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# Effects of Fluorine - Containing Insecticide Flubendiamide 39.35% SC and Entomopathogenic Fungi on the Survival Rate of Fall Armyworm, *Spodoptera frugiperda* J. E. Smith, (Noctuidae: Lepidoptera)

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**ABSTRACT**

**Purpose:** The Fall armyworm (FAW), *Spodoptera frugiperda* (Noctuidae, Lepidoptera), an invasive insect pest currently causes considerable losses in many maize growing regions across India and elsewhere. The search for newer insecticides that interact synergistically with entomopathogenic fungi (EPF) is expected to enhance the efficacy and stability of biological products against this pest. The aim of this study was to analyze the co-influence of fluorine-containing insecticide Flubendiamide 39.35% SC with two EPF, *Beauveria bassiana, Leacanicillium lecanii* on the control of FAW on maize.

**Methods:** The hypothesis of synergy betweena fluorine-based insecticide and twoEPF for the control of FAW was tested**.**We evaluated the joint action of Flubendiamide 39.35% SC with *Beauveria bassiana* or *Leacanicillium lecanii*at 1 x $10^8$ conidia/ml or their combination against third instar *S. frugiperda* larvae on maize over eleven days period under bioassay and field testing.

**Results:** Synergy between Flubendiamide and *Beauveria bassiana*, *Lecanicillium lecanii* was witnessed on the mortality of FAW. Combination treatments caused signifcantly higher mortality ofFAW larvae, compared to individual treatment, underscoring unique capacity for rapid control. The survival rate of FAW larvae at 11 DAS was estimated as 8.0 % for *B.bassiana*;8.0 % for *L. lecanni*;4.0 % for Flubendiamide;4.3 % for *B.bassiana* + Flubendiamide, 7.0 % for *L.lecanni* + Flubendiamide as against 11% in untreated control. Combination treatment of EPF with Flubendiamide caused signifcantly higher mortality compared to sole treatment with identical performance in fields.

**Conclusions:** Toxicosis caused by fluorine-containing insecticide provides stable synergistic effect between Flubendiamide, *B. Bassiana* and *L. Lecanii.* The combinations can be promising for the development of efficient microbial pesticidesfor control of FAW.It is possible that EPF will play an important role as microbial agents against FAW after confirmative research in fields.

***Key-words:*** *Flubendiamide; Beauveria bassiana; Leacanicilliumlecanii; Fall Armyworm; Synergistic effects; Toxicosis; Biological control.*

## INTRODUCTION

The Fall Armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Noctuidae: Lepidoptera)is one of the major pests in maize crops, causing signifcant economic losses [1]. The pest has rapidly spread worldwide, generating an urgent need to develop effcient and sustainable strategies for its control. Currently, the primary approach to pest control involves the application of chemical insecticides, but a high level of genetic polymorphism and the ecological plasticity of insect pests contribute to the rapid development of chemical resistance [2]. Levels of resistance to insecticides vary greatly among different populations and between FAW life stages [3]. Alternatives to chemical insecticides include biological agents based on entomopathogenic microorganisms, such as bacteria and fungi [4]. Entomopathogenic Ascomycetes (*Beauveria, Metarhizium* and *Isaria*) are widely used as biological agents around the world, including against FAW [5]; however, the effectiveness of these biopesticides is not assured and largely depends on environmental factors [6, 7]. The long latency period of the mycosis has often been observed after treatment of FAW with entomofungi [8]. Many infected larvae survive until the prepupal stage; therefore, the insects have enough time to inflict significant damage on crop plants [9]. Furthermore, generalist Ascomycetes, such as *Beauveria* and *Metarhizium*, have been adapted to kill the weakest insects, for example, those that have been stressed by different ecological factors [10, 11]. In this respect, it seems relevant to search for biological or chemical agents that would serve assynergists for the fungal entomopathogens [12]. A range of studies demonstrates that bacteria, fungi and their metabolites, and sublethal doses of chemical insecticides can act as synergists of *Beauveria* and *Metarhizium* species under treatments of FAW [13, 14]. Furthermore, it is known that some plant metabolites possess antagonistic properties against FAW [15] and increase their susceptibility to fungal pathogens [16]. This compound acts as a synergist of *Beauveria bassiana* (Bals.-Criv.) Vuill., and *Leacanicillium lecanii* (Zimm.) under treatment of FAW and wax moth *Galleria mellonella* (L.) larvae[16]. Flubendiamide(FBD) or 1, 2-benzenedicarboxamides N0-[1, 1-dimethyl-2-(methyl-sulfonyl) ethyl]-3-iodo-N-{4-[2, 2, 2tetrafluoro-1-(trifluoromethyl) ethyl]-0-tolyl} is a new insecticide belongs to phthalic acid diamides[17]. Flubendiamide is the first commercially available phthalic acid diamide that targets ryanodine receptors (RyRs) in insects, which play a key role in Lepidoptera control [17]. Certain Flourine derivatives obtained by reaction with some amines and substituted phenylhydrazines increased the susceptibility of insect pests to *B. bassiana*, but the strongest insecticidal properties and obvious synergistic interaction with fungus exhibited fluorine-containing derivatives (FUA) [18, 19]. The aim of the study was to analyze the co-influence of Flourine derived compounds and *B. bassiana* isolate,*L. Lecanii* isolates on third instars of FAW larvae and combined treatment with the fungus and Flourine derived compounds.

## MATERIAL AND METHODS

### *Insect rearing*

Eggs of FAW were collected from an established colony in the laboratory. The eggs were kept in a ventilated rectangular plastic box (28 cm × 17 cm × 18 cm). The neonate larvae were fed with fresh maize insecticides free leaves. The firstto thirdinstar larvae were kept in a rectangular plastic box (28 cm× 17 cm × 18 cm), while fourthto sixth-instar larvae were separately placed in six-well plates to prevent cannibalism until pupation. The new emerging adults were placed in cylindrical glasses. A paper towel was used to cover the top portion of the adult glasses[20], and sterile cotton balls were placed inside a plastic bottle lid soaked with a 10% concentration of honey. The larvae were kept at 25°C±2°C, with a photoperiod of 12: 12 (dark: light) and

### *Entomopathogenic fungal isolates*

Entomopathogenic fungi isolates (*Beauveria*, *Metarhizium*and *Lecanicillium*) obtained from laboratory collection at ICAR-National Beurea of Agriculturally Important Insect Resources (NBAIR), Bengaluru, Karnataka) was used. The conidia of the fungi were grown in Sabouraud Dextrose Agar with 0.25% yeast extract (SDAY) and 0.4% lactic acid over 14 days at 26°C in the dark. For insect inoculation, conidia were suspended in sterile 0.03% (v/v) aqueous Tween 80 [21]. Conidial concentrations were counted and adjusted to $1 \times 10^8$ conidia/ mLwere adjusted using h a Neubauer hemocytometer before the bioassay. The viability of conidia was verified by incubation on SDAY and determining the germination percentage. Conidial suspensions with at least 95% germination were alone used.

### *Insecticide*

The commercial product of Flubendiamide 39.35% SC(Belt® 480g active ingredient (a.i.)/L) - ryanodine receptor modulator (IRAC MoA group 28) wws provided by Bayer and used to perform bioassays.

### *Insecticidal Activity of Flubendiamide Compounds*

Insecticidal activity of the Flourine derived compounds - Flubendiamiade was evaluated on third instar larvae of *Spodoptera frugiperda.* The leaves were treated with the corresponding concentration of compounds and dried under a laminar flow hood. For evaluation of the activity, leaves were placed in plastic containers with larvae. The mortality of larvae was recorded after 1, 3, 5, and 10 days of observation. The untreated leaves served as control. Each treatment was replicated three times, and a total of 15 larvae were used for each treatment.

### *Field Bioassay*

The experiment was conducted in a maize field in the Department of Entomology, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, November 2024. The distance between the maize plants was 70-90 cm. Native FAW larvae from the field were used for the bioassay. Third instar larvae were placed into plastic boxes (1 individual per box) that were tied on maize plants, with the distance between boxes being 3.0-3.5 m. The treatment was applied using handheld sprayers in the evening (between 5:00 and 6:00). The following samples were used: (1) Flubendiamide (0.25%) in a water-Tween 20 (0.03%)-acetone (5%) solution, (2) fungus ($1\times10^9$ conidia per ml) in a water-Tween-acetone solution, (3) fungus ($1\times10^9$ conidia per ml) and Flubendiamide (0.0025%) in a water-Tween-acetone solution, and (4) a Water-Tween-acetone solution as the control. In total, 15 ml of the samples was sprayed on one plant with FAW larvae. The observations were recorded on number of live larvae per 25 plants on one day before spray and one day, three days, five days, seven days and ten days after the spray. These observations of live larvae were taken based on appearance of fresh excreta in leaf whorl of plant. The data was subjected to statistical analysis for interpretation. The daily range of relative humidity was 10-98%, and the temperature range was 20-35 °C.

## RESULTS

The results from Table 1 and Table 2 outline the efficacy of Flubendiamide and entomopathogenic fungus on the survival rate of Fall Armyworm larvae in laboratory and field settings.

**Table 1.Efficacy of Flubendiamide and Entomopathogenic fungi on the survival rate of Fall Armyworm larvae in laboratory**

| T. No. | Treatment Details | 1 DAS | 3 DAS | 5 DAS | 7 DAS | 9 DAS | 11 DAS |
|---|---|---|---|---|---|---|---|
| $T_1$ | *Beauveria bassiana* | $97.22^{a}$ (1.58) | $88.88^{b}$ (3.34) | $86.11^{bc}$ (3.29) | $77.77^{b}$ (3.13) | $72.22^{b}$ (3.02) | $66.66^{b}$ (2.91) |
| $T_2$ | *Lecanicillium lecanii* | $100^{a}$ (1.58) | $97.22^{a}$ (3.48) | $88.88^{b}$ (3.34) | $77.77^{b}$ (3.13) | $72.22^{b}$ (3.02) | $66.66^{b}$ (2.91) |
| $T_3$ | Flubendiamide | $80.55^{b}$ (1.47) | $72.22^{c}$ (3.02) | $66.66^{d}$ (2.91) | $50.00^{c}$ (2.54) | $41.66^{d}$ (2.34) | $33.33^{c}$ (2.12) |
| $T_4$ | *B. bassiana*+ Flubendiamide | $94.44^{a}$ (1.58) | $88.88^{b}$ (3.34) | $80.55^{c}$ (3.18) | $72.22^{b}$ (3.02) | $58.33^{c}$ (2.73) | $36.11^{c}$ (2.19) |
| $T_5$ | *L. lecanii*+ Flubendiamide | $100^{a}$ (1.58) | $94.44^{ab}$ (3.43) | $86.11^{bc}$ (3.29) | $80.55^{b}$ (3.18) | $69.44^{b}$ (2.97) | $58.33^{b}$ (2.73) |
| $T_6$ | Control | $100^{a}$ (1.58) | $100^{a}$ (3.53) | $97.22^{a}$ (3.48) | $97.22^{a}$ (3.48) | $94.44^{a}$ (3.43) | $91.66^{a}$ (3.39) |
| | Mean | 95.37 | 90.27 | 84.26 | 73.07 | 68.05 | 58.79 |
| | SEd | 0.9339 | 0.7923 | 0.6862 | 0.5443 | 0.4944 | 0.6086 |
| | CD (P=0.05) | 1.9906 | 1.6888 | 1.4626 | 1.1860 | 1.0538 | 1.3260 |

DAS - Days after spraying

Data on surviving no. of larvae are mean values and those in parenthesis are $\sqrt{x+0.5}$ transformed values

Figures followed by same letters are on par

NS: Non-significant at 5% level of significance

**Table 2. Efficacy of Flubendiamide and Entomopathogenic fungus on the survival rate of Fall Armyworm larvae in field**

| | | Larval count per 25 plants | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T. No.** | **Treatment Details** | **Pre-treatment count** | **1 DAS** | **Larval reduction (%)*** | **3 DAS** | **Larval reduc** | 5 DAS | **Larval reduction** | **7 DAS** | **Larval reduction (%)*** | **9 DAS** | **Larval reduction (%)*** |
| $T_1$ | *Beauveria bassiana* | 41.33$^{b}$ | 7.60$^{bc}$ (3.70) | 77.25 | 7.31$^{bc}$ (3.57) | 79.00 | 5.12$^{c}$ (3.39) | 82.49 | 4.49$^{b}$ (3.42) | 85.56 | 3.85$^{b}$ (3.24) | 83.51 |
| T2 | *Lecanicillium lecanii* | 40.22$^{b}$ | 8.20$^{ab}$ (3.90) | 72.45 | 7.91$^{ab}$ (3.77) | 74.63 | 6.62$^{b}$ (3.64) | 76.33 | 4.49$^{b}$ (3.42) | 78.44 | 3.82$^{ab}$ (3.39) | 75.78 |
| T3 | Flubendiamide | 41.11$^{a}$ | 6.60$^{d}$ (3.00) | 82.44 | 6.78$^{d}$ (2.69) | 87.13 | 4.42$^{e}$ (2.50) | 95.94 | 3.86$^{d}$ (2.34) | 89.79 | 3.34$^{d}$ (2.12) | 85.24 |
| T4 | *B. bassiana* + Flubendiamide | 40.76$^{a}$ | 7.50$^{c}$ (3.60) | 80.00 | 7.45$^{c}$ (3.50) | 82.09 | 5.00$^{d}$ (3.16) | 84.78 | 4.24$^{c}$ (3.00) | 86.76 | 3.73$^{c}$ (2.78) | 87.45 |
| T5 | *L. lecanii* + Flubendiamide | 46.22$^{b}$ | 7.59$^{bc}$ (3.84) | 78.88 | 6.85$^{bc}$ (3.70) | 80.75 | 5.32$^{bc}$ (3.53) | 81.45 | 4.49$^{b}$ (3.46) | 83.69 | 3.86$^{b}$ (3.27) | 80.33 |
| T6 | Control | 40.55$^{b}$ | 20.45$^{a}$ (4.09) | 10.23 | 18.25$^{a}$ (3.96) | 12.87 | 16.65$^{a}$ (3.84) | 13.75 | 15.31$^{a}$ (3.67) | 17.43 | 14.67$^{a}$ (3.50) | 19.55 |
| | Mean | 41.70 | | 66.71 | | 69.41 | | 72.46 | | 73.61 | | 71.98 |
| | SEd | | 0.3333 | | 0.4303 | | 0.4603 | | 0.5213 | | 0.5606 | |
| | CD (P= 0.05) | | 0.7263 | | 0.9376 | | 1.376 | | 1.1260 | | 1.4230 | |

DAS - Days after spraying
Data on surviving no. of larvae are mean values and those in parenthesis are $\sqrt{x + 0.5}$ transformed values
Figures followed by same letters are on par
NS: Non-significant at 5% level of significance

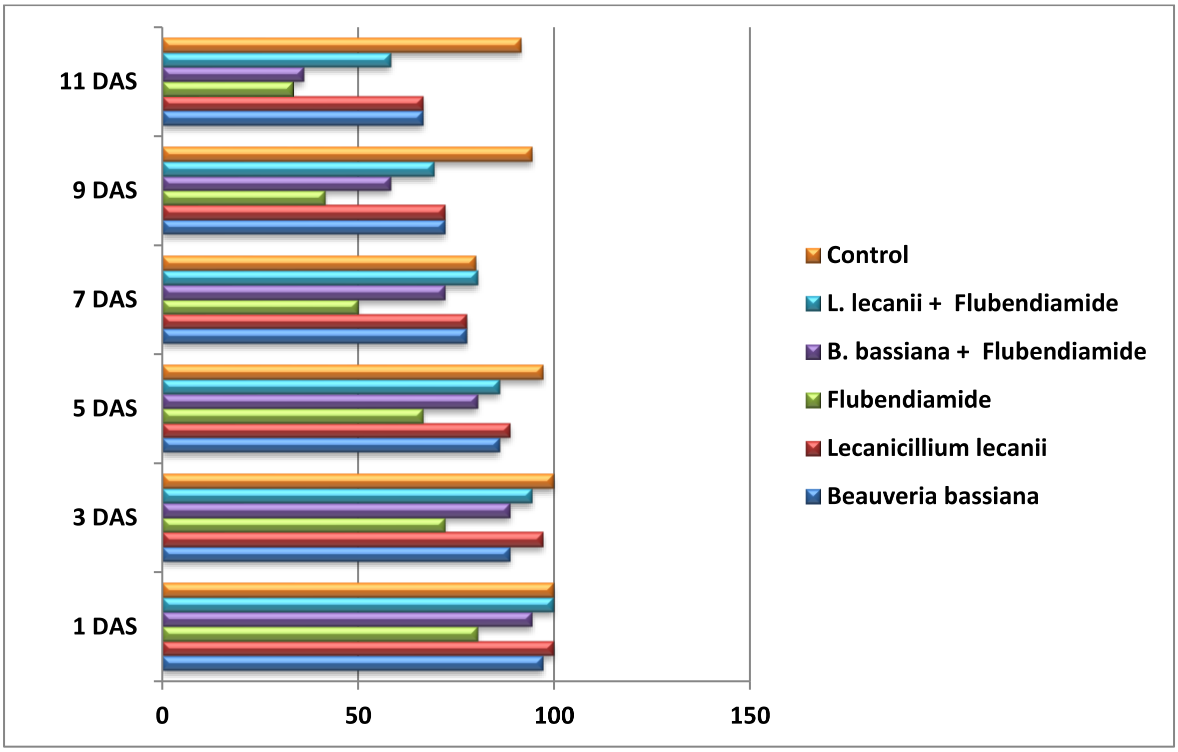


**Figure 1**. Efficacy of Flubendiamide and Entomopathogenicfungus on the survival rate of Fall Armyworm larvae in laboratory.

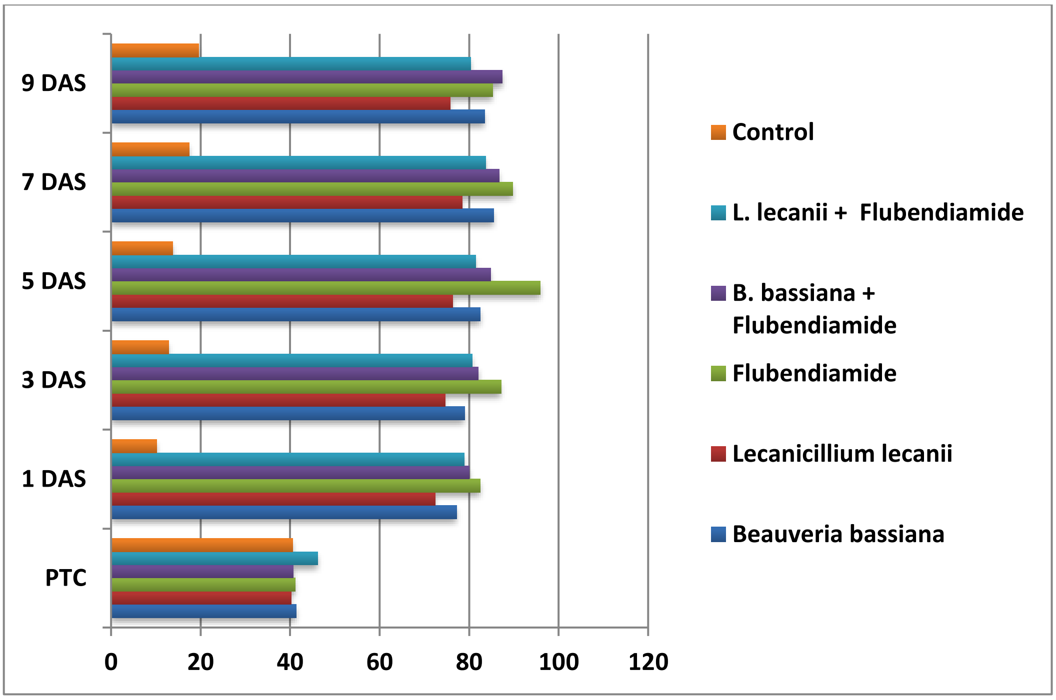


**Figure 2.** Efficacy of Flubendiamide and Entomopathogenic fungus on the survival rate of Fall Armyworm larvae in field.

***Efficacy of Flubendiamide and Entomopathogenic fungus on the survival rate of Fall Armyworm larvae in laboratory***

The table assesses the efficacy of various treatments on the survival rate of Fall Armyworm larvae measured at different days after spraying (DAS). The treatments include *Beauveria bassiana* ($T_1$), *Lecanicillium lecanii* ($T_2$), Flubendiamide ($T_3$), and their combinations ($T_4$ and $T_5$), along with a control group ($T_6$). $T_1$ and $T_2$ emerged as the most effective, starting with survival rates of 11.66 and 12.00, respectively, on Day 1, and maintaining higher rates throughout the study. In contrast, Flubendiamide ($T_3$) showed the lowest effectiveness, with survival rates decreasing sharply from 9.66 on Day 1 to just 4.00 by Day 11. Combination treatments ($T_4$ and $T_5$) demonstrated moderate effectiveness, beginning at 11.33 and 12.00 but ultimately declining over time. All treatments exhibited a general decline in survival rates, yet $T_1$ and $T_2$ consistently outperformed $T_3$, which experienced a significant drop. The control group $T_6$ remained stable, indicating no changes in untreated larvae.Statistical analysis showed that $T_1$ and $T_2$ had significantly higher survival rates than $T_3$, $T_4$, $T_5$, and $T_6$, especially in the initial days. Overall, *Beauveria bassiana* and *Lecanicillium lecanii* are identified as the most effective treatments, while Flubendiamide is less favorable. These findings can guide pest management strategies targeting Fall Armyworm larvae.

***Efficacy of Flubendiamide and Entomopathogenic fungus on the survival rate of Fall Armyworm larvae in field***

The study evaluates the effectiveness of various pest management treatments over time, focusing on both biological agents and a chemical insecticide. The treatments assessed are *Beauveria bassiana*$T_1$, *Lecanicillium lecanii* $T_2$, Flubendiamide $T_3$, combinations of these biological agents with Flubendiamide $T_4$ and $T_5$, and a control group $T_6$ with no treatment.

The control group maintained the highest initial population counts, beginning at 16.75 and declining gradually to 11.75 by day 9. This gradual decrease indicates that without intervention, pest populations can remain stable but will still decline slightly over time. It underscores the importance of treatment for effective management. Flubendiamide$T_3$had the highest initial count of 19.25 but experienced a sharp decline, reaching only 4 by day 9. While Flubendiamide is highly effective for immediate population reduction, the drastic decrease raises concerns about its sustainability. Such a rapid decline may lead to potential pest rebounds if subsequent applications are not carefully timed. The combinations of *B. bassiana* with Flubendiamide$T_4$ and *L. lecanii* with Flubendiamide$T_5$ showed intermediate effectiveness. $T_4$ started at 17.5 and decreased to 7.25, while $T_5$ began at 16.75 and ended at 10.25. These results suggest that combining biological and chemical treatments can yield effective reductions while potentially offering more sustainable outcomes compared to using Flubendiamide alone.

Both biological agents displayed more consistent effects. $T_1$ decreased from 15.75 to 10 and $T_2$ from 16.5 to 11 by day 9. These treatments maintained higher counts than Flubendiamide by the study's end, indicating that they may provide a more gradual and sustainable approach to pest management. This slower reduction could help maintain a balance in the ecosystem.

**Statistical Significance**: The use of statistical letters to denote significance highlights that Flubendiamide$T_3$ is statistically different from other treatments, underscoring its unique capacity for rapid control. However, the significant differences in population counts across treatments suggest varied efficacy, with biological agents showing a more stable and sustainable reduction.

## DISCUSSION

The combination of insecticides at a low dose and an entomopathogenic fungus can work synergistically to increase pest insect mortality. This combination is particularly advantageous because it decreases the insecticide dose applied, reduces environmental contamination, and decreases pest resistance.The present study indicated that fluorine derived Flubendiamide exhibits insecticidal properties against fall armyworm larvae (FAW).The fungus *B. bassiana, L. lecanii* and Flubendiamide interact synergistically when treating with FAW larvae. The results from both laboratory and field studies demonstrate significant differences in the efficacy of various treatments on the survival rate of Fall Armyworm (FAW) larvae, highlighting the potential of entomopathogenic fungi compared to synthetic insecticides. In the laboratory setting, *Beauveria bassiana*$T_1$and *Lecanicillium lecanii*$T_2$ consistently showed the highest survival rates across all time points, indicating their effectiveness as biological control agents. In contrast, Flubendiamide $T_3$ exhibited a marked decline in survival rates, dropping from 9.66 on Day 1 to just 4.00 by Day 11, showcasing its limited efficacy against FAW larvae. This trend aligns with findings which demonstrated that entomopathogenic fungi not only provide a sustainable approach to pest management but also exhibit lower toxicity to non-target organisms compared to chemical pesticides[22]. Flubendiamide's performance was particularly poor, with survival rates declining sharply from 19.25 at the pre-count to just 4.00 by Day 9. This decline indicates that its effectiveness wanes rapidly under field conditions, supporting the concerns raised regarding the short residual activity of synthetic insecticides and the development of resistance in pest populations [23].

Previous research has shown that natural fluorine derived compounds usnic acid possesses insecticidal properties against a variety of arthropods, including maxillopods, lepidopterans, mosquitoes, and beetles [24]. However, the enhancement of mortality was modest and required high concentrations (0.1%) [25]. The addition of fluorine to usnic acid reduced the concentration needed for a stable insecticidal effect [26]. Halogen atoms, including fluorine, enhance the efficacy and range of organic insecticides, while halogen-containing pyrethroids impact insect immunity through oxidative stress [27]. Fluoride ions act as enzymatic poisons, inhibiting various enzymes, which can disturb crucial metabolic processes like glycolysis and protein synthesis [27]. This can lead to significant weight loss in insects and decreased resistance to pathogens [28]. The combination of Flubendiamide and *B. bassiana* provided strong and stable synergy, comparable to that seen with *L. lecanii* and Flubendiamide insecticide in field conditions.

Field trials indicated that the synergy between *B. bassiana* and Flubendiamide was observed under dry and hot climate conditions, which are generally unfavorable for fungal development [29]. It was noted that while *B. bassiana* and *L. lecanii* are present in FAW populations, the percentage of naturally infected larvae remains low (<0.01%) [30]. The combined treatment significantly increased both the mortality rate and total mortality compared to treatments with the fungus alone. This combination is expected to reduce plant defoliation by disturbing larval development [31, 32]. Notably, the insecticide flubendiamide alone cause mortality or alter susceptibility to fungi in locusts or lepidopterans [33], indicating selectivity in flubendiamide effects on FAW. The combination of flubendiamide and *B. bassiana* isolate demonstrates a stable synergistic effect on the mortality of FAW larvae and field conditions. The primary reasons for this synergy appear to be the additional toxic effects and developmental delays, leading to decreased cellular immunity [34]. This combination may hold promise for developing highly efficient multicomponent products against fall armyworm larvae during their foraging period.

## CONCLUSIONS

This study demonstrates that the combination of the fluorine-containing insecticide flubendiamide and the entomopathogenic fungus *Beauveria bassiana* significantly enhances the mortality of Fall Armyworm (FAW) larvae. Our findings indicate a stable synergistic effect between flubendiamide and *B. bassiana*, leading to improved pest control under both laboratory and field conditions. While flubendiamide alone offers rapid initial mortality, its effectiveness diminishes over time, highlighting the potential for rebound pest populations. In contrast, the biological agent's *B. bassiana* and *L. lecanii* provide a more sustained reduction in larval populations.

The results suggest that the integration of flubendiamide with *B. bassiana* not only boosts immediate insecticidal effects but also disrupts larval development and immunity, enhancing overall efficacy. This combined approach could offer a promising strategy for sustainable pest management, reducing reliance on chemical insecticides and mitigating the risk of resistance development in FAW populations. Future research should explore optimal application strategies and further investigate the ecological impacts of these combined treatments, paving the way for innovative pest control solutions.

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## CONFLICT OF INTERESTS

The Authors declare that there is noconflictofinterest.

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