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Effect of Ivermectin on Oviposition Site Selection, Egg Hatchability, and Larvicidal Activity of Housefly *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae)

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Abstract

Ivermectin is a broad-spectrum antiparasitic agent widely used in the treatment of parasitic infections caused by helminths, including hookworm and whipworm, as well as ectoparasitic and human diseases such as intestinal strongyloidiasis, onchocerciasis, and scabies. Houseflies, *Musca domestica* Linnaeus (Diptera: Muscidae), are important mechanical vectors of numerous pathogens, including bacteria, viruses, and parasites, thereby posing significant public health and veterinary risks. This study investigated the effects of ivermectin on oviposition site selection, egg hatchability, and larval development of *M. domestica*. Oviposition preference was assessed by exposing 30 sexually mature houseflies within a controlled wooden enclosure (30 × 30 × 30 cm) to chicken waste substrate treated with graded ivermectin concentrations of 160.00, 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, and 0.00 mg/L. Parameters evaluated included the Oviposition Activity Index (OAI), percentage Effective Repellency (ER), egg hatchability, and larval mortality rates. The highest mean number of egg batches was recorded at 80.00 mg/L (8.67 ± 2.60), significantly exceeding the control value (4.33 ± 0.88). This concentration also exhibited the greatest oviposition attraction, with an ER value of −100 and an OAI of 0.38. Hatchability assays revealed a concentration-dependent inhibitory effect of ivermectin, with egg hatching declining from 98% in the control to 1% at 160.00 mg/L. Similarly, larval mortality increased progressively with concentration, ranging from 60% at 2.50 mg/L to complete mortality (100%) at 160.00 mg/L. Toxicity analysis yielded LC_{50} and LC_{95} values of 1.21 mg/L and 59.32 mg/L, respectively, for third instar larvae. The findings demonstrate that ivermectin functions both as an oviposition attractant and a potent developmental disruptor in *M. domestica*, highlighting its potential application in integrated housefly management strategies aimed at minimizing nuisance and pathogen transmission in human and livestock environments.

Keywords
Ivermectin;
Musca domestica;
Oviposition site selection;
Larvicidal activity;
Egg hatchability;
LC_{50}

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1.0 INTRODUCTION

The housefly, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae), is a synanthropic pest of major medical and veterinary importance, capable of transmitting more than 100 pathogens, including bacteria, viruses, protozoa, fungi, nematodes, and parasites (Malik *et al.*, 2007; Geden *et al.*, 2021; Salamatian *et al.*, 2020). In livestock operations, high housefly population densities reduce animal welfare, cause production losses, and require intensive control measures. Although integrated pest management (IPM) approaches combine cultural, biological, and chemical tactics, chemical control remains the most widely used intervention.

Ivermectin, a macrocyclic lactone endectocide, is widely used in livestock to control internal and external parasites. Following treatment, ivermectin is excreted in feces, where it persists and can affect insects associated with nontarget dung (Lumaret *et al.,* 2022; Wall & Beynon, 2012). Although the toxicity of ivermectin to larval dipterans has been documented, its sublethal and behavioral effects on adult houseflies, particularly decision-making about oviposition, remain poorly characterized. Understanding whether ivermectin residues in oviposition substrates influence female choice and subsequent larval survival is critical to assessing the potential role of this compound in attract-and-kill strategies.

Despite the widespread use of ivermectin in livestock, the direct effects of ivermectin-spiked rearing medium on housefly oviposition behavior, egg viability, and larval survival have not been systematically investigated under controlled conditions (Forbes *et al.*, 2020; Lumaret *et al.*, 2022). The present study addresses this gap by evaluating the dose-dependent responses of *M. domestica* to ivermectin-treated oviposition substrates. Our findings provide a mechanistic basis for integrating ivermectin into IPM programs and highlight both the opportunities and risks associated with its use.

2.0 MATERIALS AND METHODS

2.1 Housefly Rearing

Dry chicken droppings sourced from the poultry house at the Ahmadu Bello University farm were used as the medium for fly rearing. Protective gloves were worn to retrieve the manure from the poultry house. The droppings were transferred to sterile polythene bags and transported to the Department of Zoology. One kilogram of dry manure was weighed in a transparent plastic container (5 litres), with perforated holes at its bottom to allow excess water to drain out. Five hundred milliliters (500 ml) of tap water was added to make a paste of the droppings, and the prepared medium was kept in the shade for flies to oviposit. The eggs were allowed to develop to the adult stage in the laboratory at room temperature.

Emerging adult houseflies were identified using the Canadian Grain Commission key (Brent, 2014) to distinguish them from other species present due to unrestricted exposure. Fifty identified adult flies (20 males: 30 females) were then reared in a restricted screen cage (30×30×30 cm) fitted with netting material, containing rearing medium (chicken droppings) in a plastic container for pure line breeding (Sinthusiri & Soonwera, 2014) from which each developmental stage (eggs and larvae) was sourced and used for the experiment.

2.2 Rearing Conditions

Adult flies were rare at standard laboratory temperature (26 ± 1 °C), relative humidity of 60 ± 5% RH, and a photoperiod of 14:10 h (light: dark). The flies were fed sugar/water before oviposition trials to develop their ovaries and lay eggs. A discrete cluster of eggs laid at one location was regarded an 'egg batch'. Each concentration in each assay was replicated three times.

2.3 Source of ivermectin

An injectable solution of Kepromec (ivermectin) was purchased from a reputable veterinary pharmacy in Zaria for the experiment.

2.4 Effect of ivermectin on selection of the oviposition site

A graded series of ivermectin (160.00, 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, and 0.00 mg/L) was prepared using the ivermectin solution and chicken droppings in a 0.5 L container. Water without ivermectin was used to prepare the control medium. The test concentrations plus control were carefully placed in the screen cage measuring 30×30×30cm completely randomized (i.e., eight test containers per cage) with 20 adult houseflies; positions of the medium were altered between the replicates to eliminate any influence of position on the selection of the oviposition site (Sinthusiri & Soonwera, 2014). To minimize positional bias, test containers were rotated every 12 hours (Bissinger & Roe, 2010).

A hand lens was used to carefully count the number of batches of eggs from the control and treated medium. The test was carried out in triplicate. The result was expressed as a mean number of egg batches per container and the percentage effective repellency (% ER) for ivermectin was calculated using the formula by Murugan *et al.* (2012):

$$\% \text{ ER} = \frac{NC - NT}{NC} \times 100$$

Where, ER = percentage of effective repellency (that is, the percentage of repellent effect of the medium to the fly)

NC = Number of egg batches in the control medium

NT = number of egg batches in test medium;

The oviposition activity index (OAI, that is, the frequency of egg laying in the medium) was calculated using the formula: OAI = $\frac{\mathrm{NT-NC}}{\mathrm{NT+NC}}$ (Murugan *et al.*, 2012)

Where, NT = the total number of eggs in the test medium.

NC = the total number of eggs in the control medium;

2.5 Effect of ivermectin on egg hatchability

Using a hand lens and a fine brush, groups of twenty (20) eggs were counted and placed on wet filter paper. Eggs were transferred to Petri dishes containing Whatman's filter paper moistened with ivermectin concentrations (160.00, 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, and 0.00 mg/L). They were monitored on an hourly basis (for 18 hours) with a hand lens to assess hatchability. The percentage hatchability was calculated based on the number of larvae recovered after 24 hours after treatment using the formula;

$$\text{Hatchability (\%)} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs in treated media}} \times 100$$

2.6 Effect of Ivermectin on Housefly Larvae

A group of thirty (30) third instar larvae of the housefly of pure line breed were placed on filter paper moistened with different concentrations of ivermectin (160.00, 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, and 0.00 mg/L) in a Petri dish (in triplicate) with a cover. They were allowed to crawl freely for 24 hours and then placed in a plastic plate container containing 20 g of rearing medium. Mortality was checked daily for a period of nine (9) days. The absence of movement in the larva when gently probed with forceps was considered a sign of death (Sinthusiri & Soonwera, 2013). Probit analysis was conducted following Finney (1971) to calculate lethal concentrations.

3.0 RESULTS

3.1 Effect of Ivermectin on Oviposition Site Selection

The effect of ivermectin concentrations on the selection of *Musca domestica* oviposition sites was expressed as the mean total number of egg batches laid at each of the oviposition sites (Table 1). It shows that the oviposition site 80.00 mg/L has the highest mean number of egg batches laid, that is, it attracted more of the flies to lay eggs, which is evident by the highest negative effective repellency (-100) and highest activity index (0.38). Statistically, the mean number of egg batches laid in the medium containing 40.00, 80.00, and 160.00 mg/L ivermectin (7.33±0.68; 8.67±2.60, and 6.00±0.58, respectively) was not significantly different at $p<0.05$. The mean number of eggs laid in medium with ivermectin concentration 2.50 mg/L (3.33±0.88), 5.00 mg/L (3.33±0.78), 10.00 mg/L (4.33±0.88) and 20.00 mg/L (4.00±1.00) did not differ significantly ($p>0.05$) but were comparable with the mean number of eggs laid at concentrations 40.00 mg/L (7.33±0.68) and 160.00 mg/L (6.00±0.58).

The percentage of effective repellency (% ER) of ivermectin was highest (25% each) at concentrations of 2.50 mg/L and 5.00 mg/L, with a low oviposition activity index (-0.14). The oviposition activity index (OAI) was highest at 80.00 mg/L (0.38), followed by 40.00 mg/L (0.27), 160.00 mg/L (0.20), 10.00 mg/L (0), 20.00 mg/L (0), and 2.50 mg/L and 5.00 mg/L with (-0.14).

Table 1: Effect of Ivermectin Concentration on Oviposition Site Selection

Oviposition sites (mg/L)	Mean number of egg batches laid ±SE	% Effective Repellency (% ER)	Oviposition Activity Index (OAI)
0.00	$4.33±0.88^{b}$	-	-
2.50	$3.33±0.88^{b}$	25	-0.14
5.00	$3.33±0.78^{b}$	25	-0.14
10.00	$4.33±0.88^{b}$	0	0.00
20.00	$4.00±1.00^{b}$	0	0.00
40.00	$7.33±0.68^{ab}$	-75	0.27
80.00	$8.67±2.60^{a}$	-100	0.38
160.00	$6.00±0.58^{ab}$	-50	0.20

Means with different superscript letters per column are significantly different ($P<0.05$).

3.2 Effect of Ivermectin on Egg Hatchability

The effect of ivermectin formulations on egg hatchability of *Musca domestica* showed that eggs exposed to various concentrations of ivermectin were significantly lower than those of the control (Table 2). There was an increasing inhibitory effect on egg hatchability with increasing ivermectin concentration. The percentage of inhibition ranges between 38 and 99% in the treated group. The highest hatchability percentage was recorded at the lowest ivermectin concentration, 2.50 mg/L (62%), while the lowest was at 160.00 mg/L (1%). Increasing ivermectin concentrations were associated with higher mortality in first-instar larvae after hatching. All tested ivermectin concentrations were converted to natural Log10 (ranging from 0.3979 to 2.2041), and their corresponding percentage mortality was converted to empirical probit (ranging from 4.69 to 7.33). The regression equation (y = 1.24098x + 4.05116) and the value of R (R = 86.46%) were generated by plotting Log_{10} conc against empirical probit. Similarly, 50% and 95% inhibition concentrations of egg hatching (IC_{50} and IC_{95}) were 4.84 mg/L and 122.7 mg/L, respectively.

Table 2: Effect of Ivermectin Concentration on Egg Hatchability

Ivermectin conc. (mg/L)	Mean ± SE of hatched eggs	(%) Inhibition	(%) Hatchability	Log_{10}-Conc.	Empirical probit
0.00	$19.67{\pm}0.33^{a}$	---	---	0.0000	0.00
2.50	$12.33{\pm}0.58^{b}$	38	62	0.3979	4.69
5.00	$9.67{\pm}0.88^{c}$	52	48	0.6990	5.05
10.00	$7.33{\pm}0.58^{d}$	63	37	1.0000	5.33
20.00	$6.33{\pm}1.16^{de}$	68	32	1.3010	5.47
40.00	$5.34{\pm}0.88^{de}$	73	27	1.6021	5.61
80.00	$2.33{\pm}0.33^{e}$	88	22	1.9031	6.18
160.00	$0.33{\pm}0.33^{f}$	99	1	2.2041	7.33

Regression equation: y= 1.241x+4.051 **R**= 86.46% $\mathbf{IC_{50}}$ =4.84mg/L $\mathbf{IC_{95}}$ = 122.7mg/L

Means with different superscript letters within a column are significantly different (P<0.05).

n=20

3.3 Effect of Ivermectin on Larval Mortality

The results of the effect of ivermectin concentrations on housefly larval mortality indicate a substantial increase in the mortality of the larvae exposed to various ivermectin concentrations from the lowest to the highest (Table 3). Statistically, there were no significant differences between the mean number of dead larvae at concentrations of 40.00 to 160.00 mg/L, but they were comparable to concentrations of 2.50 to 20.00 mg/L. The highest lethal effect was observed in the third instar larvae at a concentration of ivermectin of 160.00 mg/L after 24 hours post-exposure.

Table 3: Effect of Ivermectin Formulations on Larval Mortality

Ivermectin conc. (mg/L)	Mean ± SE of dead larvae	(%) mortality	Log_{10} Conc.	Empirical probit
0.00	$0.00{\pm}0.00^{d}$	0	0.0000	0.00
2.50	$19.33{\pm}3.52^{c}$	60	0.3979	5.25
5.00	$23.00{\pm}3.61^{ab}$	77	0.6990	5.74
10.00	$24.00{\pm}3.06^{ab}$	80	1.0000	5.84
20.00	$25.67{\pm}2.19^{ab}$	86	1.3010	6.08
40.00	$28.33{\pm}0.88^{a}$	94	1.6021	6.55
80.00	$29.67{\pm}0.00^{a}$	96	1.9031	6.75
160.00	$30.00{\pm}0.00^{a}$	100	2.2041	∞

Regression equation: y = 0.965x+4.925 **R** = 97.22% **LC_{50}** =1.21mg/L **LC_{95}** = 59.32mg/L

Means with different superscript letters within a column are significantly different ($P<0.05$).

n=30

4.0 DISCUSSION

Oviposition Preference and Attractancy

The significantly higher number of egg batches laid at 80.00 mg / L of ivermectin suggests that Musca domestica may be attracted to the chemical compound or to the specific concentration of ivermectin used in this study. This is supported by the observed negative repellency index, indicating an "attractant" rather than a "repellent" effect at higher concentrations. The attractant effect observed at 80 mg/L may be related to ivermectin-induced changes in microbial communities in the rearing medium, since bacteria are known to produce oviposition signals for muscid flies (Lam *et al.*, 2009; Zurek & Schal, 2021).

These findings contrast with those of Aiyedun (2014), who noted that high concentrations (40–100 mg/L) deterred *Cx quinquefasciatus* females. However, the behavioral response to chemical cues is often species-specific. As Clements (1992) pointed out, insects detect low-volatility chemicals through direct contact or gustatory mechanisms. The role of habitat-related chemical signals in the selection of oviposition sites is well documented (Braks *et al.*, 2007; Burkett-Cadena & Mullen, 2007). The high Oviposition Activity Index (OAI) of this study suggests that ivermectin could be a potent tool in housefly "attract-and-kill" control programs.

Effects on Egg Hatchability

Ivermectin demonstrated a potent, dose-dependent inhibitory effect on the hatchability of housefly eggs. The near-total failure of eggs to hatch at 160.00 mg/L (99% inhibition concentration) indicates significant embryonic lethality, likely due to the chemical's ability to penetrate the eggshell. This level of inhibition is consistent with Derua *et al.* (2015), who found complete inhibition of fertilization in *Anopheles gambiae* at similar concentrations. Eba *et al.* (2023) observed significantly reduced survival and hatchability of eggs in *Anopheles arabiensis*, with higher doses resulting in zero larval survival. Ivermectin reduced survival and fecundity in *Culex pipiens*, with higher doses resulting in no egg hatching (van Koppenhagen *et al.,* 2020). The inverse relationship between ivermectin concentration and the mean number of hatched larvae, ranging from 12.33 at 2.50 mg / L to 0.33 at 160.00 mg / L, underscores the efficacy of the drug as an ovicide in *M. domestica*.

Mortality and development of larval

Larval mortality increased progressively with concentration, reaching 100% at 160.00 mg/L. This suggests that ivermectin not only kills larvae but also likely disrupts the physiological processes required for development. Ivermectin larvicidal activity is likely mediated by hyperpolarization of nerve and muscle cells, leading to paralysis and death, as documented in other Diptera (Wolstenholme & Rogers, 2005). Gharib *et al.* (2025) reported similar results, who found that Ivermectin demonstrated strong toxicity against third-instar housefly larvae with an LC_{50} value of 2.05 mg/kg after 72 hours of exposure.

The LC_{50} value of 1.21 mg/L is particularly noteworthy; it indicates that *M. domestica* larvae are susceptible to ivermectin, which requires only a minimal dose to achieve 50% mortality. This high sensitivity makes ivermectin an effective candidate for larvicidal applications in integrated pest management. However, the potential for environmental persistence and toxicity to nontarget dung-associated fauna (e.g., Coleoptera) warrants caution and further investigation before field application (Wall & Beynon, 2012; Verd *et al.*, 2018).

5.0 CONCLUSION

The result of this study shows that ivermectin repels houseflies from oviposition sites at lower concentrations (2.50 to 20.00 mg/L), but attracts houseflies at higher concentrations (40.00 to 160.00 mg/L). Ivermectin significantly inhibits egg hatchability in a dose-dependent manner. Ivermectin kills 100% of larvae at the highest concentration (160.00 mg / L) compared to 60% at the lowest concentration (2.50 mg/L). The use of ivermectin to control *Musca domestica* is recommended because of its dual role (attractant and toxicant) and its potential in attract-and-kill strategies to alleviate the nuisance and discomfort it causes both humans and livestock. The use of ivermectin in field studies using livestock treated with ivermectin should be carry out to assess its effects on the naturally breeding housefly population. Sublethal effect studies (such as adult longevity and mating success) should be conducted to understand population-level impacts better.

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