Development and validation of an integrated pest management strategy for the control of major insect pests on pak-choi in Cambodia

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ABSTRACT

Pak-choi is one the most important leafy vegetables, which is extensively grown and consumed in Cambodia. Insect pests, especially lepidopteran caterpillars and the flea beetles are one of the major production constraints in pak-choi. This has forced the vegetable farmers to heavily rely on calendar-based application of chemical pesticides. In order to develop an effective alternative to harmful pesticides, we evaluated the effectiveness of microbial pesticides (*Bacillus thuringiensis* and *Metarhizium anisopliae* formulations), and neem leaf extract alone and in combination (as an IPM package) against diamondback moth, common armyworm, cabbage webworm and striped flea beetle on pak-choi in three different provinces of Cambodia during 2015-early 2018. *Bacillus thuringiensis* and *M. anisopliae* formulations reduced the incidence of diamondback moth, common armyworm, cabbage webworm and the damage by striped flea beetle to the levels equivalent to chemical pesticide (abamectin). Yield was significantly higher in bio-pesticide treated plots than untreated plots in most of the trials. The performance of the IPM package was on par with Farmers' practice (calendar-based application of chemical pesticides) in reducing the damage by target pests, leading to significantly higher yield. Hence, the IPM package can be piloted and scaled out as an effective alternative to chemical pesticides to manage the insect pests on pak-choi in Cambodia.

Keywords

IPM, diamondback moth, common armyworm, cabbage webworm, striped flea beetle

INTRODUCTION

Pak-choi (*Brassica rapa* var. *chinensis*) is among the most important vegetables grown and consumed in Cambodia (Genova et al. 2010). Brassica vegetables including pak-choi are excellent sources of calcium, fiber, vitamin A (in the form of β-carotene) or pro-vitamin A, and vitamin C (Fahey 2016). Leafy brassicas are high value, short-duration and repeat-cycle crops that could lift small-scale farmers out of poverty. Since leafy vegetables account for 64% of the total vegetable area in Cambodia, their contribution to overall livelihoods is significant (Genova et al. 2010). However, their productivity is limited, mainly due to insect pests and plant diseases. Besides diamondback moth (*Plutella xylostella*), which is the dominant pest of brassicas, common armyworm (*Spodoptera litura*) and striped flea beetle (*Phyllotreta striolata*) can cause up to 100% yield losses in leafy brassicas (Srinivasan et al. 2019).

Vegetable farmers in Cambodia rely heavily on indiscriminate, repeated application of chemical pesticides to prevent the damages by pests and diseases. About 94% of the farmers in Cambodia solely rely on chemical pesticides, and the use of bio-pesticides in vegetable brassicas is almost nil (Schreinemachers et al. 2017). At least 71% of Cambodian spray applicators mixed different pesticides together in a single spray (Schreinemachers et al. 2017). Such a misuse and overuse of pesticides raises concerns for human and environmental health. In addition, development of resistance to pesticides and the pesticide residues in the harvested produce add additional dimensions to pesticide misuse. For instance, detectable levels of organophosphate and carbamate pesticides were reported in vegetable brassicas sampled from Cambodian markets (Neufeld et al. 2010). Hence, there is an urgent need to develop alternative options to reduce the use of pesticide in production of vegetable brassicas.

Bio-pesticides are an important component in integrated pest management (IPM) approaches, which can reduce the reliance on chemical pesticides. Very few studies have been carried out to assess the impact of bio-pesticides on insect pests of brassicas in Cambodia (Srinivasan et al. 2020). Bio-pesticides such as *Bacillus thuringiensis*, *Metarhizium anisopliae* and neem extract were shown to reduce the incidence of diamondback moth, common armyworm, cabbage webworm and the damage by striped flea beetle to levels equivalent to chemical pesticide (abamectin) on Chinese mustard in Cambodia (Srinivasan et al. 2020). A bio-based IPM package was demonstrated to be on par with farmers' practice of calendar-based pesticide application in reducing the damage by key pests of Chinese mustard, leading to significant yield gains in farmer participatory trials. Hence, the current study was carried out to evaluate different microbial pesticides and neem either alone or in combination (sequential applications) against major insect pests on pak-choi in different provinces of Cambodia.

MATERIALS AND METHODS

Study sites

Field studies were conducted in four Cambodian provinces (Kandal, Kampong Chhnang, Svay Rieng and Prey Veng) (Table 1) to evaluate the efficacy of individual bio-pesticides during July-September in 2015 and February-April in 2016 on pak-choi.

Table 1. Provinces and experimental locations for bio-pesticide evaluation in pak-choi during 2015-2016 in Cambodia

Province	Experimental site
Kandal	Kbal Koh Vegetable Research Station, Kbal Koh Commune, Kien Svay District
	Praek Thmey Village, Praek Thmey Commune, Koh Thum District
Kampong Chhnang	Sdork Reach Village, Andoung Snay Commune, Rolea B'ier District
	Chrey Bak Village, Chrey Bak Commune, Rolea B'ier District
Svay Rieng	Khuoch Village, Krol Kor Commune, Svay Chrum District
Prey Veng	Sdao Village, Krang Svay Commune, Preah Sdach District

Subsequently, field trials were conducted in three provinces (Kandal, Kampong Chhnang and Prey Veng) (Table 2) during October-December in 2016 and October 2017-January 2018 to evaluate the efficacy of an IPM package based on bio-pesticides in comparison with Farmers' practices, which is mainly based on calendar-based spraying of chemical pesticides.

Table 2. Provinces and experimental locations for comparing IPM vs. Farmers' practices in pak-choi in Cambodia during 2016-2018

Province	Experimental site
Kandal	Kandal Village, Banteay Daek Commune, Kien Svay District
	Praek Thmey Village, Praek Thmey Commune, Koh Thum District
Kampong Chhnang	Preal Village, Banteay Preal Commune, Rolea B'ier District
	Andong Preng Village, Krang Leav Commune, Rolea B'ier District
Prey Veng	Sdao Village, Krang Svay Commune, Preah Sdach District
	Porlos Vegetable Research Station, Preah Sdach District

Treatment and data collection

Bio-pesticide trials

Field trials were conducted during 2015 and 2016 to evaluate the efficacy of *Bacillus thuringiensis* and *Metarhizium anisopliae* formulations, and neem leaf extract against *P. xylostella*, *S. litura* and *P. striolata* on pak-choi. Seven treatments, *viz*., four bio-pesticide formulations [Xentari® (*B. thuringiensis* subsp. *aizawai*), Crymax® and E-911® (*B. thuringiensis* subsp. *kurstaki*), Real M-62® (*M. anisopliae*)], neem leaf extract, abamectin (chemical pesticide, a "positive" control), and an untreated check were used in each trial during 2015. The 2016 trials included six treatments, Crymax® was dropped. There were three replications for each treatment, and each replication was imposed on a 2 to 5 m^2 plot (depending on the farm size in the farmers' fields or in the research station) following a randomized complete block design (RCBD) with a 1 m distance between plots. The crop was monitored for damage by target pests, and the bio-pesticide treatments were initiated from one to three weeks after planting depending on the pest incidence and continued at weekly intervals. The number of larvae of *P. xylostella* and *S. litura* on five randomly selected plants in each replicate plot were counted, whereas the number of 'shot-holes' in a 4 cm^2 area in two younger leaves from each plant were counted on five randomly selected plants in each replication for *P. striolata* damage. Marketable yield was recorded during harvest.

IPM trials

Two field trials in each province were conducted during 2016 to early 2018 to evaluate the efficacy of an IPM package against *P. xylostella, S. litura*, *H. undalis* and *P. striolata* on pak-choi. The IPM package consisted of the sequential application of bio-pesticides and the chemical pesticide. The spraying order was designed based on the incidence of target pests in a given season and the location. Three treatments, *viz*., IPM package, Farmers' practice (alternate spraying of abamectin and cypermethrin) and an untreated control were used in all the trials, with six replications for each treatment, following RCBD. The individual replication size was 2.5-3.75 m^2. The crop was monitored for damage by target pests, and the treatments were initiated from one to three weeks after planting depending on the pest incidence and continued at weekly intervals until a week before the harvest. The data collection was similar to the bio-pesticide trials.

Data analysis

The data were averaged for each plot and analyzed using a combined analysis approach of several experiments (Petersen 1994; Moore and Dixon 2015). Preliminary analysis of variance was completed for each individual analysis (each location in each season), experimental errors were examined for heterogeneity and Shapiro-Wilkinson test for normality was performed in each individual analysis. The data was then analyzed using analysis of variance (ANOVA) with the Proc GLM MIXED of SAS, version 9.1 (SAS Institute, Cary, NC, USA). Each year/province/experimental site was considered a particular environment for the combined analysis. Random effects were considered for years and locations whereas treatments were fixed effects. When significant treatment differences were identified, means were separated by Tukey's HSD Test (SAS) (differences were considered significant at $\alpha = 0.05$). Data on pest incidence and *P. striolata* damage were arcsine transformed. Non-transformed data are presented in the results section.

RESULTS

Bio-pesticide trials on pak-choi (2015)

Interaction effects (Treatment*Location) showed significant difference for *P. xylostella* incidence and *P. striolata* shot-hole damage (Table 3). The *P. xylostella* population was significantly reduced by *B. thuringiensis* and neem extract, which were on par with abamectin and followed by Real M-62® in Kandal (Figure 1). In Kampong Chhnang, Xentari®, E-911® and Real M-62® recorded the lowest infestation of *P. xylostella.* Real M-62® led to significant reduction of *P. xylostella*, which was followed by *B. thuringiensis* formulations in Svay Rieng. The *P. striolata* damage (Figure 2) was significantly reduced by the E-911® treatment in Svay Rieng, which was on par with abamectin, and the bio-pesticide effects in general across locations were not so obvious in 2015 trials.

All the bio-pesticides reduced the *S. litura* incidence to significantly lower levels compared to untreated control plots (Table 4). The yield was affected by location and treatments, but not their interaction. Average yield in Svay Rieng was significantly higher (23.94 ± 3.79 t/ha), compared to Kampong Chhnang (18.64 ± 2.31 t/ha) and Kandal (16.19 ± 3.54 t/ha). Regarding the effect of treatment, the yield was significantly higher in E-911®, and Real M-62® treatments, compared to untreated control plots (Table 4). The other treatments recorded an intermediate yield and were similar to either of the extremes.

Table 3. Analyses for incidence of *P. xylostella* and *S. litura*, damage of *P. striolata* (shot-holes / 4 cm^2) and pak-choi marketable yield for target provinces in Cambodia during 2015

Source		Location	Treatment	Treatment* Location
DF		2	6	12
No. of *P. xylostella* larvae/plant	F value	53.25	38.94	5.60
	Pr>F	0.0002	<.0001	*<.0001*
No. of *S. litura* larvae/plant	F value	0.72	3.69	0.92
	Pr>F	0.526	*0.006*	0.539
No. of shot-holes/4 cm^2	F value	6.11	9.34	2.13
	Pr>F	0.036	<.0001	*0.040*
Marketable yield (t/ha)	F value	13.94	3.13	0.64
	Pr>F	*0.006*	*0.014*	0.790

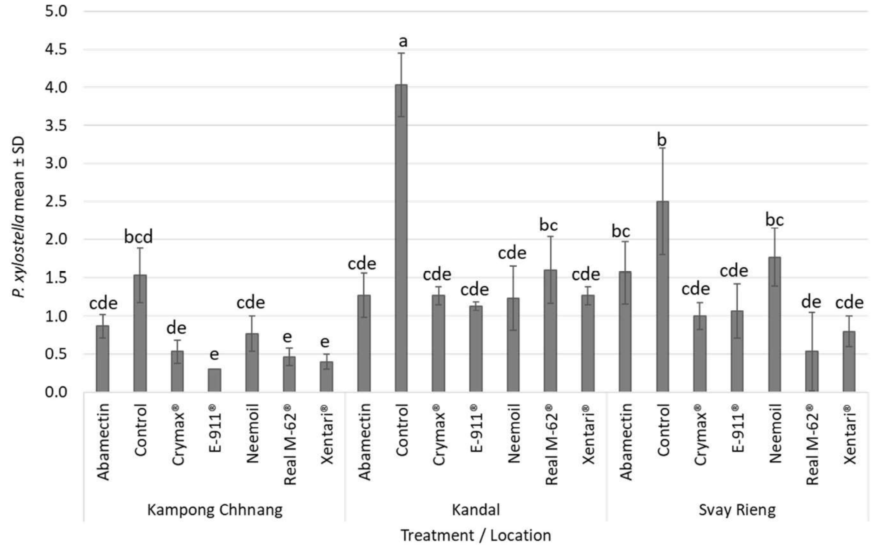

Figure 1. Mean (±SD) number of *P. xylostella* population on pak-choi in three provinces in Cambodia during 2015. Significant differences are presented for the interaction Locations*treatment. Treatments with the same letter(s) did not differ statistically across locations.

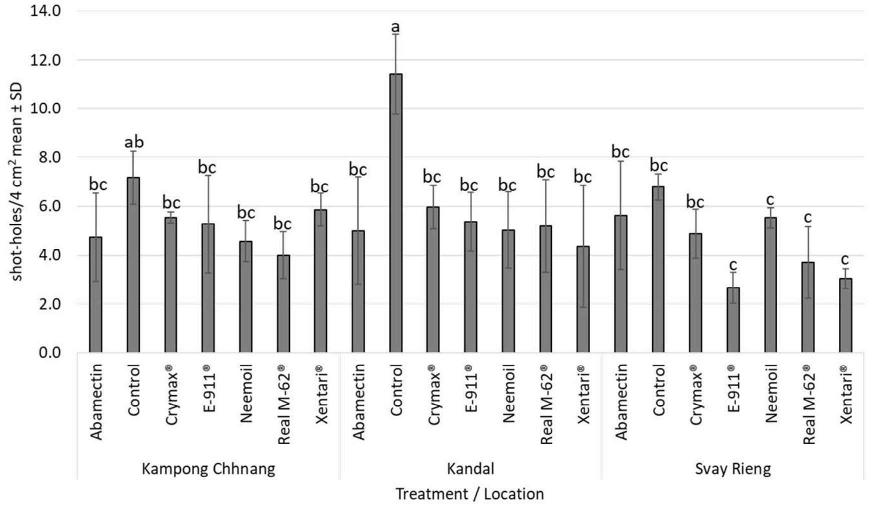

Figure 2. Mean (±SD) number of shot-holes/4 cm^2 caused by *P. striolata* on pak-choi in three provinces in Cambodia during 2015. Significant differences are presented for the interaction Locations*treatment. Treatments with the same letter(s) did not differ statistically across locations.

Table 4. Mean (±SD) number of *S. litura* and pak-choi marketable yield in Cambodia during 2015

Treatment	No. of *S. litura* larvae/plant	Marketable yield (t/ha)
Xentari®	0.24 (0.16) b	20.37 (3.95) ab
Crymax®	0.19 (0.15) b	19.01 (4.08) ab
E-911®	0.31 (0.23) b	21.06 (3.77) a
Real M-62®	0.31 (0.18) b	20.93 (4.97) a
Neem oil	0.28 (0.20) b	20.41 (5.74) ab
Abamectin	0.30 (0.21) b	19.09 (5.03) ab
Control	0.63 (0.38) a	16.28 (3.83) b

Means followed by the same letter(s) in a column are not significantly different ($p<0.05$) by Tukey's HSD

Bio-pesticide trials on pak-choi (2016)

Since the analyses for each variable (except 'shot-holes' by *P. striolata* beetles) showed significant differences among the treatments across the target provinces in 2016 (Table 5), the treatment effects in each province have been presented separately. In Kandal, E-911® treated plots recorded significantly lower *P. xylostella* larvae, followed by Real M-62® and Xentari® treatments (Figure 3). However, the treatment effects in reducing the *P. xylostella* population were not so obvious in Kampong Chhnang. In Prey Veng, Real M-62® and Xentari® treated plots recorded significantly lower *P. xylostella* population, which were followed by E-911® treated plots. *H. undalis* was absent in Prey Veng, and negligible in Kandal (Figure 3). Hence, the low incidence of *H. undalis* in some of the bio-pesticide treated plots cannot be attributed to treatment effects (Figure 4). However, *B. thuringiensis* treatments recorded significantly lower *H. undalis* larvae which were on par with abamectin treated plots in Kampong Chhnang. The shot-hole damage caused by *P. striolata* beetles was significantly higher in Kandal compared to Svay Rieng. In terms of bio-pesticide treatment, damage was lower in Real M-62® treated plots, compared to untreated control plots, whereas the other treatments recorded an intermediate damage and similar to either of the extremes (Figure 5). Significantly higher yields were recorded in E-911® and neem leaf extract treated plots in Kandal. Lower yields were recorded in the untreated control and in E-911® in Kampong Chhnang, whereas the other treatments recorded an intermediate yield (Figure 6).

Table 5. Analyses for incidences of *P. xylostella* and *H. undalis*, damage of *P. striolata* (shot-holes / 4 cm^2) and pak-choi marketable yield for each target province in Cambodia during 2016

Source		Location	Treatment	Treatment* Location
DF		2	5	10
No. of *P. xylostella* larvae/plant	F value	4.66	16.59	3.24
	Pr>F	0.060	<.0001	*0.006*
No. of *H. undalis* larvae/plant	F value	72.05	1.76	2.83
	Pr>F	<.0001	0.152	*0.013*
No. of shot-holes/4 cm²	F value	68.54	2.71	1.23
	Pr>F	*<.0001*	*0.039*	0.315
Marketable yield (t/ha)	F value	10.10	2.30	2.20
	Pr>F	0.012	0.070	*0.047*

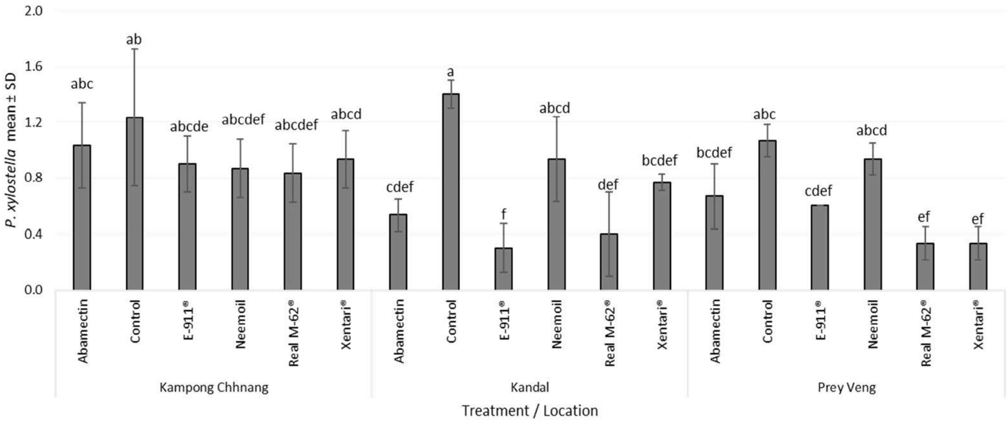

Figure 3. Mean (±SD) number of *P. xylostella* on pak-choi in three provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

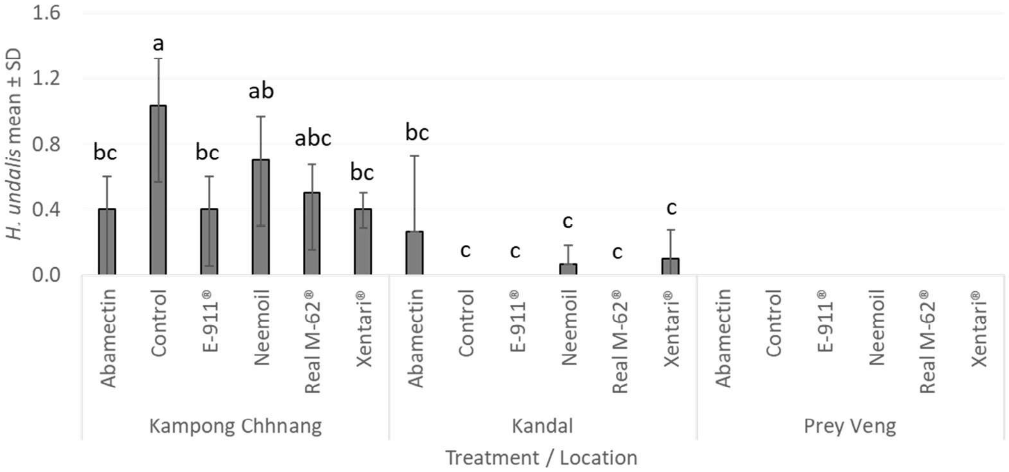

Figure 4. Mean (±SD) number of *H. undalis* on pak-choi in three provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

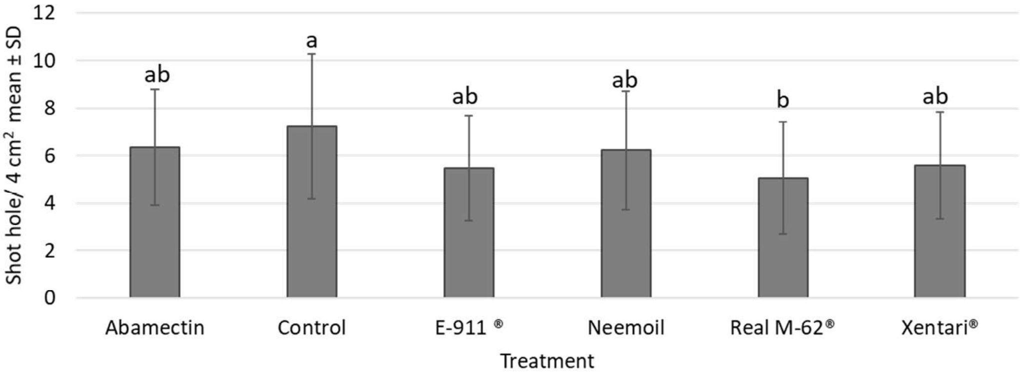

Figure 5. Mean (±SD) number of *P. striolata* shot-holes on pak-choi in target provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

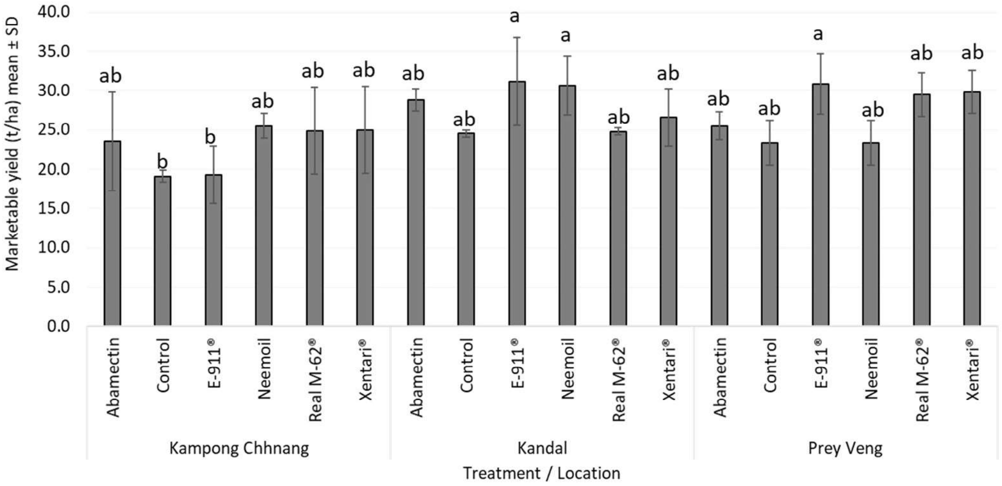

Figure 6. Mean (±SD) marketable yield of pak-choi in three provinces of Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

IPM trials on pak-choi

Based on the combined analyses for each evaluated variable, only direct effects are presented (Table 6) since no interaction effects were found in the analyses. Regarding the effect of different years and provinces, Kampong Chhnang and Prey Veng recorded less *P. xylostella* population in 2017 compared to Kampong Chhnang and Kandal in 2016 (Table 7). No. *H. undalis* populations were observed at any of the study provinces in 2017. Prey Veng showed less shot-hole damage by *P. striolata* in 2017 compared to Kampong Chhnang in 2016, where damage was two-fold higher. Interestingly, Pak-choi yield was significantly higher in Prey Veng in 2016, but 36% less in 2017 than the previous year (Table 7). The IPM treatment effects were mostly on par with the Farmers' practices. IPM treatment significantly reduced the larval population of *P. xylostella*, *S. litura* and *H. undalis*, and the shot-hole damage by *P. striolata* compared to the untreated control plots (Table 8). The marketable yield was also significantly higher in IPM treated plots than the untreated control plots.

Table 6. Combined analyses for infestation of *P. xylostella*, *S. litura*, and *H. undalis*, shot-holes damage by *P. striolata* and yield in pak-choi for six locations (2 years, 3 provinces, 1 sites/province) in Cambodia during 2016-2018

Source		Location	Treatment	Treatment* Location
DF		5	2	10
No. of *P. xylostella* larvae/plant	F value	9.44	140.82	1.69
	Pr>F	<*.0001*	<*.0001*	0.1031
No. of *S. litura*/plant	F value	0.81	14.44	0.64
	Pr>F	0.5513	<*.0001*	0.7754
No. of *H. undalis* larvae/plant	F value	8.88	3.57	0.99
	Pr>F	<*.0001*	*0.0343*	0.4582
No. of shot-holes/4 cm^2	F value	44.94	47.06	1.20
	Pr>F	<*.0001*	<*.0001*	0.3096
Marketable yield (t/ha)	F value	19.56	5.53	0.75
	Pr>F	<*.0001*	*0.0062*	0.6761

Table 7. Effect of locations [mean (±SD)] on *P. xylostella*, *H. undalis*, shot-holes damage by *P. striolata* and yield on pak-choi in Cambodia during 2016-2018

Year	2016			2017-2018		
Provinces	Kampong Chhnang	Kandal	Prey Veng	Kampong Chhnang	Kandal	Prey Veng
No. of *P. xylostella*/plant	2.62 (2.04) a	2.83 (1.50) a	2.22 (1.13) ab	1.68 (1.02) bc	2.24 (1.76) ab	1.35 (0.83) c
No. of *H. undalis*/ plant	0.41 (0.27) a	0.70 (0.15) 1	0.45 (0.25) a	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
No. of shot-holes / 4 cm^2	9.67 (0.81) a	6.54 (1.17) c	7.95 (1.09) b	5.04 (0.65) de	5.88 (1.33) cd	4.57 (0.85) e
Marketable yield (t/ha)	24.67 (1.29) ab	25.24 (2.39) ab	27.48 (2.11) a	22.67 (1.20) b	24.61 (0.74) ab	17.44 (0.48) c

Means followed by the same letter(s) in a column are not significantly different ($p<0.05$) by Tukey's HSD
Figures in parentheses are *arcsine* transformed values

Table 8. Effect of IPM treatment [mean (±SD)] on *P. xylostella*, *S. litura*, *H. undalis*, shot-holes damage by *P. striolata* and yield on pak-choi in Cambodia during 2016-2018

Treatment	Control	Farmers' practice	IPM	
No.	36	36	36	
No. of *P. xylostella*/plant	3.74 (1.22) a	1.47 (0.68) b	1.26 (0.60) b	$F_{2,107}$ = 140.82; $P<.0001$
No. of *S. litura*/plant	1.11 (0.78) a	0.52 (0.56) b	0.46 (0.46) b	$F_{2,107}$ = 14.44; $P<.0001$
No. of *H. undalis*/plant	0.38 (0.57) a	0.22 (0.42) ab	0.18 (0.38) b	$F_{2,107}$ = 3.57; $P=0.0343$
No. of shot-holes/4 cm^2	7.51 (2.04) a	6.71 (1.92) b	5.61 (1.99) c	$F_{2,107}$ = 47.06; $P<.0001$
Marketable yield (t/ha)	22.37 (3.32) b	24.02 (4.99) ab	24.67 (4.42) a	$F_{2,107}$ = 5.53; $P=0.0062$

Means followed by the same letter(s) in a column are not significantly different ($p<0.05$) by Tukey's HSD

DISCUSSION

Bio-pesticides are effective against various lepidopteran pests. *Bacillus thuringiensis* formulations were able to reduce the population of *P. xylostella* and *S. litura* on pak-choi. A study on Chinese mustard also demonstrated similar efficacy in Cambodia in the study provinces (Srinivasan et al. 2020). An earlier study confirmed that *P. xylostella* and *S. litura* were susceptible to Xentari®, Crymax® and E-911® in Taiwan (Srinivasan et al. 2017a), even though *B. thuringiensis* formulations are widely used by the brassica farmers. However, the use of *B. thuringiensis* formulations is not common in Cambodia. Hence *P. xylostella* and *S. litura* populations in our study areas were seemingly susceptible to *B. thuringiensis* foliar spraying, which suppressed the pests. Although *P. xylostella* had already developed resistance to *B. thuringiensis* formulations in different parts of the world (Díaz-Gomez et al. 2000; Ferré et al. 1991; Ghosh et al. 2011; Hama 1992; Mohan and Gujar 2003; Pérez and Shelton 1997; Shelton et al. 1993; Syed 1992; Tabashnik et al. 1990; Zhao et al. 1993), the *P. xylostella* populations resistant to *B. thuringiensis* subsp. *kurstaki* were susceptible to *B. thuringiensis* subsp. *aizawai* (Talekar and Shelton 1993; Syed 1992), since the latter produces additional toxins such as Cry1C. Hence, use of different formulations such as Xentari®, Crymax® and E-911® can last longer in field conditions in Cambodia.

Metarhizium anisopliae formulation (Real M-62®) was also found to be effective against both *P. xylostella* and *S. litura* on pak-choi. A study on Chinese mustard demonstrated similar results in Cambodia (Srinivasan et al. 2020). *Metarhizium anisopliae* isolates and formulations were found to be most effective against *P. xylostella* in Taiwan (AVRDC 1999), and against egg and/or larval stages of *S. litura* in China (Lin et al. 2007), India (Borkar et al. 2013), and Pakistan (Asi et al. 2013). We too found that *M. anisopliae* formulation can be used to manage *P. xylostella* and *S. litura* on leafy brassicas in Cambodia. In addition, Real M-62® was found to reduce the damage by *P. striolata* beetles in the current study. Few studies have demonstrated the effectiveness of entomopathogenic fungi against flea beetles on brassicas in the USA (Reddy et al. 2014) and in Cambodia (Srinivasan et al. 2020). Since the immature stages of *P. striolata* are found in the soil, future studies should focus on application of entomopathogenic fungi in soil in brassica fields. The current study found that Real M-62® could reduce the lepidopteran species (*P. xylostella* and *S. litura*) as well as the coleopteran species (*P. striolata*) on pak-choi, and hence *M. anisopliae* can be included in an overall management program for *P. xylostella*, *S. litura* and *P. striolata* on leafy brassicas in Cambodia.

Neem leaf extract reduced the population of *P. xylostella* and *S. litura* and *P. striolata* damage in 2015, but not in 2016 trials. Similar inconsistencies were recorded on Chinese mustard in Cambodia earlier (Srinivasan et al. 2020). However, neem seed kernel extract was found to be effective in reducing *P. xylostella* damage (Jayadevi and Kumar 2011), while neem was found to reduce the damage of *P. cruciferae* (Boopath et al. 2010; Reddy et al. 2014) and *P. striolata* (Hou et al. 2003). Hence, the effectiveness of neem extracts and formulations might depend on the location and climatic conditions. The yield of pak-choi was significantly higher in *B. thuringiensis* and *M. anisopliae* treated plots consistently in 2015 trials, but it did not differ significantly in 2016 trials. Hence, the bio-pesticides were incorporated into an IPM strategy instead of using them individually throughout the season, and then validated during 2016-2018.

The efficacy of an IPM package was shown to be consistent in reducing pest damage on pak-choi, which led to significant yield increases. Similar results were recorded on Chinese mustard (Srinivasan et al. 2020). A pesticide window strategy was proven effective for managing *P. xylostella* in the Asia-Pacific region (Baker 2011; Ridland and Endersby 2011; Walker et al. 2011; Feng et al. 2011; Mau and Gusukuma-Minuto 2001). Since these window strategies mainly rotated the chemical pesticides with different modes of action, bio-pesticides were substituted to reduce the amount of chemical pesticides, which was also found to be effective in lowlands of Taiwan (Srinivasan et al. 2017a). Hence, the current IPM package in Cambodia was enriched with microbial and neem pesticides, along with the chemical pesticide, and applied them sequentially. This approach achieved pest suppression levels and yields equivalent to the Farmers' traditional practice of calendar-based pesticide spraying. The reduction in the amount of chemical pesticides in the IPM fields can augment the natural enemies in brassica fields, as shown in Vietnam and Laos (Srinivasan et al. 2017b). Hence, this IPM package can be promoted for large-scale adoption, after validation in major brassica production locations in Cambodia.

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References

Asi MR, Bashir MH, Afzal M, Zia K, Akram M. 2013. Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). J. Anim. Plant Sci. 23, 913–918.

AVRDC. 1999. AVRDC Report 1998. Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan. vii+148 P.

Baker GJ. 2011. Crucifer vegetable insecticide resistance management strategies and issues in Australia,in: Srinivasan, R., Shelton, A. M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 241-247.

Boopath T, Pathak KA, Ngachan SV, Nabajyoti D. 2010. Evaluation of neem oil and insecticides against Phyllotreta cruciferae on broccoli. Ann. Plant Prot. Sci. 18: 236 -237.

Borkar AN, Deotale RO, Undirwade DB, Thakre DR. 2013. Efficacy of entomopathogenic fungal pesticides under laboratory condition against *Spodoptera litura*. J. Soils Crops. 23, 409-412.

Díaz-Gomez O, Rodríguez JC, Shelton AM, Lagunes TA, Bujanos MR. 2000. Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations in Mexico to commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 93, 963–970.

Feng X, Li ZY, Wu QJ, Chen AD, Wu YD, Hou YM, He YR, Li JH, Xie SH, Zhang JM, Wie F, Ma CS. 2011. Rising to the challenge: a national project on sustainable control of DBM in China, in: Srinivasan, R., Shelton, A. M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 15-18.

Fahey JW. 2016. Brassica: characteristics and properties. Encyclopedia of Food and Health. pp. 469-477. http://dx.doi.org/10.1016/B978-0-12-384947-2.00083-0

Ferré J, Real MD, van Rie J, Jensens S, Peferoen M. 1991. Resistance to *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. PNAS. 88, 5119–5123.

Genova C, Weinberger K, Acedo AL Jr. 2010. An assessment of market opportunities for leafy vegetables in the upland areas of Cambodia, Laos, and Vietnam. Final project report, RETA 6376. AVRDC Publication No. 10-734. AVRDC – The World Vegetable Center, Taiwan. 47 p.

Ghosh SK, Chaudhary M, Kumar P. 2011. Myco-Jaal: a novel formulation of *Beauveria bassiana* for managing diamondback moth (*Plutella xylostella*) in subtropical and tropical crucifer production systems. in: Srinivasan, R., Shelton, A.M., Collins, H.L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, 21-25 March 2011, Kasetsart University, Nakhon Pathom, Thailand. AVRDC – The World Vegetable Center, Publication No. 11-755. AVRDC – The World Vegetable Center, Taiwan. pp. 153-158.

Hama H. 1992. Insecticide resistance characteristics of the diamondback moth, in: Talekar, N.S. (Ed.), Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center, pp. 455-463.

Hou Y, Pang X, Liang G, You M. 2003. Evaluation of azadirachtin against striped flea beetle, *Phyllotreta striolata* (F.). Ying Yong Sheng Tai Xue Bao= J. Appl. Ecol. 14, 959-962.

Jayadevi HC, Kumar ARV. 2011. Why is a crude extract of neem superior to commercial neem formulations? A field test against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage, in: Srinivasan, R., Shelton, A.M., Collins, H.L., (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, 21-25 March 2011, Kasetsart University, Nakhon Pathom, Thailand. AVRDC – The World Vegetable Center, Publication No. 11-755. AVRDC – The World Vegetable Center, Taiwan. pp. 172-181.

Lin HF, Yang XJ, Gao YB, Li SG. 2007. Pathogenicity of several fungal species on *Spodoptera litura*. J. Appl. Ecol. 18, 937–940.

Mau RFL, Gusukuma-Minuto L. 2001, Diamondback moth, *Plutella xylostella* (L.), resistance management in Hawaii, in: Ridland, P.M., Endersby, N.M. (Eds.), Proceedings of the Fourth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, Melbourne, Victoria, Australia. pp. 307-311.

Mohan M, Gujar GT. 2003. Characterization and comparison of midgut proteases of *Bacillus thuringiensis* susceptible and resistant diamondback moth (Plutellidae: Lepidoptera). J. Invert. Pathol. 83, 1–11.

Moore JK, Dixon PM. 2015. Analysis of combined experiments revisited. Agron. J. 107, 763-771.

Neufeld DSG, Savoeun H, Phoeurk C, Glick A, Hernandez C. 2010. Prevalence and Persistence of Organophosphate and Carbamate Pesticides in Cambodian Market Vegetables. Asian J. Water Environ. Poll. 7, 89-98.

Pérez CJ, Shelton AM. 1997. Resistance of *Plutella xylostella* (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* Berliner in Central America. J. Econ. Entom. 90, 87–93.

Petersen RG. 1994. Agricultural field experiments: Design and analysis. Marcel Dekker, New York.

Reddy GVP, Tangtrakulwanich K, Wu S, Miller JH, Ophus VL, Prewett J. 2014. Sustainable Management Tactics for Control of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) on Canola in Montana. J. Econ. Entomol. 107, 661-666.

Ridland PM, Endersby NM. 2011. Some Australian populations of diamondback moth, *Plutella xylostella* (L.) show reduced susceptibility to fipronil, in: Srinivasan, R., Shelton, A. M. Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 207-215.

Schreinemachers P, Chen HP, Nguyen TTL, Buntong B, Bouapaoe L, Gautam S, Le NT, Pinnh T, Vilaysone P, Srinivasan R. 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. Sci. Total Env. 593-594, 470–477.

Shelton AM, Wyman JA, Cushing NL, Apfelbeck K, Dennehy TJ, Mahr SER, Eigenbrode SD. 1993. Insect resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. J. Econ. Entomol. 86, 11-19.

Srinivasan R, Lin MY, Su FC, Huang CC. 2017a. Towards developing an integrated pest management strategy for cabbage production systems in lowlands of Taiwan, Republic of China. Mysore J. Agric. Sci. 51, 185–197.

Srinivasan R, Lin MY, Hien NTT, Hai VM. 2017b. Biological control in vegetable brassica pest management in tropical Asia: where do we currently stand? In: Mason PG, Gillespie DR, Vincent C, eds. 2017. *Proceedings of the 5th International Symposium on Biological Control of Arthropods*, 11-15 September 2017, Langkawi, Malaysia. pp. 144-146.

Srinivasan R, Bhanu KRM, Lin MY, Yule S, Su FC, Huang CC, Khumsuwan C, Heng CH, Sarika S, Hai VM, Hien NTT, Khanh LD, Trang VTT, Diep NX, Phimchai V, Chansamone P, Soukhavong K. 2019. Evaluation of novel pheromone lures against striped flea beetle (*Phyllotreta striolata* Fab.) on brassicas and bean pod borer (*Maruca vitrata* Fab.) on yard-long bean in Southeast Asia. *Acta Horticulturae*. 1257: 37–46. doi: 10.17660/ActaHortic.2019.1257.6

Srinivasan R, Sotelo P, Lin MY, Heng CH, Kang S, Sarika S. 2020. Validation of a bio-based integrated pest management package for the control of major insect pests on Chinese mustard in Cambodia. *Crop Protection*. 135: 104728 https://doi.org/10.1016/j.cropro.2019.02.004

Syed AR. 1992. Insecticide resistance in the diamondback moth in Malaysia, in: Talekar, N.S. (Ed.), Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center, pp. 437-442.

Tabashnik BE, Cushing NL, Finson N, Johnson MW. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83, 1671-1676.

Walker GP, Macdonald FH, Wallace AR. 2011. Recent developments in the management of diamondback moth in New Zealand, in: Srinivasan, R., Shelton, A.M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 234-240.

Zhao JZ, Zhu GR, Ju Z, Wang W. 1993. Resistance of diamondback moth to *Bacillus thuringiensis* in China. RPMN. 5,11-12.