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Efficacy of sub lethal concentration of flubendiamide against larvae of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract
The sub lethal effects of flubendiamide (39.35% SC) were studied against the 5th instar larvae of *Spodoptera litura* under the laboratory condition. The different sub lethal concentrations used were 0.002%, 0.004%, 0.006%, and 0.008%. A significant increase in the larval mortality was observed as compared to the control with increase of sub lethal concentration of flubendiamide. Highest larval mortality of 47.33% was observed at 0.008% concentration. Longevity of both the larvae (5th and 6th) and adults increases significantly with increase in sub lethal concentration. A significant decrease in the fecundity and fertility were observed with increase in the sub lethal concentration flubendiamide as compared to the control. The inhibition of adults emergence was also observed with increase in the sub lethal concentration. Hence from this study, it was concluded that flubendiamide is highly effective insecticide at its sub lethal concentrations against the 5th instar larvae of *Spodoptera litura* under laboratory condition.

Keywords: Flubendiamide, mortality, longevity, fecundity, fertility, *Spodoptera litura*

1. Introduction
The common cutworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae), is a devastating, polyphagous insect pest having about 180 host species [1]. It showed a challenge for agricultural production throughout the world by causing damage to economically important crops like castor (*Ricinus communis* L.) tobacco (*Nicotiana tabacum* L.), soybean (*Glycine max* L.), cotton (*Gossypium sp*. L.) and groundnut (*Arachis hypogea* L.) etc. It has been reported that it is also a major pest of Black gram *Vigna mungo* (L.) commonly known as urd bean belongs to family Leguminaseae [2]. The economic loss of the crop caused by this pest varies from 25.8-100% based on the crop stage and infestation level [3].
Different groups of the insecticide have been established to be effective to control this pest; however, resistance has been developed in this pest due to extensive use of the different groups of insecticides. The problem of resistance development to different insecticides in *Spodoptera litura* has been reported by the different workers [4-8].
In the view of the fact that it has been developed resistance to different group of insecticides, novel insecticides have emerged with a different mode of action. One among them is flubendiamide having a distinctive chemical structure (N2-[1, 1-dimethyl-2-methyl sulphonyl ethyl]-3-iodo-N1-2-methyl-4-{1, 2, 2, 2-tetrafluro-1- (trifluromethyl) ethyl} phenyl), is a phthalic acid diamide insecticide, which belongs to benzene dicarboxamide group.
It is found to be effective against the broad spectrum of the lepidopterous pests and has very low or no toxicity to fish, birds, natural enemies, pollinators and mammals [9, 10]. As far as its mode of action is concerned it act on the ryanodine receptor (RyR) in insects and alter the function of Ca^{2+} channel, thus during muscle contraction stimulates the release of Ca^{2+} ions [11, 12]. However, very little information regarding the effect of flubendiamide in insects is available. Considering these views the present studies were carried out to assess the effect of sub lethal concentration of flubediamide on the 5th instar larvae of the *Spodoptera litura*.

2. Material and methods
2.1. Insecticide
Insecticide used in the studies was the commercial formulation of flubendiamide (39.35% SC) with the trade name "FAME" from Bayer Company and was purchased from the market.

2.2. Rearing of *Spodoptera litura*
Adults of the *Spodoptera litura* were collected from the Agricultural fields, faculty of Agriculture, Aligarh Muslim University, Aligarh and were maintained on the 10% glucose solution soaked in cotton which were placed in the rearing jar of size (20×15 cm), under the laboratory condition of 26±2 ºC temperature and 65-70% relative humidity12L:12D photoperiod in a B.O.D. incubator. The adult male and female after mating started laying eggs on the paper strips provided in the jar. The eggs obtained were collected daily and were kept in another jar for hatching. The larvae obtained from these eggs were reared on the castor leaves. For pupation, sixth instar larvae were transferred to another jar containing sterilized sand. The 5th instar larvae of F2 generation were used for studies of the effect of insecticide flubendiamide.

2.3. Sub lethal concentration of flubendiamide
On the basis of LC_{50} value different sub lethal concentration of flubendiamide viz. 0.002%, 0.004%, 0.006%, and 0.008% was prepared in the distilled water. For control only distilled water without insecticide was used. For each sub lethal concentration, 30 larvae were chosen including untreated control. There were five replicate for each sub lethal concentration and untreated control.

2.4. Mode of application
One µl of each sub lethal concentration of flubendiamide were taken into the micro applicator and were topically applied on the thoracic terga of each larva. After 24 hour mortality of the larvae was counted and the rest of the treated surviving larvae were kept in another jar containing sterilized sand for pupation. Other parameters like adult emergence, fecundity, fertility, larval duration and adult longevity, were also observed.

2.5. Statistical analysis
Data are expressed as mean + SEM from five independent experiments. Data obtained was analyzed by Turkey's test after One-way Analysis of variance (ANOVA) using statistical software Graph Pad Prism 5.01. (California, USA). Differences were considered statistically significant at $p \leq 0.05$.

3. Results
3.1. Sub lethal effect of flubendiamide on the mortality of 5th instar larva.
The data on the biological parameter of the Spodoptera litura treated with different sub lethal concentration of flubendiamide after 24 hours of treatment varied significantly ($p \leq 0.05$) as compared to the control. The result showed that the mortality of the larva increases as the sublethal concentration is increased (fig1.). The maximum mortality of 47.33% was observed at 0.008% concentration.

3.2. Sub lethal effect of flubendiamide on adult emergence.
Data related to sub lethal effects of flubendiamide on the adult emergence of treated 5th instar *S. litura* larvae showed a significant difference ($p \leq 0.05$) as compared to the control (fig2.). As the sub lethal concentration of insecticide increases the percent adult emergence was decreased as compared to the control.

3.3 Sub lethal effect of flubendiamide on reproduction

To estimate the effect of flubendiamide on the reproduction of *S. litura*, number of eggs laid and there hatching percentage was observed. The results showed there was a significant difference ($p \leq 0.05$) in fecundity in all sub lethal concentration as compared to control (fig3.). The fertility of eggs also varied significantly with varied sub lethal concentration (fig4.).

3.4. Sub lethal effect of flubendiamide on longevity

The longevity of both the larva and adults varied significantly after exposure to different sub lethal concentration of flubendiamide. The results showed a significant difference ($p \leq 0.05$) in the longevity of both 5th and 6th instar larvae (fig 5.and fig 6.). As far as the longevity of an adult male and adult female was concerned, it was also statistically significant at ($p \leq 0.05$) is shown in the (fig.7 and fig. 8.)

4. Discussion
Flubendiamide is a novel class of diamide insecticide, effective against a broad spectrum of lepidopterous insect pests. In muscles, it binds to the ryanodine receptors and resulting in the opening of a channel and release of Ca^{2+} ions into the cytoplasm, which causes paralysis and death [13]. In the current study, different biological parameters of *S. litura* treated with the sub lethal concentration of flubendiamide were adversely and significantly affected. The effects of different sub lethal concentration of flubendiamide after exposure to *S. litura* 5th instar larvae were increased larval mortality, increased larval duration, increased adults longevity, decreased fecundity and fertility. The larval mortality increases with the increase of sub lethal concentration of flubendiamide as shown in (fig1.). A similar type of result was also reported by [14] on *Archips rosanus*, [15] on *Brecon brevicornis*, [16] on *Aedes aegypti*, and [17, 18] on the red cotton bug.

Our results obtained showed that flubendiamide was an extremely toxic insecticide to the *S. litura.* In the present study, the fecundity and fertility of the adult emerged from the treated 5th instar larvae with sub lethal concentration of flubendiamide were significantly and adversely affected as shown in (fig.3 and fig.4). Our results showed that both fecundity and fertility was significantly reduced with the increase of sub lethal concentration in comparison to control. There were many findings of another worker which confirm our result related to reduced fecundity in insects. When the larvae of apple bud moth, *Platynota idaeusalis*, codling moth, *Cydia pomonella*, and corn earworm, *Helicoverpa zea* were fed on the diet containing the sub lethal concentration of tebufenozide resulted in the reduced fecundity and fertility in adults [19-21]. The fecundity and fertility of *Spodoptera littoralis* adults decreases in the dose dependent manner when treated orally and residually with different doses of Spinosad and methoxyfenozide. [22]

According to Indrasith *et al.* [23] it is well known that the maturation of insect's eggs is mainly dependent on the material absorbed from the surrounding hemolymph and by the material which is synthesized inside the ovary itself. These materials which are required for the embryo development are protein, lipids and carbohydrates [24]. We assumed that flubendiamide might interfere with the protein accumulation in inside the eggs, which might be the cause of fecundity and fertility reduction in *S. litura*.

The adult emergence from the surviving larvae was also affected significantly. At the higher sub lethal concentration, the inhibition of adult emergence was high as compared to the control as shown in (fig.2). A similar result was also reported by Singh and Nath [25] in which emergence of moth was affected adversely following the dietary exposure of phosphamidon, quinolphos, cypermethrin, and endosulfon. According to Abdel-Rahim *et. al.*, [26] when the larvae of the black cutworm *Agrotis ipsilon* were treated with the varying

concentration of the methomyl by using leaf dip method, the adult emergence was significantly decreased as compared to the control.

Effect of sub lethal concentration of flubendiamide on the longevity of 5th and 6th instar larvae was shown in (fig.5 and fig.6). There was a significant difference at ($p \leq 0.05$) as compared to the control. The increased larval duration after exposure to the different sub lethal concentration of insecticide may be due to starvation which was caused by the cessation of feeding [27]. The adult longevity of the *S. litura* significantly varied at ($p \leq 0.05$) after exposure to the different sub lethal concentration of fubendiamide as shown in (fig.7 and fig.8). Similar kind of result was also reported by Lai and Su [28] in which LC_{30} dose of chlorantraniliprole was given in the diet to the neonates of the beet armyworm *Sopodoptera exigua* and the longevity of adults which were emerged from the treated neonate was slightly higher as compared to the untreated control.

The results of the present study clearly showed that flubendiamide is a highly effective insecticide at its sub lethal concentration with the noble mode of action against the larvae of *S. litura*. In addition, the reproduction of this pest was also affected. From an ecological point of view these effects are essential as the sub lethal concentration reduces the offspring's number and hence the population of insect can be retained below the economic injury level. Due to these toxicological profiles, this insecticide can be used as a helpful tool in the IPM of the *S. litura*. But the thread of resistance of *S. litura* against this insecticide should not be ignored.

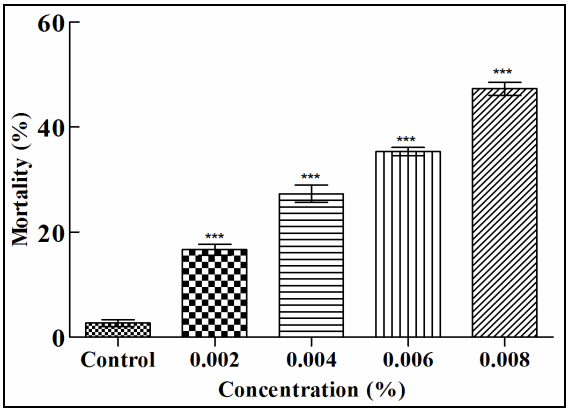

Fig 1: Graph showing nymphal mortality. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$

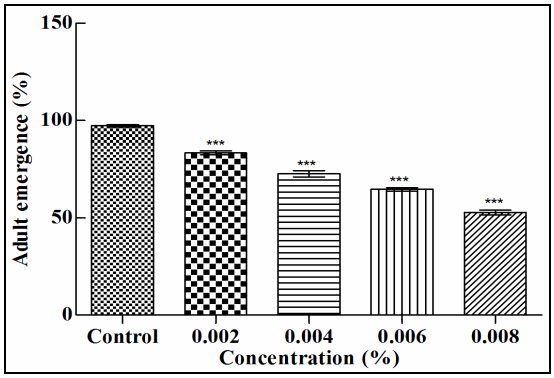

Fig 2: Graph showing the percentage of adult emergence. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$

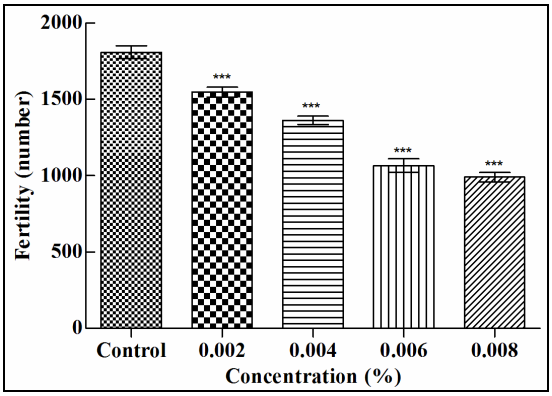

Fig 4: Graph showing the fertility of the laid eggs. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$

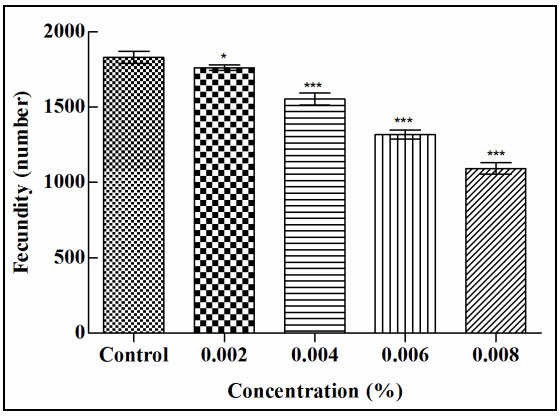

Fig 3: Graph showing the fecundity of female. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$, * represents $p \leq 0.05$

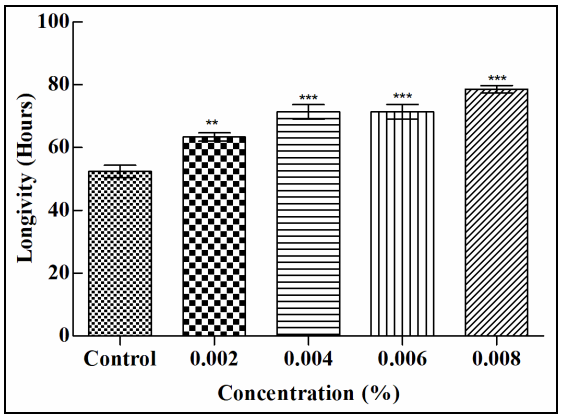

Fig 5: Graph showing the longevity of 5th instar larvae. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$, * represents $p \leq 0.05$

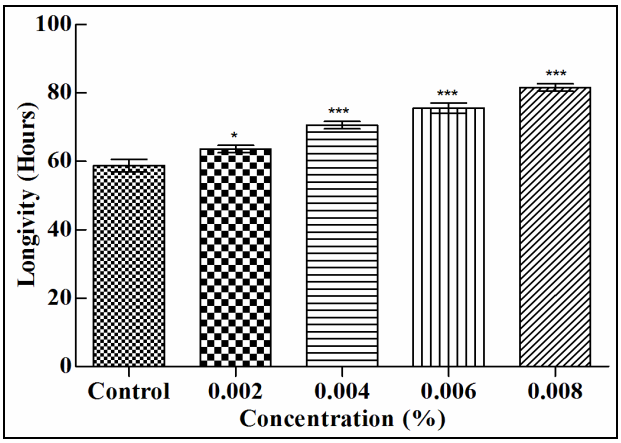

Fig 6: Graph showing the longevity of 6th instar larvae. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$, * represents $p \leq 0.05$

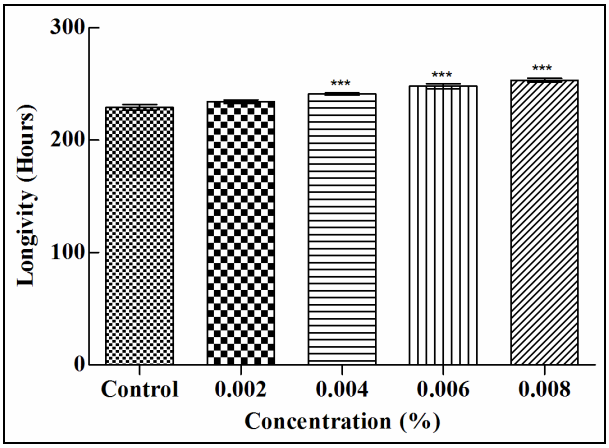

Fig 7: Graph showing the longevity of adult male. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$

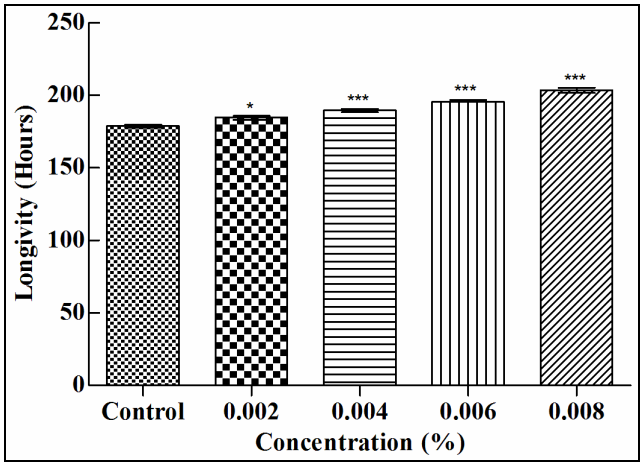

Fig 8: Graph showing the longevity of adult female. Bars represent SEM. Results are the mean of five individual experiments. *** represents $p \leq 0.001$, * represents $p \leq 0.05$

5. Conclusion

Flubendiamide at its sub lethal concentration was found to be effective against the larvae of *Spodoptera litura*. The different biological parameters of the *Spodoptera litura* were found to be affected after exposure to the varied sub lethal concentration. Therefore this insecticide can be used in an IPM program to control the pest population as well as to reduce the incidence of insecticide resistance.

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