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Anti-larvicidal Potential of Citrus aurantifolia bark extract for Sustainable control of Mechanical Vectors of human parasitic diseases

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Abstract

Musca domestica (House fly) is one of the most widespread flies of medical importance worldwide and can mechanically transmit pathogenic parasites to humans and animals. Increasing resistance to conventional insecticides and the environmental concerns associated with chemical control have prompted the search for safer alternatives. The study aimed at determining the Phytochemical constituents, anti larvicidal activity, mortality rate and lethal concentration LC50 and LC95 of Citrus aurantifolia bark extracts against M. domestica larvae. The bark samples were collected washed, shade-dried, ground into powder, and extracted by macerating 150g of the powder in 4.5L of distilled water. Housefly larvae were reared under laboratory conditions following standard procedure. The findings show that Phytochemical screening revealed the presence of alkaloids, saponins, flvonoides, and tannins, compounds known for their insecticide properties. Five extract concentration (250, 500, 750, 1000, and 1250ppm) were tested with four replicate each. Larvae mortality was recorded after 24, 48, and 72hours, respectively. The highest mortality (18 larvae, 72%) occurred at 1250ppm after 24 hours, indicating that larvicidal activity increased with concentration. Probit analysis estimated LC50 and LC95 values of 5250ppm and 7875ppm respectively at 72hours, confirming a concentration and time-dependent effect. No larval mortality was observed in the control group. These findings demonstrate that C. aurantifolia bark extract has promising larvicidal activity against M.domestica larvae and may serve as an environmentally friendly alternative to synthetic insecticides for housefly control

Keywords: ***Musca domestica, Larvicidal, Citrus aurantifolia, Mortality, Maceration***

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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 (Indriyaniet 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 (Isman 2016). This has led to an urgent demand for alternative, eco-friendly pest control techniques. Therefore, the aim of this is to determine the antilarvicidal effectiveness of the aqueous extract of Citrus aurantifolia bark against Musca domestica larvae in other to provide a natural and environmental sustainable solution for controlling houseflies, thereby reducing the risk of parasitic disease transmission caused by this mechanical vector. Citrus is a fruit that is widely consumed due to its numerous nutrient's 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 (Isman 2016). 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 bark extract for sustainable control of disease-transmitting mechanical vector, in other to provide a natural and environmentally sustainable solution for controlling housefly's population, to reducing the risk of parasitic disease transmission.

Literature review

The increasing resistance of insect vectors to synthetic insecticide has intensified the search for environmentally sustainable alternatives. Botanical larvicides are plant derived sustenance that possesses insecticidal properties capable of killing insect's larvae or disrupting their normal development. They contain numerous secondary metabolites including, alkanoid, flavonids, terpenoids, limonoids,phenolic, tannins, saponnins and essential oils, many of which interfere with insect growth, feeding behavour, respiration, nervous system function, and cuticle formation. Resent reviews have highlighted botanical larvicide as promising tools within integrated vector management programmes because they are biodegradable, readily available, relatively inexpensive, and generally safer for non-targeted organisms than conventional chemical insecticides. Sarma et al., (2019) Unlike synthentic insecticide, which often act through a single biochemical pathway, botanical extracts usually contain multiple bioactive compounds that attack reduce s the likelihood of rapid inceticide resistance while enhancing larvicidal effectiveness. Furthermore, many phyctochemicals exhibit synergetic interactions that improve insecticidal activity when present together in crude extract (Priyanka   et al 2020)

Recent studies have demonstrated that extracts from neem (Azadirachta indica), eucalyptus (Eucalyptus globules), lemongrass (Cymbopogon citrus) and citrus species posssess siginificant lavicidal activity against mosquito larvae. Among citrus plants, Citrus aurantifolia has attracted considerable interest because of its abundance of limonoids, citral, limonene, linalool, flavonoids, and phenolic compounds with documented insecticidal activity. Despite increasing evidence supporting botanical lavicides, the majority of studies have focused on leaf extract, fruit peels, and essential oils, whereas the bark of citrus aurantifolia remains poorly investigated, Additionally, very few studies have evaluated its effectiveness against larvae of mechanical vectors, creating a clear research gap that this study intends to address.

Isfandaet al., (2023) on research on Anopheles spp. larvae, 10% C. aurantifolia leaf extract led to the death of 83% of mosquito larvae six hours after exposure and at a concentration of 37.466%, the C. aurantifolia leaf juice was used to test the efficacy of bio larvicides against Ae. aegypti has a 90-percent mortality rate. Sarma et al., (2019) studies showed that Citrus aurantifolia showed a good larvicidal properties against 4th instars larvae of Aedesaegypti and it was found to have rapid toxic action. Priyanka   et al 2020 study identified the larvicidal activity of citrus aurantifolia leaves decoction against the larvae of Aedes aegpti as an effort to discover natural photochemical. The result showed that C.aurantifolia devotion significantly decreased the number of larvae. The highest mortality has proved that 30% concentration of C. aurantifolia decoction was effective in killing almost 75% of the larvae as also shown with the 90%. Isman 2006 reported that 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. (Oguh et al., 2020) Reported health risks associated with the: exposure to chemical pesticides can lead to asthma, Birth defects, neurological Effects, cancer and Hormone disruptions

Mechanical vectors are organism that transmit pathogens from one host to another without the pathogen undergoes development or multiplication within the vector, unlike a biological vector, which play essential role in the life cycle of parasite, mechanical vectors simply transport infective stages of the pathogens on contaminated body surface, mouth parts legs, or through regurgitation and defecation. Common mechanical vectors include the housefly(Musca domestica), blowflies(Calliphora spp), Cockroaches (Periplanetra Americana and Blattella germanica), and fith- breeding insects that inhabit environments contaminated with fecal maters, refused= dumps, and decaying organic materials. , These insect contribute significantly to the transmission of protozoan cyst, helmith eggs, bacteria, fungi, and viruses to humans through contaminated of food, water, and households (Ganesan,et al., 20230)
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Mechanical transmission remains a significant public health challenge, particularly in low and middle –income countries where poor sanitation, inadequate waste disposal, overcrowding and insufficient vector control measures encourages vector proliferation. According to recent reviews, mechanical vectors are responsible for the spread of several intestinal parasitic infections, including Entamoeba histolitica, Giardia duodenalis, Ascaris lumbricoides,Trichuris trichuras,and hookworms. Children are particularly vulnerable because of poor hygiene practices and frequent exposure to contaminated environment. (Ignareki, et.al., 2023). Houseflies are regarded as one of the most important mechanical vectors due to their indiscriminate feeding habits and close association with humans. Adult flies frequently move between human food and contaminated substrate such as feces, sewage, carcasses, and refuse. During feeding and movement, infective organisms adhere to the insect body, hairs, wings, legs and mouthpart or pass through the digestive tract before being deposited on food or surface through regurgitation and excreta. This mode of transmission enables pathogens to spread rapidly within household, markets, hospitals, schools, and food processing environment (Indriyan et.al. 2023).

Cockroaches also contribute substantially to mechanical transmission because they inhabit sewage system, kitchens, drains, and refuse sites where they acquire numerous pathogenic microorganisms. Their nocturnal feeding behavior allows them to contaminate food preparation areas and domestic utensils with parasite and other pathogens. Several studies have isolated protozoan cysts, helminth eggs, and enteric bacteria from cockroach's body surface and gastrointestinal tracts, emphasizing their epidemiological importance in diseases transmission. (Ignareki, et.al.,2023).

World Health Organization continues to emphasize integrated vector control management (IVM) as an effective strategy for reducing vector borne diseases through environmental management, biological control, community participation and judicious use of insecticides, Recent research has increasingly focused on interrupting vector populations at the larvae stage because larvae are concentrated in a localized breeding habitat, making them easier to target than adult insects. Effective larval control reduces adult emergence and consequently decreases pathogen transmission (Ganesan . et al., 2023).

Although considerable attention has been devoted to mosquito vectors due to their role in malaria, dengue and other arbovirus diseases, relatively few studies have investigated botanical larvicides for controlling larvae of mechanical vectors such as houseflies and other synanthropic insects involved in the transmission of human parasitic diseases. This imbalance has created an important knowledge gap that warrant further investigation (Indriyan, et.al., 2023).

Most recent studies on botanical larvicide have concentrated on mosquito larvae, especially Aedes aegypti and Anopheles gambiase, because or their public health importance.comparatively,, little attention has been given to larvae of mechanical vectors responsible for transmitting intestinal parasites. Consequently, there is limited information on plant derived larvicides that can sustainably control these vectors, thereby justifying the present study.

The evaluation of mortality rate and lethal concentrations (LC50 and LC95) is a fundamental aspect of larvicidal bioassay because it provides quantitative evidence of the potency and efficacy of the botanical insecticides. Mortality rate refers to the percentage of larvae that die following exposure to different concentrations of test extract within a specified period, while the median lethal concentration (LC50) and the 95% lethal concentration (LC50) represent the concentrations required to kill 50% and 95% of the exposed larva population, respectively. These parameters are commonly estimated using probit analysis and are internationally recognized indicators for comparing the toxicity of natural and synthetic larvicide, Lower LC50 and LC95 values indicate greater larvicidal potency because smaller quantities of the extract are needed to achieve significant mortality (Fernado,et.al.,2024).

Botanical extract generally exibit concentration and time dependent larvicidal activity. Increasing the concentration of exposure duration typically result in higher larval mortality due to greater uptake of bioactive phytochemicals. Recent reviews have shown that phytochemicals such as limonoids, flavonoids, terpernoids, alkaloids and phenolic compounds disrupt the insect nervous system, interfere with respiration, inhibit feeding, and impair molting, leading to progressive larval death. These multiple mechanism of action also reduces the likelihood of resistance development compared with conventional synthetic insect iced. (Indriyan, e.al., 2023).

Citrus aurantifolia has attracted considerable attention as a source of botanical insecticide because its tissues contain limonene, coumarins, flavonoids, and other bioactive metabolite with proven insecticidal activity, (Indriyam et,al.,2023) reviewed studies on diffent parts of C. aurantifolia and reported that larvicidal activity consistently increase with extract concentration. The review further noted that limonoids and essential oil constitutes were primarily responsible for larval mortality by disturbing physiological and metabolic processes in insects. Although most published studies focused on mosquito larvae, the findings demonstrate that Caurantifolia possesses broad spectrum larvicidal potential (kokdener, (2023).

Studies on citrus derived insecticides have also demonstrated that lethal concentration values decrease with longer exposure periods for example, a study evaluating ethanolic extract of several Citrus seed species against Culex mosquito larvae reported concentration dependent mortality, with LC50 and LC90 values decreasing markedly after 48hours of exposure. The author concluded that C. aurantifolia extract remained effective at relatively low concentratios during prolonged exposure, highlighting the potential of Citrus aurantifolia derived product as eco-friendly larvicides. (Noorazlan, et.al. 2024).

Although many investigations have focused on mosquitoes' larvae, research on Musca domestic (Housefly) larvae has also demonstrated the susceptablity of this species to plant derived essential oils and botanical extract. Kokdener(2023) reported that several essential oils produced significant larval and pupal mortality in m.domestica, with mortality increasing as concentration increased. The study emphasized that botanical that botanical products can effectively interfere with larvae development and reduce adult emergence making larval control a practical strategy for suppressing housefly population. housefly is one of the most important mechanical vectors of human parasitic and enteric diseases because it breeds in decomposing organic matter, animal manure, and refuse dumps, where larvae develop before emerging as adults capable of contaminating food and water. Targeting the larvae stage is particularly advantageous because larvae are confined to breeding habitats and are more vulnerable to toxic compounds than highly mobile adults. Consequently, determining mortality rates together with LC5and LC95 value provides essential information for assessing whether a botanical extract has sufficient potency for practical vector control application (Ojo, et.al., 2024).

Despite the growing interest in Citrus base botanical insecticides, most recent studies have evaluated extracts from leaves, fruits peels, seeds, or essential oils against mosquito vaecor such as Aedes aegypi and Culex spp. There is a scarcity of studies investigating the larvicidal efficacy of Citrus aurantifolia bark extract against Musca domestica larvae. Furthermore, limited information is on the (LC50 and LC95) of C. aurantifolia bark extract against M. domestica larvae thereby contributing evidence for the development of sustainable, plant based larvicide for the control of human parasitic diseases.

Methodology

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

Plant Materials collection and identification

Citrus aurantifolia bark was purchased from Oja Oba market in Osogbo Osun state, Nigeria and authenticated by a botanist in the department of plant Biology of Osun State university Osogbo with a specimen voucher No 34331.

Preparation of Plant Bark

3000(g) of the Citrus aurantifolia bark was cleaned under running water and once under sterilized distilled water and chopped into piece for easy drying at room 370C temperature, grinded into powdered manually with pestle and mortar and stored in a plastic container.

Extraction of aqueous extract of the Plant Material

150g of the powdered Citrus aurantifolia bark was macerated in 4.5litres 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.

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

Rearing of Housefly Colony to Obtain the Larva

The culture of housefly from poultry manure sample was maintained at a temperature of 28+2 c and at 60-70% relative humidity in a sack covered with cheese cloth, Hatched larva was checked daily until the larva stage emerge.

Larvicidal Efficacy of the Plant Bark Extract

The Larvicidal bioassay ones was carried out by following the method of Biovain (2011) and Palacinet al (2019) respectively with few 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.

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

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

$$\text{Observed mortality rate \%} = \frac{\text{control mortality \%} \times 100}{100 - \text{control mortality\%}} \times 10$$

Findings and Analysis of data

The data obtained were arranged in a spread sheet with Microsoft Excel software version 2017, Leather concentration LC50%, 95%, slope was obtained by probit analysis with the aid of the polo plus software. Statistical significant difference in the LC50 and LC95 values were obtained on non-overlap of 95% confidence interval (Hematpooret al., 2019). The analysis of variance, ANOVA, Turkish test Significance level was also carried out.

Findings

Anti-larvicidal effectiveness of the aqueous extract of Citrus aurantifolia bark against Musca domestica larvae

Table 1: The larvae mortality rate at different concentrations of bark extract of Citrus aurantifolia with exposure time rate

Citrus aurantifolia bark 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%)

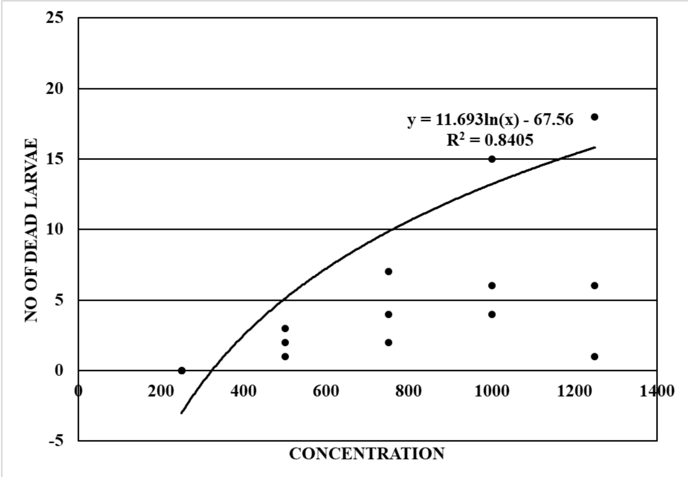
Source: The data obtained from the Quantitative analysis of the Musca domestica larvae mortality rate at different concentrations of Citrus aurantifolia bark extract with varying exposure time.

Table 2: Concentration response larvicidal bioassay of Citrus aurantifolia bark 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

Source: The Data obtained from Probit regression Analysis to determine the LC50 and LC95 Lather concentration of larvae mortality rate at 50% and 90%

Figure 1: Mortality rates of Musca domastica larvae against the concentrations (ppm)of aqueous extract of citrus aurantifolia bark



Source: The quantitative data graph of Musca domestical larvae mortality rates against the different concentrations (ppm)of aqueous extract of citrus aurantifolia bark with exposure time rate

Discussion

Larvicidal efficacy of Citrus aurantifolia bark extract was carried out in order to determine how effective aqueous lime bark extract was at killing housefly larvae. The findings of the present study categorically indicated that the larvicidal ineffectiveness of Citrus aurantifolia aqueous bark extract on Musca domestica larvae is time-dependent and dose-dependent, such that the higher the concentration of extract, the higher the mortality rate of the larvae. In addition, longer exposure durations such as 24, 48, and 72 hours, the higher the mortality rate levels even at low concentrations.

The trend in dose-dependency reveals that active photochemical compounds contained in Citrus aurantifolia, namely flavonoids, saponins, and limonoids, increasingly become toxic towards the larvae at higher concentrations. This concurs with findings by Odewoleet al. (2023), who noted high mortality of Aedes larvae exposed to higher doses of Citrus aurantifolia extract band this established that Musca domestica larval mortality was higher when concentrations of the plant extracts were raised, which was evidence of dose-dependent effect of the extract. From table 1, only 4% in 24 hours,8% in 48hours and 12% of the larvae were dead after 72 hours at the lower doses (500 ppm) and also at 750ppm,36% in 24hours,16% in48hours,8%in 72 hours death was recorded and this goes with the findings of Odewoleet al., (2023) who indicated that the lower concentration of Citrus aurantifolia had surviving larvae at a specific period.

A larger number of experimental larvae at lower concentration survived the dose while higher concentration recorded higher mortality of S. frugiperda. The maximum concentration that eliminated the larvae of Musca domestica is at 1000ppm which eliminated 60% at 24hrs and the remaining 24%at 48 hours and 16%at 72hours and also 1250 ppm which eliminated 72% at 24hrs and 18% at 48hrs and 4% at 72hours similarly, In a study conducted by Isfandaet al., (2023) using Anopheles spp. larvae, 10% C. aurantifolia leaf extract resulted to the death of 83% of mosquito larvae in 6 hours after exposure and at a concentration of 37.466%, Obembeet. al., (2022) reported findings on the larvicidal effect of citrus peels extract and the result of mortality rates induced by the different Citrus peel extracts against Culexpipiens mosquito larvae indicated that the Citrus aurantifolia showed the highest larvicidal activity against the larvae and it brought about the death of all the mosquito larvae (100%), C. aurantifolia peel extract revealed a consistently higher mortality rate at the different concentrations.

Priyanka et al., (2020) reported that the Citrus. aurantifolia decoction significantly reduction in the number of larvae such that the highest mortality rate was recorded in 30% concentration with a total of 224 in motile larvae. From the results in table 1, it shows that citrus aurantiifolia plant bark aqueous extract has a lavicidal efficacy on the larvae of housefly and can be used as an effective control and this aligns with recent studies of Sarmaet al., (2019) Who stated that Citrusaurantifolia showed a good a larvicidal properties against 4th instar larvae of Aedesaegypti and it was found to have rapid toxic action. And also, Anamika et al. (2025) examined the mosquito larvicidal properties of various Citrus species, highlighting their effectiveness against Anopheles, Aedes, and Culex larvae and it emphasized the eco-friendly nature of citrus-based larvicides, which offer an alternative to synthetic pesticides that often lead to insecticide resistance and environmental concerns.

Khanikoret al. (2021) explored the insecticidal effects of citrus essential oils, concluding that citrus exhibited moderate effectiveness compared to other botanicals, yet still contributed to larval mortality..From table 2, the lethal concentration (LC50) values in the present study indicated that the concentration required killing 50% of the larvae increases over time, with LC50 values of 896ppm at 24 hours, 1812 ppm at 48 hours, and 5250ppm at 72 hours. This suggested that prolonged exposure improves the larvicidal efficacy of citrus bark extract. The LC95 values, which represent the concentration needed for 95% mortality, were higher, with 1729ppm at 24 hours, 2250 ppm at 48 hours, and 7875 ppm at 72 hours and it goes with the findings of Ignarekiet al., (2023) who reported that Anopheles gambiae larvae treated with methanolic extracts of Citrus aurantiifolia and the LC50 and LC95 values (mg/mL) after 24 hours of exposure. Mortality rate of Citrus aurantiifoliamethanolic extract ranging from 38% at 7.5 mg/mL to 99% at 30 mg/mL with a LC50 of 9.82 mg/mL and LC95 of 27.09 mg/mL.

Similarly, methanolic extracts of C. aurantifolia peels exhibited potent larvicidal effects on Anopheles gambiae, with an LC50 of 9.82 mg/mL. The analysis of the LC50 and LC95 of all the concentrations against larvae of Musca domestica (table 2) indicated comparability of LCs values among them indicating activity of citrus aurantiifolia regardless of insect resistance and this goes in line with Sarma et al., (2019) who studied and reported that Citrus aurantifolia showed rapid effect with LC50 value of 128.81 ppm at 24 h which reduced to 106.77 ppm at 72 h while the leaf oil showed slow effect with LC50 value of 188.59 ppm, 107.37 ppm and 104.59 ppm at 24 hours, 48 hours and 72 hours respectively. In addition, Priyankaet al (2020), performed Probit analysis and reported that LC50 was 38.5% and 6.6% at 24hours and 48 hours, respectively. The highest rate of killing the larvae was taken at LC60 with 91.6% for 24 hours and LC65 64.4% for 48 hours.

In addition, the finding in figure 1 which is the time-based mortality trends observed in this study indicated that Citrus aurantifolia bark extract requires extended exposure to achieve maximum efficacy. While lower concentrations showed no immediate effect, higher concentrations exhibited a gradual increase in mortality rates, with highest effectiveness observed at 72 hours. This in line with the findings from Odewoleet al (2023), who noted that the higher the concentrations, the higher the mortality rate and the lower the concentrations, the lower the mortality rate. Therefore, this suggest that C. aurantifolia extracts have significant potential as natural bio-insecticides for house fly control, offering an alternative to synthetic insecticides and potentially reducing the risk of insect resistance.

The quantitative phytochemical screening of Citrus aurantifolia bark 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. Similarly, the detection of alkaloids and terpenoids indicate possible neurotoxic and growth inhibiting effects on insect larvae.

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 negligible or no mortality, confirming that mortality was due to the extract rather than environmental factors. Therefore, 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 require longer exposure to achieve significant mortality; the combined effect of both factors enhance overall larvicidal efficacy.

In addition, the LC50 and LC95 interpretation, by the probit regression analysis produced LC50 and LC95 values that reflect the toxicity level of the extract. The LC50 value indicates the concentration required to kill 50% of the larvae. A lower LC50 value demonstrates higher potency of the extract. The LC95 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.

Conclusion

This study evaluated the larvicidal activity of Citrus aurantifolia bark extract using a laboratory-based experimental design; the findings demonstrated that the extract possesses significant larvicidal potential, with mortality rates increasing in a dose-dependent and time dependent manner across all treatment groups. Phytochemical screening revealed the presence of important bioactive compounds including alkaloids, flavonoids, tannins, saponnin, terpenoids and phenolic compounds, 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. The probit analysis confirmed the effectiveness of the extract through LC50 and LC95 values, indicating measurable toxicity against the larvae.

Over all the study concludes that Citrus aurantifolia bark extract is a promising natural larvicidal agent that could serve as an alternative to synthetic insecticide in control of Musca domestica population.

Recommendations

Based on the findings of this study, the following recommendations are made

Further Isolation of Active compounds.

Further research should focus on isolation and characterization the specific bioactive compounds responsible for larvicidal activity in Citrus aurantifolia bark 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 evaluated under filed conditions to demine its real- world applicability in vector control program.

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