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Larvicidal Activity of Some Heterocyclic Thiosemicarbazones and Semicarbazones Derivatives Against *Anopheles arabiensis* Larvae

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تقييم النشاط اليرقي لبعض مشتقات ثيوسيميكاربازون، سيميكاربازون الحلقية غير المتجانسة ضد يرقات بعوض *Anopheles arabiensis*

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Abstract:
Malaria remains one of the most significant vector-borne diseases worldwide and is transmitted through the bites of infected female *Anopheles* mosquitoes. The increasing prevalence of insecticide resistance has substantially compromised the effectiveness of conventional chemical control measures, necessitating the development of novel, potent, and environmentally sustainable larvicides. In this study, the larvicidal activity of three thiosemicarbazone derivatives (TS1–TS3) and three semicarbazone derivatives (SC1–SC3) was evaluated against *Anopheles arabiensis* larvae following standard World Health Organization (WHO) bioassay procedures. Larvae were exposed to different concentrations of the tested compounds, and mortality rates were recorded after 24 and 48 h of exposure. Lethal concentration (LC_{50}) values were estimated using probit and logit analyses. The thiosemicarbazone derivatives exhibited clear concentration- and time-dependent larvicidal activity, whereas the semicarbazone derivatives exhibited no detectable larvicidal activity under the tested conditions. Among the active compounds, TS3 demonstrated the highest potency, with the LC_{50} value decreasing markedly from 248 ppm after 24 h to 107 ppm after 48 h of exposure. Statistical analysis revealed strong mortality–concentration relationships, with coefficients of determination (R^2) equal to or greater than 0.80. These findings indicate that the larvicidal activity observed in this study was restricted to thiosemicarbazone derivatives, while semicarbazone derivatives were inactive against *An. arabiensis* larvae under the tested conditions. Thiosemicarbazone derivatives therefore represent promising candidates for mosquito control programs, although further studies on structural optimization, mode of action, and environmental safety are required before practical application.

Keywords: *Anopheles arabiensis*, thiosemicarbazone, semicarbazone, larvicidal activity, insecticide resistance, vector control.

الملخص

تُعدّ الملاريا واحدة من أهم الأمراض المنقولة بالنواقل على مستوى العالم، وتنتقل عن طريق لدغات إناث بعوض الأنوفليس المصابة. وقد أدّى الانتشار المتزايد لمقاومة المبيدات الحشرية إلى تقليل فعالية وسائل المكافحة الكيميائية التقليدية بشكل كبير، مما يستدعي تطوير مبيدات يرقية جديدة وفعّالة وآمنة بيئيًا. هدفت هذه الدراسة إلى تقييم النشاط اليرقي لثلاثة مشتقات من الثيوسيميكاربازون (TS1–TS3) وثلاثة مشتقات من السيميكاربازون (SC1–SC3) ضد يرقات بعوض*Anopheles arabiensis* ، وذلك وفقًا لبروتوكولات منظمة الصحة العالمية القياسية. تم تعريض اليرقات لتراكيز مختلفة من المركبات المختبرة، وسُجلت معدلات الوفيات بعد 24 و48 ساعة من التعرض. وتم حساب قيم التركيز القاتل النصفي (LC_{50}) باستخدام تحليلات البروبيت واللوجيت. أظهرت مشتقات الثيوسيميكاربازون نشاطًا يرقياً واضحًا يعتمد على التركيز والزمن، في حين لم تُظهر مشتقات السيميكاربازون أي نشاط يرقي يمكن اكتشافه تحت الظروف التجريبية المستخدمة. ومن بين المركبات الفعالة، أظهر المركب TS3 أعلى فعالية، حيث انخفضت قيمة LC_{50} بشكل ملحوظ من 248 جزءًا في المليون بعد 24 ساعة إلى 107 أجزاء في المليون بعد 48 ساعة من التعرض. وأظهرت التحليلات الإحصائية وجود علاقة قوية بين الجرعة والاستجابة ($R^2 \geq 0.80$). وتشير هذه النتائج إلى أن النشاط اليرقي في هذه الدراسة كان محصورًا في مشتقات الثيوسيميكاربازون فقط، بينما كانت مشتقات السيميكاربازون غير فعّالة ضد يرقات *An. arabiensis* تحت الظروف المختبرة. وعليه، تُعد مشتقات الثيوسيميكاربازون مرشحة واعدة لبرامج مكافحة البعوض، مع ضرورة إجراء مزيد من الدراسات حول تحسين البنية الكيميائية، وآلية العمل، والسلامة البيئية قبل الاستخدام التطبيقي.

الكلمات المفتاحية: *Anopheles arabiensis*، ثيوسيميكاربازون، سيميكاربازون، فاعلية مبيدة لليرقات، مقاومة المبيدات الحشرية، مكافحة النواقل.

Introduction:

Malaria remains a life-threatening disease caused by protozoan parasites of the genus *Plasmodium*, transmitted to humans through the bites of infected *Anopheles* mosquitoes. According to the World Health Organization[1], approximately 263 million malaria cases and 597,000 associated deaths were reported globally in 2023, with nearly 95% of cases occurring in Africa. Sudan represents a major hotspot, bearing the highest malaria burden in the WHO Eastern Mediterranean Region. In 2020, the country accounted for more than half of all regional malaria cases (56%) and deaths (61%) [2]. The situation remains critical, with over 1.3 million confirmed cases and more than 850 deaths reported in 2023, including 22.3% of cases and 16% of deaths among children [1-3]. Among the principal malaria vectors, *Anopheles arabiensis* plays a pivotal role in disease transmission, where infection begins when an infected female mosquito injects *Plasmodium* sporozoites into the human bloodstream, subsequently migrating to the liver for maturation and replication.

Despite ongoing vector control efforts, malaria remains a major public health concern, and although larvicides are essential tools, their effectiveness is increasingly limited by cost, environmental impact, and the rapid development of resistance. In Sudan, resistance in *Anopheles arabiensis* has been well documented, with susceptibility to some compounds but resistance to others, alongside evidence of kdr-associated variation and reduced efficacy of pyrethroid-based interventions [4-5]

At the larval control level, similar challenges have been reported. Reduced susceptibility to the organophosphate larvicide temephos has been observed in *Anopheles arabiensis* populations from polluted habitats in Khartoum State, with elevated LD_{50} values indicating resistance linked to prolonged exposure [6]. A systematic review also documented increasing resistance to organophosphates in *Anopheles stephensi* populations in Iran [7], while field populations in India exhibited reduced operational efficacy despite apparent susceptibility at WHO-recommended doses [8]. Comparable resistance trends have been reported in *Aedes aegypti* populations across Brazil [9], Malaysia [10],, Mexico [11], and Peru [12], although certain populations of *Anopheles labranchiae* in Morocco remain largely susceptible [13]. Collectively, these findings highlight the increasing threat of larvicidal resistance and emphasize the urgent need to identify alternative compounds with novel mechanisms of action.

In this context, thiosemicarbazones, a subclass of Schiff bases, have attracted considerable attention in medicinal and coordination chemistry due to their structural versatility and wide range of biological activities. These compounds contain an azomethine (-C=N-) functional group formed through the condensation of primary amines with carbonyl compounds [14], Their molecular structure includes multiple donor atoms—oxygen, nitrogen, and sulfur—enabling strong coordination with metal ions, particularly transition metals. This chelating ability enhances their chemical stability and biological activity, making them promising candidates for pharmaceutical and pesticidal applications [15].

Thiosemicarbazones and semicarbazones are known to form metal complexes with enhanced pharmacological properties, including antibacterial and antifungal activities [16-17], antitumoral effects [18], as well as antiprotozoal, antiviral, antioxidant, and anticonvulsant activities [19]. Their applications further extend to nuclear medicine and analytical chemistry due to their strong metal-chelating capacity. Importantly, thiosemicarbazone derivatives have demonstrated promising larvicidal activity, partly attributed to

acetylcholinesterase inhibition, although their environmental safety toward non-target aquatic organisms requires careful evaluation [20]. In particular, aryl- and phenoxymethyl-thiosemicarbazones have shown potent larvicidal activity against *Aedes aegypti* larvae without cytotoxic effects on mammalian cells, suggesting relatively low non-target toxicity [21].
In this context, the present study evaluates, for the first time, the larvicidal potential of thiosemicarbazone and its corresponding semicarbazone analogues against *Anopheles arabiensis* larvae. Furthermore, a comparative assessment between thiosemicarbazone derivatives (TS1–TS3) and semicarbazone derivatives (SC1–SC3) was conducted to elucidate structure–activity relationships and determine their relative efficacy. This investigation aims to provide novel insights into alternative larvicidal agents capable of addressing emerging resistance in malaria vector control.

Materials and Methods

Tested compounds

Synthesis of Imidazolecarboxaldehyde thiosemicarbazones (TS1-TS3)

All these compounds were synthesized and characterized according to previously reported procedure as described by [22-23].

Synthesis of 4-methyl-5-imidazolecarboxaldehydethiosemicarbazone (TS1)

An equimolar amount of thiosemicarbazide (910 mg; 0.001 mol) and 4-methyl-5-imidazolecarboxaldehyde (110 mg; 0.001 mol) were dissolved in 20 mL of ethanol:water (60:40 %) and refluxed for 7 hrs in an oil bath at 78 °C. The solution was allowed to cool at room temperature and left for slow solvent evaporation. After several days pale yellow crystals were obtained. The crystals were separated, washed with cold ethanol and dried under vacuum.[49] Yield: 67%; mp 203°C. The pure crystals were suitable for X-ray analysis; data collection and structure refinement results are summarized in Table 3. For the ligand (L_1) $C_6H_9N_5S$: FT-IR (KBr cm^{-1}); $\nu(NH_2)$ 3429, 3271; ν(NH) 3134; ν(C=N) 1598; ν(C=S) 850; ν(N-N); 1101. NMR spectrum (600 MHz for ^{1}H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^{1}H NMR; δ=2.2 (3H, s, CH_3); 7.9 (1H, CH ring), 7.2-7.7(2H, s, NH_2), 8.1(1H, s, =N-NH); 11.3 (1H, s, NH), 12.3(1H, s, NH); ^{13}C NMR; δ=9.05 (CH_3); 131.35-136.34 (2Cq ring); 130.41(C-H ring), 134.82 (HC=N); 177.38(C=S). Ms (ESI.m/z): M^- 184.2; Analysis for $C_6H_9N_5S$ (Mw 183.24). UV-Vis spectrum (λ_{max} nm): 318.

Synthesis of 4-imidazolecarboxaldehyde thiosemicarbazone (TS2)

An equimolar amount of thiosemicarbazide (910 mg 0.001 mol) and 4-imidazole carboxaldehyde (960 mg 0.001 mol) were dissolved in 20mL of ethanol:water (60:40 %) and refluxed for 7 hrs in an oil bath at 78°C. The solution was allowed to cool at room temperature and left for slow solvent evaporation. After several days pale yellow crystals were obtained. The crystals were separated, washed with cold ethanol and dried under vacuum. Yield: 72%; mp 206 °C. The pure crystals were suitable for X-ray analysis; data collection and structure refinement results are summarized in Table 1. For the ligand (4-ITSC) $C_5H_7N_5S.H_2O$: FT-IR (KBr cm^{-1}); $\nu(NH_2)$ 3397, 3202; ν(NH) 3011; ν(C=N) 1606; ν(C=S) 851; NMR spectrum (600 MHz for ^{1}H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^{1}H NMR; δ=7.8-7.9 (2H, CH ring), 7.3(2H, b, NH_2), 8.2(1H, s, =N-NH); 11.3 (1H, s, NH), 12.4(1H, s, NH); ^{13}C NMR; δ=135.54(Cq); 121.60-131.13(2C-H ring), 136.60(HC=N); 177.88(C=S). Ms (ESI. m/z): M^+ 170.0; Analysis for $C_5H_7N_5S$ (Mw 169.22). UV-Vis spectrum (λ_{max} nm): 313.

Synthesis of 1-methyl-5-Imidazolecarboxaldehyde thiosemicarbazone (TS3)

An equimolar amount of thiosemicarbazide (910 mg 0.001 mol) and 1-methyl-5-imidazole carboxaldehyde (110 mg 0.001 mol) were dissolved in 20 mL ethanol:water (60:40 %) separately and then the mixture was refluxed for 7 hrs in oil bath at 78 °C. The solution was allowed to cool at room temperature and left for slow solvent evaporation. After several days pale yellow crystals were obtained. The crystals were separated, washed with cold ethanol and dried under vacuum . Yield: 67%; mp 210 °C. The pure crystals were suitable for X-ray analysis, data collection and structure refinement results are summarized in Table 1. For $C_6H_9N_5S$: FT-IR (KBr cm^{-1}); $\nu(NH_2)$ 3404, 3227; ν(NH) 3158; ν(C=N) 1605; ν(C=S) 831; ν(N-N) 1117. NMR spectrum (600 MHz for ^{1}H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^{1}H NMR; δ=3.8(3H, s, CH_3); 7.7-8.1 (2H, CH ring), 7.3-7.7(2H, s, NH_2), 8.2(1H, s,=N-NH); 11.3 (1H, s, NH); ^{13}C NMR; δ=33.99 (CH_3); 134.40 (Cq); 126.7-133.83 (2C-H ring), 142.04(HC=N); 177.41(C=S). Ms (ESI.m/z): M^+ 184.1; Analysis for $C_6H_9N_5S$ (Mw 183.24). UV-Vis spectrum (λ_{max} nm): 311.

Synthesis of Imidazolecarboxaldehyde semicarbazones (SC1-SC3)

Thee new imidazolecarboxaldehyde semicarbazones Schiff bases were obtained by following a general procedure previously reported by Hussein et al[22]. Since the semicarbazide was in its hydrochloride form (0.11153 g, 0.001 mol), prior to its condensation with aldehydes separately, it was neutralised by addition of NaOH in a 1:1 ratio. Thus, an aqueous solution (20 mL) of the neutralised semicarbazide was slowly added to a

warm (60°C) ethanolic solution (20 mL) of imidazolecarboxaldehydes (0.001 mol). The mixture was refluxed for 7 hrs and the resulting suspension was cooled at room temperature, and then allowed for slow solvent evaporation, the crystals obtained was filtrated, washed with water and ethanol, and finally dried under vacuum.

Synthesis of 4-methyl-5-imidazolecarboxaldehyde semicarbazone (SC1)
White crystals,Yield: 80%, m.p 255°C. $C_6H_9N_5O$. FT-IR (KBr cm^{-1}), 3464, 3445, *ν*(NH_2); 3140, *ν*(NH); 1584, *ν*(C=N); 1690, *ν*(C=O); 1116. *ν*(N-N). NMR spectrum (600 MHz for ^1H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^1H NMR; δ=2.2 (3H, s, CH_3); 7.6 (H, CH ring), 6.4(2H, s, NH_2), 7.8(1H, s, =N-NH); 10.0 (1H, s, NH), 12.1 (1H, s, NH ring); ^{13}C NMR; 135.54(HC=N); 156.93(C=O). Ms (ESI.m/z): M^+ 168; Analysis for $C_6H_9N_5S$ (Mw 167.17). UV-Vis spectrum (λ_{max} nm): 298.

Synthesis of 4-imidazolecarboxaldehyde semicarbazone (SC2)
White crystals, Yield: 71%, m.p 220°C. $C_6H_9N_5O$. FT-IR (KBr cm^{-1}, 3382, 3323, *ν*(NH_2); 3217, *ν*(NH); 1592, *ν*(HC=N); 1655, *ν*(C=O); 1109. *ν*(N-N). NMR spectrum (600 MHz for ^1H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^1H NMR; δ=7.3-7.8 (2H, CH ring), 6.4(2H, s, NH_2), 7.8(1H, s, =N-NH); 10.2 (1H, s, NH), 12.4 (1H, s, NH ring); ^{13}C NMR; δ=136.89 (Cq ring); 128.70-129.45(2C-H ring), 129.79(HC=N); 157.03(C=O). Ms (ESI.m/z): M^+ 154.1; Analysis for $C_6H_9N_5O$ (Mw 153.14). UV-Vis spectrum (λ_{max} nm): 283.

Synthesis of 1-methyl-5-imidazolecarboxaldehyde semicarbazone (SC3)
White crystals, Yield: 73%, m.p 240°C. $C_6H_9N_5O$. FT-IR (KBr cm^{-1}), 3471, 3309, *ν*(NH_2); 3147, *ν*(NH); 1579, *ν*(HC=N); 1691, *ν*(C=O); 1120, *ν*(N-N). NMR spectrum (600 MHz for ^1H and 151 MHz for ^{13}C, DMSO-d_6, ppm): ^1H NMR δ=3.9 (3H, s, CH_3); 7.3-7.7 (2H, CH ring), 6.3(2H, s, NH_2), 7.9(1H, s, =N-NH); 10.2 (1H, s, NH); ^{13}C NMR; δ=33.87 (CH_3);141.24 (Cq ring); 127.36-130.78(2C-H ring), 132.26(HC=N); 156.66(C=O). Ms (ESI.m/z): M^+ 168.1; Analysis for $C_6H_9N_5O$ (Mw 167.17). UV-Vis spectrum (λ_{max} nm): 288.

Preliminary Range-Finding Bioassay
Exploratory bioassays were conducted to determine the preliminary toxicity range of TS -SC derivatives and commercial temephos against late third to early fourth instar larvae of *An.arabiensis*. TS-SC compounds were initially tested using four-fold serial dilutions (1000–3.906 ppm; 15 larvae per concentration) to identify the active range, after which definitive concentrations were selected using two-fold dilutions (500–3.906 ppm) for probit analysis. For commercial temephos, exploratory log_4-spaced concentrations identified an effective range of 0.5–0.00195 ppm, and final bioassay concentrations were established using a two-fold dilution series (0.5–0.0039 ppm) suitable for probit analysis.

Preparation of Test and Control Solutions:
Stock solutions of the tested compounds were prepared and serially diluted to obtain the desired concentrations of 500, 250, and 125 ppm, following the dilution law. Distilled water containing the same volume of DMSO was used as the negative control, while temephos (0.5 ppm) served as the positive control. All solutions were freshly prepared immediately prior to testing.

Rearing and Maintenance of *Anopheles arabiensis*
Larvae were obtained from the 400th generation of a laboratory-maintained colony of the Dongola strain of *Anopheles arabiensis*, continuously reared at the Department of Entomology, National Health Laboratory, Khartoum, Sudan, since 2004. Rearing was conducted under standard insectary conditions at 27 ± 0.5 °C, 80 ± 2% relative humidity, and a 12:12 h light–dark cycle. Adults were maintained in rearing cages and provided with cotton pads soaked in 10% sucrose solution.
Females were blood-fed on bovine blood collected in glass bottles containing EDTA as an anticoagulant. The blood was aliquoted into sterile Falcon tubes, warmed to 36–38 °C in a water bath, and offered to females through a membrane feeding apparatus (International Atomic Energy Agency, Austria) fitted with a stretched parafilm membrane. Feeding was allowed for approximately 20 minutes, and a second blood meal was provided after 2–3 days to ensure complete digestion.
Oviposition was induced using paper cups lined with moist filter paper placed over cotton soaked in distilled water. Following egg deposition, the cups were transferred to enamel trays containing distilled water for larval hatching and development. Larvae were fed daily with finely powdered *Sera Vipan* fish food in small quantities to minimize water fouling. All pupae were removed and discarded immediately upon detection. Late third- and early fourth-instar larvae were used for the bioassays.

Larvicidal Bioassay:
Larvicidal activity was evaluated following standard World Health Organization (WHO) protocols[24] with minor modifications. Ten late third- or early fourth-instar larvae were introduced into plastic cups containing 20

mL of the test solution at each concentration. Each treatment was performed in triplicate, and corresponding controls were included in every assay. The cups were maintained at 28 ± 2 °C and 70 ± 10% relative humidity under laboratory conditions.
Larval mortality was recorded after 24 and 48 hours of exposure. Individuals were considered dead if they failed to respond to gentle probing or did not rise to the water surface.

Statistical Analysis:
The median lethal concentration (LC_{50}) values were calculated using probit and logit analysis performed with IBM SPSS Statistics software, version 22.

Results:
The larvicidal activity of thiosemicarbazones and semicarbazones derivatives was evaluated against *Anopheles arabiensis* larvae at varying concentrations and exposure times. After 24 hours, the highest mortality among the free ligands was observed for TS3 (93%), followed by TS3 (90%) at 250 ppm. Extending exposure to 48 hours substantially increased larval mortality across all compounds, with TS3 achieving complete mortality (100%) at 500 ppm. Mortality declined at lower concentrations (250 and 125 ppm), indicating a concentration-dependent response.
The positive control, Temephos, induced complete larval mortality (100%) at concentrations ≥125 ppm after both 24 and 48 hours, whereas the negative control showed no mortality.
Time-dependent toxicity was reflected in decreasing LC_{50} values from 24 to 48 hours. TS3 exhibited the greatest larvicidal potency among free ligands, .with LC_{50} decreasing from 248 ppm (24 h) to 107 ppm (48 h), followed by TS1 and TS2 were less active (LC_{50}: 466 → 257 ppm and 746 → 270 ppm, respectively). Temephos exhibited superior efficacy, with LC_{50} values of 0.037 ppm (24 h) and 0.028 ppm (48 h).

Tables 1a and 1b. Effect of different concentrations of thiosemicarbazone derivatives on Anopheles arabiensis larval mortality and probit analysis**.**

Code	Concentration (ppm)	Mortality% (24h)	Mortality% (48h)	Corrected Mortality % (24h)	Corrected Mortality% (48h)	Probit % (24h)	Probit % (24h)
TS1	500	50	100	50	100	5.0	8.09
	250	25	35	25	35	4.33	4.61
	125	20	25	20	25	4.16	4.33
TS2	500	20	100	20	100	4.16	8.09
	250	15	30	15	30	3.96	4.48
	125	10	20	10	20	3.72	4.16
TS3	500	93	100	93	100	6.48	8.09
	250	80	90	80	90	5.84	6.28
	125	5	80	5	80	3.36	5.84
Temephos	0.500	100	100	100	100	8.09	8.09
	0.250	100	100	100	100	8.09	8.09
	0.125	100	100	100	100	8.09	8.09

Table 2. Median lethal concentrations (LC50) of thiosemicarbazone derivatives against Anopheles arabiensis larvae.

Compound	LC_{50} (ppm, 24 h 95%)	Confidence Limits (24 h)	LC_{50} (ppm, 48 h 95%)	Confidence Limits (48 h)
TS1	466	377–627	257	215–320
TS2	746	600–830	270	220–310
TS3	248	200–290	107	90–130
Temephos	0.037	0.030–0.045	0.028	0.022–0.034

Discussion:

The present study was designed to evaluate the larvicidal potential of a selected series of chemically synthesized compounds against *An. arabiensis* larvae under controlled laboratory conditions. The primary objective was to assess the efficacy of thiosemicarbazone-based ligands (TS1–TS3) and compare their activity with structurally related semicarbazone analogues (SC1–SC3) and a conventional larvicide. This systematic selection enabled a robust comparative assessment of larvicidal activity across closely related chemical frameworks, facilitating evaluation of the influence of sulfur incorporation, ligand substitution, and molecular conformation on biological performance.

Larvicidal bioassays were performed following standardized laboratory protocols, with mortality rates and lethal concentration (LC) values determined at defined exposure intervals. The semicarbazone derivatives (SC1–SC3) exhibited no detectable larval mortality at any tested concentration throughout the experimental period. In contrast, the thiosemicarbazone derivatives (TS1–TS3) demonstrated marked larvicidal activity, with mortality clearly dependent on both concentration and exposure duration. Across all active treatments, mortality increased progressively with higher doses and extended exposure, indicating cumulative toxic effects. These results highlight the critical role of sulfur-containing functional groups in enhancing larvicidal efficacy.

The superior toxicity of thiosemicarbazones relative to semicarbazones is attributable to the presence of the sulfur atom, which increases chemical reactivity and promotes stronger interactions with biological macromolecules, particularly metal-dependent enzymatic systems. Supporting literature shows that thiosemicarbazones with sulfur-based functional groups exhibit enhanced larvicidal activity compared to other ligands. For example, Bursalı *et al*. [25] reported significant larvicidal effects of aromatic thiosemicarbazone derivatives and their Ni(II), Cu(II), and Co(II) complexes against mosquito larvae, emphasizing the importance of the C=S functional group. Similarly, Silva *et al*. [21] demonstrated that aryl- and phenoxymethyl-thiosemicarbazones with sulfur availability correlated positively with increased larvicidal potency against *Ae. aegypti*.

Among the free thiosemicarbazone ligands, TS3 exhibited the highest larvicidal potency, followed by TS1 and TS2. After 24 hours, TS3 showed strong dose- and time-dependent larvicidal activity, with mortality of 93–100% at 500 ppm, 80–90% at 250 ppm, and 5–80% at 125 ppm over 24–48 hours.

, whereas TS1 and TS2 achieved ≤50% under the same conditions. Following 48 hours, TS3 reached complete larval mortality (100%) at 500 ppm and maintained 80–90% efficacy even at 250 ppm. TS1 and TS2 remained weakly active, particularly at lower concentrations, reflecting limited larvicidal potential. These observations are consistent with previous studies indicating that structural variations within thiosemicarbazone ligands significantly influence larvicidal performance [25].

Quantitative LC_{50} assessment further confirmed these trends. Across all compounds, LC_{50} values decreased with prolonged exposure, reflecting cumulative ligand uptake and sustained toxic effects. TS3 exhibited the lowest LC_{50} values (248 → 107 ppm at 24 → 48 h), indicating higher intrinsic potency and a steeper dose–response relationship, whereas TS1 and TS2 required higher concentrations (TS1: 466 → 257 ppm; TS2: 746 → 270 ppm) to achieve equivalent mortality. This pattern correlates strongly with the balance between lipophilicity, polar surface area (PSA), and molecular planarity, which govern membrane penetration and intracellular accumulation [26].

Compared to Temephos, a widely used organophosphate larvicide, thiosemicarbazone derivatives exhibited lower absolute potency but displayed delayed, cumulative toxicity. Temephos induced complete larval mortality at 0.125 ppm and maintained activity at 0.03125 ppm, reflecting rapid acetylcholinesterase inhibition. Probit analysis confirmed the highest values for Temephos, followed by TS3, while TS1 and TS2 showed lower responses. This delayed toxic pattern in thiosemicarbazones suggests alternative mechanisms, potentially involving enzymatic inhibition, Based on 48 h data, the overall potency ranking was: Temephos > TS3 > TS1 > TS2. This comparative framework aligns with prior evidence supporting Temephos as a benchmark larvicide while highlighting the mechanistic relevance of TS derivatives for resistance management [27-7]. Silva *et al* [21] demonstrated that thiosemicarbazone compounds can exert their larvicidal effect through inhibition of the mosquito sterol carrier protein-2 (AeSCP-2), a key protein involved in cholesterol transport and uptake in mosquito larvae. Inhibition of AeSCP-2 disrupts dietary cholesterol acquisition, interferes with steroid biosynthesis, and consequently affects larval growth and development, leading to larval mortality. This mechanism may contribute to the delayed and cumulative toxic effects observed for thiosemicarbazone derivatives compared with the rapid neurotoxic action of organophosphate larvicides.

Structure–activity relationship (SAR) analysis revealed that subtle modifications on the imidazole ring of TS ligands exerted pronounced effects on larvicidal activity. TS3, with N-methyl substitution at the N1 position and the aldehyde group at C-5, exhibited enhanced planarity, reduced PSA, and increased lipophilicity, facilitating membrane permeation and π–π interactions with biological targets. TS1, bearing a C-methyl substituent rather than N-methylation, displayed moderate activity due to increased hydrogen-bond donor capacity, which may limit cuticular diffusion. TS2, the unsubstituted analogue, showed the lowest activity, consistent with higher PSA, low lipophilicity, and diminished membrane penetration. These SAR-driven trends align with previous

reports demonstrating that N-alkylation and aldehyde positioning significantly enhance thiosemicarbazone bioactivity[28-21-29-30].

In conclusion, TS derivatives demonstrated pronounced larvicidal activity relative to semicarbazones, with TS3 emerging as the most potent ligand. The observed efficacy correlates with sulfur incorporation, optimal N-methyl substitution, and aldehyde positioning, collectively influencing physicochemical properties and biological performance. These findings provide a robust experimental and SAR-based framework for prioritizing thiosemicarbazone derivatives in the development of alternative, non-neurotoxic larvicidal agents.

Conclusion:
Thiosemicarbazone derivatives demonstrated significant, concentration- and time-dependent larvicidal activity against *An. arabiensis* larvae. Among the ligands, TS3 exhibited the highest potency, while metal coordination enhanced the efficacy of TS3, likely due to increased lipophilicity and improved membrane permeability. Probit analysis supported a strong dose–response relationship ($R^2 \geq 0.8$) and homogeneous larval susceptibility. Although less potent than Temephos, the tested compounds show considerable potential as alternative larvicidal agents.

Recommendations:
Future studies should investigate the mechanism of action, environmental safety, and selectivity of these compounds toward non-target species. Optimization of structural features and formulation strategies is also recommended to enhance their stability and field performance for potential integration into mosquito control programs.

Compliance with ethical standards
Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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