Effect of *Bacillus thuringiensis*, Neem, and Karate on Diamondback Moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) Damage on Cabbage in the Central Rift Valley of Ethiopia

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Abstract: The effect of two serotypes of *Bacillus thuringiensis (Bt)*, namely, *kurstaki* and *aizawai*, which are referred to as Dipel and Xen Tari by their trade name, respectively, neem seed water extract at the rate of 25 g (Neem 25) and 50 g (Neem 50) per liter of water, neem oil (Nimbecidine) and Karate (λ-cyhalothrin) were tested on diamondback moth (DBM) (*Plutella xylostella*) at two major cabbage growing areas, Melkassa and Wonji for two seasons, October 2005 to January 2006 and March 2006 to July 2006. Dipel, Xen Tari, Neem 25 and Neem 50 were effective in controlling DBM at both locations. As a result, the yield of cabbage improved; for instance at Wonji, marketable yield ranged from 33.4 to 35.1 ton ha^{-1}, for Neem 25, Neem 50, Xen Tari and Dipel treatments; while marketable yield ranged from 19.7 to 22.5 ton ha^{-1} for Karate and Nimbecidine. This finding indicates that the use of Bt and neem seed extract should be considered in an integrated management strategy for the diamond backmoth.

Keywords: *Bacillus thuringiensis; Brassica oleracea; Plutella xylostella; Azadirachta indica*

1. Introduction

The diamondback moth (DBM) (*Plutella xylostella* L.) (Lepidoptera: Plutellidae), is one of the most significant pests of cruciferous plants throughout the world and it has developed resistance to all insecticides widely used to control it (Talekar and Shelton, 1983). In some parts of the world, economic production of crucifer crops has become increasingly difficult due to insecticides' failure to control the pest (Metcalf, 1980). Extensive uses of non-selective insecticides, which kill DBM's natural enemies, particularly parasitoids, set the insect free of its biological control agents and help the insect to attain pest status in most parts of the world (Talekar and Shelton, 1983).

In addition to posing health problems, insecticides are frequently unavailable and are expensive for subsistence farmers in Africa. Environmentally safe and economically feasible DBM control practices need to be available. Use of *Bacillus thuringiensis* (*Bt*) and neem based products have proven successful in controlling insect pests in several parts of the world (Talekar and Shelton, 1983). In addition, these products are reported to be safe for natural enemies (parasitoids and predators) and, with less danger of development of resistance, are believed to form an important integral component of IPM program (Schmutterer, 1990). This study, reports on the efficacy of *Bacillus thuringiensis,* neem and a synthetic insecticide (Karate) in controlling DBM and improving cabbage yield.

2. Materials and Methods

2.1. Description of the Experimental Sites

The experiment was conducted at two major cabbage growing sites, Melkassa (8° 24' N; 39° 21' E, 1550 m above sea level.) and Wonji (8° 27' N, 39° 13' E, 1550 m above sea level) for two seasons, October 2005 and January 2006, and March 2006 to June 2006.

2.2. Cabbage Planting

Seeds of the cabbage (*Brassica oleracea* var. *capitata*) variety, Copenhagen Market, were used. During the first season, seeds were sown on seed bed (5 m^2) on September 5, 2005 and transplanted to the experimental plot on October 21 and 22, 2005 at Melkassa and Wonji, respectively. In the second season, seeds were sown on seed bed (5 m^2) on February 10, 2006 and transplanted to the experimental plot on March 13 and March 15, 2006 at Melkassa and Wonji, respectively. Seedlings were transplanted when they attained three to four true leaves. There were 10 rows per plot. Each row was 6 m long. The spacing between rows within a plot was 60 cm. The spacing between plants within a row was 40 cm. Spacing between plots within a block and between adjacent blocks were 1 m and 1.5 m, respectively. Plots were arranged in randomized complete block design (RCBD) with three replications.

At both locations, fields were irrigated twice per week for the first three to four weeks after transplanting and once weekly thereafter. Fields at Melkassa were fertilized with diammonium phosphate (DAP) and Urea at the rate of 200 and 100 kg /ha, respectively. The entire amount of DAP was applied just before transplanting, while urea was applied by splitting the total amount into two; half of the amount was applied one week after transplanting and the remaining half at the beginning of head formation. Weeding, cultivation and maintenance of ridges were carried out as needed.

2.3. The Treatments

The treatments were, Neem 25, Neem 50, Karate, Nimbecidine, Xen Tari, Dipel and untreated control (Table 1). Two serotypes of *Bacillus thuringiensis*, *kurstaki* and *aizawai* (named Dipel and Xen Tari, respectively) were brought from the International Center of Insect Physiology and Ecology (ICIPE), Nairobi. Neem Oil (Nimbecidine) and Karate were purchased from local pesticide traders.

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Table 1. Description of treatments used against DBM at Wonji and Melkassa in 2005 and 2006 cropping seasons.

Treatment	Chemical group	Description	Rate of application
Neem 25	Botanical	Aqueous seed powder extract at the rate of 25 g/l	100 to 200 g of powder (dependent on the leaf cover)
Neem 50	Botanical	Aqueous seed extract at the rate of 50 g/l	200 to 400 g of powder (dependent on the leaf cover)
Neem oil (Nimbecidine®)	Botanical	A commercial preparation of seeds of *Azadirachta indica*	1500 ml/ 500 l of water
Karate 5 EC ® (λ-cyhalothrin)	Synthetic insecticide	a pyrethroid insecticide (Check)	320 ml/ ha
Dipel ® 2X	Microbial pesticide	*Bacillus thuringiensis* var. *kurstaki*. Wettable powder (WP)	0.5 kg/ ha
Xen Tari ™	Microbial pesticide	*Bacillus thuringiensis* var. *aizawai*. Water Dispersible Granule	0.5 kg/ ha
Water		Control	4 to 8 liters depend on leaf cover

Neem seeds were collected from neem trees grown in Dire Dawa, Eastern Ethiopia. To prepare the aqueous neem seed extract, one day before treatment, application seeds were crushed into fine powder using a wooden mortar and pestle, and sieved using wire mesh. The powder was mixed with water in a plastic container at the rate of 25 g powder (referred as Neem 25) and 50 g powder (referred as Neem 50) per liter of water. After mixing, the solution was stirred carefully until all powder aggregates were diluted and was left for about 12 hrs at room temperature (20-23 ^{0}C). The following morning the extract was filtered into the sprayer using plastic mesh. For each week's treatment, the total amount of neem seed powder mixed with water varied between 100 and 200 g for the lower rate and between 200 and 400 g for the higher rate, depending on the crop growth stage.

2.3. Treatment Application

Application of treatments started two weeks after transplanting. Treatments were applied weekly until about ten days before harvest. Spray was made using a manually operated Knapsack sprayer of 15 liters capacity with a flat fan nozzle. Sterile water was applied to the untreated control plot.

2.4. Data Collected

DBM population: To determine the effect of treatments, a day before treatment application, 10 randomly selected plants from the central six rows were examined for DBM larvae and pupae. The assessment continued weekly for nine weeks.

Leaf damage: At harvest, ten plants per plot were randomly tagged. Diamondback moth leaf damage score was taken based on a scale of 0 to 5 (0 = no leaf damaged; 1 = up to 20 % of the total leaf area damaged; 2 = 21-40% of the total leaf area damaged; 3 = 41-60% of the total leaf area damaged; 4 = 61-80 % of the total leaf area damaged; and 5 = more than 80 % leaf area damaged). Percentage leaf injury level was calculated based on the equation: $P = \frac{\sum (nv)}{5N} * 100$ (Iman *et al.,* 1990).

Where: P = percentage leaf injury level; n = total number of leaves in an infestation class; v = numerical value of infestation class 0 to 5 as described above and N = total number of leaves observed.

Yield: At harvest, marketable yield data were taken from the central six rows of each plot; the whole plant population in the six rows were assessed by removing the outer damaged leaves and discarding heads with less than 4 cm in diameter. The yield data of only the second season were collected.

2.5. Data Analysis

Number of DBM and percent leaf injury were log transformed to stabilize the variances. Yield data were not transformed. The data were subjected to analysis of variances. Mean separation was done using Student-Newman-Keuls Test (SNK). Back transformed means are presented. Data were analyzed using the statistical package SAS (SAS, 1999).

3. Results

3.1. Effects of Bt, neem and karate on Number of DBM and Leaf Injury

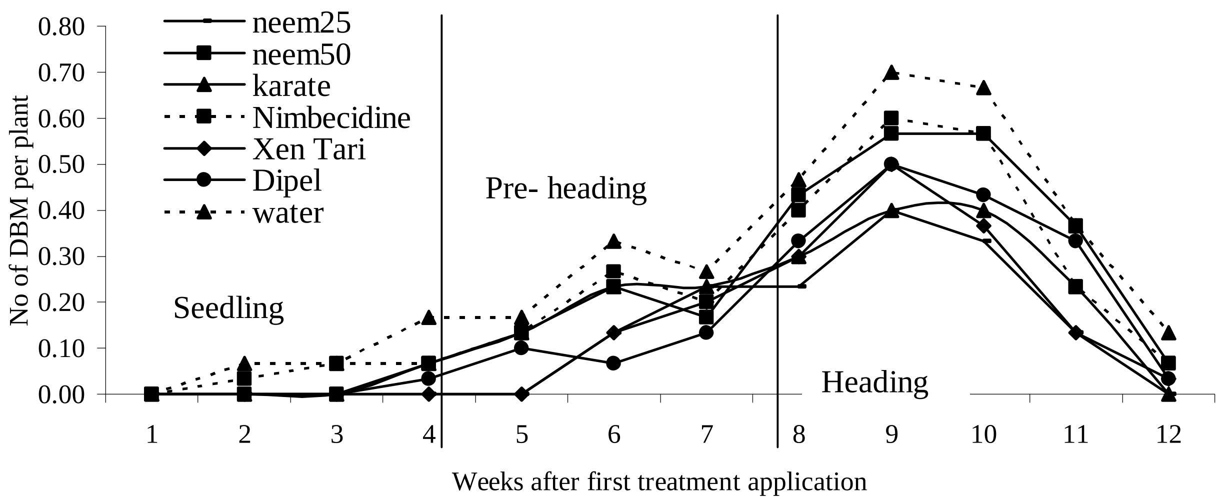
In the first season, from October 2005 to January 2006, the number of DBM at both locations was not high enough to cause significant differences between treatments (Table 2, Figureure 1 a and b). However, at Wonji, percentage leaf injury recorded from the Nimbecidine treated plot was higher than that of the Dipel treated plot.

Table 2. Mean number of diamondback moth per plant and percentage leaf injury on cabbage sprayed with neem, Bt and karate at Melkassa and Wonji, October 2005 to January 2006 (season-I).

Treatment	Melkassa		Wonji	
	Mean No. of DBM	Percentage leaf injury	Mean No. of DBM	Percentage leaf injury
Neem 25	0.23±0.07a	0.33±0.06a	0.10±0.03a	0.29±0.09ab
Neem 50	0.40±0.10a	0.26±0.10a	0.08±0.04a	0.24±0.06ab
Karate	0.30±0.04a	0.23±0.06a	0.14±0.09a	0.30±0.10ab
Nimbecidine	0.41±0.08a	0.28±0.06a	0.13±0.10a	0.33±0.06a
Xen Tari	0.39±0.04a	0.24±0.05a	0.01±0.01a	0.24±0.03ab
Dipel	0.26±0.11a	0.24±0.08a	0.07±0.03a	0.21±0.07b
Control (water)	0.52±0.09a	0.33±0.07a	0.10±0.04a	0.29±0.12ab

Means in a column followed by the same letter are not significantly different from each other at 5% significance level (Means were separated by Student-Newman-Keuls Test (SNK).

(a)



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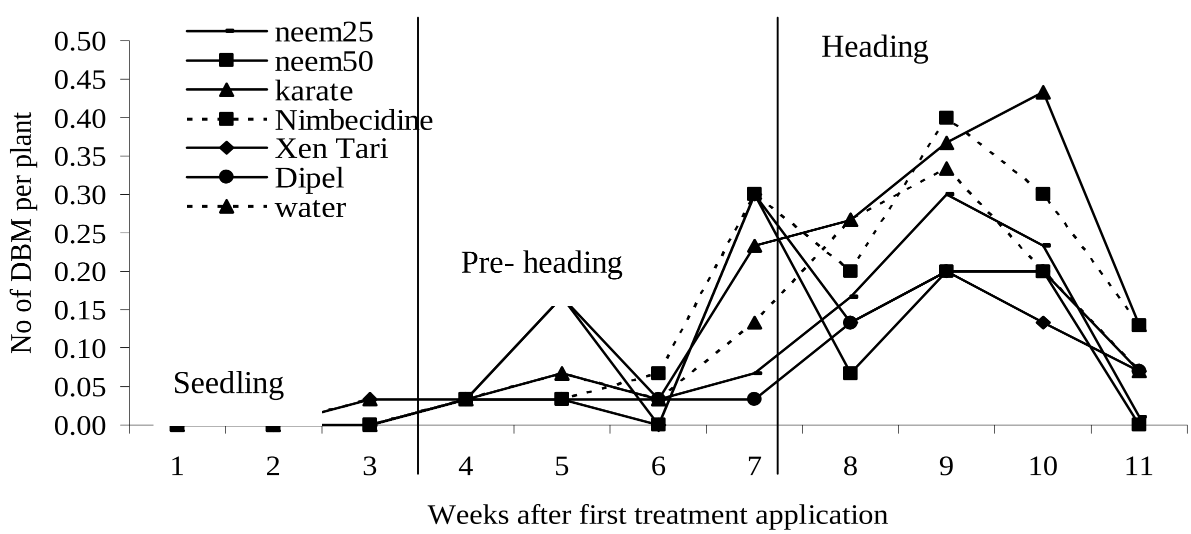


Figure 1. The effect of Neem, *Bacillus thuringiensis* and Karate on number of DBM at different crop growth stage at (a) Melkassa and (b) Wonji, from October 2005 to January 2006.

However, during the second season (March to July 2006**)**, at both locations, the number of DBM per plant varied among treatments throughout the sampling weeks (Figure. 2 a and b). At both locations, plots treated with Neem 50, Dipel and Xen Tari had the lowest number of DBM. Plots treated with Karate and Nimbicidine, however, had the highest number of DBM.

Significant differences ($P < 0.05$) were observed between treatments in the number of DBM per plant and percent leaf injury at both locations (Table 3). Plots treated with Neem 25, Neem 50, Xen Tari and Dipel, had the lowest number of DBM compared to the rest at both locations. Similarly, plots treated with Neem 50, Xen Tari and Dipel, had the lowest percent leaf injury. However, treatment with Karate and Nimbecidine appeared less effective and not apparently different from the control.

3.2. Effects of Bt, neem and karate on Marketable Yields of Cabbage

There were no significant differences ($P < 0.05$) between treatments in marketable yields at Melkassa (Table 4). However, there were significant differences ($P < 0.05$) between treatments in marketable yields at Wonji. The highest marketable yield was obtained from plots treated with Neem 25, Neem 50, Xen Tari and Dipel. Treatment with Karate and Nimbecidine, however, did not differ from the control.

Table 4. Marketable yield (tons per ha) of cabbage sprayed with different insecticides at Melkassa and Wonji, during season-II (March 2006 to July 2006).

Treatments	Melkassa	Wonji
Neem 25	20.66±3.96a	33.43±0.64a
Neem 50	25.84±4.83a	35.13±2.26a
Karate	22.03±3.96a	19.71±0.71b
Nimbecidine	15.95±3.97a	22.51±2.14b
Xen Tari	29.38±3.83a	34.09±0.13a
Dipel	23.67±3.73a	35.16±1.74a
Control (water)	22.35±1.99a	20.87±2.61b

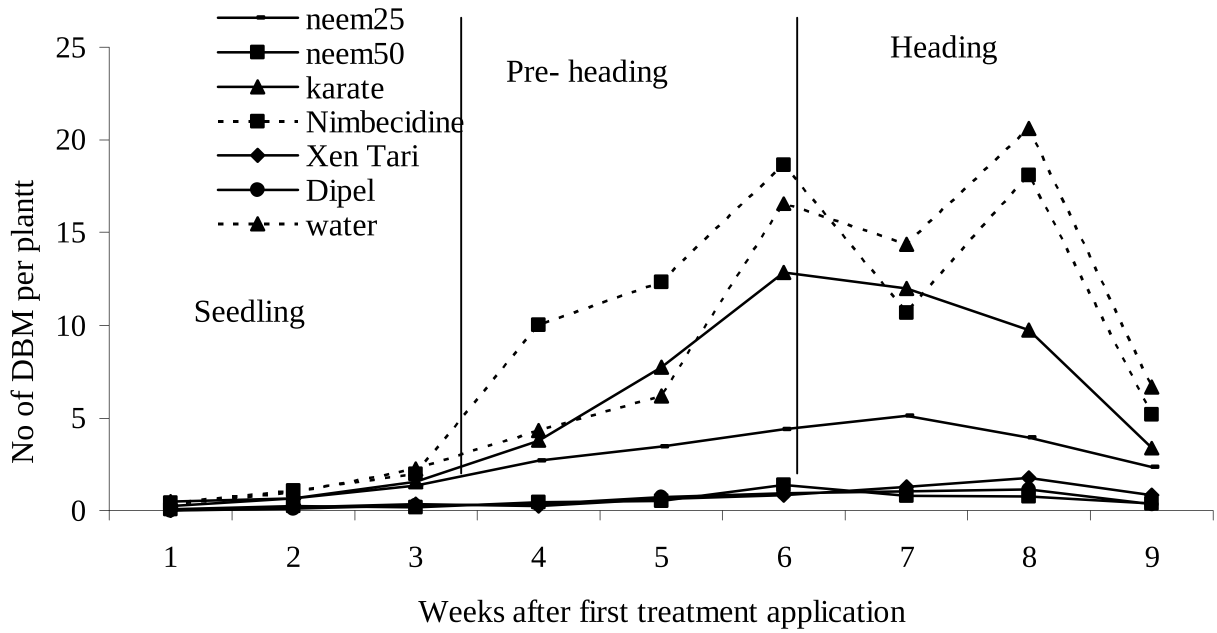
Means in a column followed by the same letter are not significantly different from each other at 5% significance level (Means were separated by Student-Newman-Keuls test (SNK).

Table 3. Mean number of Diamondback moth per plant and percentage leaf injury on cabbage sprayed with different insecticides at Melkassa and Wonji, March 2006 to July 2006 (season-II).

Treatment	Melkassa		Wonji	
	Mean No. of DBM	Percentage leaf injury	Mean No. of DBM	Percentage leaf injury
Neem 25	2.69±1.03c	10.70±2.32c	2.63±0.90c	14.4±3.61c
Neem 50	0.52±0.23c	1.00±0.06d	2.43±1.00d	6.11±1.36d
Karate	5.79±2.00b	16.00±5.08b	12.10±2.19a	35.10±4.53a
Nimbecidine	8.72±3.4a	19.20±4.40a	7.79±1.34b	25.6±6.11b
Xen Tari	0.67±0.23c	1.20±0.65d	0.68±0.37d	2.40±0.81d
Dipel	0.54±0.22c	1.40±0.46d	0.97±0.19d	6.78±2.33d
Control (water)	8.03±3.58a	17.00±4.25b	7.27±2.53b	23.20±3.15b

Means in a column followed by the same letter are not significantly different from each other at 5% significance level (Means were separated by Student-Newman-Keuls (SNK) test)

(a)



(b)

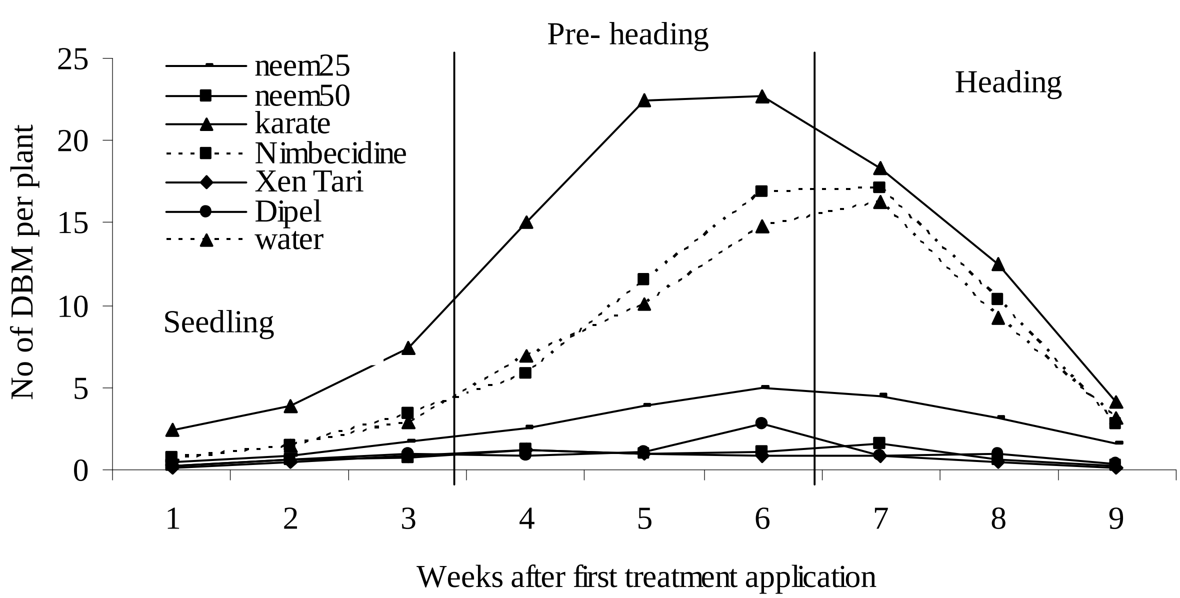


Figure 2. The effect of Neem, *Bacillus thuringiensis* and Karate on number of DBM at different crop growth at (a) Melkassa, and (b) Wonji, from March 2006 to July 2006.

4. Discussion

In general, at both locations, the population of DBM depended on cabbage growth stage. DBM showed a trend of high population during the pre-heading stage and low during the heading stage. In this study, treatment with neem seed extract and Bt had reduced the DBM population and leaf injury, and, as a result, the yield of cabbage was substantially improved. Schmutterer (1990) reported that aqueous neem extracts have proved to be effective in controlling those populations of DBM with high degrees of resistance to synthetic insecticides, even at low concentrations such as 12.5 g per liter; seed kernel extract was reported to reduce DBM adult fecundity by about 50 %. The good performance of neem in mitigating DBM infestation in areas where insecticides failed to provide control has also been reported by other researchers (Okoth, 1998; Goudegnon *et al*., 2000).

Though it is difficult to explain the observed high DBM number and percent injury level on Nimbecidine treated plots, similar reports have been made by Charleston *et al.* (2006). They observed a high level of DBM population on plots treated with a commercial preparation of *Azadirachta indica*, called Neemix 4.5® which was even higher than the untreated plot at a later stage of crop growth. Schmutterer (1990) also reported a better performance of water extracted lower concentration of neem seed kernel than formulated metanolic extract (AZT-VR-K-EC) in minimizing DBM damage.

The two Bt based products were highly effective against DBM. Tabashnik (1994) and Bauer (1995) reported that *Bt*-based products are the most promising alternative to conventional insecticides because they are highly toxic to certain pests and are compatible with IPM strategies due to their narrow host specificity, high amenability to genetic engineering, and because they cause little or no harm to humans, most beneficial insects and other non–target organisms.

The poor performance of Karate may be attributed to the presence of Karate resistant DBM population or Karate might have affected DBM natural enemies as Karate had been in use in the study areas for several years (Gashawbeza and Ogol, 2006). Frequent presence of pyrethroid resistance gene(s) in the population of DBM was reported when the pyrethroid was introduced (Hama, 1990). Host plant availability and action of its natural enemies are two key biotic factors which regulate DBM populations in the field (Harcourt, 1985). In many countries, synthetic insecticides are used to control DBM, which often eliminates natural enemies. This, in turn, can lead to continued intensive use of insecticides, eventually insecticide resistance and control failure (Sarfraz *et al.* 2005). Rowell *et al.* (2005) explained the higher DBM larval densities in the cypermetrin treated plots as a consequence of resistance to this commonly used insecticide and the probable distraction of non-resistant natural enemies.

In conclusion, neem seed extract and Bt should be encouraged for the integrated management of DBM as an alternative to synthetic insecticides.

6. References

Bauer, L.S. 1995. Resistance: a threat to insecticidal crystal proteins of *Bacillus thuringiensis*. *The Florida Entomologist* 78: 414-443.

Charleston, D.S., Rami, K., Malel, D. and Louise, E.M.V. 2006. Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: a field test of laboratory findings. *Biological Control* 39: 105-114.

Gashawbeza, A. and Ogol, C.K.P.O. 2006. Occurrence of the diamondback moth (*Plutella xylostella* L.) and its parasitoids in Ethiopia: influence of geographical region and agronomic traits. *Journal of Applied Entomology* 130: 343-348.

Goudegnon, A.E., Kirk, A.A., Schiffers, B. and Bordat, D. 2000. Comparative effects of deltametrin and neem kernel to *Plutella xylostella* and *Cotesia plutellae* populations in Cotonou Peri-urban area (Benin). *Journal of Applied Entomology* 124: 141-144.

Hama, H. 1990. Insecticide resistance characteristics of diamondback moth. *In:* Talekar, N.S. (Eds.). *Diamondback Moth and other Crucifer Pests*. Proceeding of the Second International Workshop, Tainan, Taiwan, 10-14 December 1992, Asian Vegetable Research and Development Center, Shanhua, Taiwan. pp. 455-463.

Harcourt, D.G. 1985. Population dynamics of the diamondback moth in South Ontario. *In:* Talekar, N.S. and Griggs, T.D. (Eds.). *Diamondback Moth Management*. Proceedings of the First International Workshop, Tainan, Taiwan, 11-15 March 1986. pp. 3-15.

Iman, M., Dani, S., Jestmandt, S., Adiputra, I.M. and Ishak, M. 1990. Effect of insecticides on various field strains of diamondback moth and its parasitoid in Indonesia. *In:* Talekar, N.S. (Eds.). *Diamondback Moth and other Cucifer Pests*. Proceeding of the Second International Workshop, Tainan, Taiwan, 10-14 December 1992, Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Metcalf, R.L. 1980. Changing role of insecticides in crop protection. *Annual Review of Entomolology* 25: 219-56.

Okoth, S.O. 1998. Control of diamondback moth *Plutella xylostella* (L.) (Lepepidoptera: Yoponmeutidae) in cabbage using neem based pesticides in Kenya. An M.Sc Thesis, the Kenyatta University, Ruiru, Kenya. pp. 70.

Rowell, B., Nittayaporn, B., Kosin, S., Sompian, P. and Charnnarong, D.A. 2005. Hymenopteran parasitoids of diamondback moth (Lepidoptera: Ypeunomutidae) in Northern Thailand. *Journal of Economic Entomology* 98: 449-456.

Sarfraz, M., Andrew, B.K. and Lioyd, M.D. 2005. Biological control of diamondback moth, *Plutella xylostella*: a review. *Biological Science and Technology* 15: 763-789.

SAS (Statistical Analysis System Institute). 1999. *SAS / STAT* . The SAS system for windows, version 8.0. SAS Institute, Cary, NC.

Schmutterer, H. 1990. Control of diamondback moth by application of Neem extracts. *In:* Talekar, N.S. (Eds.). *Diamondback Moth and other Crucifer Pests*. Proceeding of the Second International Workshop, Tainan, Taiwan, 10-14 December 1992, Asian Vegetable Research and Development Center, Shanhua, Taiwan. pp. 325-332.

Tabashnik, B.E. 1994. Evaluation of resistance to *Bacillus thuringiensis*. *Annual Review of Entomolology* 39: 47-79.

Talekar, N.S. and Shelton, A.M. 1983. Biology, ecology and management of diamondback moth. *Annual Review of Entomolology* 38: 275-301.