Implications Of Diflubenzuron On The Grubs Of Hadda Beetle, *Henosepilachna Vlgintioctopunctata* (Coleoptera: Coccinellidae) Infesting Eggplant

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Abstract
The present study was conducted to evaluate the implications of diflubenzuron, a chitin synthesis inhibitor, against grubs of *Henosepilachna vigintioctopunctata* following two treatment methods i.e. feeding and contact. Following feeding method, the larval mortality of 22.49±1.3 to 100% while contact treatment showed 16.67±6.7 to 100% against first and third instar grubs was recorded in different concentrations. Adult emergence was completely inhibited in 1000 ppm tested against first instars by both the treatment methods. The developmental period increased up to 22.49±1.3 days in feeding method to first instar grubs in 100 ppm as compared to control. Diflubenzuron treatment showed morphological deformity in newly emerged adults like malformed wings, elytra and legs. Comparing the LC50 values of the compound, feeding method was found to be superior to contact showing better efficacy against the grubs of hadda beetle.

Keywords: Emergence, Diflubenzuron, Developmental period, Henosepilachna vigintioctopunctata, Mortality

1. Introduction
The eggplant is heavily infested with a number of pests in which *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae), also known as hadda beetle, is one of the important pests of this crop. It is one of the most destructive pests extensively found all over India and in other countries (Rahaman et al., 2008). This pest also causes significant damage to the crops like potato, tomato, bitter gourd etc. by feeding on the leaves, fruits and flowers (Anam et al., 2006). They feed on the epidermal tissues of leaves by scraping surface and cause damage in plants (Pradhan et al., 1990).
Insect growth regulators (IGRs) are the compounds that have the strong possibility to interfere with the developmental processes of insects and are considered safe for the environment. Moreover, this class of insecticides provides an opportunity to be used as alternatives to conventional insecticides for the control of different economically important insect pests in agriculture (Mondal & Parween, 2000). Besides causing mortality, IGRs have specific targeted actions which adversely interfere with the growth and development of insects and affect reproductive physiology and metamorphosis (Khan, 2021).
The application of synthetic insecticides are generally adopted for the management of this notorious pest in standing crops. Diflubenzuron is a well known chitin synthesis inhibitor or insect growth regulator (IGR) which disrupts the normal development of insects by interrupting chitin biosynthesis process and is successfully applied in the management of many insects/pests of agricultural importance (Zhou et al., 2015; Wang et al., 2017). Thus, the present study was conducted to evaluate the implications caused by diflubenzuron following two different treatment methods against first and third instar grubs of hadda beetle.

2. Materials and methods
2.1. Culture of *H. vigintioctopunctata*
The stock culture of *H. vigintioctopunctata* was reared and maintained in Pests and Parasites Research Laboratory of the Department of Zoology, Bareilly College, Bareilly as per the method of Mehta *et al.* (1995) and Saxena and Sharma (2007). Various developmental stages of the beetle were reared on fresh and tender leaves of eggplant by changing them regularly in plastic jars (12.5cm x 25cm) and stalks of leaves were dipped in glass tubes filled with water and corked with thermacole to avoid drying as the grubs prefer fresh and green leaves for their food. The entire feeding set with leaf was kept in plastic jars covered with muslin cloth. The jars were placed in Biological Oxygen Demand (BOD) incubator maintained at 28^0C and 65±5% relative humidity (RH). The stock culture was continuously maintained to obtain sufficient number of different grub stages as per the experimental requirements.

2.2. Bioassay

For conducting the experiments, first and third instar grubs of *H. vigintioctopunctata* were procured from the nucleus culture reared and maintained in the laboratory. Various concentrations of diflubenzuron viz., 1000, 500, 250 and 100 ppm were prepared in distilled water for evaluation. The compound (Diflubenzuron, Dimilin 25%WP) was procured from M/s Gharda Chemicals Limited, Mumbai. The compound was tested on first and third instar grubs by feeding and contact methods (Balasubramanian et al., 1980). For feeding method, 2 ml of each concentration of diflubenzuron was sprayed on fresh leaf of eggplant and grubs were released on it for 24 h feeding. Thereafter, normal fresh leaves were provided to them upto the pupation. For contact method, the inner surfaces of the petridishes measuring 9 cm diameter was sprayed with 2 ml of each concentration of diflubenzuron and the grubs were allowed to crawl on it for 90 minutes. After 90 mins of crawling, fresh leaves were given to the grubs and kept in plastic jars covered with muslin cloth. The control experiments were conducted with the same manner with water. The experiment was replicated thrice having six grubs in each to evaluate the toxicity of the compound in terms of larval mortality, developmental period, adult emergence, diapause condition and other morphological deformity.

2.3. Statistical analysis

The entomological data was statistically analyzed using GraphPad Prism-5 computer software and significant differences among the mean values of different groups were analyzed by one-way ANOVA. The LC_{50} values of the compound were estimated performing probit analysis of dose response data converting the log doses and probit transformed mortality data (Finney, 1962).

3. Results

Diflubenzuron was evaluated against grubs of hadda beetle and the entomological data revealed that both the treatments on first and third instar grubs of *H. vigintioctopunctata* were found efficacious in comparison to control. The significant mortality in treated first instar grubs was in the range of 41.67±2.6 to 100 and 33.33±5.8 to 100% in feeding and contact method, respectively, with different concentrations of diflubenzuron (Table 1). However, mortality rate in treated third instar grubs ranged from 33.33±6.7 to 83.33±3.6% and 16.67±6.7 to 75.0±5.0% by the treatment methods applied in the present study (Table 2). The mortality was noticed in dose-dependent manner in both the treatments. The LC50 values of diflubenzuron were determined for first and third instar grubs as 172.4 (95% CI= 149.9-198.3) and 264.6 (95% CI= 238.4-293.7), respectively in feeding treatment while these were 258.6 (95% CI= 213.7-312.9) and 651.3 (95% CI= 561.5-755.5), respectively in contact method. During the regular experimental observation, it was noticed that mortality of most of the treated grubs happened during the molting process and paralyzed grubs were incapable of shedding off their old cuticle and later they turned black and died.

Table 1. Effect of diflubenzuron on the developmental period (days), adult emergence (%) and cumulative mortality (%) of first instar grubs of *Henosepilachna vigintioctopunctata* through contact and feeding method

Treatment Method	Conc. (ppm)	Larval period	Pupal period	Developmental period (days) (Mean ± SE)	% Adult emergence (Mean ± SE)	Mortality % (Mean ± SE)	LC50 value (95% CI)
Feeding	1000	7.33±0.88	-	7.33±0.9	0.0^{c}	$100±0.0^{c}$	172.4 (149.9-198.3)
	500	13.0±0.73	3.83±0.41	16.83±1.6	$25.0±2.5^{c}$	$75.0±2.5^{c}$	
	250	15.3±1.22	4.5±0.13	19.83±0.8	$41.67±3.3^{b}$	$58.33±3.3^{c}$	
	100	17.2±0.96	5.33±0.23	22.49±1.3	$50.0± 5.5^{b}$	$41.67±2.6^{b}$	
Contact	1000	3.17±0.04	-	3.17±0.04	0.0^{c}	$100±0^{c}$	258.6 (213.7-312.9)
	500	13.83±0.92	3.83±0.09	17.16±0.5	$16.67±3.3^{c}$	$83.33±3.3^{c}$	
	250	14.5±0.88	4.17± 0.13	18.67±1.7	$50.0±5.0^{b}$	$50.0±5.0^{c}$	
	100	15±1.05	4.5±0.07	19.5±1.5	$66.67±5.8^{a}$	$33.33±5.8^{b}$	
Control	D.W.	14±1.02	4.17±0.14	18.16±1.4	91.67±6.7	8.33±6.7	

Superscripts showing means significantly different from control at $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$

Table 2. Effect of diflubenzuron on the developmental period (days), adult emergence (%) and cumulative mortality (%) of third instar grubs of *Henosepilachna vigintioctopunctata* through contact and feeding method

Treatment Method	Conc. (ppm)	Larval period	Pupal period	Developmental period (days) (Mean ± SE)	% Adult emergence (Mean ± SE)	Mortality % (Mean ± SE)	LC50 value (95% CI)
Feeding	1000	7.33±0.76	4.0± 0.9	11.33±1.1	$16.67±3.3^{c}$	$83.33±3.6^{c}$	264.6 (238.4-293.7)
	500	7.84±0.56	4.83±0.7	12.67±1.3	$33.33±6.7^{c}$	$66.67±6.7^{c}$	
	250	8.17±1.02	4.67±0.9	12.84±0.9	$50.0±5.8^{b}$	$50.0±5.8^{c}$	
	100	9.17±1.2	5.17±0.9	14.34±1.1	$66.67±6.7^{a}$	$33.33±6.7^{a}$	
Contact	1000	9.17±0.9	5.33±0.89	14.5±1.3	$25.0±5.0^{c}$	$75.0±5.0^{c}$	651.3 (561.5-755.5)
	500	8.5±0.77	5.67±0.38	14.17±0.8	$58.33±5.8^{b}$	$41.67±5.8^{b}$	
	250	8.33±0.84	6.0±0.72	14.33±1.6	$75.0±5.0^{a}$	25.0±5.0	
	100	9.0±0.98	5.83±0.92	14.83±1.4	83.33±6.7	16.67±6.7	

Control	D.W.	8.17±1.03	4.0±0.38	12.17±1.2	100±0	0±0	

Superscripts showing means significantly different from control at [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

Data on growth period indicated that only 100 ppm of feeding method showed an increase of 6.33 days in total developmental period (22.49±1.3 days) of first instar treated grubs as compared to control (18.16±1.4 days). However, other concentrations in both the methods did not show any role in the elongation or inhibition of growth of insect when treated with these concentrations. However, the highest concentration i.e. 1000 ppm caused complete mortality during first and second instar stage when first instar grubs were treated through feeding and contact method and no grub could be able to attain pupal stage (Table 1). While the developmental period of the beetle in all the doses of diflubenzuron was increased by more than two days than control when third instars were treated through contact method (Table 2). First instar treatment by feeding 100 ppm of diflubenzuron caused diapause condition in pupae.

The diflubenzuron treatment also exhibited some adverse effects on adult emergence. Both the treatment methods on first instar grubs showed significant inhibition in adult emergence. The higher doses were very effective while the lower dose (100 ppm) caused only 50.0± 5.5 and 66.67± 5.8% adult emergence by feeding and contact method (Table 1). On the other hand, third instar grub treatments adult emergence varied from 16.67±3.3and 66.67±6.7% and 25.0±5.0 to 83.33±6.7% in feeding and contact method, respectively as against control where all the pupae emerged into normal adults (Table 2). It was realized that diflubenzuron showed dose dependent impacts on pupae to emerge into normal adult. Few treatments of diflubenzuron exhibited some morphological deformities in newly emerged adults like malformed wings, elytra and legs which make them unable to fly properly. Few of them died after one or two days of emergence.

4. Discussion

Many chitin inhibitors are used to control pest incidence in various crops in the country. Thus, in the present study, diflubenzuron was evaluated to assess toxic effects applying feeding and contact treatment methods on first and third instar grubs of *H. vigintioctopunctata*. Balasubramanian *et al*. (1980) and Bhanu and Nagalingam (2001) recorded in significant mortality of *Spodoptera litura* and *Heliothis armigera* treated with diflubenzuron. A hundred percent larval death at 1000 ppm diflubenzuron treatment on first instar larvae of *Plutella xylostella* was reported by Biradar and Dhanorkar (2001) which corroborates the present findings with the same concentration applied through feeding and contact on the first instars of hadda beetle. Similar toxicity levels of other IGRs like lufenuron, flufenoxuron and hexaflumuron in the treated larvae of house fly were reported by Tanani et al. (2022) which also confirms the mortality data obtained in the present study.

Present study showed slow rate of larval and pupal development in both the application methods on third instars, and they were attached to the lower side of the leaf for a longer time and could not pupate as normal and were smaller in size than control pupae. In contrast to the present findings made prolonged developmental process was noticed with the treatment, Chander and Bhargava (2002) investigated a decrease in developmental process of *S. litura* with the increase in doses of diflubenzuron. On the other hand, the findings made by Balasubramanian *et al* (1980) and Mule & Patil (2000) on the development and growth of grubs of other insects corroborate the present data. Similarly, Bhanu and Nagalingam (2001) recorded an increase in larval period of *H. armigera* which also supports the present results made on first instar grubs of *H. vigintioctopunctata* with 100 ppm diflubenzuron through feeding method (17.2±0.96 days). Many workers reported dose dependent inhibition in adult emergence of *S. litura, H. armigera* and *Culex pipiens pipiens* by diflubenzuron treatments (Balasubramanian *et al*., 1980; Rehimi and Soltani, 1999; Mule and Patil, 2000) which support the present findings of emergence inhibition of hadda beetle.

Thus, from the above study made on the first and third stage grubs of *H. vigintioctopunctata* treated with diflubenzuron, the feeding method was found effective on the basis of lower LC50 values of 172.4 (95% CI=149.9-198.3) and 264.6 (95% CI=238.4-293.7) ppm for first and third instar grubs, respectively.

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Conflict of interest

Authors declare no conflict of interest.

References

1. Balasubramanian, M.; R Natesan; K. Mohanvel, 1980. The effect of diflubenzuron- A chitin inhibitor on *Spodoptera litura* and *Heliothis armigera.* The Andhra Agric. J., 27(1&2): 74-79.
2. Bhanu, K.V.; B. Nagalingam, 2001. Bioefficacy of certain chitin inhibitors and botanicals on immature stages of *Heliothis armigera* (Hubner). Indian J. Environ. & Ecoplan., 5(2):361-363.

3. Biradar, V.K.; B.K. Dhanorkar, 2001. Performance of diflubenzuron, a chitin synthesis inhibitor, against diamond black moth *(Plutella xylostella* L.). Pestology, XXV(1*):* 46-48.
4. Chander, R.; M.C. Bhargava, 2002. Effect of diflubenzuron on reproduction of tobacco catterpillar, *Spodoptera litura* (Fab.). Indian J. Plant Protec., 30(1*):* 43-45.
5. Finney, D.J. 1962. Probit analysis- a statistical treatment of the response curve. Cambridge University Press, Cambridge, 1-318 PP.
6. Khan, H. A. A. (2021). Posttreatment temperature influences toxicity of insect growth regulators in *Musca domestica*. Parasitology Research., *120*, 435–441.
7. Mitsui, T.; C. Nobusawa; J. Fukami; J. Conus; L.M. Riddiford, 1980. Inhibition of chitin synthesis by diflubenzuron in *Manduca* grubs. J. Pestic. Sci., 5: 335-341.
8. Mondal, K., & Parween, S. (2000). Insect growth regulators and their potential in the management of stored-product insect pests. Integrated Pest Management Reviews., *5*, 255–295.
9. Mule, R.S.; R.S. Patil, 2000. Efficacy of diflubenzuron against tobacco leaf eating caterpillar. Journal of Maharashtra Agricultural University, 25(1): 2326.
10. Post, L.C., B.J. De Jong and W.R Vincent, 1974. 1-(2,6-disubstituted benzoyl)3-phnylurea insecticides: Inhibitors of chitin synthesis. Pestic. Biochem. Physiol., 4: 473-483.
11. Rehimi, N. and N. Soltani, 1999. Laboratory evaluation of Alsystin, a chitin synthesis inhibitor against *Culex pipiens* L. (Diptera : Culicidae) : Effects on development and cutical secretion. J. Appl. Ento., 123(7): 437-441.
12. Tanani, M.A.; Hasaballah, A.I.; Hussein, R.M., 2022. Assessment of the perturbation induced by chitin synthesis inhibitors lufenuron, flufenoxuron and hexaflumuron in the house fly, *Musca domestica* vicina (Diptera: Muscidae). The Journal of Basic and Applied Zoology, 83:29. https://doi.org/10.1186/s41936-022-00291-w
13. Wang, Z.L.; Li, C.R.; Yuan, J.J.; Li, S.X.; Wang, X.P.; Chi, H., 2017. Demographic Comparison of *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae) reared on three cultivars of *Solanum melongena* L. and a wild host plant *Solanum nigrum* L. J. Econ. Entomol. 110, 2084–2091.
14. Zhou, L.; Xie, B.G.; Wang, X.P. 20215. Population dynamic of *Henosepilachna vigintioctopunctata* in different host plants in Jianghan plain. China J. North. Hort., 11, 103–105.