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Efficacy of Abamectin against adult spider mite, *Tetranychus cinnabarinus* (Acari: Tetranychidae) on tomato in Botswana

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Abstract
The efficacy of abamectin against the carmine spider mite (CSM) on tomato was studied in the laboratory at Botswana University of Agriculture and Natural Resources, Gaborone, Botswana. The acaricide was applied at five dosages including the recommended label rate. Each treatment was replicated three times. The results were analysed using probit analysis where LD_{50} and LD_{90} values found with the acaricide were estimated at different assessment periods (24, 48, 72 and 96 hours) following application and used to determine effectiveness of the acaricide. When the treatments were assessed at 24, 48, 72 and 96 h, LD_{90} values against spider mites were 0.82, 0.71, 0.69 and 0.64. This indicated that abamectin was highly effective against spider mites. The slopes of the probit lines assessed at 24, 48, 72 and 96 h after application were 6.68, 6.97, 6.62 and 4.46. This indicates a rapid change in mortality with increase in acaricide dosage. The study shows that abamectin can be a valuable component of integrated spider mite control programme under Botswana conditions. Further research and field testing is necessary to confirm these laboratory findings.

Keywords: Abamectin, efficacy, carmine spider mite, tomato

Introduction
Tomato (*Solanum lycopersicum* var. *lycopersicum*), which belongs to the family Solanaceae, is the most popular and widely consumed vegetable crop grown in open fields, greenhouses and shade houses of the world including Botswana (Badimo 2020; Leungo *et al*. 2012; Mwandila *et al*. 2013) [2, 17, 22]. Tomato is highly valued for its economic and nutritional value (Boamah *et al.* 2010; Dube *et al.* 2020; Shiberu and Getu 2018) [5, 8, 27] and constitutes an important part of household diet and national economy. However, local farmers often lament the destruction by a wide range of invertebrate pests as a main cause of low production and quality in Botswana. Studies conducted in Botswana have identified several invertebrate pests attacking tomato which include the African bollworm (*Helicoverpa armigera*), tomato semi-looper (*Chrysodeixis acuta*), whitefly (*Bemisia tabaci*), aphids, tomato leaf miner (*Tuta absoluta*) and spider mites (Tetranychus spp.) (Baliyan 2012; Munthali *et al*. 2004; Obopile *et al*. 2008) [4, 21, 24]. One of the most serious pests of tomato in Botswana is the carmine spider mite (*Tetranychus cinnabarinus* Boisduval, 1867) (CSM). Heavy spider mite infestation causes leaf drop, loss of water and even death of the host plant, leading to severe economic losses (Bu *et al*. 2015; Jia *et al.* 2011; Liang *et al.* 2011) [13, 18].

Among the different control measures available, farmers in Botswana prefer to use chemicals to control pests because they give quick results (Baliyan and Rao 2013; Leungo *et al.* 2012) [3, 17]. Some farmers spray their crops repeatedly regardless of the presence of pests or damage symptoms. Most of the active ingredients used by farmers are classified either as extremely hazardous or highly hazardous by World Health Organization (WHO 2020) [30]. This has serious implications on the health and safety of farmers. Because of the diversity and abundance of alternative hosts, the disruption of its natural enemies, its high reproductive potential and genetic elasticity spider mites quickly develop resistance to acaricides used against them (Sato *et al*. 2005) [26]. Abamectin is one of the most commonly used acaricides for the control of spider mites in Botswana (Obopile *et al*. 2008) [24]. Abamectin is a natural fermentation product of the soil bacterium, *Streptomyces avermitilis* (Hayes and Laws 1995; Kim and Goodfellow 2002) [10. 14].

It is a Glutamate-gated chloride channel (GluCl) allosteric modulator with several effects on spider mites and insect larvae (IRAC 2020; Sato *et al*. 2005) [11, 26]. Abamectin is more effective as an ingestion toxicant with considerable contact activity. It is widely used in agriculture for the control of mites, thrips, aphids, whiteflies, psyllids and lepidopteran pest species with characteristics of high efficiency, low toxicity to non-target beneficial arthropods, low environmental persistence and lasting efficacy (Lasota and Dybas 1990) [16]. Abamectin has been a widely used acaricide worldwide, but recently it has reportedly failed to provide satisfactory spider mite control on some crops in fields and greenhouses (Memarizadeh *et al*. 2011) [19]. Abamectin resistance in spider mites has been reported worldwide (Campos *et al*. 1995; 1996; Kwon *et al*. 2010; Sato *et al*. 2005; Stumpf and Nauen 2002) [6, 7, 15. 26, 29].

The efficacy of abamectin against spider mites has not been evaluated in Botswana despite the fact that it is a popular acaricide among local tomato farmers. This study evaluated the efficacy of abamectin against adult spider mites on tomato in Botswana.

Materials and Methods

The experiment was conducted at the Botswana University of Agriculture and Natural Resources (BUAN) in Gaborone, Botswana (24º 34' 25''S, 25º 95' 0" E; altitude: 998 m) in the Crop Protection laboratory, at an average temperature of 21 ± 3 ˚C. The spider mite population used in this study was obtained from Tara farm (24º 32'39.4" S, 25º 47'57.4" E) an intensive commercial tomato producing farm in Metsimotlhabe in the greater Gaborone area. Identification of specimens was carried out in the Entomology laboratory at BUAN before use in the bioassay. Tomato seedlings were initially raised in seedling trays in the greenhouse and then transplanted into small black plastic pots filled with 1.5 kg loam soil; each pot was 12 cm in diameter and 15 cm in depth. Tomato seedlings were used to rear the spider mites and ensure adequate host substrate for oviposition and feeding. The seedlings were watered regularly adlib to prevent wilting.

Bioassay methods

Abamectin; emulsifiable concentrate (Agromectin®) Arysta LifeScience, registered for use in Botswana was used in the bioassay. The method followed the Insecticide Resistance Action Committee (IRAC) method 004 (for adults) (www.IRAC-online.org). The acaricide was applied at 5 dosages separated on a $\log_{10}$ scale, with the recommended dosages (0.6 ml/L) included as a check and distilled water as the solvent used to formulate test solutions. Abamectin was applied at 0.4, 0.5, 0.6, 0.7 and 0.8 ml/L water. Distilled water was included as a control in the experiment. The 6 treatments were arranged in a completely randomized design. Leaf discs of 2 cm diameter were cut from chemically untreated tomato plants. Each treatment had nine leaf discs. The test liquids were agitated and each leaf disc was individually dipped in one of the test liquids for 5s. The leaf discs were air dried before placing them in polystyrene cups with a layer of moist cotton wool placed over the base of the cups and tap water added to a point of saturation. A fine tooth brush was used to transfer 10 adult spider mites onto each treated leaf disc. This gave a total of 54 treated leaf discs per bioassay and 162 treated leaf discs in total. The tests were maintained at 21 ± 3˚ C and at 65 - 90% relative humidity. Each cup had a label which indicated the treatment and its date of application. The bioassay was repeated 3 times.

Assessment mortality

Spider mites were observed under a binocular microscope. A fine brush was used to stimulate individual mites. Mites that were incapable of walking were recorded as dead. The mites were assessed at intervals of 24, 48, 72 and 96 h after treatment. Results were expressed as percentage mortality and corrected for untreated mortality using Abbott's formula (Abbott 1925) [1]. Untreated mortality was also recorded.

Data analysis

Probit analysis (Finney 1971) [9] was used to analyse mortality data. The mortality data were transformed to probits while the dosages were transformed to $\log_{10}(X+1)$ before analysis. Data was analysed using $\log_{10}$ versus probit regression and analysis of variance (ANOVA). LD_{50} and LD_{90} values were estimated from the probit lines. LD_{90} values were used to compare the mortalities that the recommended dosage caused with the mortalities that were achieved by treatments at different durations after treatment. Statistical analyses were performed using the SAS statistical software (version 9.4, SAS Institute, Cary, USA). Tukey's Honestly significant difference test (Zar 1984) [31] was used to separate the means.

Results

CSM adult mortality due to abamectin

Figures 1(A - D) shows a positive linear relationship between log dose and probit mortality caused by abamectin (correlation coefficients of 0.9147, 0.9214, 0.9019 and 0.8404), when treatments were assessed at 24, 48, 72 and 96 h after acaricide application. Figure 1A shows that LD_{50} of 0.58 ml/L and LD_{90} of 0.82 ml/L were achieved 24 h after treatment. The recommended dosage (0.60 ml/L) of the abamectin showed a probit value of 0.53 (equivalent to 46.72% adult mortality) during this assessment period. Figure 1B indicates that the LD_{50} of abamectin 48 h after treatment was 0.49 ml/L, while the LD_{90} was 0.71 ml/L. At the recommended dosage, abamectin only achieved 0.705 on the probit scale, which is equivalent to 57.10% mortality. When assessed at 72 h after treatment, the LD_{50} of abamectin was 0.48 ml/L and the LD_{90} was 0.69 ml/L (Figure 1C). The recommended dosage achieved 0.73 on the probit scale, which is equivalent to 68.69% adult mortality 72 h after treatment. Figure 1D shows an LD_{50} value of 0.34 ml/L and an LD_{90} of 0.64 ml/L when the treatments were assessed at 96 h after treatment. The mortality achieved by the recommended dosage was 0.846 on the probit scale, which is equivalent to 66.89% mortality.

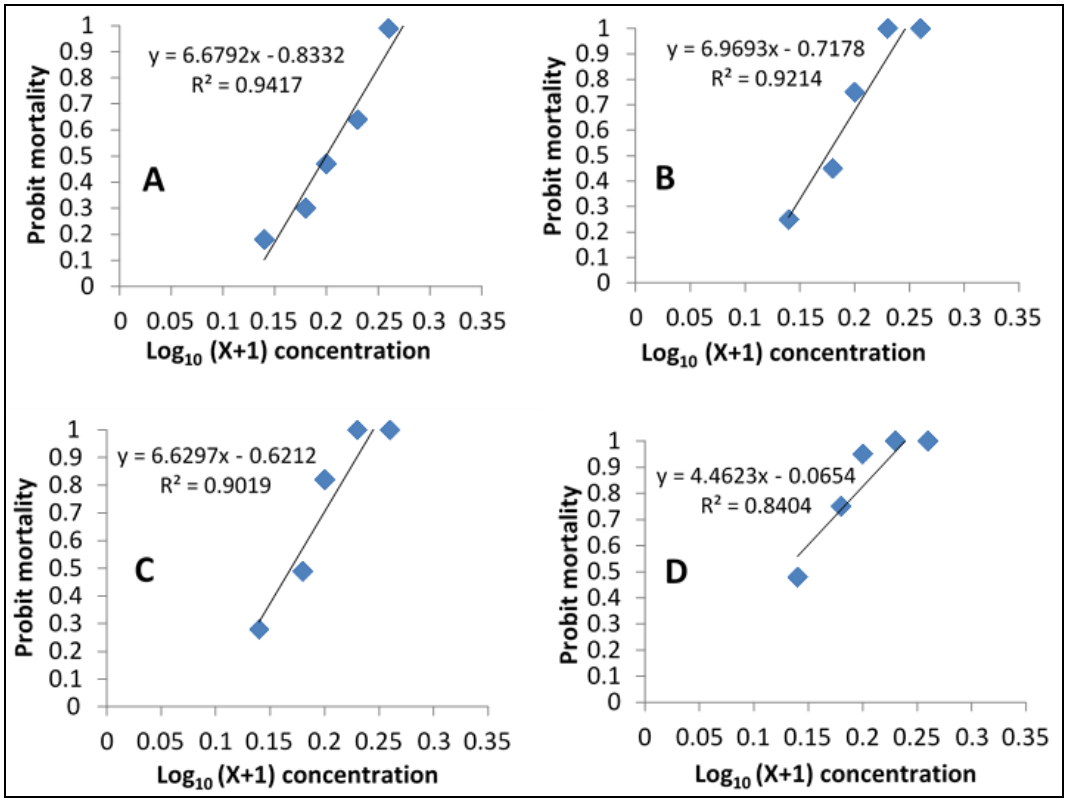

Fig 1: Probit mortality of adult spider mites assessed 24 h (A), 48 h (B), 72 h (C) and 96 h (D) after treatment with different dosages of abamectin.

The effect of abamectin dosages and time after treatment on adult spider mite mortality

Table 1 shows the effect of abamectin dosages and time after treatment on adult CSM mortality. The results of this study revealed that dosage and time after treatment interactions were significantly different ($F_{15, 46}$ = 2.71; P=0.048). When comparing the different dosages 24 h after treatment, it has been shown that control treatment was not significantly different from the 0.40 ml/L and 0.5 ml/L dosages. The recommended rate of 0.6 ml/L only achieved 43.33% mortality during the 24 h period, which is less than the required 90% mortality. The mortalities achieved by the dosages of 0.7 ml/L (53.33%) and 0.8 ml/L (83.33%) were not significantly different during the 24 h period (Table 1). These two dosages did not achieve 90% egg mortality. When the dosages were compared 48 h after treatment, control mortality was significantly different from all the other dosages except 0.40 ml/L. The recommended rate of 0.6 ml/L achieved 60.00% mortality, which was less than the required 90% egg mortality during the 48 h period. Dosages above the recommended rate of 0.6 ml/L (0.7 ml/L and 0.8 ml/L) achieved mortalities of 86.67% and 100% respectively during the 48 h period, and these were not significantly different ($F_{15, 46}$ = 2.71; P=0.048) (Table 1). When assessment was done 72 h after treatment, control mortality (3.33%) was not significantly different from the 23.33% mortality achieved at 0.40 m/L. The recommended rate of 0.6 ml/L achieved 73.33% CSM mortality during the 72 h period, which is less than the required 90% mortality. Concentrations of 0.7 ml/L and 0.8 ml/L achieved 93.33% and 100% mortality respectively, and these were not significantly different ($F_{15, 46}$ = 2.71; P=0.048) (Table 1). When assessment was done 96 h after treatment, control mortality was significantly different from the 36.67% mortality achieved at 0.40 m/L. The recommended rate of 0.6 ml/L achieved 76.67% mortality during the 96 h period, which is less than the required 90% mortality. Dosages of 0.7 ml/L and 0.8m l/L both achieved 100% mortality, and these were not significantly different ($F_{15, 46}$ = 2.71; P=0.048) (Table 1).

When comparing the dosages at different times after treatment it has been shown that mortalities achieved by the control were not significantly different ($F_{15, 46}$ = 2.71; P=0.048) from each other. When comparisons were done at the 0.4 ml/L dosage, the mortality of 6.67% achieved at 24 h exposure was significantly different from mortalities achieved at 48 h (13.33%), 72 h (23.33%) and 96 h (36.67%) (Table 1). The mortality achieved at the 0.5 ml/L dosage after 24 h was not significantly different ($F_{15, 46}$ = 2.71; P=0.048) from the mortalities achieved after 48 h (30%) and 72 h (60%). However, these were significantly different from the mortality level of 60% achieved by the 0.5 ml/L dosage after 96 h. When comparing the recommended label rate of abamectin (0.6 ml/L) at different times after treatment, the results reveal that the mortality of 43.33% achieved at 24 h exposure was significantly different from mortalities achieved at 48 h (60.00%). The mortality level achieved after 72 h (73.33%) was not significantly different from the 76.67% mortality level achieved at 96 h. These were less than the required 90% mortality. The dosage of 0.7 ml/L was able to cause 53.33% mortality at the 24 h period which was not significantly different ($F_{15, 46}$ = 2.71; P=0.048) from the 86.67% mortalities achieved after 48 h. The mortality of 93.33% achieved by the 07 ml/L dosage after 72 h was not significantly different from the 100% mortality achieved by the same dosage after 96 h (Table 1). At the 0.8 ml/L dosage, mortality of 83.33% was achieved 24 h after treatment, and this was not significantly different from 100% mortalities achieved after 48, 72 and 96 h.

Table 1: The effect of Abamectin dosages and time after treatment on adult spider mite mortality

Means ± SE						
Time after application	**Control**	**0.40 ml/L**	**0.5 ml/L**	**0.6 ml/L**	**0.7 ml/L**	**0.8 ml/L**
24 h	0.00^{cA}±0.00	6.67^{cC}±2.24	26.67^{bcB}±1.12	43.33^{bC}±0.88	53.33^{baB}±2.09	83.33^{aA}±3.16
48 h	0.00^{dA}±0.00	13.33^{cdB}±3.16	30.00^{cB}±1.83	60.00^{bB}±0.00	86.67^{aAB}±1.24	100.00^{aA}±0.00
72 h	3.33^{dA}±3.16	23.33^{cdB}±1.20	40.00^{cB}±1.58	73.33^{bA}±0.67	93.33^{abA}±1.20	100.00^{aA}±0.00
96 h	1.33^{dA}±0.50	36.67^{cA}±0.95	60.00^{bA}±2.24	76.67^{bA}±0.66	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)

Discussion

The results that abamectin became more toxic to spider mites with each increase in acaricide dosage are similar to those by Kavallieratos (2009) [23] and Mwandila *et al*. (2013) [22] where increase in dosage enhanced the efficacy of abamectin. The results that the recommended dosage did not achieve control during the assessment period indicate that higher dosages are required to achieve effective control of spider mites under Botswana conditions. Sparks *et al*. (1998) [28] reported that abamectin was a neurotoxin with a contact mode of action which suggests that spider mites adults can acquire the lethal dose through contact and ingestion of the acaricide material as they feed. Moscardini *et al*. (2013) [20] reported that abamectin causes partial paralysis of the invertebrate nervous system, which can decrease or even halt feeding, ultimately leading to death. This means that abamectin does not need to achieve high levels of mortality to achieve effective control since the spider mite may be alive but unable to feed consequently protecting the plant from damage. This mode action of abamectin is a desirable property as this is the life stage that causes the most damage to tomato. The application of abamectin as part of an integrated pest management programme can reduce the amount of active ingredient necessary to achieve effective control, reduce environmental pollution, impact on beneficial organisms, delay development of resistance to acaricides and reduce the cost to the farmer.

Conclusions and recommendations

Farmers apply pesticides against invertebrate pests at the recommended rate to safeguard the production of large quantities of high quality crop yields by using minimum amounts of active ingredient. It can be concluded from this study that abamectin offers effective protection of the tomato crop even at low spider mite mortality levels. It is recommended that abamectin should be used as part of an integrated pest management programme for the control of spider mites in Botswana. Further research is needed under field conditions to validate the results obtained in the present study.

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