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Effects of *Bacillus thuringiensis*, *Beauveria bassiana,* Neem, and a Tomato Inter-crop on Diamondback Moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) in Tharaka-Nithi County, Kenya

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Abstract

Cabbage (*Brassica oleracea* var. *capitata*) is an important crop in Kenya, ranked 3rd among the exotic vegetables. Diamondback moth (DBM) is a major cabbage pest that can cause a 100% loss in yield if not well managed. Farmers indiscriminately use synthetic pesticides associated with environmental, health, and pest resistance concerns to manage the DBM. To increase the availability of technologies for effective management of DBM in Kenya, three biopesticides; BioPower (*Beauveria bassiana* 1.0 x 108 CFUs/g), Dipel DF® (*Bacillus thuringiensis* subsp. *kurstaki*), Neemraj Super 3000 (Azadirachtin 0.3% EC), and a tomato inter-crop were evaluated. Cabbages (30 plants) spaced at 40 cm x 60 cm were established in 6 m x 6 m plots in 3 replicates per treatment. The treatments applications were; *B. bassiana* 100 g/20 L, *B. thuringiensis* 20 g/20 L, and neem 20 mL/20 L. A row of tomato inter-crop was established in every two rows of cabbage and distilled water was used on the untreated control. Data on the number of damaged leaves, live larvae, and marketable yields for two seasons was collected from five leaves of ten randomly selected cabbage plants. Results showed significant differences ($P \leq 0.05$) between the control and treatments in the mean number of live larvae and the number of damaged leaves. However, there were no significant marketable yield differences between the treatments ($P \geq 0.05$). In conclusion, the three bio-pesticides and the tomato inter-crop were effective against the DBM damage on cabbage leaves and can be part of integrated pest management (IPM) packages for DBM management on cabbage. There is a need to determine why the treatments did not result in meaningful outcomes against the DBM population and cabbage head weights. In addition, a cost-benefit analysis of each treatment is necessary.

Keywords: Cabbage, Diamondback moth, Pest resistance, Biopesticides, Integrated Pest Management.

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable crop in Kenya, ranking 3rd among the exotic vegetables (HCD 2020). The diamondback moth (*Plutella xylostella* L.) is the most destructive pest of cabbage and other cruciferous plants. Losses attributable to pest infestation in brassicas production vary from region to region. In Ontario, Canada, crop losses in cabbages and tomatoes without any pest management measures have been recorded at about 80% (Tolman et al. 2004), while in India losses are estimated at 50–

80% (Krishnamoorthy 2004). Yield losses of 60% due to diamondback moth (DBM) damage in cabbage and other brassicas have been reported in Ghana (CABI 2014). Increased pest infestation is attributed to improper agronomic practices on the farms such as poor sanitation and continuous monoculture (Odhiambo 2005). To effectively control the DBM and satisfy consumers who continue to attach high cosmetic value to cabbage, insecticides are mainly used. Reliance on insecticides has resulted in the pest developing resistance to all classes of insecticides and made the pest the most important arthropod pest worldwide (Ninsin 2015, da Silva Nunes et al. 2020). Moreover, increased pesticide use has other limitations which include food safety issues, environmental contamination, and health risks with annual fatality rates being recorded globally as 20,000 (Felsot and Rack 2007, Kumar et al. 2012, Seif and Nyambo 2013, Halimatunsadiah et al. 2016, Stamati et al. 2016). Worldwide, losses due to diseases and pests such as insects and weeds remain high. In high-income countries, this is estimated at 25–30% while the range in developing countries like Kenya is 40–50% (Macharia 2015).

In the last four decades, there has been a paradigm shift where integrated pest management (IPM) has received considerable emphasis (Grzywacz et al. 2010). Despite the advantages of the IPM approach adoption in developing countries has been low (Morse and Buhler 1997, Alwang et al. 2019). In a survey involving communities in developing countries, some of the reasons identified for failure to adopt IPM included; insufficient training and technical support to farmers, IPM technologies being too hard to implement, and lack of government support (Parsa et al. 2014). The adoption of IPM technologies in Karnataka, India, increased yields by 46%, reducing the costs of cultivation by 21%, and the net returns increased by 119% (Gajanana et al. 2006). Intercropping cabbage with non-host crops such as tomato, pepper, and onion as an IPM approach in Ghana reduced the incidence of DBM margin (Asare-Bediako et al. 2010). In agroforestry studies reported in Kenya, shade trees decreased the abundance of pests in brassica crops (Guenat et al. 2019).

In a pest constraint identification survey conducted in Tharaka-Nithi County, in 2016, the DBM was identified as the major constraint to cabbage production. Farmer practices for pest management included foliar sprays up to 8 times per season using synthetic pesticides (Ndungu et al. 2017). Biopesticides are natural pest management agents or compounds from animals, plants, and microorganisms. These products are generally safe for humans and the environment (Kumar et al. 2021). A study in Ethiopia found that neem seed extract and *Bacillus huringiensis* were effective against DBM and suggested their use as an alternative to synthetic insecticides for DBM-integrated management (Sitotaw et al. 2009). In Kenya, various biopesticides have been registered to control agricultural pests (PCPB 2019,) but most farmers have limited knowledge about them. The comparative effects of three biopesticides registered for use in the country; BioPower® (*Beauveria bassiana* 1.0 x 10^8 cfu/ml), Neemraj Super 3000 (Azadirachtin 0.3%), Dipel DF® (*Bacillus thuringiensis* subsp. *kurstaki*, Strain ABTS-351, 54% ww) (PCPB 2019) and a tomato intercrop were assessed for two seasons in a farmer's field with participation of farmers in all activities including data collection. The study anticipated that farmer participation would enhance the adoption of biopesticides in the study.

Materials and Methods

Description of the experimental site

The experiment was conducted between April 2019 and January 2020 on a farm belonging to one member of the Nthambo Horticultural Growers Self Help Group that was collaborating with the Kenya Agricultural and Livestock Research Organisation (KALRO), Horticultural Research Institute (HRI), scientists to upscale IPM technologies. The site was within the Nthambo location, Chuka Sub-County, Tharaka-Nithi County. The site was 29 m x

17 m in size within 1/2 acre of land with loam soil, previously under maize crop and located at S 0°22'9", E37°37'8", and 1460 m above the sea level. The area has a bimodal rainfall with long rains from March to June and short rains from October to December. Horticultural crop production is practiced throughout the year with supplemental irrigation from the Nthambo water project supported by the County Government to serve 800 households. Being on the slopes of Mt Kenya, the area is suited for cabbage production.

Cabbage planting

Seeds of cabbage (*Brassica oleracea* var. *capitata*) variety Queen F1 were used. A nursery bed (1 m x 3 m) was prepared by digging, levelling, raising the bed, and applying 40 kg of well-decomposed cow manure. The bed was watered and covered with a translucent 500 gauge polythene sheet for three-week solarization (to kill weed seeds, nematodes, and any soil-borne pests and/or pathogens). For the season one experiment, the bed was uncovered on 12/4/2019, furrows were made at a depth of 4 cm, 10 cm apart, and the seed was planted and then covered with soil. The nursery bed was covered with an insect-proof net to keep away insects. The seedlings were transplanted to the experimental plot three weeks after germination when they had attained three to four true leaves. The nursery for the second season was established on 18/9/2019, managed in the same way, and transplanted on 9/10/2019. The seedlings were transplanted to plots 6 m x 6 m in size with a one-metre path between plots and a 1.5 m path between blocks. The spacing was 40 cm between plants and 60 cm between rows hence 30 plants per treatment. Plots were arranged in a Randomized Complete Block Design (RCBD) with three replications. A drip irrigation system was used to irrigate the field twice per week for the first three to four weeks after transplanting and once every week thereafter. In both seasons the fields were fertilized with 5 kg cow manure per plot and 10 g diammonium phosphate (DAP) fertilizer per hole at planting, followed by a top dressing with 10 g calcium ammonium nitrate (CAN) one month after transplanting. Weeds were controlled manually twice each season.

The treatments

The treatments were; Bio-Power® (*Beauveria bassiana* 1.0 x 10^8 cfu/ml), Dipel DF (*Bacillus thuringiensis* subsp. *kurstaki*, Strain ABTS-351, 54% ww), Neemraj Super 3000 (Azadirachtin 0.3%), Tomato intercrop as a repellent (Dimsey et al. 2010) and untreated control (distilled water application) (Table 1). All the pest control products were purchased from local agrochemical traders. The tomato seedlings, variety Kilele F1, were raised by the farmer.

Table 1: Description of treatments used against diamondback moth

Treatment	Trade name of product	Description	Rate of application
1	Bio-Power® WP	*Beauveria bassiana* 1.15% at 1.0 x 10^8 CFUs/g	100 g/20 L water
2	DiPel® DF	*Bacillus thuringiensis* subsp. *kurstaki*, Strain ABTS-351, 54% ww	20 g/20 L water
3	Neemraj Super 3000 EC	Azadiractin 0.3 % EC	20 mL/20 L water
4	Tomato inter-crop	As a repellent	One row between two cabbage rows
5	Un treated control	Distilled water	N/A

Treatment applications

The plots were sectioned, and treatments were applied singly. In addition, as mentioned earlier, a 1 m distance was maintained between plots and 1.5 m between blocks. The treatments were assigned a unique tag to prevent mix-ups. The tomato inter-crop was planted two weeks before the transplanting of the cabbage seedlings to allow for establishment. Application of treatments started two weeks after transplanting. Treatments were applied weekly as foliar sprays using a classic Cooper Pegler CP3 20 L Knapsack sprayer with a conical nozzle. The rates of application for the control products were as recommended by the manufacturers. Sterile water was used in the control treatment plots and applied the same way as the test products.

Data collected

Diamondback moth population, cabbage leaf damage, and marketable yields were collected. A day before treatment application, 5 leaves of 10 plants randomly selected from the central five rows were examined for the number of DBM larvae. The data collection continued weekly for eight weeks and after which the means number of DBM larvae was calculated. At harvest, DBM damage was assessed by counting the number of damaged leaves (windowing, frass) and means calculated per treatment on ten randomly tagged plants per plot. At harvest, marketable yield data were taken from the central five rows of each plot. Marketability was determined by removing the damaged leaves of ten randomly selected cabbage heads and measuring their diameter and weighing them individually. Heads with a diameter below 4 cm were discarded. This was done for the two seasons.

Data analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model of SAS (SAS, 2017). Mean separation was done using the Student Newman-Keuls (SNK) test.

Results

Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato inter-crop on the number of diamondback moth damaged cabbage leaves

The mean number of DBM damaged cabbage leaves was significantly ($P \leq 0.05$) different among the treatments (Table 2). In season one, Neemraj Super 3000 had the highest control effect on DBM with a mean number of damaged leaves of 9.13 ± 2.40 compared to the mean of 16.19 ± 1.44 in the control treatment. However, this was not significantly different from *Beauveria bassiana*, *Bacillus thuringiensis*, and the tomato intercrop treatments. In season two, there was a similar trend with the least mean number (8.31 ± 0.84) of damaged leaves occurring in the *B. thuringiensis* treated plots. There was no significant difference between the treatments, but these were all significantly different from the control plots (Table 2).

Table 2: Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato intercrop on diamondback moth damage on cabbage leaves

	No. of damaged leaves (Mean ± SE)	
Treatment	Season 1	Season 2
Biopower 1.15EC (*Beauveria bassiana*)	11.13 ± 2.07b	9.06 ± 0.66b*
Dipel DF (*Bacillus thuringiensis* subsp. *kurstaki*)	10.63 ± 1.66b	8.31 ± 0.84b
Neemraj Super 3000 0.3EC	9.13 ± 2.40b	9.56 ± 0.60ab
Tomato intercrop	11.31 ± 1.02b	9.25 ± 0.94b
Untreated control	16.19 ± 1.44a	11.19 ± 0.91a
CV	34.48684	28.14482
P-value	0.0001	0.0497
LSD	2.85	1.89

**Means in a column followed by the same letter are not significantly different at a 5% significance level.*

Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato intercrop on mean number diamondback moth larvae on cabbage heads

The effects of the treatments on DBM damage on cabbage heads showed no significant differences (P ≥ 0.05) in both seasons. However, the highest mean number of live larvae was found in the untreated plots and those with tomato intercrop of 3.88 ± 0.76 and 2.56 ± 0.40, respectively in season one (Table 3). Season two had the same trend where there was no significant difference (P = 0.7827) in the effects of the treatments on DBM larvae but Neemraj Super gave the least mean number of larvae on cabbage leaves (Table 3).

Table 3: Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato intercrop on mean number diamondback moth larvae on cabbage heads

	DBM larvae on cabbage heads (Mean ± SE)	
Treatment	Season 1	Season 2
Biopower 1.15EC (*Beauveria bassiana*)	3.13 ± 0.64	1.00 ± 0.30
Dipel DF (*Bacillus thuringiensis* subsp. *kurstaki*)	2.56 ± 0.70	1.88 ± 0.75
Neemraj Super 3000 0.3EC	2.44 ± 0.79	0.94 ± 0.25
Tomato intercrop	2.56 ± 0.40	1.13 ± 0.24
Control (Water)	3.88 ± 0.76	1.31 ± 0.49
CV	22.90223	33.12881
P-value	0.0664	0.7827

Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato intercrop on mean number diamondback moth larvae on the weight of the cabbage heads

The effects of the treatments on DBM damage on the weight of cabbage heads showed no significant differences (P ≥ 0.05) in both seasons (Table 4). However, the highest mean weight of cabbage heads was observed in the *Beauveria bassiana* (3.31 ± 0.23) and *Bacillus thuringiensis* (3.18 ± 0.77) treated plots. The untreated plots had the least mean weight of cabbage heads (2.88 ± 0.22; 2.71 ± 0.59) in both seasons.

Table 4: Effects of *Beauveria bassiana*, *Bacillus thuringiensis*, neem, and tomato inter-crop on marketable yields of cabbage

	Weight of cabbage heads (Kg) (Mean ± SE)	
Treatment	Season 1	Season 2
Biopower 1.15EC (*Beauveria bassiana*)	3.31 ± 0.23	3.11 ± 0.33
Dipel DF (*Bacillus thuringiensis* subsp. *kurstaki*)	3.21 ± 0.81	3.18 ± 0.77
Neemraj Super 3000 0.3EC	3.24 ± 0.79	3.10 ± 0.23
Tomato inter-crop	2.95 ± 0.44	2.81 ± 0.64
Control (Water)	2.88 ± 0.22	2.71 ± 0.59
CV	12.90223	18.12881
P value	0.0763	0.084

Discussion

The study results revealed that the *Beauveria bassiana*, *Bacillus thuringiensis*, and neem treatments were effective against diamondback moth (DBM) leaf damage compared to the untreated control (distilled water) in the two seasons Though there was no significant difference in the mean number of DBM damaged cabbage leaves among the three bio-control, low mean number of DBM damaged leaves were observed in the neem treatment in the first season. Similar findings on the high efficacy of neem against DBM have been reported (Liang et al. 2003, Fening et al. 2020). According to Shannag et al. (2015), neem has an antifeedant effect as it interferes with feeding regulation and metabolism, and the anatomy and function of midgut tissues. This could be the reason as to why reduced DBM cabbage leaves damage was observed. The *B. bassian*a and *B. thuringiensis* treatments led to reduced DBM damage on cabbage leaves compared to the untreated control. The study results also indicated that all the biopesticides were efficacious on DBM in both seasons which conforms with Sitotaw et al. (2009), Rekha et al. (2011), Sharma et al. (2014), Legwaila et al. (2015), Hassan et al. (2018), Batcho et al. (2018) and Kamin et al. (2018), report on the effects of *B. bassiana*, *B. thuringiensis* and neem against DBM on cabbage.

In addition, there was no significant difference in the number of DBM among the treatment groups. Nevertheless, a higher mean number of DBM larvae on cabbage in the control group in the first season was observed. This could be attributed to variations in the environment treatment timing or inherent variations in the DBM population on cabbages during the treatment application and the time of the year the experiment was conducted. According to Xing and Zhao (2022), Daily Temperature Amplitudes (DTA) affects the *P. xylostella* phenotype acclimation response and which should be considered during insect populations studies. The planting dates have been reported to have a significant effect on DBM incidence (Vignesh and Singh 2017). The study also observed no significant differences in the marketable yields of cabbage among the treatments. This could be attributed to no significant differences in the number of DBM larvae on cabbage observed in the study, to cause injury to warrant a reasonable reduction in weight.

Thus, the cabbage may have escaped DBM injury at a pre-heading stage in the treated group which could have affected marketable yields in terms of weight. Notably, the mean cabbage head weight was least in the control group. Therefore, the *Beauveria bassiana*, *Bacillus thuringiensis*, neem treatments, and tomato intercrop had some effects on DBM hence higher weight compared to the control.

Conclusions and Recommendations

The study concluded that Dipel DF (*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351), BioPower (*Beauveria bassiana*) Neemraj Super 3000 (*Azadirachtin* 0.3%), and intercropping were effective in the management of diamondback moth (DBM) leaf damage on cabbage. The difference in the number of DBM larvae among the treatment groups was not significant but the control recorded a higher mean number of DBM larvae on cabbage specifically in the first crop season. Moreover, there were no significant differences in the marketable yields of cabbage among the treatments, but the least mean weights were recorded in the control group in both seasons. There is a need to determine why the treatments did not result in meaningful outcomes against the DBM population and cabbage head weights. In addition, a cost-benefit analysis of each treatment is necessary.

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