The African Journal of Plant Science and Biotechnology ©2010 Global Science Books

Field Evaluation in Egypt of Two Biorational Insecticides (Nimbecidine® and Bio-Power®) against the Potato Tuberworm, *Phthorimaea operculella* (Zeller)

AME Abd El-Salam[1*] • Jaime A. Teixeira da Silva[2]

[1] Pests and Plant Protection Department, National Research Centre, El-Tahrer St.12622, Dokki, Cairo, Egypt
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan

Corresponding author: * **ahmedezzat59@yahoo.com**

ABSTRACT

A field experiment was conducted to determine the efficacy of two biorational insecticides against the potato tuber worm (PTW), *Phthorimaea operculella*. Nimbecidine® was more effective than Bio-power®. However, Nimbecidine® treatments after 3 applications reduced the larval mine of PTW by 79.6 and 43.8% when applied at 5.0 and 2.5 ml/l, respectively while Bio-power® treatments reduced the larval mine of PTW by 56.7 and 30.85%, respectively after the same applications. Also, plots treated with Nimbecidine® showed increased potato (*Solanum tuberosum* var. 'Filea') tuber yield (11.53 and 9.8 kg/10 plants) more than those treated with Bio-power® (9.65 and 8.98 kg/10 plants) and compared with 7.128 kg potato tubers/10 plants in untreated plots. These results indicate that triple application with neem formulation could effectively reduce the *P. operculella* population. This study also shows that Nimbecidine® and Bio-power® could be incorporated into an integrated pest management program of potato tuber moth.

Keywords: *Azadirachta indica*, azadirachtin, *Beauveria bassiana*, neem

INTRODUCTION

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is considered one of the most economically damaging pests of potato (*Solanum tuberosum* L.) crops in regions with warm temperate to tropical climates (Radcliff 1982). Larvae mine into leaves, stems and tubers, and the latter damage results in rejection of tubers by growers and potato processors. Further, the level of *P. operculella* infestation in the foliage can influence infestation levels in tubers (Lagnaoui *et al*. 1996; Coll *et al*. 2000; Davidson *et al*. 2006). Integration of management strategies (spray insecticides, cultural methods, biological control) can help to manage this pest in the field and storage (Hanafi 1999). However, the development of resistant potato cultivars could increase the efficacy of cultural and biological methods and reduce the use of insecticides (Arnone *et al*. 1998). Populations of *P. operculella* larvae peak between February and April and are particularly prevalent in hot, dry summers (Foot 1979). They infest potato foliage before tubers are colonized. Soil moisture and tuber depth affect the ability of larvae to infest tubers (Foot 1979). Management methods emphasise the use of cultural practices such as moulding, seed depth, timing of planting and irrigation (Hanafi 1999) to reduce tuber exposure to or increase mortality of *P. operculella*. However, the *P. operculella* population can still reach levels where it is necessary to apply insecticides (Hanafi 1999). Potato cultivars resistant to *P. operculella* could eliminate the need for, or at least reduce the number of, insecticide applications to manage this pest. However, attempts to develop such cultivars using traditional breeding methods have not been successful (Arnone *et al*. 1998). Contamination of the environment by pesticides is increasing due to their use for attempted eradication of various pests and protection of agricultural crops (Matsumura 1975; Tanabe *et al*. 1983). In addition, commercial chemical insecticides are toxic to beneficial insects like parasitoids and predators (Abudulai *et al*. 2001; Keasar and Sadeh 2007). As a consequence of the concern about the environmental persistence of synthetic pesticides and their potential toxicity to humans (Linka *et al*. 2005), beneficial insects (Youn *et al*. 2003) and domestic animals (Covaci *et al*. 2004), there is renewed interest in using natural products to control pests (Weinzierl and Henn 1991). Among these natural insecticides, the botanical azadirachtin, a mixture of several structurally related tetra-nortriterpenoids isolated from the seeds of the neem tree (*Azadirachta indica* A. Juss; Meliaceae), has attracted the greatest attention (Schmutterer 1990; Hu *et al*. 1996; Immaraju 1998) and has been reviewed extensively (Mordue and Blackwell 1993; Mordue *et al.* 1998; Rong *et al*. 2000). Also, there is a search for an alternative ecofriendly strategy for the management of noxious insect pests to reduce harmful effects of chemical insecticides on humanity. In recent years, crop protection based on biological control of crop pests with microbial pathogens like viruses, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management (Bhattacharya *et al*. 2003). The appropriate use of environment-friendly microbial pesticides can play a significant role in sustainable crop production by providing a stable pest management program. Among the fungi, several asexual fungi are associated with arthropods, especially with insects. It appears that 750 fungal species (Carruthers and Soper 1987) are pathogenic to insects but only a dozen or so have been exploited for insect control. *Beauveria* is an important genus used for the management of insects. The white muscardine entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin has been recorded to infect >500 host species belonging to orders Lepidoptera, Hemiptera, Homoptera, Orthoptera and Diptera (Moore and Prior 1996). The aim of the current study was to evaluate a new potato variety that may demonstrate moderate pest resistance. Two commercial biorational insecticides, i.e., Nimbecidine® and Bio-power® were investigated against *P. operculella* larvae and potato tuber yield was finally assessed.

Received: 18 September, 2009. Accepted: 17 July, 2010.

Original Research Paper

MATERIALS AND METHODS

Field trial and design

The soil was ploughed and the recommend fertilizers were added in September. The fertilizers recommended for potatoes by the Egyptian Ministry of Agriculture per 4200 m^2 are 40 m^2 analytic manure fertilizers + 100 Kg sulfur during the preparation of the soil before planting, then 75 Kg phosphorus (P) (as super calcium phosphate (15.5%) after planting. 180 Kg nitrogen is divided as follows: at planting 60 Kg ammonium sulfate; during tuber growth (full growth) 60 Kg ammonium sulfate 40-50 days from planting. Then 100 Kg K is added as ammonium nitrate after 5 days. After 70 days, 60 Kg of nitrogen sulfate is added.

Pre-germinated *Solanum tuberosum* var. 'Filea' tubers, a Germany variety new to Egypt, were cultivated. The germinated tubers were planted in a virgin reclaimed area (Wadi El-Natron, El-Behera Governorate, Egypt) at a depth of 10 cm from the surface of the soil on the 14^{th} December 2006. There were 5 replicate plots for each treatment (Nimbecidine® or Bio-power®), and each plot contained 100 plants (treatments). One more plot served as the control (no insecticide treatments and sprayed exclusively with water). There were 5 rows (0.75 m apart) per plot, each row planted with 20 plants spaced 0.15 m apart within a row. The total area was 4200 m^2; the area of each treated plot was 380 m^2 while the area of the untreated plot was 400 m^2. The irrigation system was in the form of droplets (3 holes/plant; flow rate = 1 Drop /second), the pH of the water was 8.2 and the fertilizer system was organic (30 days before planting compost (decomposition of a waste agriculture plant incorporated into the soil). The mean daily maximum and minimum temperatures of the area were about 17.5 and 9.0°C, respectively. The mean daily maximum and minimum relative humidity of the area were about 65.4 and 55.0%, respectively.

Formulations used

Two commercial formulations were used: 1) Nimbecidine® is neem-based botanical insecticide containing 0.03% azadirachtin and other limonoids like meliantriol, salanin, nimbin and most other terpenoids in ratios that occur in natural neem (T. Stanes & Co., India; http://www.tstanes.com/pest_management.html). 2) Bio-Power® is a talc-based, liquid product and biological insecticide that contains 1×10^8 *Beauveria bassiana* spores (i.e. CFU/g) and mycelial fragments; T. Stanes & Co., India; http://www.tstanes.com/bio_power.html). The concentrations at which these compounds were applied were 5.0 and 2.5 ml/l in water, as recommended by the manufacturer for the former and through self-tested trials for the latter.

Field experiment

On the 10^{th} February 2007, the foliage of plants was examined for larval mines of *P. operculella*. Ten plants from each row within each plot were randomly examined (50 plants) and the number of larval mines was recorded weekly (either with larvae or free of larvae). The control program began on the 25^{th} February 2007. In this program, whole plants were sprayed with 25 ml of the biopesticides a total of three times at 10-day intervals. The sprayer equipment was an ECHO backpack applicator with a 13-l capacity. The plants were examined after 5 and 10 days from spraying to record the mean number of larval mines per 10 plants/treatment. The percentage reduction in the number of larval mines was calculated by the Henderson and Tilton equation (1955):

$$\%\,\text{Reduction} = \left(1 - \frac{T_a \times C_b}{T_b \times C_a}\right) \times 100$$

where T_a = mean number of living larvae mines (busy and empty mines) after treatment; T_b = mean number of living larvae mines (busy and empty mines) before treatment; C_b = mean number of living larvae mines (busy and empty mines) before treatment with water alone (control); C_a = mean number of living larvae mines (busy and empty mines) after treatment by water alone (control).

Harvest

Potato tubers were harvested on the 17–20^{th} May, 2007. Ten plants were selected randomly from each replicate for each treatment and control. The potato tubers/plant from each row within a plot were examined, weighed and scored for the presence or absence of larval mines. The number of infested and uninfested tubers were recorded and weighed for each treatment and for the control.

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) ($P \leq 0.05$) and comparisons were made based on Duncan's multiple range test using Microstat v. 2.5, 1991).

RESULTS AND DISCUSSION

Field experiment

The mean number of larval mines/10 plants was 31.4 before spraying (**Table 1**). A high dose of application (5 ml/l) was more potent than 2.5 ml/l for both commercial compounds. Also, Nimbecidine® at 5 ml/l was more effective than other treatments and the control. The efficiency of Nimbecidine® lasted until 5 days after the first spray (74.5% reduction) then decreased to 71.8% by the 10^{th} day. Furthermore, the 2^{nd} application of Nimbecidine® showed an increase in its efficiency by reducing larval mines on the 5^{th} day by 80.3%, this value decreasing to 72.3% by the 10^{th} day. The third application was very important: Nimbecidine® reducing larval mines by 94.1% on the 5^{th} day, but reducing it to 84.3% by the 10^{th} day. In general, the application of Nimbecidine® significantly reduced the percentage of larval mines compared with the untreated control. Nimbecidine® reduced larval mines by 84.3 and 46.7%, respectively when applied at 5 and 2.5 ml/l over the three applications. Other authors (Schmutterer 1990; Isman 1999; Walter 1999) also found that neem (*A. indica*)-based insecticides containing azadirachtin could control >400 species of insects, including important pests such as armyworms, leafminers, aphids, and whiteflies. In addition to controlling pests, some neem-based insecticides have negligible effects on beneficial insects, and low environmental impacts (Schmutterer 1995; Haseeb *et al.* 2004). Charleston *et al.* (2006) noted a reduced amount of damage caused by free-living larval mines in plots treated with Neemix® (4.5% azadirachtin) at a dose of 10.7 μl/100 ml water, suggesting that even during periods of high infestation, the reduced feeding by the diamondback moth, *Plutella xylostella*, larvae on plants treated with Neemix® is a more important factor than the actual population density in reducing the damage and improving the yield of cabbage seedlings, *Brassica oleraceae* var. *capitata* L. An antifeedant effect on *P. xylostella* larvae was observed on Agroneem-, Ecozin-, and Neemix®-treated cabbage leaves; larvae fed on these neem-based insecticide-treated leaves were smaller than those fed on non-treated controls (Liang *et al.* 2003). The present findings agree with those of Walter (1999) who reported that in field plots Neemix® treatment of broccoli, *B. oleracea* var. *botrytis*, had no effect on populations of beet armyworm larvae, but feeding damage was reduced to levels obtained with synthetic insecticides. Direct contact with the neem-based insecticides decreased egg survival, and larvae that fed on neem-based, insecticide-treated leaves had lower survival than the control after 1 week of exposure. Similarly, Agroneem, Ecozin and Neemix® had lethal effects on the diamondback moth larvae (Liang *et al.* 2003) and neem oil reduced egg hatching and survival larvae of *H. armigera* (Martin *et al.* 2000).

Azadirachtin is known to block molting in some insects (Mordue and Blackwell 1993). The neem-based insecticides assessed in our study show some short-term potential as a means of PTM control in potato. However, neem-based insecticides often lose effectiveness in outdoor conditions within days, as also shown by others (Koul *et al.* 1990;

Table 1 Effect of two biorational compounds against the potato tuber moth (larvae). Identical values! CHECK DATA!!!

Formulations	Rate of application ml/l	Mean № of larval mines ±SE/10 plants at indicated days				
		1st spray				
		Before spray	After (days)			
			5	% Reduction	10	% Reduction
Nimbecidine®	5.0	31.4 ± 1.7	8.6 ± 1.63 d	74.5	10.0 ± 2.20 c	71.8
	2.5	31.4 ± 1.7	21.0 ± 3.10 c	37.6	23.4 ± 1.40 b	33.9
Bio-Power®	5.0	31.4 ± 1.7	22.6 ± 1.50 bc	32.8	9.8 ± 1.15 c	72.4
	2.5	31.4 ± 1.7	29.2 ± 2.80 ab	13.2	22.2 ± 0.70 b	37.3
Control	----	31.4 ± 1.7	33.6 ± 1.74 a	-------	35.4 ± 2.90 a	-------
F		-------	17.74*	-------	32.49*	-------
L.S.D$_{05}$	-------	-------	6.66	-------	5.53	-------
		2nd spray				
		Before spray	After (days)			
			5	% Reduction	10	% Reduction
Nimbecidine®	5.0	10.0 ± 2.21 c	2.2 ± 0.53 d	80.3	3.2 ± 0.80 c	72.3
	2.5	23.4 ± 1.40 b	12.8 ± 1.90 c	50.9	17.0 ± 2.20 b	37.1
Bio-Power®	5.0	9.8 ± 1.15 c	7.5 ± 1.10 cd	31.3	4.4 ± 0.81 c	61.1
	2.5	22.2 ± 0.70 b	19.2 ± 2.30 b	22.4	17.8 ± 2.60 b	30.5
Control	----	35.4 ± 2.95 a	39.4 ± 2.90 a	-------	40.8 ± 1.50 a	-----
F		32.49*	52.48*	-------	74.9*	-------
L.S.D$_{05}$	-------	5.53	5.86	-------	5.15	-------
		3rd spray				
		Before spray	After (days)			
			5	% Reduction	10	% Reduction
Nimbecidine®	5.0	3.2 ± 0.80 c	0.2 ± 0.2 d	94.1	0.6 ± 0.40 c	84.3
	2.5	17.0 ± 2.20 b	7.8 ± 1.5 bc	56.3	10.8 ± 2.80 b	46.7
Bio-Power®	5.0	4.4 ± 0.81 c	1.6 ± 0.2 cd	65.4	1.2 ± 0.37 c	77.2
	2.5	17.8 ± 2.60 b	11.2 ± 2.3 b	40.1	12.4 ± 1.40 b	41.6
Control	----	40.8 ± 1.50 a	42.8 ± 4.2 a	----	48.62.94 a	----
F		74.9*	59.58*	-------	102.24*	-------
L.S.D$_{05}$	-------	5.15	6.65	-------	5.74	-------
		Mean % reduction				
Nimbecidine®	5.0	79.60				
	2.5	43.80				
Bio-Power®	5.0	56.70				
	2.5	30.85				
Control	----	----				
F		-------				
L.S.D$_{05}$	-------	-------				

Means in columns with the same letters are not significantly different ($P \leq 0.05$).

Schmutterer 1990; Greenberg *et al.* 2005; Dimetry *et al.* 2010). Dimetry *et al.* (2010) found that Neem Azal T/S and Neem Azal T/S + TS/fort could efficiently control *Aphis craccivora* infesting broad bean (*Vicia fabae*) under field conditions. As for leafminers, the number of living *Liriomyza trifolii* larvae decreased significantly compared to non-treated control. The number of living larvae continued to decrease until seven days post-treatment after which an increase in their number was noticed after 14 days post-treatment. Here, the effectiveness was lost after 7 days. Neem Azal T/S additive (TS/fort) ranked first in comparison to other treatments for the control of aphids and leafminers attacking broad bean in the field either by a killing, deterrent or antifeedant effect. The residual effect of neem formulations continued for at least 7 days. Unless neem-based insecticides are sufficiently cost effective, they are unlikely to receive widespread commercial use in certain crops like cotton where repetitive applications are necessary because of heavy pest pressure (Abd El-Salam and El-Hawary 2009).

Neem-based insecticides might be acceptably cost effective where the need for applications is limited, or if it can be combined with an agent that slows its degradation in field conditions. The oil of *A. indica* seeds was sprayed on the eggs of PTM at 1.3, 5 and 10%. Egg hatching was strongly inhibited at 5 and 10% (Shelke *et al.* 1987). Rama (1989) observed that in India *A. indica* oil showed both ovicidal and larvicidal actions against PTM. Sharma *et al.* (1983) reported in India that 'Neemrich' 1 (neem extracts enriched in particulate principles or properties) has high and persistent oviposition deterrence activity against adults, and short-term but high antifeedant activity against 1st-instar larvae of PTM. In a storage trial, protection against PTM was achieved for over 4 months, or longer than the usual 3-4 months period of rural storage of potatoes in various parts of India. According to Panciotto-Hediger (1985) the dried neem leaves reduced PTM infestation in stored tubers.

Shelke *et al.* (1985) tested in the laboratory the effect of *A. indica* oil (at 0.01, 0.03, 0.05 and 0.1%) against PTM adults in India. This oil provided 83.79 average percentage of oviposition deterrence action against PTM.

On the other hand, the efficiency of the entomopathogenic fungi *B. bassiana* (Bio-power®) was less potent than the botanical insecticide (Nimbecidine®). The reduction in infestation started with 32.8% at a rate of 5 ml/1 after 5 days from application then increased to 72.4% on the 10th day. After the second application and on the 5th day from application, the formulation could reduce the number of larval mines by 31.3% increasing to 61.1% on the 10th day. Bio-power®, when applied at 5 ml/l, reduced the number of larval mines by 77.2% after the third application on the 10th day from application. There were significant differences between all the treated plots with Bio-power® and untreated plots. Nimbecidine® at 5 ml/l was best: it reduced the number of larval mines by 79.6% compared to Bio-power® which caused a 56.7% reduction after three applications.

Several insect pathogenic fungi have the potential to be used in the biological control of PTM. Among these, *B. bassiana* is the most promising, being an important natural enemy of the prepupal, pupal and adult stages of the beetle in the soil (Bajan and Kmitowa 1977; Humber 1996). Although natural infections are primarily associated with these soil-dwelling stages, larvae feeding in the crop canopy are also susceptible to infection via inundative foliar spray applications. As typical with most fungal pathogens, infection and pathogenesis proceed relatively slowly compared to toxin-based control agents. Under optimal environmental conditions, infected larvae may succumb after infection within a few days, but under high-temperature or high-insolation conditions, fungal development is slowed, and the larvae may not succumb upon infection until they enter the soil to pupate. The four larval instars are equally susceptible to infection (Fargues 1972) although the speed of kill is inversely related to host age, and under field conditions most larvae infected after the 2nd instar survive until the prepupal stage. *B. bassiana* conidia are rapidly degraded by UV radiation, and residual activity of foliar sprays is limited. Early-instar larvae feeding on the lower surfaces of the leaves are difficult spray targets (Wraight and Ramos 2002) and larvae nearing moult have reduced susceptibility to fungal infection (Vey and Fargues 1977). Consequently, multiple applications are needed to provide foliage protection, even when environmental conditions are favourable for the fungus. Hafez *et al*. (1997) stated that mortalities of *P. operculella* larvae exposed to a concentration of 16.5×10^8 of *B. bassiana* were susceptible to the pathogen. The calculated LC_{50} for *B. bassiana* was 4.7×10^8 conidia/ml. The duration of the treated pupae was significantly prolonged at concentrations ranging from 16.5×10^8 to 2.06×10^8 conidia/ml compared with the control, while at low concentrations (1.03×10^8 to 0.26×10^8 conidia/ml), there was no obvious effect compared to the control. The percentage of moths that emerged progressively decreased with an increase in the concentration of *B. bassiana*: from 100% in the control to 0% at 16.5×10^8 conidia/ml. An obvious malformation was observed among the emerged moths after treatment of the prepupae with any of the used concentrations.

B. bassiana has been intensively investigated for microbial control of PTM since the 1950s, with the principal effort aimed at developing foliar application technologies to protect crops from defoliation. Not surprisingly, considering the importance of environmental conditions in *B. bassiana* efficacy, results have been highly variable (Fargues *et al*. 1980; Sikura and Sikura 1983; Anderson *et al*. 1988; Poprawski *et al*. 1997; Lacey *et al*. 1999; Martin *et al*. 2000; Wraight and Ramos 2002). Both temperature and humidity affected the survival and germination of *B. bassiana. B. bassiana* exhibited a relatively high mycelial growth at 30°C and 45% RH (Vu *et al*. 2007). Generally, under operational or pilot-scale conditions, applications of *B. bassiana* alone have failed to provide adequate early-season crop protection (Lipa 1985; Hajek *et al*. 1987). Soil inoculation is a logical alternative to foliar sprays, especially considering the long persistence of *B. bassiana* conidia in this habitat and the natural occurrence of this pathogen in populations of the soil-dwelling stages of the beetle. However, results of soil applications have been as variable as those from foliar applications (Wojciechowska *et al*. 1977; Watt and LeBrun 1984; Cantwell *et al*. 1986; Gaugler *et al*. 1989). Difficulties in delivering lethal concentrations of inoculate to insects that burrow many cm below the soil surface is an important factor. Some studies suggest that more consistent infection and mortality of prepupal and pupae might be achievable by direct spraying of late-instar or mature larvae before they enter the soil than by applying material to the soil and relying on secondary acquisition of conidia (Wraight *et al*. 2006). Biopesticides based on *B. bassiana* have been available for potato beetle control in Eastern Europe for 50 years, and similar products have been developed more recently in the US (Wraight *et al*. 2006). Despite the limited capacity of microbial control agents to compete directly with the synthetic chemical insecticides currently used for PTM control, interest in these agents persists with the demand for sustainable food production systems. Intensive use of *B. bassiana* for PTM control carries the risk that the moth will develop resistance to the endotoxin (Whalon *et al*. 1993). *B. bassiana* has a broad host range and likely poses a greater risk for negative interactions with intraguild biological control agents; however, Lacey *et al*. (1999) noted no significant effects on populations of non-target arthropods in *B. bassiana*-treated potato field plots. Because *B. bassiana* acts slowly against older larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata*, applications of the microbe for immediate foliar protection are targeted against young larvae (1st and 2nd instars). Although *B. bassiana* acts too slowly against late-instar larvae to completely protect the plants from their feeding, infected larvae have been shown to consume less foliage than healthy larvae (Fargues *et al*. 1994). Also, the fungus is as infectious against late-instar larvae as against the early instars and it may be targeted against older larvae to reduce adult populations. Healthy adult Colorado potato beetles are much less susceptible to infection than larvae (Blonska 1957). Multiple foliar applications (usually 3 or 4) are required to achieve effective control with *B. bassiana*. Applications of *B. bassiana* are most effective when applied at 3–4 day intervals (Galaini 1984; Wraight and Ramos 2002). As noted earlier, control with *B. bassiana* may be delayed under high temperature conditions, and expressed only as a reduction of the population of first-generation adults (providing little early season foliage protection). The necessary spray interval varies with rate and timing of Colorado potato beetle development; longer spray intervals may be more effective under cool weather (or cool climate) conditions, especially if spring emergence of adults occurs over an extended period. High PTM populations, rapid plant growth (which dilutes the pathogens in space), or heavy rainfall (which removes the pathogens from the target site) may necessitate additional applications. Intense insect pressure may also require integrated applications of a chemical insecticide or other control measures, especially to control adults feeding on seedlings. In small-scale field tests, researchers often report making applications of microbial control agents in late afternoon or evening with the assumption that favourable environmental conditions (moderate temperatures, high humidity, and absence of solar radiation) will enhance efficacy. This may be advantageous, especially in subtropical climates where warm conditions during the night could support high rates of fungal development and larval activity. Larval activity (feeding) is largely determined by temperature (Chlodny 1975; Wraight and Ramos 2002) and such activity likely results in secondary acquisition of inoculum from the treated foliage. Laboratory investigations on two common fungal entomopathogens, *B. bassiana* and *Metarhizium anisopliae*, have indicated potential for control of *P. operculella* larvae, especially early instars (Hafez *et al*. 1997; Sewify *et al*. 2000; Sun *et al*. 2004); however, research on use of these agents against this pest is limited. A threshold of 0.5 mines/plant at formation of leaves and stems to crop extension growth was determined by Kroschel (1995) for fenvalerate (Sumicidin) in the Republic of Yemen. Kroschel (1996) showed that yield losses were significant only when high pest pressures (> 30 larvae/plant) occurred early in the season during crop extension growth, while infestations at the end of crop extension growth and later caused yield losses between 8 and 15%. Because of the great complexity of cropping systems and crop-pest interactions, Keller (2003) emphasizes that threshold values are only one factor among many to be considered in making pest management decisions. Damage to potato foliage can be compensated to a high degree by the potato plant, so that significant differences in yields may not be detectable at harvest.

Harvest

The present study shows that Nimbecidine® formulation plots by an application rate of 5 ml/l achieved high significant results in all the parameters studied compared with untreated control plots (**Table 2**). High yield was achieved but the mean number of potato tubers/10 plants was 87.4 and the mean weight of potato tubers/10 plants was 11.534

Table 2 Mean yield of potato tubers, var. 'Filea'/10 plants from the field trial with two different biopesticides.

Formulations	Rate of application ml/l	Mean № of potato tubers ± SE	Mean № of larvae in infested potato tubers ± SE	Mean № of infested tubers ± SE	% Infestation	Mean weight of potato tubers (Kg) ± SE	Mean weight of individual potato tubers (g) ± SE
Nimbecidine	5.0	87.4 ± 1.36 a	9.2 ± 0.86 d	6.0 ± 1.37 d	6.86	11.534 ± 0.74 a	131.97 ± 10.14 a
	2.5	83.6 ± 2.13 ab	21.8 ± 1.71 b	19.0 ± 1.41 b	22.72	9.798 ± 0.43 b	117.20 ± 2.40 ab
Bio-Power	5.0	85.2 ± 1.93 ab	16.0 ± 1.00 c	13.2 ± 1.80 cd	15.49	9.650 ± 0.45 b	113.26 ± 6.84 b
	2.5	84.4 ± 2.78 ab	25.4 ± 1.50 b	18.2 ± 1.53 bc	21.56	8.980 ± 0.27 b	106.39 ± 4.66 bc
Control	----	79.2 ± 2.28 b	52.2 ± 1.59 a	28.6 ± 2.56 a	36.11	7.128 ± 0.52 c	90.00 ± 5.67 c
F	----	1.96 NS	2.02*	21.46*	-------	9.65*	5.75*
$L.S.D_{05}$	----	6.35	4.06	5.28	--------	1.51	19.08

Means in columns with the same letters are not significantly different ($P \leq 0.05$).

Kg (the mean individual tuber weight recorded 131.97 g). Also, in these treated plots the percentage of infestation decreased to the minimum value (6.86%). Bio-power® plots yielded less than Nimbecidine® plots although the product yield was 9.650 Kg (the mean individual tuber weight recorded 113.97 g). Also, the mean number of larvae in tuber infestation and the percentage of tuber infestation was 16.0 and 15.49% of 5 ml/l Bio-Power in 13.2 potato tubers, respectively. On the other hand, the untreated control plots recorded fewer potato tubers/10 plants (79.2 potato tubers) and lower yield. The percentage of potato tubers increased to 36.11%. Furthermore, product yield was the least among all treatments (7.128 Kg) and also, the mean individual potato tuber recorded only 90.0 g in the control. PTM populations occasionally reach this level of infestation in tropical or subtropical regions during mid- and late-season (*e.g.*, Egypt and Yemen), but such damaging populations are rarely seen in temperate zones. Kroschel (1995) reported that infestations after crop extension growth caused yield losses between 8 and 15%. These reductions were not statistically significant, but could nevertheless represent real losses of considerable economic significance (Nault and Kennedy 1998). Gajalakshmi and Abbasi (2004) agreed with the present findings and found that when no neem was applied to the control plot during the first three months, the different parameters of the potato plant decreased in comparison with the treated plots. On the other hand, plants in plots supplemented with neem and vermicompost had significantly better height, root length, greater biomass per unit time, quicker onset of flowering, and enhancement of fruit yield. In terms of fertility coefficient and harvest index too, in treated plots, there was statistically significant enhancement in performance. By the end of the experiment, on the 150th day, the control plants had revived so much that in terms of all parameters except for total plant weight, the difference between the test and control plants had become statistically insignificant. The differences in the modes of action of neem formulation (azadirachtin as active ingredient) and *B. bassiana* indicate a high degree of complementarity and strong potential for integrated use in biologically-based potato pest management. The rapid toxic, deterrent, repellent activity and antimetamorphosis effects of neem against PTM and/or early-instar larvae can be relied upon to protect plants from defoliation early in the season, and the slower activity of *B. bassiana* against all larval instars and epizootic potential in the soil environment can prevent successful pupation of larvae that survive neem treatments (Wraight and Ramos 2005). The combined action can greatly reduce the numbers of Colorado potato beetles surviving to the adult stage and, consequently, the size of the next generation (Groden *et al*. 2002). Excellent compatibility of these agents is further demonstrated by studies showing a low but significant level of synergism between neem and *B. bassiana* applied as a tank mix (0.25: 5.0) (Wraight and Ramos 2005). The present studies show that Nimbecidine® and Bio-power® could be incorporated into a PTM integrated pest management programme.

CONCLUDING REMARKS

From the results obtained in our work, it can be concluded that Nimbecidine® formulation was highly effective than Bio-power®. However, Nimbecidine® treatments after 3 applications achieved highly reduction in the larval mine of potato tuberworm at rate of 5.0 ml/l. Also, Nimbecidine® plots treatments produced highly potato tuber yield than the bio-power plots treatment. The results indicate that repeated application with neem formulation for 3 times was very important to reduce *Phthorimaea operculella* population. The present studies show that Nimbecidine® and bio-power could be incorporated into an integrated pest management programme of potato tuber moth.

REFERENCES

Abd El-Salam AME, El-Hawary FMA (2009) Toxic and biological effects of some bioformulations against *Spodoptera littoralis* and *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae). *Bulletin of the Entomological Society of Egypt, Economic Series* **35**, 27-38

Abudulai M, Shepard BM, Mitchell PL (2001) Parasitism and predation on eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in cowpea: impact of endosulfan sprays. *Journal Agriculture Urban Entomology* **18**, 105-115

Arnone S, Musmeci S, Bacchetta L, Cordischi N, Pucci E, Cristofaro M, Sonnino A (1998) Research in *Solanum* spp. of sources of resistance to the potato tuber moth, *Phthorimaea operculella* (Zeller). *Potato Research* **41**, 39-49

Anderson TE, Roberts DW, Soper RS (1988) Use of *Beauveria bassiana* for suppression of Colorado potato beetle populations in New York State (Coleoptera: Chrysomelidae). *Environmental Entomology* **17**, 140-145

Bajan C, Kmitowa K (1977) Contribution of entomopathogenic fungi to the natural winter reduction of Colorado potato beetle adults. *Polish Ecological Studies* **3**, 107-114

Bhattacharya AK, Mondal P, Ramamurthy VV, Srivastava RP (2003) *Beauveria bassiana*: a potential bioagent for innovative integrated pest management programme. In: Srivastava RP (Ed) *Biopesticides and Bioagents in Integrated Pest Management of Agricultural Crops*, International Book Distributing Co, Lucknow, India, pp 860-865

Blonska A (1957) Pathogenic fungi of the genus *Beauveria* from the Colorado beetle, *Leptinotarsa decemlineata* Say. *Roczniki Nauk Rolniczych Seria, A.* **74**, 359-372

Cantwell GE, Cantelo WW, Schroder FW (1986) Effect of *Beauveria bassiana* on underground stages of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Great Lakes Entomologist* **19**, 81-84

Carruthers RI, Soper RS (1987) Fungal diseases. In: Fuxa JR, Tanada Y (Eds) *Epizootiology of Insect Diseases*, Wiley, New York, pp 357-416

Charleston D, Kfir R, Dicke M, Louise E (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

Chlodny J (1975) Bioenergetics of the larval development of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in relation to temperature conditions. *Annual Zoology* (Warsaw) **33**, 149-187

Coll M, Gavish S, Dori I (2000) Population biology of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae), in two potato cropping systems in Israel. *Bulletin of Economic Research* **90**, 309-315

Covaci A, Gheorghe A, Schepens P (2004) Distribution of organochlorine pesticides, polychlorinated biphenyls and HCH enantiomers in pork tissues. *Chemosphere* **56**, 757-766

Davidson MM, Butler RC, Wratten SD, Conner AJ (2006) Field evaluation of potato plants transgenic for a cry1Ac gene conferring resistance to potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Crop Protection* **25**, 216-224

Dimetry NZ, Abd El-Salam AME, El-Hawary FMA (2010) Importance of plant extract formulations in managing different pests attacking beans in new reclaimed area and under storage conditions. *Archives of Phytopathology and Plant Protection* **34**, 700-711

Fargues J, Delmas JC, LeBrun RA (1994) Leaf consumption by larvae of the Colorado potato beetle (Coleoptera: Chrysomelidae) infected with the entomopathogen *Beauveria bassiana*. *Journal of Economic Entomology* **87**, 67-71

Fargues J, Cugier JP, Van de Weghe P (1980) Expérimentation en parcelles du champignon *Beauveria bassiana* (Hyphomycete) contre *Leptinotarsa decemlineata* (Col., Chrysomelidae). *Acta Oecologica Applicata* **1**, 49-61

Fargues J (1972) Etude des conditions d'infection des larves de doryphore, *Leptinotarsa decemlineata* Say, par *Beauveria bassiana* (Bals.) Vuill. [Fungi Imperfecti]. *Entomophaga* **17**, 319-337

Foot MA (1979) Bionomics of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae), at Pukekohoe. N. Z. *Journal Zoology* **6**, 623-636

Gajalakshmi S, Abbasi SA (2004) Neem leaves as a source of fertilizer pesticide vermicompost. *Bioresource Technology* **92**, 291-296

Galaini S (1984) The efficacy of foliar applications of *Beauveria bassiana* against *Leptinotarsa decemlineata*. MSs thesis, Cornell University, Ithaca, NY, 94 pp

Gaugler R, Costa SD, Lashomb J (1989) Stability and efficacy of *Beauveria bassiana* soil inoculations. *Environmental Entomology* **18**, 412-417

Greenberg S, Showler A, Liu T (2005) Effects of neem-based insecticides on beet armyworm (Lepidoptera: Noctuidae). *Insect Science* **12**, 17-23

Groden E, Wraight SP, Drummond FA (2002) Microbial control of Colorado potato beetle in rain-fed potato agroecosystems in the northeastern U. S. In: *VIII International Colloquium on Invertebrate Pathology and Microbial Control Proceedings*, date of meeting, location of meeting, Society for Invertebrate Pathology, pp 265-269

Hafez M, Zaki F, Moursy A, Sabbour M (1997) Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth *Phthorimaea operculella* (SeJler). *Anzeiger für schadlingskunde Pflanzenschutz Umweltschutz* **70**, 158-159

Hajek AE, Soper RS, Roberts DW, Anderson TE, Biever KD, Ferro DN, LeBrun RA, Storch RH (1987) Foliar applications of *Beauveria bassiana* (Balsamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae): an overview of pilot test results from the northern United States. *Canadian Entomology* **119**, 959-974

Hanafi A (1999) Integrated pest management of the potato tuber moth in field and storage. *Potato Research* **42**, 373-380

Haseeb M, Liu TX, Jones WA (2004) Effects of selected insecticides on *Cotesia plutellae* endoparasitoid of *Plutella xylostella. BioControl* **49**, 33-46

Henderson CF, Tilton E (1955) Tests with acaricides against the brown wheat mite. *Journal of Economic Entomology* **48**, 157-161

Hu MY, Zhao SH, Wang LC, Kuang XW (1996) Studies on the effectiveness and growth inhibitory effect of margosan-O on Diamondback moth (*Plutella xylostella*). *Journal of South China Agricultural University* **17 (4)**, 549-554

Humber RA (1996) Fungal pathogens of the Chrysomelidae and prospects for their use in biological control. In: Jolivet PHA, Cox ML (Eds) *Chrysomelidae Biology (Vol 2) Ecological Studies*, SPB Academic Publishing, Amsterdam, pp 93-115

Immaraju JA (1998) The commercial use of azadirachtin and its integration into viable pest control programmes. *Pesticide Science* **54 (3)**, 285-289

Isman MB (1999) Neem and related natural products. In: Hall FR, Menn JJ (Eds) *Biopesticides: Use and Delivery,* Humana Press, Totowa, NJ, pp 139-154

Keasar T, Sadeh A (2007) The parasitoid *Copidosoma koehleri* provides limited control of the potato tuber moth, *Phthorimaea operculella*, in stored potatoes. *Biological Control* **42**, 55-60

Keller S (2003) Integrated pest management of the potato tuber moth in cropping systems of different agro-ecological zones. In: *Advances in Crop Research 1*, Margraf-Verlag, Weikersheim, Germany, 153 pp

Koul O, Isman MB, Ketkar CM (1990) Properties and uses of neem, *Azadirachta indica. Canadian Journal of Botany* **68**, 1-11

Kroschel J, Fritsch E, Huber J (1996) Biological control of the potato tuber moth (*Phthorimaea operculella* Zeller) in the Republic of Yemen using granulosis virus: biochemical characterization, pathogenicity and stability of the virus. *Biocontrol Science and Technology* **6**, 207-216

Kroschel J (1995) Integrated pest management in potato production in the Republic of Yemen with special reference to the integrated biological control of the potato tuber moth (*Phthorimaea operculella* Zeller). In: *Tropical Agriculture 8*, Margraf Verlag, Weikersheim, Germany, 227 pp

Lacey LA, Horton DR, Chauvin RL, Stocker JM (1999) Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. *Entomologia Experimentalis Applicata* **93**, 189-200

Lagnaoui A, Salah HB, El-Bedewy R (1996) Integrated management to control potato tuber moth in North Africa and the Middle East. *CIP Circular* **22**, 10-15

Liang GM, Chen W, Liu TX (2003) Effects of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae). *Crop Protection* **22**, 333-340

Linka B, Gabrioa T, Zoellnera I, Piechotowskia I, Paepkeb O, Herrmannb T, Felder-Kennelc A, Maisnerd V, Schicke K, Schrimpff M, Schwenka M, Wuthea J (2005) Biomonitoring of persistent organochlorine pesticides, PCDD/PCDFs and dioxin-like PCBs in blood of children from South West Germany (Baden-Wuerttemberg) from 1993 to 2003. *Chemosphere* **58**, 1185-1201

Lipa JJ (1985) Progress in biological control of the Colorado beetle (*Leptinotarsa decemlineata*) in eastern Europe. *Bulletin OEPP/EPPO Bulletin* **15**, 207-211

Martin DL, Gordh G, Zalucki MP (2000) Biological effects of azadirachtin on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) fed on cotton and artificial diet. *Australian Journal of Entomology* **39**, 301-304

Matsumura F (Ed) (1975) *Toxicology of Insecticides*, Plenum, New York, 453 pp

Moore D, Prior C (1996) Mycoinsecticides. In: Upadhyay RK, Mukerjee KG, Rajak RL (Eds) *IPM system in Agriculture (Vol II) Biocontrol in Emerging Biotechnology*, Aditya Books Private Ltd, New Delhi, pp 25-56

Mordue AJ, Simmonds MSJ, Ley SV, Blaney WM, Mordue W, Nasiruddin M, Nisbet AJ (1998) Actions of azadirachtin, a plant allelochemical, against insects. *Pesticide Science* **54**, 277-284

Mordue AJ, Blackwell A (1993) Azadirachtin: an update. *Journal of Insect Physiology* **39**, 903-924

Nault BA, Kennedy GG (1998) Limitations of using regression and mean separation analyses for describing the response of crop yield to defoliation: a case of the Colorado potato beetle. *Journal of Economic Entomology* **91**, 7-20

Panciotto-Hediger E (1985) Influence of storage methods on the quality of potato seed tubers in Nepal. PhD thesis, The Swiss Federal Institute of Technology, Zurich, 210 pp

Poprawski TJ, Carruthers RI, Speese JI, Vacek DC, Wendel LE (1997) Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle. *Biological Control* **10**, 48-57

Radcliff EB (1982) Insect pests of potato. *Annual Review Entomology* **27**, 173-204

Rama HN (1989) Study on the toxic effects of plant seed oils against the potato tuber moth. *Phthorimae operculella* Zell. (Lepidoptcra: Gclcchiidac). *Mysore Journal of Agricultural Sciences* **23**, 568-560

Rong XD, Xu HH, Chiu SF (2000) Progressing on botanical insecticide-neem research. Chin. *Journal of Pesticide Science* **2**, 9-14

Schmutterer H (1995) *The Neem Tree Azadirachta indica A. Juss. and Other Meliaceous Plants: Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes*, VCH Publishers, Weinheim, Germany, 527 pp

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. *Annual Review of Entomology* **35**, 271-297

Senthil NS, Kalaivani K, Murugan K, Chung PG (2005) The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) the rice leaffolder. *Pesticide Biochemistry and Physiology* **81**, 113-122

Sewify GH, Abol-Ela S, Eldin MS (2000) Effects of the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) and granulosis virus (GV) combinations on the potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Bulletin Faculty of Agriculture Cairo University* **51**, 95-106

Sharma RN, Nagasamapgi BA, Bhosale AS, Kulakarni MM, Tungikar VB (1983) 'Neemrich'. The concept of enriched particulate fractions from neem for behavioural and physiological control of insects. *Proceedings of the Second International Neem Conference*, 25-28 May, Rauisehholzhausen, Germany, pp 115-128

Shelke SS, Jadhav LD, Salunkhe GN (1985) Ovipositional and adult repellent action of some vegetable oils/extracts against potato tuber moth. *Journal of the Maharashtra Agriculture University* **10**, 284-286

Shelke SS, Jadhav LD, Salunkhe GN (1987) Ovicidal action of some vegetable oils and extracts in the storage pest of potato, *Phthorimaea operculella* Zell. *Biovigyanam* **13**, 40-41

Sikura AI, Sikura LV (1983) Use of biopreparations. *Zasch. Rast*. **5**, 38-39

Sun YX, Li ZY, Gui FR, Yan NS, Chen B, Xu RQ, He SQ (2004) Susceptibility of *Phthorimaea operculella* (Zeller) larvae to eight isolates of *Beauveria* Southwest China. *Journal of Agriculture Science* **17**, 627-629

Tanabe S, Hidaka H, Tatsukawa R (1983) PCBs and chlorinated hydrocarbon pesticides in Antarctic atmosphere and hydrosphere. *Chemosphere* **12**, 277-288

Vey A, Fargues J (1977) Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineata* larvae during ecdysis. *Journal of Invertebrate Pathology* **30**, 207-215

Vu VH, Suk H, Keun K (2007) Selection of entomopathogenic fungi for aphid control. *Journal of Bioscience and Bioengineering* **6**, 498-505

Walter JF (1999) Commercial experience with neem products. In: In: Hall FR, Menn JJ (Eds) *Biopesticides: Use and Delivery*, Humana Press, Totowa, NJ, pp 155-170

Watt BA, LeBrun RA (1984) Soil effects of *Beauveria bassiana* on pupal populations of the Colorado potato beetle. *Environmental Entomology* **13**, 15-18

Wojciechowska M, Kmitowa K, Fedorko A, Bajan C (1977) Duration of activity of entomopathogenic organisms introduced into the soil. *Polish Ecological Studies* **3**, 141-148

Weinzierl R, Henn T (1991) Alternatives in insect management: biological and biorational approaches. North Central Regional Extension Publication 401. Cooperative Extension Service, University of Illinois at Urbana-Champaign, 73 pp

Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, Miller JR (1993) Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. *Journal of Economic Entomology* **86**, 226-233

Wraight SP, Sporleder M, Poprawski TJ, Lacey LA (2006) Application and evaluation of entomopathogens in potato. *Journal of Invertebrate Pathology* **91**, 329-359

Wraight SP, Ramos ME (2005) Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. *Journal of Invertebrate Pathology* **90**, 139-150

Wraight SP, Ramos ME (2002) Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. *Biological Control* **23**, 164-178

Youn YN, Seo MJ, Shin JG, Jang C, Yu YM (2003) Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control* **28**, 164-17