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Evaluation of toxicity and cotoxicity of biopesticides against diamondback moth, *Plutella xylostella* (L.)

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ABSTRACT

In India, the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an important pest of cruciferous vegetables and causes severe economic losses. This pest has ability to evolve resistance in laboratory and field to almost all groups of synthetic insecticides. Despite of these, management with synthetic insecticides has been considered to be the easy and favorable option by farmers in India which indeed increase the cost of cultivation and obviously reduction in the actual net profit. The use of microbial and botanicals pesticides are also available in the market and they are relatively cheap. These pesticides are eco-friendly and the residual toxicity is extremely low. In this study, we have evaluated the *in vitro* toxicity and co-toxicity of different groups of microbial and botanicals pesticides on the field derived population of *P. xylostella* using leaf dip bioassay. Among microbial pesticides Lipel® (*Bacillus thuringiensis* subsp. *kurstaki)*) was found to be very effective than MVP II (Cry1Ac) and XenTari® (*Bacillus thuringiensis* subsp. *aizawai*). Anosom® (1% EC) was found to be most effective against *P. xylostella* among botanicals as evidenced from lowest LC_{50} value of only 0.1ppm. Being a botanical pesticide, Anosom® (1% EC) could be used as a substitute to synthetic insecticides in integrated management of *P. xylostella*. Co-toxicity of pesticides against second instars larvae of *P. xylostella* was assessed using binary mixture of two botanical pesticides at three different combinations (1:1, 1:2 and 2:1 ratio) and found that all the combinations show synergistic effect against diamondback moth. Objective of present research is to investigate toxicity and co-toxicity of botanical and microbial pesticides against diamondback moth so that it could be used as substitute to chemical pesticide for integrated management of diamondback moth.

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INTRODUCTION

In India the diamondback moth occurs regularly wherever crucifers are grown and often it causes serious crop damage in cole crops (Gujar, 1999). This pest causes colossal loss to cabbage every year and it damages the crop by feeding on the foliage. Attack by a large number of larvae hinders the growth of the plant leading to significant reduction in yield. Unilateral reliance on chemical insecticide control is no longer viable because of the pest's capacity to develop insecticide resistance and spiraling costs of newer, effective insecticides. Current control strategies for DBM place greater emphasis on the integrated use of bio rational products such as botanical insecticides. In late 1980s, DBM developed resistance to all synthetic insecticides.

A botanical and microbial pesticide can be employed as an alternative source to control pests with biodegradable concern, due to the reductive contamination in environment and human health hazards (Grainge and Ahmed, 1988). Botanical pesticides are also special because they can be produced easily by farmers for sustainable agriculture and small industries. Many plant species are being investigated for their natural products to be used for *P. xylostella* control. The active management of this pest was until now based on chemical control but, the initial easy success of chemicals has followed by the reports of breakdown in control due to development of resistance. This may be a response to the realization of the potential for environmental disruption that followed the massive introduction of insecticides into the global ecosystem. The uses of mixture of pesticides have not been encouraged due the risk of development of cross resistant in target pest to the chemical present in the mixture. But in some parts of the north eastern region of the country famers have been using mixture of pesticides for management of insect pests. Unfortunately no population of *P. xylostella* from north eastern regions of India has been included for such types of studies. Hence, an important objective for research is to investigate individual and joint toxicity of botanical and microbial pesticides against diamondback moth so that it could be used as substitute to chemical pesticide for integrated pest management of diamondback moth.

MATERIALS AND METHODS

Efficacy of pesticides was studied separately and jointly (co-toxicity). All the experiments related to toxicology were carried out in the Integrated Pest Management laboratory of Division of Entomology, ICAR Research Complex for NEH Region, Umiam, Meghalaya, during the year 2012-2013.

Collection and maintenance of insect strain

A field population of DBM was collected as a larvae in April 2012 from the unsprayed field of cabbage (cv: Wonder ball) of entomology division, situated at Umiam (25^0 30' N latitude and 91^0 51' E longitude) in Meghalaya state. Healthy larvae were separated and allowed to bread for one generation under laboratory conditions at 25 ± 2°C with relative humidity of 60-70%.

In vitro efficacy of pesticides

The efficacy of microbial pesticides *viz.*, Lipel® (*Bacillus thuringiensis* subsp. *kurstaki),* Xentari® (*Bacillus thuringiensis* subsp. *aizawai*) and MVP II (Cry1Ac) was also evaluated against *P. xylostella* through leaf dip bioassay (Tabashnik and Cushing, 1987). Six day old or early second instar larvae were used for bioassay. Five to seven different concentrations of individual pesticides were used for bioassay along with control. Each concentration had three replicates and ten second instar larvae were released in each of the replicate. In order to avoid the larval escape from Petri Dishes, the Petri dishes were sealed with Para film.

Statistical analysis

The mortality caused due to the treatment was recorded from 24 hrs to 72 hrs. For botanicals and from 24hrs to 96 hrs for microbial pesticides (LC50). Mortality was determined by prodding each larva gently with a paint brush; any larva that did not respond to touch was regarded as dead. Experiments were discarded, if the mortality in control exceeds 5%. Data on mortality occurring at72 hrs and 96 hrs were subjected to probit analysis. Probit analysis the corrected mortality was used (Abbott, 1925). Probit analysis was performed using (Finney, 1952) in POLO PC (LeoRa Software; Russell *et al.*, 1977) and LC_{50} values will be obtained for each pesticide at 95% confidence interval. Co-toxicity of pesticides (botanical and microbial) on DBM was assessed using Binary mixture of two pesticides at three different combinations (1:1, 1:2 and 2:1). Five to seven concentrations were tested for each combination. The Co-toxicity coefficient was estimated using the formula of (Corbel *et al*., 2003).

RESULTS AND DISCUSSION

Efficacy of microbial pesticides

Cased on response by *P. xylostella* larvae in terms of mortality at 96hrs, Lipel® was found to be the most effective among all three microbial tested as reflected from lower LC_{50}10ppm (FL 8-10ppm) values. MVP II was the second most effective microbial pesticides against *P. xylostella*, which showed ten times higher LC_{50} 20ppm (FL 10-20ppm) values than Lipel®. Xentari was observed to be the less effective which showed higher LC_{50} 30ppm (FL30-50ppm) values than MVP II (Table 1). The estimates of LC_{70} and LC_{90} along with their fiducial limit were also calculated and as expected it gave the similar trend as observed in LC_{50} estimates.

Efficacy of botanical pesticides to *P. xylostella*

The commercially available botanicals pesticides *viz.*, Margosom® (0.015%), Anosom® (1%EC) and Derisom® (2%EC) were also evaluated for their efficacy against *P. xylostella* larvae under laboratory conditions through leaf dip bioassay method. Among three botanical pesticides tested, *P. xylostella* larvae showed high level of susceptibility to Anosom® (1%EC) as evidenced from very lower LC_{50} 0.1ppm (FL 0.06 to0.2ppm) value. Derisom® (2%EC) was the second most effective botanical pesticides against *P. xylostella* and which showed LC_{50} 380ppm (FL 240-560ppm) followed by Margosom®, LC_{50} 750 ppm (FL 360-1400ppm).

There was a significant variation in LC_{50} values of these three botanical pesticides, as evidenced from non-overlapping of fiducial limit (Table 2). LC_{70} and LC_{90} values of these three botanical pesticides ranged 0.4-3970 ppm and 1.7 to 44030 ppm respectively.

Co-toxicity of binary mixture of pesticides against *P. xylostella*

Co-toxicity of pesticides against second instars larvae of *P. xylostella* was assessed using binary mixture of two pesticides at three different combinations (1:1, 1:2 and 2:1 ratio). Binary mixture of botanicals pesticides having five to seven concentrations were tested in laboratory condition by leaf dip bioassay method and any synergistic/antagonistic effect was assessed by calculating the co-toxicity coefficient (CC). Binary mixture of Anosom + Derisom, Anosom + Margosom, Derisom + margosom,were tested for all three combinations (1:1, 1:2 and 2:1) and the results obtained are presented as below;

Co-toxicity of binary mixture of Anosom + Derisom

The observed LC_{50} values of Anosom and Derisom alone were 0.1ppm (FL 0.06-0.2ppm) and 380ppm (FL 240-560ppm) respectively. The observed LC_{50} values of binary mixture of Anosom + Derisom at 1:1, 1:2 and 2:1 combinations were 0.51ppm (FL 0.31-0.79ppm), 0.67ppm (FL 0.57-0.78ppm), 0.61ppm (FL 0.60-0.78ppm) respectively (Table 4). For all the combinations tested, the co-toxicity coefficient (CC) of this binary mixture was greater than one (>1), it indicated that this binary mixture showed synergetic interactions for all the combinations on *P. xylostella* under laboratory conditions (Table 3).

Co-toxicity of binary mixture of Anosom + Margosom

The observed LC_{50} values of binary mixture of Anosom + Margosom at 1:1, 1:2 and 2:1 combinations were 0.4ppm (FL 0.019-0.72ppm), 0.23ppm (FL 0.05-0.36ppm), 0.37ppm (FL 0.33-0.42ppm) respectively. The observed individual LC_{50} values of Anosom and Margosom were 0.1ppm (FL 0.06-0.2ppm), 750ppm (FL 360-1400ppm) respectively. The co-toxicity coefficient (CC) was greater than (>1) for all the combinations of these two botanical pesticides, which indicated that binary mixture of these two botanical pesticides at three combinations tested in this study showed synergetic interactions on *P. xylostella* (Table 3).

Co-toxicity of binary mixture of Derisom + Margosom

At 1:1, 1:2 and 2:1 combinations, the observed LC_{50} values for binary mixture of Derisom + Margosom were 56ppm (FL 16-96ppm), 52ppm (FL 29-65ppm) and 53ppm (FL 48-60ppm) respectively. The LC_{50} values of Derisom and Margosom, when tested alone were 380ppm (FL 240-560ppm) and 750ppm (FL 360-1400ppm) respectively. Even this binary mixture also showed synergistic effect as the co-toxicity coefficient (CC) was greater than one (>1) for all the tested combinations (Table 3).

Diamondback moth has a long history of eventually becoming resistant to almost all groups of insecticides used for its control. Hence, it is also one of the insect pest species in the World on which huge amount of toxicological work has been undertaken worldwide (Talekar and Shelton, 1993). The driving forces behind these changing patterns are the inventions and development of new and more effective insecticides and their subsequent lost in usefulness due to development of resistance in the target pest species (Talekar and Shelton, 1993). In India, the patterns of use of insecticides vary significant between locations to location. The insecticide use is comparatively less in north eastern region of India.

Though, many studies have conducted similar toxicological work on *P. xylostella*, present study also attempted to evaluate the *in vitro* efficacy and co-toxicity of different groups of pesticides (botanical and microbial) against the *P. xylostella* population of Meghalaya. The results obtained are presented as below;

Efficacy of microbial pesticides on *P. xylostella*

The effectiveness of *B. thuringiensis* formulations alone or in combination with other insecticides for the control of *P. xylostella* under laboratory, glasshouse and field conditions has been reported by various studies (Rabindra, *et al.*, 1995). The better efficacy of one product over other has also been reported on *P. xylostella* by various researchers (Mohan and Gujar, 2001). In present investigation single toxin (MPV II, Cry1Ac) shown intermediate effect as compared to commercial product Lipel® which has multiple toxins within the one strain (*Bacillus thuringiensis* subsp. *Kurstaki*) but similar finding has also been reported from India, where authors found that *Bt* strains having multiple toxins were more effective than *Bt* strains with single toxin (Mohan and Gujar, 2001). The LC_{50} values assessed for three treatments in this investigation are almost similar with other studies conducted on Indian populations of *P. xylostella* (Mohan and Gujar, 2002 and 2003). The microbial products mainly based on soil bacterium *Bacillus thuringiensis* offers tremendous hope for management of *P. xylostella* because of its specificity and the fact that no serious control failures in the field until the report of Tabashnik *et al.* (1990), where authors claimed to report the first case of field evolved resistance to *Bt* sprays in *P. xylostella*. In India, significant geographical variation in susceptibility of *P. xylostella* to various *Bt* toxins/products have also been reported (Mohan and Gujar, 2002), unfortunately no population of *P. xylostella* from north eastern regions of India have been included for such types of studies. Hence, the LC_{50} reported for different group of pesticides in the present study could be the first of such kind from this region of India.

Efficacy of botanical pesticides on *P. xylostella*

There is long history of the use of plants, especially medicinal plants against pests. In recent years, various reports screening medicinal plants for insect control has increased significantly. The present study attempted to evaluate the efficacy of commercially available plant products formulated from three different plant species. The results obtained based on response of *P. xylostella* larvae after 72hrs of exposure revealed that Anosom® was found to be highly toxic to *P. xylostella* as evidenced from very lower LC_{50} values of 0.1ppm (FL 0.06-0.2ppm). As compared to Anosom® (liquid formulation of *Anonin* from *Annona squamosa*), other two botanical pesticides Derisom® (Liquid formulation of *Deris indica*) and Margosom® (liquid formulation of *Azadirachta indica*) showed very high LC_{50} values of 380ppm and 750ppm respectively. The LC_{50} value obtained for Anosom® was very less even than chemical pesticide tested in present study. It clearly suggests that, Anosom® is even better than any chemical pesticide. Similar results were also reported by Londerhausen *et al*. (1991). In another study, the crude seed extract of *Annona squmosa* (Annonaceae) has also been reported to be effective against *P. xylostella* and *Spodoptera litura* (Noctuidae), where treatment showed both toxic and antifeedent activities against *Spodoptera litura* larvae (Leatemia and Isman, 2004a and 2004b). The effectiveness of the neem based products/extracts has also been reported against *P. xylostella* (Chakraborti, 2001) and other pest's species (Jayadevi and Kumar, 2002). The efficacy of products/extract of *Deris indica* has also be evaluated against various pest species (Kumar and Singh, 2002; Meera *et al*., 2003). The advantage of using botanical pesticide is that, they are easily available and have been used extensively for medicinal purpose, implying that they have low or no toxicity to humans. Additional they can be applied to insect pests in the same way as conventional insecticides.

Co-toxicity of binary mixture of pesticides against *P. xylostella*

Though the use of mixture of pesticides, have not been encouraged due the risk of development of cross resistant in target pest to the chemical present in the mixture. But in some parts of the north eastern region of the country famers have been using mixture of pesticides for management of insect pests. An important concept of examining pesticide mixture is deciphering the language of chemical interactions. In present investigation attempts were also made to assess the joint toxicity of botanicals pesticides on *P. xylostella*.

Co-toxicity of binary mixture of botanicals

The information on co-toxicity of botanicals pesticides has not been available in the literature. Given the fact, except neem based formulation, not many botanical pesticides are available in the market for management of insect pest. Thus, present study also attempted to assess the *in vitro* joint toxicity of botanicals pesticides against *P. xylostella* with the assumption that synergetic interaction may occur between the different compounds used in combination, leading to reduce cost and increased efficacy.

The observed LC_{50} values of binary mixture of Anosom + Derisom at 1:1, 1:2 and 2:1 were 0.51ppm, 0.67ppm and 0.60ppm, respectively. The observed LC_{50} values of individual Anosom and Derisom were 0.1ppm and 380ppm, respectively.

Table 1. Toxicity of microbial pesticides to *P. xylostella.*

Name of pesticides	Total number of insects	LC_{50} 90 h (ppm)	Fiducial limits (95%)		LC_{70} 90 h (ppm)	Fiducial limits (95%)		LC_{90} 90 h (ppm)	Fiducial limits (95%)		Slope ±S.E.
			Lower	Upper		Lower	Upper		Lower	Upper	
Lipe l®	180	10	8	10	20	10	25	40	31	62	2.612±0.49
XenTari ®	180	30	30	50	60	40	130	100	80	500	2.276±0.510
MVP-II	180	20	10	20	50	30	90	100	100	800	1.335±0.303

Table 2. Toxicity of botanical pesticides to *P. xylostella.*

Name of pesticides	Total number of insects	LC_{50} 72 h (ppm)	Fiducial limits (95%)		LC_{70} 72 h (ppm)	Fiducial limits (95%)		LC_{90} 72 h (ppm)	Fiducial limits (95%)		Slope ±S.E.
			Lower	Upper		Lower	Upper		Lower	Upper	
Anosom (1%EC)	180	0.1	0.06	0.2	0.4	0.2	0.6	1.7	0.9	5.4	1.215±0.25
Margosom (0.15%)	180	750	360	1400	3970	2020	13670	44030	13020	674500	0.725±0.150
Derisom 2% EC	180	380	240	560	960	640	1770	3730	1900	12600	1.289±0.231

Table 3. Co-toxicity coefficient of Anosom + Derisom, Anosom + Margosom and Derisom + Margosom to *P. xylostella.*

Treatments	Observed LC_{50} (ppm)	Fiducial limit (95%) CI	Co-toxicity Coefficient (CC)	Remark
Anosom	0.1	0.06-0.2	-	-
Derisom	380	240-560	-	-
Margosm	750	360-1400	-	-
Anosom + Derisom				
Anosom + Derisom (1:1)	0.51	0.31-0.79	>1	Synergistic effect
Anosom + Derisom (1:2)	0.67	0.57-0.78	>1	Synergistic effect
Anosom + Derisom (2:1)	0.6	0.6-0.7	>1	Synergistic effect
Anosom + Margosm				
Anosom + Margosm (1:1)	0.4	0.019-0.72	>1	Synergistic effect
Anosom + Margosm (1:2)	0.23	0.05-0.36	>1	Synergistic effect
Anosom + Margosm (2:1)	0.37	0.33-0.42	>1	Synergistic effect
Derisom + Margosm				
Derisom + Margosm (1:1)	56	16 -96	>1	Synergistic effect
Derisom + Margosm (1:2)	52	29-65	>1	Synergistic effect
Derisom + Margosm (2:1)	53	48-60	>1	Synergistic effect

The co-toxicity coefficient of binary mixture of Anosom + Derisom for all three combinations were >1. It indicated the synergetic interactions between these two botanicals pesticides at all the combinations tested in this study. The LC_{50} values of all combinations were far less than LC_{50} values of Derisom alone. Based on the combinations tested, present study suggests that, mixture of Anosom and Derisom would certainly improve the efficacy of Derisom. The lower LC_{50}.values obtained for all three combinations of these two botanicals have been mainly due to the effect of Anosom as evidenced from its very lower individual LC_{50}. Similar trend was also observed for binary mixture of Anosom + Margosom at all the combination tested. The interaction of binary mixture of Derisom + Margosom was also tested for all three combinations (1:1, 1:2 and 2:1) on *P. xylostella*. The observed individual LC_{50} values of Derisom and Margosom were 380ppm and 750ppm respectively. The observed individual LC_{50} values of these two botanicals were far less than the LC_{50} values obtained for their binary mixture at three combinations. Moreover, the co-toxicity coefficient was also greater than one (>1), it clearly indicated that, there was synergetic effects between these two botanicals when they mixed together. Many neem based products have been available in markets for managements for different insect pests; the additions of either Anosom or Dersiom in any neem based products would certainly enhance its efficacy. The finding reported in present investigation on co-toxicity should be expanded to others insect pests with additional combinations of the pesticides tested in this study or may be with other available botanical pesticides.

From the present investigation it has been concluded that botanical pesticide Anosom® was found to be very effective against. *P. xylostella*, therefore it could be used as substitute to chemical pesticide for integrated pest management of *P. xylostella*.

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