Research Square

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Engorgement and mortality of Aedes aegypti (Diptera: Culicidae) exposed to binary combinations of ivermectin and dinotefuran in Attractive Toxic Sugar Baits (ATSBs)

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Research Article

Keywords: phagostimulants, Insecticide mixture, endectocide, neonicotinoids

Posted Date: April 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9417313/v1

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Additional Declarations: No competing interests reported.

Abstract

Aedes aegypti remains a critical global threat as the primary vector for arboviruses such as Dengue and Zika. Due to widespread insecticide resistance, innovative strategies like Attractive Toxic Sugar Baits (ATSBs) and insecticide combinations are urgently needed. This study aimed to estimate the lethal concentrations (LCs) and evaluate the synergistic effects of Dinotefuran (DNT) and Ivermectin (IVM) in ATSBs against male and female *Ae. aegypti*. Mosquitoes were exposed to 70% sucrose baits containing DNT (1–100 ppm) alone or in binary combinations with IVM (0.312–100 ppm). Engorgement rates were high across all treatments (> 83% for DNT alone and > 99% for combinations). While males were generally more susceptible to DNT alone than females, the addition of IVM to DNT baits resulted in similar LC values for both sexes. Notably, the combination of IVM with a low concentration of DNT (1 ppm) yielded a pronounced synergistic effect, significantly reducing LC_{90} values for both males (SR = 7.5) and females (SR = 11.38). These findings demonstrate for the first time that combining ivermectin with low dose dinotefuran in ATSBs is highly synergistic, effectively reducing the chemical concentration required for high mortality.

Introduction

The mosquito *Aedes aegypti* remains a critical global health threat as the primary vector for major arboviruses, including Yellow Fever, Dengue (DENV), Zika (ZIKV), and Chikungunya (CHIKV). The endemic nature of these pathogens, especially in the Americas, is escalating; for instance, the region recorded historically high numbers of dengue cases in 2025 a trend that continued into early 2026, prompting an epidemiological alert from the Pan American Health Organization (PAHO). This persistent and expanding challenge underscores the urgent need for innovative and sustainable vector control strategies.

Traditional control measures, relying heavily on chemical insecticides, face increasing limitations due to widespread insecticidal resistance. To overcome this hurdle and manage resistance evolution, combining active ingredients with distinct modes of action (MoAs) has emerged as a strategic priority. This approach not only targets resistant mosquito populations but also offers the potential for synergistic effects, whereby the combined efficacy is greater than the sum of individual components. Such synergy can permit the use of lower concentrations, leading to reduced operational costs and lower environmental impact (Asidi et al. 2005; Corbel et al. 2002; Corbel et al. 2004b).

In parallel with this chemical strategy, Attractive Toxic Sugar Baits (ATSBs) are being developed as an ecologically sound delivery system. ATSBs capitalize on the mosquito's natural requirement for sugar feeding, offering a toxic compound within an attractive sugar solution. Two promising insecticides for this system are Dinotefuran (DNT) and Ivermectin (IVM). DNT, a third-generation neonicotinoid (Wakita et al. 2003), acts as a potent agonist on insect nicotinic acetylcholine receptors (Kiriyama & Nishimura, 2002). It has shown high efficacy in ATSBs against *Ae. aegypti* even in populations resistant to other chemical classes (Khallaayoune et al. 2013). IVM, conversely, interferes with invertebrate neurotransmission, primarily by acting on glutamate-gated chloride channels, causing paralysis and

death (Õmura, 2008). IVM has also recently demonstrated success when delivered via ATSBs against *Anopheles arabiensis* (Tenywa et al. 2017) and *Ae. aegypti* (Dias et al. 2025).

The success of combining different insecticides is well-documented in other vector control contexts, such as bednets (Guillet, 2001) and ectoparasite control, where formulations containing DNT and other compounds exhibit sustained high efficacy and anti-feeding effects (Franc et al. 2012; Liénard et al. 2013). Specifically, studies on house flies have shown significant synergism when IVM is combined with neonicotinoids or similar compounds in sugar cubes (Levchenko et al., 2019), underscoring the potential for a "two-in-one" toxic sugar bait approach to prevent resistance (Njoroge et al., 2023). Therefore, this study aimed to estimate the Lethal Concentrations (LCs) of Dinotefuran and Ivermectin, both individually and in combination, within Attractive Toxic Sugar Baits (ATSBs) to evaluate their synergistic effect on male and female *Aedes aegypti*.

Materials and Methods

Ethical Aspects

The procedures for mosquito rearing and human blood collection for blood feeding were conducted with the approval of the Research Ethics Committee of the Federal University of Rondônia (Universidade Federal de Rondônia, UNIR) under protocol number CAAE: 78543817.9.0000.5300.

Aedes aegypti **Rearing**

Aedes aegypti mosquitoes used in the experiments were obtained from the colony maintained at the Laboratory of Insect Bioecology (LaBEIn), Federal University of Rondônia (UNIR), and reared according to the protocol described by Dias et al. (2023). Male and female mosquitoes aged 5 to 8 days were separated and deprived of sugar feeding for 24 and 48 hours, respectively, before experimentation (Silva et al., 2021). Insectary conditions were maintained at 28°C, relative humidity of 70–80%, larval rearing tray temperature of 26 ± 1°C, and a 12:12 h light:dark photoperiod.

Insecticides

The insecticides were selected based on their distinct chemical classes and different modes of action. Dinotefuran (Pestanal®, analytical standard, 98.8%) was purchased from Sigma-Aldrich®, and ivermectin (Ivomec®, injectable, veterinary use) was obtained from a local supplier.

Feeding, Mortality Assessment, and Lethal Concentration (LC) Determination of Attractive Toxic Sugar Baits (ATSBs) Containing Dinotefuran

A 70% sucrose-based attractive sugar bait (ASB) solution was prepared according to Dias et al. (2023), consisting of guava concentrate (Dafruta®) diluted in water at a 3:1 ratio and 70% brown sugar

(Dacolônia®). The solution was allowed to ferment for 48 h and then supplemented with 3% food dye (Arcocolor®).

A stock solution of dinotefuran (DNT) was prepared at 100 ppm, and serial dilutions were made to obtain concentrations of 1, 10, 25, 50, and 100 ppm, based on previously reported toxicity data (Corbel et al., 2004a; Khallaayoune et al., 2013).

Groups of 20 mosquitoes were placed in screened disposable plastic cups (250 mL) for each concentration and the control group. In total, 2,160 mosquitoes (1,080 males and 1,080 females) were tested separately on different days. The ATSBs containing the various concentrations of dinotefuran were offered on cotton pads. Each experiment was conducted in triplicate and repeated three times across different generations.

After 6 h of exposure, mosquitoes were examined under a stereomicroscope (Zeiss®) to assess the degree of engorgement following the qualitative method described by Pilitt and Jones (1972). Engorged mosquitoes were counted and transferred to screened cages (15 × 12 × 17 cm) separated by engorgement degree for mortality assessment at 24, 48, and 72 h (WHOPES, 2005).

Evaluation of Combined Effects of Ivermectin (IVM) and Dinotefuran (DNT)

To assess the combined toxic effects, ivermectin concentrations were selected based on laboratory studies by Dias et al. (2023). A binary mixture was prepared by combining one concentration of IVM with one of DNT. The potential synergistic effect of the insecticide combination was evaluated according to the modified methodology of Corbel et al. (2004b), by testing the lowest concentration of one insecticide with increasing concentrations of the other.

The lowest DNT concentration was 1 ppm, and the lowest IVM concentration was 0.312 ppm. Increasing concentrations of DNT (1, 10, 25, 50, and 100 ppm) were each combined with 0.312 ppm IVM, and increasing concentrations of IVM (0.312, 5, 10, 25, 50, and 100 ppm) were each combined with 1 ppm DNT. The control group (ASB) contained no insecticide.

Mosquito ingestion was confirmed by the presence of red dye in the abdomen. Mosquitoes that did not ingest the bait after 6 h were discarded, and only engorged individuals were observed for mortality at 24, 48, and 72 h, with 10% sucrose solution provided daily.

Each experiment was conducted in triplicate with 20 mosquitoes per replicate, totaling 2,960 individuals (1,480 males and 1,480 females). The experiment was repeated twice with different generations. After 6 h of exposure, engorgement was assessed under a stereomicroscope (Zeiss®), and engorged mosquitoes were transferred to screened cages (15 × 12 × 17 cm) for mortality observations at 24–72 h (WHOPES, 2005).

To determine potential synergistic or antagonistic effects of the combined insecticides, the synergistic ratio (SR) was calculated according to Corbel et al. (2004b) using the following formula:

Synergistic Ratio (SR):

$$SR = \frac{\text{CL insecticide 1 without insecticide 2}}{\text{CL insecticide 1 with insecticide 2}}$$

where SR > 1 indicates synergism and SR < 1 indicates antagonism. LC = lethal concentration.

Statistical Analysis

Engorgement rates, engorment degrees and mortality of male and female *Ae. aegypti* exposed to different insecticide concentrations or their combinations were analyzed using two-way ANOVA under different variable combinations (sex and concentration; concentration and degree of engorgement; insecticide combination and concentration). Comparisons were performed using Tukey's test at a 5% significance level. Mortality data were subjected to Probit analysis (concentration × mortality) to estimate LC_{50} and LC_{90} values using Minitab 19 software (Minitab Inc., USA).

Results

The baits containing different concentrations of dinotefuran were ingested by more than 83% of the mosquitoes, and ingestion was not affected by sex (F = 0.01; P = 0.91). A significant difference in feeding rate was observed only between the baits containing 10 ppm, which had the highest ingestion (19 mosquitoes), and those with 100 ppm, which had the lowest (16 mosquitoes) (F = 2.37; P = 0.049) (Fig. 1).

The degrees of engorgement of ATSBs resulting from bait ingestion by male and female mosquitoes were not affected by the different dinotefuran concentrations (males: F = 0.006; females: F = 0.007; P > 0.9999). However, significant differences were detected among engorgement levels (0-5) within both sexes (males: F = 15.27; P < 0.0001; females: F = 27.8; P < 0.0001). Overall, the engorgement patterns were similar between sexes, with the highest mean number of engorged mosquitoes (both males and females) recorded at degrees 3 and 4 (Fig. 2).

Within the first six hours of exposure to the bait, mosquitoes with overdistended abdomens were observed lying on the cage surface (Fig. 3). These individuals did not recover and died within 24 hours.

Mosquito mortality increased with rising DNT concentrations (F = 51.4; P < 0.001), ranging from 33% (7 mosquitoes) to 82% (17 mosquitoes), and was higher in males than in females (F = 21.10; P < 0.0001), particularly at 10 and 25 ppm (F = 20.5; P < 0.001) (Fig. 4).

Different letters indicate significant differences between concentrations ($P < 0.05$). Different numbers indicate significant differences between sexes ($P < 0.05$). Two-way ANOVA (concentration x sex) and Tukey's test for multiple comparisons.

Mortality induced by ATSB–DNT was greater at engorgement degrees 2–4 in males and degrees 3–4 in females (Fig. 5).

Different letters indicate significant differences between engorgement degrees, and different numbers indicate significant differences between the tested concentrations ($P < 0.05$). Two-way ANOVA (concentration x degree) and Tukey's test for multiple comparisons.

The combined use of insecticides, regardless of the main insecticide concentration, did not affect the ingestion of ATSBs: DNT + IVM (males: $F = 1.872$; $P = 0.1127$; females: $F = 0.0267$; $P = 0.8708$) or IVM + DNT (males: $F = 0.4728$; $P = 0.8263$; females: $F = 2.671$; $P = 0.1067$). Ingestion exceeded 99% in both sexes (Fig. 6).

Mortality did not differ significantly between treatments ($F = 0.55$; $P = 0.46$) when comparing DNT alone and DNT combined with IVM in males, but increased with rising DNT concentrations ($F = 64.46$; $P < 0.0001$) (Fig. 7A). In contrast, for females, the combination DNT + IVM resulted in significantly higher mortality compared to DNT alone ($F = 72.81$; $P < 0.0001$), with mortality increasing with concentration ($F = 98.47$; $P < 0.0001$) (Fig. 7B).

Mosquito mortality with ATSB–IVM and IVM + DNT differed for males ($F = 6.71$; $P = 0.012$) (Fig. 7C) and females ($F = 4.73$; $P = 0.036$) (Fig. 7D), being higher when the insecticides were combined. Overall, significant differences were found between the lowest and all higher concentrations for both males ($F = 61.97$; $P < 0.0001$) and females ($F = 26.10$; $P < 0.0001$).

The lethal concentrations (LCs) of ATSB–DNT were lower for males than females, but the addition of IVM to DNT baits resulted in similar LC values for both sexes. Furthermore, the inclusion of IVM in DNT-based baits produced a slight synergistic effect (SR = 1.03–1.9), except for the LC_{50} in females. Conversely, no differences were found between the LCs of ATSB–IVM for males and females; however, the addition of DNT to IVM baits yielded a pronounced synergistic effect, particularly reflected in the reduction of LC_{90} values for both sexes (Table 1).

Table 1 Lethal Concentration (LC) in ppm of dinotefuran and ivermectin, alone and in combination, against male and female *Aedes aegypti* under laboratory conditions.

Insecticides	Males LC_{50} (CI L-U)	Males LC_{90} (CI L-U)	Females LC_{50} (CI L-U)	Females CL_{90} (CI L-U)
DNT	7.5 (4.02-10.49)	35.6 (30.47- 43.74)	21.8 (17.46 – 25.77)	56.0 (48.94 – 67.35)
DNT +IVM	4.5 (3.01- 5.75)	34.4 (25.52 – 51.79)	3.25 (2.41 – 4.19)	28.6 (21.90 – 39.67)
SR	1.7 (1.33 -1.82)	1.03 (1.19 – 0.84)	6.7 (7.2 – 6.15)	1.9 (2.23 -1.69)
IVM	3.06 (2.35 – 3.88)	87.23 (60.13 – 138.28)	4.54 (3.35 – 6.12)	262.36 (130.73 -712.84)
IVM +DNT	0.96 (0.66 – 1.35)	11.5 (25.52 – 51.79)	1.25 (0.77 – 1.85)	23.07 (16.64 – 33.37)
SR	3.75 (3.56 – 2.87)	7.5 (2.35 – 2.67)	3.63 (4.35 – 3.31)	11.38 (7.85 – 21.36)

CI: Confidence interval; L: lower; U: upper; DNT: Dinotefuran; IVM: Ivermectin; PPM: Parts Per Million

Discussion

During preliminary tests to estimate the lethal concentration of dinotefuran, the low number of engorged mosquitoes in 10% ASB led us to increase the sucrose concentration to 70%. This adjustment was based on findings that increasing sucrose levels can enhance engorgement in ATSBs containing IVM (Dias et al. 2023). Our results corroborated this, as ATSB-DNT achieved engorgement rates above 99% across all concentrations for both sexes, with high engorgement levels (i.e., degrees 3 and 4). In addition to sugar concentration, the use of attractants, e.g., fruit juices, must consider the primary target species, as different juices affect the engorgement of various mosquito species differently; for instance, *Culex*

quinquefasciatus fed twice as much on baits containing apple juice than *Aedes albopictus* (Zhang et al. 2025).

The mortality of *Ae. aegypti* observed in the present study is consistent with the work of Khallaayoune et al. (2013), who used sugar baits containing DNT (100 ppm) and observed mortality rates above 80% in *Ae. aegypti* and *Cx. quinquefasciatus* 24 hours after ingestion. Conversely, Zhang et al. (2025) reported that baits with concentrations lower than 1 ppm were capable of killing 90% of male and female *Cx. quinquefasciatus*, *Ae. albopictus*, and *Anopheles sinensis*, suggesting significant variation in DNT susceptibility among strains from different geographic locations.

In general, *Ae. aegypti* males were more susceptible to the DNT contained in the baits, and the estimated lethal concentrations (LCs) for this sex were approximately 70% and 40% lower than those for females. This corroborates the results of Zhang et al. (2025) using different mosquito species, i.e., *Cx. quinquefasciatus*, *Ae. albopictus*, and *An. sinensis*. Similar trends were also observed by Xue and Barnard (2003) using baits with another insecticide, i.e., boric acid, against the mosquitoes *Anopheles quadrimaculatus* Say, *Ae. albopictus* Skuse, and *Culex nigripalpus* Theobald.

The combination of different insecticides, IVM and DNT, in ATSBs was a novel approach, and the various tested concentrations did not alter the engorgement of male and female *Ae. aegypti*. This suggests that the combined use of these insecticides does not cause bait deterrence and can be used effectively. Bait engorgement is critical, as modifications to certain components can compromise feeding, as observed by Scott-Fiorenzano et al. (2017) using dual-attractant toxic sugar baits for the control of *Ae. aegypti* and *Ae. albopictus*.

Overall, mosquito mortality increased with the addition of either insecticide at low concentrations, particularly in the IVM + DNT combination. Although our literature review did not identify other studies using insecticide combinations in ATSBs for mosquitoes, the results of the combination tested in our study are promising given the synergistic effects on *Ae. aegypti* mortality. These findings support similar strategies using various insecticide combinations against other insects, including insecticide-resistant strains; for example, the combined use of insecticides showed a synergistic effect capable of killing larvae of permethrin-resistant *Cx. quinquefasciatus* strains by adding 0.1 mg/L (LC_0) of propoxur to permethrin solutions (Corbel et al. 2004). Furthermore, as in the present study, the use of ivermectin in binary combinations with the insecticides chlorfenapyr and acetamiprid applied to sugar cubes and offered to house flies (*Musca domestica*) produced the highest synergistic effects observed in that study.

More importantly than just decreasing the lethal concentrations of insecticides used in vector control, the use of combined insecticides, including neonicotinoids, appears to be a strategy that prevents the selection of resistant mosquito strains, such as *Anopheles gambiae*, allowing for the maintenance of control activities (Zoh et al. 2021). Even when used alone in sugar baits offered to deltamethrin-resistant strains of *Cx. quinquefasciatus*, DNT was equally effective at killing both resistant and susceptible strains (Gu et al. 2020).

Conclusion

Our study demonstrated that the various tested concentrations of dinotefuran in ATSBs were ingested by most of the mosquitoes, causing high mortality in both male and female *Ae. aegypti*. Furthermore, we show for the first time that the combination of ivermectin with a low concentration of dinotefuran has a high synergism ratio, significantly reducing the lethal concentrations required to kill 90% of the mosquitoes, which reduces the amount of insecticide needed in the baits

Declarations

Acknowledgements

We thank the entire team of the Laboratory of Insect Bioecology (LaBEIn) for their partnership; the **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior** (CAPES) for the scholarship.

Author contributions

AVT: Methodology, Investigation, Writing– original draft. ACAD: Supervision, Writing– review & editing. AAS: Funding, Conceptualization, Investigation, Data analysis, Writing– review & editing.

Funding

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Research Productivity Fellowship (Chamada CNPq 18/2024); Fundação Rondônia de Amparo ao Desenvolvimento das Ações Científicas e Tecnológicas (FAPERO) - CHAMADA 001/2020 PPSUS and the National Institute of Science and Technology for Epidemiology of the Western Amazon (EPIAMO) for financial support.

Data availability

Datasets used and/or analyzed during the current investigation are available from the corresponding author upon request.

Competing interest

The authors declare no competing interests

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Figures

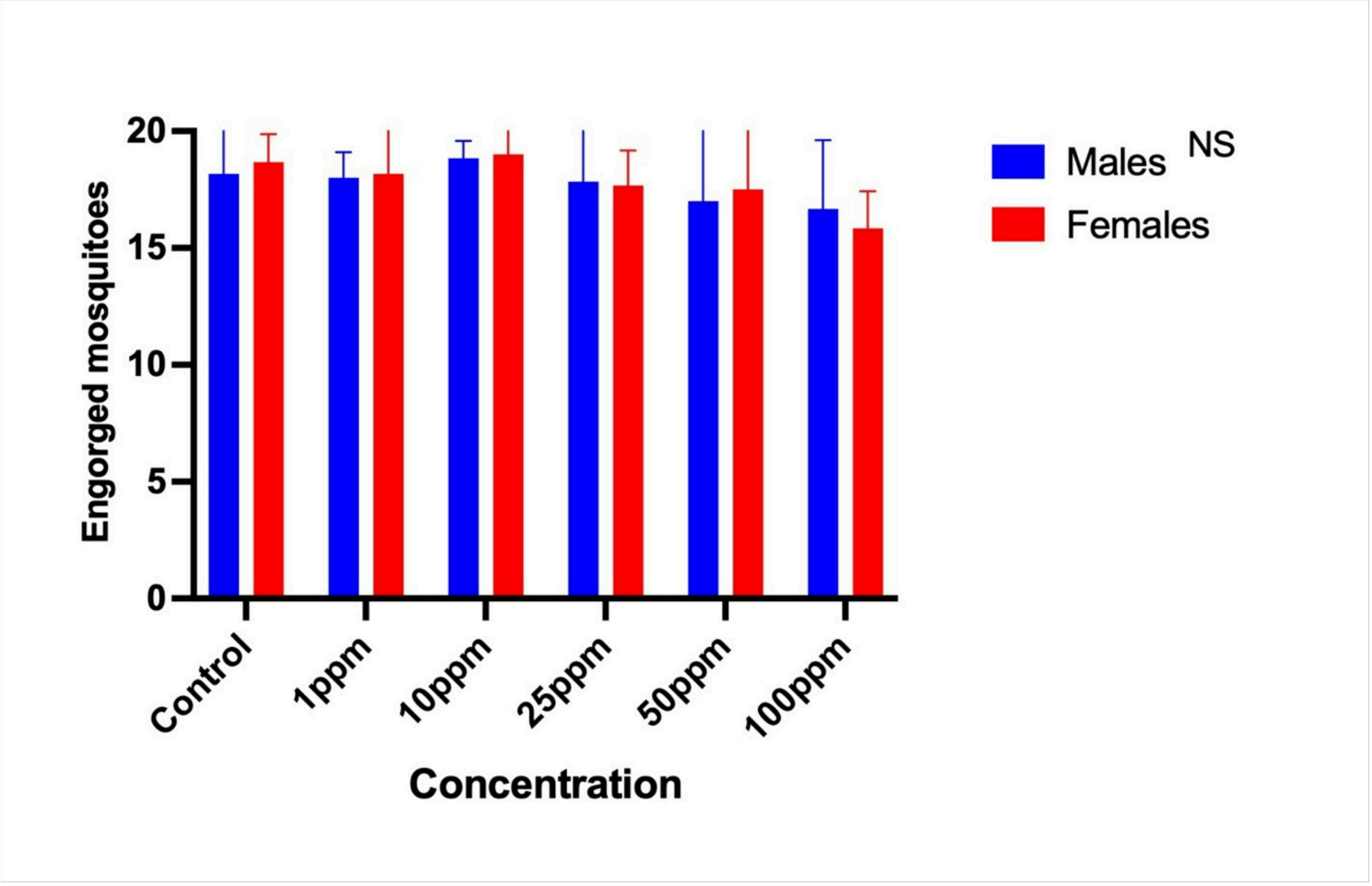


Figure 1

Engorgement of *Aedes aegypti* males and females at different dinotefuran concentrations. NS = not significant (P > 0.05).

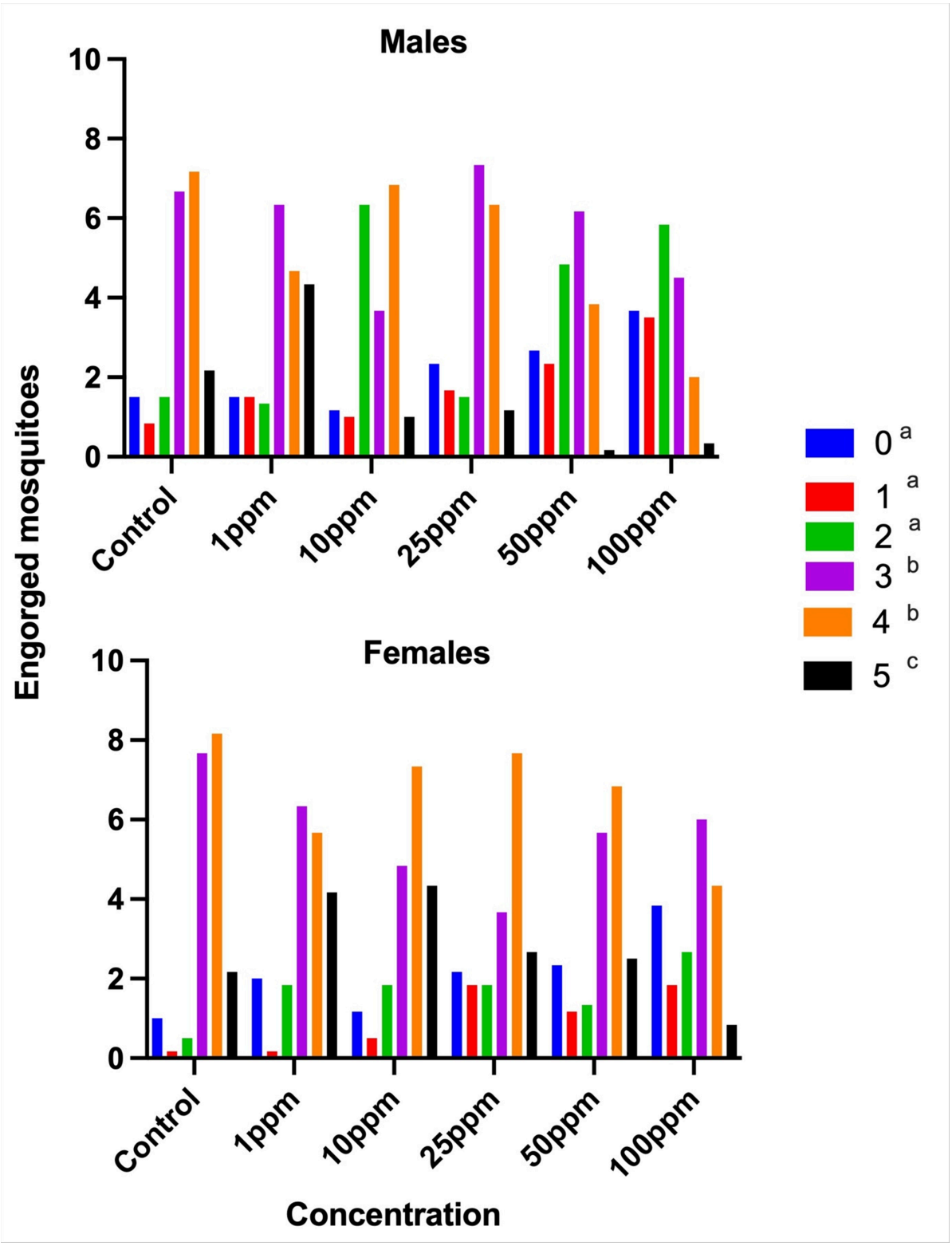


Figure 2

Degree of engorgement of ATSBs containing different dinotefuran concentrations by *Aedes aegypti*males and females. Different letters indicate significant differences ($P < 0.05$) between engorgement degrees across the different concentrations. Two-way ANOVA (concentration x degree) and Tukey's test for multiple comparisons.



Figure 3

Aedes aegypti females with hyperextended abdomens after engorging on sugar baits containing 50 ppm dinotefuran

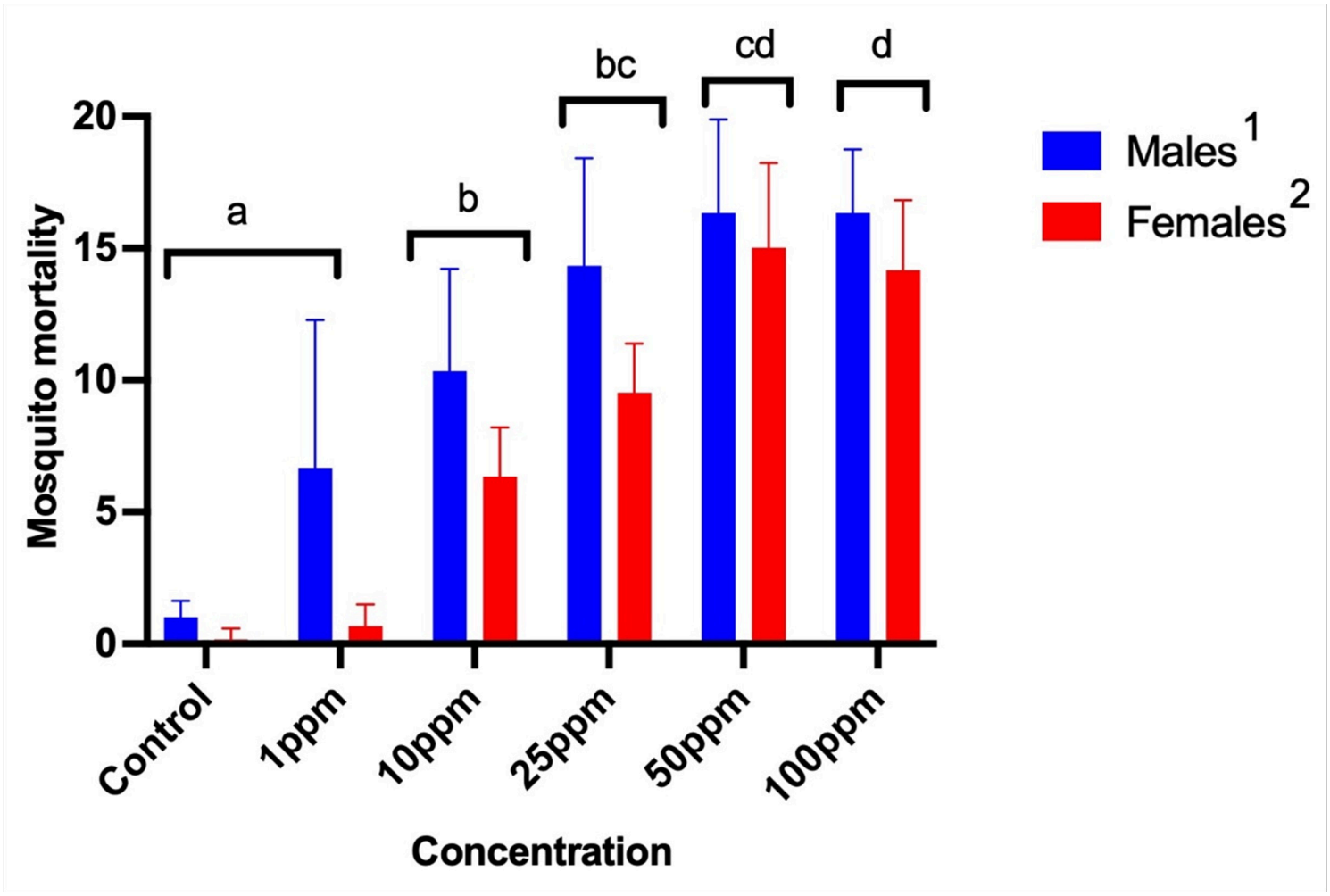


Figure 4

Mortality of *Aedes aegypti* males and females engorged with ATSB-dinotefuran.

Different letters indicate significant differences between concentrations ($P < 0.05$). Different numbers indicate significant differences between sexes ($P < 0.05$). Two-way ANOVA (concentration x sex) and Tukey's test for multiple comparisons.

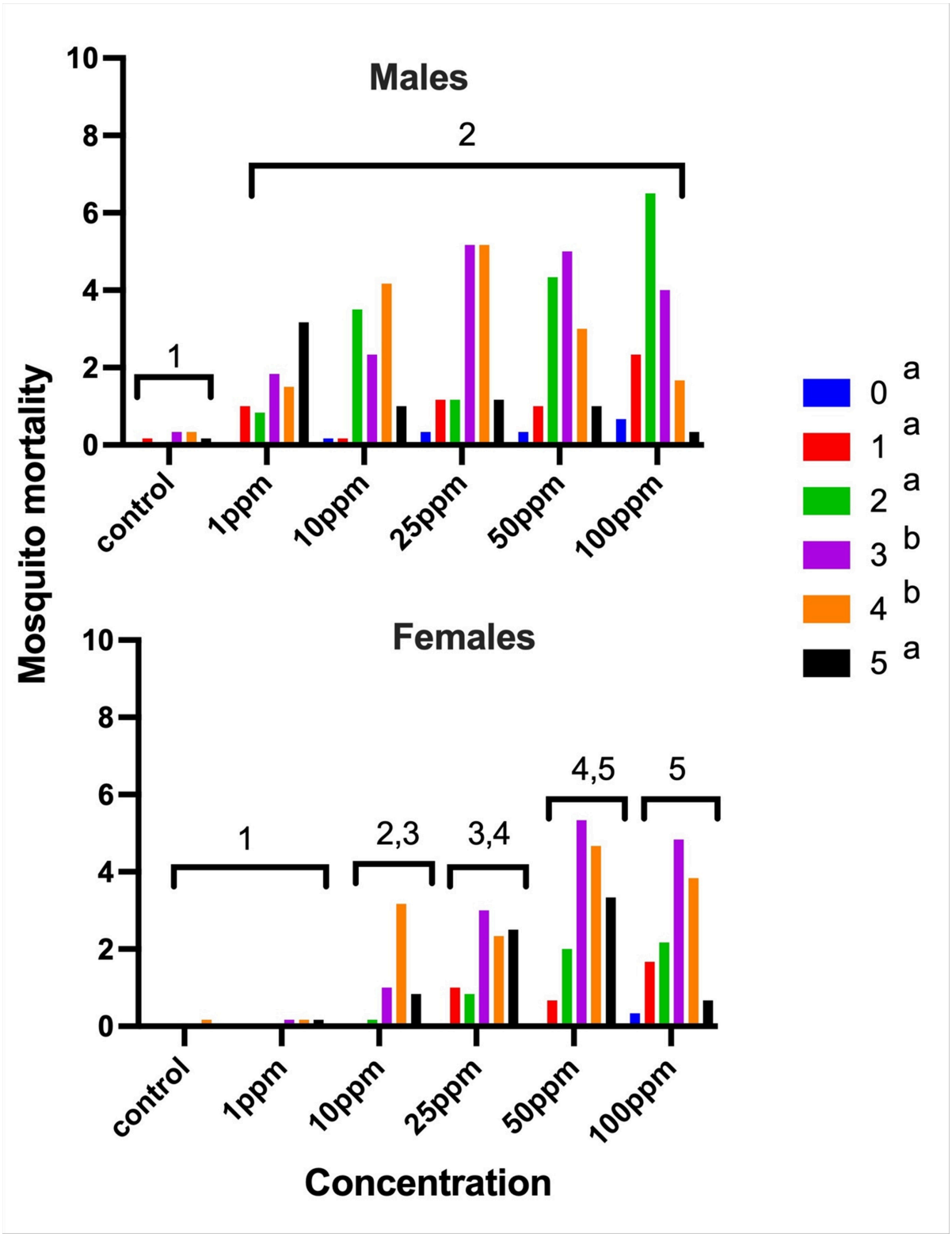


Figure 5

Mortality of *Aedes aegypti* males and females with ATSB containing different dinotefuran concentrations and at different engorgement degrees.

Different letters indicate significant differences between engorgement degrees, and different numbers indicate significant differences between the tested concentrations ($P < 0.05$). Two-way ANOVA

(concentration x degree) and Tukey's test for multiple comparisons.

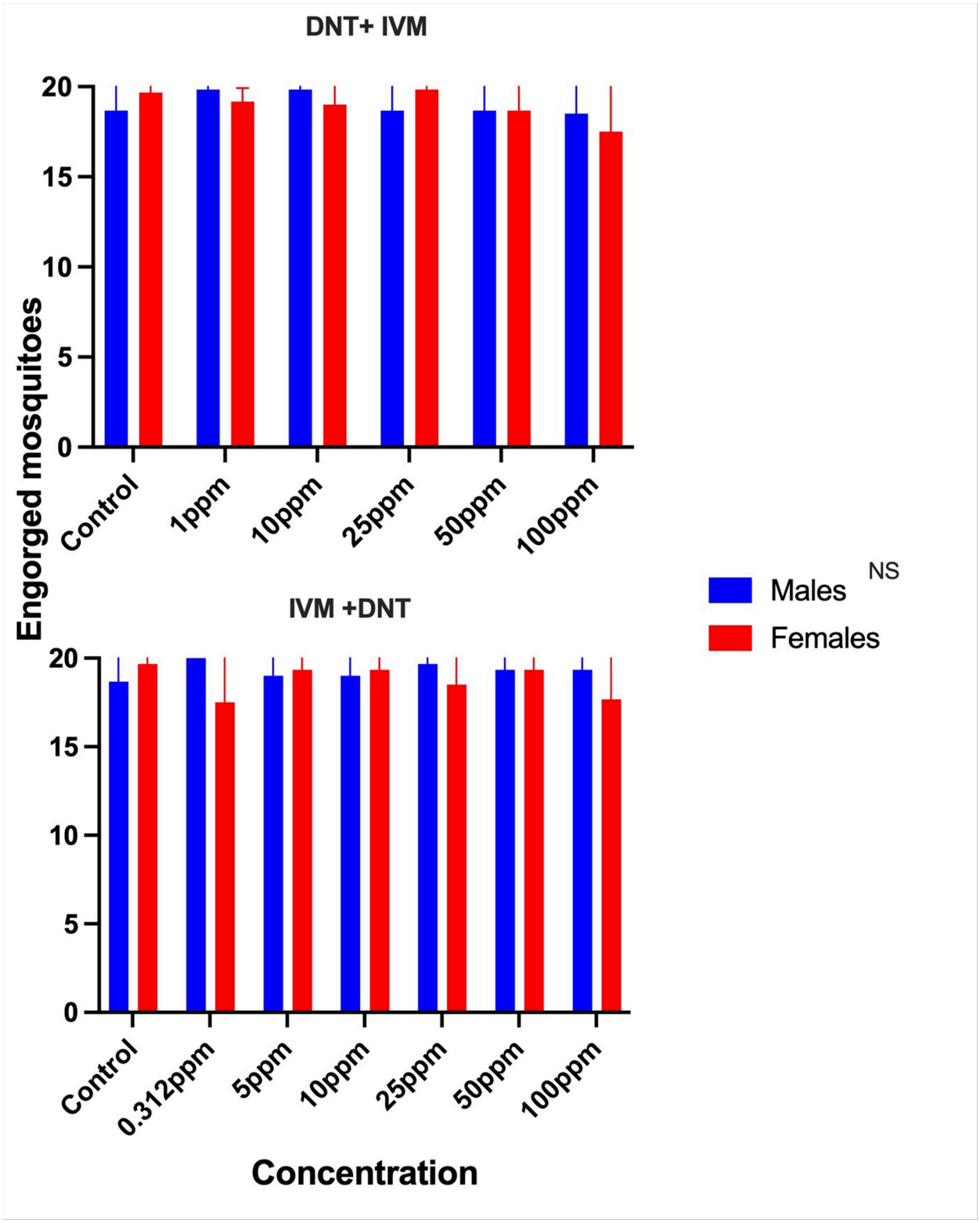


Figure 6

Engorgement of *Aedes aegypti* male and female mosquitoes on binary combinations of the insecticides dinotefuran and ivermectin.

NS = not significant (P > 0.05). DNT + IVM: Dinotefuran + Ivermectin (combined), i.e., increasing concentrations of dinotefuran with 0.312 ppm of ivermectin; IVM + DNT: Ivermectin + Dinotefuran (combined), i.e., increasing concentrations of ivermectin with 1 ppm of dinotefuran.

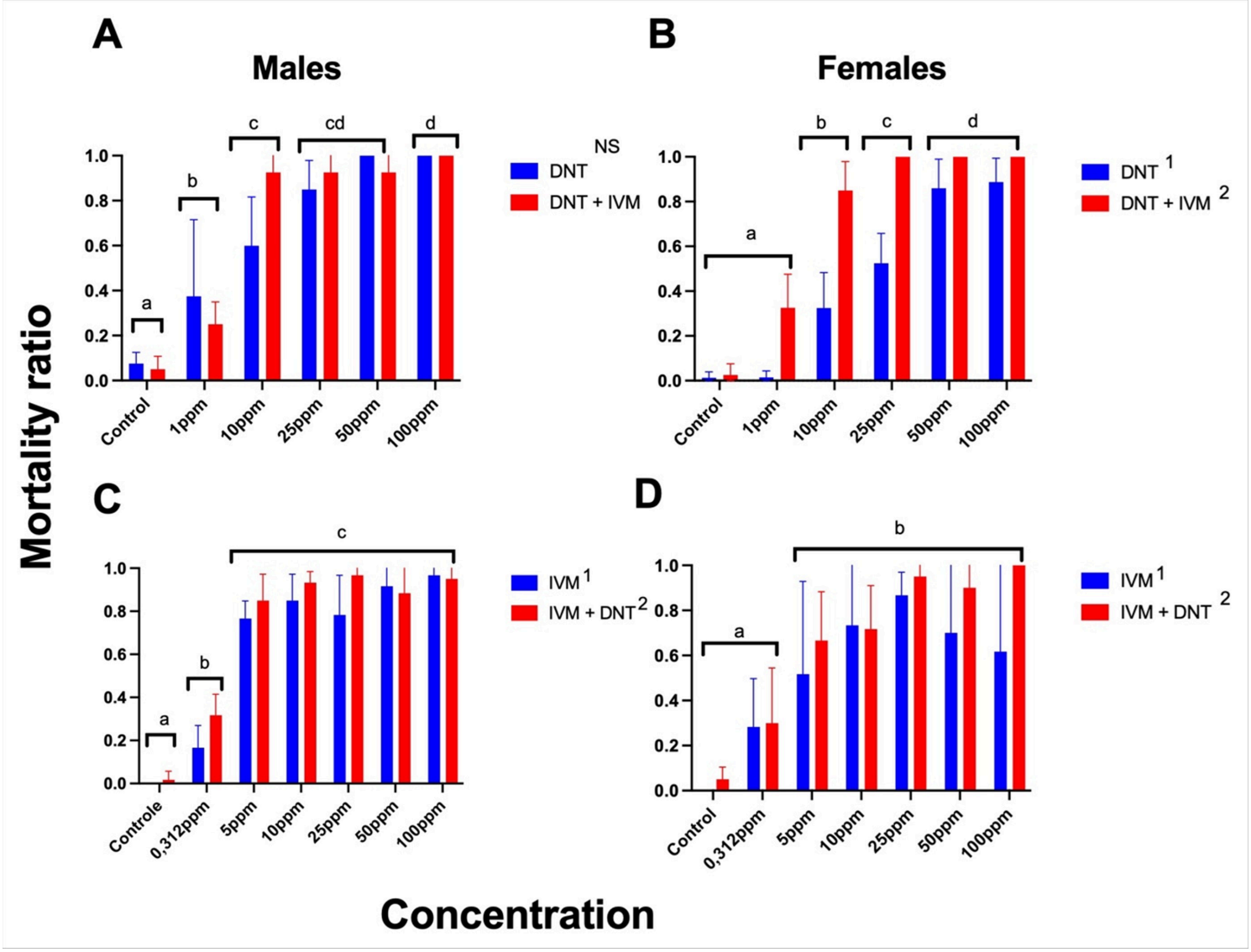


Figure 7

Mortality ratio of male and female *Aedes aegypti* 48 hours post-engorgement on attractive toxic sugar baits (ATSBs) containing dinotefuran (DNT) and ivermectin (IVM), either alone or in binary combinations.

NS: Not significant between treatments (P > 0.05). Different letters indicate significant differences between treatments (P < 0.05). Different numbers indicate significant differences between treatments (P < 0.05). DNT: Dinotefuran (alone); IVM: Ivermectin (alone); DNT + IVM: Dinotefuran + Ivermectin (combined), i.e., increasing concentrations of dinotefuran with 0.312 ppm of ivermectin; IVM + DNT: Ivermectin + Dinotefuran (combined), i.e., increasing concentrations of ivermectin with 1 ppm of dinotefuran.