, Poland, were used in the experiment. Specimens that had been subjected to prior acclimation in garden soil were tested.

**Applied formulation**

*Nuprid 200SC* (Nufarm Polska sp. z o.o.) is an insecticide belonging to the neonicotinoid group whose active substance is imidacloprid (17.8%): 1-[(6-chloro-3-pyridynyl)methyl]-N-nitro-2-imidazolidinoamine. The formulation was applied once, at the dose recommended by the manufacturer (as the equivalent of the concentration of 0.4 ml·m\(^{-2}\)).

**Course of the experiment**

The studies were carried out on a balanced biomass of mature *D. veneta* specimens. Ten earthworm specimens were placed into each plastic container (of size of 20×15×10 cm) filled with garden soil (Table 1). In order to restrict the number of enchytraeids competing with earthworms, vermicomposted kitchen waste were mixed with cellulose (in 2:1 ratio) (Kostecka 2000). The waste was provided regularly, according to the needs of the bred earthworms, and were put into large-size nylon mesh (mesh size: 5 mm).

The experiment was conducted from March to August 2017 in the climatic chamber (20±0.5°C, 24L; with soil humidity of approximately 70%) (OECD 2004). The condition of earthworm population was checked regularly, once a month, using a method of manual segregation of beddings (e.g. Pelosi et al. 2009).

**Statistical analysis**

The obtained results were presented as arithmetic means and standard deviations. *STATISTICA* software v. 13.1 (StatSoft) was used for statistical calculations. Normal distribution was checked using Shapiro-Wilk W test and Brown-Forsythe test was used to confirm the homogeneity of variance. To find significant differences between the data related to parameters of populations, the two-way ANOVA with the LSD test (post-hoc) were subjected (Stanisz 2006). Differences at the significance level α = 0.05 were considered statistically significant.

**RESULTS**

**The effect of insecticide Nuprid 200SC on *D. veneta* earthworms**

**The effect of insecticide on the number of earthworms**

The applied formulation had a negative effect on the number of earthworm populations (*F*=94.183, *p*<0.01). Starting from the second month of the experiment, neonicotinoid reduced the number of population (*H*=8.918; *p*<0.05) (Table 2).

The insecticide caused a reduction in the mean number of mature earthworms (*F*=78.460, *p*<0.0001) and immature specimens (*F*=63.370, *p*<0.001) (Fig. 3). The insecticide caused a reduction in the mean number of mature earthworms (*F*=78.460, *p*<0.0001) and immature specimens (*F*=63.370, *p*<0.001) (Fig. 4).

**The effect of insecticide on the biomass of earthworms**

Biomass of the earthworm population treated with insecticide was significantly lower compared to the control (*F*=178.918, *p*<0.0001) (Table 3) and the applied agent reduced the mean weight of an individual specimen (*F*=1.034, *p*<0.05) (Table 4).
Nuprid 200 SC reduced the biomass of reproducing specimens. It was gradually decreased – in July by 82%, and in August by 94% (p<0.0001) (Table 5). Insecticide also negatively affected the weight of an individual mature specimen (p<0.01) (Table 6).

Biomass of young specimens and the weight of individual young specimens were reduced by the insecticide as well (F=1.024, p<0.01) (Table 7).

**The effect of insecticide on the reproduction of D. veneta earthworms**

Insecticide Nuprid 200SC administered at the environmentally safe dose significantly modified the number and biomass of cocoons laid by D. veneta. The life strategy of D. veneta in the control group consisted in assigning the whole energy for reproduction. However, a decrease in the number of cocoons after the contact with Nuprid
Table 3. Mean biomass of *D. veneta* (specimens) (g· container⁻¹±SD) in experimental boxes depending on the presence of preparation during 5 months experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Start of the experiment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.5±0.1</td>
<td>18.1±0.8*</td>
<td>38.6±6.5*</td>
<td>48.7±0.1*</td>
<td>80.2±0.4*</td>
<td>85.8±0.8*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>5.7±0.2</td>
<td>13.8±0.9*</td>
<td>17.8±6.5*</td>
<td>6.7±0.1*</td>
<td>2.5±0.0*</td>
<td>1.43±0.0*</td>
</tr>
</tbody>
</table>

a, b – significant differences

Table 4. Mean biomass of *D. veneta* specimens (g· container⁻¹±SD) in experimental boxes depending on the presence of preparation during 5 months experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Start of the experiment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.47±0.1</td>
<td>0.42±0.8*</td>
<td>0.385±0.5*</td>
<td>0.378±0.1*</td>
<td>0.345±0.4*</td>
<td>0.323±0.2*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>0.52±0.2</td>
<td>0.47±0.9*</td>
<td>0.365±0.5*</td>
<td>0.342±0.1*</td>
<td>0.273±0.0*</td>
<td>0.267±0.0*</td>
</tr>
</tbody>
</table>

a, b – significant differences

Table 5. Mean biomass of adult of *D. veneta* (g· container⁻¹±SD) depending on the presence Nuprid 200SC during 5 months experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Start of the experiment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.5±0.1</td>
<td>8.1±1.8*</td>
<td>13.3±2.5*</td>
<td>13.7±2.1*</td>
<td>15.2±2.4*</td>
<td>16.8±2.8*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>5.7±0.2</td>
<td>9.2±1.9*</td>
<td>7.8±1.5*</td>
<td>2.7±0.1*</td>
<td>1.2±0.0*</td>
<td>0.53±0.0*</td>
</tr>
</tbody>
</table>

a, b – significant differences

Table 6. Mean mass of adult *D. veneta* (g· container⁻¹±SD) in experimental boxes depending on the presence of preparation during 5 months experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Start of the experiment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.55±0.05</td>
<td>0.55±0.10</td>
<td>0.55±0.03</td>
<td>0.52±0.06*</td>
<td>0.50±0.06*</td>
<td>0.51±0.05*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>0.51±0.01</td>
<td>0.52±0.18</td>
<td>0.42±0.05</td>
<td>0.37±0.03*</td>
<td>0.36±0.07*</td>
<td>0.33±0.07*</td>
</tr>
</tbody>
</table>

a, b – significant differences

Table 7. The effect of imidaclopride on biomass of immature and the individual weight of young *D. veneta* earthworms

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>8.1±0.2</td>
<td>10.±4.0</td>
<td>23.3±3.5</td>
<td>45.1±1.77</td>
<td>69.0±21.1*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>-</td>
<td>5.2±0.1</td>
<td>4.2±4.5</td>
<td>3.7±1.5</td>
<td>1.3±1.0</td>
<td>0.9±1.0</td>
</tr>
</tbody>
</table>

Individual mass of young earthworms (g· container⁻¹±SD) p<0.01

control

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>0.091±0.09</td>
<td>0.142±0.02</td>
<td>0.158±0.108</td>
<td>0.158±0.100</td>
<td>0.183±0.112*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>-</td>
<td>0.117±0.069</td>
<td>0.063±0.013</td>
<td>0.096±0.039</td>
<td>0.085±0.039</td>
<td>0.057±0.050*</td>
</tr>
</tbody>
</table>

* - after several months, a, b – significant differences

Table 8. Mean cocoon production (cocoons· mature ind⁻¹±SD) of *D. veneta* mature individual depending on the presence Nuprid 200SC during 5 months experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Start of the experiment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3±0.0*</td>
<td>3.9±0.4*</td>
<td>4.5±1.0*</td>
<td>3.8±2.2*</td>
<td>3.7±1.6*</td>
<td>2.5±1.9*</td>
<td>3.0±1.6*</td>
</tr>
<tr>
<td>Nuprid 200SC</td>
<td>0.43±0.1*</td>
<td>3.2±0.1*</td>
<td>2.1±1.5*</td>
<td>2.8±1.2*</td>
<td>0.8±0.0*</td>
<td>1.3±0.5*</td>
<td>1.8±0.1*</td>
</tr>
</tbody>
</table>

a, b – significant differences
200 SC amounted to (from July to August) from 66% to 98% (F=2.8023; p<0.01).

The number of cocoons produced by an individual mature earthworm was significantly higher in the control group (F= 2.063; p<0.05) (Table 8).

The presence of the insecticide limited the biomass of cocoons laid by *D. veneta* as well (F=3.9043, p<0.05) (Fig. 5).

**DISCUSSION**

Neonicotinoid insecticides are effective agents and thus they are competing with traditional insecticides. However, in order to obtain comprehensive and reliable results concerning toxicological risk arising from their application, it should be attempted to assess their effect on organisms, not only in the artificial soil test, but also in the natural environment and various soil types, what may be a complex study (Zhang et al. 2014; Mörtl et al. 2016). In the natural soil, unexpected synergistic and antagonistic reactions of neonicotinoids with other xenobiotics also have a significant effect on organisms. Up to now, little is known on the subject of potential toxicity of a mixture of neonicotinoids towards organisms in the environment as well (Hladik et al. 2018). Using insecticides in seed treatments may lead to their prolonged persistence in soil, which means that the non-target organisms, such as earthworms, may be exposed to them for a long time. Chronic exposure of soil organisms to xenobiotics poses a risk that is not assessed in the majority of toxicity studies (Basley & Goulson 2017). Meanwhile, it is known that earthworm reaction depends on the duration of their exposition to these agents, the dose and age of the animals as well as sensivity of the species (depending on the ecomorphological group it belongs to) (Gomez-Eyles et al. 2009; Wang et al. 2015a; Basley & Goulson 2017; Eng et al. 2017; Zhang et al. 2017; Hladik et al. 2018). Ecotoxicological studies, according to the recommendations of ISO and OECD mainly concern the epigeic species *E. fetida*, which has a short reproductive cycle and is relatively easy to breed (OECD 2004; Zhang et al. 2014; Wang et al. 2015a). According to Lukkari et al. (2005), *E. fetida* has greater tolerance to xenobiotics than the other species, so it would be indicated to apply stenotopic (with a narrow tolerance range) earthworms from three ecological groups, e.g. epigeic species – *Dendrobena octaedra*, endogeic species *Aporrectodea caliginosa* or anecic species – *Lumbricus terrestris* in ecotoxicological studies.

In the present paper, studies on the effect of imidacloprid (in the form of a formulation with trade name Nuprid 200 S.C.) on *D. veneta* species, which is more sensitive to the effect of xenobiotics than *E. fetida*, were conducted (Podolak-Machowska 2016). Administration of a single dose recommended by the manufacturer had a negative effect. A decrease in the number of all the analysed age groups and cocoons was observed. In the presence of Nuprid 200 SC, health condition of mature specimens decreased and due to this, earthworms laid cocoons only during two months following the application of the agent, whereas starting from the third month, they gradually transformed the energy obtained from food into growth. Raymann et al. (2018) claim that after the exposure to imidacloprid, earthworms become less active and feed on less intensively. This is confirmed by the observations from the present experiment. According to Eng et al. (2017), after the addition of imidacloprid, maturation of earthworms is delayed by approximately 3 weeks.

It was demonstrated (Johnston et al. 2014) that earthworms exposed to a chemical stressor are able
to modify their energy management and decrease or increase (depending on the physiological condition) the energy expenditure on detoxication. This should result in greater chances of survival in difficult conditions, but at the same time, e.g. restricts cocoon production. Sometimes a reverse situation occurs and the organisms allocate the excess energy to the production of biomass or cocoons. They may also show mixed strategies; e.g. at first, they spend a lot of energy on detoxication and then they allocate it to the production of, e.g. biomass. Garczyńska et al. (2018) have already conducted a study on the effect on detoxication and then they allocate it to the energy expenditure on detoxication. This should increase (depending on the physiological condition) the chances of survival in difficult conditions, but at the same time, e.g. restricts cocoon laying by D. veneta. The great decrease in the number of earthworms was observed in pure soil. Additionally, the authors found that the presence of clothianidin in the bedding for the first two months had the effect on weaker food intake by L. terrestris. A similar situation in the natural environment may cause a decrease in the decomposition rate of leaves treated with insecticides. The effect may be stronger if the earthworms come closer to the seeds treated with neonicotinoids.

Investigating the changes in body weight of earthworms under the effect of a stress factor e.g. insecticide, is a very good method to assess the influence of the said agent on the populations of these animals (Olvera-Velona et al. 2008). Dittbrenner et al. (2011) carried out an experiment in which they studied the effect of imidacloprid on body weight of E. fetida. The most pronounced decrease (by 37%) was noted after 7 days of the experiment, in the contact with the dose of 2.0 mg·kg⁻¹ of imidacloprid. However, after 14 days of using, the doses of 0.66 mg·kg⁻¹ and 2 mg·kg⁻¹ caused 100% mortality of the studied E. fetida specimens. A decrease in body weight of E. fetida or E. andreii earthworms in response to imidacloprid was also confirmed by Fernández-Gómez et al. (2009) and Alves et al. (2013).

The present study indicates the toxicity of Nu-prid 200SC at the dose recommended by the manufacturer as environmentally safe. Since, the applied agent had the effect on a decrease in the mean individual body weight and the mean sum of biomass of D. veneta from all the age groups.

It is worth to mention the other neonicotinoid insecticide – paichongding (IPP) that is widely used in China. Zhang et al. (2017) investigated the effect of IPP on acute toxicity in E. fetida in filter paper contact test and artificial soil test. LC50 concentrations were determined in both experiments. In filter paper contact test, the concentration amounted to 14.98 μg · cm⁻² (after 24 hours) and 7.59 μg · cm⁻² (after 48 hours). In turn, in artificial soil test, the LC50 value for IPP amounted to 541.07 mg · kg⁻¹ (after 14 days) and 238.51 mg · kg⁻¹ (after 28 days). LC50 determined in both tests for E. fetida as a response to IPP is considerably higher than in case of traditional neonicotinoid insecticides. The assessment of earthworm body weight demonstrated that earthworm growth was inhibited by the prolonged exposure to IPP in the artificial soil test. A similar relation was observed for the new cis configuration neonicotinoid insecticide called Cycloxaprid (CYC). It demonstrates high activity
against pests resistant to imidacloprid. Suzhen et al. (2018) studied acute toxicity of this compound to *E. fetida* in the artificial soil test. A negative effect on earthworms was demonstrated both under the conditions of acute and long-term exposure, however, the toxicity of this compound was lower, similar as in case of IPP, compared to the traditional neonicotinoids. In the study described in the present paper, imidacloprid disturbed the reproduction of *D. veneta*, which was manifested by a significant reduction in the number of laid cocoons, so the studied population did not develop. A similar effect was demonstrated by Wang et al. (2015a), who investigated the effect of neonicotinoids: imidacloprid, clothianidin, nitenpyram, thiacloprid and acetamiprid in relation to the mature *E. fetida* specimens in the artificial soil test. All the applied measures restricted the number and weight of cocoons and the number of earthworms hatched from one cocoon. Among the studied compounds, clothianidin was the most toxic – already at the dose of 4.34 mg·kg⁻¹. In the studies carried out by Zang et al. (2000) it was demonstrated that imidacloprid may damage DNA and causes sperm deformity in *E. fetida* (starting from the concentration of 0.5 mg·kg⁻¹) (p<0.01).

All the mentioned facts indicate that it is necessary to apply neonicotinoids very carefully and the usage of these insecticides should be restricted. In order to assess the actual risk of neonicotinoids for the biodiversity and ecosystems, it is also necessary to obtain the overall image of their distribution and transfer in the environment.

**CONCLUSIONS**

1. Results of the conducted studies with the use of concentration of 0.4 ml·m⁻², corresponding to the safe dose according to the manufacturer, indicate high toxicity of imidacloprid (trade name Nuprid 200SC) towards *D. veneta*.

2. Under the conditions applied in the present experiment, the studied insecticide:

- decreased the number of the entire population (p<0.01), the number of mature specimens (p<0.0001) and immature specimens (p<0.001). It also reduced the reproduction – by decreasing the number of laid cocoons (p<0.01),
- reduced the biomass of the population (p<0.0001), weight of an individual specimen in the entire population (p<0.05); the insecticide also disturbed biomass growth in mature specimens (p<0.0001) and the weight growth of the individual specimens (p<0.01). Similar situation was observed in immature specimens (p<0.01) and in biomass of cocoons in *D. veneta* (p<0.05).

3. The studies indicate that there is a need for further studies on the effect of neonicotinoids on earthworms as well as on the other organisms.

**REFERENCES**


