Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 7(4): 1524-1533, August 2013

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Laboratory evaluation of toxicity of *Bacillus thuringiensis*, neem oil and methamidophos against *Plutella xylostella* L. (Lepidoptera: Plutellidae) larvae

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ABSTRACT

Diamondback moth (DBM), *Plutella xylostella* (L.), remains a major pest of brassica crops worldwide. Chemical control of this pest remains difficult due to the rapid development of resistance to insecticides and to their effect on natural enemies. The objective of this study was to assess the toxicity of *Bacillus thuringiensis* (*Bt*), neem oil and methamidophos on larvae of *P. xylostella* under laboratory conditions. Leaf-dip bioassay for DBM larvae was used to assess mortality. For each treatment, three doses (low, medium and high) were applied on cabbage leaves and presented to third instar larvae. Larval mortality was performed every 24 hours for a period of eight days. The application of the three dosages of Biobit was more effective against *P. xylostella* larvae when compared to the other treatments. However, there was no significant difference in larval mortality when all three doses of Biobit were tested compared to the control. Methamidophos was the least toxic treatments with high dosage recording the lowest mortality rate of 52.5%. These results showed that *Bt*-based biopesticides and neem extracts could be of help, but their deployment should be part of an integrated pest management package, which recognizes the constraints of farmers while addressing the requirement to control of *P. xylostella* populations.

Keywords: Diamondback moth, biopesticide, *Azadirachta indica*, cabbage, bioassay.

INTRODUCTION

In Africa, cabbage remains a very important crop for smallholder farmers, providing income and nutrition and enabling small farms to remain financially viable, especially in the rapidly growing peri-urban farming sector (Grzywacz et al., 2010). The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has for many years been considered to be the most important pest of cabbages and other brassica crops worldwide (Talekar and Shelton, 1993; Sarfraz et al., 2005). The year-round brassica cultivation and short life cycle of the pest resulting in more than 25 generations of DBM a year, making it a continuous threat to production (Grzywacz et al., 2010). It can cause up to 90% crop loss (Verkerk and Wright, 1996). In Senegal, this rate is estimated between 51 and 94% according to

DOI : http://dx.doi.org/10.4314/ijbcs.v7i4.9

the direction of horticulture. The use of synthetic organic insecticides is the main method of control against this pest (Furlong et al., 2013). These insecticides can cause several consequences such as the elimination of natural enemies, the increased cost of production, emergence of resistant strains, environmental and health problems (Hooks and Johnson, 2003; Macharia et al., 2005; Sarfraz and Keddie, 2005).

However, several studies have demonstrated the efficacy of formulations of *B. thuringiensis* (Monnerat et al., 2000; Grzywacz et al., 2010) and some plant extracts like neem, *Azadirachta indica* A. Juss (Meliaceae), *Acorus calamus* L. (Araceae) and *Melia azedarach* L. (Meliaceae) against *P. xylostella* (Goudegnon et al., 2000; Patil and Goud, 2003). Despite the importance of these alternative products in controlling *P. xylostella* in other countries (Charleston et al., 2006; Ling et al., 2008; Lingathurai et al., 2011), very little information exists in literature about this in Senegal. To guide future management interventions, it is imperative to evaluate the effects of these products on *P. xylostella*. Information on the efficacy of these products is important for determining ecological safety and optimizing reliable and cost-effective management techniques for this agro-ecosystem under consideration.

As part of an ongoing larger project on integrated pest management (IPM) to reliably control the ravages of this pest, the objectives of this study was to evaluate the toxicity of *B. thuringiensis* (Biobit), extracts of neem (*Azadirachta indica*) and a chemical insecticide (methamidophos) on larvae of *P. xylostella* under laboratory conditions.

MATERIALS AND METHODS

Host plants

Two varieties of cabbage, *Brassica oleracea* var. *capitata* and cauliflower *Brassica oleracea* var. *botrytis*, were used in this study. Thirty-day old seedlings were transplanted on table (2 m x 1 m) containing a mixture of peanut hull, laterite and rice straw. Plants were fertilized with two types of fertilizers containing macronutrients (Na, K, Ca, Mg, P) and micronutrients (Fe, Zn, Cu, Mn, I) of 5 ml/l and 2 ml/l concentrations, respectively. Plants were watered weekly. No insecticide was applied in any of the plant cohorts during the entire growing season.

DBM rearing

DBM population was collected at Malika situated in the suburban area of Dakar (N: 14°47'552; W: 17°19'818 and 189 m altitude). Larvae and pupae collected were isolated in cylindrical plastic boxes (3 cm x 7 cm) with lids pierced with small holes for ventilation. Larvae are fed with the leaves of the host plant (*Brassica oleracea* var. *capitata*) and then followed until emergence of moths. Emerged adult DBM were harvested and introduced into a cubic cage of500 mm side. The eggs were collected daily on a cauliflower plant which achieves larval development. In the fourth stadium (L4), larvae were transferred on fresh leaves settled to the bottom of a large plastic box (28 cm × 27 cm) where they performed their pupation. The nymphs were collected daily. At emergence, adults were placed in the nest cage and fed with water and honey. The DBM rearing was conducted in a room with controlled climatic conditions: 25 °C, 75% RH and 12L/12D. Newly emerged third instar larvae were used in bioassay studies.

Treatments and doses

Three different treatments: Biobit, neem oil and methamidophos were used to evaluate their toxicity to *P. xylostella* under laboratory conditions. for each treatment, three doses (low, medium and high) were prepared as follows: For Biobit (*Bacillus thuringiensis* 1% WP) 5 mg, 7 mg and 10 mg were weighed and mixed with 10 ml of distilled water. for neem oil (*Azadirachta indica* 3% EC; Meliaceae), three different doses were prepared: 0.3 ml (low), 0.6 ml (medium) and 1 ml (high) per 100 ml of distilled water, for methamidophos (Metofos 600 EC; Organophosphorus), three different

doses were prepared from the volumes of 0.3 ml, 0.6 ml and 1 ml and mixed with 250 ml of distilled water. The control was applied with distilled water. A wetting agent (liquid detergent) was added to different treatments.

Toxicity bioassays

The toxicity bioassay was a leaf dip method similar to that used by Tabashnik et al. (1990). For toxicity bioassay experiment, third instar larvae were treated by oral application through cauliflower leaf discs. Leaf tissue (6 cm in diameter) was cut from uninfested cabbage plants raised in the table. Individual leaves were immersed in the prepared insecticide solution for 10 s and hung vertically to air dry for 2 h. Control leaves were treated similarly with tap water. Ten larvae from DBM rearing were placed in each petri dish (6 cm × 1.5 cm) containing a leaf disc. Larval mortality was recorded every 24 h for a period of 8 days. Larvae were considered dead if they did not move when lightly prodded with forceps (Hill and Foster, 2000). After 24 h of exposure, larvae were continuously maintained on untreated fresh cauliflower leaves. Five replicates were maintained for each treatment.

Statistical analysis

Data were normalized and transformed before subjecting them to analysis of variance (ANOVA), using a logarithmic transformation. Means were separated using Students Newman Keuls test. The statistical analysis was performed with the software XLSTAT version 2012.1.01. In all statistical analyses P values < 0.05 were considered significant.

RESULTS

Effect of doses of Biobit on larval mortality

Among the doses of Biobit, larval mortality rates were significantly different ($F = 10.50$; $df = 3, 12$; $P < 0.0001$; Table 1). At high dose, Biobit caused 100% mortality after 5 days of exposure. At medium dose, the percentage of larval mortality was constant from 5 to 7 days and was 96%. The maximum rate of 100% mortality was reached at 8 days. At low dose, the percentage of larval mortality was the highest recorded at 7 days and was 92%. For the control, the maximum mortality rate was 48% after 8 days of exposure (Figure 1). The three doses of Biobit were toxic to larvae of *P. xylostella* compared with the control. However, there was no significant difference between the three doses tested. Mortality was significantly lower in the control (Table 1).

Effect of doses of neem oil on larval mortality

Larval mortality rates were significantly different among the doses tested ($F = 8.94$; $df = 3, 12$; $P = 0.0003$; Table 1). However, there were no significant differences between high and medium doses, which were more toxic on larvae compared with the low dose and control. There were no significant differences between the low dose and the control (Table 1). At high dose, neem oil showed a percentage of larval mortality up to 92% after 7 days of exposure. At medium dose, the percentage of larval mortality reached 88% after 8 days of exposure. At low dose, the maximum larval mortality rate was 40% between 6 and 8 days of exposure. This percentage was lower compared to control where it increased from 37 to 48% over the same period (Figure 2).

Effect of doses methamidophos on larval mortality

Among the doses tested, larval mortality rates were significantly different ($F = 3.98$; $df = 3, 12$; $P = 0.018$; Table 1). However, there were no significant differences between high and medium doses that were more toxic on larvae. The low dose and control were not significantly different, they were less effective. The percentage of larval mortality recorded at high and medium doses had the same evolution. They caused 68% mortality after 5 days of exposure while in the same period, the mortality rate obtained with low dose ranged from 44 to 52%. This rate was lower in control with a maximum of

48% mortality after 8 days of exposure (Figure 3).

Effect of treatments at high dose on larval mortality

The larval mortality was significantly different among treatments at high dose (F = 10.49; df = 3, 12; P < 0.0001; Table 2). At high doses, Biobit caused 100% larval mortality after 5 days of exposure. Over the same period and at the same dose, we noted a mortality rate of 84% for neem oil with a maximum rate of 92% at 7 days, 68% for methamidophos and 27% in control. At high dose, Biobit was more toxic than neem oil and methamidophos (Figure 4).

Effect of treatments at medium dose on larval mortality

The larval mortality was significantly different among treatments at medium dose (F = 8.69; df = 3, 12; P = 0.0003; Table 2). At medium dose, the mortality rate for Biobit ranged from 96 to 100% between 5 and 8 days of exposure while for neem oil over the same period, the rate varied from 80 to 88% and for methamidophos, it reached 68% mortality. At medium dose, Biobit and neem oil were more toxic than methamidophos (Figure 5).

Effect of treatments at low dose on larval mortality

The larval mortality was significantly different among treatments at low dose (F = 7.60; df = 3, 12; P = 0.0007; Table 2). At low dose, the maximum mortality recorded after 7 days of exposure was 92% for Biobit. For neem oil, the percentage mortality reached 40% after 6 days and a mortality rate of 52% obtained for methamidophos after 8 days of exposure. However, there were no significant differences in mortality that occurred among the neem oil, methamidophos and control (Figure 6). At low dose, Biobit was more effective than neem oil and methamidophos.

Table 1: Mean larval mortality of *P. xylostella* among treatments.

Treatments	Biobit	Neem	Methamidophos
High dose	84 [a]	70 [a]	52.5 [a]
Medium dose	82 [a]	61.5 [a]	51 [a]
Low dose	67.5 [a]	24 [b]	36 a[b]
Control	23.5 [b]	23.5 [b]	23.5 [b]
ANOVA F	10.50	8.94	3.98
P	< 0.0001	0.0003	0.018

Means in columns followed by the same letters are not significantly different by Student – Newman – Keuls test.

Table 2: Mean larval mortality of *P. xylostella* among doses tested.

Doses	High dose	Medium dose	Low dose
Biobit	84 [a]	82 [a]	67.5 [a]
Neem	70 [ab]	61.5 [a]	24 [b]
Methamidophos	52.3 [bc]	51 [ab]	36 [b]
Control	23.5 [c]	23.5 [b]	23.5 [b]
ANOVA F	10.49	8.69	7.60
P	< 0.0001	0.0003	0.0007

Means in columns followed by the same letters are not significantly different by Student – Newman – Keuls test.

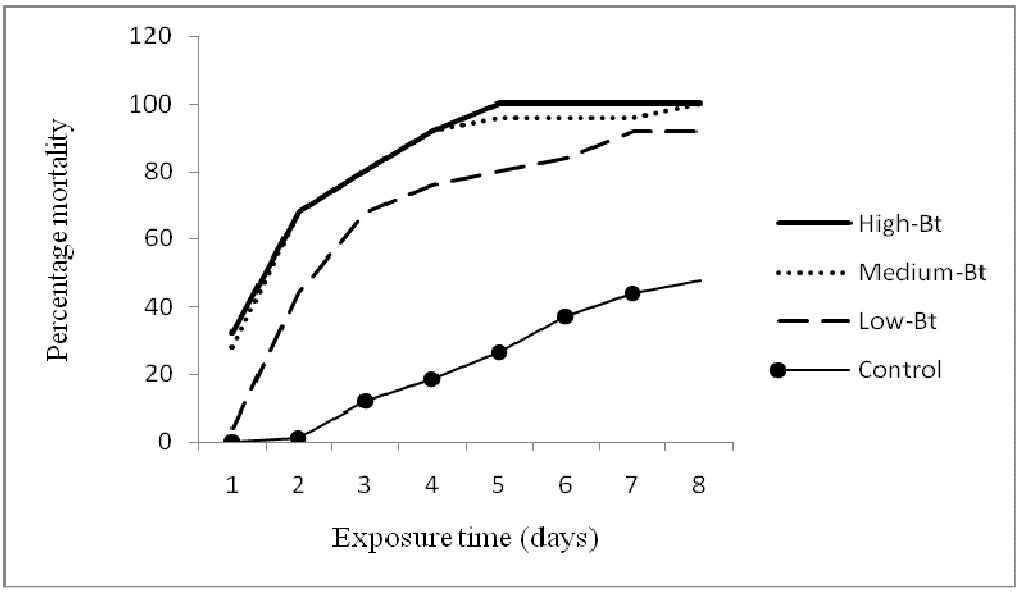

Figure 1: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of Biobit (Bt).

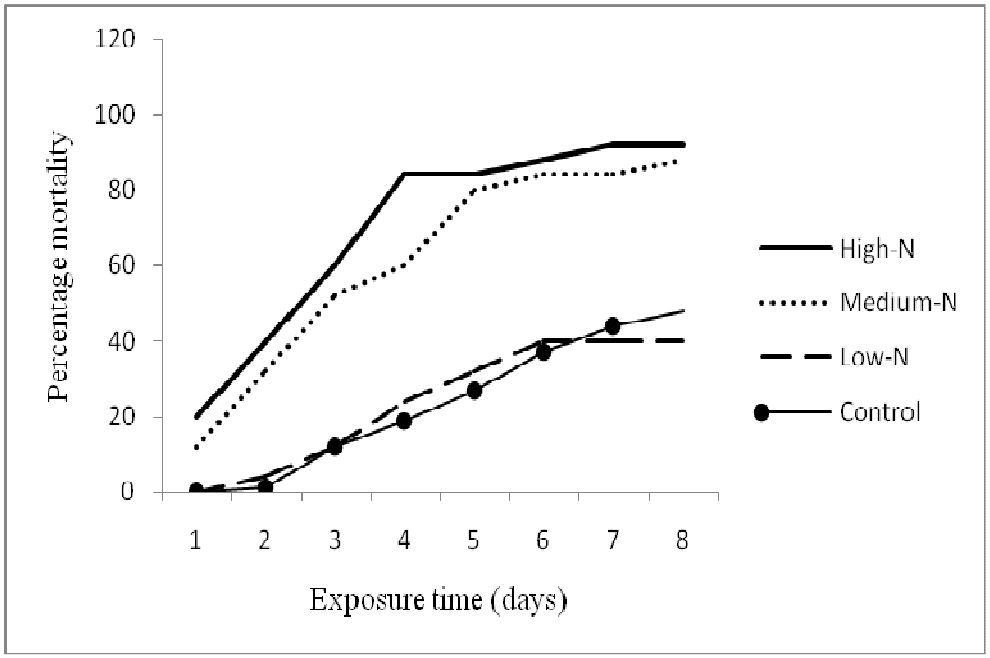

Figure 2: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of Neem (N).

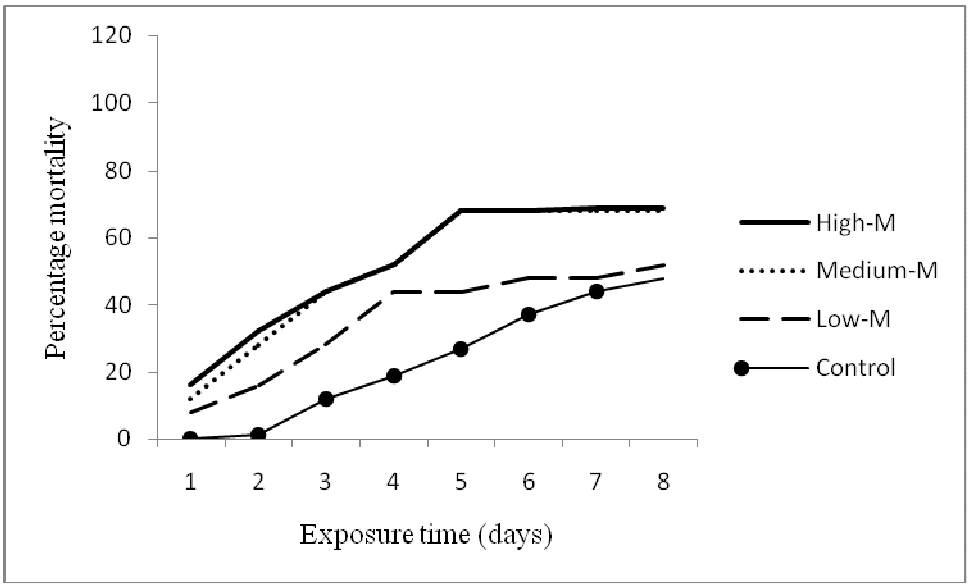

Figure 3: Percentage mortality of *P. xylostella* larvae in terms of the exposure time and doses of methamidophos (M).

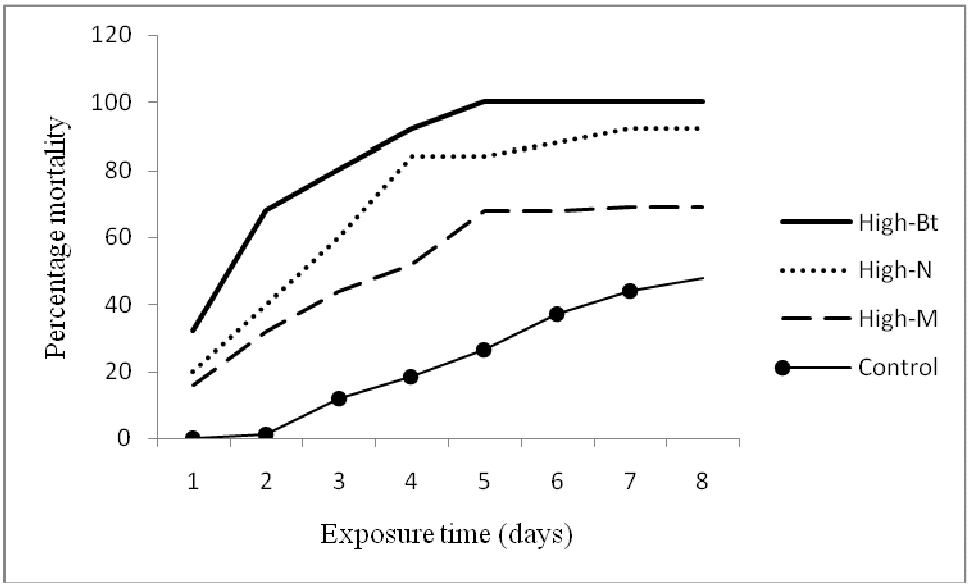

Figure 4: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at high dose (Bt= Biobit; N= Neem; M= Methamidophos).

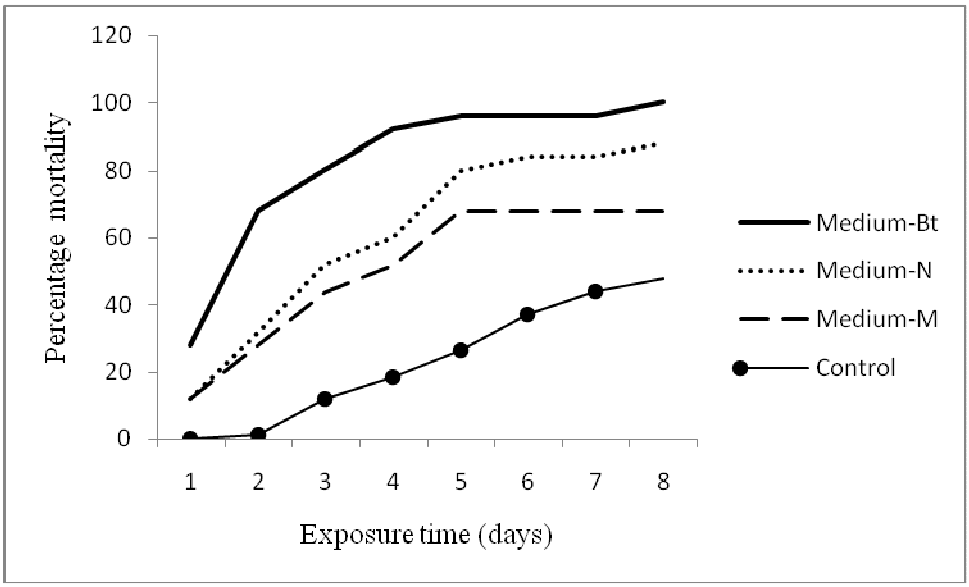

Figure 5: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at medium dose (Bt= Biobit; N= Neem; M= Methamidophos).

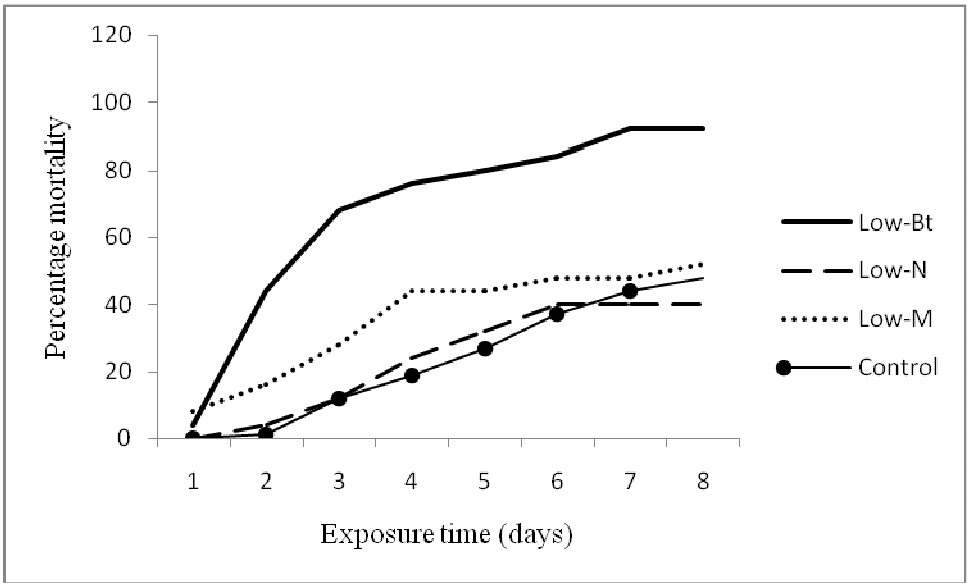

Figure 6: Percentage mortality of *P. xylostella* larvae depending on the exposure time and treatments at low dose (Bt= Biobit; N= Neem; M= Methamidophos).

DISCUSSION

The findings from this study revealed that Biobit was the most effective treatment for *P. xylostella* L3 larvae under laboratory conditions. The toxicity of Biobit has been reported to be primarily due to the presence of delta-endotoxin (Lereclus et al., 1993). According to our observations, the efficacy of Biobit can be attributed to its high degree of larval mortality when different dosages (low, medium and high) are evaluated. Our results are similar to that reported by Monnerat et al. (2000), who reported that Biobit is effective on larvae of *P. xylostella*, irrespective of the applied dosage. The high mortality recorded when low doses of Biobit was applied in this study is also supported by Ketoh et al. (2004), who reported that effective biopesticides are those that cause high mortality in the population even at extremely low concentrations. González-Cabrera et al. (2010) has also shown the efficacy of *B. thuringiensis* in the laboratory control of the tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). According to Regnault-Roger (2005), the effectiveness of this biopesticide is due to its rapid mode of action.

Neem oil is effective when high and medium doses are applied, whereas low doses are completely ineffective and recorded very low larval mortality in our study. These results are not surprising given that the toxicity of insecticides of plant extracts has been shown to vary with dose and duration of exposure (Bouchikhi et al., 2010). The results from this study are similar to that reported by Charleston et al. (2005), who studied the impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies. Several other studies have demonstrated the impact of neem on mortality of *P. xylostella* (Perera et al., 2000; Liang et al., 2003). However, the low toxicity of neem oil compared to Biobit can be attributed to its anti-palatable and repellant effect on insects (Patil and Goud, 2003). This anti-palatable effect of neem extracts has been reported to be accompanied by a significant reduction in food consumption by the herbivorous insect (Liang et al., 2003), which might be the cause of its low toxicity in this study. They contain an active ingredient, azadirachtin, which has anti appetizing, disgusting and sterile properties that inhibits molting, growth and larval development (Patil and Goud, 2003).

Methamidophos on the other hand recorded the lowest larval mortality rates when compared to Biobit and neem oil extract. This reduced mortality effect is not uncommon, given that several studies have demonstrated that *P. xylostella* is resistant to Methamidophos (Sereda et al., 1997). This pattern of introduction and failure of insecticides continues today because of the widespread lack of adoption of any insect resistance management (IRM) strategy by farmers (Wright, 2004; Mau and Gusukuma-Minuto, 2004).

Our results showed that biopesticides based on *Bacillus thuringiensis* and neem oil extracts were more effective against *P. xylostella* compared to synthetic organic insecticide (Methamidophos). The use of these two products could provide an alternative approach to conventional insecticides. Further experiments are needed to clarify the nature of the compounds involved in their larvicidal activity to optimize the effective doses. Despite the results obtained from this study, the effectiveness of these treatments remains to be demonstrated in the field before their deployment as part of an integrated pest management (IPM) package.

ACKNOWLEDGEMENTS

The authors are grateful to the support from AUF through the program “Horizons Francophones”. We also thank two

anonymous reviewers for comments on the manuscript.

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