

Comparative laboratory efficacy of three commercial organophosphate insecticides against fall armyworm (*Spodoptera frugiperda*) larvae

Perbandingan efikasi laboratorium tiga insektisida organofosfat komersial terhadap larva ulat grayak (*Spodoptera frugiperda*)

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ABSTRACT

Fall armyworm, *Spodoptera frugiperda*, is a maize pest whose management remains dependent on synthetic insecticides. This study compared three organophosphate insecticides against third-instar *S. frugiperda* larvae. Chlorpyrifos, profenofos, and diazinon were evaluated at 5%, 20%, 50%, 75%, and 95% of the maximum label dose using a factorial completely randomized design with three replications of six larvae. Mortality was recorded for 168 h and corrected using Abbott's formula based on pooled control mortality. Insecticide formulation significantly affected mortality ($F_{2,30} = 39.06$, $P < 0.001$), whereas concentration ($F_{4,30} = 0.57$, $P = 0.687$) and the insecticide $\times$ concentration interaction ($F_{8,30} = 1.62$, $P = 0.162$) were not significant. Across concentrations, profenofos and diazinon caused mean mortalities of 90.4% and 93.2%, respectively, and both were more effective than chlorpyrifos, which caused 32.2% mortality. Exploratory probit analysis estimated LC_{50} values of 2.01% for profenofos and 0.71% for diazinon, but both were below the lowest concentration and had wide confidence intervals. Chlorpyrifos LC values were not estimable because its concentration–mortality slope was negative and non-significant. At 95% of the label dose, profenofos reached 50% corrected mortality within 24 h and diazinon within 48 h, whereas chlorpyrifos did not reach this threshold within 168 h. Profenofos and diazinon showed comparably high efficacy and outperformed chlorpyrifos.

ABSTRAK

Ulat grayak, *Spodoptera frugiperda*, merupakan hama jagung yang pengendaliannya masih bergantung pada insektisida sintesis. Penelitian ini membandingkan tiga insektisida organofosfat terhadap larva instar ketiga *S. frugiperda*. Klorpirifos, profenofos, dan diazinon diuji pada 5%, 20%, 50%, 75%, dan 95% dari dosis label maksimum menggunakan rancangan acak lengkap faktorial dengan tiga ulangan, masing-masing terdiri atas enam larva. Mortalitas diamati selama 168 jam dan dikoreksi menggunakan rumus Abbott berdasarkan mortalitas kontrol gabungan. Formulasi insektisida berpengaruh nyata terhadap mortalitas ($F_{2,30} = 39,06$; $P < 0,001$), sedangkan konsentrasi ($F_{4,30} = 0,57$; $P = 0,687$) dan interaksi insektisida $\times$ konsentrasi ($F_{8,30} = 1,62$; $P = 0,162$) tidak berpengaruh nyata. Pada seluruh konsentrasi, profenofos dan diazinon menghasilkan mortalitas rata-rata masing-masing sebesar 90,4% dan 93,2%, serta keduanya lebih efektif dibandingkan klorpirifos yang hanya menghasilkan mortalitas 32,2%. Analisis probit eksploratif memperkirakan LC_{50} sebesar 2,01% untuk profenofos dan 0,71% untuk diazinon, tetapi keduanya berada di bawah konsentrasi terendah yang diuji dan memiliki selang kepercayaan yang lebar. Nilai LC klorpirifos tidak dapat diestimasi karena kemiringan hubungan konsentrasi–mortalitasnya negatif dan tidak signifikan. Pada 95% dosis label, profenofos mencapai mortalitas terkoreksi 50% dalam 24 jam dan diazinon dalam 48 jam, sedangkan klorpirifos tidak mencapai ambang tersebut dalam 168 jam. Profenofos dan diazinon menunjukkan efikasi tinggi yang sebanding dan melampaui klorpirifos.

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INTRODUCTION

Maize (*Zea mays* L.) is an important food and feed crop whose production is frequently constrained by insect pests. Among these, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), has become a major threat to maize cultivation because of its broad host range, high reproductive capacity, strong dispersal ability, and intensive larval feeding. Following its expansion beyond the Americas, *S. frugiperda* spread rapidly across Africa and Asia, where it has caused substantial crop damage and created persistent challenges for maize pest management (Goergen et al., 2016; Day et al., 2017).

Synthetic insecticides remain an important component of fall armyworm management, particularly when rapid population suppression is required. Organophosphate insecticides are commonly used because of their broad-spectrum activity, relatively rapid action, and accessibility. These compounds inhibit acetylcholinesterase, resulting in the accumulation of acetylcholine at neuronal synapses, disruption of normal nervous-system function, and eventual insect death. However, repeated exposure to insecticides with the same mode of action imposes selection pressure on pest populations and may reduce their long-term effectiveness. Reduced susceptibility and resistance to several insecticide classes have already been documented in geographically distinct populations of *S. frugiperda* (Sparks & Nauen, 2015; Gutiérrez-Moreno et al., 2019; Zhang et al., 2021; Van den Berg & du Plessis, 2022).

Insecticide performance may vary among pest populations because of differences in prior insecticide exposure, cropping systems, local application practices, and other ecological or operational factors. Consequently, efficacy results obtained from one geographical population cannot necessarily be generalized to another. Periodic laboratory evaluation of locally collected populations is therefore important for identifying formulations that remain effective and for supporting more rational insecticide selection. Recent resistance monitoring has demonstrated spatial variation among Indonesian *S. frugiperda* populations, while field surveys in Banten Province have documented infestation intensities ranging from light to severe (Rubiana et al., 2026; Subagyo et al., 2025).

Previous studies have demonstrated substantial geographical variation in the susceptibility of *Spodoptera frugiperda* to synthetic insecticides. Populations from Puerto Rico and different regions of Mexico exhibited markedly different resistance levels to several insecticide classes, including chlorpyrifos (Gutiérrez-Moreno et al., 2019). Similarly, laboratory monitoring of five invasive populations from different regions of China revealed considerable variation in susceptibility to 14 insecticides and particularly high resistance ratios to chlorpyrifos (Zhang et al., 2021). Such variation may reflect differences in local selection pressure, insecticide-use patterns, pest-management practices, and other operational factors affecting insecticide performance (Van den Berg & du Plessis, 2022). These international findings, together with emerging evidence of spatial variation in Indonesia, reinforce the need to assess insecticide performance using locally derived populations and clearly defined commercial formulations. However, comparative evidence on the laboratory efficacy of chlorpyrifos-, profenofos-, and diazinon-based formulations against an Indonesian *S. frugiperda* population remains scarce. In particular, their relative performance has not been adequately characterized within a single experimental framework using equivalent fractions of their respective recommended label doses. Such a direct comparison is needed to distinguish formulation-level efficacy while avoiding unsupported conclusions about intrinsic active-ingredient toxicity or resistance status.

This study therefore compared the laboratory efficacy of three commercial organophosphate insecticides: chlorpyrifos, profenofos, and diazinon, against third-instar *S. frugiperda* larvae derived from a field population in Indonesia. The formulations were tested side by side at five standardized fractions of their respective maximum recommended label doses. Efficacy was assessed using Abbott-corrected mortality at 168 h, supported by exploratory concentration-mortality analysis and directly observed mortality development over time. By integrating these complementary response measures in a single bioassay, this study provides locally relevant comparative evidence for distinguishing the performance of commonly available commercial organophosphate formulations. Because the tested products differed in

active-ingredient concentration and formulation characteristics, the results represent differences in commercial-formulation efficacy rather than intrinsic active-ingredient toxicity. Moreover, because no susceptible reference strain was included, the findings were not intended to establish resistance status.

MATERIALS & METHODS

Study site and insect material

The study was conducted from December 2025 to May 2026 at the Basic Science and Plant Protection Laboratory, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Banten, Indonesia. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), larvae were collected from maize fields in Cimaung Village, Cikeusal District, Serang Regency, Banten, Indonesia. The field-collected population was maintained under laboratory conditions until the third generation, and third-instar larvae from this generation were used in the bioassay. The insect colony and bioassays were maintained under ambient laboratory conditions at approximately 24–34 °C with a natural photoperiod of approximately 12:12 h light:dark. Relative humidity was not monitored. The use of third-instar larvae from an early laboratory generation was consistent with established susceptibility-monitoring approaches for field-derived *S. frugiperda* populations, in which F1–F3 larvae are used to minimize prolonged laboratory adaptation while providing insects of a relatively uniform developmental stage (Bird et al., 2022; Thirawut et al., 2023).

The collection site represented a maize-growing area in which organophosphate insecticides were commonly used for fall armyworm management. However, complete records of previous insecticide applications and the exact number of founder insects were unavailable because the larvae were collected from farmers’ production fields rather than from a monitored experimental site.

Commercial insecticides and treatment preparation

Three commercial emulsifiable-concentrate organophosphate formulations were evaluated: chlorpyrifos 200 EC (200 g active ingredient [a.i.] L⁻¹; Dow AgroSciences, Indianapolis, IN, USA), profenofos 500 EC (500 g a.i. L⁻¹; Syngenta Crop Protection AG, Basel, Switzerland), and diazinon 600 EC (600 g a.i. L⁻¹; PT Petrokimia Kayaku, Gresik, Indonesia).

Table 1. Preparation of the commercial insecticide treatments

Standardized label-dose fraction (%)	Formulation concentration (mL L ⁻¹)	Formulation volume in 31.25 mL solution (μL)	Chlorpyrifos (mg a.i. L ⁻¹)	Profenofos (mg a.i. L ⁻¹)	Diazinon (mg a.i. L ⁻¹)
5	0.10	3.1	20	50	60
20	0.40	12.5	80	200	240
50	1.00	31.3	200	500	600
75	1.50	46.9	300	750	900
95	1.90	59.4	380	950	1,140

Note: The 100% label-dose level was standardized as 2.0 mL commercial formulation L⁻¹. Active-ingredient concentrations were calculated from the nominal concentrations of the respective commercial formulations.

For each formulation, the maximum recommended label rate was standardized as 2.0 mL formulation L⁻¹ and designated as the 100% label-dose level. Five treatment levels: 5%, 20%, 50%, 75%, and 95% of this standardized rate, were prepared, corresponding to formulation concentrations of 0.10, 0.40, 1.00, 1.50, and 1.90 mL L⁻¹, respectively. To prepare 31.25 mL of each treatment solution, 3.1, 12.5, 31.3, 46.9, or 59.4 μL of the respective commercial formulation was added to distilled water (Table 1). Treatment solutions were prepared immediately before use.

Experimental design

The experiment was arranged as a 3 × 5 factorial completely randomized design. The first factor was commercial insecticide formulation, consisting of chlorpyrifos, profenofos, and diazinon. The second factor was the standardized label-dose fraction, consisting of 5%, 20%, 50%, 75%, and 95%. Each of the 15 insecticide × concentration combinations was replicated three times. Five corresponding control combinations were included, one for each nominal concentration level, and each control was also replicated three times. The controls received distilled water without insecticide and were used to quantify natural mortality. Control treatments were not included as levels in the factorial analysis.

Each experimental unit consisted of six third-instar larvae, resulting in 18 larvae per insecticide × concentration combination. The study therefore comprised 60 experimental units: 45 insecticide-treated units and 15 control units, containing a total of 360 larvae.

Laboratory bioassay

The general bioassay structure, use of formulated insecticides, third-instar larvae, individual exposure containers, a distilled-water control, and mortality as a quantal response, was consistent with procedures commonly used in laboratory susceptibility studies of *S. frugiperda* (Bird et al., 2022; Thirawut et al., 2023). The food-treatment procedure used here, however, was specific to the present study.

Freshly prepared insecticide solutions were used to treat maize kernels by immersion for approximately 30 s. The kernels were then removed and allowed to air-dry briefly until no visible excess solution remained. Control kernels were treated with distilled water using the same procedure.

Each larva was maintained individually in a 60-mL plastic container to prevent interaction among larvae. Two treated maize kernels were supplied to each larva as food. Larval mortality was recorded at 24, 48, 72, 96, 120, 144, and 168 h after treatment. A larva was considered dead when it showed no coordinated movement after gentle stimulation with a fine brush. The number of dead and surviving larvae was recorded separately for each experimental unit. Individual containment, use of a water-treated control, and mortality assessment at the level of each exposed larva are consistent with established fall armyworm bioassay procedures (Bird et al., 2022; Thirawut et al., 2023).

Changes in larval movement, feeding activity, body coloration, and post-mortem appearance were documented descriptively. The morphological observations were not subjected to statistical analysis.

Mortality correction

Control mortality was pooled across the five control combinations because the authors confirmed that pooled rather than concentration-specific control mortality was used. At 168 h, 17 of 90 control larvae had died, corresponding to pooled control mortality of 18.89%. Mortality in each insecticide-treated experimental unit was corrected using Abbott's formula (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{T-C}{100-C} \times 100 \quad (1)$$

where *T* is the observed mortality in an insecticide-treated experimental unit (%) and *C* is the pooled control mortality (%). Corrected values below zero were set to zero.

For the analysis of final mortality, *C* was the pooled control mortality recorded at 168 h. For the descriptive time-course analysis, mortality at each observation time was corrected using the pooled control mortality recorded at the corresponding time.

Statistical analyses

Abbott-corrected mortality at 168 h was analyzed using a two-way analysis of variance to evaluate the effects of insecticide formulation, standardized label-dose fraction, and their interaction. The experimental unit was the replicate comprising six individually maintained larvae. When the insecticide main effect was significant, formulation means were compared using Tukey's honestly significant difference test at $P < 0.05$. Concentration means were not compared because the concentration main effect was not significant. Results are presented as mean $\pm$ standard deviation of three experimental units.

Exploratory concentration–mortality relationships were examined separately for each commercial formulation using probit regression in SPSS version 27. The cumulative number of dead larvae out of 18 exposed larvae at each concentration was used as the response, and concentration was transformed to $\log_{10}$ scale. The natural-response proportion was fixed at 0.1889, corresponding to pooled control mortality at 168 h. Median and 90% lethal concentrations (LC_{50} and LC_{90}) and their 95% confidence intervals were reported only when the fitted concentration–mortality slope was positive and statistically supported. Estimates falling outside the tested concentration range or associated with wide confidence intervals were regarded as exploratory.

Mortality development over time was summarized descriptively at the common 95% label-dose fraction. At each observation time, pooled mortality for each insecticide was corrected using pooled control mortality recorded at the same time. The first observed time at which corrected mortality reached or exceeded 50% was recorded as a descriptive indicator of the relative speed of action. No extrapolated LT_{50} or LT_{90} values were used as primary inferential outcomes.

The supplementary material provides replicate-level mortality data at 168 h, pooled cumulative time-course data, Abbott-correction calculations, and the statistical inputs and outputs used to generate the tables.

RESULTS & DISCUSSION

Effects of commercial insecticide formulation on larval mortality

Pooled control mortality increased from 0% at 24 h to 18.89% at 168 h. Because control mortality at the final observation exceeded 10%, treatment mortality at 168 h was corrected using Abbott's formula before statistical analysis. The corrected mortality of *Spodoptera frugiperda* larvae differed markedly among the three commercial organophosphate formulations (Table 2).

Table 2. Abbott-corrected mortality of *Spodoptera frugiperda* larvae at 168 h

Insecticide	5%	20%	50%	75%	95%	Mean across
Chlorpyrifos	52.1 $\pm$ 11.9	46.1 $\pm$ 50.4	18.7 $\pm$ 19.2	5.9 $\pm$ 10.3	38.4 $\pm$ 20.5	32.2 $\pm$ 28.9 ^b
Profenofos	72.6 $\pm$ 47.5	86.3 $\pm$ 23.7	93.2 $\pm$ 11.9	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	90.4 $\pm$ 23.1 ^a
Diazinon	79.5 $\pm$ 0.0	93.2 $\pm$ 11.9	100.0 $\pm$ 0.0	93.2 $\pm$ 11.9	100.0 $\pm$ 0.0	93.2 $\pm$ 10.0 ^a

Note: Values are mean $\pm$ SD of three experimental units, each containing six larvae. Concentrations represent percentages of the standardized maximum recommended label rate of 2.0 mL commercial formulation L⁻¹. Mortality was corrected using pooled control mortality at 168 h (17/90 larvae; 18.89%). Means in the overall-mean column followed by different letters differ significantly according to Tukey's HSD test at $P < 0.05$.

Insecticide formulation significantly affected corrected larval mortality ($F_{2,30} = 39.06$, $P < 0.001$), whereas standardized label-dose fraction ($F_{4,30} = 0.57$, $P = 0.687$) and the insecticide $\times$ concentration interaction ($F_{8,30} = 1.62$, $P = 0.162$) were not significant. Across the five concentrations, diazinon and profenofos produced mean mortalities of 93.2% and 90.4%, respectively. Tukey's HSD test showed no significant difference between these formulations ($P = 0.939$), whereas both caused significantly greater mortality than chlorpyrifos ($P < 0.001$).

The absence of a significant overall concentration effect should not be interpreted as evidence that insecticide concentration was biologically irrelevant. Rather, it reflects the contrasting concentration–mortality patterns of the tested formulations. Profenofos and diazinon already caused high mortality at the lowest tested concentration and approached a ceiling response at the higher concentrations. Chlorpyrifos, by contrast, showed low and highly variable mortality without a consistent increase across the concentration range. These different response patterns resulted in no uniform concentration effect across all three formulations.

The high mortality caused by profenofos and diazinon indicates that both commercial formulations remained effective against the tested laboratory-reared population under the present bioassay conditions. In contrast, chlorpyrifos produced lower and less consistent mortality. Previous studies have similarly demonstrated substantial differences in the performance of insecticides against *S. frugiperda*. Deshmukh et al. (2020) found that emamectin benzoate, chlorantraniliprole, and spinetoram were among the most effective insecticides against second-instar larvae under laboratory and field conditions in India. In Nepal, Karki et al. (2023) reported that a cypermethrin–chlorpyrifos mixture was less effective than chlorantraniliprole and emamectin benzoate under field conditions. Profenofos also showed measurable toxicity against Egyptian field populations, although its relative performance varied between populations and among the insecticide classes evaluated (Moustafa et al., 2024). These findings emphasize that insecticide performance depends on the tested population, formulation, exposure method, and local pest-management context.

Comparisons among studies must nevertheless be made cautiously because insect populations, larval stages, exposure methods, formulations, environmental conditions, and application rates may differ. In the present study, the products also contained different concentrations of active ingredient. The observed differences therefore represent the relative laboratory efficacy of the tested commercial formulations at equivalent fractions of their respective label rates, rather than a direct comparison of the intrinsic toxicity of chlorpyrifos, profenofos, and diazinon as pure active ingredients.

The low performance of chlorpyrifos may reflect product-specific characteristics, biological variability in the tested population, previous selection pressure, or differences in ingestion and exposure under the kernel-dipping bioassay. Resistance cannot be inferred because the study did not include a known susceptible reference strain, diagnostic concentrations, or a documented insecticide-use history for the source population. Consequently, the results demonstrate reduced relative efficacy of the tested chlorpyrifos formulation, but they do not establish chlorpyrifos resistance.

Exploratory concentration–mortality relationships

Probit analysis was conducted as a supplementary assessment of the concentration–mortality relationships at 168 h. Significant positive slopes were obtained for profenofos and diazinon, whereas chlorpyrifos produced a negative slope that was not statistically significant (Table 3). Probit analysis is commonly used for quantal insecticide-bioassay data because it relates the proportion of dead individuals to the logarithm of concentration and provides estimates of lethal concentrations, slopes, confidence intervals, and goodness-of-fit statistics (Finney, 1971; Robertson et al., 2007; Burgess et al., 2020).

The positive slopes for profenofos ($P = 0.008$) and diazinon ($P = 0.042$) indicated increasing predicted mortality with increasing formulation concentration. The Pearson goodness-of-fit tests were not significant, providing no evidence of gross lack of fit for either model. Nevertheless, this result should not be interpreted as proof that the models were highly precise because each model was based on only five concentration levels and relatively few intermediate mortality responses. Standard reporting of concentration–mortality bioassays includes the fitted slope and its standard error, LC estimates with confidence intervals, and goodness-of-fit statistics, as presented here (Burgess et al., 2020).

Table 3. Exploratory concentration–mortality probit estimates at 168 h

Insecticide	Slope $\pm$ SE	<i>P</i> for slope	Pearson χ^2 (df)	<i>P</i> for fit	LC ₅₀ , % label rate (95% CI)	LC ₉₀ , % label rate (95% CI)
Chlorpyrifos	-0.785 $\pm$ 0.408	0.054	4.500 (3)	0.212	Not estimable	Not estimable
Profenofos	1.283 $\pm$ 0.487	0.008	1.253 (3)	0.740	2.01 (0.004–6.05)	20.05 (7.28–91.13)
Diazinon	0.999 $\pm$ 0.491	0.042	2.265 (3)	0.519	0.71 (0.000–4.14)	13.57 (0.002–112.46)

Note: Concentrations are expressed as percentages of the standardized maximum recommended label rate. LC estimates are exploratory. The LC₅₀ estimates for profenofos and diazinon were below the lowest tested concentration of 5%, and several confidence intervals were wide. LC values were not reported for chlorpyrifos because its concentration–mortality slope was negative and not statistically significant.

The estimated LC₅₀ values were 2.01% of the standardized label rate for profenofos and 0.71% for diazinon. Both estimates were below the lowest concentration actually tested, which was 5% of the label rate. They therefore represent extrapolations beyond the experimental concentration range rather than interpolations from observations that bracketed 50% mortality. Robust concentration–mortality bioassays normally use a concentration series that spans low to high mortality and adequately defines the central portion of the sigmoidal response curve. For example, dose–response studies of *S. frugiperda* have used six to eight serial concentrations selected to produce responses ranging approximately from 0% to 100% mortality (Bird et al., 2022).

The wide confidence intervals further demonstrate the limited precision of the present estimates. This was particularly evident for diazinon, whose LC₉₀ confidence interval extended from 0.002% to 112.46% of the label rate. Confidence intervals around LC estimates become more precise when the tested concentrations generate sufficient observations near the target response level, especially around 50% mortality. Conversely, concentration levels that produce predominantly near-zero or near-complete mortality provide relatively little information for estimating the central portion and slope of a dose–response curve (Burgess et al., 2020).

The ceiling responses observed for profenofos and diazinon were consistent with their high efficacy in the factorial mortality analysis. Even at the lowest tested concentration, profenofos and diazinon produced corrected mortalities of 72.6% and 79.5%, respectively. Consequently, the study did not include enough low-concentration treatments to define the lower portion of their concentration–mortality curves. Future bioassays should therefore include several concentrations below 5% of the label rate and a larger number of larvae per concentration to obtain more stable LC₅₀ and LC₉₀ estimates.

A previous bioassay of profenofos against susceptible and field-derived Egyptian *S. frugiperda* populations produced positive concentration–mortality slopes and finite LC₅₀ estimates when five concentrations ranging from 2.5 to 100 mg L⁻¹ were tested using four replicates of 25 second-instar larvae. Profenofos LC₅₀ values were approximately 25.7 mg L⁻¹ across the susceptible, Fayoum, and Giza populations, although the associated LC₉₀ confidence limits were also relatively wide in some populations (Moustafa et al., 2024). Direct numerical comparison with the present results is inappropriate because that study expressed exposure as active-ingredient concentration, used a leaf-dipping method, evaluated second-instar larvae after 96 h, and included a susceptible reference strain. Nevertheless, it demonstrates that reliable probit estimation requires a concentration range capable of generating graded mortality rather than predominantly ceiling responses.

Chlorpyrifos showed a different response pattern. Mortality fluctuated across the tested concentrations and did not increase monotonically, resulting in a negative fitted slope of -0.785. Although its slope test produced *P* = 0.054, the principal problem was not merely that the value narrowly exceeded the conventional significance threshold; rather, the estimated direction of the relationship was biologically inconsistent with a conventional concentration-dependent toxic response. Accordingly, calculation and interpretation of LC₅₀ and LC₉₀ values for chlorpyrifos were not justified.

Variation in chlorpyrifos response among *S. frugiperda* populations has also been reported elsewhere. Bird et al. (2022) found approximately 2.1-fold variation in chlorpyrifos response among 11 Australian field populations and lower chlorpyrifos sensitivity in *S. frugiperda* than in a susceptible laboratory strain of *Helicoverpa armigera*. However, their study used technical-grade chlorpyrifos applied topically and therefore cannot be directly compared with the present kernel-dipping bioassay of a commercial formulation. The earlier findings nevertheless reinforce that organophosphate response can vary among populations and experimental systems.

The probit results support the high activity of profenofos and diazinon but do not provide sufficiently precise LC benchmarks for direct comparison between the two products. The LC estimates should therefore be regarded as exploratory indicators of formulation performance. They should not be used as diagnostic concentrations, resistance baselines, or evidence of susceptibility status because the experiment lacked a susceptible reference strain, did not adequately bracket 50% mortality for profenofos and diazinon, and produced no interpretable concentration–mortality relationship for chlorpyrifos.

Development of mortality over time

To describe the relative speed of action without relying on unstable extrapolated lethal-time estimates, mortality development was evaluated at the common 95% label-dose fraction. Mortality at each observation time was corrected using the pooled control mortality recorded at that same time (Table 4).

Table 4. Abbott-corrected mortality over time at 95% of the standardized label rate

Insecticide	24 h	48 h	72 h	96 h	120 h	144 h	168 h	First observation with ≥50% corrected mortality
Chlorpyrifos	11.1	9.1	30.2	28.6	30.4	30.4	38.4	>168 h
Profenofos	50.0	65.9	94.2	94.0	100.0	100.0	100.0	24 h
Diazinon	33.3	54.5	65.1	64.3	87.3	93.7	100.0	48 h

Note: Values are pooled Abbott-corrected mortality percentages based on 18 larvae per insecticide at each observation time. The pooled control mortalities used for correction were 0.0%, 2.2%, 4.4%, 6.7%, 12.2%, 12.2%, and 18.9% at 24, 48, 72, 96, 120, 144, and 168 h, respectively. The small decreases between some consecutive corrected values resulted from changes in pooled control mortality and do not indicate recovery of dead larvae. The designation >168 h indicates that corrected mortality did not reach 50% during the observation period.

At the 95% label-dose fraction, profenofos produced the earliest accumulation of mortality. Corrected mortality reached 50.0% at 24 h, exceeded 94% at 72 h, and reached 100% at 120 h. Diazinon reached 54.5% corrected mortality at 48 h and subsequently increased to 100% at 168 h. Chlorpyrifos did not reach 50% corrected mortality during the observation period and produced only 38.4% mortality at 168 h.

The increase in mortality with observation time demonstrates that insecticide efficacy cannot be characterized adequately from a single early assessment. Recent laboratory bioassays with *Spodoptera frugiperda* have likewise shown that cumulative mortality generally increases as the post-treatment interval is extended and that the apparent ranking of insecticides may change between 24, 48, and 72 h (Idrees et al., 2022). Fiaboe et al. (2023) also reported formulation-dependent mortality development over 24–96 h, demonstrating that insecticides causing similar final mortality may differ substantially in the timing of their effects. These findings support the use of repeated mortality observations when comparing insecticides with potentially different temporal response patterns.

The rapid mortality associated with profenofos in the present study is broadly consistent with its demonstrated activity against *S. frugiperda* in other laboratory bioassays. Mumtaz et al. (2024), using second-instar larvae and label-recommended application rates, recorded profenofos mortalities of approximately 48%, 61%, 69%, and 83.5% at 12, 24, 48, and 72 h, respectively. In the present study, corrected mortality reached 50.0% at 24 h and 94.2% at 72 h. Although the

numerical values cannot be compared directly because the studies differed in larval instar, insect population, formulation, exposure procedure, and dose expression, both datasets indicate progressive mortality following profenofos exposure rather than an exclusively immediate response.

The chlorpyrifos response contrasted sharply with the findings of Mumtaz et al. (2024), who reported approximately 52% mortality at 12 h and 98% at 72 h at the recommended dose. In the present study, chlorpyrifos produced only 11.1% corrected mortality at 24 h and remained below 40% at 168 h. This discrepancy indicates that chlorpyrifos should not be characterized universally as either rapidly or slowly acting solely on the basis of one bioassay. Instead, its observed efficacy may depend on the susceptibility of the tested population, larval developmental stage, formulation characteristics, exposure route, consumed dose, and other experimental conditions.

Substantial variation in chlorpyrifos susceptibility among *S. frugiperda* populations has been documented elsewhere. Kulye et al. (2021) evaluated 47 field populations from India and reported chlorpyrifos EC_{50} values ranging from 3.62 to 49.1 $\mu\text{g mL}^{-1}$, indicating considerable geographical variation in response. This variability provides a plausible biological context for differences among studies, but it does not establish resistance in the present population because no susceptible reference strain or resistance ratio was included.

Diazinon showed an intermediate temporal pattern, reaching the 50% corrected-mortality threshold later than profenofos but eventually causing complete mortality. This pattern indicates that final mortality alone would obscure the earlier difference between the two formulations. Profenofos and diazinon produced similar final efficacy, but profenofos generated a larger early response. The result is relevant operationally because delayed mortality may allow surviving larvae to continue feeding for a longer period before death. Nevertheless, feeding damage was not quantitatively measured in this experiment, so the consequences of the observed difference for maize protection cannot be determined from mortality data alone.

The timing of mortality assessment is also important for interpreting insecticide bioassays. Mortality endpoints are inherently conditional on the specified post-exposure period, and extending the assessment interval can alter apparent product efficacy and estimated toxicity parameters (Burgess et al., 2020; Leong et al., 2020). Consequently, the first observed time at which corrected mortality reached or exceeded 50% in Table 4 should be regarded only as a descriptive threshold within the scheduled observation intervals. It is not equivalent to a statistically estimated LT_{50} because mortality was assessed only at discrete times and no formal time-to-event or survival model was fitted.

The descriptive threshold was retained instead of the earlier probit-derived LT_{50} and LT_{90} values because several model estimates extended beyond the 168-h experimental period or had extremely wide confidence intervals. Estimates outside the observed time range depend predominantly on extrapolation and can imply greater precision than the data support. Thus, Table 4 provides a direct and transparent description of mortality development under the present bioassay conditions, but the reported thresholds should not be generalized as intrinsic lethal-time parameters for the three active ingredients.

Visual symptoms and post-mortem changes

Control larvae remained active and retained their normal body shape and coloration throughout the observation period. Insecticide-exposed larvae commonly showed reduced movement and feeding activity before death. Dead larvae subsequently exhibited body darkening, shrinkage, tissue deterioration, and loss of responsiveness (Figure 1).

Reduced movement and feeding are biologically plausible responses to neuroactive insecticide exposure. In *Spodoptera frugiperda*, Vinha et al. (2021) demonstrated that deltamethrin exposure reduced locomotor activity, inhibited food consumption, altered respiration, and caused midgut injury. That study involved a pyrethroid rather than an

organophosphate, so it does not establish that the symptoms observed here resulted from the same mechanism. It nevertheless shows that impaired movement and feeding can accompany insecticide intoxication in *S. frugiperda*.

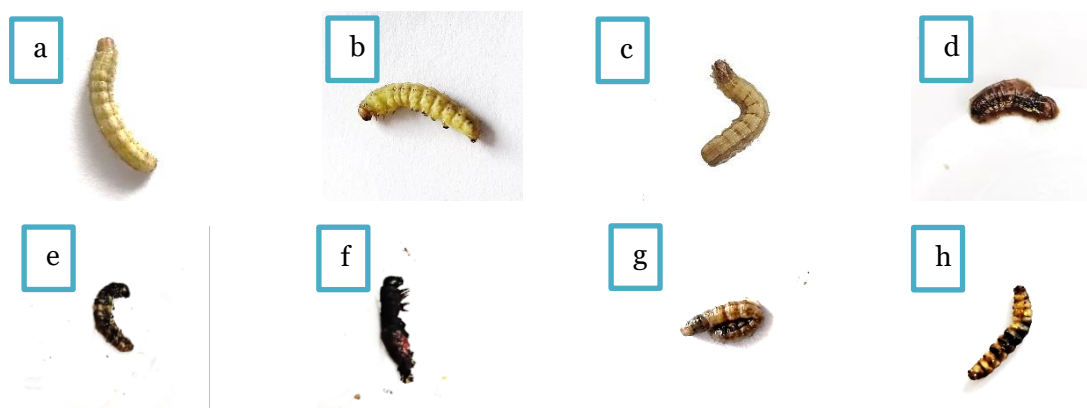


Figure 1. Representative appearance of *S. frugiperda* larvae during the laboratory bioassay. (a) Healthy control larva; (b) larva exposed to profenofos at 5% of the standardized label rate; (c) larva exposed to profenofos at 50%; (d) larva exposed to chlorpyrifos at 5%; (e, f) larvae exposed to diazinon at 75%; and (g, h) larvae exposed to chlorpyrifos at 20%.

The photographs are presented for descriptive purposes and do not represent a quantitative assessment of morphological effects.

The observed darkening, shrinkage, and tissue deterioration should not be interpreted as diagnostic symptoms of chlorpyrifos, profenofos, or diazinon exposure. Altered body coloration has also been reported in *S. frugiperda* larvae killed by biologically unrelated agents, including entomopathogenic nematodes (Chen et al., 2023). This supports the interpretation that discoloration and post-mortem deformation are nonspecific indicators of larval deterioration rather than evidence of a formulation-specific toxicological pathway.

Because movement, food consumption, coloration, and body condition were not assessed using predefined scores or quantitative measurements, no statistical comparison among formulations is possible. Figure 1 should therefore be interpreted solely as visual documentation of representative larval conditions during the bioassay. Claims concerning formulation-specific symptoms, pathological mechanisms, or severity differences should be avoided.

Implications and study limitations

The present findings identify profenofos- and diazinon-based commercial formulations as the most effective products under the tested laboratory conditions. Both formulations caused substantially greater final mortality than chlorpyrifos, while profenofos produced the most rapid observed mortality at 95% of the standardized label rate. These results may help prioritize products for subsequent field evaluation. However, they should not be interpreted as a direct recommendation for routine field application or as evidence that increasing application rates will improve control. Concentration had no significant overall effect in the factorial analysis, and the laboratory bioassay did not account for spray coverage, environmental degradation, maize-canopy architecture, larval concealment within the whorl, or other operational factors that influence field performance.

The higher efficacy of profenofos and diazinon also does not justify their repeated or exclusive use. Chlorpyrifos, profenofos, and diazinon are all organophosphate acetylcholinesterase inhibitors belonging to the same IRAC mode-of-action group, Group 1B. Consequently, alternating only among these three insecticides would not constitute an effective mode-of-action rotation and may maintain similar selection pressure on the pest population. Effective resistance management should instead combine need-based insecticide application with rotation or alternation involving

insecticides from genuinely different mode-of-action groups, together with non-chemical measures and regular monitoring of control performance. Integrated pest management is particularly important for *Spodoptera frugiperda*, because repeated insecticide exposure, inappropriate application timing, treatment of larger larvae, and sublethal exposure can contribute to poor control and the evolution of resistance (Sparks & Nauen, 2015; Van den Berg & du Plessis, 2022).

Environmental and food-safety considerations should also accompany efficacy-based insecticide selection. The relatively poor performance of chlorpyrifos in the present bioassay does not itself demonstrate resistance, but its continued use may provide limited pest-control benefits while contributing to environmental loading. In Indonesian peatland rice systems, chlorpyrifos was among the most frequently detected pesticide residues in rice and remained detectable in 50% of the examined peat-soil samples despite its reported discontinuation for more than one year (Purnama et al., 2025). Although those results were obtained from a different crop and agroecosystem and cannot be directly extrapolated to maize production, they demonstrate that pesticide selection should consider environmental persistence and residue risk in addition to short-term mortality.

Diversification of fall armyworm management may include biological control, conservation of natural enemies, cultural practices, botanical products, and biomass-derived pest-control materials. Wood vinegar produced from torrefied coconut shells, for example, has shown concentration-dependent insecticidal activity against third-instar *S. frugiperda* larvae under laboratory conditions (Purnama et al., 2024). That study used a different product, exposure protocol, and concentration range and therefore cannot be directly compared with the commercial organophosphate formulations evaluated here. Nevertheless, it illustrates the potential contribution of locally available bio-based products to reducing exclusive dependence on conventional insecticides. Further work is required to standardize such products, establish effective and crop-safe application rates, determine their compatibility with natural enemies, and validate their performance under field conditions.

Several limitations constrain the interpretation and generalization of the present results. First, the experiment used a single field-derived population collected from one location and maintained until the third laboratory generation. The number of founder larvae and their previous insecticide-exposure history were not documented; therefore, the tested population may not represent the genetic and susceptibility variation occurring across Serang Regency or other Indonesian maize-growing regions. Laboratory rearing may also alter population structure or reduce field-derived variation. Second, no known susceptible reference strain was included. The lower mortality produced by chlorpyrifos can therefore be interpreted only as lower relative efficacy of the tested formulation and cannot be used to calculate a resistance ratio or establish resistance status.

Third, each insecticide × concentration combination contained only 18 larvae distributed among three experimental units. This relatively small sample size contributed to substantial variation among replicates and wide confidence intervals. Pooled control mortality reached 18.89% at 168 h, necessitating Abbott correction and adding uncertainty to the corrected treatment responses. The control data were pooled across the five nominal concentration groups, and the analysis consequently assumed that background mortality was sufficiently comparable among those groups.

Fourth, the selected concentration range did not adequately characterize the complete concentration–mortality relationship. Profenofos and diazinon caused high mortality even at the lowest tested concentration, producing ceiling effects at higher concentrations. Their LC_{50} estimates therefore fell below the experimental range and were based on extrapolation rather than interpolation. Chlorpyrifos showed a non-monotonic response and a negative, non-significant probit slope, preventing reliable LC estimation. Future dose–response studies should include additional concentrations below 5% of the label rate for profenofos and diazinon and a redesigned range for chlorpyrifos, with larger numbers of larvae to improve model precision.

Fifth, the study compared commercial products at equivalent fractions of their respective recommended label rates. Because the products differed in active-ingredient concentration and potentially in solvents, emulsifiers, and other formulation components, the results represent formulation-level efficacy rather than the intrinsic toxicity of chlorpyrifos, profenofos, and diazinon. Direct comparison of active ingredients would require exposure based on equivalent active-ingredient concentrations, preferably using technical-grade compounds or otherwise standardized formulations.

Sixth, the kernel-immersion method combined ingestion and possible contact exposure but did not quantify the amount of insecticide deposited on or consumed from each kernel. Variation in feeding activity, kernel surface retention, and formulation adherence may therefore have influenced larval exposure. Only third-instar larvae were evaluated, although susceptibility and field control may vary with larval age and size. The morphological symptoms were also recorded descriptively without quantitative scoring and should not be interpreted as compound-specific toxicological effects.

Finally, mortality development over time was summarized descriptively because the same larvae were observed repeatedly and several probit-derived lethal-time estimates were unstable or extended beyond the 168-h observation period. The observed time required to reach 50% corrected mortality at the 95% label-rate fraction provides a transparent comparison within this experiment, but it is not equivalent to a generalized LT_{50} estimate. Future studies should record individual time-to-death data and apply survival-analysis methods that account for censoring and repeated observation.

Taken together, broader validation should include multiple field populations, a susceptible reference strain, more informative concentration ranges, larger sample sizes, individual time-to-death records, and field trials conducted under realistic maize-production conditions. Evaluation of crop injury, residual activity, effects on natural enemies and other non-target organisms, pesticide residues, and yield protection would further support evidence-based integration of effective insecticides into sustainable fall armyworm management.

CONCLUSIONS

The laboratory efficacy of the three commercial organophosphate formulations differed significantly, whereas the standardized label-dose fraction and the insecticide $\times$ concentration interaction had no significant effects on larval mortality. Across the tested concentrations, profenofos and diazinon produced comparably high Abbott-corrected mortalities of 90.4% and 93.2%, respectively, and both were significantly more effective than chlorpyrifos, which produced 32.2% mortality. Exploratory probit analysis indicated positive concentration–mortality relationships for profenofos and diazinon, but their LC_{50} estimates fell below the lowest tested concentration and were associated with wide confidence intervals. Chlorpyrifos LC values were not estimable because its concentration–mortality relationship was inconsistent. At 95% of the standardized label rate, profenofos reached 50% corrected mortality within 24 h and diazinon within 48 h, whereas chlorpyrifos did not reach this threshold during the 168-h observation period. Overall, profenofos and diazinon showed substantially greater and faster laboratory efficacy than chlorpyrifos. These findings represent formulation-level performance under controlled conditions and should not be interpreted as evidence of resistance. Further studies should include susceptible reference strains, additional field populations, lower concentration ranges, and field validation.

ETHICAL STATEMENT

This study involved only the fall armyworm, *Spodoptera frugiperda*, an invertebrate agricultural pest, and did not involve human participants or vertebrate animals. Therefore, institutional animal-ethics approval was not required. The insects were handled and maintained under standard laboratory conditions, and all experimental procedures were conducted solely for scientific evaluation of insecticide efficacy.

DATA AVAILABILITY

All data supporting the findings of this study are included in the article and its supplementary material. The supplementary material contains the replicate-level mortality data, pooled control mortality, Abbott-correction calculations, factorial ANOVA results, Tukey HSD comparisons, concentration-mortality probit inputs and outputs, and cumulative mortality observations recorded from 24 to 168 h.

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