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Effectiveness of Spinosad Against Diamondback Moth (*Plutella xylostella* L.) Eggs and Larvae on Cabbage Under Botswana Conditions

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ABSTRACT: The efficacy of spinosad against the diamondback moth (DBM) on cabbage was studied at Botswana College of Agriculture, Gaborone, Botswana in 2011. Using five concentrations of spinosad: 0.12, 0.36, 0.60, 0.84 and 1.08 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 LD_{50} and LD_{90} values for the treatments against eggs and larvae. When the treatments were assessed at 72 and 96 hours, LD_{90} values against larvae were 0.74 and 0.59 g/L, whereas they were 0.35 and 0.32 g/L against eggs. This indicated that spinosad was more effective against eggs than against larvae. The slopes of the probit lines for larvae assessed at 48, 72 and 96 hours after application were 3.519, 3.810 and 3.427, while those against eggs were 1.725, 1.316 and 1.086. This indicates that there was a more rapid change in larval mortality with increase in pesticide dosage than in egg mortality. The study shows that spinosad can achieve effective control of DBM eggs and larvae under Botswana conditions.

KEYWORDS: spinosad efficacy, diamondback moth, cabbage

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Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is an extensively grown vegetable in the world.[1] It is among the most popular food crops in Botswana households; it grows well in many parts of the country.[2] However, cabbage production in Botswana is greatly constrained by a number of insect pests. These include the cabbage aphid (*Brevicoryne brassicae* L.), cabbage webworm (*Hellula undalis* (Fabricius)), cutworm (*Agrostis* spp.), bagrada bug (*Bagrada cruciferanum* Kirk) and diamondback moth (DBM) *Plutella xylostella* (L.).[2–4] 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.[5] DBM was first recorded as an important pest of cabbage in Southern Africa as early as 1917.[6] It is highly migratory, and its seasonal movements have been well documented.[5] 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 more than 20 generations per year in the tropics), and its genetic elasticity, which leads to rapid development of resistance to insecticides.[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.[3] However, it has been demonstrated that DBM quickly develops resistance to many new insecticides.[8,9] It has reportedly developed resistance to most synthetic pyrethroids, organophosphates, carbamates, and actinomycetes in many cabbage growing areas of the world.[10,11] 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.[10] Such pesticides are

not environmentally friendly because they affect non-target organisms in both treated and untreated fields. Therefore, efforts to promote the use of environmentally friendly pesticides as alternatives are continually being made; one such alternative is the use of spinosad.

Spinosad, the first of the naturalyte class of insecticides, consists of two macrolytic lactones: spinosyn A and spinosyn D. These are fermentation products of the soil inhabiting actinomycete *Saccharopolyspora spinosa*.[12,13] Spinosad is a neurotoxin that has both contact and gastric activity.[14–16] The mode of action of spinosyns is primarily through altering the function of GABA-gated chloride channels.[12] Spinosad is highly effective against many insect pests and compatible with many insect natural enemies.[17] It has low mammalian toxicity. Compared to conventional synthetic insecticides, spinosad poses minimum risk to humans and wildlife.[16] It degrades quickly in the environment, with little or no impact on beneficial fauna.[17] It is currently registered for use on more than 100 crops against insects that belong to the Lepidoptera, Diptera, Thysanoptera, Coleoptera and Orthoptera in the United States and 24 countries (including Botswana).[18–20] The target species among the Lepidoptera include army worms (*Spodoptera* sp.), cutworms (*Agrostis* sp.), fruit worms (*Heliothis* sp.), leaf rollers (Tortricidae) and DBM. Because of its low toxicity to most beneficial insects, spinosad is suitable for use in integrated pest management (IPM) programs, especially where pests have developed resistance to other insecticides.[21] The application of spinosad as a component of an IPM program could reduce environmental pollution and its deleterious impact on beneficial entomofauna, and could delay expression of resistance to pesticides.[7] It has been shown that the application of spinosad against DBM female adults causes them to lay significantly smaller eggs compared to untreated females.[22] However, little is known about its effects when applied against DBM eggs and larvae. This study evaluated the efficacy of spinosad on 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: 998m) 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, then 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 diamondback moth to ensure adequate host substrate for oviposition of eggs by adults. The seedlings were watered regularly as needed 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 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. The spinosad Tracer® (Dow AgroSciences, Indianapolis, USA), 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 spinosad concentrations (0.12, 0.36, 0.60, 0.84 and 1.08 g/L water) and distilled water were used. The recommended rate (0.60 g/L) was included as a check. The six 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 three times. This gave a total of 54 treated plants per bioassay and 162 sprayed plants all together. Each pot had a label that 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, not susceptible to a pesticide with a contact and stomach poison mode of action such as spinosad.) 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 10x magnification, and the eggs laid on the leaves were counted. The artificially infested seedlings were sprayed to runoff with five 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 4d to hatch at 25 ± 5°C), treatments against eggs were applied 3d after oviposition.[23] 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 eggs were allowed to hatch into first instars and to develop into second instar larvae. Because first instar larvae are leaf miners and second instar larvae are surface feeders, the stages were easy to differentiate. The larvae were counted before treatment and 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 14d after DBM eggs had hatched. The total number of leaves per plant was recorded, and the number of leaves with damage symptoms were

counted. 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 three times.

Data analysis. Probit analysis was used to analyze mortality results.[24,25] 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 the mortalities achieved by treatments at different periods of exposure to spinosad.

The results on percentage seedling damage were transformed to arcsines before analysis in order to normalize them. Using the MSTATC statistical package,[26] Analysis of Variance (ANOVA) was used to analyze the data. Averages were separated using the Tukey's Honestly significant difference test where significant effects were found.[27]

Results

DBM larval mortality. Figures 1 and 2 show positive linear relationships while figures 3, 4, 5 and 6 show positive curvilinear relationships between log dose and probit mortality caused by spinosad (correlation coefficients of 0.915, 0.917, 0.982, 0.998, 0.992 and 0.917), when treatments were assessed at 24, 48, 72, 96, 120 and 144 h after pesticide application. Figure 1 shows that LD_{50} of 0.59 g/L and LD_{90} of 1.04 g/L were achieved 24 h after application. The recommended dose (0.60 g/L) of the pesticide showed a probit value of 0.620 (equivalent to 51.94% larval mortality) during this exposure period. Figure 2 indicates that the LD_{50} of spinosad after 48 h exposure was 0.47 g/L, while the LD_{90} was 0.95 g/L. At the recommended dose,

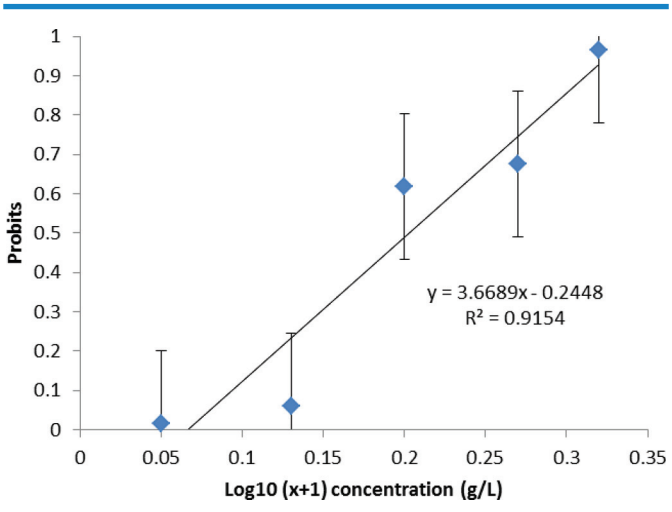

Figure 1. The probit mortality of DBM larvae 24 h after application of different doses of spinosad.

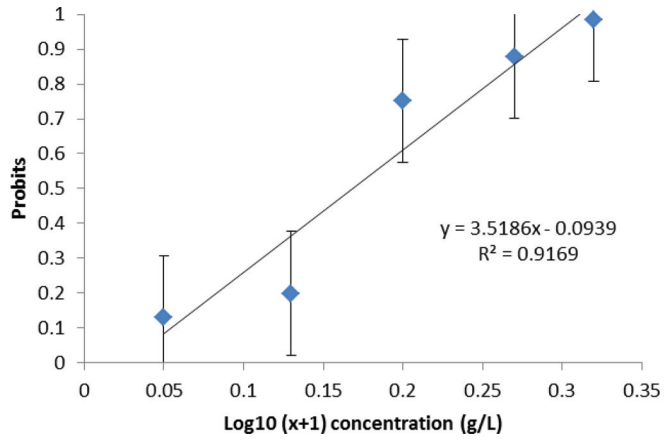

Figure 2. The probit mortality of DBM larvae 48 h after application of different doses of spinosad.

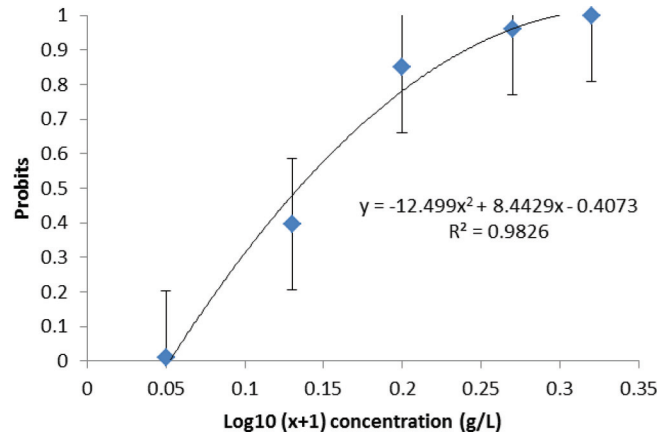

Figure 3. The probit mortality of DBM larvae 72 h after application of different doses of spinosad.

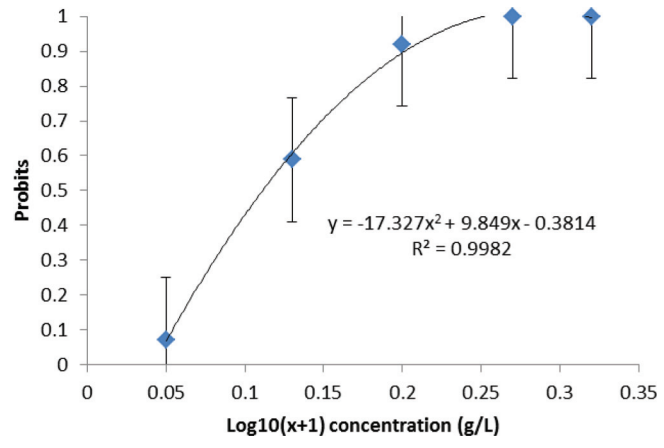

Figure 4. The probit mortality of DBM larvae 96 h after application of different doses of spinosad.

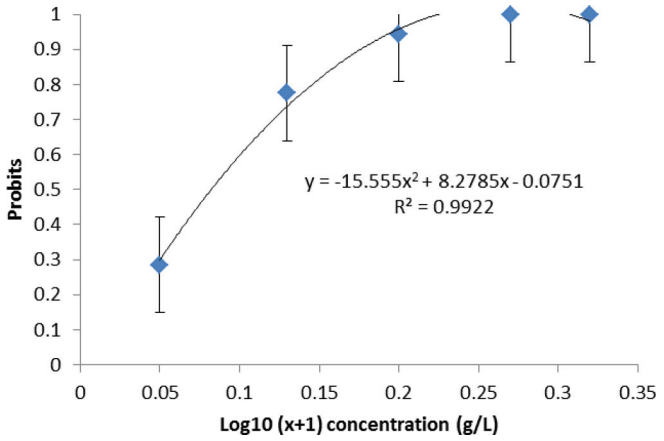

Figure 5. The probit mortality of DBM larvae 120 h after application of different doses of spinosad.

spinosad only achieved 0.752 on the probit scale, which is equivalent to 60.13% larval mortality. When assessed at 72 h after application, the LD_{50} of spinosad was 0.35 g/L and the LD_{90} was 0.74 g/L (Fig. 3). The recommended dosage achieved 0.851 on the probit scale, which is equivalent to 67.29% larval mortality after 72 h exposure. Figure 4 shows an LD_{50} value of 0.29 g/L and an LD_{90} of 0.59 g/L when the treatments were assessed at 96 h after application. The mortality achieved by the recommended dose was 0.919 on the probit scale, which is equivalent to 73.46% larval mortality. Figure 5 shows that when assessed at 120 h after application, the LD_{50} was 0.20 g/L and the LD_{90} was 0.50 g/L. The exposure to the recommended dose for 120 h achieved a probit value of 0.943, which is equivalent to 76.19% larval mortality. The assessment after 144 h exposure showed LD_{50} and LD_{90} values of 0.15 and 0.38 g/L (Fig. 6). During this period, the recommended dose achieved 1.00 on the probit scale, which is equivalent to 100% larval mortality.

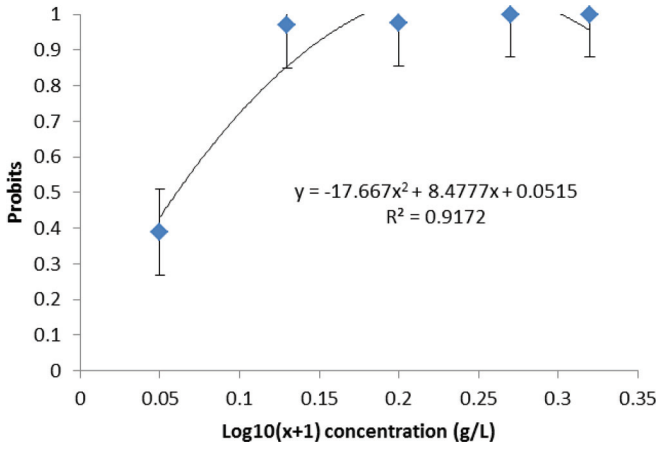

Figure 6. The probit mortality of DBM larvae 144 h after application of different doses of spinosad.

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 (91.1 to 100.0%) occurred at 96 h after the application of 0.84 g/L and at 72 h after the application of 1.08 g/L concentration. The recommended dose of 0.60 g/L did not achieve 90% larval mortality during the 144 h assessment period. The results also show that the lowest mortality of 4.5 to 8.9% per plant occurred in the control treatment throughout the assessment period. The mortalities that occurred in the control treatment at 144 h were similar to the mortalities achieved by 0.12 g/L spinosad solution at 96 h, and the mortalities caused by 0.36 g/L at 24 h (Tukey, $P < 0.05$). The overall treatment averages show that spinosad concentrations also had a significant effect (Tukey, $P < 0.05$) on the mortality of larvae. The mortalities differed significantly from each other and increased in the order of 6.1 <26.8 <47.4 <70.7 <83.1 < 91.2% on plants treated with 0.0, 0.12, 0.36, 0.60, 0.84 and 1.08 g/L. The results of overall exposure period were also significantly different (Tukey, $P < 0.05$), and increased in the order of 36.9 <46.0 <49.7 <57.6 <66.0 <69.2 when assessed at 24, 48, 72, 96, 120 and 144 h.

DBM egg mortality. Figures 7, 8 and 9 show a positive curvilinear relationship between the log dose and the mortality of DBM eggs (r values of 0.971, 0.981 and 0.981). The LD_{50} and LD_{90} of spinosad against eggs were 0.12 and 0.62 g/L respectively when assessed at 48 h (Fig. 7). During this period, the recommended dose of 0.60 g/L gave a probit value of 1.0, which is equivalent to 100% egg mortality. When assessment was done at 72 h, the LD_{90} was 0.35 g/L (Fig. 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 0.32 g/L (Fig. 9). These results show that the toxicity of spinosad to eggs increased with each increase in dosage. The LD_{90} values obtained when spinosad treatments were assessed at 48, 72 and 96 h show that spinosad can cause 90–100% egg mortalities at dosages lower than the recommended dose, provided that longer periods of exposure are allowed.

Table 2 shows that the spinosad 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 treated with 0.84 and 1.08 g/L and assessed at 48 h. The lowest egg mortality (44.0%) was on plants treated with 0.12 g/L and assessed at 48 h (Tukey, $P < 0.05$). The overall treatment averages indicate that concentrations higher than 0.84 g/L caused the greatest mortality (100%) and the lowest concentration (0.12 g/L) caused the least mortality (50.7%). The overall period averages indicate that spinosad caused the greatest mortality (84.4%) when treatments were assessed at 96 h and the lowest mortality (80.2%) when treatments were assessed at 48 h.

Table 1. The effect of spinosad concentrations and period of exposure on DBM larval mortality.

PERIOD AFTER APPLICATION	0 g/L	0.12 g/L	0.36 g/L	0.60 g/L	0.84 g/L	1.08 g/L	OVERALL PERIOD AVERAGES
24 h	4.5n*	10.1mn	17.0lmn	53.3ghi	56.7fghi	79.5bcde	36.9d**
48 h	4.5n	25.0jklm	30.0jkl	62.0efgh	71.0defg	83.7abcd	46.0c
72 h	4.5n	11.7mn	42.7ij	69.3defg	80.0bcd	90.0abc	49.7c
96 h	5.6n	23.9klm	55.0ghi	75.7cde	91.1abc	94.0ab	57.6b
120 h	8.9mn	41.1ijk	66.7defgh	79.3bcde	100.0a	100.0a	66.0a
144 h	8.9mn	48.9hi	73.3cdef	84.3abcd	100.0a	100.0a	69.2a
Overall treatment averages	6.1f***	26.8e	47.4d	70.7c	83.1b	91.2a	54.2

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

DBM damage on cabbage plants. Table 3 shows that damage caused by DBM larvae on plants was significantly affected (Tukey, $P < 0.05$) by the concentration of spinosad. DBM larvae caused 72.3% leaf damage on untreated plants, but on plants treated with spinosad concentrations of 0.12 and 0.36 g/L the leaf damage caused was 41% and 23%, respectively. DBM larvae on plants treated with the recommended dose of 0.6 g/L caused only 8.7% leaf damage, while there was no leaf damage on plants treated with higher dosages.

Discussion

Effect of spinosad dosage on larval mortality. From the results in Figures 1, 2, 3, 4, 5 and 6 and Tables 1, 2 and 3, several observations can be made. When exposure periods increased, lower doses of spinosad were able to cause 90 to 100% larval mortality. The recommended dose of spinosad effectively protected the crop from serious damage, and higher dosages achieved total protection of the crop from larval damage. When LD_{90}s are used alone to assess the effectiveness of spinosad, the mortality level of 84.3% caused by the recommended dose during the 144 h study period appears to be too low to achieve effective control. The level of pest decline was sufficient to significantly (Tukey, < 0.05) reduce crop damage to levels achieved by higher dosages. It is therefore

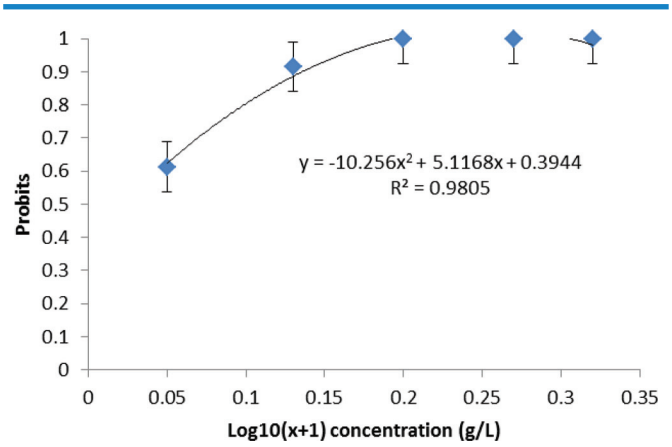

Figure 8. Probit mortality of DBM eggs exposed to different doses of spinosad assessed 72 h after expected time of hatching.

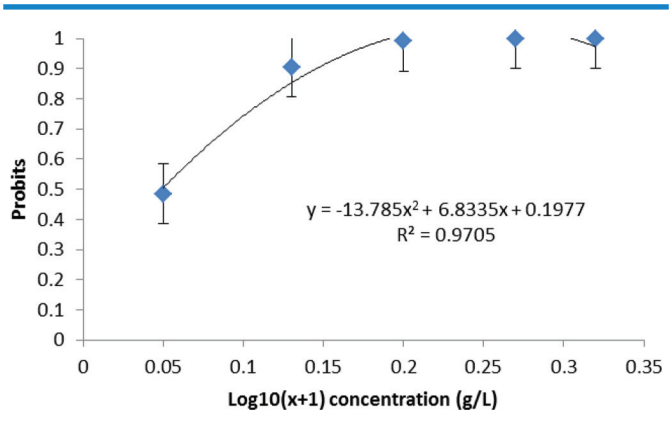

Figure 7. Probit mortality of DBM eggs exposed to different doses of spinosad assessed 48 h after expected time of hatching.

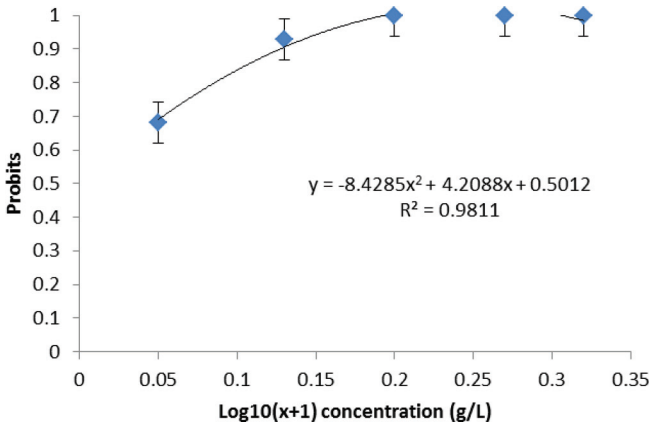

Figure 9. Probit mortality of DBM eggs exposed to different doses of spinosad assessed 96 h after expected time of hatching.

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

PERIOD AFTER EXPECTED DATE OF HATCHING	0.12 g/L	0.36 g/L	0.60 g/L	0.84 g/L	1.08 g/L	OVERALL PERIOD AVERAGES
48 h	44.0f*	72.0d	85.0bc	100.0a	100.0a	80.2b**
72 h	52.0e	73.0d	90.0ab	100.0a	100.0a	83.0ab
96 h	56.0e	75.0cd	91.0ab	100.0a	100.0a	84.4a
Overall treatment averages	50.7d***	73.3c	88.7b	100.0a	100.0a	82.5

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

patent that LD_{90} values alone do not provide sufficient indication of the effectiveness of spinosad against DBM larvae.

The slopes of the probit lines in Figures 1, 2, 3, 4, 5 and 6 show that spinosad became more toxic to DBM larvae with each increase in pesticide concentration. These results are similar to those by Adan et al where mortality of *Ceratitis capitata* (Wiedemann) adults also depended on the concentration of spinosad used.[28] 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 corroborates findings by Sparks et al who reported that spinosad was a neurotoxin with a contact mode of action, and Bret et al who found that in addition to contact action, spinosad is a stomach poison, and that mortality due to ingestion was five to ten times greater than through contact.[29,30] This may explain the relatively faster mortality of DBM larvae compared to that of eggs. The fast action of spinosad against larvae is a desirable property as this is the damaging developmental stage of the pest.

Effect of spinosad dosage on egg mortality. The results that doses lower than the recommended dose achieved 90–100% egg mortality, when exposed to the insecticide for over 72 and 96 h (Figs. 7, 8 and 9), suggest that spinosad is highly effective against DBM eggs. As spinosad is a contact insecticide,[30] 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 spinosad 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 spinosad against DBM, the egg is the most susceptible stage to target.

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

SPINOSAD CONCENTRATION	0.0 g/L	0.12 g/L	0.36 g/L	0.6 g/L	0.84 g/L	1.08 g/L
Treatment averages	72.3a*	41.0b	23.0bc	8.7cd	0.0d	0.0d

*Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant different 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 active ingredient. It can be concluded from this study that spinosad 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. The results in this study show that spinosad does not require total larval mortality to provide effective protection of cabbage against DBM damage. Spinosad is a potentially effective alternative to the synthetic insecticides that are currently being used in Botswana. Spinosad can also be used in combination with natural enemies in an integrated pest management program. Because this study was conducted under greenhouse conditions, effectiveness of spinosad might need to be verified in extensive field trials.

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Author Contributions

Conceived and designed the experiments: MML, DCM. Co-supervised the research work: DCM, MO and BCK. Jointly developed the structure and arguments for the paper: MML, DCM. All authors reviewed and approved the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither

under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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