Toxic effect of neem, *Azadirachta indica* (A. Juss) foliage extracts against diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera, Plutellidae)

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ABSTRACT

Present study was taken up to evaluate the toxic effect of methanol and hexane extracts of neem leaf against diamondback moth (DBM), *Plutella xylostella.* The toxic effect of the two extracts was evaluated at six different concentrations, viz. 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. Mortality of larvae was significantly higher at even the lowest concentration of 0.5% (61.67%) as compared to control (13.33%). Complete larval mortality with neem methanol extract (NME) was recorded at 3% concentration. Mortality during early larval stages was significantly higher as compared to control at all the NME concentrations, and more than 50% larvae died during first two larval stages at 3%, 2.5% and 2% NME concentrations. The mortality was mainly due to the failure to moult successfully into next stage. 52.8% mortality was observed during intermoult. At 0.5% NME, 63.64% mortality was recorded during pupal-adult moult. Hexane extract from neem leaves (NHE) had a significant lethal effect at 1.5% concentration and above as compared to control. Maximum mortality recorded with this extract was 51.67% at 3% concentration. At 2.5% and 3% NHE concentrations significant mortality was recorded during early larval stages. The estimated LC_{50} calculated for total mortality for NME and NHE extracts were 0.61% and 3.95% respectively. The results suggest that extract from neem leaves may potentially be used for the management of *P. xylostella.*

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Key words: Diamondback moth, *Plutella xylostella,* neem leaf extract, survival, toxicity.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidopetra: Plutellidae) is a serious pest and a major constraint in the production of cruciferous crops throughout the world. This pest has been reported to cause more than 90% crop loss in the area of their outbreaks (Verkerk and Wright, 1996). The economic loss due to this pest has been estimated worldwide to be US$4-US$5 billion (Zalucki *et al.,* 2012) and $16 million annually in India (Mohan and Gujar, 2003). In addition, US $1.0 billion is spent annually for the management of this pest globally (Talekar and Shelton, 1993). DBM is the first insect to develop resistance against DDT (Johnson, 1953). The genetic elasticity of DBM has enabled this pest to develop resistance against almost all insecticide (Mota-Sanchez *et al.*, 2002), and consequently its management with insecticide is gradually becoming difficult (Shelton *et al.*, 1993).The potential alternative for the sustainable management of this insect may be natural plant products, which have been successfully used for centuries (Crosby, 1971). The crude plant extract consists of complex mixtures of active compounds. The complex mixtures act synergistically (Berenbaum, 1985), and show greater overall bioactivity compared to the individual components (Berenbaum *et al.,* 1991; Chen *et al.,* 1995). Also, there is less likelihood for insect to develop resistance against such mixtures (Feng and Isman, 1995; Shukla and Toke, 2013). Product of neem tree, *Azadirachta indica* (A. Juss) (Meliaceae), has been reported to contain a plethora of chemical compounds. Extracts from the seeds and kernels have been reported to adversely affect biology of many pests (Verkerk and Wright, 1993; Schmutterer, 1997; Das *et al.,* 2010; Naveena *et al.,* 2010; Wondafrash *et al.,* 2012). The most active compound in neem is 'azadirachtin (AZA)' which has been reported to produce varied effects, including insecticidal activity, oviposition deterrent, antifeedant, growth retardant, moulting inhibitor,

sterilant etc. (Prakash and Rao, 1997). Several studies have demonstrated the efficacy of neem seed and kernel extracts against DBM (Dreyer, 1987; Schmutterer, 1992; Shin-Foon and Yu-Tong, 1993; Verkerk and Wright, 1993; Javaid *et al.,* 2000; Liang *et al.,* 2003). However, there are only a few reports about the effect of neem leaf extracts on DBM. Keeping these points in mind the present work was initiated to evaluate the effect of polar and non-polar extracts of neem leaves on development and survival of *P. xylostella.*

MATERIALS AND METHODS

Plant extracts preparation: Freshly excised young neem leaves were collected from the Delhi University campus. The leaves were washed thoroughly and spread on a blotting paper to drain out excess water. Two different types of extracts were prepared from neem leaves, *viz.,* neem methanol extract (NME) and neem hexane extract (NHE). Leaves were weighed and transferred in large glass jar and filled with methanol/hexane. Solvents were decanted after 24 h and the leaves were rinsed three times with solvent, and decanted in the jar so as to extract maximum of compounds from the leaves. The pooled extract was then filtered using a Watmann filter paper No.1. The extracts were then concentrated in rotary evaporator at 40°C under reduced pressure to near dryness. The extracts were refrigerated until use. A 5% stock solution was prepared by dissolving 0.5 g of extract in 10 ml of control solution (methanol control: prepared by mixing 94.5% water, 5% methanol and 0.5% Triton X, as emulsifier and hexane control: prepared by mixing 97.5% water, 2% hexane and 0.5% Triton X, as emulsifier). Lower extract concentrations, 3%, 2.5%, 2%, 1.5%, 1%, and 0.5% were prepared by serial dilution using control solution.

Insects rearing: *Plutella xylostella* larvae were reared on freshly excised cauliflower leaves which has been reported most suitable for its growth and development (Charleston and Kfir, 2000; Golizadeh *et al.,* 2007). Seeds of the host plant, i.e. cauliflower (var. Poosi spl.) were obtained from National Seed Bank, IARI, New Delhi. Cauliflower plants were grown in the field plots of Zoology Department, Delhi University, under pesticide free conditions, following standard farm practices. Newly emerged adults were provided with a mixture of 10% sucrose solution and vitamins and kept in a plexiglass cage for the purpose of mating and oviposition. Insect rearing as well as all experimental bioassays were maintained under controlled laboratory conditions (Temp. 26±2 ^{0}C; 65±5% Relative Humidity and photoperiod regime of 14D:10L)

Toxicity bioassay: Freshly excised cauliflower (*Brassica oleraceae* var. *botrytis*) leaves of approximately same age were taken to study the toxic effect of extracts. Leaf surfaces were applied with a particular concentration of extract on both sides using Hamilton syringe at the rate 2µl/cm^2, and air dried. Control leaves were similarly applied with same quantity of control solution. 10 neonate larvae were released on the treated and control leaf surface that were subsequently placed in individual plastic boxes (10 cm dia x 3 cm height), having bottom lined with moist filter paper. Larvae were transferred every alternate day on freshly excised cauliflower leaf, applied with extract, which in turn was placed in clean box. This was done for 6 days, and thereafter, larvae were reared on non treated leaf till pupation. Since first instar larvae are leaf miners and remain inside the leaves for approximately two days, the first observation was recorded on completion of the second day. Thereafter, observations for their mortality, moulting, pupation, and adult emergence were recorded daily at a fixed time of day. The experiment was replicated six times, each replicate consisting of response of 10 larvae.

Statistical analysis: Significant differences between the mean percentage mortalities at different extract concentrations and between different extracts were analyzed by one way analysis of variance (ANOVA) and means were separated using Tukey's test (Sigma Stat). Dose response mortality data were analyzed using linear regression analysis to calculate LC_{50} values (Zar, 1999). Expected probits were obtained from the best fit in the regression analysis and chi-squared values for both the applications calculated thereafter. Abbott's (1925) formula was used to correct for control mortality.

RESULTS AND DISCUSSION

The larvae fed on cauliflower leaf, smeared with 3% Neem Methanol Extract (NME), caused mortality (86.67%) in the early larval stages (ELM-

1st & 2nd), which was significantly higher as compared to control, and all lower extract conc., except 2.5% (df = 6, 41; F= 16.19; P< 0.05). Significant difference in ELM was also recorded between all tested conc. of NME and control (df = 6, 41; F= 16.19; P< 0.05). Moreover, 1st instar larvae did not show their normal mining behaviour, but exhibited wandering activity at the periphery of the rearing boxes. The mortality of DBM larvae was also significantly higher as compared to control in late larval (LLM- 3rd & 4th) and pupal stages that were fed on untreated leaves after initially feeding on treated leaves during 1st and 2nd larval stages. This clearly indicates carry over of NME toxicity beyond feeding period. Such toxic effect beyond feeding period has also been observed for hexane and ethanolic extracts of *Muntingia calabura* fruit against DBM larvae (Bandeira *et al.*, 2013).

Total mortalities of DBM larvae fed on leaves, smeared with 2.5%, 2% and 1.5% NME were significantly higher as compared to control (Fig. 1) (df = 6, 41; F= 49.11; P< 0.05).

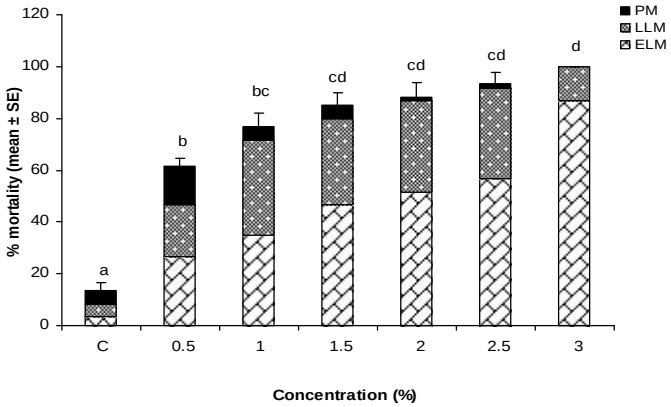

Fig. 1. Percentage mortality (mean ± SE) of *P. xylostella* fed on cauliflower leaves treated with NME extract. Bars capped by different letters are statistically significant (P<0.05). ELM, early larval mortality; LLM, late larval mortality; PM, pupal mortality.

The larvae that were fed on leaf treated with 3% NME failed to reach pupal stage, and died before pupation. Such mortality has also been observed when DBM larvae were fed leaves treated with methanolic extract of *Melia azedarach* (Chen *et al.*, 1996), ethanolic extract from the twigs of *M. azedarach* (Rani *et al.*, 1999) or crude ethanolic leaf extract from *Annona muricata* (Trindade *et al.*, 2011). Toxicity of polar neem foliage extract has also been observed against *Spodoptera frugiperda* (Viana and Prates, 2003), *Spodoptera exigua* (Hubner) (Greenberg *et al.*, 2005), *Rastrococcus invadens* (Ande and Olowojolu, 1999) and *Dysdercus cingulatus* (Sharma *et al.*, 2010). Martinez and van Emden (2001) recorded mortality in *Spodoptera littoralis* larvae that were fed diet, mixed with azadirachtin.

The toxicity of methanol extract of neem leaves against DBM in the present study is comparable to the toxicity observed for neem seed extract and neem oil (Ahmad, 1999, Parera *et al.*, 2000, Sow and Diarra, 2013). Three neem based formulations, i.e. Agroneem™, Ecozin™ and Neemix™ have been reported to cause mortality of DBM larvae (Liang *et al.*, 2003). Verkerk and Wright (1993) observed complete mortality of DBM larvae, fed on Chinese cabbage leaf disc treated with azdirachtin (AZ) and formulated neem product (NEEM-AZAL).

The anterior portion of larvae that died due to consumption of extract mixed diet was stretched as compared to control food (Plate 1).

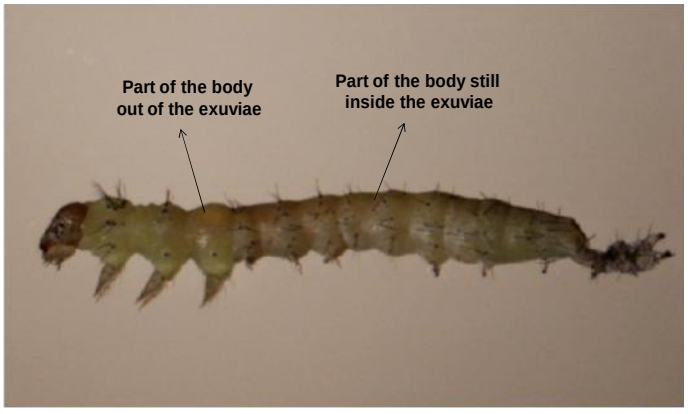

Plate 1. . *P. xylostella* larvae dead during L3-L4 moult.

It appears that larvae failed to come out of the old exuviae, and died after remaining in this condition for several days. Moult related deformities due to application of neem seed extract has also been reported in *Trichoplusia ni* and *Spodoptera exigua* (Prabhakar *et al.*, 1986) and *Oncopeltus fasciatus* (Aerts and Mordue (Luntz), 1997). The mortality of

larvae due to feeding on NME smeared leaf surface was significantly higher during moulting into next stage. It appears that NME disturbs neuroendocrine system of larvae that is responsible for moulting and metamorphosis. Mortality of DBM larvae during moulting due neem formulations has been observed earlier (Schmutterer, 1990; Isman, 1995). Azadirachtin, active neem constituent, has been reported to interfere with ecdysis of insects (Sieber and Rembold 1983; Singh and Bhathal, 1994). Such moulting disruption due to neem constituents has been observed in *Spodoptera frugiperda*, *Pectinophora gossypiella*, *Heliothis virescens* and *H. zea* (Kubo and Klocke, 1982), *Spodoptera exempta* (Tanzubil and McCaffery, 1990), *Spodoptera littoralis* (Martinez and van Emden, 2001).

The mortality of larvae fed on leaf, smeared with 3% neem hexane extract (NHE) was significantly higher as compared to mortality recorded at all the other tested concentrations of extract and control (Fig. 2) (df = 6, 41; F=23.06; P < 0.05).

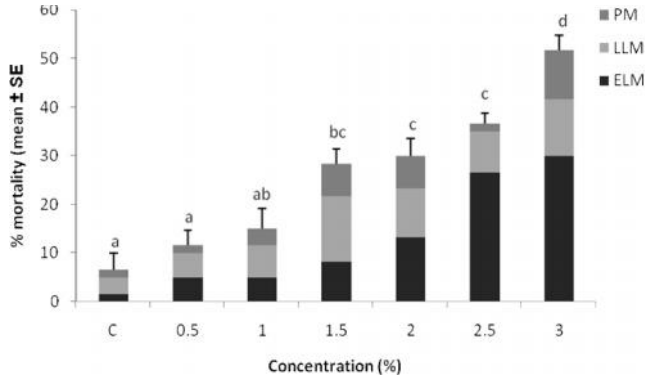

Fig. 2. Percentage mortality (mean ± SE) of *P. xylostella* fed on cauliflower leaves treated with NHE extract. Bars represented by different letters are significantly (P<0.05) different. ELM, early larval mortality; LLM, late larval mortality; PM, pupal mortality.

However, difference in mortality between 1.5%, 2% and 2.5% conc. of NHE was not significant, but statistically higher as compared to control (df = 6, 41; F=23.06; P < 0.05). Mortality of larvae at 3% and 2.5% during early stages were 30% and 26.67% respectively, which were statistically similar, but significantly higher than other lower tested conc. of extract and control (df= 6, 41; F= 19.79, P < 0.05).

Also, mortality between different tested conc. of NHE and control were significantly not different during late larval stages (df= 6, 41; F= 1.58; P>0.05) and pupal period (df = 6, 41; F= 1.82, P>0.05). Neem hexane extract appears to have lower toxicity as compared to methanol extract, as mortality recorded at the highest concentration of hexane extract (3%) is lower than mortality at lowest concentration of methanolic extract (0.5%) (Fig.3). This is further evident by the observation that the mortality of larvae at each individual conc. of extract, is higher in polar than in non-polar extract (Fig. 3).

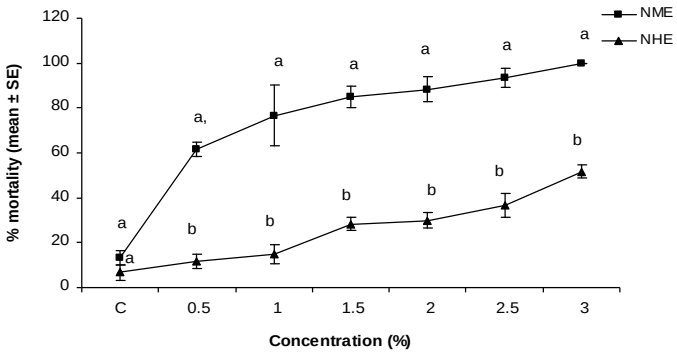

Fig. 3. Mortality of DBM larvae with methanol and hexane extracts of neem foliage. Values denoted with different letters at same conc. are significantly different.

This contention is further supported by the estimated LC_{50} value for total mortality of larvae, which is 0.61% for NME and 3.95% for NHE (Table 1). The 95% Confidence Interval (CI) for LC_{50} values of both the extracts do not overlap

Table 1. Lethal concentration (LC_{50}) of methanol and hexane extracts of neem leaves on *P. xylostella* following feeding on treated cauliflower leaves for 6 days.

Extract	Slope ± S.E.	LC_{50} (%; 95% CI)	X^2-value	P-value
NME	3.382 ±1.359	0.61 (0.43-0.86)	0.405	0.982
NHE	1.932 ± 0.253	3.95 (2.15-7.27)	0.02	1.000

which further indicated that NME was more toxic than NHE. The results of the present study clearly indicate the toxicity of neem leaf extract against DBM larvae, the polar being more effective than non-polar. There are reports about toxic effect of different components of neem against DBM larvae, but no such report is available for neem foliage extract. The present study indicates the potentialities of neem leaves for the management of *P. xylostella.* The neem plants are locally available in India, having plenty of leaves and as such preparing polar extract of leaves will be economical and sustainable. However, further research is required to isolate the active components of foliage extract for their effective use in the IPM of this insect pest.

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REFERENCES

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology,* **18**: 265–267.

Aerts, R. J. and Mordue (Luntz), J. 1997. Feeding deterrence and toxicity of neem triterpenoids. *Journal of Chemical Ecology,* **23**: 2117-2132.

Ahmad, I. 1999. Dosage mortality studies with *Bacillus thuringiensis* and neem extract on diamondback moth, *Plutella xylostella* (L.) (Lepidoptera:Plutellidae). *Indonesian Journal of Plant Protection,* **5** (2): 67-71.

Ande, A. T. and Olowojolu, B. I. 1999. Biocidal efficacy of the extracts from various neem parts against the mealy bugs, *Rastrococcus invadens*. *Bioscience Research Communication,* **11**(1): 41-51.

Bandeira, G. N., da Camara, C. A. G., de Moraes, M. M., Barros, R., Muhammad, S. and Akhtar, Y. 2013. Insecticidal activity of *Muntingia calabura* extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae). *Journal of King Saud University-Science,* **25**: 83-89.

Berenbaum, M. 1985. Brementown revisted: interactions among allelochemicals in plants. *Rec. Adv. Phytochemicals,* **19**: 39-169.

Berenbaum, M. R., Niato, J. K. and Zangerl, A. R. 1991. Adaptive significance of furanocoumarin diversity in *Pastinaca sativa*. *Journal of Chemical Ecology,* **17**: 207-215.

Charleston, D. S. and Kfir, R. 2000. The possibility of using Indian mustard, *Brassica juncea*, as a trap crop for diamondback moth, *Plutella xylostella*, in South Africa. *Crop Protection,* **19**: 455-460.

Chen, C. C., Chang, S., Chen, L. L. and Hou, R. F. 1996. Effects of chinaberry fruit extract on feeding, growth and fecundity of the diamondback moth, *Plutella xylostella* L. (Lep., Yponomeutidae). *Journal of Applied Entomology,* **120**: 341-345.

Chen, W., Isman, M. B. and Chiu, S. F. 1995. Antifeedant and growth inhibitory effects of the limonoid toosendanin and *Melia toosendan* extracts on the variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae). *Journal of Applied Entomology,* **119**: 367-370.

Crosby, D. G. 1971. Minor Insecticides of Plant Origin. In: Naturally Occurring, Insecticide (Jacobson, M. and Crosby, D.G eds.), Marcel Dekker Inc., New York, **PP,** 17-239.

Das, R., Chutia, B. C., Sarmah, M. and Rahman, A. 2010. Effect of neem kernel aqueous extract (NKAE) on growth and development of red slug caterpillar, *Eterusia magnifica* butl in tea in North- East India, India. *Journal of Biopesticide,* **3**(2): 489-494.

Dreyer, M. 1987. Field and laboratory trials with simple neem products as protectants against pest of vegetables and field crops in Togo. In: *Natural pesticides from the neem tree (Azadirachta indica* A. Juss*.) Proceedings of the third International neem conference.* (Schmutterer, H. and Acher, K.R.S. eds.). GTZ, Eschborn, FRG, 431-447 **PP**.

Feng, R. and Isman, M. B. 1995. Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experimentia,* **51**: 831-833.

Golizadeh, A., Kamali, K., Fathipour, Y. and Abbasipour, H. 2007. Temperature-dependent development of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on two brassicaceous host plants. *Insect Science,* **14**: 309-316.

Greenberg, S. M., Showler, A. T. and Liu, T-X. 2005. Effect of neem based insecticides on beet armyworm (Lepidoptera: Noctuidae). *Insect Science*, **12**(1): 17-23.

Isman, M. B. 1995. Leads and prospects for the development of new botanical insecticides. *Reviews in Pesticide Toxicology*, **3**: 1-20.

Javaid, I., Saifudine, N., Tombolane, L. and Rafael, E. 2000. Efficacy of aqueous neem extracts in the control of diamondback moth, *Plutella xylostella* (L.) on cabbage. *Insect Science and its Application*, **20**: 167-170.

Johnson, D. R. 1953. *Plutella maculipennis* resistance to DDT in Java. *Journal of Economic Entomology*, **46**: 176.

Kubo, I. and Klocke, J. A., 1982. Azadirachtin, insect ecdysis inhibitor. *Agricultural and Biological Chemistry*, **46** (7): 1951-1953.

Liang, G. M., Chen, W. and Liu, T. X. 2003. Effects of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae). *Crop Protection*, **22**: 333-340.

Martinez, S. S. and Van Emden, H. F. 2001. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* caused by azadirachtin. *Neotropical Entomology*, **30**: 113-125.

Mohan, M. and Gujar, G. T. 2003. Local variation in susceptibility of the diamondback moth *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. *Crop Protection.*, **22**: 495-504.

Mota-Sanchez, D., Bills, P. S. and Whalon, M. E. 2002. Arthropod resistance to pesticides in agriculture and the environment (Wheller, W.B. ed.), Dekker Incorporation, **PP,** 241-272.

Naveena, N. L., Jagadeesh Babu, C. S. and Byregowda, M. 2010. Evaluation of biopesticide against field infestation of bruchid, *Callosobruchus theobromi* L. on fieldbean, *Dolichos lablab. Journal of Biopesticide*, **3**(3): 622-623.

Perera, D. R., Armstrong, G. and Senanayake, N. 2000. Effect of antifeedants on the diamondback moth (*Plutella xylostella)* nad its parasitoid *Cotesia plutellae. Pest Management Science*, **56:** 486-490.

Prabhakar, N., Cougriet, D. L., Kishaba, A. N. and Meyerdirk, D. E. 1986. Laboratory evaluation of neem seed extract against larvae of the cabbage looper and beat armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **79**: 39-41.

Prakash, A. and Rao, J. 1997. Botanical pesticides in agriculture. CRC Lewis Publisher. Boca Raton. USA. 481 **PP**.

Rani, M., Suhag, P., Kumar, R., Singh, R. and Kalidhar, S. B. 1999. Chemical components and biological efficacy of *Melia azedarach* stems. *Journal of Medical and Aromatic Plant Science*, **21**: 1043–1047.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica. Annual Review Entomology*, **35**: 271-297.

Schmutterer, H. 1992. Control of diamondback moth by application of neem extracts. In: *Diamondback moth and other crucifer pests* (Talekar, N.S. ed.). Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990, Asian Vegetable Research and Development Center, Shanhua, Taiwan, AVRDC Publication No. 92-368, **PP,** 325-332.

Schmutterer, V. H. 1997. Side-effects of neem (*Azadirachta indica*) products on insect pathogens and natural enemies of spider mites and insects. *Journal of Applied Entomology*, **121**: 121-128.

Sharma, T., Qamar, A. and Khan, A. M. 2010. Evaluation of neem (*Azadirachta indica*) against the eggs and adults of *Dysdercus cingulatus* (Fabricius). *World Applied Science Journal*, **9**(4): 398-402.

Shelton, A. M., Wyman, J. A., Cushing, N. L., Apfelbeck, K., Denney, T. J., Mahr, S. E. R. and Eigenbrode, S. D. 1993. Insecticide resistance of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae), in North America. *Journal of Economic Entomology*, **86**: 11-19.

Shin-Foon, C. and Yu-Tong, Q. 1993. Experiments on the application of botanical insecticides for the control of diamondback moth in south china. *Journal of Applied Entomology*, **116**: 479-486.

Shukla, A. and Toke, N. R. 2013. Plant products as a potential stored product insect management agents. *Indian Journal of Research*, **2**(2): 4-6.

Sieber, K. P. and Rembold, H. 1983. The effect of azadirachtin on the endocrine control of moulting in *Locusta migratoria. Journal of Insect Physiology,* **29**: 523-527.

Singh, D. and Bhathal, S. S. 1994. Role of insect growth regulators in integrated pest management. *Journal of Insect Science,* **7**: 1-9.

Sow, G. and Diarra, K. 2013. Laboratory evaluation of toxicity of *Bacillus thuringiensis*, neem oil and methamidophos against *Plutella xylostella* L. (Lepidoptera: Plutellidae) larvae. *International Journal of Biological and Chemical Sciences,* **7**(4): 1524-1533.

Talekar, B. E. and Shelton, A. M. 1993. Biology, ecology and management of diamondback moth. *Annual Review of Entomology,* **38**:275-301.

Tanzubil, P. B. and Mc Caffery, A. R. 1990. Effect of azadirachtin and aqueous neem seed extracts on survival, growth and development of the African armyworm, *Spodoptera exempta. Crop Protection,* **9**: 383-386.

Trindade, R. C. P., Luna, J. DE-S, De-lima, 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 of Colombo Entomology,* **37** (2): 0120–0488.

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**: 83-91.

Verkerk, R. H. J. and Wright, D. J. 1996. Multitrophic interactions and management of the diamondback moth: a review. *Bulletin of Entomological Research,* **86**: 205-216.

Viana, P. A. and Prates, H. T. 2003. Larval development and mortality of *Spodoptera frugiperda* fed on corn leaves treated with aqueous extract from *Azadirachta indica* leaves. *Bragantia,* **62**(1): 69-74.

Wondafrash, M., Getu, E. and Terefe, G. 2012. Neem, *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African Bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Academic Journal of Entomology,* **5**(1): 22-27.

Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L. and Furlong, M. J. 2012. Estimating the economic cost of one of the world's major insects pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string? *Journal of Economic Entomology,* **105**(4): 1115-1129.

Zar, J. H. 1999. Simple linear regression. In: *Biostatistical analysis.* Pearson Education, Inc., Singapore, **PP,** 324–359.

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