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Efficacy of chitin synthesis inhibitors against fall armyworm *Spodoptera frugiperda* (J. E. Smith) under laboratory conditions

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Abstract
Experiment was conducted to find out the relative toxicity of chitin synthesis inhibitors *viz*., lufenuron and novaluron with 25, 50 and 75 ppm concentration against third instar larvae of *Spodoptera frugiperda* (J. E. Smith) under laboratory conditions at College of Agriculture, Kolhapur, (Maharashtra) during *summer* 2024-25.
Among chitin synthesis inhibitors with three different concentrations tested for their larvicidal action revealed that Novaluron @ 75 ppm was found most effective and superior, it was followed by Novaluron @ 50 ppm. The percent mortality recorded at 6, 24, 48 and 72 hours after treatment. There was no mortality found 6 hours after treatment. After 24 hours, mortality ranged between 17.78 to 28.89 percent. After 48 hours, mortality increased considerably and ranged between 41.11 to 68.89 percent. At 72 hours after treatment, all the treatments showed its maximum efficacy and recorded Percent mortality of novaluron @ 25, 50 and 75 ppm were 86.67, 92.22 and 97.78; While, Percent mortality of lufenuron @ 25, 50 and 75 ppm were 77.78%, 84.33% and 88.67% respectively.

Keywords: Chitin synthesis inhibitors, *Spodoptera frugiperda*, novaluron, lufenuron, mortality

1. Introduction
The Fall Armyworm (FAW), an economically significant pest native to tropical and subtropical regions of the Americas, has recently invaded India, causing substantial damage to crops like maize and sorghum. Its high dispersal capacity, broad host range and high fecundity make it one of the most destructive economic pests. Fall armyworm is recognized as a globally distributed pest of maize (Wiseman *et al.* 1966) [18]. It infests the crop at all developmental stages, though its incidence is more prominent in the whorl region of young plants up to 45 days old. The larvae consume substantial quantities of foliage and may occasionally damage the growing point. Females deposit eggs in clusters that are irregularly scattered across the crop, and under summer conditions, these eggs usually hatch within three days. The upper surface of the leaves is home to the first and second instar larvae, which cause elongated papery windows on the leaves by scraping the epidermis. The third to fifth instar larvae settle in the whorl and produce a number of holes and feces in the unfolding leaves as a result of their feeding. Larvae in their sixth instar leave behind a lot of feces in the plant whorl and undergo significant defoliation. These older larvae can occasionally carry the early whorl stage of maize's growing internodes, which kills the plant.
Fall armyworm has been reported to inflict severe damage on maize and other crops. Yield reductions of nearly 40% were documented in Honduras (Wyckhuys and O'Neil, 2006) [19] and as high as 72% in Argentina (Murua *et al.* 2006). In African countries, estimated losses were 22% in Ghana, 32% in Zambia, 47% in Kenya, and 67% in Ethiopia (Kumela *et al.* 2018) [8]. In India, maize
yield losses due to FAW were recorded at 33% in Telangana and 34% in Karnataka (Mooventhan *et al.* 2019) [11].
For effective management, broad-spectrum insecticides are currently recommended. Pest resurgence, insecticide resistance, environmental contamination, and toxicity concerns to nontarget animals are only a few of the serious problems that have resulted from the use of pesticides alone. The current tendency in integrated pest control is to

discourage reliance on hazardous substances and to reduce their use. This is only possible with the use of compounds that are more economical, safe, selective, eco-friendly, and suitable for the integrated pest control program (Ganguly *et al.* 2020) [4]. Benzoylphenylureas (BPUs) are a class of insect growth and development inhibitors that prevent insects from synthesizing chitin (Post L. C. and Vincent W. R. 1973) [17].

Chitin is the second most abundant natural biopolymer in the world, after cellulose. It is a polymer of N-acetyl glucosamine which is absent in mammals, but present in the exoskeleton of arthropods, the cell walls of filamentous fungi, and is also found in crustaceans, algae, and yeast (Javed *et al.* 2013) [5]. As a key structural element in the insect cuticle, chitin helps protect insects from microbial infections, water loss, and physical damage. It is crucial for the growth and development of arthropods, particularly during the moulting process, where old chitin is replaced with newly formed chitin (Doucet D. and Retnakaran A, 2012) [3]. The breakdown and formation of chitin are tightly regulated by enzymes, especially chitinases. Because of its essential role in insect development, chitin synthesis has become a valuable target for developing pest control agents that interfere with the formation of the cuticle (Merzendorfer H, 2013) [9]. Chitin Synthesis Inhibitors (CSIs) act as insect growth regulators by disrupting the production and deposition of chitin, leading to death during the moulting process. In cases where insects manage to moult after CSI treatment, they are often unable to feed and eventually die, likely due to starvation (Mulder and Gijswijt, 1973; Neuman and Guyer, 1987) [12, 15]. Novaluron and Lufenuron are benzoylphenylurea- based insecticides classified under group Chitin Synthesis Inhibitors. They show strong effectiveness against the larval stages of several economically significant lepidopteran pests. It works by blocking chitin production in insects, interfering with the development of their exoskeleton during moulting, ultimately causing their death (Oberlander and Silhacek, 1997) [7]. Therefore, keeping this in view, these two CSIs were tested in laboratory against fall armyworm (*S. frugiperda*).

2. Materials and Methods

Methodology

The experiment was carried out at R. C. S. M. College of Agriculture, Kolhapur (Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra) during year 2024-25. The experiment was performed in Completely Randomized Design with seven treatments and three replications. The total 30 third instar larvae of *S. frugiperda* used in each treatment.

Collection of Caterpillars

Approximately 100 third and fourth instar caterpillars of *S. frugiperda* were collected from infested field.

Mass Rearing of Fall Armyworm

Material required

Rearing cages, Maize leaves, plastic vials, camel hair brush, honey/sugar solution, cotton, formalin, measuring cylinder, micropipette, etc.

Rearing and Maintenance

Field collected larvae were reared under laboratory condition at 28± 2 ^{0}C temperature and 70± 5% relative humidity. Larvae were placed into ventilated plastic vials individually to avoid cannibalism into and fed with fresh maize leaves collected from 15-30 days old plants. The food was changed daily to maintain sanitation. After pupation of larvae, the pupae were collected in separate cleaned vials. The pupae of male and female were kept in separate cages after determining their sex for the emergence of adults. After emergence of adult from pupa, male and female were released in separate oviposition cage for egg laying. Sterile cotton dipped in a sugar solution was placed in the oviposition cage as source of food for the emerging adults. The young maize seedling was placed in the cage for egg laying. After approximately 2-3 days old egg batches were removed from the oviposition cages and used for further study.

Table 1: List of chitin synthesis inhibitors along with their concentrations and doses

Treatment No.	Treatments	Dose (PPM)
T_1	Lufenuron 5.40 EC	25
T_2	Lufenuron 5.40 EC	50
T_3	Lufenuron 5.40 EC	75
T_4	Novaluron 10 EC	25
T_5	Novaluron 10 EC	50
T_6	Novaluron 10 EC	75
T_7	Untreated control	-

Bioassay Method (Leaf-dip Method)

Leaf discs (6 cm diameter) of maize were used for bioassay tests, after washing it with tap water. The leaf discs were dipped in treatment concentrations for 20 seconds with gentle agitation, air dried at room temperature and kept in petri plates separately containing wet filtered paper to avoid drying of leaf disc. The single pre starved (24 hrs) third instar larvae were allowed to feed on each treated leaf discs and observations were taken 6 HAT and thereafter 24, 48 and 72 HAT accordingly. Leaf discs treated with water were considered as control. Dead larvae were separated immediately and alive larvae were provided with fresh untreated maize leaves.

$$\% \text{ Mortality } = \frac{\text{Number of larvae died due to treatment application}}{\text{Total number of larvae in a treatment}} \times 100$$

3. Statistical Analysis

The experimental data of percent bio-efficacy were analysed by applying Completely Randomized Design (CRD), and results obtained were subjected to angular transformation. The significance of treatments was assessed by determining the critical difference (CD) at 1% level of significance. Data pertaining to larval count after their transformation was statistically analysed as per the standard analysis of variance.

Result and Discussion

The data on per cent mortality of third instar larvae of fall armyworm in a laboratory condition with 25, 50 and 75 ppm concentration of lufenuron and novaluron including control treatment and observations recorded at 6 HAT, 24 HAT, 48 HAT and 72 HAT reveal that (Table 2) there was no mortality at 6 hours after treatment from any of the treatments. Significant variations between treatments started to show up after 24 hours. Mortality varied from 17.78 (Novaluron @ 25 ppm) to 28.89 percent (Novaluron @ 75 ppm) at 24 HAT. At 48 HAT, larval mortality ranged from

41.11% (novaluron @ 50 ppm) to 68.89% (novaluron @ 75 ppm).

At 72 HAT, all the treatments showed its maximum efficacy. Novaluron @ 75 ppm had the highest larval mortality (97.78%), followed by novaluron @ 50 ppm (92.22%). Lufenuron @ 75 ppm (88.67%), novaluron @25 ppm (86.67%) and lufenuron @50 ppm (84.33%) was showed moderate mortality rate as compared to superior treatment. The lowest rate of mortality was recorded at lufenuron @ 25 ppm (77.78%). There was no recorded death in the untreated control.

The progressive rise in larval mortality observed in the present study corresponds with the typical action of chitin synthesis inhibitors, which hinder chitin deposition during the moulting process. The mortality tends to occur during ecdysis rather than through immediate toxicity. Similar delayed lethal effects of benzoylurea insecticides on *S. frugiperda* were reported by Mansoor *et al.* (2022) [20], who documented moulting disruption, incomplete shedding of the cuticle, and the appearance of larval-pupal intermediates following treatment with novaluron. The effect of lufenuron and novaluron was influenced by both the dosage applied and the duration after treatment. Alve *et al.* (2001) [1] reported that increasing concentrations of novaluron resulted in high mortality across all larval instars of *S. litura*, with values ranging from 86.67% to 100%. The study further emphasized that larval mortality was positively correlated with the dose of novaluron applied. Moreover, the clear dose-dependent trend observed in this study (25 < 50 < 75 ppm) is consistent with earlier laboratory evaluations, where higher concentrations of benzoylurea compounds resulted in enhanced moulting disruption and increased larval mortality demonstrated by *Mansoor et al.* (2022) [20]. The results of novaluron @ 75 and 50 ppm (97.78 and 92.22%) obtained after 72 hrs. were similar with Hardake *et al.* (2011) [11] who reported that novaluron caused an average larval mortality of 92.20% in fall armyworm after 72 hours of exposure. Also, Islam *et al.* (2015) [7] concluded that lufenuron has been found highest mortality @ 75 ppm followed by 50 ppm and 25 ppm. against *S. litura*, with its stomach-mediated activity. The superior efficacy of novaluron over lufenuron recorded in the present investigation is in agreement with previous findings. Deshmukh *et al.* (2020) [2] also highlighted the strong activity of novaluron against fall armyworm under Indian field conditions. In the current study, a similar pattern was evident, with relatively low mortality at 24 hours after treatment, which progressively increased and reached near-complete control by 72 hours.

Table 2: Efficacy of Chitin Synthesis Inhibitors against fall armyworm in laboratory conditions

Sr No.	Treatments	Dose (PPM)	Per cent mortality			
			6 HAT	24 HAT	48 HAT	72 HAT
1	Lufenuron 5.40 EC	25	0.00	21.11 (27.34)*	41.22 (39.94)	77.78 (61.89)
2	Lufenuron 5.40 EC	50	0.00	24.48 (29.64)	53.33 (46.91)	84.33 (66.70)
3	Lufenuron 5.40 EC	75	0.00	22.22 (28.11)	64.44 (53.43)	88.67 (70.55)
4	Novaluron 10 EC	25	0.00	17.78 (24.92)	41.11 (39.88)	86.67 (68.59)
5	Novaluron 10 EC	50	0.00	27.81 (31.82)	66.67 (54.74)	92.22 (73.88)
6	Novaluron 10 EC	75	0.00	28.89 (32.50)	68.89 (56.14)	97.78 (82.99)
7	Untreated control	-	0.00	0.00	0.00	0.00
	SE(m)±	-	-	0.70	1.18	1.69
	CD at 1%	-	-	2.95	4.99	7.10
	CV%	-	-	4.87	4.93	4.81

HAT - Hours after treatment *Figures in the parentheses are arcsine (x+0.5) transformed values.

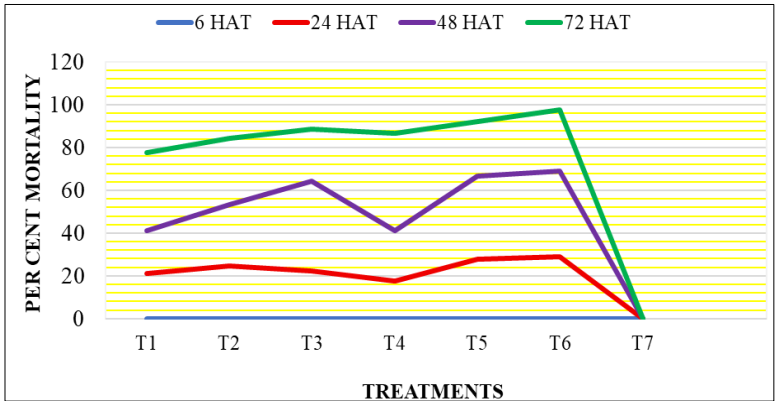

Fig 1: Efficacy of Chitin Synthesis Inhibitors against Fall armyworm in laboratory conditions

Conclusion

Novaluron and lufenuron are part of the benzoylphenyl urea group of insect growth regulators, which act by disrupting chitin formation, a key process in insect growth and development. Novaluron with 75 ppm concentration was found very effective in laboratory conditions. Overall, this study demonstrates that both the chitin synthesis inhibitors novaluron and lufenuron exhibited efficacy in suppressing the larval population. Their impact was influenced by both the dosage applied and the duration after treatment. Higher concentrations resulted in the greatest larval mortality, while lower concentrations produced comparatively lesser effect.

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