BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

Farmers' knowledge of the carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae), its susceptibility to acaricides and activities of detoxifying enzymes in Botswana

A thesis presented in fulfillment of the degree of Doctor of Philosophy in Crop Science (Crop Protection)

By

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CERTIFICATION

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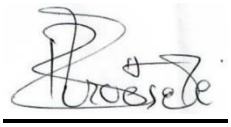
PROF. BAMPHITLHI TIROESELE		**04/07/2023**

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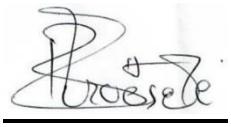
PROF. BAMPHITLHI TIROESELE		**04/07/2023**

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STATEMENT OF ORIGINALITY

I, Mosimanegape Mitch Legwaila, declare that this dissertation is my original work except where due reference is made. The work contained in this thesis/dissertation was completed at the Botswana University of Agriculture and Natural Resources (BUAN) and it is an original work and neither has been nor will be submitted for the award of any other University.

Mosimanegape Mitch Legwaila **04/07/2023**

AUTHOR'S NAME AND SIGNATURE DATE

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DEDICATION

This work is a dedication to my dearest loving mother, Leano Gladys Legwaila, for all the sacrifice you made to ensure that I become a responsible man. Since the passing of our father, Ponatshego Victor Legwaila, you have remained our pillar of strength. Thank you for being there when I felt like giving up. I will always be grateful. May the good Lord continue to protect you.

GENERAL ABSTRACT

Tomato (*Solanum lycopersicon* L.) is an important vegetable due to its nutritional and economic value. It's production has become very difficult and costly due to the prevalence of pests and diseases. Spider mites are among the most damaging pests of tomato in Botswana. The overarching objective of this study was to assess farmers' knowledge of the carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae), its susceptibility to acaricides and activities of detoxifying enzymes in Botswana. An oral questionnaire was used to evaluate farmers' knowledge, perceptions and management of the carmine spider mite (CSM) on tomato in Botswana. The second study evaluated the effectiveness of selected pesticides for the control of CSM in Botswana. In a third study, the economic injury levels and yield loss were assessed for CSM on tomato. The fourth study evaluated pesticide detoxification enzyme activities in CSM strains collected from different locations in Botswana. The results of the survey showed that most farmers (75.7%) identified spider mites as a major constraint to profitable tomato production. Spider mites reduce the quality and quantity of tomatoes resulting in loss of income. The red form of the spider mites was more prevalent in farms across the country. All the farmers interviewed had prior knowledge of spider mites and their sources of pest information were personal experiences, agro-traders and fellow farmers. Demographic characteristics did not have a significant effect on the seriousness of the spider mite problem. The use of chemical pesticides was the most common method of controlling spider mites. An array of pesticides are applied, some of which are not in the list of chemicals recommended for spider mite control. Some farmers report reduced effectiveness of some of the pesticides used for its control. This has made its control very difficult since most farmers are resource poor and can not afford the cost associated with controlling spider mites. The second study evaluated the efficacy of abamectin, methomyl and

chlorfenapyr against CSM eggs and adults in the laboratory. The treatments were each replicated three times. The toxic effect was evaluated in the laboratory bioassay after 24, 48, 72 and 96 h of application of pesticides. Chlorfenapyr and methomyl were highly effective in the control of eggs and adult spider mites. Although abamectin required longer exposure periods to achieve effective control of both eggs and adults it did not need to achieve high mortalities to offer adequate protection to the tomato crop. The study showed that the pesticides evaluated can be used as part of an integrated management programme to reduce resistance development by using one pesticide. The third study was conducted over two cropping seasons, 2018/2019 and 2019/2020, to evaluate the economic injury level and assess yield loss for carmine spider mite on tomato in Botswana. Tomato plants were infested with adult spider mites for periods of 0 (no exposure), 1, 2, 3, 4, 5, 6 and 7 weeks (complete exposure). The corresponding treatments were 7, 6, 5, 4, 3, 2, 1 and 0 sprays with abamectin. The results showed a significant reduction in the spider mite populations per plant as the frequency of spraying increased. An inverse relationship between spider mite exposure and yield was also observed following three weeks exposure. Yield loss increased to more than 50% when the pesticide was not applied to control spider mites. Economic decision levels are important components of cost saving integrated pest management programs and can be effective tools for making decisions about the application of pesticides against carmine spider mite in Botswana. The fourth study was conducted to establish pesticide metabolism enzymes activity among CSM strains collected from tomato fields in seven geographical locations of Botswana. Activities of metabolic enzymes, namely, esterases (α and β- esterases), cytochrome P_{450} monooxygenases and glutathione-*S*-transferases were estimated. The highest levels of α-esterase activity (nmol/min/mg of protein^{-1}) were observed in the Bela-bela strain (1.966 nmol/min/mg of protein^{-1}), followed by Sikwane (1.008nmol/min/mg of protein^{-1}). The Sikwane strain (3.276

nmol/min/mg of protein^{-1}) registered enhanced β-esterase activity, followed by the Glen Valley strain (1.966 nmol/min/mg of protein^{-1}) and Francistown (1.102 nmol/min/mg of protein^{-1}) strain. Elevated level of GSTs were observed in the Francistown (20.026 nmol/min/mg of protein^{-1}), followed by the Moshupa (15.655 nmol/min/mg of protein^{-1}) and Bela-bela (15.371 nmol/min/mg of protein^{-1}) strains. The Francistown strain showed the highest (0.222 nmol/min/mg of protein^{-1}) P_{450} monooxygenase activity followed by Bobonong (0.193 nmol/min/mg of protein^{-1}) and Sikwane (0.135 nmol/min/mg of protein^{-1}) strains. Variation in detoxification enzymes activity among CSM strains can be attributed to differing pesticide application regimes. These findings will be helpful in the selection of acaricides and in formulating resistance management strategies for effective management of spider mite in tomato fields in Botswana.

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LIST OF ABBREVIATIONS/ACRONYMS

ANOVA	analysis of variance
BSA	bovine serum albumin
cm	centimetre
CSM	carmine spider mite
TSSM	two-spotted spider mite
^{o}C	degree celcius
EC	emulsifiable concentrate
EIL	economic injury level
ET	economic thresholds
g	grams
GE	general esterases
GST	glutathione S- transferease
IRAC	Insecticides Resistance Action Committee
LC_{50}/LD_{50}	concentation/dosage required to cause 50% mortality
LC_{90}/LD_{90}	concentration/dosage required to cause 90% motality
LEA	Local enterprise authority
mg	miligrams
ml	mililitres
M	Moles
pp.	pages
%	percentage
PCR	polychain reaction
R.A	relative activity
SAS	statistical analysis system
Sc	soluble concentrate
SE	standard error
SPSS	statistical package for social sciences
STDEV	standard deviation
var.	variety

CHAPTER 1 – GENERAL INTRODUCTION

1.1 Background

Tomato (*Solanum lycopersicum* L., Solanaceae) is commonly grown and consumed in Sub-Saharan Africa. It is among the highly popular vegetable crops due to its nutrient density and economic value (Olaoye *et al.*, 2006; Nicola *et al.*, 2009; Baliyan & Rao, 2013; Mwandila *et al.*, 2013;). In most rural suburban communities, tomato is mostly grown as a cash crop serving as an important source of economic sustenance for the population (Fufa *et al.*, 2011; Boukar *et al.*, 2016; Ochilo *et al.*, 2019). Tomato is a multipurpose vegetable consumed in a variety of ways including in salads, or as an ingredient in many dishes (Ahmed, 2004; Dube *et al.*, 2010; Badimo, 2020). It is also processed into soups, pastes, concentrates, juices, sauces and ketchup (Bergougnoux, 2014) making it the most versatile and widely consumed vegetable worldwide (Shakeel *et al.*, 2012; FAO, 2016; USDA, 2016). Tomato is an important source of iron, phosphorus and vitamins (Cheema and Dhaliwal, 2005). It contains significant quantities of B complex vitamins, niacin, riboflavin and thiamin. Tomatoes are a major food source of important phytochemicals, carotenoids, phenolic acid, phytosterols, polyphenols, ascorbic acid, flavonoids and calcium (Wilcox *et al.*, 2003; Hedges & Lister, 2005; Luthria *et al.*, 2006; Erba *et al.*, 2013) therefore contributing to overall health (Hanson and Yang, 2016). Lycopene, the most well-known and potent antioxidant with high anti-carcinogenic potential is abundant in tomatoes (Hedges & Lister, 2005; Erba *et al.*, 2013; Kumar, 2014). Antioxidants in tomato are responsible for inactivating volatile oxygen molecules and, consequently delay or even prevent oxidative damage (Dube *et al.*, 2020). Tomato is easily digestible and its bright color stimulates appetite (Sainju and Dris, 2006).

According to data from Faostat, the world produced over 186 million metric tonnes of fresh tomatoes on more than five million hectares of land in 2020. The major producers of tomato are China, United States, Turkey, Egypt, Italy, Spain and India (FAO, 2020). Africa contributes about 11.8% of total global production of tomato with Egypt and Nigeria as the largest producers in Africa (Dube *et al.,* 2020). In the year 2021, the government of Botswana introduced a vegetable import ban in an attempt to encourage local production. However, the initiative was met with sceptisism as local producers could not meet local demand. In 2018, the monthly demand for tomato was estimated at 12 000 tonnes, however local productivity ranged between 60 and 100 tonnes owing to variety and prevailing growing conditions (Disele, 2018), and this presents a serious shortfall. Local production can only satisfy about 40% of local demand (LEA, 2015).

The low productivity of tomato in Botswana is caused by several factors, which include unfavorable climatic conditions, low yielding varieties, pests and diseases (Baliyan and Rao, 2013). Botswana experiences extremely high temperatures which can sometimes reach 45^{o}C, which make vegetable production very difficult. Under these conditions, vegetable crops are prone to a variety of arthropod and disease infestations (Baliyan, 2012). Furthermore, the majority of production in Botswana is undertaken by resource poor farmers who do not have access to finance, electricity, water, transport, and marketing facilities (Madisa *et al.,* 2010). The nature and magnitude of these constraints differ across geographic regions. However, the most challenging constraints for the production of tomatoes in Botswana are invertebrate pests (Munthali, 2004; Obopile *et al.,* 2008; Baliyan and Rao, 2013), including the African bollworm (*Helicoverpa armigera)*, tomato leaf miner (*Tuta absoluta*), cutworms (Agrotis spp.), semi-looper (*Chrysodeixis acuta*), leaf miners (*Liriomyza* spp.), root-knot nematodes and red spider mites (*Tetranychus* spp.).

The Carmine spider mite (CSM) (Acarina: Tetranychidae), is among the most economically damaging vegetable pests globally (Auger *et al.*, 2013; Chen *et al.*, 2021). CSM are among the most devastating invertebrate pest of tomatoes and a major factor contributing to loss of quality and yield of tomatoes (Hennebery *et al*., 1991; Shakeel *et al*. 2011; Mwandila *et al.*, 2013). It causes yield losses to many vegetable crops grown in open fields and protected environments around the world (Kielkiewicz, 1996). Spider mites penetrate their stylet into the leaf to feed by and sucking out the cell contents (Murungi *et al.*, 2014). This results in reduction in chlorophyll and photosynthetic efficiency of the leaves (Park and Lee, 2005). They also cause speckling on leaves and fruits which reduces the aesthetic appeal of the fruits to consumers. High CSM population levels can cause crop losses of over 80% (Sibanda *et al.*, 2000). These pests are normally present annually causing crop losses and may be the reason why Botswana is not among the top producers of tomatoes in Sub-Saran Africa. In Botswana as well as in most southern African countries, the management of invertebrate pests, including CSM, is heavily reliant on the usage of synthetic pesticides (Munthali, 2004; Obopile *et al.*, 2008). Pesticides with different mechanisms of action are used to manage pests of tomato in Botswana and these include avermectins, pyrethroids, organochlorines, pyrazoles, organophophates and carbamates. According to Obopile *et al*. (2008), the provocation to apply control measure is premised on noticing the pest or pest symptoms on the crop.

1.2 Problem statement

In Botswana, the productivity of tomato is generally low because of pests and diseases, unfavorable weather conditions, water unavailability, poor soils, lack of market and lack of transport (Munthali, 2004; Obopile *et al*., 2008; Baliyan, 2012; Madisa *et al.*, 2012). Several researchers (Obopile *et al*., 2008; Mwandila *et al*., 2009; Madisa *et al*., 2010a; Baliyan, 2012) have stated that most farmers in Botswana perceived invertebrate pests and diseases as the main limitations to vegetable productivity. The attack by a variety of pests is a major factor discouraging profitable yields and is the main cause of low-quality tomatoes, which translates to losses to the farmer (Hennebery *et al*., 1991; Shakeel *et al*. 2011). Obopile *et al.* (2008) also found that spider mites are among the most damaging pests hindering tomato production in Botswana.

Although other control methods are available, chemical pesticides play an important role in arthropod pest management (Munthali, 2004; Obopile *et al*., 2008; Wang *et al*., 2015). As pesticides are heavily and frequently applied there is great chance that the pesticides will increase the cost of production (Mukiipi, 2001) and have a negative impact on the environment (Tusiime, 2014). Pesticides can also lead to health problems since farmers apply them without appropriate personal protective clothing and use defective spray equipment (personal observation). The frequent and indiscriminate use of pesticides, however, often places selective pressure on spider mites (Van Leeuwen *et al*., 2010; Wang *et al*., 2015). Farmers in Botswana have reported reduced effectiveness of pesticides commonly applied to manage spider mites in their farms. However, no study has been undertaken to determine the usefulness of current management actions against spider mite effecting tomato production in Botswana. The strategy for increasing tomato productivity and profitability could be by development of an effective spider mite management programme. The current management strategies can be enhanced by adopting an integrated pest management (IPM) approach to manage spider mites and pest complexes on vegetables which will

also help delay resistance development. The use of economic decision levels will also guide on the timing of application of control measures and reduce unnecessary wastage.

1.3 Justification of study

The cultivated tomato is an important source of livelihood and nutrition for rural and suburban households (Fufa *et al.,* 2011; Ochilo *et al.,* 2019; Dube *et al.,* 2020). Invertebrate pests have been cited as a significant constraint to tomato production with spider mites documented to be an important pest of tomato production in Botswana. The management of spider mites mainly depends on the usage of synthetic acaricides with different pesticide chemistries and mechanisms of action. These chemicals are often not properly applied and the decision to apply them is upon seeing pest or damage symptoms on the crop. The indiscriminate and improper application of pesticides has its negative consequences including environmental pollution, harm to non-target organisms, effect to human health and evolution of resistance by the pest (Al-Zyoud, 2014). Spider mites have a documented propensity to rapidly evolve resistance to most pesticides employed for their management. Local farmers have reported declining efficacies of previously effective chemical formulations presumably due to resistance development. Additionally, pesticides are very expensive therefore increase the production costs of the farmer. The current situation of reduced effectiveness of pesticides against spider mites justifies research on the effectiveness of pesticide formulations used against local strains of spider mites and economic decision levels need to be established for effective and efficient use of pesticides. This is an important step towards avoiding resistance development, reducing spider mite management costs and improving the profitability of tomato production in Botswana.

1.4 Objectives

1.4.1 General Objective

The overarching objective of this study was to assess farmer's knowledge of the carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae), its susceptibility to acaricides and activities of detoxifying enzymes in Botswana.

1.4.2 Specific objectives

1. To assess farmers' knowledge, perceptions and management strategies for spider mites in six geographical locations in Botswana.
2. To determine the effectiveness of commonly applied pesticides in the control of spider mite populations in tomato production in Botswana.
3. To develop economic injury levels and yield loss assessment for spider mite on tomato.
4. To compare the activities of detoxifying enzymes in spider mite strains from different geographical locations in Botswana.

1.5 Research Hypotheses

The study tested the hypotheses:

Null hypothesis (H_o)

1. Farmers' knowledge and perceptions influence their management of spider mites in Botswana.
2. The pesticides evaluated (Abamectin, methomyl and chlorfenapyr) are equally effective for the management of spider mites on tomato.
3. There is a critical population density of spider mites that causes economic damage to tomatoes.
4. There is a significant difference between detoxifying enzyme activities in spider mite strains collected from different geographical locations in Botswana.

Alternative hypothesis (H_a)

1. Farmers' knowledge and perceptions do not influence their management of spider mites in Botswana.
2. The pesticides evaluated (Abamectin, methomyl and chlorfenapyr) are not equally effective in the control spider mites on tomato.
3. There is a no critical population density of spider mites that causes economic damage to tomatoes.
4. There is no significant difference between detoxifying enzyme activities in spider mite strains collected from different geographical locations in Botswana.

CHAPTER 2 - LITERATURE REVIEW

2.1 Ecology of carmine spider mite (CSM)

Spider mites are considered the most destructive family of phytophagous mites in the world (Alatawi & Kamran, 2018; Marić, 2018). They belong to the class Arachnida and the order Acari, which is the most diverse taxon in the subphylum Chelicerata, comprising over 40, 000 determined species that display significant differences in lifestyle, including predatory, parasitic and plant-feeding mites (Grbic *et al*., 2011). Two of the most damaging families to crop plants are in the families Eriophydae and Tetranychidae (Hoy, 2011).

Mites of the family Tetranychidae are commonly referred to as spider mites. The name underscores their ability to spin silken webs and establish and maintain a colonial micro-habitat to shelter them from abiotic agents, protect them from natural enemies, provide a mode for pheromonal communication and provide a means for dispersal (Grbic *et al*., 2011). The genus *Tetranychus* hosts three very destructive spider mite species, *T. cinnabarinus* (Boisduval) (Carmine spider mite) (CSM), *T. urticae* (Koch) (Two-spotted spider mite) (TSSM) and *T. evansi* (Baker & Prichard) (Tobacco spider mite) (TSM) (Visser, 2005). TSSM and CSM are both notorious pests of agricultural importance that feed on numerous economically important plants worldwide (Bi *et al*., 2016; Lu *et al*., 2017).

Attempts to distinguish between the TSSM and CSM have brought about a lot of dialogue. Researchers have had great difficulty attempting to separate between the two based on morphological divergence. This proved difficult because both species are polymorphic and they presented variations among populations found on different host plants and geographical locations. Specialists of the family Tetranychidae considered both TSSM and CSM to be the same species (Baker and Tuttle, 1994; Bolland *et al.*, 1998; Ehara, 1999). Several other scientists showed significant differences between the two in that TSSM females have 10 setae on tibia 1 while CSM has 10 – 13 setae (an addition of up to three solenidia) on tibia 1 (Kung and Cheng, 1990). Zhi-

quiang and Jacobson (2000) suggested that the two species cannot be reliably differentiated using colour. The lack of a distinctive clarification scheme has now resulted in the red coloured spider mite being referred to as CSM and the green form TSSM (Baker and Tuttle, 1994; Bok *et al*., 2006; Mwandila *et al.,* 2013).

Spider mites are extensively distributed and considered among the most polyphagous arthropods shown to feed on over 1,100 plant species across over 180 plant families (Agrawal, 2000; Naher *et al*., 2008; Lin *et al*., 2009; Grbic *et al*., 2011; Van Leeuwen *et al*., 2012; Xu *et al*., 2014; Hossain *et al*., 2022). They feed on food crops, trees, and ornamental plants among others, causing serious economic injury and sometimes plant death (Jhonson & Lyon, 1991). Spider mites attack plant species of the nightshade family all over the world including Africa (Varela *et al*., 2003). It is regarded as the principal pest of tomatoes across Southern Africa most damaging during the dry season (Knapp *et al*., 2003). In Botswana, spider mites are considered among the most damaging pests of tomato everywhere tomatoes are grown (Munthali *et al*., 2004; Mwandila *et al*., 2013; Obopile *et al*., 2008). CSM is more prevalent in tomato fields across Botswana (Munthali *et al*., 2004; Bok *et al.,* 2006; Anonymous, 2019) and is consequently the main focus of this study.

Figure 1. Adult carmine spider mite (Nikon photo microscope- X2000 magnification)

2.2 Origin and distribution of the carmine spider mite

Spider mites possibly originate in the South Americas from where they spread to other parts of the world (Boubou, 2011). In Africa, spider mites were first reported in tobacco fields in Zimbabwe in 1979 before spreading to other African countries (Azandeme -Hounmalon *et al.*, 2015). It was also discovered to have spread from Mauritius to Reunion in the 1970s. Spider mites have been recorded in most countries including Africa, Asia, Europe, America, Australia, the Pacific and Caribbean islands (Bolland *et al*., 1998; CABI, Undated; Tsagkarakou, 2007).

2.3 Lifecycle of spider mites

Spider mite development follows the typical lifecycle of warm weather spider mites and progresses through four stages of development: (1) the oval translucent egg; (2) the six-legged translucent larva; (3) the eight-legged immature stage (protonymph and deutonymph) and (4) the eight-legged adult. The stages are each separated by a resting stage prior to moulting into the adult stage (Klubertanz *et al*., 1991; Mwandila *et al*., 2013).

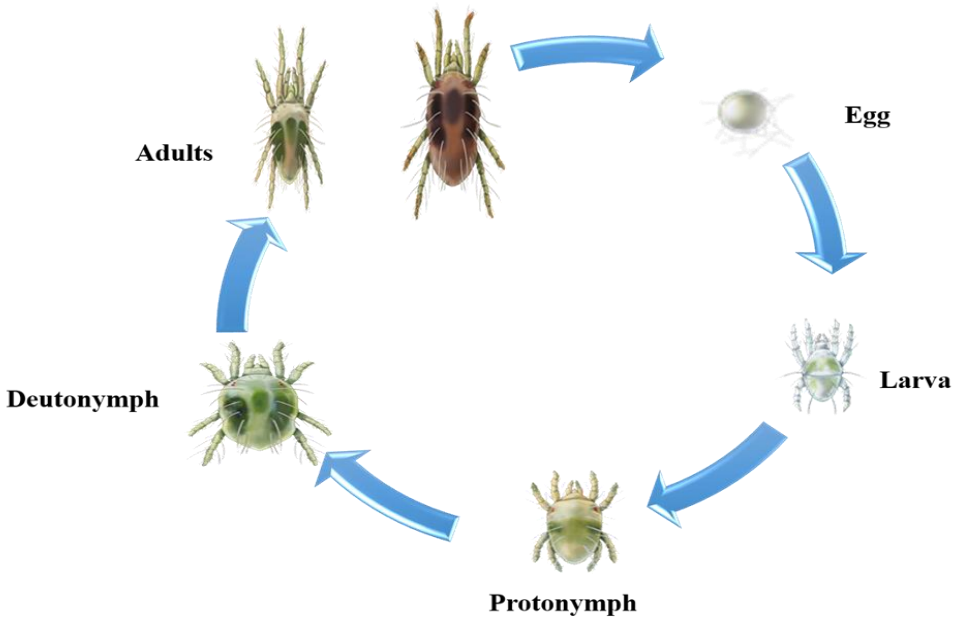

Figure 2. Lifecycle of carmine spider mite (the larger adult is the female). Adapted from Wikipedia, the free encyclopedia (Online). Accessed 6th September 2019.

At optimal temperatures of 30 ºC spider mites complete their lifecycle within 8 - 12 days. All life stages are typically present all through the year, depending on the environmental conditions (Zhang, 2003; Naher *et al*., 2008). The lifecycle begins with small oval, translucent eggs (Kaimal and Ramani, 2011), about 0.14mm in diameter that are laid individually (Van Leeuwen *et al*., 2010). The developmental duration for eggs ranges betwwen 2 and 3 days depending on prevailing temperatures. The eggs slowly develop a bright orange colour and later hatch into pink six-legged translucent larvae that begin to feed immediately. Spider mites display a haplo-diploid breeding system – sexual and arhenotokous reproduction. Eggs laid by copulated females develop into either males or females (diploid) while eggs from un-fertilized females develop into males (haploid) (Kaimal and Ramani, 2011; Tehri, 2014; Ding *et al.*, 2018). The haploid genetic system allows an individual spider mite female to start a new colony and initiate an outbreak. Spider mites lay an average of five to six eggs daily, with a lifetime total of up to 200 (Mwandila *et al*., 2013). The period of incubation is longer (2.73 days) for sexual development in contrast with parthenogenesis (2.69 days) (Kaimal and Ramani, 2011). The larvae are small, hexapod and yellowish with slight sexual dimorphism especially at the hysterosomal region. The larva of spider mites passes through the 1st quiescent stage and subsequently moults into the 1st nympal stage (protonymph). The protonymph is somewhat larger than the larvae, reddish brown with four pairs of limbs and is more active than the larva (Knapp *et al*., 2009; Kaimal and Ramani, 2011). At the completion of each active developmental stage, spider mites enter into a period of quiescence when they penetrate their stylet into the leaf and stay inactive near the mid-rib. At the end of quiescence, the cuticle becomes transparent. A slit appears at the dorsal region below the propodosoma, and is widened

by vigorous movements, leading to the appearance of the subsequent instar. The entire process takes under 30 minutes to complete (Kaimal and Ramani, 2011; Piraneo, 2013). The deutonymph is smaller and lighter in colour but resembles the adult with a difference in setation (Kaimal and Ramani, 2011). Male spider mites mature earlier and copulation takes place as soon as the adult female emerges. Females begin laying eggs as soon as they emerge as adults (Knapp *et al*., 2003; Piraneo, 2013).

Outbreaks most commonly occur during summer months with spider mite populations increasing rapidly, and with many overlapping generations at a time (Naher *et al*., 2008). Spider mite development takes longer at low temperatures, averaging up to a month. Plant characteristics including; age of foliage, plant nutrition, and moisture stress have an effect on spider mite development (Klubertanz *et al*., 1991). Adult males are very vigorous, smaller, with a wedge shaped hystrosoma, narrowing at the posterior end (Kaimal and Ramani, 2011; Piraneo, 2013) while females are sizeable, reddish and more oval in shape (Mwandila *et al*., 2013). Female spider mites enter a state of reproductive facultative diapause (pause their development) when immature stages are exposed to decreased photoperiod (Piraneo, 2013; Byron *et al*., 2017), thus allowing spider mites to survive hostile conditions (Ghazy & Suzuki, 2014). Once spider mites enter diapause they move to resting sites, such as dried litter, ground cover, soil and tree bark (Kim & Lee, 2003). Diapause causes several molecular, physiological and morphological changes characterised by a lowered metabolic rate, reduced energy expenditure, heightened stress tolerance and the synthesis of cryoprotectants that delays freezing and desiccation (Popov & Veerman, 1996; Piraneo, 2013). The intense orange colour of diapausing females is attributable to a rise in ketocarotenoids. As the environmental conditions improve, spider mites look for host plants for food and oviposition sites (Kim & Lee, 2003; Piraneo, 2013).

2.4 Feeding behavior and associated damage

Figure 3. Symptoms of carmine spidermite (a) feeding and (b) webbing on the tomato plant (Photo: Legwaila M. M.).

Spider mites mainly feed on the leaves by piercing a retractable stylet through the open stomata or between epidermal cells. Spider mites inject saliva into the mesophyll cell to predigest the contents and then draw out the liquid contents (Murungi *et al*., 2014; Santamaria *et al*., 2020). A mature spider mite ingests up to 50 percent of its body weight with each hour that passes. Numerous chloroplast containing leaf cells are pierced and emptied by each spider mite every minute (Chhillar, 2007). This results in loss of chlorophyll consequently reducing the photosynthetic rate (Park & Lee, 2005) and causing the development of chlorotic lesions (Bensoussan *et al*., 2016). This also negatively affects transpiration (Park & Lee, 2005) leading to reduced vigour, growth, flowering and consequently reduction in yield (Mathews and Tunstall, 1994; Tehri, 2014). Under severe infestations, the stippled lesions coalesce and then the leaf crumbles and dies (Park & Lee, 2005; Nyoike & Liburd, 2013; Bensoussan *et al*., 2016). When food becomes limited due to over infestation, spider mites produce a silken web as a medium to disperse by either wind or animal

dispersal to colonise new plants (Clotuche *et al*., 2011). The silk webbing also protects the spider mite colony from adverse elements and functions as a pheromone substrate (Meyer, 1996; Santamaria *et al*., 2020).

High temperature and low humidity conditions that are prevalent in Botswana allow spider mite populations to increase rapidly causing serious damage to plants (Nyoike & Liburd, 2013; Bensoussan *et al*., 2016). Extreme level of damage usually causes leaf and fruit failure and eventually death of the tomato plant (Knapp *et al*., 2003; Van Leeuwen *et al*., 2012). At high population levels, spider mites may cause irrepairable damage to the plant leading to total crop loss (Nyoike & Liburd, 2013). Moreover, the defoliation, webbing, and build up of faeces can affect the tomato plant's aesthetics and commercial worth (Jhonson & Lyon, 1991). These factors can reduce the preference of customers and may lead to severe economic losses.

2.5 Pest status and host plants of carmine spider mite

Spider mites are considered a principal pest of tomatoes in many African communities (Saunyama and Knapp, 2003). It has a wide host plant range worldwide (Meyer, 1996; Nauen *et al*., 2001; Farouk & Osman, 2011) and is documented to infest more than 1,100 plant species most of which are commercially significant (Zhang, 2003; Xie *et al*., 2006; Chillar *et al*., 2007). The pest status of spider mites on greenhouse vegetables, ornamental and horticultural crops is well reported worldwide (James & Price, 2002; Irigaray *et al*., 2003; Islam *et al*., 2008; Parvin & Haque, 2008). Spider mites have shown an obvious proclivity for solanaceous crops which include tomato,

potato, nightshade and tobacco (Leite *et al*., 2003). CSM has been shown to have a high reproductive capacity and a more social behavior in contrast with TSSM (Azandeme -Hounmalon *et al*., 2014). Under Botswana's climate change situation comprised of hot and dry conditions, spider mite life cycle is shorter and produces more offsprings per year.

2. 6 Management of tetranychid mites

Historically, spider mite infestations have been kept at a minimum by natural enemies, diseases, and poor plant nutrition until the 1950s when pest management became intensely reliant on chemical pesticides which were deemed very effective (Guo *et al*., 1998; Piraneo, 2013).

2.6.1 Chemical control measures

Several pesticide formulations are registered in Botswana for use in the management of invertebrate pests affecting vegetable production. The pesticides registered and used in Botswana for control pests are shown in Table 1. The control of spider mites exclusively by application of synthetic pesticides has risen globally (Sundaram & Sloane, 1995; Van Leeuwen *et al*., 2010). A wide variety of pesticides with varying chemical structures and mechanisms of action are employed in the management of spider mites (Knowles, 1997; Fahnbulleh, 2007; Attia *et al*., 2013). Mitochondrial electron transport inhibitors (METI) which act by uncoupling electron transport from the phosphorylation of ADP to ATP, thus wasting the energy as heat in process; or by inhibiting ATP synthase, an enzyme that converts ADP to ATP (Ahammadsahib, 1995; Wood *et al*., 1996; Hollingworth & Tomlin, 2000). The other group of pesticides/acaricides employed in spider mite control is referred to as the mite growth inhibitors affect mostly nymphs by preventing moulting and have little effect on adult females, apart from causing them to lay fewer viable eggs (Fahnbulleh, 2007). The mectins accelerate the movement of chloride-ions leading to paralysis

and death of the spider mite. Chlorine channel activators are ɣ- Aminobutyric acid (GABA) receptor antagonists still used in spider mite control in many countries. Most of the pesticides used in Botswana are featured in the list of extremely hazardous or highly hazardous chemicals by the World Health Organisation (WHO, 2019).

Table 1. Some pesticides used in Botswana to control pests and diseases

Active ingredient	WHO hazard class	Crop	Target pest/disease
Cypermethrin (Pyr) (i)	II	Tomato, onions *brassicae	*H. armigera, P. xylostella, B. brassicae, H. undalis, B. tabaci, B. hilaris*
Malathion (OP) (i)	II	Butternuts, brassicae, onion	*Bactrocera* spp., *B. tabaci, P. xylostella*
Alpha – cypermethrin (Pyr) (i)	II	Tomato, brassicae	*H. armigera, B. hilaris, B. brassicae*
Dimethoate (OP) (i)	II	Brassicae, onion	*B. brassicae, B. tabaci*
Chlorpyrifos (OP)(i)	II	Tomato, cabbage	*H. armigera, P. xylostella*
Methomyl (Carb) (i)	1B	Brassicae, tomato	*P. xylostella, H. armigera*
Carbaryl (Carb)(i)	II	Tomato, cabbage	*Agrotis* spp.
Fenthion (OP)(i)	II	Butternuts	*Bactrocera* spp.
Diazinon (OP)(i)	II	Butternuts, onion	*Bactrocera* spp.
Demeton- s-methyl (OP)(i)	1B	Brassicae	*B. brassicae*
Trichlorfon (OP)(i)	II	Tomato	*L. trifolii*
Endosulfan (OC)(i)	II	Tomato, onion, cabbage	*H. armigera, B. tabaci*
Deltamethrin (Pyr)(i)	II	Brassicae, onion	*B. tabaci, B. hilaris*
Parathion (OP) (i)	1A	Cabbage, onion	*B. hilaris*
Dichlorvos (OP)(i)	1B	Brassicae	*P. xylostella, B. brassicae*
Methamidophos (OP)(i)	1B	Tomato, cabbage	*B. brassicae*
Beta-yhalothrin (Pyr)(i)	II	Tomato	*Tetranynchus* spp.
Dicofol (OC)(a)	III	Tomato	*Tetranynchus* spp.
Chlrofenapyr(Prz)(i)(a)	II	Tomato, cabbage	*Tetranynchus* spp. *P. xylostella*
Abamectin (Avermectin) (a)	not listed	Tomato	*Tetranynchus* spp.
Fenamiphos (OP)(i)(n)	1B	Tomato, spinach	*Meloidogyne* spp.
Carbofuran (Carb)(i)(n)	1B	Cabbage	*B. hilaris*
Mancozeb (Dithio)(f)	U	Swiss chard	Leaf spot
Copper oxychloride (Cu)(f)	III	Cabbage	Powdery mildew

Carb = carbamates; Cu = inorganic-copper; OC = organochlorine; OP = organophosphate; Dithio = dithiocarbamate; Prz = Pyrazole; Pyr = pyrethroid; a = acaricide; f = fungicide; I = insecticide; n = nematicide; 1A = extremely hazardous; 1B = highly hazardous; II = moderately hazardous; III = slightly hazardous; U = unlikely to present acute hazard in normal use; *brassicae = cabbage, choumollier, rape (Source: Obopile *et al*., 2008)

2.6.2 Spider mite resistance to pesticides

The intensive and unselective application of synthetic chemicals to control vegetable pests has been the major contributor to development of resistance in arthropod species globally such that their control has become extremely difficult (Guo *et al*., 1998; Stumpf & Nauen, 2001; He *et al*., 2009; Van Leeuwen *et al.,* 2010). Resistance to a synthetic pesticide occurs when an arthropod population becomes less sensitive to pesticide applications that were previously effective against

it (Price & Nagle, 2012). Outbreaks of spider mites are mostly attributable to their ability to rapidly evolve pesticide resistance (Grbic *et al*., 2011; Piraneo, 2013). Several characteristics including its minuscule individual size, high fecundity, short developmental period, high mutation rate, strong adaptability, extensive dispersal behavior, polyphagous feeding habit, and frequent application of pesticides, have allowed spider mites to quickly develop resistance against most pesticides used in their control (Stumpf & Nauen, 2001; Fahnbulleh, 2007; Reddy & Dolma, 2018; Choi *et al.*, 2020). Spider mite control failures as a result of resistance development have been documented for numerous chemical compounds, including organophosphates, organotins, hexythiazox, abamectin and clofentezine, following their introduction (Van Leeuwen *et al*., 2006). This has earned it the notorious reputation of the "most resistant species" in terms of the aggregate number of chemicals to which it has evolved resistance (Van Leeuwen *et al.*, 2010).

Spider mites, as with other arthropods, possess several biological mechanisms that allow them to survive lethal doses of a pesticide which include reduced target-site sensitivity, elevated metabolism and movement of the pesticide active ingredient (Feyereisen *et al.*, 2015; Riga *et al.*, 2017). The mechanisms often involve alteration in the sensitivity of the target site owing to point mutations, or increased metabolism of the pesticide before it reaches the site of action (Li *et al.*, 2007; Van Leeuwen *et al.*, 2010; Feyereisen *et al.*, 2015). Metabolic resistance, or elevated detoxification of pesticides is the most studied and common type of resistance (Li *et al.*, 2007). It is a mode of resistance development that comprises the breaking down of the pesticide lethal component prior to it reaching the target site, caused by the qualitative or quantitative changes in key detoxification enzymes, due to the up-regulation of detoxifying enzyme genes (Van Leeuwen *et al.*, 2010; Roy *et al.*, 2018). The key detoxification enzymes in spider mites are Carboxylesterase (CarE), mixed function oxidase (MFO), and glutathione-S-transferase (GST) which can detoxify

and excrete exogenous compounds through specific pathways to minimise damage and to develop pesticides resistance (He *et al.,* 2009; Dias *et al.,* 2016). The significance of these enzymes is that they detoxify xenobiotics into non-toxic compounds (Khan *et al.,* 2020). These enzymes are responsible for spider mite resistance development to almost all classes of pesticides. When spider mites come in contact with the pesticide material, several changes take place in the activities of metabolic enzymes which affect the normal metabolism of spider mites (Liu *et al.,* 2014). Resistance mechanisms in *Tetranychus urticae* Koch are widely reported (Kumral *et al*., 2009). However, that information is not available for CSM in Botswana. Therefore, assessment of these detoxification enzymes in CSM can provide a foundation for reducing the development of spider mite resistance to pesticides. The second most common type of resistance, target site resistance, involves the adjustment of the structure of the pesticide receptor molecule, which decreases the binding of the chemical to its receptor, therefore reducing the effectiveness of a pesticide that acts on that site (Russel, 2004; Khan *et al.,* 2020). This mechanism of resistance usually confers some level of cross-resistance to all pesticides acting on that particular site. Principal target site changes leading to resistance are voltage sensitive sodium channels to DDT and pyrethroids, GABA-chloride channels to cyclodienes and related compounds, and acetylcholinesterases to organophosphates and carbamates (Clark and Yamaguchi, 2001). Four different types of target site resistance mechanisms exist in insects/pests namely; nicotinic Acetylene choline receptor-based resistance (nAChRs); modified acetylcholine esterase-based resistance (MACE) resistance; knock-down resistance (KDR); and duplication of resistance to dieldrin (RDL) Gamma-Amino Butyric Acid (GABA) Receptor (Dang *et al.,* 2017; Khan *et al.,* 2020). Behavioral resistance (deterrence), the third mechanism of pesticide resistance, arises when arthropods are capable of avoiding (escaping) contact with the pesticide, or their behavior is modified such that they cannot

come into contact with it, even when they cannot sense it (Grieco and Achee, 2007). This type of resistance is further categorised into direct contact irritation (excitation) and non-contact (spatial) repellency. Contact excitation involves the arthropod escaping the treated area only after making physical contact with the pesticide, whereas non-contact repellency' is when the pest avoids the pesticide-treated zone without making direct contact (Roberts *et al.,* 1997; Chareonviriyaphap *et al.,* 2013).

Cuticular penetration resistance involves the thickening of the arthropod cuticle, and alteration of cuticular components and the physical structure of the cuticle layers to reduce the rate of absorbance of the pesticide material through the cuticle or gut lining (Liu *et al.,* 2015; Dang *et al.,* 2017). Although this is the least studied resistance mechanism, it is reported to be responsible for delaying pesticide molecules from reaching their target proteins (Chen *et al.,* 2019). The arthropod cuticle is a complex of matrix of chitin fibrils covered by an epicuticular lipid layer on the outside body surface (Moussian, 2013). It serves as the first line of defense against the penetration of pesticides (Balabanidou *et al.,* 2019). Cuticular thickening is suspected to be due to an increase in biosynthesis and/or the transport of cuticle building materials including cuticular lipids (Balabanidou *et al.,* 2016), structural proteins and chitin layers (Lin *et al.,* 2012; Fang *et al.,* 2015; Vannini *et al.,* 2015). The knockdown efficacy of pesticides has been shown to be positively related to cuticular thickness (Lilly *et al.,* 2016).

Spider mite resistance was first documented in Japanese tea fields in the *Tetranychus kanzawa* K. (Kanzawa spider mite) (Ozawa, 1994). Resistance to METI pesticides was reported for *T. urticae* in Australia, Belgium and England (Devine *et al*., 2001; Sato *et al*., 2005) and was associated with overproduction of metabolic enzymes (Stumpf & Nauen, 2001; Sato *et al*., 2005). However, such studies while done somewhere else have not been carried out in Botswana. TSSM control failures

with abamectin have been reported in Carlifornia, Florida, Netherlands (Campos *et al*., 1996) and Washington (Beers *et al*., 1998). Farmers in Sao Paulo reported reduced effectiveness with abamectin (Sato *et al*., 2005). Resistance of pider mite to dicofol was documented in USA, Europe, New Zealand and Japan (Fegusson-Kolmes *et al*., 1991). Herron & Rophail (2003) documented control failures with chlorfenapyr in a nectarine orchard. Spider mites can evolve tolerance to new pesticides within fewer than four years, meaning that management of multiple - insecticide tolerant spider mite is becoming increasingly difficult (Grbic *et al*., 2011). Spider mite control failures have been reported for pesticides including organophosphates, hexythiazox, bifenthrin, fenpyroximate, bifenzate, dicofol and abamectin (Fergusson-Kolmes *et al*., 1991; Farnharm *et al*., 1992; Stumpf & Nauen, 2001; Sato *et al*., 2005; Van Leeuwen *et al*., 2006). While the quantity of effective pesticides registered for spider mite control diminishes, fewer new formulations enter the market due to the high cost associated with their usage and registration restrictions (Dekeyser, 2005).

Tetranychid mites usually remain at low to unobservable densities until pesticides are applied (Prischman *et al*., 2005). Generally, outbreaks of pests following pesticide usage are believed to be caused by a number of mechanisms. The pesticides have been reported to either eliminate natural enemies or reduce of their foraging abilities, cause an increase in spider mite fecundity either through hormoligosis, or by trophobiosis, and a shift toward female-biased sex ratio that results in greater number of eggs (Hardin *et al*., 1995). Extensive and non-selective application of pesticides to control herbivorous arthropods is known to cause the resurgence of primary pests and outbreaks of secondary pests (Frampton & Dorne, 2007; Liang *et al*., 2011). In 1975, it was found that mosquito-fogging caused an increase in population of pine needle scale, *Chionaspis pinifoliae* (Luck & Dahlsten, 1975). Increased abundance of secondary pests was also documented for citrus red mite, woolly whitefly, honeylocust, spider mite, citrus mealybug and purple scale (Raupp *et*

al., 2001). Increased abundance of spider mites due to the usage of chemicals have been documented widely (Li & Harmsen, 1993). Van de Vrie *et al*. (1972) documented approximately 300 cases where the abundance of spider mites on several plant species was linked to various pesticide applications, including insecticides, acaricides and fungicides. Synthetic formulations have been shown to cause a reduction in foraging ability of natural enemies (Szczepaniec, 2009). Pesticides eliminate natural enemies and release spider mites from their regulating pressure allowing for outbreaks. Trichilo & Wilson (1993) reported that pyrethroids also gave rise to a 12 times increase in population of spider mites and elimination of natural enemies and an increase in fertility of spider mites were responsible for outbreaks. Treatments of grapes vines with organophosphates have led to outbreaks which were associated with reduced populations of phytoseiid mites (McMurty & Croft, 1997; Prischmann *et al*., 2005). High spider mite populations coupled with lower numbers of phytoseiid mites were also observed on grapes treated with imidacloprid treated (Stavrinides & Mills, 2009).

There are numerous reports of pesticides with direct effect on spider mites. Spider mites have been shown to develop resistance to pesticides by lowering the lethal dose at the target site, through several mechanisms including metabolic detoxification, lowered penetration or absorption at the cuticlar level, sequestration, and behavioral resistance (Van Leeuwen *et al*., 2010). Szczepaniec (2009) reported that DDT treated spider mites showed increased oviposition and increased female-biased sex ratio. Methyl carbamate applications were also reported to have a stimulatory effect on spider mites (Calabrese & Blain, 1999). Synthetic pyrethroids were also reported to cause a high fertility, female-biased sex ratio, rapid developmental time and delayed diapause in spider mites treated with synthetic pyrethroids (Ayyappath, 1997). Pesticides have been shown to promote plant health and vigor leading to outbreaks of spider mites (Gonias *et al*., 2008; Tenczar & Krischik,

2006). Several researchers suggest that alterations in the physiology of the plant may have a direct influence on spider mite abundance. Boykin and Campbell discovered that physiological changes in peanut plants due to carbaryl treatments resulted in spider mite outbreaks (Szczepaniec, 2009). Moreover, similar effects were observed by Mellors *et al*. (1984) on soybean plants treated with carbofuran. Gupta & Krischik (2007) observed elevated indices of chlorophyll and leaf area in rose plants treated with imidacloprid and consequently housed higher populations of spider mites than control plants.

Although it may not be easy to avoid resistance development altogether, the judicious application of established pesticide resistance management principles has the potential to avoid resistance development or to maintain current resistance traits at low enough levels that the effectiveness of valuable pesticides can be sustained for a very long time. The incidence of spider mite resistance against currently used pesticides has not been investigated in Botswana. Early detection of spider mite tolerance to pesticides is critical for the formulation of an effective resistance management system (Osakabe *et al*., 2009; Kwon *et al*., 2015). In field populations, the effectiveness of a pesticide can be evaluated through toxicity tests with discriminating concentrations. These concentrations cause mortality in most of the susceptible individuals of a population, leaving out resistant individuals (Monteiro *et al*., 2015). The discriminating concentration is expressed by values that are usually between the lethal concentrations (LCs) LC_{95} and LC_{99} of susceptible populations (Halliday & Burnham, 1990). Currently the main emphasis in resistance research is to investigate the rudimentary molecular mechanisms leading to resistance development, with a view to use this knowledge to control the establishment and spread of resistant populations. Molecular analysis permits the precise identification of alterations at the genomic level and the consequent development of robust diagnostics which are crucial in resistance management, while the

characterization of detoxification enzymes in resistance guides the development of add-ons and ‘’resistance-breaking’’ compounds for pesticide formulations (Van Leeuwen *et al.,* 2010). While considerable advances have been made in terms of understanding the pesticide resistance mechanisms in economically important spider mites elsewhere, knowledge on the underlying mechanisms involved in Botswana has not kept pace.

2.6.3 Alternatives to chemical control

2.6.3.1 Biological control

Biological control using natural enemies has been identified as a viable alternative to pesticides in crop production systems (Pakyari *et al*., 2011). Acarophagous predators play a critical role in reducing outbreaks of phytophagous mites (Meyer, 1996; Piraneo, 2013). *Phytoseilus persimilis* Athias-Henriot is a well-known specialist predator of Tetranychidae (Van Lenteren *et al*., 1992; Moraes *et al*., 2004; Duso *et al*., 2008; Cakmak *et al*., 2009). At adequate densities these acarophagous mite species are capable of rapidly suppressing spider mite populations rapidly (Jansen and Sabelis, 1992). However, their reproduction is dependent on the abundance of spider mites on the host plant, and therefore when spider mite populations on the host plant are low, they tend to disperse (Cakmak *et al*., 2009). New predator releases are needed to combat new spider mite infestations. Researchers have deliberated on combining releases of *P. persimilis* with another predatory mite *Neoseiulus carlifornicus* Mcgregor (Walzeret *et al*., 2001; Barber *et al*., 2003; Rhodes et al., 2006). *N. carlifornicus* has a wider diet, which includes some phytophagous mites and pollen and are able to survive at low spider mite populations and low humidity (Bakker *et al*., 1993; Castagnoli, 2005), therefore being a good biological control candidate for spider mites. However, the effectiveness of combining the two agent species depends on their influence on each

other through exploitative competition and interference, including intraguild predation (Cakmak *et al*., 2006; Rhodes *et al*., 2006). Intraguild predation is skewed in favour of *N. carlifornicus* (Walzer and Schausberger, 1999a).

Amblyseius californicus McGregor (Acari: Phytoseiidae), a predatory mite, is an efficient generalist predator of *T. urtcae* and *T. cinnabarinus* and is preferred for its ability to survive and thrive in hot, dry conditions (Hart, 2002; Gotoh *et al*., 2004). Ladybird beetles belonging to the Stethorus genus (Coleoptera: Coccinellidae) are specialist and key predators of tetranychid mites and have been reported to be proficient biological control agents for spider mites (Rott and Ponsonby, 2000; Ullah, 2000; Gotoh *et al*., 2004; Mori *et al*., 2005; Biddinger *et al*., 2009; Kishimoto, 2011). They have been found to be voracious predators feeding on all spider mite life stages, with a high host locating, high dispersal ability and adult longevity (Roy *et al*., 2005). The predatory lady bug *Stethorus japonicas* Kamiya is an efficient natural enemy of *T. urticae* and *T. kanzawai* Kishida in apple, pear, citrus, tea, hydrangea, and lima bean (Gotoh and Gomi, 2000; Kishimoto, 2002; Gotoh *et al*., 2004). The predacious ladybird beetle *Stethorus tridens* Gordon (Coleoptera: Coccinellidae) promises to be an effecient biocontrol agent for all lifestages of *T. evansi* in tomato fields (Fiaboe *et al*., 2007; Britto *et al*., 2009; Dayoub *et al*., 2020). The predatory thrips, *Scolothrips takahashii* and *Scolothrips longicornis* (Thysanoptera: Thripidae), are important predators and good biological control candidates for spider mites (Aydemir and Toros, 1990). *S. takahashi*i has been reported to be an effective control agent for *T. urticae* Koch in integrated pest management programs (Gotoh *et al*., 2004; Yanagita *et al*., 2014). Several studies have verified the effectiveness of *S. longicornis* in controlling *T. urticae* (Gotoh *et al*. 2004b; Pakyari *et al*., 2011). Pathogenic fungi in the order Entomophthorales ahave been known to cause high infection in spider mites. *Neozygites floridana* Weiser and Muma (Zygomycetes:

Entomophthorales), a specialist parasite of spider mites has been documented to infect at least 18 species of tetranychids worldwide including on *T. evansi* on tomato, *T. ludeni* Zacher on bean, *O. hondoensis* Ehara on cedar, *T. urticae* on corn, *M. tanajoa* Bondar, in Venezuela, Brazil, India and Kenya (Van der Geest *et al*., 2000; Ribeiro *et al*., 2009). *N. floridana* isolates have been studied for biological control of *T. evansi* and *T. urticae* in Brazil. These studies have also shown that *N. floridana* can co-exist with the predatory mite *P. longipes* without producing any epizootics (Wekesa *et al*., 2007). This means that both fungal pathogens and predators can be employed concurrently in biological control programs for spider mites.

2.6.3.2 Cultural control

Cultural control practices including crop rotation, crop free-periods, planting dates, field sanitation, pruning and trellising, living barriers, companion crops, crop residue disposal and sprinkler irrigation have been used for management of spider mites (Hilje *et al*., 2001). Cultural controls are preventative in nature and are intended to create a less conducive environment for pest reproduction and survival. These work by deliberately manipulating some component of the agroecosystem (soil, associated plants, and the crop) (Hilje *et al*., 2001). Therefore, cultural management involves the manipulation of current and new components of the agroecosytem to reduce pest damage to non-economic levels (Hilje, 2000b). Pruning and trellising of tomatoes had a positive effect on tomato yield and quality; and consequently profit margin of tomato production in Zimbabwe (Saunyama and Knapp, 2003). Some cultural practices such as early planting after the closed season and stalk destruction had a positive impact role in the contol of some major cotton pests such as red bollworm (*Diparopsis castanea*), pink bollworm (*Pectinophora gossypiella*) and cotton jassid (Empoasca sp.) respectively (Javaid, 1995). High temperature conditions are favorable for spider mite outbreaks therefore increasing the planting space and

sprinkler irrigation opposes these conditions and thus depresses spider mite populations. Regular watering with decreasing temperature and increasing humidity has been reported to impede pest infestations (Latifian *et al*., 2014). A high relative humidity has been found to decrease the adult female life span and causes spider mites to lay fewer eggs. Varying fertiliser regimes have been shown to reduce build up of spider mite populations. Cultural practices are easily integrated with other management practices and thus have a tremendous potential for the management of spider mites in many countries. They can reduce pesticide usage to sustain tomato production in Africa, especially for small-scale farmers (Javaid, 1995). The Integrated effects of cultural factors are better than application of each of them alone and reduce pest damage lower than the economic injury level (Latifian *et al*., 2014). There are relatively few references to cultural control of spider mites in literature, compared to other management tactics. Cultural techniques remain under-utilized in favor of pesticide application. Constraints to adoption include: (a) the amount of changes the farmer has to make to his conventional cropping practices for effective implementation of tactics such as living barriers, trap cropping and floating covers; (b) the scale required to implement crop-free periods, planting dates, crop rotation and weed disposal; (c) difficulty in experimentally quantifying and demonstrating effectiveness by means of experiments, due to interference between treatments caused by pest mobility; and (d) inability of most cultural practices to provide adequate control when not in combination with other management tactics (Hilje *et al*., 2001). More research and adoption of culturally-oriented insect pest management of spider mites in Botswana is necessary.

2.6.3.3 Host Plant Resistance

Plant resistance against arthropod pests is defined as the sum of the heritable traits which influence the ultimate level of damage induced by the arthropod pest on the plant (Mitchell *et al*., 2016; Gajger & Dar, 2021). Plant resistance reactions to invertebrate pests are founded on heritable characters and are usually grouped into three: antixenosis (non-preference), antibiosis and tolerance (Smith, 2005; Tabari *et al*., 2016; Santamaria *et al*., 2020). These mechanisms can also either be constitutive or induced. Antixenosis mechanism deters arthropod pests from ovipositing, feeding, seeking shelter, and colonization (Oyetunji *et al*., 2014; Tabari *et al*., 2016). It is a clear non-preference of a plant by a pest indicated by the occurrence of repulsive factors texture, odour, colour) that cause the arthropod to move to a different host. Antibiosis on the other hand refers to the antagonistic effect the plant imposes on the pest physiology such as its survival, development and reproduction (Wiseman, 1994). Antibiosis may be mild or cause larval mortality, disturbance of lifecycle and the reduction in fertility and fecundity of the pest (Oyetunji *et al*., 2014; Gajger & Dar, 2021). Antixenosis and antibiosis resistance reactions can occur simultaneously in the same host plant across many taxa (Smith, 2005; Sharma, 2009).

These plant tactics can occur singularly or simultaneously mechanistically and functionally. Their self-protective purpose is plant specific and reliant on the arthropod pest and its feeding style (Santamaria *et al*., 2020). The plant's physical structures that may alter the pest's behavior include surface waxes, tissue hardness and plant trichomes. Plant trichomes vary in structure from simple, erect hairs to complex multicellular glandular structures (Smith, 2005). Simple plant trichomes inhibit the ability of the pest to adhere to the plant surface, so as to feed. Hook shaped and complex glandular trichomes trap or pierce the arthropod body leading to desiccation and death. Maluf *et al*. (2001) found that the wild tomato accession *L. hirsutum f. glabratum* consists of leaf hairs that confer antixenotic effects to *T. evansi* and *T. urticae*. Patterson *et al* (1974) also revealed that

resistance against *T. urticae* in *Nicotiana* spp. was caused by a combination of antixenosis and antibiosis through a sticky exudation from trichomes that was lethal and also trapped the spider mites. However, some arthropod pests (e.g *Phthorimaea operculella* Zeller) may prefer hairy surfaces for oviposition making pubescence a characteristic not always useful for suppression of pests. Surface waxes are also vital in the resistance of some plants to arthropod pests especially when the pest perceives negative chemical stimuli from the leaf surface (Smith, 2005). For example, and brussel sprouts and glossy leafed kale lacking surface wax tolerate less feeding by *B. brassicae* L. than wax coated varieties (Eigenbrode *et al*., 1991). The thickness of the plant physical structures such as leaves, shoots, stems and pods influences the extent of resistance in most crop varieties (Smith, 2005). Leite *et al*. (2003) found that the adults and nymphs of spider mites prefer to feed on the younger upper and middle leaves of the host compared to the harder lower leaves. Jiang and Ridsdill-Smith (1996) found that the sturdiness of cotyledons of several clover varieties is directly responsible for resistance by antixenosis to feeding by the red legged earth mite, *H. destructor* Tucker. Antibiosis may also involve quick multiplication of cells activated by pest feeding or elevated discharge of plant exudates that cause mortality of eggs or young larvae inside the host plant (Panda and Khush, 1995). Mustard plants were shown to cause dessication of the eggs of cabbage worm *Artogeia rapae* L. by producing a necrotized zone around them. Allelochemicals serve as growth inhibitors, repellents, feeding deterrents and feeding inhibitors for smell navigating arthropod arthropods (Gajger & Dar, 2021). Volatile emissions from the leaves of resistant plants contain an array of arthropod repellents. Dabrowski and Rodriguez (1971) found that volatile emmissions from strawberry plants with a high essential oil content are able to repel feeding by the two-spotted spider mite, *T. urticae* and the strawberry spider mite, *T. turkestani* Ugarov and Nikolsik. Guo *et al*. (1993) and Snyder *et al*. (1993)

established that the glandular trichomes of wild tomato, *L. hirsutum F. glabratum* produces a distinctive volatile organic acid, 2, 3-dihydrofarnesoic acid that suppresses feeding by *T. urticae*. Allelochemicals reported to frequently cause deterrence and toxicity include terpene lactones, ketones alkaloids, organic acids, flavonoids and phenols synthesized and stored in leaves, vacuoles, trichomes and waxes (Smith, 2005). For instance, leaf glycoalkaloids in wild *Solanum* species prevent feeding of the potato leafhopper, *Empoasca fabae* Harris (Sinden *et al*., 1986; Medeiros *et al*., 2004). Assessments of the contact toxicity of the methyl ketone 2-tridecanone in wild tomato species on revealed strong acaricidal properties against *T. urticae* (Chatzivasileiadis and Sabelis 1997, 1998).

Plant resistance studies have largely concentrated on antixenosis, whereby the plant is able to deter the pest away from it and antibiosis, whereby the plant imposed a harmful effect on the pest. Although these two types of resistance have been shown to reduce injury and yield loss, they may produce selection pressures on the pest and lead to pest tolerance (Peterson *et al*., 2017). Tolerance is resistance in which the plant is able to endure or recover (through growth and compensation) from damage caused by the pest abundance equal to that damaging a plant without resistance characters (susceptible). Tolerance takes place when plant characters lessen the adverse effects of pest damage on crop yield (Smith, 2005; Mitchell *et al*., 2016). Tolerance is a more suitable pest management strategy because it depends only on the plant's response and therefore does not cause development of resistance in pests (Peterson *et al*., 2017). Strauss and Agrawal (1999) identified several physiological mechanisms responsible for tolerance including increased growth rates, high photosynthetic rate after injury, increased branching following release of apical dominance, increased levels of carbon storage in roots, and ability to reallocate carbon after injury from roots to shoots. Two physiological mechanisms have been documented as common for tolerant plants;

increased photosynthetic activity (Botha *et al*., 2006; Franzen *et al*., 2007; Murugan *et al*., 2010; Luo *et al*., 2014; Cao *et al*., 2015) and up-regulation of detoxification mechanisms to counter the harmful effects of hemipteran pests (Passardi *et al*., 2005; Gutsche *et al*., 2009; Kerchev *et al*., 2012; Ramm *et al*., 2013). Several species of tomato can tolerate a significant amount of leaf damage by spider mites but with yields comparable to those without damage (Gilbert *et al*., 1966; Keskin & Kumral, 2015).

Host plant resistance can be a vital component of an integrated pest management (IPM) strategy for tomato spider mite management since it is an environmentally friendly pest control approach (Nwilene *et al*., 2009; Karimi *et al*., 2012; Tabari *et al*., 2016; Hondelmann *et al*., 2020). It is considered one of the key tactics for arthropod pest management, mostly in resource poor communities where application of other control methods such as pesticides is often challenging or imprudent (Bosque-Pérez & Buddenhagen, 1992). However, tolerance has been poorly studied and understood globally (Peterson *et al*., 2017). Research focused on the mechanisms of plant tolerance has been scanty, probably due to the hesitancy of plant breeders and producers to use crop varieties which are able to withstand and host high pest populations (Smith, 2005). Several studies have revealed the direct involvement of hormones, photosynthesis and physical structures in the expression of plant tolerance. For instance, Gawronska and Kielkiewicz (1999) reported that tolerant tomato plants infested by *T. cinnabarinus* had higher leaf abscisic acid (ABA) content compared to susceptible varieties. Tolerance is a complex genetic characteristic, therefore it is crucial to identify the gene sequences of several different components in order to fully comprehend the contributions of each to the phenotypic effect marked as plant tolerance to arthropod pests (Smith, 2005).

2.6.3.4 Integrated Pest Management

According to FAO (2017), Integrated Pest Management (IPM) is the prudent consideration of all available pest management practices and their consequent integration in order to depress the development of pest populations whilst keeping pesticides and other interventions at levels that are economically acceptable and lessen the risks to human and animal health as well as the environment. IPM underscores the production of a healthy crop with minimal disturbance to agro-ecosystems and encourages natural pest control mechanisms.

2.7 Summary

Tomato is an important economic crop extensively grown all over the world for human consumption. Tomato is primarily grown as a cash crop providing an important source of income for the rural and suburban population. It is a nutrient dense food which provides important components such as iron, phosphorus and vitamins, niacin, riboflavin and thiamin. Tomatoes are a major food source of important phytochemicals, carotenoids, ascorbic acid, phenolic acid, phytosterols, polyphenols, flavonoids, calcium and lycopene therefore contributing to overall health. Despite its importance, local tomato production does not satisfy domestic demand. Conditions affecting tomato production in Botswana include poor soils, water shortage, lack of marketing infrastructure, weeds, pest and disease attacks. Arthropod pests are commonly cited as the main constraint to profitable tomato production. Farmers in Botswana considered spider mites among the most important pest of tomato and the red coloured form of spider mite is more rampant in tomato fields across the country. Farmers lament that spider mites reduce the yield and quality of tomatoes produced and increase their production costs. Farmers normally apply chemical pesticides to manage arthropod pests on their crops and their response is primarily based on the

sight of the pest or symptoms on the tomato plant. However, spider mites quickly evolve resistance to pesticides used frequently for their management. Farmers report that most pesticides are no longer affective against spider mites and presume it could be due to development of resistance. An improved spider mite management programme is the only solution to achieve optimum tomato production. Alternatives to synthetic pesticides, which can reasonably replace the more expensive chemicals, need to be researched and developed. The application of an integrated pest management approach to manage spider mites and pest complexes on vegetables can help delay development of resistance and reduce the damaging effects of pesticides on humans and non-target organisms and the environment.

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CHAPTER 3 - FARMERS' KNOWLEDGE, PERCEPTIONS AND MANAGEMENT OF SPIDER MITES (T*etranychus* spp.) ON TOMATO IN BOTSWANA

ABSTRACT

This paper reports on farmers' knowledge, perceptions and management practices for spider mites (Tetranychus spp.) affecting tomatoes in Botswana. A survey of 120 tomato farmers was conducted using questionnaires during the period of March to June 2019. The study findings revealed that farmers producing tomatoes considered spider mites among the most important pest of tomatoes with the red form of the spider mite being more prevalent than the green form in tomato fields across Botswana. The farmers reported that spider mites affect the quality and quantity of tomatoes and increased the cost of production. Farmers typically apply synthetic pesticides to control invertebrate pests on their crops and their action is mainly premised on the

presence of the pest or symptoms of damage on the crop. Unlike most pests, spider mites quickly develop resistance to formulations employed for their control. Farmers report reduced effectiveness of the current management tactics against spider mite infestations on their tomato crop and presume it could be due to resistance development. The current management practices can be improved by sensitizing and training farmers on the use of appropriate pesticides and spray regimes for spider mites. Tomato farmers need to know the importance of integrated pest management to control spider mites and other pests on vegetables whilst delaying resistance development and avoiding harmful effects of pesticides on the environment humans and non-target organisms.

3.1 INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.), a herbaceous fruiting plant with origins in Latin America (Peralta *et al*., 2006; Nebuzale, 2014), is a ecomically high-value vegetable that is commonly grown and utilised in most countries in the world (Naika *et al*., 2005; Mutamiswa *et al*., 2017). It is a multi-use fruit consumed in many ways; as an ingredient in soups, sauces, fish or meat dishes, and in fresh salads (Badimo, 2020), making it the most consumed fruit in the world. The economic significance of the crop has increased owing to the global rise in production and economic value (Bodunde *et al*., 1993). It is the world's third most common vegetable crop behind the potato and sweet potato (Olaniyi *et al*., 2010) and first in terms of processing volumes. Tomato production is an important income earning activity and a source of food security for small-holder farming households (Braesco, 2019; Melomey, 2019). Nutritional studies have found that tomatoes have many health benefits and contribute to the attainment of a well-balanced diet (Tusiime, 2014).

Tomatoes are an important source of vital nutrients (Beecher, 1998), providing up to 20 and 40% of the recommended daily allowance of vitamin A and C respectively (Wilcox *et al*., 2003; Kelly and Boyhan, 2010). They also provide lycopene, which has strong natural anti-oxidant properties (Shi and Manguer, 2000; Melomey, 2019) protecting the body from cancer and cardiovascular diseases. The tomato fruit contains significant quantities of water, calcium and niacin, essential for metabolism (Olaniyi *et al*., 2010; Melomey, 2019). In Botswana, tomatoes are among the most popular vegetable crops grown for home consumption, retail sale and for sale in the local market (Madisa *et al*., 2010). Production of tomatoes has provided employment opportunities for the rural populace and improved the livelihoods of those involved in the tomato value chain. Tomatoes are mostly grown in backyard gardens, open fields and lately in protected structures (greenhouses, tunnels and shade nets) of different makes and sizes. Most cultivation of tomatoes is by farmers who own two hectares of land or less often with limited knowledge of the new technologies necessary to improve production levels, and insufficient access to the formal market (Munthali *et al*., 2004; Bok *et al*., 2006; Obopile *et al*., 2008). Although government has introduced various interventions to promote agricultural development (Madisa *et al*., 2010), the productivity of tomato production remains low when likened to major tomato producers in Africa. Egypt is the leading producer of tomato in Africa at 7 297 108t, followed by Nigeria (4,100 000t), Morocco (1, 293 761t), Tunisia (1,298 000t), Cameroon (1,279 853t), Algeria (1,286 286t) and South Africa (608 306t) (Dube *et al*., 2020). Tomato demand in Botswana is estimated at 12000t monthly while local productivity ranges from 60-100t per hectare subject to the variety and predominant production conditions, and this presents a shortfall. Local production only manages to satisfy about 40% of local demand (Badimo, 2020).

Farmers in Botswana are faced with numerous difficulties during production of vegetables which include; water scarcity, infertile soils, lack of farm inputs, labour shortage, poor farming practices, income, lack of proper market, infrastructure, pests and disease outbreaks (Madisa *et al*., 2010). Most of these farmers consider invertebrate pest problems as the most important constraint to their production (Obopile *et al*., 2008; Munthali, 2009; Madisa *et al*., 2010; Baliyan, 2012). Major pests associated with tomato production in Botswana include the cutworm (Agrotis spp.), whitefly (*Bemisia tabaci*), African bollworm (*Helicoverpa armigera*), tomato semi-looper (*Chrysodeixis acuta*), tomato leaf miner (*Tuta absoluta*), and spider mites (Tetranychus spp.) (Obopile *et al*., 2008; Munthali, 2009; Leungo *et al*., 2012). Spider mites are reported to be one of the most serious pests of tomato production in Botswana (Munthalli, 2004; Obopile *et al*., 2008; Munthali *et al*., 2009). Two sibling species; *Tetranychus cinnabarinus* (Carmine spider mite - CSM) and *Tetranychus urticae* (Two- spotted spider mite - TSSM), have been shown to occur in a majority of tomato production settings in Botswana. These spider mites have a high deleterious effect on tomatoes and are a menace to food production and consequently national food security.

Several methods have been used to control spider mites in Botswana, but few have been considered satisfactory. The ease and speed of control offered by chemical pesticides have led to the indiscriminate and excessive application of these products (Obopile *et al*., 2008; Madisa *et al*., 2010; Ghaderi *et al*., 2019). The over-reliance on and over use of pesticides increase the costs of production for farmers (Mwaule, 1995; Mukiibi, 2001). Improper use of pesticides has an adverse effect on agricultural sustainability by instigating environmental problems such as surface and underground water contamination. The excessive use of pesticides also eliminates useful and non-target organisms, development of pest resistance and has harmful effects on the health of humans

and animals (Pedigo and Rice, 2006; Leungo *et al*., 2012; Pelaez *et al*., 2013; Roditakis *et al*., 2017).

In Southern Africa and in Botswana specifically, there has not been any comprehensive study to investigate the knowledge, perceptions and practices of farmers regarding spider mite management. Farmer-level information is the foundation for introducing new interventions since farmers' experiences, perceptions and knowledge form part of the framework on which new decisions are made (Abdollahzadeh *et al*., 2015; Fan *et al*., 2015). Agricultural development needs to build on farmers' knowledge systems to enable the tailoring of innovative solutions to local situations (Salembier *et al*., 2021).

3.1. 1 Research Problem

Spider mites are amongst the key constraints to profitable tomato production in Botswana and the world (Munthali, 2004; Obopile *et al*., 2008). Tomatoes are very susceptible to spider mite infestations which can inflict serious damage to tomatoes and therefore affecting production levels, markets, income levels and livelihoods. Despite the high value placed on tomatoes, there is no investigation on how farmers perceive and control spider mites in Botswana. It is important to conduct this study so as to understand farmers' knowledge, perceptions and control actions for spider mites affecting their tomato crop.

We conducted this study to find answers to the following research questions: What knowledge, attitudes and perceptions do the farmers hold pertaining to the management of spider mites on tomato? This question sought to find out whether respondents are knowledgeable about spider

mites, What spider mite species is most prevalent on their tomato crop, What are the effects of the spider mite on the farming communities and how spider mites on tomatoes are managed in Botswana? This question focused on the effect of spider mites on crop yields, income levels as well as their economic effects on farmer's livelihoods. What strategies do the farmers use to manage spider mites in tomatoes? This question sought to find out the management strategies the farmers use to manage spider mites and their effectiveness.

3. 1. 2 Study Objectives

The general objective of the chapter was to assess the effect of farmers' knowledge and perceptions on management spider mites on tomato in Botswana.

3.1. 2.1 Specific Objectives

Here we present results of a questionnaire survey conducted in six geographical locations in Botswana. The objectives of the survey were to;

1) To assess farmers' knowledge and perceptions of the constraints to tomato production in Botswana.
2) To identify invertebrate pests that farmers perceive as important in tomato production in Botswana.

3) To identify the spider mite species most prevalent in tomato producing farms in Botswana.

4) To examine the effect of spider mites on farmers' livelihoods.

5) To identify farmers' management actions and their perceived effectiveness for controlling spider mites in Botswana.

3.1.3 Research Hypotheses

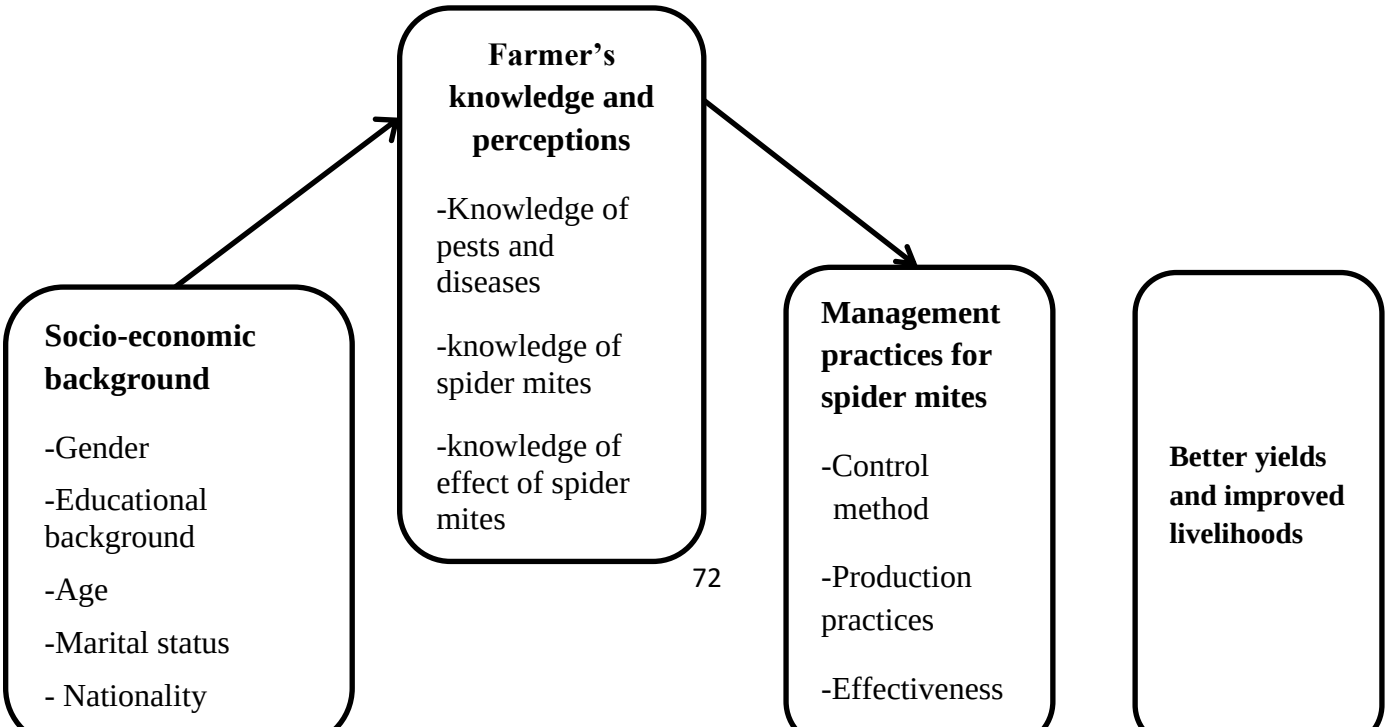
1) Gender significantly influences farmers' perceptions of the seriousness of spider mite infestations.

2) Age significantly influences farmers' perceptions of the economic impact of spider mites.

3) Educational level significantly influences farmers' management actions against spider mites.

4) Level of experience significantly influences the effectiveness of management actions against spider mites.

3.1.4 Conceptual Framework



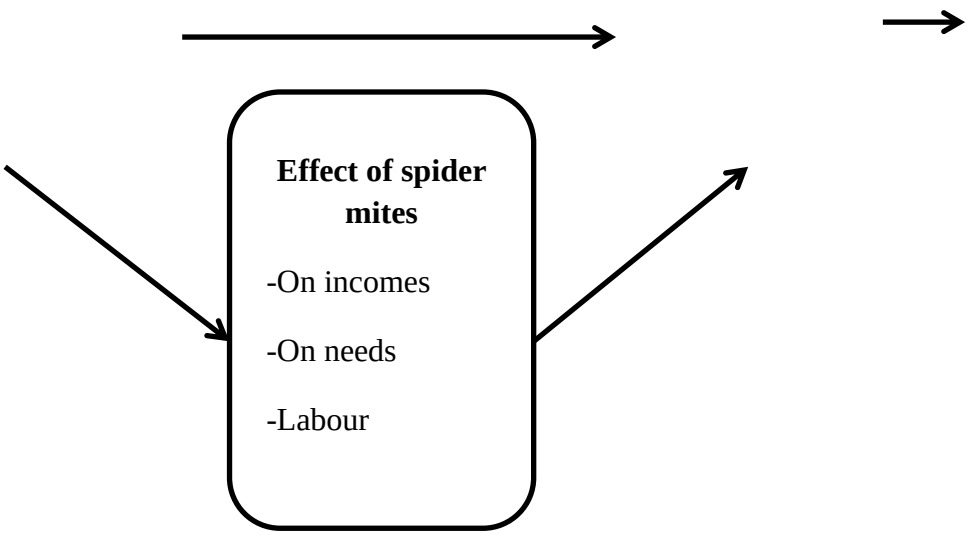


Figure 4. Conceptual framework

The socio-economic background affects the farmers' knowledge, perceptions and management actions towards pests and diseases. The management actions are dependent on the farmers' knowledge and perceptions of spider mites in tomato production. Alternatively, socio-economic variables are the intermediate factors affecting both the independent and the dependent variables. Farmers with knowledge about spider mites and their effect on livelihoods are most likely to manage the pest better thus achieving profitable yields which will bring better incomes and result in improved livelihoods.

3.1.5 Scope of the study

The study focused on farmers' knowledge, perceptions and management practices for spider mites affecting tomato production and the consequent effect on farmers' livelihoods. It also explored management practices that are applied in controlling spider mites. Earlier studies by other researchers pertaining to the management of vegetable pests was also consulted.

3.1.6 Significance of the study

New information will add to policy formulation. The Ministry of Agriculture Development and Food Security may utilize the findings of this study during policy development and formulation for improved extension services for better pest and disease management. The findings will afford farmers improved knowledge and influence a shift of perceptions towards spider mites and vegetable pests in general therefore adopting better pest and disease management practices. This study will contribute to development of interventions geared toward improving pest management, increasing yields, and consequently their incomes and livelihoods. The results will add to recent literature for researchers on vegetable pests and spider mites affecting tomato farmers' livelihoods in Botswana.

3.1.7 Theoretical framework

During policy formulation it is important to have access to dependable scientific information. Therefore it would be wise to integrate active farmer contribution into research and development programs. The direct exchange of information and advice is an important component of linear technology transfer models. Up to date information will make it easier to come up with new training procedures, education and information dissemination but will need to be augmented by other extension approaches. For small-scale farming to thrive and be sustainable agricultural production systems and management must be advanced and new farming practices must be employed (Govaerts *et al*., 2009; Ntshangase *et al*., 2018). Therefore, new management practices

must be incorporated into conventional farming practices and this requires an understanding of farmers' knowledge; perceptions and management practises for pests and diseases.

This study recognizes the importance of linear knowledge transfer as well as understanding community perceptions through a participatory 'bottom up' approach where farmers provide hands-on experience information. Information exchange allows for knowledge integration as perceived by the farmers themselves as opposed to the researcher's viewpoint. During farmer training and resourcing it is of vital importance to grasp the concepts of pest management from a farmers' viewpoint as one would learn more from the farmer than they would learn from him. This theory forms the foundation of this study that it is crucial to have an understanding of farmers' knowledge and perceptions for development of improved management practices for spider mites (Black, 2000).

3. 2 MATERIALS AND METHODS

3.2.1 Study design

A cross sectional study design was used to assess farmers' knowledge, perceptions and management actions against spider mites. A cross-sectional research design involves data collection of at least two variables at a given point in time with the objective of assessing the existence of a relationship between the variables in question (Bryman and Bell, 2007). A questionnaire survey is usually the best method of data collection in such a design. Both quantitative and qualitative data was collected. The design allowed the researcher an in-depth inquiry into the problem under investigation. The objectives of the study were accomplished from respondents spread over an extensive geographic area randomly chosen as a representative sample.

Both primary and secondary data was collected on knowledge, perceptions and attitudes as far as management of spider mites was concerned.

3.2.2: Area of Study

The study was undertaken in six geographical locations of Botswana namely Gaborone, Southern district, Central district, Francistown, Maun and Gantsi (Figure 5).

3.2.3 Population of the study

Farmers (males and females) actively producing tomatoes comprised the population of the study. These people were included because they are better positioned to provide complete information about production of tomato. The number of participants interviewed interviewed per region varied according to the number of active tomato farmers in that particular location. The highest number of farmers interviewed were from greater Gaborone (38.33%) followed by Southern (20.83%), Central (20.00%), Francistown (13.33%), Maun (4.17%) and Gantsi (3.33%).

Figure 5. A map showing the location of different agricultural regions of Botswana. (Source: Mokibelo, 2015).

3.2.4 Sample Size and Procedure

With the assistance of Agricultural extension officers, a preliminary survey was conducted to obtain information on the total number of tomato farmers in each district. Six agricultural regions were consequently identified within which a total of 600 active tomato farms were identified. According to Levy and Lemeshow (1999) and Strydon and Devos (2000), a twenty percent sample of the population is sufficient to control error. Therefore twenty percent of the total population was assumed to constitute the sample. A total of 120 participants from the six agricultural regions constituted the sample of the study. The qualification of the selected farms was based on the fact that they have been actively growing tomatoes for atleast five years. The number of respondents

interviewed varied depending on the number of active tomato farms in a particular region with the highest number of farmers interviewed being from greater Gaborone (n = 46) followed by Southern (n = 25), Central (n = 24), Francistown (n = 16), Maun (n = 5) and Gantsi (n = 4). The respondents were purposively identified through the help of extension officers in each district. The farmers were visited for face-to-face interviews. In the absence of the farm owner, the interview was carried out with the farm managers and farm workers provided they had long experience and detailed knowledge of farm management practices. A mostly open ended questionnaire allowed farmers to freely give their answers. The interviews were conducted in Setswana and or English. Most of the questions focused on farmers' level of awareness of vegetable pests and the management strategies with some on their demographic background. After noting down their demographic profiles, they were asked to outline and rank the major constraints to tomato production. They were then asked to rank pests of tomatoes starting with the most important. Farmers were asked to list the management tactics used to control them. Where respondents mentioned the use of chemical pesticides they were asked to name the pesticides or avail the pesticide container label to the interviewer for confirmation and recording purposes. In addition, farmers were asked to state how frequently they applied pesticide to their crop and whether their tactics were effective. Farmers were also asked to suggest the solutions to pest problems in tomato production. For each question, the proportion of farmers who gave similar responses was calculated and percentages were computed based on the total number of farmers who responded to each question. Farmers who did not respond to certain questions were excluded from the analysis. Where a farmer selected more than one option, percentages were calculated for each group of similar responses. Farmers were asked to help in identifying other tomato growers in their region so as to increase the sample size.

3.2.5 Data Processing and Analysis

Data from the questionnaire were coded and checked before analysis. Percentages may not add to 100 since some farmers provided multiple responses to the same questions. The findings are based on the data that were analysed statistical using (SPSS) IBM Version 28.0 to test the hypotheses set for the study and responses from open ended and closed ended questions in the questionnaire. Comparative statistical tools including Chi-square and one-way analysis of variance (ANOVA) were conducted to assess differences regarding socio-demographic and farm characteristics, knowledge and perceptions of spider mites and their management practices. Means were separated using Tukey's Honestly Significant Difference test. All hypotheses were converted to the null and tested at alpha level of 0.05.

3.2.6 Quality Control

The questionnaire instrument was pretested prior to the actual data collection. This was necessary to identify shortcomings, ambiguous questions and other vital information that may have been overlooked during the design of the questionnaire. Farmers were consulted for permission before the interviews and participation was voluntary. During data collection, responses were recorded, photographs were taken, and verbatim accounts were written for further report writing.

3.2.7 Validity of the instrument

To ensure validity, the questionnaire was given to experts in the Research and Evaluation Department at the University of Botswana to examine and make suggestions for items needing revision. Experts at the Department of Crop and Soil Sciences at the Botswana University of Agriculture and Natural Resources were given the opportunity to assess the items and make comments. The revisions were based on the feedback given.

3.2.8 Reliability of the instrument

The internal consistency of the measuring instrument was tested using Statistical Package for Social Science (SPSS) IBM version 20.0. The Cronbach's Alpha coefficient was determined to find out if the instrument was appropriate for collection of data used in the study. A value of .804 was obtained.

3.2.9 Ethical Considerations

The participants' consent was requested and the objectives of study were clearly communicated to them prior to administering the questionnaire. They were informed that their participation was on a voluntary basis. The participants also were assured of the anonymity and confidentiality of the information they provided during the study. The respondents were given numerical tags for identification and their names were not used in the final report.

3.2. 10 Limitations of the study

During data collection, several challenges were encountered as had been expected. Some of the respondents were not willing to be interviewed regardless of the level of assurance of anonymity. Those respondents were excluded and therefore prolonged the duration of data collection as

replacements had to be sought. Language barrier was also a limitation however, the researcher comfortably translated some difficult words which the participant has difficulty with, in order to gather meaningful data. Some of the respondents did not keep records so they were not sure about some of the information that they were giving therefore to overcome this limitation, follow-up questions were asked to ascertain the answers given.

3.3. RESULTS

3.3.1. Farmers' demographic background and farm characteristics

The demographic characteristics recorded in this study include gender, age, education level and marital status of the respondents as indicated in table 2. The findings indicate that tomato production in Botswana is male dominated with 73.3 % of the farmers being males and 26.7% being females. The respondents' age ranged from 20 as a minimum to above 60 years. Most farmers (32.5%) were aged 41-50 years and 50.0% were married. All of the farmers had formal education. 48.3% of the respondents had completed their secondary education and 45.9% had attended tertiary school. Most (74.2%) farmers were Batswana while 25.8% were expatriates. 65.5% of the farms interviewed were owner managed and 14.2% employed a farm manager. Most (79.2%) of the respondents were in charge of the farm (owner/manager) while 9.2% and 10.8% were farm hands and gardeners respectively. Most (68.3%) of the interviewees had more than 10 years of experience growing vegetables while 64.2% had the same level of experience growing tomatoes. Most farmers (87.5%) grew tomatoes for retail sale followed by 10.0% who produced for street vending and 2.5% for home consumption. Most farmers (80.8%) produced their tomatoes in open fields, while fewer grew them in greenhouses (13.3%), tunnels (5.0%) and hydroponics (0.8%). There was a significant relationship (at a 5% significance level) between the age of the

farmer and the type of production system used (χ^2= 26.81, *df*= 12, *P*=0.008). The farmers who used greenhouses were in the age bracket 41 -50 years (10) while those who used tunnels were in the bracket 51- 60 years (4), hydroponics in the bracket 20-30 years (1) and open fields 41-60 years (54). Most (92.5%) farmers produced tomatoes over an area less than 5 ha, followed by 6-10 ha (7.5%). However, there was an insignificant relationship (χ^2= 4.112, *df*= 4, *P*=0.391) between age of farmer and size of field plot. The gender of the farmer did not influence size of field plot (χ^2= 1.204, *df*= 1, *P*=0.273). Similarly, there was no significant relationship (χ^2= 12.23, *df* = 5, *P* = 0.032) between educational background and area planted. Educational background also did not have a significant relationship (χ^2= 20.49, *df* = 15, *P* = 0.154) with the production system used.

Table 2. Demographic characteristics of respondents (n=120)

Demographic characteristics	Frequency	Percent (%)	Cum. %
1. Gender			
Male	88	73.3	73.3
Female	32	26.7	100.0
2. Age of respondent (years)			
20-30	9	7.5	7.5
31-40	20	16.7	24.2
41-50	39	32.5	56.7
51-60	32	26.7	83.4
Above 60 years	20	16.7	100.0
3. Marital status			
Single	52	43.3	43.3
Married	60	50	93.3
Divorced	4	3.3	96.6
Widowed	4	3.3	100.0
4. Educational level			
Primary education	7	5.8	5.8
Secondary education	58	48.3	54.1
Diploma	31	25.8	79.9
Bachelor's degree	20	16.7	96.6
Master's degree	2	1.7	98.3
Doctorate	2	1.7	100.0
5. Nationality			
Motswana	89	74.2	74.2
Expatriate	31	25.8	100.0
6. Management of the farm			
Farm Manager	17	14.2	14.2
Owner managed	75	62.5	76.7
Employee	28	23.3	100.0
7. Responsibilities			
Farm hand	11	9.2	9.2
Gardener	13	10.8	20.0
Manager	95	79.2	99.2
Labourer	1	0.8	100.0
8. Experience growing vegetables			
Less than 10 years	38	31.7	31.7
10-20years	73	60.8	92.5
21-30 years	9	7.5	100.0
9. Experience growing tomatoes			
Less than 10 years	43	35.8	35.8
10-20years	74	61.7	97.5
21-30 years	3	2.5	100.0
10. Purpose for growing tomatoes			
Home consumption	3	2.5	2.5
Retail sale	105	87.5	90.0
Street vending	12	10	100.0
11. Production system for tomatoes			
Greenhouse	16	13.3	13.3
Open field	97	80.8	94.1
Tunnels	6	5	99.1
Hydropnics	1	0.8	100.0
13. Area planted with tomatoes			
1-5ha	111	92.5	92.5
6-10 ha	9	7.5	100.0

3.3.2 Farmers' knowledge and perceptions of constraints to tomato production

The farmers ranked the constraints basing on the impact of that constraint on their production (Table 3). Most farmers (90.0%) mentioned invertebrate pests as the most important constraint to tomato production followed by diseases (47.5%), infertile soils (18.3%), unavailability of water (12.5%), unavailability of market (12.5%), lack of irrigation facilities (5%) and poor managerial skills (1.7%). The farmers named tomato pests as shown in Figure 6. Most farmers (75.7%) identified spider mites as a major pest hindering economic production of tomato in Botswana followed by tomato leaf miner (56.5%), aphids (38.3%) and white fly (30.4%). Other pests reported as less damaging to tomato production were African bollworm (21.7%), semi-looper (8.7%) and variegated grasshopper (8.7%).

Table 3. Constraints to tomato production in Botswana

Constraint	Frequency	% responses
Invertebrate pests	108	90.0
Diseases	57	47.5
Water unavailability	15	12.5
Lack of capital	4	3.3
Unavailability of market	15	12.5
Transport	3	2.5
Infertile soils	22	18.3
Poor management skills	2	1.7
Irrigation facilities	6	5.0

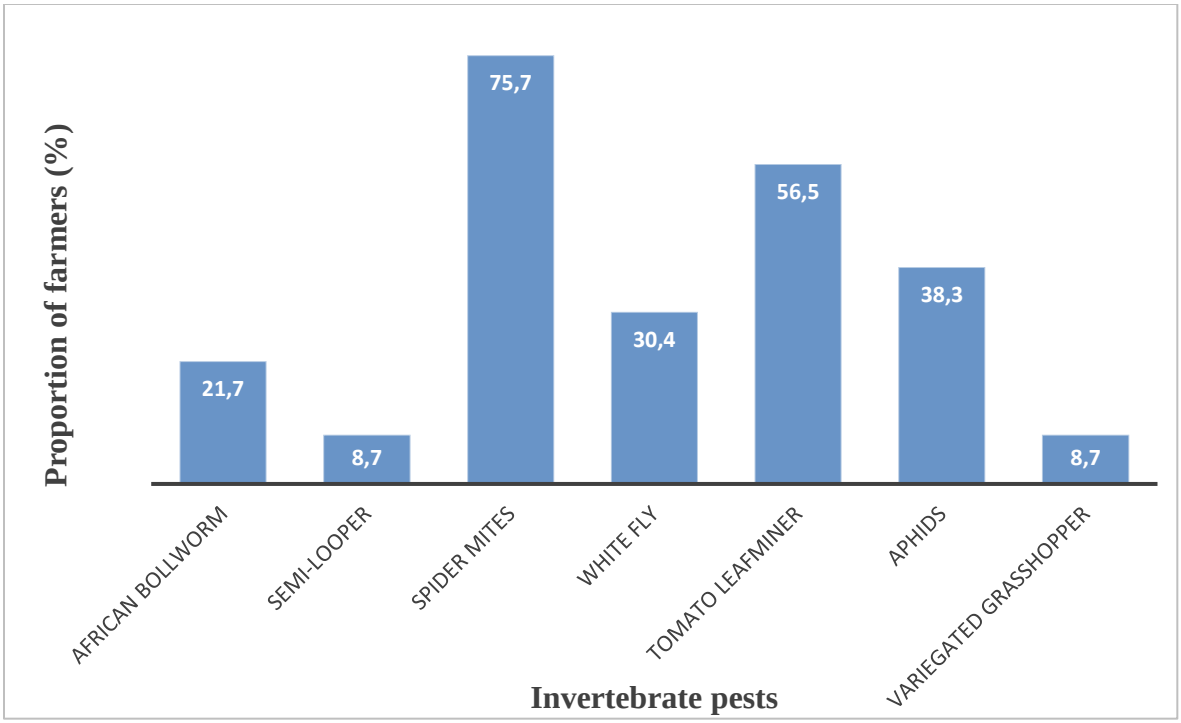


Figure 6. Percentage of farmers who mentioned an invertebrate pest

3.3.4 Farmers' knowledge and perceptions of spidermites

All (100%) of the farmers interviewed reported having prior knowledge of tomato spider mites (Table 4). 48.30% reported having personal experience with spider mites in their farms while others mentioned agro-traders (23.3%) and fellow farmers (20%) as the most important sources of vegetable pest information. Other less important sources of information mentioned were agricultural extension officers (4.2%) and radio/television (3.3%). Educational background did not have a significant (χ^2= 20.07, *df* = 25, *P* = 0.743) effect on the farmers' source of vegetable pest information.

Spider mites were reported as a very serious (35.8%) constraint in most of the farms, serious (50%), moderate (8.3%) and not serious (5%) in other farms. Demographic characteristics (area

planted and production system) did not have a significant effect on the seriousness of spider mite problem. However, the seriousness of the spider mite problem differed significantly ($P<0.001$) across districts. Most farmers in Gaborone (n = 46) reported that spider mites were very serious followed by Southern district (n = 25), Central (n = 24), Francistown (n = 16), Maun (n = 5) and lastly Gantsi (n = 4). The primary features farmers used to identify spider mites were colour (87.5%), followed by shape (6.7%) and size (5.8%). The red form of the spider mite was reported as the most prevalent (76.7%) followed by the green form (23.3%) by respondents. The farmers referred to the spider mite most prevalent in their localities as red spider mite (68.3%), carmine spider mite (8.3%) and two spotted spider mite (23.3%). Respondents attributed the seriousness of the spider mite problem to pesticide resistance development (55.0%), fast reproduction (19.2%), high population densities (13.3%) and climatic conditions (12.5%). Most farmers associated spider mites with sucking (80%) and yellowing (20%) damage to tomato plants.

Table 4. Farmers' knowledge and perceptions of spider mites

	Frequency	Percent (%)	Cum. %
3. Ever heard of spider mites before?			
Yes	120	100.0	100.0
No	0	0	100.0
4. Source of information			
Own experiences	58	48.3	48.3
Fellow farmers	24	20.0	68.3
Agro-traders	28	23.3	91.6
Agricultural extension officers	5	4.2	95.8
Researchers	1	0.8	96.6
Radio/television	4	3.3	100.0
5. Seriousness of the spider mites problem in your area			
Very serious	43	35.8	35.8
Serious	61	50.8	86.6
Moderate	10	8.3	94.9
Not serious	6	5.0	100.0
6. What do you call the spider mite prevalent in your area?			
Red spider mite	82	68.3	68.3
Carmine spider mite	10	8.3	76.6
Two-spotted spider mite	28	23.3	100.0
7. What feature do you identify spider mites with?			
Size	7	5.8	5.8
Shape	8	6.7	12.7
Colour	105	87.5	100.0
8. What is the colour of the spider mite prevalent in your area?			
Yellow	0	0	0
Green	28	23.3	23.3
White	0	0	23.3
Red	92	76.7	100.0
Blue	0	0	100.0
9. Cause of seriousness of spider mite problem.			
Climate	15	12.5	12.5
Fast reproduction	23	19.2	31.7
Pesticide resistance	66	55.0	86.7
High population	16	13.3	100.0
10. What type of damage do you associate with spider mite			
Sucking	38	31.7	31.7
Yellowing	73	60.8	92.5
Leaf cutting	0	0.0	0.0
Stem boring	0	0.0	0.0
Leaf mining	9	7.5	100.0

3.3.5 Farmers' perceptions of damage and economic impact of spider mites

Results in Table 5 indicate farmers' perceptions of damage and economic impact of spider mites in their enterprises. Most (47.5%) of the farmers interviewed strongly agree that spider mite infestations are characterised by yellowing of plant leaves followed by agree (38.3%), slightly agree (10%), slightly disagree (1.7%), disagree (1.7%) and strongly disagree (0.8%). 42.5% of respondents strongly agree with the statement that spider mites suck sap from plant leaves while 40.8 % agree followed by slightly agree (7.5%), slightly disagree (4.2%) and disagree (5%). The majority of farmers (44.2%) strongly agree that spider mites multiply quickly followed by those who agree (40.8%), slightly agree (11.7%), slightly disagree (0.8%) and disagree (2.5%). Most (43.3%) of farmers strongly agree that spider mite outbreaks are common during summer months followed by those who agree (40.8%), slightly agree (14.2%) and disagree (1.7%). 45.8% strongly agree that spider mite infestations negatively affect farmers' income followed by agree (45%), slightly agree (8.3%) and slightly disagree (0.8%). Majority (50%) of the farmers strongly agree that spider mites are a threat to the horticulture industry with agree (40.8%), slightly agree (6.7%) and disagree (2.5%). 49.2% strongly agree that spider mites reduce fruit quality followed by agree (44.2%), slightly agree (5.8%) and slightly disagree (0.8%). Half (50.0%) of the respondents strongly agree that spider mite infested tomatoes attract poor market followed by those who agree (45.8%) and slightly agree (4.2%). More than half (53.3%) of interviewees strongly agree that infested tomato plants usually bear less fruit followed by those who agree (41.7%), slightly agree (2.5%) and slightly disagree (2.5%). Majority (46.7%) of farmers agree that spider mites transmit plant diseases followed by those who agree (45.0%), slightly agree (5.8%), slightly disagree (1.7%) and disagree (0.8%). More than half (55.0%) of the farmers strongly agree that spider mites are difficult to control followed by those who agree (42.5%) and slightly agree (2.5%). Majority

(51.7%) of the farmers strongly believe that spider mite infestations increase the cost of production followed by those who agree (37.5%), slightly agree (10.0%) and disagree (0.8%).

Table 5. Farmers' knowledge and perceptions towards spider mite damage and economic impact

Statements	Rating scale					
	SD	D	SLD	SLA	A	SA
1. Spider mite causes yellowing of plant leaves.	1 (0.8%)	2 (1.7%)	2 (1.7%)	12 (10.0%)	46 (38.3%)	57 (47.5%)
2. Spider mite damage plants by sucking sap.	0 (0%)	6 (5.0%)	5 (4.2%)	9 (7.5%)	49 (40.8%)	51 (42.5%)
3. Spider mites reproduce and multiply quickly.	0 (0%)	3 (2.5%)	1 (0.8%)	14 (11.7%)	49 (40.8%)	53 (44.2%)
4. Spider mite outbreaks occur during summer months.	0 (0%)	2 (1.7%)	0 (0%)	17 (14.2%)	49 (40.8%)	52 (43.3%)
5. Spider mites reduce farmers' income.	0 (0%)	0 (0%)	1 (0.8%)	10 (8.3%)	54 (45.0%)	55 (45.8%)
6. Spider mites are a threat to the horticulture industry.	0 (0%)	3 (2.5%)	0 (0%)	8 (6.7%)	49 (40.8%)	60 (50.0%)
7. Spider mites reduces fruit quality.	0 (0%)	0 (0%)	1 (0.8%)	7 (5.8%)	53 (44.2%)	59 (49.2%)
8. Spider mite infested tomatoes attract poor market.	0 (0%)	0 (0%)	0 (0%)	5 (4.2%)	55 (45.8%)	60 (50.0%)
9. Infested plants usually bear less fruit.	0 (0%)	0 (0%)	3 (2.5%)	3 (2.5%)	50 (41.7%)	64 (53.3%)
10. Spider mite can transmit plant diseases.	0 (0%)	1 (0.8%)	2 (1.7%)	7 (5.8%)	54 (45.0%)	56 (46.7%)
11. Spider mites are difficult to control.	0 (0%)	0 (0%)	0 (0%)	3 (2.5%)	51 (42.5%)	66 (55.0%)
12. Spider mite increases the cost of production.	0 (0%)	1 (0.8%)	0 (0%)	12 (10.0%)	45 (37.5%)	62 (51.7%)

3.3.6. Farmers' management practices for spider mites

The use of chemical pesticides was by far the most commonly mentioned (85.8%) method of controlling spider mites followed by water sprinkling (23.3%), controlled entry (16.7%), crop rotation (15.8%) and weeding (13.3%) (Figure 7). Other management tactics mentioned but were perceived as less important were the use of bio-pesticides, intercropping, sanitation, tillage and burning crop residues.

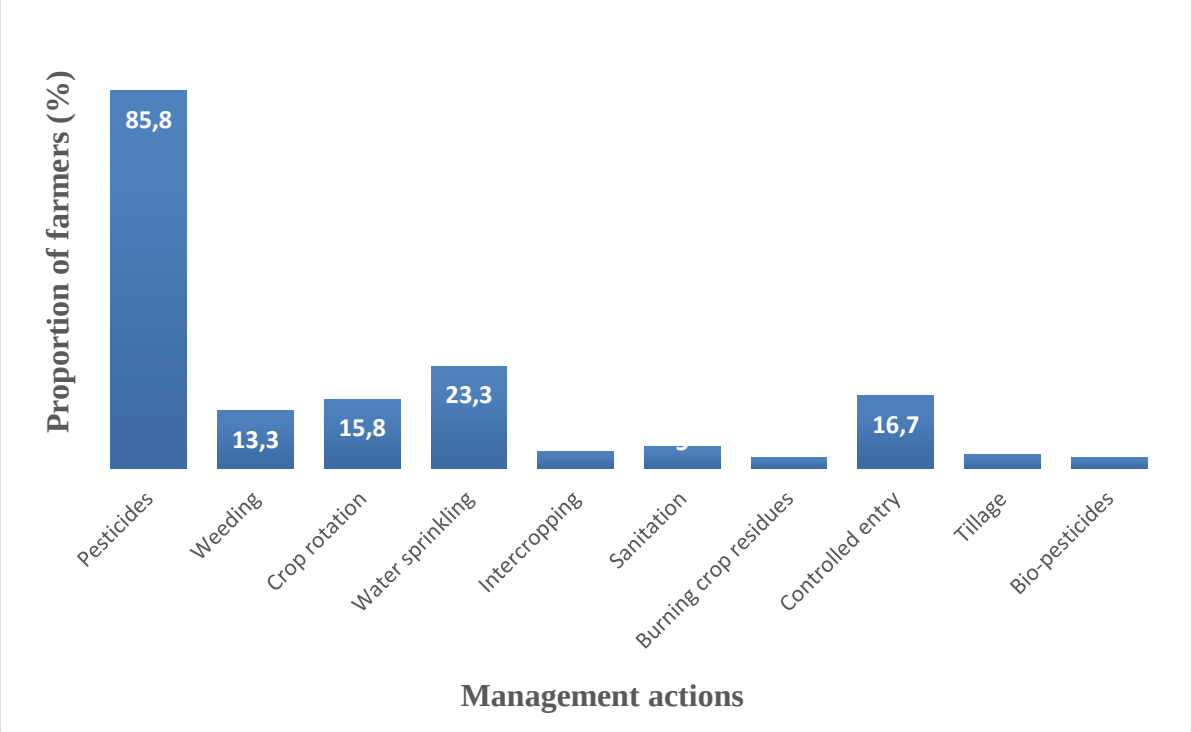


Figure 7. Percentage of farmers who mentioned a pest management action

3.3.7 Pesticides used by vegetable farmers to control pests of tomato in Botswana

The results of the survey show that 29 pesticide active ingredients were used by the farmers to control spider mites on tomatoes in Botswana (Table 6). The chemical groups most cited were organophosphates (11), pyrethroids (5), carbamates (3), avermectins (2), organochlorines (2), pyrazole (1). The type of pesticides included 18 insecticides, 3 acaricides, 3 insecticides/ acaricides, 2 insecticide/ nematicides and 1 fungicide. The most commonly used pesticide was

abamectin (79.2%) followed by methomyl (61.7%), chlorfenapyr (69.2%), emmamectin benzoate (61.7%), cypermethrin (55.8%), dicofol (52.5%), carbaryl (50.8%), beta-cyhalothrin (46.7%), lambda-cyhalothrin (41.7%), endosulfan (39.2%), chlorpyrifos (33.3%) and trichlofon (33.3%). Other pesticides which were mentioned are shown in Table 5. Among the commonly used pesticides, eight are classified as extremely (class 1a) or highly hazardous (class 1b) by the World Health Organisation (WHO 2020).

Table 6. Pesticides used in Botswana to control pests of tomato

Active ingredient	Chemical type	WHO hazard class	Count	%
Cypermethrin (I)	PY	II	67	55.8%
Emmamectin–Benzoate (Avermectin)(I)		II	74	61.7%
Alpha – Cypermethrin (I)	PY	II	43	35.8%
Abamectin (Avermectin)(AC)		1B	95	79.2%
Lambda-cyhalothrin(I)	PY	II	50	41.7%
Chlorfenapyr (I/AC)	PZ	II	83	69.2%
Chlorpyrifos (I)	OP	II	40	33.3%
Malathion (I)	OP	III	13	10.8%
Dimethoate (I)	OP	II	15	12.5%
Methomyl (I)	C	1B	74	61.7%
Carbaryl (I)	C	II	61	50.8%
Fenthion (I)	OP	II	9	7.5%
Diazinon (I)	OP	II	6	5.0%
Demeton-s-methyl (I)	OP	1B	10	8.3%
Trichlorfon (I)	OP	II	40	33.3%
Endosulfan (I)	OC	II	47	39.2%
Deltamethrin (I)	PY	II	11	9.2%
Parathion (I)	OP	1A	6	5.0%
Dichlovos (I)	OP	1B	3	2.5%
Methamidophos (I)	OP	1B	37	30.8%
Dicofol (AC)	OC	II	63	52.5%
Fenamiphos (I/N)	OP	1B	7	5.8%
Carbofuran (I/N)	C	1B	1	0.8%
Mancozeb (Dithio)(F)		U	1	0.8%
Bifenazate (Bifenazate)(AC)		U	29	24.2%
Etoxazole (Pyrroles)(I/AC)		III	30	25.0%
Clofentezine (chlorobenzene) (I/AC)		III	23	19.2%
Beta-cyhalothrin	PY	II	56	46.7%

OP = organophosphate; PY = pyrethroid; OC = organochlorine; Carb = carbamates; Dithio = dithiocarbamate; PZ = Pyrazole; AC = acaricide; F = fungicide; H = herbicide, I = insecticide; N = nematicide; 1A = extremely hazardous; 1B = highly hazardous; II = moderately hazardous; III = slightly hazardous; U = unlikely to present acute hazard in normal use (WHO, 2020).

3.3.8 Farmers' management protocols for spider mites

Most (54.2%) of the respondents mentioned that they apply management measures when they discover the pest on their plants while 15.8% based their decision on an existing spray program, 22.5% on noticing damage symptoms and 7.5% on economic decision levels (Table 7). There was no significant relationship between age and the decision to spray. Regarding their spray frequencies, 38.3% mentioned that they spray on weekly basis, 29.2% spray once a month, 23.3% spray every two weeks and 9.2% spray twice a week. The frequency of spraying was significantly ($P = 0.001$) associated with the age of the farmer. When asked about the effectiveness of their management actions, 59% of the farmers indicated that they were moderate, slightly effective (28%), not effective (20%) and only 13% indicated that they were effective. Most farmers (49%) mentioned that in order to avoid resistance development they alternate several different pesticides, 20% use integrated pest management, 10% reduce spray frequency, 9.2% increase pesticide dosage, 8.3% increase spray frequency and only 3.3% observe economic thresholds.

Table 7. Farmers' management protocols for spider mites on tomato

	Frequency	Percent (%)	Cum. %
3. Frequency of application of pesticides.			
Weekly	46	38.3	38.3
Twice a week	11	9.2	47.5
Every two weeks	28	23.3	70.8
Once a month	35	29.2	100.0
4. What influences your decision to spray?			
Pest presence	65	54.2	54.2
Spray programme	19	15.8	70.0
Damage symptoms	27	22.5	92.5
Economic thresholds	9	7.5	100.0
5. How effective are your current management tactics?			
Very effective	13	10.8	10.8
Slightly effective	28	23.3	34.1
Moderate	59	49.2	83.3
Not effective	20	16.7	100.0
6. Does spider mite develop resistance?			
Yes	120	100.0	100.0
No	0	0.0	100.0
7. When did you notice?			
This year	15	12.5	12.5
One year ago	77	64.2	76.7
Two or more years back	28	23.3	100.0
8. How do you avoid resistance development?			
Increase dosage	11	9.2	9.2
Alternate pesticides	59	49.2	58.4
Increase spray frequency	10	8.3	66.7
Reduce spray frequency	12	10.0	76.7
Integrated pest management	24	20.0	96.7
Observe economic thresholds	4	3.3	100.0

3. 4 DISCUSSION

This study established that invertebrate pests are a major limiting factor to profitable tomato production in Botswana. This perception is in agreement with Munthali *et al*. (2004), Obopile *et al*. (2008) and Baliyan and Rao (2013) that pests and diseases are important constraints to vegetable production. According to the farmers', spider mites and tomato leaf miner are the most damaging arthropod pests of tomatoes in Botswana. This is in agreement with reports from other parts of Southern Africa (Sibanda *et al*., 2000; Munthali *et al*., 2004; Obopile *et al*., 2008; Grzywacz *et al*., 2010). Spider mites are an invasive species in Southern Africa and these findings emphasise its growing importance in the region. Migeon *et al*. (2009) predicted its widespread expansion to more parts of Africa, therefore new technologies that can be accessed by resource deficient farmers should be developed to manage this pest. These results also suggest that the spider mite problem was more pronounced in the greater Gaborone, Southern, Central and Francistown areas than Maun and Gantsi. This may be due to the fact that farmers in these areas undertake intensive production of tomato for the urban market. Spider mites cause serious damage to the tomato crop thereby affecting the income level of the farmer. These results are similar to Bok *et al*. (2006) and Mwandila *et al*. (2013) where heavy spider mite infestations drastically reduce yield. Apart from affecting yield levels, tomatoes with spider mite streaks on them fetch very low prices and are more likely to be discarded. The study revealed that most of the farmers depend on pesticides to control invertebrate pests including spider mites. This is consistent with other studies which report of the growing reliance on synthetic pesticides to manage vegetable pest infestations (Orr and Ritchie, 2004; Munthali, 2009; Grzywacz *et al*., 2010; Leungo, 2012). Most of the pesticides were mentioned in the Gaborone, Southern, Central and Francistown study areas. This is consistent with the level of production in these areas and consequently high pesticide usage. The high value placed on tomatoes coupled with the ease of application and accessibility of

pesticides make them the first choice for farmers to ensuring the production of good yields. Most farmers report reduced effectiveness of some pesticides in controlling spider mites. This is consistent with several reports (Van Leeuwen *et al*., 2010; Badieinia *et al*., 2020) that indicate that spider mites quickly develop resistance to most new formulations used against them. Farmers' perceived development of resistance forces them to seek new, more toxic and more expensive formulations. Labour that would otherwise be used for other aspects of production is diverted to spraying and management of spider mites. Not all pesticides mentioned by farmers are recommended for spider mite control. The reality that farmers used pesticides that were not recommended for spider mite control is tell-tale of their desperation to eradicate spider mites. Furthermore, Palikhe (2002) outlined some detrimental effects of some of the formulations to human health, environment and non-target organisms. Several of the pesticides mentioned are classified under the highly hazardous and extremely hazardous (1A and 1B) categories by the World Health Organisation (WHO). This has harmful consequences on the health of farmers, livestock and the environment (Ngowi *et al*., 2007). Similar results have been reported in Malawi (Nyirenda, 2015). Apart from their environmental and health effects, pesticides are becoming increasingly expensive to use. More focus should be put on educating farmers on proper spray regimes to reduce the amount of active ingredients and frequency applied to crops. The development of economic decision levels can help farmers reduce the amount of pesticide applied and reduce the cost to the farmer. Farmers can be assisted to develop spray schedules for their fields to reduce the risk of resistance development. Since farmers do not seem to realise that weeds serve as alternate hosts for spider mites they should be made aware of the importance of weeding and general hygiene in controlling this pest. Integrated pest management (IPM) remains a vital tool for the management of vegetable pests therefore emphasis should be placed on the use, in

addition to chemical control, of cultural and biological control to reduce dependence on synthetic pesticides for spider mite control. Farmers indicated that pest information was gained from personal experiences, agro-traders and fellow farmers. Our results that agricultural extension officers play a small role in information dissemination to vegetable farmers are similar to those of TAHAAL (2000) and Madisa *et al*. (2010) who reported low delivery of advisory services to farmers. This has a negative impact on vegetable production since agricultural extension officers have a critical role to play in increasing farmers' awareness of pest management and pesticides usage. According to Abdollahzadeh *et al*. (2015), farmers in close contact with extension officers are most likely to employ alternative methods of pest control, such as integrated pest management. Extension and education programs are critical in pest management, providing farmers with the knowledge for the selection of the appropriate pest control options (Prudent *et al*. 2007). An effective way of addressing the problems that hamper the productivity of the vegetable sub-sector is to develop and disseminate appropriate technologies and policies to farmers according to their social and economic situations (DAR, 2000). Government should take into consideration farmers' sentiments and put emphasis on extension services, particularly for the vegetable sub-sector.

3.5 CONCLUSIONS

Farmers in Botswana perceive spider mites as an important constraint to tomato production. Most of the farmers possess considerable educational background and experience in growing tomatoes. Extension services played the least role in providing valuable pest information to farmers. The primary characteristic that the farmers use to distinguish between the spider mites is colour and the red form is most prevalent across fields in Botswana. Spider mites were reported to reduce the quantity and quality of tomato yields and therefore affect farmers' incomes. Growers stated that spider mites are very difficult and increase the cost of production. Pesticide use is the most

common method of spider mite control with numerous chemical formulations in use across the country. Some of the pesticide formulations applied are classified as highly or extremely harzadous by the world health organisation. Farmers' decision to spray pesticides was mainly upon seeing the pest or pest symptoms on the crop. Most farmers report reduced effectiveness of their current management practices in controlling spider mites and assume it may have developed resistance. In conclusion, innovations designed to control spider mites on tomatoes in Botswana should consider farmers' knowledge of the pest, socio-economic circumstances and current management practices.

3.6 RECOMMENDATIONS

The study suggests that the current extension programmes should be strengthened to better disseminate information on proper pest management practices to vegetable farmers. Intensified agronomic information sharing through short courses, workshops, and hands-on training would be ideal for producers across the country. Vegetable farmers should be advised to employ an integrated pest management strategy for pests and diseases. IPM can help reduce their use of pesticides and avoid development of pest resistance. It is mostly utilised by growers who employ multiple cultural methods to reduce pesticide use. Instead of applying pesticides at the sight of the pest or damage symptoms, farmers can be advised to minimise the risks of pest residues, resistance development and deleterious effects on non target organisms. The development of economic decision levels can help guide farmers on taking appropriate actions in pest management and to apply pesticides in the most economical manner. Government through the registrar of agrochemicals should restrict the availability of the most hazardous formulations and regulate the use of pesticides. More work on farmers' management practices for spidermites should be carried out in the future.

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Appendix 1: Individual Survey Questionnaire

20th March 2019

Dear participant

RE: LETTER OF CONSENT FOR PARTICIPATING IN THE RESEARCH STUDY

I am a student at the Botswana University of Agriculture and Natural Resources undertaking a study as part of the requirements for my PhD degree. The title of my survey is "Farmers' Knowledge, Perceptions and Management of Spider Mites (Tetranychus spp.) on Tomato in Botswana".

The purpose of this survey is to gather information on farmers' knowledge and perceptions of tomato spider mites and the management practices they employ for its control. To develop integrated and sustainable pest management strategies for this pest, it is highly necessary to have adequate information about farmers' management strategies. Therefore, during our search for effective control measures, it is important to carry out surveys that can provide farmers' alternative viewpoint of the pest.

Participation in this study is voluntary and you are allowed to withdraw any time you want. Anonymity and confidentiality is ensured. The researcher will make every effort that the information you provide is confidential. The questionnaires will be labeled using pseudonyms. You will be given a study identification number for recording your responses in order to protect your privacy.

In signing this letter, you are making a declaration that you are participating in this study. If there is something you do not understand kindly ask the researcher for clarification.

Name and Surname of Participant:

Sign: Date:

Yours faithfully

Mitch M. Legwaila

FARMERS' KNOWLEDGE, PERCEPTIONS AND MANAGEMENT OF SPIDERMITES (T*etranychus* spp.) (ACARI: TETRANYCHIDAE) ON TOMATO IN BOTSWANA

QUESTIONNAIRE

The questionnaire consists of eight (8) printed pages and will take about 10 minutes to complete

Mitch M. Legwaila

Email: mitchlaila@yahoo.com

Tel: 3973860

Cell: 72362059/ 73278558

SECTION A

DEMOGRAPHIC CHARACTERISTICS

Information from this section will be helpful in the interpretation of this study. Please circle the correct letter for your response.

1. What is your gender?
    A. Male
    B. Female

2. How old are you?
    A. Less than 20 years
    B. 20-30 years
    C. 31-40 years
    D. 41-50 years
    E. Above 50 years

3. What is your marital status?
    A. Single
    B. Married
    C. Divorced
    D. Widowed

4. What is your highest educational qualification?
    A. Primary education
    B. Secondary education
    C. Diploma
    D. First degree
    E. Master's degree
    F. Doctorate
    G. Other please specify _____

5. Who is in charge of the farm?
    A. Farm manager
    B. Owner
    C. Other please specify _____

6. What are your responsibilities in the farm?
    A. Farm hand
    B. Gardener
    C. Manager
    D. Labourer
    E. Other please specify _____

7. How long have you been growing vegetables?
    A. Less than 10 years
    B. 10-20 years
    C. 21-30 years
    D. 31-40 years
    E. Above 40 years
8. How long have you been growing tomatoes?
    A. Less than 10 years
    B. 10-20 years
    C. 21-30 years
    D. 31-40 years
    E. Above 40 years

9. For what purpose do you grow tomatoes?
    A. Home consumption
    B. Retail sale
    C. Market
    D. Donation
    E. Other please specify _____

10. What type of production system do you use to grow tomatoes?
    A. Greenhouse
    B. Open field
    C. Tunnels
    D. Hydroponics
    E. Other please specify _____

11. What is the area planted with tomato?
    A. Less than 10ha
    B. 10- 15 ha
    C. 15-20ha
    D. 20-25ha
    E. Other please specify _____

SECTION B

Farmers knowledge and perceptions towards spider mites

Attempt all questions in this section in the spaces provided

1. Kindly list in order of importance the production constraints in your tomato production.

_____
_____
_____
_____
_____
_____

2. Kindly list in order of importance your major insect pests of tomatoes?

_____
_____
_____
_____
_____
_____

Please circle the correct letter for your response.

3. Have you ever heard of tomato spider mites before?

   A. Yes

   B. No

4. If your answer is "Yes" to the above, what is your source of information on tomato spider mites?

A. Own experiences B. Fellow farmers

C. Agro-traders D. Agricultural extension officers

E. Researchers F. Radio/television

G. Others please specify _____

5. How serious is the tomato spider mite problem in your area?

A. Very serious B. Serious

C. Moderate D. Not serious

6. What is the name given to the tomato spider mite most prevalent in your area?

A. Red spider mite D. Tobacco spider mite

B. Carmine spider mite E. Other please specify _____

C. Two-spotted spider mite

7. What important features do you use to identify the spider mite named above?

A. Size D. Reproduction

B. Shape E. Other please specify _____

C. Colour

8. What is the colour of the tomato spider mite indicated as the most prevalent pest of tomato in your area?

A. Yellow B. Green

C. White D. Red

E. Blue F. Other please specify _____

9. What do you think causes the spider mite indicated above to be a serious pest of tomato? Tick the correct answer from the options below

A. Climate B. Fast reproduction

C. Pesticide resistance D. High population

H. Other please specify _____

10. What type of damage do you associate with the identified spider mite?

A. Sucking E. Leaf mining

B. Yellowing F. Other please specify _____

C. Leaf cutting

D. Stem boring

SECTION C

Farmers' Knowledge and Perceptions towards Tomato Spider Mite damage and economic impact.

Please indicate your level of agreement or disagreement with each of the statements by ticking (√) the appropriate box in the rating scale that best corresponds with your perception regarding the statement.

Rating scale

1. Strongly Disagree (SD) 4. Slightly Agree (SLA)

2. Disagree (D)
3. Slightly Disagree (SD)
5. Agree (A)
6. Strongly Agree (SA)

STATEMENTS	**RATING SCALE**					
1. Spider mites cause yellowing of plant leaves	SD	D	SLD	SLA	A	SA
2. Spider mites damage plants through sucking of sap						
3. Spider mites reproduce and multiply quickly						
4. Spider mite outbreaks occur during summer months						
5. Spider mite reduces farmers' income						
6. Spider mites are a threat to the horticulture industry.						
7. Spider mite infested tomatoes attract poor market						
8. Spider mite reduces fruit quality						
9. Infested plants usually bear less fruit						
10. Spider mite can transmit diseases to plants						
11. Spider mites are difficult to control						
12. Spider mite increases the cost of production						

SECTION C

Management Practices for Tomato Spider Mite on tomato

1. Kindly list the pest management actions you use for control of insect pests of tomato in your farm.

_____
_____
_____
_____
_____

2. Starting with the most common, kindly list the chemical formulations you use to control spider mites in your operation.

_____
_____
_____
_____
_____

3. What is the frequency of application of the pesticides indicated above?

A. Every week

B. Every two weeks

C. Every three weeks

D. Monthly

E. Other please specify _____

4. What determines the frequency of application of the pesticides indicated above?

A. Weather

B. Pest population

C. Plant age

D. Damage symptoms

E. Other please specify _____

5. How effective is the method you indicated above for the control of this pest?

A. Very effective

B. Slightly effective

C. Moderate

D. Not effective

6. Does the spider mite develop resistance to acaricides?

A. Yes

B. No

7. When did you notice?

A. This year

B. One year ago

C. Two or more years ago

8. What measures do you take to avoid resistance development?

Choose one or more options.

A. Increase pesticide dosage

B. Alternate pesticides

C. Increase spray frequency

D. Reduce spray frequency

E. Employ integrated pest management

F. Observe economic thresholds

Thank you for your participation!

CHAPTER 4 - EFFECTIVENESS OF THREE PESTICIDES AGAINST CARMINE SPIDER MITE (*TETRANYCHUS CINNABARINUS* BOISDUVAL) ON TOMATO IN BOTSWANA

ABSTRACT

The carmine spider mite (CSM; *Tetranychus cinnabarinus* Bois.) is among the most destructive pests of vegetables, especially tomatoes. Its management in Botswana has, for years, depended on

the application of synthetic pesticides. This study evaluated the efficacy of abamectin, methomyl and chlorfenapyr against CSM eggs and adults under laboratory conditions in Botswana. Each treatment was replicated three times. The toxic effect was evaluated in the laboratory bioassay after 24, 48, 72h and 96 h following application of pesticides. This study revealed that chlorfenapyr was relatively more effective against eggs since it had lower LD_{50} values than those for abamectin and methomyl. It was further revealed that at recommended rates, 90% mortalities occurred 48 h after application of methomyl and chlorfenapyr, while abamectin did not achieve 90% egg mortality throughout the study period. This implies that abamectin requires extra dosages to achieve mortalities comparable to those of the other two pesticides. The study has found that chlorfenapyr was the most effective pesticide followed by methomyl and then abamectin when applied to CSM eggs. When treatments were applied against adults, chlorfenapyr was relatively more effective since it had lower LD_{50} values than those for abamectin and methomyl. The study revealed that abamectin requires extra dosages or longer application periods to achieve mortalities comparable to chlorfenapyr and methomyl. Chlorfenapyr was the most effective of the three pesticides followed by methomyl and then abamectin. Further investigations and field tests are needed to confirm these laboratory findings.

4.1 INTRODUCTION

The carmine spider mite, *Tetranychus cinnabarinus* (Boisduval, 1867) (CSM) is among the most destructive pests of crops and vegetables in the world. It has a broad host plant range (Sokeli *et al*., 2007) including cultivated crops such as pepper, tomatoes, cucurbits, maize, tobacco, soy, cotton, beans, eggplant, and many others (Bu *et al*., 2015). It causes great economic impact wherever tomatoes are grown (Mwandila *et al*., 2013). CSM are a parenchyma cell feeding pest where the adults and nymphs suck sap mostly from the mature leaves. Heavy infestations result in

loss of foliage thereby significantly lowering yields, causing unbearable economic losses (Xu *et al*., 2014). Its remarkable pest status is due to the abundance and diversity of host plants, its high reproductive ability and its genetic elasticity that leads to a quick development of resistance to pesticides (He *et al*., 2009). Many researchers in other parts of the world have demonstrated that CSM has a high proclivity to evolve resistance to most new formulations introduced for its management (Stumpf and Nauen, 2001; Sato *et al*., 2005). Control failures have been reported in several countries for formulations such as dicofol, hexythiazox, fenpyroximate, clofentezine, abamectin, organophosphates and organotins (Nauen *et al*., 2001; Kim *et al*., 2004; Sato *et al*., 2005; Van Leeuwen *et al*., 2005). This presents a serious impediment to its effective management. In Botswana, the management of CSM is profoundly reliant on conventional chemical pesticides. Abamectin and Chlorfenapyr are among the most widely used pesticides in the control of arthropod pests of vegetables in Botswana (Obopile *et al*., 2008) while Methomyl has been used worldwide as an acaricide for the control of insects and mite pests of vegetables (Tomlin, 2000).

Abamectin (Mode of Action Group 6 - Glutamate-gated chloride channel (GluCl) allosteric modulator) (IRAC, 2018) is a macrolytic lactone obtained from the soil bacterium *Streptomyces avertimilis* (Kim and Goodfellow, 2002). It is more effective as an ingestion toxicant, but also has some contact activity. It acts on the γ-aminobutyric acid (GABA) gated chlorine channels, glutamate-gated chlorine channels causing paralysis and death of the pest (Sato *et al*., 2005). It has been shown to be active against all motile life stages of spider mites (Ismail *et al*., 2007). However, the widespread and indiscriminate use of abamectin applies heavy selection pressure on the pest population, resulting in resistance development (Sato *et al*., 2005). Many researchers report that many strains and field populations around the world have developed resistance to abamectin (Stumpf and Nauen, 2002). Methomyl, S-methyl (EZ)-N- (methylcarbamoyloxy) thioacetimidate

is an oxime carbamate toxicant used as an acaricide to control ticks and spiders. It is also used for foliar treatment of vegetables, fruits and field crops, as well as cotton and commercial ornamentals (Gaete *et al*., 2013). Methomyl is effective in two ways; contact because it kills target insects upon direct contact and also as a systemic because of its capacity to cause overall systemic poisoning of the pest (Aktar *et al*., 2010) and acts as an acetylcholinesterase inhibitor. Chlorfenapyr, 4-bromo-2- (4-chloropheny)-1-ethoxymethyl-5 trifluoro methylpyrrole-3-carbonitrile, is a broad-spectrum pesticide/ acaricide employed for the control of various species of arthropod pests. It is a chitin synthesis inhibitor used against pests in cotton, vegetables, citrus and soy (Tomlin, 2000). It is an N-substituted halogenated pyrrole obtained from the natural product dioxapyrrolomycin (Hunt and Treacy, 1998). Once the formed metabolite has inhibited oxidative phosphorylation by disrupting the proton gradient across mitochondrial membranes, the ability of cells to produce ATP and ADP is affected, ultimately resulting in cell death and death of the pest (Rand, 2004; Rahman *et al*., 2012). Although the pesticides evaluated in this study have been used to control vegetable pests in Botswana for over a decade, their effectiveness has not been evaluated on local strains of CSM. Determination of relative effectiveness of pesticides used in the control of CSM is a crucial element for development of suitable and affordable strategies for its effective management. This study evaluated the effectiveness of abamectin, methomyl and chlorfenapyr against CSM eggs and adults under laboratory conditions in Botswana.

4.2 MATERIALS AND METHODS

The bioassay was conducted at the Botswana University of Agriculture and Natural Resources (BUAN) in Gaborone, Botswana (24°35'29.04" S, 25°56'29.40" E; altitude: 998 m) in the Crop Protection laboratory, at an average temperature of 25 ± 3°C. CSM population used in this study

was obtained from a commercial tomato producing farm in Metsimotlhabe just outside Gaborone and identified in the Crop Protection laboratory at BUAN before use in the bioassay. Tomato seedlings (var. Rodade) were raised in the greenhouse in seedling trays and transplanted into black plastic pots filled with 1.5 kg loam soil; each pot was 12 cm wide and 15 cm deep. CSM were reared on tomato seedlings and adults were allowed to oviposit eggs on the plants. The seedlings were watered on a regular basis (ad-lib) to avoid wilting.

4.2.1 Bioassay methods

Three commercially available pesticides, Abamectin (Agromectin®) Arysta LifeScience, Methomyl (Spitfire® 900SP) Bitrad Consulting (PTY) LTD and Chlorfenapyr (Savage 360® SC) registered in Botswana, were used in the laboratory study. The method followed the IRAC method 003 (for eggs) and IRAC method 004 (for adults) (IRAC, 2009). Each pesticide was applied at 5 concentrations separated on a $\log_{10}$ scale, with the recommended rates (0.6 ml/L for abamectin; 0.5 g/L for methomyl; and 0.4 ml/L for chlorfenapyr) included as a check. Distilled water was included as a control and 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; methomyl: 0.3, 0.4, 0.5, 0.6 and 0.7 g/L; chlorfenapyr: 0.2, 0.3, 0.4, 0.5 and 0.6 ml/L water. The experiment was arranged in a completely randomized design. 2 cm diameter leaf discs were cut from chemically untreated tomato leaves using a 2 cm diameter aluminium pipe. 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 5 s. The surface liquid was allowed to dry from the leaves 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.

For the egg bioassay: A fine brush was used to transfer CSM eggs (not older than 48 h) onto each treated leaf disc. Eggs (10 - 20) were placed on each treated leaf disc. Each bioassay had a total of 54 treated leaf discs and 162 treated leaf discs in total. The tests were maintained at 25 ± 3°C and at 65 to 90% relative humidity. Each cup had a label to indicate the treatment and date of application. The experiment was repeated 3 times.

For the adult bioassay: A fine brush was used to transfer spider mites onto each treated leaf disc. 10 adults were placed on each treated leaf disc. Each bioassay had a total of 54 treated leaf discs 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 showing the treatment and its date of application. The experiment was repeated 3 times.

4.2.2 Assessment of egg mortality

Each leaf disc was observed daily (24, 48 and 72 h after treatment) under a binocular microscope until complete (or nearly complete) hatching on the control leaf discs (water only) was observed. The number of un-hatched eggs on treated and untreated leaf sections was recorded. Results were expressed as percentage mortality and corrected for untreated mortality using Abbott's correctional formula (Abbott, 1925). Untreated mortality was also recorded.

4.2.3 Assessment of adult 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. Assessments for mortality were aundertaken at intervals of 24, 48, 72 and 96 h following treatment. Results were

expressed as percentage mortality and corrected for untreated mortality using Abbott's correctional formula (Abbott 1925). Untreated mortality was also recorded.

4.2.4 Data analysis

Probit analysis (Finney, 1971) was used to analyse the results. The mortality data were transformed to probits while the dosages were transformed to $\log_{10}$ (X+1) prior to analysis. Data was analysed using $\log_{10}$ of dosage versus probit mortality regression and analysis of variance (ANOVA). Probit lines were used to estimate LD_{50} and LD_{90} values. Comparative susceptibilities of eggs and adults were estimated using LD_{50} values and slopes of probit lines. LD_{90} values were used to compare the mortality caused by the recommended dosage with the mortalities achieved by the treatments following different durations of exposure to the pesticides. Averages were separated using the Tukey's Honestly significant difference test (Zar, 1984).

4.3 RESULTS

4.3.1 CSM egg mortality due to abamectin

Figures 8 (A-C) shows the probit mortality of CSM eggs exposed to different dosages of abamectin assessed at 24 (A), 48 (B) and 72 h (C) after treatment. The figures show transformed dose and mortality data. These figures reveal a positive linear relationship between log dose and probit mortality caused by abamectin (correlation coefficients of 0.91, 0.90 and 0.75, respectively).

Figure 8A shows that LD_{50} of 0.55 ml/L and LD_{90} of 0.72 ml/L were achieved 24 h after application. The recommended dose (0.60 ml/L), abamectin showed a probit value of 0.58 (equivalent to 49.60% egg mortality) during this period. Figure 8B indicates that the LD_{50} of abamectin after 48 h exposure was 0.51 ml/L, while the LD_{90} was 0.69 ml/L. At the recommended dose (0.60 ml/L), abamectin achieved 0.67 on the probit scale, which is equivalent to 54.94% egg mortality. When assessed at 72 h after application, the LD_{50} of abamectin was 0.44 ml/L and the LD_{90} was 0.66 ml/L (Figure 8C). The recommended dosage (0.60 ml/L) of abamectin achieved 0.77 on the probit scale, which is equivalent to 61.34% egg mortality after 72 h exposure.

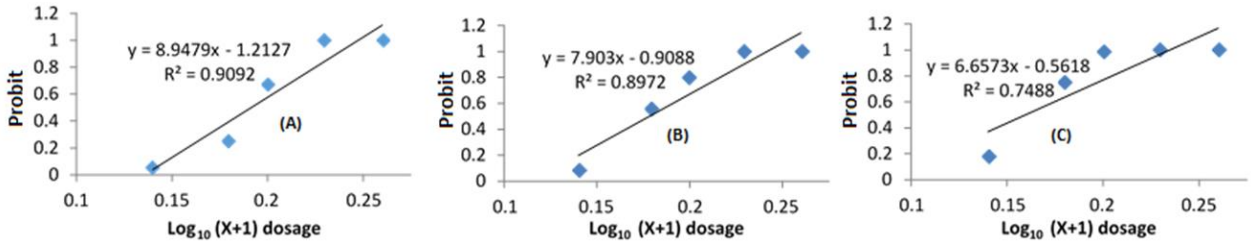

Figure 8. Probit mortality of CSM eggs exposed to different doses of Abamectin 24 h (A), 48h (B) and 72h (C) following application.

4.3.2 CSM egg mortality due to methomyl

The probit mortality of CSM eggs exposed to different dosages of methomyl assessed 24 (A), 48 (B) and 72 h (C) after treatment. These figures reveal a positive linear relationship between log dose and probit mortality caused by methomyl (correlation coefficients of 0.85, 0.65 and 0.61, respectively) (Figure 9A-C) during the assessment periods. Figure 9A shows that LD_{50} of 0.40 g/L and LD_{90} of 0.57 g/L were achieved 24 h after application. The recommended dose (0.50 g/L) of the pesticide showed a probit value of 0.76 (equivalent to 60.67% egg mortality) during this

exposure period. Figure 9B indicates that the LD_{50} of methomyl at the 48 h assessment period was 0.27 g/L, while the LD_{90} was 0.52 g/L. At the recommended dose, methomyl achieved 0.89 on the probit scale, which is equivalent to 70.63% egg mortality. When assessed at 72 h, the LD_{50} of methomyl was 0.12 g/L and the LD_{90} was 0.48 g/L (Figure 9C). The recommended dosage achieved 0.93 on the probit scale, which is equivalent to 74.66% egg mortality at the 72 h period.

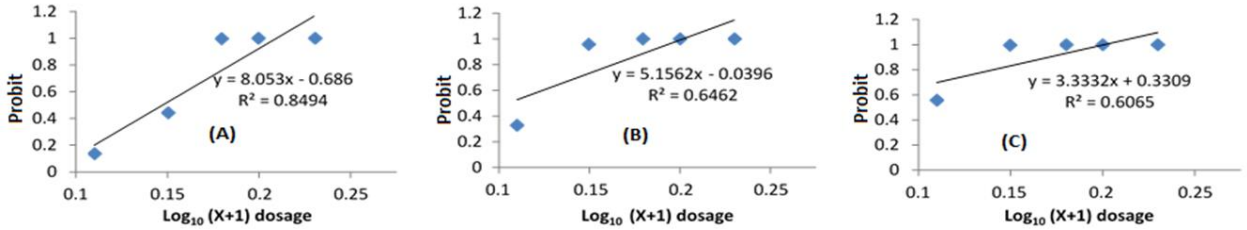

Figure 9. Probit mortality of CSM eggs exposed to different doses of Methomyl 24 h (A), 48 h (B) and 72 h (C) following application.

4.3.3 CSM egg mortality due to chlorfenapyr

Figure 10A to C shows probit mortality of CSM eggs exposed to different dosages of chlorfenapyr assessed 24 (A), 48 (B) and 72 h (C) after treatment. This figure revealed positive linear relationship between log dose and probit mortality caused by chlorfenapyr (correlation coefficients of 0.58, 0.5579 and 0.5367), when treatments were assessed at 24, 48 and 72 after treatment. Figure 10A shows that LD_{50} of 0.15 ml/L and LD_{90} of 0.42 ml/L were achieved 24 h after application. The recommended dose (0.40 ml/L) of the pesticide showed a probit value of 0.89 (equivalent to 70.63% egg mortality) during this assessment period. Figure 10B indicates that the LD_{50} of

chlorfenapyr after 48 h exposure was 0.08 ml/L, while the LD_{90} was 0.40 ml/L. At the recommended dose, chlorfenapyr achieved 0.91 on the probit scale, which is equivalent to 72.54% mortality. When assessed at 72 h after application, the LD_{90} was 0.33 ml/l (Figure 10C). The recommended dosage achieved 0.95 on the probit scale, which is equivalent to 77.08% egg mortality during the 72 h assessment period.

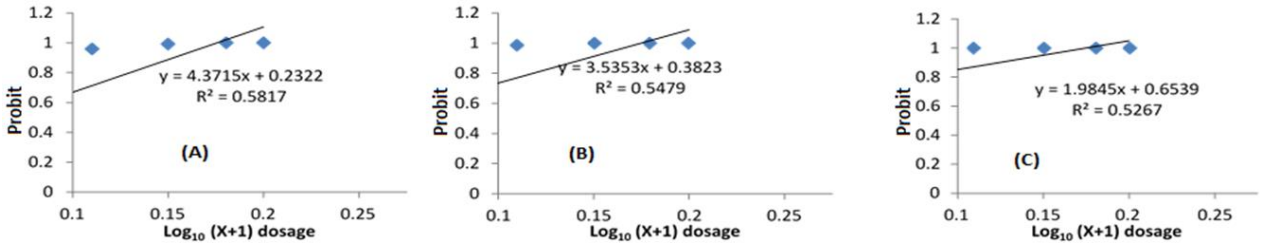

Figure 10. Probit mortality of CSM eggs exposed to different doses of Chlorfenapyr 24 h (A), 48 h (B) and 72 h (C) following application.

4.3.4 CSM adult mortality due to abamectin

Figures 11(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 pesticide application. Figure 11A 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 (0.60ml/L) of the abamectin showed a probit value of 0.53 (equivalent to 46.72% adult mortality) during this assessment period. Figure 11B 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 11C). The recommended dosage achieved 0.73 on the probit scale, which is equivalent to 68.69% adult mortality 72 h after treatment. Figure 11D 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.

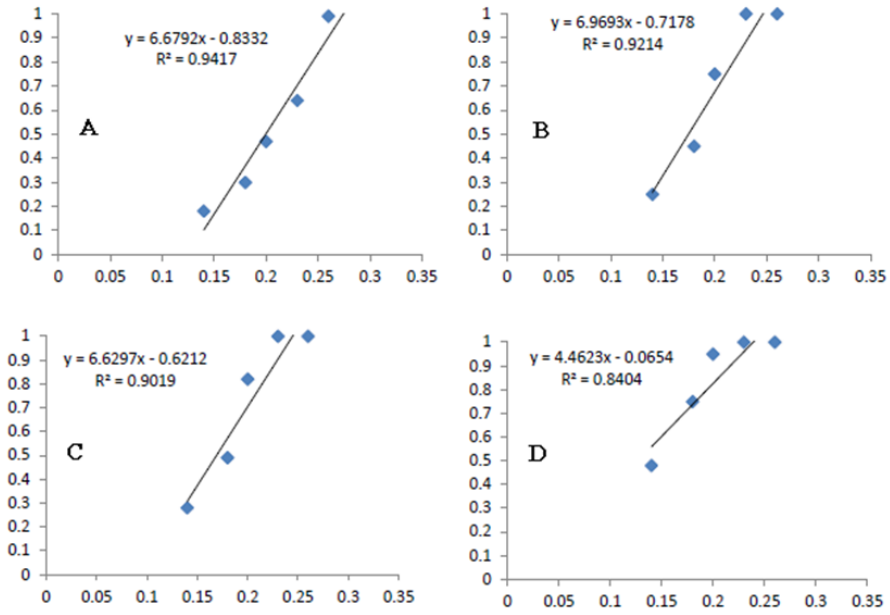

Figure 11. Probit mortality of CSM adults assessed 24 h (A), 48 h (B), 72 h (C) and 96 h (D) after treatment with different dosages of abamectin.

4.3.5 CSM adult mortality due to Methomyl

Figures 12 (A - D) shows positive curvilinear relationships between log dose and probit mortality caused by methomyl (correlation coefficients of 0.8554, 0.7313, 0.6009 and 0.6009), when treatments were assessed at 24, 48, 72 and 96 h after treatment. Figure 12A shows that LD_{50} of

0.39 g/L and LD_{90} of 0.57 g/L were achieved 24 h after treatment. The recommended dose (0.50 g/L) of methomyl showed a probit value of 0.758 (equivalent to 60.53% mortality) during this assessment period. Figure 12B indicates that the LD_{50} of methomyl after 48 h was 0.27 g/L, while the LD_{90} was 0.52 g/L. At the recommended dosage, methomyl achieved 0.859 on the probit scale, which is equivalent to 68.03% adult mortality. When assessed 72 h after treatment, the LD_{50} of methomyl was 0.19 g/L and the LD_{90} was 0.50 g/L (Figure 12C). The recommended dosage achieved 0.902 on the probit scale, which is equivalent to 71.76% mortality after 72 h. Figure 12D shows an LD_{50} value of 0.04 g/L and an LD_{90} of 0.46 g/L when the treatments were assessed at 96 h after treatment. The mortality achieved by the recommended dose was 0.934 on the probit scale, which is equivalent to 75.11% mortality.

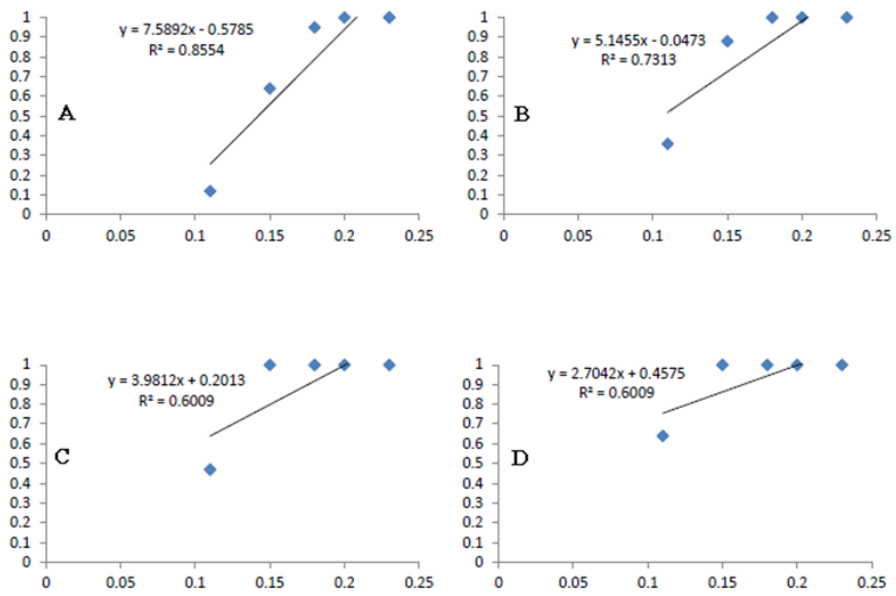

Figure 12. Probit mortality of CSM adults assessed 24 h (A), 48 h (B), 72 h (C) and 96 h (D) after treatment with different dosages of methomyl.

4.3.6 CSM adult mortality due to Chlorfenapyr

Figures 13 (A - D) show positive curvilinear relationships between log dose and probit mortality caused by chlorfenapyr (correlation coefficients of 0.9877, 0.9188, 0.6954 and 0.5267), when treatments were assessed at 24, 48, 72 and 96 h after treatment. Figure 13A shows that LD_{50} of 0.36 ml/L and LD_{90} of 0.52 ml/L were achieved 24 h after treatment. The recommended dosage (0.40 ml/L) of the chlorfenapyr showed a probit value of 0.597 (equivalent to 50.59% adult mortality) during the 24 h period. Figure 13B indicates that the LD_{50} of chlorfenapyr after 48 h was 0.31 ml/L, while the LD_{90} was 0.50 ml/L. At the recommended dosage, chlorfenapyr achieved 0.597 on the probit scale, which is equivalent to 50.59% mortality. When assessed at 72 h after treatment, the LD_{50} of chlorfenapyr was 0.22 ml/L and the LD_{90} was 0.44 ml/L (Figure 13C). The recommended dosage achieved 0.830 on the probit scale, which is equivalent to 65.65% adult mortality after 72 h. Figure 13D shows an LD_{50} value of 0.01 ml/L and an LD_{90} of 0.38 ml/L when the treatments were assessed at 96 h after treatment. The mortality achieved by the recommended dosage was 0.916 on the probit scale, which is equivalent to 73.15% CSM mortality.

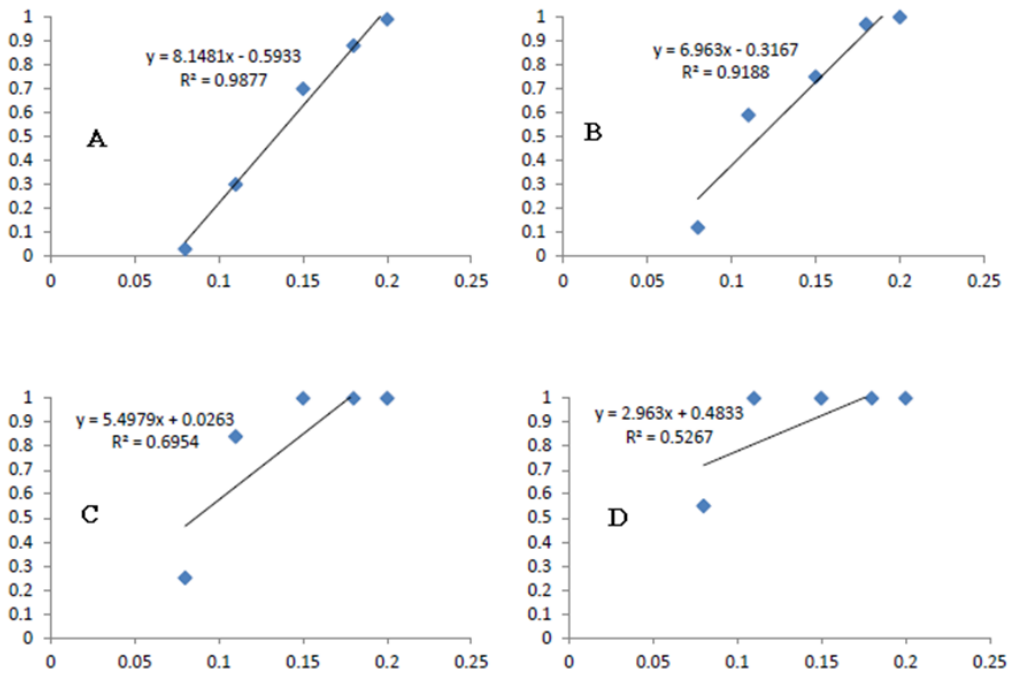

Figure 13. Probit mortality of CSM adults assessed 24 h (A), 48 h (B), 72 h (C) and 96 h (D) after treatment with different dosages of chlorfenapyr

4.3.7 The effect of abamectin concentrations and duration of exposure on CSM egg mortality

Table 8 shows the effect of abamectin concentrations and duration of exposure on CSM egg mortality. This study showed that concentration and period of exposure interactions were significantly different ($F_{10, 34} = 4.77$; P=0.0003). When comparing the different concentrations at 24 h period of exposure it has been shown that control was significantly different from all the other concentrations except 0.40 ml/L. The recommended rate of 0.6 ml/L only achieved 55% mortality during the 24 h period, which is less than the required 90% mortality. Concentrations of 0.7 and 0.8 ml/L were not significantly different in egg mortality at 24 h period (Table 8). These two concentrations had more than 90% egg mortality and this was significantly higher than the

mortality achieved at the label rate of 0.6 ml/L. When the concentrations were compared at 48 h period of exposure, control mortality was significantly different from all the other concentrations except 0.40 ml/L. The recommended rate of 0.6 ml/L achieved 63.33% mortality, which was less than the required 90% egg mortality during the 48 h period. Concentrations above the recommended rate of 0.6 ml/L (0.7 and 0.8 ml/L) achieved mortalities of 100% during the 48 h period, and these were not significantly different (Table 8). When assessment was done following 72 h exposure, control mortality (18.33%) was not significantly different from the 25% mortality achieved at 0.40 m/L. The recommended rate of 0.6 ml/L achieved 83.33% CSM egg mortality during the 72 h period, which is less than the required 90% mortality. Concentrations of 0.7 and 0.8 ml/L achieved 100% egg mortality, and these were not significantly different (Table 8). When comparing the concentrations at different periods of exposure it has been shown that mortalities achieved by the control were not significantly different from each other ($F_{10, 34} = 4.77$; P=0.0003). When comparisons were done at the concentration rate of 0.4 ml/L, the mortality of 13.33% achieved at 24 h exposure was not significantly different from mortalities achieved at 48 h (16.67%) and 72 h (25%) (Table 8). When comparing the recommended rate of abamectin (0.6 ml/L) at different periods of exposure, it has been shown that the mortality of 55% achieved at 24 h exposure was not significantly different from mortalities achieved at 48 h (63.33%) and 72 h (83.33%) exposure periods. These were less than the required 90% egg mortality. The concentration of 0.7 ml/L was able to cause 96.67% mortality at the 24 h period which was not significantly different ($F_{10, 34} = 4.77$; P=0.0003) from the 100% egg mortalities achieved at the 48 and 72 h periods of exposure (Table 8).

Table 8. The effect of Abamectin concentrations and duration of exposure on CSM egg mortality

			Means ± SE			
Period of application	Control	0.40 ml/L	0.5 ml/L	0.6 ml/L	0.7 ml/L	0.8 ml/L
24 h	$5.00^{dB} \pm 2.89$	$13.33^{dcB} \pm 1.67$	$30.00^{cC} \pm 2.89$	$55.00^{bB} \pm 7.64$	$96.67^{aA} \pm 3.33$	$100.00^{aA} \pm 0.00$
48 h	$11.67^{dAB} \pm 6.01$	$16.67^{dB} \pm 3.33$	$48.33^{cB} \pm 1.67$	$63.33^{bAB} \pm 3.33$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
72 h	$18.33^{dA} \pm 6.67$	$25.00^{dA} \pm 2.89$	$60.00^{cA} \pm 2.89$	$83.33^{bA} \pm 3.33$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$

** Means followed by the same small letter within a row are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)
** Means followed by the same capital letter within a column are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)

4.3.8 The effect of methomyl concentrations and duration of exposure on CSM egg mortality

Table 9 shows the effect of methomyl concentrations and duration of exposure on CSM egg mortality. This study showed that concentration and period of exposure interactions were significantly different ($F_{10,\ 34}$ =14.68; P=0.0001). When comparing the different concentrations at 24 h period of exposure it has been shown that control mortality (15.00%) was significantly different from all the other concentrations (Table 9). The recommended rate of 0.50 g/L achieved 86.67% egg mortality during the 24 h period, which is not significantly different ($F_{10,\ 34}$ = 14.68; P=0.0001) from the 100% mortalities achieved by 0.60 and 0.70 g/L concentrations. When the concentrations were compared at 48 h period of exposure, control mortality was significantly different from all the other concentrations ($F_{10,\ 34}$ = 14.68; P=0.0001). The recommended rate of 0.50 g/L achieved 96.67% egg mortality, which was not significantly different from the 100% egg mortalities achieved by the 0.6 and 0.7 g/L during the 48 h assessment period (Table 9). When assessment was done following 72 h exposure, control mortality (20.00%) was significantly different from all the other concentrations ($F_{10,\ 34}$ = 14.68; P=0.0001). The recommended rate of 0.50 g/L achieved 96.67%CSM egg mortality during the 72 h period, which is similar to mortalities achieved by the two higher concentrations of 0.6 and 0.7 g/L. Concentrations of 0.7 and 0.8 ml/L achieved 100% egg mortality, and these were not significantly different (Table 9). When comparing the concentrations at different periods of exposure it has been shown that mortality achieved by the control at 24 h was not significantly different ($F_{10,\ 34}$ = 14.68; P=0.0001) from the mortality achieved after 48 h (Table 9). However, mortality achieved by the control at 24 h period were not similar to mortality after 72 h ($F_{10,\ 34}$ = 14.68; P=0.0001). When comparisons were done at the concentration rate of 0.30 g/L, the mortality of 21.67% achieved at 24 h exposure was not significantly different from mortalities achieved at 48 h (35.00%) and 72 h (48.33%) (Table 9).

When comparing the recommended rate of methomyl (0.50 g/L) at different periods of exposure, it has been shown that the mortality of 86.67% achieved at 24 h exposure was not significantly different from 96.67% mortalities achieved at 48 and 72 h exposure periods. The concentration of 0.60 g/L was able to cause 100% mortality at the 24 h period which was not significantly different ($F_{10, 34}$ = 14.68; P=0.0001) from the 100% egg mortalities achieved at the 48 and 72 h periods of exposure (Table 9).

Table 9. The effect of Methomyl concentrations and duration of exposure on CSM egg mortality

	Means ± SE					
Period of application	Control	0.30 g/L	0.40 g/L	0.50 g/L	0.60 g/L	0.70 g/L
24 h	$5.00^{dB} \pm 2.89$	$21.67^{cC} \pm 1.67$	$41.67^{bB} \pm 3.33$	$86.67^{aA} \pm 7.26$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
48 h	$15.00^{dAB} \pm 0.00$	$35.00^{cB} \pm 2.89$	$78.33^{bA} \pm 3.33$	$96.67^{aA} \pm 3.33$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
72 h	$20.00^{dA} \pm 2.89$	$48.33^{cA} \pm 1.67$	$86.67^{bA} \pm 2.89$	$96.67^{aA} \pm 3.33$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$

** Means followed by the same small letter within a row are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)
** Means followed by the same capital letter within a column are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)

4.3.9 The effect of chlorfenapyr concentrations and duration of exposure on CSM egg mortality

Table 10 shows the effect of chlorfenapyr concentrations and duration of exposure on CSM egg mortality. This study showed that concentration and period of exposure interactions were significantly different ($F_{10, 34}$ = 1.84; P=0.09). When comparing the different concentrations at 24 h period of exposure it has been shown that control mortality was significantly different from all the other concentrations. The recommended rate of 0.40 ml/L achieved 85.00% egg mortality during the 24 h period, which is not significantly different from the 93.33% mortality achieved at the higher concentration of 0.50 ml/L during the same period. Concentrations of 0.50 and 0.60 ml/L achieved 93.33 and 100% egg mortality during the 24 h assessment period ($F_{10, 34}$ = 1.84; P=0.09) (Table 10). When the concentrations were compared at the 48 h period of exposure, control mortality was significantly different from all the other concentrations. The recommended rate of 0.40 ml/L achieved 93.33% egg mortality, which was not significantly different from the 100% egg mortality achieved by higher concentrations of 0.50 and 0.6 ml/L during the same period (Table 10). When assessment was done following 72 h exposure, control mortality was significantly different from mortalities achieved at all other concentrations. The recommended rate of 0.40 ml/L achieved 96.67% CSM egg mortality during the 72 h period, which is similar to 100% mortalities achieved at higher concentrations of 0.50 and 0.6 ml/L during the same period (Table 10). When comparing the concentrations at different periods of exposure it has been revealed that mortalities achieved by the control treatment at 24 h were significantly different from mortality achieved at 72 h ($F_{10, 34}$ = 1.84; P=0.09). When comparisons were done at the concentration rate of 0.30 ml/L, the mortality of 78.33% achieved at 24 h exposure was not significantly different from mortalities achieved at 48 h (83.33%) (Table 10). The concentration of 0.30 ml/L achieved 96.67%

mortality at 72 h. When comparing the recommended rate of chlorfenapyr (0.40 ml/L) at different periods of exposure, it has been shown that the mortality of 85.00% achieved at 24 h exposure was not significantly different ($F_{10, 34} = 1.84$; P=0.09) from mortalities achieved at 48 h (93.33%) and 72 h (96.67%) exposure periods. The concentration of 0.50 ml/L was able to cause 93.33% mortality at the 24 h period which was not significantly different ($F_{10, 34} = 1.84$; P=0.09) from the 100% egg mortalities achieved at the 48 and 72 h periods of exposure (Table 10).

Table 10. The effect of Chlorfenapyr concentrations and duration of exposure on CSM egg mortality

	Means ± SE					
Period of application	Control	0.20 ml/L	0.30 ml/L	0.40 ml/L	0.50 ml/L	0.60 ml/L
24 h	$11.67^{dB} \pm 1.67$	$36.67^{cB} \pm 3.33$	$78.33^{bA} \pm 3.33$	$85.00^{abA} \pm 5.00$	$93.33^{abA} \pm 6.67$	$100.00^{aA} \pm 0.00$
48 h	$11.67^{dB} \pm 1.67$	$43.33^{cAB} \pm 3.33$	$83.33^{bA} \pm 4.41$	$93.33^{abA} \pm 4.41$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
72 h	$20.00^{cA} \pm 2.89$	$56.67^{bA} \pm 3.33$	$96.67^{aA} \pm 3.33$	$96.67^{aA} \pm 3.33$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$

** Means followed by the same small letter within a row are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)
** Means followed by the same capital letter within a column are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)

4.3.10 The effect of abamectin dosages and duration of exposure on adult CSM mortality

Table 11 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 0.40ml/L and 0.5ml/L dosages. The recommended rate of 0.6ml/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 0.7ml/L (53.33%) and 0.8ml/L (83.33%) were not significantly different during the 24 h period (Table 11). 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 0.40ml/L. The recommended rate of 0.6ml/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 0.6ml/L (0.7ml/L and 0.8ml/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 11). When assessment was done 72 h after treatment, control mortality (3.33%) was not significantly different from the 23.33% mortality achieved at 0.40m/L. The recommended rate of 0.6ml/L achieved 73.33% CSM mortality during the 72 h period, which is less than the required 90% mortality. Concentrations of 0.7ml/L and 0.8ml/L achieved 93.33% and 100% mortality respectively, and these were not significantly different($F_{15, 46}$ = 2.71; P=0.048) (Table 11). When assessment was done 96 h after treatment, control mortality was significantly different from the 36.67% mortality achieved at 0.40m/L. The recommended rate of 0.6ml/L achieved 76.67% mortality during the 96 h period, which is less than the required 90% mortality. Dosages

of 0.7ml/L and 0.8ml/L both achieved 100% and 100% mortality, and these were not significantly different ($F_{15, 46}$ = 2.71; P=0.048) (Table 11).

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 0.4ml/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 11). The mortality achieved at the 0.5ml/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 0.5ml/L dosage after 96 h. When comparing the recommended label rate of abamectin (0.6ml/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 at the 48 h. The mortality of 93.33% achieved by the 07ml/L dosage after 72 h was not significantly different from the 100% mortality achieved by the same dosage after 96 h (Table 11). At the 0.8ml/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 11. The effect of Abamectin dosages and duration of exposure on adult CSM 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	$0.00^{cA} \pm 0.00$	$6.67^{cC} \pm 2.24$	$26.67^{bcB} \pm 1.12$	$43.33^{bC} \pm 0.88$	$53.33^{baB} \pm 2.09$	$83.33^{aA} \pm 3.16$
48 h	$0.00^{dA} \pm 0.00$	$13.33^{cdB} \pm 3.16$	$30.00^{cB} \pm 1.83$	$60.00^{bB} \pm 0.00$	$86.67^{aAB} \pm 1.24$	$100.00^{aA} \pm 0.00$
72 h	$3.33^{dA} \pm 3.16$	$23.33^{cdB} \pm 1.20$	$40.00^{cB} \pm 1.58$	$73.33^{bA} \pm 0.67$	$93.33^{abA} \pm 1.20$	$100.00^{aA} \pm 0.00$
96 h	$1.33^{dA} \pm 0.50$	$36.67^{cA} \pm 0.95$	$60.00^{bA} \pm 2.24$	$76.67^{bA} \pm 0.66$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$

** Means followed by the same small letter within a row are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)
** Means followed by the same capital letter within a column are not significantly different, P≤0.05 (Tukey's Honestly significant difference test)

4.3.11 The effect of Methomyl dosages and duration of exposure on adult CSM mortality

Table 12 shows the effect of Methomyl dosages and time after treatment on adult CSM mortality. The results revealed that dosage and time after application interactions were significantly different ($F_{15, 46}$ = 3.98; P=0.0001). When comparing the different dosages 24 h after application it has been shown that control mortality (0.33%) was significantly different from all the other dosages except the 20% mortality achieved at 0.30 m/L (Table 12). The recommended rate of 0.50g/L achieved 76.67% mortality during the 24 h period, which is not significantly different ($F_{15, 46}$ = 3.98; P=0.0001) from the 86.67% mortality achieved by 0.60g/L during the same period. The CSM mortality achieved at 0.6g/L (86.67%) was not significantly different from the 100% mortality achieved by the 0.7g/L dosage during the 24 h period. When the dosages were compared 48 h after treatment, control mortality was significantly different from all the other dosages ($F_{15, 46}$ = 3.98; P=0.0001). The recommended rate of 0.50g/L achieved 86.67% mortality, which was not significantly different from the 93.33 % and 100% mortalities achieved by the 0.6g/L and 0.7g/L during the 48 h assessment period ($F_{15, 46}$ = 3.98; P=0.0001) (Table 12). When assessment was done following 72 h after treatment, control mortality (1.33%) was significantly different ($F_{15, 46}$ = 3.98; P=0.0001) from all the other dosages. The recommended rate of 0.50g/L achieved 96.67% mortality during the 72 h period, which is similar to 100% mortalities achieved by the two higher dosage levels of 0.6g/L and 0.7g/L. dosages of 0.7ml/L and 0.8ml/L achieved 100% egg mortality, and these were not significantly different (Table 12). When assessment was done following 96 h after treatment, control mortality (16.67%) was significantly different ($F_{15, 46}$ = 3.98; P=0.0001) from all the other dosages. The 93.33% mortality achieved by the 0.40g/L dosage during the 96 h period was similar to 100% mortalities achieved by the recommended dosage (0.50g/L), 0.6g/L and 0.7g/L during the same period. The recommended dosage achieved 100% mortality during 96

h after treatment which was not significantly different from mortalities achieved by higher dosages. Dosages of 0.7ml/L and 0.8ml/L achieved 100% egg mortality, and these were not significantly different (Table 12).

When comparing the dosages at different times after treatment it has been revealed that 0.33% mortality achieved by the control at 24 h was significantly different ($F_{15, 46} = 3.98$; P=0.0001) from the 3.33% mortality achieved after 48 h (Table 12). When comparisons were done at the concentration rate of 0.30g/L, the mortality of 20.00% achieved at 24 h exposure was significantly different from mortalities achieved at 48 h (36.67%), which was also significantly different ($F_{15, 46} = 3.98$; P=0.0001) from mortality achieved after 72 h (43.33%) and 96 h (53.33%) (Table 12). When comparing the recommended dosage of methomyl (0.50g/L) at different times after treatment, it has been shown that the mortality of 76.67% achieved after 24 h was not significantly different ($F_{15, 46} = 3.98$; P=0.0001) from 86.67, 96.67 and 100.00% mortalities achieved at 48, 72 and 96 h respectively. The dosage of 0.60 g/L caused 86.67% mortality at the 24 h period which was not significantly different ($F_{15, 46} = 3.98$; P=0.0001) from the 93.33 100.00 and 100.00% mortalities achieved at the 48, 72 and 96 h periods of assessment (Table 12).

Table 12. The effect of Methomyl dosages and duration of exposure on adult CSM mortality

	Means ± SE					
Time after application	Control	0.30 g/L	0.40 g/L	0.50 g/L	0.60 g/L	0.70 g/L
24 h	$0.33^{dB} \pm 1.00$	$20.00^{dC} \pm 2.24$	$53.33^{cC} \pm 0.79$	$76.67^{bA} \pm 0.66$	$86.67^{abA} \pm 1.64$	$100.00^{aA} \pm 0.00$
48 h	$3.33^{dA} \pm 3.16$	$36.67^{cB} \pm 0.95$	$70.00^{bB} \pm 1.20$	$86.67^{abAB} \pm 1.24$	$93.33^{aA} \pm 0.60$	$100.00^{aA} \pm 0.00$
72 h	$1.33^{dB} \pm 0.50$	$43.33^{cA} \pm 0.88$	$86.67^{bA} \pm 0.62$	$96.67^{abA} \pm 0.59$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
96 h	$16.67^{cA} \pm 1.41$	$53.33^{bA} \pm 0.79$	$93.33^{aA} \pm 0.60$	$100.00^{aA} \pm 0.00$	100.00aA ± 0.00	$100.00^{aA} \pm 0.00$

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

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

4.3.12 The effect of Chlorfenapyr dosages and duration of exposure on adult CSM mortality

Table 13 shows the effect of chlorfenapyr dosages and time after treatment on CSM mortality. The results of this study revealed that dosages and time after treatment interactions were significantly different ($F_{15, 46} = 8.73$; P = 0.0001). When comparing the different dosages 24 h after treatment it has been shown that control mortality was significantly different from all the other dosages except 0.20ml/L (10.00%). The recommended rate of 0.40ml/L achieved 56.67% mortality during the 24 h period, which is not significantly different ($F_{15, 46} = 8.73$; P = 0.0001) from the 70.00% mortality achieved at the higher dosage of 0.50 ml/L during the same period. Dosages of 0.50ml/L and 0.60 ml/L achieved 70.0% and 83.33% mortality during the 24 h assessment period (Table 13). When the dosages were compared 48 h after treatment, the recommended dosage of 0.40 ml/L achieved 60.00% mortality, which was significantly different ($F_{15, 46} = 8.73$; P = 0.0001) from the 80.00% mortality achieved by higher dosage of 0.50ml/L during the same period (Table 13). The mortality level of 80.00% achieved by the dosage of 0.50ml/L 48 h after treatment was significantly different from the 100.00% mortality achieved by 0.60ml/L dosage during the same period. When assessment was done following 72 h exposure, control mortality was significantly different ($F_{15, 46} = 8.73$; P = 0.0001) from mortalities achieved at all other dosages. The recommended rate of 0.40 ml/L achieved 90.00% CSM mortality during the 72 h period, which is similar ($F_{15, 46} = 8.73$; P = 0.0001) to 100% mortalities achieved at higher concentrations of 0.50 ml/L and 0.6 ml/L during the same period (Table 13).

When comparing the dosages at different times after treatment it has been revealed that mortalities achieved by the control treatment at 48 h were not significantly different ($F_{15, 46} = 8.73$; P = 0.0001) from mortality achieved 72 and 96 h after treatment. When comparisons were done at the dosage rate of 0.30ml/L, the mortality of 33.33% achieved at 24 h exposure was not significantly different

from mortality achieved after 48 h. The mortalities level of 50.00% achieved by 0.30ml/L chlorfenpyr after 48 h was not significantly different from 66.67% mortality achieved by the same dosage after 72 h (Table 13). The dosage of 0.30ml/L achieved 93.33% mortality 96 h after treatment. When comparing the recommended rate of chlorfenapyr (0.40ml/L) at different times after treatment, it has been shown that the mortality of 56.67% achieved 24 h after treatment was not significantly different ($F_{15, 46} = 8.73$; $P = 0.0001$) from mortality achieved at 48 h (60.00%). The dosage of 0.40ml/L was able to cause 90.00% and 93.33% mortality 72 and 96h respectively. The dosage of 0.50 ml/L caused 70.00% mortality at the 24 h period which was not significantly different ($F_{15, 46} = 8.73$; $P = 0.0001$) from the 80.00% mortalities achieved after 48 h (Table 13). At the 72 and 96 h period after treatment, the dosage level of 0.50ml/L was able to cause 100.00%, which were not significantly different ($F_{15, 46} = 8.73$; $P = 0.0001$). When comparisons were done at the dosage rate of 0.60ml/L, the mortality of 83.33% achieved 24 h after treatment was not significantly different from the 100.00% mortality achieved after 48 h. The dosage level of 0.60ml/L achieved 100.00% mortality at 48, 72 and 96 h after treatment and these were not significantly different ($F_{15, 46} = 8.73$; $P = 0.0001$) (Table 13).

Table 13. The effect of Chlorfenapyr dosages and duration of exposure on adult CSM 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	$0.00^{dB} \pm 0.00$	$10.00^{dB} \pm 3.16$	$33.33^{cC} \pm 1.00$	$56.67^{bB} \pm 0.77$	$70.00^{abB} \pm 0.00$	$83.33^{a} \pm 0.63$
48 h	$0.67^{eAB} \pm 0.71$	$20.00^{dB} \pm 2.24$	$50.00^{cCB} \pm 0.00$	$60.00^{cB} \pm 0.00$	$80.00^{bB} \pm 1.12$	$100.00^{aB} \pm 0.00$
72 h	$1.33^{dA} \pm 0.50$	$30.00^{cA} \pm 1.83$	$66.67^{bB} \pm 1.41$	$90.00^{aA} \pm 1.05$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$
96 h	$1.33^{cA} \pm 0.50$	$46.67^{bA} \pm 0.85$	$93.33^{aA} \pm 0.60$	$93.33^{aA} \pm 0.60$	$100.00^{aA} \pm 0.00$	$100.00^{aA} \pm 0.00$

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

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

4.4 DISCUSSION

4.4.1 Toxicity of different abamectin dosages to CSM eggs

CSM eggs can only acquire the lethal dose of abamectin through contact with the pesticide material. Even though, generally, abamectin was effective on eggs, its effectiveness was affected by dosage. Higher concentrations, above recommended 0.60 ml/L of abamectin are required to achieve effective control on eggs under laboratory conditions in Botswana. This has been revealed when higher doses than the recommended dose of abamectin were required to achieve 90% (LD_{90}= 0.69 ml/L), 96.67 (0.70 ml/L) and 100% (0.80 ml/L) egg mortality in 24 h. In addition, the mortality level of 83.33% was achieved by the recommended dose during the 72 h study period (Figure 8A-C and Table 8). The probit line slopes show that abamectin became more lethal to CSM eggs with each increase in pesticide concentration. Since the recommended dosage of abamectin only achieved 83.33% CSM egg mortality during the assessment period, it suggests that the eggs have lowered sensitivity to the pesticide. The explanation to this loss of sensitivity has to be explored further. Some researchers have stated that abamectin is effective on eggs when the embryo is full-grown, that is when the newly formed larvae are visible through the chorion of treated eggs (Schuster and Everett, 1983). This implies that abamectin has little or no effect on the early stages of embryo development. Generally, abamectin at the recommended dosage required exposure periods longer than 72 h to cause 90 to 100% CSM egg mortality as demonstrated in this research. There are several reports on the effect of abamectin on the eggs of spider mites. Ismail *et al*. (2007) reported that abamectin had no effect on eggs of the TSSM at different dosages. Massoud *et al*. (2018) demonstrated that abamectin was highly effective against adult TSSM on strawberry.

4.4.2 Toxicity of different abamectin dosages to CSM adults

From the results in Figures 11 (A - D) and Table 11 several annotations have been made: when time after treatment increased, higher dosages than the recommended of abamectin were able to cause 90 to 100% adult mortality; when LD_{90}s are used alone to assess the effectiveness of abamectin, the mortality level of 76.67% caused by the recommended dose during the 96 hours study period is too low to achieve effective control. The slopes of the probit lines in Figure 11 (A - D) show that abamectin became more toxic to CSM with each increase in pesticide dosage. These results are similar to those by Kavallieratos (2009) and Mwandila *et al*. (2013) where increase in dosage enhanced the efficacy of abamectin. The results that the recommended dosage did not achieve 80% CSM adult mortality during the assessment period indicate that higher dosages are required to achieve effective control under Botswana conditions. CSM adults can acquire the lethal dose of abamectin through contact and ingestion of the pesticide material as they feed. This corroborates findings by Sparks *et al*. (1998) who reported that abamectin was a neurotoxin with a contact mode of action. Abamectin is known to cause partial paralysis of the insect nervous system, which can reduce food consumption, eventually leading to death (Moscardini *et al*. 2013). Therefore abamectin does not need to achieve high levels of mortality to achieve effective control since the mite may be alive but unable to feed on the host consequently protecting the plant from damage. Therefore LD_{90} values alone do not provide sufficient indication of the effectiveness of a pesticide with a stomach mode of action like abamectin against CSM motile stages. This mode action of abamectin is a desirable property as this is the damaging developmental stage of the pest.

4.4.3 Toxicity of different methomyl dosages to CSM eggs

Ovicidal activity of methomyl has been demonstrated against eggs of several pest species such as cabbage looper, *Trichoplusia ni* (Hübner, 1803) (Chalfant *et al*., 1979), tobacco budworm, *Heliothis virescens* Fabricius 1777 (Pitts and Peters, 1980), and budworm, *Helicoverpa punctiger* (Wallengren, 1860) (Wiate, 1981). However, since it does not have fumigant activity, the eggs have to be covered by the pesticide for it to be effective. Therefore, CSM eggs can only acquire the lethal dose through direct hits or contact with the pesticide material on the leaf surface. The recommended dose of methomyl was able to cause 86.67% egg mortality in 24 h. In addition, the mortality level of 96.67% caused by the recommended dose (0.50 g/L) during the 48 and 72 h study period, appears to be sufficient to achieve effective control of CSM eggs (Figure 9A-C and Table 9). The probit line slopes showed that methomyl became more lethal to CSM eggs with each increase in pesticide concentration. Similar observations were recorded by Silva *et al*. (2018) who stated that methomyl, at the recommended doses, exhibited high toxicity against the eggs of *Neoleucinodes elegantalis* (Guenée, 1854) with higher than 80% of mortality. This pesticide has embryo toxic activity, causing malformations in neonates of *Daphnia obtusa* Kürz (1874) suggesting that it can be used to inhibit reproduction in spider mites therefore reducing outbreaks (Gaete *et al*., 2013). The high egg mortality achieved with methomyl means that buildup of adult populations from hatching eggs would be reduced, thereby minimizing subsequent damage to the host plants. Therefore, when using methomyl against CSM, the egg stage is a suitable stage to target.

4.4.4 Toxicity of different methomyl dosages to CSM adults

From the results in 12 (A - D) and Table 12 several observations can be made: when time after treatment increased, lower dosages of methomyl were able to cause 90 to 100% adult mortality; when LD_{90}s are used alone to assess the effectiveness of methomyl, the mortality level of 75.11% caused by the recommended dosage during the 96 hours study period, appears to be too low to achieve effective control. The slopes of the probit lines in 12 (A - D) show that methomyl became more toxic to CSM with each increase in pesticide dosage. Similar results were obtained by Afzal *et al*. (2000) where better control of cotton whitefly (*Bemisia tabaci* Genn.) was observed at higher dosages of methomyl. CSM adults can acquire the lethal dose through contact with the pesticide material as they move on the leaf surface. Methomyl has been reported to be effective though direct contact and also as a systemic (Pohanish 2015). This may explain the relatively faster mortality of CSM adults. Gaete *et al*. (2013) observed that this pesticide has ebryotoxic activity, causing malformations in neonates of *D. obtusa* suggesting that it can be used to inhibit reproduction in spider mites therefore reducing outbreaks.

4.4.5 Toxicity of different chlorfenapyr dosages to CSM eggs

Chlorfenapyr is a slow acting pesticide and belongs to the pyrrole group. The suggested mechanism for chlorfenapyr metabolism is conversion of the pro-insecticide chlorfenapyr to toxic form CL30328 by monooxygenases and this toxic form inhibits ATP synthesis in the mitochondria leading to inhibition of oxidative phosphorylation and resulting in the effect on CSM eggs (embryo in eggs) (Verma *et al*., 2015). The study showed that doses lower than the recommended dosage of chlorfenapyr achieved 90 to 100% egg mortality during the study period (Figure 10A-C and Table 10). This suggests that chlorfenapyr is highly effective against CSM eggs. Ullah and Gotoh (2013) reported that eggs of spider mites, *Tetranychus macfarlanei* and *Tetranychus truncatus* were highly susceptible to chlorfenapyr at LD_{50} level; therefore, the results were expected. CSM

egg mortalities were probably due to direct hits or contact with the active ingredient on the leaf surface. When LD_{90}s are used alone to assess the effectiveness of chlorfenapyr, the mortality level caused by the recommended dose during the 72 h study period, appears to be sufficient to achieve effective control. This is in agreement with Amjad *et al*. (2012) where chlorfenapyr was highly effective against *T. urticae* compared to dicofol, fenpyroximate, azocyclotin, propergite and pyrabiden. However, LD_{90} values alone do not provide sufficient indication of the effectiveness of chlorfenapyr against CSM eggs. The slopes of the probit lines in Figure 10A-C shows that chlorfenapyr became more toxic to CSM eggs with each increase in pesticide concentration. These results are similar to those by Amjad *et al*. (2012) where mortality of *T. urticae* also depended on the concentration of chlorfenapyr used. The action of chlorfenapyr against eggs is a desirable property since the buildup of pest populations from hatching eggs would be reduced thereby minimizing subsequent damage to tomato plants. When applying chlorfenapyr to control CSM, the egg stage would therefore be a suitable stage to target. This is a desirable property since it affects reproduction of the mites consequently preventing pest outbreaks. Chlorfenapyr has been shown to have little or no toxicity to beneficial insects therefore suitable for use in integrated pest management programs (IPM).

4.4.6 Toxicity of different chlorfenapyr dosages to CSM adults

From the results in 13 (A - D) and Table 13 several observations can be made: when time after treatment increased, lower doses of chlorfenapyr were able to cause 90 to 100% adult mite mortality; when LD_{90}s are used alone to assess the effectiveness of chlorfenapyr, the mortality level caused by the recommended dose during the 96 hours study period, appears to be sufficient to achieve effective control. This is in agreement with Amjad *et al*. (2012) where chlorfenapyr caused the highest mortality of *Tetranychus urticae* compared to dicofol, fenpyroximate,

azocyclotin, propergite and pyrabiden. LD_{90} values alone do not provide sufficient indication of the effectiveness of chlorfenapyr against CSM adults.

The slopes of the probit lines in 13 (A - D) show that chlorfenapyr became more toxic to CSM adults with each increase in pesticide dosage. These results are similar to those by Amjad *et al*. (2012) where mortality of *T. urticae* also depended on the dosage of chlorfenapyr used. CSM adults can acquire the lethal dose through contact and ingestion of the pesticide material as they feed. N'Guessan *et al*. (2007) and Oxborough *et al*. (2015) also reported that pyrroles act at the cellular level and disrupt respiratory pathways and proton gradients through the uncoupling of oxidative phosphorylation in mitochondria. Because of its unique mode of action, chlorfenapyr does not show any cross resistance to mechanisms that confer resistance to standard neurotoxic pesticides. The fast action of chlorfenapyr against adults is a desirable property as this is the damaging developmental stage of the pest. Chlorfenapyr has also been shown to possess ovicidal properties which are desirable since it affects reproduction of the mites.

4.5 CONCLUSIONS

Farmers apply pesticides against crop pests at the recommended dosage to ensure production of high amounts of good quality crop yields using minimum amounts of active ingredient. In this study chlorfenapyr was the most effective followed by methomyl and then abamectin when applied on CSM eggs. Chlorfenapyr was able to achieve 90% mortality faster, followed by methomyl and then abamectin. Chlorfenapyr was also able to achieve 90% egg mortality at a dosage lower than the label rate. Therefore, chlorfenapyr offers the most effective and timely control of CSM eggs. The results also suggest that dosages below the label rate can be applied without seriously affecting the level of protection of the crop especially when targeting the egg stage. The results also show

that the recommended rate of abamectin did not achieve high egg mortality throughout the duration of the study. However, it was found to achieve effective control at dosages above the recommended rate. This means that higher dosages of abamectin are required for effective control of CSM eggs.

Chlorfenapyr and methomyl proved to be very effective miticides compared to abamectin when applied against adult spider mites. Methomyl and chlorfenapyr were both able to achieve high adult mortality at the recommended rates whilst abamectin did not for the duration of the bioassay. They were also able to achieve high adult mortality dosages lower than the recommended. Therefore, methomyl and chlorfenapyr offer the most effective and timely control of CSM adults. The results also indicate that methomyl and chlorfenapyr can be applied at lower dosages and still achieve the same level of protection of the crop. Abamectin was found to achieve effective control at dosages only above the recommended rate of application. Therefore, higher dosages of abamectin would be required to achieve effective control of CSM adults comparable to the other two pesticides. However, an increase in dosage of abamectin may be undesirable from an economic viewpoint, and may not receive much practical adoption amongst resource poor farmers. Additionally, this would expose farmers to the harmful effects of the pesticide. It can also be concluded from the findings of this study that abamectin can offer adequate control of CSM and avoid serious damage to tomato plants if long durations of exposure are allowed.

4.6 RECOMMENDATIONS

The current findings demonstrated that the three products are effective for the control of CSM eggs and adults. It can be recommended that chlorfenapyr and methomyl be used to target the eggs before they hatch in order to avoid any damage by subsequent damaging stages. Chlorfenapyr and

methomyl were also more effective against adults than abamectin. Abamectin was found to offer effective control of CSM and prevents serious damage to tomato plants following long exposure periods. Farmers can be advised to allow for longer exposure periods to achieve required results when using abamectin. The CSM population in this study did not show any signs of methomyl and chlorfenapyr resistance, therefore it can be recommended that methomyl and chlorfenapyr use be continued. Furthermore, lower dosages need to be evaluated to confirm their effectiveness in the protection of tomato plants. Reduction in dosage would result in reduction in the cost of controlling CSM by farmers. The results of the study also suggest that the three pesticides can be used in a CSM management rotation program and thereby avoid the selection of resistant CSM populations by using only one product. More detailed studies and field testing are necessary to confirm these laboratory findings and determine the sources of variation in effectiveness of the tested materials.

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CHAPTER 5 - ECONOMIC INJURY LEVELS AND YIELD LOSS ASSESSMENT FOR CARMINE SPIDER MITE *TETRANYCHUS CINNABARINUS* BOISDUVAL (ACARI: TETRANYCHIDAE) ON TOMATO (*SOLANUM LYCOPERSICUM*) UNDER GREENHOUSE CONDITIONS

ABSTRACT

A study was undertaken over two cropping seasons, 2018/2019 and 2019/2020, to determine the economic injury level for carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (CSM) on tomato, *Solanum lycopersicum* in Botswana. Tomato plants were infested with adult CSM for durations of 0 (no exposure), 1, 2, 3, 4, 5, 6 and 7 weeks (complete exposure). The corresponding treatments were 7, 6, 5, 4, 3, 2, 1 and 0 sprays with Abamectin. The results showed a significant reduction in the number of spider mites per plant as the frequency of spraying increased. An inverse relationship between spider mite exposure and yield was also observed following three weeks exposure. In this study, tomato yield per hectare decreased as spider mite populations increased and ranged from 5.19 tonnes ha^{-1} at the lowest spider mite population to 7.32 tonnes ha^{-1} at the highest population during the 2018/19 and from 4.32 tonnes ha^{-1} at the lowest spider mite

population to 6.93 tonnes ha^{-1} at the highest spider mite population in 2019/20. Yield loss increased to more than 50% when the pesticide was not applied. The gain threshold increased with frequency of spraying, ranging from 0.087 to 0.606 in 2018/19 and 0.060 to 0.420 in 2019/20. However, an increase in spraying frequency resulted in an increase in cost of protection from BWP 780 to BWP 5,460 in both 2018/19 and 2019/20 seasons. The results indicate that the application of pesticide at lower pest population will have less gain threshold and, therefore, be uneconomical. An increase in spray frequency reduced the level of exposure of host plants to spider mites by reducing their densities. Maximum possible yield loss was observed when complete exposure to spider mite feeding was allowed. The results also reveal that regardless of how high spider mite populations reached, the tomato plants were still able to produce minimum yield levels. The EILs are 136 and 182 spider mites per plant for pesticide application, every three to four weeks. The results obtained in this study will be useful in future spider mite resistance monitoring research. Economic decision levels are fundamental components of cost effective IPM programs and can be effective tools for making decisions about the application of pesticides against carmine spider mite in Botswana.

5.1 INTRODUCTION

Tomato (*Solanum lycopersicum var. lycopersicum*) is one of the most commonly grown vegetables in Botswana (Madisa *et al*., 2010) and is among the most widely consumed vegetable crops globally (Retta & Berhe, 2015; Ghaderi *et al*., 2019). It is a nutritionally well-balanced and dense food containing significant quantities of vitamin A and vitamin C, therefore contributing enormously to food security and nutrition (Brasesco *et al*., 2019; FAO, 2020). Tomato is a valuable product for smallholder farmers and large scale commercial producers, serving mainly as a commercial crop and grown in shade nets and open spaces in many parts of the country.

At only 60 -100 tonnes per hectare, tomato production and productivity in Botswana is low when compared to other tomato producing countries in Africa (Badimo, 2000). Egypt is the leading producer of tomato in Africa at 7 297 108 tonnes, followed by Nigeria (4,100 000t), Morocco (1, 293 761t), Tunisia (1,298 000t), Cameroon (1,279 853t), Algeria (1,286 286t) and South Africa (608 306t) (Dube *et al*., 2020). Among the major constraints to tomato production in Botswana invertebrate pests are frequently cited as the most serious (Madisa *et al*., 2010a; Baliyan, 2012). These include the cutworm (*Agrotis* spp.), whitefly (*Bemisia tabaci*), African bollworm (*Helicoverpa armigera*), tomato semi-looper (*Chrysodeixis acuta*), tomato leafminer (*Tuta absoluta*), and spider mites (Bok *et al*., 2006; Obopile *et al*., 2008; Munthali, 2009; Leungo *et al*., 2012). Spider mites are the most notorious family of phytophagous mites in the world (Cobanoglu

et al., 2015). Two sibling species, the two - spotted spider mite, *Tetranychus urticae* Koch and the carmine spider mite, *Tetranychus cinnabarinus* Boisduval are economically important pests of many vegetable species worldwide (Bi *et al*., 2016; Lu *et al*., 2018).They have been shown to affect tomatoes wherever they are grown (Bok *et al*., 2006; Obopile *et al*., 2008). The carmine spider mite (CSM), *Tetranychus cinnabarinus* (Boisduval, 1867) has been shown to attack tomato plants in almost all production systems in Botswana. CSM are polyphagous plant pests that feed on over 1100 plant species, constituting over 140 crop families (Migeon and Dorkeld, 2006 – 2017; Grbic *et al*., 2011). Its adults and juveniles typically feed on the underside of the leaves by inserting their stylets and sucking cell contents thereby damaging protective leaf surface, palisade layers and causing yellowing and curling of the leaves (Figure 14A). CSM spin thick webs that cover foliage (Fig. 14B), thereby impeding photosynthetic ability and transpiration of host plants. Heavy spider mite infestations result in stunted growth; delay in flowering and fruit set, and in severe cases, death of the plant (Kaimal & Ramani, 2011).

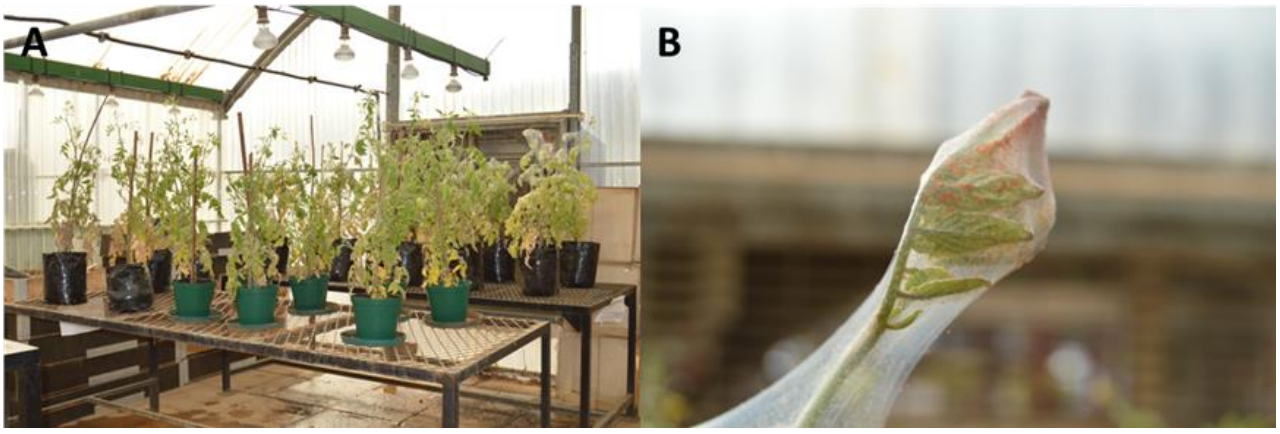

Figure 14. A greenhouse experiment; (**A)** tomato plants under heavy infestation by spider mites. (**B)** tomato leaves covered by heavy spider mite webbing. (Pic. Legwaila M. M.).

In Botswana, the majority of farmers depend on pesticides to control a medley of invertebrate pests affecting their crops (Obopile *et al*., 2008; Madisa *et al*., 2010a) and the decision to apply is mainly upon the sight of the pest on the crop (Munthali *et al*., 2004). The ease and speed of control provided by pesticides have promoted their widespread use, which is often followed by countless complications including development of resistance, toxic effects on animals, humans and beneficial fauna (Pimentel, 2009; Roditakis *et al*., 2017). Moreover, spider mites have a documented predisposition to rapidly evolve resistance to most pesticides employed for their control (Van Leeuwen *et al*., 2010; Grbic *et al*., 2011; Dermauw *et al*., 2013; Bu *et al*., 2015). Large volumes of active ingredients are repeatedly applied to crops thereby, over and above environmental damage and human health effects, increasing the cost of production to the farmer. Therefore, the effective use of pesticides requires that they be applied only when economic loss occurs to minimize the cost to the farmer and the effect on beneficial insects and the environment (Obopile, 2006). Pest management should therefore be based on proper economic decision making to ensure that proper amounts of pesticides are applied economically to control pests and avoid unnecessary wastage (Ghaderi *et al*., 2018). Determining the economic injury level (EIL) for spider mites is one of the fundamentals to the formulation of an integrated pest management (IPM) program for spider mites (Pedigo *et al*., 1986; Higley & Pedigo, 1993).

Considering the seriousness of the spider mite problem in Botswana, it is necessary to carry out studies to determine yield loss due to CSM and develop economic injury levels for this pest under local conditions. This will help farmers to minimize the use of pesticides since they will only be applied when necessary. Despite its importance, few or no research studies have been carried out to estimate the EILs for spider mites in Botswana. This study was therefore conducted to

investigate the relationship between tomato infestation and yield loss to CSM and consequently determine the EIL for CSM so that pesticide usage can be economically justified.

5.2 MATERIALS AND METHODS

The study was carried out in a greenhouse at the Botswana University of Agriculture and Natural Resources (BUAN) in Gaborone, Botswana (24º34'25" S, 25º95'0" E) at 30 ± 5ºC. The experiment was laid out in a randomized complete block design with 4 replicates. Each replicate consisted of 8 tomato plants. Tomato seedlings (var. Rodade) initially sown in seedling trays were transplanted into plastic pots (12 cm wide, 15 cm deep) filled with 1.5 kg garden soil mixed with potting soil and left to establish for a week before the commencing of the experiment. The pots were watered ad-lib and no fertilizer was added. The pots were kept free of weeds by manual weeding. The CSM colony used in this study was obtained from a commercial tomato producing farm in Metsimotlhabe just outside of Gaborone, identified using taxonomic keys in the Crop protection laboratory at BUAN and then reared in the greenhouse. Tomato seedlings were used to rear CSM and provide adequate plant material for reproduction.

At the beginning of the experiment, each seedling was infested with 4 pre-ovipositional adults using a fine brush. Seedlings were exposed to feeding by CSM for duration of 0 (no exposure), 1, 2, 3, 4, 5, 6 weeks and complete exposure (unsprayed control) following the procedure of Singh and Sachan (1997) and Obopile (2006). The corresponding treatments consisted of sequential

applications of Agromectin (abamectin 18 g/L emulsifiable concentrate, Arysta Lifescience, South Africa) at 6, 5, 4, 3, 2, 1 and 0 sprays at 1.2 L ha^{-1}.

5.2.1 Data collection and analysis

CSM population counts were conducted a day before each spray application on all plants per replicate. During harvest, all tomatoes from all treatments were weighed and yield data recorded. Fresh weight of tomatoes from each pot was expressed as yield in tonnes ha^{-1}. Data sets were transformed to stabilize the variance before analysis. Yield data were transformed to square root and percentage infestation subjected to arcsine transformation (Sokal & Rohlf, 1995), and spider mite counts to log (X+1) (Mosweu *et al*., 2015). Yield data and number of spider mites per plant in a treatment were used to calculate the linear regression,

$$Y = a - bx$$

where Y = potential yield, a = expected yield loss at zero level of infestation, b = regression coefficient or yield loss in tonnes ha^{-1} caused by one mite per plant, and x = number of mites per plant.

The economic injury levels for CSM were determined based on estimation of the gain-threshold (GT), defined in terms of tonnes ha^{-1} as suggested by Pedigo and Higley (1992).

Gain threshold was calculated for each treatment using the equation

$$\text{Gain threshold (GT)} = \frac{\text{Cost of protection (BWP/ha)}}{\text{Market value (BWP.tonne/ha)}}$$

The market price of Tomato in Botswana ranged from BWP 9, 000 per tonne in 2018/19 to BWP 13, 000 per tonne in 2019/20. The cost of insecticide applied by knapsack sprayer was on average BWP 780 per hectare for both 2018/19 and 2019/20 cropping seasons.

$$\text{Economic injury level (EIL)} = \frac{\text{Gain threshold } \left(\frac{\text{BWP}}{\text{ha}}\right)}{\text{b (regression coefficient)}}$$

Statistical analyses were performed using the SAS statistical software (version 9.4, SAS Institute, Cary, USA). Tukey's Honestly significant difference test (Zar 1984) was used to separate means.

5.3 RESULTS

5.3.1 Effect of exposure period and spray frequency on CSM population

During the 2018/19 season the number of CSM per plant varied significantly between exposure period and number of insecticide sprays ($F_{42, 165} = 20.18$; $P<0.0001$). The results in Table 14 revealed a significant reduction in spider mite population per plant as the frequency of spraying increased. The mean number of spider mites ranged from 0 where there was no exposure to 1134 where there was full exposure. Yield varied significantly among the exposure periods and also between the number of sprays. The mean yield ranged from 5.19 tonnes ha^{-1} where there was full exposure to 7.32 tonnes ha^{-1} where there was no exposure. The yield infestation regression equation was obtained as $Y = -0.0013x + 6.2531$ (Figure 15A). Regression analysis showed an inverse relationship between spider mite exposure and yield (Figure 16A). The gain threshold (GT), Economic injury levels (EIL) for CSM in respect of the different treatment modules are depicted in Table 14. The GT was computed on the basis of market price for tomato at BWP 9000/ ha and increased significantly with the number of spraying and ranged from 0.087 for one spray to 0.606 for 7 sprays. The EIL values ranged from as low as 66.92 for 1 spray to 466.15 for 7 sprays. There was also a direct relationship between the costs of protection, the gain threshold and economic injury level (Figures 17A & 18A). An increase in spray frequency resulted in cost of protection ranging from BWP 780.00 for one spray to BWP 5,460.00 in a period of 7 weeks.

During the 2019/20 season the results revealed a significant reduction in CSM population per plant as the frequency of spraying increased (Table 14) ($F_{42, 165} = 64.90$; $P<0.0001$). The average number of spider mites ranged from 0 where there was no exposure to 1188 where there was full exposure. Yield varied significantly among the exposure periods and also between the number of sprays and ranged from 4.32 tonnes ha^{-1} where there was full exposure to 6.93 tonnes ha^{-1} where there was no exposure (Table

14). Regression analysis showed an inverse relationship between spider mite exposure and yield (Figure 16B) and the yield infestation regression equation was obtained as $Y = -0.0011x + 5.2316$ (Figure 15B). The gain threshold (GT) computed on the basis of market price for tomato at BWP 13,000/ha increased significantly with the number of spraying and ranged from 0.060 for one spray to 0.420 for 7 sprays (no exposure). The corresponding values of EIL ranged from as low as 54.55 for 1 spray to 381.82 for 7 sprays (no exposure) (Table 14). There was also a direct relationship between the costs of protection, the gain threshold and economic injury level (Figure 17B & 18B). An increase in spray frequency also resulted in cost of protection from BWP 780.00 for one spray to BWP 5,460.00 in a period of 7 weeks.

Table 14. Infestation, yield and economic injury level for CSM on tomato at different durations of exposure

Mite exposure (weeks)	No. of sprays	Number of spider mites		Yield (tonnes ha^{-1})		Cost of Protection (BWP/ha)	Gain threshold (GT)		Economic injury level (EIL)	
		2018/19	2019/20	2018/19	2019/20		2018/19	2019/20	2018/19	2019/20
Full exposure	0	1134.00±41.86^{a}	1188.00±49.30^{a}	5.19^{bc}	4.32^{cd}	-	-	-	-	-
6	1	381.25±141.86^{b}	621.00±88.94^{b}	5.10^{bc}	4.26^{cd}	780.00	0.087	0.060	66.92	54.55
5	2	192.75±55.61^{bc}	292.50±85.50^{c}	4.98^{c}	4.14^{cd}	1,560.00	0.173	0.120	133.08	109.09
4	3	114.50±19.39^{c}	220.50±59.87^{cd}	4.86^{c}	4.05^{cd}	2,340.00	0.260	0.180	200.00	163.64
3	4	79.00±12.56^{c}	139.50±38.45^{cd}	6.33^{ab}	5.28^{bc}	3,120.00	0.347	0.240	266.92	218.18
2	5	12.00±12.00^{c}	112.50±22.50^{cd}	7.17^{a}	5.97^{ab}	3,900.00	0.433	0.300	333.08	272.73
1	6	0.00±0.00^{c}	54.00±19.44^{d}	4.71^{c}	3.93^{d}	4,600.00	0.511	0.354	393.08	321.82
0	7	0.00±0.00^{c}	27.00±11.62^{d}	7.32^{a}	6.93^{a}	5,460.00	0.606	0.420	466.15	381.82

** Means followed by the same letter within a column are not significantly different ($P \leq 0.05$, Tukey).

Table 15 shows the effect of exposure period and spray frequency on CSM population density assessed at weekly intervals during the 2018/19 growing season. The study revealed that exposure period and spray frequency interactions were significantly different ($F_{42, 165} = 20.18$: $P = 0.0001$). When assessments were done at 7 weeks the following observations were made; the highest spider mite population of 1134 was found in treatments exposed to full exposure (0 sprays) and was significantly different from spider mite population level of 381.25 found following 6 weeks exposure (1 spray) (Tukey, $P \leq 0.005$). The spider mite population of 79.00 found following 3 weeks exposure (4 sprays) was not significantly different from 0.00 population found with 1 week exposure (6 sprays) and 0 weeks exposure (7 sprays) ($F_{42,165} = 20.18$: $P = 0.0001$). When assessments were made at 6 weeks the following observations were made; spider mite population was 1034.50 which was similar to population of 1028.50 attained following 6 weeks exposure (1 spray) but significantly different from spider mite population (170.50) following 5 weeks exposure (Tukey, $P \leq 0.005$). When assessment was done at 4 weeks; the highest spider mite population (782.00) was found at full exposure (no spray) and was not significantly different from 769.50, 767.50 and 700.25 spider mite populations following 6 (1 spray), 5 (2 sprays) and 4 weeks (3 sprays) exposure respectively (Table 15). The lowest population was 0.00 which was not significantly different from spider mite population of 41.50 and 12.00 achieved with 2 (5 sprays) and 1 week (6 sprays) exposure respectively during the assessment period.

Table 15. Effect of exposure period and spray frequency on CSM population per plant (2018/2019 season) ($F_{42, 165} = 20.18$; $P = 0.0001$)

		Means ± SE						
Exposure	sprays	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7
Full	0	232.50^{eA}±40.77	408.25^{deA}±76.14	552.75^{cdAB}±56.32	782.00^{bcA}±70.26	944.25^{abA}±29.69	1034.50^{aA}±29.72	1134.00^{aA}±41.86
6	1	191.75^{dA}±35.69	375.75^{cdA}±81.16	649.50^{bcA}±25.08	769.50^{abA}±13.43	912.50^{aA}±42.24	1028.50^{aA}±54.55	381.25^{cdB}±141.86
5	2	210.75^{bA}±7.89	378.75^{abA}±23.13	551.00^{abAB}±30.76	767.50^{aA}±31.63	755.25^{aA}±229.52	170.50^{bB}±10.60	192.75^{bBC}±55.61
4	3	194.50^{dA}±14.27	345.00^{cA}±31.77	566.75^{bAB}±31.33	700.25^{aA}±39.27	144.50^{dB}±18.57	124.75^{dBC}±16.26	114.50^{dC}±19.39
3	4	149.00^{bcdA}±16.90	233.25^{bAB}±23.14	472.00^{aB}±24.17	202.75^{bcB}±25.72	119.75^{cdB}±21.00	77.50^{dBCD}±29.71	79.00^{dC}±12.56
2	5	181.00^{bA}±15.74	301.75^{aA}±36.94	70.00^{cC}±7.07	41.50^{cC}±15.46	31.00^{cB}±16.49	24.75^{cCD}±14.81	12.00^{cC}±12.00
1	6	171.00^{aA}±1.79	60.50^{bBC}±5.72	27.00^{cC}±10.60	12.00^{cdC}±5.61	5.00^{cdB}±5.00	5.00^{cdD}±5.00	0.00^{dC}±0.00
0	7	105.00^{aA}±57.82	1.50^{bC}±1.50	0.00^{bC}±0.00	0.00^{bC}±0.00	0.00^{bB}±0.00	0.00^{bD}±0.00	0.00^{bC}±0.00

** Means followed by the same small letter within a row are not significantly different ($P \leq 0.05$, Tukey).
** Means followed by the same capital letter within a column are not significantly different ($P \leq 0.05$, Tukey).

Table 16 shows the effect of exposure period and spray frequency on CSM population density assessed at weekly intervals during the 2019/20 growing season. The study revealed that exposure period and spray frequency interactions were significantly different ($F_{42, 165} = 64.90$: $P = 0.0001$). When assessments were done at 7 weeks the following observations were made; the highest spider mite population (1188) was found in treatments exposed to full exposure (0 sprays) and was significantly different from spider mite population level of 621 found following 6 weeks exposure (1 spray) (Tukey, $P \leq 0.005$). The spider mite population of 220.50 found following 4 weeks exposure (3 sprays) was not significantly different from 139.50 and 112.50 found with 3 (4 sprays) and 2 weeks (5 sprays) exposure ($F_{42,165} = 64.90$: $P = 0.0001$). When assessments were made at 6 weeks the following observations were made; the highest spider mite population (1111.50) was found at full spider mite exposure (no spray) and was not significantly different from spider mite population (1125) following 6 weeks exposure. The lowest population density was 0.00 was attained following 0 weeks exposure (7 sprays) and was not significantly different from spider mite population following 2 (5 sprays) and 1(6 sprays) week exposure during the same assessment period (Tukey, $P \leq 0.005$). When assessment was done at 4 weeks; the spider mite population was 900.00 at full exposure (no spray) which was not significantly different from 936.00 spider mite population following 6 (1 spray) (Table 16). The lowest spider mite population of 0.00 was not significantly different from 18.00 and 45.00 spider mite populations found following 2 (sprays) and 1 (6 sprays) weeks exposure.

Table 16. Effect of exposure period and spray frequency on CSM population per plant (2019/2020 season) ($F_{42, 165} = 64.90$; $P = 0.0001$)

		Means ± SE						
Exposure	sprays	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7
Full	0	256.50^{eA}±59.87	396.00^{deA}±64.06	540.00^{dBC}±12.73	900.00^{cAB}±12.73	1008.00^{bcA}±41.57	1111.50^{aA}±22.50	1188.00^{aA}±49.30
6	1	198.00^{cAB}±18.00	297.00^{cAB}±15.59	702.00^{bA}±55.48	936.00^{aA}±72.75	1044.00^{aA}±36.00	1125.00^{aA}±18.74	621.00^{bB}±88.94
5	2	216.00^{deAB}±12.73	373.50^{dA}±25.85	558.00^{cBC}±25.46	760.25^{bBC}±28.57	1003.00^{aA}±26.88	184.50^{eB}±4.50	292.50^{deC}±85.50
4	3	220.50^{bcAB}±18.55	337.50^{bAB}±18.55	616.50^{aAB}±11.33	693.00^{aC}±9.00	180.00^{cB}±12.73	130.50^{cC}±4.50	220.50^{bcCD}±59.87
3	4	144.25^{bcdAB}±12.85	225.00^{bcBC}±21.42	508.50^{aC}±19.96	234.00^{bD}±14.70	130.50^{cdB}±8.62	63.00^{dD}±9.00	139.50^{bcdCD}±38.45
2	5	202.50^{bAB}±22.50	355.50^{aAB}±4.50	67.50^{cdD}±8.62	18.00^{deE}±10.39	9.00^{deC}±9.00	0.00^{eE}±0.00	112.50^{cCD}±22.50
1	6	207.00^{aAB}±27.00	90.00^{bCD}±0.00	40.50^{bcD}±4.50	45.00^{bcE}±9.00	18.00^{cC}±12.73	4.50^{cE}±4.50	54.00^{bcD}±19.44
0	7	108.00^{aB}±36.00	0.00^{bD}±0.00	0.00^{bD}±0.00	0.00^{bE}±0.00	0.00^{bC}±0.00	0.00^{bE}±0.00	27.00^{bD}±11.62

** Means followed by the same small letter within a row are not significantly different ($P \leq 0.05$, Tukey).
** Means followed by the same capital letter within a column are not significantly different ($P \leq 0.05$, Tukey).

Figures 15 (A, B) show the yield in tomato versus spider mite infestation relationships for the 2018/19 (15A) and 2019/20 (15B) growing seasons. The figures reveal a negative relationship between spider mite infestation and tomato yield during the assessment (correlation coefficients of 0.26 and 0.162 respectively) and gave yield infestation relationship regression equations of Y = -0.0013x +6.2531 and Y = 0.0011x + 5.2316 respectively. Figure 15 (A) shows that during 2018/19 season, at infestation rates of 0, 12, 79, 114.5, 1134 spider mites per plant, average yields of 7.32, 7.17, 6.33, 4.86 and 5.19 tonnes ha^{-1} respectively were attained. During the 2019/20 season, infestation rates of 27, 112.5, 139.5, 220.5 and 1188 spider mites per plant gave average yields of 6.93, 5.97, 5.28, 4.05 and 4.32 tonnes ha^{-1} respectively. There was a significant reduction in yield as the number of spider mites increased during both seasons.

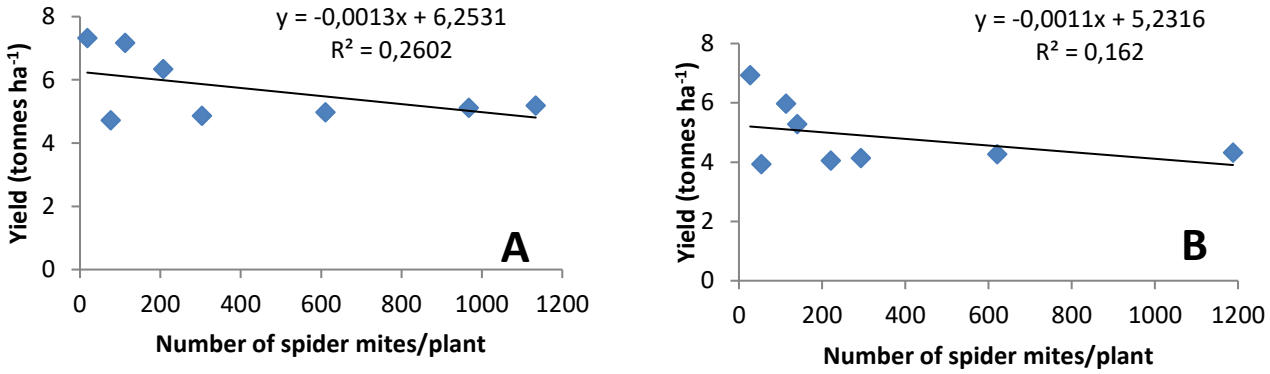

Figure 15. Relationship between tomato yield and rate of infestation by CSM (2018/2019 (A) and 2019/2020 (B) seasons).

Figures 16 (A, B) reveal a negative relationship between spider mite exposure (weeks) and tomato yield when assessments were done during the 2018/19 and 2019/20 seasons (correlation coefficients of 0.2602 and 0.162 respectively). These figures show that yields of 7.32, 4.71, 6.33, 4.98 and 5.19 tonnes ha^{-1} were achieved following spider mite exposure periods of 0, 1, 3 , 5 and 7 weeks during the 2018/19 season

while yields of 6.93, 3.93, 5.28, 4.14 and 4.32 tonnes ha^{-1} were achieved during the 2019/20 season following the same exposure periods.

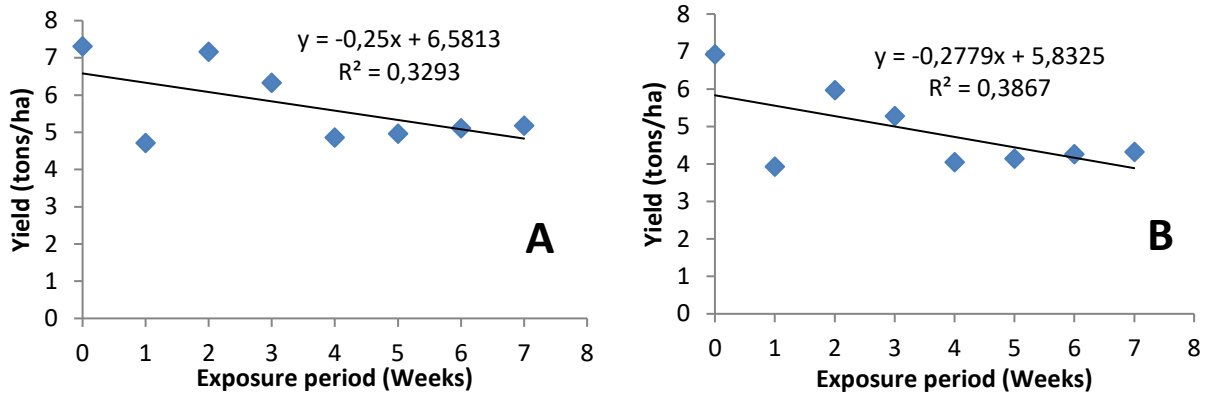

Figure 16. Relationship between CSM exposure period and yield of tomato assessed during the 2018/2019 (A) and 2019/2020 (B) seasons.

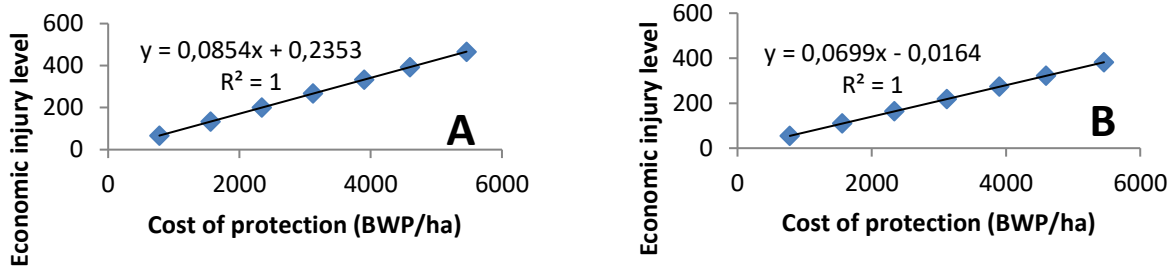

Figure 17. Relationship between cost of protection and Economic injury level assessed during 2018/19 (A) and 2019/20 (B) seasons.

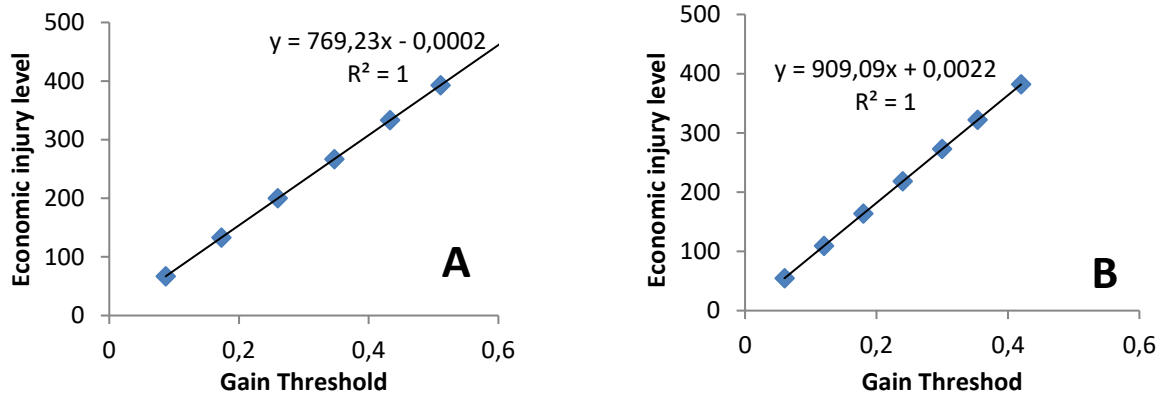

Fig 18. Relationship between gain threshold and economic injury level assessed during 2018/19 (A) and 2019/20 (B) seasons.

5.4 DISCUSSION

Farmers invest in crop protection to prevent crop losses due to agricultural pests both on the field and in storage (Oerke, 2016). Whereas economic thresholds for spider mites have been developed for most crops elsewhere (Shelton *et al*., 1987; Rueda *et al*., 2007; Pereira *et al*., 2017; Paes *et al*., 2019; Diamantino *et al*., 2021) they have not been developed for tomato in Botswana. This is despite the economic importance placed on tomatoes (Zeiss & Klubertanz, 2020). Collection of yield loss data is vital for economic management of pests and for assessing the effectiveness of the current crop management practices. Based on these data, strategies for the prudent use of scarce resources may be developed in order to optimize productivity (Nutter *et al*., 1993; Cooke, 1998). The present paper focuses on pre-harvest losses, that is, the effect of CSM on tomato production in the field, and the effectiveness of control measures applied by farmers to reduce losses to an acceptable level. In this study, tomato yield per hectare decreased as spider mite populations increased and ranged from 5.19 tonnes ha^{-1} at the lowest spider mite population to 7.32 tonnes ha^{-1} at the highest population during the 2018/19 and from 4.32 tonnes ha^{-1} at the lowest spider mite population to 6.93 tonnes ha^{-1} at the highest spider mite population in 2019/20. The findings of this study are in line with those of earlier studies that found that yield reduction caused to tomato plants by spider mites can be correlated to spider mite population densities. Fadini *et al.* (2004) and Kalmosh (2016) observed that the injury caused by *T. urticae* results from puncture of the lower epidermis cells. High infestations by spider mites have been reported to diminish the rate of photosynthesis and also damage the leaf mesophyll and cause the stomata to close. This reduces the efficiency of the leaves to manufacture sufficient nutrition for desired development of the fruit (Fathipour and Maleknia, 2016). According to a study by Ghaderi *et al*. (2019) on tomato leaf miner, the average number of fruits per tomato plant could be correlated with the percentage leaf

damage inflicted on the plant.

The gain threshold increased with frequency of spraying, ranging from 0.087 to 0.606 in 2018/19 and 0.060 to 0.420 in 2019/20. However, an increase in spraying frequency resulted in an increase in cost of protection from BWP 780 to BWP 5,460 in both 2018/19 and 2019/20 seasons. This is consistent with findings of of other authors (Obopile, 2006; Neves *et al*., 2022) where an increase in spray frequency resulted in an increase in cost of protection. The results indicate that the application of pesticide at lower pest population will have less gain threshold and, therefore, be uneconomical. The cost of protection and gain threshold were directly related. An increase in spray frequency reduced the level of exposure of host plants to spider mites by reducing their densities. Maximum possible yield loss was observed when complete exposure to spider mite feeding was allowed. The results also reveal that regardless of how high spider mite populations reached, the tomato plants were still able to produce minimum yield levels. Similar results were observed by other studies on spider mites (Padilha *et al*., 2020). Suekane *et al*. (2012) working with two spotted spider mite, TSSM observed reduction in yield as spider mite exposure increased.

The deleterious impact of spider mites on tomato production components mostly occurred when the plants were at the reproductive stage, suggesting that it is the tomato plant's growth stage where most damage happens. Thus, CSM at the observed densities (i.e., up to 182 spider mites plant^{-1}) caused tomato plants to abort flowers. Here, the economic injury levels for spider mites in tomato were determined for the first time. The economic injury levels were developed for insecticide applications using knapsack sprayer, which is one of the most widely used methods in tomato production in Botswana (Legwaila *et al*., 2022). In addition, these economic injury levels were determined in the greenhouse, reflecting the reality of greenhouse tomato production. In integrated pest management programs, control measures should only be initiated when the spider

mite population is equal to or greater than the economic injury level to prevent economic damage (Moura *et al*., 2018). This avoids unnecessary pesticide applications, which increase the cost of production and exposure of non-target organisms to pesticides (Paes *et al*., 2019). Furthermore, this approach helps alleviate the harmful effects of pesticides on human health and the environment (Carvalho, 2017) which are mostly caused by spray drift (Hladik *et al*., 2014; Bueno *et al*., 2017).

5.5 CONCLUSION

The EIL determined in this study for CSM will help in decision making and can be incorporated into integrated pest management programs in tomato production. The EILs are 136 and 182 spider mites per plant for pesticide application, every three to four weeks. The results obtained in this study will contribute to future research involving the monitoring of these pests in the field.

5.6 RECOMMENDATIONS

For tomato growers producing tomatoes under greenhouse conditions, these results suggest that initiation control measures three to four weeks after appearance of CSM can reduce economic losses associated with the spider mites. High yield losses were observed during this period, suggesting that this was the most economical time to apply the pesticide. Pedigo and Rice (2006) recommended the economic threshold (ET) to be 75% of the EIL, therefore between 136 and 182 spider mites per plant would necessitate control actions, every three to four weeks. Since the EIL is dependent on changes in market price of tomato, cost of pest management, pest injury inflicted on the plant by the pest, and susceptibility of the host plant to spider mite injury, this recommendation is expected to change depending on location and time. There is also need to develop EILs based on different growth stages of the tomato plant and under field conditions.

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CHAPTER 6 – DETOXIFYING ENZYME ACTIVITIES IN THE CARMINE SPIDER MITE, *TETRANYCHUS CINNABARINUS* BOISDUVAL COLLECTED FROM DIFFERENT GEOGRAPHICAL LOCATIONS IN BOTSWANA

ABSTRACT

A study was conducted to establish pesticide metabolism enzymes activity among carmine spider mite (Acari: Tetranychidae) strains collected from tomato fields in seven geographical locations of Botswana. Metabolic enzymes, namely, esterases, Glutathion-*S*-transferase and Cytochrome P_{450} monooxygenases were estimated using standard methodology. The highest levels of α-esterase activity (nmol/min/mg of protein^{-1}) were observed in the Bela-bela strain (1.966 nmol/min/mg of protein^{-1}) followed by Sikwane (1.008nmol/min/mg of protein^{-1}). The Sikwane strain (3.276 nmol/min/mg of protein^{-1}) registered enhanced β-esterase activity, followed by the Glen Valley strain (1.966 nmol/min/mg of protein^{-1}) and Francistown (1.102 nmol/min/mg of protein^{-1}) strain. Elevated level of GST were observed in the Francistown (20.026 nmol/min/mg of protein^{-1}), followed by the Moshupa (15.655 nmol/min/mg of protein^{-1}) and Bela-bela (15.371 nmol/min/mg of protein^{-1}) strains. The Francistown (0.222 nmol/min/mg of protein^{-1}) strain showed the highest P_{450} monooxigenase activity followed by Bobonong (0.193 nmol/min/mg of protein^{-1}) and Sikwane (0.135 nmol/min/mg of protein^{-1}) strains. Variation in detoxification enzymes activity among CSM strains could be attributed to differing pesticide usage patterns. These findings will be useful during the selection of acaricides and in the development of resistance management strategies for an effective spider mite management program for tomato growers in Botswana.

6.1 INTRODUCTION

The Carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae) (CSM), is an extremely damaging pest that affects many economically important crops worldwide (Migeon *et al*., 2010; Auger *et al*., 2013; Roy *et al*., 2014a). It is a pest of great agricultural significance that attacks crops including tomatoes, cucumbers, peppers, maize, grapes, apples, strawberries and

citrus in both open fields and protected habitats (Grbic *et al*., 2011). The principal host of CSM in Botswana is tomato (*Solanum lycopersicum var. lycopersicum*) (Solanaceae), upon which it inflicts overwhelming crop damage and production losses (Herrman *et al*., 2012; Roy *et al*., 2014a). CSM nymphs and adults feed by puncturing the leaves with a retractable stylet and drawing the cell contents out. They rupture photosynthetically active cells, lowering the photosynthetic and transpiration capacity of the plant (Park and Lee, 2002). The resultant chlorotic lesions coalese causing the leaves to die, resulting in leaf abscission which in turn causes decreased vigour, flowering and ultimately reduction in yield (Gotoh *et al*., 2010; Tehri, 2014). Presently, the control of spider mites is achieved through the use of synthetic acaricides (Obopile *et al*., 2008; Demaeght, 2015). The severity of infestations has also led to frequent and indiscriminate use of pesticides on tomatoes. Pesticide compounds commonly used by farmers include organochlorines, organophosphates, carbamates and pyrethroids (Obopile *et al*., 2008). Most of the pesticides are unselective and harmful to both spider mites and non-target organisms. The overuse and misuse of synthetic pesticides, through the elimination of susceptible individuals, has resulted in a reduction in spider mite susceptibility and a subsequent failure to control spidermites in many countries. Resistance does not only reduce the effectiveness of the selecting compound but also confers cross-resistance between formulations with a shared mechanism of action (Demaeght, 2015). Numerous instances of pesticide resistance in spider mites have been documented globally (Grbic *et al*., 2011; Bu *et al*., 2015; APRD, 2017). Spider mites quickly have a high tenedency to become resistant to commonly used pesticides due to their high reproductive rate, high inbreeding rate, short developmental time, cross-fertilization, high mutation rates and arrhenotokous reproduction (Singh, 2010; Roy *et al*., 2014a; Xu *et al*., 2014; Demaeght, 2015). This has rendered the management of spider mites more challenging for local tomato growers. Resistance

mechanisms in spider mites are common to most arthropods and include reduced cuticular penetration, target site insensitivity and metabolic resistance (Ramasubramanian *et al*., 2005). At the molecular level, two mechanisms are involved in pesticide resistance development in arthropods, namely, amplified sequestration and increased pesticide metabolism, reducing the lethal dose that reaches the target site (Demaeght, 2015; Namin 2017; Khan *et al*., 2020; Adesanya *et al*., 2021; Naveena *et al*., 2022). This type of resistance confers resistance to almost all groups of pesticides (Van Leeuwen *et al*., 2010; Roy *et al*., 2018) and is closely linked to increased activity of three principal detoxifyication metabolism enzymes in mites, which include esterases (GE), glutathione-S-transferases (GSTs) and Cytochrome P_{450} monooxygenases (CYP_{450}) (Leeuwen *et al*., 2010; Shen *et al*., 2014; Pavlidi *et al*., 2015; Han *et al*., 2016; Ru *et al*., 2017; Bisset *et al*., 2020; Epelboin *et al*., 2021). These enzymes can metabolize and excrete exogenous chemicals through specialised pathways to minimise damage and to develop pesticide resistance to almost all classes of pesticides (Chen *et al*., 2021).

Esterases are a group of functionally different enzymes that hydrolyze exogenous and endogenous esters in biological organisms (Oakshott *et al*., 2005; Wheelock *et al*., 2005; Demaeght, 2015). Most esterases are within the carboxyl/cholinesterase (CCE) superfamily. They are proteins which contain a hydrolase fold where hydrolysis involves a catalytic triad with serine, an acidic residue and a histidine (either aspartate or glutamate) (Oakshott *et al*., 2005). The contribution of esterases in organophosphates resistance in various arthropods is well researched (Grisales *et al*., 2013; Poupardin *et al*., 2014; Bhatt *et al*., 2021). Esterases appear to play a part in detoxification, neurodevelopmental processes and signal transduction of hormones (Oakshott *et al*., 2005; Farnsworth *et al*., 2010). Adhikari and Khanikor (2021) found that in resistant arthropod strains, esterase protein makes up to three percent of the overall body protein compared to 0.4 percent in

the susceptible strain. This is due to the rapid amplification of the esterase genes in response to the pesticide (Hemmingway *et al*., 2004). Esterases have been implicated in resistance development in *Panonychus ulmi* and *T. urticae* (Van Pottelberge *et al*., 2008; Kramer and Nauen, 2011).

Glutathione S-transferases (GSTs) are functionally diverse intracellular enzymes that detoxify electrophilic compounds via glutathione conjugation, dehydrochlorination, glutathione peroxidase (GPx) activity, or passive/sacrificial binding, resulting in more water-soluble and excretable products than non-conjugated substrates (Gharib *et al*., 2001; Yang *et al*., 2001; Gao *et al*., 2021). GSTs can also function as non-enzymatic binding proteins in intracellular transport, detoxification of endogenous and exogenous compounds, hormone biosynthesis, signaling processes, and protection from oxidative stress (Adler *et al*., 1999, Oakley *et al*., 2011). Most organisms' genetic capacity to encode different GST isoforms is related to their diversity of enzymatic and non - enzymatic functions. Increased levels of GST activity have been linked to pesticide resistance in a variety of insects. Increased GST activity has been linked with resistance to organophosphates in *Musca domestica* (Wei *et al*., 2001), organochlorines 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT) in *Drosophila melanogaster* (Tang and Tu, 1994), and pyrethroid resistance in *Nilaparvata lugens* (Vontas *et al*., 2002). GST is the primary DDT-resistance detoxification enzyme in mosquitos (Hemingway and Ranson, 2000). Twelve Mu-class GSTs have been associated with spider mite resistance development (Grbic *et al*., 2011).

Cytochrome P450 monooxygenases are responsible for the breakdown of agrochemicals in almost all agricultural pests and are crucial in pesticide resistance development (Shi *et al*., 2016). P450s are documented to catalyze numerous reactions and act as reductases, oxidases, and isomerases. Some are essential for the synthesis, activation, and catabolism of ecdysteroids, juvenile hormones, pheromones, fatty acids, and lipids. Above being involved in the metabolization of endogenous

substrates (lipids, hormones), P450s are important in the metabolism of xenobiotic compounds (drugs, pesticides), making them prime suspects in most insecticide resistance cases (Feyereisen, 2012). The elevations in the activities of P450s is responsible for the metabolism of neonicotinoids, organophosphates and growth regulator acaricides (Fahnbulleh, 2007). P450s were found to induce tolerance to spirodiclofen in *T. urticae* (Rauch and Nauen, 2004) and have also been linked to resistance development in *Panonychus ulmi* (Van Pottelberge *et al*., 2008; Kramer and Nauen, 2011). Acaricide detoxification through enzyme activity can result in cross-resistance to several classes of acaricides (Ranson *et al*., 2011).

Currently, synthetic compounds are used extensively against spider mites, and control failures noticed mostly implicated the possibility of development of resistance to pesticides. Therefore, it is important to study the underlying metabolic resistance mechanisms and susceptibility status of CSM to commonly used pesticides. Although the resistance mechanisms of *T. urticae* have been extensively studied around the world (Kumral *et al*., 2009), the same knowledge is not available for CSM, particularly from tomato fields in Botswana. Understanding the activity of detoxifying enzymes in CSM may lay the groundwork for reducing resistance development against effective acaricides. This will give clear picture of detoxification enzymes associated with different strains and could be a useful tool to monitor development of reistance in field strains. More information could provide a better understanding of resistance development, the most appropriate strategies to avoid resistance and better ways of managing spider mites when resistance has already developed. The purpose of this study was to compare the activity of detoxifying enzymes in CSM populations from tomato fields across Botswana. The objective of this study was to provide vital information for efficient CSM management on tomatoes and development of viable alternative means to

minimize resistance to commonly used pesticides and ensure the effective management of spider mites.

6.2 MATERIALS AND METHODS

Sample collection

CSM samples were collected from intensive tomato producing farms in seven different geographical locations across Botswana in 2022. The areas sampled were Bela- Bela farms (24º 28’52” S 26º 01’01” E), Glen Valley LEA horticulture incubator (24º 36’05” S 25º 58’24” E), Moshupa (24º 43’13” S 25º 28’ 43” E), Sikwane (24º38’30” S 26º24’47” E), Bobonong (22º03’03” S 28º21’05” E), Letlhakane (21º 26’28” S 25º 36’06” E), and Francistown Impala Station (21º 09’48” S 27º 35’16” E). Morphological identification of specimens was carried out using Taxonomic keys (Seeman and Beard, 2005; 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 intensive tomato production and therefore intensive application of synthetic pesticides. The biochemical assays were undertaken in the molecular biology laboratory at the University of Botswana.

6.2.1 Detoxifying enzymes assays

6.2.1.1 Sample preparation

Individual adult spider mites were homogenized in Eppendorf tubes containing 200µl of ice-cold distilled water. The homogenates were then centrifuged at 14000xg for 60 seconds and the tubes were placed back on ice.

Total protein quantification

Total protein was measured using Pierce Bicinchoninic acid (BCA) protein assay kit. Briefly, 25µl of CSM homogenates were transferred in duplicates into a microtiter plate. The same amount of water was used as a blank while 10µl of bovine serum albumin (BSA) was used as a positive control. This was followed by addition of 200µl of 50: l BCA copper sulphate solution (prepared by mixing 24.5 milliters of BCA and 0.5ml of copper sulphate solution). The plate was then covered and incubated at room temperature for 1 hour. After incubation the absorbance was read at 560nm in a Thermo-Scientific Multiskan FC microtiter plate reader. A standard curve of BSA using different amounts (0, 2.5, 5, 7.5 and 10µg) was also prepared for use in protein estimation.

Esterase activity determination

10 µl of each of the homogenates was distributed in duplicates in two microtiter plates; for alpha and beta esterases. 10 µl of water in duplicate was used as blanks while 10 µl of the solution α/β naphthol at 0.5 µg/µl (0.5mg/ml) in each well (total: 5 µg or ~35 nmoles) of respective plates was used as positive controls. To each well 200 µl of α/β-naphthyl acetate/ sodium phosphate working solution (working solution prepared by adding 250 µl of 30 mM α/β-naphthyl acetate (0.03M) to 24.75 ml of 20mM (0.02M) sodium phosphate buffer pH 7.2) was added in respective plates covered with a lid and incubated at room temperature for 20 minutes. The 50 µl of Fast Blue (prepared by dissolving 45 mg of fast blue salt in 4, 5 ml water and adding 10.5 of 5% sodium dodecyl salt solution) was added. The plate was then incubated at room temperature for another 5 minutes and read at 570 nm.

Glutathione s-transferases activity determination

10 µl of each of the homogenates in duplicate was mixed with 200 µl of reduced glutathione (GSH)/1-chloro-2,4 dinitrobenzene (CDNB) working solution (0.060 g of reduced glutathione (GSH) in 20 ml of sodium phosphate buffer 0.1 M pH 6.5 + 0.013 g of CDNB diluted in 1 ml of methanol) in a microtiter plate well. 10µl of water was used as a blank. The reaction was read at 340 nm immediately and after 10 minutes. An extinction coefficient of 0.0096 $\mu M^{-1}cm^{-1}$ was used to convert absorbance values to moles of product with 0.6 cm used as the path length. GST specific activity was reported as µmol CDNB conjugated/ min/ mg protein.

Monooxygenases activity determination

For monooxygenases, 20µl of the homogenates were distributed in duplicate in a microtiter plate. Same amount of 0.0625 M was used as a blank while cytochrome c (0.2µg) from bovine heart was used as a positive control. To each well, 60µl potassium phosphate buffer was added. This was followed by addition of 200 µl of 3,3',5,5' tetramethylbenzidine and sodium acetate working solution. Lastly, 25 µl of 3% H_2O_2 was added. The plate was covered with a lid and incubated at room temperature in the dark for 30 minutes. The plate was read at 620 nm. This assay does not measure the monooxygenase activity, but titrates the amount of bound haem in the homogenate (Brogdon, McAllister, & Vulule, 1997). Since haem is present in the active site of monooxygenases, major changes in the amount (0, 0.01, 0.02, 0.05, 0.1 and 0.2 µg) of monooxygenases produce a measurable increase in the haem. A standard curve using different amounts of cytochrome c was prepared to estimate of the amount of the monooxygenases present. This was obtained and expressed as nmol cytochrome P_{450}/ mg protein.

Calculation of Enzyme Activity

Esterase and cytochrome P_{450} activity were calculated using a protocol by Safi *et al*. (2017). The enzyme was calculated using the standard alpha/beta esterase alpha/beta naphthol and TMBZ for cytochrome P_{450}. Protein quantification was calculated from the mean OD using the BSA standard curve. The formula below was used to calculate enzyme activity.

$$\frac{Enzyme\ X\ 0.0069\ (esterase)/0.00113\ (cyt\ P450)}{Incubation\ period}$$

The product obtained was divided by the protein estimated to give the enzyme activity.
GST enzyme activity was calculated using the formula

$$C = \frac{A}{\varepsilon l}$$

Where: C = Concentration

A = Absorbance

Ɛ = CDNB coefficient

I = path length

The product obtained was divided by estimated protein.

Data analysis

Analysis was performed using GraphPad Prism software version 8.0. The activity of detoxification enzymes were subjected to one-way analysis of variance (ANOVA) and Tukey's Honestly significant difference test ($P < 0.05$) (Zar, 1984) using SAS statistical software (version 9.4. SAS institute, Cary, USA)

6.3 RESULTS

1. Esterases

Figures 19A and 19B show results of α and β –esterase activity in CSM samples collected from the seven geographical locations in Botswana. A statistically significant difference in α – esterase activity was observed between the CSM strains ($F_{51, 270} = 4.88$; $P<0.001$) (Fig. 1A). CSM strains from Bela-bela showed the highest activity of α – esterase (1.966 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), followed by Sikwane (1.008 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Francistown (0.946 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Glen Valley (0.739 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Moshupa (0.615 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Bobonong (0.607 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and Letlhakane (0.495 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) (Fig. 19A). 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 only 1.23 times in the Bobonong strain as compared to the Letlhakane strain (Table 17). A statistically significant difference in β-esterase activity was also observed among the strains ($F_{51, 270} = 4.76$; $P<0.0001$) (Fig. 19B). The greatest β - esterase activity was recorded in the Sikwane strain (3.276 nmoles ml^{-1} min^{-1} mg of protein-1), followed by Glen Valley (1.966 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Bobonong (1.653 nmoles ml^{-1} min-1 mg of $protein^{-1}$), Francistown (1.102 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Letlhakane (0.953nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Moshupa (0.825 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and Bela-bela (0.784 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) (Fig. 19B). The relative activity of β - esterase was 4.18 times more in the strain from the Sikwane, 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 population as compared to the Bela-bela strain (Table 17).

2. Glutathione-S-transferase

Glutathione-S-transferase (GST) activity in CSM adults collected from different locations is given in Fig 19C. Results indicate that there was a significant difference in GST activity among the strains ($F_{49,\ 180}$ = 1.46; P<0.0393). However, the highest GST activity was observed in the Fracistown strain (20.026 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$). This was followed by Moshupa (15.655 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Bela-bela (15.371 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Glen Valley (14.506 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), and Bobonong (10.638 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) (Fig. 19C). 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, as compared to the Bobonong strain (Table 17).

3. Cytochrome P_{450} monooxygenase

Figure 19D presents results of cytochrome P_{450} monooxygenase activity in CSM adults collected from different localities in Botswana. From the results, a significant difference in P_{450} activity was observed among the strains ($F_{51,\ 270}$ = 2.85; P<0.0001). It is also evident that Francistown had the highest P_{450} mooxygenase activity (0.222 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), followed by Bobonong (0.193 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Sikwane (0.135 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Glen Valley (0.124 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Moshupa (0.098 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$), Letlhakane (0.069 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) and Bela-bela (0.016 nmoles ml^{-1} min^{-1} mg of $protein^{-1}$) ((Fig. 19D). The relative activity of cytochrome P_{450} 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 (Table 17).

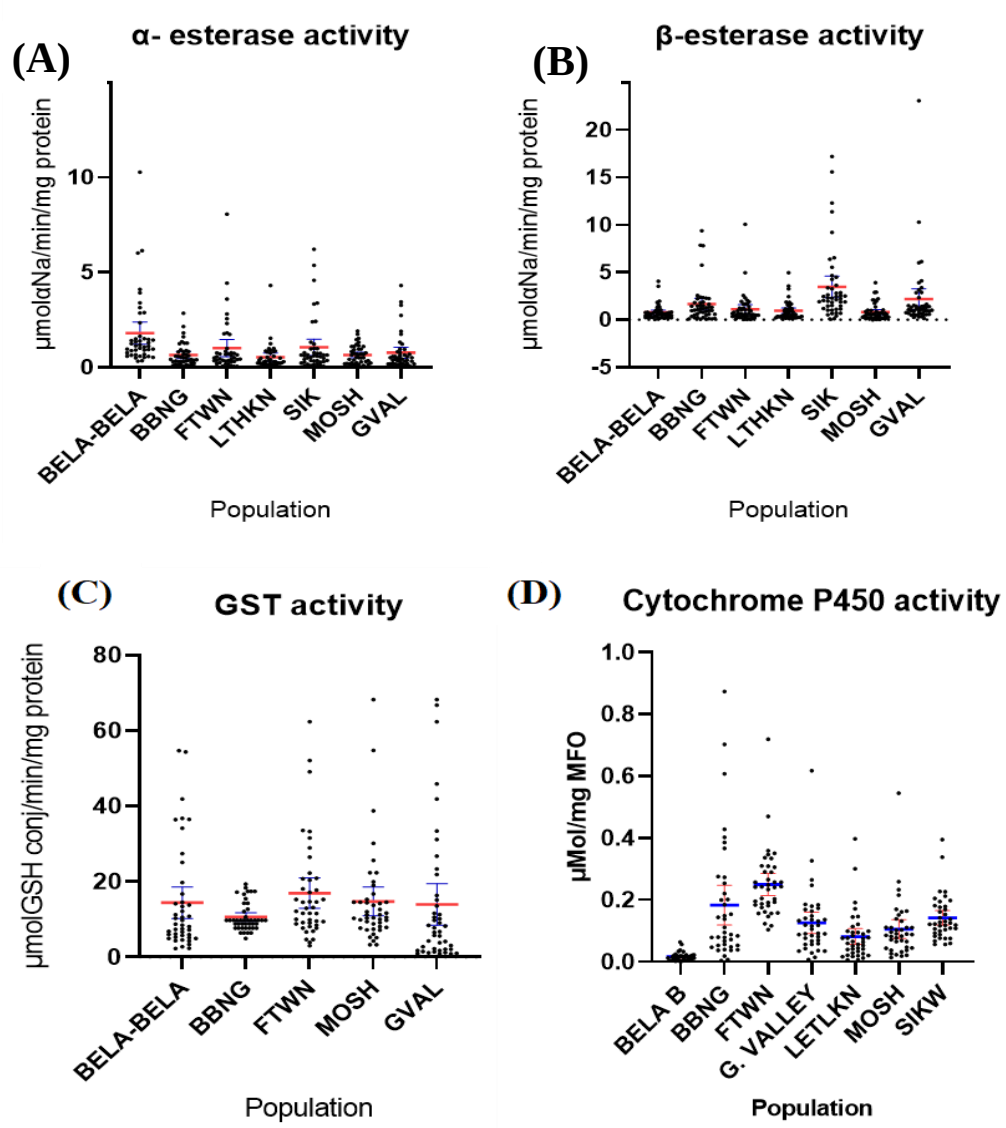

Figure 19. Detoxifying enzyme activities in spider mite populations form different geographical locations of Botswana.

Table 17. Relative activity of pesticide detoxification enzymes in CSM populations from different geographical locations.

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

Enzyme activity in nmol/min/mg of protein^{-1}) (±SE)
R.A* Relative activity = 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

6.4 DISCUSSION

In Botswana, spider mite control relies primarily on the application of chemical pesticides. Resistance development in spider mites is a consequence of prolonged exposure to frequently applied chemicals or pesticides with similar chemical profiles (Das *et al*., 2017; Roy *et al*., 2018). An increase in tolerance to pesticides in field strains could be attributable to the overexpression of metabolic enzymes combined with target site insensitivity (Karuppaiah *et al*., 2017). Results of this study are in agreement with those observations. Metabolic detoxification plays an crucial role in resistance development in spider mites and the activities of metabolic detoxifying enzymes in relation to the development of resistance in spider mite populations are widely documented (Van Leeuwen & Tirry, 2007; Das *et al*., 2017). Esterases, glutathione S-transferase (GST) and cytochrome P_{450}s are the major detoxification enzymes in arthropod systems (Martin *et al*., 2002;

Zhang *et al*., 2010). In the present study, spider mite strains collected from Bela-bela showed the highest α- esterase activity followed by Sikwane, Francistown, Glen Valley, Moshupa, Bobonong and Letlhakane. The highest β- esterase activity were recorded in Sikwane followed by Glen-Valley, Bobonong, Francistown, Letlhakane, Moshupa and Bela-bela. Elevated esterase activity has been documented in many arthropod species with pronounced resistance to organophophates (Ay & Gurkan, 2005). Other authors (Young *et al*., 2006; Mohan *et al*., 2007; Grisales *et al*., 2013; Poupardin *et al*., 2014; Adhikari & Khanikor, 2021) observed that elevated activity of esterases is a major resistance mechanism for pyrethroid and organophosphate resistance in insects. The elevated esterase activities recorded in Sikwane, Francistown, and Glen- Valley strains might be due to indiscriminate use of pyrethroid and organophosphate pesticides in these locations. These pesticides are highly favoured because of their quick knock down effect. These farms are also in close proximity to the city Gaborone where there is a high demand for high quality and quantity of blemish free vegetables, therefore pressure to apply pesticides. The variation in esterase activity could be due to the prevailing pre-adaptive occurrence in spider mite strains collected from different geographical locations as well as pesticide usage patterns. Elevated esterase activity has been reported in oxydemeton methyl, deltamethrin, monocrotophos, fenvalerate and endosulfan resistant 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 Letlhakane and Bela-bela strains showed low titre of α and β –esterase activities respectively as compared with other spider mite strains in this study. The detoxification enzymes, cytochrome P_{450} monooxygenases are responsible for the hydrolysis of various compounds by breaking the ester linkage (Bhatt *et al*., 2021), thereby breaking down several organophosphate and pyrethroid pesticides with carboxyl or amide groups (Wu *et al*., 2011; Das *et al*., 2017). Enhanced activity of

cytochrome P_{450} monooxygenase was found to be responsible for pyrethroid resistance in *S. litura* (Young *et al*., 2006). The present study showed that Francistown had the highest P_{450} monooxygenase activity followed by Bobonong, Sikwane, Glen Valley, Moshupa, Letlhakane and Bela - bela. Elevated levels of cytochrome P_{450}s could be responsible for greater tolerance to pyrethroids (Karuppaiah *et al*., 2017). Enhanced activity of P_{450} monooxygenase may indicate that synthetic pyrethroids are overused in these farming locations. The Bela-bela strain showed low titre of cytochrome P_{450} monooxygenase as compared with other strains in this study suggesting that pyrethroids are not oversused. Elevated levels of GSTs were observed in the Francistown strain. The descending order of GST activity was Francistown, Moshupa, Bela-bela, Glen Valley and Bobonong. The Sikwane, Letlhakane and Bobonong strains showed low titre of GST activity when compared with spider mite strains from other locations. GSTs are documented to confer resistance to organophosphates, organochlorines and pyrethroid pesticides. They act by conjugation of polar products with several endogenous compounds which include sulphates, phosphates, sugars, amino acids, or glutathione (Kostaropoulos *et al*., 2001; Hemmingway *et al*., 2004; Che-Mendoza *et al*., 2009; Nehare *et al*., 2010; Roy *et al*., 2018). However, GST activity has been associated with resistance to all main classes of pesticides. Enhanced activity of GSTs may indicate that organophosphates, organochlorines and pyrethroids are overused in these farming locations. This study observed that the activities of detoxification enzymes varied across geographic locations. It is expected that resistance development in spidermite populations becomes more pronounced in spidermite strains from fields with high pesticide usage. Navarro-Roldan *et al*. (2020) observed that enzymatic activity is directly related to pesticide pressure and thus it can vary in locations due to the pesticide application regimes. Forrester (1990) and 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 pesticide active ingredient. Pesticides used in agriculture can contaminate water sources through run-off, 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. However, the results in this study highlight the need for development of an elaborate pesticide resistance management program. 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 pesticides for spider mite control. The present study was conducted on spidermite populations collected from only seven geographic locations. Additional studies are required to investigate resistance development in other geographical locations and to elucidate the pace at which spider mite strains evolve resistance. Such a study will inform better pesticide usage programmes that consider the role of detoxification enzymes in pesticide breakdown. Additionally, the present study focuses only metabolic resistance, therefore other mechanisms of resistance development in spider mites such as target site insensitivity and transcriptomic alteration due to frequent pesticide applications must be investigated to develop substantial understanding of the effect of prolonged pesticide exposure in local spider mite populations.

6.5 CONCLUSION

This study provides important information on the status of major pesticide detoxification enzymes in spider mite strains from different geographical locations in major tomato growing districts of Botswana. Resistance development is an adaptive mechanism due to prolonged exposure to pesticides for many generations and use of pesticides having similar mode of action to control spider mites by tomato farmers. Detoxification enzymes are documented to be responsible for differential susceptibility of arthropods to pesticides. Detoxification enzymes are specific to a certain group of pesticides, however, the multiple enzymes role may be the reason for the mixed response of spider mites in this study. It is imperative to consider the level and role of various detoxification enzymes in a population when formulating management strategies against spider mites and they should be location specific. Metabolic resistance is an irreversible process that could potentially limit pesticide use once it occurs. Monitoring the biochemical mechanism of pesticide metabolism will be helpful when substituting the existing conventional formulations with those which are not affected by these enzymes. Farmers should be trained to implement proper spider mite management and pesticide usage programs that focus on prioritization and understanding of the potential risks of resistance development. Appropriate and efficient use of pesticides could help growers derive use from a pesticide for a longer period. This would also provide researchers with the necessary time to develop and register new pesticide formulations for efficient pest control. In addition, there is a need for more confined enzymatic analyses for spider mites in order to provide comparable results from various geographical locations. Results obtained in this study would give the susceptibility status of spider mite to most commonly used pesticides and the role of detoxification enzymes in resistance development. The data can be used as a

baseline for resistance monitoring and development of appropriate strategies to manage spider mites on tomato. More research should be undertaken to measure detoxification enzyme levels for effective CSM control in Botswana.

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CHAPTER 7 – GENERAL DISCUSSION, IMPLICATIONS AND RECOMMENDATIONS

7.1 General discussion

This research fmocused on farmers knowledge and perceptionjs towards spider mites, its susceptibility to acaricides and activities of detoxifying enzyme activities in Botswana. The first study (chapter 3), which was a survey of vegetable farmers, found that farmers had considerable knowledge about pests of vegetable crops in their fields. They were also knowledgeable about

spider mites. They considered spider mites as major constraints to profitable tomato production. The study revealed that farmers use colour to identify between spider mite species infesting their crops and the red form is more prevalent across fields in Botswana. Most farmers reported prior knowledge of spider mites. Most of the pest information was from personal experiences and agro-suppliers. Extension service played a lesser role in pest information dissemination. Spider mites are highly problematic and costly in terms of the damage they inflict on the tomato crop and the resultant losses affect their livelihoods as crop production was their primary source of sustenance. Farmers employ several tactics to protect their crop from pest damage but none is more popular than the use of synthetic pesticides. Farmers are heavily reliant on conventional pesticides for crop protection from pests including spider mites. The perceived simplicity of pesticide application coupled with their quick knock down effect has make them a popular choice for vegetable farmers. Their decision to apply pesticides is mostly based upon seeing the pest or pest symptoms on the crop. The study revealed that an assortment of pesticides are used for vegetable pest control by farmers, however some of them are not recommended for spider mite control. Some are classified as either highly hazardous or extremely harzadous as per the World Health Organisation criterion (WHO, 2019). The indiscriminate use of pesticides may indicate the farmers' desperation to contain the spider mite problem. Pesticides are not only harmful to the farmer but may have a deleterious effect of beneficial entomofauna and the environment. Pesticide residues may also affect the consumers' health. Spider mites are documented to rapidly develop resistance to most new formulations developed for its control which may be the reason why farmers report reduced effectiveness of their current tactics.

The second study (chapter 4) evaluated the effectiveness of three pesticides in the control of carmine spider mite of tomato. Since CSM eggs can only acquire the lethal dose of the pesticide

through contact activity, it is expected that pesticides with contact mode of action can have an effect on eggs. The study revealed that amongst the three pesticides evaluated, chlorfenapyr was the most effective ovicide followed by methomyl and then abamectin. Chrlorfenapyr was able to cause high egg mortality at dosages lower than the recommended label rate. The toxic effect of chlorfenapyr active ingredient was mainly through direct contact with the eggs on the leaf surface. The study also showed that the efficacy of chlorfenapyr increased with each increase in dosage. The findings that chlorfenapyr was highly effective against CSM eggs are highly welcome since the accumulation of spider mite populations from hatching eggs would be suppressed thereby reducing subsequent damage to the crop. Therefore, when using chlorfenapyr to control CSM, the egg stage would be ideal to target. Abamectin is mainly effective through ingestion but with negligible contact activity therefore the findings that abamectin was less effective against CSM eggs were expected since eggs can only acquire the lethal dose of the abamectin through contact on the leaf surface. Only abamectin dosages above the recommended label rate were able to achieve high egg mortality. The study also revealed that abamectin became more effective with increase in dosage. Abamectin at the recommended dosage required longer exposure periods to achieve adequate mortality. Methomyl is effective in two ways, as a contact and systemic acaricide. It has also been documented to have ovicidal and embryotoxic properties. Therefore the results that methomyl was highly effective against CSM eggs in this study were anticipated. The lethal dose of methomyl was acquired by CSM eggs through direct hits or contact with the active ingredient on the leaf surface. The study revealed that the recommended label rate of methomyl was sufficient to achieve effective control of CSM eggs. The results also showed that methomyl became more toxic to CSM eggs with each increase in dosage. The high egg mortalities achieved with methomyl are desirable since the build up of damaging motile stages would be minimised,

thereby reducing subsequent damage to tomato plants. Therefore, eggs are a suitable target when using methomyl to control CSM. The study also evaluated the effectiveness of the three pesticides against CSM adults. It was revealed that chlorfenapyr was the most effective, followed by methomyl and abamectin. Chlorfenapyr became more toxic with each increase in dosage. CSM adults can acquire the lethal active ingredient through contact and ingestion as they move and feed on the leaf surface. The dual mode of action may explain the the relatively quick action of chlorfenapyr against CSM adults. The fast action of chlorfenapyr is very desirable since this is the most damaging and reproductive stage. Methomyl was able to cause high mortality of CSM adults with dosages lower than the recommended label rate. Since methomyl is effective in two ways, as a contact and systemic acaricide, the high CSM mortalities in this study were expected. CSM adults can acquire the lethal dose of methomyl through direct hits from droplets or contact as they move and forage on the leaf. Abamectin became more toxic to CSM adults with each increase in dosage. Abamectin was reported to cause partial paralysis of the pest nervous system which can reduce feeding and eventually lead to death. This means that although mortality levels achieved by abamectin are low, the pest may be alive but unable to cause damage through feeding. The study also showed that the three pesticides can be used as components of an integrated pest management programme for spider mites and reduce resistance development by using only one pesticide. The third study (chapter 5) dteremined the economic injury levels and yield loss for CSM on tomato over the 2018/2019 and 2019/2020 cropping seasons. The study revealed that the yield of tomato decreased when spider mite populations increased. Spider mites puncture the lower epidermal leaf cells amd suck the cell contents causing speckling and eventual death of the leaf. This reduces the photosynthetic capacity of the plant thereby affecting yield. The study also revealed that the gain threshold increased with the frequency of spraying through the two cropping seasons. However,

increasing the spraying frequency resulted in a significant increase in the cost of protection. The study also showed that the application of control measure at low densities will have a low gain threshold and be uneconomical. The maximum possible loss of yield was observed when complete exposure of plants to spider mite feeding was allowed. The results showed that regardless of how high the spider mite densities accumulated per plant, the tomato plants were still able to produce minimum yields. The highest negative impact of spider mites was observed when the plant was at the reproductive stage, suggesting that this is the plant's phenological growth stage where the most damage occurs. High spider mite densities at this stage caused the plants to abort flowers. In integrated pest managemet programmes, control measures should only be initiated when the spider mite populations is equal or greater than the economic injury level to avoid economic damage. This avoids excessive usage of pesticides, which increase the cost of protection, exposure of non-target organisms and environmental damage. The fourth study (chapter 6) sought to establish the activity of pesticide detoxifying enzymes in spider mite strains collected from different geographical locations in Botswana. Spider mites are reported to rapidly evolve resistance to most new pesticides and metabolic detoxification is an important mechanism by which they develop resistance. An increase in tolerance to pesticides in field strains could be attributable to the overexpression of detoxification enzymes owing to the prolonged exposure and frequent application of pesticides with similar chemical profiles. General esterases, glutathione S-transferase (GST) and cytochrome P_{450}s are the key detoxification enzymes in arthropod systems. In the present study, spider mite strains collected from Bela-bela showed the highest α- esterase activity followed by Sikwane, Francistown, Glen Valley, Moshupa, Bobonong and Letlhakane. The highest β- esterase activity was recorded in Sikwane followed by Glen-Valley, Bobonong, Francistown, Letlhakane, Moshupa and Bela-bela. Elevated activity of esterases is a major

resistance mechanism for pyrethroid and organophosphate resistance in insects. The elevated esterase activities recorded in Sikwane, Francistown, and Glen- Valley strains might be due to unselective use of pyrethroid and organophosphate pesticides in these locations. These pesticides are highly favoured because of their quick knock down effect. These farms are also in close proximity to the city Gaborone where there is a demand for high quality and quantity of 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. Esterases are also responsible for cross resistance to carbamate, organophosphate and pyrethroid pesticides. The Letlhakane and Bela-bela strains showed low titre of α and β –esterase activities respectively as compared with other spider mite strains in this study. The detoxification enzymes, cytochrome P_{450} monooxygenases are responsible for the hydrolysis of several compounds by breaking the ester linkage, thereby degrading various organophosphate and pyrethroid pesticides with carboxyl or amide groups. Enhanced activity of cytochrome P_{450} monooxygenase was found to be responsible for pyrethroid resistance in arthropods. The present study showed that Francistown had the highest P_{450} monooxygenase activity followed by Bobonong, Sikwane, Glen Valley, Moshupa, Letlhakane and Moshupa. Enhanced activity of P_{450} monooxygenase may indicate that synthetic pyrethroids are overused in these farming locations. The Bela-bela strain showed low titre of cytochrome P_{450} monooxygenase as compared with other strains in this study suggesting that pyrethroids are not oversused. Elevated levels of GSTs were observed in the Francistown strain. The descending order of GST activity was Francistown, Moshupa, Bela-bela, Glen Valley and Bobonong. The Sikwane, Letlhakane and Bobonong strains showed low titre of GST activity when compared with other strains from other locations. GSTs are documented to confer resistance to organophosphates, organochlorines and pyrethroid

pesticides. However, GST activity has been associated with resistance to all main classes of pesticides. Enhanced activity of GSTs may indicate that organophosphates, organochlorines and pyrethroids are overused in these farming locations. This study observed that the activities of detoxification enzymes varied across geographic locations. It is expected that resistance development in spider mite populations becomes more pronounced in spider mite strains from fields with high pesticide usage. Enzyme activity is directly related to pesticide pressure and thus it can vary in locations due to the pesticide application regimes. 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 pesticide active ingredient. Pesticides used in agriculture can contaminate water sources through run-off, 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.

7.2 RECOMMENDATIONS

The study suggests that the current extension programmes should be strengthened to better disseminate information on proper pest management practices to vegetable farmers. Intensified agronomic information dissemination through workshops, short courses, and in-field training would be ideal for producers across the country. Instead of applying pesticides at the sight of the

pest or damage symptoms, farmers can be advised use economic decision levels to apply pesticides in the most economical manner. Government through the registrar of agrochemicals should restrict the availability of the most hazardous formulations and regulate the use of pesticides. Vegetable farmers should be advised to employ an integrated pest management strategy for pests and diseases. IPM can help reduce their use of pesticides and avoid development of pest resistance. ore work on farmers' management practices for spidermites should be carried out in the future. The study demonstrated that the three products evaluated are effective for the control of CSM eggs and adults. It can be recommended that chlorfenapyr and methomyl be used to target the eggs before they hatch in order to avoid any damage by subsequent damaging stages. Farmers can be advised to allow for longer exposure periods to achieve required results when using abamectin. The CSM population in this study did not show any signs of methomyl and chlorfenapyr resistance, therefore it can be recommended that methomyl and chlorfenapyr use be continued. Furthermore, lower dosages need to be evaluated to confirm their effectiveness in the protection of tomato plants. Reduction in dosage would result in reduction in the cost of controlling CSM by farmers. The results of the study also suggest that the three pesticides can be used in an integrated management program for CSM and thereby avoid the selection of resistant populations by using only one product. More detailed studies and field testing are necessary to confirm these laboratory findings and determine the sources of variation in effectiveness of the tested materials. It is important to consider the level and role of various detoxification enzymes in a population when formulating management strategies against spider mites and they should be location specific. Understanding the biochemical mechanisms of pesticide metabolism will prove helpful when replacing the existing pesticide formulations with those which are not affected by these enzymes. Farmers should be trained to implement proper spider mite management and pesticide application programs

that focus on prioritization and understanding of the potential risks of resistance development. Proper and efficient use of pesticides could help growers derive use from a pesticide for a longer period. This would also provide researchers with the necessary time to develop and register new pesticide formulations for efficient spider mite control. In addition, there is a need for more confined enzymatic analyses for spider mites in order to provide comparable results from various geographical locations. The data obtained in this study can be used as a baseline for resistance monitoring and development of appropriate strategies to manage spider mites on tomato. More research should be undertaken to measure detoxification enzyme levels for effective CSM control in Botswana.