UTTAR PRADESH JOURNAL OF ZOOLOGY

42(10): 10-18, 2021
ISSN: 0256-971X (P)

LARVICIDAL EFFECT OF *Coffea arabica* L., *Camellia sinensis* (L.) Kuntz, AND *Punica granatum* L. ON *Aedes albopictus* (Skuse), THE VECTOR OF DENGUE AND CHIKUNGUNYA

DIPAK KUMAR SOM[1*], PALLABI LAHA[1] AND SUPRIYA MONDAL[1]

[1]Post Graduate Department of Zoology, Maulana Azad College, 8 Rafi Ahmed Kidwai Road, Kolkata 700 013, West Bengal, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author DKS has made substantial contributions to conception, design of experiment, interpretation of data and final checking of manuscript. Author PL has performed the experiment and analysed the data and wrote the first draft of manuscript with literature survey. Author SM has assisted in performing the experiment, data analysis and statistical analyses. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Sirigireddy Sivajothi, Sri Venkateswara Veterinary University, India.
Reviewers:
(1) Charles S. Apperson, North Carolina State University, North Carolina.
(2) Anupam Ghosh, Bankura Christian College, India.
(3) Raimundo N. Colares Camargo Júnior, Federal Institute of Education, Science and Technology of Pará, Brazil.

Received: 23 February 2021
Accepted: 01 May 2021
Published: 06 May 2021

Original Research Article

ABSTRACT

The purpose of the present study is to envisage the larvicidal activities of three common plant extracts in controlling *Aedes albopictus* (Skuse), a potential vector of Dengue and Chikungunya. Chloroform extract of green coffee beans, *Coffea arabica* L.; methanol extract of green tea leaves, *Camellia sinensis* (L.) Kuntz and chloroform extract of pomegranate fruit peel *Punica granatum* L. were tested against late 3rd instar/ early 4th instar larvae of *Ae. albopictus* under laboratory condition following WHO methodology, 2005. LC_{50} values were determined using the probit analysis with 95% confidence limit. The result indicated that after 24 hours and 48 hours of treatment, LC_{50} values for green coffee bean extract, green tea leaves extract and pomegranate fruit peel extract against *Ae. albopictus* larvae were 0.08, and 0.06, 0.13 and 0.06, 0.09 and 0.06 respectively. Our findings revealed that chloroform extract of green coffee beans and methanol extract of green tea leaves are the most effective in controlling *Aedes albopictus* larvae showing 100% mortality after 48 hours of exposure than chloroform extract of pomegranate fruit peel that shows 66% mortality after 48 hours of treatment. The overall larvicidal trend with reference to LC_{50} after 24 hours and 48 hours was green coffee > green tea > pomegranate. The larvae exposed to green coffee bean

**Corresponding author: Email: dipaksom@gmail.com;*

crude extract showed deformities related to moulting behavior and larval morphology. As these plants are widely distributed in India, their formulation might manifest effective eco-friendly alternatives for combating this upcoming potential vector of Dengue and Chikungunya.

Keywords: *Aedes albopictus*; botanicals as larvicides; dengue; Chikungunya; plant extract; vector control.

1. INTRODUCTION

The medical importance of mosquitoes as vectors for the transmission of life threatening diseases that causes morbidity, mortality, economic loss and social disruption such as dengue, Chikungunya, malaria, Japanese B encephalitis, lymphatic filariasis etc. are well recorded. Dengue is regarded as one of the most important arboviral infections in the world transmitted by *Aedes* mosquitoes, mainly *Aedes (Stegomyia) aegypti* and *Aedes albopictus* (Skuse) [1]. The incidence of Dengue has grown dramatically around the world in recent decades. Over 40% of world's population is now at risk from dengue [2]. *Aedes aegypti* is of supreme concern because of its wide distribution and close association with human [3]. *Aedes albopictus* (Skuse), commonly called 'the banded mosquito of Bengal', is now playing the role of secondary vector of Dengue and Chikungunya in rural and suburban regions of West Bengal [4]. This vector is now proliferating at a very fast rate due to high survivality in a wide range of environmental parameters throughout the world including India [5]. *Ae. albopictus*, is generally associated with human-outdoor areas but are gradually becoming a much potential vector for transmission of Dengue and Chikungunya virus in West Bengal due to rapid urbanization and deforestation [6].

Use of chemical insecticides has several drawbacks, such as these produce resistance among vectors [7], produce undesirable effects on non-target organisms and also foster environmental and human health concern [8,9], whereas, plant derived compounds are target specific and biodegradable [10]. Resistance to various synthetic insecticides [11] and detrimental effect on human health are compelling us to search for alternative weapons especially natural products of plant origin as vector controlling agents [12]. Many plant-based products are widely used for their insecticidal properties to control the vector mosquitoes [3,10]. In recent years, interest in phytochemicals has been revived because of the development of resistance, cross resistance and toxicity hazards associated with synthetic insecticides [4]. A large number of pant products have been reported to have mosquito larvicidal activity [12,13, 14]. This result may be observed due to presence of various active components like steroids, saponins, proanthocyanidins, glycosides and phenolic compounds etc. inside those plant extracts [15, 16].

The present endeavour is to envisage the larvicidal activities of Chloroform extract of green coffee beans, *Coffea arabica* L., methanol extract of green tea leaves, *Camellia sinensis* (L.) Kuntz and chloroform extract of pomegranate fruit peel, *Punica granatum* L., as, botanicals are likely to cause the least damage to anthropological environment in controlling this up surging vector of Dengue virus in the regional-national-international context [17,18,19].

2. MATERIALS AND METHODS

2.1 Procurement of Plant Parts

The three plants were selected randomly from a list of plants primarily sorted on the basis of their ethno medicinal value. Green tea leave and Pomegranate fruits were collected from local market. The Green coffee beans were procured from online market (Table 1).

2.2 Extraction Method of Plant Material

The extraction method of Freedman et al. [20] is followed here with few modifications.

Table 1. Common name and scientific name of three plants, parts used for bioassay and the extraction media

Common name	Botanical name	Parts used for extraction	Extraction medium
Green Coffee	*Coffea arabica* L.	Seed	Chloroform
Green Tea	*Camellia sinensis* (L.) Kuntz	Leaf	Methanol
Pomegranate	*Punica granatum* L.	Fruit peel	Chloroform

Green coffee beans were ground. An amount of 62.5 gm of green coffee bean dust was dissolved in 250 ml of chloroform and kept for 72 hours before getting filtrate. Then the filtrate was evaporated and dried. 1.25 gm of dry green coffee bean crude extract was collected in this process. The material was then dissolved in 95% ethanol. After ethanol extraction, post dried material was further sequentially extracted using distilled water with 5% ethanol for next 7 days. The extract was filtered using Whatmann No. 1 filter paper and collected.

Green tea leaves were ground in an electric grinder. Then the ground material was dissolved in 90% methanol (12.5 gm in 50 ml), stirred well and kept for 72 hours. Then the extract was filtered and the filtrate was kept for subsequent evaporation. We get 8.01 gm of crude extract of green tea from 50 gm of ground tea leaves.

The fruit peel of *Punica granatum* was prepared by macerating, dissolving in absolute ethanol for about 10-15 days. It was filtered using Whatmann No. 1 filter paper and dried in hot air oven. The material was then processed for chloroform extraction following the process as that of coffee bean extraction.

All crude dried extracts were kept in small vials in a deep freezer (-20^{o}C) until used.

2.3 Collection and Rearing of Mosquito

Eggs of *Aedes albopictus* mosquito were collected from natural habitat at Nalpur (22°31′45″N 88°11′10″E), Howrah, West Bengal, India. Larvae were reared inside the entomology laboratory of Maulana Azad College, with utmost precautions following WHO methodology [21]. Third instar/early fourth instar larvae were kept in a deep plastic container [400 larvae per 3L of rain water] in the Air conditioned room maintaining the temperature and humidity of 27±2^{o}C and 80±5% respectively. The pH of water was recorded as 6.5.

2.4 Preparation of Stock Solution

To prepare three separate stock solutions for three plant parts, 1g of each extract were added in three separate beakers containing 50 mL of 5% ethanol each and were considered as 2% stock solutions (20,000 ppm) [14].

2.5 Quantification of Larval Mortality Rates

Equal starting numbers of larvae (n = 30 larvae) were placed into each plastic bowl containing different concentrations of extract. We used pipette to measure the desired amount of plant extracts from stock solution. The concentrations for Green coffee bean extract were 0.4, 0.5, 0.6, 0.7 and 0.8 (%v/v which are equivalent to 80ppm, 100ppm, 120ppm, 140ppm and 160ppm) respectively; for Green tea leaves extract, concentration were 0.6, 0.7, 0.8, 0.9, 1.0, and 1.2 (%v/v which are equivalent to 120 ppm, 140 ppm, 160 ppm, 180 ppm, and 200 ppm, and 240 ppm) respectively and for Pomegranate fruit peel extract, concentration were 0.6, 0.7, 0.8, 0.9, and 1.0 (%v/v which are equivalent to 120ppm, 140ppm, 160ppm, 180ppm, and 200ppm) respectively. Mortality rates of treated larvae were quantified at 24hour and 48hour intervals. Each larva was examined and considered dead if it did not respond to probing with a dropper. Treatment-induced morphological deformities relative to control were analysed using light microscopy at 25x magnifications and recorded for further analysis.

2.6 Larvicidal Bioassay

The extracted plant materials were used in different concentration against *Aedes albopictus* and their efficacy was evaluated as per standard WHO method [7]. Each replicate contained 100ml of seasonal water (rain water) and above mentioned concentration of plant material in plastic bowls. Three replicas were conducted for each concentration and against each concentration along with a control were set [22]. Batches of 30 late 3rd instar/early 4th instar larvae were exposed in each container. The numbers of dead larvae were counted after 24 hour and 48 hour intervals. The experiment was conducted under laboratory condition at 27±2˚C and 80±5% RH.

2.7 Analysis of Data

The data, obtained in this experiment was analysed with special reference to Probit analysis, $LC_{50,}$ LC_{90}, Regression graph and homogeneity Chi-square using SPSS version 22.0.0.0 software.

3. RESULTS AND DISCUSSION

Larvae of *Aedes albopictus* were subjected against plant extracts of *Coffea arabica* (Green coffee), *Camellia sinensis* (Green tea), and *Punica granatum* (Pomegranate). Five different concentrations of crude plant extracts were tested. Table 2 and Graph 1 showed that *Coffea arabica* (Green coffee) was considered the best with LC_{50} values 0.08 with >90% mortality at 160ppm after 24 hours, followed by *Punica granatum* (Pomegranate) and *Camellia sinensis* (Green tea) with LC_{50} values 0.09 and 0.13 with 33% and 67% mortality at 200ppm and 240ppm respectively after 24 hours. The total mortality percentages of *Ae. albopictus* larvae at each concentration after 24 hours are shown in Fig. 1.

Table 2. Result summary of different plant extracts against *Aedes albopictus* larvae after 24 hours of exposure

LC_{50} -Lethal concentration 50 at which 50% of target population died.
LC_{90} - Lethal concentration 90 at which 90% of target population died.
P value - Level of significance $p \leq 0.05$, $p \geq 0.05$ non-significant
LFL = Lower fiducial limit UFL = Upper fiducial limit
SE = Standard Error, $\chi 2$ = Chi-square

Plant extract	Lethal concentration		LFL	UFL	Slope ±SE	χ^2	P-value	Regression equation
Green coffee	LC_{50}	0.08	-1.07	6.23	2.58±2.14	1.1	0.12	Y= - 0.53+2.58X
(*Coffea arabica*)	LC_{90}	0.14	-1.07	6.23	2.58±2.14	1.1	0.12	Y= - 0.53+2.58X
Pomegranate	LC_{50}	0.09	1.99	2.42	2.21±0.13	0.3	0.08	Y= - 0.0189+2.21X
(*Punica granatum*)	LC_{90}	0.17	1.99	2.42	2.21±0.13	0.3	0.08	Y= - 0.0189+2.21X
Green tea	LC_{50}	0.13	-0.28	4.49	2.1±1.67	1.2	0.07	Y= - 0.378+2.10X
(*Camellia sinensis*)	LC_{90}	0.24	-0.28	4.49	2.1±1.67	1.2	0.07	Y= - 0.378+2.10X

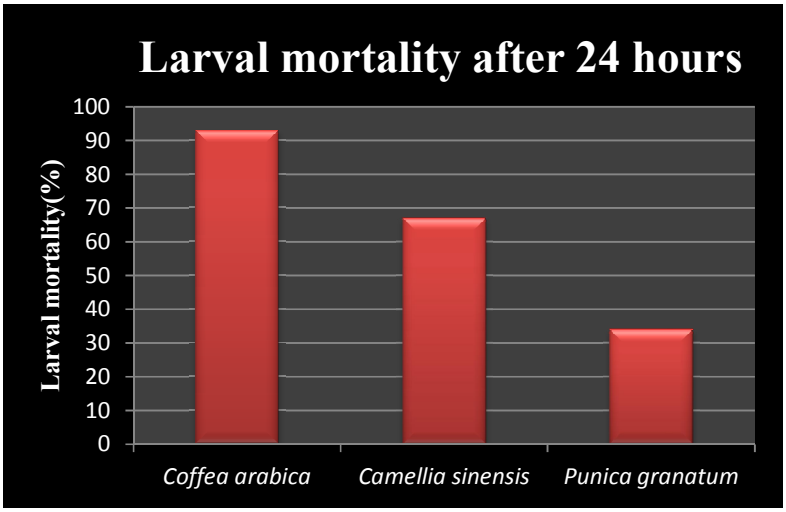


Fig. 1. Total larval mortality after 24 hours

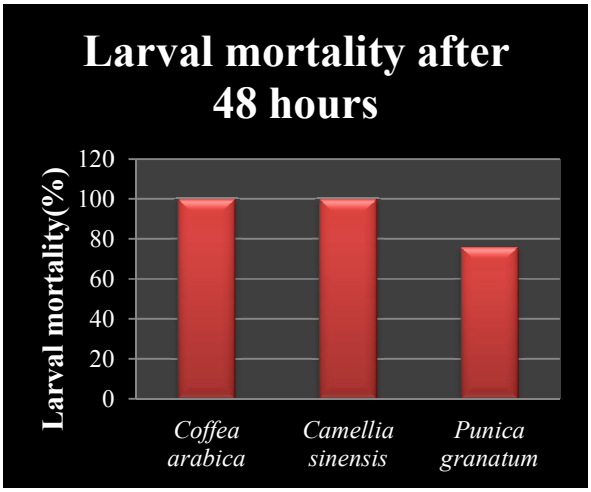
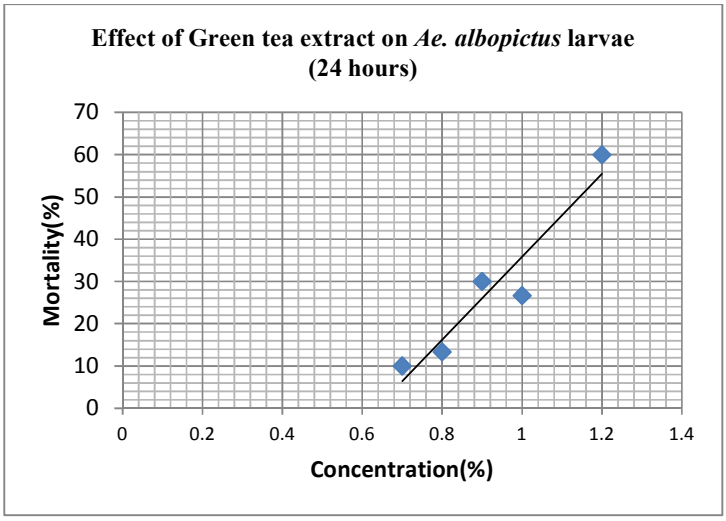
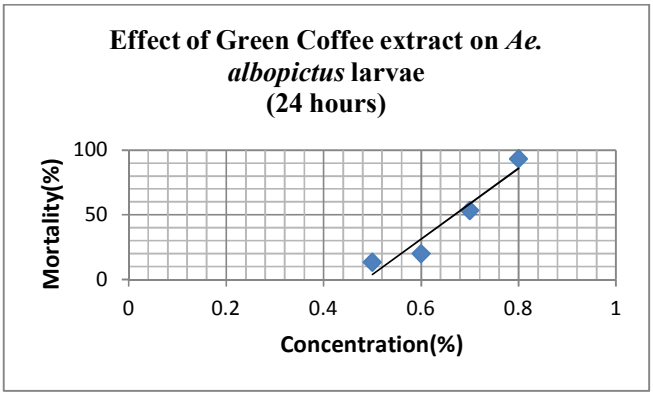
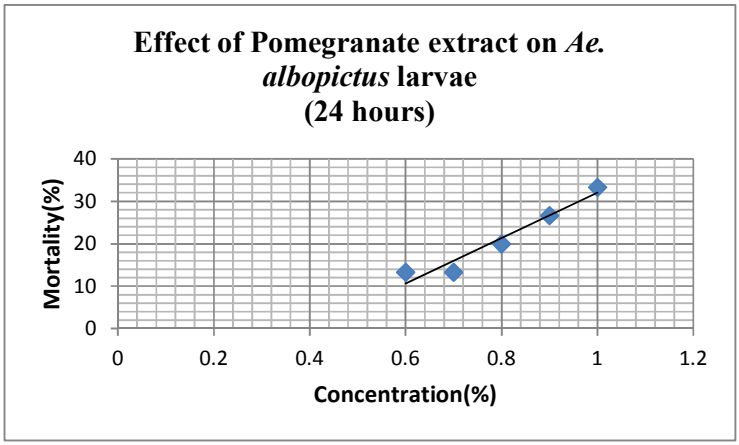


Fig. 2. Total larval mortality after 48 hours







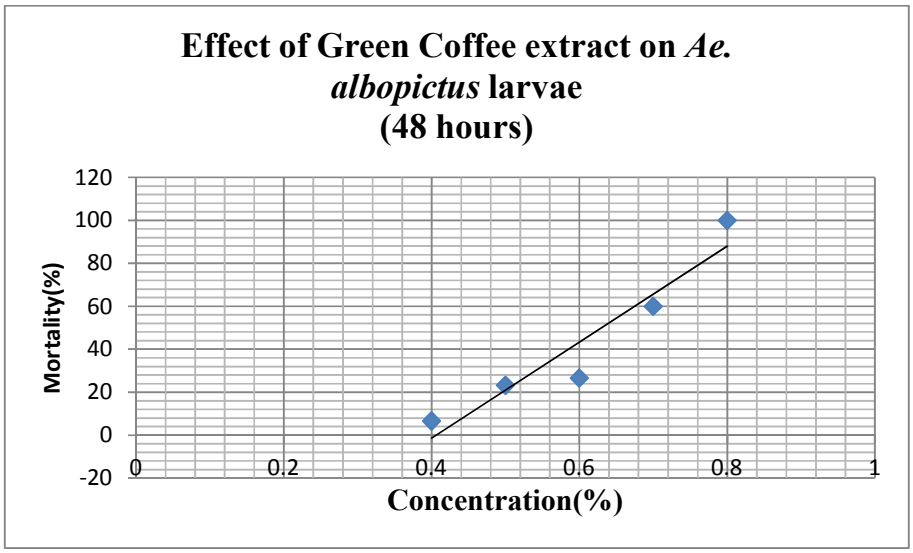
Graph 1. Regression of mortality on different concentration of three plant extract after 24 hours of exposure

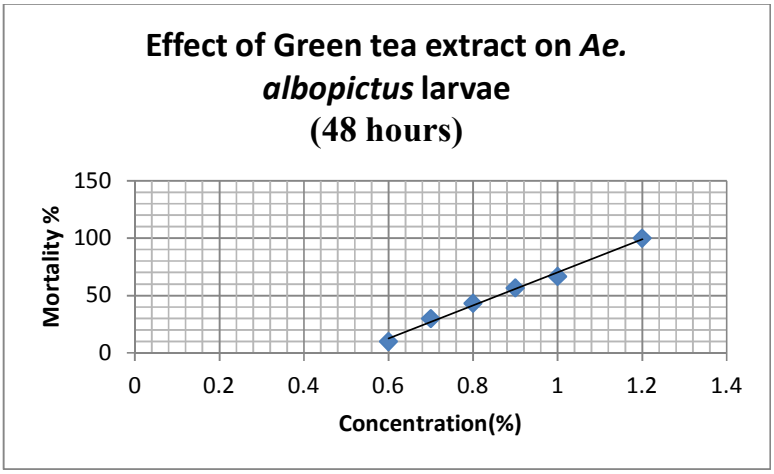
The *Coffea arabica* (Green coffee) and *Camellia sinensis* (Green tea) were considered the best with LC_{50} values 0.06 and 0.06 respectively showing 100% mortality at 160ppm and 240ppm respectively after 48 hours, followed by *Punica granatum* (Pomegranate) with LC_{50} values 0.06 exhibiting 66% mortality at 200ppm after 48 hours of treatment (Table 3 and Graph 2). The total mortality percentage of *Ae. albopictus* larvae at each concentration of plant extract after 48 hours of exposure are shown in Fig. 2.

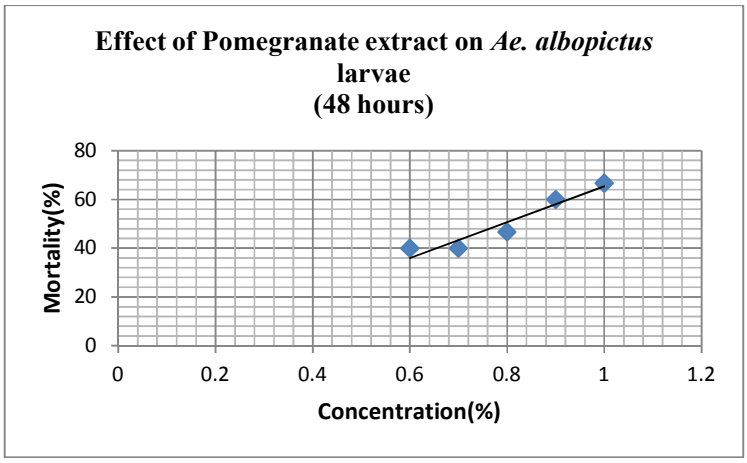
Table 3. Result summary of different plant extracts against *Aedes albopictus* larvae after 48 hours of exposure

LC_{50} -Lethal concentration 50 at which 50% of target population died.
LC_{90} - Lethal concentration 90 $_{at}$ which 90% of target population died.
P value - Level of significance p≤ 0.05, p≥ 0.05 non-significant
FL = Lower fiducial limit UFL = Upper fiducial limit
SE = Standard Error, χ 2 = Chi-square

Plant extract	Lethal concentration		LFL	UFL	Slope ±SE	χ^2	P-value	Regression equation
Green coffee	LC_{50}	0.06	0.79	5.1	2.95±1.26	0.23	0.02	Y= - 0.288+2.95X
(*Coffea arabica*)	LC_{90}	0.09	0.79	5.1	2.95±1.26	0.23	0.02	Y= - 0.288+2.95X
Pomegranate	LC_{50}	0.06	2.41	2.85	2.63±0.13	0.22	0.05	Y= - 0.0139+2.63X
(*Punica granatum*)	LC_{90}	0.30	2.41	2.85	2.63±0.13	0.22	0.05	Y= - 0.0139+2.63X
Green tea	LC_{50}	0.06	1.36	4.25	2.79±1.01	0.3	0.004	Y= - 0.266+2.79X
(*Camellia sinensis*)	LC_{90}	0.10	1.36	4.25	2.79±1.01	0.3	0.004	Y= - 0.266+2.79X







Graph 2. Regression of mortality on different concentration of three plant extract after 48 hours of exposure

Several authors [22,23,24] reported morphological aberrations in mosquito larvae induced by plant extracts. Saranya et al. [23] observed that aqueous leaf extract of *Spathodea campanulata* affect *Aedes aegypti* larval morphology such as dechitinized larva with damaged digestive tract and exuviae of the proceeding instar attached to the dead. Similarly, Arivoli and Tennyson [24] found that after treated with crude leaf extracts of *Abutilon indicum*, larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* had striated sclerotization, which appeared to be a feature of pupal cuticle. *Ae. albopictus* larvae in our experiment, when exposed to sub-lethal concentration of crude extract with special reference to green coffee bean showed severe deformities (Fig. 3A-D). Some of the distinct aberrations so far noticed were dechitinized larvae, damaged digestive tract with special reference to twisted digestive tract and appearance of early malformed pupae. During first 24 hours, larvae were flexing to clean their siphon with mouthparts & they stay at bottom of containers. Larvae also showed some kind of restless movement during 48 hours & after this they died in the treated solution.

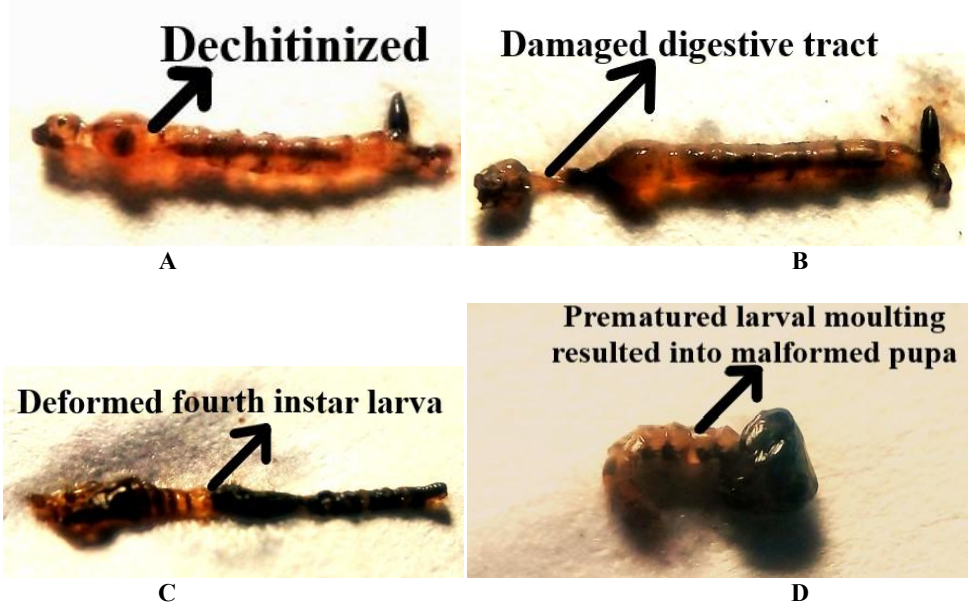


Fig. 3. *Aedes albopictus* larval deformities when treated with green coffee bean extract. A. Dechitinized larva B. Larva with deformed digestive tract. C. Deformed 4th instar larva. Exuviae of the proceeding instar attached to the dead larvae. D. Premature last larval moulting resulted in malformed pupa with some melanisation

4. CONCLUSION

A large number of pant products have been reported to have mosquito larvicidal activity. The present result revealed that chloroform extract of green coffee beans, *Coffea arabica* and methanol extract of green tea leaves, *Camellia sinensis* were the most effective against *Aedes albopictus* larvae showing 100% mortality after 48 hours of exposure. The overall larvicidal trend with reference to LC_{50} after 24 hours and 48 hours was green coffee > green tea > pomegranate. Larval survival and adult emergence was significantly reduced in different sub-lethal concentrations of above mentioned plant extracts over time. The result may be observed due to presence of phytochemicals, having mosquito lavicidal property. Exact phytochemical component and its mechanism of larvicidal activity can be studied further.

In present study, *Ae. albopictus* larvae, exposed to green tea, green coffee & pomegranate extract showed some kinds of deformities like browning of abdomen and twisted abdomen. During first 24 hours larvae were flexing to clean their siphon with mouthparts & they stay at bottom of containers. Larvae showed some kind of restless movement during 48 hours before they died. Exuviae of the late 3rd & late 4th instar larvae attached to the dead larvae were also observed. After 48 hours some dechitinized larvae were found with damaged digestive tract.

In search of alternative and safe methods for controlling larvae of Aedes *albopictus,* the potential vector of Dengue and Chikungunya, these eco-friendly phytochemicals especially green coffee bean extract might prove to be a good vector control tool as safe and cost effective chemicals over more resistant synthetic insecticides.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Subir Chandra Dasgupta, Professor and Head, Department of Zoology, Maulana Azad College, Kolkata, West Bengal, India, for providing necessary laboratory facilities in the execution of this work. The authors also express their sincere thanks to Dr. D. K. Nath, Ex-State Entomologist, Government of West Bengal, for rendering his valuable technical expertise.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gratz NG. Critical review of the vector status of *Aedes albopictus.* Medical and Veterinary Entomology. 2004;18:215–227.
2. World Health Organisation: Dengue fact sheet . Available:http://www.searo.who.int/entity/vector_borne_tropical_diseases/data/data_factsheet/en/ 2018
3. Beula JM, Ravikumar S, Ali MS. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. Asian Pacific journal of Tropical Biomedicine. 2011;1(2):143-146.
4. Bharati M, Saha D. Insecticide susceptibility status and major detoxifying enzymes' activity in *Aedes albopictus* (Skuse), vector of dengue and chikungunya in Northern part of West Bengal, India. Acta Tropica. 2017;170:112-119.
5. Hawley WA. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 1988;1:1-39.
6. Tandon N, Raychoudhury S. Reinvasion of Calcutta city by *Aedes albopictus*: the proven vector of Dengue in Suburban areas. Indian J Public Health. 1998;42(1):24–5. PMID: 10389503.
7. World Health Organisation: Insecticide resistance and vector control. XVII report of WHO Expert Committee on Insecticides. WHO Tech Rep Ser. 1970;443:279.
8. Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. Journal Vector Borne Disease. 2007;44:145-148.
9. Hayes JB Jr., Laws ER Jr. Handbook of pesticide toxicology. v.1. San Diego, CA: Academic Press; 1991.
10. Hamouda AB, Mechi A, Zarred K, Chaieb I, Laarif A. Insecticidal activities of fruit peel extracts of Pomegranate (*Punica granatum*) against red flour beetle *Tribolium castaneum.* Tunisian Journal of Plant Protection. 2014;9(1):91-100.
11. World Health Organization Global plan for insecticide resistance management in malaria program; 2012. Available:Http://www.who.int/malaria/publications/atoz/gpirm/en/
12. Mukhtar MU, Mushtaq S, Arslan A, Zaki AB, Hammad M, Bhatti A. Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti.* International Journal of Mosquito Research. 2015;2(3):127-130. ISSN: 2348-5906.

13. Mansour SA, Bakr RFA, Hamouda LSA, Mohamed RI. Toxic and synergistic properties of several botanical extracts against larval and adult stages of mosquito, *Anopheles pharoensis*. Biopestic. Int. 2010;6(2):129-145.
14. Murgan K, Murgan P, Noortheen A. Larvicidal and repellent potential of *Albizziaamara Boivin* and *Ocimum basilicum* Linn against dengue vector, *Aedes aegypti*. Bioresource Technology. 2007;98:198-201.
15. Bhandary SK, Sucheta Kumari N, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. Nitte University Journal of Health Science, NUJHS. 2012; 2(4):34-38.
ISSN: 2249-7110.
16. Hostettmann K, Marson A. Saponins. Chemistry and pharmacology of natural products Cambridge: Cambridge University Press. 1995;132.
17. Rampurawala J, Vedamurthy AB, Joy Hoskeri H. A Report on a potent antihelminthic agent – Unbaked *Coffea arabica* bean extracts. Asian Journal of Pharmaceutical and Clinical Research. 2013;6(5):119-121.
18. Muema JM, Bargul JL, Nyanjom SG, Mutunga JM, Njeru SN. Potential of *Camellia sinensis* proanthocyanidines-rich fraction for controlling malaria mosquito populations through disruption of larval development. Parasites and Vectors. 2016;9:512.
19. Radwan SF, Sekina SE. Insecticidal activities of Pomegranate Peels and leave crude juices. Bulletin of Environment. Pharmacology and Life Sciences. 2016;5(3):68-75.
20. Freedman B, Nowak LJ, Kwalek WF, Berry EC, Gutnrie WD. A bioassay for plant derived pest control agents using the European corn borer. J. Econ. Entomol. 1979;72:541-545.
21. WHO. Guidelines for Laboratory and field testing of mosquito larvicides. 2005; WHO/CDS/WHOPES/GCDPP/2005.13.
22. Sharma P, Mohan L, Srivastava C. Phytoextract-induced developmental deformities in malaria vector. Bioresource Technology. 2006;97(14):1599-1604.
23. Saranya M, Mohanraj RS, Dhanakkodi B. Larvicidal, pupicidal activities and morphological deformities of *Spathodea campanulata* aqueous leaf extract against the dengue vector *Aedes aegypti*. Eur. J. Exp. Biol. 2013;3:205–213.
24. Arivoli S, Tennyson S. Larvicidal and adult emergence inhibition activity of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). J. Biopestic. 2011;4:27–35.