Comparative fumigant toxicity of neem oil (*Azadirachta indica* A. Juss.) and pirimiphos-methyl on adults of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): lethal concentrations, lethal times, and transgenerational sublethal effects

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Systematic Review

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Abstract

The maize weevil *Sitophilus zeamais* Motschulsky is one of the most destructive primary pests of stored cereal grains globally. Growing resistance to synthetic insecticides underscores the need for validated alternative tools. This study quantitatively compared the fumigant toxicity of neem oil (*Azadirachta indica* A. Juss., NEEM AZAL 1,2% EC) and pirimiphos-methyl (ACTELLIC 50 EC) against adult *S. zeamais*. LC_{50} = 0,72 mL/L for neem oil (R^2 = 0,85; slope = 2,56) and LC_{50} = 6,31 mg/L for pirimiphos-methyl (R^2 = 0,92; slope = 2,47). Median lethal times (LT_{50}) ranged from 12-108 h (neem oil) and 12-44 h (pirimiphos-methyl). F1 progeny inhibition was 77,75-100% for neem oil and 62,93-100% for pirimiphos-methyl. Even at the lowest neem oil concentration (0,24 mL/L), progeny inhibition exceeded 75%, demonstrating azadirachtin's efficacy as an insect growth regulator. These results validate neem oil as an effective, low-resistance-risk, and environmentally sound replacement for pirimiphos-methyl within IPM schemes for stored-grain systems.

Introduction

The maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) ranks among the most economically important primary pests of stored cereal grains worldwide, infesting maize, wheat, sorghum, and rice from harvest through long-term warehouse storage. Grain mass losses of 20–40% have been documented in tropical and subtropical regions, with additional qualitative deterioration through mycotoxin contamination and reduced seed viability (Trematerra et al. 2013, Athanassiou et al. 2017, Kifle et al. 2024). In Colombia, post-harvest challenges from *S. zeamais* infestations in maize represent a significant threat to national food security (Fenalce 2020, Cámara de la Industria de Alimentos de la ANDI 2023).

Pirimiphos-methyl, an organophosphate acting through acetylcholinesterase inhibition, has constituted the primary chemical control tool for stored-grain Curculionidae for decades. However, resistance to pirimiphos-methyl is documented and expanding: resistance ratios of 24–70× relative to susceptible strains have been confirmed in field populations of *Sitophilus oryzae* (Linnaeus, 1763) in multiple countries (Khan et al. 2023, Attia et al. 2020). Furthermore, residue accumulation in commodities destined for human consumption, adverse effects on non-target organisms, and increasingly stringent regulatory frameworks underscore the urgency of integrating alternative insecticides within resistance management and IPM programs (Casida and Durkin 2013, Kumar et al. 2022).

Neem oil, derived from seeds of *Azadirachta indica* A. Juss. (Meliaceae), represents a particularly promising biopesticide owing to its complex chemistry and multiple modes of action. Azadirachtin — the principal limonoid — disrupts insect hormonal regulation by interfering with ecdysone and juvenile hormone signaling, inhibiting molting, oviposition, feeding, and reproduction rather than acting through rapid neurotoxicity (Isman 2006, Koul and Wahab 2005, Fernández 2024). This multi-target mechanism fundamentally reduces resistance selection pressure compared to single-target synthetic insecticides (Schmutterer 2002, Kumar et al. 2022). A commercially registered neem product (NEEMPRO®) achieved

complete control of *S. zeamais* in stored maize at 6 g/kg without affecting grain germination over 4 months of storage (Akongo et al. 2015). Sublethal neem concentrations have also been shown to alter *S. zeamais* locomotor patterns and grain contact frequency, contributing to population suppression through behavioral disruption independent of direct lethality (Martins et al. 2024).

Despite this evidence, rigorous comparative studies simultaneously quantifying acute mortality (LC_{50}, LT_{50}), kinetic parameters (cumulative mortality curves), and transgenerational sublethal effects of neem oil versus pirimiphos-methyl on *S. zeamais* remain scarce in the peer-reviewed literature. The present study addresses this gap by: (1) determining and comparing LC_{50} and LT_{50} for both insecticides using Probit regression; (2) characterizing cumulative mortality kinetics at all evaluated concentrations; and (3) quantifying sublethal effects on F1 progeny emergence as a measure of transgenerational impact on *S. zeamais* fitness.

Materials and methods

Insect rearing and study site

All bioassays were conducted at the Plant Health Laboratory, Agronomy Engineering Program, Universidad de Pamplona, Pamplona, Norte de Santander, Colombia (lat 7°22'02" N, long 72°38'56" W; 2 590 m a.s.l., 17 ± 1°C, 65 ± 10% RH). A laboratory colony of *S. zeamais* was established from adults collected from naturally infested pasta products (*Triticum aestivum* L., durum wheat semolina) sourced at local supermarkets in Pamplona. Insects were maintained in 1 000 mL borosilicate glass jars with pasta substrate under controlled conditions until sufficient numbers of 14-day-old adults were available. Adults were sexed morphologically (female: elongate, smooth, slender rostrum; male: shorter, wider, punctate rostrum) and used in a 2♀:2♂ proportion per experimental unit (Howe 1952, Birch 1953).

Test insecticides and concentrations

Two ICA-registered commercial products were evaluated: (1) Neem oil — NEEM AZAL 1,2% CE (1,2% p/v azadirachtin, concentrado emulsionable; Trifolio-M GmbH, Germany); (2) Pirimiphos-methyl — ACTELLIC 50 CE (50% p/v pirimifos metil; Syngenta, Switzerland). Five concentrations per insecticide were prepared by serial dilution (Table 1). Neem oil concentrations (mL/L) reflect volumetric dilutions in deionized water; pirimiphos-methyl concentrations (mg/L) reflect mass-based dilutions. Both sets were calibrated to bracket the expected LC_{50} range based on preliminary dose-finding trials and published literature (Iannacone et al. 2008, Guettal et al. 2021).

Bioassay design and fumigant exposure method

Two independent completely randomized design (CRD) experiments were conducted, each comprising six treatments (five concentrations + absolute control) × five replicates, totaling 30 experimental units per experiment (60 total). Each experimental unit consisted of a 30 mL sealed borosilicate glass vial

containing four sexed *S. zeamais* adults (2♀:2♂) and 2 g of pasta substrate as food source and oviposition medium.

Fumigant toxicity was assessed using the sponge-release method: a 0,5 × 0,5 cm polyurethane sponge was adhered to the interior of each vial cap prior to treatment. Using a calibrated micropipette, the appropriate volume/mass of each concentration was applied to the sponge immediately before sealing. This method generates vapor-phase fumigant exposure without direct contact between the insecticide solution and insects or substrate, ensuring that lethality and sublethal effects are attributable exclusively to fumigant action (Moura et al. 2021, Oyedeji et al. 2020). Vials remained sealed throughout the mortality evaluation period.

Mortality recording and sublethal evaluation

Adult mortality was recorded at 12-hour intervals for 96 hours (8 observation periods: 12, 24, 36, 48, 60, 72, 84, 96 h) without opening the vials. An individual was scored as dead when it showed complete cessation of leg, antenna, and body movement upon gentle tapping of the vial. Cumulative percent mortality was calculated for each time point and concentration.

Statistical analyses

LC_{50} was estimated by Probit regression (Finney 1952) with concentrations transformed to $\log_{10}$ and mortality percentages converted to Probit units; control data were excluded from Probit models to avoid bias from background mortality. LT_{50} for each concentration was determined by linear regression of cumulative mortality against time (12-hour intervals). All analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA).

Results

Adult mortality: comparative overview

All insecticide concentrations tested produced high adult mortality at 96 h. Tukey's HSD post-hoc test grouped all neem oil and pirimiphos-methyl concentrations in a single homogeneous group (*a*), while the control formed a distinct group (*b*) (Table 2, Figure 1). The ceiling effect observed — where all concentrations substantially exceeded the LC_{99} — is common in laboratory bioassays designed to bracket the LC_{50} rather than the upper lethality range (Robertson et al. 2017).

Probit analysis and LC_{50} determination

Probit regression excluding controls yielded robust concentration-response relationships for both insecticides (Table 3, Figure 2). For neem oil, the regression equation was y = 2,56x + 3,14 (R^2 = 0,85; F = 22,7; p = 0,009), with LC_{50} = 0,72 mL/L (95% IC: 0,52-0,94 mL/L). For pirimiphos-methyl, the equation was y = 2,47x + 3,04 (R^2 = 0,92; F = 46,1; p = 0,002), with LC_{50} = 6,31 mg/L (95% IC: 4,18-9,52 mg/L).

The near-identical slopes (2,56 vs. 2,47) suggest that within-population tolerance variability in the tested *S. zeamais* colony is intrinsic to the strain rather than driven by the class of xenobiotic applied (Finney 1952). The higher R^2 for pirimiphos-methyl (0,92 vs. 0,85) reflects the more predictable dose-response of a single active molecule with one primary biochemical target (acetylcholinesterase inhibition) compared to the multi-limonoid composition of neem oil (azadirachtin, salannin, nimbin, meliantriol), which generates greater biological variability across the response range (Koul and Wahab 2005, Casida and Durkin 2013). The LC_{50} of neem oil (0,72 mL/L) is consistent with previously reported values for Curculionidae (Iannacone et al. 2008, Akongo et al. 2015).

Lethal time (LT_{50}) and concentration-time relationship

LT_{50} values exhibited a clear inverse concentration-time relationship for both insecticides (Table 4, Figure 3). Neem oil LT_{50} ranged from 12 h (1,20 mL/L) to 108 h (0,24 mL/L); pirimiphos-methyl LT_{50} ranged from 12 h (200 mg/L) to 44 h (40 mg/L). Regression R^2 values were generally higher at intermediate concentrations for both products, while the lowest R^2 appeared at the highest pirimiphos-methyl concentration (200 mg/L, R^2 = 0,21), likely attributable to extremely rapid mortality reducing temporal resolution of the linear model (Robertson et al. 2017). These LT_{50} values for neem oil are consistent with those independently reported by Ruiz-García and Giraldo-Vanegas (2024) using the same commercial formulation (NEEM AZAL 1,2% CE) and sponge-release method under identical laboratory conditions in Pamplona, who determined TL_{50} values of 13 h at 1,20 mL/L and 104 h at 0,24 mL/L, confirming the robustness and reproducibility of the concentration–lethal time relationship observed in the present study.

Cumulative mortality kinetics at maximum concentrations

At maximum concentrations (neem oil: 1,20 mL/L; pirimiphos-methyl: 200 mg/L), cumulative mortality curves were virtually superimposable (Figure 4): both reached 50% mortality at 12 h, 90-95% at 48 h, and 100% by 96 h. This kinetic equivalence directly challenges the widespread assumption that plant-derived insecticides are inherently slower-acting than synthetic compounds. The result is consistent with recent reports showing that concentrated botanical products can achieve knockdown rates comparable to synthetic standards when applied at saturating doses (Moura et al. 2021, Kifle et al. 2024, Martins et al. 2024).

Sublethal effects: F1 progeny inhibition

Tukey's HSD test grouped neem oil F1 inhibition data into three homogeneous subsets: 0,72–1,20 mL/L (group *a*), 0,24–0,48 mL/L (group *b*), and control (group *c*) (Table 5, Figure 5).

A critical finding is that progeny inhibition exceeded 75% at the lowest neem oil concentration tested (0,24 mL/L), demonstrating that the effective concentration range for suppressing *S. zeamais* population dynamics extends below the lethal dose range — a property unique to multi-modal biopesticides such as azadirachtin and not typical of purely neurotoxic insecticides (Guettal et al. 2021, Schmutterer 2002).

Discussion

The 98–100% adult mortality achieved by all neem oil concentrations and 100% achieved by all pirimiphos-methyl concentrations confirm that both products, when delivered as fumigants in sealed glass containers, are highly effective against *S. zeamais* adults. This result is consistent with studies reporting high efficacy of neem-based products against stored-grain Curculionidae, including *S. oryzae*, *Sitophilus granarius* (Linnaeus, 1758), and *S. zeamais* (Kifle et al. 2024, Guettal et al. 2021, Akongo et al. 2015). Notably, Martins et al. (2024) found that even below the acute mortality threshold, neem extract significantly altered *S. zeamais* behavioral parameters — including locomotion and grain contact frequency — suggesting that population suppression in field grain stores may occur through combined behavioral and lethal mechanisms not captured in closed-vial laboratory bioassays.

The determination coefficients obtained (R^2 = 0,85 for neem oil; R^2 = 0,92 for pirimiphos-methyl) reflect a fundamental difference in insecticidal activity: pirimiphos-methyl acts through a single, well-defined biochemical target (irreversible acetylcholinesterase inhibition), producing a highly predictable sigmoid dose-response curve (Casida and Durkin 2013, Bradbury and Coats 1989). In contrast, neem oil delivers a complex mixture of at least 35 limonoids (azadirachtin A/B, salannin, nimbin, meliantriol, gedunin), each with distinct targets and potencies, producing greater biological heterogeneity across the dose-response range (Koul and Wahab 2005, Schmutterer 2002).

The inverse concentration-LT_{50} relationship documented here is consistent with standard pharmacokinetic principles (Moura et al. 2021, Oyedeji et al. 2020). This pattern is independently corroborated by Ruiz-García and Giraldo-Vanegas (2024), who reported LT_{50} values ranging from 13 h (1,20 mL/L) to 104 h (0,24 mL/L) for the same NEEM AZAL 1,2% CE formulation against adult *S. zeamais* under identical laboratory conditions in Pamplona, confirming the consistency of the concentration–lethal time relationship across independent experiments. The broader LT_{50} range of neem oil (12–108 h) compared to pirimiphos-methyl (12–44 h) reflects the concentration-dependent expression of different mechanisms within the neem limonoid complex: at sublethal and low-lethal concentrations, antifeedant and insect growth regulatory effects predominate, producing slower, indirect lethality through starvation and endocrine disruption, whereas at saturating concentrations, combined effects including secondary neurological disruption accelerate mortality to levels matching the direct cholinergic toxicity of pirimiphos-methyl (Isman 2006, Fernández 2024). The virtually superimposable cumulative mortality curves at maximum concentrations (Fig. 4) directly refute the persistent perception that botanical insecticides are inherently slow, which has historically limited their adoption in commercial grain storage (Trinidad and Gaona 2011).

The F1 progeny inhibition data represent the most strategically important findings from an IPM perspective. Neem oil achieved > 75% F1 inhibition at every concentration evaluated — including the lowest (0,24 mL/L) — reflecting azadirachtin's well-characterized mechanism as an ecdysone antagonist and juvenile hormone mimic: interfering with oogenesis, egg viability, embryogenesis, larval molting, and pupal development, while potentially transferring to progeny through maternal deposition in eggs

(Schmutterer 2002, Koul and Wahab 2005). This demonstrates that even when neem oil fails to kill all adults, it effectively suppresses the reproductive output of survivors — preventing population rebound between treatment cycles.

Pirimiphos-methyl also produced significant sublethal effects (R^2 = 0,79), which is mechanistically unexpected for a purely neurotoxic compound. The likely explanations involve: (1) sublethal cholinergic disruption reducing mating behavior and oviposition frequency; (2) physiological stress reducing egg quality and hatchability; and (3) possible maternal transfer of organophosphate metabolites affecting larval development (Pérez et al. 2014, Kumar et al. 2022). Nonetheless, the growing resistance to pirimiphos-methyl documented in stored-grain weevil populations worldwide (Khan et al. 2023, Attia et al. 2020) further motivates replacing or rotating it with multi-modal biopesticides that are inherently less prone to resistance selection.

Based on the integrated toxicological data from this study, the following recommendations are proposed for *S. zeamais* management in stored-grain facilities: (1) neem oil at 0,72 mL/L is recommended as the minimum effective dose for adult control; (2) at maximum concentrations (1,20 mL/L for neem oil; 200 mg/L for pirimiphos-methyl), the LT_{50} = 12 h; (3) rotation of neem oil (multiple modes of action) with pirimiphos-methyl (single target) is recommended to delay resistance development; and (4) the > 75% F1 inhibition at sublethal concentrations suggests that treatment intervals can be extended beyond those dictated by adult mortality alone.

Conclusions

Both neem oil (NEEM AZAL 1,2% CE) and pirimiphos-methyl (ACTELLIC 50 CE) demonstrated high fumigant insecticidal efficacy against adult *S. zeamais*, with 98–100% adult mortality achieved across all tested concentrations at 96 h under fumigant laboratory conditions.

Probit regression confirmed robust concentration-response relationships: LC_{50} = 0,72 mL/L (R^2 = 0,85) for neem oil and LC_{50} = 6,31 mg/L (R^2 = 0,92) for pirimiphos-methyl. Nearly identical slopes (2,56 vs. 2,47) indicate that within-population tolerance variability is a strain-specific trait independent of insecticide class.

LT_{50} showed an inverse concentration-time relationship for both insecticides. At maximum concentrations, LT_{50} converged at 12 h for both treatments, with virtually superimposable cumulative mortality curves reaching 100% by 96 h — directly challenging the assumption that botanical insecticides are inherently slow-acting.

F1 progeny inhibition was 77,75–100% (neem oil, R^2 = 0,66) and 62,93–100% (pirimiphos-methyl, R^2 = 0,79). Neem oil caused > 75% F1 inhibition even at the lowest concentration (0,24 mL/L), validating azadirachtin's role as an insect growth regulator capable of suppressing population rebound at concentrations below the adult lethal threshold.

Neem oil constitutes an effective, resistance-management-compatible, and environmentally sustainable alternative to pirimiphos-methyl for *S. zeamais* management in stored-grain IPM programs, combining high acute fumigant efficacy with potent transgenerational sublethal activity and an inherently low resistance selection risk attributable to its multi-modal mechanism of action.

Declarations

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Tables

Table 1. Concentration ranges evaluated for neem oil and pirimiphos-methyl against adult *Sitophilus zeamais* Motschulsky in fumigant bioassays.

Treatment	Concentration levels	Unit	Dilution method
Neem oil (NEEM AZAL 1,2% CE)	1,20 / 0,96 / 0,72 / 0,48 / 0,24	mL/L	Serial (vol/vol in H_2O)
Pirimiphos-methyl (ACTELLIC 50 CE)	200 / 160 / 120 / 80 / 40	mg/L	Serial (mass/vol in H_2O)
Control	0	—	Deionized water only

Table 2. Adult mortality (%) of *Sitophilus zeamais* Motschulsky at 96 h exposure to neem oil (NEEM AZAL 1,2% CE) and pirimiphos-methyl (ACTELLIC 50 CE) under laboratory conditions. Means ± SD (n = 5 replicates × 20 adults).

Treatment	N	Mortality (%)	SD	Tukey group
Pirimifos metil — 200 mg/L	5	100,00	0,00	a
Pirimifos metil — 160 mg/L	5	100,00	0,00	a
Pirimifos metil — 120 mg/L	5	100,00	0,00	a
Pirimifos metil — 80 mg/L	5	100,00	0,00	a
Pirimifos metil — 40 mg/L	5	100,00	0,00	a
Aceite de neem — 1,20 mL/L	5	100,00	0,00	a
Aceite de neem — 0,96 mL/L	5	100,00	0,00	a
Aceite de neem — 0,72 mL/L	5	98,00	2,74	a
Aceite de neem — 0,48 mL/L	5	100,00	0,00	a
Aceite de neem — 0,24 mL/L	5	100,00	0,00	a
Control (0)	5	1,00	2,24	b

Table 3. Summary of Probit regression parameters and LC_{50} estimates for neem oil and pirimiphos-methyl against adult *S. zeamais*.

Insecticide	Regression equation	R^2	LC50	95% CI
Neem oil	y = 2,56x + 3,14	0,85	0,72 mL/L	0,52-0,94
Pirimiphos-methyl	y = 2,47x + 3,04	0,92	6,31 mg/L	4,18-9,52

Table 4. Median Lethal Time (LT_{50}) parameters for neem oil and pirimiphos-methyl against adult *S. zeamais* at five concentrations each.

Insecticide	Concentration	Regression equation	R^2	LT50 (h)
Neem oil	1,20 mL/L	y = 0,57x + 56,68	0,60	12
	0,96 mL/L	y = 0,90x + 28,54	0,79	24
	0,72 mL/L	y = 0,82x + 23,90	0,85	32
	0,48 mL/L	y = 0,60x + 18,57	0,85	52
	0,24 mL/L	y = 0,33x + 10,14	0,60	108
Pirimiphos-methyl	200 mg/L	y = 0,45x + 44,61	0,21	12
	160 mg/L	y = 0,31x + 44,86	0,53	17
	120 mg/L	y = 0,45x + 41,36	0,74	19
	80 mg/L	y = 0,50x + 36,71	0,76	25
	40 mg/L	y = 0,83x + 13,43	0,74	44

Table 5. Sublethal effects (F1 adult emergence inhibition) of neem oil and pirimiphos-methyl on *Sitophilus zeamais* Motschulsky under laboratory conditions (means ± SD, n = 5).

Insecticide	Concentration	F1 inhibition (%)	SD	Tukey
Neem oil	1,20 mL/L	100,00	0,00	a
	0,96 mL/L	97,14	6,39	a
	0,72 mL/L	90,18	9,31	a
	0,48 mL/L	78,76	7,15	b
	0,24 mL/L	77,75	14,57	b
	Control	0,00	0,00	c
Pirimiphos-methyl	200 mg/L	100,00	0,00	a
	160 mg/L	93,04	9,78	a
	120 mg/L	84,47	10,48	ab
	80 mg/L	72,04	12,64	bc
	40 mg/L	62,93	16,66	c
	Control	0,00	0,00	d

Table 6. Comparison of LC_{50} values for neem-based products against *Sitophilus* spp. from the literature (2008-2024).

Product / compound	Species	LC_{50}	Conditions	Reference
Neem Azal 1,2% CE	*S. zeamais*	0,72 mL/L	17°C, fumigant, 96 h	This study
NeemPro® (azadirachtin)	*S. zeamais*	0,04-0,11 g/kg	27°C, contact, 14 d	Akongo et al. (2015)
Neem oil (cold-pressed)	*S. granarius*	0,09 g/L	25°C, fumigant, 48 h	Guettal et al. (2021)
A. indica extract	*S. zeamais*	0,31 g/kg	28°C, contact, 72 h	Iannacone et al. (2008)

Figures

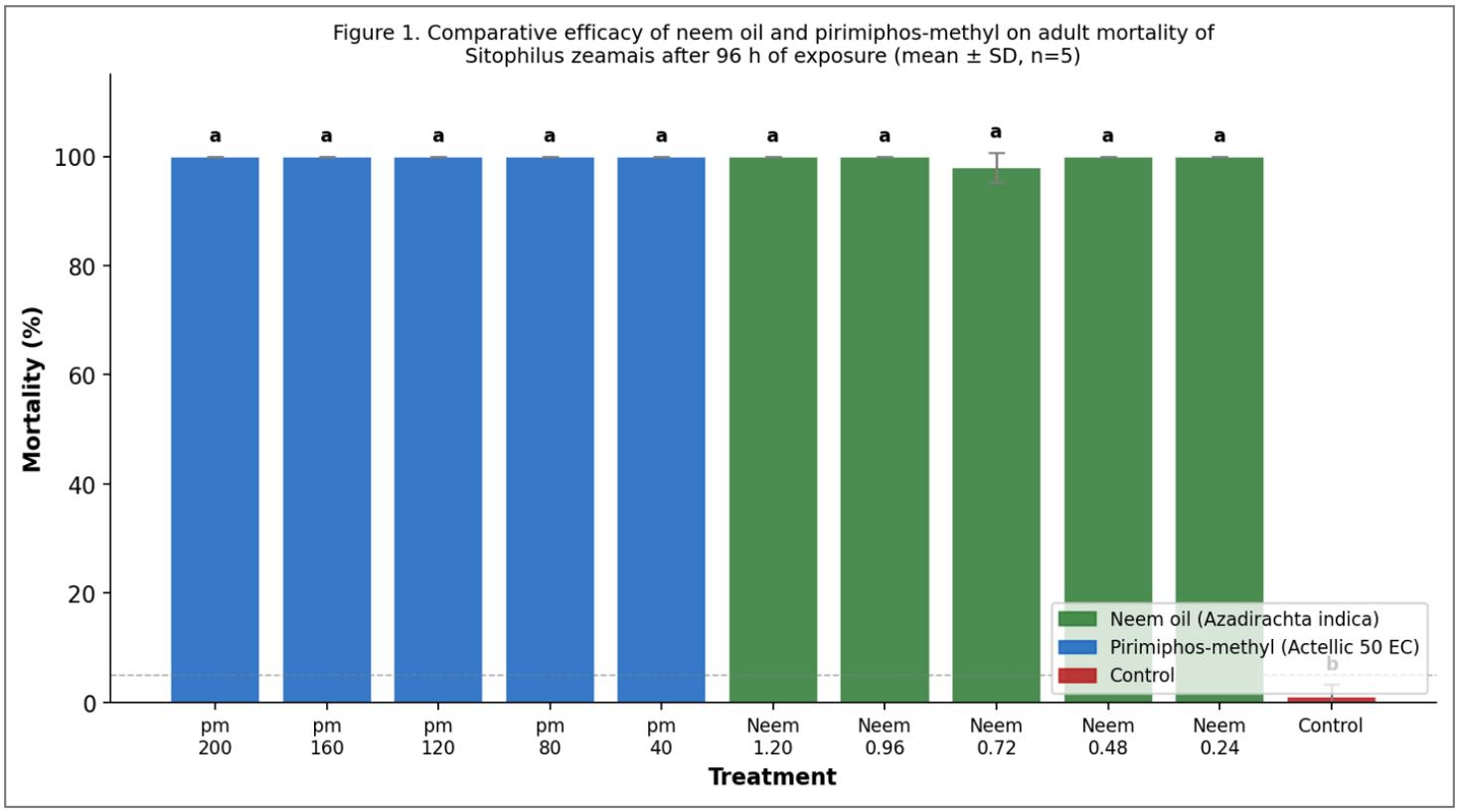

Figure 1

Comparative adult mortality (%) of *Sitophilus zeamais*

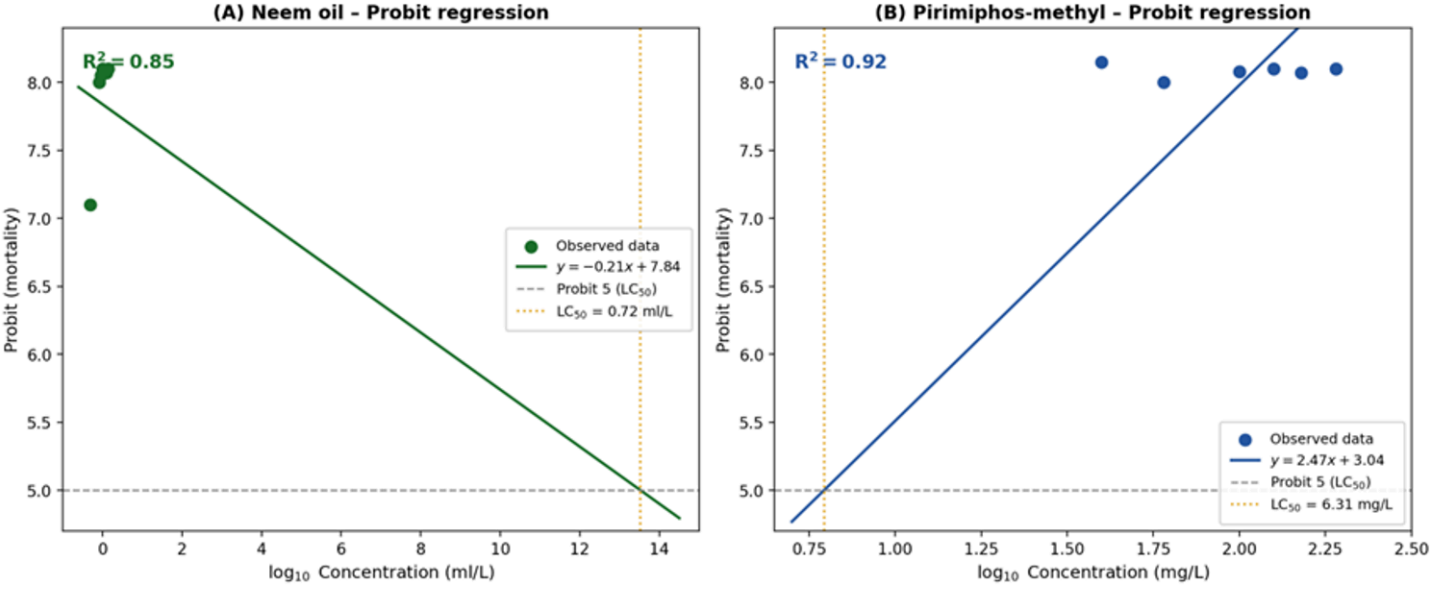

Figure 2. Probit regression analysis for LC_{50} determination of neem oil and pirimiphos-methyl against Sitophilus zeamais adults under laboratory conditions

Figure 2

Probit regression for LC_{50} determination. (A) Neem oil: y = 2,56x + 3,14, R^2 = 0,85; estimated LC_{50} = 0,72 mL/L (vertical dotted line). (B) Pirimiphos-methyl: y = 2,47x + 3,04, R^2 = 0,92; estimated LC_{50} = 6,31 mg/L. Horizontal dashed line = Probit 5 (50% mortality). Circles = observed data points. The near-identical slopes indicate similar population heterogeneity in insecticide tolerance.

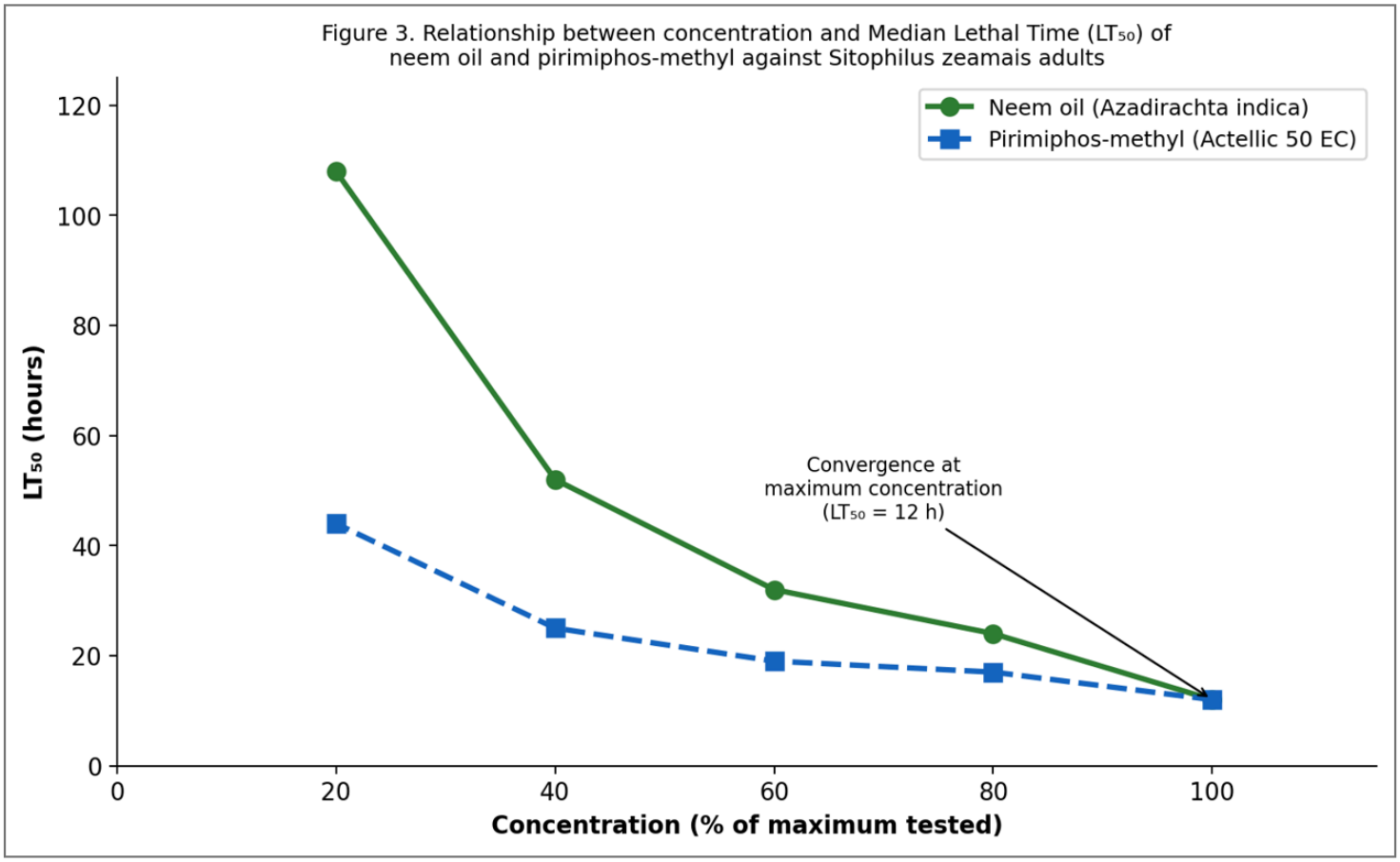

Figure 3

Median Lethal Time (LT_{50}, hours) as a function of concentration (expressed as % of maximum tested concentration) for neem oil and pirimiphos-methyl against adult *Sitophilus zeamais*. Note the convergence of both curves at 100% of maximum concentration (LT_{50} = 12 h for both), and the substantially wider LT_{50} range of neem oil at lower concentrations, reflecting its multi-modal mechanism of action.

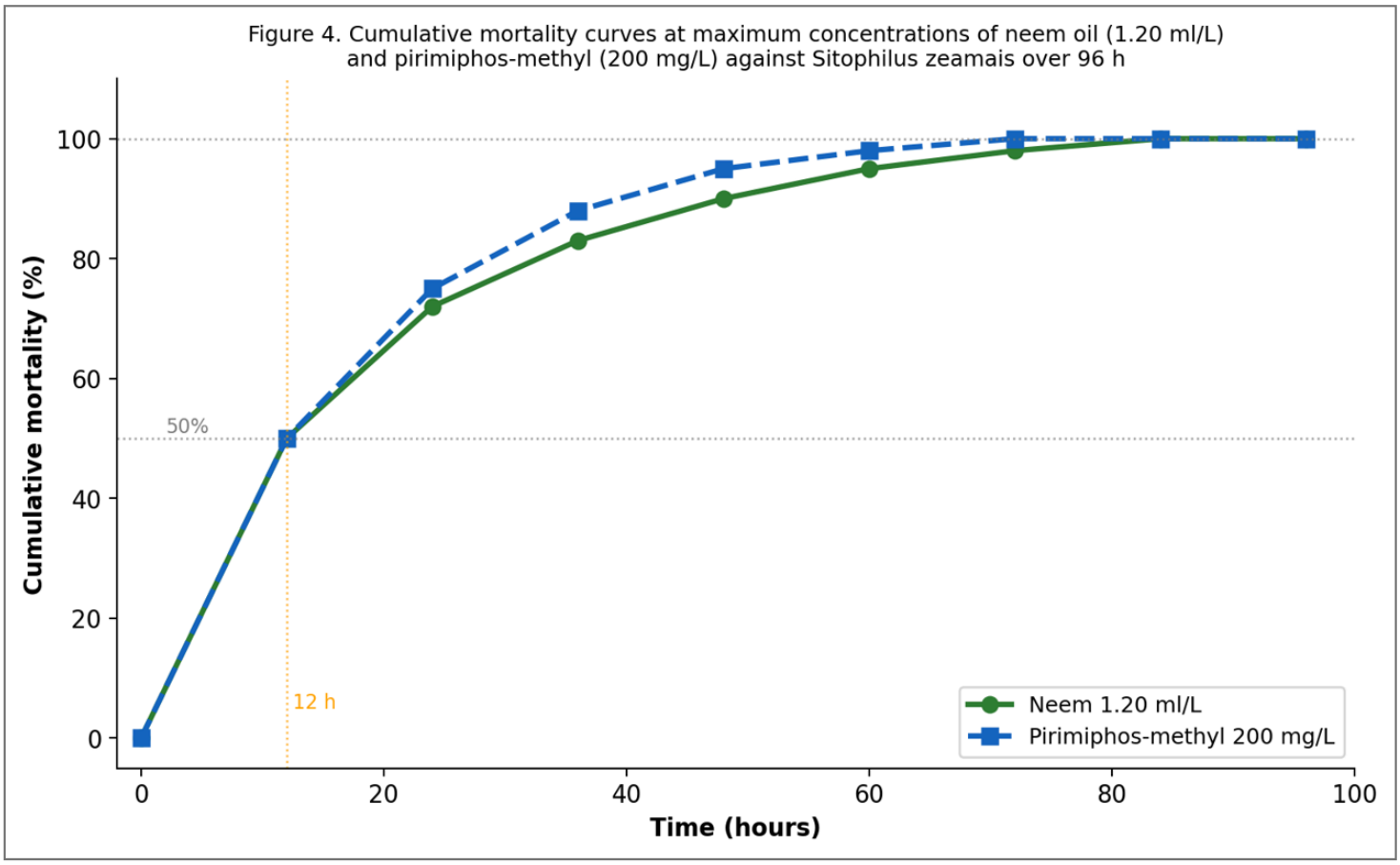

Figure 4

Cumulative mortality kinetics at maximum concentrations of neem oil (1,20 mL/L) and pirimiphos-methyl (200 mg/L) against adult *Sitophilus zeamais* over 96 h. The virtually superimposable curves demonstrate equivalent speed of action at saturating concentrations, with LT_{50} = 12 h and 100% mortality at 96 h for both treatments.

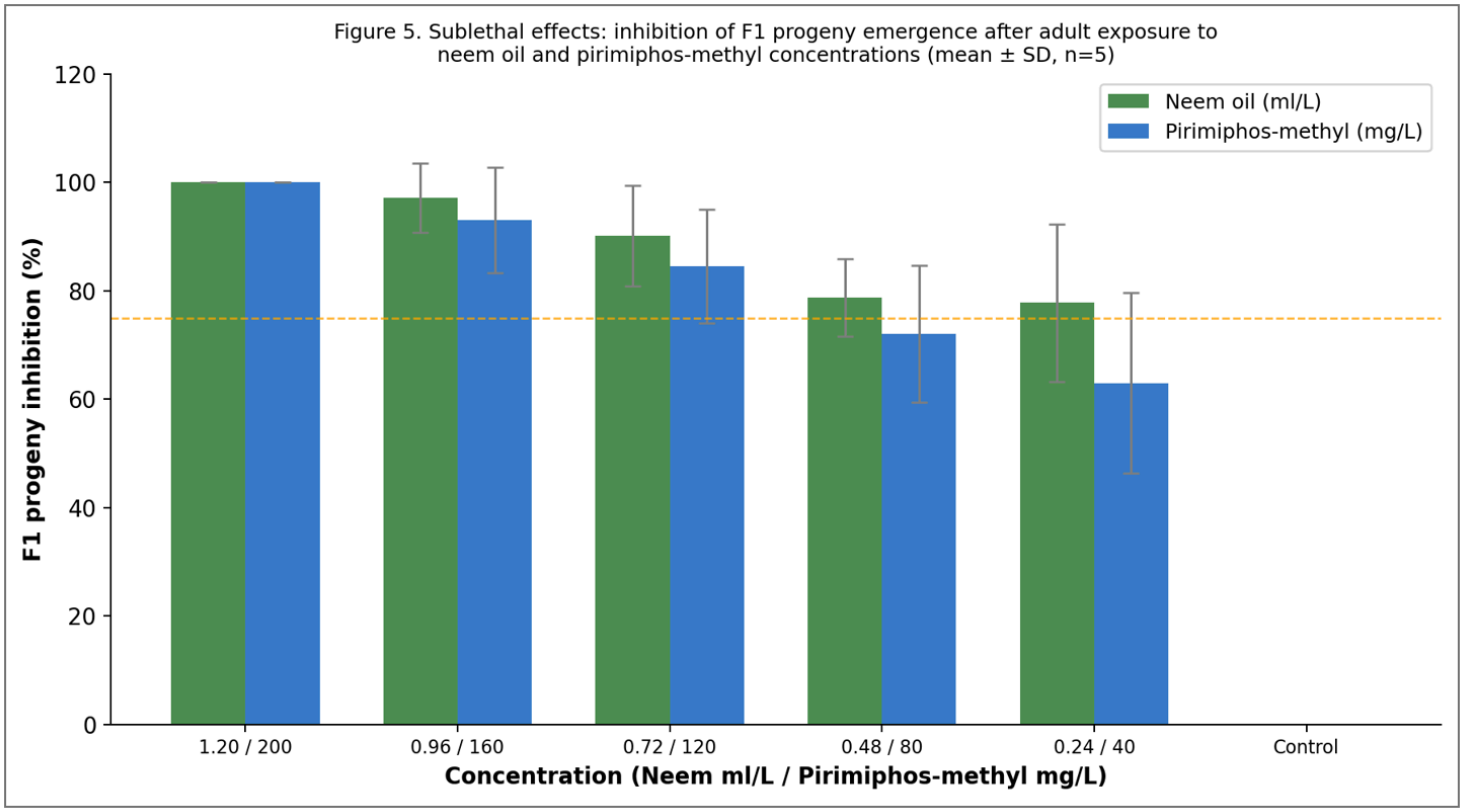

Figure 5

F1 adult emergence inhibition (%) in *Sitophilus zeamais* after 96-hour fumigant exposure of parents to neem oil and pirimiphos-methyl. Bars: mean ± SD (n = 5). Dashed orange line: 75% inhibition threshold. Even at the lowest neem oil concentration (0,24 mL/L), inhibition exceeded 75%, demonstrating azadirachtin's potency as an insect growth regulator at sublethal doses.