

## Comparative evaluation of the larvicidal activity of aqueous and ethanolic extracts of selected vegetable peel wastes against *Culex* spp. mosquito larvae

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### Abstract

Mosquitoes of the genus *Culex* mosquitoes are important vectors of several human diseases and the increasing resistance to synthetic insecticides has highlighted the need for environmentally safe and sustainable larvicides. In the present study, the aqueous and ethanolic extracts of peels of potato (*Solanum tuberosum*), banana (*Musa acuminata*), pointed gourd (*Trichosanthes dioica*), sponge gourd (*Luffa acutangula*), bottle gourd (*Lagenaria siceraria*) and cucumber (*Cucumis sativus*) were assayed for larvicidal potential against third and fourth instars of *Culex* spp. The peel powders after oven drying were extracted by maceration and the extracts subjected to qualitative phytochemical screening for the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, coumarins and quinones using standard protocols. Larvicidal bioassays were carried out at five concentrations (50, 150, 250, 350 and 450 ppm) and mortality was recorded at 24 h, 48 h, 72 h and 96 h. The  $LC_{50}$ ,  $LC_{90}$ , regression equations and coefficients of determination ( $R^2$ ) were calculated using Abbott's correction and log-probit regression analysis.

Phytochemical analysis revealed the presence of several bioactive secondary metabolites and banana and potato peel extracts exhibited the richest phytochemical profiles. Larval mortality increased significantly with increase in concentration and time of exposure. On the other hand,  $LC_{50}$  values decreased progressively showing the increase in toxicity with the prolonged exposure. Ethanolic extracts were always more larvicidal than water extracts. Among all the treatments, ethanolic extract of potato peel was found to be most effective followed by banana and pointed gourd extracts whereas cucumber peel extracts showed lowest effectiveness.

The results showed that vegetable peel extracts, in particular ethanolic extracts of potato and banana peels, can be potential eco-friendly larvicides. Their rich phytochemical composition and considerable larvicidal activity indicate their potential as sustainable alternatives to synthetic insecticides for integrated mosquito vector management.

**Keywords:** *Culex* spp., larvicidal activity, vegetable peel waste, botanical insecticides, phytochemical analysis, secondary metabolites, aqueous extract, ethanolic extract,  $LC_{50}$ , integrated vector management

### Introduction

Mosquitoes are among the most important vectors of infectious disease of humans and animals worldwide. They are among the most difficult groups of insects to control because of their rapid rate of reproduction, wide geographical distribution and remarkable adaptability to a variety of environmental conditions. Mosquito larvae, especially those from the genus *Culex*, commonly referred to as house mosquitoes, are known vectors for the pathogens responsible for lymphatic filariasis, Japanese encephalitis, West Nile fever, and various other arboviral infections. Their capacity to breed in standing water pooled in drains, ponds, irrigation canals, discarded containers and domestic water-storage vessels allows for rapid population growth and makes them abundant in both urban and rural habitats. The growing incidence of mosquito-borne diseases has increased the need for effective vector control strategies. Larval management is one of the most efficient and economical approaches because mosquito larvae are confined to aquatic habitats before emerging as adults.

Mosquito control programmes have extensively utilized synthetic larvicides such as organophosphates, pyrethroids, insect growth regulators and microbial formulations because of their rapid action and high efficacy. But the long-term and indiscriminate use of these insecticides has led to the development of resistance, environmental pollution, bioaccumulation and harmful effects on non-target

organisms (Senthil-Nathan, 2020) <sup>[10]</sup>. Therefore, more attention has been paid to eco-friendly and sustainable approaches to mosquito control by researchers.

Botanical insecticides are promising alternatives to synthetic chemicals because they are biodegradable, renewable and have a wide range of biologically active secondary metabolites. Plant-derived compounds like alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides have exhibited insecticidal, larvicidal, ovicidal, repellent and growth-regulating actions against mosquito vectors (Pavela, 2015; Pavela *et al.*, 2019; Senthil-Nathan, 2020) <sup>[6, 7, 10]</sup>. Botanical compounds generally act through multiple modes of action, unlike common insecticides which generally act on only one physiological pathway, thus reducing the chances of resistance development (Pavela *et al.*, 2019; Senthil-Nathan, 2020) <sup>[7, 10]</sup>.

Vegetable peels generated from households and agricultural activities have drawn much attention in recent years as low-cost and sustainable sources of bioactive phytochemicals. Vegetable peels are important part of food processing waste and are rich in phenolic compounds, flavonoids, antioxidants, vitamins, tannins and many other secondary metabolites with various biological activities (Sagar *et al.*, 2018) <sup>[8]</sup>. Several studies have reported that vegetable peels often contain higher concentrations of bioactive compounds than their edible portions and thus are promising candidates for developing botanical larvicides. Their application not

only offers an eco-friendly method for mosquito control, but also contributes to sustainable waste management and value addition to agricultural by-products (Sagar *et al.*, 2018)<sup>[8]</sup>.

Common vegetables like potato (*Solanum tuberosum*), banana (*Musa paradisiaca*), pointed gourd (*Trichosanthes dioica*), sponge gourd (*Luffa acutangula*), bottle gourd (*Lagenaria siceraria*) and cucumber (*Cucumis sativus*) are rich sources of phytochemicals with insecticidal potential. Steroidal glycoalkaloids like  $\alpha$ -solanine and  $\alpha$ -chaconine are found in potato peels, whereas banana peels are rich in flavonoids, tannins, phenolics, and saponins. Similarly, cucurbit vegetables contain cucurbitacins, triterpenoids, flavonoids and phenolic compounds which may be responsible for larvicidal activity. However, comparative studies on the larvicidal efficacy of aqueous and ethanolic extracts of these commonly discarded vegetable peels against *Culex* spp. require careful evaluation before practical application (Sagar *et al.*, 2018; Senthil-Nathan, 2020)<sup>[8, 10]</sup>.

The solvent used for extraction has a significant influence on the yield and composition of phytochemicals extracted from plant materials. Polar compounds are extracted with distilled water, whilst aqueous ethanol can extract both polar and moderately polar phytochemicals, and to some extent, extracts with improved biological activity (Azwanida, 2015)<sup>[1]</sup>. Hence it is important to compare the aqueous and ethanolic extracts for identification of the most efficient method of extraction to recover the larvicidal constituents.

In the present study, the efficacy of the aqueous and ethanolic extracts was compared by determining the larval mortality at varying concentrations and time intervals and determining LC<sub>50</sub> values by log-probit analysis. The results are expected to aid in the development of environmentally sustainable botanical larvicides and to ease the use of vegetable peel waste as an environmentally sustainable resource for integrated mosquito management.

## Materials and Methods

### Study Design

The study evaluated the larvicidal potential of aqueous and ethanolic extracts of peels of potato (*Solanum tuberosum*), banana (*Musa acuminata*), pointed gourd (*Trichosanthes dioica*), sponge gourd (*Luffa acutangula*), bottle gourd (*Lagenaria siceraria*) and cucumber (*Cucumis sativus*) against third and fourth instar *Culex* spp. larvae. Phytochemical screening of the extracts was done for qualitative screening of the presence of major secondary metabolites. The larvicidal activity was then assessed at five concentrations (50–450 ppm) for 24, 48, 72, and 96 hours. Mortality data were subjected to Abbott's correction and log-probit analysis to determine LC<sub>50</sub>, regression equations and R<sup>2</sup> values.

### Experiment location

The experiment was conducted in the entomology laboratory of Vidyasagar College, Salt Lake Campus, Kolkata under ambient laboratory conditions (30 ± 2°C temperature, 75 ± 5% relative humidity).

### Collection and Identification of Mosquito Larvae

Third and fourth instar larvae of *Culex* spp. were collected from stagnant water of drains and water bodies of different areas. To reduce transportation stress larvae and water from

the original breeding habitats were collected in a plastic container.

Larvae were kept in the laboratory under ambient environmental conditions until the start of the bioassay. Only healthy and active third and fourth instar larvae were selected for experiments and damaged, injured or inactive individuals were discarded. The larvae were identified by the use of standard morphological characteristics described in mosquito identification manuals and other mosquito species were discarded.

### Collection of Vegetable Peel Samples

Fresh potato, banana, pointed gourd, sponge gourd, bottle gourd and cucumber were collected from the local markets. The vegetables were washed thoroughly with tap water and rinsed with distilled water to remove adhering soil particles, dust and other surface contaminants. The peels were gently peeled off with a sterile knife, cut into small pieces and prepared for drying.

### Preparation of Peel Powder

The vegetable peels were dried in hot air oven at a constant temperature till all the moisture was removed. Oven drying reduced microbial contamination and preserved the stability of bioactive phytochemicals in the plant materials.

The peels were dried separately and ground to a fine powder in an electric grinder. All powdered samples were stored in clean, labelled, airtight containers at room temperature until extraction.

### Preparation of Plant Extracts Materials

The powdered samples were placed in each of conical flasks. Then the solution was homogenized with two suitable solvents distilled water and ethanol. The solutions were kept at room temperature in the laboratory for 3 days. The conical flasks were shaken gently 3 times a day. After 3 days, the supernatant was carefully discarded and the remaining residue was collected in petri dish. The mixture collected in petri dish was dried in incubator 2-3 days. Then dried extract was obtained after 2-3 days.

### Phytochemical Screening

Some quantities of the residue were used for phytochemical screening. Qualitative phytochemical analysis was carried out for the presence of major secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, coumarins, glycosides and quinones by standard procedures. Flavonoids, phenols, terpenoids, coumarins, glycosides, quinones, alkaloids, saponins and tannins were identified by standard colorimetric or precipitation tests using concentrated sulphuric acid, ferric chloride, Mayer's reagent, lead acetate, sodium hydroxide, hydrochloric acid and glacial acetic acid. The appearance of characteristic colours, frothing or precipitates was taken as an indication of the presence of respective phytochemical constituents. The phytochemical profiles of the aqueous and ethanolic extracts were then correlated with their larvicidal activities against *Culex* spp. larvae.

### Preparation of Test Concentrations

The prepared different concentrations of vegetable peel extract were 50ppm, 150ppm, 250ppm, 350ppm and 450ppm. Each concentration was replicated in triplicate (R1, R2, R3). 1000ppm stock solution was prepared by mixing 0.1g peel extract powder with 100ml distilled water.

The 50 ppm solution was prepared by adding 5 ml of stock solution to 95 ml of distilled water, the 150 ppm solution was prepared by adding 15 ml of stock solution to 85 ml of distilled water, the 250 ppm solution was prepared by adding 25 ml of stock solution to 75 ml of distilled water, the 350 ppm solution was prepared by adding 35 ml of stock solution to 65 ml of distilled water, the 450 ppm solution was prepared by adding 45 ml stock solution to 55 ml distilled water.

### Larvicidal Bioassay

The larvicidal activity was evaluated under laboratory conditions using *Culex* spp. larvae of third and fourth instar. For each concentration three cups were prepared (labelled R1, R2, R3). Then, 10 *Culex* spp. larvae were transferred into each cup containing the concentration of peel extract using a dropper. After this procedure, all the cups were placed in a large tray, covered with newspaper (with some holes in it for proper ventilation) (figure 2).

Controls were kept separate during the experiment. The control for aqueous extracts was distilled water and for ethanolic extracts was ethanol but without the plant extract. During the exposure period, no larval food was supplied to avoid any possible interference with the bioassay. The experimental containers were kept under the same laboratory conditions throughout the study.

The cups were observed after 24, 48 72 and 96hrs. Larvae that did not respond to gentle stimulation with a fine glass rod were considered dead. The number of dead larvae was then counted and recorded after 24, 48, 72 and 96hrs respectively.

### Determination of Larval Mortality

Percentage larval mortality was calculated using the following formula:

Larval mortality (%) = (No. of dead larvae/Total no. of larvae exposed) x 100

Treatments resulting in total mortality (100%) of the thirty larvae tested were corrected using Abbott's correction to avoid computational errors during probit analysis. The equivalent probit value for 100% mortality was calculated to be 7.40.

### Log–Probit Analysis

Mortality percentages were transformed to corresponding probit values and the test concentrations to logarithmic (log 10) values.

Then probit mortality was plotted against log concentration according to the equation and linear regression analysis was performed. The equation is:

$Y = aX + b$

where:

- Y = probit mortality,
- X = logarithm (base 10) of concentration,
- a = regression slope,
- b = regression intercept.

For each vegetable peel extract and exposure time a different regression equation was developed. Regression

equations were then used to estimate LC<sub>50</sub> values as the concentrations that produce 50% of larval mortality.

The goodness-of-fit of each regression model was determined by calculating the coefficient of determination (R<sup>2</sup>). Lower LC<sub>50</sub> values were considered as indicators of higher larvicidal potency.

### Statistical Analysis

All experimental data were entered in Microsoft Excel and statistically analysed. From these, percentage mortality values were calculated for each treatment. The Probit transformation, the conversion of the concentration into logarithmic form, the linear regression analysis, the regression equations, the coefficients of determination (R<sup>2</sup>) and the LC<sub>50</sub> values were calculated separately for each exposure period (24, 48, 72 and 96 h) for each vegetable peel extract.

The larvicidal efficacy of the different vegetable peel extracts was evaluated by comparison of mortality percentages, regression characteristics, LC<sub>50</sub> values and relative performance of aqueous and ethanolic extracts.

## Results

### Preliminary phytochemical screening

The qualitative phytochemical analysis exhibited a significant variation in the composition of secondary metabolites among the six vegetable peel extracts and between the two extraction solvents. Aqueous and ethanolic extracts of banana (*Musa acuminata*) contained flavonoids, phenols, alkaloids, glycosides, saponins and tannins while quinones were only present in ethanolic extract and terpenoids were only present in aqueous extract (table 2). Phytochemical screening of potato (*Solanum tuberosum*) peel extracts revealed a rich profile of phytochemicals with flavonoids, phenols, alkaloids, glycosides and tannins in both solvents, while quinones were only present in the aqueous extract (table 1).

Phenols, alkaloids, glycosides and saponins were found in pointed gourd (*Trichosanthes dioica*) extracts in both solvents. The findings showed that terpenoids were present in both ethanolic and aqueous extracts whereas flavonoids and tannins were only present in the aqueous extract (table3). Sponge gourd (*Luffa acutangula*) exhibited comparatively lower phytochemical constituents and presence of terpenoids, coumarins, alkaloids, tannins and quinones were observed mainly in ethanolic extract whereas saponins and quinones were present in aqueous extract (table 4). The extracts of bottle gourd (*Lagenaria siceraria*) showed the presence of phenols, alkaloids, glycosides and saponins irrespective of the solvent used while coumarins and tannins varied with the solvent (table 5). Both solvent extracts of cucumber (*Cucumis sativus*) peel showed the presence of flavonoids, phenols, terpenoids, coumarins and saponins (table 6). Only alkaloids and quinones were detected in ethanolic extract and only glycosides were detected in aqueous extract (Sagata *et al.*, 2026) [9].

Overall, banana and potato peels extracts exhibited the widest range of phytochemical constituents whereas sponge gourd and bottle gourd had relatively lower metabolites detected. The results also showed that the extraction solvent affected the recovery of several phytochemical groups.

## Preliminary Phytochemical Analysis of Fruit and Vegetable Peel Extracts

**Table 1:** Preliminary phytochemical analysis of *S. tuberosum* peel extract

| Phytochemicals | <i>S. tuberosum</i> peel extract |                 |
|----------------|----------------------------------|-----------------|
|                | Ethanol                          | Distilled water |
| Flavonoids     | +                                | +               |
| Phenols        | +                                | +               |
| Terpenoids     | -                                | -               |
| Coumarins      | -                                | -               |
| Alkaloids      | +                                | +               |
| Glycoside      | +                                | +               |
| Saponin        | -                                | -               |
| Tannins        | +                                | +               |
| Quinones       | -                                | +               |

**Table 2:** Preliminary phytochemical analysis of *M. acuminata* peel extract

| Phytochemicals | <i>M. acuminata</i> peel extract |                 |
|----------------|----------------------------------|-----------------|
|                | Ethanol                          | Distilled water |
| Flavonoids     | +                                | +               |
| Phenols        | +                                | +               |
| Terpenoids     | -                                | +               |
| Coumarins      | -                                | -               |
| Alkaloids      | +                                | +               |
| Glycoside      | +                                | +               |
| Saponin        | +                                | +               |
| Tannins        | +                                | +               |
| Quinones       | +                                | -               |

**Table 3:** Preliminary phytochemical analysis of *T. dioica* peel extract

| Phytochemicals | <i>T. dioica</i> peel extract |                 |
|----------------|-------------------------------|-----------------|
|                | Ethanol                       | Distilled water |
| Flavonoids     | +                             | +               |
| Phenols        | +                             | +               |
| Terpenoids     | +                             | +               |
| Coumarins      | -                             | -               |
| Alkaloids      | +                             | +               |
| Glycoside      | +                             | +               |
| Saponin        | +                             | +               |
| Tannins        | -                             | -               |
| Quinones       | -                             | -               |

**Table 4:** Preliminary phytochemical analysis of *L. acutangula* peel extract

| Phytochemicals | <i>L. acutangula</i> peel extract |                 |
|----------------|-----------------------------------|-----------------|
|                | Ethanol                           | Distilled water |
| Flavonoids     | -                                 | -               |
| Phenols        | -                                 | -               |
| Terpenoids     | +                                 | -               |
| Coumarins      | +                                 | +               |
| Alkaloids      | +                                 | +               |
| Glycoside      | -                                 | -               |
| Saponin        | -                                 | +               |
| Tannins        | +                                 | -               |
| Quinones       | +                                 | +               |

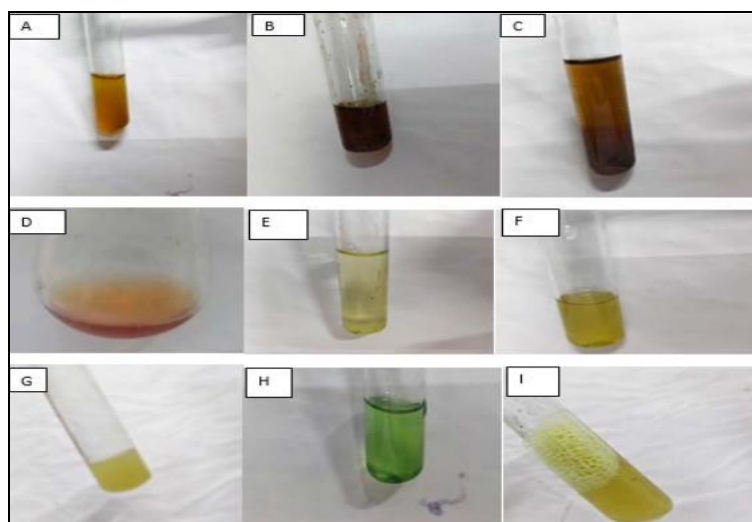
**Table 5:** Preliminary phytochemical analysis of *L. siceraria* peel extract

| Phytochemicals | <i>L. siceraria</i> peel extract |                 |
|----------------|----------------------------------|-----------------|
|                | Ethanol                          | Distilled water |
| Flavonoids     | -                                | -               |
| Phenols        | +                                | +               |
| Terpenoids     | -                                | +               |
| Coumarins      | +                                | -               |
| Alkaloids      | +                                | +               |
| Glycoside      | +                                | +               |
| Saponin        | +                                | +               |
| Tannins        | +                                | -               |
| Quinones       | -                                | +               |

**Table 6:** Preliminary phytochemical analysis of *C. sativus* peel extract

| Phytochemicals | <i>C. sativus</i> peel extract |                 |
|----------------|--------------------------------|-----------------|
|                | Ethanol                        | Distilled water |
| Flavonoids     | +                              | +               |
| Phenols        | +                              | +               |
| Terpenoids     | +                              | +               |
| Coumarins      | +                              | +               |
| Alkaloids      | +                              | -               |
| Glycoside      | -                              | +               |
| Saponin        | +                              | -               |
| Tannins        | -                              | -               |
| Quinones       | +                              | -               |

Where '+' indicates presence of phytochemicals and '-' indicates absence of phytochemicals.



**Fig 1:** Phytochemicals estimation of peels. [A]. Flavonoid [B]. Terpenoid [C]. Glycoside [D]. Tannin [E]. Alkaloid [F]. Coumarin [G]. Quinone [H]. Phenol [I]. Saponin

### Larvicidal activity of vegetable peel extracts against *Culex* spp. larvae

Larvicidal activity of aqueous and ethanolic vegetable peel extracts against third and fourth instar larvae of *Culex* spp. increased progressively with increase in concentration and exposure period (Table 7).

Among the aqueous extracts, potato peel was observed to have the highest larvicidal activity. Mortality increased from 33.33% at 50 ppm after 24 h to 86.67% at 450 ppm after 96 h. Banana peel extract showed the second highest activity among the aqueous extracts with mortality rates of 20.00 to 80.00% at the tested concentrations and exposure periods. Extracts of pointed gourd, sponge gourd and bottle gourd showed moderate larvicidal activity with maximum mortality of 76.67, 66.67 and 66.67 percent, respectively after 96 h exposure at 450 ppm.

In contrast, in the experiment, the minimum larvae killing activity was obtained by cucumber peel aqueous extract. The mortality rate ranged from as low as 3.33% at 50 ppm after 24 h to 36.67% at 450 ppm after 96 h, showing a relatively weak larvicidal efficacy.

The ethanolic extracts consistently yielded higher larval mortality than their respective aqueous extracts. The most effective treatment was the ethanolic extract of potato peel, causing 60.00% mortality at the lowest concentration (50 ppm) after 24 h and reaching total (100%) mortality at 450 ppm after 72 and 96 h. Mortality was greater than 90% at 350 and 450 ppm concentrations after prolonged exposure showing strong larvicidal potential.

The ethanolic extract of banana peel showed a significant larvicidal activity. The mortality increased from 26.67% at 50 ppm after 24 h to complete mortality (100%) at 450 ppm after 96 h. Mortality greater than 90 % was obtained at concentrations of 250 ppm and above after 96 h of exposure. The ethanolic extract of pointed gourd showed moderate to high larvicidal activity. Mortality was significantly increased with increasing concentration and exposure duration and reached 80.00% at 350 and 450 ppm after 96 h. Ethanolic extract of sponge gourd exhibited a similar concentration dependent response with a maximum mortality of 76.67% at 96 h.

The ethanolic extract of bottle gourd exhibited moderate larvicidal activity during the experiment with the mortality rate increasing from 20.00% at 50 ppm after 24 h to 66.67% at 450 ppm after 96 h. Ethanol extraction enhanced the activity in comparison with the aqueous extract but complete larval mortality was not achieved.

The ethanolic extract of cucumber peel was the least effective among all ethanolic extracts. The larvicidal potential was relatively low as the mortality was only 43.33% even at the highest concentration (450 ppm) and longest exposure period (96 h).

The effectiveness of ethanolic extracts after 96 h based on the overall mortality percentages was in the order:

Potato > Banana > Pointed gourd > Sponge gourd > Bottle gourd > Cucumber.

The same trend was observed for aqueous extracts although in general mortality rates were significantly lower (figure 3; figure 4; figure 5; figure 6; figure 7; figure 8)

### Log-probit regression analysis

Log-probit regression analysis showed a gradual decline in  $LC_{50}$  values with an increase in the exposure period for

almost all vegetable peel extracts (Table 8). Thus, the larvicidal activity increased after prolonged period of exposure.

Of the aqueous extracts, potato peel showed the greatest improvement over time, from an  $LC_{50}$  of 727.09 ppm at 24 h to only 24.07 ppm after 96 h. The banana peel showed similar trend with the  $LC_{50}$  values decreased from 2036.61 ppm to 31.23 ppm from 24 h to 96 h.  $LC_{50}$  values of pointed gourd, sponge gourd and bottle gourd extracts also showed significant reduction in exposure period, however their toxicities were less than potato peel. In contrast, cucumber peel extract had constantly very high  $LC_{50}$  values throughout the study, indicating poor larvicidal efficacy.

The ethanolic extracts showed generally much lower  $LC_{50}$  values than the aqueous extracts. The minimum  $LC_{50}$  values were observed for ethanolic extract of potato peels during the experiment, from 39.72 ppm (after 24 h) to 18.02 ppm (after 96 h). The banana peel ethanolic extract also showed increasing toxicity over time with the  $LC_{50}$  decreasing from 293.79 ppm to 25.79 ppm. Ethanolic extracts of pointed gourd, sponge gourd and bottle gourd showed moderate toxicity. The least toxic was the ethanolic extract of cucumber peel with  $LC_{50}$  values > 2,000 ppm even after 96 h.

The regression coefficients ( $R^2$ ) were in the range of 0.5567 to 0.9930. Most treatments showed acceptable goodness-of-fit ( $R^2 > 0.80$ ), indicating strong concentration response relationships. The regression models proved to be more significant for bottle gourd aqueous extract (72 h,  $R^2 = 0.9930$ ), banana aqueous extract (96 h,  $R^2 = 0.9864$ ), sponge gourd aqueous extract (96 h,  $R^2 = 0.9870$ ) and pointed gourd ethanolic extract (72 h,  $R^2 = 0.9673$ ). Some treatments exhibited lower  $R^2$  values, especially for potato and cucumber ethanolic extracts during the early exposure periods, suggesting comparatively weaker dose-response relationships.

### Comparative efficacy of aqueous and ethanolic extracts

A comparison of extraction solvents showed that the larvicidal efficacy of most of the vegetable peel extracts was consistently increased by ethanol. The ethanolic extracts showed higher percent mortality, lower  $LC_{50}$  values and generally more steep concentration response relationship than the corresponding aqueous extracts.

The ethanolic extract of potato peel was the most effective larvicide against *Culex* spp. larvae among all treatments, causing complete mortality at higher concentrations within 72–96 h and exhibiting the lowest  $LC_{50}$  values. The second most effective treatment was ethanolic extract of banana peel which also showed complete larval mortality after 96 h at 450 ppm. Pointed gourd and sponge gourd showed intermediate activity while bottle gourd was moderately effective. The cucumber peel showed consistently poor larvicidal performance irrespective of the extraction solvent. The results show that the extraction solvent and the exposure time had significant effect on the larvicidal efficacy with ethanolic extracts showing better mosquito larval control than aqueous extracts.

### Discussion

The present work showed that vegetable peel extracts have appreciable larvicidal activity against third and fourth instar larvae of *Culex* spp. However, their activity was highly

variable based on the plant species, extraction solvent, concentration and time of exposure. In general, larval mortality increased gradually with increasing extract concentration and duration of exposure and the LC<sub>50</sub> values decreased accordingly. These results indicate a definite dose-dependent and time-dependent toxic effect. This is typical of many plant-derived larvicides (Pavela, 2015; Benelli & Pavela, 2018; Benelli *et al.*, 2018) [2, 6].

Of the six vegetable peels tested, potato (*Solanum tuberosum*) had the highest larvicidal activity, particularly in the ethanolic extract. The ethanolic potato peel extract caused 100% larval mortality at 450 ppm after 72 and 96 hours and recorded lowest LC<sub>50</sub> value of 18.02 ppm after 96 hours. The extract of banana (*Musa acuminata*) peel also showed significant larvicidal potential, at the highest concentration, after 96 h, with a concomitant LC<sub>50</sub> of 25.79 ppm, resulting in 100% mortality. In contrast, *C. sativus* peel extracts were always found to have the least mortality and highest LC<sub>50</sub> values, suggesting relatively poor larvicidal efficacy. The efficacy of the tested vegetable peels based on the general mortality and toxicity data can be ranked as potato > banana > pointed gourd > sponge gourd > bottle gourd > cucumber.

Phytochemical composition of potato and banana peel extracts can account for their higher performance. Qualitative screening revealed the presence of a wide range of bioactive secondary metabolites in these peels including flavonoids, phenolic compounds, alkaloids, glycosides, tannins and saponins. Potato peel contains high levels of steroidal glycoalkaloids, including  $\alpha$ -solanine and  $\alpha$ -chaconine, which have been reported to disrupt cell membrane integrity, interfere with digestive physiology, and impair nervous system function in insects (Friedman, 2006; Friedman, 2015) [4, 5]. Banana peel is also found to be rich in phenolics, flavonoids and tannins which have insecticidal, antioxidant and antimicrobial activities (Pavela *et al.*, 2019; Senthil-Nathan, 2020) [7, 10]. These metabolites likely contributed to the high larvicidal activity observed in the present investigation.

A consistent result observed throughout the study was the better efficacy of ethanolic extracts than the respective aqueous extracts. Ethanolic extraction of most of the vegetable peels were higher in percentage mortality and lower in LC<sub>50</sub> values than extraction with distilled water. This difference is probably due to better extraction efficiency of ethanol which can dissolve a wider range of polar and semi-polar phytochemicals compared to water (Azwanida, 2015; Pavela *et al.*, 2019) [1, 7]. Similarly, previous studies have shown that hydroalcoholic solvents extract higher concentrations of phenolics, flavonoids, alkaloids and terpenoids and extracts with higher insecticidal activity (Pavela *et al.*, 2019) [7]. The present results indicate that the choice of solvent is important for increasing the larvicidal potential of plant materials.

In nearly all treatments, the LC<sub>50</sub> values decreased from 24 to 96 hours indicating that the susceptibility of larvae increased with longer exposure. Longer contact may have allowed for more absorption and accumulation of the phytochemicals in the larval tissues causing interference with vital physiological processes and eventual death. Similar decreases in LC<sub>50</sub> with increasing exposure time have been reported for many botanical extracts tested against mosquito larvae, indicating that many plant-derived compounds exert cumulative toxic effects rather than rapid

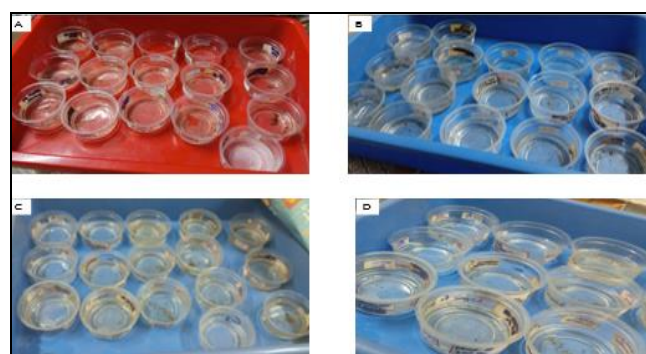
knockdown activity (Benelli & Pavela, 2018; Benelli *et al.*, 2018; Pavela *et al.*, 2019) [2, 7].

The regression analysis also supported the reliability of the bioassay. The majority of treatments gave a good coefficient of determination ( $R^2 > 0.80$ ), which shows a good correlation between concentration of extract and mortality of larvae.

Based on the observed larvicidal activity, it can be concluded that this activity is due to the combined effect of several phytochemicals rather than a single compound. Oxidative stress and inhibition of digestive enzymes are caused by phenolics and flavonoids. Tannins decrease nutrient utilization by precipitating proteins, saponins increase membrane permeability, alkaloids interfere with neurotransmission and terpenoids regulate respiration and hormones in insect larvae. The synergism between these compounds could explain why extracts containing a greater diversity of secondary metabolites, like potato and banana peels, have higher larvicidal activity than those with fewer phytochemical constituents.

From an applied point of view, the use of vegetable peels as botanical larvicides has many environmental and economic advantages. These peels are cheap, easily available and produced in large quantities as household and agricultural waste. Their conversion to mosquito control agents provides an eco-friendly approach for waste utilization and reduction in dependence on synthetic insecticides. Plant-based larvicides are usually biodegradable and less harmful to non-target organisms, and thus represent promising alternatives for inclusion in integrated mosquito management programmes.

The laboratory results are encouraging, but certain limitations need to be acknowledged. The study was conducted in laboratory conditions under controlled conditions using crude extracts and the active compounds responsible for the larvicidal activity were not isolated and quantified. Additional studies on phytochemical characterization, toxicity to non-target organisms, formulation development and field trials are needed before these extracts can be recommended for large-scale vector control.



**Fig 2:** Setup for mortality assessment of different vegetable peel extract on larvae of *Culex* spp. Larvae treated with A) *S. tuberosum*; B) *M. acuminata*; C) *T. diocia*; D) *L. acutangula*

The present study reveals that vegetable peel extracts especially ethanolic extracts of potato and banana peels possess significant larvicidal activity against *Culex* spp. larvae. These findings highlight the potential of agricultural waste as a renewable source of eco-friendly larvicides which deserves further investigation in the development of sustainable plant-based alternatives for mosquito control.

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