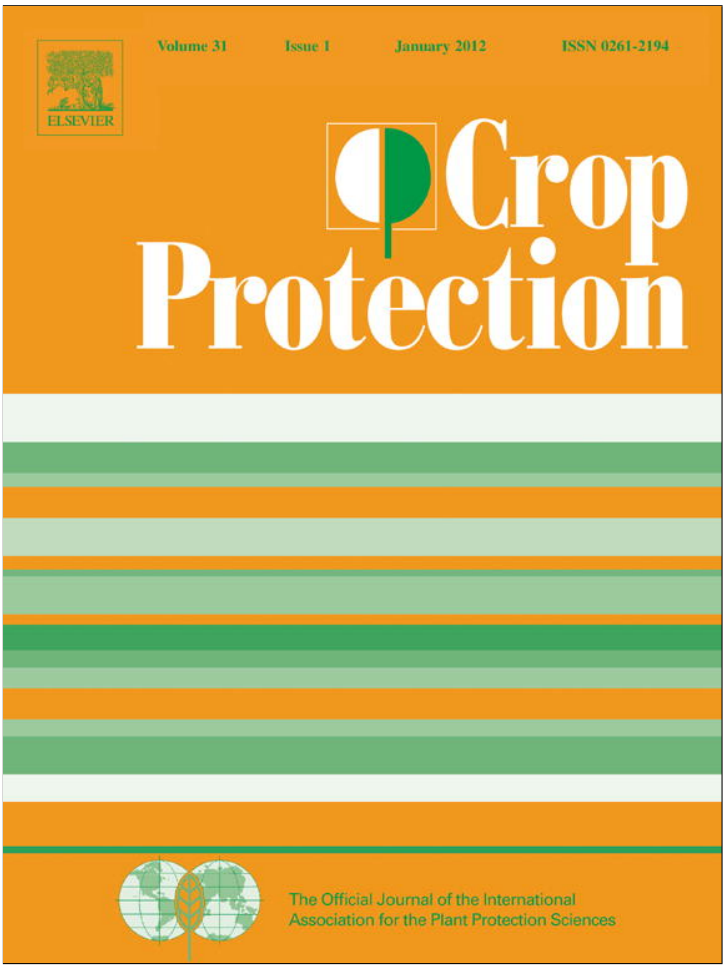

Crop Protection 52 (2013) 91–96

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Insecticides for control of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Pakistan and factors that affect their toxicity

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ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form
20 May 2013
Accepted 21 May 2013

Keywords:
Insecticides
Cruciferous plants
Synergists
Toxicity

ABSTRACT

The diamondback moth, *Plutella xylostella* (L.), is one of the most destructive insect pests of crucifer crops in Pakistan and other parts of the world where crucifers are grown. It has displayed an ability to develop resistance to most insecticides rapidly due to a range of biochemical and behavioral factors. Two factors affecting toxicity of insecticides, host plants and insecticide synergists, were assessed under laboratory conditions. The LC_{50} values of different insecticides varied significantly and feeding by *P. xylostella* on different host plants sometimes significantly affected their toxicity. Against *P. xylostella* collected in Pakistan, the insect growth regulator chlorfluazuron was the most toxic compound (LC_{50} of 0.0006 mg a.i. ml^{-1}) and dimethoate was the least toxic (LC_{50} of 76.6 mg a.i. ml^{-1}). Feeding on different hosts significantly affected toxicity of some insecticides. For example, when larvae were fed rocket plants, *Eurica sativa*, the LC_{50} of λ-cyhalothrin was 0.105 mg a.i. ml^{-1} whilst it was 0.035 a.i. ml^{-1} when larvae were fed cabbage, *Brassica oleracea* var. *capitata*. The LC_{50} values of lufenuron, profenofos, λ-cyhalothrin, spinosad and avermectin alone were 1.14, 8.67, 0.0418, 0.37, and 0.013 mg a.i. ml^{-1}, respectively. With some, but not all insecticides, a low but sometimes significant level of synergism was recorded with use of the synergists piperonyl butoxide and S,S,S-tributyl phosphorotrithioate.

1. Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most destructive insects of cruciferous plants throughout the world. *P. xylostella* occurs wherever crucifer crops are grown, and is believed to be the most universally distributed of all Lepidoptera (Talekar and Shelton, 1993; Grzywacz et al., 2010). Nearly two decades ago, the annual cost of controlling *P. xylostella* on a worldwide basis was estimated to be US$ 1 billion (Talekar and Shelton, 1993) but in a more recent study the overall management costs were estimated at US$ 4–5 billion (Zalucki et al., 2012). In the Indo-Pakistan sub-continent, *P. xylostella* was first recorded in 1914 on cruciferous vegetables (Fletcher, 1914). Mohyuddin and Mushtaque (1983) were able to collect *P. xylostella* from throughout Pakistan and Abro et al. (1994) found *P. xylostella* caused serious damage to cruciferous vegetables in Southern Sindh, Pakistan.

There are many crucifer hosts of *P. xylostella* and many insecticides are used to control it. Host plants can modify the susceptibility of herbivorous insects to insecticides (Yu, 1986; Brattesten, 1988), because physiological responses of herbivores to plant allelochemicals may lead to enhanced levels of metabolizing enzymes that may also detoxify insecticides (Yang et al., 2001). Many enzymes involved in detoxification pathways act on a broad array of substrates and synthetic insecticides (Yu, 2008) and differences in insecticide susceptibilities have been recorded for insects feeding on different host plants (Yu, 1983; Tan and Guo, 1996). Synergists can help determine the mechanism(s) of insecticide resistance, potentially render a resistant population susceptible, prevent the development of resistance, improve the efficacy of an insecticide and lower the insecticide dose required (Raffa and Priester, 1985). The synergist piperonyl butoxide (PBO) is an inhibitor of cytochrome P450 monooxygenases (microsomal oxidases) (Sayyed and Wright, 2006), while the synergist S,S,S-

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http://dx.doi.org/10.1016/j.cropro.2013.05.017

tributyl phosphorotrithioate (DEF) is an inhibitor of insecticide-metabolizing esterases in insects (Aperson and Georghiou, 1975). Both are commonly used in laboratory diagnostic tests to reveal the presence of esterase-mediated resistance (Horowitz et al., 1988).

Cabbage (*Brassica oleracea* var. *capitata*) and cauliflower (*B. oleracea* var. *botrytis*) are preferred hosts of *P. xylostella* and important vegetables worldwide. These vegetables are high value crops with high cosmetic standards; therefore, effective control of the pest is necessary. Insecticides are the most common strategy to control *P. xylostella* on vegetable crops and farmers are always in need of new effective insecticides due to *P. xylostella*'s long history of eventually becoming resistant to every insecticide used extensively against it. The first record of insecticide resistance in *P. xylostella* was reported in 1953 from Java, Indonesia (Ankersmith, 1953) and this was also the first crop pest in the world to develop resistance to DDT. It also has the distinction of being the first agricultural insect to develop resistance in the field to sprays of *Bacillus thuringiensis* Berlinar (Tabashnik et al., 1990; Shelton et al., 1993) and more recently it has developed resistance to newer insecticides such as spinosad, indoxicarb and emamectin benzoate (Zhao et al., 2006). These multiple cases of resistance could be due to microsomal oxidases of *P. xylostella* that are considered to be unusually versatile in dealing with new types of xenobiotics (Sun et al., 1986). This could also be due to inherent differences in physiological and behavioral responses of *P. xylostella* larvae and adults to insecticides (Moore and Tabasink, 1989; Head et al., 1995; Eziah et al., 2009). Perng et al. (1988) suggested that the presence of different microsomal oxidases in *P. xylostella* influences metabolism of different insecticides.

The objectives of the present study were to: 1) investigate the toxicity of commonly used insecticides and insecticide mixtures marketed by different chemical companies against *P. xylostella* in Pakistan; 2) examine the influence of different host plants on toxicity of these insecticides, and 3) examine the effect of synergists on toxicity of some of the recently developed and widely-used insecticides in Pakistan. This may help in elucidating the role of enzymes in detoxification of these insecticides.

2. Materials and methods

2.1. Insects

Three sets of experiments were conducted and, for each experiment, *P. xylostella* larvae were collected in Pakistan from farmer fields during 2008–2009. For each collection, at least 100 larvae were collected from cauliflower fields that had been treated with insecticides commonly used in Pakistan and in accord with typical grower practices. After their collection, each population was maintained separately under laboratory conditions at 21.0 ± 2.0 °C and 62.0 ± 2.0% relative humidity under a 16:8 h light: dark cycle in Petri dishes (15 cm diam). Larvae were fed insecticide-free fresh cauliflower leaves until pupation. Petri dishes were placed in wooden chambers measuring 40 × 40 × 65 cm with fine mesh wire gauze on all sides. After adult emergence, adults were moved to an egg-laying wooden chamber measuring 20 × 20 × 30 cm having two sides and the top with fine mesh wire gauze and two sides with glass panels. Adults were fed a 10% glucose solution impregnated into a small piece of cotton placed in a Petri dish (5 cm diam) that was changed daily to avoid fungal growth. A fresh young cauliflower leaf with its petiole in a vial containing water was provided as an egg-laying substrate. The leaf was inspected for eggs and changed daily. Eggs were placed in Petri dishes and kept separately for hatching. Larvae used in bioassays were laboratory-reared first generation insects.

2.2. Insecticides

The insecticides tested, representing different classes and mixtures of insecticides marketed by different chemical companies against *P. xylostella* in Pakistan, are shown in Table 1.

2.3. Bioassays

The insecticide solutions were prepared in water and a cauliflower leaf was dipped in the insecticide solution for 10 s and allowed to dry for 1–2 h at room temperature. A leaf disc (10 cm diam) was cut from the treated leaf and placed in a Petri dish (15 cm diam) lined with moistened Whatman® no.1 filter paper. Ten fourth instar *P. xylostella* were introduced into each Petri dish (a total of 50 insects per insecticide concentration) and allowed to feed for 48 h. After 48 h, an untreated fresh cauliflower leaf was provided to surviving larvae. Larval mortality was recorded 96 h after treatment. For every insecticide, eight concentrations were tested. The concentrations were determined based on preliminary studies using a wide range of doses to estimate the proper dose range to use in the formal assay. The surfactant Triton X-100® (50 μg ml^{-1}) was added to different neem solutions (Abro et al., 1988; Sayyed and Wright, 2006). Control insects were kept on cauliflower leaf discs dipped in water.

2.4. Effects of different host plants on toxicity of insecticides

Newly hatched *P. xylostella* larvae (<10 h old) were transferred to different host plants in rearing jars: cauliflower, *B. oleracea* var. *botrytis* (cv. Snowdrift White Mountain); cabbage, *B. oleracea* var. *capitata* (cv. Golden Acre); radish, *Raphanus sativus* (cv. Mino Early Long White) and rocket, *Eurica sativa* (cv. Desi). Upon reaching the fourth instar, the toxicity of different insecticides was tested. Five concentrations of each insecticide were tested and, for each concentration, 50 fourth instars were used. A control treatment for every insecticide and every host plant was included. Different

Table 1
Description of insecticides used in the study.

Common name and formulation	Trade name and company
Abamectin 18 g l^{-1} EC	Showgun®, Pan-Pacific company, Karachi, PK
Acephate 750 g kg^{-1} SP	Orthene®, R.B. Avari, Karachi, PK
Chlorfluazuron 50 g l^{-1} EC	Atabron®, American Cyanamid company, Karachi, PK
Cyfluthrin 50 g l^{-1} EC	Baythroid®, Bayer, Karachi, PK
Cyfluthrin + methamidophos 525 g l^{-1} EC	Baythroid TM®, Bayer, Karachi, PK
Cypermethrin 100 g l^{-1} EC	Cymbush®, ICI Pakistan, Karachi, PK
Cypermethrin + profenophos 440 g l^{-1} EC	Poltrin C® Ciba-Giegy, Karachi, PK
Decamethrin 25 g l^{-1} EC	Permethrin®, ICI Pakistan, Karachi, PK
Dimethoate 400 g l^{-1} EC	Rogor®, American Cyanamid company, Karachi, PK
Endosulfan 350 g l^{-1} EC	Thiodan®, AgrEvo, Pak. Ltd, Karachi, PK.
Flucythrinate 1000 g l^{-1} EC	Payoff®, American Cyanamid Company, Karachi, PK
Flucythrinate + dimethoate 400 g l^{-1} EC	Payoff D®, Ciba-Giegy, Karachi, PK
Flufenoxuron 500 g l^{-1} EC	Cascade®, American Cyanamid Company, Karachi, PK
λ-cyhalothrin 25 g l^{-1} EC	Karate®, ICI Pakistan, Karachi, PK
Lufenuron 500 g l^{-1} EC	Match®, Sygenta Pakistan, Karachi, PK
Malathion 570 g l^{-1} EC	Emmatos®, Biochem.Pvt.Ltd., Karachi, PK
Methamidophos 600 g l^{-1} SL	Tamaron®, Bayer, Karachi
Neem oil 100%	Neem oil
Profenofos 500 g l^{-1} EC	Curacron®, Ciba-Giegy, Karachi, PK
Spinosad 240 g l^{-1} EC	Tracer®, Dow Agro Sciences, Karachi, PK

concentrations of insecticides were prepared in water and a host plant leaf was dipped into the insecticide solution for 10 s and allowed to dry in the laboratory. Leaf discs (10 cm diam) were cut from treated leaves and placed in Petri dishes (15 cm diam). Ten fourth instars were introduced into each Petri dish and allowed to feed for 48 h. The surviving larvae were transferred to untreated fresh food and mortality was recorded after 96 h.

2.5. Effect of different synergists on toxicity of insecticides

The synergists, piperonyl butoxide (PBO, 980 g L^{-1}, Sigma Ltd, UK) and S,S,S- trybutyl phosphorotrithioate (DEF 6.0 E, Mobay Chemical Corp., Kansas City, Mo. USA) were used in this study. The insecticide solutions were prepared in water as mentioned above. The insecticides used in the synergism studies had not been widely used for control of *P. xylostella*, since they were newly introduced into Pakistan at the time of the study. Nine to ten concentrations of each insecticide were prepared and tested. Thirty *P. xylostella* larvae were tested for each concentration of an insecticide. *P. xylostella* larvae were held on treated leaves for 48 h and then transferred to untreated fresh cauliflower leaf discs. Mortality was recorded 96 h after treatment. The synergists (i.e., PBO at 1.2 μg/ml and DEF at 2.8 μg/ml) were prepared in ethyl methyl ketone and stored at −20 °C. Each synergist was applied topically to fourth instar *P. xylostella* using a micro applicator. A 1 μl drop was applied to the dorsal region of larvae 1 h prior to exposing the larvae to insecticides. After applying the synergists, the larvae were placed in Petri dishes containing cauliflower leaf discs with different concentrations of insecticides.

2.6. Analysis of data

The data were subjected to probit analysis using the Raymond (1985) program for the calculation of LC_{50} values, slope and χ^2. The χ^2 value is used for testing the heterogeneity of discrepancies between observed and expected numbers (Yu, 2008). The smaller the value of χ^2, the better the fit. If the calculated value of χ^2 exceeds that given in table, the line is not a good fit (Matsumura, 1985). Differences in toxicity were considered significant when the 95% CL of LC_{50} values did not overlap. The ratio of toxicity was calculated by dividing the LC_{50} of the insecticide on cauliflower by the LC_{50} of the same insecticide on another host plant (Yang et al., 2001). The synergism ratio (SR) was calculated by dividing the LC_{50} of the insecticide alone by the LC_{50} of the insecticide + synergist (Table 1).

3. Results

3.1. Toxicity of insecticides on cauliflower as a host

Based on the non-overlap of the LC_{50} values, there were many statistical differences in toxicity of the insecticides tested (Table 2). Among IGRs, chlorfluazuron was the most toxic compound with a LC_{50} of 0.0006 mg a.i. ml^{-1} followed by lufenuron and flufenoxuron with LC_{50} values of 0.004 and 0.32 mg a.i. ml^{-1}, respectively. Cyfluthrin + methamidophos (Baythroid TM®) was the most toxic mixture to *P. xylostella* with an LC_{50} of 1.02 mg a.i. ml^{-1} and methamidophos was the most toxic organophosphate insecticide with an LC_{50} of 0.54 mg a.i. ml^{-1}. Cyfluthrin and dimethoate were the least toxic insecticides tested, with LC_{50} values of 35.1 and 76.6 mg a.i. ml^{-1}, respectively.

3.2. Effects of different host plants on toxicity of insecticides

Host plants affected the LC_{50} values, but not always in a consistent way (Table 3). There were some significant differences in LC_{50} values of an insecticide on different host plants. For example, the LC_{50} of decamethrin was significantly lower on rocket compared to cauliflower or cabbage, but not radish. λ-cyhalothrin was significantly more toxic when *P. xylostella* fed on cauliflower than on radish or rocket. With malathion there were no significant differences in toxicity of *P. xylostella* when it fed on different host plants. Neem oil was significantly more toxic to *P. xylostella* when it fed on rocket than on cabbage, but was not significantly different from the other plants.

3.3. Effects of different synergists on toxicity of insecticides

3.3.1. Lufenuron

The LC_{50} of lufenuron alone was 1.14 mg a.i. ml^{-1} (Table 4). The addition of PBO reduced the LC_{50} to 0.65 mg a.i. ml^{-1} and with DEF the LC_{50} was 0.74 mg a.i. ml^{-1}. However, none of these differences were significant. Application of both synergists together further reduced the LC_{50} to 0.57 mg a.i. ml^{-1} but this was again not statistically significant.

3.3.2. Profenofos

The LC_{50} of profenofos when used alone was 8.67 mg a.i. ml^{-1}. Application of synergists reduced the LC_{50} of profenophos but not always significantly so. Based on non-overlap of the 95% CL of the LC_{50} values, only DEF provided a significantly lower LC_{50} value. The

Table 2
Toxicity of insecticides to *Plutella xylostella* larvae in Pakistan.

Insecticides	N[a]	LC_{50} (mg a.i. ml^{-1}) (95% CL)[b]	Slope	SE	χ^2 (df)[c]
Endosulfan	300	21.73 (17.67–26.05)	3.11	0.50	1.41 (4)
Acephate	300	15.0 (13.1–17.1)	3.08	0.44	5.02 (4)
Dimethoate	300	76.58 (64.63–107.89)	3.22	0.73	3.49 (4)
Profenofos	300	34.73 (31.30–38.70)	3.60	0.44	1.08 (4)
Methamidophos	300	0.54 (0.36–0.80)	0.91	0.96	4.88 (4)
Flucythrinate	300	13.50 (10.90–16.50)	1.92	0.20	6.18 (4)
Cyfluthrin	300	35.10 (29.70–43.2)	2.30	0.32	2.01 (4)
Cypermethrin	300	11.10 (8.55–15.12)	1.56	0.26	2.47 (4)
Flucythrinate + dimethoate	300	24.28 (22.29–26.51)	7.47	1.39	28.83 (4)
Cyfluthrin + methamidophos	300	1.02 (0.73–1.34)	1.72	0.27	10.07 (4)
Cypermethrin + profenophos	300	20.43 (17.32–24.41)	2.37	0.32	1.94 (4)
Flufenoxuron	300	0.32 (0.17–0.46)	1.38	0.23	1.49 (4)
Lufenuron	300	0.004 (0.001–0.007)	0.65	0.10	17.93 (4)
Chlorofluazuron	300	0.0006 (0.0005–0.007)	1.86	0.36	13.64 (4)

[a] Number of larvae tested, including control.
[b] CL, confidence limits.
[c] df, degrees of freedom.

Table 3
Effect of host plants on toxicity of insecticides to *Plutella xylostella* larvae.

Insecticide	Host[a]	N[b]	LC_{50} (mg a.i. ml^{-1}) (95% CL)[c]	Slope	SE	χ^2 (df)[d]	Toxicity ratio[e]
Decamethrin	*B. oleracea botrytis*	250	0.052 (0.039–0.064)	1.97	0.30	2.93 (3)	–
	B. oleracea capitata	250	0.062 (0.055–0.074)	4.34	0.76	0.34 (3)	0.84
	R. sativus	250	0.039 (0.032–0.045)	4.01	0.69	2.34 (3)	1.33
	E. sativa	250	0.029 (0.019–0.038)	2.19	0.42	1.13 (3)	1.79
λ-cyhalothrin	*B. oleracea botrytis*	250	0.034 (0.021–0.047)	1.68	0.27	1.25 (3)	–
	B. oleracea capitata	250	0.035 (0.011–0.061)	1.26	0.34	9.07 (3)	0.97
	R. sativus	250	0.093 (0.074–0.102)	3.22	0.65	7.84 (3)	0.36
	E. sativa	250	0.105 (0.082–0.12)	3.56	0.82	2.82 (3)	0.32
Malathion	*B. oleracea botrytis*	250	1.35 (0.57–2.988)	1.76	0.40	8.22 (3)	–
	R. sativus	250	1.37 (0.55–1.94)	1.82	0.51	0.64 (3)	0.98
	E. sativa	250	2.02 (1.31–2.65)	1.96	0.39	0.75 (3)	0.67
Neem oil	*B. oleracea botrytis*	250	6.34 (4.63–7.77)	2.36	0.48	0.19 (3)	–
	B. oleracea capitata	250	8.17 (5.40–9.18)	3.04	0.91	4.02 (3)	0.78
	R. sativus	250	4.36 (2.98–5.56)	2.37	0.41	1.60 (3)	1.45
	E. sativa	250	3.66 (2.52–4.67)	2.81	0.49	9.54 (3)	1.73

[a] Host plants are cauliflower (*Brassica oleracea* var. *botryis*), cabbage (*Brassica oleracea* var. *capitata*), radish (*Raphanus sativus*) and rocket (*Eurica sativa*).
[b] Number of larvae tested, including control.
[c] CL, confidence limits.
[d] df, degrees of freedom.
[e] Toxicity Ratio, LC_{50} of insecticide on cauliflower/LC_{50} of same insecticide on other host plant.

maximum synergism of 2.32 was recorded with the application of DEF.

3.3.3. λ-cyhalothrin

When λ-cyhalothrin was used alone, its LC_{50} against *P. xylostella* was 0.0418 mg a.i. ml^{-1}. Although synergists increased toxicity, the differences were not significant based on non-overlap of the 95% CL of the LC_{50} values.

3.3.4. Spinosad

The LC_{50} of spinosad was 0.37 mg a.i. ml^{-1} when used alone. Although synergists increased toxicity, the differences were not significant based on non-overlap of the 95% CL of the LC_{50} values.

3.3.5. Avermectin

The LC_{50} of avermectin was 0.013 mg a.i. ml^{-1}. A slight increase in toxicity occurred with the synergists, but this was not significant.

4. Discussion

The only approach hitherto employed by farmers in Pakistan to control *P. xylostella* has been the use of traditional classes of insecticides. Insecticides with new modes of action need to be introduced into Pakistan for control of this severe pest. Our study indicated that the toxicity of organophosphate (OP) and synthetic pyrethroid (SP) compounds varied considerably but insect growth regulators were the most toxic class of compounds followed by insecticide mixtures. Several studies have evaluated newer insecticides against *P. xylostella* in developing countries. Abro et al. (1988) tested different insecticides against *P. xylostella* and found avermectin considerably more active than cypermethrin. Diaz-Gomez et al. (1994) determined the susceptibility of three Mexican populations of *P. xylostella* by leaf residue feeding bioassay to Novo-Biobit, Dipel 2X, Javelin, Thuricide and Cutlass (all *B. thuringiensis* subsp. *kurstaki* formulations) and by topical application to

Table 4
Effect of synergists on toxicity of insecticides to *Plutella xylostella* larvae in Pakistan.

Insecticide	N[a]	LC_{50} (mg a.i. ml^{-1}) (95% CL)[b]	Slope	S.E	χ^2 (df)[c]	SR[d]
Lufenuron	300	1.14 (0.76–1.95)	1.14	0.21	3.69 (7)	–
Lufenuron + PBO	300	0.65 (0.45–0.96)	1.23	0.20	1.56 (7)	1.75
Lufenuron + DEF	300	0.74 (0.49–1.16)	1.26	0.22	1.41 (7)	1.54
Lufenuron + PB0 + DEF	300	0.57 (0.38–0.98)	1.26	0.22	0.77 (7)	2.00
Profenophos	300	8.67 (5.46–15.18)	0.99	0.19	7.28 (7)	–
Profenophos + PBO	300	4.62 (2.94–6.95)	1.11	0.20	3.61 (7)	1.87
Profenophos + DEF	300	3.73 (2.41–5.34)	1.23	0.20	2.03 (7)	2.32
Profenophos + PBO + DEF	300	4.12 (2.68–5.95)	1.18	0.20	1.41 (7)	2.10
λ-cyhalothrin	300	0.0418(0.028–0.067)	1.15	0.2	7.588 (7)	–
λ-cyhalothrin + PBO	300	0.028 (0.019–0.041)	1.20	0.19	2.11 (7)	1.47
λ-cyhalothrin + DEF	300	0.021 (0.013–0.032)	0.97	0.18	1.54 (7)	1.99
λ-cyhalothrin + PBO + DEF	300	0.021 (0.013–0.030)	1.22	0.20	3.84 (7)	1.99
Spinosad	300	0.37 (0.24–0.55)	1.08	0.17	2.76 (7)	–
Spinosad + PBO	300	0.22 (0.15–0.29)	1.42	0.19	8.58 (7)	1.68
Spinosad + DEF	300	0.22 (0.15–0.31)	1.40	0.19	0.85 (7)	1.68
Spinosad + PBO + DEF	300	0.19 (0.12–0.28)	1.19	0.22	7.09 (7)	1.95
Avermectin	300	0.013 (0.008–0.020)	1.01	0.16	6.48 (7)	–
Avermectin + PBO	300	0.011 (0.008–0.016)	1.34	0.22	3.68 (7)	1.18
Avermectin + DEF	300	0.012 (0.008–0018)	1.04	0.16	4.68 (7)	1.08
Avermectin + PBO + DEF	300	0.011 (0.007–0.015)	1.23	0.21	2.53 (7)	1.18

[a] Number of larvae tested, including control.
[b] CL, confidence limits.
[c] df, degrees of freedom.
[d] SR, synergism ratio, LC_{50} of insecticide alone/LC_{50} of insecticide + synergist.

avermectin. *P. xylostella* was more susceptible to avermectin than to formulations of *B. thuringiensis*. Raju et al. (1994) conducted laboratory studies to evaluate the relative toxicity of different insecticides against *P. xylostella* and found cypermethrin and fenvalerate almost 10–15 times more toxic, respectively, than endosulfan. Williams and Mansingh (1996) reported that the compounds isolated from the neem plant manifested their effects on test organisms in many ways such as antifeedants, growth regulators, repellents, toxicants and chemosterilants. Wang et al. (1994) tested the toxicity of several chitin synthesis inhibitors to fourth instar *P. xylostella* in the laboratory and found them to be much more toxic than some conventional insecticides.

Insecticide toxicity is influenced by many factors including feeding behavior, the physical–chemical properties of an insecticide, the method of testing, and the host plant on which insects feed. Insect age at the time of test may cause variation due to factors such as larval weight, detoxification enzymes, insecticide penetration, uptake and rate of excretion (Wells et al., 1983; Aikins and Wright, 1985), temperature (Sparks et al., 1982; Scott and Georghiou, 1984), sex, population density (Yu, 2008) and insecticide selection pressure. *P. xylostella* first instars feed by leaf mining (Liu et al., 1995) while later (second, third and fourth) instars feed on the lower epidermis of leaves and may escape toxic residues of insecticides unless insecticides have translaminar activity (Abro et al., 1989). Similarly, different methods (leaf dip, foliar spray or topical) of assessing toxicity of insecticides can also affect the results (Tabashnik and Cushing, 1987; Zhao and Grafius, 1993). Among crucifer crops, cauliflower and cabbage are preferred host plants of *P. xylostella* and toxicity of different insecticides on cauliflower and cabbage was comparable and did not vary much compared to radish and rocket. Variation in toxicity of insecticides to *P. xylostella* feeding on radish and rocket may be due to interaction between phytochemical profiles of these hosts and insecticides and resultant induction of enzymes (Yu and Hsu, 1993). The toxicity of any insecticide against a phytophagous insect may be significantly affected by feeding on different host plants, since plants contain secondary plant substances that induce detoxification enzymes of insects enabling them to metabolize insecticides and other xenobiotics (Lindroth, 1989). Differences in toxicity on host plants may also be due to larvae consuming more plant tissue, and hence more insecticide, on certain plants but this has not been thoroughly investigated. Berry et al. (1980) found midgut microsomal aldrin epoxidase of the variegated cutworm, *Peridroma saucia* (Hub.), fed peppermint, *Mentha piperita* L., leaves up to nine times more active and tolerant of acephate, methomyl and malathion than larvae fed leaves of other host plants. Similarly, observations of induction and enhancement of detoxification enzymes in insects due to feeding on different host plants have been reported for fall armyworm, *Spodoptera furgiperda* (J.E. Smith) (Yu, 1986), *Heliothis virescens* F. (Riskallah et al., 1986), Colorado potato beetle, *Leptinotarsa decamlineata* Say (Ghidiu et al., 1990), soybean looper, *Pseudoplusia includins* (Walker) (Thomas and Boethel, 1993), diamondback moth, *P. xylostella* (Abro and Wright, 1989) and two-spotted spider mite, *Tetranychus urticae* Koch (Yang et al., 2001).

Both synergists enhanced the toxicity of some insecticides, indicating that *P. xylostella* populations had increased levels of detoxification enzymes. Although not always significant, the levels of synergism recorded in our study by PBO or DEF, individually and combined, ranged from 1.08 to 2.32. Fauziah et al. (1992) reported that treatment with the synergists PBO or DEF to a susceptible strain of *P. xylostella* had no effect on the toxicity of chlorofluazuron and teflubenzuron. However, with the ATA-SEL and NON-SEL subpopulations, both synergists significantly increased the toxicity of chlorofluazuron and teflubenzuron. The hydrolytic metabolism by esterases plays an important role in the degradation of OP insecticides (Motoyama and Dauterman, 1974). In the present study, the synergism ratio of profenofos with DEF was 2.32, which was more than with PBO and suggests the importance of esterases in metabolism of OP insecticides. Jao and Casida (1974) demonstrated that synergism of pyrethroids by DEF in several insects was due to inhibition of pyrethroid-hydrolyzing esterases. Other studies have reported synergism of pyrethroids in the Egyptian leafworm (Isharya et al., 1983) and sweet potato whitefly (Horowitz et al., 1988) through inhibition of esterases. In the present study, we could not determine whether esterases played a role in the hydrolysis of λ-cyhalothrin in *P. xylostella*. Abro et al. (1988) reported synergism of abamectin with PBO indicating the involvement of oxidation in the metabolism of abamectin.

P. xylostella has a history of developing populations resistant to insecticides throughout its broad geographic range. Intense use of insecticides makes some of most potent insecticides ineffective over time. Successful Integrated Pest Management (IPM) of *P. xylostella* in many countries is based on judicious use of insecticides that are regarded as relatively harmless to natural enemies, along with different cultural practices. Use of such a strategy will be helpful for IPM of *P. xylostella* in Pakistan.

Acknowledgment

Authors are thankful to Professor G. P. Georghiou for generous gifts of the synergist, DEF and the Probit Analysis Program (Raymond, 1985).

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