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Terrestrial short-term ecotoxicity of a green formicide

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ABSTRACT

When ants become annoying, large quantities of formicide are applied to terrestrial ecosystems in tropical regions, but awareness of the health and environmental impacts related to the use of synthetic pesticides has been increasing. The use of green pesticides to combat target organisms could reduce these impacts. In this regard, terrestrial ecotoxicity tests with higher plants (*Brassica olaracea*, *Lactuca sativa* and *Mucuna aterrima*), annelids (*Eisenia foetida*), Collembola (*Folsomia candida*) and soil enzyme activity analysis (diacetate fluorescein hydrolysis) were used to evaluate short-term terrestrial ecotoxicity of a green pesticide prepared from naturally-occurring organic compounds. At the highest formicide concentration tested in these experiments (i.e., 50 g kg^{-1} soil) no toxicity toward terrestrial organisms was observed. The lack of short-term terrestrial ecotoxicity suggest that this green formicide can be classed as an environmentally friendly product as compared to the ecotoxicity of the most commonly used commercialized formicides.

1. Introduction

In recent years there has been increasing appreciation of the role that terrestrial organisms play in the flow of energy and materials through the various compartments of different ecosystems (Chapin et al., 2002). Some environmental impacts of anthropogenic activity directly affect terrestrial ecosystems, e.g., agrochemical application. Before the physico-chemical dispersion or (bio) transformation of pesticides, they can act as an important element in the disruption of trophic interactions of the terrestrial ecology.

Pest control is an expensive global problem when toxic pesticides are necessary to protect food production. Thus, large quantities of pesticides are used to combat pests, but the introduction of xenobiotic molecules into the environment can damage ecosystem equilibrium because synthetic pesticides could have high toxicity and, generally, low biodegradability, which can lead to persistent toxic action, as reported, for example, by Wiktelius et al. (1999). These authors concluded that African countries need to develop procedures for testing pesticides in order to arrive at the correct conclusions concerning the adverse side effects from pesticide use. In general, the environmental impacts of pesticides are evaluated using models developed to support comparative assertions in the context of the reduction of risks associated with agrochemical use in plant protection (Humbert et al., 2007; OECD/FAO, 1996; Polidoro et al., 2008). A more specific problem concerning terrestrial ecosystems is the use of formicide to kill some undesirable ants where these organisms could damage food production. Ants are a diverse group of more than 12,000 species, with a higher diversity in the tropical regions, which invade homes, yards, gardens and fields. These eusocial insects of the family Formicidae are known for their highly organized colonies and nests, which sometimes consist of millions of individuals. In terrestrial ecosystems, ants may constitute up to 15–25% of the total terrestrial animal biomass (Schultz, 2000). Ant colonies are sometimes described as super-organisms because the colony appears to operate as a single entity. The social structure of the colonies is based on a complex communication system, these organisms being biologically and economically important members of natural and agro-ecosystems where they exhibit diverse biologies, mating systems, and semiochemical pheromones (Majer, 1983; Ayasse et al., 2001; Holway et al., 2002; Hara et al., 2005).

According to Forti and Boaretto (1997), several mechanical (e.g., excavating their nests for queen removal), cultural (e.g., conventional soil preparation by ploughing and harrowing), biological (e.g., use of predators, parasitoids and pathogenic microorganisms) and chemical methods have been studied as early as the 50 s for controlling leaf-cutting ants. Currently used formicides are chlorpyrifos, fenthion, endosulfan, deltamethrin, and sulfluramid. Among these, chlorpyrifos and fenthion have active ingredients more toxic to mammals, water organisms, fish and bees than sulfluramid. Ant baits (ready-to-use formulation),

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practically impede risks to humans and reduce environmental impact (Forti and Boaretto, 1997).

Ideally, a good formicide must be environmental friendly, i.e., not disrupting the ecosystem equilibrium, while it must be effective against the target species. It is expected that the ecotoxicity of this new (and necessary) class of "green pesticides" is low or non-existent (Kähkönen and Nordström, 2008). The term "green pesticides" is not new, but it is not well defined in available literature. Thus, in this paper, the term "green pesticide" must be understood as a natural or synthesized biocide produced according to the green chemistry principles and acting specifically and effectively upon a precise target, with no harmful or deleterious effects on the non-target ecosystem components. It is clear from the above definition that green chemistry principles (Anastas and Warner, 1998) and biocide regulation trends (Kähkönen and Nordström, 2008) are the driving forces that must guide the development of new environmentally friendly pesticides. However, the lack of data on the ecotoxicity of these new pesticides is surprising, as the soil ecosystem is the first compartment affected by formicide application. In this compartment there are high rates of energy conversion by the primary producers and decomposer organisms. This energy conversion is important to the structure and maintenance of the soil ecosystem and energy flow efficiency can be increased if the producer and decomposer organisms are benefited by invertebrate organism action, which improves soil productivity (Chapin et al., 2002).

Thus, the objective of this study was to evaluate the short-term terrestrial ecotoxicity of a green formicide candidate commercialized as Macex®, which is a mixture of caffeine and common fatty acids with apple and citric pulp as attractive agents. To achieve this objective, a battery of short-term terrestrial ecotoxicity tests was carried out with bacteria, earthworms, Collembola and higher plants. These organisms belonging to different trophic levels are representative (or co-participants) of the energy flow system in the terrestrial ecosystem.

2. Materials and methods

2.1. Soil characterization and contamination

The red yellow Podzolic soil used in this study was collected from the A–B horizons (0 and 15 cm depth) in the rural area of Brusque (SC, Brazil). The area had not been exposed to the soil pollutants and the soil sample was characterized in terms of pH (6.5), texture (% sand:silt:clay=22:16:62), cation exchange capacity (8.3 mEq 100 g^{-1}), N_{total} (0.2%), organic $matter_{total}$ (10.8%) and C:N mass ratio (4:1) according to published methodologies (EMBRAPA, 1999). The natural formicide Macex® is a mixture of caffeine (40 mg kg^{-1}) and common fatty acids (palmitic—1.5%, oleic—1.9%, linoleic—6.2% and estearic—0.4% mass/mass basis) with apple and citric pulp as attractive agents. The granulated formicide Macex® was crushed using a mortar and pestle after that the powder was thoroughly homogenized with soil and placed in the vials used in the biotests. Formicide soil contamination was carried out with the following concentrations (dry weight basis): 50.0; 25.0; 12.5; 6.25 and 3.12 g kg^{-1} dry soil, which are higher than manufacturer recommended concentrations used in the field (5 g kg^{-1}—Macex do Brasil Ind. e Com. de Produtos Químicos e Biológicos Ltda).

2.2. Ecotoxicity tests

2.2.1. Plant growth test

Phytotoxicity tests were conducted with cabbage (*Brassica oleracea* L. var. *capitata*), lettuce (*Lactuca sativa* L), and black mucuna (*Mucuna aterrima* Piper and Tracy). Seeds were supplied by the SC state agricultural research company EPAGRI (Empresa de Pesquisa Agropecuária e Difusão de Tecnologia de Santa Catarina, Florianópolis, SC, Brazil). Assays were performed according to the ISO (1995) guidelines. The reference (control) soil was the same red yellow Podzolic soil used in the other tests. Three pots (disposable plastic, 7-cm diameter, 5-cm height) were used for each test concentration. The phytotest was performed at two different times. Twenty-five cabbage or lettuce or black mucuna seeds were sown in each pot containing 200 g of soil or soil with powdered formicide. Dechlorinated tap water was gently pipetted onto the soil surface at 66% (i.e., 62 mL) of the maximum water-holding capacity (52 mL water per 100 g soil). Water loss through evapo-transpiration was determined by daily weighing of the pots and replaced by addition of distilled water. Plants were grown under controlled conditions at a daytime temperature of 25 ± 2 °C and a nighttime temperature of 16 ± 2 °C, with lighting of 60 mE m^{-2} s^{-1} (cool white fluorescent lamps) under a 16:8 h light:dark photoperiod. To test the phytotoxic potential of the green formicide two endpoints were assessed, i.e., germination and biomass growth. Sixteen days after planting, the number of germinated seeds was reported and the shoot plants were cut at soil level and the wet weight of the plant material was immediately determined using an analytical scale. For each pot, the total weight of germinated plants (expressed in grams wet weight) was divided by the number of germinated plants. As positive control, a 0.001 M of sodium trichloroacetate (97% purity; Acros Organics, 18415-82) was used in these experiments to check organism sensitivity.

2.2.2. Earthworm test

Annelid specimens (*Eisenia fetida* Savigny, 1826) were purchased 2 weeks prior to use and stored in commercial worm bedding with worm food. The tests were performed in accordance with the ISO (1993) methodology. Adult earthworms, at least 2 months old with clitella, were selected and used in all the experiments. The experiments were conducted in a culture chamber with constant light intensity at a temperature of 20 °C. Earthworms were acclimatized to the experimental conditions in the culture chamber for 1 week before the tests. Five adult earthworms were placed into 600 g (dry weight) of natural soil (control) or soil formicide mixtures, at different test concentrations, which were previously hydrated to 25% water-holding capacity in 1000-mL polypropylene wide-mouthed jars with loose-fitting lids to minimize water evaporation and animal escape. The jar lids were punched with holes to allow ventilation and oxygen supply. Sixteen days after the start of the test, the earthworms were collected and the wet weight of individuals was immediately determined using an analytical scale. For each pot, the total weight of earthworms (expressed in grams wet weight) was divided by the number of individuals. Earthworm tests were performed at two different times with triplicate samples for each concentration. Carbendazim (ISO-French; 5 mg kg^{-1} dry soil) was used as positive control in these experiments.

2.2.3. Collembola test

The growth inhibition test was carried out on Collembola (*Folsomia candida* Willem, 1902) following the ISO (1999) protocol with minor modifications. Briefly, the animals (10–12-day-old juveniles) were placed in experimental pots (triplicate per concentration, 100 mL capacity; 10 animals and 50 g wet soil per pot) containing the soil and litter with different formicide concentrations tested under laboratory conditions (temperature: 25 ± 2 °C, light-dark cycle: 16:8 h 700 Lux, relative humidity: 80%, sterilized granulated dry yeast as food). The formicide was macerated and well-mixed with the soil litter before organism transplantation. The test duration was 28 days. The pots were opened twice a week for aeration and every week for feeding with yeast. At the end of the exposure period, the water content and pH of the soil samples were determined. Water was added and the animals floating on top of the suspension were photographed and analyzed. The test was carried out twice with triplicates for each concentration. Carbendazim (ISO-French; 5 mg g^{-1} dry soil) was used as positive control in these experiments.

2.2.4. Hydrolytic activity of soil microorganism consortium

Total hydrolytic activity was assessed by the fluorescein diacetate hydrolysis (FDA) method of Schnürer and Rosswall (1982), with slight modifications. The formicide was macerated and well mixed with the soil, and the microorganisms (e.g., bacteria, fungi, algae, protozoa) present in the soil were then exposed to the formicide for 2 days. Soil at field moisture content was placed in 50 mL polycarbonate centrifuge tubes and 20 mL of 30 mM Na_2HPO_4 buffer (adjusted to pH 7.5) was added. Tubes were shaken for 40 min before adding 100 μL of a 2 mg L^{-1} FDA solution in acetone. A further 2 h-period of shaking was then carried out before terminating the reaction by the addition of 20 mL of acetone. Test tubes were then centrifuged at 500 rpm for 15 min followed by filtration of the supernatant (Whatman 0.45 μm). The filtrate was analyzed for FDA by measurement of florescence using a Turner fluorimeter. Microbial analysis was conducted twice in triplicate from composite samples representing the three pots for each concentration.

2.3. Statistical analysis

Statistical analysis was carried out on a microcomputer using the TOXSTAT 3.0 software. Responses were presented with a mean (X) and coefficient of variation (CV). William's test ($\alpha \leq 0.05$) was used to obtain the lowest-observed-effect concentration (LOEC) after applying Shapiro–Wilk's test for normality and Hartley's test for homogeneity of variance. As no EC50 values were available in the preliminary tests, even when high formicide concentrations were used, we chose LOEC values as the measured values, since the assessed endpoints used to measure effects are very sensitive.