The effects of two pesticides on the mortality and reproduction of *Folsomia candida*

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Abstract: The aim of our study was to determine the effects of two pesticides (a fungicide and an insecticide) after the administration as single compounds and in combination on the mortality and reproduction of springtails. The test organism was the *Folsomia candida* (*Collembola, Isopotomidae*), which is widely used for testing the toxicity of soils. Springtails have a significant role in decomposition and mineralization of soils. Testing was carried out using the OECD 232 guideline. The examined insecticide contains lambda-cyhalothrin active ingredients and the fungicide consists of a combination of 3 active ingredients. Five concentrations of both test items have been investigated (insecticide: 0.166, 0.299, 0.538, 0.968, 1.742 mL/L; fungicide: 1.157, 2.083, 3.75, 6.75, 12.15 mL/L). One-way ANOVA test was used for the statistical analysis. In both cases, obviously, the Collembolan species are non-target organisms. However, our results showed that there is significantly higher mortality in 2 concentrations of the insecticide (2nd and 5th concentration, $p<0.001$), in 3 concentrations of the fungicide (2nd, 3rd and 4th concentration, $p<0.001$), and in the middle concentration ($p<0.05$) of the combined treatment, compared to the control. The reproduction was found to be significantly lower ($p<0.001$) in all of the examined concentrations compared to the control, except the lowest concentration of the single administrations. These results can indicate that pesticides may also have effect on the non-target organisms.

Keywords: insecticide, herbicide, *Collembola*, *Folsomia candida*, reproduction, mortality

Introduction

Pesticides have been used with different scopes in significant quantity for decades. Despite of their well-known negative effects and high costs, farmers apply them in increasing amounts. The use and application of pesticides (besides others) are regulated by legal regulations based on the results of variety of previous tests. However, it is clearly known that the residues of pesticides can accumulate in the environment, can get into the ground-water, or the wind can take them far away from the application area. But there are other important and good-to-examine effects, like the effects of the combination of pesticides and the effects on non-target organisms that belong to the ecosystem of the agricultural area or the puffer area.

Folsomia candida is a wingless, eyeless, unpigmented and parthenogenetic arthropod, and belongs to *Collembola* subclass. It can be found worldwide, except Africa and India (Hopkin, 1997). It is not so common in natural soils; it prefers humus rich areas (Krogh, 2008). It has a furca, "jumping organ" that helps its active running movement and jumping, when it is suddenly disturbed (OECD, 2009). It is a widely used organism for ecotoxicological soil tests for almost 40 years, it has a short generation time, easy to keep in laboratory and its life cycle is well-known (Fountain and Hopkin, 2005). This springtail is omnivorous, fungal hyphae, bacteria, protozoa and detritus can be found in its food. *Folsomia candida* has an important role in microbial ecology, soil fertility due to the contribution of decomposition processes and the regulation of nutrient cycling (Alves et al., 2014; OECD, 2009; Culik and Zeppelini, 2003). So it can be used as a good indicator of soil pollution (Achazi et al., 2000; Greenslade and Vaughan, 2003; Heupel, 2002).The aim of this study was to assess the effect of different types of pesticides on a non-target soil organism, *Folsomia candida*.

Materials and methods

The effect of two pesticides and their combination were tested on mortality and reproduction of *Folsomia candida*.

The Karate Zeon 5 CS (insecticide) contains a synthetic pyrethroid, lambda-cyhalothrin as active ingredient that has broad spectrum and it is used for controlling biting, chewing and sucking insect pests in cereal crops, fruits, vegetables, maize, sugar beets, alfalfa, sunflower etc. The Cherokee (fungicide) contains the combination of 3 active ingredients: chlorothalonil, propiconazole and cyproconazole. It is applied for the control of fungal diseases in winter wheat and both winter and spring barley.

Synchronous (9-12 days) female juveniles of *Folsomia candida* were used for the test. In the breeding period the springtails were kept in plastic Petri dishes on a mixture of plaster of Paris and activated charcoal layer, ensuring the required temperature (20±2ºC) and they were nourished with granulated dry yeast and water was dripped on the surface for the proper humidity once a week.

The 28-day reproduction test was carried out by the standard OECD 232 Guideline (OECD, 2009). The modified OECD artificial soil was composed 5% air-dried sphagnum peat, 20% kaolin clay, approximately 74% air-dried industrial sand and $CaCO_3$. The plastic test vessels contained 30 g moist soil (24.5 g dry soil + 5.5 ml solution). In the case of the simultaneous application of the pesticides 2.75 ml/each concentration was added to the soil. The calculation of concentrations was based on the application procedure of the pesticides, five concentrations in a geometric series (by a factor 1.8) were calculated, and the middle concentration was the application concentration used in practice (*Table 1*). Four replicates for each test concentration treatment and eight controls were set. Ten individuals were placed in each vessel. For nourishing granulated dry yeast was used. The vessels were kept under the same conditions as the culture. At the end of the test, 200 ml distilled water and ink was added to the test vessels and the adults and juveniles were counted.

Table 1. Concentrations of pesticides used in the experiment (mL/L)

	Concentration of pesticides (ml/l)	
	Insecticide	Fungicide
I	0.166	1.157
II	0.299	2.083
III	0.538	3.75
IV	0.968	6.75
V	1.742	12.15

The normality of the data was determined by Shapiro-Wilk normality test and Q-Q plot. Significant differences between treatments were tested with one-way analysis of variance (ANOVA), and the comparison of treatments with the control was carried out by post-hoc Tukey test, using the R software package (version 3.3.2).

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Results and discussion

According to the OECD 232 Guideline, in a valid experiment the following 3 criteria are required: (1) Mean mortality of adult should not exceed 20% at the end of the test; (2) The average number of juveniles per vessel should be minimum 100 at the end of the test; (3) The coefficient of variation of reproduction should be less than 30% in the control.

In our test, (1) the mean mortality of adults was 5.27±4.44, so less than 20%; (2) the mean number of juveniles was over 100 in every vessel, (3) the CV% was 26.97, which is less than 30%. Overall, the experiment meets the validity criteria (*Table 2*).

Table 2. Number of individuals in the control group

Mark of control vessels	Adults	Juveniles
C1	10	437
C2	10	287
C3	10	631
C4	9	411
C5	10	584
C6	10	615
C7	9	295
C8	9	510
C9	9	513

Only by seeing the mean number of juveniles (*Table 3*), a decreasing trend can be observed in all of the 3 treatments. This can be particularly observed due to the effect of the simultaneous application, where there were no juveniles from the 2nd to the 5th concentration. In the highest concentration of fungicide can be seen, that although in lower number, but juveniles were found. This different result (13 juveniles) was only in one vessel of the four replicates. So this outlier can be explained by an error made during setting the experiment. Because compared with the number of juveniles in the other vessels, this is significantly lower.

Table 3. Average number of individuals

	Mean number of individuals					
	Insecticide		**Fungicide**		**Combined**	
Concentration	**Adult**	**Juvenile**	**Adult**	**Juvenile**	**Adult**	**Juvenile**
Control	9.56	475.89	9.56	475.89	9.56	475.89
I	9.00	310.75	8.75	317.25	7.25	113.00
II	7.00	208.50	7.25	105.00	5.50	0.00
III	9.50	135.25	5.75	0.00	4.25	0.00
IV	7.75	51.50	6.75	0.00	6.50	0.00
V	3.75	13.00	8.75	3.25	6.25	0.00

Significant differences were detected at the 2nd (p<0.05) and the 5th concentration (p<0.001) of the insecticide, at the 2nd (p<0.1), 3rd (p<0.001) and 4th concentration (p<0.05) of the fungicide, and at the middle concentration of the combined treatment (p<0.05), compared to the control (*Figure 1*). The results of the treatments were significantly higher compared to the control group

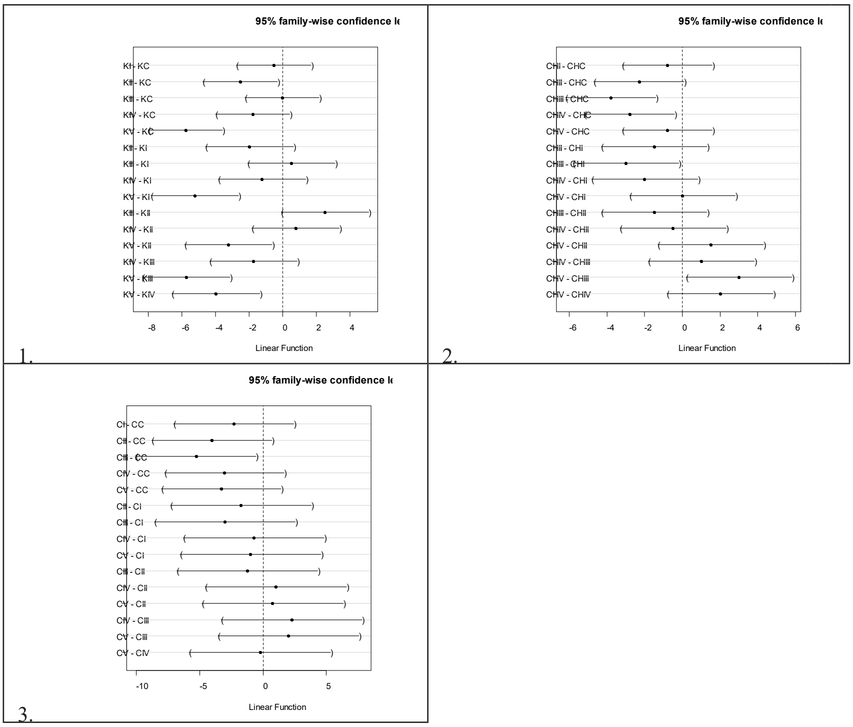

Figure 1. Significant differences in mortality between the treatments and compared with the control according to the Tukey test in the case of insecticide (1.), fungicide (2.) and the combined treatment (3.)

It can be stated, that the mortality due to the single administrations and the combined treatment was not dose-dependent apart from the significant differences.

In the case of reproduction, all of the examined concentrations showed significant difference compared with the control, except the lowest concentration of the single administrations (*Figure 2*).

The results of the 4 significantly different treatments of the insecticide (2nd concentration (p<0.01), 3rd, 4th and 5th (p<0.001)) and of the fungicide (2nd, 3rd, 4th and 5th (p<0.001)) and all of the treatments of the combined concentrations (p<0.001) were lower compared with the control.

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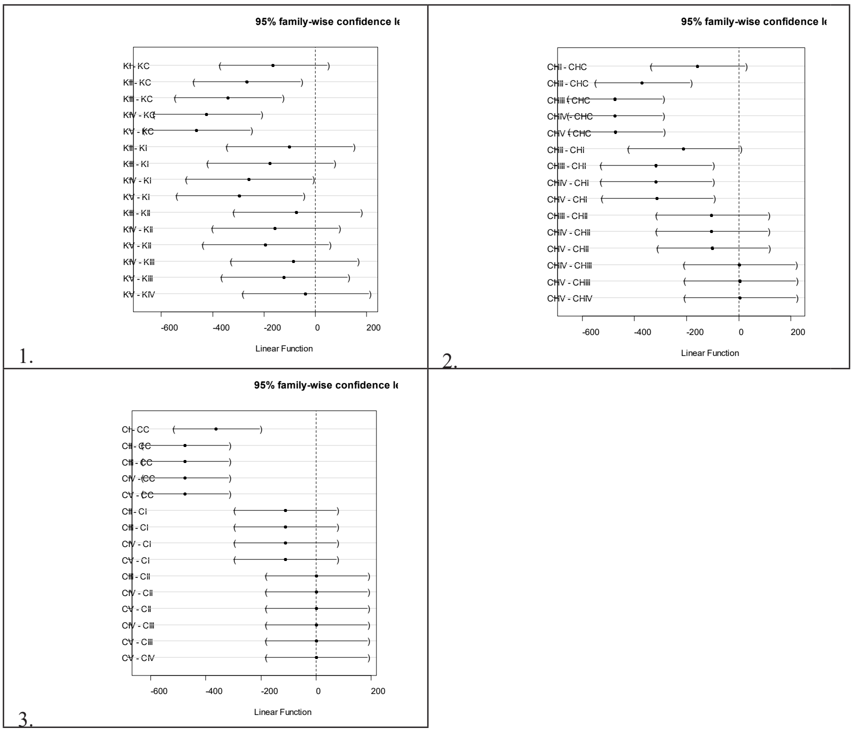

Figure 2. Significant differences in reproduction between the treatments and compared to the control according to the Tukey test in the case of insecticide (1.), fungicide (2.) and the combined treatment (3.)

Conclusions

The mean number of juveniles compared with the control was reduced with 72% in case of fungicide and with 100% in case of the insecticide and the simultaneous treatment at the practically application rate. Decreasing (or a stagnating) trend can be observed with the increasing of the concentration. Moreover, the 2nd concentration of the combined treatment caused already 100% reduction in the reproduction. However, the same effect cannot be seen in the mortality. So based on our results it can be stated that the reproduction of the *F. candida* has a higher sensitivity to pesticides than the mortality and gives us more information. Similar conclusions have been drawn by Krogh and Petersen (1995) and Crouau et al. (1999).

These results can indicate that these pesticides may also have effect on the non-target organisms; however, more information and experiments are needed for examining the effect of different pesticides on non-target organisms.

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