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Vol. 9(51), pp. 3704-3711, 18 December, 2014
DOI: 10.5897/AJAR2014.8679
Article Number: A9F0F1F49071
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effectiveness of cypermethrin against diamondback moth (*Plutella xylostella* L.) eggs and larvae on cabbage under Botswana conditions

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Received 11 March, 2014; Accepted 7 November, 2014

The efficacy of cypermethrin against the diamondback moth (DBM) on cabbage was studied at Botswana College of Agriculture, Gaborone, Botswana. Using five concentrations of cypermethrin: 1.2, 1.6, 2.0, 2.4 and 2.8 g/L, bioassays were conducted against DBM eggs and second instar larvae at 30±5°C. Each treatment was replicated three times. Probit analysis was used to determine LD_{50} and LD_{90} values for the treatments against eggs and larvae. When the treatments were assessed at 48, 72 and 96 h, LD_{90} values against larvae were 2.01, 1.82 and 1.19 g/L, whereas they were 1.69, 1.63 and 1.40 g/L against eggs. This indicated that cypermethrin was highly effective against both eggs and larvae. The slopes of the probit lines for larvae assessed at 48, 72 and 96 h after application were 0.999, 0.995 and 0.949, while those against eggs were 0.973, 0.961 and 0.945. This indicates a rapid change in mortality with increase in pesticide dosage for both eggs and larvae. The study shows that cypermethrin can still be used to achieve effective control of DBM eggs and larvae under Botswana conditions especially when used in combination with other control methods in an integrated pest management programme.

Key words: Cypermethrin, efficacy, diamondback moth, cabbage.

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is an extensively grown vegetable in the world (Sances, 2000). It is among the most popular food crops in Botswana households; it grows well in many parts of the country (Bok et al., 2006). However, its production is seriously affected by a wide range of pests including the diamondback moth (*Plutella xylostella* L.), bagrada bug (*Bagrada hiliaris* Burn) and the cabbage aphid (*Brevicoryne brassicae* L.) (Munthali, 2009). The most serious among these is DBM, which has a cosmopolitan distribution; it is believed to be the most universally distributed species among the Lepidoptera; and it occurs wherever brassicas are grown (Talekar and Shelton, 1993). DBM was first recorded as an important pest of cabbage in Southern Africa as early as 1917 (Charleston and Kfir, 2000). It is highly migratory; and its seasonal movements have been well documented (Talekar and Shelton, 1993). Its exceptional pest status is due to

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several factors: The diversity and abundance of host plants, the disruption of its natural enemies, its high reproductive potential (with over 20 generations per year in the tropics), and its genetic elasticity which leads to rapid development of resistance to insecticides (Kahuthia-Gathu et al., 2009; Shelton, 2004). DBM is most destructive in areas where there is frequent application of insecticides. In Botswana and other Southern African countries the control of DBM relies heavily on the use of synthetic insecticides (Talekar et al., 1990). However, it has been demonstrated that DBM quickly develops resistance to many new insecticides (Fahmy and Miyata, 1991; Shelton and Nault, 2004). It has reportedly developed resistance to most synthetic pyrethroids, organophosphates, carbamates, and actinomycetes in many cabbage growing areas of the world (Talekar et al., 1990; Sereda et al., 1997); this represents a serious threat to its effective management.

Cypermethrin is one of the most widely used insecticides in Botswana (Obopile et al., 2008). Like other synthetic pyrethroids, cypermethrin has a chemical structure that is based on natural pyrethrum extracted from flowers of chrysanthenum (Ware and Whitacre, 2004). It is a mixture of eight isomers (USDA, 1995). Cox (1996) reported that cypermethrin was used worldwide to control many pests, including lepidopteran pests of cotton, fruit, and vegetable crops. It affects target insects by disrupting normal functioning of the nervous system (Cox, 1996). Cypermethrin delays the closing of the "gate" that allows the sodium flow along the nerve. This results in multiple nerve impulses instead of the usual single impulse. In turn, these impulses cause the nerve to release the neurotransmitter acetylcholine which stimulates other nerves (Eells, 1992).

Cypermethrin inhibits the γ-aminobutyric acid receptor, causing excitability and convulsions (Cox 1996). It also inhibits calcium uptake by the nerves. Cypermethrin affects the enzyme adenosine triphosphate, which is not directly involved with the nervous system; but is involved in cellular energy production, transport of metal ions and muscle contraction (El-Toukhy and Girgis, 1993).

Although cypermethrin is the most popular insecticide for the control of DBM in Botswana, its broad spectrum characteristic causes mortality of non-target beneficial arthropods in the field; and reduction in invertebrate biodiversity. This loss of biodiversity is undesirable, especially because it can lead to insecticide induced pest resurgence (Hardin et al., 1995).

Farmers chose to use cypermethrin against destructive pests such as DBM because they believe that it provides rapid, effective and economic control. However, the widespread and frequent use exerts a heavy selection pressure on the pest population which has resulted in the development of pest resistance to it (Baek et al., 2010; Furlong et al., 2008). The efficacy of cypermethrin against DBM has not been evaluated in Botswana despite the fact that the pesticide has been used to control the pest for over 10 years. This study evaluated the efficacy of cypermethrin against DBM eggs and larvae under Botswana conditions.

MATERIALS AND METHODS

The experiment was conducted at the Botswana College of Agriculture in Gaborone, Botswana (24° 34' 25''S, 25° 95' 0" E; altitude: 998 m) in cages that were placed in a greenhouse, at an average temperature of 30 ± 5°C. The cabbage seedlings were initially raised in nursery trays and transplanted into small black plastic sleeve pots filled with loam soil; each pot was 12 cm in diameter and 15 cm in depth. Cabbage seedlings at the 5 leaf stage were used to rear the diamond back moth to ensure adequate host substrate for oviposition of eggs by adults. The seedlings were watered regularly adlib to prevent wilting. Nine potted plants were placed in each of six insect rearing cages. Each cage was 45 cm long, 45 cm wide and 40 cm high; it was covered with clear lumite netting of 32 mesh size; this was to prevent pest infestation from natural populations or escape of insects from the artificially infested plants in the cage. Every cage had a door with a sleeve that was used during the watering of plants and their artificial infestation, the application of sprays, feeding of adult insects and the removal of plants at each pest assessment.

Bioassay methods

Cypermethrin; emulsifiable concentrate (Avi-sipermetrin®), registered for use in Botswana, was used in the bioassay experiment. A small hand held trigger sprayer that produced a fine spray of a relatively narrow range of droplet sizes was used to apply spray solutions. Six treatments comprising five cypermethrin concentrations (1.2, 1.6, 2.0, 2.4 and 2.8 g/L water) and distilled water were used. The recommended rate (2.0 g/L) was included as a check. The 6 treatments were arranged in a completely randomized design. Each treatment had nine seedlings. The sprays against eggs were applied when each plant had more than 50 eggs; and those against larvae were made when plants had more than 30 larvae each. Each seedling was sprayed separately. The bioassay was repeated 3 times. This gave a total of 54 treated plants per bioassay and 162 sprayed plants all together. Each pot had a label which indicated the treatment and its date of application. The bioassay was conducted on eggs and second instar larvae (the first instar larvae are leaf miners which are not susceptible to a pesticide with a contact and stomach poison mode of action such as cypermethrin). DBM eggs used in the bioassay were obtained by placing 50 laboratory bred pupae in each of six insect rearing cages that contained 9 potted cabbage seedlings. Adults emerging from the pupae were left to oviposit on the seedlings for 4 days before they were removed from the cages. Each seedling was examined using a hand lens at 10x magnification; the eggs laid on the leaves were counted. The artificially infested seedlings were sprayed with 5 concentrations of the insecticide and water which was the control treatment.

Assessment of egg and larval mortality

As viable DBM eggs take an average of 4 day to hatch at 25±5°C (Chan et al., 2008), treatments against eggs were applied 3 days after oviposition. The eggs oviposited on each plant were counted immediately before application of treatments followed by counts at 48, 72 and 96 h intervals. Egg mortality was determined by comparing the number of eggs prior to application of treatments with numbers found after treatment. The eggs found unhatched after each treatment were considered dead. For larval mortality, the

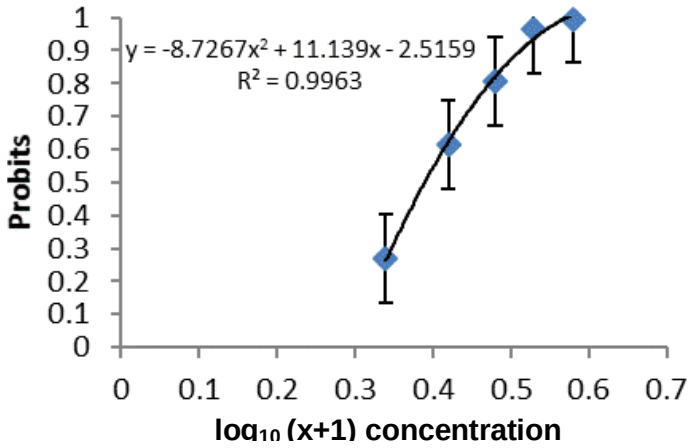

Figure 1. The Probit mortality of DBM larvae 24 h after application of different doses of cypermethrin.

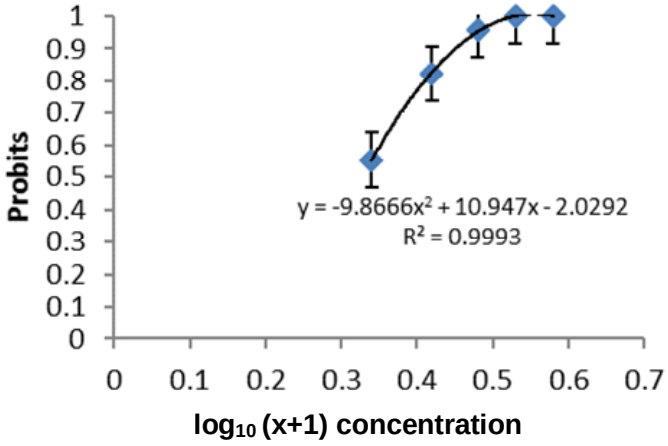

Figure 2. The Probit mortality of DBM larvae 48 h after application of different doses of cypermethrin.

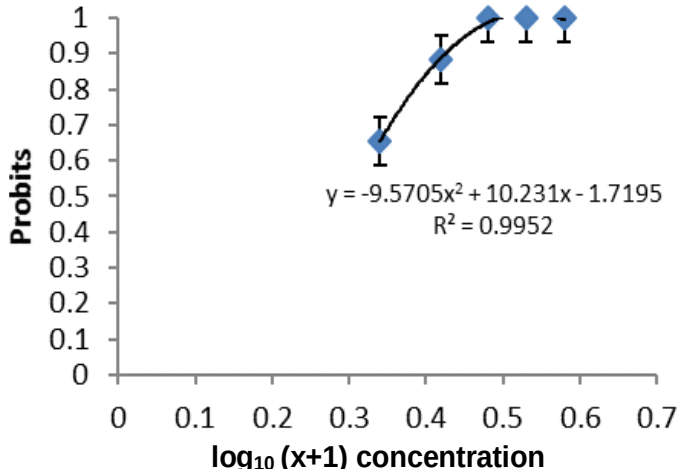

Figure 3. The Probit mortality of DBM larvae 72 h after application of different doses of cypermethrin.

eggs were allowed to hatch into first instars and to develop into second instar larvae; first instar larvae are leaf miners and second instar larvae are surface feeders therefore they were easy to differentiate; these were counted before treatment. The larvae were assessed at intervals of 24, 48, 72, 96, 120 and 144 h after treatment. Any larvae that did not show signs of life after prodding with a needle were counted as dead.

Plant damage assessment

Plant damage assessments in each treatment were conducted 14 days after DBM eggs had hatched. The total number of leaves per plant was recorded; the number of leaves with damage symptoms was counted; and the results were used to calculate the percentage of damaged leaves per plant. The number of windows per leaf for each plant was also recorded and used to estimate the intensity of damage caused per plant. The experiment was repeated 3 times.

Data analysis

Probit analysis (Finney, 1971; Mead and Curnow, 1983) was used to analyse mortality results. The mortality data were transformed to probits while the dosages were transformed to $\log_{10}$ (x+1) before analysis. LD_{50} and LD_{90} values were estimated from the probit lines. Relative susceptibilities of eggs and second instar larvae were compared using LD_{50} values and slopes of probit lines. LD_{90} values were used to compare the mortalities that the recommended dosage caused to the mortalities that were achieved by treatments at different periods of exposure to cypermethrin.

The results on percentage seedling damage were transformed to arcsines before analysis in order to normalize them. Using the MSTATC (1985) statistical package, analysis of variance (ANOVA) was used to analyse the data. Averages were separated using the Tukey's Honestly significant difference test (Zar, 1984) where significant effects were found.

RESULTS

DBM larval mortality

Figures 1 to 4 show positive curvilinear relationships between log dose and probit mortality caused by cypermethrin (correlation coefficients of 0.996, 0.999, 0.995 and 0.949), when treatments were assessed at 24, 48, 72 and 96 h after pesticide application. Figure 1 shows that LD_{50} of 1.50 g/L and LD_{90} of 2.31g/L were achieved 24 h after application. The recommended dose (2.0 g/L) of the pesticide showed a probit value of 0.806 (equivalent to 63.87% larval mortality) during this exposure period. Figure 2 indicates that the LD_{90} of cypermethrin after 48 h exposure was 2.01 g/L. At the recommended dose, cypermethrin only achieved 0.959 on the probit scale, which is equivalent to 78.32% larval mortality. When assessed at 72 h after application, the LD_{90} of cypermethrin was 1.82 g/L (Figure 3). The recommended dosage achieved 1.0 on the probit scale, which is equivalent to 100% larval mortality after 72 h exposure. Figure 4 shows an LD_{90} value of 1.19 g/L when the treatments were assessed at 96 h after application. The mortality achieved by the recommended dose was 1.0 on the probit scale, which is equivalent to 100% larval

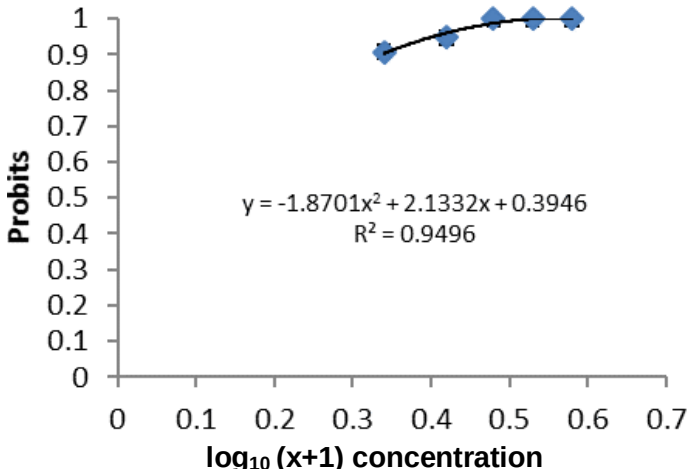

Figure 4. The probit mortality of DBM larvae 96 h after application of different doses of cypermethrin.

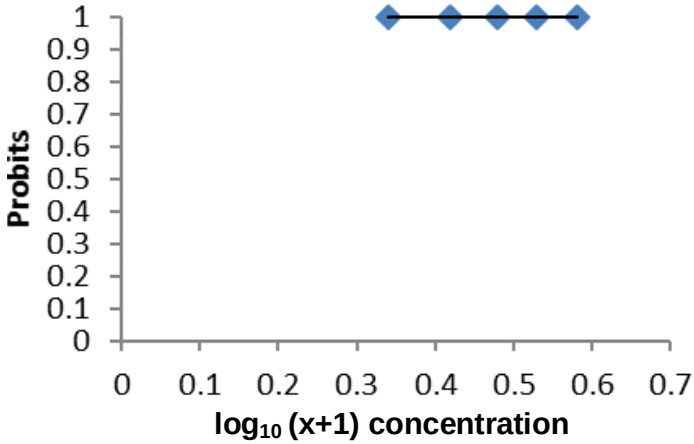

Figure 5. The probit mortality of DBM larvae 120h after application of different doses of cypermethrin.

mortality. Figures 5 and 6 show that when assessed 120 and 144 h after application all cypermethrin concentrations achieved 100% larval mortality.

The results in Table 1 show that both the concentration and the period after pesticide application significantly affected average mortality of DBM larvae per plant (ANOVA, P < 0.05%). The interactions were also significant. The greatest mortality (91.7 to 100.0%) occurred at 120 h after the application of 1.2 and 1.6 g/L concentrations. The recommended dose of 2.0 g/L achieved 91.3% larval mortality during the 72 h assessment period. The results also show that the lowest mortality of 5.0 to 15.0% per plant occurred in the control treatment throughout the assessment period. The overall treatment averages show that cypermethrin concentrations also had a significant (Tukey, P<0.05) effect on the mortality of larvae. The mortalities differed significantly from each other and increased in the order of 11.1 < 67.9 < 77.3 < 89.3 < 95.7 = 96.9% on plants treated with 0.0, 1.2, 1.6, 2.0, 2.4 and 2.8 g/L. The results of overall exposure period were also significantly (Tukey, P<0.05) different, and increased in the order of 53.9 < 65.9 < 71.9 ≤ 77.6 ≤ 82.9 < 85.8 when assessed at 24, 48, 72, 96, 120 and 144 h.

DBM egg mortality

Figures 7 to 9 show a positive curvilinear relationship between the log dose and the mortality of DBM eggs (r values of 0.973, 0.960 and 0.945). The LD_{90} of cypermethrin against eggs was 1.69 g/L when assessed at 48 h (Figure 7). During this period, the recommended dose of 2.0g/L gave a probit value of 1.0, which is equivalent to 100% egg mortality. When assessment was done at 72h, the LD_{90} was 1.63 g/L (Figure 8). The mortality caused by the recommended dose was 1.00 on the probit scale, which is equivalent to 100% egg mortality. The LD_{90} value at 96 h was 1.40 g/L (Figure 9). These results show that the toxicity of cypermethrin to eggs increased with each increase in dosage.

Table 2 shows that the cypermethrin concentration and the period after application significantly affected the average mortality of DBM eggs per plant (ANOVA, P < 0.05%). The interactions were also significant (ANOVA, P<0.05). The greatest egg mortality (100%) occurred on plants which were treated with 2.0 g/L and assessed at 48 h; the lowest egg mortality (60.0%) was on plants treated with 1.2 g/L and assessed at 48 h (Tukey, P,<0.05). The overall treatment averages indicate that concentrations higher than 2.0 g/L caused the greatest mortality (100%) and the lowest concentration (1.2 g/L) caused the least mortality (62.3%). The overall period averages indicate that cyprmethrin caused the greatest mortality (89.8%) when treatments were assessed at 96 h and the lowest mortality (87.9%) when treatments were assessed at 48 h.

DBM damage on cabbage plants

Table 3 shows that damage caused by DBM larvae on plants was significantly (Tukey, P<0.05) affected by the concentration of cypermethrin. DBM larvae caused 79.0% leaf damage on untreated plants; but on plants treated with cypermethrin concentrations of 1.2 and 1.6 g/L the leaf damage caused was 10.3 and 1.7%. DBM larvae on plants treated with the recommended (2.0 g/L) or higher dose did not cause any leaf damage.

DISCUSSION

From the results in Figures 1 to 6 and Tables 1 to 3 several observations can be made: When exposure

Table 1. Effect of cypermethrin concentration and period of exposure on larval mortality.

Period after application (h)	0 g/L	1.2 g/L	1.6 g/L	2.0 g/L	2.4 g/L	2.8 g/L	Overall period averages
24	5.0^{k§}	33.3^{ij}	53.3^{gh}	65.0^{efgh}	80.0^{bcde}	86.7^{abcd}	53.9$^{e¥}$
48	10.0^{k}	50.6^{hi}	66.7^{efg}h	79.3^{bcde}	93.9^{abc}	95.0^{ab}	65.9^{d}
72	11.7^{k}	56.7^{fgh}	72.0^{defg}	91.3^{abcd}	100.0^{a}	100.0^{a}	71.9^{c}
96	11.7^{k}	75.0^{cdef}	79.0^{bcde}	100.0^{a}	100.0^{a}	100.0^{a}	77.6^{bc}
120	13.3^{k}	91.7^{abc}	92.8^{abc}	100.0^{a}	100.0^{a}	100.0^{a}	82.9^{ab}
144	15.0^{jk}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	85.8^{a}
Overall treatment averages	11.1$^{e\$}$	67.9^{d}	77.3^{c}	89.3^{b}	95.7^{a}	96.9^{a}	73

§Interaction averages in the body of the table followed by the same letters are not significantly different (Tukey's Honestly significant difference test (P<0.05). $^{¥}$Averages in the column followed by the same letters are not significantly different (Tukey's Honestly significant difference test (P<0.05). $^{\$}$Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test (P<0.05).

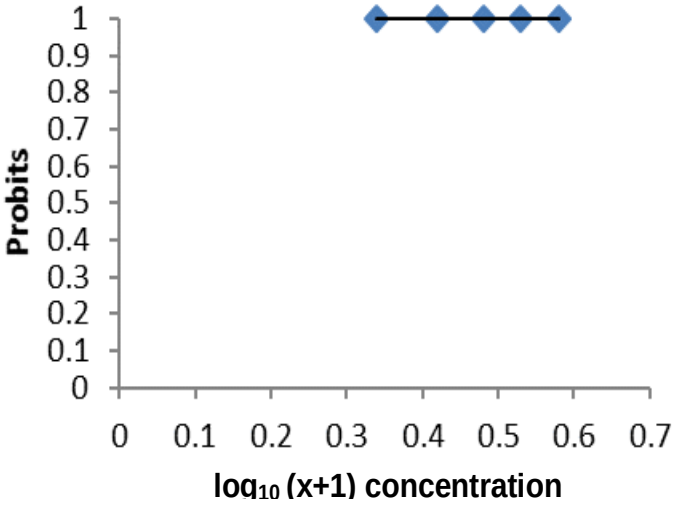

Figure 6. The probit mortality of DBM larvae 144h after application of different doses of cypermethrin.

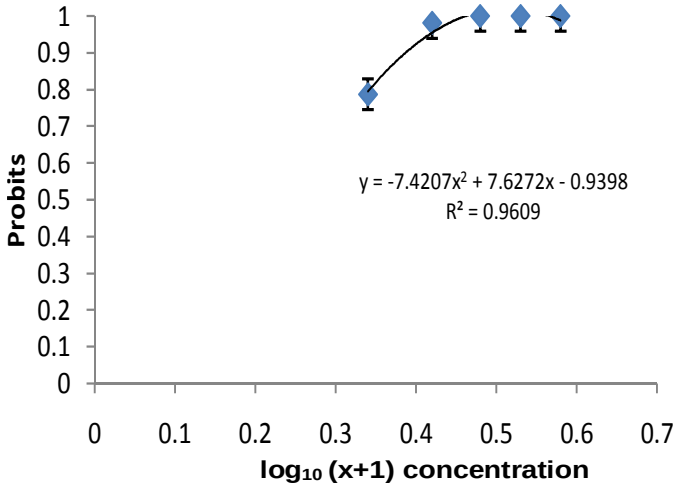

Figure 8. Probit mortality of DBM eggs exposed to different doses of cypermethrin assessed 72 h after expected time of hatching.

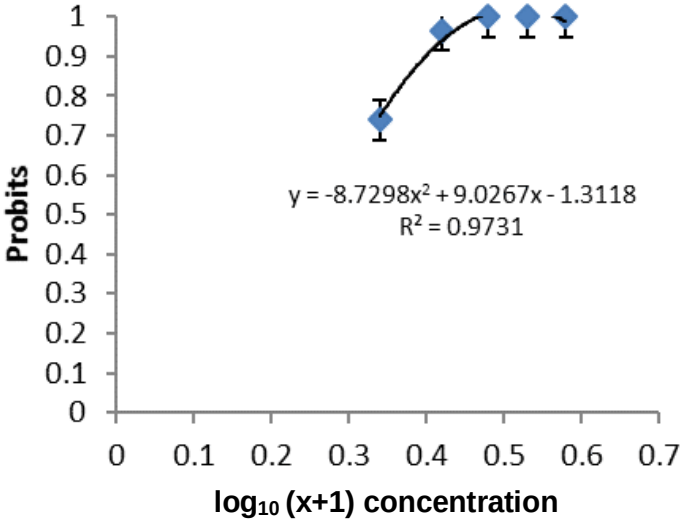

Figure 7. Probit mortality of DBM eggs exposed to different doses of cypermethrin assessed 48 h after expected time of hatching.

periods increased, dosages lower than the recommended dose of cypermethrin were able to cause 90 to 100% larval mortality; the recommended and higher dosages of cypermethrin achieved total protection of the crop from larval damage; when LD_{90}s are used alone to assess the effectiveness of cypermethrin, the mortality level caused by the lowest dose during the 144 h study period, appears to be sufficient to achieve effective control; the level of pest decline was sufficient to significantly (Tukey, $P < 0.05$) reduce crop damage to levels achieved by higher dosages.

The slopes of the probit lines in Figures 1 to 6 shows that only slight increases in the dosage of cypermethrin are needed to cause large increases in mortality of DBM larvae. Cypermethrin provided a rapid pest control per unit concentration of the pesticide. The fact that dosages higher than the recommended dosage of cypermethrin only took 48 h to achieve 90 to 100% mortality shows that higher concentrations can be used to achieve earlier control of DBM larvae. One of the desirable properties of

Table 2. Effect of cypermethrin concentrations and period of exposure on egg mortality.

Period after expected date of hatching (h)	1.2 g/L	1.6 g/L	2.0 g/L	2.4 g/L	2.8 g/L	Overall time averages
48	60.0^{d§}	79.0^{c}	100.0^{a}	100.0^{a}	100.0^{a}	87.8$^{b¥}$
72	63.0^{d}	82.0^{bc}	100.0^{a}	100.0^{a}	100.0^{a}	89.0^{ab}
96	64.0^{d}	85.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	89.8^{a}
Overall treatment averages	62.3$^{c\$}$	82.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	88.9

§Interaction averages in the body of the table followed by the same letters are not significantly different (Tukey's Honestly significant difference test, P<0.05). ¥Averages in the column followed by the same letters are not significantly different (Tukey's Honestly significant difference test, P<0.05). $Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test, P<0.05).

Table 3. Leaf damage caused by DBM larvae on cabbage plants treated with different cypermethrin dosages.

Cypermethrin concentration	0 g/L	1.2 g/L	1.6 g/L	2 g/L	2.4 g/L	2.8 g/L
Treatment averages	79.0^{a§}	10.3^{b}	1.7^{b}	0.0^{b}	0.0^{b}	0.0^{b}

§Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test, P<0.05).

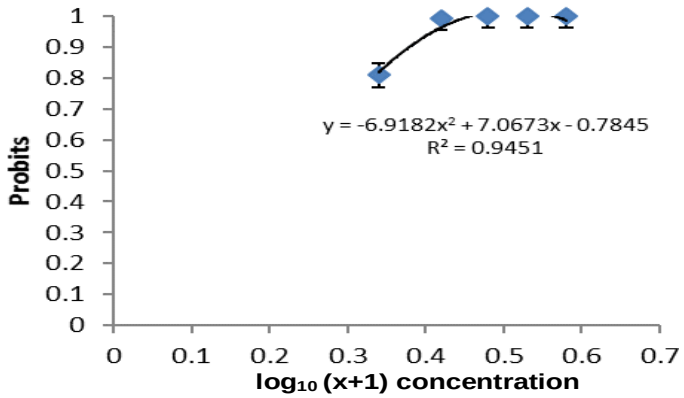

Figure 9. Probit mortality of DBM eggs exposed to different doses of cypermethrin assessed 96h after expected time of hatching.

pyrethroids (including cypermethrin) is that they have a quick knockdown effect (Ware and Whitacre, 2004). The quick knockdown effect can be attributed to the dual mode of action (contact and stomach poison) of cypermethrin (Tomlin, 1994). While DBM eggs can only acquire the lethal dose through contact, larvae can acquire the lethal dose through contact and ingestion of the pesticide material as they feed. This may explain the relatively faster mortality of DBM larvae compared to that of eggs. The fast action of cypermethrin against larvae is a desirable property as this is the damaging developmental stage of the pest.

In this study, the recommended dose achieved 100% egg mortality, when exposed for only 48 h (Figure 7), suggesting that cypermethrin is highly effective against DBM eggs. As cypermethrin is both a contact and stomach poison (Tomlin, 1994), the egg mortalities were due to direct hit or contact with the active ingredient which spread from deposits on the leaf surface to the eggs. The high egg mortality achieved with cypermethrin sprays means that the buildup of larval populations from hatching eggs would be reduced, thereby minimizing subsequent damage by DBM larvae on host plants. Therefore, when using cypermethrin against DBM, the egg is the most susceptible stage to target.

CONCLUSIONS AND RECOMMENDATIONS

The objective of applying insecticides against crop pests at the recommended dose is to ensure the production of large quantities of high quality crop yields by using minimum amounts of active ingredient. It can be concluded from this study that cypermethrin can offer effective control of DBM eggs and larvae and prevent serious damage to cabbage. Lower dosages than those recommended can be used to control DBM, particularly when applications target the egg stage and when long exposure periods are allowed. Since the population in this study did not show any signs of cypermethrin resistance, it is recommended that the use of cypermethrin for the control of DBM in Botswana should continue. However, lower dosages need to be evaluated to validate their effectiveness under field conditions. Reduction in dosages would result in reduction in cost of controlling DBM by farmers and slow down the development of resistance in subsequent populations.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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