Efficacy of botanicals alone and in combination with insecticide against *Trilocha varians* (Lepidoptera: Bombycidae) in controlled conditions

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Abstract

Ficus benjamina (Rosales: Moraceae), commonly known as weeping fig, is not only planted alongside the road as decorative plant in Pakistan but also has chemicals which used to treat cancer and ulcer. A leaf eating caterpillar, *Trilocha varians* (Lepidoptera: Bombycidae) is a serious pest of *Ficus* spp. especially *Ficus benjamina*. This is a new emerging pest of horticultural and agricultural crops all over the world. The pest is causing 100% defoliation and even death of plant. The severe attack of this pest has seen on *F. bejamina*. The current pest is invading to other ornamental plants, especially jackfruit

(*Artocarpus heterophyllus*) in the world and becoming risk for other crops. There is need to minimize the pest population, but still no management strategies have adopted to control this pest in the world. The current experimental study was conducted to check the efficacy of botanicals alone and in combine application with insecticides against 2nd instar larvae of leaf eating caterpillar, *T. varians* under laboratory conditions. Results showed that among botanicals, *Azadirachta indica* was showed more toxicity as compared to *Eucalyptus globulus*. *A. indica* was showed the highest mortality (6.40%) of larvae at 72 hours of post treatment, while minimum mortality (0.40%) at 24 hours. The combine application of *A. indica* and Emamectin benzoate was given maximum mortality (9.20±9.20) at 72 hours and minimum (1.80±3.20) at 24 hours. The mixture of emamectin benzoate and *E. globulus* was also found effective and toxic against second instar larvae but less than combine application of *A. indica* and Emamectin benzoate. In response to this new pest issue in the ornamental plants, control strategies including biological control alone and in combine with others chemicals are being developed in Pakistan.

Keywords: *Ficus benjamina;* Moraceae; *Trilocha varians*;

Bomycid moth; Insecticides; Toxicity

Introduction

Ficus benjamina commonly known as weeping fig is an ornamental plant planted alongside the road and landscape purposes. The beauty of the country enhances with the growth and development of these 60 ft tall trees. There is great diversity of fig species such as *F. tinctoria, F. religiosa, F. vasculosa, F. benghalensis, F. depressa, F. elastica, F. infectoria. F. deltoidei, F. macrocarpa, F. carica, F. rumphii, F. lyrata, F. hispida, F. racemose, F. annulate, F. palmata, F. macrophylla,* and *F. virens* (Kumar et al., 2021). Not only these plants increase the aesthetic value of the country but also use as medicine to treat the various diseases such as cancer and ulcer. Several compounds such as ascorbic acid, alkaloids, flavonoids and triterpenoids are found in *F. benjamina* that show anticancer, antimicrobial and antidiabetic characters (Sirisha et al., 2010; Mousa et al., 1994; Lansky et al., 2008; Kim et al., 2008).

Several insect pests (whitefly, thrips, mealy bugs, Ficus leaf rolling Psyllid and leaf eating caterpillar) attack on reproductive and vegetative organs of *F. benjamina* (Ramzan et al., 2019a; Arya, 2020). Among insect pests, leaf eating caterpillar, *Trilocha varians* is a serious pest of *F. benjamina* in the globe especially Pakistani areas (Ramzan et al., 2019b; Ramzan et al., 2020). The larvae of the pest attack on new leaves of the host and then invade the whole plant. Larvae feed quickly and caused 100% defoliation even death of the plant occurred due to severe pest attack (Navasero et al., 2013; Ramzan et al., 2021). Several integrated pest management (IPM) strategies have been adopted by scientists and farmers at national and international level to reduce the pest population on various agricultural and horticultural crops throughout the world. The current pest is new and emerging now a day which becoming serious threat to economy of Pakistan (Ramzan et al., 2023). There is need to control this pest to protect the economy of the country. For this purpose, the current study is carried out to check the efficacy of botanicals alone and in combination with insecticide against this pest. The results of current study will help the farmers and researchers in future to control this pest.

Material and Methods

Insect rearing and culture maintenance

Rearing of test organism was carried out at Institute of Plant protection (IPP2)-MNS-University of Agriculture, Multan during February 2019-July 2019 on *F. benjamina* leaves under controlled environmental conditions (25 ± 2 ◦ C and 65% ± 5%

relative humidity, 16:10 h L:D). The culture of insect was maintained up to five generations. Rearing procedure of Naeem-Ullah et al., (2020) was followed to rear the current pest.

Treatments and procedure of applications

Two botanicals and one control were used and each with three concentrations (1, 2 and 3%) with three replications. There were seven treatments, each with five replications. Only one insecticide (emamectin benzoate) was used @150ul in the combination of each botanical treatment. The bioassay study was conducted by using CRD design.

T1: 1% Azadirachtin indica

T2: 2% Azadirachtin indica

T3: 3% Azadirachtin indica

T4:1% Eucalyptus globulus

T5: 2% Eucalyptus globulus

T6: 3% Eucalyptus globulus

T7: Control

Different concentrations of each botanical were prepared in distilled water and acetone also mixed to dissolve the botanical in water. Equal size *F. benjamina* leaves were dipped in prepared solution for 10 second, dried for an hour and after drying two leaves placed into petri dishes per replication. To perform this experiment, ten second instar larvae of *T. varians* were selected and put into petri dishes containing treated leaves to check their mortality against these botanicals.

Data collection and statistical analysis

Mortality data of each treatment were recorded after 24, 48 and 72 hours of post treatment. Collected data were analyzed statistically following ANOVA techniques by using Statistix-10 computer package.

Results

A. indica was showed the highest mortality (17%) of larvae at 72 hours of post treatment, while minimum mortality (4.40) at 24 hours. It was showed 17.60%, 23.40, and 33.60%, mortality after

24, 48 and 72 hours of post treatment, respectively **(Table 1)**.

Table 1. Toxicity *A. indica* on 2nd larval instar of *T. varians*

Botanical	Percentage (%age) mortality		
A. indica	24 h	48 h	72 h
	Mean±SE	Mean±SE	Mean±SE
1%	4.40±1.60a	9.00±4.00a	17.00±7.00a
2%	9.01±1.00b	15.80±3.80b	22.40±6.40b
3%	17.60±0.40ab	23.40±3.40ab	33.60±8.60ab
Control	0.00±0.00c	0.00±0.00c	0.00±0.00c

The similar numbers showed significant differences between means in a column.

The mixture of *A. indica* and Emamectin benzoate was found more toxic as compared to alone application. The mixture of *A. indica*+ Emamectin benzoate was given maximum mortality at 72 hours and minimum at 24 hours. The mortality rate increased with increased in time. 37.0, 54.80 and 61.90% mortality was recorded at 3% concentration of A. indica with insecticide after 24, 48 and 72 h of post treatment (**Table 2**).

Table 2: Toxicity of mixtures of Emamectin benzoate and *A. indica* on 2nd larval instar of *T. varians*

Mixture	Percentage (%age) mortality		
A. indica +EB	24 h	48 h	72 h
	Mean±SE	Mean±SE	Mean±SE
1% *A. indica*+EB	19.80±3.20a	25.60±5.60a	35.80±7.80a
2% *A. indica*+EB	27.00±3.00b	38.00±7.0b	47.20±9.20b
3% *A. indica*+EB	37.20±3.2ab	54.80±6.80ab	61.90±9.00ab
Control	0.20±0.20c	0.00±0.00c	0.20±0.20c

EB: Emamectin benzoate. The similar numbers showed significant differences between means in a column.

E. globulus showed 3.01, 7.16, and 15.01% mortalities at 1% concentration after 24, 48, and 72 hours of applications, respectively. The highest mortality (27.14%) of pest was recorded at the highest dose of plant extracts (*E. globulus*) after 72 h of application **(Table 3)**. The mixture of emamectin benzoate and *E. globulus* showed 15.60 and 56.80% mortality of larvae at 24 and 72 h of application, respectively **(Table 4).**

Table 3: Toxicity of *E. globulus* on 2nd larval instar of *T. varians*

Botanical	Percentage (%age) mortality		
E. globulus	24 h	48 h	72 h
	Mean±SE	Mean±SE	Mean±SE
1%	3.01±1.0a	7.16±2.6a	15.01±4.0a
2%	7.11±1.6a	13.22±3.2b	20.02±5.0b
3%	13.10±1.0ab	19.8±3.8ab	27.14±6.4ab
Control	0.0±0.0c	0.0±0.0c	0.0±0.0c

The similar numbers showed significant differences between means in a column.

Table 4: Toxicity of mixtures of Emamectin benzoate and *E. globulus* on 2nd larval instar of *T. varians*

Mixture	Percentage (%age) mortality		
E. globulus+EB	24 h	48 h	72 h
	Mean±SE	Mean±SE	Mean±SE
1% E. *globulus*+EB	15.60±2.60a	22.40±5.40a	30.40±7.40a
2% E. *globulus*+EB	23.60±2.40b	33.40±3.40b	44.20±5.20b
3% E. *globulus*+EB	34.40±1.6ab	47.80±4.80ab	56.80±7.80ab
Control	0.40±0.40b	0.40±0.40b	0.00±0.00b

EB: Emamectin benzoate**.** The similar numbers showed significant differences between means in a column.

Discussion

The plant-based insecticides (botanicals) like *A. indica* have been practiced since many centuries (Parakash and Srivastava, 2008) to control insect pests (Hashmi, 2001) all over the globe (Mordue and Blackwell, 1993; Lale and Mustapha, 2000; Ahmed et al., 2001) due to eco-friendly and safe for natural enemies. The various toxic chemicals like campesterol, galactose, stigmasterol, sitosterol, oleic acid and ascorbic acid (Murugan et al., 1998) glucose, are present on the botanicals that cause the death of various insect pests such as *T. varians.* The chemical such as meliantriol, deacetyl azadirachtinol, salannin, vepol and sulfur compounds having insect repellant, deterrent and anti- ovipositional and anti-feedant properties (Atawodi and Atawodi, 2009; Diaz et al., 2010).

The similar observations have been reported by (Reddy, 2000; Debaraj et al., 2002; Rajesh Kumar and Elangovan, 2012). The improper and excessive application of chemicals (insecticides) against insect pests cause insecticidal resistance and environmental pollution. The mixture of both botanical and insecticides can give better insect control and harmless for non-target organism (Prabhakar et al., 2003) like silkworm. The combine effect of some insecticides like Takumi (flubendiamide), proclaim (Emamectin benzoate), Vertimec (Abamectin) and the plant-based products like Achook (*Azadirachtin*) against the 4th larval instar of *Bombyx mori* under the laboratory conditions (Kordy, 2014). *B. mori* is an important economic insect due to silk production. The plant extracts can affect the growth and development of *B. mori* larvae. The larval development can reduce due to application of botanicals. The significant difference in the larval weight has been reported by early researchers (Narayanamma et al., 2012). A study was conducted to check the effect of botanicals, *viz. Pongamia pinnata*, *Occimum sanctum* and *A. indicia* against *B. mori* for silk production (Naik et al., 2013). The current study was revealed the same results. Insecticides like dichlorvos have found an effective control against insect pests of mulberry, especially *B. mori.* The study resulted that dichlorvos oxidative response of *B. mori* larvae induced (Deponte, 2013). The oxidative stress induced by increasing the dose of dichlorvos (Muthusamy and Rajakumar, 2016).

Conflict of interest

Authors have no conflict of interest.

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References

1. Ahmed, K.S., Yasui, Y. and Ichikawa, T., 2001. Effect of neem oil on mating and oviposition behavior of azuki bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). Pak. J. biol. Sci. 4: 1371-1373.
2. Atawodi, S.E. and Atawodi. J.C., 2009. *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. Phytochem. Rev., 8: 601- 620.
3. Debaraj, Y., Datta, R. N., Das, P. K. and Benchamin, K. V. 2002. Eri silkworm crop improvement – A review. Indian Journal of Sericulture, 41:100-105.
4. Deponte M. 2013. Glutathione catalyses and the reaction mechanisms of glutathione dependent enzymes. Biochem et Biophy Acta. 1830(4): 3217-66.
5. Diaz, G.E., Collado, J.L., Jimenez, A.V., Acosta, F.O., Colina G.O. and Diaz E.C., 2010. *Azadirachtin* concentration, insecticide efficacy and phytotoxicity of four neem extracts. Agrocienc., 44: 821-833
6. Hashmi, A.A., 2001. Integrated pest management in the 21st century, PARC publication, Islamabad, pp. 27.
7. Kordy, A. M. (2014). Residual effect of certain pesticides on the mulberry silkworm (Bombyx mori L.). *Middle East J. Appl. Sci*, *4*(3), 711-717.
8. Lale, N.E.S. and Mustapha, A., 2000. Potential of combining neem (*Azadirachta indica* A. Juss) seed oil with varietal resistance for the management of the cowpea

bruchid, *Callosobruchus maculatus* (F.). J. Stored Prod. Res., 36: 215–22MORDUE, A. J. AND BLACKWELL, A. ,1993. *Azadirachtin*, an update. J. Insect Physiol., 39: 903-924

9. Lansky, E.P., Helena, M.P., Alison, D.P. and Robert, A.N. (2008) Ficus spp. (fig): Ethnobotany and potential as anti cancer and anti inflammatory agents, Journal of Ethnopharmacology, 119: 195-213.
10. Mordue, A. J. and Blqackwell, A. ,1993. Azadirachtin, an update. J. Insect Physiol., 39: 903-924.
11. Mousa, O., Vuorela, P., Kiviranta, I., Abdel Wahab, S., Hiltunen, R. and Vuorela, H. (1994) Bioactivity of certain Egyptian *Ficus* species. Journal of Ethnopharmacology 41: 71-76.
12. Murugan, K., Jeyabalan, D, Senthil Kumar, N., Senthilnathan, S. and Sivaprakasam, N. 1998. Growth promoting effects of plant products on silkworm – A biotechnological approach. Journal of Science Industrial Research, 57: 740-745.
13. Muthusamy, R., & Rajakumar, S. (2016). Antioxidative Response in a Silkworm, Bombyx mori larvae to Dichlorvos insecticide. *Free Radicals and Antioxidants*, *6*(1), 58.
14. Naeem-Ullah, U., Ramzan, M., Saeed, S., Iqbal, N., Sarwar, Z. M., Ali, M., ... & Ghramh, H. A. (2020). Toxicity of four different insecticides against *Trilocha varians* (Bombycidae: Lepidoptera). *Journal of King Saud University-Science*, *32*(3), 1853-1855.
15. Naik, SA., Suresh, B., Ravi babu, B., Naik, MJ. 2013. Impact of Botanical Extracts on the Incidence of Major Pest (tukra) in Mulberry leaves on Carbohydrate metabolism in Silkworm, Bombyx Mori L. Bull. Env. Pharmacol. Life Sci., 2 (11):110-114.
16. Narayanamma, VL., Reddy, KD., Reddy, AV. 2012. Effect of botanical insecticides on the growth and silk production of Ambagoan and Lakhimpur strains of eri silkworm, *Samia cynthia ricini* (Boisduval). Journal of Biopesticides, 6(1):41-45.
17. Navasero, M.V., Navasero, M.M., Roxas, M.C., and Calumpang, S., 2013.

Occurrence of the moraceae feeding bombycid, *Trilocha varians* (Walker) (Bombycid, Lepidoptera) as pestof jackfruit and some ornamental species ofFicus in the Philippines. Journal of International Society of Southeast Asian Agriculture Science,19(2): 41-48.

18. Prabhakar, M., Rao, M. S. and Prasad, Y. G. 2003. Evaluation of bio-intensive integrated pest management modules against castor semilooper, Achaea janata Linn. Indian Journal of Plant protection, 31(1): 56-58.
19. Rajesh Kumar and Elangovan, V. 2012. Rearing performance of different eco-races of eri silkworm (*Philosamia ricini*) Donovan during Summer season of Uttar Pradesh. Journal of Experimental Zoology, 15(1): 163-168.
20. Ramzan, M., U. Naeem-Ullah, M. Javaid, M.Nadeem, N. Iqbal and S. Saeed. 2019b.Comparative biology of *Trilocha varians* (walker, 1855) (Lepidoptera: Bombycidae), anew pest of Ficus plant in Punjab, Pakistan. Pak.J. Sci., 71(4): 220-225.
21. Ramzan, M., U. Naeem-Ullah, N. Iqbal, Z.Rasheed, S. Saba, H. Ghaffar and S. Saeed.2019a. Effect of temperature on the lifecycle of *Trilocha varians* (Lepidoptera:Bombycidae) in Pakistan. Pure Appl. Biol.,9(1): 436-442.
22. Ramzan, M., U.N. Ullah, M.U. Sial, N. Iqbal and S. Saeed. 2021. Distribution, biology and management strategies about a less studied insect pest (*Trilocha varians*) of Ficus: A review. Pakistan Journal of Agricultural Research, 34(3): 638-642.
23. Ramzan, M., U.N. Ullah, S. Saba, M.M. Khan, U. Faheem, A. Rehman, N.A. Maan and W. Hassan. 2023. First record of *Trilocha varians* (Bombycidae: Lepidoptera) from Pakistan. Journal of Innovative Sciences, 9(1): 61-64.
24. Ramzan, M., Naeem-Ullah, U, Ali, M. and Riaz, H., 2020. Biological and morphological parameters of *Trilocha varians* (Lepidoptera:Bombycidae) in Pakistan. Punjab Univ. J. Zool., 35(2): 255-259.
25. Reddy, D. N. R. 2000. On the nomenclature of eri silkworm. Sericologia, 40: 665-667.

26. Sirisha, N., Sreenivasulu, M., Sangeeta, K. and Chetty, C. M. (2010) Antioxidant Properties of *Ficus* Species – A Review. International Journal of Pharm. Tech Research 2: 2174-2182.