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Sustainable Approach for the Management of the Pod Borers on Bean in Mauritius

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Abstract

Bean (*Phaseolus vulgaris* L.) is grown on about 360 hectares in Mauritius. Pod borers (*Lampides boeticus* L., *Maruca vitrata* F. and *Etiella zinckenella* Tr.) are reported to cause serious damage to floral parts and pods. They are difficult to control with insecticides because they remain concealed in feeding sites. Farmers apply insecticides excessively and this leads to problems that threaten production, sustainability, health and the environment. This paper reports on results so far obtained on the first attempt at development of a sustainable pod borer management strategy in Mauritius.

The importance of pod borers in bean cultivation was first determined. *Maruca vitrata* was the most important species. It represented 99.6% of field collected pod borer larvae and accounted for the 42% of pod damage in untreated bean fields. *Lampides boeticus* was not important (0.1%) while *E. zinckenella* was not even present. *Helicoverpa armigera* Hubner was recorded on bean (0.3%) for the first time. Two species of larval parasitoids were recovered from collected larvae of *M. vitrata* and parasitism in treated and untreated fields was 0.7% and 2.4% respectively.

Three biocontrol agents (egg parasitoid, entomopathogenic nematode and pathogenic fungus) were recorded for the first time during the inventory of natural enemies of *M. vitrata*. The egg parasitoid (*Trichogrammma chilonis* Ishii) and entomopathogenic nematode (unidentified) were retrieved by exposure method and the pathogenic fungus (*Metarhizium* sp.) was detected in diseased *M. vitrata* larvae from the wild legume, *Pueraria phaseoloides* (Roxb.) Benyh. Parasitism in exposed eggs in an untreated pigeon pea plot was 62%. 52% of exposed pupae were infected with nematodes and 14.6% of collected *M. vitrata* larvae were infected with *Metarhizium* sp.

Commercial biopesticides were tested against *M. vitrata*. *Bacillus thuringiensis* var. *kurstaki* was effective against larvae while *Beauveria bassiana* was not. Azadirachtin was effective in reducing pod damage by *M. vitrata* in bean fields.

Keywords: Pod borers, bean, *Maruca vitrata*, egg parasitoid, entomopathogenic nematode, pathogenic fungus, biopesticides.

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INTRODUCTION

Bean (*Phaeseolus vulgaris* L.) is grown on about 360 hectares in Mauritius as a cash crop by small farmers with an annual production of about 1826 tonnes (Anon, 2004). In Mauritius, 21 arthropod pests are recorded on the bean plant. Three of them are pod borers (*Lampides boeticus* L., *Maruca vitrata* Fabricius and *Etiella zinckenella* Tr.) and are reported to cause significant damage to flowers and pods (Moutia, 1955). Their damage has so far been quantified only for pigeon pea crops (Anon, 1958).

Until recently, research focus was on screening of insecticides and recommended ones are mainly contact in action (Abeeluck *et al.*, 2004). Pod borers are difficult to control with contact insecticides because larvae are well protected in floral parts and pods. To achieve satisfactory control, farmers do not follow the prescribed recommendations; they spray their fields more often and at higher dosages than recommended (Fagoonee, 1984; Abeeluck *et al.*, 1997). Such misuse of insecticides has caused human toxicity due to drift in the past decade. Other negative impacts may be development of insecticide resistance as reported in Africa and Philippines (Ekesi, 1999 and Ulrichs *et al.*, 2002), decline of beneficial insect populations and the presence of pesticide residues on harvested produce. These negative impacts can eventually render chemical control socially unacceptable. Furthermore, existing export opportunities to European Union (EU) countries can be put at risk. EU has imposed strict phytosanitary regulations to ensure safe food production through adoption of Good Agricultural Practice and compliance with Maximum Residue Levels (ECDB, 2005). Mauritius is a member of the World Trade Organisation, therefore its producers involved in export of agricultural commodities has to comply under such phytosanitary agreement (MAIFPS, 2000).

Legume production is likely to expand (Anon, 2008) and this increasing intensity in production will no doubt increase the crop vulnerability to pod borers. It has therefore become imperative to develop an integrated pest management (IPM) programme against pod borers. IPM as such is environmentally safe and sustainable and is the most viable alternative to chemical control.

This paper presents results so far obtained on the first attempt at development of a sustainable pod borer management strategy. As a prerequisite for development of a sustainable pod borer management, it was first necessary to identify the major species among the 3 pod borers. Research was then directed to select non chemical control methods against the identified target pest.

MATERIALS AND METHODS

These studies were conducted during January 2007-March 2008.

Determination of the economic importance of pod borers on bean plant
During a 10-month survey, pod borer larvae were collected from farmers' fields (n=27, treated) in 3 bean growing areas and from untreated plots (n=5) at the Crop

Research Station (CRS) of the Agricultural Research and Extension Unit (AREU) at Réduit. At each visit to a study site, a farmer's field ($\leq$ 0.25 hectare and at flowering and fruiting stage) was selected. 50 flower buds and 50 flowers were collected from 10 randomly selected plants every 15 days. On each harvest occasion, 50 pods (5/plant) were also examined from these plants.

An untreated bean plot (9 m^2) was set at Reduit CRS every 4-5 weeks. Every week, flower buds and flowers were sampled as per above method. One row within the plot was not sampled and pods from 5 randomly selected plants were examined at every harvest. Such sampling was undertaken during 5 crop cycles.

Sampled floral parts and pods from treated and untreated fields were examined in laboratory and kept in individual plastic containers for 7 days to allow hatching of recently laid eggs that could not be visually detected. Collected larvae from floral parts and pods were raised on mung bean sprouts.

Laboratory bioassay and field trial for testing the efficacy of non-chemical products

2 commercial formulations of microbial (*Bacillus thuringiensis var. kurstaki, Beauveria bassiana*) and 1 botanical (*Azadirachtin*) insecticides were tested against *M. vitrata*. *Bacillus thuringiensis* (Dipel 16000 I.U./mg from Abbott Laboratories, USA), *B. bassiana* (Biofix larvo-guard 2 x 10^9 CFU/mL from Ajay Biotech Ltd, India) and *Azadirachtin* (Bioking 0.15% EC from Scientific Fertiliser Company Pvt. Ltd, India) were obtained from local suppliers. Larvae (4 and 7-day old) for the bioassay were procured from a laboratory colony (F1 generation). They were raised on mung bean sprouts. Bioassays were run in laboratory at 27 $\pm$ 2ºC and relative humidity 75% - 80%.

Laboratory dose response tests for Bacillus thuringiensis (Dipel) and Beauveria bassania against Maruca vitrata larvae

The two products were tested at three rates against 4 and 7-day old larvae. Dipel was tested at the rate of 1.0, 1.5 and 2.0 g/L of water while *B. bassania* at 2.0, 3.0 and 4.0 g/L of water.

Sprouts were dipped for 10 seconds in a prepared solution at a given rate and air dried. 20 of such treated sprouts were placed in a paper cup lined with tissue paper. Five larvae (4-day old and starved for 1 hour) were transferred to the cup and covered with a lid. Sprouts were dipped in water and air dried. Twenty of them were kept in a cup with 5 larvae as described above and kept as control. Cups were checked every 24 hours and dead larvae recorded until pupation in control cups.

Six replicates were set for each of Dipel, *B. bassania* rates and control. The same procedure was followed to test the product against 7-day old larvae.

Field assessment of the efficacy of azadirachtin (Bioking) for control of M. vitrata on bean

The experiment was set in a Randomised Block Design with 4 block replicates of bean plants raised under recommended agronomic practices (Abeeluck et al, 2004). Each block was split into 4 plots. Each plot (9 m^2) had 7 rows of bean plants. Three

plots from each block were treated with Bioking at 3 rates: 5.0 (T1), 7.5 (T2) and 10.0 mL/L of water (T3) with a knapsack sprayer every 7 days as from flowering. An untreated plot (T4) was kept as control.

From each plot, 10 flower buds and 10 flowers were sampled from 5 randomly selected plants from the 3rd and 5th row. The 4th row was not sampled and all pods (damaged and undamaged) from 5 selected plants were recorded at every harvest. Collected flower buds and flowers were examined in laboratory and held in plastic containers for 7 days to allow hatching of recently laid eggs.

Identification of existing natural enemies

Egg parasitoids

Eggs of *M. vitrata* are minute and laid scattered on leaves and floral parts of plants (Jackai, 1981) and direct observation of parasitism in the field is not possible. Arodokoun's method (1996) was used to detect egg parasitoids in an untreated pigeon pea plot at Reduit CRS.

Potted bean plants were placed in a rearing cage with 25 mated females for 24 hours. A plant with freshly laid eggs was placed in the pigeon pea plot for 48 hours. Leaves with eggs from the exposed plant were excised in laboratory, held in a glass jar and observed daily. Parasitised eggs (n=32) were kept in individual glass vials. Emerging parasitoids were sexed under a binocular microscope.

Plant exposure was undertaken on 4 occasions (one plant/occasion) at weekly intervals.

Entomopathogenic nematodes (EPN)

Pupae of *M. vitrata* (n=50) were placed at a depth of 3 cm in soil in a bean field and brought to the laboratory after 7 days. They were washed with water to remove soil particles and placed in individual petri dishes with 5 mL of water. Each pupa was macerated and examined under a binocular microscope. When nematodes were detected, the macerated content was filtered with cheesecloth. A stock solution was thus prepared.

Nematodes were multiplied by method of Kaya and Stock (1997) with White's trap. Five *M. vitrata* larvae (5th instar) were placed in a petri dish (5 cm dia.) to which 2 mL of the stock solution was added. After two days, the petri dish was placed on a bigger one (9 cm dia.) with a layer of water. Larvae were examined daily under a binocular microscope.

Fungal pathogen

Larvae of *M. vitrata* were collected from 5 cultivated legumes (bean, ground nut, pea, pigeon pea, cowpea and lima bean) and a wild one (*Pueraria phaseoloides*) from study sites. Flowers were sampled from legumes and leaves from ground nut. Collected larvae were raised individually on sprouts in sterile plastic cups and examined daily. Diseased larvae were forwarded to the Plant Pathology Division of AREU to identify the pathogenic organism.

STATISTICAL ANALYSIS

In the neem trial, larval numbers on floral parts was converted as proportion of larvae after treatment over number of larvae before treatment. Percentage pod damage was arcsine transformed to stabilize variances and analysed by one way Analysis of variance (ANOVA) with means for different treatments separated by Fisher's Least Significant Difference test (SPSS 11.5).

For the evaluation of biopesticides (Dipel and Biofix larvo-guard), larval mortality was corrected using Abbott's formula:
$P_T = P_O - P_C / 100 - P_C \times 100$
where
P_T – Corrected mortality in terms of %
P_O – Observed mortality in terms of %
P_C – Control mortality in terms of %

The percentage mortality was arcsine transformed and analysed by one way ANOVA with means separated by Tukey's Studentized Range Test (SAS 2004).

RESULTS

Determination of the economic importance of pod borers on bean plant
Maruca vitrata constituted the major proportion (99.6%) of pod borer larvae (n=1886) collected at the 4 study sites. *Lampides boeticus* larvae were present only on flower buds and flowers at Reduit CRS and in very low percentage (0.1%). *Etiella zinckenella* was not found at any site. Larvae of *H. armigera* were recovered (0.3%) from flower buds, flowers and pods in the North and East of the island. 42% of pods (n=3153) from untreated fields were damaged by *M. vitrata* only. In treated fields, 22% of pods (n=1000) were damaged by *M. vitrata* (99.5%) and *H. armigera* (0.5%).

1.5% of *M. vitrata* larvae (n=1879) collected from the 4 sites were parasitized. No parasitism was observed in larvae (n=244) from the East. Two species of larval parasitoids (*Bracon* sp. (Hymenoptera: Braconidae) and *Eiphosoma annulatum* Cress (Hymenoptera: Braconidae)) were recovered from *M. vitrata* larvae from the North, South and Reduit CRS. Parasitism in untreated fields was comparatively higher (2.4%) than that (0.7%) in treated ones. Deltamethrin, cypermethrin and lambda cyhalothrin were commonly used by farmers during the whole crop cycle.

Efficacy of Bacillus thuringiensis (Dipel) against Maruca vitrata
Larvae stopped feeding on Dipel treated sprouts after 24 hours of exposure. Mortality was first observed in young larvae but at low percentage (10.3% at 24 hours) and then in mature larvae (12.2% at 48 hours). However, it reached up to above 70% after 7 days in both young and mature larvae in Dipel treatment at the rate of 2.0 g/L. Mortality in younger larvae, as such, was higher than that in mature larvae, particularly at higher dosages of Dipel (Fig. 1).

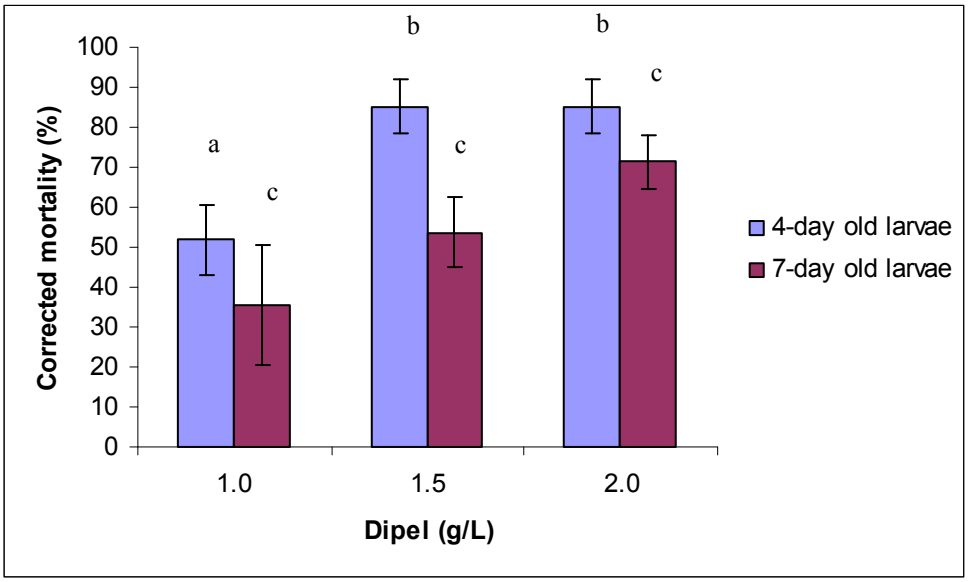

Figure 1: Percentage mortality (± SE) in sprouts treated with 3 rates of Dipel; bars with different letters are significantly different (P<0.05), one-way ANOVA, Tukey's Studentized Range Test

Efficacy of Beauveria bassiania against larvae of M. vitrata

Mortalities in larvae (4 and 7-day old) fed on sprouts treated with *B. bassiana* at the 3 rates were very low (<15%). There was no significant difference in larval mortalities between treated and untreated sprouts.

Efficacy of azadirachtin (Bioking) for control of M. vitrata on bean

Bioking application, irrespective of rates, did not reduce larval numbers in floral parts. Although, larval numbers were numerically highest in control plots compared to Bioking treatments, differences were not statistically significant (P>0.05) (Fig. 2).

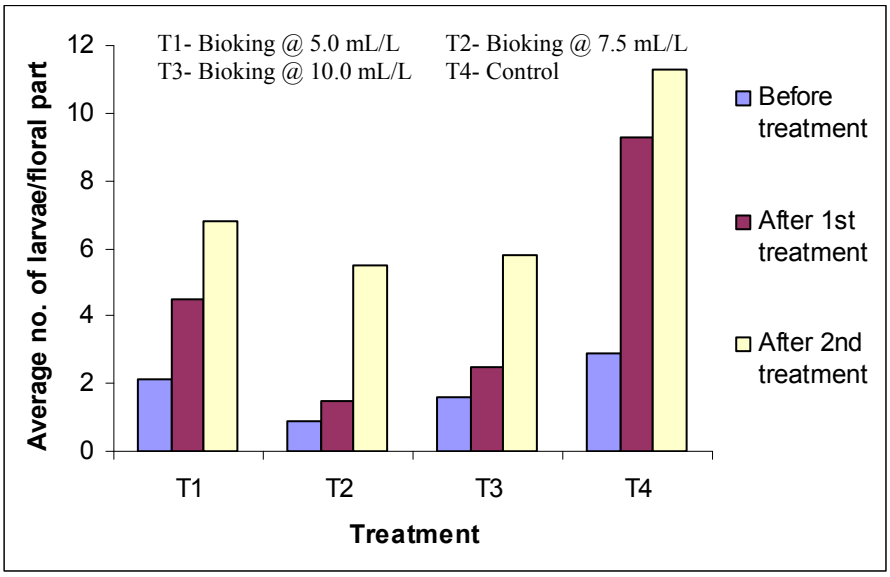

Figure 2: Average number of larvae on floral parts (per flower bud and flower) in treated and control plots before and after Bioking application

On the other hand, pod damage was significantly reduced with Bioking treatments, irrespective of rates compared to control plots from which highest pod damage was recorded ($F=22.6$; $df= 3, 31$; $P<0.05$) (Fig. 3). Pod damage was numerically lowest in plots treated with Bioking at 7.5 mL/L (T2) but there was no significant difference among Bioking treatments.

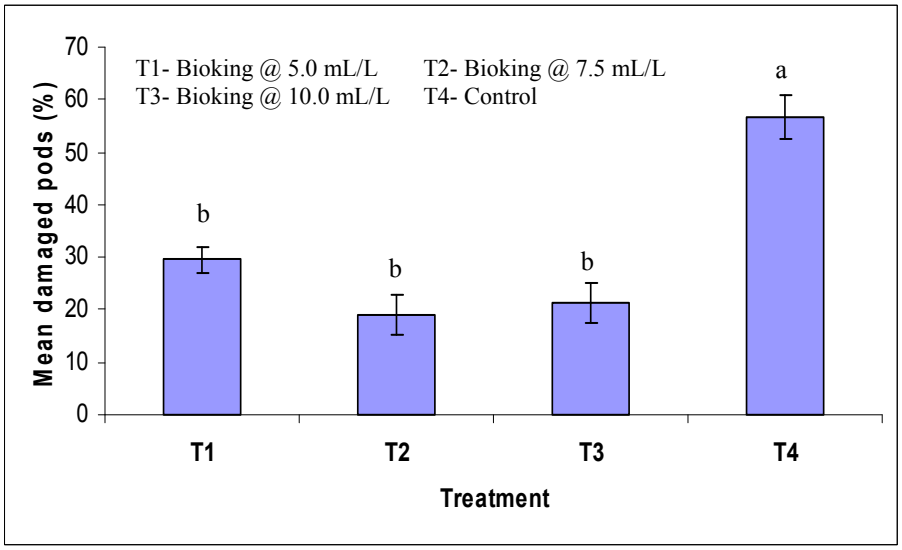

Figure 3: Mean (± SE) percentage pod damage in Bioking treated and control plots; bars with different letters are significantly different ($P<0.05$), one-way ANOVA, Fisher's LSD test.

Identification of existing natural enemies

Egg parasitoids

1217 eggs were exposed on 4 occasions and 750 were parasitised. Parasitised eggs turned black on the 4th day of observation and could be easily differentiated from unparastiised ones. Only one individual emerged from a parasitised egg. 69% of the emerged parasitoids (n=32) were females. The egg parasitoid was identified as *Trichogramma chilonis* Ishii by a specialist from Natural History Museum. This is the first record of *T. chilonis* parasitising *M. vitrata* eggs in Mauritius.

Entomopathogenic nematodes (EPN)

56% of exposed pupae were infected with nematodes. Recovered nematodes were successfully multiplied in laboratory. The isolated EPN was pathogenic to healthy larvae. Infected larvae died on day 3. The nematodes multiplied within the dead body and leave it as from day 4 until day 10. This is a first record of EPN infecting *M. vitrata* pupae in Mauritius.

Fungal pathogen

Diseased larvae were recorded on the wild legume, *P. phaseoloides* only (Table1). 14.6% of the collected larvae (n=287) were infected with a pathogenic fungus that was identified as *Metarhizium* sp.

Crop	No. of *M. vitrata* collected	% of larvae diseased
Bean	1125	0
Groundnut	25	0
Pea	11	0
Pigeon pea	78	0
Cowpea	20	0
Lima bean	360	0
Pueraria phaseoloides	287	14.6%

Table 1. Percentage of diseased *Maruca vitrata* larvae collected from legumes

DISCUSSION

Findings of this study are not in conformity with Moutia's (1955) report that *L. boeticus*, *M. vitrata* and *E. zinckenella* cause serious damage to bean. During the 10-month survey, *M. vitrata* and *L. boeticus* and *H. armigera* (but not *E. zinckenella*) were recorded. The latter is a first record on bean in Mauritius.

Larvae of *M. vitrata* were more abundant (99.6%) than those of *H. armigera* (0.3%) and *L. boeticus* (0.1%) at all sites over several crop cycles. In the present study, pod damage was mostly due to *M. vitrata* and reached up to 42% in

untreated plots. However, this study needs to be pursued over successive years to verify the abundance of *M. vitrata* and other pod borers on bean.

Four types of biological control agents (larval and egg parasitoid, entomopathogenic nematode and pathogenic fungus) were encountered during the inventory of natural enemies. The egg parasitoid, entomopathogenic nematode and pathogenic fungus were not previously recorded on *M. vitrata* in Mauritius.

Two larval parasitoids were recovered. But their activity seems to be greatly reduced in farmers' fields probably because of weekly application of broad spectrum insecticides. Such negative impact of commonly used chemical insecticides was observed by Ulrichs *et al.* (2002) on a larval parasitoid in the Philippines.

Exposure of freshly laid eggs in the field was effective in recovering the egg parasitoid, *T. chilonis*. This 'sentry method' was previously used by Tamo *et al.* (2002) and Ulrichs *et al.* (2002) to record egg parasitoids of *M. vitrata* (*Trichogrammatoidea eldanae* Viggiani and *Trichogramma evanescens* Westwood (Trichogrammatidae)) in West Africa and Philippines in cowpea and yardlong beans fields respectively. In this study, *T. chilonis* was recovered on all 4 occasions with an average of 62% parasitism. In similar studies, Tamo *et al.* (2002) recorded 53.9% parasitism in exposed *M. vitrata* eggs. As per Arodokoun (1996), egg parasitoids can reduce feeding damage of larvae because they exert control at egg stage and consequently reducing larval numbers. In contrast, Ulrichs and Mewis (2004) did not find such reduction when they undertook inundative releases of *T. evanescens* in yardlong bean fields.

The EPN induced a high rate of infection in exposed pupae. Mraeek and Beevao (2000) related such high incidence to host aggregations. These nematodes normally penetrate insects and release their bacterial symbionts that kill their hosts within 3 days. Larval death in this study occurred 3 days after initial inoculation. This shows that EPN can be highly effective as pointed out by Poinar (1990). Despite their high virulence, EPN have shown no mammalian pathogenicity (Gaugler and Boush, 1979) and are reported to be safe to vertebrates, plants, earthworms, honey bees, and other non target organisms (Kaya and Gaugler, 1993). Conservation and augmentation of natural nematode populations through proper management practices and periodic releases can contribute in the campaign at biological control of *M. vitrata*.

Metarrhizium sp. occurred on the unsprayed wild legume, *P. phaseoloides* but not in cultivated ones because the latter were sprayed with fungicides against economically important pathogens. Besides, *P. phaseoloides* has an extended crop canopy (leafy and totally covering soil surface) that provides a humid microclimate conducive to the development of the fungus. Fungicides are reported to contribute to the disappearance of epizootics of entomopathogenic fungi that exert control on key pests in apple orchards (Jaques and Patterson,1962; Picco, 1978). In Taiwan, *Fusarium* sp., *Paecilomyces* sp. and *Beauveria bassiana* were recovered by Huang *et al.* (2003) from diseased *M. vitrata* larvae on the wild legume *Sesbania cannabina* but their potential as a biocontrol agent were not evaluated. Further

studies of *Metarrhizium* sp. are warranted to evaluate its potential against *M. vitrata*. The commercial *Beauveria* formulation was not effective against larvae in laboratory. Further testing with this formulation along with other isolates is required to determine their ovicidal effect. Such ovicidal effect of *B. bassiana* and *M. anisopliae* on eggs of *M. vitrata* has been reported in Nigeria by Ekesi *et al.* (2002). However, the use of *Metarrhizium* sp. and other fungi against *M. vitrata* in cultivated legumes is largely limited by the regular application of fungicides application in bean fields.

Dipel was toxic to young and mature larvae in laboratory bioassay. They stopped feeding probably due to gut paralysis caused by the bacterium spores and crystal protein, as reported by Deacon (1983). However, larval mortality was related to Dipel rates and instars. Young larvae were more susceptible to Dipel than mature ones. Similar results were obtained by Taylor (1968). Field trials need to be undertaken to determine whether pod damage is significantly reduced on bean.

No economic injury level (EIL) of *M. vitrata* larvae on bean has been worked out in Mauritius. Azadirachtin was applied as from plant flowering, a stage at which larval infestation starts building up (Unmole, 2007). Larval infestation was not reduced but there was a 3-fold reduction in pod damage in treated plots compared to untreated ones. This demonstrates the antifeedant property of azadirachtin. Similar results were obtained by Tanzubil (1991) and Jagginavar *et al.* (1991). The former observed live larvae on the surface of undamaged treated pods and attributed this to the antifeedant activity of neem. In the present study, Bioking gave moderate pod damage reduction (19-30%) compared to the untreated control (57%). Similar results were obtained by Chandrayudu *et al.* (2008) during their field evaluation of *B. thuringiensis*, azadirachtin and other insecticides against *M. vitrata* in cowpea. Azadirachtin was found to offer moderate control of *M. vitrata* with a corresponding moderate benefit-cost ratio while they found Bt to be very effective resulting in less pod damage which was associated with high yields and high benefit-cost ratio.

CONCLUSION

Maruca vitrata is identified as the major pod borer on bean causing up to 42% of pod damage. Biopesticides (*B. thuringiensis* and Azadirachtin) are appropriate substitutes for chemical insecticides. Their efficacy against *M. vitrata* needs to be demonstrated to farmers to avoid the use of broad spectrum insecticides. Such a shift to biopesticides will eventually enhance the activity of existing parasitoids. Further research is warranted on the newly identified biocontrol agents (egg parasitoid, EPN and fungal pathogen) for their possible use in pod borer management.

In the long run, an IPM strategy against *M. vitrata* could be developed. It could be based on augmentative releases of parasitoids and effective entomopathogens as a preventive measure and application of biopesticides as curative. Such a strategy should enable farmers to sustainably manage *M. vitrata* in their bean fields.

It is also highly desirable to establish the economic injury level for *M. vitrata* larvae on bean to rationalize the use of insecticides for its control. This concept is based on economic considerations and will be helpful to farmers when making insecticide treatment decisions.

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