



DETOXIFICATION ENZYMES IN THE CARMINE SPIDER MITE *TETRANYCHUS CINNABARINUS* BOISDUVAL

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ABSTRACT

A study was conducted to establish acaricide detoxification enzymes activity among carmine spider mite (Acari: Tetranychidae) strains collected from commercial tomato fields in seven geographical locations of Botswana. The highest levels of α -esterase activity (nmol/ min/ mg of protein⁻¹) were observed in the Bela-bela strain, followed by the Sikwane strain. The Sikwane strain showed enhanced β -esterase activity, followed by the Glen Valley and Francistown strains. Elevated levels of GSTs were observed in the Francistown strain, Moshupa and Bela-bela farms. The Francistown strain showed the highest CYP450 activity followed by Bobonong and Sikwane. Variation in detoxification enzyme activities among *T. cinnabarinus* strains can be attributed to differing pesticide application regimes in these regions. These findings may be helpful in the selection of acaricides and in developing effective resistance management strategies for *T. cinnabarinus* in tomato fields in Botswana.

Key words: Acaricide resistance, esterases, cytochrome P450 monooxygenases, glutathione S- transferases, *Tetranychus cinnabarinus*, tomato, resistance management

The carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae), is an extremely damaging polyphagous pest of many economically important crops worldwide (Roy et al., 2014). Currently, the control of spider mites is mainly achieved through the application of synthetic acaricides (Demaeght, 2015). Acaricides are generally non-selective and detrimental to both spider mites, non-target organisms, and the environment. The overuse of synthetic acaricides has given rise to resistant populations, and has resulted in control failures globally (Demaeght, 2015; APRD, 2017). Resistance mechanisms in spider mites are similar to those found in other arthropods and include reduced penetration through the cuticle, metabolic resistance, and target site insensitivity (Adesanya et al., 2021; Khan et al., 2020; Naveena et al., 2022). Metabolic resistance is attributed to increased activity of three principal detoxification enzymes in spider mites, which include esterases (GE), glutathione-S-transferases (GSTs) and Cytochrome P450 monooxygenases (CYP450) (Bisset et al., 2020; Epelboin et al., 2021; Ou et al., 2023). Pest control failures very often indicate the possibility of acaricide resistance development. It is important to study the underlying metabolic resistance mechanisms and susceptibility status of *T. cinnabarinus* to commonly used acaricides. The purpose of this study

was to compare the activity of detoxifying enzymes in *T. cinnabarinus* populations from across Botswana and to provide information for effective *T. cinnabarinus* management on tomatoes while developing viable alternative strategies to minimize resistance to commonly used acaricides and ensure successful management of this pest.

MATERIALS AND METHODS

The *T. cinnabarinus* samples were collected from tomato farms in seven different geographical locations across Botswana in 2022. The areas sampled were Bela-Bela farms (24° 28'52" S 26° 01'01" E), Glen Valley LEA horticulture incubator (24° 36'05" S 25° 58'24" E), Moshupa (24° 43'13" S 25° 28' 43" E), Sikwane (24°38'30" S 26°24'47" E), Bobonong (22°03'03" S 28°21'05" E), Letlhakane (21° 26'28" S 25° 36'06" E) and Francistown Impala Research Station (21° 09'48" S 27° 35'16" E). Morphological identification of specimens was carried out using taxonomic keys (NAPPO, 2014) in the Entomology laboratory at Botswana University of Agriculture and Natural Resources (BUAN) before use in the bioassay. The fields sampled were under commercial tomato production and consequently intensive application

Table 1. Relative activity of acaricide detoxification enzymes in *T. cinnabarinus* populations

	GST ($\pm$ SE)	R.A*	Cytochrome P ₄₅₀ ($\pm$ SE)	R.A*	α -esterase ($\pm$ SE)	R.A*	β -esterase ($\pm$ SE)	R.A*
Bela-bela	15.371 $\pm$ 2.23 ^{ab}	1.45	0.016 $\pm$ 0.002 ^d	1.0	1.966 $\pm$ 0.37 ^a	3.97	0.784 $\pm$ 0.12 ^c	1.0
Bobonong	10.638 $\pm$ 0.62 ^b	1.0	0.193 $\pm$ 0.03 ^{ab}	12.06	0.607 $\pm$ 0.09 ^b	1.23	1.653 $\pm$ 0.30 ^{bc}	2.11
Francistown	20.026 $\pm$ 3.13 ^a	1.88	0.222 $\pm$ 0.02 ^a	13.88	0.946 $\pm$ 0.21 ^b	1.91	1.102 $\pm$ 0.094 ^{bc}	1.41
Glen Valley	14.506 $\pm$ 2.48 ^{ab}	1.36	0.124 $\pm$ 0.02 ^{bc}	7.75	0.739 $\pm$ 0.14 ^b	1.49	1.966 $\pm$ 0.91 ^b	2.51
Letlhakane	-	-	0.069 $\pm$ 0.011 ^{cd}	4.31	0.495 $\pm$ 0.07 ^b	1.0	0.953 $\pm$ 0.18 ^{bc}	1.22
Moshupa	15.655 $\pm$ 2.16 ^{ab}	1.47	0.098 $\pm$ 0.014 ^c	6.13	0.615 $\pm$ 0.07 ^b	1.24	0.825 $\pm$ 0.51 ^c	1.05
Sikwane	-	-	0.135 $\pm$ 0.014 ^{bc}	8.44	1.008 $\pm$ 0.21 ^b	2.04	3.276 $\pm$ 0.15 ^a	4.18

Enzyme activity in nmol/ min/ mg of protein⁻¹ ($\pm$ SE); *Relative activity (R.A) = Activity of enzyme in respective population/ Least activity observed in each locality strain; Means in the same column followed by the same letters are not significantly different at $p=0.05$

of synthetic pesticides. The biochemical assays were undertaken in the molecular biology laboratory at the University of Botswana. Individual *T. cinnabarinus* adults were homogenized in Eppendorf tubes containing 200 μ l of ice-cold distilled water. The homogenates were then centrifuged at 14000 $\times$ g for 60 sec and the tubes were placed on ice. Total protein on enzyme suspension was measured using Pierce Bicinchoninic acid (BCA) protein assay kit following the method of Lowry et al. (1951). Activities of α / β - esterases were estimated following a protocol by Safi et al (2017). CYP 450 and GST activities were measured using the method described by Roy et al (2018). The activities of detoxification enzymes were subjected to one-way analysis of variance (ANOVA) and Tukey's Honestly significant difference test ($P < 0.05$) (Zar, 1984) was used to separate the means using SAS statistical software (version 9.4. SAS institute, Cary, USA).

RESULTS AND DISCUSSION

There was a statistically significant difference in α - esterase activity observed between the *T. cinnabarinus* strains ($F_{51, 270} = 4.88$; $P < 0.001$). The relative activity of α - esterase was 3.97 times more in the Bela-bela strain, 2.04 in the Sikwane strain, 1.91 in the Francistown strain, 1.49 in the Glen Valley strain 1.24 in the Moshupa strain, and 1.23 times in the Bobonong strain as compared to the Letlhakane strain. A statistically significant difference in β -esterase activity was also observed among the strains ($F_{51, 270} = 4.76$; $P < 0.0001$). The relative activity of β - esterase was 4.18 times more in the Sikwane strain, 2.51 in Glen Valley, 2.11 in Bobonong, 1.41 in Francistown, 1.22 in the Letlhakane and 1.05 times in the Moshupa strain as compared to the Bela-bela strain. Enhanced activity of esterases is linked to resistance development against pyrethroids and organophosphates in insects (Adhikari & Khanikar, 2021; Grisales et al., 2013; Poupardin et

al., 2014), therefore the elevated esterase activities recorded in Sikwane, Francistown and Glen- Valley strains might be due to indiscriminate use of pyrethroids and organophosphates in these locations. Pyrethroids and organophosphates are highly favoured because of their quick knock down effect. These farms are also in close proximity to urban centres where there is a high demand for high quality and quantity of blemish free vegetables, therefore increased pressure to apply pesticides. The variation in esterase activity could be due to the prevailing pre-adaptive occurrence in spider mite strains from different geographical locations as well as pesticide usage patterns. Elevated esterase activity has been reported in oxydemeton methyl, deltamethrin, monocrotophos, fenvalerate and endosulfan tolerant strains of *H. armigera* (Mohan et al., 2007). Zhao et al. (1996) also found esterases to be responsible for cross resistance to carbamate, organophosphate and pyrethroid pesticides.

The results indicate that there was a significant difference in GST activity among the strains ($F_{49, 180} = 1.46$; $p < 0.0393$). The relative activity of GST was 1.88 times more in the Francistown strain, 1.47 times in the Moshupa strain, 1.45 times in the Bela-bela strain, 1.36 in the Glen Valley strain, when compared to the Bobonong strain. GST activity has been associated with resistance to all main classes of pesticides (Nehare et al., 2010; Roy et al., 2018). Enhanced activity of GSTs may indicate that several different compounds are overused in these farms, possibly to control multiple pest species associated with tomato. A significant difference in CYP450 activity was observed among the strains ($F_{51, 270} = 2.85$; $p < 0.0001$). CYP450 activity was 13.88 times more in the Francistown strain, 12.06 in the Bobonong strain, 8.44 in the Sikwane strain, 7.75 times in Glen Valley, 6.13 times in the Moshupa strain and 4.31 times in the Letlhakane strain as compared to the Bela-bela strain. CYP450s are responsible for the hydrolysis of

organophosphates and pyrethroids by breaking the ester linkage (Das et al., 2017; Bhatt et al., 2021). The high CYP450 activities found in farms in these locations may indicate that pyrethroids are overused in these farming locations (Karuppaiah et al., 2017).

The development of resistance in spider mites is a consequence of prolonged exposure to frequently applied pesticides with similar chemical profiles (Das et al., 2017; Roy et al., 2018). Navarro-Roldan et al. (2020) observed that enzyme activity is directly related to pesticide pressure and it can vary over locations due to the pesticide application regimes. Roy et al. (2018) observed that resistance development in insects and mites increases when pesticides are used thus exerting a high selective pressure on resistant genotypes, but the resistance development is not observed in populations unexposed to pesticides. The results that elevated detoxification enzyme levels were observed in strains collected from areas with low production levels were not expected. This suggests another mode of exposure of the pest to the acaricide active ingredient. Pesticides used in agriculture can contaminate water sources through run-off (Kim et al., 2023), therefore some exposure of the pest to the pesticide active ingredient may be through irrigation with pesticide contaminated water. Although elevated levels of detoxifying enzymes were observed in this study, they generally indicate low activity that may be attributed to the seasonality of planting on a subsistence basis. Additionally, with low levels of production in Botswana, farmers have less pressure to apply pesticides compared with major producers in the region and the world.

The results in this study highlight the need for development of an immediate acaricide resistance management program for *T. cinnabarinus*. Integrated pest management (IPM) remains a vital tool for the management of vegetable pests therefore emphasis should be on the use, in addition to chemical control, of cultural and biological control to reduce dependence on synthetic acaricides for *T. cinnabarinus* management. This study provides important information on the status of major pesticide detoxification enzymes in *T. cinnabarinus* strains from different geographical locations in Botswana. This data can be used as a baseline for resistance monitoring and development of appropriate strategies to manage *T. cinnabarinus* on tomato. More research should be undertaken to measure detoxification enzyme levels for effective *T. cinnabarinus* control in Botswana.

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AUTHOR CONTRIBUTION STATEMENT

MML, TK, NM conceived and designed the experiments. MML, NM and TK contributed new reagents and/or analytical tools. MML and TK analysed the data. MML wrote the first draft of the manuscript. MML and TK contributed to the writing of the manuscript. MML, NM, TK and BT agreed with the manuscript results and conclusions. MML and TK developed the structure and arguments for the paper. MML, NM and BT made critical revisions and approved final version. All authors reviewed and approved of the final manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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