Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.12, n.4, p.470-474, 2017
Recife, PE, UFRPE. www.agraria.ufrpe.br
DOI:10.5039/agraria.v12i4a5480
Protocolo 5480 - 21/03/2017 • Aprovado em 16/08/2017

Ecotoxicological potential of bordeaux mixture and Neem oil on non-target soil organisms

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ABSTRACT

The use of alternative pesticides has as main function to maximize agricultural production with little or no environmental impact. However, due to the lack of studies and repeated application in certain cultures it is important to check whether there are adverse effects on non-target soil organisms. Thus, the aim of this study was to evaluate the effect of Neem oil and Bordeaux mixture on survival and reproduction *Enchytraeus crypticus* and *Folsomia candida*. The tests followed the relevant standards, where the pesticides are applied separately in tropical artificial soil, the tested doses 0, 0.3, 0.5, 1, 3 and 5%, corresponding to concentrations of 0.41, 0.69, 1.38, 4.15 and 6.91 mg g^{-1} soil. Effects on the organisms survival were only observed at doses equal to or higher than 3%. However, doses equal to or less than recommended (1%) had negative effects on reproduction. These results have a potential impact of Neem oil and Bordeaux mixture on non-target soil organisms, which must be proven with field studies.

Key words: organic agriculture; alternative defensive; terrestrial ecotoxicology

Potencial ecotoxicológico da calda bordalesa e óleo de Neem sobre organismos não-alvo do solo

RESUMO

O uso de defensivos alternativos tem como principais funções a maximização da produção agrícola com baixos impactos ecológicos, contudo é importante comprovar se há efeitos adversos sobre organismos não-alvo do solo. Com isso, o objetivo deste trabalho foi avaliar o efeito do óleo Neem e da calda Bordalesa sobre a sobrevivência e reprodução de *Enchytraeus crypticus* e *Folsomia candida*. Os ensaios seguiram as normas da Associação Brasileira de Normas Técnicas, onde os defensivos foram aplicados separadamente em solo artificial tropical, nas doses 0, 0,3, 0,5, 1, 3 e 5%, que correspondem a concentração de 0,41, 0,69, 1,38, 4,15 e 6,91 mg g^{-1} no solo. Os efeitos na sobrevivência dos organismos foram observados apenas em doses iguais ou superiores a 3%. Entretanto, doses iguais ou inferiores a recomendada (1%) apresentaram efeitos negativos na reprodução de ambos organismos. Estes resultados apresentam um potencial impacto do óleo Neem e da calda Bordalesa sobre organismos não-alvo do solo, o que deve ser comprovado com estudos realizados no campo.

Palavras-chave: agricultura orgânica; defensivo alternativo; ecotoxicologia terrestre

Introduction

Alternative measures can be used to maximize production and control of pests in agriculture that combine chemical and biological management to minimize ecological damage (Barbosa et al., 2006; Pavlovic, 2011; Archana et al., 2017). Alternative pesticides, such as Neem oil and Bordeaux mixture are products prepared from substances not harmful to human health and the environment, aimed at the control of pests and diseases in agriculture (Fernandes, 2013). When used within the agroecological perspective of production, they reduce costs, the use of conventional agrochemicals, environmental impacts and risks (Fernandes et al., 2006; Fernandes, 2013).

The Bordeaux mixture has fungicidal and bactericidal action on crops such as potatoes, tomatoes, onions, garlic, strawberries and other vegetables, as well as having a repellent and fertilizer action (Weingartner et al., 2006; Kungolos et al., 2009). It is a colloidal suspension obtained by the mixture of copper sulfate solution with virgin lime (Fernandes et al., 2006). The recommended dosage of the Bordeaux mixture determines the time of its absorption by the plant, varying from 0.2 to 4.0% (Weingartner et al., 2006)

Neem oil is produced from a fast-growing perennial plant (*Azadirachta indica* A Juss) belonging to the Meliaceae family. Originally from India, it is currently growing in many places in the world, where it spreads rapidly. It is recognized and used mainly as an alternative control method for organisms considered pest (insects, nematodes, and fungi), but also in human medicine, reforestation of degraded areas and firewood production (Fernandes et al., 2006; Archana et al., 2017). Among the active principles, the most important is the azadirachtin, found in greater quantity in the seed of ripe fruits. This is the main component responsible for anti-food and toxic effects on insects. It also causes deformities in the pupae, increasing in the instar period (which causes aging of the larvae) and reproductive effects (Souza et al., 2015, Zanuncio et al., 2016, Archana et al., 2017). Neem applications ranging from 0.5 to 7% in the control of *Bemisia argentifolli, Keratoma tingomarianus, Liriomyza sativae, Sitophilus zeamais and Zabrotes subfasciatus; Gyropsylla spegazziniana* are recommended (Fernandes et al., 2006, Silva et al., 2015, Formentini et al., 2016).

Generally, substances used in organic agriculture, especially those derived from plant extracts are considered safe. The risks of Bordeaux mixture for non-target arthropods are considered low (Bengochea et al., 2014). Neem oil has been shown exceptionally safe for beneficial organisms, mainly because it is very selective (Zanuncio et al., 2016; Archana et al., 2017).

However, this is not always true (Raguraman & Kannan, 2014), especially when these products are not used correctly, which requires studies that assess their toxicological potential. Considering the importance of proving the adverse effects of these alternative pesticides on non-target organisms, the aim of this study was to evaluate the toxicity of the commercial insecticide Neem and the fungicide Bordeaux mixture on the survival and reproduction of *Enchytraeus crypticus* and *Folsomia candida*.

Materials and Methods

The pesticides used in the tests are (1) commercial Neem oil commonly used in the control of agricultural pests and repellent action (Nim-I-Go®), with azadirachtin as the active principle; (2) Bordeaux mixture that has fungicidal and bactericidal action based on copper sulfate and calcium oxide. The evaluations of the pesticides were carried out using standardized ecotoxicological tests (ABNT NBR ISO) of survival and reproduction of *Enchytraeus crypticus* and *Folsomia candida*, through the application of increasing doses of homogenized defenses in “tropical artificial soil - TAS” (ABNT, 2014), consisting of 70% industrial (fine) sand, 20% kaolinite clay, and 10% coconut fiber (dried and sieved).

The experiment was conducted in a completely randomized design with 4 replicates. The doses were determined based on the manufacturer´s recommendation (Neem oil) and on information in the literature (Fernandes et al., 2006; Fernandes, 2013) of 1% for both pesticides, being 0%, 0.3% , 0.5%, 1%, 3% and 5%, corresponding to the concentration of 0.41, 0.69, 1.38, 4.15 and 6.91 mg g^{-1} in the soil, respectively. The pesticides were applied to 65% of the maximum soil retention capacity (CMR). The pH of the TAS was adjusted to 6.0 + 0.5 by the addition of $CaCO_3$. The fifth replicate of each treatment, without food and organisms, was used to evaluate the suitability of the environmental conditions of the tests, such as luminosity, temperature, humidity and soil pH.

The trials with *F. candida* and *E. crypticus* followed the mandatory and recommended standards of NBR ISO 11267 (ABNT, 2011) and NBR ISO 16387 (ABNT, 2012), respectively. It was conducted in incubation chamber with temperature control (20 ± 2 ºC), photoperiod (16:8 h light:dark) and luminous intensity (400 to 800 lux). Soil moisture was maintained constant throughout the experiment and the pH variation between the beginning and the end for all doses was equal or less than 0.3.

The individuals of *E. crypticus* were raised in Petri dishes containing agar medium in an environment of a constant temperature of 20 ± 2 ºC. Transparent cylindrical containers (40 mL) with a lid containing 30 g of moist soil and 50 mg of thin oats flakes (feed) were used. In each container, 10 individuals adults with clitellum were inserted, selected and collected in a stereomicroscope. The containers were opened weekly for aeration and all replicas were weighed weekly for moisture loss replenishment and 25 mg of food added if needed on soil surfaces in containers with organisms. The assay lasted 28 days. At the end, organisms were fixed with alcohol and stained with Bengal red before counting, facilitating the counting of adults and juveniles under a stereomicroscope.

The individuals of *F. candida* were raised on a substrate consisting mixture of plaster and activated charcoal, in the proportion of 8:1, in an environment of a constant temperature of 20 ± 2 ºC. In the assay, clear cylindrical containers (80 mL) containing 30 g of moist soil and 2 mg of dry granulated yeast (feed) were used. In each recipient, 10 individuals synchronized with 10 to 12 days aged were added. The containers were opened weekly for aeration and ll replicas were weighed weekly for moisture loss replenishment and 2 mg of dry granulated yeast added if needed, on the soil surface in containers with organisms. The assay lasted 28 days. At the end, the soil containing the organisms was placed in a larger container (500 mL), distilled water was added until it was completely covered, promoting slight agitation with a spatula, promoting the flotation of living organisms. A few drops of dark blue ink were used to highlight the organisms. Adult were counted visually and juveniles, by manually counting photographs in ImageToll 3.0 software.

The data obtained from reproduction and survival tests were submitted to analysis of variance and later to the Dunnett test, for the calculation of No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The values of EC_{20} and EC_{50} (Effect Concentration causing effects in 50 and 20% of juveniles) were obtained using the Exponential nonlinear model ($p < 0.05$). Both analyses were performed by STATISTICA 7.0 software.

Results

All the tests were validated according to the criteria established by the ABNT NBR ISO standards in the control. In natural soil without the application of pesticides, there was no mortality of the adults of *E. crypticus*, the average number of juveniles was 735 and the coefficient of variation for reproduction was 21.8%. For *F. candida* there was also no adult mortality the mean number of juveniles was 791 and the coefficient of variation for reproduction was 16.2%.

For both species and alternative defenses, there was a significant effect on adult survival from the dose of 4.15 mg g^{-1}, except for survival of *E. crypticus* at the highest dose of Neem oil (6.91 mg g^{-1}) (Figure 1).

Considering the nominal dose, Neem oil caused different effects on reproduction of the organisms tested. For *E. crypticus*, the lowest dose with an effect on reproduction (LOEC) is the lowest dose tested, whereas for *F. candida* the LOEC was 0.69 mg g^{-1}. The Bordeaux mixture similarly influenced the reproduction of both organisms, with the highest dose that had no reproductive effect (LOEC) of 0.41 mg g^{-1} and the LOEC at 0.69 mg g^{-1} (Figure 1).

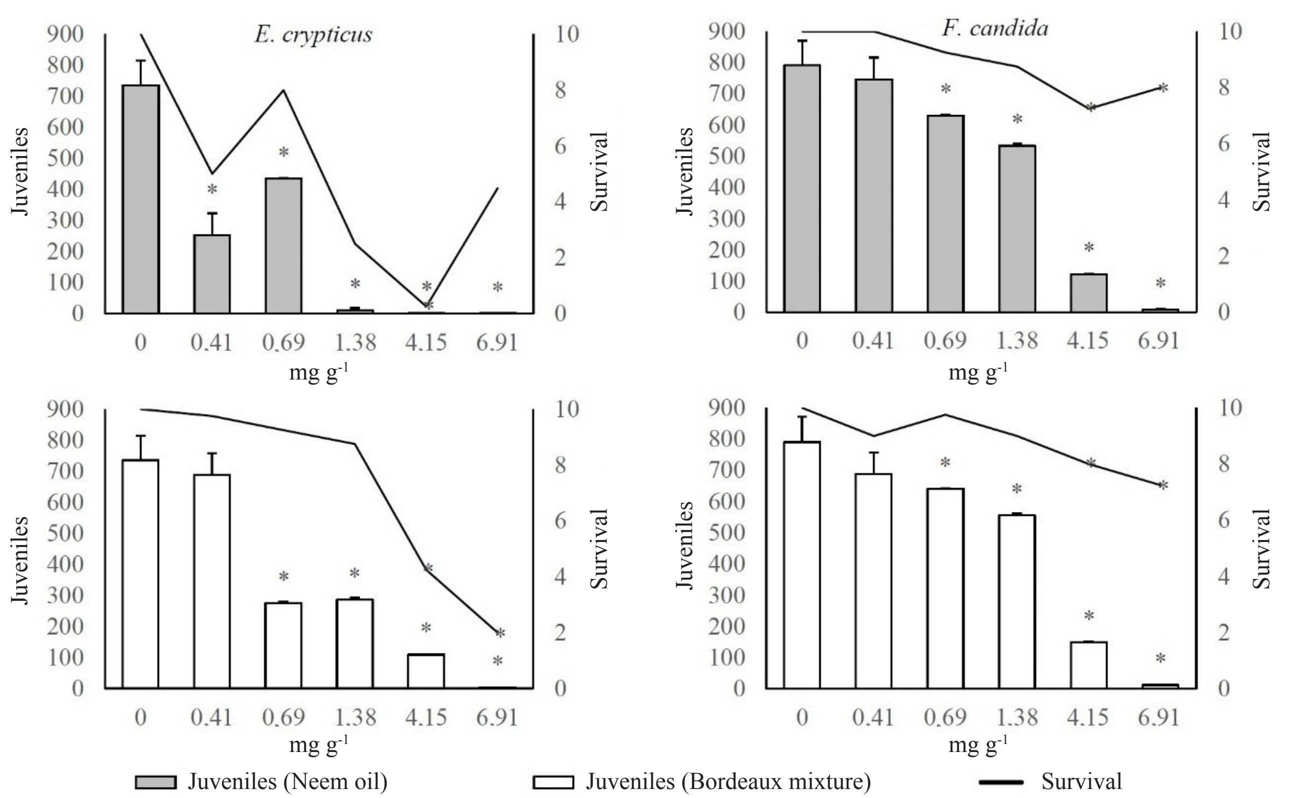
For the effective dose, determined by the exponential model, the doses of the pesticides that affected 50% of the reproduction (EC_{50}) of *E. crypticus* were smaller than those verified for *F. candida*. The effective doses that affected the reproduction of *F. candida* were more than five times higher than those found for *E. crypticus*, even superior to the LOEC. For Bordeaux mixture, the result was similar, with the effective dose for *F. candida* superior by more than three times compared to *E. crypticus*. In this case, the LOEC for *E. crypticus* was lower than the EC_{50} (Figure 2).

Discussion

The effects of Neem oil and Bordeaux mixture on non-target organisms are poorly evaluated, especially in terrestrial ecotoxicology where there are no studies. Probably this is due to a greater focus on conventional pesticides because they have a high spectrum of action and are known to affect target and non-target organisms (Raguraman & Kannan, 2014). Also, it is expected that if there is any impact of pesticides used in organic agriculture on non-target organisms, they are only at the time of application (Bengochea et al., 2013).

This study revealed that only doses greater or equal to 3% (4.15 mg g^{-1}) of Neem oil, which is three times higher than recommended, caused significant mortality of the adult organisms used in the trials. It is known that some insecticides derived from plants cause mild to moderate effect on beneficial and non-target organisms associated with the aerial part of plants, such as parasitoids and predators. Among them, some orders are more sensitive to the active principle of Neem oil, azadirachtins, such as Coleoptera, Hemiptera, Hymenoptera, and Lepidoptera (Raguraman & Kannan, 2014).

However, doses equal or less than the recommended (1%) of the Neem oil affected the reproduction of the tested organisms. The nominal dose evaluation found that 0.69 mg g^{-1} Neem oil per gram of soil affected reproduction for both



* It differs from control by Dunnet test at 5%.

Figure 1. Survival and reproduction of *E. crypticus* and *F. candida* submitted to increasing doses of Neem oil and Bordeaux mixture.

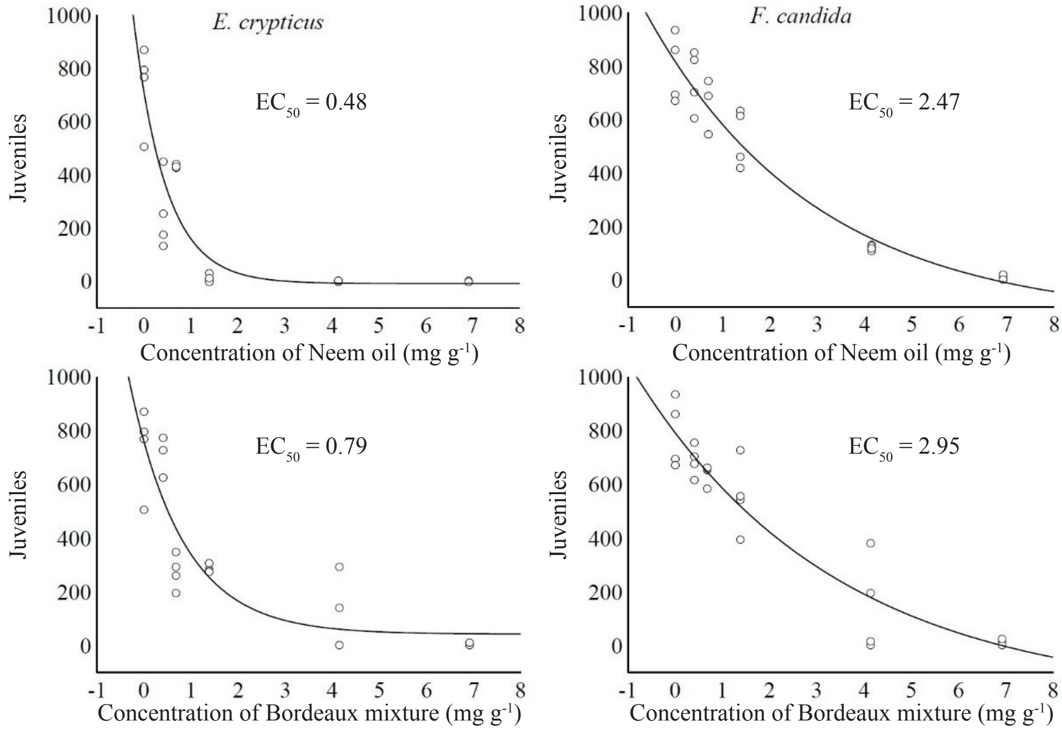


Figure 2. Exponential regression and effective concentration values (EC_{50}) for juveniles of *E. crypticus* and *F. candida* submitted to increasing doses of Neem oil and Bordeaux mixture. Values of the confidence interval of the estimates (CL95%): Neem oil in *E. crypticus* (0.25-0.70) and *F. candida* (0.88 - 4.06); Bordeaux mixture in *E. crypticus* (0.36 - 1.21) and *F. candida* (0.32 - 5.58).

species, but for *E. crypticus*, the effect occurred at lower doses, from 0.41 (LOEC) to 0.48 mg g^{-1} of soil (EC_{50}). Other studies have reported negative effects on non-target organisms. Neem oil mortality was confirmed in juveniles and adults of the non-target predator *Podisus nigrispinus*, depending on concentration applied, and they could be compared to a neurotoxic insecticide and growth inhibitor (Zanuncio et al., 2016). It also verified the toxicity of this oil for adults and nymphs of *Aphis gossypii* (target) and the larva of its natural predator *Cycloneda sanguine* (non-target). Therefore it is necessary to prove the real need of application in the agricultural crop (Souza et al., 2015)

Regarding the Bordeaux mixture, the survival of adults from both organism tests was also significantly affected from the 4.15 mg g^{-1} dose. According to the literature, soil organisms such as springtail, mites, ants and earthworms also had negative effects on the application of Bordeaux mixture in the soil (Pozzebon et al., 2010; Hammad & Gurkan, 2012), since copper is a metal and high doses becomes toxic to soil invertebrates (Ardestani et al., 2013). In the aquatic environment, the copper-based fungicide was also toxic, even at doses lower than 0.1 mg L^{-1}. Thus, it is concluded that this metal exhibits an environmental risk to non-target organisms (Kungolos et al., 2009).

On reproduction, the recommended dose of 1% (0.69 mg g^{-1}) of Bordeaux mixture was the lowest dose that affected reproduction (LOEC) for both organism tests, although the effective dose for *E. crypticus* (0.79 mg g^{-1}) and *F. candida* (2.95 mg g^{-1}) are higher. Copper-based defensive agents generally have negative effects on the reproduction or life cycle of beneficial organisms. In the predator *Chrysoperla carnea*, the Bordeaux mixture induced a small reduction in fecundity when it was residually exposed to larvae or adults (Bengochea et al., 2013).

Another problem associated with the Bordeaux mixture is the possibility of contamination of the soil by copper because it is a heavy metal (Pavlovic, 2011; Gutiérrez-Barranquero et al., 2012). In severe cases, it can lead to problems of phytotoxicity to crops (Pavlovic, 2011). Therefore, Gutiérrez-Barranquero et al. (2012) recommend the application of compounds containing copper should be replaced by an environmentally friendly alternative and compatible with organic farming.

Comparing the sensitivity between the tested organisms, it was showen the toxicity EC_{50} which it is the main endpoint criterium (ABNT, 2010; Ardestani et al., 2013). This difference in sensitivity is provided by the standard and for this reason, it is recommended to use more than one species for testing. Although the *F. candida* clamp exhibits high sensitivity to a wide range of pesticides (Hammad & Gurkan, 2012), their reproduction in this study resulted in EC_{50} values of three to five times greater than *E. crypticus*. This is probably due to the route of exposure to the contaminant, where the springtail is exposed mainly by the dermal route, whereas for oligochaetes the direct intake of soil is greater (ABNT, 2012).

The results of the ecotoxicological tests presented are the first for Neem oil and Bordeaux mixture, revealing that the dose currently used (1%) affects the reproduction of non-target organisms in the soil. However, it is important to note that the tests were conducted in an artificial soil and performed under controlled laboratory conditions. For a complete evaluation of pesticides tocixity and understanding the environmental aspect, studies should be carried out in other tiers, such as greenhouse and field conditions (Pozzebon et al., 2010).

Besides, another important issue to consider is the high biodegradability of these pesticides, and that's why they are constantly applied in the field (Archana et al., 2017). Moreover it is also very important to inform the producers about the rational use of pesticides and to monitor the indiscriminate use of pesticides in the environment.

Conclusions

Neem oil and Bordeaux mixture on tropical artificial soil have a potential toxicity effetcs on non-target organisms.

Under the test conditions, the recommended 1% dose for pest control in most crops causes significant effects on reproduction of organism tested. If proven in additional studies under field conditions, this effect represents a potential impact on the biodiversity of soil organisms and the ecosystem services they provide.

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