

Comparative Laboratory and Field Bioefficacy of Seven Binary Insecticide Formulations Against a Field Population of *Spodoptera frugiperda* in Punjab, Pakistan

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ABSTRACT

Fall armyworm (*Spodoptera frugiperda*) threatens maize in Pakistan, and growers rely on binary premixed insecticides for which local efficacy data are scarce. We evaluated seven binary formulations against a Sahiwal-derived field population in laboratory bioassays and a field experiment. Third-, fourth- and fifth-instar larvae were exposed to five concentrations (0.25–4× the recommended field rate; three cups of ten larvae per treatment), and mortality was recorded up to 168 h. Field plots (three blocks) were assessed 3, 7 and 14 days after treatment (DAT); blocks and repeated measures were not modeled. Third-instar mortality rose from 22.7% at 0.25× to 77.7% at 4× (partial $\eta^2 = 0.74$ –0.78 across instars). LC_{50} values ranged from 0.86 to 1.74× the recommended rate with overlapping fiducial limits; because exposure route (leaf dip versus diet) is confounded with formulation, no product is ranked as more potent, and LC_{90} estimates lie beyond the tested range. Pupation of fifth-instar survivors fell from 82.3% (control) to 33–38%. In the field, treated plots had 1.02–1.15 larvae per plant at 14 DAT versus 4.37 in controls (about 73–77% fewer), without differences among formulations when pooled; significant insecticide × concentration interactions make this pooled comparison incomplete. Control mortality (up to 17.7%) and unrecovered field-method details limit interpretation. These results are a local benchmark, not evidence of resistance status, synergism or yield protection.

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1. INTRODUCTION

Maize (*Zea mays* L.) is among the most widely grown cereals worldwide (Prasanna et al., 2018). In Pakistan it contributes about 2.9% of agricultural value added and 0.7% of gross domestic product (Government of Pakistan, 2024) and is widely grown in Punjab province.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is native to the Americas and has invaded Africa and Asia since 2016 (Goergen et al., 2016; Sharanabasappa et al., 2018). It was first recorded in Pakistan in 2019 (Gilal et al., 2020; Khan & Ali, 2025). Larvae feed inside the maize whorl, which limits contact with foliar sprays, and insecticides remain the main tool available to growers in many regions (Prasanna et al., 2018).

Intensive insecticide use has selected for resistance in *S. frugiperda* on several continents, including resistance to diamide insecticides (Richardson et al., 2020; Tay et al., 2023). Field-evolved resistance to insecticides has now been reported from Punjab, Pakistan (Khan & Ali, 2025), and chlorantraniliprole resistance and its fitness costs have been described in recent studies (Abbas et al., 2025; Salamat et al., 2025).

Binary premixed insecticides, which combine two active ingredients in one product, are widely marketed for fall armyworm control. They are intended to broaden the spectrum or speed of action, but a mixture is not necessarily

synergistic: unless each component is tested alone, additive, antagonistic and synergistic interactions cannot be distinguished (Zhang et al., 2022). Repeated use of products that share a mode of action (MoA) maintains selection pressure on that target, even when the second partner differs (Insecticide Resistance Action Committee [IRAC], 2026). Laboratory and field studies in India, East Africa and Pakistan have reported the efficacy of several insecticide groups against *S. frugiperda*, with substantial differences among products and populations (Deshmukh et al., 2020; Sisay et al., 2019; Tajdar et al., 2025).

It is therefore known that insecticides remain central to fall armyworm management in Pakistan and that resistance is emerging. It remains unknown how commercially available binary formulations perform against a Sahiwal-derived population when compared on a common basis across larval instars, and whether laboratory toxicity corresponds to suppression of the pest in the field. We addressed this gap with laboratory dose–response bioassays on third-, fourth- and fifth-instar larvae and a replicated field experiment with seven binary formulations. The objectives were to (a) estimate dose-dependent mortality and lethal concentrations and times, (b) quantify sublethal effects on growth, pupation, adult emergence and deformity, and (c) measure suppression of larval abundance and leaf injury in the field. These objectives correspond to three research questions: (1) How do dose-dependent mortality and lethal concentrations, expressed relative to each product’s label rate, vary among the seven formulations and across larval instars? (2) What sublethal effects do survivors show in pupation, adult emergence and deformity? (3) Does laboratory toxicity correspond with suppression of larval abundance and leaf injury in the field? Because single-active-ingredient treatments were not included, the study was not designed to test synergism.

2. MATERIALS AND METHODS

2.1 Study Sites and Experimental Design

Laboratory work was done in the Integrated Pest Management (IPM) Laboratory, Department of Entomology, University of Agriculture, Faisalabad (UAF). The field experiment was done at the Entomology Research Farm of the same department (31.43° N, 73.07° E, elevation 185 m), which is representative of the agroecological conditions of central Punjab. Both components were conducted between November 2025 and February 2026. The laboratory bioassay followed a completely randomized design (CRD) with three replications per treatment, and the field experiment followed a randomized complete block design (RCBD) with three replications (Gomez & Gomez, 1984). The experimental unit was the cup in the laboratory (ten larvae per cup, $n = 3$ true replicates per treatment) and the plot in the field ($n = 3$ true block replicates per treatment); larvae within cups and plants within plots were subsamples to avoid pseudoreplication.

2.2 Insect Collection and Culture

Larvae and infested plants were collected from maize fields in the Sahiwal and Chichawatni tehsils of district Sahiwal and taken to the IPM Laboratory, where larvae were isolated and reared at 25 ± 2 °C, $65 \pm 5\%$ relative humidity and a 14:10 h light:dark photoperiod. The field-collected generation was fed untreated maize leaves, and later generations were reared on an artificial diet (25–30 g chickpea flour, 2 mL linseed oil, 15 g brewer’s yeast, 1 g sorbic acid, 1 g ascorbic acid, two multivitamin capsules and 25–30 g agar in 700 mL boiling water). Because no genetically defined strain was established, the insects are referred to as the Sahiwal field population. Collection dates, the number of fields and larvae sampled, crop stage, pesticide history of the sampled fields, species confirmation, the generation tested and the criteria used to assign instars are not documented in our records and are therefore not reported.

2.3 Insecticides and Concentrations

Seven commercially available binary premixed insecticides representing different chemical combinations were tested (Table 1). Products were stored at 15–25 °C, away from direct sunlight, in accordance with label recommendations. Five concentrations were prepared for each product by serial dilution of a stock solution with distilled water. The middle concentration (1×) corresponds to the label rate per acre diluted in 100 L of water for liquid and water-dispersible products (e.g., 70 mL per 100 L = 0.07%) and to the corresponding amount for granules; the tested range was therefore 0.25–4× the label rate. The 2× and 4× concentrations were used to construct complete dose–response curves and are not application recommendations.

Table 1. Binary Premixed Insecticides Tested, with IRAC Mode-of-Action (MoA) Groups and Tested Concentrations

Product (company)	Formulation	Active ingredients (IRAC MoA group)	Label rate per acre	1× concentration (tested range, 0.25–4×)
Forward (Four Brothers Group)	40% SC	Chlorantraniliprole 20% (28) + lufenuron 20% (15)	70 mL	0.07% (0.0175–0.28%)
Rector Super (Ag Pharma)	0.35% G	Fipronil 0.3% (2B) + emamectin benzoate 0.05% (6)	8 kg	45 mg (11.25–180 mg)
Spinal (Ag Pharma)	14% WDG	Chlorantraniliprole 4% (28) + thiamethoxam 10% (4A)	200 g	0.20% (0.05–0.80%)
Sphire (Evyol Group)	0.26% G	Chlorantraniliprole 0.2% (28) + emamectin benzoate 0.06% (6)	8 kg	55 mg (13.75–220 mg)
Taragen (Tara Group)	11.6% SC	Chlorantraniliprole 9% (28) + emamectin benzoate 2.6% (6)	80 mL	0.08% (0.02–0.32%)
Astris Gold (Swat Agro)	20% SC	Lambda-cyhalothrin 10% (3A) + lufenuron 10% (15)	40 mL	0.04% (0.01–0.16%)
Milzon (Tara Group)	0.30% G	Chlorantraniliprole 0.25% (28) + emamectin benzoate 0.05% (6)	7 kg	80 mg (20–320 mg)

Note: SC = suspension concentrate; G = granules; WDG = water-dispersible granules. IRAC MoA groups: 28 = diamides (ryanodine receptor modulators); 6 = glutamate-gated chloride channel allosteric modulators; 15 = benzoylureas (chitin biosynthesis inhibitors, type 0); 2B = phenylpyrazoles (GABA-gated chloride channel blockers); 4A = neonicotinoids (nicotinic acetylcholine receptor competitive modulators); 3A = pyrethroids (sodium channel modulators) (IRAC, 2026). Percent values for liquid and water-dispersible products are % of formulated product.

2.4 Laboratory Bioassays

Liquid and water-dispersible formulations were tested by a leaf-dip method: maize leaf sections were dipped in the test solution for 10–15 s, air-dried and placed in plastic cups lined with moist filter paper. Granular formulations (Rector Super, Sphire and Milzon) were mixed into the artificial diet. Ten larvae of a given instar were placed in each cup, with three cups per treatment combination ($n = 3$ true replicates, 30 larvae total per concentration) and an untreated control, which entered the analysis as the eighth level of the insecticide factor. Formulation is therefore completely and inherently confounded with exposure route (leaf residue for liquid and water-dispersible products, diet incorporation for granules), and direct potency comparisons between these groups are therefore not valid. Mortality of third-, fourth- and fifth-instar larvae was recorded at 12, 24, 48, 72 and 168 h, and larvae that did not respond to gentle probing were scored as dead. The diet mass per cup in which granule amounts were mixed is not documented in our records. Mortality was corrected for control mortality with Abbott's (1925) formula before probit analysis. Control mortality up to 20% is commonly accepted when Abbott's correction is applied; the acceptance criterion of the bioassay protocol followed here, and how runs exceeding it would have been handled, are not documented in our records. All control values in this study were below 20% (Table 2). Surviving larvae were transferred to untreated leaves or diet and followed for growth, pupation, adult emergence and deformity (fifth instar only for the last three).

2.5 Growth and Sublethal Measurements

Relative growth rate (RGR) and efficiency of conversion of ingested food (ECI) were calculated for surviving third-, fourth- and fifth-instar larvae following Waldbauer (1968). RGR was calculated as weight gained divided by the product of mean larval weight and time, and ECI as weight gained divided by weight of food ingested, multiplied by 100. The relative consumption index (RCI), efficiency of growth on ingested food (EGI) and efficiency of larval inhibition (ELI) were excluded because their calculation formulas could not be recovered or independently verified from the experimental records. Percent larval/pupal deformity, pupation and adult emergence were recorded for fifth-instar larvae based on the true experimental units (cups).

2.6 Field Experiment

The seven formulations at five concentrations and an untreated control were applied to maize plots in a factorial arrangement with three replicates. Liquid and water-dispersible products were applied with a calibrated knapsack sprayer, and granules were applied into the plant whorl. Larval density and leaf injury were recorded before treatment and at 3, 7 and 14 DAT. Plot dimensions, row and plant spacing, the number of plants sampled per plot, crop stage and infestation level at application, spray volume, weather conditions and the scoring scale behind the leaf infestation severity index (recorded with a standard fall armyworm damage-rating scale) are not documented in our records and are not reported. Percent reduction in larval density was calculated relative to the pre-treatment count and relative to the untreated control.

2.7 Statistical Analysis

Percent mortality, growth indices, sublethal variables and field variables were analyzed by two-way ANOVA (insecticide $\times$ concentration) with Tukey's honestly significant difference (HSD) test (Tukey, 1953) at $P < 0.05$ in Statistix 8.1. Each observation time was analyzed separately; repeated observations on the same cups or plots are therefore not independent, and the 168-h endpoint is used as the primary laboratory outcome. Separate analyses by date were retained because the analyses were originally run for each date and pest abundance and plant growth changed non-linearly over the 14-day field period; repeated-measures or mixed-effects models were not fitted for this report (see Limitations). Because the untreated control is a level of the insecticide factor, a significant insecticide effect mainly reflects the contrast between treated and untreated units; differences among the seven formulations are judged from Tukey's test and from the ANOVA of percent reduction (seven formulations only). Effect sizes are reported as partial η^2 (SS effect / [SS effect + SS error]). Block was not included as a term in the historical field ANOVAs, so the field analysis was structured as a completely randomized design by date; a re-analysis with a block term was not performed for this report (see Limitations). Concentration–mortality data (30 larvae per concentration, pooled over the three cups) were analyzed by probit analysis in SPSS to estimate LC_{50} , LC_{90} , LT_{50} , slope and 95% fiducial limits (FL) (Finney, 1971). Because concentrations are expressed in different units across products, LC estimates are also reported as multiples of each product's recommended field rate ($\times$ FR). Relative-potency ratios and their approximate 95% confidence intervals were derived from the reported fiducial limits (standard error of $\ln LC_{50} = [\ln \text{upper} - \ln \text{lower}] / [2 \times 1.96]$, covariance ignored) and are indicative only.

3. RESULTS

3.1 Control Mortality

Mean control mortality remained below 20% at every interval (Table 2), reaching 17.7% at 168 h in third and fourth instars and 7.7% in fifth instars. Control values at 168 h varied widely among cups in third and fourth instars (SE = 7.62–7.65), meaning that baseline uncertainty is high; control-corrected estimates for these early instars at late endpoints should therefore be interpreted with caution, because 17.7% approaches the 20% level commonly accepted with Abbott's correction (Section 2.4).

Table 2. Mean ($\pm$ SE) Percent Mortality in the Untreated Control, by Instar and Time after Treatment ($n = 3$ Cups per Instar)

Instar	12 h	24 h	48 h	72 h	168 h
Third	1.33 $\pm$ 0.77	3.33 $\pm$ 1.35	7.67 $\pm$ 3.51	12.00 $\pm$ 5.36	17.67 $\pm$ 7.65
Fourth	1.33 $\pm$ 0.77	3.33 $\pm$ 1.35	7.00 $\pm$ 3.55	12.33 $\pm$ 5.32	17.67 $\pm$ 7.62
Fifth	1.00 $\pm$ 0.53	2.33 $\pm$ 0.83	2.67 $\pm$ 0.83	5.33 $\pm$ 0.91	7.67 $\pm$ 0.96

Note: SE = standard error. High control mortality and wide standard errors at 168 h in third and fourth instars require cautious interpretation of corrected mortality outcomes.

3.2 Mortality, Concentration, and Formulation

At 168 h, both the insecticide factor and concentration had significant effects in all three instars (Table 3). The insecticide $\times$ concentration interaction was not significant in any instar ($P = 0.334, 0.503$ and 0.051), so no difference in dose–response slope among formulations could be detected. Concentration had a large effect (partial $\eta^2 = 0.74$ – 0.78). The insecticide effect (partial $\eta^2 = 0.51$ – 0.72) includes the untreated control, and Tukey's HSD detected no significant pairwise differences among the seven formulations in any instar at 168 h when pooled across doses; all differed significantly from the untreated control.

Pooled over the seven formulations, mean mortality at 168 h rose with concentration from 22.7% at $0.25\times$ to 77.7% at $4\times$ in third instars, from 22.1% to 77.7% in fourth instars and from 22.1% to 71.9% in fifth instars. Mortality at the label rate ($1\times$) was about 52.5–52.7% in all three instars. In third instars at $4\times$, mortality accumulated from 17.1% at 12 h to 77.7% at 168 h.

Table 3. Two-Way ANOVA of Percent Mortality at 168 h (Error df = 80; Cups as Experimental Units)

Instar	Insecticide $F(7, 80)$	P	Partial η^2	Concentration $F(4, 80)$	P	Partial η^2	Interaction $F(28, 80)$	P
Third	12.01	<0.001	0.51	55.79	<0.001	0.74	1.12	0.334
Fourth	12.56	<0.001	0.52	58.44	<0.001	0.75	0.98	0.503
Fifth	29.92	<0.001	0.72	71.86	<0.001	0.78	1.61	0.051

Note: The insecticide factor comprises the seven formulations and the untreated control. F values, degrees of freedom, and partial η^2 are based on cups as experimental units.

3.3 Lethal Concentrations

Probit models fitted adequately in all cases (χ^2 goodness-of-fit $P > 0.05$; Table S2). Expressed relative to each product's recommended rate, 168-h LC_{50} values ranged from 0.86 to 1.74 $\times$ across products and instars (Table 4, Figure 1), that is, close to the label rate. In third instars, LC_{50} was 1.05 $\times$ for Taragen, 1.24 $\times$ for Astris Gold and Spinal, 1.41 $\times$ for Forward (all leaf dip), and 1.34 $\times$ for Rector Super, 1.64 $\times$ for Milzon and 1.74 $\times$ for Sphire (all diet incorporation), and the 95% fiducial limits overlapped broadly. By the approximate ratio method, only Sphire differed from Taragen (ratio 1.67, 95% CI 1.03–2.71); all other comparisons included 1. This contrast is between a diet-incorporated granular product and a leaf-dip product and is therefore confounded with exposure route. In original units Astris Gold had the lowest LC_{50} (0.0495%), but its label rate (0.04%) is lower than those of the other liquid products (0.07–0.20%), so this does not indicate higher potency relative to the label rate. Estimated LC_{90} values, ranging from 5.5 to 29 $\times$ FR, lie completely beyond the highest concentration tested (4 $\times$); they are mathematical extrapolations and must not be interpreted as experimentally demonstrated toxicity levels.

Table 4. LC_{50} at 168 h as a Multiple of the Recommended Field Rate ($\times$ FR) with 95% Fiducial Limits, Grouped by Exposure Route

Product	Third instar	Fourth instar	Fifth instar
Leaf dip (liquid and water-dispersible formulations)			
Taragen	1.05 (0.78–1.40)	1.14 (0.83–1.55)	0.86 (0.60–1.24)
Astris Gold	1.24 (0.90–1.70)	1.17 (0.86–1.58)	0.89 (0.63–1.26)
Spinal	1.24 (0.94–1.64)	1.27 (0.96–1.67)	1.02 (0.73–1.42)
Forward	1.41 (1.09–1.83)	1.41 (1.10–1.82)	1.11 (0.83–1.48)
Diet incorporation (granular formulations)			
Rector Super	1.34 (1.01–1.78)	1.46 (1.08–1.99)	1.10 (0.78–1.54)
Sphire	1.74 (1.18–2.57)	1.54 (1.08–2.20)	1.11 (0.76–1.60)
Milzon	1.64 (0.98–2.75)	1.62 (1.02–2.58)	1.11 (0.69–1.79)

Note: $\times$ FR = multiple of the recommended field rate (Table 1). Granular products (Rector Super, Sphire, Milzon) were tested by diet incorporation and the other products by leaf dip, so formulation and exposure route are completely confounded and cross-group potency comparisons are not valid. LC_{90} estimates lie beyond the highest concentration tested (4 $\times$ FR) and are mathematical extrapolations, not observed toxicity levels.

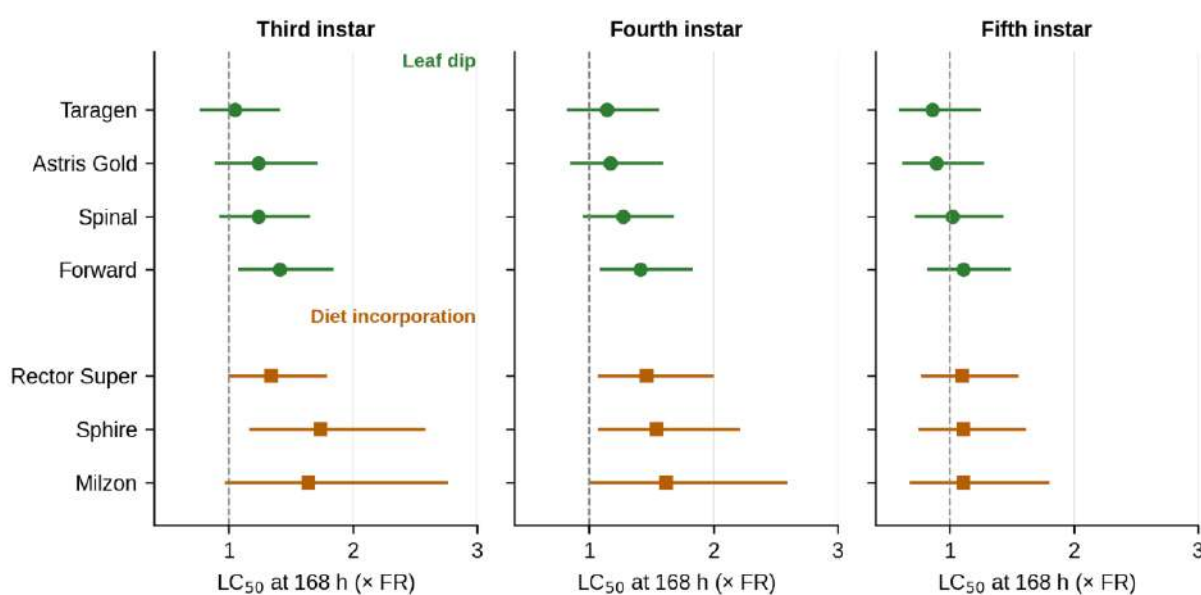


Figure 1. LC_{50} Values at 168 h ($\times$ Recommended Field Rate, FR) with 95% Fiducial Limits for Third-, Fourth-, and Fifth-Instar *S. frugiperda* Exposed to Seven Binary Formulations

Note: Circles = leaf-dip products; squares = diet-incorporated granular products. Formulation and exposure route are confounded, so the two groups should not be compared directly. The dashed line marks 1 $\times$ FR. Values are plotted from Table 4. LC_{90} estimates lie beyond the highest concentration tested (4 $\times$ FR) and are not shown.

3.4 Comparison among Instars

LC₅₀ point estimates were lower in fifth than in third instars for all seven formulations (third-to-fifth ratios 1.21–1.58), but the 95% fiducial limits overlapped for every product and the approximate ratio intervals included 1. Mean mortality at each concentration was similar across instars. The lethal time (LT₅₀) at 4× was 43.8–67.3 h in fifth instars. Third-instar LT₅₀ at 4× was estimable within 168 h for only Rector Super (70.2 h) and Taragen (78.2 h); the other estimates exceeded the observation period or were imprecise.

3.5 Growth and Sublethal Effects

In fifth-instar survivors, pupation (insecticide $F(7, 80) = 31.48$; concentration $F(4, 80) = 78.52$; both $P < 0.001$; interaction $P = 0.029$) and adult emergence ($F(7, 80) = 11.48$ and $F(4, 80) = 79.84$; both $P < 0.001$; interaction $P = 0.002$) were strongly reduced (Table 5). Pupation was 33.3–38.3% in treated larvae versus 82.3% in the control, and adult emergence was 22.7–32.2% versus 55.5%. Larval/pupal deformity was numerically higher in treated groups (12.2–27.2%) than in the control (4.6%), but the insecticide effect was not significant ($F(7, 80) = 1.30$, $P = 0.262$), the concentration effect was ($F(4, 80) = 8.01$, $P < 0.001$), and all treatments shared one Tukey group because variances were large.

Growth and nutritional indices of survivors are restricted to verified metrics. RGR was lower in treated than in control larvae in third instars (0.02 versus 0.04) and fourth instars (0.03–0.04 versus 0.05) but not in fifth instars (0.01 versus 0.02; shared Tukey group). ECI was lower than the control for six of seven formulations in third instars (7.76–8.69 versus 11.36; Sphire did not differ) and did not differ from the control in fourth or fifth instars.

Table 5. Mean ($\pm$ SE) Percent Deformity, Pupation, and Adult Emergence of Fifth-Instar Larvae, Pooled over Five Concentrations (Cups as Experimental Units)

Treatment	Larval/pupal deformity (%)	Pupation (%)	Adult emergence (%)
Forward	17.66 $\pm$ 7.05 a	38.33 $\pm$ 7.05 b	32.18 $\pm$ 9.12 b
Rector Super	26.39 $\pm$ 10.54 a	37.33 $\pm$ 6.62 b	26.64 $\pm$ 6.82 b
Spinal	27.19 $\pm$ 10.69 a	36.00 $\pm$ 6.16 b	23.65 $\pm$ 7.47 b
Sphire	21.79 $\pm$ 8.76 a	36.67 $\pm$ 6.13 b	25.43 $\pm$ 6.52 b
Taragen	12.22 $\pm$ 5.00 a	33.33 $\pm$ 5.97 b	22.77 $\pm$ 7.01 b
Astris Gold	19.02 $\pm$ 7.84 a	34.33 $\pm$ 5.71 b	22.71 $\pm$ 6.35 b
Milzon	24.63 $\pm$ 8.87 a	37.00 $\pm$ 5.71 b	25.29 $\pm$ 5.65 b
Control	4.58 $\pm$ 1.22 a	82.33 $\pm$ 0.96 a	55.49 $\pm$ 0.98 a

Note: Means within a column sharing a letter are not significantly different (Tukey's HSD, $P < 0.05$). All values are based on cups as the experimental units ($n = 3$).

3.6 Field Experiment

Plots were similar before treatment (larvae per plant: insecticide $F(7, 79) = 1.29$, $P = 0.266$; leaf severity index: $F(7, 79) = 1.16$, $P = 0.333$). After treatment, larval abundance fell in all treated plots and was 1.02–1.15 larvae per plant at 14 DAT, compared with 4.37 in the untreated control (Table 6). The percent reduction in larvae relative to the untreated control at 14 DAT was 73.3–76.7%, with no significant difference among the seven formulations ($F(6, 70) = 0.33$, $P = 0.920$) when pooled across doses, but a large concentration effect ($F(4, 70) = 72.88$, $P < 0.001$; partial $\eta^2 = 0.81$). The leaf infestation severity index at 14 DAT was 5.00–5.15 in treated plots versus 15.79 in the control; concentration had a very large effect on this index ($F(4, 80) = 608.74$, $P < 0.001$; partial $\eta^2 = 0.97$). Highly significant insecticide $\times$ concentration interactions were noted for both larval abundance ($P = 0.002$) and leaf injury severity ($P < 0.001$) at 14 DAT, which indicates that concentration responses were not identical across products. Because product-by-dose interaction means were unavailable from original software printouts, results pooled over concentrations must be treated as indicative only, because pooled formulation means may mask product-specific concentration responses.

Table 6. Field Results at 14 Days after Treatment (DAT) by Formulation, Pooled over Five Concentrations (Mean $\pm$ SE; Plots as Experimental Units, $n = 3$)

Formulation	Larvae per plant, 14 DAT	Leaf severity index, 14 DAT	Reduction in larvae vs. control, 14 DAT (%)
Forward	1.03 $\pm$ 0.17 b	5.13 $\pm$ 0.76 b	75.83 $\pm$ 4.09 a
Rector Super	1.12 $\pm$ 0.16 b	5.15 $\pm$ 0.82 b	74.34 $\pm$ 3.63 a
Spinal	1.08 $\pm$ 0.19 b	5.15 $\pm$ 0.80 b	75.03 $\pm$ 4.06 a
Sphire	1.07 $\pm$ 0.17 b	5.00 $\pm$ 0.73 b	75.83 $\pm$ 3.88 a
Taragen	1.10 $\pm$ 0.17 b	5.04 $\pm$ 0.74 b	74.76 $\pm$ 3.77 a
Astris Gold	1.15 $\pm$ 0.15 b	5.09 $\pm$ 0.76 b	73.34 $\pm$ 3.57 a
Milzon	1.02 $\pm$ 0.17 b	5.04 $\pm$ 0.72 b	76.72 $\pm$ 3.86 a
Untreated control	4.37 $\pm$ 0.12 a	15.79 $\pm$ 0.19 a	—

Note: Means within a column sharing a letter are not significantly different (Tukey's HSD, $P < 0.05$). Because the insecticide $\times$ concentration interactions were significant, comparisons among products based on pooled means are strictly indicative. The scoring scale of the leaf severity index is not documented.

4. DISCUSSION

4.1 Dose Dependence and the Limits of Comparing Formulations

Concentration was the main source of variation in mortality, with large effects in every instar and no detectable difference in dose–response slope among formulations. At the label rate, mortality of third- to fifth-instar larvae at 168 h was about 52–53% under laboratory exposure, and 90% mortality was not reached within the tested range (LC_{90} 5.5–29 $\times$ the label rate, extrapolated). Laboratory exposure on treated leaves or in diet is not equivalent to field application, so these values should not be read as predicted field control.

The seven formulations could not be separated by Tukey's test, and ranking by LC_{50} depends on how concentration is expressed. In original units Astris Gold had the lowest LC_{50} , but this largely reflects its low label rate; relative to label rates, LC_{50} values were similar (0.86–1.74 $\times$) and their fiducial limits overlapped, with only Sphire differing from Taragen by an approximate ratio test. Sphire was also tested in diet, so exposure route and formulation cannot be separated. No formulation is therefore ranked as more potent. Because the study did not include each active ingredient alone, it cannot show whether the mixtures were additive, synergistic or antagonistic, or that complementary modes of action caused the observed mortality; designs that test components individually and estimate a co-toxicity coefficient are needed for that purpose (Zhang et al., 2022).

4.2 Larval Instar

Lower LC_{50} point estimates in fifth instars did not constitute clear evidence of greater susceptibility. Fiducial limits overlapped for every product, raw mean mortality at each concentration was similar across instars, and control mortality at 168 h was lower in fifth instars (7.7%) than in third and fourth instars (17.7%), which raises control-corrected mortality in fifth instars for the same raw mortality. The shorter LT_{50} at 4 $\times$ in fifth instars (43.8–67.3 h) may indicate faster action at high concentration, but larger larvae also eat more treated tissue, which can raise the dose ingested (Waldbauer, 1968). Contributions from cuticular penetration, cytochrome P450 monooxygenases, carboxylesterases, glutathione S-transferases and target-site differences at the ryanodine receptor were not measured and remain hypotheses for enzyme and molecular assays (Guo et al., 2024; Richardson et al., 2020).

4.3 Sublethal Effects

Survivors of treatment had strongly reduced pupation and adult emergence, consistent with developmental costs beyond acute mortality (Guedes et al., 2016). Deformity was numerically higher in treated groups but not significantly different among treatments, and growth indices responded differently among instars (RGR lower than the control in third and fourth but not fifth instars), so these data support an effect on pupation and emergence more clearly than on growth.

4.4 Field Performance

In the field, treated plots carried about one larva per plant at 14 DAT, a reduction of roughly three quarters relative to the control, and leaf injury scores fell from about 15 to about 5. Reductions did not differ among formulations when pooled, and concentration explained most of the variation in percent reduction. The significant insecticide $\times$ concentration interactions indicate that response to dose was not identical across products, but means by product and dose were not available. Because the field analyses did not model repeated measurements or blocks, and only three

replicates were used, the results are best viewed as indicative. No grain yield was analyzed, so effects on crop production cannot be inferred.

4.5 Resistance Context and Mode-of-Action Management

High mortality at high concentration does not establish susceptibility. Resistance status requires comparison with a susceptible reference strain or baseline and calculation of resistance ratios, which were not available here. Recent reports of field-evolved resistance and diamide resistance in Pakistan and elsewhere (Abbas et al., 2025; Khan & Ali, 2025; Salamat et al., 2025) mean that the present estimates should be treated as an initial dose–response benchmark for this population, to be compared with future monitoring.

Five of the seven products contain the diamide chlorantraniliprole (IRAC group 28), and three of these also contain emamectin benzoate (group 6); Spinal adds a neonicotinoid (4A) and Forward a benzoylurea (15) (Table 1). Alternating among brands that share group 28 is not MoA rotation. Astris Gold (3A + 15) and Rector Super (2B + 6) contain no diamide. IRAC advises alternating MoA groups between treatment windows rather than relying repeatedly on one group, including within mixtures (IRAC, 2026). The 2× and 4× concentrations were experimental; applications should follow label rates.

4.6 Limitations

The study used one geographic population collected once; findings may not apply to other Pakistani populations. No susceptible reference strain or historical baseline was available, so resistance ratios could not be calculated. Formulation and exposure route are completely confounded (leaf dip for liquid and water-dispersible products, diet incorporation for granules). No single-active-ingredient treatments were included, so synergism could not be assessed. Repeated observations on the same cups and plots were analyzed separately by time; neither blocks nor repeated measures were modeled, and the untreated control was entered as a level of the insecticide factor. Replicate-level re-analysis with binomial mixed models or survival models is a priority. Probit estimates and fiducial limits were derived from larvae pooled over cups (30 per concentration) rather than from cups as the experimental units, so the limits may be narrower than a cup-level analysis would give. Control mortality at 168 h in third and fourth instars (17.7%) approached the 20% level commonly accepted with Abbott's correction, and the acceptance criterion of the protocol followed is not documented. Field results come from a single experiment with three replicates; means by formulation, concentration and date were not available, and no yield or economic data were analyzed. No biochemical or molecular measurements were made, so mechanisms are hypothetical. Several methodological details (collection dates and numbers, instar assignment, generation tested, plot layout, application volume, the severity-index scale and the formulas for RCI, EGI and ELI) are not documented in our records, which limits reproducibility; in particular, because the scoring scale of the severity index could not be recovered, absolute severity values cannot be compared with those of other studies.

5. CONCLUSION

Under laboratory conditions, mortality of third- to fifth-instar *S. frugiperda* from Sahiwal increased strongly with concentration, with LC₅₀ values at 168 h close to the label rate (0.86–1.74×). Because liquid and water-dispersible products were tested by leaf dip and granular products in diet, no formulation can be ranked as more potent, and LC₉₀ estimates beyond the tested range must not be read as observed toxicity. Evidence that fifth instars were more susceptible than third instars was weak, and the relatively high control mortality of third and fourth instars at 168 h calls for cautious interpretation of late endpoints. Survivors showed reduced pupation and adult emergence. In the field, treated plots had about 73–77% fewer larvae than the control at 14 DAT without differences among formulations when pooled over doses; however, significant insecticide × concentration interactions, the absence of block and repeated-measures terms in the analysis, and unrecovered field-method details (including the severity-index scale) mean that these field results are indicative only. In answer to the research questions, no differences among formulations in dose-dependent toxicity were detected, and because no laboratory ranking was established, correspondence between laboratory and field rankings could not be assessed. These results provide an initial local benchmark; they do not show absence of resistance, synergism or protection of yield. Priorities are comparison with a susceptible reference strain, single-active-ingredient controls, replicate-level re-analysis with block and repeated-measures terms, recovery or re-measurement of the field-method details, enzyme and molecular assays, multi-site and multi-season trials with yield assessment, and label-compliant, MoA-based resistance management.

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