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Combined Effects of Soil Moisture and Carbaryl on Earthworms and Plants

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Abstract—The species *Brassica rapa*, *Triticum aestivum* (plants) and *Eisenia andrei* (Oligochaeta) were exposed to a natural stressor (soil moisture) and to the chemical stressor carbaryl. These stress factors were tested individually and in combination, and acute and chronic tests were performed. Discussion was raised upon the responses of organisms to the combination of natural and chemical stressors and mathematical models that have been described for chemical mixture will be used.

Keywords: soil moisture, carbaryl, independent action, synergism and antagonism

INTRODUCTION

The study of interactions between toxic chemicals and extreme climatic events is very important for ecological risk assessment procedures. Their joint action can cause an increase on the toxicity of the contaminant or an increase on its availability in soil, or even decrease organisms' tolerance to abiotic factors. This work analyses the combined effect of soil moisture content and carbaryl. Toxicological tests were performed using different species of soil fauna and flora. To evaluate the combined effects of soil moisture and the pesticide carbaryl to *Eisenia andrei*, *Brassica rapa* and *Triticum aestivum*. For that, models that describe interactions between different mixtures were also tested.

MATERIALS AND METHODS

Test substance and test species

Carbaryl, (1-naphthol N-methyl carbamate) is a common insecticide of the carbamate family, a potential neurotoxicant to non-target species. The inhibition of cholinesterase (ChE) activity by carbaryl is documented on invertebrate species like earthworms by several authors (e.g. Valbonesi *et al.*, 2003; Caselli *et al.*, 2006; Gambi *et al.*, 2007).

All the tests were performed with Standard soil LUFA 2.2, a sandy soil from Speyer, Germany (for details in characterization please see Loureiro *et al.*, 2009).

The earthworm *Eisenia andrei* (Bouché, 1972) was kept in laboratory cultures, at 16:8 h light:dark photoperiod, at 20 ± 2°C. Seeds of *B. rapa* were purchased from Carolina Biological Supply Company (US) and *T. aestivum* in a local supplier (Aveiro, Portugal). For both single and combined exposures, tests with earthworm and plants were carried out in accordance to the standard protocols from OECD 207 (1984) and ISO 11269-2 (1995).

Exposure to chemical stressor

Earthworms were exposed to concentrations of 20, 40, 60, 80 and 100 mgCarbaryl/Kgsoil, with four replicates per treatment. The experiment was kept at 20 ± 2°C; 16 h light/8 h dark, and soil moisture equivalent to 60% water holding capacity (WHC). After 7 days of exposure surviving worms were counted, and after 14 days survival and loss biomass were reported.

For the plant tests the concentrations of carbaryl used were 50, 75, 100, 125 and 150 mg/Kg, with four replicates per treatments. Bioassays were carried out at 20 ± 2°C, 12000 lx; 16:8 (light:dark). The soil moisture was maintained by capillary action, through a fiberglas wick located at the pot's bottom (see Loureiro *et al.*, 2006, for details). After 14 days, growth (shoot length) and biomass (fresh and dry weight) were recorded and the hydric content also calculated.

Exposure to natural stressor

The organisms were exposed to drought conditions 10%, 20%, 40% of the WHC and flood conditions, with 80%, 100 and 120% of WHC. The control was set with 60% of WHC. The pots were weighted every day and were irrigated to obtain field capacity wanted.

Combined exposure

The dry soil was contaminated with different carbaryl concentrations and afterwards moistened to obtain a water content of 10%, 20%, 40%, 80%, 100% and 120% of the WHC.

Statistical analysis

For the statistical analysis, One Way ANOVA was used to determine if there were significant differences between treatments. Whenever differences were attained, a Dunnett test was run to compare treatments with the control situation. EC_{50} values were calculated using a sigmoidal (logistic, 3 parameter) equation (Sigma Plot 10.0). LC_{50} values were calculated through the Probit Method.

To address the toxicity effects of combination of stressors, the MixTox model was used (Jonker *et al.*, 2005). A stepwise approach was done, initialized with the fit of data to the Independent Action model (Bliss, 1939) and then, when possible deviations for synergism or antagonism evaluated. It is a multiplicative model, which can be used to analyze the effects of combined treatments in detail (Jensen *et al.*, 2009).

Table 1. Summary of carbaryl and soil moisture effect on *Eisenia andrei*, *Brassica rapa* and *Triticum aestivum*. IA is independent action; SS is the objective function used for continuous data; r^2 is the coefficient of determination; a and b are parameters of the deviation functions; S/A is synergism/antagonism; DR and DL are "dose ratio" and "dose level" deviation from the reference model.

Parameter	Carbaryl and natural stressor type	IA model fit		Deviation from IA model				
		SS	r^2	Deviation type	SS	r^2	a	b
E. andrei survival	drought	1.44	0.39	S	1.44	0.39	–0.05	—
	flood	42.0	0.65	A	42	0.65	102.2	—
E. andrei biomass	drought	0.15	0.96	S	0.15	0.96	–6.8	—
	flood	0.3	0.78	A	0.3	0.78	3.10	—
B. rapa length	drought	3.96	0.69					
	flood	2770	0.44	DL	2770	0.44	7.15	1.4
B. rapa FW	drought	149398	0.78					
	flood	16.59×10^5	0.68	A	16.59×10^5	0.68	2.5	—
B. rapa DW	drought	1879	0.51					
	flood	8307	0.66	A	8307	066	1.7	—
T. aestivum length	drought	8.36	0.89	DR	8.63	0.89	3.9	–4.6
	flood	1972	0.88	DL	1972	0.88	0.04	–49
T. aestivum FW	drought	82744	0.9	A	82744	0.90	1.15	—
	flood	691305	0.75	A	691305	0.76	0.65	–31
T. aestivum DW	drought	2395	0.85	A	2395	0.86	1.13	—
	flood	16734	0.66	A	16734	0.66	3.0	—

RESULTS AND DISCUSSION

To understand the response of soil organisms to combined stressors the IA reference model was used because we know that carbaryl and content moisture have dissimilar physiological modes of action.

After the fit to the IA model, deviations from the conceptual model were observed in some cases and results from the Mixtox model fit depicted in Table 1.

Soil moisture showed a strong influence on soil organisms and in toxicity of carbaryl. Drought stress showed to increase the deleterious effect of carbaryl to *E. andrei*, where a synergism could be observed on survival and biomass of earthworms exposed to carbaryl in drought conditions. On the other hand water excess (simulating flood scenarios) led to a decreased on toxic effect of this pesticide to earthworms.

In soil flora the drought stress showed different responses. For *B. rapa* the reference model IA (independent action) gave a valid estimation of the overall toxicity for combined chemical and drought stressors, but in *T. aestivum* an antagonism and dose ratio deviations were observed. In the flood stress, deviations from reference models for dose level and ratio dependency were found, when evaluating length and fresh weight and antagonistc deviation when dry weight data was modelled.

For *T. aestivum* length, in drought conditions our results indicate that there is a strong synergism related to the presence of carbaryl. When levels for drought are high and carbaryl is present in low levels, antagonistic patterns are observed.

For flood situations, at low level of both stressors (i.e. low carbaryl concentrations and low levels of flood), antagonism is observed; however when both stressors are increased, synergism occurs.

The influence of soil moisture in soil fauna and flora is described in several studies (Reinecke and Venter, 1987; Domínguez and Edwards, 1997; Gunadi *et al.*, 2003) and several studies have reported the great importance of research in climate changes (Reilly *et al.*, 2003; Ortiz *et al.*, 2008). This study showed that soil moisture has a strong influence on the toxicity of carbaryl. The increase of deleterious effect on earthworms by carbaryl when exposed to drought soil condition showed the importance of climate change in the performance of pesticides in soil organisms. However the decrease of deleterious effects than those expected in flood stress for earthworms and plants may be due to carbaryl dilution in soil pore water. Therefore lixiviation of carbaryl must be considered and evaluated in future studies as it can state a potential risk to underground water.

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