Journal of the Egyptian Society of Parasitology, Vol. 54, No. 3, December 2024
J. Egypt. Soc. Parasitol. (JESP), 54(3), 2024: 491– 498
Online: 2090-2549

LARVICIDAL EFFICACY OF *HYPTIS PECTINATA* LEAF EXTRACTS AGAINST *AEDES AEGYPTI* 4TH INSTAR LARVAE IN NIGERIA

By
BLESSING C. NWASO[1], ADENIRAN J. IKUESAN[1], ESE S. IZEKOR[1], EZE E. AJAEGBU[2]*, ABDULRASHEED M. BELLO[1], IJEOMA O. OKOLO[1], NNYENEIME U. BASSEY[1], UKACH-UKWU C. EZEH[1], ADAOBI J. DIEKE[1], CHINENYE A. NWOBODO[1] and FLORENCE O. NDUKA[1]
[1]Department of Applied Sciences, Federal College of Dental Technology and Therapy, Trans-Ekulu Enugu State, and [2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu Ebonyi State, Nigeria
(*Correspondence: ajaegbuee@yahoo.com)

Abstract

Yellow fever, Dengue fever, Chikungunya, and Zika virus are risky *Aedes aegypti*-borne infectious diseases among natives and travelers who travel to endemic countries globally. A total of 10g of each rigorously weighed dried leaf of *Hyptis pectinata* and four extracts (hexane, DCM, acetone, &ethanol) at concentrations of 125, 250, 500, & 1000ppm were evaluated for larvicidal activities against *Ae. aegypti* 4th instar larvae.

LC_{50} mortality increased to 100%, 20%, 60%, & 60% at 500ppm of hexane, DCM, acetone, and ethanol extract (171, 203, 419 & 449 ppm, respectively), after 24 hours laboratory exposure. This inexpensive medicinal plant reduced mosquitoes and thus can control their infectious parasitic and viral diseases.

Keywords: *Aedes aegypti, Hyptis pectinata,* extracts, larvicidal, control, mortality.

Introduction

Arthropod-borne diseases are diseases acquired through arthropod vectors, most often from the bite of infected mosquitoes, ticks, sandflies, or fleas, which infectious diseases are predominantly carried by developing countries, including countries of African continent (Gossner *et al,* 2023). Five of the most important arboviruses affecting communities in Africa are chikungunya, dengue, West Nile, yellow fever and Zika viruses (El Bahnasawy *et al*, 2015). With the exception of yellow fever vaccine and Ebola vaccine, other vaccines for viral hemorrhagic fevers were generally not available (Ergönül *et al,* 2018). Also, malaria is an acute febrile illness caused by five zoonotic *Plasmodium* species that are spread to people by the bites of infected female *Anopheles* mosquitoes (WHO, 2024). Malaria and human immunodeficiency virus are risky infectious diseases that desire more national collaborations (Saleh *et al,* 2019). No doubt, protection against these risky diseases is based on the combination of chemically-treated gear, clothing and effective chemical repellent (Morsy, 2012). Generally, the chemical insecticides are used against mosq-uitoes, ticks, mites and other pests affecting hum an welfare, but with marked significant in alt-ering ecosystems; and toxic to humans, edib-le animals, vegetation and others (El Bahnasawy *et al*, 2014). Mosquito-borne diseases still the major source of human morbidity and mortality globally particularly only in the year 2017, it has been reported that approximately 700,000 deaths occurred as a result of mosquito bites from *Anopheles*, *Aedes* and *Culex*) mosquitoes (Bhatt *et al,* 2013, WHO, 2017). But, in developing countries worldwide, there are specific medications available commercially and patients still die of malaria health-related problems every day that is even preventable or curable due to lack of simple health care not to mention unpreventable or incurable health problems, for example, dengue fever the carrier of this disease which is majorly transmitted by *A. aegypti* (Berhanu *et al,* 2006).

Mosquito control interventions are indicated to suppress their infectious diseases and reducing mortality not only in Nigeria, but also globally (Al-Agroudi *et al,* 2017).

Many intervention strategies and measures have been implemented worldwide in order to reduce mosquito-transmitted diseases in humans such as repelling mosquitoes by the means of insecticides, mosquito nets, or by disrupting the mosquito lifecycle via the removal of stagnant water, with various degrees of success (Von *et al,* 2017).

Many chemical insecticide resistance of *A. aegypti* such as DDT, pyrethroids, organophosphate, bendiocarb and carbamates (Moyes *et al,* 2017) apart from being risky (Kamgang *et al,* 2017). The research was conducted on natural Bti, temephos and spinosad which were susceptible without resistance to *A. aegypti* was reported in Africa whereas temephos resistance was extremely common in Latin America and throughout Asia (Mazzarri and Georghiou 1995). Naturally used spinosad retained larvicidal activity against anopheline, as a result, the problem of degradation of the environment, hazards and resistance can be totally eradicated with this natural product (Marina *et al,* 2014).

No doubt, essential oils from plant extracts have mosquito larvicidal and repellant efficiency without posing any human toxicity or any hazardous (Amer and Mehlhorn, 2006). Besides, medicinal plant extracts as garlic, ginger and *Commiphora molmol* were safely used in treating intestinal protozoan, cryptosporidiosis (Abouel-Nour *et al,* 2016), Egyptian phenolic plants treated tissue protozoan, cutaneous leishmaniasis (Abdel-Hady *et al,* 2011), *Calotropis procera* latex controlled of *Musca domestica* 3rd instar larvae (Morsy *et al,* 2001) and camphor oil successfully treated human *Sarcoptes scabiei* (Morsy *et al,* 2003).

However, mosquito control by *H. pectinata* plants has not been sought at a large scale leading to the availability of limited reports as regards the importance of the use of certain *H. pectinata* against *A. aegypti* (Warikoo and Kumar, 2014).

The present study aimed to assess the leaf extracts *Hyptis pectinata* using four solvents against *Aedes aegypti* larvae. Hopping that the outcome data of this plant, which is available, inexpensive, safe, and friendly insecticide for mosquitoes borne risky diseases.

Materials and Methods

Collection of plant: Freshly harvested leaves of *H. pectinata* were collected from the Institute of Management and Technology (IMT), Enugu State, in December 2020, and kindly identified by Mr. Alfred Ozioko, a taxonomist of the Bio-resources Development and Conservation Programme (BDCP), Nsukka, Enugu State. Cleaned *H. pectinata* leaves were dried for 14 days at a room temperature of 25-27°C & a relative humidity of 75-81%. The dried leaves were powdered using an electric grinder, sieved with a 0.4 mm mesh cloth, and stored in a refrigerator at -4°C an opaque container until needed.

Preparation of extracts: 10g of each rigorously weighed dried leaf part was extracted in hexane, DCM, acetone and ethanol by cold maceration process with constant shaking for 48 hours in the laboratory, School of Preliminary Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, and filtered in a Buchner funnel by Whatman® No. 1 size 24 cm to remove suspended particles. The n-hexane, DCM, acetone, and ethanol crude the leaf extracts were concentrated to dryness in rotary vacuum evaporator RE300 (ROTAFLO, England) at a temperature of (40±5°C). The extracts were kept in the refrigerator at –4°C before use to deactivate bacteria (Ajaegbu *et al,* 2022)

Rearing**:** Larvae of *A. aegypti* larvae were collected (National Arbovirus and Vectors Research Centre Enugu) reared, rose in tap water, colonized and allowed chicken feed (grower), and fish in a ratio of 3:1. Before 4th instar larvae were employed for the bioassays accomplished, the culture medium water was changed on each alternative day. A sugar to water (1 to 9) was allowed to adults for five days and blood of a Guinea pig sequentially. They were maintained at 26±3°C, 80±4% RH& 12:12 L/D photoperiod (Ajaegbu *et al,* 2014).

Extracts larvicidal bioassay against *A. ae-*

gypti: N-hexane, DCM, acetone & ethanol extracts were carried out at 26±2°C and relative humidity of 81±2%. The stock of each extract was made using tween 80 an emulsifier to assist dissolving in water. Bioassays were carried out by 1g of powdered plant passed into a solution of 2ml of Tween 80 as stock solution and diluted to 100ml of tap water. Serial dilution was prepared in order to have from 125-1000ppm. For negative control, 1ml of Tween 80 and 99ml of water was used for each replicate.

A dash insecticide (Dichlorvos 100% EC weight/volume) with 2500ppm was used as a positive control. A total of 25 early 4^{th} instar larvae were collected with a pipette, put into each 250ml beaker of 100ml of aliquot and the larval mortality was recorded 24hr post-treatment as well as in negative control. For each dose, four replicates were done side by side with its corresponding control. During the experiments, larvae in tests and controls were not allowed food. Larval % mortality at each concentration was corrected by Abbott's formula where negative control mortality ranged from 5-20%. Experiments were discarded and repeated when it showed > 20% negative control mortality or larvae didn't respond to gentle touch with a fine needle (Ibe *et al,* 2020).

Procedures were adopted for synergistic activities of three solvent extracts of *H. pectinata* leaf extracts. Effects were carried out for mixture of (DCM & acetone, acetone & ethanol and DCM & ethanol) showed ratios of 50%:50% respectively. Synergistic factor (SF) using formula if value was >1 interpreted as synergism, & < 1 indicated antagonism (Kalyanasundaram and Das, 1985).

$$SF = \frac{\text{LC50 value of insecticide alone}}{LC_{50} \text{ of insecticide with assumed synergist}}$$

Statistical analysis: Data were collected, computerized and analyzed by statistical ANOVA and Regression using computerized Statistical Package for Social Sciences (SPSS version 23.0). Mean ± standard deviation and F-value were calculated using the Student Newman Keuls (SNK) test significantly at (p=0.05). LC_{50} & LC_{90} larval mortality 24hr post-exposure, and other statistics included 95% lower and upper confidence limit (LCL & UCL), slope and Chi-square by Probit analysis evaluated lethal mortality dosages (Onah *et al,* 2022).

Results

Extracts of *H. pectinata* showed that ethanol gave the highest values (9.1%), followed by DCM (7.4%), acetone (7.2%), but N-hexane extract gave 6%

The mean and percentage mortality with the various concentrations showed concentration ranged between (125-1000ppm) for N-hexane, acetone and ethanol leaf extracts were effective, but DCM extract at a concentration between (125-250 ppm & 500-1000ppm) was ineffective and effective respectively. Mortality (20%) was at I- IV instar larvae treated with n-hexane extract at least concentration of 125ppm, mortality (80%) at 250 ppm and 100% at 500 and 1000ppm. DCM extract was exposed to I-IV instar larvae of showed a mortality (20%) with a concentration (500, 1000ppm), respectively, but none activity (125, 250ppm). Acetone extract showed a mortality (12%) and increased to 24%, 60, & 64% at concentrations of 250, 500 & 1000ppm respectively. Mortality (16%) was noted at I- IV instar larvae by ethanol extract and increased by (20%, 60% & 76%) at concentrations of 250, 500 & 1000ppm respectively. LC_{50} of DCM extract was 2034.117 more toxic as compared to acetone, ethanol and/or n-hexane extracts with values of 519.859, 449.679 & 171.374 respectively. Chi-square of different concentrations extracts showed that the mortality was highly significant.

The combinations of DCM & acetone, acetone & ethanol and DCM & ethanol extract at concentrations of 125, 250, 500 & 1000ppm showed that LC_{50} mortality were 2597.86 and 737.97 respectively. The combination of DCM & ethanol was more toxic followed by DCM & acetone, but without significant difference from acetone & etha-

nol as to mortality rate. The LC_{90} were 13520.955 for DCM & acetone more toxic as compared to 60754.62 (DCM & ethanol), but without significant difference from acetone & ethanol as to mortality rate. Combined effective mortality was (52% & 32%) for DCM & ethanol and DCM & acetone at 1000ppm respectively, and (12%) with acetone & ethanol was at 125-1000ppm.

Details were shown in tables (1, 2 & 3).

Table 1: Extraction and yields of *H. pectinata* leaf extracts.

Extracts	Yield (%w/w)
N-hexane	6.0
DCM	7.4
Acetone	7.2
Ethanol	9.1

% yield = yield divided by 10g of plant material multiplied by 100

Table 2: Larvicidal activity of different leaf extract of *H. pectinata* against *A. aegypti.*

Solvent	Conc (ppm)	Mortality %	LC_{50}(UCL–LCL)	LC_{90} (UCL–LCL)	Slope ± SE	χ^2
N-hexane	125 250 500 1000 F-value	20±1.73[a] 84±3.61[b] 100± 0[c] 100±0[c] 1088.00	171.374 (200.709- 144.597)	275.170 (391.891-229.264)	6.231±1.279	0.057
DCM	125 250 500 1000 F-value	0±0[a] 0 ±0[a] 20±1[b] 20 ± 3.61[b] 114.286	2034.117	8425.013	2.076 ± 0.767	3.943
Acetone	125 250 500 1000 F-value	12± 1.73[a] 24 ± 2[b] 60± 5.57[c] 64 ± 1.73[c] 196.683	519.859 (838.901- 371.734)	2598.649 (13286.217- 1353.863)	1.834 ± 0.425	1.887
Ethanol	125 250 500 1000 F-value	16 ± 2.65[a] 20±2[a] 60 ±5.30[b] 76 ± 4.58[c] 175.733	449.679 (647.809 - 332.913)	1829.931 (5654.374- 1090.855)	2.103 ± 0.433	1.845

Means within a product followed by same letter without significant at $p = 0.05$,*$p < 0.05$; LC50 & LC90: Lethal concentration killed 50 & 90% of larvae, respectively; LCL: Lower confidence limit; UCL: Upper confidence limit (–): No confidence limit estimated; $\chi 2$: Chi-square; Replicates no.: 4.

Table 3: Synergistic factor (SF) of DCM, acetone, of *H. pectinata* ethanol extracts against *A. aegypti larvae.*

Mixed extract	Conc. (ppm)	% Mortality	LC_{50}(LCL -UCL) (ppm)	LC_{90}(LCL-(UCL) (ppm)	(SF) at LC_{50}	Slope ±SE	χ^2
DCM & Acetone	125 250 500 1000 F-value	12±1[a] 12±2[a] 32±3.61[b] 32± 4.36[b] 43.243	2597.86 (935.31-6.125E+11)	60754.62 (5310.82-1.965E+11)	0.8	0.936 ± 0.436	1.202
Acetone & Ethanol	125 250 500 1000 F-value	12±2[a] 12±1.73[a] 12±1.53[a] 12± 2.65[a] 0.020	-	-	-	-	-
DCM & Ethanol	125 250 500 1000 F-value	20±1[a] 32±1.73[b] 48±2.65[c] 52±3[c] 131.20	737.968 (411.840-8404.068)	13520.955 (2707.509- 1304905415)	2.7	1.015 ±0.394	0.391

Discussion

Chemical insecticides are extensively used in order to eliminate or to reduce the mosquitoes, ticks and other pests, which threatened human welfare (Ghosal, 2018). Nowadays, mosquitoes' control of oval and larval stages and even adults targeted the use of medicinal plants and herbs extracts to kill them and to stop adult-vectors emergency (Ghosh *et al,* 2012; Torres *et al,* 2014).

At least 10% of all vascular plants are used as medicinal plants (Fonnegra, 2007), and

there were estimated to be between 350,000 (Joppa *et al*, 2011), and almost half a million species of them (Pimm *et al,* 2014). Since the ancient times, plants have been used in medicine and are still used today (Salmerón-Manzano *et al,* 2020). Medicinal plants have long been used to treat or prevent diseases and are already well documented in ancient Egypt, India, and China (Xia *et al,* 2022)

In the present study, hexane, ethanol, acetone & dichloromethane used to extract larvicidal active agents from *H. pectinata* gave various extractions. This may be due to difference in extraction solvents polarity caused wide activities' variations. A higher extraction was in ethanol and dichloromethane compared to acetone and hexane extracts, with highly polar solvents favors during extraction process. This agreed with Dhawan and Gupta (2017), who reported that the plant *Datura metel* was more soluble in acetone extracts with a higher percentage as compared to hexane Also, this agreed with Truong *et al.* (2019), who found that plant *Severinia buxifolia* with more polar compounds highly soluble and high polarity in solvents and with ethanol gave greater compared to acetone and dichloromethane.

In the present study, the potency of 125-1000ppm hexane, DCM, acetone and ethanol leaf extracts of *H. pectinata* against early 4^{th} instars of *A. aegypti*, at 125ppm concentration of hexane, acetone and ethanol extracts caused significant larvicidal efficacy of 20%, 12% & 16% larval mortality. But, the DCM extract didn't show larvicidal activity. At 250ppm concentration larvicidal mortality increased by 84%, 24%, & 20% by hexane, acetone and ethanol extracts against *Ae. aegypti* larvae. Mortality markedly increased to 100%, 20%, 60%, & 60% at 500ppm concentration of hexane, DCM, acetone and ethanol extracts. At 1000ppm concentration, larvicidal mortality was 100%, 20%, 64% & 76% for hexane, DCM, acetone and ethanol respectively. This agreed with Kumar *et al.* (2012), who evaluated the larvicidal efficiency of stem, roots and leaves of *Parthenium hysterophorus* (Family: Asteraceae) against *A. aegypti* larvae, extracted in different solvents found that only hexane and petroleum ether extracts caused 100% mortality Also, this agreed with Sharma *et al.* (2016), they evaluated larvicidal efficacy of five indigenous weeds against an Indian strain of *A. aegypti* showed 100% mortality by hexane extract at1000ppm.

In the present study, larvicidal activity of hexane, DCM, acetone and ethanol of leaf *H. pectinata* various extracts with concentrations from (125-1000ppm) against larvae showed that hexane extract gave highest toxicity with least LC_{50} of 171.37ppm followed by ethanol toxicity with 449.67ppm. Acetone extract showed moderate toxicity with LC_{50} of 519.86ppm and least one was DCM extract with highest LC_{50} of 2034.12ppm. This agreed with Kamaraj *et al.* (2011), who reported that LC_{50} gave highest toxic effect of bark, leaf ethyl acetate extract of *C. indicum*, leaf acetone extract of *T. procumbens* against the *Anopheles subpictus* larvae when compared with methanol extract for *An. squamosal*. Besides, Oluah and Ezeabiakwa (2011), reported that four differ-ent concentrations (0.3, 0.6, 0.9 & 1.2g/l) of aqueous and ethanolic leaf extracts of *Lantana camara* of both extracts against the 4^{th} instar larvae of *A. aegypti* for 24hr in laboratory conditions showed that mortality rates ranged from 91.66% to 96.66 % in larvae treated with 0.3 to 1.2g/l, respectively, and added that 24hr lethal time and LC_{50} were 6.8hr and 0.48g/l, respectively.

In the present study, the 48hr bioassay effect of DCM & acetone, acetone & ethanol and DCM & ethanol at s of 125, 250, 500, & 1000 ppm concentration showed the highest toxicity with least LC_{50} of 737.968ppm as compared to DCM & acetone with the highest LC_{50} value of 66419.62ppm. Mixtures of different solvent extracts showed dose-dependent mortality of larvae. This agreed with Mgbemena (2016), who reported that combined ethanolic extracts of *L. camara*, *Stachytarpheta indica* and *Allamanda blanchetii*

against *A. aegypti* larvae showed 100% mortality with a powerful synergist effect with combinations. Also, Dimitri *et al.* (2022) reported that combined *L. camara & L. chevalieri*, *L. chevalieri & C. schoenanthus* and *L. multiflora & L. chevalieri* showed LC_{50} of 33.16, 12.08 & 20.61ppm respectively for *Culex quinquefasciatus* with best larvicidal activity than LC_{50} value of each plant alone, but LC_{50} of 44.05 ppm was detected for combination of *Cx. schoenanthus & L. multiflora* on *An. funestus* with less toxicity compared to LC_{50} value of each plant alone. Again, Thangam and Kathirasan (1997) reported the synergistic of root of *R. apiculata* mixed with pyrethrum at 5mg/l reduced to 0.107mg/l gave a synergistic factor of 0.81 against *Cx. quinquefasciatus* larvae. The extract reduced the pyrethrum consumption by 19% without change in $LC_{50}$when the extract was mixed at 1mg/l. Also, Megha *et al.* (2012) determined synergistic effects of both Neem and Karanja oil larvicidal activity for mosquito control. They found that the mortality of active ingredient increased (20-77%) after 48hr exposure showed LC_{50} of 3.1g/ml against *A. aegypti,* which was more effective than either individual application.

Conclusion

The outcome data showed that leaf extract of *H. pectinata* can be developed and used as a larvicide to provide an opportunity for an effective and inexpensive approach for controlling dengue and yellow fever diseases in Nigeria since the plant shows larvicidal activity and is available in all country areas.

Recommendations

No doubt, the medicinal plants have always played an important role in the history of human welfare. Nevertheless, humans' migration, and/or travelling abroad, and climatic changes altered the world ecosystem as to infectious diseases, arthropod-vectors, and even plants' values, especially medical ones.

Data availability: Data supported the study are available within the paper and its supplementary information.

Authors' contributions: BCN, AJI, ESI, EEA, AMB, IOO, NUB, UCE, AJD, CAN and FON contributed to the study conception and design. Material was done by BCN, EEA, AMB, ESI, IOO, NUB, UCE, AJD, CAN and FON, data collection and analysis were performed by AJI and EEA. The first manuscript draft was written by AJI and all authors revised it, and approved the manuscript publication.

Authors' declarations: They declared that neither have any conflicts of interest nor received any funds.

References

Abdel-Hady, NM, Dawoud, GT, El-Hela, AA, Morsy, TA, 2011: Interrelation of antioxidant, anticancer and anti-*Leishmania* effects of some selected Egyptian plants and their phenolic constituents. J. Egypt. Soc. Parasitol. 41, 3:785-800.

Abouel-Nour, MF, El-Shewehy, DMM, Hamada, SF, Morsy, TA, 2016: The efficacy of three medicinal plants; garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum*: ii- Histological changes. JESP 46, 1:185-200.

Ajaegbu, EE, Danga, SPY, Okoye, FBC, 2014: Larvicidal activity of the leaf extracts of *Spondias mombin* Linn. (Anacardiaceae) from various solvents against malarial, dengue and filarial vector mosquitoes (Diptera: Culicidae). J Vector Borne Disease 51:1

Ajaegbu, EE, Onah, GT, Ikuesan, AJ, Bello, AM, 2022: Larvicidal synergistic efficacy of plant parts of *Lantana camara* against *Aedes aegypti*. J. Entomol. Zool. Stud. 10:187-94

Al-Agroudi, MA, Megahed, LA, Banda, LT, Morsy, TA, 2017: An overview on malaria in Sub-Saharan with special reference to Tanzania. JESP 47, 2:273-92

Amer, A, Mehlhorn, H, 2006: Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and *Culex* larvae (Diptera, Culicidae). Parasitol. Res. 99:466-77

Berhanu, A, Asfaw, Z, Kelbessa, E, 2006: Ethnobotany of plants used as insecticides, repellents and antimalarial agents in Jabitehnan District, West Gojjam. Ethiopian J. Sci. 29:87-92.

Bhatt, S, Gething, PW, Brady, OJ, *et al*, 2013: The global distribution and burden of dengue. Nature 496:504-**9.**

Dhawan, D, Gupta, J, 2017: Research article comparison of different solvents for phytochem-

ical extraction potential from *Datura metel* plant leaves. Inter. J. Biol. Chem. 11:17

Dimitri, WW, Jackline, K, Maxwell, M, *et al*, 2022: Larvicidal activities and synergistic effects of essential oils against *Anopheles funestus* and *Culex quinquefasciatus* (Diptera: Culicidae) from Kisumu, Kenya. Psyche J. Entomol. 2022: 8302696.

El-Bahnasawy, MM, Mohammad, AH, Morsy, TA, 2014: Hydrocarbon insecticides: Their risks for environment and human health. J. Egypt. Soc. Parasitol. 44, 2:361-72.

El-Bahnasawy, MM, Megahed, LA, Saleh, H AA, Morsy, TA, 2015: Training program for the nursing staff regarding the viral hemorrhagic fevers (VHFs) in a fever hospital. J. Egypt. Soc. Parasitol. 45, 2:249-72.

Ergönül, Ö, Keske, Ş, Çeldir, MG, Kara, İA, Pshenichnaya, N, *et al,* 2018: Systematic review and meta-analysis of post-exposure prophylaxis for crimean-congo hemorrhagic fever virus among healthcare workers. Emerg. Infect. Dis. 24, 9:1642-8.

Fonnegra, FG, 2007: Plantas Medicinales Aprobadas en Colombia, University of Antioquia, Antioquia, Colombia.

Gossner, CM, Hallmaier-Wacker, L, Briet, O, Haussig, JM, de Valk, H, *et al,* 2023: Arthropod-borne diseases among travellers arriving in Europe from Africa, 2015 to 2019. Euro Surveill. 2023 Feb;28(7):2200270.

Ghosal, A, 2018: Mode of action of insecticides. Appl. Entomol. Zool. 1:1-17.

Ghosh, A, Chowdhury, N, Chandra, G, 2012: Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 135, 5:581-98.

Ibe, IC, Ajaegbu, EE, Younoussa, L, Danga, S PY, Ezugwu, CO, 2020: Larvicidal property of the extract and fractions of *Hannoa klaineana* against larvae of *Aedes aegypti.* Curr. J. Appl. Sci. Technol. 39, 17:127-32.

Joppa, LN, Roberts, DL, Myersm, N, Pimm, SL, 2011: Biodiversity hotspots house most undiscovered plant species. Proc. Natl. Acad. Sci. USA. 108:13171-6.

Kalyanasundaram, M, Das, PK, 1985: Larvicidal and synergistic activity of plant extracts for mosquito control. Indian J. Med. Res. 82:19-23.

Kamaraj, C, Bagavan, A, Elango, G, *et al,* 2011: Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. Indian J Med Res, vol. 134:101-6.

Kamgang, B, Yougang, AP, Tchoupo, M, Riveron, JM, Wondji, C, 2017: Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. Parasit. Vectors 10:469;

Kumar, S, Nair, G, Singh, AP, *et al*, 2012: Evaluation of the larvicidal efficiency of stem, roots and leaves of the weed, *Parthenium hysteronphorus* (Family: Asteraceae) against *Aedes aegypti* L. Asian Pacific J. Trop. Dis. 2, 5:395-400.

Marina, CF, Bond, JG, Muñoz, J, Valle, J, Novelo-Gutiérrez, R, *et al,* 2014: Efficacy and non-target impact of spinosad, Bti and temephos larvicides for control of *Anopheles* spp. in an endemic malaria region of Southern Mexico. Parasit. Vectors 7, 55:

Megha, P, Saurabh, D, Raza, SK, Pantanjali, PK, 2012: Encapsulation of Neen and karanja oil mixture for synergistic as well as larvicidal activity for mosquito control. J. Sci. Indus. Res. 71:348-52.

Mgbemena, IC, 2016: The synergistic larvicidal activities of three local plants on *Aedes aegypti*. Glob. J. Sci. Front. Res. C Biol. Sci. 16, 3:1-11.

Morsy, TA, 2012: Insect bites and what is eating you? J. Egypt. Soc. Parasitol. 42, 2: 291-308.

Morsy, TA, Abdel Rahem, MA, Allam, KAM, 2001: Control of *Musca domestica* 3rd instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). J. Egypt. Soc. Parasitol. 31, 1: 107-10.

Morsy, TA, Abdel Rahem, MA, El Sharkawy, EMA, Shatat, MA, 2003: *Eucalyptus globules* (camphor oil) against the zoonotic scabies *Sarcoptes scabiei*. J. Egypt. Soc. Parasitol. 33, 1:47-53.

Moyes, CL, Vontas, J, Martins, AJ, *et al,* 2017: Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. PLoS Negl. Trop. Dis. 11: e0005625.

Oluah, NS, Ezeabiakwa, OB, 2011: Evaluation of larvicidal activity of leaf extract of *Lantana camara* (Family: Verbenaceae) Against the *Aedes aegypti* Mosquito. Bio-Res. 9, 2:768-74.

Onah, GT, Ajaegbu, EE, Ezeagha, CC, *et al,* 2022: Larvicidal and synergistic potentials of some plant extracts against *Aedes aegypti.* J. Entomol. Zool. Stud. 10, 2:177-80.

Pimm, SL, Jenkins, CN, Abell, R, Brooks, T

M, Gittleman, JL, *et al,* 2014: The biodiversity of species and their rates of extinction, distribution, and protection. Science 344:1246752. doi: 10.1126/science.1246752.

Rahuman, AA, Bagavan, A, Kamaraj, C, *et al,* 2009: Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res. 104, 3:637-43.

Saleh, A.MA, El Nakib, MM, Malek, DMA, Morsy, TA, 2019: Malaria and human immunodeficiency virus (HIV) in Republic of South Sudan: A mini-overview. JESP 49, 2:313-20.

Salmerón-Manzano, E, **Garrido-Cardenas, J A, Manzano-Agugliaro, F, 2020:** Worldwide Research Trends on Medicinal Plants. Int. J. Environ. Res. Publi. Hlth. 2020 May 12;17 (10): 3376. doi: 10.3390/ijerph17103376.

Sharma, A, Kumar, S, Tripathi, P, 2016: Evaluation of the larvicidal efficacy of five indigenous weeds against an Indian strain of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). J. Parasitol. Res. Article ID2857089.

Thangam, ST, Kathiresan, K, 1997, Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of *Rhizophora apiculata* with pyrethrum against *Culex quinquefasciatus*. Inter. J. Pharmaceut. 35, 1:69-71.

Torres, RC, Garbo, AG, Walde, RZML, 2014: Larvicidal activity of *Persea americana* Mill. against *Aedes aegypti*. Asian Pac. J. Trop. Med. 7, 1:S167-70.

Truong, D, Nguyen, D, Ta, NTA, *et al,* 2019: Evaluation of the Use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. J. Food Qual. Article ID 8178294.

Von, SL, Kekule, AS, Strickman, D, 2017: Novel vector control approaches: The future for prevention of Zika Virus transmission. PLoS Med. 14:e1002219.

Warikoo, R, Kumar, S, 2014: Oviposition altering and ovicidal efficacy of root extracts of *Argemone mexicana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae). J. Entomol. Zool. Stud. 2, 4:11-7.

WHO, 2017: Vector-borne diseases fact sheet. Geneva, Switzerland. Https://www.whoint/en/news-room/fact-sheet/detail/vector-borne-diseases.

Xia, C, Huang, Y, Qi, Y, Yang, X, Xue, T, *et al,* 2022: Developing long-term conservation priority planning for medicinal plants in China by combining conservation status with diversity hotspot analyses and climate change prediction. BMC Biol. 2022 Apr 21; 20:89. Doi: 10.1186/s 12915-022-01285-4

.