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Laboratory evaluation of chlorfenapyr for control of carmine spider mite, *Tetranychus cinnabarinus* on tomato in Botswana

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Abstract
The efficacy of chlorfenapyr was studied in the laboratory against the carmine spider mite on tomato. The acaricide was applied at five concentrations, including the label rate, separated on a $\log_{10}$ scale. Each treatment was replicated three times. LC_{50} and LC_{90} values were determined by probit analysis during different periods after application and were used to determine the efficacy of the acaricide. LC_{90} values were 0.52, 0.50, 0.44 and 0.38 ml/L when treatments were evaluated at 24, 48, 72 and 96 hours after treatment. This indicated that chlorfenapyr was highly effective against *T. cinnabarinus* adults. Probit line slopes were 8.15, 6.96, 5.50 and 2.96 at 24, 48, 72 and 96 hours after application. This indicates a fast change in spider mite mortality with increasing in acaricide concentrations. This study found that chlorfenapyr can be used to effectively control *T. cinnabarinus* under Botswana conditions, especially in combination with other control methods in integrated pest management programs.

Keywords: Chlorfenapyr, carmine spider mite, *Tetranychus cinnabarinus*, efficacy, tomato

Introduction
The cultivated tomato (*Solanum lycopersicum* var. *lycopersicum*) is among the top major economic vegetable crops in the world including Botswana (Jones 1999; Mwandila *et al*. 2013) [39, 23]. It is highly valued for its economic and nutritional value. It is a nutritious food as it is rich in essential phytonutrients, minerals, vitamins and dietary fiber (Boamah *et al*. 2010; FAO 2020) [7, 10]. Compared with other tomato producing countries in Africa, local tomato yields and productivity are very low, ranging from 60 -100 tonnes per hectare (Badimo 2020) [5]. The major tomato producers in Africa are Egypt (7, 297 108 tons), Nigeria (4, 100 000 tons), Morocco (1, 293 761 tons), Tunisia (1, 298 000 tons), Cameroon (1, 279 853 tons), Algeria (1, 286 286 tons) and South Africa (608 306 tons) (Dube *et al*. 2020) [9]. Tomato growers in Botswana often report that invertebrate pests are the main constraint to production and the main cause of low productivity and quality (Baliyan and Rao 2013; Obopile *et al*. 2008) [6, 26]. Among the various pests affecting tomato production, the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) is the most damaging and prevalent. Several studies conducted in Botswana have also identified *T. cinnabarinus* as one of the most important invertebrate pests of tomato in Botswana (Munthali *et al*. 2004; Obopile *et al*. 2008) [22, 26]. *T. cinnabarinus* is found in most tomato growing regions of the world (Sun and Meng 2001; Zhang *et al*. 2003) [33, 38]. Spider mite outbreaks cause leaf defoliation, water loss and eventual host plant death, resulting in severe economic losses (Bu *et al*. 2015; Jia *et al*. 2011; Liang *et al*. 2011) [14, 20].
Several factors contribute to the spider mite pest status and these include the abundance and diversity of host plants, the removal of its predators, its high reproduction rate, rapid developmental rate, and arrhenotokous parthenogenesis which lead to fast development of acaricide resistance (Sato *et al*. 2005) [30]. Among the many control measures available, Botswana growers prefer synthetic pesticides to control pests (Baliyan and Rao 2013; Leungo *et al*. 2012) [6, 19]. Some farmers repeatedly spray their crops regardless of the presence of pests on them. Various synthetic acaricides from different chemical groups, including avermectins, pyrethroids, organophosphates, organochlorines, pyrroles and carbamates, have been used to control spider mites on various crops.

Some of the pesticides used by farmers are categorized in the extremely hazardous or highly hazardous classes by World Health Organization (WHO 2020) [36]. These chemicals pose serious threats to human health, the environment, and non-target organisms, and can lead to resistance development. *T. cinnabarinus* has reportedly developed resistance to many new acaricides shortly after their introduction (Kim *et al*. 2004; Nauen *et al*. 2001; Sato *et al*. 2005; Stumpf and Nauen 2001; Van Leeuwen 2005) [16, 20, 24, 30, 32, 35]. This study tested chlorfenapyr, a pyrrole class acaricide, for use in control programs for spider mites in Botswana. Chlorfenapyr is used commercially to control termites and a variety of invertebrate pests (Raghavendra *et al*. 2011; Sheppard and Joyce 1998) [28, 31]. It is among the acaricides used to control tomato spider mites in Botswana (Obopile *et al*. 2008) [26]. This acaricide has low toxicity to mammals and is categorized as slightly hazardous (class II) by the World Health Organisation (WHO 2020) [36]. Acaricides with novel mechanisms of action, such as chlorfenapyr, have less toxic effects on non-target organisms, predators, mammals, and the environment due to their 'environmentally friendly' chemical origin and low application rates (Leonard 2000) [18]. The current focus is on the development of environmentally friendly pesticides with novel mechanisms of action, such as chlorfenapyr, to control pests and prevent the development of resistance. Despite the beneficial properties of chlorfenapyr, its efficacy against spider mites has not been evaluated in Botswana. This study evaluated the efficacy of chlorfenapyr against spider mites in the laboratory.

Materials and Methods

The experiment was conducted at the Botswana University of Agriculture and Natural Resources (BUAN), Gaborone, Botswana (24º3425''S, 25º 95'0" E; 998 m altitude). Crop Protection laboratory, average temperature 21±3˚ C. The spider mite population used in this study was collected from Tara farm (24º32'39.4" S, 25º47'57.4" E) an intensive vegetable farm in Metsimotlhabe. Spider mite samples were identified in BUAN's entomology laboratory prior to starting the bioassay. Tomato seedlings, originally grown in seedling trays in the greenhouse, were transplanted into plastic pots filled with 1.5 kg of loam soil. Spider mites were cultured on tomato seedlings in the greenhouse. The seedlings were watered ad libitum to prevent wilting.

Bioassay method

A locally available acaricide, chlorfenapyr (Savage 360® SC), was used in the bioassay. The method followed the Insecticide Resistance Action Committee (IRAC) Method 004 (for adult spider mites) (www.irac-online.org). The acaricide was applied at 5 concentrations separated on a $\log_{10}$ scale, including the recommended label rate (0.4 ml/L) as a check. The acaricide was applied at 0.2, 0.3, 0.4, 0.5 and 0.6 ml/L water. Distilled water was included in the experiment as a control. Six treatments were arranged in a fully randomized design. Leaf discs 2cm in diameter were cut from tomato leaves that had not been chemically sprayed. Each treatment contained nine leaf discs. Leaf discs were separately immersed in one of the test liquids for approximately 5 seconds. A layer of cotton wool was placed at the bottom of each polystyrene cup and tap water was added to saturate it. The leaf discs were allowed to dry and placed on the cotton wool in the polystyrene cups. A fine brush was used to place spider mites onto each treated leaf disc. Ten adult spider mites were transferred onto each leaf disc. This gave 54 treated leaf discs per bioassay and 162 treated leaf discs in total. Testing was performed at 21±3 ˚C and 65-90% relative humidity. Each cup was marked to indicate the treatment level and date of application. The bioassay was repeated 3 times.

Mortality Assessment

Spider mites were observed under a binocular microscope. A fine detail brush was used to rouse spider mites into moving. Spider mites that were unable to walk were recorded as dead. Spider mites were recorded at intervals of 24, 48, 72 and 96 hours following treatment. Results were converted to percentage mortality and corrected for control mortality using Abbott's formula (Abbott 1925) [1]. Mortality in the control was also recorded.

Data analysis

Results were analyzed using probit analysis (Finney 1971). Mortality data were converted to probits and concentrations were transformed to $\log_{10}$ (X+1) prior to analysis. Data were analyzed using $\log_{10}$ versus probit regression and analysis of variance (ANOVA). Median lethal concentration values (LC_{50} and LC_{90}) were estimated from probit plots. The comparative susceptibility of spider mites was compared using the LC_{50} values and slopes of probit lines. LC_{90} values were used to compare the mortalities induced by the label application rate and the mortality achieved by treatments at different time points after treatment. Data analysis was performed using the statistical software SAS (version 9.4, SAS Institute, Cary, USA). Means were separated using Tukey's Honestly significant difference test (Zar 1984) [37].

Results

Spider mite mortality after chlorfenapyr application

Figures 1 (A - D) show the mortality of spider mites after treatment with various chlorfenapyr concentrations measured at different time intervals. There was a positive relationship between log dose and chlrofenapyr induced probit mortality (correlation coefficients of 0.9877, 0.9188, 0.6954 and 0.5267), when treatments were assessed at 24, 48, 72 and 96 hours after treatment. Figure 3A shows an LC_{50} of 0.36 ml/L and an LC_{90} of 0.52 ml/L were achieved 24 hours after treatment. The recommended dose of chlorfenapyr (0.40 ml/L) showed a probit value of 0.597 (equivalent to 50.59% adult mortality) at 24 hours. Figure 3B shows that chlorfenapyr had an LC_{50} of 0.31 ml/L and an LC_{90} of 0.50 ml/L after 48 hours. At the recommended dose rate, chlorfenapyr scored 0.597 on the probit scale, corresponding to a mortality rate of 50.59%. Chlorfenapyr had an LC_{50} of 0.22 ml/L and an LC_{90} of 0.44 ml/L when assessed 72 hours after treatment (Figure 3C). The recommended label rate reached 0.830 on the probit scale. This corresponds to the spider mite mortality rate of 65.65%. Figure 3D shows an LC_{50} value of 0.01 ml/L and an LC_{90} of 0.38 ml/L when assessed 96 hours after treatment. The mortality rate achieved at the recommended label rate was 0.916 on the probit scale, corresponding to 73.15% mortality.

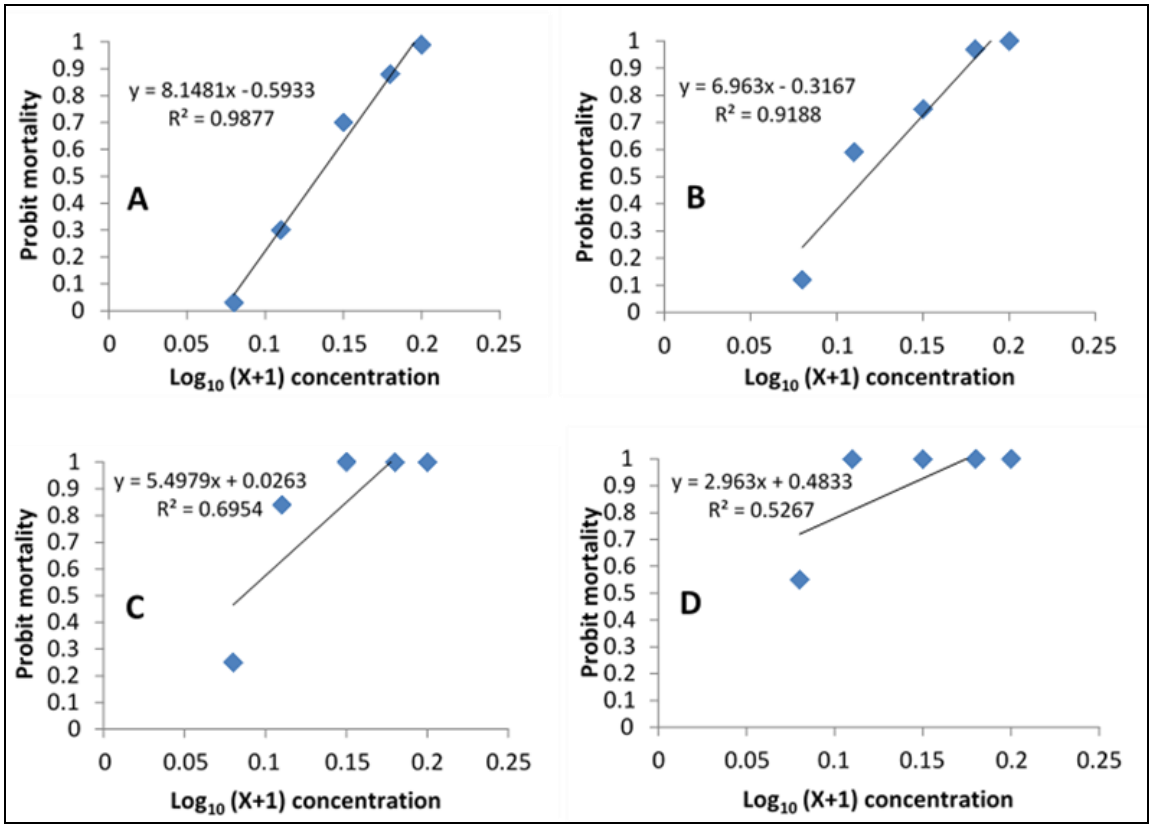

Fig 1: Probit mortality of spider mites 24 h (A), 48 h (B), 72 h (C) and 96 h (D) after treatment with various concentrations of chlorfenapyr

Effect of Chlorfenapyr concentration and exposure on spider mite mortality

Table 1: The effect of Chlorfenapyr concentrations and exposure on spider mite mortality

	Means±SE					
Time after application	**Control**	**0.20 ml/L**	**0.30 ml/L**	**0.40 ml/L**	**0.50 ml/L**	**0.60 ml/L**
24 h	0.00^{dB}±0.00	10.00^{dB}±3.16	33.33^{cC}±1.00	56.67^{bB}±0.77	70.00^{abB}±0.00	83.33^{a}±0.63
48 h	0.67^{eAB}±0.71	20.00^{dB}±2.24	50.00 ^{cCB}±0.00	60.00^{cB}±0.00	80.00^{bB}±1.12	100.00^{aB}±0.00
72 h	1.33^{dA}±0.50	30.00^{cA}±1.83	66.67^{bB}±1.41	90.00^{aA}±1.05	100.00^{aA}±0.00	100.00^{aA}±0.00
96 h	1.33^{cA}±0.50	46.67^{bA}±0.85	93.33^{aA}±0.60	93.33^{aA}±0.60	100.00^{aA}±0.00	100.00^{aA}±0.00

** Means followed by the same small letter within a row are not significantly different, $p \leq 0.05$ (Tukey's Honestly significant difference test)

** Means followed by the same capital letter within a column are not significantly different, $p \leq 0.05$ (Tukey's Honestly significant difference test)

Table 1 shows the effect of chlorfenapyr concentration and time after treatment on *T. cinnabarinus* mortality. The results of this study revealed that concentrations and periods after treatment interaction were significantly different ($F_{15, 46}$ = 8.73; P = 0.0001). Comparing different concentrations 24 hours after treatment showed that mortality in the control was significantly different from all the other concentrations except 0.20ml/L (10.00%). The recommended rate of 0.40ml/L achieved 56.67% mortality in 24 hours. This was not significantly different from the 70.00% mortality achieved at a higher concentration of 0.50 ml/L during the same period ($F_{15, 46}$ = 8.73; P = 0.0001). Concentrations of 0.50ml/L and 0.60 ml/L achieved 70.0% and 83.33% mortality during the 24 hour assessment period (Table 1). Comparing concentrations after 48 hours, the recommended rate of 0.40 ml/L achieved a mortality rate of 60.00%, which was significantly different from the 80.00% mortality achieved by a higher concentration of 0.50ml/L during the same period ($F_{15, 46}$ = 8.73; P = 0.0001). The 80.00% mortality achieved with the 0.50ml/L concentration 48 hours after treatment was significantly different from the 100.00% mortality achieved with the 0.60ml/L concentration during the same period. When assessed after 72 hours of exposure, control mortality was significantly different ($F_{15, 46}$ = 8.73; P = 0.0001) from that achieved at all other concentrations. The recommended rate of 0.40 ml/L achieved a mortality rate of 90.00% over 72 hours. This was similar to 100% mortality achieved at higher concentrations of 0.50 ml/L and 0.6 ml/L during the same period ($F_{15, 46}$ = 8.73; P = 0.0001).

Comparing concentrations at different periods after treatment, it was found that mortality achieved by the control treatment at 48 hours was not significantly different from that achieved at 72 h and 96 hours after treatment ($F_{15, 46}$ = 8.73; P = 0.0001). When compared with an application rate of 0.30ml/L, the 33.33% mortality achieved after 24 hours of exposure was not significantly different from that achieved after 48 hours. The 50.00% mortality achieved at 48 hours with 0.30ml/L chlorfenapyr was not significantly different from the 66.67% mortality achieved with the same concentration at 72 hours (Table 1). A concentration of 0.30ml/L achieved 93.33% mortality 96 hours after treatment. Comparing the recommended dose of chlorfenapyr

(0.40ml/L) at different time points after treatment showed no significant difference between the 56.67% mortality achieved at 24 hours and the 60.00% mortality achieved at 48 hours ($F_{15, 46}$ = 8.73; P = 0.0001). A concentration of 0.40ml/L was able to cause 90.00% and 93.33% mortality in 72 and 96 hours respectively. At a concentration of 0.50 ml/L, 70.00% mortality occurred at 24 hours, which was not significantly different from the 80.00% mortality reached after 48 hours ($F_{15, 46}$ = 8.73; P = 0.0001). At both 72 and 96 hours post treatment, the concentration level of 0.50ml/L caused the same mortality of 100.00%, which was not significantly different ($F_{15, 46}$ = 8.73; P = 0.0001). When compared with an application rate of 0.60ml/L, the 83.33% mortality achieved 24 hours after treatment was not significantly different from the 100.00% mortality achieved at 48 hours. The concentration level of 0.60ml/L achieved 100.00% mortality 48, 72 and 96 hours after treatment, which were not significantly different ($F_{15, 46}$ = 8.73; P = 0.0001) (Table 1).

Discussion

The mortality level due to the recommended label rate during the study period appears to be sufficient to achieve effective control of spider mites. These results corroborate those by Amjad *et al*. (2012) [2] who found that compared with other acaricides, chlorfenapyr caused very high mortality in *Tetranychus urticae*. The results that chlorfenapyr became more lethal to spider mites with increasing acaricide concentrations are also similar to those by Amjad *et al*. (2012) [2] where mortality of *T. urticae* was dependent on the applied concentration of chlorfenapyr. Chlorfenapyr is an acaricidal pyrrole, and the primary mode of action is to affect oxidative phosphorylation in the mitochondria, which results in the death of the cell through inhibition of ATP synthesis and eventual death of the pest organism (Arthur 2009; McLeod *et al*. 2002) [3, 21]. Arthur (2008) [4] reported that the mechanism of action of chlorfenapyr differs from that of conventional neurotoxins, and mortality of the pest as a result of exposure to chlorfenapyr is not immediate but is delayed for several days after the initial exposure (Arthur, 2008) [4]. Therefore the results of this study that chlorfenapyr caused rapid mortality of spider mites were unexpected. Leonard (2000) [18] found that chlorfenapyr was primarily active through ingestion with considerable contact activity. This means that active life stages of spider mites can therefore acquire the lethal concentrations through both feeding and contact with the acaricide material as they move and forage on the plant. This may explain the fast mortality of spider mites obtained in this study. The rapid action of chlorfenapyr against spider mite adults is a desirable trait as this is the most harmful and reproductive life stage of this pest. N'Guessan *et al*. (2007) [25] and Oxborough *et al*. (2015) [27] also reported that chlorfenapyr does not exhibit any cross-resistance to mechanisms that confer resistance to standard neurotoxins due to the exclusive mechanism of action of pyrroles. This novel mechanism of action makes it a suitable candidate for targeting multi-acaricide-resistant spider mite strains. Kumari *et al*. (2015) [17]; Ullah and Gotoh (2013) [34] reported that spider mites eggs were highly susceptible to chlorfenapyr. This is a welcome trait as it means that the upsurge of nymphal populations from hatching eggs would be reduced, thereby minimizing consequent damage from spider mite larvae.

Conclusions and Recommendations

Farmers use pesticides against crop pests at the recommended dose to safeguard the production of large quantities of high quality crop harvests with minimum amounts of the active ingredient. From the results of this study, it can be concluded that chlorfenapyr can offer effective and timely control of spider mites at minimal levels of the active ingredient without compromising the protection of the crop. The population in this study did not exhibit any signs of resistance, therefore continued use of chlorfenapyr can be safely recommended. With a completely unique mechanism of action, chlorfenapyr can be used as a resistance management component of integrated spider mite control programs. Since this study was conducted in the laboratory, further research and field testing is needed to verify these test results.

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