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Side effects of copper fungicides on *Amblyseius cucumeris* by laboratory bioassays

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Abstract

The predatory mite, *Amblyseius cucumeris* (Oudemans) (Acari Phytoseiidae), is an important biological control agent of thrips and pest mites in many various crops. To understand the side effect of copper fungicides on *A. cucumeris*, the toxicity of three copper fungicides, namely 77% copper hydroxide WP, 30% DT (copper succinate + copper glutarate+ copper adipate) WP and 95% copper gluconate, on the predatory mite were determined using "Slide-dip immersion method" in the laboratory. The results showed that the LC_{50} of 77% copper hydroxide WP, 30% DT WP, and 95% copper gluconate to *A. cucumeris* was 16.5032×10^3 mg/l, 19.6118×10^3 mg/l, and 92.3287×10^3 mg/l, respectively; based on the toxicity rating and the recommended field concentration, it was suggested that the three copper formulations had low or moderate toxicity to *A. cucumeris*.

Key words: copper fungicide, copper gluconate, toxicity, *Amblyseius cucumeris*.

Introduction

The predatory mite, *Amblyseius cucumeris* (Oudemans) (Acari Phytoseiidae), is an important biological control agent of various species of insect pests and mites, such as the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouse vegetables and ornamentals (Gillespie, 1989; Larsen *et al*., 1998; Williams, 2001; van Houten *et al*., 2005). The *A. cucumeris* has becomes one of the most widely used bio-control agents due to the success of large-scale commercial production. In China, It is also called a "biological pesticide" (Zhang *et al*., 2002). Moreover, the field studies suggested that *A. cucumeris* is a desirable bio-control agent to control the important citrus pest mites, *Panonychus citri* (McGegor) (Acari Tetranychidae) and *Phyllocoptruta oleivora* (Ashmead) (Acari Eriophyidae) (Zhang *et al*., 2003; 2004).

Copper compounds have been widely used in citrus pest management (McCoy *et al*., 2010), especially for control of citrus fungal diseases (Albrigo *et al*., 2005), like melanose, greasy spot and canker etc. (Schutte *et al*., 1997). For example, copper-based fungicides are a standard control measure worldwide for controlling citrus canker (Leite and Mohan, 1990; Das, 2003). Citrus is an important economic fruit crop in southern China. Numerous species of insects, mites and disease pathogens are being threatened its production. Copper hydroxide, a common inorganic copper fungicide, was found to be very effective in controlling the disease. Organic copper fungicides, such as DT, Copper ammonium (Chen, 1998), cupric acetate (copper acetate) (Chen, 2008), copper abietate etc were also used to control the citrus canker.

Integrated pest management programs (IPM) require pesticides to be effective against target species and at the same time to be relatively harmless to non-target parasitic and predatory arthropods (Lee *et al*., 2002). Evaluation of the effects of pesticides on natural enemies and other non-target organisms is an essential requirement in IPM programs (Kavousi and Talebi, 2003). Most fungicides often have very subtle toxic effects on predacious mites and they must be thoroughly evaluated before they are implemented in IPM programs (Bostanian *et al*., 2009).

Compared with conventional insecticides and acaricides, copper fungicides were considered to be more compatible with the natural enemies of pests (e.g. Metcalf, 1980). However, various species of predatory mites responded differently to a certain copper formulation. Copper hydroxides is the harmless to some predatory mites (Bernard *et al*., 2004), but its adverse impacts were found on the predatory mite, *Agistemus industani* Gonzalez (Childers *et al*., 2001). Concerning other predator such as the coccinellid beetles, laboratory study conclude that copper-sulfate fungicides are unlikely to disrupt biological control processes in citrus groves that rely on the coccinellid beetles, *Curinus coeruleus* Mulsant, *Harmonia axyridis* (Pallas), and *Olla v-nigrum* (Mulsant) (Michaud *et al*., 2003), whereas copper fungicide formulation reduced predation of *Halmus chalybeus* (Boisduval) larva on *Ceroplastes destructor* Newstead by 70-90% compared with untreated citrus leaves (Lo and Blank, 1992).

Our overall goal of the study was to determine the compatibility of copper fungicides with *A. cucumeris* in laboratory conditions. The specifically objectives were to 1) test the LC_{50} of 77% copper hydroxide WP, 30% DT, and 95% copper gluconate on *A. cucumeris* in laboratory conditions; 2) made the comparison of the toxicity of three copper fungicides to *A. cucumeris*. The results will provide a fundament basis for the use of copper-based fungicides in bio-control orchards with mite predators, which may eventually improve the prevention and treatment of citrus diseases and insect pests.

Materials and methods

The predatory mite

A susceptible strain colony of *A. cucumeris* was reared and maintained with *Tetranychus viennensis* Zacher collected from apple trees in He'yang, Shaanxi, China in rearing room conditions (26 ± 2 °C, 60 ± 10% RH and a photoperiod of 16L:8D), original population was provided by the Institute of Plant Protection of Fujian Academy of Agricultural Sciences, China.

Experiment design and treatments

77% copper hydroxide wettable powder (WP) (DuPont Agricultural Chemicals, Shanghai) and 30% DT (copper succinate + copper glutarate+ copper adipate) is representative of inorganic and organic copper fungicides widely used in China to control the plant disease. Copper gluconate was designed as a potential copper fungicide in this study for evaluation of its use in plant protection. Copper gluconate, containing the inorganic acid radical gluconic, is safe for the environment and plants, and capable of promoting Cu^{2+} absorption and diffusion. The experiment was designated to have following treatments:

a). 77% copper hydroxide WP: KOCIDE® 101, containing 50.16% metallic copper; dissolved in distilled water at five concentration: 20, 10, 5, 2.5, and 1.25×10^3 mg/l;
b). 30% DT WP: 9.88% metallic copper was dissolved in distilled water at five concentrations: 20, 10, 5, 2.5, and 1.25×10^3 mg/l;
c). 95% copper gluconate: water soluble powder (Cinochem, Beijing), 13.30% metallic copper were dissolved in distilled water at five concentrations: 8, 4, 2, 1, and 0.5×10^3 mg/l;
d). Distilled water was used as the control.

Experimental methods

The toxicity experiment was conducted using the "Slide-dip immersion method" recommended by FAO (Busvine, 1980). Briefly, double-sided sticky tape (Wingtai Co. Ltd., Zhongshan) was cut into 2cm-long pieces, and attached to one end of the glass slide. Healthy adult female *A. cucumeris* mites were gently glued to the tape on their back by using a small brush. The work carefully was to avoid sticking their feet, mouthparts, and pedipalps. A total of 20 *A. cucumeris* adult female mites in several rows were glued on each slide. Subsequently, slides with mites were placed on big porcelain containing a large sponge filled with water. After 24 h, the slides were examined under binocular microscope, and the dead or injured individuals were replaced to keep the 20 mites on each slide. The slides were then immersed and gently shaken for 5 seconds in copper fungicide solutions at different concentrations. After excess liquid was removed by absorbent paper, the slides were covered by translucent plastic film and placed on porcelain plates. 24h later, the number of dead mites was counted under binocular microscope; a mite was determined as dead if it showed no reaction to gentle touch from a small brush. Each concentration was replicated 4 times (four slides), with 20 mites per slide, for a total of 80 mites per concentration.

Analysis and calculation of LC_{50}

The corrected mortality (%) was calculated by the formula according to Abbott (1925): (mortality in treatment − mortality in control) / (1 − mortality in control) × 100.

The variance of mortality percentage after arcsine transformation were compared using one-way ANOVA and Tukey multiple range tests (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL, USA), with a significance level of a = 0.05. For calculation of LC_{50}, probit analysis were performed using SPSS 13.0 for Windows.

The grading criteria for pesticide's toxicity on predatory mites were assigned based on IOBC toxicity ratings (Sterk *et al*., 1999): low toxicity: percentage of mortality was 0-25%, moderate toxicity: 25-50%, high toxicity: 50-75%, and severe toxicity: > 75%.

Table 1. Mortality of *A. cucumeris* females at the treatments of the three copper fungicides. 80 *A. cucumeris* for each concentration (4 replicates with 20 mites per slide).

Fungicides	Concentration (10^3 mg/l)	Mortality ± SE (%)	Grade of toxicity
77% copper hydroxide	20	59.46 ± 1.39 a	High
"	10	29.73 ± 1.45 b	Moderate
"	5	18.92 ± 1.06 cd	Low
"	2.5	9.46 ± 0.93 ef	Low
"	1.25	4.05 ± 0.55 f	Low
30% DT WP	20	52.78 ± 1.42 a	High
"	10	30.56 ± 1.34 b	Moderate
"	5	16.67 ± 1.63 cd	Low
"	2.5	9.72 ± 1.13 ef	Low
"	1.25	5.56 ± 0.81 f	Low
95% copper gluconate	8	21.92 ± 1.85 c	Low
"	4	21.92 ± 1.67 c	Low
"	2	15.07 ± 0.80 de	Low
"	1	12.33 ± 1.48 de	Low
"	0.5	4.11 ± 0.80 f	Low

Means within the same columns followed by the same letter are not significantly different (P > 0.05) using Tukey test.

Results

Laboratory test results showed that the corrected mortality (%) of *A. cucumeris* treated by 77% copper hydroxide WP with concentration from 20 × 10^3 mg/l to 1.25 × 10^3 mg/l ranged from 29.73% to 4.05%. Based on toxicity standard rating, dilutions at 20 × 10^3 mg/l and at 10 × 10^3 mg/l were scored as high and moderate toxicity, respectively, with the other dilutions corresponding to low toxicity (table 1). Similar to the treatments by 77% copper hydroxide WP, the corrected mortality (%) at the treatment of 30% DT with concentration from 20 × 10^3 mg/l to 1.25 × 10^3 mg/l ranged from 52.78% to 5.56%; only dilution at 20 × 10^3 mg/l was highly toxic, with the other dilutions showing moderate or low toxicity (table 1). All dilutions of 95% copper gluconate from 1 × 10^3 mg/l to 8 × 10^3 mg/l showed low toxicity to *A. cucumeris*. The corrected mortality of *A. cucumeris* ranged from 4.11% to 21.92% (table 1).

The Log concentration-probit regression curve of toxicity on *A. cucumeris* female adult mites was generated based on probit analysis shown as figure 1. The toxicity of 77% copper hydroxide WP, 30% DT WP, and 95% copper gluconate on *A. cucumeris* adult females, LC_{50} was determined to be 16.5032 × 10^3 mg/l, 19.6118 × 10^3 mg/l, and 92.3287 × 10^3 mg/l, respectively. These results indicated that 95% copper gluconate was least toxic to *A. cucumeris*, followed by 30% DT. In comparison, 77% copper hydroxide WP showed the highest toxicity.

Discussion

In this laboratory bioassay, all dilutions of the three copper fungicides had low or moderate toxicity to *A. cucumeris*, except that 20 × 10^3 mg/l copper hydroxide and 20 × 10^3 mg/l 30% DT WP had high toxicity. The maximum recommended concentration limit of both copper hydroxide (KOCIDE® 101, WP) and 30% DT WP are 2 × 10^3 mg/l. Based on our results, the three copper fungicides are safe to *A. cucumeris* at the recommended field concentration. Similar results were reported about low toxicity cuprous oxide to *A. cucumeris* (Chen *et al*., 2007). Copper hydroxide has no effect on predatory phytoseiid mites in the field (Bernard *et al*., 2004). However, few literature data is available about effects of 30% DT and 95% copper gluconate on predatory mites. Because of its short generation time and high reproduction rate, *A. cucumeris* only needs seven to eight days to produce one generation with adequate food at 25 °C (Gillespie and Ramey, 1988), potentially allowing rapid recovery in the field from the moderate toxicity of these fungicides is very important.

Due to the different strains and other factors, it needs to be confirmed in field conditions that toxicity of these three copper fungicides to *A. cucumeris*. Insects only make sporadic contact with these deposits while foraging, as opposed to the continuous contact they had with coated surfaces in experiments (Michaud *et al*., 2003). On the other hand, Pozzebon *et al*. (2010) presented evidence to indicate that the impacts of copper on the predatory mites, *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) can be mediated by availability of plant pathogens as alternate food resource.

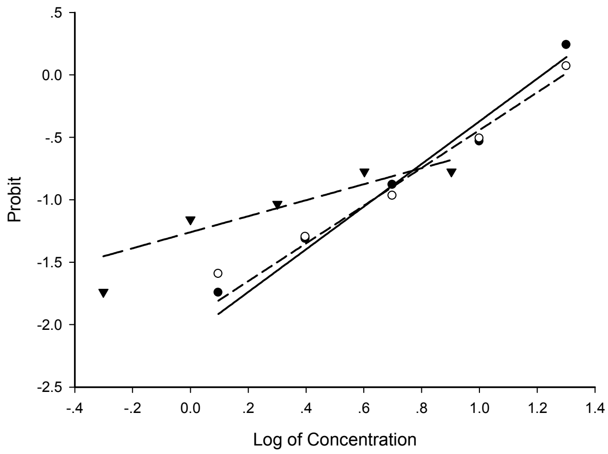

Figure 1. The concentration-probit (mg/ml) curves of the three copper formulations against *A. cucumeris*. 77% copper hydroxide WP: $Y = -2.0806 + 1.7088x$ (P = 0.663; solid ●); 30% DT WP, $Y = -1.9545 + 1.5122x$ (P = 0.882, short dash ○); 95% copper gluconate: $Y = -1.2600 + 0.6411x$ (P = 0.743, long dash ▼).

Finally, this study provided useful and definite information that the three copper formulations had low or moderate toxicity to *A. cucumeris*. 95% copper gluconate, similar to 77% copper hydroxide WP and 30% DT that have been widely used in citrus, is also an effective fungicide to control citrus canker. In summary, these three copper fungicides can be applied to control citrus canker in bio-control citrus orchards relied on *A. cucumeris*.

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