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Evaluation of the Effectiveness of Citrus aurantifolia Leaf extract as a Botanical Larvicide against mechanical vector of Human Parasitic Diseases

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Abstract
Musca domestica (housefly) is a medically important insect that act as a mechanical vector of many parasitic diseases affecting humans and animals worldwide. Increasing resistance to conventional chemical insecticide and their negative environmental impacts have created the need for eco-friendly alternatives. This study evaluated the larvicidal potential of Citrus aurantifolia leave extract against housefly larvae. Plant leave sample were collected, washed, air-dried under shade, ground into powder, and extracted using maceration by dissolving 250g of powdered bark in 4.5L of distilled water. Photochemical screening revealed the presence of alkaloid, saponins, flavonoids, and tannins, which are known for insecticidal properties. Laboratory reared larvae were exposed to different concentration of the extract (250-1250ppm) with four replicate each and mortality was recorded after 24, 48, and 72 hours. Result showed concentration and time- dependent larvicidal activity. The highest mortality (72%) was observed at 1250ppm after 24hhours, while lower concentration produced reduced mortality rate.LC_{50} and LC_{95} values indicated high larvicidal potency. No mortality was observed in the control group. The findings suggest that citrus aurantifolia leave extract has promising anti-larvicidal properties and could serve as a sustainable, eco-friendly alternative for controlling housefly population.

Keywords: Housefly, Larvicidal, Citrus aurantifolia, Mortality, Maceration

1. Introduction and Background of the Study

Citrus is a fruit that is widely consumed due to its numerous nutrients' benefits. C. aurantifolia, is a species that widely utilized, as a raw material for cosmetics, food flavouring, beverage flavour enhancement, and traditional medicine. It is a member of the Rutaceae plant family, which has 156 genera and 900species, and cultivated widely throughout the world (Indriyani et al., 2023). The most prevalent fly species worldwide is the housefly, or Musca

domestica. Over 100 pathogenic parasites have the potential to infect humans and animals and cause various parasitic diseases in which housefly serves as a mechanical vector of transmitting the parasite to human. Houseflies (Musca domestica) are known to transmit pathogens that cause cholera, typhoid, and dysentery in Nigeria. These diseases continue to be common, especially in places with open defecation and inadequate sanitation, creating ongoing public health problems (Banjo et al., 2010). Synthetic pesticides, which are the mainstay of common housefly management techniques, have over time led to resistance, environmental contamination, and harm to non-target species (Izah,2016) . (Adebayo, et al 2015). This has led to an urgent demand for alternative, eco-friendly pest control techniques. Therefore, the objective of this is to determine the Larvicidal potential of Citrus aurantifolia leave extract for sustainable control of disease-transmitting mechanical vector, in other to provide a natural and environmental sustainable solution for controlling housefly's population, to reducing the risk of parasitic disease transmission.

2. Methodology

2.1 Study Design

The study adopted a laboratory based experimental design to evaluate the larviciadl activity of Citrus aurantifolia leave extract against Musca domestica larvae. The experiment was involve different concentrations of the extract and varying exposure time (24, 48, and 72hours) with mortality recorded and statistically analyzed

2.2 Collection and Preparation of the Plant Material

Citrus aurantifolia leave was purchased from Oja Oba market in Osogbo Osun state, because the market is a major plant material market authenticated by (Adeyemi, et al 2020) in Nigeria and the plant material also authenticated by a botanist in the department of plant Biology of Osun State university Osogbo with a specimen voucher No 34331.
3000(g) of the citrus aurantifolia leaves was washed with clean water to remove debris, Air –dried under shade for 7-14 days to preserved phytochemicals, Ground into fine powered using a sterile electric blender, stored in airtight container until extraction.

2.3 Preparation of Citrus aurantifolia leave Extract (Maceration Method).

250g of the powdered Citrus aurantifolia leaves was macerated in 3.5litres of distillated water and vigorously stirred at one (1) hour interval for a period of four (4) hours, and it was allowed to stand on bench for an hour without disturbance.
The solution obtained was refrigerated for (24) hours and sieved with a laboratory sieve of 0.5usize,and allowed to stand for 1hour for its heavy particles to settle down then the supernatant was decanted and filtered using whatman filter paper and the residue was transferred into an open tray and dried in the oven at 100 degree for 2 days scraped with spatula and grinded into fine powder using laboratory pestle and mortar (Abiodun et al., 2021)
Percentage yield was calculated using this formula.

$$\%\ \text{yield} = \frac{\text{weight of the extract}}{\text{weight of powdered seed}}\ x\ \frac{100}{1}$$

Phytochemical Screening of Extract
Qualitative phytochemical analysis was conducted using standard procedure to detect major bioactive compound
Test of Major Phytochemicals
Alkalods (Mayer Test)
Extract + Mayer reagent
Cream precipitate indicates presence of alkanoid
Flavonodis (Alkaline Test)
Extract + NaOH solution
Yellow coloration that disappears with acid confirm flavonods
Tannins (Ferric Chloride Test)
Extract + 1% $FeCl_2$
Blue- black coloration indicated tannins
Saponins (Froth Test)
The extract was shaking with distilled water
Persistent froth indicated saponins

Terpenoids (Salkowski Test)
Extract + Chloroform + Concentrated H_2S0_4
Reddish brown interface indicated terpenoids
Phenols (Ferric Chloride Test)
Dark coloration indicated presence of phenolic compound

My scientific note: These tests provide preliminary qualitative confirmation of bioactive compounds responsible for larviciadl activity, although they do not concentration quantified

Rearing of Musca domeditca Larvae

The housefly larvae was collected from poultry decomposed manure sample and it was maintained under laboratory conditions at a temperature of 28+2 c and 60-70% relative humidity feed standard larvae diet (Wheat brain+ yeast+ water) in a sack covered with cheese cloth, The third instars larvae was used for bioassay due t their high sensitivity and uniform development stage. (Ahmed, et al., 2018).

Larvicidal bioassay Procedure

The Larvicidal bioassay ones were carried out by following the method of Biovain (2011) and Palacin et al (2019) respectively with little modification. The aqueous extract was dissolved in 1000ml of distilled water to form different concentrations such as 250ppm, 500ppm, 750ppm, 1000ppm; 1250ppm for plant extract in the plastic cup, 25 larvae was transferred into each test solution prepared. Four replicates were maintained for different. Concentrations and mortality of the larva was recorded after 24, 48 and 72 hours for the plant extract. Dead larvae were detected when appendages did not move when probed with needle. The mortality rate of the tested larva was then calculated after 72 hours by using the below formula.

$$\text{mortality percentage} = \frac{\text{No of dead larvae}}{\text{No of larvae tested}}\ x\ 100$$

$$\text{Corrected mortality \% } == \frac{\text{observed mortality rate } - \text{ control mortality rate}}{100 - \text{control mortality rate}}$$

$$\text{Observed mortality rate \% } = \frac{\text{control mortality \%} \times 100}{100 - \text{control mortality\%}}\ x\ 10$$ Eneojor,(2015)

Determination of LC_{50} and LC_{95}
Data preparation
Percentage mortality was corrected using Abbort's formular

Corrected mortality (%) = $\frac{\text{Expected} - \text{Control}}{(100 - \text{Control}} \times 100$

Probit Analysis
LC_{50} (Lather concentration for 50% mortality) and LC_{95} value was determined using probit regression analysis
Scientific note: LC_{50} is the most reliable indicator of larviciadal potency where lower LC_{50} indicate higher toxicity.
Statistical Analysis
A Two-way Analysis of variance (Two-Way ANOVA) was used
Factors:
Factor A: Extract concentration 250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm
Factor B: Exposure time: (24, 48,72hours)
Dependent Variable:
Larvae Mortality percentage

Model Structure
The ANOVA model
$Y_{ijk} = \mu + C_1 + T_j + (CT)_{ij} + \Sigma_{ijk}$
Y_{ijk} = Observed mortality
μ = Overall mean
C_1 = Effect of concentration
T_j = Effect of Time
$(CT)_{ij}$ = Interaction effect
Σ_{ijk} = Error term

Post Hoc Test
Turkey's HSD test was used to separate the means when the significant difference are observed at P<0.05)
Significant Level
Statistical significance was set at P<0.05)

Ethical and Safety consideration

Proper handling of biological specimen was ensured
Waste disposal was followed laboratory bioassay guideline
No vertebrate animal are involved hence ethical approval is minimal but institutional guideline was observed

Scientific Summary of methodology used for this study

This Methodology ensured a rigorous evaluation of the larvicidal potential of Citrus aurantifolia leave extract by intergrading phytochemical profiling dose- response bioassay and robust statistical analysis. The combined use of LD50 determination and ANOVA ensure both toxicity quantification and statistical validation of efficacy trends foster the reliability of findings

3. Results

Table 1: Summary of detected Phytochemicals

Photochemical Groups	Presence
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenol	+
Glycoside	-
Terpenoids	+

Interpretation of phytochemicals result

The quantitative phytochemical screening of Citrus aurantifolia leave extract revealed the presence of several biological active compounds including Alkaloids, Flavonoid, Tannins, saponins, Phenol, and terpenoids while Glycoside was not detected. The presence of Flavonoids, Phenols, and Tannin suggests strong antioxidant and insecticidal potential as these compounds are known to interfere with insect metabolic and neurological functions

Citrus aurantifolia leave Extract effect on Musca domestica larvae

Table 2: Larvae mortality rate at different concentrations of leave extract of Citrus aurantifolia with time rate of exposure

Citrus aurantifolia leave extract Concentration (ppm)	**No of larvae of Musca domestica**	**Mortality rate % at 24hrs**	**Mortality rate % at 48hrs**	**Mortality rate % at 72hrs**	**No(%) of alive larvae**
250 ppm	25	0(0.00%)	0(0.00%)	0(0.00%)	25(100%)
500 ppm	25	1(4%)	2(8%)	3(12%)	19(76%)
750 ppm	25	7(36%)	4(16%)	2(8%)	12(48%)
1000 ppm	25	15(60%)	6(24%)	4(16%)	0(0.00%)
1250 ppm	25	18(72%)	6(24%)	1(4%)	0(0.00%)
MEAN	25.00	8.2(32.8%)	3.6(14.4%)	2(8%)	11.2(44.8%)
CONTROL 0.00mg/L	25.00	0.00	0.00	0.00	25.00(100%)

Table 3: Concentration response larvicidal bioassay of Citrus aurantifolia leave extract against larvae of Musca domestica

Citrus aurantifolia bark	Time	%mortality	LC50(ppm)	LC95(ppm)
Aqueous extract	24hrs	8.2(32.8%)	896	1729
	48hrs	3.6(14.4%)	1812	2250
	72hrs	2(8%)	5250	7875

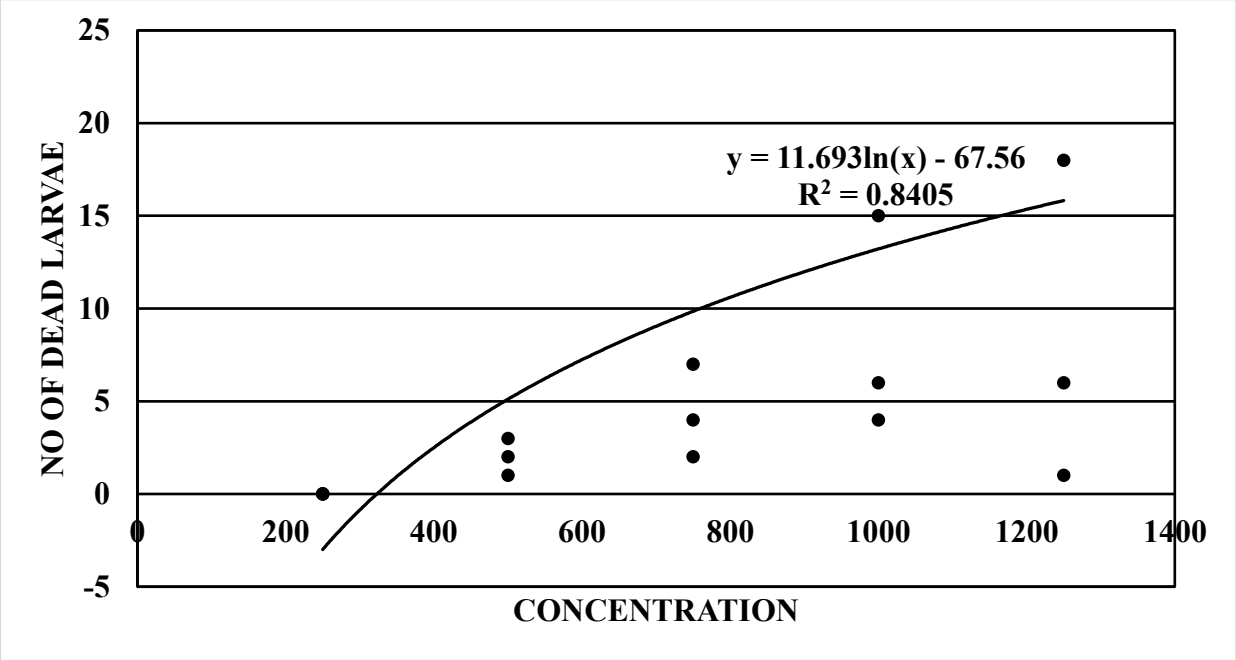

Figure 1: Mortality rates of larvae against the concentration (ppm)of aqueous extract of citrus aurantifolia leave and time period

4. Discussion

Overview of findings

This study evaluated the larvicidal activity of Citrus aurantifolia leave extract against Musca domesica larvae across different concentrations and exposure times, The results are discussed in relation to mortality trends, dose-dependent response relationship, LD50 values.

Table 1: Phytochemical Composition and Bioactivity Implications

- The qualitative phytochemical screening indicated the presence of key bioactive compounds such as alkanoids, Flavonoids, tannins, saponins, terpenoid and phenolic compounds these secondary metabolites are widely associated with insecticidal and larvicidal effect
- The observed Larvicidal activity can be attributed to these compounds through several mechanism
- Alkanoids may interfere with nervous system signaling in larvse
- Flavonoids and Phenols can distrust cellular metabolism and induce oxidative stress
- Ternnims may bind to protein and digestive enzyme reducing nutrient absorption
- Terpenoids are known to affect insect hormonal regulation and cuticle integrity

These findings support the hypothesis that Citrus aurantifolia bark contains biological active compound capable of inducing larval mortality

Table 2: Effect of Concentration on Larval Mortality and exposure time

A clear dose –dependent increase in larval mortality was observed across all exposure periods. Higher concentrations consistently produced greater mortality rates compared to lower concentration and control. This trend suggests that the extract exhibits strong concentration-dependent toxicity increasing concentration likely increase the availability of active phytochemicals interaction with larval physiological systems. The control group showed no mortality, which confirmed that mortality was due to the extract rather than environmental factors.

Furthermore, on the effect of exposure time rate on mortality increased progressively from 24 to 72 hours across all concentrations this signified a time-dependent lavicidal effect of the extract, where prolonged exposure enhances toxicity. The possible explanations include: gradual absorption of bioactive compounds through the larval cuticle Accumulation of toxic metabolites within the larval tissues over time. and delayed physiological disruption leading to eventual death.

The interaction effect of time reveled a significant interaction between concentration and exposure time ($p=<0.05$), indicating that the effect of concentration depends on the duration of exposure. This suggests that higher concentration is more effective when exposure time is extended. Lower concentrations may required longer exposure to achieve significant mortality, the combined effect of both factors enhance overall larvicidal efficacy

Table 3 and Figure 1, LC_{50} and LC_{95} interpretation, the probit analysis produced LC_{50} and LC_{95} values that reflect the toxicity level of the extract. The LC_{50} value indicates the concentration required to kill 50% of the larvae. A lower LC_{50} value demonstrates higher potency of the extract. The LC_{95} value provides insight into near complete population control efficiency. The regression line between log concentration and probit mortality showed a strong positive correlation, confirming a reliable dose response relationship

5. Comparison with Previous Studies

The larvicidal activity observed in the study is consistent with previous research on plant based insecticides. Many citrus species have demonstrated insecticidal properties due to their rich phytochemical composition, particularly terpernoids and flavonoids. When compared to similar botanical extract, Citrus aurantifolia leave extract shows a promising potential as a natural larvicide, although variation in the potency may deepen on method of extraction, environmental factors and solvent type.

6. Summary of Discussion

Overall, the results demonstrated Citrus aurantifolia leave extract exhibited a significant larvicidal activity that was characteristized by strong dose-dependent and time dependent effect. With statistically significant mortality difference and a reliable LC_{50} and LC_{95} toxicity indicator, bioactivity also supported by phytochemical constituents, these findings, support the potential use of the extract as a natural bio- insecticide vector control strategies.

Conclusion: This study evaluated the larvicidal activity of Citrus aurantifolia leave extract with the adoption of laboratory-based experimental design; the findings demonstrated that the extract has a significant larvicidal potential, with mortality rates increasing in a dose-dependent and time dependent manner across all treatment groups. Phytochemical screening also revealed the presence of important bioactive compounds including alkaloids, flavonoids, saponnin, tannins, phenolic compounds and terpenoids. These secondary metabolites are likely responsible for the observed lrvicidal activity through mechanisms such as enzyme inhibition, metabolic disruption, and interference with larval physiological processes. Also, probit analysis confirmed the effectiveness of the extract through LC_{50} and LC_{95} values, indicating measurable toxicity against the larvae.

It can be concluded from the study that Citrus aurantifolia leave extract is a well promising natural larvicidal agent that could serve as an alternative to synthetic insecticide in control of Musca domestica population.

Recommendations

Following the findings of this study, the following recommendations are made:

Further Isolation of Active Compounds

There is need for more research to focus on isolation and characterization of the specific bioactive compounds responsible for larvicidal effect of the Citrus aurantifolia leave extract.

Toxicological Evaluation on Non- Target Organisms
Additional studies should assess the safety of the extract on non-target organisms, beneficial insects, and humans, to ensure environmental safety.

Field Trials for Practical Application
The larvicidal effectiveness of the extract should be carried out under filed conditions to demine its real- world applicability in vector control program**.**

Formulation Development
There is need to develop the extract into stable formulations such sprays, granules, or emulssifiable concentrate to improve ease application and shelf life.

Integration into integrated Pest Management (IPM)
The leave extract should be considered as part of integrated pest management strategies to reduce more reliance on synthetic insecticides and minimize environment pollution**.**

Does Standardization Studies
There is need for further studies to established standardized effective dosages for large scale application to ensure consistent larvicidal performance

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