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Phytochemical Screening and Comparative Larvicidal Activity of *Albizia lebbeck* and *Tamarindus indica* Leaf Extracts against *Culexquinquefasciatus* and *Aedesaegypti*

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ABSTRACT: Different species of mosquitoes have been implicated in the transmission of various diseases to humans and livestock, including malaria, filariasis, dengue fever, encephalitis, etc. This study aimed to evaluate the *in vitro* larvicidal potentials of *Albizia lebbeck* and *Tamarindus indica* against the third instar larvae of two mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. Fresh leaves of the plants were collected, dried, grounded, and extracted separately with petroleum ether in a soxhlet apparatus. The powdered samples were screened for phytochemical constituents using standard methods. The larvicidal potential of both extracts was evaluated using the WHO protocols. Results of the phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, and triterpenoids. The extracts of both plants exhibited significant and dose-dependent mortality of *C. quinquefasciatus* larvae at 97.33% with LC_{50} of 0.3092 for *A. lebbeck* and 98.67%, with LC_{50} of 0.1729 mL/L for *T. indica* at extracts concentration of 1.6 mL/L. Also, the extracts showed significant and dose-dependent mortality of *A. aegypti* larvae with 25-92% mortality and LC_{50} = 0.2735 mL/L for *A. lebbeck* and 32-92% for *T. indica* leaf extracts with LC_{50} = 0.2889 µg/mL ($p \leq 0.006621$). We conclude that the extracts of these plants possess larvicidal potentials and could be developed into natural larvicides for mosquito control programs.

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Insect-transmitted diseases remain a major cause of illness and death worldwide with an attendant toll on the public healthcare budget of most countries. Vector-borne diseases have been reported to contribute to about 17% of the global disease burden resulting in over 1.4 million deaths annually, posing a serious challenge to public health with enormous social and economic impact especially in subtropical and tropical countries of the world(Townson et al., 2005; Campbell-Lendrum et al., 2005; Klempner and Unnasch (2007). Mosquitoes are well known for their public health importance. They act as vectors for many tropical and subtropical diseases, causing a nuisance by their bites and also transmit deadly diseases like malaria, filariasis, yellow fever, dengue fever, and Japanese encephalitis, which contribute significantly to poverty, social debility, and disease burden mostly in tropical countries (Jang et al., 2002 and Tiwaryet al., 2007). The WHO declared mosquitoes as "public enemy number one" (WHO, 1996). Dengue fever is a pathogenic disease (Cime-Castillo, 2015) transmitted through a bite of an infected female mosquito, *Aedes aegypti*, during a blood meal. *A. aegypti* is also responsible for Zika fever, yellow fever, Venezuelan Equine Encephalitis virus (Larsen et al., 1971), and also a vector for the West Nile virus (Turell et al.,

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2005). Dengue fever is one of the most prevalent viral diseases that affect over 100 million people worldwide with over 21,000 deaths annually (WHO, 2009). On the other hand, *Culex quinquefasciatus* is the vector of avian filariasis, lymphatic filariasis, systemic lupus erythematosus, and Eastern Equine Encephalitis Virus (EEEV), and others(Dacko et al., 2020). *Culex* spp. facilitates the outbreak of serious and emerging diseases (Gorris et al., 2021; Hamer et al., 2009). The outbreaks of EEEV in the USA in 2009 resulted in 34 infections and 11 deaths(Lindsey et al., 2019).

Albizia lebbeck (*L.*) Benth, belong to the family Mimosaceae and has been used as a traditional medicine in most countries of Asia, Africa, and South America to cure several ailments. It is a fast-growing medium size deciduous tree that is native to India and Africa. Phytochemical screening of different parts of the plant (leaves, seeds, and stem bark) revealed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, fatty acids, and glycosides (Pal, 1995; Sharma, 2015; Fazil, 2019). Aqueous extracts and decoctions of the leaves and stem bark of the plant have been used in the treatment of acute inflammations, boils, wounds, cough, pruritis, scorpion bite, and asthma (Balkrishna*et al*., 2022; Sina, 1998; Khan, 2012).

Tamarindus indica (Linn.), family Fabaceae, is an important medicinal plant in folkloric medicine. It is indigenous to tropical Africa, and North/South America, and cultivated in subtropical China, Pakistan, Java, and Spain (Alkofahi and Atta, 1999). In Nigeria, it is commonly known as Tsamiya in the North amongst the Hausas (Ajayiet al., 2006), Ichekuoyibo among the Ibos in the East, and Ajagbon among the Yoruba in the Southwest (Iwu, 2014). Their fruits are often eaten directly from the pod but the seeds are very hard and not edible. It is commonly known as 'tamarind' (Yusueet al., 2007). Phytochemical studies of the plant revealed the presence of alkaloids, saponins, and glycosides. In traditional medicine, extracts of this plant are used for the treatment of various diseases including gastric disorders, vomiting, scurvy, scabies, pharyngitis, constipation, hemorrhoids (Panara et al., 2014), malarial fever (Timyan, 1996), and shown to possess Hypolipidemic, anti-inflammatory, anti-fungal and anti-bacterial activities (John et al., 2004). Different strategies have been employed in the control, prevention, and treatment of vector-borne diseases. The use of synthetic agents (chemicals) in the control of these vectors poses environmental hazards to humans and agricultural products. Natural products of plant origin have been a major reservoir of useful insecticides and larvicides, with significant potency and no toxic or harmful effects on the environment, humans, and livestock. In our search for potent bio-organic agents of plant origin, we investigated the petroleum ether leaf extracts of *Albizia lebbeck* and *Tamarindus indica* for larvicidal activity against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Collection, identification, and preparation of plant materials: The leaves of *A. lebbeck* and *T. indica* were collected from flowering trees within Zaria regions (located between longitude 7º 36´ and 7º42´ E and latitude 11º 00´ and 11º 10´ N of the equator), Nigeria, in May and June 2020. The plant samples were identified by a taxonomist at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria, where also a voucher specimen was deposited. The leaves were shade dried completely and ground into powder using a mechanical blender. The powdered samples were stored in separate labeled polythene bags until further use.

Extraction of plant materials: The powdered plant samples (50 g each) were separately extracted with petroleum ether (4 x 250 mL) by cold maceration for 48 hrs with intermittent shaking. The macerate was filtered with Whatman No.1 filter paper and the filtrates were concentrated to dryness *in vacuo* with a rotary evaporator at 40 °C. The crude extracts were then stored in a refrigerator at 4 °C until use.

Collection and identification of mosquito larvae for bioassays: Blood-fed indoor resting *C. quinquefasciatus* mosquitoes were collected from homes of consenting owners within the Zaria environs using test tubes and released to oviposit into entomological cages containing bowls of borehole water in the research laboratory. The eggs laid were maintained to hatch and develop into larvae under 27±2ºC and 70±10% relative humidity (RH). They were maintained at approximately 12 hrs of light and dark photoperiodic cycle conditions during the rainy season (May-October). The larvae were fed with biscuits and baker's yeast (ratio 3:1) until they developed into a third instar and then used for the bioassay (Tiwary et al., 2007). Similarly, the larvae of *Aedes aegypti* were collected from rock pools at Dumbiinselberg (latitude 10° 57.7´ N and longitude 7° 39.3´ E at an elevation of 111.56m above the surrounding) and on the Wusasainselberg (latitude 11° 04.597' N and longitude 7° 40.475´ E at an elevation of 32meters above the surrounding), during the rainy season (June) within Zaria (North West, Nigeria) where they are predominantly known to breed (Adeboteet al., 2008). All larvae collected were identified based on observable characteristics of their

siphon, antenna, seta, thorn-like scales, and colour with the aid of a dissecting microscope using the keys of Hopkins (Hopkins, 1952).

Qualitative Phytochemical screening of the leaf extracts of A. lebbeck and T. indica: Powdered samples (75 g each) of the plants were boiled in water for 30 minutes and filtered. The filtrates were tested for the presence of secondary metabolites (alkaloids, tannins, saponins, anthraquinones, phenolics, etc) using standard methods previously described (Trease and Evans, 1989).

Larvicidal Bioassays: The larvicidal activities of both plant samples were evaluated according to WHO-established protocols (WHO, 2005). The experimental bowls (500 mL-sized) were filled with 100 mL of distilled water. Twenty-five third instar larvae of each of the two mosquito species were then isolated from the breeding media (as described above) using a dropping pipette into 100 mL distilled water in the 500 mL sized experimental bowls under an average temperature of 26 ^{o}C and 70% relative humidity. The leaf extracts of *A. lebbeck and T. indica* were diluted with dist. water into six concentrations (0.01, 0.02, 0.04, 0.08, and 0.16 mg/mL) of 100 mL volume. Twenty-five third instar larvae of the *A. aegypti* and *C. quinquefasciatus* were each administered into each experimental plastic bowl containing the test concentrations according to Mohtaret al., (1999). A control test that consisted of twenty-five third instar mosquito larvae in 100 mL distilled water was also set up. Treatments and controls were replicated thrice for each species of mosquito larvae. Treatment and control experimental bowls were labeled according to the mosquito species, concentration, and time of inoculation of plant extract. The mortality of larvae in each experimental bowl was determined and recorded after 24 hours post treatments. The larvae were considered dead when they failed to move when prodded with a needle (Ali et al.,2013). The mortality of larvae was determined using the formula of Abbott (1925) as reported in Fleming and Retnakaran (1985).

Statistical analysis: Mortality means for each treatment were compared using paired t-test while LSD (least significant difference) was used to separate different means. Then, Median lethal concentrations (LC_{50}) of the extracts against the two mosquito species were accessed using probit based on the SPSS 21.0, version 2013.

RESULTS AND DISCUSSION

Mosquito-borne diseases are on the increase in recent times due to the growing resistance of insects to currently used synthetic insecticides (Soltani et al., 2015). Different strategies have been explored to prevent the transmission of mosquito-borne diseases: targeting the vector (Niang et al., 2018), and the use of larvicides, insecticides, and repellants (Derua et al., 2018; Derua et al., 2019). Natural products have been investigated for larvicidal, insecticidal, and repellant activities due to their environmental safety, non-toxicity, specificity, and lack of damage to valuable natural insects (Yohana et al., 2022). In this study, phytochemical screening of the two plants revealed the presence of secondary plant metabolites as shown in table 1. Plant secondary metabolites have been shown to be responsible for the various biological activities exhibited by the plants' extracts (Vishnu et al., 2013).

Table 1. Phytochemical characteristics of the leaf extract of *A. lebbeck and T. indica*

Physical/chemical	*A. lebbeck*	*T. indica*
Carbohydrate	+	+
Anthraquinones	-	-
Glycosides	+	+
cardiac glycosides	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
steroids and triterpenoids	+	+

Keys: + = Present, + absent

Different parts (root, stem bark, leaves, and flowers) of plants are a natural reservoir of larvicides(Kumar &Dube, 2015; Govindarajan et al., 2012; Varun et al., 2013). The petroleum ether extracts of *A. lebbeck* and *T. indica* were screened for larvicidal activity against the third instar larvae of *A. aegypti* and *C. quinquefasciatus* using the WHO protocols (WHO, 2015). Our results are in agreement with those reported by (Aisha et al., 2020; Abukakar et al., 2008). These plants have been reported to exhibit different pharmacological activities (antibacterial, antioxidant, antimalarial, hypolipidemic, and antidiabetic, etc), (Abukakar et al., 2008; Bhadoriya et al., 2011). Results of the exposure of the third instar larvae of *C. quinquefasciatus* to concentrations (0 - 1.6 ml/L) of *A. lebbeck* and *T. indica* leaf extracts after 24 hrs are shown in Table 2. The mean mortality of *C. quinquefasciatus* larvae ranges from 0% in the control as against 97.33% (24.33/25 larvae population) with LC_{50} of 0.3092 for *A. lebbeck* and 98.67% (24.67/25), with LC_{50} of 0.1729 µg/mL for *T. indica*, respectively, at the highest extract concentration of 1.6 mL. The mean larval mortality was observed to be concentration dependent. Extracts concentrations between 0.2 and 0.4 mL yielded no significance in mean mortality of *C. quinquefasciatus* larvae ($p \geq 0.05$), Table 2.

Table 2: Mortality caused by median lethal concentrations (LC_{50}) of *A. lebbeck* and *T. indica* leaf extracts against third instar larvae of *C. quinquefasciatus* under laboratory conditions by probit analysis.

Conc. of extract (ml/L)	Log of conc.	% mortality	Empirical probit of mortality	% mortality	Empirical probit of mortality
		A. lebbeck leaf extract		*T. indica* leaf extract	
1.6	0.20412	97^a	6.88	98^a	7.05
0.8	-0.09691	61^b	5.28	86^c	6.08
0.4	-0.39794	46^d	4.90	74^e	5.64
0.2	-0.69897	37^f	4.67	60^g	5.25
0.1	-1	25^h	4.33	32^i	4.53
0.0	-	0	0	0	0
		LC_{50} = 0.3092 ml/mL		**LC_{50} = 0.1729ml/mL**	

Note: The different superscript in the same row shows differences in insects' percentage mortality at the same concentration of plant extracts.

The LC_{50} of the extracts was determined from a plot of the probit of kills against logarithms of concentration from the regression equations, y = 1.8968x + 5.9668, R^2 = 0.8242 and y = 1.95x + 6.486, R^2 = 0.973 for A. lebbeck and T. indica, respectively. The results of the percentage mortality of the third instar larvae of *A. aegypti* mosquito by the leaf extracts of *A. lebbeck* and *T. indica* are shown in Table 3. Similarly, the larvicidal efficacy of the plants of study against *C. quinquefasciatus* larvae was compared (Table 4). The mean mortality of *A. lebbeck* against *C. quinquefasciatus* larvae across all experimental concentrations ranged from 6.33 to 24.33 from probit analysis, while that of *T. indica* is from 8.00 to 24.67. The mean mortality of the larvae when exposed to *T. indica* leaf extract was significantly higher ($p \leq$ 0.006621) than that of *A. lebbeck*. However, variations in larvicidal efficacy of *A. lebbeck* and *T. indica* against *C. quinquefasciatus* under treatment conditions were statistically insignificant ($p \geq$ 0.05) with increasing concentrations. Also, there was a significant difference in larvae mortality with concentrations of 0.2, 0.4, and 0.8 ml/mL ($p \leq 0.05$) compared to 0.1 and 1.6 mL/L (Table 4).

Table 3: Mortality caused by median lethal concentrations (LC_{50}) of *A. lebbeck* and *T. indica* leaf extracts against third instar larvae of *A. aegypti*.

Conc. of extract (mg/L)	Log of conc.	% mortality	Empirical probit of mortality	% mortality	Empirical probit of mortality
		A. lebbeck leaf extract		*T. indica* leaf extract	
1.6	0.20412	92^a	6.41	92^a	6.41
0.8	-0.09691	81^b	5.88	72^c	5.58
0.4	-0.39794	61^d	5.28	53^e	5.08
0.2	-0.69897	37^f	4.80	32^g	5.53
0.1	-1	25^h	4.16	32^i	4.53
0.0	-	0	0	0	0
		LC_{50} = 0.2735 µg/mL		**LC_{50} = 0.2889µg/mL**	

Note: The different superscript in the same row shows differences in insects' percentage mortality at the same concentration of plant extracts.

The LC_{50} values of larvae mortality of the extracts of A. lebbeck and T. indica were generated from a plot of a probit of kills against logarithms of concentration with regression equation of y = 1.8536x + 6.0436, R^2 = 0.9985 and y = 1.5978x + 5.8618, R^2 = 0.9191, respectively. A comparison of the larvicidal efficacy of *A. lebbeck* and *T. indica* against *A. aegypti* is presented in Table 4. When the larvae of *A. aegypti* were exposed to *A. lebbeck* leaf extract, larvae mortality ranged from 5 to 23, compared to *T. indica* leaf extract, which range from 8 to 23. The variation in larvicidal efficacy with varying concentrations was found to be statistically significant ($p \leq 0.023054$).

Table 4: Comparison of larvicidal efficacy of *A. lebbeck* and *T. indica* on *C. quinquefasciatus*.

Conc. (ml/L)	Mean (mortality ± SE) After 24 hrs		
	A. lebbeck	*T. indica*	*p*- Value
0.1	$6.33^a \pm 0.8819$	$8.00^a \pm 1$	0.006621
0.2	$9.33^b \pm 1.2019$	$15.00^a \pm 1.1547$	
0.4	$11.67^b \pm 1.3333$	$18.67^a \pm 0.8819$	
0.8	$15.33^b \pm 0.8819$	$21.67^a \pm 0.8819$	
1.6	$24.33^a \pm 0.6667$	$24.67^a \pm 0.3333$	

Means followed by the same superscript along the same row are statistically insignificant ($p \geq 0.05$)

The larvicidal efficacy of both plants' extracts was compared against the larvae of *A.* a*egypti* (Table 5). Against the larvae of *A.* a*egypti*, the extract of *A. lebbeck* showed a mean mortality range between 5 and 23, but 8 to 23 when exposed to *T. indica*. The larvicidal efficacy of both extracts varies significantly with varying concentrations ($p \leq 0.023054$), but not very much when compared to concentrations of 0.2 – 1.6 ml/L.

Table 5: Comparison in larvicidal efficacy of *A. lebbeck* and *T. indica* on *A. aegypti*.

Conc. (ml/L)	Mean (mortality ± SE) after 24 hrs		P- Value
	A. lebbeck	*T. indica*	
0.1	$5^{b} \pm 0.57735$	$8^{a} \pm 0.57735$	0.023054
0.2	$10.67^{a} \pm 1.1547$	$8^{a} \pm 1.1547$	
0.4	$15.33^{a} \pm 0.8819$	$13.33^{a} \pm 0.8819$	
0.8	$20.33^{a} \pm 1.1547$	$18^{a} \pm 1.1547$	
1.6	$23^{a} \pm 0.57735$	$23^{a} \pm 0.57735$	

Means followed by the same superscript along the same row are statically insignificant ($p \geq 0.05$)

Plants' secondary metabolites, glycosides, saponins, tannins, flavonoids, steroids, and terpenoids are known for their numerous biological and therapeutic properties(Vishnu et al., 2013). The qualitative phytochemical screening revealed the presence of different metabolites in the extracts of *A. lebbeck* and *T. indica* which may have been responsible for the larvicidal activity exhibited against the larva of *C. quinquefasciatus* and *A.* a*egypti*. Morrissey et al.reported that saponins interact with the cuticle membrane of the larvae which ultimately interferes with larvae membrane integrity and eventual death (Morrissey, 1999). Crude plant extracts often consist of a complex mixture of bioactive compounds and this complex mix may act synergistically with greater overall bioactivity compared to the individual constituents (Sumroiphon et al., 2006). It has been advocated that insect resistance to crude plant extract is much less likely to develop with mixtures of active compounds since there is a possibility of each component of the mix, exerts a different mechanism of action (Mandal, 2012; Samidurai, 2012). Modern mosquito control programs have continuously been focused and targeted at the larvae stages using natural extract and this is attributed to the inability of the larvae to escape from breeding sites until adult stages are attained (Kanba, 2015). With effective management of insects' development stages and timely application of bioactive plant materials, the larva control of mosquitoes holds a promising future that ensures success to combat mosquito vectors. Our study shows that the leaf extract of *A. lebbeck* and *T. indica* exhibits excellent larvicidal effects against both *A. aegypti*, and *C. quinquefasciatus* mosquitoes implicated in the transmission of various diseases. The extracts of both plants in this study produced significant mortality of the test mosquito larvae compared to the controls (tables 2-5). This is similar to the findings of (Kanba, 2015)who reported increasing larvicidal activities of *Parkiabiglobosa*against *A.* a*egypti* with increasing concentration. Adebote et al. also reported a dose-dependent mortality response of *C. quinquefasciatus* to treatments of *Bobyguniamadagascariensis* stem bark extract (Adebote et al., 2008). Kabadiya, reported the efficacy of the seed oil of *T. indica* oil against the larvae of *A. aegypti* with IC_{50} values of 1.248 mL/L (99%) and 1.359 mL/L (95%) pupae at relatively low concentrations which presents an alternative to the use of synthetic pesticides for control of mosquitoes(Kabadiya, 2016). This technique is environmentally friendly, biodegradable, less expensive, and locally available in mosquito endemic areas. Leaves extract of *Azadirachtaindica* and *D. melei* has been suggested as natural larvicides after a study on controlling mosquitoes in India. Both plants were said to be economically safe and less expensive to control mosquitoes (Chakkaravarthy et al., 2011). Also, a study suggested that a formulation, NeemAzal T/S 1.2 percent EC produced significant mortalityorinhibitionofemergenceagainstthethirdinstarlarvae of *A. stephensi*, *C. quinquefasciatus* and *A. aegypti* when treated with concentrations ranging from 0.046 - 0.866ppm (Gunasekaran et al., 2009). Neem oil formulation was also shown to be effective in controlling mosquito larvae in different breeding sites under natural field conditions (Dua et al., 2009).

The mean mortality of *C. quinquefasciatus* larvae when exposed to extract of *A. lebbeck* at concentrations of 0.8 -1.6 mL/L was significant ($p \leq 0.05$) statistically at the LC_{50} value of 0.3092 ml/L required to achieve 50% mortality. *T. indica* on other hand caused 50% larvae mortality at LC_{50} = 0.1729 ml/L. Adeyemi and Adebote reported that methanolic extracts of various parts of *Bobgunniamadagascariensis* have shown superior antifeedant and contact toxicity effects on the rust-red

flour beetle, *Triboliumcastaneum* (Adeyemi &Adebote, 2010). Studies have shown that almost all parts (leaves, bark, seeds) of *T. indica* are used in traditional medicine in Africa (Kuru, 2014). The various plant parts are used as a panacea to treat diseases, malaria, stomach ache, fever, microbial infections, diarrhea, anemia, dysentery, and nausea among others (Nguta et al., 2010). The median lethal concentration LC_{50} values of both plant extracts, *A. lebbeck* (LC_{50} =0.3092) and *T. indica* LC_{50} =0.1729) against *C. quinquefasciatus* and *A. lebbeck* LC_{50}=0.2735) and *T. indica* LC_{50} =0.2889) against *A. aegypti* were low indicating that very low concentrations of both extracts are required to cause appreciable larval mortality. A literature search shows that the findings of this study were superior to other studies. For instance, Elangovanet al. recorded LC_{50} values of 42.17 ml/L, 30.32 ml/L, and 35.95 ml/L for the seed oil of *T. indica* against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*, respectively(Elangovanet al., 2008). Waseemand Low, also indicated that out of the total citrus seed extracts tested, rough lemon and lemon had the lowest LC_{50} values 119.99 ml/L and 137.26 ml/L, respectively, after 24 hours of exposure, followed by red blood orange (295.63 ml/L), chakutra (334.87 ml/L), galgal (644.25 ml/L), Brazilian sour (905.96 ml/L) and kinnow (1022.67 ml/L). Narangi had the highest LC_{50} value (2069.12 ml/L) after 24h of exposure followed by grapefruit (1598.15 ml/L) and musambi (1389.16 ml/L) (Waseem& Low, 2015). Zewdnehet al. reported LC_{50} value of crude methanol seed extract of *J. curcas*tested against *Anopheles arabiensis* to be 92.09 ml/L. The findings in this study revealed that phytochemicals are suitable alternatives to synthetic insecticides and may be used in the future as they are relatively safe, inexpensive, and readily available in many areas of the world (Zewdneh et al., 2011). According to Bowers et al. the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products, and stimulate local efforts to enhance public health (Bowers et al., 1995). The study has further demonstrated the ability of the leaf extracts of tropical plants to elicit larvicidal effects against noxious mosquito species and their potential to be adopted for mosquito control operations. Oils extracted from the leaves block the respiratory siphon of the larvae resulting in their suffocation and eventual death (Rotimi&Ekperusi, 2012).

Conclusion: This study reported a concentration dependent pattern in larvae mortality rates in the two species of mosquito investigated. The leaf extracts of *A. lebbeck* and *T. indica* showed potent larvicidal activities against *C. quinquefasciatus* and *A. aegypti* mosquitoes under laboratory conditions. The result suggests that the leaf extracts have the potentials that can be exploited to develop larvicides as bioresource agents that can supplement synthetic chemical pesticides for mosquito control programs.

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