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Toxicity of Biorational Insectoacaricides to Cassava Red Spider Mite *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) and its Phytoseiid Predator *Amblyseius longispinosus* Evans (Acari: Phytoseiidae)

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ABSTRACT

The minimum effective concentrations of biorational insectoacaricides such as abamectin and emamectin benzoate on the female adults of *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) was investigated *in vitro*. Diluted concentrations for each biorational insectoacaricides were prepared by a factor of 10: 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, and 1,000 mg of active ingredient/L of solution. The adulticidal bioassay revealed that 100 % mortality of the female adults of *Tetranychus kanzawai* could be achieved at concentrations of 100 mg/L and 0.1 mg/L of emamectin benzoate and abamectin, respectively. The residual toxicity test revealed that emamectin benzoate is highly persistent with a residual toxicity of 28 days compared to 1 mg/L abamectin in which its residual toxicity wears off after 24 hours of treatment. However, the 100 mg/L emamectin benzoate was highly toxic for the adults of *Amblyseius longispinosus*, whereas 1 mg/L abamectin showed no toxicity under laboratory conditions.

Keywords: *Tetranychus kanzawai*; *Amblyseius longispinosus*, biorational insectoacaricides

INTRODUCTION

Spider mites are serious pest with over 1,300 species which cause considerable losses in yield of economically important crops (Migeon & Dorkeld, 2006-2018; Van Leeuwen et al 2015). Its management has required the creation of cutting-edge technology for millennia due to the need to feed a growing global population and handle the long-standing risks of mite-borne plant diseases (Godfray et al 2010). Although there are already existing several techniques and technologies for pest mite control, including the use of biological control, host plant resistance, cultural controls, and biopesticides, chemical control remains an integral part of many crop-pest-geography scenarios (Lacey 2016; Shin et al 2020; Miyazaki et al 2012; Merton et al 1995; Sundaram & Sloane 1995).

The Tetranychidae family includes the spider mites, a widespread arthropod pest. In Indonesia and the Philippines, cassava red spidermite (*Tetranychus*

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kanzawai) is one of the most prevalent mite pests (Sanjaya et al 2013). It is a polyphagous species that infest various plants, including crops. In East and South Asia, it infests cassava and papaya plants (Gavarra 1981) and hundreds of other species, including strawberries, peppers, tomatoes, potatoes, beans, and corn (Takafuji et al 2005).

They devour the chloroplasts on the underside of the leaf, leaving white or yellowish stippling on the upper surface. Stippling coalesces into brownish lesions as mite feeding continues (Cheng et al 2009). Heavy mite infestation eventually leads to withering and defoliation, which further lowers plant development resulting to the plant's diminished photosynthetic activity.

A predacious mite, *Amblyseius longispinosus* is one of the promising predators of spider mites (Hamamura 1987; Mori & Saito 1979). It serves as a biological control agent for *Tetranychus kanzawai* (Vasquez 1994; Hamamura 1987), *Tetranychus urticae* (Mori & Saito 1979), *Eotetranychus cendanai* (Thongtab et al 2001), *Aponychus corpuzae* (Zhang et al 1998), and *Schizotetranychus nanjingensis* (Zhang et al 1998). However, since they are generally susceptible to pesticides, their effectiveness in controlling the population of spider mites might be reduced in agricultural ecosystems that use many chemical pesticides (Zhao et al 2013). Predatory mites should be given a considerable attention nowadays because of the development of acaricide-resistant spider mites (Xu et al 2018; Fotoukkiaii, 2020; Namin et al 2020; Simma et al 2020; Maeoka et al 2021).

Abamectin and emamectin benzoate are the only biorational insectoacaricides that are approved by FPA for commercial use. Several studies have been conducted on their effects on certain phytophagous and predatory mite species, however there are still no research on cassava red spider mites *T. kanzawai* and the predatory mite *A. longispinosus* in the Philippines or other countries. Considering the importance of *A. longispinosus* as a biological control agent of *T. kanzawai*, this study assesses the minimum effective concentration of emamectin benzoate and abamectin recommended for controlling spider mites and its toxicity to *A. longispinosus*. This information is critical for constructing management plans targeted at the conservation and enhancement of biological control agents and ensuring the effectiveness of cassava IPM programs.

MATERIALS AND METHODS

Mass Rearing

Cassava Red Spider Mite (*Tetranychus kanzawai* Kishida)

The cassava red spider mite *T. kanzawai* colonies were obtained from susceptible varieties of cassava plants that have not been treated with pesticides. Cassava leaves infested with spider mites were transported to the Pest Management and Natural Products laboratory in polyethylene bags and reared on one 2-month-old potted cassava (Rayong/NSIC Cv 30) in the screenhouse. The mites produced were used for the adulticidal bioassay.

Predatory Phytoseiid Mite *(Amblysieus longispinosus* Evans)

Field collected adults and nymphs of the predatory mite *Amblyseius longispinosus* were brought to the laboratory and were used for the initial culture for mass rearing using the technique of Vasquez (1994). The species was identified based on its long hairs and smooth dorsal shield. Another distinctive feature is the single microseta on Leg IV of the basitarsus (Gapud & Raros 1986).

Cassava leaflets heavily infested with red spider mites were placed in a plastic rearing tray. Layers of wire rack were placed on top of the leaf of mite-infested cassava leaves and adults of *Amblyseius longispinosus* were introduced and allowed to reproduce. After two days, a new set of freshly taken mite-infested leaves was placed on the wire rack allowing the mixed culture to transfer to the freshly provided host. One-week-old adults were used for the toxicity tests. Adult predators were identified based on their thicker body walls relative to the younger ones. The female adults are less than 0.5 mm long and pear-shaped, whereas males are slightly smaller with narrower body than females and appear almost triangular with tapering idiosoma (Vasquez 2016). Both males and females are translucent and turn peach after feeding, the latter is slightly darker (Vasquez 2016).

Adulticidal Activity on Female Adults of *Tetranychus kanzawai*

The acaricides were evaluated for their toxicity against the female individuals of *T. kanzawai*. It was performed following the fourth method of the series of methods for susceptibility tests of the Insecticide Resistance Action Committee (IRAC 2009). Female mites were used because they are the least sensitive (Vasquez 1994). Thus, the dosage toxic to them is also toxic to immature mites. Female cassava red spider mites are carmine red in color with dark marks on the sides while the males are lighter, orange red (Corpuz-Raros 1986). Diluted concentrations for each of the acaricides were prepared by a factor of 10: 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, and 1,000 mg of active ingredient/L of solution and distilled water for the control. The experimental unit was a cassava leaf disc a 30-mm in diameter, immersed for 5 seconds in the acaricide solution or water. Twenty female mites were placed on each cassava leaf disc laid on a Petri dish (160 mm x 10 mm) lined with saturated tissue paper. This experimental unit represented one replicate. Each treatment had three repetitions consisting of 20 individuals per repetition and a total of 60 mites in each acaricide concentration. The number of live and dead mites was quantified every 24 hours for three days. Mites were considered dead if no movement was seen for 5 seconds after poking with a No. 000 brush (Helle & Overmeer 1985). The concentration range that produced 0 to 100 % mortality was determined for each acaricide.

Determination of Residual Toxicity

The residual toxicity of the acaricides was determined using the methods of Cocco and Hoy (2008). Using a hand-held sprayer, one-month-old potted cassava plants were sprayed with emamectin benzoate (Proclaim Opti) and abamectin (Agriguard) until approximately 90 % of the adaxial and abaxial surface of the leaf

was covered. The minimum effective concentrations (MEC) and concentrations that are ten times higher than the MEC were used (Table 1). One hour after spraying, leaves were taken randomly from the treated plants and placed at the top (with the abaxial surface upward) of a Petri dish lined with moist tissue paper. Twenty female mites were introduced to each leaf sample, and the number of dead individuals was counted after 24 hours. Another set of treated leaves was taken 24 hours after spraying and again used to test for the presence of acaricide residues. This process was repeated every 24 hours until the mite mortality due to acaricide residues in the experimental group was no longer observed. Three replications were used for each treatment.

Table 1. Acaricide treatments for the residual toxicity experiment

TREATMENTS	CONCENTRATIONS (mg/L)
T_1 - Untreated/ Negative Control	None
T_2 - Abamectin (Agriguard®)	0.1
T_3 - Abamectin (Agriguard®)	1
T_4 - Emamectin benzoate (Proclaim Opti ®)	10
T_5 - Emamectin benzoate (Proclaim Opti ®)	100

Toxicity of Acaricides to *Amblyseius longispinosus*

The most effective acaricide concentrations against the cassava red spider mites *T. kanzawai* identified from the *in vitro* bioassay were tested against the female adults of the predatory mite *A. longispinosus*. Female adults were used because they are the least sensitive to pesticides (Vasquez 1994). Similar to the adulticidal bioassay for *T. kanzawai,* leaf-dip method was also followed. A total of 60 spider mites were then placed on the treated leaf disc daily as food source. These were added in three batches – at 0 HAT, 5 HAT and 10 HAT, and repeated the next day at 24 HAT, 29 HAT and 34 HAT. To avoid overcrowding, dead cassava red spider mites were removed from the leaf disks before adding a new batch of preys. The zero mortality rate in the control served as an indicator that the amount of food for the predatory mites in the experimental groups was sufficient and that mortality was solely due to the acaricide treatments.

Statistical Analysis

A Probit analysis (Finney 1971) was performed using the mortality data after it had been corrected in the control (Abbott 1925). Analysis of variance (ANOVA) was performed to determine whether there was a significant difference in the mean % mortality and % hatchability between different acaricides and concentrations at a 5 % significance level. These were carried out using the R software version 4.0.2.

RESULTS AND DISCUSSION

Acaricide Toxicity to *Tetranychus kanzawai*

Table 2 shows that at 24, 48 and 72 HAT, emamectin benzoate and abamectin significantly vary in terms of their ability to kill cassava red spider mites (CRSM) across concentrations. Abamectin, having the lowest LC_{50} value of 0.01674 mg/L, is the most toxic to the female adults of *T. kanzawai* (Table 2). *T. kanzawai* was most susceptible to Abamectin concentrations of ≥ 0.1 with 100 % mortality, whereas 0.01 to 0.0001 mg/L did not show lethal toxicity against CRSM because the percent mortality values at these concentrations were statistically similar to the control (Table 2). In the study of Lagziri and Elamrani (2009), *T. urticae* mortality was 54 % and 100 % when the abamectin trade product was used at 2 and 9 mg/L, respectively. Abamectin trade product at 0.417 mg/L (0.010 mg/L active ingredient) was utilized by Ebrahimi and Shiri (2018) in their study to obtain a 50 % mortality of *T. urticae*, which is 8.4 times less than what Lagziri and Elamrani (2009) used. The LC50 value for the German susceptible reference strain (GSS) of *T. urticae* collected on bean *Phaseolus vulgaris* L. was reported by Vassiliou and Kitsis (2013) to be between 0.02 to 0.03 mg/L of abamectin. The LC50 value of abamectin against *T. kanzawai* in the present study is lower than in these three studies, indicating that the population utilized in the present study is more susceptible to abamectin (Table 2). However, sources of variation such as the product formulation and the environmental conditions should also be considered. Their feeding may significantly impact phytophagous pests' susceptibility to any pesticide on different host plants because the secondary plant metabolites stimulate the insect's detoxifying enzymes, which affect the pesticides' rapid metabolism (Abro et al 2013). Additionally, differences in susceptibility may result from variations in insect feeding rates, which are positively correlated with pesticide consumption.

Emamectin benzoate was also very effective in female adults of *T. kanzawai* based on the *in vitro* assay. This acaricide is a semi-synthetic derivative of abamectin and is also a GABA agonist (IRAC 2019). Its miticidal activity was observed at concentrations of 0.1 mg/L, although 100 % mortality was only observed at concentrations ≥100 mg/L with an LC50 value of 0.29 mg/L (Table 2). This active ingredient is mainly used as an insecticide. It is even indicated on the product label (Proclaim Opti®) that this pesticide only suppresses the mite population. As defined by its manufacturer, suppression refers to either inconsistent control (good to poor) or consistent control at a level below that is generally considered acceptable for commercial control. As it turned out, results showed that it was very effective in *T. kanzawai*. This study's results are similar to that of Islam (2018) but differ from Sun et al (2010), who claimed that emamectin benzoate was more harmful than abamectin against *T. urticae*. In studies conducted on the leaves of bean, papaya, and jute, respectively, Islam (2018) discovered that abamectin was around 60, 70, and 68 times more toxic than emamectin benzoate.

Table 2. Mean percent mortality (± SD) of female adults of *T. kanzawai* hours after treatment (HAT)

AI CONCENTRATION (mg/L)	HOURS AFTER TREATMENT		
	24	48	72
Abamectin			
0	0 ±0 b	1.67 ±3 b	3.33 ±3 b
0.0001	3.33 ±3 b	8.32 ±6 b	9.97 ±0 b
0.001	10 ±13 b	13.32 ±14 b	14.98 ±22 b
0.01	6.67 ±3 b	38.32 ±53 b	41.63 ±51 b
0.1	100 ±0 a	100 ±0 a	100 ±0 a
1	100 ±0 a	100 ±0 a	100 ±0 a
10	100 ±0 a	100 ±0 a	100 ±0 a
100	100 ±0 a	100 ±0 a	100 ±0 a
1,000	100 ±0 a	100 ±0 a	100 ±0 a
P-value	<0.0001 ***	<0.0001 ***	<0.0001 ***
LC50 (mg/L)	0.01674	0.00696	0.00598
Emamectin benzoate			
0	0 ±0 d	1.67 ±3 b	3.33 ±3 c
0.0001	1.67 ±3 d	3.33 ±3 b	4.98 ±5 c
0.001	5 ±5 d	3.33 ±3 b	29.98 ±44 c
0.01	3.33 ±6 d	21.67 ±29 b	38.32 ±34 bc
0.1	61.67 ±28 bc	81.65 ±24 a	98.32 ±3 a
1	41.67 ±13 c	84.98 ±26 a	91.65 ±14 ab
10	88.33 ±20 ab	93.33 ±12 a	96.67 ±6 a
100	100 ±0 a	100 ±0 a	100 ±0 a
1,000	100 ±0 a	100 ±0 a	100 ±0 a
P-value	<0.0001 ***	<0.0001 ***	<0.0001 ***
LC50 (mg/L)	0.292	0.043597	0.00792

Means on the same row in a particular time and acaricide with the same letter assignment are not significantly different at 5 %.
ns- Not Significant; *-Significant at 5 % level; **-Significant at 1 % level; ***-Significant at 0.1 % level
probit(p) = b0 + b1×log10 (Concentration)

RESIDUAL TOXICITY

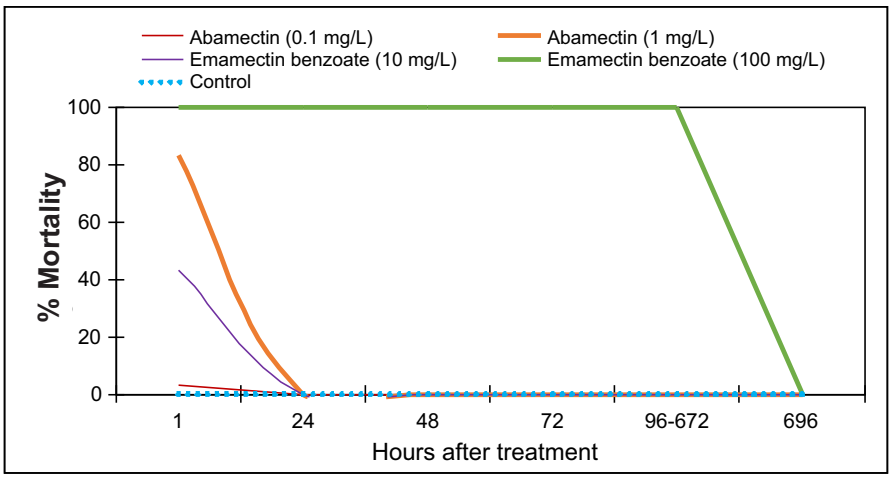

Figure 1. Mean % mortality of *T. kanzawai* after 24-hour exposure to acaricide residues aged 1 to 696 hours (29 days) after treatment

The residual activity of the top two lowest and most effective concentrations of abamectin and emamectin benzoate is presented in Figure 1. The treatments used in this experiment were chosen based on their ability to kill 100 % of T. kanzawai adults in the *in vitro* assay. Hence, it is expected that 100 % of *T. kanzawai* adults should also be dead when exposed to a 1-hour-old residue. However, the results from the laboratory experiment did not fully coincide with the screenhouse test because only the 1-hour-old residue of 10 mg/L abamectin and 100 mg/L emamectin benzoate showed high activity (83-100 % mortality). The 0.1 mg/L abamectin and 10 mg/L emamectin benzoate, which were supposedly the minimum effective concentrations based on the *in vivo* assay, could not deliver the same results when their 1-hour old residue was treated to *T. kanzawai* on 1-month old cassava. The data showed that the % mortality of *T. kanzawai* at these treatments was not significantly different from the control (Table 7). Lower concentrations of these acaricides may be less stable in the environment. Also, abamectin is easily degraded by UV light. Photodegradation of abamectin, when exposed to UV light, was first reported by Wislocki et al (1989) and was further supported by Escalada et al (2008). They stated that abamectin's degradation is highly efficient under 254 nm UV light. In the study of Ebrahimi and Shir (2018), a day-old residue of abamectin was able to create 100 % mortality of Trichogramma brassicae, but this mortality decreased by 55.62 % 21 days after spraying. Lagziri and Elamrani (2009) showed that the mortality of T. urticae on strawberries remained high at 85-96 % 49 days after treatment. In contrast, Cloyd et al (2009) proved the inefficacy of abamectin in cotton plants. According to Putter et al (1981) and Lasota and Dybas (1991), the amount of abamectin sprayed is not entirely degraded by UV light since some of it is absorbed by the plant itself a few hours after spraying. The sensitivity of abamectin to UV degradation is influenced by plant species and age (Putter et al 1981; Lasota & Dybas 1991). On the other hand, emamectin benzoate penetrates the leaf tissue and forms a reservoir within

activity against pests that ingest the substance when feeding (US EPA 2009).

Acaricide Toxicity to *Amblyseius longispinosus*

As shown in Table 3, 1 mg/L abamectin is not toxic to *A. longisponosus,* while 100 mg/L emamectin benzoate was slightly toxic (15 % mortality) 24 hours after treatment under laboratory conditions. At 48 hours after treatment, high mortality (88.3 %) of A. longispinosus was observed in 100 mg/L emamectin benzoate (Table 3). On the other hand, no toxicity was observed in 1 mg/L abamectin because the mortality value (6.7 %) is not significantly different from the control (Table 3). This result suggests that only the minimum effective concentration of abamectin was safe for *Amblyseius longispinosus*. Similarly, Zhang and Anderson (1990) reported that abamectin was not toxic to *Phytoseiulus persimilis* while Zhao et al (2013) found that three strains of *Amblyseius longispinosus* collected from South China were resistant to abamectin under laboratory bioassay conditions. Conversely, other studies reported that abamectin was very toxic to the female adults of predatory mites such as Amblyseius swirskii (Doker & Kazak 2019) and *Neoseiulus longispinosus* (Ibrahim & Yee 2000).

The recommended rate for emamectin benzoate was also very toxic to the female adults of the predatory mite, *Phytoseiulus persimilis*, dying within fifth day of the test (Kuk & Kim 2018). Also, Bernard et al (2010) observed that emamectin benzoate was highly toxic to juveniles of *Euseius victoriensis*. In contrast, Halloum and Qerhaili (2013) revealed that emamectin benzoate exhibited high toxicity only to T. urticae but not to the two species of predatory mites, *Neoseiulus fallacies* and *Typhlodromus cotoneastri.*

Table 3. Mean percent mortality of *Amblyseius longispinosus* adults 24 and 48 hours after treatment (HAT)

TREATMENTS	HOURS AFTER TREATMENT	
	24	48
Control	0 ± 0 a	0 ± 0 a
Abamectin (1 mg/L)	5 ± 5 ab	6.7 ± 3 a
Emamectin benzoate (100 m	15 ± 5 b	88.3 ± 8 b

Means on the same column in a particular time and treatment with the same letter assignment are not significantly different at 5 %.

CONCLUSIONS

The minimum effective concentration for abamectin and emamectin benzoate in the *in vitro* assay which are 0.1 mg/L and 10 mg/L, respectively, were not effective against *T. kanzawai* on screenhouse-grown cassava. The residual toxicity of a 100 mg/L emamectin benzoate to *T. kanzawai* is 28 days while the 1 mg/L abamectin is only 1 day on screenhouse conditions. The 1g/L abamectin is safe for *A. longispinosus* while 100 mg/L emamectin benzoate is highly toxic to A. *longispinosus* 48 hours after treatment under laboratory conditions.

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