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Evaluation of *Raphanus sativus* (Radish) Leaf Extract and Its Insecticidal Potential against *Drosophila melanogaster* (Fruit Fly)

Keran Tomson[1], Amrutha Thanku[2], Varada Gigi[3], Niranjana S. Kumar[4], Shaik Mohammad Ashwaq[5], Chris Paul P. Pagaoa[6]
University of Northern Philippines, Philippines, Philippines
[1]tomson.keran@unp.edu.ph
[2]thanku.amrutha@unp.edu.ph
[3]gigi.varada@unp.edu.ph
[4]meds_niranjana.sajeevkumar@unp.edu.ph
[5]shaikmohammad.ashwaq@unp.edu.ph
[6]chrispaul.pagaoa@unp.edu.ph

ABSTRACT

Insects pose significant ecological threats and act as vectors for pathogenic microorganisms. In addition, insects damage crops, leading to substantial economic losses. Traditionally, chemical insecticides have been employed to manage insect populations; however, their detrimental effects on the environment and human health have raised concerns. The study evaluated the potential of Raphanus sativus as an alternative natural insecticide. Contact toxicity and mortality assays were used to evaluate the insecticidal efficacy of R. sativus leaf extract in varying concentrations against D. melanogaster larvae and adults. The insecticidal activity was assessed by measuring percentage mortality at three different time intervals (30, 45, and 60 minutes). Statistical analyses, including ANOVA and Scheffe's post-hoc tests, were conducted to identify significant differences between treatments. The findings demonstrated that R. sativus leaf extract exhibited insecticidal activity against D. melanogaster larvae after 45 and 60 minutes of exposure. The extract showed promising efficacy against adult D. melanogaster, with insecticidal activity comparable to the chemical insecticide malathion at all exposure times (30, 45, and 60 minutes). These results suggest that R. sativus leaf extract holds potential as an effective and environmentally safer alternative to chemical insecticides in pest management.

Keywords: *insecticidal, contact toxicity, mortality test*

INTRODUCTION

Agriculture plays a crucial role in the global economy by providing humanity with the basic needs and raw materials required for industrial development (Navarro et al., 2020). Pest insects negatively impact agriculture, livestock, human health, and the environment by damaging crops, disrupting food production, parasitizing livestock, and posing health hazards (Corpuz & Savella, 2019). Globally, pathogens and pests, including viruses, bacteria, fungi, nematodes, arthropods, and parasitic plants, significantly reduce the yield of major crops,

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causing losses of 10-28% in wheat, 45-41% in maize, 8-21% in potatoes, and 11-32% in soybeans (Kan-Rice, 2019).

Several management approaches have been conducted to minimize these losses, including chemical, biological, physical, and cultural methods. The use of pesticides has contributed immensely to the increase in agricultural productivity and improving human health, particularly to eradicating vector-borne diseases. However, it has been established that the use of synthetic organic pesticides, particularly chlorinated hydrocarbons, leads to severe environmental pollution (water, air, and soil), affecting human health and causing the death of non-target organisms, plants, and animals, mainly fishes (Biswas et al., 2014).

There is an increasing trend in the use of botanicals, with more than 2400 plant species identified for their insecticidal and anti-pathogenic properties (Karunamoorthi, 2012). Plant extracts and essential oils are safe, eco-friendly, and more compatible with environmental components than synthetic pesticides. On the other hand, plant-based products are cheap, bio-degradable, and therefore environmentally friendly (Souto, 2021).

Raphanus sativus (Radish) is grown mainly for its thickened fleshy root. The spice of radishes depends on the content of isothiocyanates, which varies with cultivar and environmental conditions. Its main compound is 4-methylthio-3-trans-butenyl isothiocyanate. Glucosinolates, which are the precursors of isothiocyanates, are also present. These compounds have long been known for their fungicidal, bactericidal, nematicidal, and allelopathic properties and have recently attracted attention because of their chemoprotective attributes against cancer (Schippers, 2004).

R. sativus has been studied for its potential as an alternative insecticide. The plant root extracts have promising insecticidal properties against *A. gossypii* (Ibrahim et al., 2020). However, no studies have been conducted yet to evaluate the insecticidal potential of the *R. sativus* leaf extract against *Drosophila melanogaster*.

Objectives of the Study

This study aimed to evaluate the potential of *Raphanus sativus* leaf extract as a natural insecticide by investigating its contact toxicity and insecticidal efficacy against *Drosophila melanogaster* larvae and adults. Specifically, it sought to determine the mortality rates of *D. melanogaster* larvae and adults when exposed to varying concentrations of *R. sativus* leaf extract and to assess whether significant differences existed in the percentage mortality of *D. melanogaster* larvae based on contact toxicity and mortality tests.

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METHODOLOGY

The study was conducted to evaluate the insecticidal activity of *R. sativus* aqueous leaf extract against *D. melanogaster*. The research used a quantitative design using experimental methods in a laboratory setup.

Preparation of Plant Extract

The leaves of *R. sativus* were collected and washed with distilled water and air-dried under shade for 2-3 days at room temperature (Alkan et al., 2015). The dried leaves were cut into fine pieces using scissors and ground using a grinder machine into fine powder (Riaz et al., 2018). The aqueous extract was prepared at a ratio of 20 g of dried plant material to 100 ml of distilled water. The extract was filtered by using Whatman No.1 filter paper. The filtrate was stirred and heated until a yield of 20 ml was obtained. The extracts were stored in an amber glass and protected from light for up to 24 hours before bioassays (Nascimento, 2022). The *R. sativus* leaves aqueous crude extract was prepared for different concentrations. The concentrations used were 25% (v/v), 50% (v/v), 75% (v/v), and 100% (v/v). These extract concentrations were prepared as follows: The 25% (v/v) concentration was prepared by diluting 1 ml of the stock solution with 3 ml of solvent to make up 4 ml. The 50% (v/v) concentration was prepared by diluting 2 ml of the stock solution with 2 ml of the solvent to make up 4 ml, while for the 75% (v/v) concentration, 1 ml of the solvent was added to 3 ml of stock solution to make up 4 ml (Gitahi et al., 2021).

Culture of the Test organism

Wild-type *D. melanogaster* was obtained from the Vigan Market, Vigan City fruit section, using ripe bananas as bait and primary food source. The culture was raised and maintained on an artificial diet with a photoperiod of 16:8 (light: dark) hrs. The artificial diet contains brewers' yeast (60 g), glucose (80 g), and agar (12 g) in 1000 mL of distilled water (Nair & Kavrekar, 2017). After collection, wild *D. melanogaster* was placed inside the culture containers containing the artificial diet to mate and leave for five days to obtain the third instar larvae. The third instar larvae were identified based on their life cycle. Adults and larvae of *D. melanogaster* were used in the experiment.

Contact Toxicity Test of the Plant Extract against D. melanogaster Larvae

A mortality bioassay was conducted under standard room conditions (Temp. 25∘C). Fifteen late-third instar larvae of *D. melanogaster* were selected for each set of treatments. Larvae were dipped into the different treatments for two minutes and then transferred back into the rearing medium. Mortality of *D. melanogaster* larvae was observed after time intervals of 30, 45, and 60 minutes. Each treatment was replicated three times. Further, positive control, Malathion, was used to compare its efficacy with plant extract (Alkan et al.,

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2015). The insects were considered dead after observing no movements following gentle probing with a needle (Sousa et al., 2024).

Insecticidal Activity of Plant Extract against Adult D. melanogaster

Filter papers were saturated with one drop of the different treatments (Khan et al., 2017). The saturated filter papers were then placed in Petri dishes. After drying, five adult *D. melanogaster* were introduced in each petri dish with treated filter papers. Mortality was assessed by direct observation. The insects were considered dead after observing no leg or antennal movements following gentle probing with a needle. The mortality of the adult *D. melanogaster* with different treatments was recorded after 30, 45, and 60 minutes (Nair et al., 2017).

Percentage mortality was calculated by dividing the number of dead by the total number of test organisms introduced multiplied by 100 (Abraham et al., 2015).

$$\text{Mortality (\%)} = \frac{\text{Number of dead test organisms in sample} * 100}{\text{Total number of test organisms used in each treatment}} \quad (1)$$

Statistical Treatment of Data

One-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of three or more independent (unrelated) groups. Lastly, the Scheffe test was used to determine which compared pairs significantly differed.

RESULTS AND DISCUSSIONS

Mortality rates of *D. melanogaster*

The effect of various treatments on the larval mortality of *D. melanogaster* is presented in Table 1. The data indicates that Treatment 1 (T_1), the positive control utilizing Malathion, achieved the highest larval mortality rate, with 100% mortality recorded at 30, 45, and 60 minutes of exposure. Following T_1, Treatment 5 (T_5), which consisted of the crude extract of *R. sativus* (radish), demonstrated substantial insecticidal activity, with larval mortality rates of 80%, 86.7%, and 100% after 30, 45, and 60 minutes, respectively.

These findings highlight the promising insecticidal activity of *R. sativus* extract, particularly at higher concentrations, against *D. melanogaster* larvae. The ability of the extract to match the efficacy of a widely used chemical pesticide like Malathion underscores its potential application as a sustainable and environmentally friendly alternative in pest management practices.

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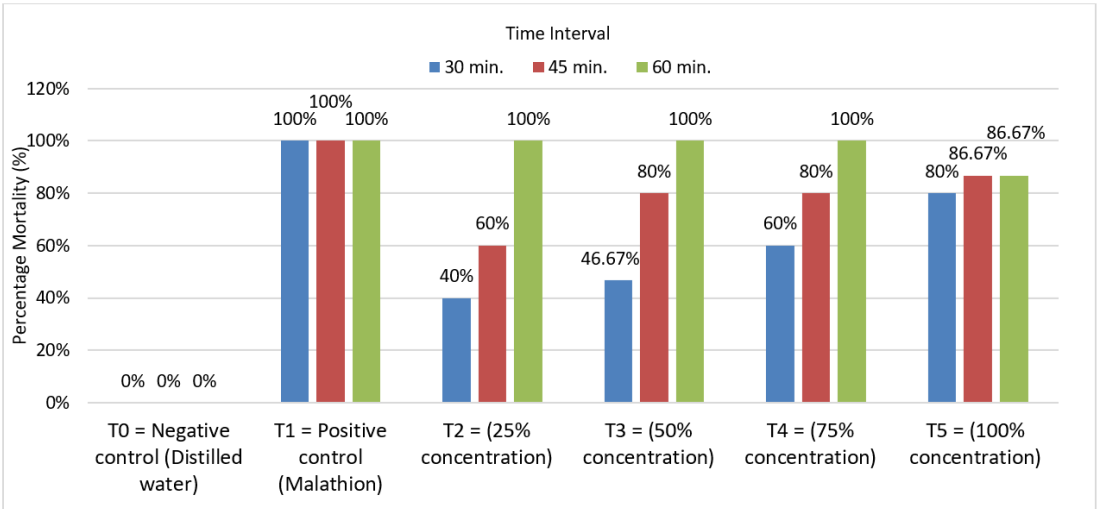
R. sativus contains various bioactive compounds, including flavonoids and glucosinolates, contributing to its insecticidal and pesticidal properties (Seimandi et al., 2023). These compounds are known for their antimicrobial, antioxidant, and insecticidal activities, making *R. sativus* a promising candidate for natural pest control. Flavonoids and glucosinolates are natural compounds found in various plants and are known for their potential insecticidal properties. These compounds are being explored as alternatives to synthetic pesticides due to their environmental and health benefits. Flavonoids such as quercetin and kaempferol have been identified in several plants and exhibit significant insecticidal activities. For instance, quercetin 3-O-β-D-glucoside from *Annona mucosa* showed high activity against pests like *S. zeamais* and *P. truncatus*, although it was less effective than commercial insecticides like deltamethrin (Makenzi et al., 2019). Similarly, flavonoids from *Ricinus communis* demonstrated potential insecticidal and ovicidal activities against *Callosobruchus chinensis* (Upasani et al., 2003). Flavonoids can inhibit detoxification enzymes such as glutathione S-transferases (GSTs) and esterases, which are crucial for insecticide resistance. For instance, taxifolin from conifer trees enhances the efficacy of insecticides like Guthion by inhibiting these enzymes, thereby increasing insect mortality (Wang, 2016).

Table 1

Insecticidal Activity of the Different Treatments against D. Melanogaster Larvae Using Contact Toxicity Test with the Time Intervals of 30, 45, and 60 Minutes terms of Percentage Mortality



On the other hand, glucosinolates, extracted from plants like *Tropaeolum tuberosum*, have shown effective insecticidal properties. They were found to cause high mortality rates in

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aphid pests when used alone or in combination with capsaicinoids (Cuadrado et al., 2019). The insecticidal activity of glucosinolates is linked to their breakdown products, which have toxic effects on herbivores. Insecticides can upregulate the biosynthesis of glucosinolates, thereby boosting the plant's natural defense system.

The observed insecticidal activity of *R. sativus* extract against *D. melanogaster* larvae aligns with similar studies investigating plant-derived insecticides. For instance, a study by Khan et al. (2017) demonstrated the larvicidal efficacy of various plant extracts against *D. melanogaster*, with *R. sativus* showing significant mortality effects. The methylene chloride fraction of R. sativus root extracts exhibited the highest insecticidal activity against both adults and second-instar nymphs of *A. gossypii*. Similarly, Khater (2015) reported that plant extracts from *R. sativus* exhibited comparable or even superior insecticidal activity to synthetic chemicals like Malathion. In another study, Jbilou et al. (2006) found that plant-derived insecticides can effectively control pest populations, supporting the notion that *R. sativus* extract holds promise as an alternative to chemical pesticides. Notably, the application of these plant extracts, including those from *R. sativus*, did not exhibit phytotoxic effects on plants such as *B. campestris*, indicating their safety for use in agricultural settings without harming non-target plant species (Choi et al., 2013). These results, in conjunction with the findings, suggest that *R. sativus* extract has the potential to serve as a sustainable biopesticide with comparable efficacy to conventional chemical insecticides like Malathion.

Significant Difference Between and Among the Percentage Mortality of *D. melanogaster* Larvae

As shown in Table 2, the computed F-ratio is 161.800 and is significant at 0.05 level. This implies that the percentage mortality of *D. melanogaster* larvae differs after 30 minutes of contact toxicity test with the other treatments.

Table 2

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 30 minutes with the Different Treatments

Source of Variation	Some of Squares	Degrees of Freedom	Mean Square	F – ratio	Interpretation
Between Source	44.944	5	8.989		
Within Source	0.667	12	0.056	161.800	Significant
Total	**45.611**	**17**			

*Legend: Significant at 0.01 level**

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Table 3 reveals a significant difference in the percentage mortality of *D. melanogaster* larvae between T_0 and $T_1 – T_5$, T_1 and $T_2 – T_5$, T_3 and T_5, and T_4 and T_5. T_1 has the highest percentage mortality of *D. melanogaster* after 30 minutes of exposure compared to those with the other treatments.

As shown in Table 4, the computed F-ratio is 143.500 and is significant at 0.05 level. The percentage mortality of *D. melanogaster* larvae differs after 45 minutes of contact toxicity test with the different treatments.

Table 3

Scheffe's Test on the Significant Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 30 minutes with the Different Treatments

SCHEFFE' SUMMARY					
	T_1	T_2	T_3	T_4	T_5
T_0	-5.00*	-2.00*	-2.33*	-3.00*	-4.00*
T_1		3.00*	2.67*	2.00*	1.00*
T_2			-0.33	-1.00*	-2.00*
T_3				-0.67	-1.67*
T_4					-1.00*

Table 4

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 45 minutes with the Different Treatments

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F – ratio	Interpretation
Between Source	38.267	4	9.567		
Within Source	0.667	10	0.067	143.500	Significant
Total	38.933	14			

*Legend: Significant at 0.01 level**

Table 5 reveals a significant difference in the percentage mortality of *D. melanogaster* larvae between T_0 and $T_1 – T_5$, T_2 and T_5. No significant difference was noted between T_1, T_3, T_4-T_5, and T_4 and T_5. However, T1 still has the highest percentage of mortality, killing all D. melanogaster larvae after 30 minutes and even after 45 minutes of exposure, compared to those with the other treatments.

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Table 5

Scheffe's Test on the Significant Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 45 minutes with the Different Treatments

SCHEFFE' SUMMARY					
	T_1	T_2	T_3	T_4	T_5
T_0	-5.00*	-3.00*	-4.00*	-4.00*	-4.33*
T_1					
T_2			-1.00	-1.00	-1.33*
T_3				0.00	-0.33
T_4					0.33

**Significant at the 0.05 level.*

Table 6

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 60 minutes with the Different Treatments

Source of variation	Some of Squares	Degrees of Freedom	Mean Square	F – ratio	Interpretation
Between Source	60.000	4	9.567		
Within Source	0.000	10	0.067	143.500	Significant
Total	60.000	14			

*Legend: Significant at 0.01 level**

Table 7

Scheffe's Test on the Significant Difference Between and Among the Percentage Mortality of D. melanogaster Larvae using Contact Toxicity Test after 60 minutes with the Different Treatments

SCHEFFE' SUMMARY					
	T_1	T_2	T_3	T_4	T_5
T_0	-5.00*	-5.00*	-5.00*	-5.00*	-5.00*
T_2			0.00	0.00	0.00*
T_3				0.00	0.00
T_4					0.00

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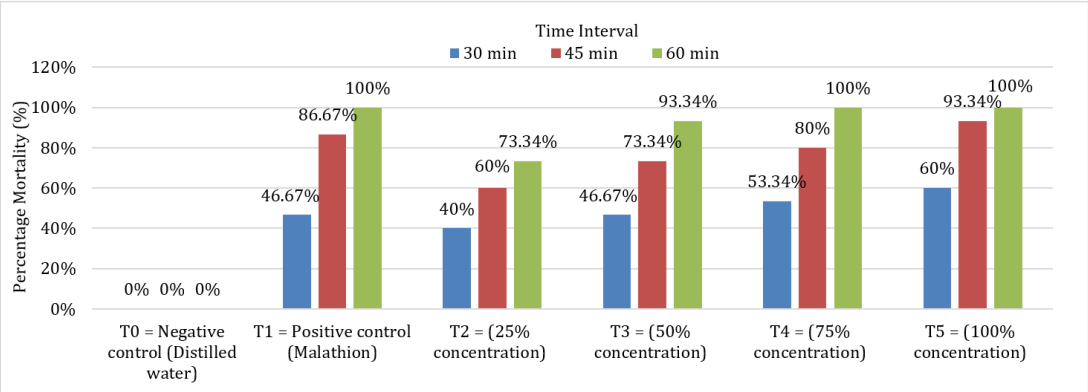
As shown in Table 6, the computed F-ratio is 143.500 and is significant at 0.05. The percentage mortality of *D. melanogaster* larvae differs after 60 minutes of contact toxicity test with the different treatments.

Table 7 reveals a significant difference in the percentage mortality of D. melanogaster larvae between T_0 and $T_1 – T_5$, as well as T_2 and T_5. No significant difference was noted between T_1, T_3, T_4-T_5, and T_4 and T_5.

However, T_1 still has the highest percentage mortality of *D. melanogaster* after 60 minutes of exposure compared to those with the other treatments. Scheffe's test results disproved the similarities in insecticidal activity of the different treatments, as shown in Table 1.

Table 8

Insecticidal Activity of the Different Treatments against Adult D. melanogaster using Contact Toxicity Test with the Time Interval of 30, 45, and 60 minutes in terms of Percentage Mortality



The mortality rates of adult *D. melanogaster* subjected to various treatments are summarized in Table 8. The data indicate that Treatment 5 (T_5), utilizing *R. sativus* crude extract, resulted in the highest percentage mortality of adult *D. melanogaster* after 30 minutes (60%), 45 minutes (93.34%), and 60 minutes (100%) of exposure. Treatments 4 (T_4) and 1 (T_1) followed, with mortality rates of 53.34% and 46.67% at 30 minutes, 80% and 86.67% at 45 minutes, and both reaching 100% mortality by 60 minutes. In contrast, the negative control (T_0) exhibited no mortality throughout the experimental period, demonstrating 0% mortality at all time points.

These findings suggest that T_5, containing the *R. sativus* crude extract, exhibited superior insecticidal efficacy compared to the positive control, particularly within the 30- and 45-minute exposure periods. Furthermore, T_4 demonstrated comparable insecticidal activity

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to T_1, with a minimal difference in mortality rates (6.67%) at the 30- and 45-minute intervals. The results underscore the potential of *R. sativus* crude extract as an effective bioinsecticide against adult *D. melanogaster*, with rapid action observed in the initial phases of exposure.

As shown in Table 9, the computed F-ratio is 2.259 and insignificant at the 0.05 level. The percentage mortality of adult *D. melanogaster* is similar to previous data. This data supports the results obtained from Table 8 and shows the promising potential of *R. sativus* leaf extract's insecticidal activity against adult *D. melanogaster*, comparable to the effect of Malathion after 30 minutes.

Table 9

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of adult D. melanogaster using Mortality Test after 30 minutes with the Different Treatments

Source of variation	Sum of Squares	Df	Mean Square	F – ratio	Interpretation
Between Source	16.944	5	3.389		
Within Source	18.000	12	1.500	2.259	Not Significant
Total	34.944	17			

*Legend: Significant at 0.01 level**

As shown in Table 10, the computed F-ratio is 13.083 and is significant at 0.05 level. This implies that the percentage mortality of adult D. melanogaster differs after 45 minutes of mortality test with the other treatments.

Table 10

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of adult D. melanogaster using Mortality Test after 45 minutes with the Different Treatments

Source of variation	Some of Squares	Df	Mean Square	F – ratio	Interpretation
Between Source	43.611	5	8.722		
Within Source	8.000	12	0.667	13.083	Significant
Total	51.611	17			

*Legend: Significant at 0.01 level**

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Table 11 reveals a significant difference in the percentage mortality of adult *D. melanogaster* between T_0 and $T_1 – T_5$. No significant difference was observed between T_1 and T_2 - T_5, T_2 and $T_3 – T_5$, T_3 and $T_4 – T_5$, T_4, and T_5.

This data supports the results obtained from Table 8. It showed the promising potential of *R. sativus* leaf extract's ($T_2 – T_5$) insecticidal activity against adult *D. melanogaster*, comparable to the effect of the synthetic insecticide Malathion after 45 minutes.

Table 11

Scheffe's Test on the Significant Difference Between and Among the Percentage Mortality of Adult D. melanogaster using Mortality Test after 45 minutes with the Different Treatments

SCHEFFE' SUMMARY					
	T_1	T_2	T_3	T_4	T_5
T_0	-4.33*	-3.000*	-3.67*	-4.000*	-4.67*
T_1		1.33	0.67	0.33	0.33
T_2			-0.67	-1.00	-1.67
T_3				-0.33	-1.00
T_4					-0.67

As shown in Table 12, the computed F-ratio is 105.200 and is significant at 0.05 level. This nullifies the hypothesis that there is no significant difference between and among the average mortality of adult D. melanogaster with the different treatments after 60 minutes. This implies that the percentage mortality of adult *D. melanogaster* differs after 60 minutes of mortality test with the other treatments.

Table 12

Summary of ANOVA on the Difference Between and Among the Percentage Mortality of adult D. melanogaster using Mortality Test after 60 minutes with the Different Treatments

Source of variation	Sum of Squares	Df	Mean Square	F - ratio	Interpretation
Between Source	58.444	5	11.689		
Within Source	1.333	12	0.111	105.200	Significant
Total	59.777	17			

*Legend: Significant at 0.01 level**

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Table 13 reveals a significant difference in adult *D. melanogaster* larvae percentage mortality between T_0 and $T_1 – T_5$. No significant difference was observed between T_1 and T_2 - T_5, T_2 and $T_3 – T_5$, T_3 and $T_4 – T_5$, T_4, and T_5.

Table 13
Scheffe's Test on the Significant Difference Between and Among the Percentage Mortality of Adult D. melanogaster using Mortality Test after 60 minutes with the Different Treatments

SCHEFFE' SUMMARY					
	T_1	T_2	T_3	T_4	T_5
T_0	-5.00*	-3.67*	-4.67*	-5.00*	-5.00*
T_1		1.33	0.33	0.00	0.00
T_2			-1.00	-1.33	-1.33
T_3				-0.33	-0.33
T_4					0.00

This data also supports the results obtained from Table 3 and shows the promising potential of *R. sativus* leaf extract's ($T_2 – T_5$) insecticidal activity against adult *D. melanogaster*, which is comparable to the effect of the synthetic insecticide, Malathion, after 60 m

CONCLUSIONS

Based on the results, T_1 exhibited the most effective insecticidal activity against *D. melanogaster* larvae across all exposure times (30, 45, and 60 minutes) under the contact toxicity test. Additionally, *R. sativus* leaf extract, tested at varying concentrations ($T_2–T_5$), demonstrated notable insecticidal properties against *D. melanogaster* larvae, with observable efficacy beginning at 45 minutes for treatments $T_3–T_5$ and extending to all concentrations ($T_2–T_5$) at 60 minutes. The study further reveals that the *R. sativus* leaf extract at different concentrations ($T_2–T_5$) exhibited promising insecticidal activity against adult *D. melanogaster*. Notably, the effectiveness of *R. sativus* leaf extract was comparable to that of Malathion, a widely used synthetic insecticide, at all observed time points (30, 45, and 60 minutes). These findings suggest that *R. sativus* leaf extract has significant potential as a bioinsecticide, providing an alternative to synthetic chemical insecticides in controlling *D. melanogaster*. The results highlight its rapid and sustained efficacy, making it a viable candidate for further development in pest management strategies.

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RECOMMENDATIONS

The findings indicate that *R. sativus* leaf extract holds significant potential as a natural alternative to synthetic insecticides for managing *D. melanogaster*. Proper identification and authentication of the plant are critical to ensuring consistency and reliability in its use. Phytochemical analyses should focus on isolating and identifying the bioactive compounds responsible for their insecticidal properties, while pharmacological studies could explore additional medicinal applications. Toxicological evaluations are essential to determine the safety of the extract for non-target organisms and its environmental impact. Future studies should also include field trials to assess its effectiveness in real-world scenarios, as well as cost-benefit analyses to evaluate its practicality and economic viability. Additionally, optimizing extraction techniques, determining ideal concentrations, and refining application methods will be crucial for its integration into sustainable pest management strategies. These steps will enhance the utility and applicability of *R. sativus* extract as an eco-friendly and effective solution in pest control.

ETHICAL STATEMENT

This study adhered to the ethical standards established by the University of Northern Philippines (UNP). No conflicts of interest were identified in the course of this research.

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