1st December, 2024

Original Article

EXTRACTS FROM KINNOW PEEL WASTE AS LARVICIDAL AGENTS AGAINST DENGUE SPREADING VECTOR, *AEDES AEGYPTI*

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Received : 9th October, 2024

Accepted : 07th November, 2024

ABSTRACT

Background: Mosquitoes, in particular, *Aedes aegypti* pose a serious threat to human health as they act as vectors for the transmission of several deadly diseases namely dengue, chikungunya, yellow fever and Zika. The present research is targeted towards the evaluation of larvicidal property of extracts prepared from kinnow peel waste against *Ae. aegypti*.

Methods: Ethanolic and aqueous extracts were prepared from kinnow peel waste followed by agitation for 24 hours in an orbital shaker. Larvae of *Ae. aegypti* (L4) were exposed to laboratory prepared five different concentrations of kinnow peel

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Cite this article as:

Kaur Arshkamaljot, Kochar Devinder Kaur; Extracts from kinnow peel waste as larvicidal agents against dengue spreading vector, *Aedes aegypti*. *J Med Arthropodol & Public Health.* 2024; 4(2): 29-44.

ethanolic extract @ 0.05, 1.0, 1.5, 2.0 and 2.5% and aqueous extract @ 6.0, 7.0, 8.0, 9.0 and 10.0%. Effective larvicidal concentration of each of the kinnow peel extracts along with their storage and retention efficacy was also determined.

Results: Out of the tested concentrations, 2.0% ethanolic extract and 9.0% of aqueous extract from kinnow peel waste were found to be the effective larvicidal concentrations against *Ae. aegypti*. After 12 hours of exposure, LC_{50} and LC_{90} toxicity values were calculated to be 0.07 and 0.16% for ethanolic and 6.41 and 8.23% for aqueous extracts, respectively. Larvicidal retention efficacy of the effective concentration of these extracts persisted up to 12 hours. A significant delay in development (from L4 to adult) was also observed after placing new larvae in the left over effective concentration of these extracts. No effect of storage (for 6 months) on the larvicidal potential of kinnow peel extracts was observed.

Conclusion: Present study showed the larvicidal property of extracts prepared from kinnow peel waste against *Ae. aegypti* revealing their potential to be used as eco-friendly mosquito control agents in future.

Keywords: *Aedes aegypti*; development duration; kinnow peel waste; larvicidal potential; mosquito control; retention efficacy

INTRODUCTION

Aedes mosquito serves as vector for some of the world's threatening diseases like dengue, chikungunya, yellow fever and zika fever[1]. The two primary vectors responsible for the proliferation of viral disease dengue are *Aedes aegypti* and *Ae. albopictus* and among both, *Ae. aegypti* has higher rate of transmitting this disease in India[2]. *Ae. aegypti* is abundantly present in small freshwater collections like roadside ditches, earthen pots, desert coolers, gardens, small containers etc, lying in peri-domestic areas[3]. Last year, 2,89,235 dengue cases have been recorded in India by National Vector Borne Disease Control Programme[4]. The control and prevention of dengue disease entirely depends on the effectiveness of strategies and measures being implemented towards the control of this dreadful vector. Use of insecticides is a common practice in mosquito control and many synthetic/chemical insecticides are being widely used for controlling adult and larval mosquito population at their breeding locations. Synthetic insecticides used for mosquito control include permethrin, allethrin and malathion along with some insect repellents like DEET

(N, N-Diethyl-meta-toluamide) and picaridin[5]. However, studies have shown that these compounds when used intensively results in affecting humans, the non-target organisms, and the environment, due to their residual effects along with increasing resistance among mosquito population[6]. Because of their potentially virulent effects, high operational costs and environmental contamination, there is an urgent need for the development and implementation of effective, economic and eco-friendly alternative ways to control mosquitoes and associated diseases.

The plant based products are found to have insecticidal properties which has resulted in significant interest of researchers in botanicals as potential sources of natural mosquito control material in recent years. Nearly 86 compounds extracted from plants have been observed to have larvicidal potential against *Ae. Aegypti*[7,8]. Essential oil (EOs) and extracts of plant origin are the mixtures of major as well as minor constituents, which act synergistically in the target individuals in contrast to synthetic insecticides. Moreover, these plant based products are environmentally safe and biodegradable[9] and also have been found to be potent against those species of insects which are resistant to synthetic products[10]. Citrus is an important fruit crop that is mostly grown in tropical or subtropical climates[11]. Kinnow, a high yielding mandarin hybrid variety (a cross between *Citrus nobilis* and *C. deliciosa*) grown in Punjab, has become well-known among citrus fruits. The peel of this fruit, which accounts for around 30-34% of the total weight[12] is typically considered as trash, although it is more valuable due to the presence of numerous active phytochemicals with potential larvicidal characteristics[13]. By utilizing kinnow peel, the plant wastes can be reduced and assessed for further usage in a beneficial manner. Keeping this in mind, the current study was planned to evaluate the larvicidal potential of ethanolic and aqueous extracts derived from kinnow peel waste against *Ae. aegypti*.

MATERIAL AND METHODS

***Collection of Ae. aegypti larvae*:** Water samples were taken from various small fresh water collections such as desert coolers, roadside ditches, plates under pots, rubber tyres, plastic containers and earthen pots located in peri-domestic areas of urban regions in the Ludhiana district of Punjab state (India). *Ae. aegypti* larvae were identified and separated from other types of mosquito larvae (if present) from these collected water samples using standard keys based on their morphological characters[1].

Collection of kinnow peels**:** Fresh kinnow peels were collected from the various local fruit vendors of Ludhiana city. The collected peels were cleaned and separated from the pulp, leaves and stem of the fruit. Peels that were infected were discarded.

Preparation of kinnow peel extracts**:** The collected fresh kinnow peels were shade dried on filter paper at room temperature for 5-6 days till they got brittle and completely dried. Dried peels were finely pulverized using a grinder to make fine powder. For preparing kinnow peel extracts, 20 g of kinnow peel powder was taken in conical flask, followed by addition of 100 ml ethanol for ethanolic extract and 100 ml distilled water for the preparation of aqueous extract. The flasks were covered with aluminum foil and then kept in an orbital shaker for frequent agitation at 25° C temperature at 80 rpm for mixing over a period of 24 hours. Then the mixture was filtered using muslin cloth followed by filter paper and filtrate/extracts so obtained were used for further testing.

Dose response larvicidal bioassay of kinnow peel extracts against Ae. aegypti**:** To determine the larvicidal potential, preliminary testing of freshly prepared kinnow peel ethanolic and aqueous extracts was carried out against *Ae. aegypti* larvae (L4) by random selection of higher and lower concentrations of these extracts. On the basis of preliminary screening, five different concentrations of kinnow peel ethanolic extract @ 0.05, 1.0, 1.5, 2.0 and 2.5% and aqueous extract @ 6.0, 7.0, 8.0, 9.0 and 10.0% were prepared by mixing the required volume of each of kinnow peel extract in 1 ml of dimethyl sulphoxide (DMSO, a non-polar and non-toxic emulsifying agent) and total volume was made up to 250 ml with de-chlorinated water (prepared by keeping the tap water in opened buckets for overnight). *Ae. aegypti* L4 larvae (n=20) were exposed to five different concentrations of the prepared kinnow peel ethanolic and aqueous extracts in plastic beakers of 250 ml capacity covered with muslin cloth using a rubber band. A control set (having 250 ml of de-chlorinated water) and a vehicle-control set (having 1 ml DMSO and 249 ml of de-chlorinated water) were also run simultaneously. All experimental sets were run in triplicate and larvae were fed adequately with dog biscuits and yeast ground in ratio 3:1 (added @ 2mg/100ml) till their transformation to next non-feeding pupal stage. Larval mortality was recorded after 3, 6, 9, 12, 24, 36 and 48 hours of treatment in ethanolic and aqueous extract treated, control and vehicle-control sets. The larvae were considered dead, if they were unable to respond or move when stimulated using a brush. The dead larvae in each set were counted and

removed from the experimental sets. Out of the tested concentrations, the minimum concentration of ethanolic and aqueous extracts showing maximum mortality was considered as the effective concentration, which was used for further experimentation purpose. For calculating LC_{50} and LC_{90} after 12 hours of post-exposure, the log concentration-mortality regression was worked out by log probit technique[14] employing the computer programme POLO[15].

***Retention activity period of kinnow peel extracts*: For assessing the larvicidal retention activity period of kinnow peel extracts, fresh *Ae. aegypti* L4 larvae (n=20) were kept in the leftover tested solution of effective concentration of kinnow peel ethanolic and aqueous extracts (assessed during larvicidal bioassay of respective extracts) after removing all the dead larvae from the beakers. After 3, 6, 9, 12, 24, 36 and 48 hours, dead larvae (if any) were counted and replenished with the same number of the new larvae so as to have total number of larvae as 20 (which were taken initially). Control and vehicle-control sets were also kept simultaneously along with treatment trials in triplicate. In another experiment, fresh *Ae. aegypti* L4 larvae (n=20) were introduced in properly covered beakers containing the leftover tested solution of the effective concentration of kinnow peel ethanolic and aqueous extracts and were further monitored to determine the retention activity effect of these extracts (after removing all the dead larvae of the larvicidal bioassays) on larval development duration i.e. time taken by L4 larvae to pupae and from pupae till adult emergence. The muslin cloth was removed from the beakers when the larvae got transformed into pupae and then these beakers were kept in mosquito rearing cages. Emerged adults were fed on sugary juice of deseeded water-soaked raisins kept in a sterilized petri plate already placed inside mosquito rearing cages. To provide them water, a moist cotton swab was also placed on the top of each cage. After the completion of experiments, emerged mosquitoes were killed by keeping chloroform dipped cotton swabs inside the mosquito rearing cages. Control and vehicle-control sets were also kept simultaneously along with treatment sets in triplicate. The time taken for each transformation (i.e. from L4 to pupa and pupa to adult) was recorded along with recording of per cent adult emergence in all the sets.

***Larvicidal potential of stored kinnow peel extracts*: Kinnow peel ethanolic extract was kept in clean glass vials covered with aluminum foil and placed at 4º C in the refrigerator, while aqueous extract was kept in properly covered plastic vials at -18º C in deep freezer and both the extracts were stored for 2, 4 and 6 months. The

already determined effective larvicidal concentrations (tested during larvicidal bioassay) were tested again for the freshly prepared and stored (2, 4 and 6 months old) ethanolic and aqueous extracts against *A*e. *aegypti* by following the same procedure and larval mortality was recorded at regular intervals of three hours in all the sets.

***Statistical analysis*: ** The data was statistically analyzed by comparing the larval mortality and developmental duration recorded in kinnow peel ethanolic and aqueous extract treated sets with that of control and vehicle-control sets in their respective experimental trials and larvicidal potential of stored kinnow peel ethanolic and aqueous extract treated sets with that of their respective freshly prepared extracts by one way analysis of variance (Duncan multiple range test).

RESULTS

Dose response larvicidal bioassay of kinnow peel extracts against Ae. aegypti

When *Ae. aegypti* larvae (L4) were exposed to 0.05% of kinnow peel ethanolic extract, 13.33±2.88% mortality was observed within 3 hours, which was observed to increase up to 26.67±5.77% till 12 hours and after that, no further larval mortality was found. A similar trend in larval mortality was observed after exposing the larvae to 0.10 and 0.15% concentrations of ethanolic extract. However, exposure of larvae to 0.20 and 0.25% of ethanolic extracts resulted in 100%mortality within 6 and 3 hours respectively. Per cent larval mortality was found to increase statistically with increase in the concentration of ethanolic extract at the respective hours of each treatment. However, no larval mortality was observed in control and vehicle-control set (Table 1). Exposure of L4 *Ae. aegypti* larvae to 6.0% kinnow peel aqueous extract resulted in 28.33±7.63% mortality which increased up to 41.67±5.77% till 12 hours and there after that no further larval killing was reported. Increase in larval mortality was recorded with increase in concentration of aqueous extract and at 9.0 and 10.0% concentrations, 100% larval killing was recorded respectively within 12 and 9 hours of exposure (Table 2). Various toxicity values i.e. LC_{50} and LC_{90} of kinnow peel extracts computed for *Ae. aegypti* larvae based on record of their mortality for the exposure of 12 hours were worked out to be 0.07 and 0.16% for ethanolic extract and 6.41 and 8.23% for aqueous extract, respectively (Tables 1 and 2). Kinnow peel ethanolic extract @ 0.20% and aqueous extract @ 9.0% were found to be the effective larvicidal

concentratons, as these resulted in maximum larval mortality (100%) with minimum concentration in comparison to that of all the respective tested concentrations (Fig.1).

Retention activity period of kinnow peel extracts

Freshly added *Ae. aegypti* L4 larvae exposed to the effective concentration of ethanolic (0.20%) and aqueous (9.0%) extract (determined during larvicidal bioassay in Tables 1 and 2) showed no larval killing after 3, 6, 9, 12, 24, 36 and 48 hours in any of the triplicate sets. However, a significant delay in duration of time taken from L4 to adult emergence was recorded during the development of these larvae exposed to each of kinnow peel extract. In control set the average time taken for the transformation of L4 larval stage to pupa and from pupa to adult was recorded to be 2.66±1.15 and 1.16±0.28 days and in vehicle-control set it was found to be 2.5±0.86 and 1.33±0.28 days. Retention efficacy effect in terms of delayed development that is exposure of leftover effective concentration (0.20%) of kinnow peel ethanolic extract resulted in significant delay in development of L4 to pupa and pupa to adult, as it took 5.00±0.00 and 1.83±0.28 days respectively. Similarly, exposure of L4 stages of *Ae. aegypti* to leftover effective concentration of kinnow peel aqueous extract (9.0%) resulted in significant delay in development of L4 to pupa and pupa to adult i.e. 4.83±0.28 and 2.00±0.00 days respectively. Overall development from L4 stage till adult formation in ethanolic and aqueous extract treated sets was observed to take statistically longer duration almost double time i.e. 6.83±0.28 and 6.50±0.50 days respectively as compared to control (3.82±1.43 days) and vehicle-control sets (3.83±1.14 days). However, there was no effect of treatment of kinnow peel extracts on the adult emergence, as it was found to be 100% in all the sets (Table 3).

Table 1. Mortality of *Aedes aegypti* larvae being exposed to different concentrations of kinnow peel ethanolic extract

Concentration (%)	Per cent mortality up to (Mean±S.D)							Maximum mortality (%)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
0.05	13.33±2.88[a]	16.67±5.77[a]	21.67±2.88[a]	26.67±5.77[a]	NFM	NFM	NFM	26.67±5.77[a]
0.10	31.67±5.77[b]	51.67±7.63[b]	53.33±5.77[b]	63.33±2.88[b]	NFM	NFM	NFM	63.33±2.88[b]
0.15	58.33±5.77[c]	61.67±2.88[b]	66.67±2.88[c]	76.67±7.63[c]	NFM	NFM	NFM	76.67±7.63[c]
0.20	76.67±2.88[d]	100.00±0.00[c]	-	-	-	-	-	100.00±0.00[c]
0.25	100.00±0.00[e]	-	-	-	-	-	-	100.00±0.00[e]
0 (Control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0 (Vehicle-control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Toxicity Value (%)	Fiducial limits		$\chi 2$
	Lower limit	Upper limit	
LC_{50}= 0.07	0.3	0.11	11.25
LC_{90}= 0.16	0.11	0.45	

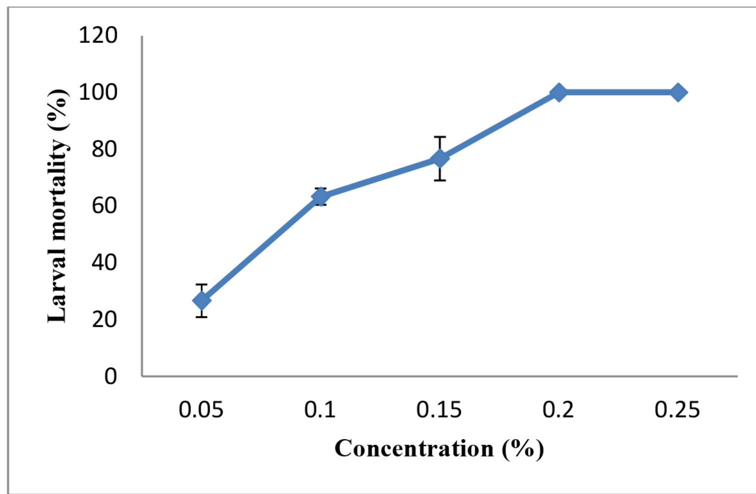
- NFM represents no further mortality
- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to different treatments by using Duncan multiple range test

Table 2. Mortality of *Aedes aegypti* larvae being exposed to different concentrations of kinnow peel aqueous extract

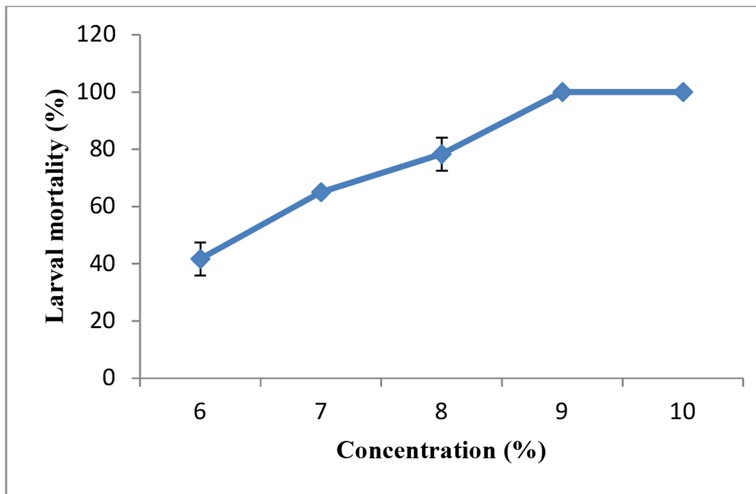
Concentration (%)	Per cent mortality up to (Mean±S.D)							Maximum mortality (%)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
6.0	28.33±7.63^{a}	33.33±7.63^{a}	38.33±2.88^{a}	41.67±5.77^{a}	NFM	NFM	NFM	41.67±5.77^{a}
7.0	31.67±7.63^{a}	36.67±2.88^{a}	43.33±5.77^{a}	65.00±0.00^{b}	NFM	NFM	NFM	65.00±0.00^{b}
8.0	40.00±5.00^{b}	46.67±2.88^{b}	53.33±2.88^{b}	78.33±5.77^{c}	NFM	NFM	NFM	78.33±5.77^{c}
9.0	51.67±2.88^{c}	58.33±7.63^{c}	83.33±7.63^{c}	100.00±0.00^{d}	-	-	-	100.00±0.00^{d}
10.0	66.67±2.88^{d}	81.67±5.77^{d}	100.00±0.00^{d}	-	-	-	-	100.00±0.00^{d}
0 (Control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0 (Vehicle-control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Toxicity Value (%)	Fiducial limits		$\chi2$
	Lower limit	Upper limit	
LC_{50}= 6.41	4.99	7.06	8.18
LC_{90}= 8.23	7.44	11.31	

- NFM represents no further mortality
- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to different treatments by using Duncan multiple range test



(A)



(B)

Fig. 1. Maximum mortality (%) of *Aedes aegypti* larvae after exposing to different concentrations of kinnow peel extracts. (A) Ethanolic extract and (B) Aqueous extract

Table 3. Retention activity of effective concentration of kinnow peel extracts in terms of development and emergence of *Aedes aegypti*

Experimental set	Duration of developmental period in days (Mean±S.D)			Per cent adult emergence (Mean±S.D)
	L4-Pupa	Pupa-Adult	L4-Adult	
Control	2.66±1.15a	1.16±0.28a	3.82±1.43a	100.00±0.00a
Vehicle-control	2.5±0.86a	1.33±0.28a	3.83±1.14a	100.00±0.00a
Ethanolic extract (0.20%)	5.00±0.00b	1.83±0.28b	6.83±0.28b	100.00±0.00a
Aqueous extract (9.0%)	4.83±0.28b	2.00±0.00b	6.50±0.50b	100.00±0.00a

- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to Control and Vehicle-control sets by using Duncan multiple range test

Larvicidal potential of stored kinnow peel extracts

When *Ae. aegypti* L4 larvae were exposed to effective concentration i.e. 0.20% of fresh and stored (2, 4 and 6 months) ethanolic kinnow peel extract, statistically similar per cent larval mortality after 3 hours and 100% mortality after 6 hours was observed. Similarly, effective concentration of aqueous kinnow peel extract i.e. 9.0% of fresh and those stored for 2, 4 and 6 months, resulted in statistically similar per cent mortality up to 9 hours and 100% killing of *Ae. aegypti* larvae after 12 hours in all cases. Results clearly indicated no effect of storage on larvicidal activity of both the types of prepared kinnow peel extracts.

DISCUSSION

Citrus species are well known for their economic importance and thus are widely cultivated fruits globally. Citrus plant contains several important phytochemicals[16] out of these limonoids exhibit a wide range of biological activities including insect repellent and larvicidal[17]. Kaur *et al.*[18] have observed limonene as the most abundant compound (64.82%) in kinnow peel oil which is mainly considered to be responsible for its larvicidal action by acute toxicity against *Ae. aegypti*,being mainly involved in arresting the metabolic activities of larvae. In the

same study, the other major constituents of the kinnow peel oil reported were elemol (8.28%), β-citronellal (4.34%), geraniol (3.58%), viridifloral (2%), N, N-dimethylacetamide (DMA) (1.91%), β-elemene (1.64%), β-citronellol (1.52%), β-myrcene (1.34%) and germacra-1,4,5-triene (1.07%). Ethanolic extract from the pulp of *C. aurantifolia* when tested against *Ae. albopictus*, was found to possess larvicidal property[19]. In another finding *Cambly carpa* ethanolic extract exhibited promising larvicidal effects against *Ae. aegypti*[20]. The larvicidal activity of prepared kinnow peel extracts observed during the present study is due to the presence of such secondary metabolites. During the present study, it was observed that both the type of extracts (ethanolic and aqueous kinnow peel extracts) showed no larval killing after 12 hours of exposure, indicating highly volatile characteristics of these extracts. Actually kinnow peel has high content of limonene, which is unstable in light and is highly volatile in nature[18]. Due to this reason very low retention efficacy of the prepared extracts was observed. Volatility of plant based products (oils/extracts) vary from plant to plant species, as certain phytochemicals are stable for few hours and others may show their stability up to several days. However, rapid action of these phytochemicals cause acute neurotoxicity, resulting in knockdown effect on the larvicidal populations[21] as also seen during the present research.

Plant-based products exhibit growth inhibiting effects on the various developmental stages of different mosquito species due to a variety of pre-emergent defects such as prolongation of instars and pupal duration, inhibition of larval and pupal moulting, morphological abnormalities and mortality due to toxicity during the developmental phases[12]. Inhibition/delay in development of *Ae. aegypti* larvae along with morphological and histological alterations have been observed after their exposure to curcumin/d-mannitol[22]. Similarly, the current study also found a significant delay in duration of time taken from L4 to adult emergence during the developmental period of the larvae after exposure to kinnow peel extracts. Though the larvicidal retention activity of kinnow peel extracts was found to be only up to 12 hours, but after that freshly added L4 instars of *Ae. aegypti* in the leftover extract solutions of treatment trials showed significant delay in overall development (L4 to adult) indicating retention activity of these extracts in terms of increasing the development duration. Delayed development have also been recorded in *Ae. aegypti* larvae after treatment with extracts from seeds of *Adenanthera pavonina*, which possibly may be because of defective proteolysis in the larvae's midgut[22]. Extract

from *Brucea* spp leaves[24]and *Eucalyptus* oil nanoemulsion[13]have been observed to cause developmental delay in *Ae. aegypti* larvae. Actually, moulting and metamorphosis in insects depends on several growth hormones and disruption of growth hormone production may cause inhibition and delay in larval growth and development to pupa and then to adult[25].

Oils/extracts of plant origin get easily degraded, when stored at room temperature and to a lesser extent when kept in the dark. Thus, if these extracts are properly stored to prevent oxidation and polymerization caused by air and light, they remained stable and effective too. During the present study, it was observed that the larvicidal potential of kinnow peel ethanolic and aqueous extracts was found to remain unaffected even kept for 6 months because of their proper storage in cold and dark conditions in closed vials which effectively prevented autoxidation. Santos *et al*.[26] have observed that larvicidal properties of EOs against *Ae. aegypti* remained unaffected even up to three years of storage kept under proper conditions.

CONCLUSION

The present study concluded that kinnow peel ethanolic extract @ 0.20% and aqueous extract @ 9.0% were found to have efficient larvicidal potential against *Ae. aegypti* L4 instars. These extracts showed retention activity in terms of significantly delaying the development duration (L4 to adult). No effect of storage (even up to six months) on the larvicidal activity of kinnow peel ethanolic and aqueous extracts was observed. This study highlighted the significance of kinnow peel (generally treated as waste byproduct) as an important source of different bioactive compounds exhibiting larvicidal properties. However, the issue of high volatility of kinnow peel extracts necessitates the addition of a stabiliser or fixative to enhance the sustainability and stability of these extracts leading to the preparation of any formulation in future. Thus, such extracts can be exploited for managing the problem of dengue transmitting vector, *Ae. aegypti*.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Department of Zoology, Punjab agricultural University, Ludhiana for providing all the necessary facilities and

Department of Science and Technology, Government of India, New Delhi, for providing infrastructural facilities under FIST grant.

AUTHOR CONTRIBUTION STATEMENT

DK conceived and designed research problem. AK conducted experiments. AK and DK analyzed data. DK and AK prepared, edited and finalized the manuscript.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES

1. Becker N, Petrić D, Zgomba M, et al. *Mosquitoes: Identification, ecology and control*. Berlin, Germany: Springer Nature; 2020.
2. Shinde L. Distribution of *Aedes aegypti* and *Aedes albopictus* from Jalna District (MS) India. Biosci Discov. 2014; 5: 11-14.
3. Kaur K, Jamwal S, Kocher DK. Monitoring of mosquito larvae from temporary water collections of Ludhiana, Punjab. Indian J Ecol. 2016; 43(1): 357-58.
4. National Vector Borne Disease Control Programme. Dengue situation in India. 2023. Available from: https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715, assessed on October 2024.
5. Thatheyus AJ, Selvam AG. Synthetic pyrethroids: Toxicity and biodegradation. Applied Ecol Environ Sci. 2013; 1(3): 33-36.
6. Choudhary S, Yamini NR, Yadav SK, Kamboj M, Sharma A. A review: pesticide residue: Cause of many animal health problems. J Entomol Zool Stud. 2018; 6(3): 330-33.
7. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012; 135(5): 581-91.
8. Kocher DK, Riat AK. Larvicidal potential of *Eucalyptus gloublus* oil against *Aedes* and *Culex* mosquitoes. J Insect Sci. 2017; 30(1): 5-9.
9. Kabaru JM, Gichia L. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. African J Sci Technol. 2001; 2(2): 44-49.
10. Asadollahi A, Khoobdel M, Ramazani AZ, Azarmi S, Mosawi SH. Effectiveness of plant-based repellents against different *Anopheles* species: A systematic review. Malaria J. 2019; 18(1): 1-20.

11. Kamal GM, Anwar F, Hussain AI, Sarri N, Ashraf MY. Yield and chemical composition of citrus essential oils as affected by drying pretreatment of peels. Int Food Res J. 2011; 18(4): 1275-82.
12. Rafiq S, Kaul R, Sofi S, Bashir N, Nazir F, Nayik GA. Citrus peel as a source of functional ingredient: A review. J Saudi Soc Agric Sci. 2018; 17: 351-58.
13. Kaur N, Kocher DK. Effect of nanoemulsified *Eucalyptus globulus* oil on development, emergence and survival of *Aedes aegypti* L. Indian J Entomol. 2022; 84(4): 804-08.
14. Finney DJ. *Probit analysis*, III edn. Cambridge: Cambridge University Press; 1971. p. 68-72.
15. Robertson JL, Russel RM, Savin N. *POLO: A user's guide to probit or logit analysis*. Pacific South-West forest and range experiment station, Berkeley, USA; 1980.
16. Satari B, Karimi K. Citrus processing wastes: environmental impacts, recent advances, and future perspectives in total valorization. Resour Conserv Recycl. 2018; 129: 153-67.
17. Gomes PRB, Oliveira MB, De Sousa DA, Da Silva JC, Fernandes RP, Filho VEM. Larvicidal activity, molluscicide and toxicity of the essential oil of *Citrus limon* peels against *Aedes aegypti*, *Biomphalaria glabrata* and *Artemia salina*. Eclét Quím. 2019; 44(4): 85-95.
18. Kaur A, Kocher DK, Kumar R. Larvicidal potential and residual activity effect of kinnow peel oil against *Aedes aegypti* L. J Vector Borne Dis. 2022; 59(3): 228-35.
19. Nishan M, Subramanian P, Saahatish R, Jesudoss VAS. Toxicity of *Citrus aurantifolia* and *Citrus hystrix* against *Aedes albopictus* larvae. Int J Biosci. 2017; 10(6): 48-54.
20. Ishak NI, Hastutiek P, Suprihati E. Potential extract ethanol *Citrus amblycarpa* as a bioinsecticide against *Aedes aegypti* larvae. Systematic Rev Pharm. 2021; 12(1): 1614-18.
21. Bibi R, Tariq RM, Rasheed M. Toxic assessment, growth disrupting and neurotoxic effects of red seaweeds' botanicals against the dengue vector mosquito *Aedes aegypti* L. Ecotoxicol Environ Saf. 2020; 195: 1-12.
22. Mezzacappo NF, De Souza LM, Inada NM, et al. Curcumin/d-mannitol as photolarvicide: induced delay in larval development time, changes in sex ratio and reduced longevity of *Aedes aegypti*. Pest Manag Sci. 2021; 77(5): 2530-38.
23. Sasaki DY, Jacobowski AC, Souza AP, Cardoso MH, Franco OL, Macedo MLR. Effects of proteinase inhibitor from *Adenanthera pavonina* seeds on short-and long term larval development of *Aedes aegypti*. Biochimie. 2015; 112: 172-86.
24. Sutiningsih D, Mustofa M, Satoto TB, Martono E. Inhibitory effects of bruceine A biolarvicide on growth and development of *Aedes aegypti* larvae. J Entomol. 2017; 14: 104-11.
25. Ghoneim K, Hamadah K, Waheeb H. Bioefficacy of farnesol, a common sesquiterpene, on the survival, growth, development, and morphogenesis of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Egyptian Acad J Biol Sci. 2020; 12(1): 71-99.

26. Santos GK, Dutra KA, Lira CS, et al. Effects of *Croton rhamnifolioides* essential oil on *Aedes aegypti* oviposition, larval toxicity and trypsin activity. Molecules. 2014; 19(10): 16573-87.