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BIO-EFFICACY OF *Bacillus thuringiensis* BERLINER WITH INSECTICIDES AGAINST FALL ARMYWORM, *Spodoptera frugiperda* (J. E. SMITH) UNDER LABORATORY CONDITIONS

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ABSTRACT

A laboratory experiment was conducted at the Biological Control Research Laboratory, ICAR Unit-9, Anand Agricultural University, Anand, Gujarat, during November 2025 to evaluate the bio-efficacy of *Bacillus thuringiensis*, applied alone and in combination with selected insecticides, against fall armyworm. Insecticides were evaluated individually at their recommended concentration and in combination with *B. thuringiensis* at half of their recommended concentration. The combinations of *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC, *B. thuringiensis* 1% WP + broflanilide 20 SC, as well as the individual applications of chlorantraniliprole 18.5 SC, tetraniliprole 18.18 SC, isocycloseram 18.1 SC and broflanilide 20 SC, were the most effective treatments, each resulting in cent per cent larval mortality after 96 hours. The combination of *B. thuringiensis* 1% WP + isocycloseram 18.1 SC recorded 96.67 per cent larval mortality, followed by *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC with 90.00 per cent mortality, whereas *B. thuringiensis* 1% WP applied alone resulted in 70.00 per cent larval mortality.
***Keywords* :** *Bacillus thuringiensis*, *Spodoptera frugiperda*, Fall armyworm, Bio-efficacy.

Introduction

Maize (*Zea mays* Linnaeus) is often regarded as the "Queen of Cereals" owing to its wide adaptability and superior genetic yield potential among cereal crops (Jeyaraman, 2017). Globally, it ranks as the third most important cereal crop, extensively grown across various agro-climatic regions. It is grown in roughly 165 countries (Badhai *et al.*, 2020). Globally, maize occupied 204.23 million hectares during 2023-24, with production of 1,241.56 million tonnes and an average productivity of 5,962 kg/ha (Anonymous, 2024). In India, during 2025-26, maize was cultivated on 14.41 million hectares, including 9.86 million hectares under *kharif* and 3.49 million hectares under *rabi*. The country produced 55.09 million tonnes of maize with an average productivity of 3,824 kg/ha (Anonymous, 2026).

Maize cultivation is constrained by several biotic and abiotic stresses, among which insect pests are the major biotic factor responsible for substantial yield losses. In India, nearly 130 insect pest species infest maize (Atwal & Dhaliwal, 2002). The major pests include stem borer, *Chilo partellus* (Swinhoe); pink stem borer, *Sesamia inferens* (Walker); armyworm, *Mythimna separata* (Walker); cob borer, *Helicoverpa armigera* (Hübner); bark beetle, *Anthracophora crucifera* (Olivier); leaf-eating caterpillar, *Spodoptera litura* (Fabricius); blister beetle, *Cylindrothorax audouini* (Haag-Rutenberg); grasshopper, *Epacromia dorsalis* (Thunberg); surface grasshopper, *Chrotogonus* sp.; aphid, *Rhopalosiphum maidis* (Fitch); white grub, *Holotrichia consanguinea* (Blanchard); termites, *Odontotermes* sp. and *Microtermes* sp. along with several other economically important insect pests.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is an invasive alien pest and one of the most destructive insect pests of maize. Native to the tropical and subtropical regions of the Americas, it is a highly migratory, nocturnal species characterized by high reproductive potential, voracious feeding behaviour and the absence of diapause (Sah *et al.*, 2019; Naganna *et al.*, 2020). Since its first report in West and Central Africa in 2016 (Goergen *et al.*, 2016), the pest has rapidly spread across more than 40 African countries, causing annual crop losses exceeding 6.2 billion USD (Khan & Midega, 2019). The pest was first reported in India on maize in Karnataka during 2018 (Sharanabasappa *et al.*, 2018a) and subsequently spread to several Indian states as well as other Asian countries, posing a serious threat to maize production across the region (Balla *et al.*, 2019; Chen *et al.*, 2023).

Following its infestation of maize, fall armyworm was also reported on sugarcane in Maharashtra (Chormule *et al.*, 2019). It is an extremely polyphagous pest, feeding on 353 host plant species belonging to 76 families, with a preference for Poaceae, Asteraceae and Fabaceae, and infesting cereals, pulses, vegetables, fibre crops and several other economically important crops (Montezano *et al.*, 2018; Anonymous, 2018). Females lay around 1,000 eggs in clusters on maize plants and the larvae pass through six instars over 14-19 days, causing severe feeding damage before pupating in the soil. Adult females generally survive longer than males, with the complete life cycle lasting about 32-46 days depending on environmental conditions (Prasanna *et al.*, 2018a, 2018b; Sharanabasappa *et al.*, 2018b).

Heavy reliance on chemical insecticides for maize pest management has raised concerns regarding environmental safety, human health and insecticide resistance. *Bacillus thuringiensis* Berliner, a Gram-positive entomopathogenic bacterium producing insecticidal crystal proteins, is an effective and environmentally benign alternative for the management of several insect pests, particularly lepidopteran larvae (Palma *et al.*, 2014). Its integration with compatible insecticides may enhance pest control, reduce chemical inputs and delay resistance development.

In view of the serious damage caused by fall armyworm, *S. frugiperda,* to maize, growing concern over the excessive reliance on chemical insecticides and the need for sustainable pest management, the present study was undertaken to evaluate the bio-efficacy of *B. thuringiensis* in combination with insecticides against the pest under laboratory conditions. The findings of the study will contribute to the development of effective and environmentally sustainable management strategies for fall armyworm in maize.

Materials and Methods

The study on the bio-efficacy of *B. thuringiensis* in combination with selected insecticides against fall armyworm, *S. frugiperda* was conducted at the Biological Control Research Laboratory, ICAR Unit-9, Anand Agricultural University, Anand, Gujarat, India, during 2025. The experiment was conducted in a Completely Randomized Design comprising ten treatments, including one untreated control. Three repetitions were maintained for each treatment. The details of the treatments are presented below:

Table 1 : Treatments detail

Tr. No.	Treatments	Quantity of formulations required (ml or g/L of water)
T_1	*B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC	2 + 0.15
T_2	*B. thuringiensis* 1% WP + tetraniliprole 18.18 SC	2 + 0.25
T_3	*B. thuringiensis* 1% WP + isocycloseram 18.1 SC	2 + 0.3
T_4	*B. thuringiensis* 1% WP + broflanilide 20 SC	2 + 0.13
T_5	Chlorantraniliprole 18.5 SC	0.3
T_6	Tetraniliprole 18.18 SC	0.5
T_7	Isocycloseram 18.1 SC	0.6
T_8	Broflanilide 20 SC	0.25
T_9	*B. thuringiensis* 1% WP	4.0
T_{10}	Control	-

Mass rearing of fall armyworm

In order to establish the initial culture, the larvae of the fall armyworm were collected from the field and brought to the Biological Control Research Laboratory, ICAR Unit-9, AAU, Anand. Each of them was placed in a separate plastic vial (Diameter: 50 mm, height: 40 mm) having pin-sized holes on the cap. Every day, the larvae were fed pieces of fresh maize leaves and frass was routinely disposed of. After pupation, the pupae were collected and transferred to Petri plates (Diameter: 90 mm). Before adult emergence, they were placed inside transparent oviposition cages (length: 30 cm, breadth: 30 cm, height: 30 cm). Approximately 30-

day-old maize plants were placed in a conical flask (capacity: 250 ml) containing 150 ml of water. At the neck region of the conical flask, cotton wool was inserted around the plant to prevent the fall of adults into the conical flask. The conical flask containing the maize plant was placed in the cages to serve as a substrate for oviposition. A cotton swab soaked in a 5 per cent honey solution was placed in the cage to provide food for the adult moths. Maize plants were replaced every two days and examined for the presence of eggs. Egg masses were carefully transferred to cylindrical plastic containers (Diameter: 20 cm, height: 7 cm) covered at the top with black muslin cloth to prevent escape of larvae and to maintain favourable conditions. Food was provided daily to the larvae and replaced every 24 hours. After reaching the third instar, the larvae were individually transferred into plastic vials (Diameter: 50 mm, height: 40 mm) to prevent cannibalism and subsequently used for mass culturing and bio-efficacy trial.

Bio-efficacy of *B. thuringiensis* in combination with insecticides

The spray solution was prepared by combining the required quantity of *B. thuringiensis* spore suspension (1×10^8 spores/ml) with the selected insecticide. Fresh maize leaves were cut into small pieces and treated with the respective spray solutions with the help of a hand sprayer having a one-litre capacity. The treated leaves were then allowed to dry under a fan. Treated leaves were then placed inside plastic vials (Diameter: 50 mm, height: 40 mm). Ten third-instar larvae of fall armyworm were sourced from the mass culture and introduced into plastic vials. To prevent cannibalism, a single larva was placed in each plastic vial. Before being placed in plastic vials, the larvae were starved for 10 hours. Larval mortality was recorded after 24, 48, 72 and 96 hours.

The per cent larval mortality was calculated using the following formula.

$$\text{Larval mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

Statistical Analysis

The data on per cent larval mortality were analyzed using analysis of variance (ANOVA). Prior to statistical analysis, the percentage mortality data were subjected to arcsine transformation. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at the 5 per cent level of significance.

Results and Discussion

The data on larval mortality of *S. frugiperda* recorded at 24, 48, 72 and 96 hours after treatment are presented in Table-2.

Larval Mortality after 24 hours

At 24 hours after exposure of larvae to treated leaves, mortality data showed a significant difference among treatments. The highest mortality of 56.67 per cent was recorded in tetraniliprole 18.18 SC, which was statistically at par with isocycloseram 18.1 SC (53.33%) and broflanilide 20 SC (50.00%). The latter treatment was also statistically similar to chlorantraniliprole 18.5 SC (43.33%). The treatment of chlorantraniliprole 18.5 SC did not differ significantly from *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC (40.00%), *B. thuringiensis* 1% WP + broflanilide 20 SC (40.00%) and *B. thuringiensis* 1% WP + isocycloseram 18.1 SC (36.67%). The larval mortality in the treatment of *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC was 26.67 per cent, whereas mortality in *B. thuringiensis* 1% WP alone was 20.00 per cent. The untreated control recorded no larval mortality (0.00%).

Larval Mortality after 48 hours

At 48 hours post-treatment, larval mortality differed significantly among treatments (Table-1). The highest mortality (76.67%) was recorded in tetraniliprole 18.18 SC, which was statistically comparable to isocycloseram 18.1 SC (70.00%). The treatment of isocycloseram 18.1 SC was also at par with broflanilide 20 SC (66.67%) and chlorantraniliprole 18.5 SC (63.33%). The latter treatment was statistically at par with *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC (56.67%). The treatment of *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC was statistically not different from *B. thuringiensis* 1% WP + broflanilide 20 SC (53.33%) and *B. thuringiensis* 1% WP + isocycloseram 18.1 SC (50.00%). Moreover, *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC (40.00%) and *B. thuringiensis* 1% WP alone (36.67%) were at par and exhibited comparatively lower larval mortality. The untreated control recorded nil larval mortality.

Table 2: Effect of *B. thuringiensis* in combination with insecticides on mortality of fall armyworm, *S. frugiperda*

Tr. No.	Treatments	Quantity of formulations required (ml or g/L of water)	Larval mortality (%) at indicated hours			
			24	48	72	96
T_1	*B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC	2.00 + 0.15	30.98^{d} (26.67)	39.22^{e} (40.00)	52.75^{de} (63.33)	74.64^{b} (90.00)
T_2	*B. thuringiensis* 1% WP + tetraniliprole 18.18 SC	2.00 + 0.25	39.22^{c} (40.00)	48.83^{cd} (56.67)	58.98^{cd} (73.33)	88.97^{a} (100.00)
T_3	*B. thuringiensis* 1% WP + isocycloseram 18.1 SC	2.00 + 0.30	37.21^{c} (36.67)	44.99^{d} (50.00)	56.77^{de} (70.00)	83.16^{ab} (96.66)
T_4	*B. thuringiensis* 1% WP + broflanilide 20 SC	2.00 + 0.13	39.22^{c} (40.00)	46.90^{d} (53.33)	58.98^{cd} (73.33)	88.97^{a} (100.00)
T_5	Chlorantraniliprole 18.5 SC	0.30	41.14^{bc} (43.33)	52.75^{bc} (63.33)	66.12^{bc} (83.33)	88.97^{a} (100.00)
T_6	Tetraniliprole 18.18 SC	0.50	48.83^{a} (56.67)	61.20^{a} (76.67)	77.35^{a} (93.33)	88.97^{a} (100.00)
T_7	Isocycloseram 18.1 SC	0.60	46.90^{a} (53.33)	56.77^{ab} (70.00)	68.83^{ab} (86.67)	88.97^{a} (100.00)
T_8	Broflanilide 20 SC	0.25	44.98^{ab} (50.00)	54.76^{b} (66.67)	68.83^{ab} (86.67)	88.97^{a} (100.00)
T_9	*B. thuringiensis* 1% WP	4.00	26.55^{e} (20.00)	37.21^{e} (36.67)	48.83^{e} (56.67)	56.77^{c} (70.00)
T_{10}	Control	-	0.91^{f} (0.00)	0.91^{f} (0.00)	0.91^{f} (0.00)	0.91^{d} (0.00)
S.Em. ±			1.42	1.56	2.71	3.01
CD at 5%			4.17	4.61	7.99	8.88
C.V. (%)			6.88	6.10	8.40	6.96

Notes: (1) Figures outside the parentheses are arcsine transformed values and those inside the parentheses are original values
(2) Treatment means followed by the same letter within a column are not significantly different by Duncan's New Multiple Range Test at 5% level of significance

Larval Mortality after 72 hours

At 72 hours after exposure of larvae to treated food, significant variations in larval mortality were observed among the treatments. Tetraniliprole 18.18 SC (93.33%) emerged as the most effective treatment, as it recorded the highest larval mortality. However, it was at par with isocycloseram 18.1 SC (86.67%) and broflanilide 20 SC (86.67%), both of them were also statistically similar to chlorantraniliprole 18.5 SC (83.33%). Furthermore, chlorantraniliprole 18.5 SC was statistically comparable with *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC (73.33%) and *B. thuringiensis* 1% WP + broflanilide 20 SC (73.33%), which were also statistically at par with *B. thuringiensis* 1% WP + isocycloseram 18.1 SC (70.00) and *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC (63.33%). The last two treatments were at par with *B. thuringiensis* 1% WP alone (56.67%), which showed comparatively lower mortality. The untreated control did not exhibit any larval mortality (0.00%).

Larval Mortality after 96 hours

The data on larval mortality recorded at 96 hours after exposure of larvae to treated leaves are presented in Table-1, which revealed that *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC, *B. thuringiensis* 1% WP + broflanilide 20 SC, chlorantraniliprole 18.5 SC, tetraniliprole 18.18 SC, isocycloseram 18.1 SC and broflanilide 20 SC were the most effective treatments, each recording cent per cent larval mortality. All of them remained statistically at par with *B. thuringiensis* 1% WP + isocycloseram 18.1 SC (96.67%). The last treatment was statistically comparable with *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC (90.00%). The treatment of *B. thuringiensis* 1% WP (70.00%) showed comparatively lower mortality, whereas the untreated control recorded no larval mortality (0.00%).

Earlier, Idrees *et al*. (2022) reported that broflanilide 100 SC caused maximum larval mortality of *S. frugiperda*. Belay *et al.* (2012) found that larval mortality of *S. frugiperda* by chlorantraniliprole exceeded 80 per cent by 96 hours after treatment. Gowtham *et al.* (2022) also reported that the combined application of *B. thuringiensis* and chlorantraniliprole was more effective against *S. frugiperda* than the application of *B. thuringiensis* alone, indicating the superiority of the combined treatment. Hence, the present findings corroborate earlier findings.

Conclusion

The laboratory bioassay conducted against third-instar larvae of *S. frugiperda* revealed significant differences in larval mortality among the treatments throughout the observation period. Larval mortality increased progressively with the increase in exposure time in all the treatments. After 72 hours, Tetraniliprole 18.18 SC (93.33%), isocycloseram 18.1 SC (86.67%) and broflanilide 20 SC (86.67%) recorded significantly higher larval mortality of *S. frugiperda* as compared to other treatments. Among the combination treatments, *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC and *B. thuringiensis* 1% WP + broflanilide 20 SC recorded 73.33 per cent mortality and they were at par with *B. thuringiensis* 1% WP + isocycloseram 18.1 SC (70.00%) and *B. thuringiensis* 1% WP + chlorantraniliprole 18.5 SC (63.33%). The treatment of *B. thuringiensis* 1% WP alone recorded 56.67 per cent mortality, whereas no larval mortality was observed in the untreated control. At 96 hours after treatment, cent per cent larval mortality was recorded in tetraniliprole 18.18 SC, broflanilide 20 SC, isocycloseram 18.1 SC, chlorantraniliprole 18.5 SC, *B. thuringiensis* 1% WP + tetraniliprole 18.18 SC and *B. thuringiensis* 1% WP + broflanilide 20 SC.

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