VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 418-424 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Assessment of Lethal Dose of Frequently used Synthetic Insecticides Alphamethrin and Chlorpyrifos against the Larva of Diamondback Moth (*Plutella xylostella* L.)

Ansari Taheera and Kumar Sushil*

Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur 273009, Uttar Pradesh, India

**Corresponding Author*

Received: 2nd June, 2024; **Accepted:** 10th August, 2024; **Published online:** 18th August, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.039

Abstract: Diamondback moth (DBM), *Plutella xylostella* (Plutellidae; Lepidoptera), is the major pest inflicting significant losses to cauliflower and cabbage plants in India. We attempted to identify the suitable dilutions of alphamethrin and chlorpyrifos for the mortality of second instar larvae. The larvea of DBM were collected from the cauliflower field in Rajahicamp, Kushmhi, Gorakhpur, Uttar Pradesh, India. Leaf disc method for toxicity bioassay was prepared from the cauliflower leaf from unsprayed field. After determining the mortality of larvae, a narrower range of 6 dilutions as 10, 20, 40, 60, 80 and 100 ppm of each pesticide was used for the period of 24, 48, 72 and 96 h, respectively. The experiment was conducted in six replicates. A control was also run simultaneously without any pesticide. Alphamethrin showed the LD_{10}, LD_{50} and LD_{90} values as 12.28, 8.83, 5.39, 4.06 and 56.55, 47.34, 34.00, 24.35 and 260.27, 253.75, 214.59, 146.06; ppm for 24, 48, 72 and 96 h, respectively. LD_{10}, LD_{50} and LD_{90} values for chlorpyrifos were 10.80, 8.11, 5.04, 3.93 and 52.87, 43.88, 31.69, 22.60 and 258.89, 237.48, 199.46, 129.87 ppm for 24, 48, 72 and 96 h, respectively. The slopes values were 1.93±0.23 to 1.65±0.21 for alphamethrin and 1.86±0.22 to 1.69±0.21 for chlorpyrifos for 24-96 h. This indicates a rapid change in mortality with increase in pesticide dosage especially the chlorpyrifos. Our study suggests that these pesticides especially chlorpyrifos upto amount 60 ppm diluted solution during 48-96 h were the sufficient dose against the DBM larva of cruciferous cultivars particularly cauliflower and cabbage crops and minimal the insecticide resistance capability.

Keywords: Diamondback moth, Synthetic insecticide, Brassicaceae, Cauliflower, Cabbage, Mortality, Chlorpyrifos, Alphamethrin, LD_{50}

Citation: Ansari Taheera and Kumar Sushil: Assessment of lethal dose of frequently used synthetic insecticides alphamethrin and chlorpyrifos against the larva of diamondback moth (*Plutella xylostella* L.). Intern. J. Zool. Invest. 10(2): 418-424, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.039

Introduction

The diamondback moth (DBM), *Plutella xylostella* L., is the most serious constraint in the profitable cultivation of cruciferous crops wherever they are grown (Talekar and Shelton, 1993). The larvae of

DBM infest the leaves, leading to a reduction in the plant's photosynthetic surface area, hindering its growth and productivity. DBM was initially documented in India in 1914, infesting cruciferous vegetables (Regupathy, 1996). This insect primarily thrives on host plants within the Brassicaceae family which are characterized by having sulfur-containing secondary plant components known as glucosinolates. The presence of glucosinolates may be toxic to generalist insects, but in the case of DBM, certain glucosinolates, particularly cardenolides, plant volatiles, and waxes, as well as host plant nutritional quality, leaf morphology, and leaf color, or a combination of these factors, may trigger DBM reproductive and feeding behaviors (Sarfraz *et al*., 2006).

DBM is known to cause yield losses of 31–10% (Abraham and Padmanabhan, 1968). Agriculturists frequently use synthetic insecticides to reduce the loss. Chemical control represents an affordable, convenient, and efficacious approach for managing pest insects, offering a practical solution for farmers seeking to mitigate crop damage and enhance agricultural productivity (Matthews, 2008; Tomenson and Matthews, 2009). However, overuse of insecticides has fostered resistance and eradicated natural predators of the pest, exacerbated control challenges, and disrupted the ecological balance within affected ecosystems (Ankersmit, 1953; Johnson 1953). The initial approach entails employing insecticides that pose greater harm to the pest compared to their impact on natural enemies (Goulart *et al*., 2012; Pereira *et al*., 2014). Conversely, this approach involves reducing the exposure of non-target organisms, achieved through meticulous selection, timing, dosage, placement, and formulation. Dose- and time-dependent assessment can minimize collateral damage to beneficial organisms while effectively controlling the target pest population. Frequently, the use of chemical insecticides at elevated levels has led to the emergence of resistance among DBM offspring against various insecticides across different regions of India, including eastern Uttar Pradesh.

Alphamethrin and chlorpyrifos are both insecticides widely utilized in farming and public health for pest control. Alphamethrin is a synthetic pyrethroid insecticide derived from natural pyrethrins found in chrysanthemum flowers. It is a neurotoxin that disrupts the neural system of insects (Thatheyus and Selvam, 2013). On the other hand, chlorpyrifos is an organophosphate insecticide, containing phosphorus as a central component. It is a broad-spectrum pesticide with systemic and contact activity. It inhibits the enzyme acetylcholinesterase, leading to the accumulation of acetylcholine in the nervous system, which disrupts neurotransmission, causing paralysis and the eventual death of insects (Nandi *et al*., 2022). Both insecticides are generally used to manage a variety of pests in agriculture, such as aphids, caterpillars, thrips, beetles, and mites (Bielza, 2016). It is also used in public health programs to control termites and mosquitoes.

Thus, there is a need to test the efficiency of commercially used and newer chemical molecules with higher insecticidal properties, lower mammalian toxicity, and a lower dosage of application to monitor the level of resistance. Therefore, the present study was aimed at evaluating the bio-efficacy of suitable doses of these synthetic insecticides for the management of DBM.

Materials and Methods

Collection and rearing of larvae:

The larvae of DBM were collected from Village Rajahicamp, Kushmhi, Gorakhpur district, UP, India, and kept in a box lined with blotting paper. Then, they were transported to the laboratory of the Department of Zoology at DDU Gorakhpur University for further experiments. Collected samples were kept in jars and provided with fresh Cabbage (*Brassica oleracea*) leaves and covered with muslin cloth tightly fixed by a rubber band until adult emergence started. Ten per cent (10%)

honey solution was provided as food to adults on petri plates. The day before hatching, fresh cabbage leaves were introduced amidst older leaves to serve as food for newly emerged larvae. Each larval stage was nurtured separately in its own rearing tray. We have used our own-made oviposition chamber for rearing purposes, which is made up of wood, light plastic, and muslin cloth. When the larvae reached full maturity (2nd instar), strips of corrugated paper were placed to serve as pupation sites. The culture was maintained under controlled conditions at 27±2ºC and 70±5% relative humidity. The bioassays were started after three generations of DBM.

Larvicidal bioassay:

The larvicidal bioassay was carried out in accordance with the World Health Organization's (WHO's) guidelines for laboratory and field testing of DBM larvicide with slight modifications (WHO, 2005). Larvae were transferred by means of a soft camel hair brush to the jars, each containing insecticide-free cabbage or cauliflower leaves. Six replicates for each dilution and an equal number of controls were simultaneously used for the experiments. Firstly, DBM insects were held up to late second instars using a standard leaf disc bioassay (Tabashnik, 1987). Each leaf disc of 4.5 cm in diameter was made from the cauliflower leaf collected from the unsprayed field. Firstly, we have treated the cauliflower leaves with streptomycin for the removal of foreign organisms. These were carefully washed, dried, immersed, or dipped in a stock solution of alphamethrin or chlorpyrifos for 30 sec and allowed to dry at room temperature for 1–1.5 h. The test solution of insecticides was freshly prepared in distilled water as a surfactant. For the controls, the leaf disc was only immersed in distilled water and stapled in Petri dishes. We tested six concentrations: 10, 20, 40, 60, 80, and 100 ppm concentrations over replicated six times, including controls against late second instar larvae. On drying, the leaf discs were placed in individual Petri dishes containing moistened filter paper. Larval survivability was assessed after 24, 48, 72 and 96 h, respectively. Larvae were considered dead if they could not show any movement.

Statistical Analysis:

Larvicidal activities of synthetic insecticides were carried out for lethal dose (LD) values with lower and upper confidence limits (LDL and UDL), slope value (mean ± SE), t-ratio, heterogeneity, and chi-square test. $P < 0.05$ was taken as significant. Using probit analysis, the POLO Plus program (LeOra Software version 2.0) determined the LD_{10}, LD_{50}, and LD_{90} values.

Results

The treated larvae become slowly inactive after 11–12 h, and then they can not show any movement. Determinations on the effective dose of the toxicity of the dilutions were obtained in terms of ppm (parts per million) with a mean ± SE for the slope value.

Assessment of toxicity of alphamethrin:

Alphamethrin was significantly efficient for control of DBM larvae control. We have found that 60 and 80 ppm were sufficient sub-lethal doses for larvicidal activity. However, 10 and 20 ppm dilutions did not show toxic doses against DBM larvae. We have found that LD_{10}, LD_{50}, and LD_{90} values were 12.28, 56.55, and 260.27 ppm for 24 h; 8.83, 47.34, and 253.75 ppm for 48 h; 5.39, 34.00, and 214.59 ppm for 72 h; and 4.06, 24.35, and 146.06 ppm for 96 h, respectively (Table 1). The suitable dose of dilutions of alphamethrin against the larvicidal activity of DBM showed 78.33–93.33% mortality at 100 ppm in 24 to 96 h of exposure (Fig. 1a).

Larvicidal efficacy of Chlorpyrifos:

Chlorpyrifos showed effective as well as sufficient DBM larval activity at the experimental dilutions of 10, 20, 40, 60, 80, and 100 ppm. The LD_{10}, LD_{50}, and LD_{90} values were 10.80, 52.87, and 258.89 ppm for 24 h; 8.11, 43.88, and 237.48 ppm for 48 h; 5.04, 31.69, and 199.46 ppm for 72 h; and 3.93, 22.60, and 129.87 ppm for 96 h, respectively (Table 2). The larvicidal activity of chlorpyrifos

Table 1: Toxicity (LD values) of different dilutions of alphamethrin against DBM larvae after 24 to 96 h exposure period

Exposure Period (Hours)	Effective dose (ppm)	Limits (PPM)		Slope Value	't' ratio	Hetero-geneity	Chi-square value
		LCL	UCL				
24	LD_{10} = 12.28	7.88	16.45	1.93±0.23	8.38	0.61	20.84
	LD_{50} = 56.55	47.77	68.61				
	LD_{90} = 260.27	180.96	457.90				
48	LD_{10} = 8.83	5.10	12.53	1.76±0.22	8.05	0.49	16.62
	LD_{50} = 47.34	39.45	57.54				
	LD_{90} = 253.75	172.72	466.36				
72	LD_{10} = 5.39	2.64	8.36	1.60±0.21	7.72	0.58	19.77
	LD_{50} = 34.00	27.36	41.43				
	LD_{90} = 214.59	145.73	399.34				
96	LD_{10} = 4.06	1.90	6.50	1.65±0.21	7.88	0.59	20.08
	LD_{50} = 24.35	18.87	29.79				
	LD_{90} = 146.06	105.14	243.70				

Batches of ten larvae were exposed to four different dilutions in ppm (parts per million) in the boxes. Each set of experiments was replicated six times, and mortality was recorded after every 24 h up to 96 h. Regression analysis showed that a significant value of $p<0.05$ with confidence limits (LCL = lower confidence limit, UCL = upper confidence limit), slope value (±SE), and t-ratio were applied to locate significant changes with control. Here, LD_{10}, LC_{50}, and LC_{90} mean lethal concentrations that kill 10%, 50%, and 90% of DBM larvae.

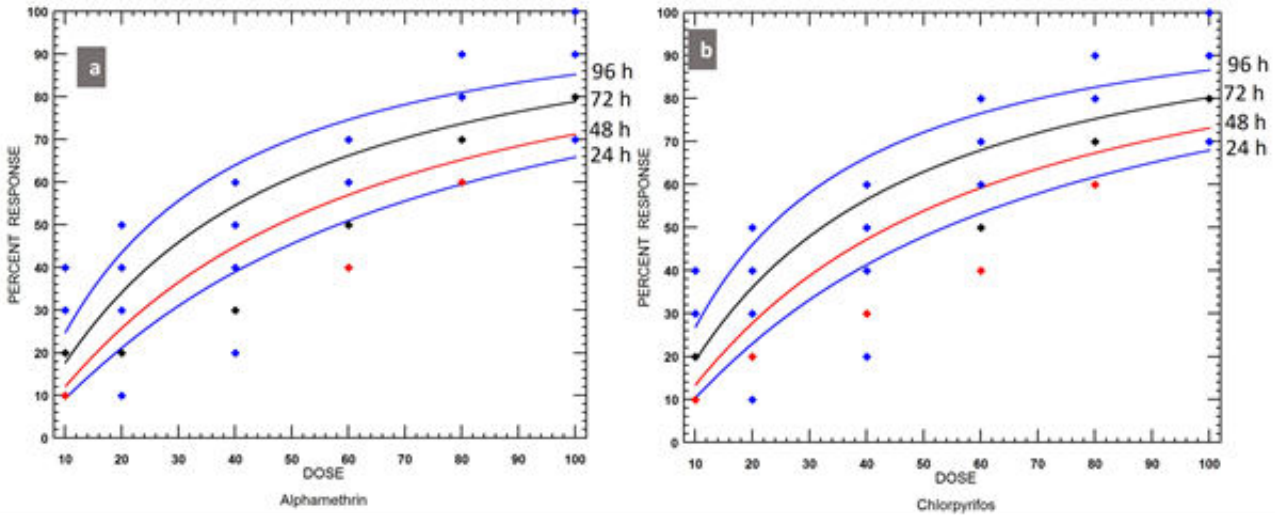

Fig. 1**:** Effective dose of different dilutions of **(a)** alphamethrin and **(b)** chlorpyrifos at 24 h, 48 h, 72 h, and 96 h, where dose is denoted in parts per million (ppm) and larvicidal capability is denoted by per cent response in DBM mortality.

dilutions showed 80–95% mortality at 100 ppm in 24–96 h of exposure periods against DBM (Fig. 1b).

Discussion

In the present study, the sub-lethal doses of insecticides were 24.35 ppm of alphamethrin and 22.60 ppm of chlorpyrifos, in which chlorpyrifos up to 60 ppm diluted solution during 48–96 h were the sufficient dose against the DBM larvae of cruciferous cultivars. DBM is the most important pest, causing severe loss of cruciferous crop yield (Ansari and Kumar, 2022). Worldwide direct losses from brassica and control expenses from

Table 2: Toxicity (LD values) of different dilution of chlorpyrifos against DBM larvae after 24 to 96 h exposure period

Exposure period (Hours)	Effective dose (ppm)	Limits (PPM)		Slope Value	't' ratio	Hetero-geneity	Chi-square value
		LCL	UCL				
24	LD_{10} = 10.80	6.67	14.77	1.86±0.22	8.27	0.63	21.36
	LD_{50} = 52.87	44.45	64.21				
	LD_{90} = 258.89	178.37	463.55				
48	LD_{10} = 8.11	4.60	11.63	1.75±0.22	8.09	0.52	17.54
	LD_{50} = 43.88	36.46	53.09				
	LD_{90} = 237.48	163.12	429.07				
72	LD_{10} = 5.04	2.43	7.88	1.60±0.21	7.74	0.65	22.14
	LD_{50} = 31.69	25.32	38.61				
	LD_{90} = 199.46	136.92	363.84				
96	LD_{10} = 3.93	1.86	6.27	1.69±0.21	8.01	0.59	20.10
	LD_{50} = 22.60	17.42	27.67				
	LD_{90} = 129.87	95.32	208.71				

Batches of ten larvae were exposed to four different dilutions in ppm (parts per million) in the boxes. Each set of experiments was replicated six times, and mortality was recorded after every 24 h up to 96 h. Regression analysis showed that a significant value of $p < 0.05$ with confidence limits (LCL = lower confidence limit, UCL = upper confidence limit), slope value (±SE), and t-ratio were applied to locate significant changes with control. Here, LC_{10}, LC_{50}, and LC_{90} mean the lethal concentration that kills 10%, 50%, and 90% of DBM larvae.

DBM are expected to be $1 billion (Grzywacz *et al*., 2010). Originating in Europe and the Mediterranean region, cabbage is the second most important Cole crop, after cauliflower. It is one of the most commonly cultivated winter vegetables in India and the second-largest producer of cabbage in the world after China (Wikipedia contributors, 2024). The other most important cruciferous cultivar is cauliflower, and it is reported that 52% of the yield loss on cauliflower is due to DBM in India (Krishnamoorthy, 2004). So, our findings suggest the required pesticide amount to reduce the yield loss of cauliflower and cabbage through DBM.

The toxicity of most of the organophosphates and pyrethroids is positively correlated with temperature (Normant and Chambers, 1970). The use of synthetic insecticides constitutes the primary control strategy against DBM (Kibata, 1996). This pest has become resistant to every major class of pesticide, including biopesticides based on the *Bacillus thuringiensis* bacterium (Tabashnik *et al*., 1990; Zhou *et al*., 2011). The infected cultivar treated with the chemical insecticide Anthsuper (chloropyrifos 16% A.I. + alphacypermethrin 1% E.C.) was found to significantly reduce the larval population of DBM (Bhattarai and Tiwari, 2021). The present observation is also supported by Leibee and Savage (1992), where the combination of chlorpyrifos and cytomethrin was most effective to control DBM and *Trichoplusia ni* (cabbage looper) in field conditions. Treatment with chlorpyriphos 50% EC and Cypermethrin 5% EC was found to fully control DBM larvae after 15 days (Boopathi *et al*., 2010).

In the present study, Alphamethrin was also efficient to control DBM larvae. Under laboratory conditions the application of sublethal doses of alphamethrin at concentrations of 0.03% and 0.01% on 8-day-old larvae of the tobacco caterpillar *Spodoptera litura* fed continuously on treated leaf discs for 2 days resulted in a significant decrease in feeding activity and larval weight at 2 days after treatment compared to the control group (Kumar and Srivastava, 2008).

Insecticides are frequently the effective solution for swiftly and cost-effectively reducing pest populations below economically damaging levels. When crops are under pest attack, it is imperative to employ suitable methods to prevent damage. Over recent decades, the utilization of toxic chemicals and bio-pesticides for pest control has seen a significant rise. Concerns over the toxicity of insecticides to humans and wildlife have encouraged efforts to adopt more precise, target-specific chemicals (Begum *et al*., 2017). Alphamethrin and chlorpyrifos effectively manage agricultural pest species while minimizing impacts on natural enemies. Furthermore, we concluded that, in general, alphamethrin and chlorpyrifos have wider safety margins for parasitoids and predators, although higher concentrations can be lethal to certain beneficial arthropods. To maintain their effectiveness, these insecticides should be rotated judiciously with other suitable options in spray programs, limiting the number of applications. Therefore, it is crucial to identify the most effective chemicals against specific insect pests that are environmentally friendly and biodegradable. Farmers find themselves obligated to utilize chemical insecticides for profitable cultivation, as traditional and cultural methods alone often fail to provide adequate control over pest infestations. This scenario has spurred the adoption of alternative insecticide doses to ensure effective control of DBM. Additionally, resistance to these traditional insecticides can be addressed by utilizing precise application rates.

Conclusion

In the present study we assessed the mortality caused by insecticides on DBM larvae. 100 ppm diluted alphamethrin and chlorpyrifos showed 80–95% mortality in DBM larvae after the durations of 24 h and 96 h. The sub-lethal doses were recorded in our study as 24.35 ppm for alphamethrin and 22.60 ppm for chlorpyrifos. So, our recommended dilutions of the above insecticides are permissible doses for protection of cruciferous cultivars, particularly cauliflower and cabbage crops, from DBM and minimal insecticide resistance capability.

Acknowledgements

Ansari Taheera thanks Prof. Vinay Kumar Singh, Dr. Ram Pratap Yadav, and Director, Research and Development Cell, DDU Gorakhpur University, for their suggestions and smooth cooperation as a part of her Ph.D. synopsis. The authors are also thankful to the Vice Chancellor and HOD, Department of Zoology, DDU Gorakhpur University, Gorakhpur, for providing the research facility.

References

Abraham EV and Padmanabhan MD. (1968) Bionomics and control of the diamondback moth, *Plutella xylostella* Linn. Indian J Agricult Sci. 20: 513-519.

Ankersmit GW. (1953) DDT-resistance in *Plutella maculipennis* (Curt.) (Lep.) in Java. Bull Entomol Res. 44(3): 421-425.

Ansari T and Kumar S. (2022) Influence of insecticides on the development of pest resistance of diamondback moth *(Plutella xylostella*). Front Crop Improvement 10: 969-976.

Begum A, Alam SN and Jalal Uddin M. (2017) Management of pesticides: Purposes, uses, and concerns. In: Pesticide Residue in Foods, (eds.) Khan M., and Rahman M., Springer, Cham. https://doi.org/10.1007/978-3-319-52683-6_4

Bhattarai AM and Tiwari S. (2021) Effects of different bio-rational compounds on mortality of diamond back moth (*Plutella xylostella L.*) larva under laboratory condition. J Agricult Environ. 22: 31-40.

Bielza P. (2016) Insecticide resistance in natural enemies. In: Advances in insect control and resistance management, Springer, Cham. https://doi.org/10.1007/978-3-319-31800-4_16

Boopathi T, Pathak KA, Ngachan SV and Das N. (2010) Evaluation of insecticides and biopesticides against diamond back moth, *Plutella xylostella* (L.) on Broccoli. Pest Managem Horticult Ecosystems 16(1): 69-72.

Goulart RM, Volpe HX, Vacari AM, Thuler RT and De Bortoli SA. (2012) Insecticide selectivity to two species of *Trichogramma* in three different hosts, as determined by IOBC/WPRS methodology. Pest Manag Sci. 68: 240-244.

Grzywacz D, Rossbach A, Rauf A, Russel DA, Srinivasan R and Shelton AM. (2010) Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management

with lepidopteran– resistant Bt vegetable brassicas in Asia and Africa. Crop Protection 29: 68-79.

Johnson DR. (1953) *Plutella-maculipennis* resistance to DDT in Java. J Economic Entomol. 46(1): 176-176.

Kibata GN. (1996) Diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), a problem pest of brassica crops in Kenya. Proc 1st Biennial Crop Protection Conference, 27-28 March, Nairobi, Kenya, pp.1-11.

Krishnamoorty A. (2004) Biological control of diamondback moth *Plutella xylotella* (L.), an Indian scenario with reference to past and future strategies. Proc Int *Plutella xylostella* Symp., Montpellier, pp. 204-2l I.

Kumar Y and Srivastava RP. (2008) Effect of sublethal doses of some synthetic pyrethroids on biological parameters of tobacco caterpillar, *Spodoptera litura* (Fab.). J Entomol Res. 32(3): 211-216.

Leibee GL and Savage KE. (1992) Evaluation of selected insecticides for control of diamondback moth and cabbage looper in cabbage in Central Florida with observations on insecticide resistance in the diamondback moth. Florida Entomologist 1: 585-591.

Matthews GA. (2008) Attitudes and behaviours regarding use of crop protection products-- A survey of more than 8500 smallholders in 26 countries. Crop Prot. 27: 834-846.

Nandi NK, Vyas A, Akhtar MJ and Kumar B. (2022) The growing concern of chlorpyrifos exposures on human and environmental health. Pesticide Biochem Physiol. 185:105138.

Norment BR and Chambeas HW. (1970) Temperature relationships in organophosphorus poisoning in boll weevils. J Economic Entomol. 63(2): 502-504.

Pereira RR, Picanço MC, Santana PA, Moreira SS, Guedes RNC and Corrêa AS. (2014) Insecticide toxicity and walking response of three pirate bug predators of the tomato leaf miner *Tuta absoluta*: Insecticide impact on predatory bugs. Agricult Forest Entomol. 16: 293-301.

Regupathy A. (1996) Insecticide resistance in diamondback moth (DBM), *Plutella xylostella* (L.): Status and prospects for its management in India. In: Proc 3rd International Workshop, Kaulalampur, pp. 233-239.

Sarfraz M, Dosdall LM and Keddie BA. (2006) Diamondback moth–host plant interactions: implications for pest management. Crop Protection 25(7): 625-639.

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

Tabashnik BE, Cushing NL and Johnson MW. (1987) Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. J Economic Entomol. 80(6):1091-1099.

Talekar NS and Shelton AM. (1993) Biology, ecology, and management of the diamondback moth. Ann Rev Entomol. 38(1): 275-301.

Thatheyus AJ and Selvam AG. (2013) Synthetic pyrethroids: toxicity and biodegradation. Appl Ecol Environ Sci. 1(3): 33-36.

Tomenson JA and Matthews GA. (2009) Causes and types of health effects during the use of crop protection chemicals: Data from a survey of over 6,300 smallholder applicators in 24 different countries. Int Arch Occup Environ Health 82: 935-949.

Wikipedia contributors (2024) Cabbage. In: Wikipedia, The Free Encyclopedia.

World Health Organization (2005) Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization.

Zhou L, Huang J and Xu H. (2011) Monitoring resistance of field populations of diamondback moth *Plutella xylostella L.* (Lepidoptera: Yponomeutidae) to five insecticides in South China: A ten-year case study. Crop Protection 30: 272-278.