

## Ecotoxicological potential of copper-based organic fungicide in non-target soil organisms

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### ABSTRACT

The Viçosa (Blooming) mixture is an important product for pest and disease control in agriculture. It is considered as an alternative fungicide, and no effects are expected when exposed to humans and non-target organisms, which may not be true because it contains copper in its composition and may be applied multiple times in certain crops. Thus, the objective of this study was to verify its ecotoxicological potential through survival and reproduction trials of the invertebrates of the soil *Enchytraeus crypticus* and *Folsomia candida*. Both trials lasted 28 days in a completely randomized design, in tropical artificial (TAS) and natural soils. From the recommended 0.5% concentration of Viçosa mixture, increasing doses equivalent to 0% (control), 0.1, 0.3, 0.5, 1 and 3% were tested. There were effects on survival and reproduction of soil invertebrates on TAS and natural soils from the lowest dose of 0.1%. Therefore, it is concluded that the use of this copper-based mixture presents toxic potential over the life cycle of invertebrates living in the soil, and there is need of new studies that evaluate the effects in natural environment.

**Key words:** terrestrial ecotoxicology, organic fungicide, soil invertebrates

### Potencial ecotoxicológico de fungicida orgânico a base de cobre em organismos não-alvo do solo

### RESUMO

A calda Viçosa é um importante produto de controle de pragas e doenças na agricultura. Considerado como um fungicida alternativo, não se espera efeitos quando expostos ao homem e organismos não alvos, o que pode não ser verdadeiro por conter cobre em sua composição e se aplicado múltiplas vezes em determinadas culturas. Com isso, objetivou-se neste estudo verificar o seu potencial ecotoxicológico através de ensaios de sobrevivência e reprodução dos invertebrados do solo *Enchytraeus crypticus* e *Folsomia candida*. Ambos ensaios duraram 28 dias em delineamento inteiramente casualizado, nos solos artificial tropical (SAT) e natural. A partir da concentração recomendada da Calda Viçosa de 0,5%, foram testadas doses crescentes equivalentes a 0% (controle), 0,1; 0,3; 0,5; 1 e 3%. Verificaram-se efeitos na sobrevivência e na reprodução dos invertebrados do solo, nos solos SAT e natural em ambos os solos, a partir na menor dose de 0,1%. Com isso, conclui-se que a utilização da calda a base de cobre apresenta potencial tóxico sobre o ciclo de vida de invertebrados do solo, o que somente pode ser concretizado com novos estudos que avaliem os efeitos em ambiente natural.

**Palavras-chave:** ecotoxicologia terrestre, fungicida orgânico, invertebrados do solo

## Introduction

In the agroecological perspective, alternative fungicides are important tools in the control of pests and diseases in agriculture, promoting improvements in food production. Their main positive aspects are the reduction of costs and minimum damages to the environment and human health due to their non-toxicity (Penteado, 2001; Fernandes et al., 2006; Pavlovic, 2011, Fernandes, 2013).

Among the less toxic fungicides for man, the copper-based ones were the first to be tested and adapted to Brazilian conditions (Carvalho et al., 2012). The *Viçosa* mixture is an example, improved from the existing Bordeaux mixture, differs in that it contains in its formulation other nutrients beneficial to plants (Schwengber et al., 2007; Aquino et al., 2008). Classified as a “fertiprotector”, it promotes a triple action for the plants, as it provides nutrients in the form of foliar fertilizer, positively influences the metabolic process with increased resistance to diseases and contributes directly to the control of parasites (Carvalho et al., 2012; Fernandes, 2013).

The efficiency of the *viçosa* mixture in disease control is probably due to copper in association with virgin lime in its composition, which have a preventive effect against fungi (Penteado, 2001; Carvalho et al., 2012) that cause some diseases such as pustule, brown eyespot (*Cercospora coffeicola*), early blight (*Alternaria solani*), grey leaf spot (*Cercospora zeae-maydis*) and late blight (*Phytophthora infestans*) (Schwengber et al., 2007). In addition, this mixture has also been applied in the control of invertebrates that are harmful to agriculture, such as the butterfly *Leucoptera coffeela* (Venzon et al., 2013) and the white mite *Polyphagotarsonemus latus* (Venzon et al., 2006).

In general, substances used in organic agriculture are considered safe by society for not presenting toxic potential to non-target organisms and to ecological processes in which they participate. However, this is not always true (Raguraman & Kannan, 2014), especially when these products are not used correctly. In view of this, studies, among others aspects, are necessary to evaluate its ecotoxicological potential, starting from the recommended dose. For these assays, annelids of the genus *Enchytraeus* and colobus of the species *Folsomia candida* can be used. Both are ecologically relevant because they are present in high population densities in the soil of the world, have high skin permeability and participate actively in soil decomposition processes (Fountain & Hopkin, 2005; ABNT, 2012).

Therefore, considering that alternative copper-based fungicides, such as the *Viçosa* mixture, do not cause negative effects on non-target biota organisms, the objective of this study was to verify its ecotoxicological potential on survival and reproduction in *Enchytraeus crypticus* and *Folsomia candida*, on artificial and natural soil.

## Material and Methods

The *Viçosa* mixture fungicide was evaluated by means of ecotoxicological tests contained in the norms NBR ISO 11267 (ABNT, 2011) and NBR ISO 16387 (ABNT, 2012). The tests

were carried out in two soils: tropical artificial soil (TAS) consisting of 70% fine industrial sand, 20% kaolinite clay and 10% coconut fiber (ABNT NBR 15537, 2014); natural soil is a Red-Yellow Argisol collected in the superficial layer (0-20 cm), consisting of 36.6% sand, 56.5% of clay and 6.9% of silt and pH<sub>(water)</sub> 5,6. In the laboratory, the natural soil was initially sieved in a 2 mm mesh and subjected to two cycles of freezing and thawing, 24 hours each, aiming at the complete elimination of organisms from the edaphic fauna.

The mixture was prepared based on the recommendations of Fernandes et al. (2006), composed of 22.7% of virgin lime, 22.7% of copper sulfate PA (CuSO<sub>4</sub>), 9,1% of boric acid PA (H<sub>3</sub>BO<sub>3</sub>), 9,1% of zinc sulphate PA (ZnSO<sub>4</sub>) and 36.4% of magnesium sulfate PA (MgSO<sub>4</sub>). Increasing doses were tested, based on the 0.5% recommendation for most cultures (Fernandes et al., 2006), being 0% (control), 0.1%, 0.3%, 0.5%, 1% and 3%, corresponding to 0.15, 0.44, 0.73, 1.46 and 4.37mg g<sup>-1</sup> for the artificial soil and 0.12, 0.36, 0.59, 1.19 and 3.56mg g<sup>-1</sup> for the natural soil, respectively. The difference of concentration in mg g<sup>-1</sup> between soils is due to the distinct maximum retention capacity, which was determined to 65%.

The experiments were carried out in a completely randomized experimental design with five replications. A sixth replication of each treatment without food and organisms was used to assess the pH and the moisture at the end of the test. These were conducted in incubation chamber with control of temperature (18 to 22°C), photoperiod (16 hours of light for 8 hours of darkness) and luminous intensity (400 to 800 lux). The soil moisture was maintained constant throughout the experiment. The pH of the artificial soil was corrected to 6.0 ± 0.5 by the addition of CaCO<sub>3</sub>.

The *F. candida* individuals were raised on a substrate consisting of gypsum and activated charcoal (8: 1) at 20 ± 2°C. Transparent cylindrical vessels (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 of life were added. Weekly aeration was promoted and addition of 2 mg food. The assay lasted 28 days. For the counting of adult and juvenile individuals, the soil containing the organisms was placed in a larger container containing water and a few drops of stamp paint. Adult subjects were counted visually and juveniles were counted manually in photographs in ImageToll 3.0 software.

*E. crypticus* individuals were raised in petri dishes containing agar media at 20 ± 2° C. Transparent cylindrical containers (40 mL) were capped, containing 30 g of moist soil and 50 mg of oats in fine flakes (feed). In each vessel, researchers put 10 ovate individuals, selected and collected in a stereomicroscope. Weekly, the pots were opened for aeration and for adding 25 mg of food. The assay lasted 28 days. At the end, the containers were filled with 1% Bengal Rose solution of ethanol, which promoted the staining of the organisms and facilitated the counting of adults and juveniles under stereomicroscope.

The data obtained regarding reproduction and survival were submitted to analysis of variance and then to the Dunnett test at 5%, for the calculation of the concentration of non-observed effect (CNOE - higher dose that does not cause

effect on reproduction) and concentration of observed effect (COE - lower dose that causes effect on reproduction). The EC<sub>50</sub> values (effective concentration causing effects in 50% of juveniles) were obtained by using the non-linear exponential model ( $p < 0.01$ ).

## Results

The ecotoxicological tests were validated according to the criteria of the cited standards. For *F. candida* in artificial and natural soil, respectively, the mortality in the control was 15% and 6%, the average number of juveniles was 315 and 592 and the coefficient of variation was 13.6% and 20.4%. For *E. crypticus* in artificial and natural soil, respectively, the mortality in the control was 6% and 12%, the average number of juveniles was 149 and 393 and the coefficient of variation was 33.0% and 9.1%. The pH variation between the beginning and end of the trials for both soils did not exceed one unit.

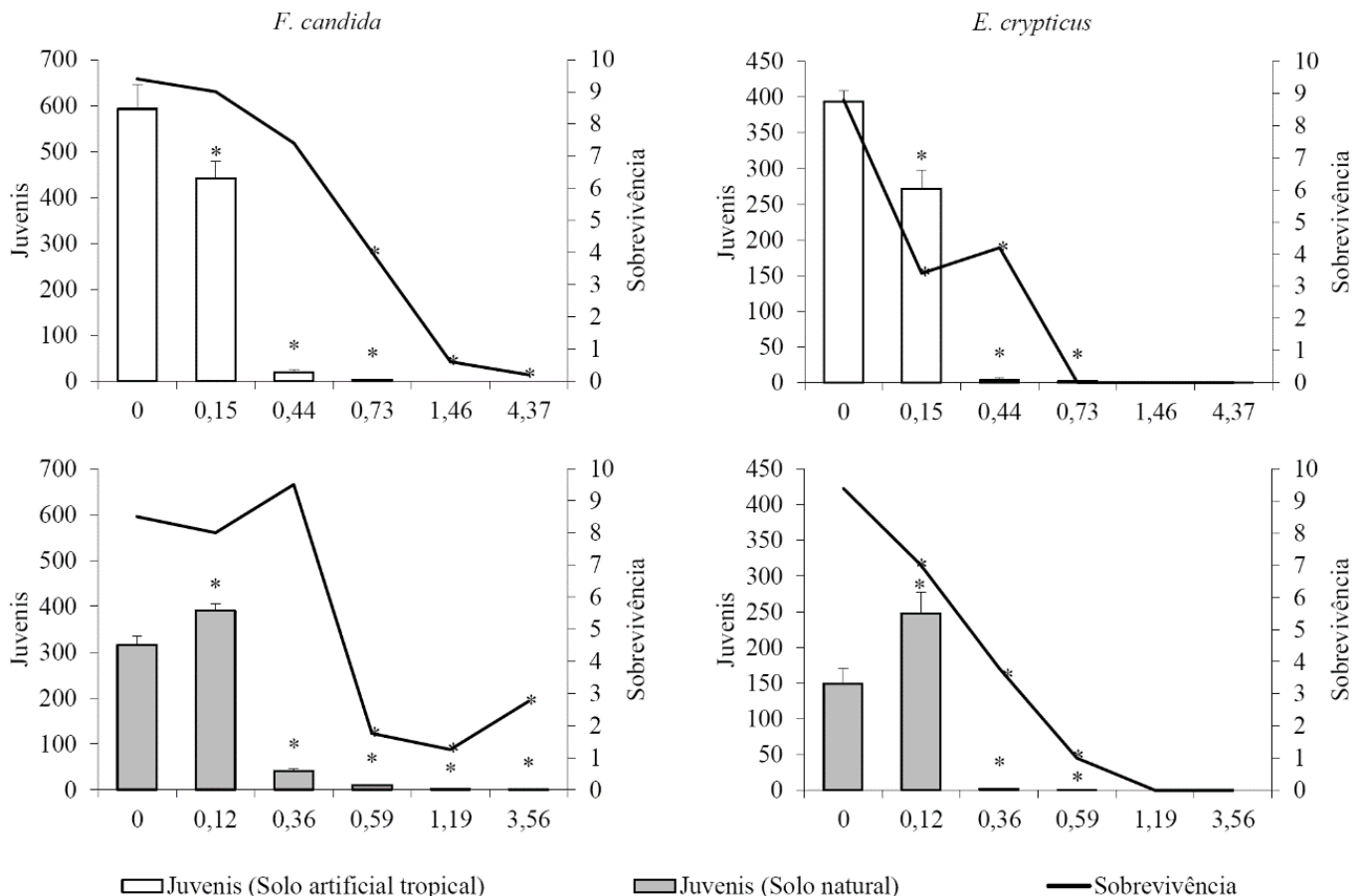
The *Viçosa* mixture proved to be capable of affecting the survival of *F. candida* and *E. crypticus* in the artificial and natural soil. The effects on the survival of *F. candida* were observed from the dose 0.73 mg g<sup>-1</sup> for the artificial soil and 0.59 mg g<sup>-1</sup> for the natural soil, corresponding to the dose of 0.5%. For *E. crypticus* a reduction in survival was observed from the first dose evaluated in both soils (Figure 1).

The reproduction of both organisms is similarly affected by *Viçosa* mixture, presenting variations according to soil type. The number of juveniles of *F. candida* and *E. crypticus* presented differences in relation to the control starting from the first dose, with reduction in the artificial soil and increase in the natural soil (Figure 1). For the artificial soil there is no concentration of non-observed effect (CNOE) and it is not possible to determine precisely the lowest dose that causes effect on reproduction (COE), being necessary the evaluation of doses lower than 0.1%. For the natural soil, even with differences in relation to the control, it can be considered that the CNOE of *Viçosa* mixture corresponds to 0.12 mg g<sup>-1</sup> and the COE corresponds to 0.36 mg g<sup>-1</sup>, which correspond to 0.1% and 0.3% respectively.

The evaluation of the effective concentration that causes effects in 50% of juveniles (EC<sub>50</sub>) also showed similar results for *F. candida* and *E. crypticus*. There was a slight difference between the soils used, with values varying between 0.17 and 0.23 mg g<sup>-1</sup>, which are intermediate concentrations between CNOE and COE for the natural soil (Figure 2).

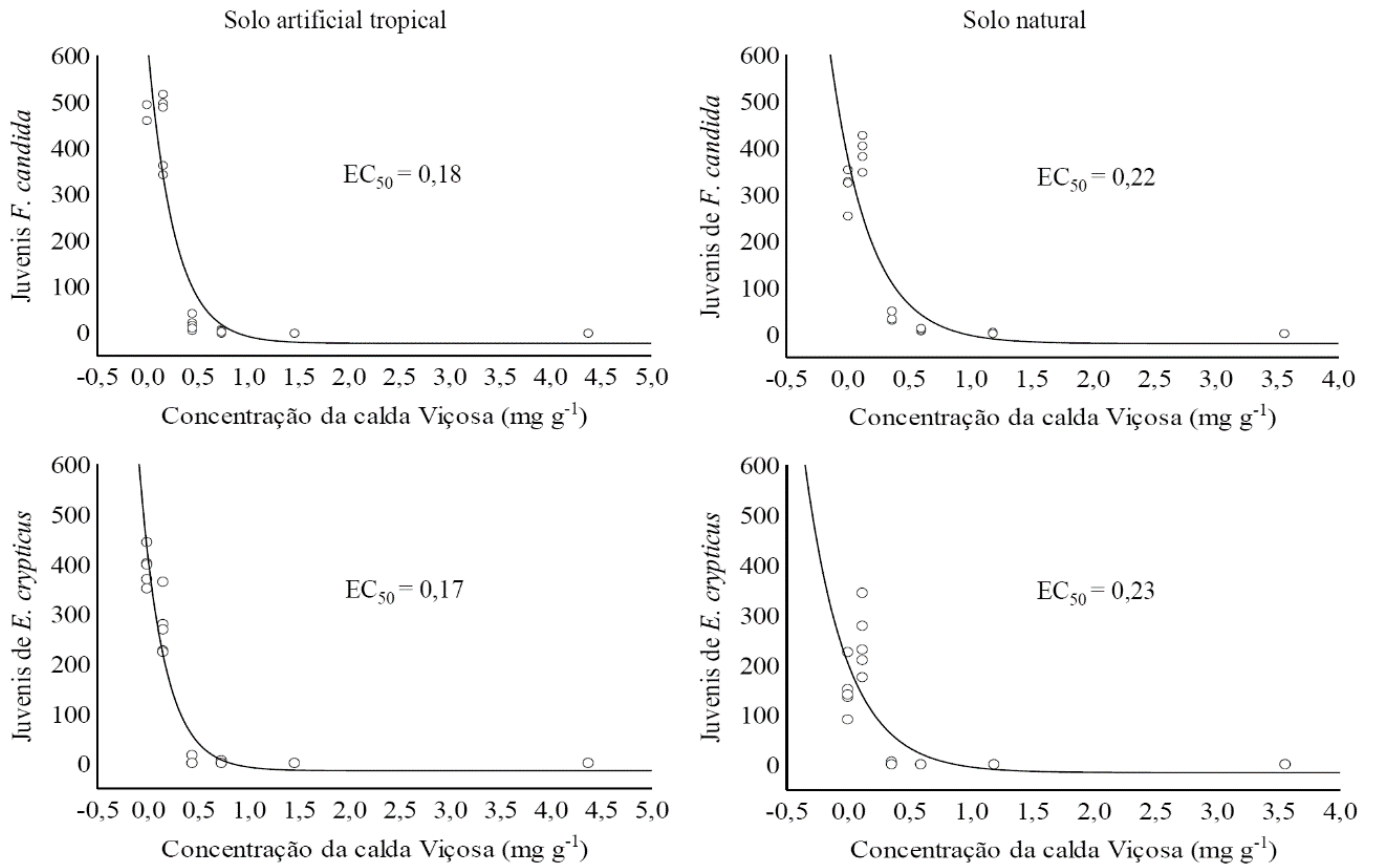
## Discussion

There are few studies on the toxic effects of *Viçosa* mixture on invertebrates, and the existing ones are focused on the control of organisms considered pests in agricultural crops



\* It differs from the control according to Dunnett test, at 5%

**Figure 1.** Survival and reproduction (juveniles) of *F. candida* and *E. crypticus* in tropical artificial and natural soil, submitted to increasing doses of *Viçosa* mixture



**Figure 2.** Exponential regression of effective concentration ( $EC_{50}$ ) for reproduction (juveniles) of *F. candida* and *E. crypticus* in artificial and natural soil, submitted to increasing concentrations of Viçosa mixture. Values of the regression confidence interval for: *F. candida* in artificial (0.12 – 0.24) and natural soil (0.10 – 0.35); *E. crypticus* in artificial (0.12 – 0.22) and natural soil (0.04 – 0.42)

(Venzon et al., 2006; Venzon et al., 2013). This is probably related to what is expected of alternative fungicides, that is, little or no impact on non-target organisms (Bengochea et al., 2013). However, the copper present in the Viçosa mixture composition, when at high doses, has toxic potential for soil invertebrates (Amorim et al., 2005). Therefore, it is necessary to investigate the possible adverse effects of this mixture on organisms (Venzon et al., 2006).

The effects of Viçosa mixture on the survival of *F. candida* and *E. crypticus*, even at lower doses than the recommended, which is 0.5%, shows that this alternative fungicide has great potential to cause direct impacts on non-target organisms and, consequently, on the ecosystem services performed by these. Although *F. candida* is one of the most sensitive species to different types of pesticides, including copper-based fungicides (Hammad & Gurkan, 2012), *E. crypticus* showed to be more sensitive to the Viçosa mixture, since it presented decreased survival from the first concentration evaluated, of 0.1%. Greater sensitivity of species of *Enchytraeus* was also observed by Amorim et al. (2005) when subjecting organisms to copper. This is probably due to the exposure route to the contaminant agent, in which the springtails are exposed mainly by the dermal route, whereas the oligochaetes have a greater direct intake of soil (Lanno et al., 2004; ABNT, 2012).

The reproduction of both organisms was similarly affected by the Viçosa mixture, converging to similar values of CNOE, COE and  $EC_{50}$  and lower than the recommended dose. Copper-

based fungicides have generally caused negative effects on the reproduction or life cycle of beneficial organisms (Bengochea et al., 2013). In the predator *Chrysoperla carnea* the cupric fungicide caused a small reduction in fecundity when it was residually exposed to larvae or adults (Bengochea et al., 2014).

In evaluating the reproduction of *E. crypticus* and *F. candida*, there was a small difference in the response for the artificial and natural soil, in which the nominal concentration of the mixture that caused a decrease in the number of juveniles corresponded to the concentration of 0.1 and 0.3%, respectively. However, the values of  $EC_{50}$ , which is the main toxicity criterion for these organisms, presented very similar values, corresponding to a dose between 0.12 to 0.19%. The use of a natural soil in the ecotoxicological assay helps to determine the existence of interaction between the evaluated substance and the soil matrix, approaching the representativeness of the results to what would occur in the natural environment (ABNT NBR ISO 17616, 2010). Thus, according to the data obtained, although both soils are predominantly sandy, it can be stated primarily that the effects of Viçosa mixture on the reproduction of the organisms depend little on the characteristics of the soil. However, for a more assertive statement, there is need of new tests with soils with different textural characteristics.

The results of the ecotoxicological tests presented are the first for Viçosa mixture. It is important to emphasize that

they come from tests that were conducted under controlled laboratory conditions. For a complete understanding of the influence of the environmental aspect on the toxicity of this mixture, it is necessary to carry out new tests that include other phases, such as greenhouse and field (Pozzebon et al., 2010). Nevertheless, the results show that the recommended dose of 0.5% for most crops has great potential of affecting the survival and reproduction of non-target organisms in the soil. In view of this, there is the concern of applying this product in nature, which can be in higher doses than the recommended, as of 2% for the control of larvae of *Leucoptera coffeella* (Venzon et al., 2013) or a high frequency of applications, which occurs to prevent fungi, with biweekly intervals in some vegetables (Fernandes, 2013).

## Conclusions

The use of the copper-based mixture (*Viçosa*) presented a toxic potential to non-target organisms of the soil.

At doses equal to or lower than the recommended (0.5%) there were significant effects on the survival and reproduction of these organisms, which represents a direct impact on the life cycle and ecosystem functions of soil organisms. However, for the purpose of proving these results, new studies should be carried out to evaluate the effects of this fungicide in a natural environment.

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