Chapter 8

Utilization of Fruit Peel Wastes for the Management of Chikungunya Vector, *Aedes aegypti*

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Additional information is available at the end of the chapter

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Abstract

Chikungunya, a widely spread viral disease transmitted to human beings by *Aedes aegypti*, is on rise in India, Africa and Asian subcontinent since last decade. Although chemical insecticides are used at a large scale for the control of Chikungunya vector, their applications have led to several undesirable effects including insecticide resistance, revival of pests species, appearance of secondary pests, environmental pollution, noxious hazards to human beings and non-target organisms forcing investigators to explore unconventional alternate strategies. As an environment-friendly approach, there is increased attention to devise and adopt suitable methods to utilize wastes as value-added products to reduce the problem of environmental pollution. Consequently, the larvicidal and adult irritant potential of hexane and petroleum ether peel extracts of three different *Citrus* species, *C. limetta*, *C. sinensis* and *C. Limon,* were assessed against *Ae. aegypti*. The results showed the larvicidal potential of all the three peels, *C. limetta* peel extracts exhibiting the least activity. Furthermore, hexane extracts were more effective than petroleum ether extracts, *C. sinensis* peels hexane extract being most effectual ($LC_{50,}$ 39.51 ppm) while petroleum ether peels extract of *C. limon* was the most effective larvicide with LC_{50} value of 51.25 ppm. All the extracts also exhibited significant elicit response and irritant potential against adults signifying their potential role in reduced mosquito bites and disease transmission. The qualitative phytochemical analysis of the extracts showed presence of certain components suggesting their probable role in bioefficacy of extracts. Further studies are needed to isolate and identify the active ingredient to formulate strategies for mosquito control.

Keywords: *Aedes aegypti*, citrus, larvicidal, peel waste, repellent

1. Introduction

Mosquitoes and the related mosquito-borne diseases are a growing menace around the globe. Prevalence of mosquito-borne diseases is one of the world's most health hazardous problems.

Several mosquito species belonging to genera *Culex*, *Anopheles* and *Aedes* are vectors for the pathogens of various diseases like filariasis, Japanese encephalitis, malaria, dengue, dengue haemorrhagic fever, yellow fever and Chikungunya [1]. *Ae. aegypti*, the primary carrier for viruses, that cause dengue and Chikungunya fever, is prevalent over wide regions of the tropical and subtropical world. Currently, no effectual vaccine is offered for dengue and Chikunguya fever. Recently, Zika virus disease transmitted by *Aedes* mosquitoes, especially *Ae. aegypti* in tropical world, has been the cause of concern in the world. In today's scenario, the only approach that can be employed to reduce the incidence of *Aedes*-borne diseases is by vector control, which is recurrently reliant on the application of conventional synthetic chemical insecticides [2].

Ae. aegypti, thus has engrossed extensive interest at universal level because of rapid spread of these diseases. Chikungunya is primarily centred across the urban and peri-urban areas, having severe effects in the Asian subcontinent. WHO reports state the massive-fold increase in the number of reported cases over the last decade. Also, the unavailability of a vaccine against the disease further makes the scenario graver.

The first documented outbreak of Chikungunya took place in 1952 in Southern Tanzania, East Africa [3]. Subsequently, epidemics were recorded soon in several other countries : Cambodia, Thailand, Philippines, India, Vietnam, Burma and Sri Lanka. Ever since 2003, there have been outbreaks in the islands of the Pacific Ocean, including Comoros, Mauritius, Madagascar and Reunion Island [4].

The increasing population of Chikungunya vector has urged scientists to come up with effective methods of mosquito control. Several methods have been adopted for controlling mosquito population. In the past, control measures for insect pests and disease vectors utilizing synthetic organic chemical insecticides have caused development of resistance to these insecticides in the disease vector of Chikungunya and dengue fever [5]. It has been reported that insecticide resistance may develop due to changes in the biochemical machinery of mosquitoes, resulting in the quick detoxification or appropriation of the insecticide, or because of the mutation in the target site inhibiting its interaction with insecticide [6]. Progressively, the problem of insecticide resistance is augmenting leading to collapse of many vector control programs. Moreover, the recurrent use of insecticides to manage insect pests and vectors is causing deterioration of the ecosystem [7]. These insecticides are thus, now not a preferred choice for mosquito control because of the environmental degradation they usher. Thus, scientists are now on the look-out for alternative strategies to circumvent the problems posed by the use of synthetic chemical-based insecticides.

Botanicals are one such alternative; researchers are banking upon to alleviate these problems. Although using botanicals seems to be a relatively newer form of mosquito control but its history dates back to the first century AD when a Greek philosopher, Pliny the Elder, wrote ''Natural History'' and recorded all the pest control methods known at that time. Simultaneously, the Chinese discovered the use of *Chrysanthemum* flowers as an effective insecticide and many others reported a few important phytochemical insecticides, including pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene, camphor and

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turpentine, widely used earlier in developed countries before the introduction of synthetic organic insecticides.

Though with the advent of DDT in 1939, the researchers' quest for mosquito control agents was over and phytochemicals saw their downfall, yet there was a re-focus on phytochemicals because of several problems faced due to injudicious and over application of synthetic insecticides in nature. The scientists have always appreciated the easily biodegradable nature of the phytochemicals leading to no ill-effects on non-target organism. Almost two decades back, phytochemicals could make up only 1 % of world's pesticide market but now, there is a continuous rise in the use of these environment-friendly chemicals.

By definition, phytochemicals are non-nutritive plant chemicals with defensive characteristics. They assist the plants to endure the incessant selection pressure from herbivorous pests, predators and other environmental factors. They are known to be the chemical factories of nature, producing many chemicals, some of which have medicinal and pesticidal properties [8]. Researchers have identified several groups of phytochemicals which possess insecticidal activities. It has been reported that approximately 2000 plant species produce secondary metabolites of importance in pest control programs [9]. Amongst these plant species, metabolites of approximately 344 species have been reported to have a range of activity against mosquitoes [10]. In 2000, Liu et al. suggested these natural plant products as possible alternatives to synthetic chemical insecticides [11]. Some of such phytochemicals include alkaloids, steroids, terpenoids, essential oils and phenolic compounds. The intensity of the insecticidal effect of a particular plant is not static and varies with plant species, mosquito species, geographical varieties, part of the plant used, extraction method adopted, etc. Bio-pesticides provide an alternative to synthetic pesticides as they are effective, easily available, safe for manufacturers and users, biodegradable, inexpensive and environment-friendly.

Recently, there is always an increased attention in bringing useful products from waste materials and fruit wastes are no exceptions. With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Citrus is one of the most important commercial fruit crops grown in all continents of the world, the fruits primarily used by juice processing industries while the peels are considered a waste material [12]. Since the juice yield of citrus is less half of the fruit weight, enormous quantity of peel wastes are formed every year. Unfortunately, the peels though perishable and seasonal, they pose a big problem of disposal to the processing industries and pollution monitoring agencies. There is a growing interest to adopt appropriate methods for utilizing them for the conversion into value-added products and improve the overall economics of processing units and reduce the problem of environmental pollution considerably.

Recycling of fruit waste is one of the most imperative means of exploiting it in a number of pioneering and ground-breaking ways leading to the yield of novel products and meeting the requirements of indispensable products required in pharmaceutical industry. The reports are available which confirmed the presence of nutrients and several secondary metabolites in the citrus peels in high magnitude. This clearly indicates the probable use of citrus peel products as drugs and harmless alternate to synthetic chemicals. Consequently, citrus plants have attracted the attention of many workers, and thus organic extracts or essential oils of the leaves and fruit peels are under investigations for various purposes, especially against control of mosquitoes. Suitable methods have to be adopted to utilize them for the conversion into value-added products to improve the overall economics of processing units and reduce the problem of environmental pollution considerably [13]. Many plants have been found to contain chemicals, which are helpful for the control of insects and are useful for field applications in mosquito control programs [14]. Limonene isolated from abraded fresh peels of *Citrus aurantium* has been investigated for its toxicity against larvae of *Cx. quinquefasciatus* and found effective [15]. In 2006, Lee assessed the larvicidal efficacy of *Citrus bergamia* essential oil against *Ae. aegypti* and *Cx. Pipiens* [16], while Amer and Mehlhorn investigated essential oil extracted from *C. limon* against three mosquito species : *Ae. aegypti*, *Cx. quinquefasciatus* and *Anopheles stephensi* [17].

It is well known that the approach to combat mosquito-borne diseases largely relies on interruption of the disease transmission cycle by either targeting the mosquito larvae at breeding sites through spraying of stagnant water or by killing/repelling the adult mosquitoes using insecticides. Thus, investigations were carried out to explore the efficient anti-mosquito potential of citrus peels obtained from three species of *Citrus* : *C. limetta*, *C. limon* and *C. sinensis* to control *Ae. aegypti* at larval stage and to repel at adult stage keeping in mind that such a study would not only devise alternate strategies for mosquito control but also would focus towards recycling of wastes, managing littering and waste-related environmental degradation.

2. Review of literature

Citrus plants have attracted the attention of many scientists. Consequently, researchers have carried out investigations on the organic extracts and essential oils extracted from the leaves and fruit peels of citrus plants against different species of insect pests including mosquitoes [18–21]. There are reports available which confirm the larvicidal and repellent activities of the essential oil isolated from *C. sinensis* against *Ae. aegypti* mosquitoes [22].

The larvicidal efficacy of *C. sinensis* peels has been documented against various species of mosquitoes [21]. They reported the respective LC_{50} and LC_{90} values of 58.25 and 298.31 ppm when the larvae of *An. subpictus* were exposed to the chloroform extract of *C. sinensis* peels. On the other hand, assays with methanol extract of *C. sinensis* peels resulted in lower LC_{50} and LC_{90} values of 38.15 and 184.67 ppm, respectively against the larvae of *Cx. tritaeniorhynchus*. The effectiveness of the ethanolic extracts of the *C. sinensis* has been established by Amusan et al. against the larvae of *Ae. aegypti* [23]. Likewise, Michaelakis et al. reported the strong toxicity of essential oils extracted from peels of *C. limon* and *C. sinensis* against larvae of *Cx. pipiens* resulting in the LC_{50} values ranging from 30.1 to 51.5 mg/l, respectively [24]. Earlier investigations have shown that the peel oil extracts of *C. sinensis*, *C. aurantium* and *C. limon* possessed outstanding larvicidal potential against *Cx. quinquefasciatus* with lemon peel oil exhibiting the maximum larvicidal efficacy [18,19]. The volatile extracts of *C. sinensis* and *C. aurantifolia* (lime) peels were also observed to have cidal activity against mosquitoes [25].

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It has been reported earlier that phytochemicals play a foremost role in the mosquito control programmes. Different secondary metabolites of plants, such as alkaloids, steroids, phenolics, terpenoids, saponins, essential oil, etc., are coupled with an extensive range of biological activities. Marston et al. have reported the efficacy of prenylated xanthones, tetracyclic phenols and saponins in controlling the mosquito *Ae. aegypti* [26]. Likewise, the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins has been observed in the plant extract exhibiting mosquito larvicidal activity [27]. Furthermore, the citrus fruit peel has been found to be a rich source of flavanones and many polymethoxylated flavones, rare in other plants [28]. Kumar et al. have showed the presence of alkaloids, flavonoids and terpenoids in the petroleum ether extracts of *C. sinensis* peels [29]. Table 1 summarizes the larvicidal activity of certain *Citrus* species against different species of mosquitoes.

S. no.	Plant species	Plant product	Mosquito species	Reference
1.	*Citrus aurantium*	Fruit peel oil	*Cx. quinquefasciatus*	Mwaiko (1992)
2.	*Citrus limon*	Fruit peel oil	*Cx. pipiens*	Dakhil and Morsy (1999) Shalaby et al. (1998)
3.	*Citrus sinensis*	Essential oil	*Cx. pipiens molestus*	Traboulsi et al. (2002)
4.	*Citrus reticulata*	Seed extracts	*Cx. quinquefasciatus; Ae. aegypti*	Sumroiphon et al. (2006)
5.	*Citrus sinensis*	Crude peel extracts	*Cx. tritaeniorhynchus, An. subpictus*	Bagavan et al. (2008)
6.	*Citrus sinensis, Citrus limon*, *Citrus aurantium*	Enantiomers and essential oils	*Cx. pipiens*	Michaelakis et al. (2009)
7.	*Citrus grandis*; *Citrus mitis*, *Citrus jhambiri*, *Citrus paradise*, *Citrus pseudolimon*	Seed extract	*Ae. albopictus*	Akram et al. (2010)
8.	*Citrus limetta*	Fruit peel extract	*Ae. aegypti*	Kumar et al. (2011c)
9.	*Citrus sinensis*	Leaf extract	*Ae. aegypti*	Kumar et al. (2012)

Table 1. Larvicidal activity of certain *Citrus* species against different species of mosquitoes

3. Materials and methods

3.1. Collection of the larvae and adults of *Ae. aegypti*

The present investigations employ the Chikungunya fever mosquito, *Ae. aegypti* that originated from fields of Delhi and surrounding areas. The larvae were collected from seven locations spread across Delhi and NCR (National Capital Region) (Figure 1). The locations selected were:

- **Okhla, New Delhi** (latitude: 28° N 34′ 2.583″, longitude: 77° E 17′ 32.039″, altitude: 214 m)
- **Alipur, New Delhi** (latitude : 28° N 48′, longitude : 77° E 8′ 59.999″, altitude : 211 m)
- **Dwarka, New Delhi** (latitude: 28° N 35′ 31.704″, longitude: 77° E 2′ 45.773″, altitude: 212 m)
- **Faridabad, Haryana** (latitude: 28° N 24′ 32.08″, longitude: 77° E 19′ 4.041″, altitude: 205 m)
- **Shahdara, New Delhi** (latitude: 28° N 41′ 10.29″, longitude: 77° E 16′ 29.987″, altitude: 211 m)
- **Noida, Uttar Pradesh** (latitude: 28° N 35′ 12.99″, longitude: 77° E 20′ 27.61″, altitude: 201 m)
- **Ghaziabad, Uttar Pradesh** (latitude: 28° N 38′ 11″, longitude: 77° E 25′ 38.1″, altitude: 384 m)

The larvae and adults collected from different locations were brought to the laboratory. The stages were critically observed and those of *Aedes* were segregated and bought back to the laboratory for rearing.

3.2. Laboratory colonization and maintenance of *Ae. aegypti*

The colony of *Ae. aegypti* was maintained in an insectary under controlled conditions of 28 ± 1°C, 80 ± 5% relative humidity and 14:10 light/ dark photoperiod [30]. Adult mosquitoes were kept in screened cloth cages (45 cm × 40 cm × 40 cm) with a long cloth sleeve on one side to prevent the escape of any mosquito from the cage. A wet cotton pad was kept on the top of each cage to provide water to the mosquitoes. Deseeded, water-soaked split raisins kept in a Petri plate were placed in the cage, primarily as a source of food for the male mosquitoes. Female mosquitoes were provided with blood meal on alternate days by keeping a restrained albino rat in the cage for at least 2 h during the day. The day following blood meal, an ovitrap consisting of an enamel bowl lined on all sides with the Whatman filter paper and half-filled with dechlorinated water was kept in the cage for the collection of eggs. Dechlorinated water was prepared by storing tap water in large plastic buckets for 24 h. The strips of the filter paper laden with the eggs were taken out on every alternate day and kept moist for 2 days to allow embryonation of eggs. A few strips were subsequently dried and stored in sterilized bottles. The bottles were stocked in the refrigerator at 4°C. The eggs, thus stored, were viable for at least 6 months. Whenever required, the stored egg strips were dipped into deoxygenated water, which was prepared by boiling distilled water in an Erlenmeyer flask and then cooling it without access to the air. This resulted in synchronous and immediate hatching of larvae within 20– 25 min.

The remaining egg strips were dipped in the dechlorinated water for maintenance of culture. The newly emerged larvae were transferred in dechlorinated water-filled enamel trays (25 cm × 30 cm × 5 cm) for rearing. Each day, food consisting of finely ground dog biscuits and yeast (3:1 by weight) was provided to the larvae. Paramount care was taken to prevent scum formation on the surface of water. The larval period of *Ae. aegypti* comprising four instars, lasted for 9– 10 days. Pupae formed were isolated into an enamel bowl containing water which was then kept in the cloth cage for adult emergence. Blood meal was provided to the female mosquitoes on the third day after emergence.

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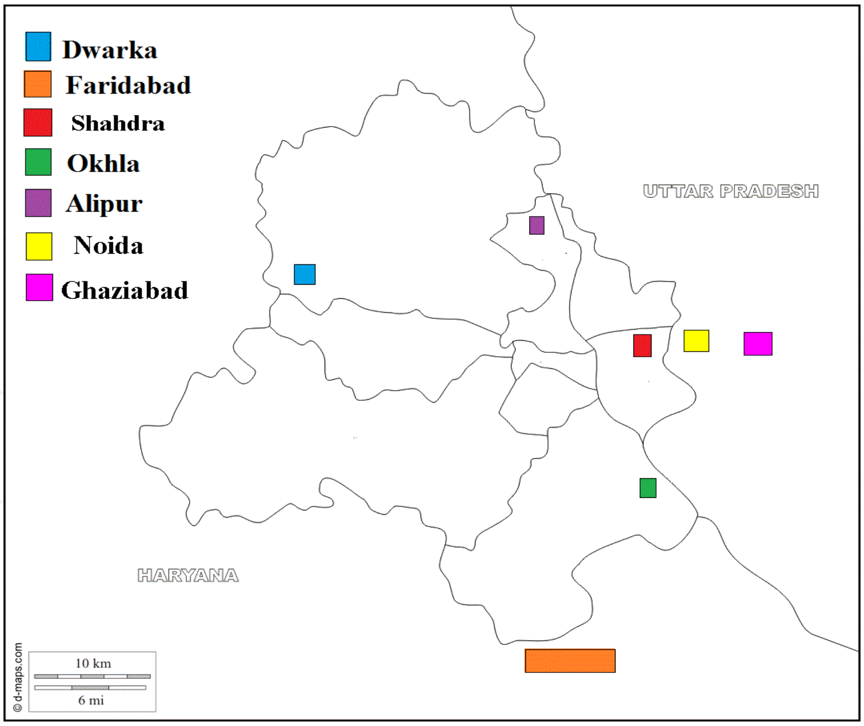
(a)



(b)

Figure 1. Areas selected in Delhi and NCR for the collection of *Aedes aegypti* larvae:(a) overview; (b) enlarged view.

3.3. Collection of plant material

The fruits of three citrus plants *C. sinensis*, *C. limon* and *C. limetta* were gathered from the surrounding areas of New Delhi, India in the sterilized polythene bags. The peels were separated from all the three species of fruits and were thoroughly washed with tap water. Care was taken to clean dust, particles or any unwanted material stuck to them. The peels were also observed cautiously to detect any kind of disease or infection. The infected peels were discarded while the selected peels were kept for drying under shade at room temperature (27 ± 2°C). The peels were dried completely ensuring to prevent fungal or bacterial growth.

3.4. Preparation of peel extract

The dried peels were mechanically grinded using a small blender. The grounded material was sieved to get fine powder. The 15 g of each dried and powdered citrus peels was extracted with 200 ml of hexane and petroleum ether, separately. The extraction was carried out for 8 h per day and continued for consecutive 3 days, using Soxhlet extractor at a temperature not exceeding the boiling point of the solvent. The six extracts obtained were concentrated using a vacuum evaporator at 45°C under low pressure and stored in a refrigerator at 4°C as the stock solution of 1000 ppm for further use.

3.5. Larvicidal bioassay

The larvicidal bioassay was performed at 28 ± 1°C on the *Ae. aegypti* early fourth instars in agreement with the methodology described by WHO with minor modifications [31]. The graded series of each peel extract was prepared using ethanol as the solvent. The early fourth instars of *Ae. aegypti* were separated, in groups of 20, in plastic bowls filled with 99 ml of distilled water. The larvae were transferred to a glass jar containing 1 ml of the particular concentration of extract added to 100 ml of distilled water. For each dilution, four simultaneous replicates were carried out making a total of 80 larvae for each extract concentration. On the other hand, in controls, ethanol was added to water instead of peel extract. During the exposure period, the larvae were not provided with any food. The dead and moribund larvae were recorded after 24 h as larval mortality.

3.6. Data analysis

The tests with more than 20% mortality in control assays and 20% pupae formed were rejected and performed again. In case, the control mortality ranged between 5% and 20%, it was rectified using Abbott's formula [32].

$$\text{Corrected mortality} = \frac{\%\ \text{Test Mortality} - \%\ \text{Control Mortality}}{100 - \%\ \text{Control Mortality}} \times 100$$

The data was subjected to the regression analysis using computerized SPSS 18.0 Programme. The LC_{50} and LC_{90} values with 95% fiducial limits were calculated in each bioassay to measure difference between the test samples. Other statistical parameters, such as standard error and regression coefficient, were also recorded.

3.7. Contact irritancy assays

Whatman filter paper circles were impregnated with 50 ppm hexane and petroleum ether peel extracts of *C. sinensis*, *C. limon* and *C. limetta,* separately. These papers were completely dried and used afresh for contact irritancy assays. Each paper was placed on a glass plate and a Perspex funnel with a hole on the top was kept inverted over them. The 3-day- old unfed female adults of *Ae. aegypti* were separated. Single female was released inside the funnel on the paper. The opening on the top of funnel was plugged with a cotton swab. The adult was left undisturbed to settle for 3 min after which the time taken for the first take-off flight was recorded. The assay was continued for 15 min during which the total number of flights undertaken by that mosquito was scored. Similar parallel control tests were performed with ethanol-impregnated papers. Each treatment had three replicates. Data was analysed and the relative irritability of the extract in each case was calculated with respect to control.

3.8. Phytochemical analysis

All the extracts were subjected to preliminary screening of phytochemical investigation and the components in each extract were identified using standard protocols as documented by Harborne et al. [33]. Various qualitative tests were performed to discover the presence of alkaloids, phenolic compounds, carbohydrates, flavonoids, tannins, phlobatannins, saponins and terpenoids in the extracts.

4. Results

Present investigations were performed with an objective to formulate a safe and environment-friendly strategy for minimization of waste in fruit juice processing industry by employing citrus fruit peel waste as the larvicidal agent against *Ae. aegypti*. Keeping this in view, the hexane and petroleum ether extracts of *C. Sinensis*, *C. limon* and *C. limetta* peels were assessed for their larvicidal and irritant potential against early fourth instar larvae and non-bleed fed females of *Ae. aegypti*. The comparative result of larvicidal bioassays of peel extracts performed on early fourth instar larvae of *Ae. aegypti* with hexane and petroleum ether extracts of *C. sinensis*, *C. limon* and *C. limetta* peels are presented in Tables 2– 4, respectively. The results prove and establish the efficacy of all the extracts of citrus peels against the mosquito larvae.

Our investigations showed that the hexane extracts of peels were more effective than petroleum ether extracts irrespective of the citrus species. The hexane extract of *C. sinensis* peels proved to be more effectual larvicidal agent against *Ae. aegypti* resulting in LC_{50} values as low as 39.51 ppm. Nevertheless, petroleum ether peels extract of *C. limon* was the most effective larvicidal agent against *Ae. aegypti* with LC_{50} value of 51.25 ppm, while that of *C. limetta* peel wastes showed least activity exhibiting LC_{50} value of 145.50 ppm. All treatments resulted in complete mortality without any pupa or adult emergence. The control or untreated groups did not show any mortality within 24 h. The larvae developed into pupae and then adults within 48–72 h (Figure 2).

Extraction solvent	LC_{50} (ppm)	95% Fiducial limits	LC_{90} (ppm)	95% Fiducial limits	S.E.	χ^2 (*df*)	Regression coefficient
Hexane	39.51	35.22–43.66	61.90	54.73–74.99	0.97	2.16 (5)	6.57
Petroleum ether	55.58	50.22–60.62	85.05	75.56–104.51	1.15	7.06 (5)	6.93

Table 2. Larvicidal activity of extracts prepared from the peels of *Citrus sinensis* against early fourth instars of *Aedes aegypti*

Extract	LC_{50} (ppm)	95% Fiducial limits	LC_{90} (ppm)	95% Fiducial limits	S.E.	χ^2 (*df*)	Regression coefficient
Hexane	46.08	40.10–52.07	85.34	72.83–109.29	0.68	4.78 (5)	4.78
Petroleum ether	51.25	45.37–57.83	93.50	79.01–122.97	0.71	3.36 (6)	4.90

Table 3. Larvicidal activity of extracts prepared from the peels of *Citrus limon* against early fourth instars of *Aedes aegypti*

Extraction solvent	LC_{50} (ppm)	95% Fiducial limits	LC_{90} (ppm)	95% Fiducial limits	S.E.	χ^2 (*df*)	Regression coefficient
Hexane	96.15	83.52–112.11	163.27	139.21–241.30	1.08	7.58 (6)	5.57
Petroleum ether	145.50	118.05–180.58	371.48	278.83–596.47	0.47	6.52 (4)	3.14

Table 4. Larvicidal activity of extracts prepared from the peels of *Citrus limetta* against early fourth instars of *Aedes aegypti*

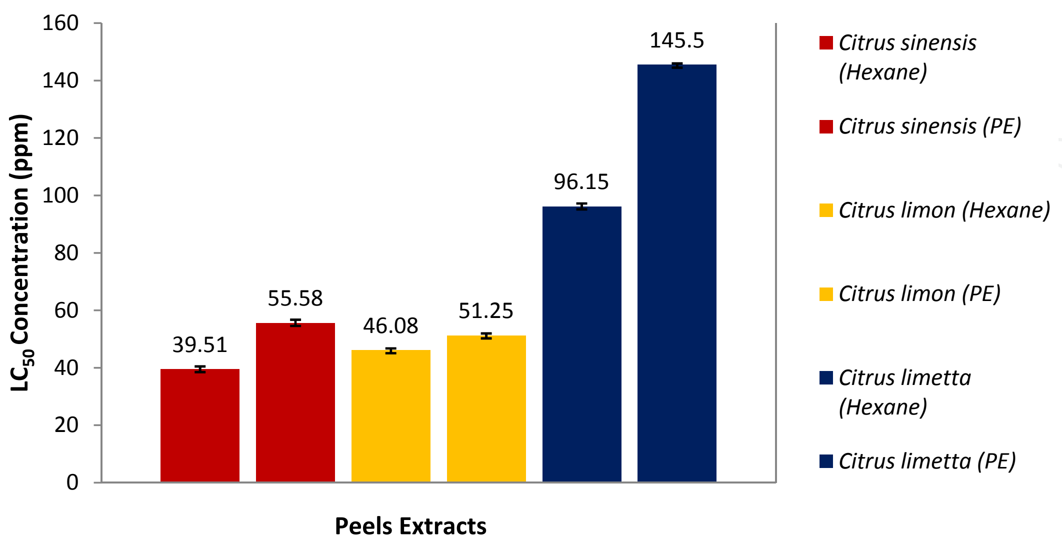


Figure 2. Comparison of LC_{50} value of hexane and petroleum ether extracts prepared from the peels of *Citrus sinensis*, *Citrus limon* and *Citrus limetta* against early fourth instars of *Aedes aegypti*.

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When the females of *Ae. aegypti* were subjected to contact irritancy assays, a significant response was observed (Table 5). The results revealed the more irritant potential of petroleum ether extracts as compared to hexane extracts. The petroleum ether extract of *C. limon* was found to be the most effective against *Ae. aegypti* with first time of flight recorded as 14s with maximum irritability effect of 25-fold (Table 5).

Solvent of extraction	Mean time lapse before first take-off (in s)	Mean no. of take- offs for females (in 15 min)	Relative irritability
	Control		
–	410.00 (258.30)	1.00 (1.00)	1.00
	(a) *Citrus sinensis*		
Hexane	29.66 (33.8)	6.00 (4.35)	6.00
Petroleum ether	38.60 (21.00)	10.00 (2.00)	10.00
	(b) *Citrus limon*		
Hexane	39.33 (35.55)	12.00 (11.26)	12.00
Petroleum ether	14.00 (4.00)	25.33 (9.60)	25.33
	(c) *Citrus limetta*		
Hexane	66.33 (54.81)	18.30 (6.50)	18.30
Petroleum ether	53.00 (13.07)	18.60 (4.16)	18.60

Figures in parentheses indicate standard deviation.

Table 5. Response of of non-blood fed females of *Ae. aegypti* to papers impregnated with extracts of *Citrus sinensis*, *Citrus limon* and *Citrus limetta* in the contact irritancy assays

S. no.	Phytochemical constituents	Hexane extract	Petroleum ether extract
1	Alkaloid	–	+
2	Carbohydrates	–	–
3	Saponins	–	–
4	Phenolic compounds	–	–
5	Tannins	–	–
6	Flavonoids	+	+
7	Terpenoids	+	+
8	Phlobatannins	–	–

Table 6. Phytochemical screening of *Citrus* peels

The preliminary qualitative phytochemical analysis of the peel extracts of all the three *Citrus* sp. discovered the occurrence of terpenoids and flavonoids in both the hexane and petroleum ether extracts (Table 6). Other components, i.e., carbohydrates, phenolic compounds, saponins, tannins and phlobatannins were not noticed in any of these extracts. Nevertheless, the petroleum ether extract exhibited alkaloids, the presence of which was not observed in the hexane extracts.

5. Discussion

It is well documented that the control of mosquito-borne diseases can be achieved either at adult stage preventing mosquitoes to bite human beings by using repellents/irritants or at larval stage causing mortality at a large scale in the breeding areas. Nonetheless, the wide-spread use of synthetic insecticides has caused environmental risks and the development of insecticide resistance in the vector species. The adverse impact of insecticides has caused concern and necessitated the search and development of environmentally safe, biodegradable, economically effective and indigenous methods for mosquito control, which can be employed with least care by human beings and communities [1].

A number of reports documented in the field of mosquito control reveal the worthiness of diverse phytochemicals procured from various plant species against different mosquito species. Sukumar et al. made an extensive review of botanical derivatives which have been investigated against mosquitoes [10]. They reported a large number of plant extracts which possess cidal or repellent activities against mosquito vectors, but indicated that very few plant products have shown practical value for mosquito control. Plants are known to be rich sources of complex mixtures of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. Nevertheless, natural pesticides derived from plants are a promising tool especially for targeting mosquitoes in larval stage [21]. Our experiments reveal the hexane extracts of the peels of all the three *Citrus* species were highly effective against *Ae. aegypti* as compared to petroleum ether extracts.

Very few reports are available regarding larvicidal and contact irritancy of citrus peels against *Ae. aegypti.* Our results are in agreement with that of Murugan et al. who had tested the efficacy of *C. sinensis* ethanolic peel extract against *Ae. aegypti* and *Cx. quinquefasciatus* and found it to be more effective against *Ae. aegypti* having LC_{50} value of 92.27 ppm whereas against *Cx. quinquefasciatus* the value reported was 244.70 ppm [34]. The essential oils extracted from peels of *C. limon* and *C. sinensis* have been reported to exhibit strong toxicity against larvae of *Cx. pipiens* with the LC_{50} values ranging from 30.1 to 51.5 mg/l [24]. Akram et al. had found extracts prepared from the seeds of rough lemon and lemon as effective larvicides with LC_{50} values of 119.993 and 137.258 ppm, respectively, after 24 h of exposure and 108.85 and 119.853 ppm, respectively, after 48 h of exposure [35]. The volatile peel extracts of *C. sinensis* and *C. aurantifolia* (lime) were also reported to have insecticidal activity against mosquitoes [25].

Our studies also showed significant irritant potential of petroleum ether extract of *C. limon* against *Ae. aegypti* as compared to *C. sinensis* and *C. limetta* extracts. Similar repellency behaviour in *Ae. aegypti* was reported by Kumar et al. when adults were exposed to the leaf extracts of *Parthenium hysterophorus* prepared in different solvents [36]. Essential oils from a few verbenaceae plants have also shown repellent activity against *Ae. aegypti*, *An. stephensi* and

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Cx. quinquefasciatus [17]. Similarly repellency of aromatic plants and pure components was studied by Abdallah et al. against *Cx. pipiens molestus* [37]. The potential of volatile oils extracted from turmeric, citronella grass and hairy basil as topical repellents have also been reported against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* [38]. Thangam also studied repellent activity of acetonic extracts of four seaweeds *Caulerpa peltata*, *C. racemosa*, *C. scalpelliformis* and *Diclyota dichotoma* and eleven mangrove plant samples, *Avicennia marina*, *A. officinalis*, *Excoecaria agallocha*, *Lumnitzera racemosa*, *Rhizophora apiculata*, *R. lamarckii*, *R. mucronata*, *Salicornia brachiata*, *Sonneratia apetala*, *Xylocarpus granatum*, against *Ae. aegypti* out of which he found the stilt root of *Rhizophora apiculata* extract to be most effective [39]. Petroleum ether extract of *Vitex negundo* leaves offered bite protection for 8 h (2 mg/cm^2) by different mosquitoes in the field [40]. This suggests that natural pesticides derived from plants are a promising tool for mosquito control programs.

Earlier studies have showed that phytochemicals play a major role in mosquito control programme. The secondary metabolites of plants (such as steroids, alkaloids, terpenoids, saponins, phenolics, essential oil, etc.) are associated with a wide range of biological activities. Marston et al. have reported the efficacy of prenylated xanthones, tetracyclic phenols and saponins in controlling *Ae. aegypti* [26]. Likewise, the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins have been observed in the plant extract exhibiting mosquito larvicidal activity [27]. Furthermore, the citrus fruit peel has been found to be a rich source of flavanones and many polymethoxylated flavones, rare in other plants [28].

The present study showed the presence of flavonoids and terpenoids as the common constituents in the hexane and petroleum ether extracts of *Citrus* sp. These results are in conformity with that of Kumar et al. who showed the presence of alkaloids, flavonoids and terpenoids in the petroleum ether extracts of *C. sinensis* peels [29]. The presence of flavonoids and cardiac glycosides in methanol extract of *Lantana camara* leaves and flowers, flavonoids in leaf and terpenoids in the flower of ethanol extract has been shown by Sathish and Maneemegalai [41]. Rawani et al. suggested that the presence of bioactive principles such as steroids, alkaloids, terpenes, saponins, etc. may be responsible for the larvicidal properties of crude extracts of three plants, *viz. Carica papaya*, *Murraya paniculata* and *Cleistanthus collinus* against *Cx. quinquefasciatus* [42]. Vinayachandra et al. concluded that either the presence of saponins, phenolics, steroids or terpenoids in the plant extracts of *Knema attenuata* or a combination of two or more of these metabolites might be the cause for larvicidal efficacy against the Indian strains of *An. stephensi* and *Ae. albopictus* [43]. Although our investigations have revealed the occurrence of only two phytochemical constituents, the larvicidal potential of the extracts might be because of the synergistic effects of other phytochemical constituents present in the extracts, identified or unidentified in the present investigation.

6. Conclusion

Our studies have clearly demonstrated that waste peels of citrus fruits can be utilized as the effective agents of mosquito control. The utilization of wastes as beneficial products would not only assist to reduce waste load by managing intractable waste discharge but would also diminish the pollution load and improve the environmental profile of fruit juice processing industry.

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