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Behavioural Response of Cabbage White Butterfly (*Pieris rapae)* as Affected by Different Levels of Deltamethrin Insecticide

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ABSTRACT

The cabbage white butterfly (*Pieris rapae* L.) is a major pest of cruciferous crops, causing significant yield losses through larval feeding. Although synthetic pyrethroids such as deltamethrin are widely used for control, their efficacy may be compromised by larval behavioural responses that reduce lethal exposure. This study quantified the behavioural and toxic effects of deltamethrin on *P. rapae* larvae using a standardised one-way no-choice laboratory bioassay. The experiment was performed in a completely randomised design with three replications for each treatment under controlled conditions (20-23 °C; 30-60% relative humidity). Cabbage leaves were subjected to three coverage levels: water-treated control, 50% insecticide coverage, and 100% insecticide coverage at the recommended field rate (0.65 mL/L). Late-instar larvae were exposed to treated leaves. Larval survival, movement, and leaf area damage were recorded at multiple time points up to 72 hours. Complete insecticide coverage significantly reduced larval survival, locomotion, and feeding damage compared with partial coverage and controls, resulting in near-complete mortality by 72 hours. Partial coverage produced moderate but highly variable suppression, indicating inconsistent exposure. Early reductions in larval movement and feeding suggest avoidance of treated surfaces, while progressive declines over

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time indicate cumulative neurotoxic effects. These findings demonstrate that uniformity in insecticide coverage strongly influences both behavioural responses and control efficacy. Hence, optimising spray coverage is critical for effective *P. rapae* management and for reducing the risk of resistance development associated with sublethal exposure.

Keywords: Behavioural avoidance, deltamethrin, feeding deterrence, insecticide exposure, neurotoxic effects, *Pieris rapae*

INTRODUCTION

The cabbage white butterfly (*Pieris rapae* L.) is a globally significant pest of cruciferous crops, causing substantial economic losses through larval feeding on leaves and developing heads (Gautam et al., 2022). In the Philippines and across Southeast Asia, synthetic pyrethroids such as deltamethrin are widely used for *P. rapae* management due to their broad-spectrum efficacy, rapid action, and cost-effectiveness (Soltan et al., 2020). However, the long-term sustainability of pyrethroid-based control is threatened by the evolution of resistance, which can arise through physiological adaptations (e.g., target-site insensitivity, enhanced detoxification) and behavioural changes (e.g., avoidance of treated surfaces) (Haddi et al., 2018; Zalucki & Furlong, 2017).

According to Gómez-Guzmán et al. (2022), behavioural resistance is defined as the ability of insects to detect and avoid insecticide exposure before reaching lethal doses, thereby representing a critical but often overlooked mechanism of pest survival. Sublethal exposure to pyrethroids can induce altered feeding patterns, increased movement, or repellency, allowing insects to minimise contact with insecticides and survive (Muller et al., 2019; Nansen et al., 2016). For *P. rapae*, such behaviours may reduce the efficacy of foliar applications, particularly if larvae avoid treated leaves or exhibit reduced feeding. Notwithstanding its importance, few studies have quantitatively linked insecticide coverage levels to behavioural responses in *P. rapae* under controlled conditions.

Insecticide spray coverage in field conditions is often heterogeneous due to canopy structure, spray drift, and application inefficiencies. To approximate this variability under controlled laboratory conditions, a comparative assessment in this study was conducted between partial (50%) and full (100%) insecticide application coverage. This approach allows evaluation of larval behavioural responses under contrasting but field-relevant exposure scenarios. Hence, this study aimed to quantify the behavioural avoidance of *P. rapae* larvae in response to the different application coverage levels of deltamethrin on cabbage leaves, using a standardised "one-way no-choice" laboratory bioassay. Specifically, the experiment evaluated larval survival, movement, and leaf area damage in three experimental treatments: water-treated leaves (control), 50% insecticide coverage, and 100% insecticide coverage. It also addresses the study's limitations and recommended

directions for future research, including comparisons with resistant populations, dose-response assays, and field validation of laboratory observations.

METHODOLOGY

Study Area and Collection of Specimens

The experiment was conducted in November 2023 at the Entomology Laboratory, College of Agriculture, Benguet State University (BSU), La Trinidad, Benguet, Philippines. The experiment was conducted under consistent laboratory lighting (12-hour light: dark cycle) to ensure standardised conditions throughout the observation period. This is also to minimise circadian effects on larval feeding and movement. Late instar larvae of *Pieris rapae* were hand-collected from cabbage fields in the vicinity of BSU and transported to the laboratory, where they were contained in rectangular containers with mesh covers to ensure adequate ventilation. Cabbage leaves used in the bioassay were standardised by selecting similarly aged, fully expanded leaves from comparable canopy positions to minimise variation in nutritional content and leaf structure.

Experimental Design and Treatments

The experiment employed a "one-way no-choice" method (*Figure 1*), designed to simulate forced exposure scenarios common in dense crop plantings. The setup consisted of a dual-container arena connected by a sealed bridge, preventing larval escape while allowing movement between treated and untreated zones. Cabbage leaves were arranged on one side of the arena and treated according to the assigned treatment, while the opposite side remained untreated.

Figure 1. Experimental design using a "One-way no-choice method" in a dual-container setup to assess larval movement and feeding behaviour

This design allowed us to isolate the effects of insecticide coverage on larval behaviour, though it does not fully replicate field conditions where larvae may disperse or encounter heterogeneous spray patterns.

The same *Pieris rapae* larvae were observed across all time points (15, 30, and 60 minutes, and 2, 24, 48, and 72 hours) to monitor the progression of behavioural and toxic responses to deltamethrin exposure. Naïve larvae with no prior exposure to the insecticide or experimental conditions were used at the start of each replicate to standardise baseline conditions. Further, to ensure uniformity, larvae were introduced into the arenas 3-5 minutes post-treatment.

The bioassay was conducted under controlled ambient conditions (20-23°C, 30-60% relative humidity) following a Completely Randomised Design (CRD) with three replications per treatment. For each replicate, it contains two-late instar larvae housed in a shared container, and their responses (e.g., movement, survival, leaf damage) were recorded across all sampling time points. Meanwhile, despite a limited sample size per replicate, repeated measurements of the same larvae over time increased the robustness of the statistical analyses by capturing individual progression rather than cross-sectional comparisons. Treatments included T1 (water-treated control), T2 (50% insecticide coverage) and T3 (100% insecticide coverage, 0.65 mL/L deltamethrin, Decis 25 EC, Bayer).

Unfortunately, the use of late-instar larvae and a single recommended field rate of deltamethrin in this study may not capture the full range of behavioural or dose-dependent responses observed in earlier instars or at sublethal concentrations. Because this design focuses on immediate (short-term) and cumulative (long-term) responses to insecticide exposure, without confounding effects from repeated insecticide contact or prior experience.

Insecticide Application and Data Collection

Deltamethrin (Decis 25 EC, Bayer, 25 g/L active ingredient) was dissolved in 1 L of water at the manufacturer's recommended field rate (0.65 mL/L) using a beaker, then transferred to a 1.5-litre spray bottle. To avoid leaf saturation and ensure even coverage, a fine-nozzle atomiser was used for application. Two larvae were assigned to each treatment arena immediately after spraying.

Behavioural responses of the treated larvae per replicate were observed and recorded at 15, 30, and 60 minutes, and at 2, 24, 48, and 72 hours post-exposure. The observation intervals were strategically selected to capture both the immediate neurotoxic effects (e.g., hyperactivity, paralysis) and cumulative sublethal/lethal effects (e.g., feeding suppression, mortality) of deltamethrin on *Pieris rapae*, based on its known mode of action targeting insect sodium channels (Narahashi, 2000; Wolansky & Harrill, 2008) and documented residual toxicity in Lepidoptera (Desneux et al., 2007). Intermediate time points were excluded to minimise larval stress, reduce experimental complexity, and focus on the critical phases of insecticide response, acute symptoms and long-term outcomes,

while aligning with standard bioassay protocols for balancing biological relevance with practical feasibility (Leite et al., 2022; Wondafrash et al., 2012). The design used in this study ensures robust detection of treatment effects while adhering to ethical and logistical constraints.

Movement was measured objectively using pre-marked distances on the arena floor and standardised observation intervals to minimise observer bias during manual movement tracking. In addition, multiple observers cross-checked measurements to ensure consistency and reduce potential bias. The following parameters were measured:

i. Survival: Percent mortality was calculated using Abbott's corrected formula as shown below (Abbott, 1925; Fleming & Retnakaran, 1985). Though control mortality was negligible, Abbott's correction was applied as a standard practice.

   % Mortality (corrected) = [(% survival in control - % survival in treatment) / % survival in control] × 100

ii. Movement: The total distance travelled by each larva from the leaf centre was measured using marked reference points in the arena.
iii. Leaf Area Damage: Damage severity was scored using a modified categorical scale by Sorgini et al. (2019): 1 (sound): No damage, 2 (slight): 1-10 % damage, 3 (moderate): 11-20 % damage, 4 (heavy): 21-30 % damage and, 5 (severe): >31 % damage.

While linear displacement was used as a simplified measure of movement, future studies could employ video tracking to analyse path complexity and turning behaviour for a more nuanced understanding of larval responses.

Statistical Analysis

Leaf Area Damage (1-5 Ordinal Scale)

Kruskal-Wallis non-parametric ANOVA was performed to compare differences across treatments and time points, since the given nature of the leaf area damage ratings (1-5 scale) is ordinal. This statistical test was selected because it does not assume normality or equal intervals between ratings, making it appropriate for ordered categorical data (Siegel & Castellan, 1988). For post hoc analysis, Dwass-Steel-Critchlow-Fligner pairwise comparisons were used to control for multiple comparisons and identify specific treatment effects. Further, ordinal logistic regression was used to model the effect of treatment and time on damage ratings, as it accounts for the ordered nature of the response variable and provides odds ratios for interpreting treatment effects (McCullagh, 1980).

Larval Survival (Percentage Data)

Larval survival data were initially analysed using a two-way ANOVA after arcsine transformation, which is a common practice for proportional data to stabilise variance (Zar, 2010). However, Shapiro-Wilk tests indicated consistent deviations from normality ($p < 0.001$), and Levene's tests revealed heterogeneous variances ($p < 0.05$), even after transformation. These violations were likely due to the bounded nature of survival data (0-100%), small sample sizes ($n = 3$ per treatment/time combination), and zero-inflated values in high-dose treatments (e.g., 100% insecticide coverage). Consequently, Kruskal-Wallis non-parametric ANOVA was employed, followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons, as non-parametric tests are robust to non-normality and heterogeneity of variance (Conover, 1999).

Distance Travelled (Continuous Data)

Shapiro-Wilk tests indicated non-normality ($p <0.001$), and Levene's tests confirmed heterogeneous variances ($p <0.05$). These violations were attributed to skewed distributions (e.g., clustering of lower values in high-dose treatments) and limited sample sizes, which constrained the robustness of parametric tests. Consequently, Kruskal-Wallis non-parametric ANOVA was also employed, followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons to ensure valid inferences without relying on parametric assumptions (Dunn, 1964). Standard deviation (SD) was calculated to quantify within-group variability, while estimated marginal means were reported for treatment effects.

All analyses were conducted in jamovi (Version 2.6.13), which ensures both descriptive and inferential rigour.

Limitations of the Study

Several limitations must be acknowledged in this study. First, the focus on late-instar larvae and a single field rate of deltamethrin (0.65 mL/L) may not capture the full spectrum of instar-specific or dose-dependent responses, particularly in earlier, more susceptible stages (Zalucki & Furlong, 2017).

Second, the "one-way no-choice" design may be effective for isolating coverage effects, but it does not fully replicate field conditions, where larvae may disperse or encounter heterogeneous spray patterns. Likewise, the design does not disentangle contact toxicity from ingestion effects, as both routes were evaluated jointly.

Third, the sample size of two larvae per replicate (with three replicates per treatment) may limit statistical power for detecting subtle behavioural differences, although repeated measurements over 72 hours enhanced robustness.

Fourth, the study only focused on immediate and cumulative behavioural responses within 72 hours without assessing longer-term effects (e.g., recovery from sublethal exposure or delayed mortality).

Fifth, the laboratory setting fails to account for environmental factors (e.g., temperature, humidity) that could influence larval behaviour under natural conditions (Desneux et al., 2007).

Sixth, a solvent control was omitted, as the commercial formulation was compared to a water control, but future studies could include one to isolate formulation effects.

Finally, while insecticide degradation may occur over time, the progressive mortality and behavioural suppression observed suggest cumulative rather than diminishing effects. Hence, future research should incorporate physiological biomarkers, resistant strains, and field validation to further elucidate these mechanisms.

Ethical Considerations

This study adhered to standard entomological practices for insect bioassays, ensuring that all procedures were conducted with scientific rigour and ethical responsibility. *Pieris rapae* larvae were exposed to deltamethrin under controlled laboratory conditions, and prolonged exposure assays were terminated at 72 hours. After the experiment, surviving larvae were humanely euthanised via freezing at -20°C to prevent potential environmental contamination and align with standard entomological practices. No recovery assessment was conducted, as the study's objective was to evaluate the immediate and cumulative effects of insecticide exposure on larval survival, movement, and feeding behaviour. This approach aligns with guidelines for invertebrate research, which prioritise minimising unnecessary suffering while balancing scientific objectives.

RESULTS AND DISCUSSION

Larval Survival of *Pieris rapae* Under Varying Deltamethrin Insecticide Exposure

Table 1 presents the result of the mean larval survival of *Pieris rapae* exposed to three treatment levels, T1 (water control), T2 (50% deltamethrin coverage), and T3 (100% deltamethrin coverage), across seven-time intervals (0.25, 0.5, 1, 2, 24, 48, and 72 hours). Result revealed that the water control (T1) maintained consistently high survival (1.57 ± 0.00) across the first 24 hours, with a slight decline to 1.37 ± 0.196 at 48 and 72 hours. In contrast, T2 (50% coverage) showed no mortality (1.57 ± 0.00) during the first 2 hours, followed by a gradual decline to 1.37 ± 0.196 at 24 hours and 0.594 ± 0.373 at 48 and 72 hours, indicating partial but inconsistent suppression. Meanwhile, T3 (100% coverage) exhibited no mortality (1.57 ± 0.00) during the first hour, but survival dropped sharply to 0.592 ± 0.194 at 2 hours, ultimately reaching near-complete mortality (0.204 ± 0.194) by 72 hours, demonstrating the potent effect of full insecticide coverage.

Table 1
Mean (± SEM) larval survival, distance travelled (movement), and leaf area damage of pieris rapae *late-instar larvae exposed to varying deltamethrin coverage across seven time intervals*

Time Interval (hr)	Larval Survival			Distance Travelled (Movement)			Leaf Area Damage		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
0.25	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	43.8 ± 6.25	46.3 ± 2.98	36.9 ± 5.14	1.75 ± 0.250	1.75 ± 0.250	1.25 ± 0.250
0.50	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	53.3 ± 3.33	41.9 ± 4.93	36.9 ± 5.14	1.75 ± 0.250	1.50 ± 0.289	1.25 ± 0.250
1	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	64.0 ± 16.6	44.8 ± 3.77	36.9 ± 5.14	2.00 ± 0.00	1.75 ± 0.250	1.25 ± 0.250
2	1.57 ± 0.00	1.57 ± 0.00	0.592 ± 0.194	67.5 ± 21.0	36.3 ± 10.6	32.5 ± 11.4	2.00 ± 0.00	1.75 ± 0.250	1.25 ± 0.250
24	1.57 ± 0.00	1.37 ± 0.196	0.592 ± 0.194	69.3 ± 19.9	40.8 ± 13.9	32.5 ± 11.4	2.25 ± 0.250	2.00 ± 0.408	1.25 ± 0.250
48	1.37 ± 0.196	0.594 ± 0.373	0.592 ± 0.194	87.5 ± 37.5	35.6 ± 12.4	35.5 ± 11.9	2.25 ± 0.250	2.25 ± 0.629	1.25 ± 0.250
72	1.37 ± 0.196	0.594 ± 0.373	0.204 ± 0.194				2.25 ± 0.250	2.25 ± 0.629	1.25 ± 0.250

Statistical analysis using two-way ANOVA on arcsine-transformed larval survival data as shown in Table 2 revealed highly significant effects of treatment ($F_{2,63} = 21.14$, $p < 0.001$, $\eta^2 = 0.178$), time ($F_{6,63} = 14.41$, $p < 0.001$, $\eta^2 = 0.363$), and their interaction ($F_{12,63} = 3.85$, $p < 0.001$, $\eta^2 = 0.194$), explaining 17.8%, 36.3%, and 19.4% of the total variability, respectively. However, violated assumptions of homogeneity of variances (Levene's test, $p < 0.001$) and non-normality (Shapiro-Wilk, $p < 0.001$) necessitated the use of Kruskal-Wallis non-parametric ANOVA ($\chi^2_2 = 16.6$, $p < 0.001$, $\varepsilon^2 = 0.200$, Table 4), followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons (Table 5). Post-hoc analyses confirmed that T3 significantly reduced larval survival compared to T1 ($p < 0.001$), while T2 did not differ significantly from T1 ($p = 0.139$) or T3 ($p = 0.082$), which highlights the critical role of complete coverage in achieving mortality (Table 3).

Further, the significant interaction effect ($\eta^2 = 0.194$) indicates that mortality dynamics vary over time, with T3s lethality becoming pronounced after 24 hours, likely due to cumulative toxicity and residual effects of deltamethrin (Leite et al., 2022). The immediate survival rates (15 minutes to 2 hours) were comparable across treatments, indicating that initial exposure to deltamethrin does not cause rapid mortality. However, survival diverged sharply from the control after 24 hours, particularly in T3, where full surface coverage and prolonged exposure led to cumulative toxicity, aligning with studies on plant-derived insecticides by Amongi (2016) and Viteri Jumbo et al. (2018).

The biological variability in T2 survival suggests that partial coverage may result in uneven insecticide distribution, allowing some larvae to avoid lethal exposure. This variability underscores the importance of consistent development (Desneux et al., 2007). While Abbott's correction was applied, its impact was minimal due to negligible control mortality, reinforcing the robustness of these trends.

To further elucidate mortality dynamics, time to event analyses (e.g., Kaplan-Meier survival curves) could be employed in future studies. Likewise, larger sample sizes and continuous survival metrics (e.g., video tracking) may improve statistical power and resolution.

Table 2
Two-way ANOVA results for arcsine-transformed percent larval survival of Pieris rapae *under varying deltamethrin coverage (T1: water control; T2: 50% coverage; T3: 100% coverage) across seven-time intervals (15 min–72 h)*

	Sum of Squares	df	Mean Square	F	p	η^2
Treatment	4.39	2	2.194	21.14	<.001*	0.178
Time	8.97	6	1.495	14.41	<.001*	0.363
Treatment ∗ Time	4.79	12	0.399	3.85	<.001*	0.194
Residuals	6.54	63	0.104			

Note. Significant differences ($p<0.05$) are indicated in asterisks (*). η^2 (eta-squared) represents the proportion of total variance in the dependent variable (e.g., distance travelled or survival) that is explained by the independent variable (e.g., treatment or time). Values range from 0 to 1, where 0.01 = small effect; 0.06 = medium effect; and ≥0.14 = large effect (Cohen, 1988)

Table 3
Tukey HSD post hoc comparisons of estimated marginal means for percent larval survival among treatments (T1: water control; T2: 50% deltamethrin coverage; T3: 100% deltamethrin coverage)

Comparison							
Treatment		Treatment	Mean Difference	SE	df	t	p_{tukey}
T1	-	T2	0.251	0.0861	63.0	2.92	0.013
	-	T3	0.559	0.0861	63.0	6.49	<.001*
T2	-	T3	0.308	0.0861	63.0	3.58	0.002*

Note. Comparisons are based on estimated marginal means. Significant differences ($p<0.05$) are indicated in asterisks (*)

Table 4
Kruskal-Wallis non-parametric ANOVA results for arcsine-transformed percent larval survival of Pieris rapae *under varying deltamethrin coverage*

	χ^2	df	p	ε^2
Larval Survival_Arcsine transformed	16.6	2	<.001*	0.200

Note. Significant differences ($p<0.05$) are indicated in asterisks (*). ε^2 (epsilon squared) represents the estimates of the proportion of variability in the ranked data

Table 5
Dwass-Steel-Critchlow-Fligner pairwise comparisons of percent larval survival among treatments (T1: water control; T2: 50% deltamethrin coverage; T3: 100% deltamethrin coverage)

		W	p
T1	T2	-2.69	0.139
T1	T3	-5.69	<.001*
T2	T3	-3.02	0.082

Note. Significant differences (p<0.05) are indicated in asterisks (*). 'W' represents the standardised difference between the rank sums of the two groups

Larval Movement Behaviour of *Pieris rapae* Under Varying Deltamethrin Insecticide Exposure

Larval distance travelled data is presented in Table 1, where movement behaviour of *Pieris rapae* was assessed across seven-time intervals (0.25-72 hours) under three treatments: T1 (water control), T2 (50% deltamethrin coverage), and T3 (100% deltamethrin coverage). Result revealed that T1 larvae exhibited the highest movement (e.g., 87.5 ± 37.5 mm at 48 hours), reflecting normal exploratory behaviour, while T3 larvae showed the lowest movement (e.g., 32.5 ± 11.4 mm at 24-48 hours), indicating strong behavioural suppression or neurotoxic effects. T2 larvae displayed intermediate movement (e.g., 40.8 ± 13.9 mm at 24 hours), which may demonstrate a dose-dependent response.

Statistical analyses were presented in Tables 6 and 7 for the two-way ANOVA, while Tables 8 & 9 were for the Kruskal-Wallis test. Results consistently confirmed that T1 larvae travelled significantly farther than T2 and T3 ($p < 0.05$), while T2 and T3 did not differ significantly ($p > 0.05$). This consistency between parametric and non-parametric results reinforces the reliability of the findings, highlighting that full insecticide coverage (T3) most effectively suppresses larval movement, likely due to combined behavioural avoidance and neurotoxic impairment.

In this study, behavioural resistance is operationally defined as the adaptive avoidance of deltamethrin-treated surfaces by *Pieris rapae* larvae, manifesting as reduced locomotor activity or repellency in response to chemical cues (Foster et al., 2007; Haynes, 1988; Hubbard & Murillo, 2024). Meanwhile, neurotoxic impairment refers to the physiologically mediated inhibition of locomotion or feeding, arising from deltamethrin's disruptive effects on neural function (Desneux et al., 2007; Wolansky & Harrill, 2008).

Observations obtained in the bioassay reveal a distinct temporal and dose-dependent pattern in larval movement: larvae in the control group (T1) exhibited highly variable and extensive displacement (e.g., 64.0 ± 16.6 mm at 1 hour) at early time points, reflecting uninhibited exploratory and foraging behaviour characteristic of undisturbed *P. rapae* (Tsuji et al., 2018). This absence of insecticide stress in T1 permitted natural locomotor activity, consistent with baseline observations in lepidopteran larvae.

Table 6
Two-way ANOVA results for distance travelled by Pieris rapae *larvae under varying deltamethrin coverage (T1: water control; T2: 50% coverage; T3: 100% coverage) across six-time intervals*

	Sum of Squares	df	Mean Square	F	p	η^2
Treatment	11192	2	5596	6.734	0.002*	0.184
Time	837	5	167	0.202	0.960	0.014
Treatment ∗ Time	4016	10	402	0.483	0.894	0.066
Residuals	44875	54	831			

Note. Significant differences ($p<0.05$) are indicated in asterisks (*). η^2 (eta-squared) represents the proportion of total variance in the dependent variable (e.g., distance traveled or survival) that is explained by the independent variable (e.g., treatment or time). Values range from 0 to 1, where 0.01 = small effect; 0.06 = medium effect; and ≥0.14 = large effect (Cohen, 1988)

Table 7
Tukey HSD post hoc comparisons of estimated marginal means for distance travelled by Pieris rapae *larvae among treatments (T1: water control; T2: 50% deltamethrin coverage; T3: 100% deltamethrin coverage)*

Treatment		Treatment	Mean Difference	SE	df	t	p_{tukey}
T1	-	T2	23.31	8.37	54.0	2.785	0.020
	-	T3	29.03	8.37	54.0	3.470	0.003*
T2	-	T3	5.73	8.32	54.0	0.688	0.771

Note. Comparisons are based on estimated marginal means. Significant differences ($p<0.05$) are indicated in asterisks (*)

Table 8
Kruskal-Wallis non-parametric ANOVA results for distance travelled by Pieris rapae *larvae under varying deltamethrin coverage*

	χ^2	df	p	ε^2
Distance travelled by the larvae	16.4	2	<.001*	0.231

Note. Significant differences ($p<0.05$) are indicated in asterisks (*). ε^2 (epsilon squared) represents the estimates of the proportion of variability in the ranked data

Table 9
Dwass-Steel-Critchlow-Fligner pairwise comparisons of distance travelled by Pieris rapae *larvae among treatments (T1: water control; T2: 50% deltamethrin coverage; T3: 100% deltamethrin coverage)*

		W	p
T1	T2	-3.68	0.025
T1	T3	-5.49	<.001*
T2	T3	-2.47	0.188

Note. Significant differences ($p<0.05$) are indicated in asterisks (*). 'W' represents the standardised difference between the rank sums of the two groups

In contrast, larvae exposed 100 % deltamethrin coverage (T3) displayed minimal movement (e.g., 32.5 ± 11.4 mm at 24 hours) and low variability, indicating strong behavioural avoidance and/or neurotoxic impairment. The reduced variability observed under full insecticide coverage (T3) reflects biologically constrained responses near zero movement and feeding rather than statistical compression. Such patterns are consistent with strong toxic or deterrent effects limiting behavioural expression.

Meanwhile, the intermediate movement (e.g., 40.8 ± 13.9 mm at 24 hours) in T2 (50% coverage), where larvae travelled farther than in T3 but significantly less than in T1, supports a dose-dependent response, consistent with findings by Wondafrash et al. (2012). Such behaviour is underpinned by their sensory capacity to detect volatile and contact chemical cues from both host plants and insecticides (Miles et al., 2005; Tsuji et al., 2018).

Understanding the dual mechanisms of behavioural resistance and neurotoxic impairment can guide the development of more effective insecticide application strategies, ensuring both immediate deterrence and sustained larval suppression in integrated pest management (IPM) programs. The suppressed movement in T3 has critical implications for pest management, as reduced larval dispersal can limit both direct damage and the spread of infestations.

Future studies could employ video tracking to analyse path complexity, turning behaviour, and velocity, offering deeper insights into the mechanisms of avoidance and impairment (Charreton et al., 2015). This would enhance our understanding of behavioural resistance and neurotoxic effects, guiding the development of more effective insecticide application strategies in IPM programs.

Effect of Insecticide Coverage on *Pieris rapae* Feeding Damage

Mean leaf area damage is presented in Table 1, wherein the effect of deltamethrin coverage on *Pieris rapae* feeding behaviour was assessed by measuring mean leaf area damage across seven-time intervals (0.25-72 hours) under three treatments: T1 (water control), T2 (50% coverage), and T3 (100% coverage). Result revealed a clear treatment-dependent suppression where T1 larvae (control) caused increasing damage over time, with mean ratings rising from 1.75 ± 0.25 at 0.25 hours to 2.25 ± 0.25 at 24-72 hours, reflecting unrestricted feeding. In contrast, T3 larvae (100% coverage) showed minimal and consistent damage (mean = 1.25 ± 0.25 across all time points), indicating near-complete feeding suppression. T2 larvae (50% coverage) exhibited intermediate damage, with ratings increasing from 1.75 ± 0.25 at 0.25 hours to 2.25 ± 0.63 at 48-72 hours, suggesting partial but inconsistent feeding deterrence.

Statistical analyses confirmed these patterns as shown in Table 10, where Kruskal-Wallis ANOVA revealed a highly significant effect of treatment on leaf area damage ($\chi^2_2 = 27.6$, $p < 0.001$, $\varepsilon^2 = 0.332$), explaining 33.2% of total variability.

Table 10
Kruskal-Wallis non-parametric ANOVA results for leaf area damage ratings (1–5 scale) caused by Pieris rapae *larvae under varying deltamethrin coverage (T1: water control; T2: 50% coverage; T3: 100% coverage)*

	χ^2	df	p	ε^2
Damage_Scale	27.6	2	<.001*	0.332

Note. Significant differences (p<0.05) are indicated in asterisks (*). ε^2 (epsilon squared) represents the estimates of the proportion of variability in the ranked data

Table 11
Dwass-Steel-Critchlow-Fligner pairwise comparisons of leaf area damage ratings (1–5 scale) among treatments (T1: water control; T2: 50% deltamethrin coverage; T3: 100% deltamethrin coverage)

		W	p
T1	T2	-2.10	0.297
T1	T3	-7.27	<.001*
T2	T3	-5.04	0.001*

Note. Significant differences (p<0.05) are indicated in asterisks (*). 'W' represents the standardised difference between the rank sums of the two groups

Table 12
Ordinal logistic regression model fit measures for leaf area damage ratings (1–5 scale) caused by Pieris rapae *larvae, modelling the effects of treatment and time.*

Model	Deviance	AIC	R^2_{McF}
1	122	134	0.214

Note. Models estimated using a sample size of N=84. The dependent variable 'Damage_Scale' has the following order: 1 | 2 | 3 | 4

Table 13
Ordinal logistic regression coefficients for leaf area damage ratings (1–5 scale) caused by Pieris rapae *larvae, modelling the effects of treatment and time*

Predictor	Estimate	SE	Z	p
Time	0.0163	0.00950	1.72	0.085
Treatment:				
T2 – T1	-0.9117	0.62624	-1.46	0.145
T3 – T1	-3.2810	0.68904	-4.76	<.001*

Note. Significant differences (p < 0.05) are indicated in asterisks (*)

Pairwise comparisons (Dwass-Steel-Critchlow-Fligner, Table 11) showed that T3 caused significantly lower damage than T1 ($p < 0.001$) and T2 ($p = 0.001$), while T1 and T2 did not differ ($p = 0.297$). Ordinal logistic regression further supported these results (Tables 12-13), with T3 significantly reducing damage compared to T1 ($p < 0.001$) and no significant effect of time ($p = 0.085$) or T2 ($p = 0.145$). The large effect size ($\varepsilon^2 = 0.332$) and moderate McFadden's R^2 (0.214) underscore the biological significance of full coverage (T3) in suppressing feeding, while partial coverage (T2) failed to reduce damage relative to controls.

The low variability in T3 suggests a uniform larval response, likely due to high mortality or deterrence, whereas the higher and more variable damage in T1 and T2 reflects inconsistent larval behaviour and uneven exposure to untreated leaf areas. This aligns with the studies of Desneux et al. (2007) and Nansen et al. (2016), highlighting that suboptimal coverage leaves gaps in protection, leading to variable and suboptimal pest control outcomes.

These results further highlight the critical need for uniform insecticide application, as suboptimal coverage (T2) leads to inconsistent feeding suppression and risks accelerated resistance development due to sublethal exposure (Desneux et al., 2007; Nansen et al., 2016).

Despite the focus on *P. rapae* in this study, the behavioural responses to pyrethroids, such as avoidance and locomotor inhibition, are likely conserved across related crucifer-feeding Lepidoptera (Haynes, 1988). Hence, future work should prioritise larger sample sizes and continuous damage metrics (e.g., digital image analysis) to address distributional challenges and refine these findings.

CONCLUSION

The result of this study demonstrates that insecticide coverage level strongly influences both the behavioural and toxic responses of *Pieris rapae* larvae to deltamethrin. Complete coverage at the recommended field rate resulted in near-total mortality, minimal larval movement, and consistently low feeding damage. This implies an effective suppression through combined behavioural avoidance and cumulative neurotoxic effects. In contrast, partial coverage produced variable outcomes, with moderate reductions in survival and feeding that were inconsistent across replicates, highlighting the limitations of incomplete exposure.

The result also suggests that behavioural responses contribute to early avoidance of treated leaf surfaces, as shown in the rapid suppression of larval movement and feeding shortly after exposure. Conversely, the sustained declines in activity and survival of *P. rapae* larvae over time reflect progressive toxic effects; thus, both behavioural avoidance and toxicity contribute to insecticide efficacy and should be interpreted as potential

precursors to resistance development under prolonged field selection pressure rather than direct evidence of resistance. Hence, these findings emphasise that suboptimal insecticide coverage can reduce control reliability and potentially promote resistance by allowing survival under sublethal exposure.

This study underscores the importance of uniform insecticide application for effective *P. rapae* management. Incorporating behavioural assessments into insecticide evaluation can improve understanding of control failures and guide the development of more sustainable pest management strategies. Future research should expand on these findings by examining dose-response relationships, resistance populations, and field-scale validation to better integrate behavioural dynamics into integrated pest management programs.

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DISCLAIMER

AI tools were not employed during the preparation of this manuscript; particularly, the core intellectual contributions, including study design, data collection, analysis, interpretation of results, and conclusions, were independently developed and validated by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the manuscript and confirm that AI functioned solely as a supportive language correction aid rather than a source of original scientific content for improved clarity and enhanced readability. In addition, any AI-assisted vocabulary in the manuscript was critically reviewed, selectively adapted, and integrated by the authors to ensure that the wording accurately reflects the intended scientific meaning and disciplinary context.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*(3), 265–267. https://doi.org/10.1093/jee/18.2.265a

Amongi, E. S. (2016). *Efficacy of neem tree extract on white cabbage aphid, Brevicoryne brassicae control* [Doctoral dissertation, Uganda Martyrs University]. http://dissertations.umu.ac.ug/xmlui/handle/123456789/393

Charreton, M., Decourtye, A., Henry, M., Rodet, G., Sandoz, J. C., Charnet, P., & Collet, C. (2015). A locomotor deficit induced by sublethal doses of pyrethroid and neonicotinoid insecticides in the honeybee *Apis mellifera*. *PLOS ONE*, *10*(12), Article e0144879. https://doi.org/10.1371/journal.pone.0144879

Conover, W. J. (1999). *Practical nonparametric statistics* (3rd ed.). John Wiley & Sons.

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, *52*, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics*, *6*(3), 241–252. https://doi.org/10.1080/00401706.1964.10490181

Fleming, R. A., & Retnakaran, A. (1985). Evaluating single treatment data using Abbott's formulae with reference to insecticides. *Journal of Economic Entomology*, *78*(6), 1179–1181. https://doi.org/10.1093/jee/78.6.1179

Foster, S. P., Tomiczek, M., Thompson, R., Denholm, I., Poppy, G., Kraaijeveld, A. R., & Powell, W. (2007). Behavioural side-effects of insecticide resistance in aphids increase their vulnerability to parasitoid attack. *Animal Behaviour*, *74*(3), 621–632. https://doi.org/10.1016/j.anbehav.2006.12.018

Gautam, B., Tiwari, S., & Thapa, R. B. (2022). Efficacy of insecticides against *Pieris brassica nepalensis* (Doubleday) on cabbage in Chitwan, Nepal. *International Journal of Recent Advances in Multidisciplinary Topics*, *3*(8), 12–18. https://www.ijramt.com

Gómez-Guzmán, J. A., Sainz-Pérez, M., & González-Ruiz, G. (2022). Monitoring and inference of behavioural resistance in behavioural insects to insecticides in two pest control systems: IPM and organic. *Agronomy*, *12*(2), Article 538. https://doi.org/10.3390/agronomy12020538

Haddi, K., Valbon, W. R., Viteri-Jumbo, L. O., De Oliveira, L. O., Guedes, R. N. C., & Oliveira, E. E. (2018). Diversity and convergence of mechanisms involved in pyrethroid resistance in the stored grain weevils, *Sitophilus* spp. *Scientific Reports*, *8*, Article 16361. https://doi.org/10.1038/s41598-018-34513-5

Haynes, K. F. (1988). Sublethal effects of neurotoxic insecticides on insect behaviour. *Annual Review of Entomology*, *33*, 149–168. https://doi.org/10.1146/annurev.en.33.010188.001053

Hubbard, C. B., & Murillo, A. C. (2024). Behavioural resistance to insecticides: Current understanding, challenges, and future directions. *Current Opinion in Insect Science*, *63*, Article 101177. https://doi.org/10.1016/j.cois.2024.101177

Leite, W. D. A., Jacobowski, A. C., & Macedo, M. L. R. (2022). Insecticide activity of a peptidase inhibitor isolated from *Anadenanthera macrocarpa* seeds against *Anagasta kuehniella*. *Ciência e Agrotecnologia*, *46*, Article e006822. https://doi.org/10.1590/1413-7054202246006822

McCullagh, P. (1980). Regression models for ordinal data. *Journal of the Royal Statistical Society: Series B (Methodological)*, *42*(2), 109–127. https://doi.org/10.1111/j.2517-6161.1980.tb01109.x

Miles, C. I., Campo, M. L. D., & Renwick, J. A. A. (2005). Behavioural and chemosensory responses to a host recognition cue by larvae of *Pieris rapae*. *Journal of Comparative Physiology A*, *191*(2), 147–155. https://doi.org/10.1007/s00359-004-0580-x

Muller, T., Romer, C. I., & Muller, C. (2019). Parental sublethal insecticide exposure prolongs mating response and decreases reproductive output in offspring. *Journal of Applied Ecology*, *56*(7), 1529–1538. https://doi.org/10.1111/1365-2664.13398

Nansen, C., Baissac, O., Nansen, M., Powis, K., & Baker, G. (2016). Behavioural avoidance - Will physiological insecticide resistance level of insect strains affect their oviposition and movement responses? *PLOS ONE*, *11*(3), Article e0149994. https://doi.org/10.1371/journal.pone.0149994

Narahashi, T. (2000). Neuroreceptors and ion channels as the basis for drug action: Past, present, and future. *Journal of Pharmacology and Experimental Therapeutics*, *294*(1), 1–26. https://doi.org/10.1016/S0022-3565(24)39034-2

Siegel, S., & Castellan, N. J. (1988). *Nonparametric statistics for the behavioural sciences* (2nd ed.). McGraw-Hill.

Soltan, H. R., ElBakary, A. S., Shawir, M. S., & AlHomudi, T. (2020). Comparative acute toxicity of five insecticide against rice weevil. *Modern Concepts & Developments in Agronomy*, *7*(3), Article 000665. https://doi.org/10.31031/MCDA.2020.07.000665

Tsuji, J., Logan, T., & Russo, A. (2018). A hierarchy of cues directs the foraging of *Pieris rapae* (Lepidoptera: Pieridae) larvae. *Environmental Entomology*, *47*(6), 1485–1492. https://doi.org/10.1093/ee/nvy124

Viteri Jumbo, L. O., Haddi, K., Faroni, L. R. D., Heleno, F. F., Pinto, F. G., & Oliveira, E. E. (2018). Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils. *PLOS ONE*, *13*(11), Article e0207618. https://doi.org/10.1371/journal.pone.0207618

Wolansky, M. J., & Harrill, J. A. (2008). Neurobehavioural toxicology of pyrethroid insecticides in adult animals: A critical review. *Neurotoxicology and Teratology*, *30*(2), 55–78. https://doi.org/10.1016/j.ntt.2007.10.005

Wondafrash, M., Getu, E., & Terefe, G. (2012). Neem, *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Academic Journal of Entomology*, *5*(1), 22–27. https://doi.org/10.5829/idosi.aje.2012.5.1.6218

Zalucki, M. P., & Furlong, M. J. (2017). Behaviour as a mechanism of insecticide resistance: Evaluation of the evidence. *Current Opinion in Insect Science*, *21*, 1–7. https://doi.org/10.1016/j.cois.2017.05.006

Zar, H. J., Connell, T. G., & Nicol, M. (2010). Diagnosis of pulmonary tuberculosis in children: New advances. *Expert Review of Anti-infective Therapy*, *8*(3), 277–288. https://doi.org/10.1586/eri.10.9