Assessing the effects of plant protection products on enchytraeids under field conditions: test of carbendazim as a toxic reference

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Introduction

According to the Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (EFSA PPR Panel, 2017), **Specific Protection Goal** options have been identified for different groups of soil organisms. For Enchytraeidae, *"the maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be small effects less than 35% for months on the ecological entity 'populations of different enchytraeid species' or medium effects less than 65% for weeks."* However, to validate magnitudes of effects on enchytraeids under field conditions, no active substance for the negative control is clearly identified. Our study aimed at testing under field conditions the effects of carbendazim - a fungicide already used as a toxic reference in earthworm field studies – also on enchytraeids.

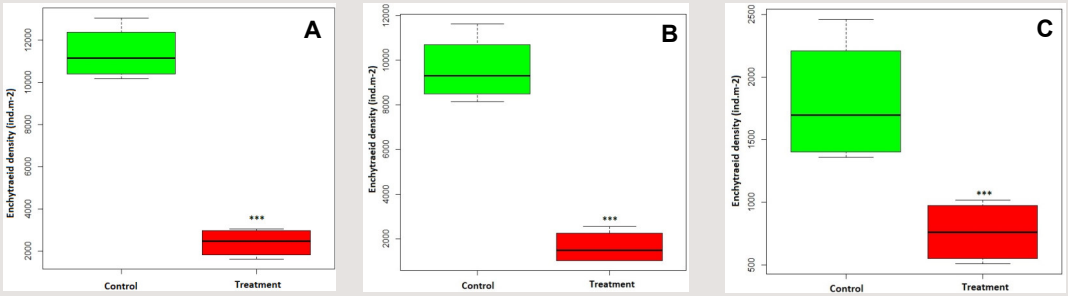
Material and methods

The field study was conducted in a meadow in Central Germany in 2018. Eight study plots (4 control plots and 4 plots treated once with carbendazim at 10 kg a.s./ha in March) with a size of 10 m x 10 m each were randomly located. Seven months after carbendazim application (in November), six soil cores (10 cm depth, 5 cm in diameter) were taken using a split corer from a 6 m x 6 m core area in the central part of each study plot. Soil samples were horizontally divided in two equal parts corresponding to two soil layers (0-5 cm and 5-10 cm). Sampling, heat-gradient extraction and counting of Enchytraeidae were conducted in accordance with ISO norm 23611-3 (2018). Enchytraeid densities were calculated from the sum of the six cores and expressed as density (ind/m^2) for each plot. Mean values of densities were averaged from the four replicates.

Results and discussion

Enchytraeids – control versus treatment plots

- The total density of enchytraeids (10 cm soil depth) was significantly lower in the treated compared to control plots (A)
- The enchytraeid density in the upper 5 cm soil was significantly lower in the treated compared to control plots (B)
- Similarly, the enchytraeid density in the layer 5-10 cm was significantly lower in the treated compared to control plots (C)



*** p<0.001, using one way ANOVA followed by a post-hoc t-Test after validation of the homogeneity of variance (Bartlett-test) and the normality of residuals (Shapiro test).
All analyses were carried out with R statistical software (R Development Core Team, 2016)

- **Control plots**: The mean total density (10 cm soil depth) was 11 395 ± 1 290 ind/m^2. This enchytraeid density is comparable to enchytraeid densities generally found in meadows in Germany. For example, Römbke et al. (2013) recorded on average 13 000 ind/m^2 in two grassland sites. In the studied control plots, the mean densities were 9 592 ± 1 510 ind/m^2 and 1 804 ± 511 ind/m^2 in the soil layers 0-5 cm and 5-10 cm, respectively.

- **Treatment plots**: The mean total density (10 cm soil depth) was 2 398 ± 687 ind/m^2. Within the layers the mean densities were 1 634 ± 751 ind/m^2 and 764 ± 250 ind/m^2 in 0-5 cm and 5-10 cm depth, respectively.

Our results are in accordance with another field study conducted in a grassland in Germany where the enchytraeid abundance was lower eight and sixteen weeks after single carbendazim applications of 9.72, 29 and 87.48 kg a.s/ha, compared to the control plots (Moser et al., 2004). Moreover, several laboratory studies showed that carbendazim is highly toxic to enchytraeids (Collado et al., 1999; Römbke and Moser, 1999). Chronic effects were even observed at low carbendazim concentrations with NOEC values calculated as 1.2 mg a.s/kg and 2.7 mg a.s/kg for *Enchytraeus albidus* (Henle, 1873) and *Enchytraeus buchholzi* (Vejdovský, 1879), respectively (Collado et al., 1999).

Scientific and regulatory conclusions

With the significant differences of enchytraeid densities observed between the control and the treatment plots seven months after application, carbendazim could be considered as a relevant active substance for toxic reference in enchytraeid studies. Tests of the effects of carbendazim on enchytraeid density one to three months after application would confirm carbendazim as a toxic reference at 10 kg a.s./ha. Moreover, enchytraeid field studies could be adapted from the ISO standard method 11268-3 (2014) for earthworm field studies. Investigation of enchytraeid community composition (i.e. genus/species levels or r/K strategy types) would deliver further key information on community change, biodiversity loss and potential impact on the soil functioning in soils of agroecosystems treated with plant protection products.

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