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Comparative Analysis: Larvicidal efficacy of traditional Saudi Arabian herbs and boric acid against *Aedes aegypti* larvae, the Dengue fever vector

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Abstract

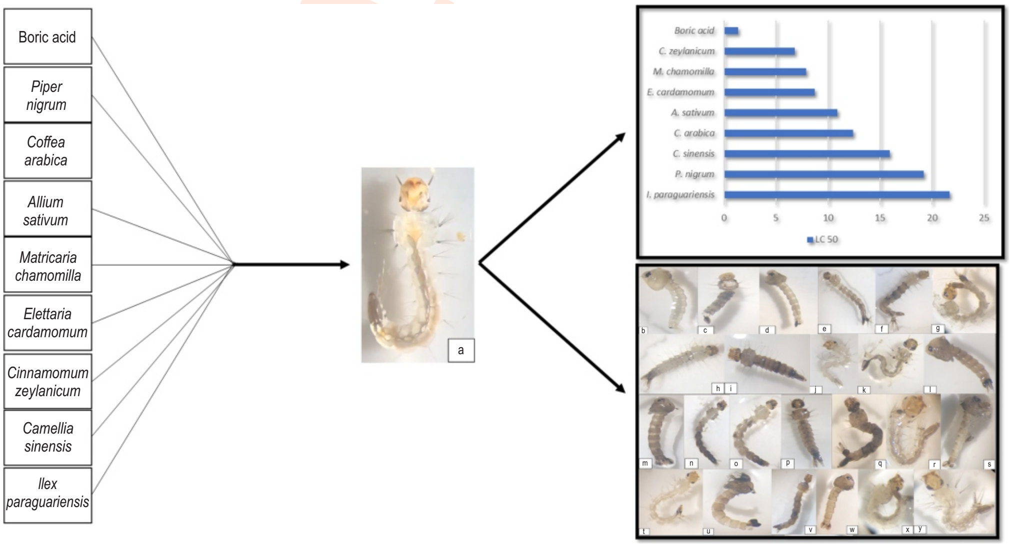
Aim: This study evaluates the larvicidal properties of traditional Saudi Arabian herbs compared to Boric acid against the *Aedes aegypti* larval stage.

Methodology: An evaluation of the larvicidal activity of eight plant extracts, as well as Boric acid, was conducted with *Ae. aegypti* larvae in the 3rd and 4th larval stages. Through stereo-dissecting microscope, morphological and death defects in larvae treated with Boric acid were documented.

Results: The results showed that the mortality rates ranged from 59.3 to 98.3% at 30% concentration. Particularly noteworthy were Boric acid, *M. chamomilla,* and *C. zeylanicum,* demonstrating mortality rates of 100 to 89%, falling within the highly effective category. *E. cardamomum, C. arabica,* and *A. sativum* exhibited moderate efficacy (88 to 70%) whereas *C. sinensis, P. nigrum* and *I. paraguariensis* manifested relatively lower effectiveness (50 to 69%). Significantly, Boric acid showcased remarkable potency, inducing mortality rates of 28.3% even at an infinitesimal concentration of 1 ppm, accompanied by distinct malformation effects on treated larvae. These findings underscore the unparalleled larvicidal prowess of Boric acid, positioning it as a paramount choice for integrated mosquito control strategies.

Interpretation: Furthermore, Boric acid accessibility, cost-effectiveness, and minimal impact on ecological systems and human health make it a compelling solution for mitigating *Ae. aegypti* populations, thereby fortifying our arsenal against mosquito-borne diseases.

Key words: *Aedes aegypti*, Biological control, Boric acid, Plant extract



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Introduction

Mosquitoes (Culicidae family) distinguish themselves as significant disease vectors within the order Diptera, affecting both human and veterinary health (Guzman *et al.,* 2010). Their close proximity to humans makes them a significant public health concern, particularly in the regions like Saudi Arabia, where dengue fever, transmitted by *Aedes aegypti* mosquitoes, is endemic (Aziz *et al.,* 2014). Recently, Tangsathapornpong and Thisyakorn (2023), have reported a rising prevalence of dengue fever in the Middle East, with a considerable number of reported cases, emphasizing the urgency for effective control strategies. *Ae. aegypti,* a highly efficient vector, spreads various diseases, including Dengue fever, Zika virus, Chikungunya and Yellow fever, posing substantial global public health threats (Gubler, 2011). The spread of these diseases is influenced by factors like climate, urbanization, international travel, and mosquitoes' adaptability to diverse environments amplifies their role in rapid disease transmission. In outbreaks, the use of organophosphates and insect growth regulators has demonstrated short-term effectiveness. Rahuman *et al.* (2008), however, raised concerns about the implications of their application on the environment and health. Moreover, mosquitoes, like other insects, develop resistance to these insecticides after exposure, necessitating exploration of alternative methods.

Plants and their derivatives have emerged as promising alternatives due to their effectiveness and lower toxicity to both humans and the environment compared to conventional insecticides. Various extraction techniques, including maceration, are employed to obtain bioactive plant compounds for industrial and commercial use (Saqib *et al.,* 2022). Several plants, such as *Ilex paraguariensis* (yerba mate), *Camellia sinensis* (tea plant), *Cinnamomum zeylanicum* (cinnamon), *Elettaria cardamomum* (cardamom), *Matricaria chamomilla* (chamomile), *Allium sativum* (garlic), *Coffea arabica* (Arabica coffee), and *Piper nigrum* (black pepper), exhibit potent larvicidal properties against *Ae. aegypti* larvae. *I. paraguariensis* disrupts the physiological processes of mosquito larvae, leading to their mortality (Oliveira *et al.,* 2018). *C. sinensis* contain bioactive compounds like catechins and flavonoids, which impede larval growth and reduce mosquito populations (Ferreira *et al.,* 2009). *C. zeylanicum's* larvicidal efficacy is attributed to active compounds such as cinnamaldehyde (Pavela *et al.,* 2019).

Similarly, *E. cardamomum,* with constituents like eucalyptol and 1,8-cineole, exhibits notable larvicidal effects (Pavela *et al.,* 2019). Active compounds like coumarin and herniarin in *M. chamomilla* demonstrate potent insecticidal effects (Cheraghi *et al.,* 2016). *A. sativum,* rich in allicin, disrupts larval development, inhibiting pupation. *C. arabica* and *P. nigrum*, with their unique compounds, effectively control mosquito larvae, making them valuable natural solutions for vector control (Govindarajan *et al.,* 2013). Inorganic compounds like Boric acid, recognized for their low toxicity and non-volatility, offer an alternative to conventional neurotoxic insecticides (Quarles, 2001). Though historically used in adult-stage mosquito control, limited research have been conducted on Boric acid impact on mosquito larvae. Therefore, this study investigates the larvicidal effects of plant extracts from traditional Saudi Arabian plants, comparing their efficacy with Boric acid against *Ae. aegypti's* at 3rd and 4th larval stages. This research seeks to identify safer and more effective methods for mosquito control, crucial for public health and environmental sustainability.

Materials and Methods

Mosquito culture: A research experiment was undertaken to assess the larvicidal effect of eight plant extracts on 3rd and 4th stage larvae of *Ae. aegypti.* The experiments were carried out at the Dengue Mosquito Research Station in Saudi Arabia, located at King Abdelaziz University in Jeddah, during the period from March to June 2023 according to Asahina (1964). Laboratory strains of *Ae. aegypti* were used for the study, and the laboratory conditions were maintained at temperature 27 ± 1°C, relative humidity 70 ± 5%, and a light-dark cycle of 14:10 hrs. The larvae were nourished with a mixture of fish food or dry bread powder combined in A 1:1 ratio with dried milk. To ensure accuracy, mosquito larvae collected were meticulously examined for species identification and viability by a senior entomologist, serving as a quality control measure for the study.

Plant extraction: Plants of yerba mate (*I. paraguariensis*), green tea (*C. sinensis*), cinnamon (*C. zeylanicum*), cardamom (*E. cardamomum*), chamomile (*M. chamomilla*), garlic (*A. sativum*), coffee (*C. arabica*), and black pepper (*P. nigrum*) were procured from local markets. The extraction methos was carried according to Abubakar and Haque (2020). Subsequently, 20 g of each plant sample were soaked in 100 ml distilled water for 24 hr at room temperature (37 °C). After soaking period, the solutions were diluted to concentration of 1, 5, 10, 20, and 30 ppm respectively.

Boric acid preparation: Boric acid was purchased from AL-Shafei Medica & Scientific Equipment Exh. Jeddah, Saudi Arabia. The choice of the Boric acid inorganic compounds was based on the fact that these insecticides are easily available, cost effective and tested less against *Ae. aegypti* larvae in Jeddah Province. A stock solution of Boric acid was prepared by adding 1 ml of Boric acid to 99 ml distilled water and ensuring that Boric acid was completely soluble in water. Five concentrations of Boric acid (1, 1.5, 2, 2.5, and 3 ppm) were prepared for further experiments.

Mortality bioassay: Mortality bioassay was conducted according to World Health Organization (1996). Plant stock solutions and Boric acid were prepared by mixing 1 ml of solution with 99 ml of distilled water. Twenty larvae were placed in small cups containing 50 ml of each test concentration. In addition, 20 larvae were placed in a separate cup containing 50 ml of distilled water which as a control. Mortality was studied for both plant extracts and Boric acid after 24 hr of exposure by counting dead and live insects during March-June 2023. Larvae were considered dead when they settled at the bottom without any movement. Experiments were conducted at room temperature (30-37°C). Each experiment was repeated thrice.

Statistical analyses: The experiments were conducted in a random design. Mortality in the treatment groups was corrected using Abbott's formula if the mortality of the control was between 5 and 20%. The LC_{50} and LC_{90} were calculated by Probit Analysis program with lower and upper confidence limits and the inclination of toxicity line and Chi-square. Larval mortality was recorded daily. All the experimental photographs were taken on a dissecting microscope with a digital camera as an attachment.

Results and Discussion

In this study, the larvicidal activity of eight extracts were obtained from eight different plants against *Ae. aegypti,* a vector of dengue, was evaluated as a safe means of combating it. We Aqueous solutions were used as test solutions because it is the most polar solvent, used to extract polar compounds, dissolves a variety of substances, is in expensive, non toxic, non flammable, and highly polar (Majekodunmi, 2015). The results obtained showed differences in the level of effectiveness of these extracts depending on the type of plant from which they were extracted as the effective concentrations ranged from 1 to 30 ppm, and the corresponding mortality rates ranged from 13.5 to 59.3 for *I. paraguariensis,* 5.2 to 84.3 for *A. sativum,* 9.3 to 71.8 for *C. arabica,* 8.3 to 66.6 for *C. sinensis,* 9.3 to 96.8 for *C. zeylanicum,* 12.5 to 87.5 for *E. cardamomum,* 4.4 to 90.6 for *M. chamomilla* and 6.2 to 63.5 for *P. nigrum* extracts, respectively. (Table 1). For Boric acid, the effective concentrations ranged from 1 to 3 ppm, and the corresponding mortality rates ranged from 28.3 to 98.3 (Table 2). On the other hand, the laboratory toxicity lines for plant extracts and Boric acid were drawn (Fig. 1-9) and all statistical constants were derived, such as the 50 and 90% lethal concentration of the larvae treated with the test solutions and their corresponding confidence intervals with their lower and upper

Table 1: Mortality rate of *Ae. aegypti* larvae treated with different plant extracts

Extracts		Concentration (ppm)					
		1	**5**	**10**	**20**	**30**	**Control**
I. paraguariensis	Mortality (%) ±SD	13.5 ±0	20.8 ±1.7	34.3 ±3.2	50 ± 2.1	59.3 ±1	0
A. sativum	Mortality (%) ±SD	5.2 ±3.79	14.5 ±1	51.0 ±2.1	67.7±3.1	84.3±1.5	0
C. arabica	Mortality (%) ±SD	9.3 ±1.15	26.0 ±3.06	45.8 ±2.65	59.3±1	71.8 ±1.2	0
C. sinensis	Mortality (%) ±SD	8.3 ±1	21.8 ±0.6	35.4 ±1.5	57.2 ±2.5	66.6 ±1	0
C. zeylanicum	Mortality (%) ±SD	9.3 ±0	33.3 ±3.1	52.0 ±3.1	84.3±0.6	96.8 ±1.2	0
E. cardamomum	Mortality (%) ±SD	12.5 ±1.1	25 ±2.1	47.9 ±4	71.8±1.7	87.5±0	0
M. chamomilla	Mortality (%) ±SD	4.4 ±1.2	32.2 ±0.6	57.2 ±1	78.1±1	90.6 ±0	0
P. nigrum	Mortality (%) ±SD	6.2 ±0.6	18.7 ±1.2	31.2±1.2	52.0±0.6	63.5 ±0.6	0

Table 2: Mortality rate of *Ae. aegypti* larvae treated with Boric acid

Material		Concentration (ppm)					
		1	**1.5**	**2**	**2.5**	**3**	**Control**
Boric acid	Mortality (%) ±SD	28.3±1.1	56.6±0.57	76.6±2.0	88.3±1.5	98.3±1.5	0

Table 3: Mortality rate of *Ae. aegypti* larvae treated with different plant extracts

Extracts	LC_{50} (ppm)	LCI-UCI	LC_{90} (ppm)	LCI-UCI	χ^2	Slope	p-value
Boric acid	1.36	1237.3-1387.4	2.52	2372.6- 2854.7	6.7	±0.24.3	P < 0.002
I. paraguariensis	21.7	15.89-33.3	472.9	202-1990.9	4.5	0.96±0.2	P<0.2
A. sativum	10.9	5.8-20	49.4	49.4-255.8	12.7	1.9±0.2	P<0.005
C. arabica	12.4	10.1-15.6	118.6	74.6-234.4	1.7	1.3±0.1	P<0.6
C. sinensis	15.95	12.8-20.6	155.3	92.9-336.9	3.07	1.3±0.1	P<0.38
C. zeylanicum	6.8	2.8-12.2	29.9	25-143.2	16.8	2 ± 0.2	P<0.0008
E. cardamomum	8.75	3.67-18.6	57.4	56.6-599.599.8	14.17	1.6±0.1	P<0.003
M. chamomilla	7.89	6.7-9.1	33.6	26.98-44.7	1.31	2.04±0.17	P<0.7
P. nigrum	19.2	15.4-25.2	172.4	101.7-385.9	2.7	1.3±0.2	P<0.4

Lc_{50}: 50% lethal concentration; LC_{90}: 90% lethal concentration, LCI: lower Confidence Interval; UCI: Upper Confidence Interval.

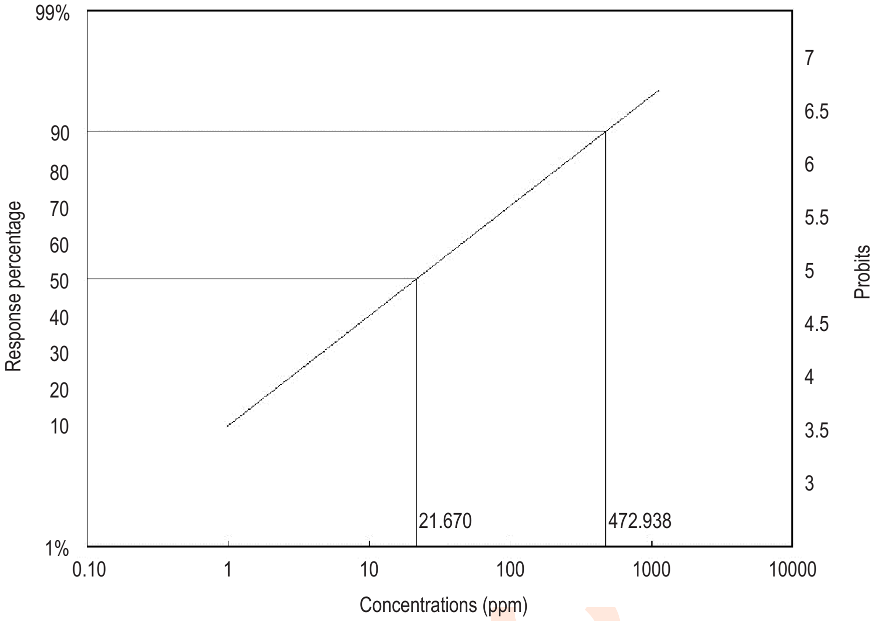

Fig. 1: LC$_{50}$ and LC$_{90}$ of *I. paraguariensis* against *Ae. aegypti* larvae after 24 hr.

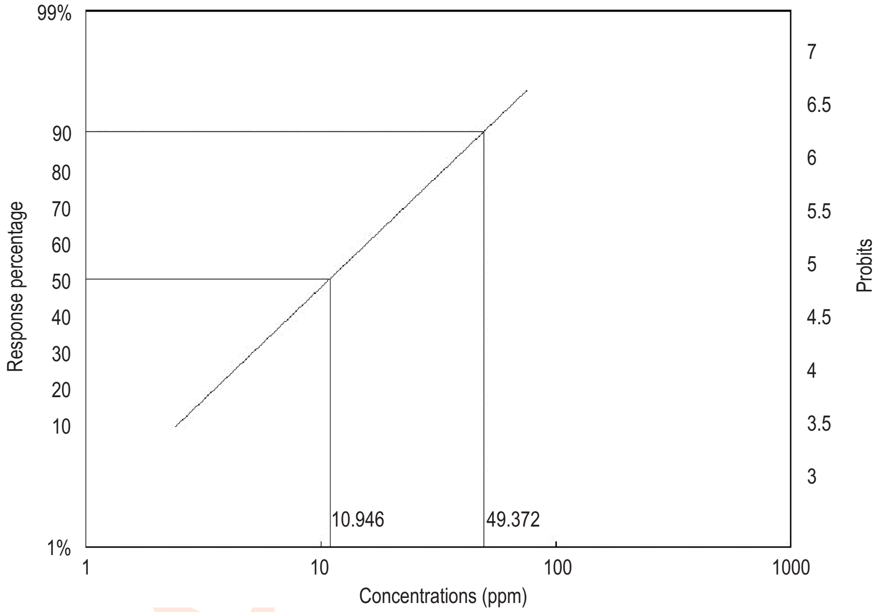

Fig. 2: LC$_{50}$ and LC$_{90}$ of *A. sativum* against *Ae. aegypti* larvae after 24 hr.

limits, as well as the values of Chi-square, the slope of the laboratory toxicity line, and others. Based on the calculated LC$_{50}$ for Boric acid (1.36) and C zeylanicum extract (6.2ppm), the materials were arranged according to their degree of effectiveness, with Boric acid (LC$_{50}$=1.36) being the most effective, followed by *C zeylanicum* extract (LC$_{50}$=6.8ppm), whereas *I paraguariensis* extract (LC$_{50}$=21.7ppm) was the least effective (Fig. 10; Table 3). The perusal also showed malformation effects of Boric acid against treated larvae as compared to the control group (Fig. 11). These malformations manifested in Albino pupa (Fig. 12, b), Pigmentation (Fig. 12, c, h, i, n, o, q, v), Neck elongation (Fig. 12, v), Segment contraction

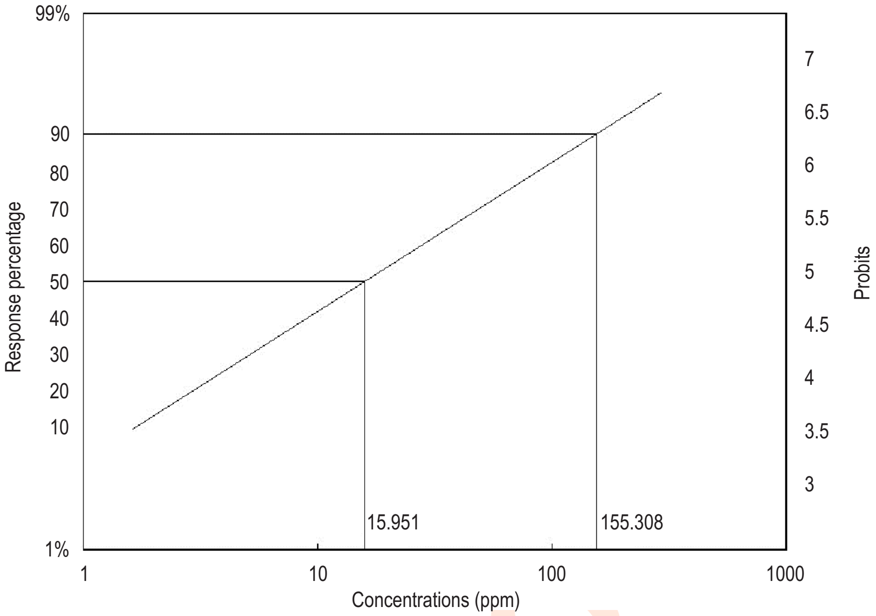

Fig. 3: LC$_{50}$ and LC$_{90}$ of *C. sinensis* against *Ae. aegypti* larvae after 24 hr.

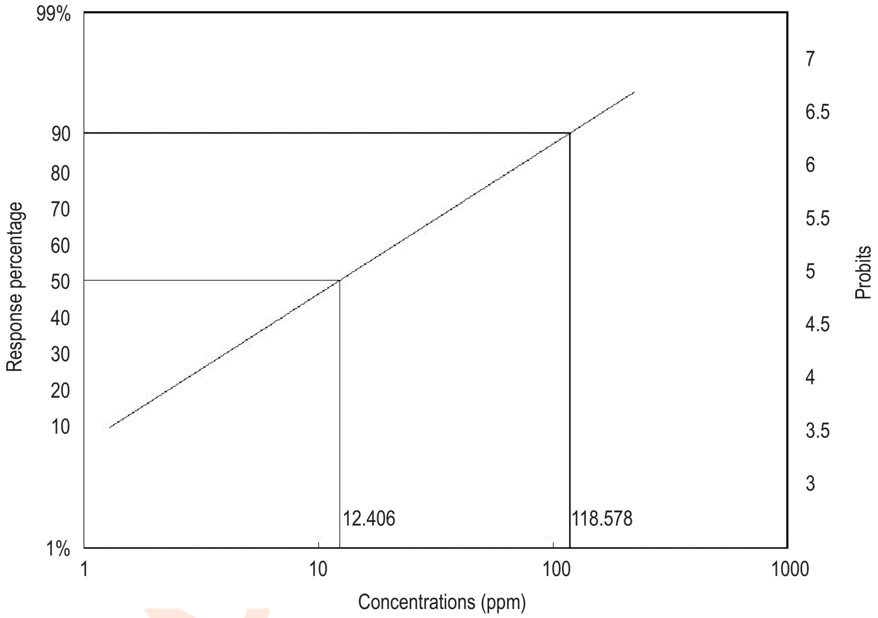

Fig. 4: LC$_{50}$ and LC$_{90}$ of *C. arabica* against *Ae. aegypti* larvae after 24 hr.

(Fig. 12, h, i, p), Deformed cuticles (Fig. 12, j, k, r, x, z), Opaque swelling on thorax (Fig. 12, f, g, n, o, r, t), Black coloration at the posterior end (Fig. 12, e, p, t, u), Larval-pupal intermediate (Fig. 12, d, l, m, s, u, w). The utilization of various plant parts for the purpose of controlling mosquito larvae has been a subject of extensive investigation in the past. Previous studies have demonstrated that extracts derived from the leaves, flowers, and roots of various plants exhibit larvicidal activity against mosquitoes. Lim *et al.* (2023) conducted experiments with aqueous extracts of three aromatic plants, *Curcuma longa* (turmeric), *Ocimum americanum* (hoary basil), and *Petroselinum crispum* (parsley) and observed significant mortality rate among

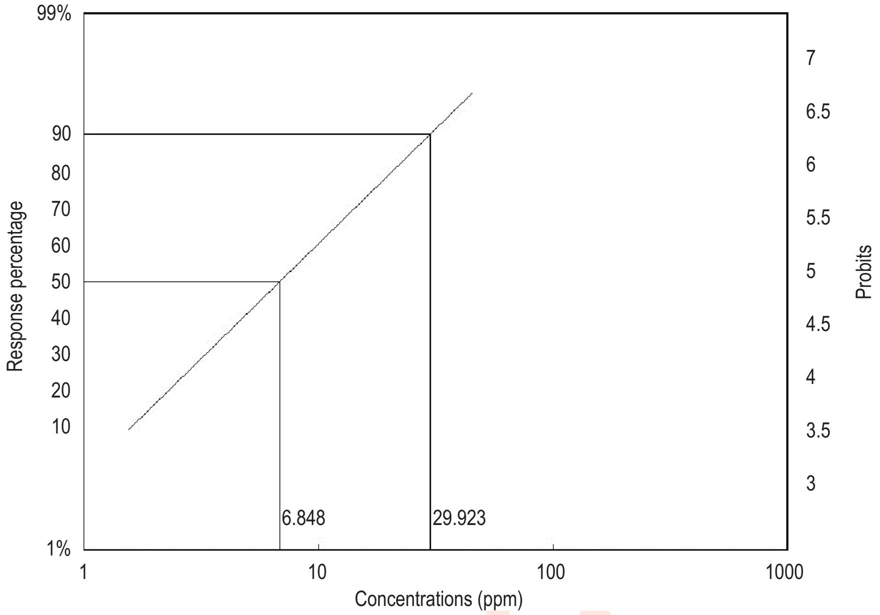

Fig. 5: LC_{50} and LC_{90} of *C. zeylanicum* against *Ae. aegypti* larvae after 24 hr.

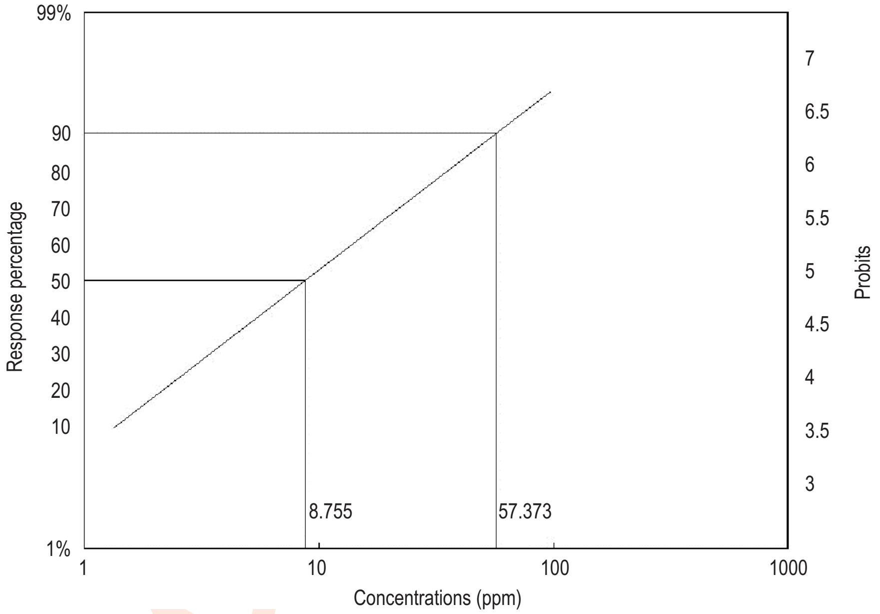

Fig. 6: LC_{50} and LC_{90} of *E. cardamomum* against *Ae. aegypti* larvae after 24 hr.

the mosquito larvae. Furthermore, Tchoumbougnang *et al.* (2009) studied the insecticidal properties of *Thymus vulgaris* against *Aedes* mosquitoes. It is worth mentioning that the leaves of *I. paraguariensis* are commonly used in tea infusions in the Middle East countries. In the current study, *I. paraguariensis* exhibited a relatively modest effect against *Ae. aegypti* larval stages, achieving a mortality rate of 59.3% at the highest concentration of 30 ppm (Table 1 and 3). However, there has been limited exploration of the larvicidal efficacy of aqueous extracts from *I. paraguariensis* plant against *Ae. aegypti* larvae. In line with the present study findings, Busato *et al.* (2015) reported low larvicidal effect of *I. paraguariensis* extract against *Ae. aegypti* larvae. This

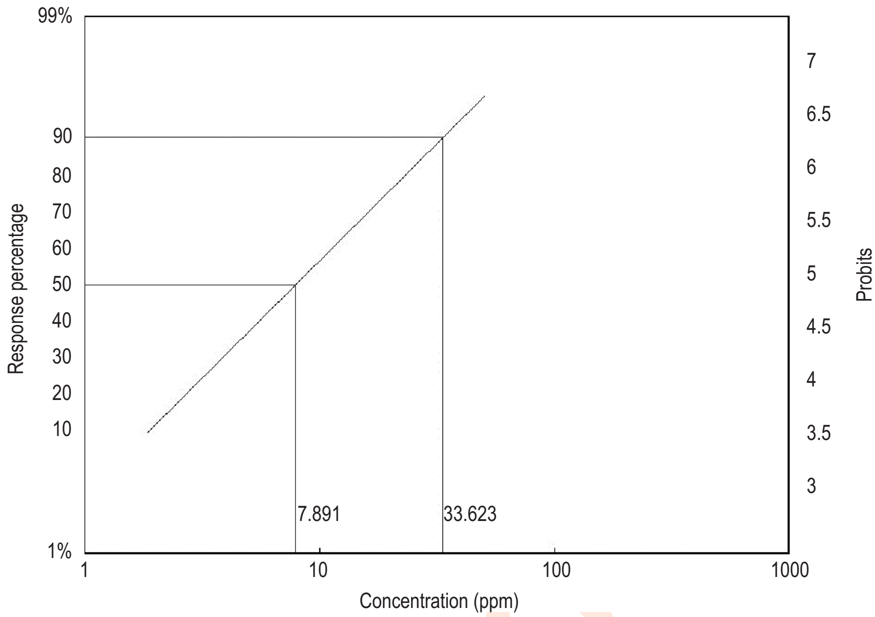

Fig. 7: LC_{50} and LC_{90} of *M. chamomilla* against *Ae. aegypti* larvae after 24 hr.

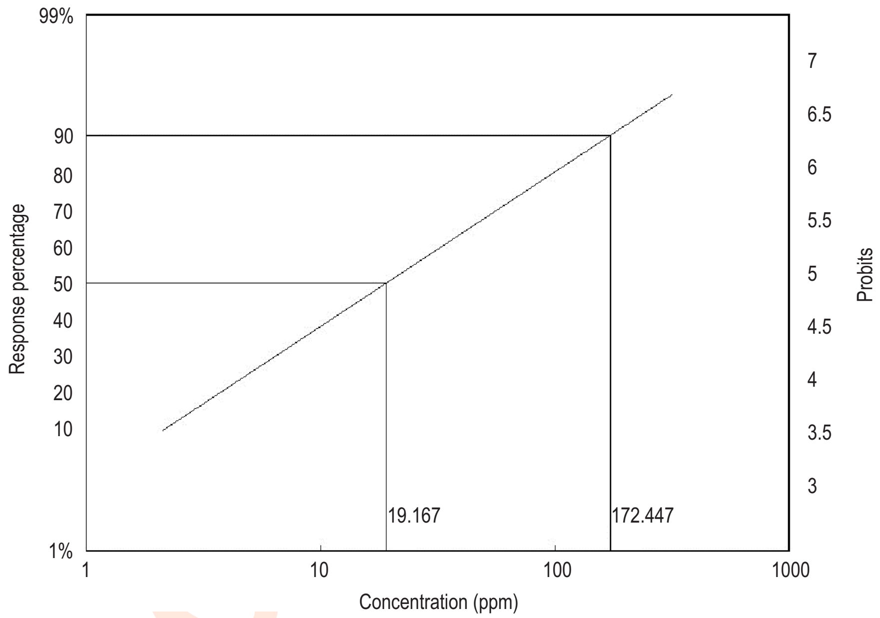

Fig. 8: LC_{50} and LC_{90} of *P. nigrum* against *Ae. aegypti* larvae after 24 hr.

suggests the necessity for further research on this particular plant. Our study also determined the mortality rate of *C. sinensis,* which, exhibited (Table 1 and 3), relatively low efficiency in controlling *Ae. aegypti* larvae, achieving a mortality rate of 66.6% at highest concentration of 30 ppm. Muema *et al.* (2016) reported larvicidal activity of *C. sinensis* extract along with growth-disrupting effects, implying the presence of biologically active phytochemicals. Additionally, a study on *C. sinensis* confirmed our findings, revealing that the exposure of *Anopheles gambiae* larvae to a crude *C. sinensis* extract at 250 ppm and 500 ppm for 24 hr resulted in larval mortality rates exceeding 90%, as well as 75% in *Anopheles arabiensis* (Muema *et al.,* 2016). Similarly, in

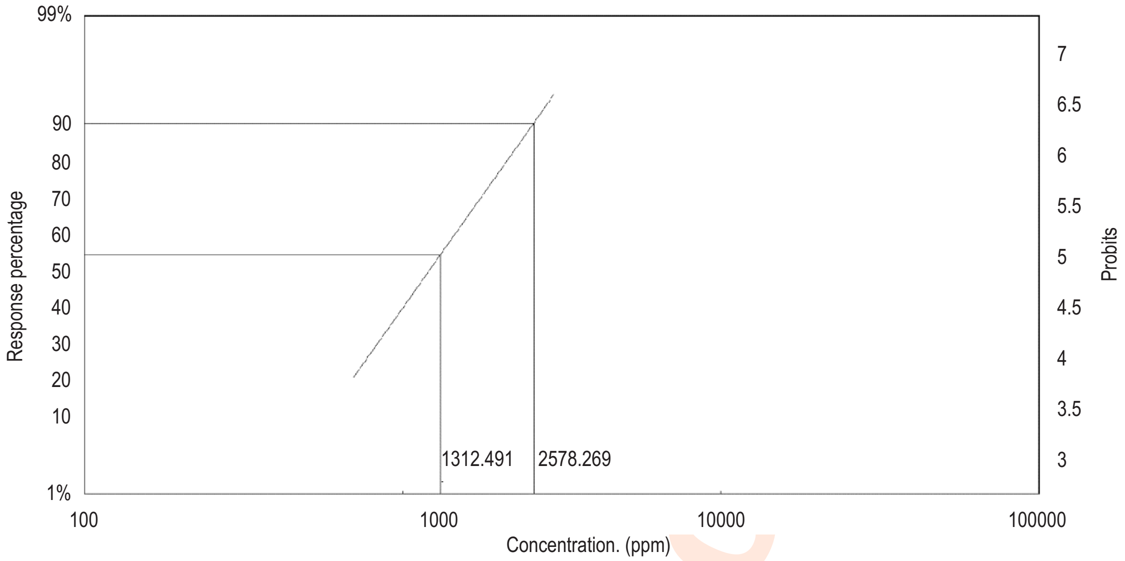

Fig. 9: LC_{50} and LC_{90} of Boric acid against *Ae. aegypti* larvae after 24 hr.

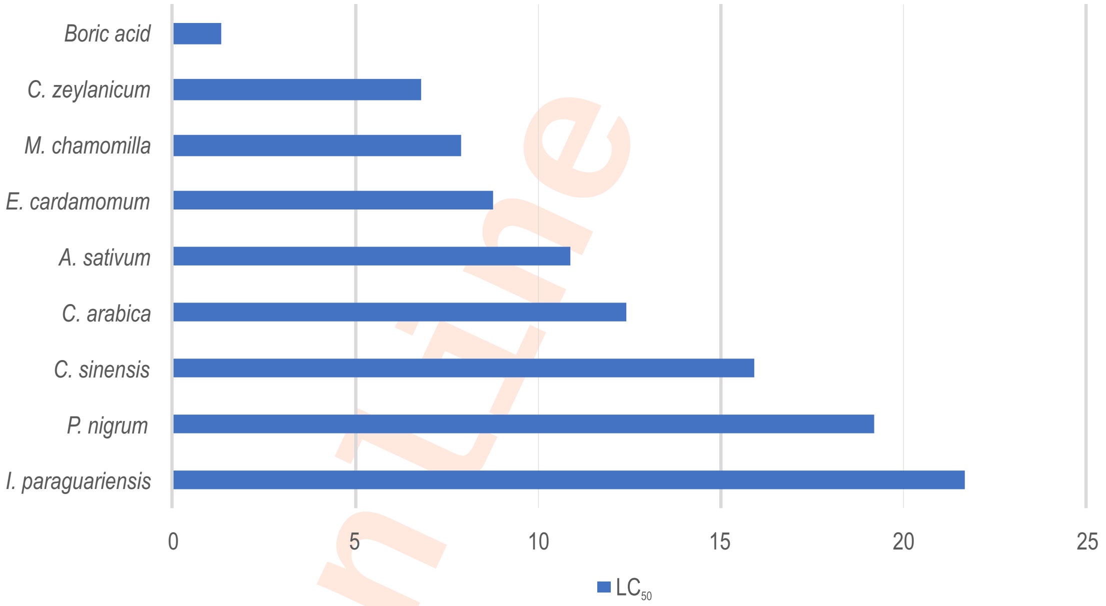

Fig. 10: LC_{50} of plant extracts against *Ae. aegypti* larvae after 24 hr.

this study, *C. zeylanicum* exhibited a potent larvicidal effect, achieving a mortality rate of 96.8% at 30 ppm, concentration. Aromatic plant *E. cardamomum,* commonly known as the "Queen of Spices,"has been traditionally used as a culinary and flavoring agent. In this study, *E. cardamomum* led to a moderate mortality rate of 87.5% against *Ae. aegypti* larvae at 30 ppm (Table 1 and 3). Consistent with the findings of this study, Sanei-Dehkordi *et al.* (2022) reported that essential oils extract from *C. zeylanicum* and *E. cardamomum* resulted in 100% larval mortality in *Anopheles stephensi* at a concentration of 25 μg ml^{-1}. *M. chamomilla* is a traditional plant commonly used in Saudi Arabia to cure a number of diseases such as gastrointestinal disorders, common cold and some respiratory problems. In this study, *M. chamomilla* caused a high mortality rate of 90.6% at 30 ppm and exhibited an LC_{50} value of 7.89 against *Ae. aegypti* larvae after 24 hr. however, the extract of *A. sativum* showed moderate potency (84.3%) at 30 ppm, with an LC_{50} value of 10.9 against *Ae. aegypti* larvael. Bhami and Das (2015) noted that the mortality of *Culex* and *Anopheles*

Fig. 11: Normal 4th larvae stage of *Ae. aegypti*.

mosquitoes increased with longer exposure period and higher extract concentrations, with the highest mortality observed at 3.0 mg ml^{-1} after 3 hr of exposure. *C. arabica* is a popular and traditional plant in Saudi Arabia used for medicinal purposes and as a basic beverage in Saudi Arabia. In this study, *C. arabica* exhibited a moderate mortality rate, with an LC$_{50}$ value of 12.4 at 30 ppm, *P. nigrum,* a traditional Saudi plant used as a food additive and spice, was also studied. However, it demonstrated low larvicidal efficacy, resulting in a mortality rate of 63.5% and an LC$_{50}$ of 19.2. Several studies have (Rattan 2010; Lim 2023; Rawani 2017; Radwan 2022) documented physiological disruptions caused by plants on insect bodies, including inhibition of acetylcholinesterase, GABA-gated chloride channels, sodium and potassium ion exchange disruptions, and inhibition of cellular respiration, and as part of such disruption, calcium channels, nerve cell membranes, octopamine receptors, hormonal balance, mitotic poisoning, disruptions of morphogenesis molecular events, as well as changes in cholinergic behavior and memory. Notably, boric acid, a frequently used pesticide in Saudi Arabia, exhibited the highest mortality rate (98.3%) at 30 ppm among all the tested phytochemicals, with corresponding LC$_{50}$ and LC$_{90}$ values of 1.36% and 2.52%, respectively, (Tables 2 and 3).

This aligns with Xue and Barnard (2003), who reported that 1% boric acid mixture with sugar effectively lured and eliminated adult mosquitoes. Bhami and Das (2015) also found that a 1% concentration of boric acid resulted in 100% mortality of certain *Aedes* species' eggs such as *Ae. aegypti* and *Ae. albopictus*. Nonetheless, it's important to note that boric acid can induce diverse biological effects on mosquito larvae, including malformation. This may be attributed to the chemical components of boric acid, which include derivatives of minerals commonly used for insect control, as suggested by See *et al.* (2010). Xue and Barnard (2003) mentioned that boric acid can be effective even at concentrations as low as 1%. These effects may indicate that boric acid induces complex alterations within exposed individuals, affecting various organs, tissues and cells, possibly due to its rapid transport to haemolymph.

Accordingly, substance concentration is important to consider. Higher concentrations generally leads to higher mortality rates across of Boric acid studied, indicating a dose-dependent response in plants. *C. zeylanicum, A. sativum,* and *M. chamomilla* appeared to be more sensitive, showing higher mortality rates even at lower concentrations against *Ae. aegypti* larvae. Boric acid displayed exceptionally high toxicity, making it a potent substance even at low concentrations. Further, using Boric acid as a safe method for controlling larval stages of *Ae. aegypti* can be recommended as well studying its mode of action on different insect cells, moreover, it is available, cost-effective, and less harmful to humans, animals, and the environment.

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Authors' contribution: S. Sharawi: Conceived and designed the study conducted research, provided research materials, and collected and organized data. Also, analyzed and interpreted data, wrote the initial and final draft of the article, and provided logistic support. The author has critically reviewed and approved the final draft and is responsible for the content and similarity index of the manuscript.

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Ethical approval: There is no ethical issue.

Fig. 12: Several abnormalities were observed in Ae. aegypti larvae. These abnormalities included:b: Albino pupa, c: Pigmentation irregularities, d: Larval-pupal intermediate stage, e: Black coloration at the posterior end of the larvae, f & g:Opaque swelling on the thorax, h & i: Irregular pigmentation and segment contraction, j & k: Deformed cuticles, l & m: Intermediate stage between larva and pupa, n & o: Pigmentation issues and opaque swelling on the thorax, p: Segment contraction and black coloration at the posterior end, q: Pigmentation abnormalities, r: Deformed cuticles and opaque swelling on the thorax, s: Intermediate stage between larva and pupa, t: Opaque swelling on the thorax and black coloration at the posterior end, u: Intermediate stage between larva and pupa with black coloration at the posterior end, v:, Pigmentation irregularities and elongation of the nick, w: Intermediate stage between larva and pupa, x-z: Deformed cuticles and opaque swelling on the thorax.

Conflict of interest: There is no interest in conflict among authors.

Data availability: Not applicable.

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