

Evaluation of larvicidal efficacy of indigenous plant extracts against *Culex quinquefasciatus* (Say) under laboratory conditions

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Abstract: The present study focused on the insecticidal potential of easily available local botanicals using a simple but effective method. Seven indigenous plants (*Cinnamomum tamala* (taiz pat), *Aloe vera* (aloe vera), *Datura alba* (datura), *Allium sativum* (garlic), *Allium cepa* (onion), *Zingiber officinale* (ginger), and *Ocimum basilicum* (niazbo/basil) were tested for their larvicidal efficacy against *Culex quinquefasciatus* (Say) under laboratory conditions. The evaluation of a series of five concentrations (1%, 2%, 3%, 4%, and 5%) of aqueous plant extracts against the 4th instar larvae revealed convincing larval mortality effects at 24 and 48 h after exposure. Larval mortality showed a significant concentration-dependent correlation. No mortality was observed in the control. The LC₅₀ values demonstrated garlic as the most effective (1.37%), followed by taiz pat (1.48%) and aloe vera (1.96%), at 24 h. Moreover, the LC₅₀ at 48 h showed high efficiency by aloe vera (0.37%), followed by garlic (0.55%) and taiz pat (0.98%). The sequence of LC₅₀ values for the other plants were onion (2.20%) < datura (2.49%) < niazbo (5.32%) < ginger (7.48%) after 24 h and datura (1.13%) < niazbo (1.17%) < onion (1.24%) < ginger (2.43%) after 48 h. Taken together, the aqueous extracts of all plants exhibited potential efficacy against *C. quinquefasciatus* larvae and could be considered as potent natural larvicidal agents. These plants may be recommended for use in mosquito management programs as potential alternatives to synthetic insecticides. The simple aqueous extraction method is easy and inexpensive and can be used at the home level for mosquito management.

Key words: Aqueous extracts, botanicals, larvicidal activity, mosquito

1. Introduction

Mosquitoes (Insecta: Diptera) are a serious threat to public health, as they transmit many vector-borne diseases and cause millions of deaths every year (Rozendaal, 1997; Service, 2004; WHO, 2006). Diseases such as dengue fever, malaria, filariasis, Japanese encephalitis, West Nile fever, and yellow fever are transmitted among humans and animals mainly by four genera (*Culex*, *Anopheles*, *Mansonia*, and *Aedes*) of mosquitoes (Crans, 2004; Samidurani et al., 2009; Remia and Logaswamy, 2010; Arivoli et al., 2011). Thus, both vectors and vector-borne diseases have become challenging problems that have social and economic impacts (Raveen et al., 2014).

Culex mosquitoes (Diptera: Culicidae) are pantropical pests that are likely the most abundant house mosquitoes prevailing in urban and rural areas (Samuel et al., 2007; Wajihha et al., 2017). *Culex quinquefasciatus* (Say) is the most dominant mosquito species in Pakistan (Ashfaq et al., 2014). It is a vector of filarial fever and Japanese

encephalitis, and it is a major public health concern in many developing countries (Arivoli et al., 2011; Ashfaq and Ashfaq, 2012). One of the major strategies is to control the vector or immediate host, which can minimize the spread of disease, and this strategy may be applied against immature or adult insect stages.

Chemical insecticides are commonly considered to be the most effective control strategy against mosquitoes. However, public concern has increased significantly regarding their negative effects, such as potential health hazards, water contamination, environmental pollution, toxicity to nontarget organisms, the development of resistance in insects, and residual effects (Lee et al., 2001; Azmi et al., 2009; Zacharia, 2011; Bilal and Hassan, 2012; Ndakidemi et al., 2016). Approximately 355,000 deaths per year are associated with pesticide poisoning globally (Tariq et al., 2007; Carvalho, 2017).

Scientists have begun to look toward traditional botanicals as an alternative for managing insects (Khanna

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and Kannabiran, 2007; Eliman et al., 2009). Plants contain a wide range of potential larvicidal phytochemicals (tannins, isoflavonoids, terpenes, steroids, saponins, etc.) that are target specific, rapidly biodegradable, ecofriendly, and less toxic to human health (Joseph et al., 2004; Isman, 2006; Zhu et al., 2008; Ghosh et al., 2012; Shivakuma et al., 2013). Thus, attention has been steadily diverted toward plant-based chemicals for insect control.

The present study evaluated the larvicidal potential of seven locally available botanicals against the 4th instar *C. quinquefasciatus* larvae under controlled conditions.

2. Materials and methods

2.1. Collection of plants and extract preparation

The plants (Table 1) were purchased from a local market, washed, air-dried in the shade at room temperature for 7 days, and powdered with an electrical stainless steel blender (Kalimuthu et al., 2012). The powder was stored in glass jars for subsequent solution preparations. Ten grams of powdered material from each plant was dissolved in water to a total volume of exactly 100 mL and stirred for 45 min at 25 °C with a magnetic stirrer. Afterwards, the mixture was filtered through Whatman No. 1 filter paper (Whatman, UK) (Ashfaq and Ashfaq, 2012). The stock aqueous solutions (10%) from each plant were refrigerated at 4 °C until the subsequent larvicidal bioassay. The stock solution of each plant was further diluted with water to prepare five concentrations (1%, 2%, 3%, 4%, and 5%).

2.2. Mosquito culture

Culex quinquefasciatus larvae were collected from different locations in Bahawalpur, Pakistan (29.37°N, 71.76°E; altitude 121 m) in 2015 with an aquatic net and transferred to the laboratory. Larvae were reared in 1000-mL beakers containing water for adult emergence at 28 ± 2 °C and 65 ± 5% relative humidity (Akram et al., 2010). Larvae were fed dry chicken liver powder (artificial diet), and emerged adult mosquitoes were given cotton balls

soaked in 10% sucrose solution (Arivoli et al., 2011) in a plastic cage of 75 × 75 × 75 cm. The cotton balls were suspended in the middle of the cage and changed daily. The rearing of larvae was carried out using the protocol of Das et al. (2007) with slight modifications. Adult female mosquitoes were periodically blood-fed on a restrained living rabbit in a small mesh bag inside the cage to avoid its frequent movement. Female mosquitoes began to lay eggs 2 days after feeding on blood. For egg deposition, a small filter paper wrapped in a conical shape was placed in a small cup of water to keep the filter paper moist. The filter paper containing eggs was placed in a plastic tray with 300 mL of water for larval emergence. The plastic tray was placed inside a separate cage to obtain new progeny. The cyclic generation of larvae was maintained by keeping all emerged adults in separate cages following a similar protocol.

2.3. Bioassay test for larvicidal activity

Bioassays were carried out on laboratory-reared 4th instar larvae of *C. quinquefasciatus*. The 4th instar larvae were visually detected by relatively bigger size. Larvicidal activity (percentage of mortality) and LC₅₀ values were calculated using the WHO (2005) bioassay protocol with slight modifications. The tested larvae were free from any exposure to insecticides or chemicals. A series of five concentrations (1%, 2%, 3%, 4%, and 5%) from the stock solutions was prepared. Twenty-five larvae were released by means of a dropper into a 500-mL beaker containing 250 mL of distilled water with each concentration of solution. A control (distilled water only) was also included with each concentration. For each concentration, three replicates were conducted to check the mortality in a completely randomized design. Larvae were considered dead if they showed no sign of movement even after being touched with a glass rod (Langat et al., 2012). The percentage of larval mortality was recorded after 24 and 48 h and corrected using Abbott's formula (Abbott, 1925):

Table 1. Indigenous plants used to prepare aqueous extracts for *Culex quinquefasciatus* larval mortality.

Botanical name	Local name	Family	Plant parts used for the aqueous extract
<i>Cinnamomum tamala</i>	Taiz pat	Lauraceae	Dried leaves
<i>Aloe vera</i>	Aloe vera	Asphodelaceae	Dried leaves
<i>Datura alba</i>	Datura	Solanaceae	Dried fruits
<i>Allium sativum</i>	Garlic	Amaryllidaceae	Dried bulbs
<i>Allium cepa</i>	Onion	Amaryllidaceae	Dried bulbs
<i>Zingiber officinale</i>	Ginger	Zingiberaceae	Dried fruits
<i>Ocimum basilicum</i>	Niazbo/basil	Lamiaceae	Dried leaves
Control	Water	-	-

$$\text{Corrected mortality (\%)} = \frac{\% \text{ mortality in treated} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

2.4. Statistical analysis

The LC₅₀ and LC₉₀ values were estimated using probit analysis (Finney, 1971) with LdP Line software (<http://www.ehabsoft.com/ldpline/>). The mortality data were analyzed using analysis of variance (ANOVA), and means were separated with the least significant difference (LSD) test at P < 0.05 in Statistix version 8.1 software.

3. Results and discussion

The aqueous extracts of all the tested plants exhibited prominent larvicidal activity at different levels against the 4th instar larvae of *C. quinquefasciatus*. The percentages of larval mortality at 24 and 48 h after exposure to the five different concentrations of each aqueous plant extract are presented in Table 2. Mortalities increased with an increase in the concentration of the aqueous plant extracts. The correlation analysis showed a strong concentration-dependent positive correlation (r) with larval mortality after 24 and 48 h (Figures 1 and 2). This gradient of positive dependency between mortality and concentration is supported by previous studies showing that an increase in the concentration of aqueous *Blighia sapida* leaf (Ubulom et al., 2012), *Citrus sinensis* peel extract (Sattar et al., 2016), and *Melia azedarach* ethanolic extracts (Al-Mehmadi and Al-Khalaf, 2010) resulted in increased mortality in *C. quinquefasciatus*. The same was reported in *Aedes aegypti* with ethanolic extracts from *Delonix elata* (Vasugi et al., 2013).

The LC₅₀ and LC₉₀ values of seven aqueous plant extracts against the 4th instar *Culex* larvae were determined (Table 3). Among them, *Allium sativum* (garlic), *Cinnamomum tamala* (taiz pat), and *Aloe vera* (aloe vera) exhibited the highest larvicidal activity, with the lowest LC₅₀ values at 24 h (1.37%, 1.48%, and 1.96%) and 48 h (0.55%, 0.98%, and 0.37%), respectively (Figure 3; Table 3). Based on the LC₅₀ values, the order of other plants was as follows: onion (2.20%) < datura (2.49%) < niazbo (5.32%) < ginger (7.48%) after 24 h and datura (1.13%) < niazbo (1.17%) < onion (1.24%) < ginger (2.43%) after 48 h. The LC₉₀ values for the three effective plants (*A. sativum*, *C. tamala*, and *A. vera*) were 3.98%, 2.98%, and 11.93% at 24 h and 1.77%, 2.20%, and 1.72% at 48 h, respectively (Table 3). Thus, all aqueous plant extracts demonstrated larvicidal activity against *Culex* larvae after a time period of 24–48 h. The *A. sativum* (garlic) aqueous extract was the most effective after 24 h and this result is in accordance with a study by Singha and Chandra (2011), which showed that the same extract exhibited the highest larval mortality. *A. vera* was effective against *Culex* larvae even at low concentrations (LC₅₀: 1.96% at 24 h and 0.37% at 48 h), whereas high

aqueous concentrations (10% and 20%) were reported as having high mortality against *C. salinarius* larvae by Verma et al. (2013).

In the literature, the organic solvent-based plant derivatives of other plants were generally evaluated for their potential mosquitocidal activity against different *Culex* spp. (Isman, 2006; Zhu et al., 2008; Ashfaq and Ashfaq, 2012; Ghosh et al., 2012; Kishore et al., 2013; Pavela, 2016). The extract of *A. vera* (aloe vera) in an organic solvent showed good larvicidal activity against *A. aegypti* (Subramaniam et al., 2012), and the extract of *A. sativum* (garlic) in ethanol (Kalu et al., 2010), the extract of *Ocimum sanctum* in an organic solvent (Gokulakrishnan et al., 2015), and *Cinnamomum osmophloeum* essential oil (Cheng et al., 2009) showed good larvicidal activity against *C. quinquefasciatus*. Likewise, the ethanol extract of *Datura stramonium* (datura) (Swathi et al., 2012) and the essential oils of *Ocimum canum* (basil), *O. basilicum* (niazbo), and *Z. officinale* (ginger) (Singh et al., 2003; Pushpanathan et al., 2008a, 2008b) act as good control agents against *C. quinquefasciatus*. Moreover, *Z. officinale* (ginger) aqueous extract was the least potent, with the highest LC₅₀ (7.48%) at 24 h. However, this result contradicts the study of Nasir et al. (2015), in which *Z. officinale* essential oil was more potent against *Culex* larvae at 24 h, followed by *Mentha piperita* (peppermint), *O. basilicum* (basil), *A. sativum* (garlic), and *Azadirachta indica* (neem) (Nasir et al., 2015).

Rather than sophisticated extraction, an easy and inexpensive aqueous method was used for the plant extract preparation in the present study. Our results provide experimental evidence that the local tested plants have significant larvicidal effects against *C. quinquefasciatus*. Plants are rich in bioactive phytochemicals and compounds with insecticidal properties, which could be the main reason for the high larval mortality (Maurya et al., 2009; Khairul-Bariyah et al., 2012; Mdoe et al., 2014; Pavela, 2016). Phytoextracts can also induce developmental changes and alterations to the midgut epithelium in mosquito vectors (Sharma et al., 2006; Al-Mekhlafi, 2018). Thus, these plants might be potent natural larvicidal agents that are effective in aqueous form within 24 h. Consequently, these plants may be recommended for mosquito management using this easy method and could be a better alternative to synthetic insecticides. However, further investigations are needed on the toxicological activity of these crude plant extracts against all stages of mosquito species as well as the identification of the active ingredients responsible for larvicidal activity. Evaluation of these aqueous plant extracts in field trials at breeding sites of mosquitoes may also strengthen these findings.

Table 2. Larval mortality (%) (mean ± SEM) of *Culex quinquefasciatus* at 24 h and 48 h after exposure to aqueous plant extracts.

Plant extracts		Larval mortality (%) ± SEM											
		1%		2%		3%		4%		5%			
Botanical name	Local name	24 h*	48 h*	24 h*	48 h*	24 h*	48 h*	24 h*	48 h*	24 h*	48 h*		
<i>Cinnamomum tamala</i>	Taiz pat	20.00 ± 2.31 c	48.00 ± 2.31 b	80.00 ± 2.31 a	93.33 ± 1.33 a	88.00 ± 2.31 a	96.00 ± 0.00 a	93.33 ± 1.33 a	96.00 ± 2.31 a	100.00 ± 0.00 a	100.00 ± 0.00 a		
<i>Aloe vera</i>	Aloe vera	36.00 ± 2.31 a	80.00 ± 2.31 a	45.33 ± 1.33 c	92.00 ± 2.31 ab	60.00 ± 2.31 b	96.00 ± 2.31 a	64.00 ± 2.31 c	96.00 ± 0.00 a	82.67 ± 1.33 c	100.00 ± 0.00 b		
<i>Datura alba</i>	Datura	24.00 ± 2.31 bc	42.67 ± 2.67 bc	45.33 ± 1.33 c	77.33 ± 2.67 c	60.00 ± 2.31 b	89.33 ± 1.33 b	62.67 ± 1.33 c	94.67 ± 1.33 a	65.33 ± 1.33 d	100.00 ± 0.00 a		
<i>Allium sativum</i>	Garlic	34.67 ± 1.33 a	77.33 ± 2.67 a	64.00 ± 2.31 b	86.67 ± 1.33 b	89.33 ± 1.33 a	98.67 ± 1.33 a	90.67 ± 1.33 a	98.67 ± 1.33 a	94.67 ± 2.67 b	100.00 ± 0.00 a		
<i>Allium cepa</i>	Onion	20.00 ± 2.31 c	42.67 ± 2.67 bc	41.33 ± 1.33 cd	69.33 ± 2.67 d	56.00 ± 2.31 b	81.33 ± 2.67 c	76.00 ± 2.31 b	82.67 ± 2.67 b	94.67 ± 1.33 b	98.67 ± 1.33 a		
<i>Zingiber officinale</i>	Ginger	25.33 ± 2.67 bc	38.67 ± 1.33 c	26.67 ± 1.33 c	41.33 ± 1.33 f	28.00 ± 2.31 d	52.00 ± 2.31 e	40.00 ± 2.31 d	58.67 ± 1.33 e	50.67 ± 2.67 e	65.33 ± 1.33 b		
<i>Ocimum basilicum</i>	Niazbo/ basil	28.00 ± 2.31 b	46.67 ± 1.33 b	40.00 ± 2.31 d	58.67 ± 1.33 e	41.33 ± 1.33 c	62.67 ± 1.33 d	44.00 ± 2.31 d	64.00 ± 2.31 c	50.67 ± 1.33 e	66.67 ± 1.33 b		
Control	Water	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 g	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 c		
LSD		6.32	6.32	4.47	6.48	4.69	5.10	4.24	5.10	4.90	2.83		

*Means followed by the same letter in the same column do not differ based on the LSD test at P ≤ 0.05, SEM: standard error of the mean.

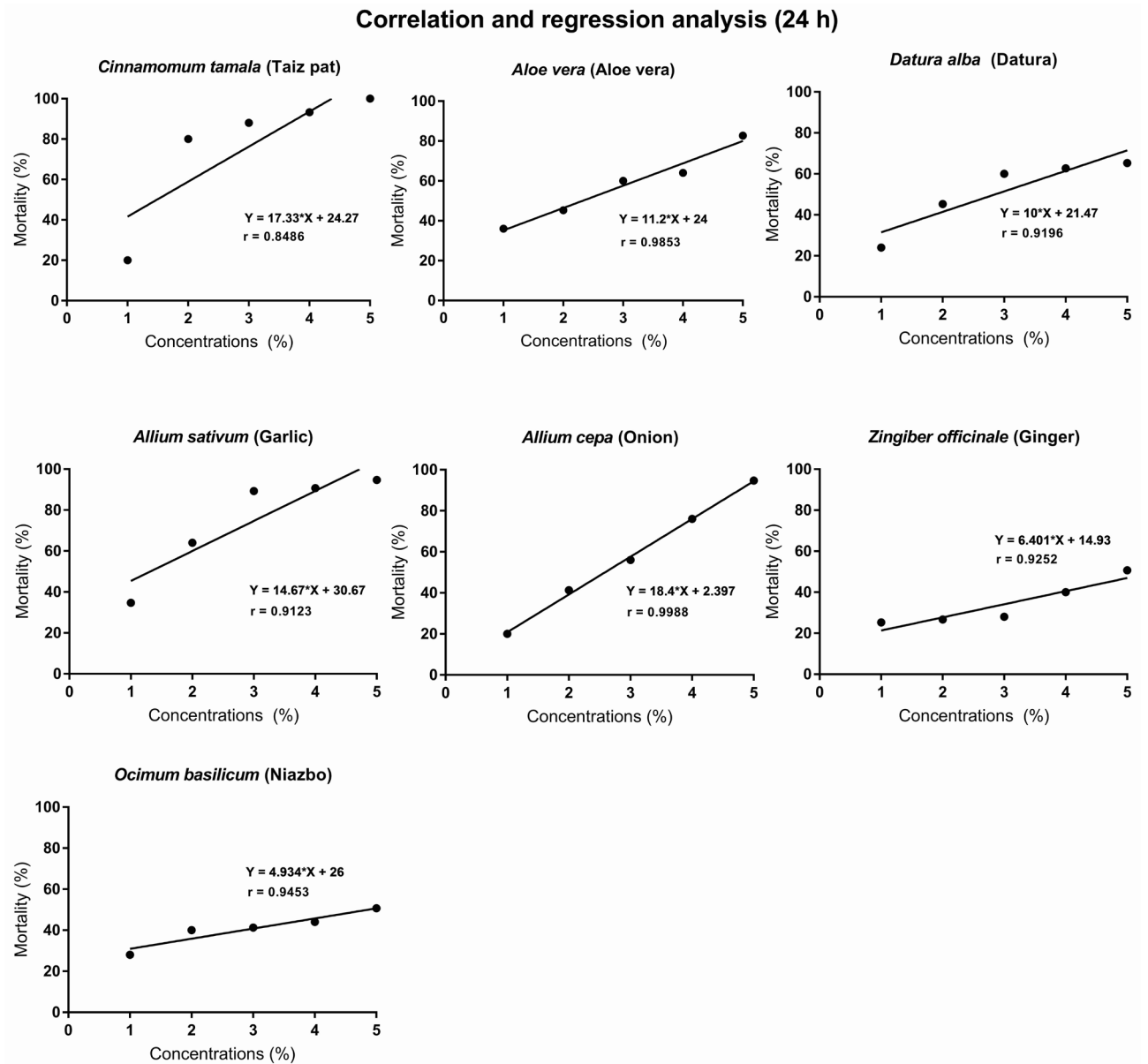


Figure 1. Linear regression equation (Y) and correlation coefficient (r) between plant extract concentration and larval mortality at 24 h after exposure. The mortality percentage increased with increasing concentrations at 24 h and showed a positive correlation.

Correlation and regression analysis (48 h)

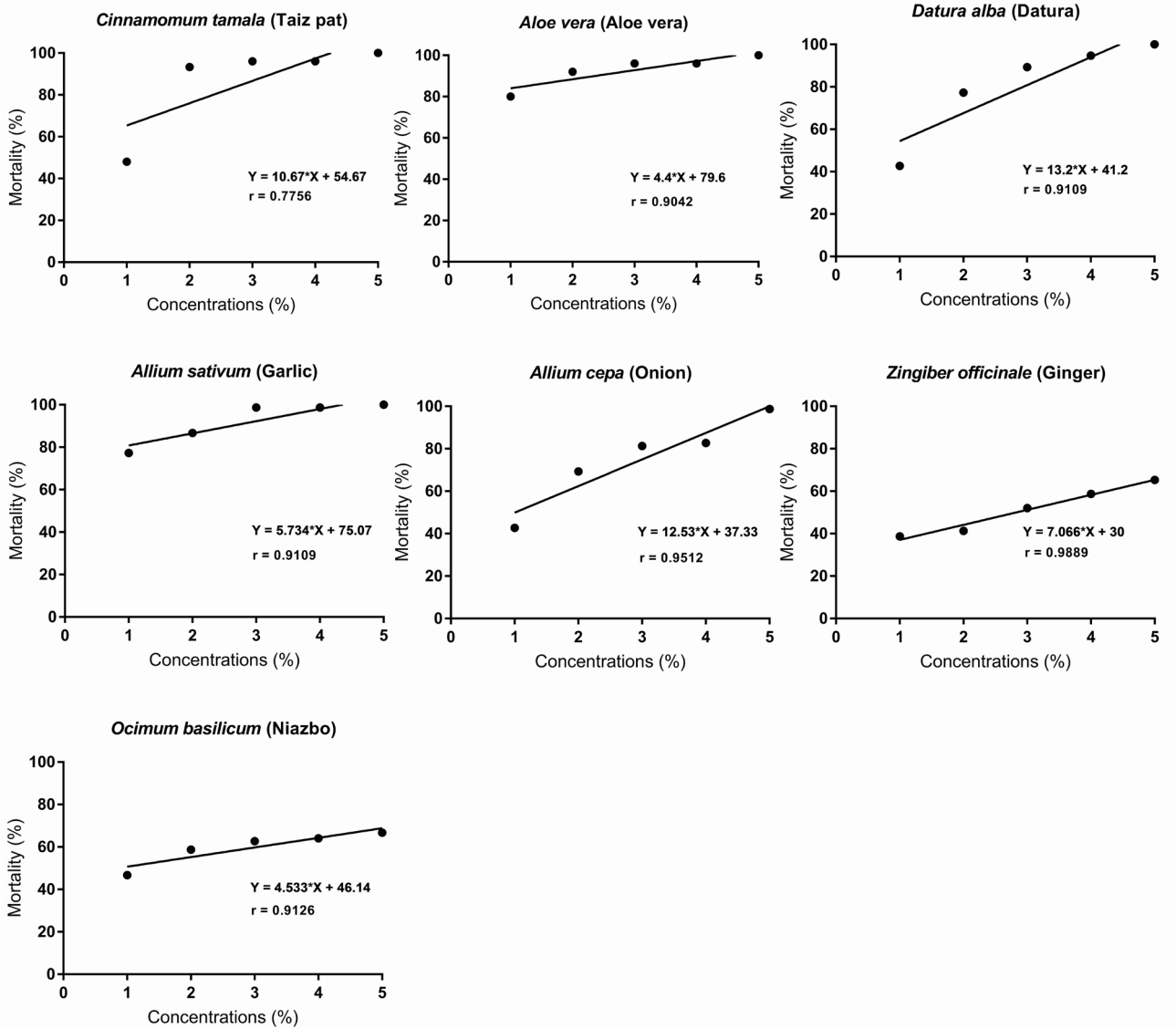


Figure 2. Linear regression equation (Y) and correlation coefficient (r) between plant extract concentration and larval mortality at 48 h after exposure. The mortality percentage increased with increasing concentrations at 48 h and showed a positive correlation.

Table 3. Probit analysis of the larvicidal efficacy of plant extracts against *Culex quinquefasciatus*.

Plant extracts		Time (h)	LC ₅₀ (%)	Lower limit	Upper limit	LC ₉₀ (%)	Slope ± SE	χ ²
Botanical name	Local name							
<i>Cinnamomum tamala</i>	Taiz pat	24	1.48	1.03	1.82	2.98	4.23 ± 0.34	9.65
		48	0.98	0.44	1.17	2.20	3.62 ± 0.38	9.90
<i>Aloe vera</i>	Aloe vera	24	1.96	1.58	2.30	11.93	1.63 ± 0.24	6.85
		48	0.37	0.14	0.60	1.72	1.93 ± 0.33	2.56
<i>Datura alba</i>	Datura	24	2.49	2.09	2.93	15.51	1.61 ± 0.24	1.80
		48	1.13	0.44	1.33	3.07	2.97 ± 0.29	12.65
<i>Allium sativum</i>	Garlic	24	1.37	1.17	1.56	3.98	2.78 ± 0.27	5.30
		48	0.55	0.33	0.75	1.77	2.55 ± 0.39	6.39
<i>Allium cepa</i>	Onion	24	2.20	1.33	3.01	5.89	3.00 ± 0.27	13.29
		48	1.24	0.48	1.50	4.20	2.41 ± 0.26	9.55
<i>Zingiber officinale</i>	Ginger	24	7.48	4.91	24.31	187.52	0.92 ± 0.24	6.23
		48	2.43	1.77	3.20	48.54	0.99 ± 0.23	2.22
<i>Ocimum basilicum</i>	Niazbo / basil	24	5.32	3.62	17.01	250.33	0.77 ± 0.23	0.71
		48	1.17	0.27	1.79	71.54	0.72 ± 0.23	0.37

LC₅₀: Lethal concentration to kill 50% of the exposed population, LC₉₀: lethal concentration to kill 90% of the exposed population; chi-square test (χ²) at P ≤ 0.05.

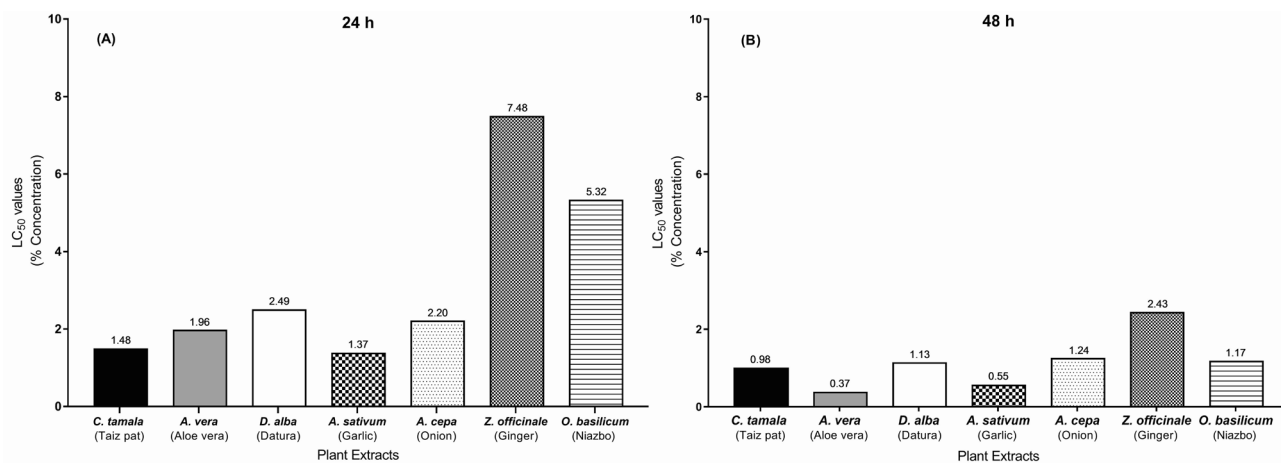


Figure 3. Graphical summary for LC₅₀ values of plant extracts at 24 h (A) and 48 h (B) after exposure.

In conclusion, all the tested plants showed larvicidal activity against *C. quinquefasciatus* larvae after an easy and inexpensive aqueous extraction within 24 h and they may be recommended for mosquito control. Among them, *A. sativum* (garlic), *C. tamala* (taiz pat), and *A. vera* (aloe vera) showed the highest larvicidal activity, with the lowest LC₅₀ values. These aqueous plant extracts may be directly used at the breeding sites of mosquitoes in stagnant water and in localized conditions.

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