EFFECTS OF DIFFERENT BIO-RATIONAL COMPOUNDS ON MORTALITY OF DIAMOND BACK MOTH (*Plutella xylostella* L.) LARVA UNDER LABORATORY CONDITION

A.M. Bhattarai[1*] and S. Tiwari[2]

ABSTRACT

A leaf dip technique of bioassay for mortality of DBM larvae was conducted in laboratory condition with room temperature of 25±2°C, relative humidity of 80±3% and 13:11 ratio of Light: dark period at Department of Entomology, Agriculture and Forestry University, Rampur, Chitwan with three replication and eight treatments; i.e. i) Lipel (Bacillus thuriengiensis var. kursataki) 2gm/l, ii) Racer (Beauveria bassiana1.15% WP) 2gm/l, iii) Derisom (Fractions of Derris indica) 2ml/l, iv) Anosom (Extracts of Annona spp. 1%) 2ml/l, v) Neemix (Neem oil 60% w/w, Azadiractin content less than 300 ppm) 2ml/l, vi) Anthsuper (Chloropyrifos 16% A.I. + Alphacypermethrin 1%EC (w/w) 2ml/l, vii) cow urine (1:5 with water) and viii) control (water spray) in Completely Randomized Design (CRD). The larval mortality was taken after 3, 9, 21, 33, 57 and 93 hours after the treatment application. The larval mortality was found to be significantly higher in Anthsuper treated with 100% mortality of larvae within 33 hours after treatment application followed by cow urine, botanicals (Neemix, Derisom and Anosom) and microbials (Racer and Lipel) where the larval mortality over control was found to ranging from 10% to 47.57% during the experimental period. The larval mortality was 47.57% for Cow urine and Neemix followed by 38.14% for Anosom, 33.29% for Racer, 28.57% for Derisom and 19% for Lipel.it is concluded that chemical pesticide Anthsuper is superior for immediate control of the pest but considering the safety of environment and human health; for long-term control of the pest botanicals and microbials would be more efficient.

Key Words: Anthsuper, botanicals, crucifers, DBM, microbials

INTRODUCTION

Cabbage is one of the most important crucifers vegetables in the context of Nepal, where crucifers is contributing 28% of economy among the vegetable production. It covers an area of 28,071 ha, 468,836 mt production and 16.7 mt/ha average productivity in Nepal (VDD, 2016). Different factors like varieties, quality seeds, cultivation practices, fertilizers, diseases and insect pests are the limiting factors for their production.

Diamondback moth (*Plutella xylostella* L.) is one of the major leading pest factors for low productivity of cruciferous crops (RPPL, 2003; NARC, 2016;

1 Mahendra Ratna Multiple Campus, Institute of Agriculture and Animal Sciences, TU, Nepal

2 Agriculture and Forestry University, Rampur, Chitwan, Nepal

* Corresponding Author: ananta.bhattarai@mrmc.tu.edu.np / palpali.ananta@gmail.com

PPD, 2018) and has caused heavy loss in cole crops in Nepal. Larvae are the damaging stage of the pest where they damage the crop by feeding on foliage parts of the crop and hinder the growth of the plant leading significant reduction in yield. DBM is known to cause yield loss from 31 percent (Abraham and Padmanabhan, 1968) to 100 percent (Cardleron and Hare, 1986) and the conservative estimate of total costs for managing this pest is estimated to be US$4 billion to US$5 billion (Zalucki *et al.,* 2012).

The essential oils extracted from the botanical plants like *Corymbia* spp. And *Eucalyptus* spp. have insecticidal activity and caused up to 80% larval mortality against *Plutella xylostella* L. (Filomeno, et. al., 2017).

Diamondback moth is the most serious one because of the development of resistance with many common commercially available chemical pesticides including DDT (Ankersmit, 1953), bacterial insecticide, *Bacillus thuringiensis* (Tabashnik, Cushing, Finson, and Johnson, 1990), diamide (Gong, Yan, Gao, Guo, and Xue, 2014) and many others commercially available chemical pesticides due to intensive use of those insecticides (Rindland and Enderby, 2011).

The objective of the study was to find out the effect of different bio-rational compounds (chemical and bio-pesticide) on mortality of diamond back (*Plutella xylostella* L.) larvae in laboratory condition.

MATERIALS AND METHODS

LOCATION OF EXPERIMENTAL SET UP, PERIOD, DESIGN AND UNIT

The experimental location for bioassay was laid out in the IPM laboratory of Department of Entomology, Agriculture and Forestry University, Rampur, Chitwan.

The experiment was conducted in April 2015 in Completely Randomized Design (CRD).

Each experimental unit consisted of 10 third instar larvae of *P. xylostella* (L.) which was replicated three times with eight treatments. In total there were twenty-four experimental units. The treatments were allocated randomly using three-digit random numbers selected from random number table

Plutella xylostella L. adult was reared on cages; where biology was observed and let them bred. The experimental unit of larvae was taken from the cages which were free of any treatments to be applied during the experimental period.

TREATMENTS

200 ml solution of insecticide/ bio-pesticide with distilled water was made for each treatment. The leaf dip bioassay technique was applied. The treated leaf size has a diameter of 4 cm and was changed every 24 hours. All this bioassay activity was conducted under the controlled room condition in Entomology Laboratory with room temperature of 25±2°C, relative humidity of 80±3% and 13:11 ratio of Light: Dark period. The treatment consisted as given below:

Table 1. Treatment details for bioassay of *P. xylostella* (L.) in Laboratory, Rampur, Chitwan, 2015

Treatment	Common Name	Chemical/Scientific Name	Dose
1	Lipel	*Bacillus thuringiensis* var. *kurstaki* (SP)	2gm/l
2	Racer	*Beauveria bassiana* 1.15% WP	2gm/l
3	Derisom	Fractions of *Derris indica*	2ml/l
4	Annosom	Extracts of *Annona* spp. 1%	2ml/l
5	Neemix	Neem oil 60%w/w, Azadiractin content less than 300 ppm	2ml/l
6	Anthsuper	Chloropyrifos 16% A.I. + Alphacypermethrin 1%EC (w/w)	2ml/l
7	Cow urine	-	1:5 with water
8	Control (Water spray)	-	-

OBSERVATION, DATA RECORDING AND ANALYSIS

The mortality record of DBM larvae were taken at an interval of 3, 9, 21, 33, 57 and 93 hours after the setup of the experiment.

Data were tabulated using Microsoft Excel software. The larval population was transformed by square root transformation (SQRT ~$\sqrt{}$(x+0.5)) and analyzed using R-Studio (Version 4.0.0) software package to see the effect of bio-pesticide on diamondback moth (*Plutella xylostella* L.) third instar larvae. Means separation was done by DMRT at 5% level of significance (Gomez and Gomez, 1984).

RESULT AND DISCUSSION

LARVAL MORTALITY BY CHEMICAL TREATMENT

The larvae treated with chemical insecticide Anthsuper (Chloropyrifos 16% A.I. + Alphacypermethrin 1%EC) was found to be significantly at par with

other (botanicals, cow urine and control) treatments during the whole period of experiment on the mortality of DBM larvae in the laboratory in different duration with range of more than 65% mortality after three (3) hours treatment up to 100% mortality rate within a duration of 33 hours of treatment. Comparatively higher reduction of larval population of *Plutella xylostella* (L.) was found with Anthsuper treated plots immediately after application containing Chloropyrifos 16% A.I. and Alphacypermethrin 1%EC; which was supported by Leibee and Savage (1992) where the combination of Chloropyrifos and Cypermethrin was most effective to control *Plutella xylostella* and *Trichoplusia ni* in field condition. The larval population of DBM was found to be controlled by cent percent even 15 days after treatment application when Chlorpyriphos 50% EC + Cypermethrin 5% EC was sprayed (Boopathi, Pathak, Agachan, and Das, 2010).

Table 2. Bioassay of *P. xylostella* (L.) larvae in laboratory for calculating mortality incurred by different treatments (Relative Humidity=80±3%, temperature= 25±2°C and 14:10 Light: Dark Period) in Rampur, Chitwan, 2015

Treatments	Pre-Treatment Population of larvae	Average number of Dead Larvae					
		3 Hours after Treatment Application	9 Hours after Treatment Application	21 Hours after Treatment Application	33 Hours after Treatment Application	57 Hours after Treatment Application	93 Hours after Treatment Application
Lipel@2 gm/l	10	0.00^{B}±0.00	1.67^{BC}±0.33	2.00^{BC}±0.00	2.67^{CD}±0.67	3.67^{B}±0.33	4.33^{C}±0.33
		(0.7071068)	(1.4623408)	(1.5811388)	(1.761199)	(2.037823)	(2.195950)
Racer@ 2gm/l	10	0.00^{B}±0.00	1.00^{BCD}±0.58	1.33^{C}±0.33	2.00^{D}±0.00	4.33^{B}±0.33	5.33^{BC}±0.33
		(0.7071068)	(1.1709968)	(1.3435429)	(1.581139)	(2.195950)	(2.413309)
Derisom @2ml/l	10	0.67^{B}±0.67	1.67^{BC}±0.67	2.67^{B}±0.33	2.67^{CD}±0.33	4.00^{B}±0.58	5.00^{BC}±0.58
		(0.9984508)	(1.4401061)	(1.7742654)	(1.774265)	(2.112452)	(2.338679)
Anosom @2ml/l	10	0.67^{B}±0.67	2.67^{B}±0.33	3.33^{B}±0.33	3.33^{BC}±0.33	4.33^{B}±0.67	5.67^{BC}±0.88
		(0.9984508)	(1.7742654)	(1.9543259)	(1.954326)	(2.187081)	(2.469814)
Neemix @2ml/l	10	0.67^{B}±0.67	1.67^{BCD}±1.20	2.67^{B}±0.67	4.00^{B}±0.00	5.00^{B}±0.58	6.33^{B}±0.33
		(0.9984508)	(1.3510573)	(1.7611993)	(2.121320)	(2.338679)	(2.612544)
Anthsup er@2ml /l	10	6.67^{A}±0.88	7.67^{A}±0.33	9.67^{A}±0.33	10.00^{A}±0.00	10.00^{A}±0.0	10.00^{A}±0.00
		(2.6664322)	(2.8565216)	(3.1876492)	(3.240370)	(3.240370)	(3.240370)
Cow urine@ 1:5 with water	10	0.00^{B}±0.00	0.33^{CD}±0.33	2.00^{BC}±0.58	3.67^{BC}±0.33	5.33^{B}±0.88	6.33^{B}±0.67
		(0.7071068)	(0.8796528)	(1.5589041)	(2.037823)	(2.401714)	(2.607478)

Control (Water spray)	10	0.00^{B}±0.00 (0.7071068)	0.00^{D}±0.00 (0.7071068)	0.33^{D}±0.33 (0.8796528)	2.00^{D}±0.58 (1.558904)	2.33^{C}±0.33 (1.677702)	3.00^{D}±0.00 (1.870829)
LSD		0.56383	0.616382	0.382776	0.319176	0.357269	0.299813
CV		30.69	24.47	12.60	9.20	9.08	7.02
F-test		'***'	'***'	'***'	'***'	'***'	'***'
Mean		1.06	1.46	1.76	2.01	2.27	2.47
MS Error		0.1061	0.1268	0.0489	0.034	0.0426	0.03

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

CV: Coefficient of Variation; S: Significant; LSD: Least Significant Difference; values with the same letters in a column are not significantly different at 5% by DMRT (Duncan's Multiple Range Test); figures after ± indicate standard error and figure in parenthesis indicate mean value of $\sqrt{(x+0.5)}$ transformation data

LARVAL MORTALITY WITH BOTANICALS

The mortality of *P. xylostella* (L.) larvae were not significantly higher than control in the initial period of experiment but found to be increasing and were significantly at par in long duration (21 hours, 33 hours, 57 hours and 93 hours after treatment) than control with percentage reduction over control by 24%, 31% and 24% in21 hours after treatment application for derisom, anosom and neemix respectively and 28%, 38% and 47% in 93 hours after treatment for derisom, anosom and neemix respectively.

Azadirachtin extract was evident to have significant larval mortality in leaf-dip bioassay against second instar larvae over synthetic azadirachtin and have high anti-feedant and repellent effects with ovicidal activity against *P. xylostella* L. at relatively higher dose range (Verkerk and Wright, 1993). Neem based pesticides are effective against a wide range of pests (Singh and Singh, 2009) with very low toxicity to non-target organisms; without toxic residue left to contaminate the environment and insects do not develop resistance to it (Prakash and Rao, 1997).

An aqueous emulsion of an ethanolic seed of *Annona squamosa* was found to be 2.5 times more effective than 1% Rotenone against diamondback moth, *Plutella xylostella* L. in an experiment conducted in three greenhouse trials in Maluku, Indonesia (Leatemia and Isman, 2004). 100% larval mortality was observed when larvae of DBM were treated with higher concentration of ethanolic leaf extract of *Annona muricata* (5mg/ml) and larval survival was significantly reduced when treated with low concentration (Trindade, Luna, De Lim, Da Silva, and Sant'ana, 2011). Similarly, the efficacy of ethanolic leaf extract of *Annona muricata* was tested in a greenhouse against DBM larvae on cabbage plants, where aqueous extract gives 90% mortality of larvae, 1.3-fold higher than mortality caused by 1% rotenone dust (Leatemia and Isman, 2001).

Similarly, 100% mortality of DBM larvae was found when ethanolic leaf extract of *Annona muricata* (5 mg/l) was applied. The extract of *Derris* sps was found to cause up to 69.74% mortality of *Lipaphis erysimi* when a concentration of 1 mg/ml was applied (Hu *et al.*, 2005).

LARVAL MORTALITY WITH COW URINE

The mortality of *P.xylostella* (L.) larvae were not significantly higher than control in first 9 hours after treatment application of experiment but found to be increasing and were significantly at par in long time; 21 hours, 33 hours, 57 hours and 93 hours after treatment application with percentage reduction over control by 17%, 20%, 39% and 47% for 21 hours, 33 hours, 57 hours and 93 hours after treatment application respectively. Results from previous studies suggested that 75% of larval population of flea beetle was reduced by application of cow urine (Subedi and Vaidya, 2003). Foliar spray of cattle urine 1:7 ratio of water is beneficial for controlling hairy caterpillar and larval insects (Poudel, 2008). Solution prepared from cow urine and water in ratio of 1:5 as foliar spray was beneficial for controlling aphids, larva and mites in cucurbits vegetables (GC and Neupane, 2009).

LARVAL MORTALITY WITH MICROBIALS (ENTOMO-PATHOGENS).

The larval mortality of *P. xylostella* (L.) larvae was found to be with mixed result when compared to control and was not found to be significantly at par. In the initial stage of the experiment lipel; bacteria-based insecticide causes higher mortality than that of Racer a fungus- based insecticide. But in long duration i.e. 57 hours and 93 hours after treatment application in experiment the microbial pesticide cause significantly higher larval mortality compared to control. The percentage controls of racer and lipel over control were 26% & 17% and 33% & 19% for 57 hours and 93 hours of treatment respectively.

The activity of the fungus was effective at ideal temperature of 25°C and relative humidity of 80% (G.C. and Keller, 2013). The different strains of *Beauveria bassiana* can cause mortality of the 3rd instar larvae of *Plutella xylostella* ranging from 41-64% in laboratory condition (Agrawal, Simon and Tayde, 2017). Sood, Mehta, Kashyap and Lal (2001) studied bio-efficacy of *B. bassiana* (Bals.) under laboratory condition and reported that larval mortality of diamondback moth (DBM) varied from 6.7% to 86.7%. Application of *Bt.* at sub-lethal doses deter the pupal weight, pupal duration and adult emergence of DBM and was practically significant in pest control in Taiwan; and was found to be more effective than conventional chemical insecticides for controlling DBM on cruciferous vegetables in winter (Hou and Chou, 1993).Highly susceptible strain of *Plutella xylostella* to *Bacillus thuringiensis* lost its susceptibility after 10 insecticide sprays in two generations in the field with a 36-fold increase in its LC50 (Branco and Gatehouse, 2001).

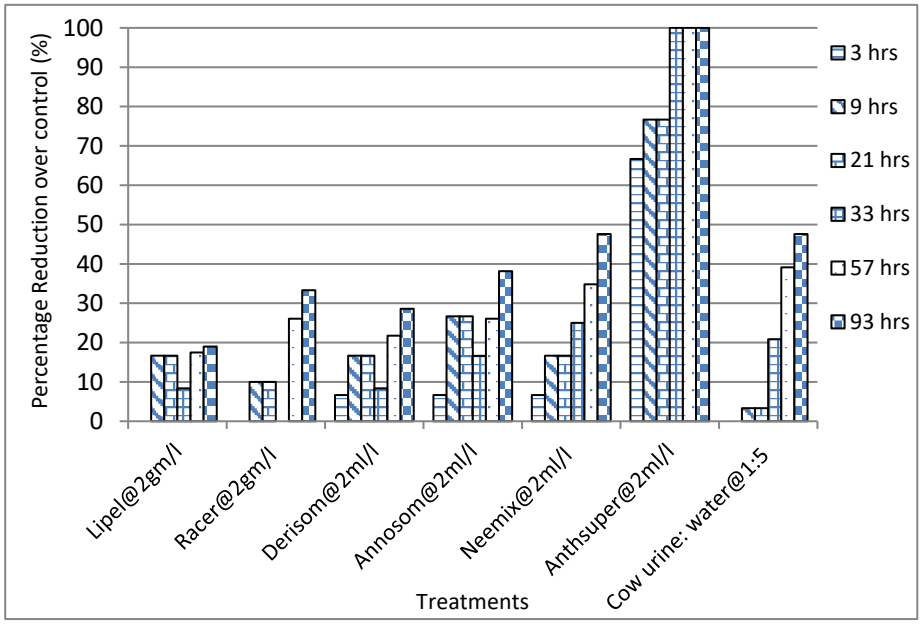

Fig 1: Percentage Population Reduction over Control (PROC) of different treatments at different time intervals

CONCLUSION

As the mortality of DBM larvae on bioassay was found to be significantly higher in Anthsuper than those of other compared treatments; Anthsuper (Chloropyrifos 16% A.I. + Alphacypermethrin 1% EC) can be recommended to apply for immediate control of the pest when they are above the economic threshold level. Considering the significantly higher mortality rate of cow urine, neemix, anosom and derisom followed by racer and lipel when compared to control can be applied in the field when the insect appears in the field because cow urine, botanicals and microbials are safer to the natural enemies when compared to anthsuper; which kills natural enemy upto 100% and also the pest DBM has been found to develop resistance to Anthsuper (Chloropyrifos 16% A.I. + Alphacypermethrin 1%EC). The use of cow urine, botanicals and microbials are found to be environmentally friendly and was found to control the pest significantly in longer duration also. Similarly, the use of cow urine, botanicals and microbial are encouraged to control/ manage the pest in Integrated Pest Management (IPM).

ACKNOWLEDGEMENT

I would like to acknowledge Nepal Public Health Foundation (NPHF)/Dialogos Team, Nepal for providing fund for research and faculties and staff of Department of Entomology/Agriculture and Forestry University (AFU) for technical and administrative support during my research period.

REFERENCES

Abraham, E.V., and Padmanabhan, M.D., 1968. Bionomics and control of the Diamondback Moth, *Plutella xylostella* (L.).*Indian Journal of Agricultural Science,* 20, 513-519.

Agrawal, S., Simon, S., and Tayde, A.R., 2017. Survey of *Beauveria bassiana* from cadaver of *Indarbela quadrinotata* and its virulence against the *Plutella xylostella* (Diamond back moth) in various places of Allahabad and Koushambi District. Journal of Entomology and Zoology Studies, 5(6): 797-799

Ankersmit, G.W., 1953. DDT-Resistance in *Plutella maculipennis* (Curt.)(Lepidoptera) in Java.*Bulletin of Entomological Research*, *44*(3), 421-425.

Boopathi, T., Pathak, K.A., Ngachan, S.V., and Das, N., 2010. Evaluation of insecticides and biopesticides against Diamondback moth, *Plutella xylostella* (L.) on broccoli.*Pest Management in Horticultural Ecosystems*, *16*(1), 69-72.

Branco, M.C., and Gatehouse, A.G., 2001. A Survey of Insecticide Susceptibility in *Plutellaxylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil.*Neotropical Entomology*, *30*(2), 327-332.

Cardleron, J.J., and Hare, C.J., 1986. Control of Diamondback Moth in South East Asia by profenofos. In *Diamondback Moth Management: Proceedings of the First International Workshop* (pp. 347-357). Shanhua, Taiwan: Asian Vegetable Research and Development Center.

Filomeno, C.A., Barbosaa, L.C.A., Teixeies, R.R., Pinherio, A.L., de Sa Farias, E., de Paula Silva, E.M., and Picanco, M.C., 2017. *Corymbia* spp. and *Eucalyptus* spp. essential oils have insecticidal activity against *Plutella xylostella*. Industrial Crops & Products. 109, 374-383.http://dx.doi.org/10.1016/j.indcrop. 2017.08.033

GC., Y. D., and Neupane, A., 2009. Use of botanical and biopesticides for insect pests and disease control. World Vision International, Lamjung, Nepal.

GC, Y. D. and Keller, S., 2013. *Crop Pests of Nepal and their Management*. Kathmandu, Nepal.

Gomez, K. A. and A.A. Gomez., 1984. *Statistical procedures for agriculture research*.2nd ed. John Wiley and Sons, Inc., New York. 680.

Gong, W., Yan, H.H., Gao, L., Guo, Y.Y., and Xue, C.B., 2014. Chlorantraniliprole Resistance in the Diamondback Moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 107, 808-814.

Hou, R.F., and Chou, Tao-mei., 1993. Applications of *Bacillus thuringiensis* preparation against the Diamondback Moth, *Plutella xylostella* (L.), in Taiwan. 167-178.

Hu, M.Y., Zhong, G.H., Sun, Zh. T., Sh, G., Liu, H.M., and Liu, X.Q., 2005. Insecticidal activities of secondary metabolites of endophytic *Pencillium* sp. in *Derris elliptica* Benth. *Journal of Applied Entomology, 129*(8), 413-417.

Leatemia, J.A, and Isman, M.B., 2001. Crude seed extract of *Annona squamosa* (Annonaceae) as a potential botanical insecticide. (Available at: https://esa.confex. com/ esa/2001/ techprogram/paper_3973.htm Retrived on: May 14: 2015).

Leatemia, J.A., and Isman, M.B. (2004). Efficacy of crude seed extracts of *Annona squamosa* against diamondback moth, *Plutella xylostella* L. in the greenhouse. *International Journal of Pest Management*, *50*(2), 129-133.

Leibee, G.L., and Savage, K.E., 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. *The Florida Entomologist*, *75*(4), 585-591.

NARC., 2016. *Annual report*. Khumaltar, Nepal: Entomology Division, Nepal Agriculture Research Council.

Poudel, M.S., 2008. Local technologies used in Plant Protection. Unpublished article.

PPD., 2018. Annual Report.Plant Protection Directorate (PPD), Department of agriculture, Hariharbhawan, Lalitpur, Nepal. 151.

Prakash, A., and Rao, P., 1997. *Applied Entomology*.Wiley Eastern Limited, New Delhi, India. 298.

Ridland, P.M., & Endersby, N.M., 2011. Some Australian populations of diamondback moth, *Plutella xylostella* (L.) show reduced susceptibility to fipronil. In: *Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests* (pp. 21-25). Shanhua, Taiwan: Asian Vegetable Research and Development Center.

RPPL., 2003. Annual report. Regional Plant Protection Laboratory, Hariharbhawan, Nepal. 59.

Singh, K.I., and Singh, N.J., 2009. Environment and ecology. *Journal of Environment Ecology, 27*(2A): 752-756.

Sood, A.K., Mehta, P.K, Kashyap, N.P., and Lal, R., 2001. Bioefficacy of *Beauveria bassiana* against diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *Journal of Entomological Resistance,25*(4), 267-271.

Subedi, I.P., and Vaidya, K., 2003. Control of flea beetle, *Phyllotreta nemorum* (L.) (Coleoptera: Chrysomelidae) using locally available natural resources. *Himalayan Journal of Science, 1*(2), 111-114.

Tabashnik, B.E., and Cushing, N.L., 1987. Leaf residue Vs Tropical bioassay for assessing insecticide resistance in the Diamondback Moth *Plutella xylostella* (L.) *DAO Plant Protection Bulletin*, 35, 11-14.

Trindade, R.C.P., Luna, J. de Souza., De Lima, M.R.F., Da Silva, P.P., and Sant'ana, A.E.G., 2011. Larvicidal activity and seasonal variation of *Annona muricata* (Annonaceae) extract on *Plutella xylostella* (Lepidoptera: Plutellidae). *Review Colombia Entomology, 37*(2): 223-227.

VDD., 2016. Vegetable Development Directorate. Khumaltar, Lalitpur.

Verkerk, R.H.J., and Wright, D.J., 1993. Biological activity of neem seed kernel extracts and synthetic azadirachtin against larvae of *Plutella xylostella* L. *Pesticide Science, 37*(1), 83-91.

Zalucki, M.P., Shabbir, A., Silva, R., Adamson, D., Sheng, L.S., and Furlong, M.J., 2012. Estimating the Economic Cost of One of the World's Major Insect Pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just How Long Is a Piece of String? *Entomological Society of America, 105(4),* 1115-1129

Zhang, L.J., Jing, Y.P., Li, X.H., Li, C.W., Bourguet, D., and Wu, G., 2015. Temperature-sensitive fitness cost of insecticide resistance in Chinese populations of the diamondback moth *Plutella xylostella*. *Molecular Ecology*, *24*(7), 1611-1627