Efficacy of Bacillus Thuringiensis (var. Kurstaki) against Diamondback Moth (Plutella Xylostella L.) Eggs and Larvae on Cabbage Under Semi-Controlled Greenhouse Conditions

Authors: Legwaila, Mitch M., Munthali, David C., Kwerepe, Baone C., and Obopile, Motshwari

Source: International Journal of Insect Science, 7(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/IJIS.S23637

Efficacy of *Bacillus thuringiensis* (var. kurstaki) Against Diamondback Moth (*Plutella xylostella* L.) Eggs and Larvae on Cabbage Under Semi-Controlled Greenhouse Conditions

Mitch M. Legwaila[1], David C. Munthali[2], Baone C. Kwerepe[2] and Motshwari Obopile[2]

[1]Botswana National Museum, [2]Botswana College of Agriculture, Gaborone, Botswana.

ABSTRACT: The efficacy of *Bacillus thuringiensis* (var. kurstaki) (*Btk*) against the diamondback moth (DBM) on cabbage was studied at Botswana College of Agriculture, Gaborone, Botswana. Using five concentrations of *Btk*: 2, 4, 6, 8, and 10 g/L, bioassays were conducted against DBM eggs and second instar larvae at 30°C ± 5°C. Each treatment was replicated three times. Probit analysis was used to determine the LD_{50} and LD_{90} values for the treatments against eggs and larvae. When the treatments were assessed at 72, 96, 120, and 144 hours, LD_{90} values against larvae were 11.02, 10.22, 5.92, and 4.01 g/L, whereas they were 7.71, 6.94, and 6.24 g/L against eggs when assessed 48, 72, and 96 hours after the expected time of hatching. This indicated that *Btk* was effective against both eggs and larvae when exposed for long periods. The slopes of the probit lines for larvae assessed at 24, 48, 72, 96, 120, and 144 hours after application were 0.250, 1.064, 0.910, 0.383, 0.453, and 0.414, while those against eggs were 1.153, 1.246, and 0.933 when assessed 48, 72, and 96 hours after the expected time of hatching. This indicates a smaller change in mortality with increase in pesticide dosage for both eggs and larvae. *Btk* treatments achieved 85.7%–94.6% reduction in DBM damage on cabbage. Therefore, *Btk* can be used to achieve effective control of DBM eggs and larvae and reduce damage on cabbage under greenhouse conditions.

KEYWORDS: *Bacillus thuringiensis*, efficacy, diamondback moth, cabbage

CITATION: Legwaila et al. Efficacy of *Bacillus thuringiensis* (var. kurstaki) Against Diamond-back Moth (*Plutella xylostella* L.) Eggs and Larvae on Cabbage Under Semi-Controlled Greenhouse Conditions. *International Journal of Insect Science* 2015:7 39–45 doi:10.4137/IJIS.S23637.

RECEIVED: January 7, 2015. **RESUBMITTED:** March 22, 2015. **ACCEPTED FOR PUBLICATION:** April 13, 2015.

ACADEMIC EDITOR: Vicky Schneider, Editor in Chief

TYPE: Original Research

FUNDING: The funding for this study was through a self-sponsored MSc scholarship for the first author and through support by the Botswana College of Agriculture to the other three co-authors. The authors confirm that the funders had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: dc.munthali@yahoo.com

Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is an extensively grown vegetable around the world.[1] It is among the most popular food crops in Botswana and grows well in many parts of the country.[2] However, its production is seriously affected by a wide range of pests including the diamondback moth (*Plutella xylostella* L.) (DBM), bagrada bug (*Bagrada hiliaris* Burn), and the cabbage aphid (*Brevicoryne brassicae* L.).[3] 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 occurs wherever brassicas are grown.[4] DBM was first recorded as an important pest of cabbage in Southern Africa as early as 1917.[5] It is highly migratory, and its seasonal movements have been well documented.[4] Its exceptional pest status is due to 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.[6,7] DBM is most destructive in areas where there is frequent application of insecticides. In Botswana, the control of DBM relies heavily on the use of synthetic insecticides.[8] However, it has been demonstrated that DBM quickly develops resistance to many new insecticides.[9,10] It has reportedly developed resistance to most synthetic pyrethroids, organophosphates, carbamates, and actinomycetes in many cabbage growing areas of the world;[8,11] this represents a serious threat to its effective management. Unfortunately, in Southern Africa, control of DBM is still heavily dependent on these conventional synthetic pesticides.[8] Such pesticides can affect nontarget organisms in both treated and untreated fields. Therefore, efforts to promote the use of microbial insecticides as alternatives are continually being made, and one such alternative is the use of *Bacillus thuringiensis*.

The most successful of the microbial products are those based on *Bacillus thuringiensis* Berliner (*Bt*). *B. thuringiensis* (*Bt*) var. *aizawai* and var. *kurstaki* (*Btk*) products are recommended for the control of DBM in South Africa.[12] *Bt* is a spore-forming, soil-inhabiting bacterium that produces a selectively toxic protein in the form of a crystal within the cell. This protein inclusion is the active component in *Bt* products. It consists of a protoxin form of one or more α-endotoxins. When this inclusion is ingested by a susceptible host, it is solubilized and the protoxin is processed to the

active δ-endotoxin form. The insecticidal crystal proteins are divided into five major classes, namely cry 1, cry 2, cry 3, cry 4, and cry 5, with specific insecticidal activity against Lepidoptera, Lepidoptera and Diptera, Coleoptera, Diptera and Lepidoptera, and Coleoptera, respectively.[13] The active toxin binds to specific receptor sites on the gut epithelium, leading to slow degradation of the gut lining and starvation. *Bt* slows development, lowers survival, and reduces rates of larval feeding. The toxins also affect the muscular and nervous systems.[14] Thus, several days are required to kill insects that have ingested *Bt* products.[14] Death generally occurs within 1–3 days.[15] Effective commercial *Bt* products for the control of caterpillars have been available all over the world for over 20 years and have been used against lepidopteran pests of brassicae crops for many years.[13]

With the increasing costs and risks posed by synthetic pesticides, advances in biotechnology have facilitated efficient production of microbial insecticides. As a result, the use of *Bt* in pest control has become an important tool. The use of *Bt* in most cabbage growing areas of the world is likely to increase in the future. Munthali et al[16] suggested that the use of *Bt* is the most effective method of controlling DBM in areas where resistance has not yet been reported.

Microbial insecticides, particularly those derived from *Bt*, offer a great deal of promise for pest management. *Bt* insecticides are highly effective against certain pests, yet they do not harm humans, most beneficial insects, and other nontarget organisms.[17] Thus *Bt* insecticides do not pose the serious environmental hazards associated with conventional pesticides.

Although resistance to conventional insecticides is widespread,[18] few cases of resistance to *Bt* have been reported.[17] However, potential for development of pest resistance is a threat to the continued success of *Bt*.[15] Increased use of *Bt* is likely to intensify selection for resistance in the field. Development of substantial levels of resistance to *Bt* in DBM has been documented for two field populations from watercress and one from cabbage in Hawaii.[17]

The usefulness of *Bt* for pest management has been increased by the development of new strains and recent advances in genetic engineering, including the insertion and expression of *Bt* toxin genes in major crop plants and in plant-colonizing bacteria.[15]

Several factors influence the effectiveness of this entomogenous bacterium when it is employed in microbial insect control. During the application of the bacterium, a suspension of minute particles is deposited on the plant foliage. From the time the bacterium leaves the application equipment until it is consumed by the target species, it is exposed to all the fluctuations of the environmental conditions. The period of exposure will vary depending on the feeding habits and activities of the target insect. During this period, sunlight and temperature appear to be major deleterious environmental factors.[19] Burges and Hussey[19] found that low temperatures in the field would allow more exposure of the bacterium to solar radiation, rainfall, etc, and would reduce the effectiveness of the bacterium. Higher temperatures would allow the bacterium to kill the insect before solar radiation could have its effect.

Because of its low toxicity to many beneficial insects, *Bt* is suitable for use in integrated pest management (IPM) programs, especially where pests have developed resistance to other insecticides.[20] The application of *Bt* as a component of an IPM program can reduce environmental pollution, deleterious impact on beneficial entomofauna, and delay the expression of resistance to other pesticides.[7] However, little is known about its effects when applied against DBM eggs and larvae. This study evaluated the efficacy of *Btk* on DBM eggs and larvae under semicontrolled greenhouse conditions.

Materials and Methods

Experiments were conducted at the Botswana College of Agriculture, Gaborone, Botswana (24°34′ 25″S, 25°95′0″E; altitude: 998 m) from June to December 2010 in cages that were placed in a greenhouse at an average temperature of 30°C ± 5°C and a day length average of 9.8 h per day. 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 five-leaf stage were used to rear the DBM 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, and was covered with clear lumite netting of 32 mesh size 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 watering of plants, artificial infestation of the plants, feeding of the adult insects, the application of sprays, and the removal of plants during pest and plant damage assessments.

Bioassay methods. *Btk*, Vectobac® (3000 ITU/mg) (soluble granules), registered and locally available 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 the spray solutions. Six treatments comprising five *Btk* concentrations (2, 4, 6, 8, and 10 g/L water) and distilled water were used. The manufacturer's recommended rate (4 g/L) was included as a check. Each treatment had nine seedlings. The sprays against eggs were applied when each plant had between 50 and 70 eggs, and those against larvae were made when plants had between 30 and 40 larvae each. Each seedling was sprayed separately. The bioassay was repeated three times. This gave a total of 54 plants per bioassay and 162 sprayed plants altogether. 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 stomach poison mode of action such as *Btk*). DBM eggs used in the bioassay were obtained by

placing 50 laboratory-bred pupae in each of six insect rearing cages that contained nine 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 ×10 magnification, and the eggs laid on the leaves were counted. Each artificially infested seedling was sprayed with one of five different insecticide concentrations or distilled water for control.

Assessment of egg and larval mortality. As viable DBM eggs take an average of 4 days to hatch at 25 ± 5°C,[21] 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 hours. Egg mortality was determined by comparing the number of viable eggs prior to application of treatments with the numbers found after treatment. The eggs found unhatched after each treatment were considered dead. For larval mortality, eggs were allowed to hatch into first instars and develop into second instar larvae. The 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 hours 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 the 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. When the tiny DBM larvae hatch from the eggs, they penetrate the leaf and immediately begin to feed between the upper and lower epidermis, removing the mesophyll tissue and the chlorophyll and leaving the clear upper and lower epidermis intact, which is called a "window". The number of windows per leaf for each plant was recorded and used to estimate the intensity of damage caused per plant.

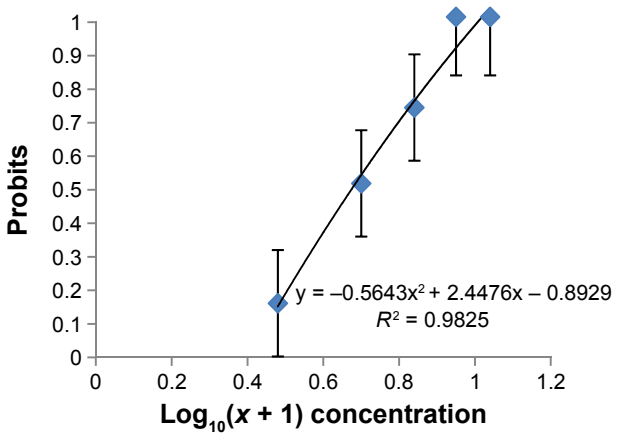

Figure 1. Probit mortality of DBM eggs exposed to different doses of *Btk* assessed 48 hours after expected time of hatching.

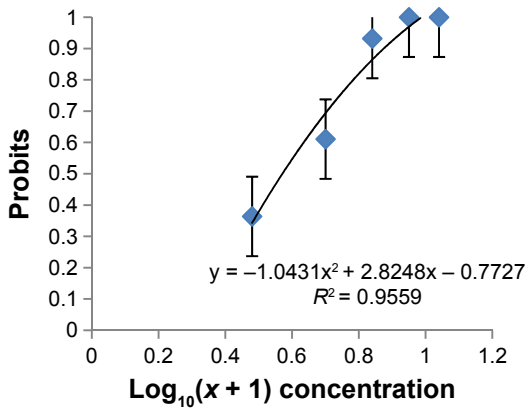

Figure 2. Probit mortality of DBM eggs exposed to different doses of *Btk* assessed 72 hours after expected time of hatching.

The experiment was repeated three times. A single assessment was conducted at day 14 of each of the three trials.

Data analysis. Probit analysis[22,23] was used to analyze the 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 caused by the recommended dosage to those that were achieved by the other dosage levels at different periods of exposure to *Btk*.

The results on percentage seedling damage were transformed to arcsines before analysis in order to normalize them. Using the MSTATC[24] statistical package, analysis of variance (ANOVA) was used to analyze the plant damage data. Averages were separated using the Tukey's Honestly significant difference test[25] where significant effects were found.

Results

DBM egg mortality. Figures 1–3 show a positive linear relationship between log dose and mortality (transformed to probits) caused by *Btk* on DBM eggs (r^2-values of 0.983, 0.956,

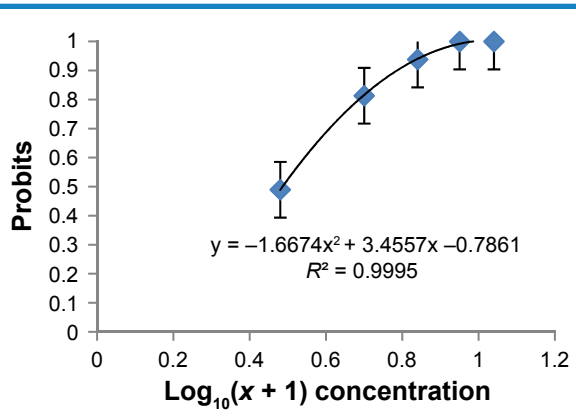

Figure 3. Probit mortality of DBM eggs exposed to different doses of *Btk* assessed 96 hours after expected time of hatching.

Table 1. Effect of *Btk* concentrations and period of exposure on egg mortality.

PERIOD AFTER EXPECTED DATE OF HATCHING (HOURS)	2 g/L	4 g/L	6 g/L	8 g/L	10 g/L	OVERALL PERIOD AVERAGES
48	24.0g*	46.0de	60.0c	100.0a	100.0a	66.0c**
72	38.0f	51.0d	75.0b	100.0a	100.0a	72.8b
96	44.0ef	64.0c	76.0b	100.0a	100.0a	76.8a
Overall treatment averages	35.3d***	53.7c	70.3b	100.0a	100.0a	71.9

Notes: *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 ($P < 0.05$).

and 0.999, respectively). Figure 1 indicates that the LD_{50} and LD_{90} of *Btk* against DBM eggs when assessed 48 hours after expected hatching of eggs was 3.79 and 7.71 g/L, respectively. The recommended dose (4.0 g/L) of *Btk* gave a probit value of 0.52 (equivalent to 46.15% egg mortality) during this period. When the assessment was done at 72 hours after expected hatching period, the LD_{50} and LD_{90} values of *Btk* were 2.60 and 6.94 g/L, respectively (Fig. 2). The mortality caused by the recommended dose was 0.61 on the probit scale (which is equivalent to 51.35% egg mortality). The LD_{50} and LD_{90} values of *Btk* were 1.75 and 6.24 g/L, respectively, 96 hours after they were expected to have hatched (Fig. 3). The recommended dosage achieved 0.81 on the probit scale (equivalent to 64.16% egg mortality).

Table 1 shows that the average mortality of DBM eggs per plant was significantly affected by both *Btk* concentration and period after application. The interactions were significant (ANOVA, $P < 0.05\%$). The greatest mortality (100%) occurred on plants treated with 8 and 10 g/L dose assessed 48 hours after the eggs were expected to have hatched, while the lowest (24.0%) was on plants treated with 2.0 g/L assessed 48 hours after the expected incubation period of eggs (Tukey $P = 0.05$) (Table 1). The overall treatment averages show that *Btk* caused the greatest average egg mortalities (76.8%) when treatments were assessed 96 hours after expected egg incubation period and that the lowest (66.0%) occurred 48 hours after the expected egg incubation period.

DBM larval mortality. Figures 4–9 show a positive linear relationship between the log dose and probit mortality caused by *Btk*. Figure 4 indicates that *Btk* insecticide did not achieve 50% larval mortality 24 hours after application at all the concentrations. The recommended dose (4 g/L) achieved 0.074 (equivalent to 15.79%), 0.157 (equivalent to 23.34%), 0.563 (equivalent to 48.62), 0.740 (equivalent to 59.34%), 0.832 (equivalent to 65.80%), and 1.0 (equivalent to 100%) larval mortalities 24, 48, 72, 96, 120, and 144 hours after exposure, respectively. The LD_{50} values achieved with application of *Btk* when assessed 48 and 72 hours (Figs. 5 and 6) were 6.71 and 3.07 g/L, respectively. The LD_{90} values achieved with application of *Btk* when assessed 72, 96, 120, and 144 hours were 11.02, 10.22, 5.92, and 4.01 g/L, respectively.

Results in Table 2 show that both concentration and period after *Btk* application significantly affected the average mortality of DBM larvae per plant (ANOVA, $P < 0.05\%$). The interactions were also significant. The greatest mortality (90.8, 92.3, 93.0, and 93.3% per plant) occurred 144 hours after application of 4.0, 6.0, 8.0, and 10.0 g/L *Btk* solutions, while 91.7% mortality occurred 120 hours after application of 10 g/L. The recommended dose (4 g/L) took 144 hours to achieve 90% larval mortality. The results also show that the

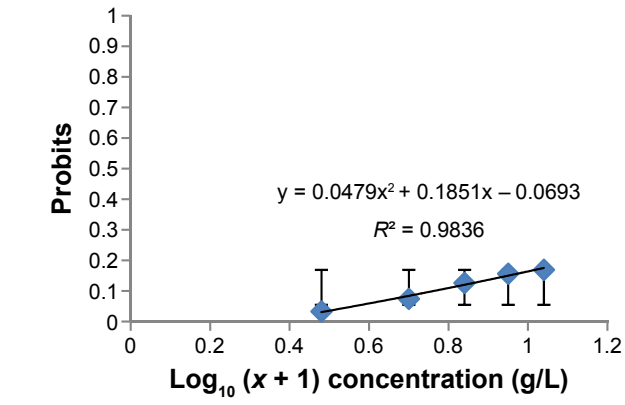

Figure 4. Probit mortality of DBM larvae 24 hours after application of different doses of *Btk*.

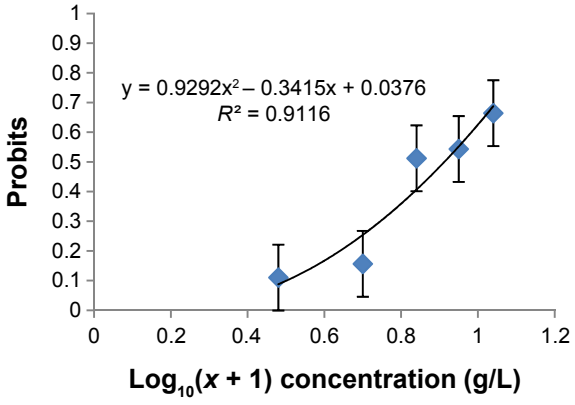

Figure 5. Probit mortality of DBM larvae 48 hours after application of different doses of *Btk*.

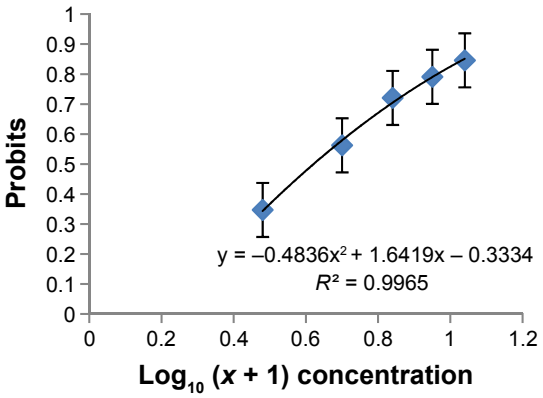

Figure 6. Probit mortality of DBM larvae 72 hours after application of different doses of *Btk*.

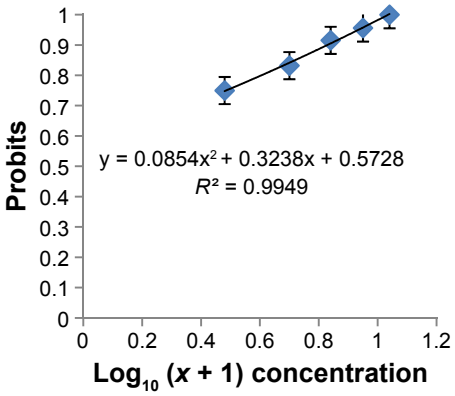

Figure 8. Probit mortality of DBM larvae 120 hours after application of different doses of *Btk*.

least mortality (0.0–26.7% per plant) occurred in the control treatment throughout the study period. Mortalities that occurred in the control treatment 144 hours after application of treatments were similar to those achieved 72 hours after application of 2.0 g/L, 48 hours after application of 4.0 g/L solution, and 24 hours after application of 6.0, 8.0, and 10.0 g/L *Btk* solution (Tukey, $P < 0.05$). The overall treatment averages show that *Btk* concentrations also had a significant effect on the mortality of larvae. Overall larval mortalities differed significantly from each other and increased in the order $44.8 < 53.6 < 61.5 < 63.9 < 68.7\%$ on plants treated with 2.0, 4.0, 6.0, 8.0, and 10.0 g/L, respectively. The overall exposure period results were also significantly different and increased in the order $17.9 < 34.2 < 48.1 < 57.8 < 66.9 < 77.1$ when assessment was done 24, 48, 72, 96, 120, and 144 hours after application.

DBM damage on cabbage plants. Table 3 shows the effect of different insecticide concentrations on the intensity of cabbage leaf damage per plant. One-way ANOVA shows that the average damage was significantly (ANOVA, $P < 0.05$) affected by the insecticide concentrations used. The least damage (14.3%, 12.7%, and 3.3% per plant) was achieved with application of 6, 8, and 10 g/L of *Btk*, while the greatest damage (81.3%) occurred on untreated plants followed by plants treated with 2 and 4 g/L, respectively (Tukey, $P < 0.05$).

Discussion

The finding in this study that *Btk* caused egg mortality is unexpected because other researchers (eg, Gould)[26] found that *Btk* requires to be ingested to be effective. Since eggs cannot acquire the lethal dose of an insecticide like *Btk* through feeding, it can be deduced from the findings that *Btk* uses other modes of action to cause mortality of DBM eggs. The results found in the present study, where application of the recommended dose of *Btk* did not achieve effective control over the 96-hour period, suggests that longer periods are required to achieve high egg mortality. Since the bulk of *Btk* activity is lost within 2–3 days of application under field conditions,[15] effectiveness of *Btk* for periods longer than 72 hours would be expected to decline. However, applications higher than the recommended dose achieved 100% control 48 hours after the expected period to hatching. Toxicity data with *Btk* insecticides is limited, but Burges and Hussey[19] found that *Btk* had insecticidal activity on DBM

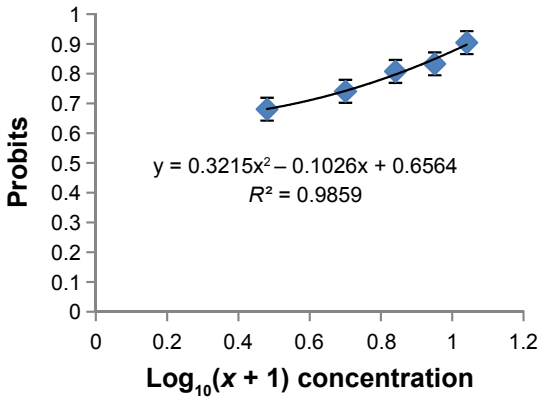

Figure 7. Probit mortality of DBM larvae 96 hours after application of different doses of *Btk*.

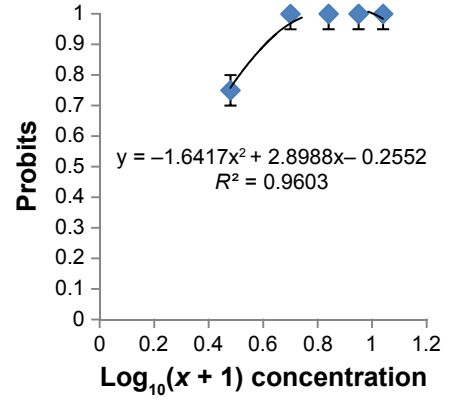

Figure 9. Probit mortality of DBM larvae 144 hours after application of different doses of *Btk*.

Table 2. Effect of *Btk* spray concentration and period of exposure on DBM larval mortality.

PERIOD AFTER APPLICATION (HOURS)	CONTROL	2 g/L	4 g/L	6 g/L	8 g/L	10 g/L	OVERALL PERIOD AVERAGES
24	0.0j*	13.3ij	18.4hij	23.3ghij	25.8ghij	26.7fghij	17.9f**
48	0.0j	23.3ghij	26.7fghij	48.3defgh	50.0defg	56.8cdef	34.2e
72	2.2j	40.0efghij	51.7cdefg	60.7bcde	65.1abcde	68.9abcde	48.1d
96	12.2ij	60.0cde	63.4abcde	67.5abcde	69.2abcde	74.7abcd	57.8c
120	16.7ij	65.6abcde	70.3abcde	76.7abcd	80.8abc	91.7a	66.9b
144	26.7fghij	66.7abcde	90.8ab	92.3a	93.0a	93.3a	77.1a
Overall treatment averages	9.6d***	44.8c	53.6bc	61.5ab	63.9a	68.7a	50.4

Notes: *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 ($P < 0.05$).

through inhibition of the development of the embryo and that the insecticidal effects against eggs were considerably lower than against larvae. Bond and Boyce[27] demonstrated that, when viable DBM eggs on cabbage leaves were dipped in solutions of *Btk*, mortality increased with increase in *Btk* concentration. This explains the results found in this study, where egg mortalities increased significantly with increase in *Btk* concentration.

From the results in Figures 4–9 and Tables 2 and 3, several observations can be made. Longer exposure periods are required to achieve 90%–100% DBM larval mortality; the recommended dose did not achieve 90% larval mortality during the study period, Higher dosages than the recommended dosage are required to effectively protect cabbage plants from DBM damage. It is therefore logical to assume that the longer it takes to kill sufficient numbers of larvae, the greater the damage caused by the larvae on the crop. After ingestion of *Btk*, the active toxin is known to bind to and destroy the midgut epithelium, resulting in rapid gut paralysis, which causes the larva to stop feeding within hours in the most sensitive species.[15] *Btk*-affected larvae die from starvation, which may take several days. Since *Btk* does not kill rapidly, users may incorrectly assume that it is ineffective if treatments are assessed a day or two after application. The finding that the use of higher concentrations of *Btk* achieved 90% larval mortality 120 hours after application shows that much higher concentrations of *Btk* are required to achieve adequate control of the pest and protect the crop from serious damage in a greenhouse. The results in this study show that, apart from the dosage that should be used against a pest species, information on the label of an insecticide should include periods of exposure required to achieve the required level of control of the target pest. Such information would enable farmers to decide on the periods between spray applications.

Comparisons of slopes of probit lines can also provide an indication of relative toxicities of insecticides.[28] An insecticide with a steep slope would therefore be expected to provide faster pest mortality with change in dose. The slopes of the probit lines in Figures 4–9 show that large increases in the dosages are needed to cause significant increases in mortality of DBM larvae when *Btk* is applied against DBM larvae. The fact that dosages higher than the recommended dosage of *Btk* took longer than 120 hours to achieve 90%–100% mortality shows that higher concentrations can be used to achieve effective control of DBM provided longer exposure periods are provided. This suggested that the recommended dosage was too low to achieve effective DBM larval mortalities. These results are similar to those found by Talekar,[15] who suggested that use of higher doses of *Btk* than those previously used in control programs might reduce pest populations sufficiently and avoid the need for repeat applications in the following year. Burges and Hussey[19] also reported that control efforts with *Bt* failed because of insufficient doses and differences in potency of the preparations used.

In order to determine the effectiveness of the pesticide in the management of DBM, it is important to consider the level of pest reduction achieved as well as the reduction in crop damage as a result of application of the pesticide. When results in Table 2 are considered together with those in Table 3, it appears that 90.8% larval mortality is required to effectively protect cabbage using *Btk*. This shows that the level of mortality that resulted in effective protection of cabbage plants required a dosage higher than the recommended dosage of *Btk* (of 4.0 g/L).

Table 3. Effect of *Btk* spray concentrations on intensity of cabbage leaf damage per plant.

	0 g/L	2.0 g/L	4.0 g/L	6.0 g/L	8.0 g/L	10.0 g/L
Treatment averages	81.3a*	46.0b	40.7b	14.3c	12.7c	3.3c

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

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 the active ingredient. It can be concluded from this study that *Btk* can offer effective control of DBM eggs and larvae and prevent serious damage to cabbage provided long exposure periods are allowed. Higher *Btk* dosages than recommended by the manufacturer are required to offer effective control of DBM under greenhouse conditions. Since *Btk* sprays caused a considerable level of mortality of DBM eggs, it can be concluded that apart from requiring ingestion, *Btk* also uses other unidentified mechanisms of killing target pests. Further research is needed to investigate the mode of action used by *Btk* sprays against sessile, nonfeeding life stages of DBM. This study also showed that, when applied under high temperature conditions, *Btk* persisted and caused mortality of eggs for up to 3 days after application. Since this study was conducted under semicontrolled greenhouse conditions, further research is needed under field conditions to validate the results obtained in the present study.

Author Contributions

Conceived and designed the experiments: MML, DCM. Analysed the data: MML, DCM. Wrote the first draft of the manuscript: MML. Contributed to the writing of the manuscript: MML, DCM. Agree with the manuscript results and conclusions: MML, DCM, BCK, MO. Jointly developed the structure and arguments for the paper: MML, DCM. Made critical revisions and approved final version: MML, DCM. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Sances FV. Crop profile for cabbage in Carlifornia. 2000. Available at: http://www.ipmcenters.org/cropprofiles/docs/cabbage.html. Accessed September 16, 2006.
2. Bok I, Madisa M, Machacha D, Moamongwe M, More K. *Manual for Vegetable Production in Botswana*. Botswana; Gaborone: Department of Agricultural Research, Ministry of Agriculture; 2006:52.
3. Munthali DC. Evaluation of cabbage varieties for resistance to the cabbage aphid. *Afr Entomol*. 2009;17(1):1–7.
4. Talekar NS, Shelton AM. Biology, ecology and management of the DBM. *Annu Rev Entomol*. 1993;38:275–301.
5. Charleston DS, Kfir R. The possibility of using Indian mustard, *Brassica juncea*, as a trap crop for the DBM, *Plutella xylostella* in South Africa. *Crop Prot*. 2000;19:455–460.
6. Kahuthia-Gathu R, Löhr B, Poehling HM, Mbugua PK. Diversity, distribution and role of wild crucifers in major cabbage and kale growing areas of Kenya. *Bull Entomol Res*. 2009;99:287–297.
7. Shelton AM. Management of the Diamondback moth: déjà vu all over again? In: Endersby NM, Ridland PM, eds. The management of Diamondback moth and other crucifer pests. Proceedings of the fourth international workshop, November 26–29, 2001. Melbourne, Australia: Department of Natural Resources and Environment; 2004:3–8.
8. Talekar NS, Yang JC, Liu MY, Ong PC. Use of parasitoids to control the diamondback moth, *Plutella xylostella*. In: Mochida O, Kiritani K, eds. *The use of Natural Enemies to control Pests*. Taipei, Taiwan: Food and Fertilizer Technology Center for the Asian and Pacific Region; 1990:254.
9. Fahmy RA, Miyata T. Development and reversion of Chlorfluazuron resistance in diamondback moth. In: Talekar, NS, ed. *Diamondback moth and other crucifer pests: Proceedings of the second international workshop*, Tainan, Taiwan, December 10–14, 1990. Asian Vegetable Research and Development Center. 1992;603. [Publication No. 92–368].
10. Shelton AM, Nault BA. Dead-end trap cropping: a technique to improve management of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Prot*. 2004;23:497–503.
11. Sereda B, Basson NCJ, Marais P. Bioassay of insecticide resistance in *Plutella xylostella* (L.) in South Africa. *Afr Plant Prot*. 1997;3:67–72.
12. Nel A, Krause M, Ramautar N, van Zyl K. *A guide for the Control of Plant Pests*. Republic of South Africa: National Department of Agriculture; 1999.
13. Crickmore N, Zeigler DR, Feitelson J, et al. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol Mol Biol Rev*. 1998;62:807–813.
14. Ware GW, Whitacre DM. *The Pesticide Book*. USA: Freeman and Co; 2004:1.
15. Talekar NS. Diamondback moth and other crucifer pests: Proceedings of the second international workshop; December 10–14, 1990, Asian Vegetable Research and Development Center, Tainan, Taiwan, 1992;92–368, 603.
16. Munthali DC, Obopile M, Modise DM, et al. *A Guide to Commercial Production of Selected Vegetables in Botswana*. Gaborone, Botswana: Printing and Publishing Company; 2004.
17. Tabashnik BE. Evolution of resistance to *Bacillus thuringiensis*. *Annu Rev Entomol*. 1994;39:47–49.
18. Roush RT, Tabashnik BE. *Pesticide Resistance in Arthropods*. London: Chapman and Hall; 1990:303.
19. Burges HD, Hussey NW. *Microbial Control of Insects and Mites*. London: Academic Press Inc; 1971:247–273.
20. Cameron PJ. Comparative insecticide resistance of New Zealand and North American populations of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *N Z J Crop Hortic Sci*. 1997;25:117–122.
21. Chan NW, Moe KT, Weine NN. Study on the biology of diamondback moth, *Plutella xylostella* (L.) on cabbage. In: International Conference on Sustainable Development: Issues and Prospects for the GMS, November 12–14, 2008. Kunming, China.
22. Finney DJ. *Probit Analysis*. 3rd ed. London: Cambridge University Press; 1971:20–63.
23. Mead R, Curnow RN. *Statistical Methods in Agriculture and Experimental Biology*. London and New York: Chapman and Hall; 1983.
24. MSTATC. *Microcomputer Statistical Program, Experimental Design, Data Management, Data Analysis*. East Lansing, Michigan: Michigan State University; 1985.
25. Zar JH. *Biostatistical Analysis*. 2nd ed. Englewood Cliff, NJ: Prentice—Hall, Inc; 1984:718.
26. Gould F. Arthropod behavior and the efficacy of plant protectants. *Annu Rev Entomol*. 1991;36:305–330.
27. Bond RP, Boyce CB. *The Thermostable Exotoxin of Bacillus thuringiensis*. Kent, England: Shell Research Limited, Milstead Laboratory of Chemical Enzymology; 1971.
28. Walstrom RJ. *The Hive and The Honey Bee. A New Book on Beekeeping which Continues Tradition of "Langstroth on the Hive and the Honeybee"*. Hamilton, Ill: Dadant and Sons; 1980.