

Toxicity effect of *Ricinus communis* methanolic extracts against *Bactrocera cucurbitae* (Diptera: Tephritidae)

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Abstract: *Bactrocera cucurbitae*, commonly known as the melon fruit fly, stands as a formidable threat to global agriculture, particularly in the cultivation of cucurbitaceous crops. The adaptability, high reproductive capacity, and broad host range of *B. cucurbitae* make it a persistent challenge for growers worldwide. Conventional control methods, often reliant on chemical pesticides, pose environmental and ecological concerns, necessitating the exploration of alternative strategies for sustainable pest management. Invasive plants often exert deleterious effects on ecosystems, and the castor bean plant, *Ricinus communis*, is no exception. To explore the efficacy of *R. communis*, a methanol extract was tested to find the toxicity effect against *B. cucurbitae*. In this study, different bioactive compounds were isolated from *R. communis*. The crude extract of *R. communis* was subjected to fractionating using different organic solvents in an increasing order of polarity, where the fraction indicating maximum activity was then taken for the isolation of the bioactive compounds using various chromatographic and spectroscopic techniques such as column chromatography, thin layer chromatography (TLC), gas chromatography-mass

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spectrometry (GC-MS). The concentrations of *R. communis* extracts at 0.5, 1.0, 1.5 and 2% methanol were used. Pure methanol was used as the control. The experimental conditions were maintained at 28 ± 20 °C and 65 ± 5% relative humidity. The experiment was laid out in a complete randomised design having five replications. A probit analysis was used to find the LC50 and LC90. The results showed that, as the concentration of the plant extracts increases, the mortalities of *B. cucurbitae* also increased. After a 72 h exposure period, the crude extracts exhibited the lowest LC50 at 0.30% and LC90 at 0.60%. This study investigates the potential of methanolic extracts derived from various parts of *R. communis* to serve as a biopesticide against *B. cucurbitae* which can be easily available, economically feasible, socially acceptable and environmentally friendly.

Keywords: *Bactrocera cucurbitae*; *Ricinus communis*; isolation and purification

Crop pests pose a growing and severe threat to food security (Singh et al. 2023). Among various problems, fruit flies are one of the significant pests. Fruit flies are, as implied by their name, true flies that feed on fruits. The fruit flies *Bactrocera cucurbitae* are economically important pests belonging to the order Diptera and the family Tephritidae, which consist of about 4 000 species in 481 genera (Ganie et al. 2022). These can be found in any habitat with suitable life on the planet, ranging from rainforests to open savannahs (Hudiwaku et al. 2021). Except for Antarctica, the distribution of these insects is global (Bartlett et al. 2020). The female *B. cucurbitae* typically select soft, delicate fruit tissues for laying their eggs. Fruit flies are highly energetic flying insects. Within the fruit, the eggs hatch into maggots that burrow through the fruit and consume its pulp. The infected fruits eventually dry out, decay, and shed before their time.

It has been reported that cucurbit fruit flies can infest between 41 and 95% of the crop in bitter melon crops (Sami et al. 2023). Depending on the crop variety's susceptibility and the environmental conditions, losses can range from 30 to 100% (John et al. 2023). According to reports, *B. cucurbitae* can infest 90% of snake gourds, 60 to 87% of pumpkins, and 95% of bitter melon fruits in Papua New Guinea (Mondal et al. 2020). In California, they cause a total of 910 mil. USD in annual losses; in Australia, the losses have been estimated to be 65.778 USD (100 mil. AUD). The situation is worse than this in developing countries, for example, Egypt spends millions of dollars on controlling fruit flies. Furthermore, the continuous use of chemical pesticides has resulted in adverse effects including pollution, health risks, and biodiversity loss, while the application of botanical pesticides promotes a healthy environment and sustainable agriculture

(Souto et al. 2021). Moreover, the use of synthetic pesticides by export-oriented farmers in the cultivation of horticulture crops has had a detrimental impact on farmers (Demi & Sicchia 2021). Growers and exporters in developing nations have reported losing their market share and revenue as a result of the discovery of prohibited pesticides or traces of them exceeding the legal residue limits (Nader et al. 2020). Due to their effectiveness, biodegradability, and variety of modes of action including repellent action, inhibition, protein denaturation, contact toxicity, antifeedant, and other activities depending on the type of compounds in the botanical pesticide, botanical pesticides are currently recommended for use in pest management (Dar et al. 2022). *Ricinus communis* is a common weed in regions including Asia, South Africa, Brazil, and Russia, which is a member of the Euphorbiaceae family (Landoni et al. 2023). The plant was chosen due to its accessibility and the presence of bioactive components that have been shown to disrupt insect pest life cycles (Chaudhari et al. 2021). Research on the plant's aerial portions revealed the existence of flavonoids, ricin, ricinine, and N-demethyl ricinine, among other active ingredients (Amandeep Singh 2016). Although ricinine, an efficient pesticide, is found throughout the plant, ricin is the most poisonous bioactive component found in the seeds. These chemicals have demonstrated exceptional insecticidal, antifeedant, and repellent effects (Cheikhoussef & Cheikhoussef 2023). Research has documented the detrimental impacts of the *R. communis* extract on arthropod vectors, including ticks, mites, and mosquitoes.

The significance of investigating the toxicity effect of the invasive plant *R. communis* methanolic extracts against *B. cucurbitae* lies in addressing the ecological and agricultural challenges posed

by invasive species and fruit fly pests. *R. communis* is recognised for its invasiveness and allelopathic potential. Understanding the toxic effects of its methanolic extracts on *B. cucurbitae*, a notorious fruit fly pest, is crucial for developing sustainable and eco-friendly pest management strategies. Previous research has emphasised the allelopathic properties of *R. communis* (Saadaoui et al. 2015), and this study contributes to expanding our knowledge by assessing its specific impact on *B. cucurbitae*. The findings could have implications for integrated pest management, offering a novel and potentially effective botanical solution to control *B. cucurbitae* infestations while addressing concerns about the environmental impact of synthetic pesticides (Rupawate et al. 2023).

We hypothesise that the methanolic extracts derived from the invasive plant *R. communis* exhibit significant toxicity against *B. cucurbitae* potentially impacting its survival, development, and reproductive capabilities. This hypothesis builds on previous studies highlighting the toxic properties of *R. communis* (Saadaoui et al. 2015) and specifically extends the investigation to the interaction with *B. cucurbitae*. The study aims to explore the potential biopesticidal effects of *R. communis* extracts on *B. cucurbitae*, contributing to the understanding of their eco-friendly pest control strategies. The research questions for the study on the toxicity effect of invasive plant *R. communis* ethanolic extracts against *B. cucurbitae* are multifaceted. Firstly, how do various concentrations of *R. communis* methanolic extracts affect the mortality rates of *B. cucurbitae*? Secondly, are there specific chemical compounds within the methanolic extracts of *R. communis* that can be identified as being responsible for the observed toxic effects on *B. cucurbitae*?

MATERIAL AND METHODS

The experiment was conducted in the Entomology laboratory at the University of Haripur Khyber Pakhtunkhwa Pakistan during 2023. *B. cucurbitae* were used in this experiment and were obtained from the culture maintained in the laboratory at $28 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity (RH) and a 14:10 h light and dark cycle. A mixture of sugar, yeast and water was placed in a Petri dish as the food supplement in the fruitflies rearing cage.

Adult rearing cage

The mature *B. cucurbitae* specimens were chosen at random from the bulk culture to be utilised for further investigations. To facilitate mating, two hundred and fifty (250) fruit fly pairs with similar sex ratios were transferred from plastic containers to adult rearing cages measuring $1 \times 1 \times 1$ m. For the adult diet, the cage included water, sugar, and yeast. To keep the interior damp, moist cotton was arranged in a basin. The culture was maintained in the laboratory at controlled conditions ($27 \pm 1.5^\circ\text{C}$, $60 \pm 5.5\%$ humidity and a 14 : 10 L : D photoperiod (Saeed et al. 2022)).

Plants materials and crude extract preparation

R. communis leaves were collected from a region of the district Haripur. The leaves were identified by a botanist at the department of Biology, the University of Haripur. After being cleaned with distilled water, the leaves were left to dry in the shade for a period of seven to fifteen days. An electrical grinder was then used to create powders, which were then sieved through a sieve with a mesh size of 60 mm. After that, the powder was stored at room temperature in airtight jars to avoid quality deterioration (Akbar et al. 2022a; Akbar et al. 2022b). For preparation of the crude extracts, we followed the methods of (Akbar & Khan 2021). The shade dried fine powder of the plant weighing 10 g were mixed in 100 mL of methanol, was then placed in an orbit shaker for one hour at 150 rpm (revolutions per minute). Then, it was kept at room temperature for 24 h. After 24 h, it was filtered through Whatman No. 1 filter paper (Maidstone, United Kingdom). the filtration was repeated twice for the maximum yield. The filtrate was concentrated by using a rotary evaporator. The stock solution, i.e., 10%, was further diluted for the experiment as shown in Table 1.

Table 1. Different concentrations of *Ricinus communis* extracts for the insecticidal bioassay

Concentrations (%)	Stock solution (mL)	Solvent (methanol) (mL)	Total volume (v/v) (mL)
0.5	0.5	9.5	10
1.0	1.0	9.0	10
1.5	1.5	8.5	10
2.0	2.0	8.0	10

Toxicity of *R. communis* against *B. cucurbitae* adults

In this study, *R. communis* extracts with four different concentrations along with the control (Table 1) were tested, following the completely randomised design (CRD) design with factorial arrangement with five replicates. A toxicity test was performed for the adult insecticidal activity. The insects were chilled for a period of 1 min at -2°C . Then, the immobilised insects were individually picked up with the help of a camel brush and transferred to the 9 cm Petri dish. Then, 1.00 μL solutions of three solvents of four different concentrations of *R. communis* were applied to the dorsal surface of the thorax of each insect by using a microlitre syringe (Hamilton 700, Germany). Ten *B. cucurbitae* were used in each treatment. The numbers of dead *B. cucurbitae* were counted after the treatment and the percent mortality was calculated for each concentration after 24, 48 and 72 h of exposure. All the experiments were conducted in a completely randomised design with three replications.

$$\text{Corrected \% mortality} = \frac{(\% \text{ mortality in treatment} - \% \text{ mortality in control}) \times 100}{100 - \% \text{ mortality in control}}$$

Preparation of plant extracts for chromatography

The extraction from the plant material was performed by the solid-liquid extraction method. Methanol was used as a solvent. Twenty (20) g of the powdered plant material was soaked in 100 mL of methanol in a beaker (500 mL) and was placed on the orbital shaker at 150 rpm for 2 h, then it was placed at room temperature for 72 h. The mixture was then filtered through Whatman No. 1 filter paper (Maidstone, United Kingdom) and the stock solution was collected in a conical flask (Akbar et al. 2024). After extraction with the methanol, the excessive solvent was evaporated in a rotary evaporator under vacuum at a temperature of 20°C to get the crude extract in the form of a viscous semi-solid mass. The dried crude extracts were stored in glass Petri dishes covered with aluminium foil and were placed at room temperature, i.e., $27 \pm 2^{\circ}\text{C}$.

Column chromatography

Procedure of column chromatography. A glass column with dimensions of 600 mm \times 15 mm with a stopper at the bottom end was used for the gravitational column chromatography. A first disc was al-

ready fitted above the stopcock to prevent the silica gel from escaping the column. Silica gel 60 [100–200 mesh size, Merck (Merck, Germany)] was used as the stationary phase (Mishra et al. 2019).

Packing the column. To pack the column for the chromatography, the dry packing method is used. Silica gel was added from the top of the glass column with concurrent tapping on the side of the column to ensure free air bubbles. After the silica gel was settled, it was covered with a 0.5 cm layer of fine sand.

Pouring column and collection of fractions. Thereafter, we dissolved the crude mixture of the compound in the chosen eluent. With the help of a Pasteur pipette, the mixture was loaded onto a packed chromatography column. Then the column was eluted with the selected solvents. With the stopcock closed for 24 h, we let the mixture run slowly down the inner side of the column. The eluent flows down the column from the top under gravity. After 24 h, we opened the stopcock and the solvents (mobile phase) were then allowed to flow down the column until the upper level of solvent reached the top layer of the sand. Then, solvents of different polarities (gradient elution) were passed through the column to purify the sample extract, 100 mL of each eluent was added to column. A three solvent gradient elution was carried out using pure n-hexane followed by pure ethyl acetate and pure methanol as the mobile phase in the case of the methanol plant extract. The chemicals in the crude mixture interact differently with the mobile phase and stationary phase (silica gel), which causes them to move along the mobile phase at various rates. Polar solvents elute polar chemicals from the column mixture, while non-polar solvents elute non-polar compounds. In this way, the separation of the compounds from the mixture was achieved. The purified fractions were collected in glass test tubes at a time interval of 30 min with a speed of 20–30 mL/30 min. The test tubes were labelled in order. Then, these fractions were more concentrated by the removal of excess solvent through a rotary evaporator. All the collected fractions were subjected to thin layer chromatography (TLC) to determine the purity of the eluted fractions. The insecticidal activity of the collected fractions was tested against *B. cucurbitae*. A direct toxicity test was performed for the insecticidal activity. Ten *B. cucurbitae* per replication were treated. The numbers of dead *B. cucurbitae* were counted after treatment and the percent mortality was calculated for each concentration after 24, 48 and 72 h of exposure.

Table 2. Toxicological effect of the *Ricinus communis* methanol extracts against *Bactrocera cucurbitae* after 24 h of exposure

Concentration (%)	Mortality	<i>n</i>	LC 50 (%)	LC 90 (%)	Slope
0.5	6.66 ^b	50			
1.0	13.33 ^{ab}	50	1.12	2.12	
1.5	16.66 ^{ab}	50			1.10 + 0.44
2.0	23.33 ^a	50	(0.04–1.78)	(0.78–3.24)	

LSD for *Ricinus communis* concentration = 12.00

n – number of insects used in the test; LC – lethal concentration – indicated with 95% confidence limits (CLs) or the LC 90 (%) of the plant extract

GC-MS [Gas Chromatography – Mass Spectrometry (GC–MS)] analysis of the isolated compounds. Using helium gas as the carrier, an Agilent Technologies GC 5890 (Shimadzu GC17-A Gas Chromatograph Mass Spectrometer Ultra, Japan) was used in conjunction with an auto sampler injection system and electron spray ionisation. The analysis used the Acquired Method Default, with a temperature range of 40–340 °C and a run time of 28 min (Nisa et al. 2022).

FTIR (Fourier Transform Infrared) analysis of the isolated compounds. To analyse the chemicals, a Thermo Nicolet 380 FT-IR Spectrophotometer was used. It was run on sodium chloride discs with Thermo Electron Corporation's OMNIC (version 7.3) (IRTracer-100, Shimadzu, Japan) controllers and processing software. There was a coating of the absorption bands in wave numbers (cm⁻¹). For the analysis, the separated chemicals were combined with the Nujol mull technique after being dissolved in chloroform (Pharmawati & Wrasati 2020).

Data analyses

Significant differences between the treatments were found when the data was subjected to an analysis of variance (ANOVA). Using the least significant difference (LSD) test at a 5% level of sig-

nificance, mean values were significantly separated (Gray 2012). Subsequently, the adult *B. cucurbitae* percentage mortality was analysed using the Log-Probit model in order to calculate the 50% and 90% lethal concentrations (LC50/LC90) (Queiroz de Oliveira et al. 2010). The analysis of variance and Probit analysis were conducted using the Statistical Package for the Social Sciences (SPSS) (version 20).

RESULTS

R. communis methanol extract toxicity against *B. cucurbitae*

The data of the bioassay experiments of the methanol extracts of *R. communis* on the adults of *B. cucurbitae* exposed to different concentrations through the direct method in a Petri dish after 24 h, 48 h, and 72 h, are shown in Tables 2, 3 and 4, respectively.

The results in Table 2 show that after 24 h of exposure, the mortalities of *B. cucurbitae* increased as the concentration of the plant extracts increased. The mortalities of *B. cucurbitae* were significantly higher at the 2% concentration, i.e., 23.33% and lower mortalities of *B. cucurbitae* were seen with the 0.5% concentration, i.e., 6.67% (*df* = 3, *F* = 4.00 and

Table 3. Toxicological effect of the *Ricinus communis* methanol extracts against *Bactrocera cucurbitae* after 48 h of exposure

Concentration (%)	Mortality	<i>n</i>	LC 50 (%)	LC 90 (%)	Slope
0.5	13.03 ^b	50			
1.0	16.39 ^b	50			
1.5	19.73 ^{ab}	50	0.50	1.20	
2.0	29.79 ^a	50	(0.00–1.15)	(0.00–1.93)	0.80 + 0.38

LSD for *Ricinus communis* concentration = 11.17

n – number of insects used in the test; LC – lethal concentration – indicated with 95% confidence limits (CLs) or the LC 90 (%) of the plant extract

Table 4. Toxicological effect of the *Ricinus communis* methanol extracts against *Bactrocera cucurbitae* after 72 h of exposure

Concentration (%)	Mortality	<i>n</i>	LC 50 (%)	LC 90 (%)	Slope
0.5	19.19 ^b	50			
1.0	22.55 ^b	50			
1.5	29.29 ^{ab}	50	0.30 (0.00–0.78)	0.66 (0.00–1.25)	0.889 + 0.35
2.0	42.75 ^a	50			

LSD for *Ricinus communis* concentration = 14.66

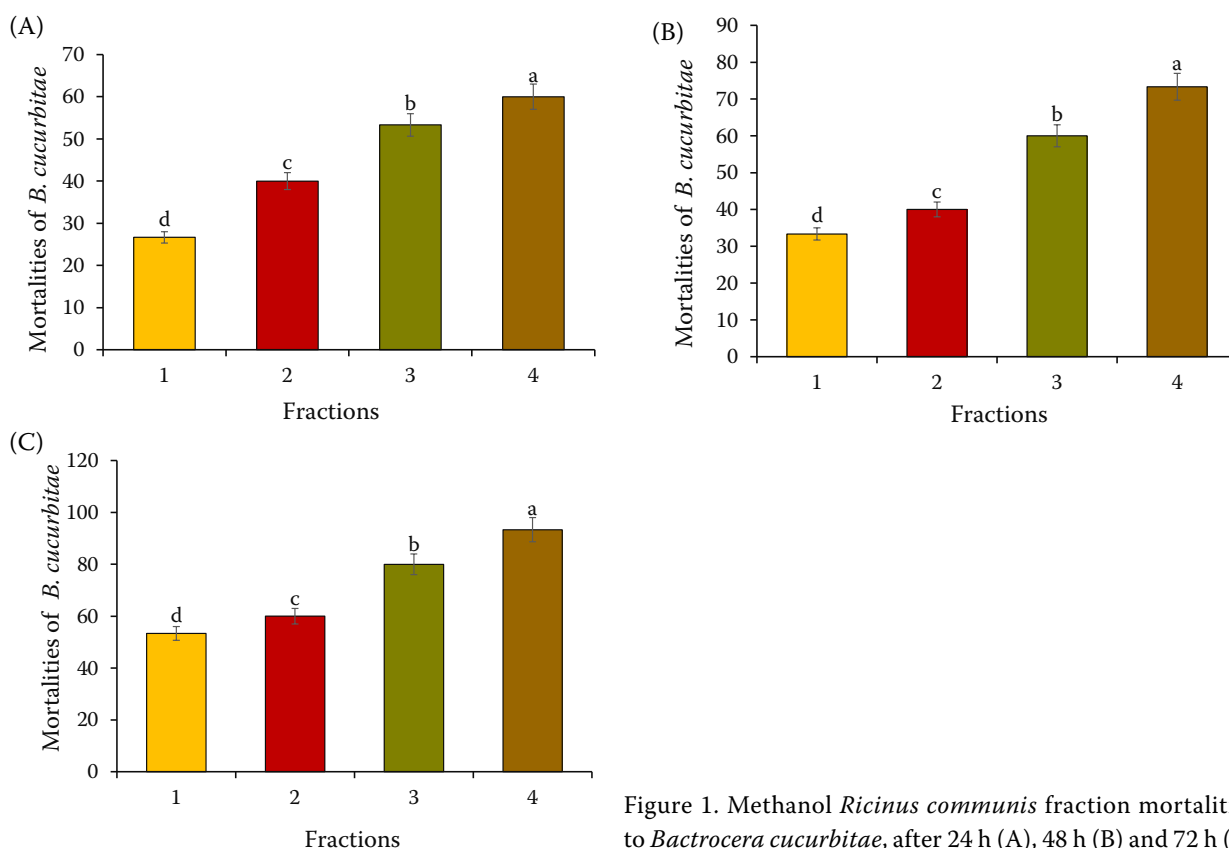
n – number of insects used in the test; LC – lethal concentration – indicated with 95% confidence limits (CLs) or the LC 90 (%) of the plant extract

$P = 0.070$). It was also observed that the methanol extract of *R. communis* was effective against *B. cucurbitae*, i.e., the LC50, was 1.12 % and the LC90 was 2.12% having a slope of 1.106. From (Table 3), it is clear that, at the lowest concentration, 13.03% mortalities were observed with the methanol extract of *R. communis*, while, at the highest concentrations, significantly, 29.797% mortalities were observed to *B. cucurbitae* after 48 h of exposure ($df = 3$, $F = 5.03$ and $P = 0.0446$). In the case of LC50 and LC90, it was noted that the methanol extract of *R. communis* was effective, i.e., 0.50% and 1.20%, respectively, having a slope of 0.81. After 72 h of exposure, the mortalities of *B. cucur-*

bitae increases as the concentration of the methanol plant extract increased and also with the time interval. It was observed that the minimum mortalities were recorded with 0.5% concentrations, i.e., 19.19% and, significantly, the maximum mortalities at 42.75% of *B. cucurbitae* were recorded with the 2.5% concentrations ($df = 3$, $F = 6.05$ and $P = 0.03$) having an LC50 of 0.30% and an LC90 of 0.66% with a slope of 0.88 as shown in Table 4.

R. communis fraction mortalities to *B. cucurbitae*

From Figure 1, it can be seen that there is direct relationship of the *B. cucurbitae* mortalities to both

Figure 1. Methanol *Ricinus communis* fraction mortalities to *Bactrocera cucurbitae*, after 24 h (A), 48 h (B) and 72 h (C)

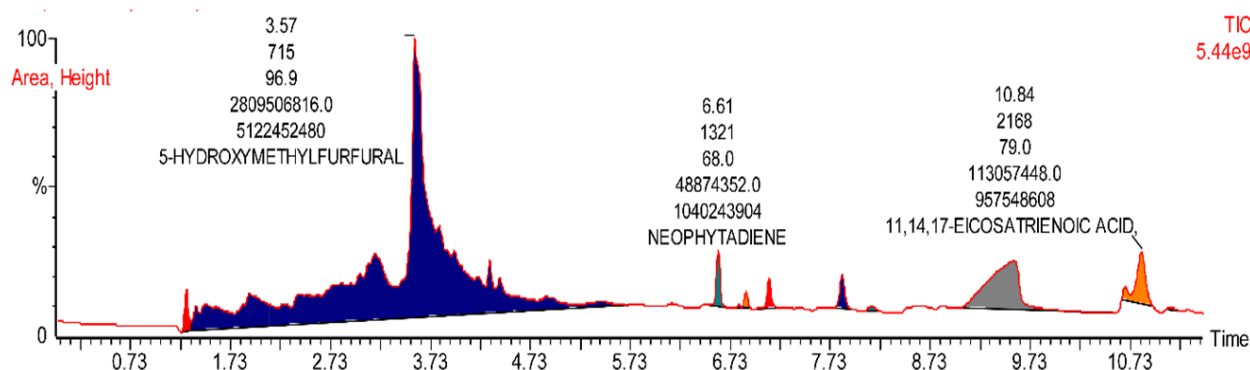


Figure 2. Gas Chromatography – Mass Spectrometry analysis of the methanol extract of *Ricinus communis*

the concentrations and time period. As the concentrations of the fractions of *R. communis* increases, the mortalities of *B. cucurbitae* increases with the time period. The methanol fraction (A) after 24 h of plant exposure, recorded the maximum mortality, i.e., 60%, at the highest concentration, while the lowest mortality was recorded at 0.5%, i.e., 26.67%. After 48 h of exposure, the minimum mortality of *B. cucurbitae* was observed at the 0.5% concentration, i.e., 33.33%, while the highest was recorded at the 2% concentration, i.e., 73.33%. Similarly, after 72 h of plant exposure, higher mortalities, i.e., 93.33%, were recorded at the higher concentration, while lower mortalities were recorded at the 0.5% or lowest concentration, i.e., 53.33%.

GC–MS analysis of the methanol extract of *R. communis*

Figure 2 depicts the GC–MS analysis of the methanol extract of *R. communis*. It can be seen that there are seven distinct peaks representing some bioactive compounds in the extract. Each peak corresponds to a particular compound. Peak number 1 with a retention time of 3.57, corresponds to the 5-hydroxymethylfurfural. Similarly, another peak eluting at a retention time of 6.61 corresponds to another compound neophytadiene. Peak number 3, with a retention time of 10.84, corresponds to another compound which is 11,14,17-eicosatrienoic acid according to the GC–MS Turbo Mass 5.4 online library.

These three primary bioactive compounds, namely 5-hydroxymethylfurfural, neophytadiene, and 11,14,17-eicosatrienoic acid, are present in the methanol extract. The data are input into the Turbo Mass software (version 5.4.2), and this software assigns names to these compounds. The details of these compounds are given below:

(i) 5-Hydroxymethylfurfural. 5-Hydroxymethylfurfural (5-HMF) in Table 5 is a chemical compound with the molecular formula $C_6H_6O_3$. HMF is soluble in water and various organic solvents, including ethanol and methanol. 5-Hydroxymethylfurfural belongs to the furan family of organic compounds. 5-HMF is classified as an aldehyde due to the presence of a carbonyl group ($C=O$) in its structure. Aldehydes are a class of organic compounds containing this functional group. They are known for their reactivity in various chemical reactions.

(ii) Neophytadiene. Neophytadiene is a natural organic compound belonging to the class of compounds known as diterpenes. It is a hydrocarbon, meaning it consists solely of carbon and hydrogen atoms, and it is classified as a $C_{20}H_{32}$ molecule. Diterpenes like neophytadiene, as shown in Table 5, are often found in essential oils and resins produced by plants. Neophytadiene may have antioxidant properties and could play a role in the defence mechanisms of plants against environmental stressors.

(iii) 11, 14, 17-Eicosatrienoic acid. The term "11, 14, 17-eicosatrienoic acid" refers to a type of fatty acid. Specifically, it is an omega-3 polyunsaturated fatty acid, as seen in Table 5. The numbers 11, 14, and 17 in the name refer to the positions of the double bonds in the carbon chain of the fatty acid. Omega-3 fatty acids, including eicosatrienoic acid, are essential for various biological processes in the body. They are known for their anti-inflammatory and cardio protective properties.

FTIR Analysis of methanol of *R. communis*

The FTIR spectrum of the methanol extract in Figure 3 indicate the presence of a peak at 3400 cm^{-1} ,

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Table 5. Chemical components identified in the methanolic plant extracts based on the Gas Chromatography – Mass Spectrometry analysis

Name	Retention time	Percent area	Composition (%)
Hentriacontane	0.16	11 259.90	0.00
Hentriacontane	0.18	25 642.23	0.00
Hentriacontane	0.21	9 537.64	0.00
Hentriacontane	0.24	2 966.68	0.00
Hentriacontane	0.25	4 244.54	0.00
Hentriacontane	0.26	3 089.49	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	0.27	5 803.87	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	0.33	2 104.18	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	0.34	2 863.88	0.00
Hentriacontane	0.36	13 630.15	0.00
Hentriacontane	0.38	9 888.39	0.00
Hentriacontane	0.41	2 524.72	0.00
Hentriacontane	0.43	21 237.56	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	0.60	4 633.65	0.00
Hentriacontane	0.63	43 454.09	0.00
3-Methyldotriacontane	0.65	4 622.47	0.00
Hentriacontane	0.68	3 913.55	0.00
Hentriacontane	0.70	14 803.40	0.00
3-Methyldotriacontane	0.73	3 171.14	0.00
Pentatriacontane	0.86	598.45	0.00
Hentriacontane	0.89	21 693.04	0.00
Hentriacontane	0.91	7 702.09	0.00
Hentriacontane	0.95	38 730.58	0.00
Hentriacontane	0.99	46 411.97	0.00
Hentriacontane	1.03	13 407.09	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	1.08	12 791.81	0.00
Hentriacontane	1.13	84871.04	0.00
1,1,3,6-Tetramethyl-2-(3,6,10,	1.18	35667.56	0.00
Isobutyl (3-(Methylthio)Propyl	1.29	34 784 736.00	1.01
5-Hydroxymethylfurfural	3.58	2 809 506 816.00	81.79
Neophytadiene	6.61	48 874 352.00	1.42
3-Methyl-2-(2-Oxopropyl)Furan	6.82	1 746 191.13	0.05
Phytol Tetradecanoate	6.89	11 496 019.00	0.33
Trans,Cis-1,8-Dimethylspiro[4.	7.12	29 370 840.00	0.86
Tetradecanoic Acid, 10,13-Dime	7.85	39 661 132.00	1.15
2,6,10,14-Tetramethyl-7-(3-Met	8.15	7 045 127.50	0.21
N-Hexadecanoic Acid	9.57	335 654 304.00	9.77
11,14,17-Eicosatrienoic Acid,	10.84	113 057 448.00	3.29
3-Methyl-2-(2-Oxopropyl)Furan	11.13	3 235 609.50	0.09

and $3\,000\text{ cm}^{-1}$ indicates the presence of alcohol. Another peak at $2\,850\text{ cm}^{-1}$ corresponds to the presence of alkane (C-H). The peaks at $1\,650$ and $1\,500\text{ cm}^{-1}$

indicate the presence of (N-H), a secondary amine. Other peaks at $1\,150$ and $1\,030\text{ cm}^{-1}$ correspond to the presence of (C-F), aliphatic fluoro compounds.

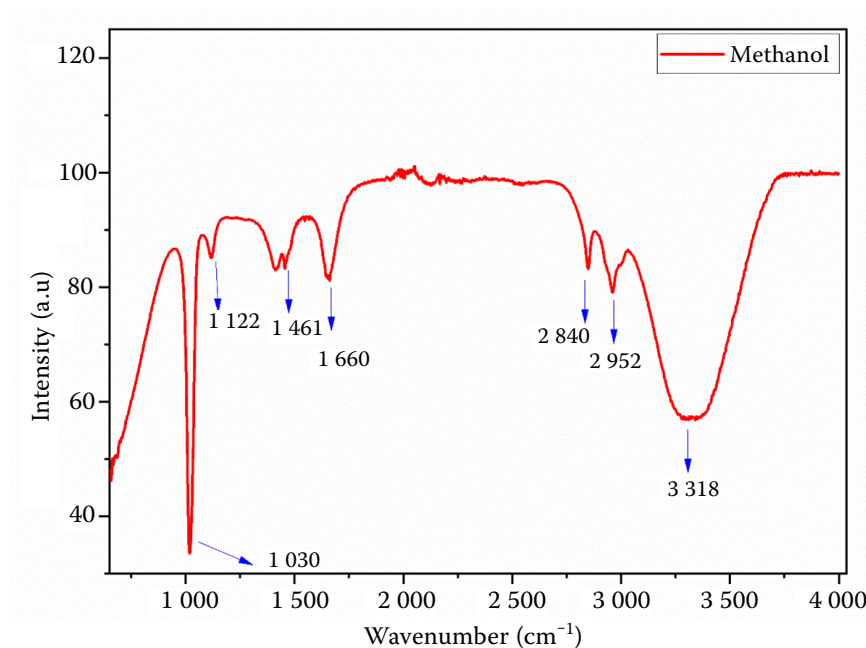


Figure 3. FTIR spectra of the methanol extracts of the *Ricinus communis* leaves

DISCUSSION

The plant extracts tested in our experiment showed positive and significant effects of toxicity against adult *B. cucurbitae* in a laboratory natural environment. Plant extracts of *R. communis* showed maximum toxicity effects against adult *B. cucurbitae* up to 72 h after exposure. Previous studies have reported on the bioactivity of the plant extracts of *R. communis*. It has been reported that the feeding behaviour of the larvae was altered due to the adverse effects caused by the active plant parts that damaged the gut's epithelial lining. The adult mortality may be due to the bioactive components of the plant extracts penetrating into the adult body through the oral route or the body wall in the direct toxicity method (Abdel-Shafy et al. 2009). Similar outcomes were noted in a study assessing the toxicity of the castor plant against *Costelytra zealandica*, an adult grass grub that exhibits the highest activity in a chloroform extract. Using mass spectrometry, ricinine was found to be the primary poisonous material (Zheng et al. 2023). The powerful insecticidal component of *R. communis*, ricinine, has been observed to have the highest solubility in chloroform, which could account for the maximum efficacy of chloroform extracts (Khan et al. 2015; Kaur et al. 2020). A neurotoxic alkaloid called ricinine has the ability to paralyse and kill insects (Nwaji et al. 2022). According to reports, it possesses an insecticidal effect against pest insects

such as *Myzus persicae* (Homoptera: Aphididae), *Atta sexdens rubropilosa* (Hymenoptera: Formicidae), and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Ramos-López et al. 2010; Djillali et al. 2022). According to (Ramos-López et al. 2010), at 24 000 ppm, a methanol leaf extract of *R. communis* demonstrated 100% mortality against *S. frugiperda* larvae, while the activity began at 560 ppm. A petroleum ether extract showed the least mortality among all the four extracts. Batabyal et al. (2009) reported that the most effective *R. communis* toxicity against *Culex quinquefasciatus* was a carbon tetrachloride extract (LC50 144.11 ppm), which was followed by a methanol extract (LC50 91.62 ppm). The petroleum ether extract was the least efficient with the LC50 at 390.26 ppm. In our recent results, as the concentration of the tested plant extracts increased, the mortalities of *B. cucurbitae* also increased. These results also corroborated with the earlier findings of (Akbar, et al. 2022; Akbar et al. 2024) who stated that as the concentrations of plant extracts increased, the mortalities of *C. maculatus* also increased and vice versa. The results from this study have been encouraged by many researchers (Rampadarath & Puchooa 2016) who have shown that larvicidal activities against *B. zonata* of three different concentrations of *R. communis* extracts (0.2, 0.4, and 0.8 mg/L) were effective, significantly reducing larval survival rates in a dose-dependent manner. The plant extracts showed lethal activities at a concentration

as low as 0.2 mg/L. The mean mortality at 24 h was 41.67% at 0.2 mg/L with a maximum of 75.00% at 0.8 mg/L for fully mature leaves ethyl acetate extract. The finding of this study shows that mortality increases with the increase in the concentration. The GC-MS analysis also revealed that there are 47 active compounds present in methanol extracts of *R. communis* in which there are three primary bioactive compounds, namely 5-hydroxymethylfurfural, neophytadiene, and 11, 14, 17-eicosatrienoic acid. These bioactive compounds showed toxicity effect against *B. cucurbitae*. Our results are similar with the findings of (Nour et al. 2023) who performed a GC-MS technique that revealed that six major compounds were detected in the n-hexane extract. Isophytol, n-Hexadecanoic acid, 9, 12, 15-octadecatrienoic acid, oleic acid, octadecanoic acid and tributyl acetylcitrate. The highest percentages of n-Hexadecanoic acid and 9, 12, 15-Octadecatrienoic acid were recorded. In the present study, we also show that there are three main bioactive d compounds which were isolated in the n-hexane extract namely, neophytadiene, 1-(+)-ascorbic acid 2, 6-dihexa, 1-propyl 9, 12, 15-octadecatrien, and 2-methyl-3(3-methyl-but-2-enyl). Steroids, saponins, alkaloids, flavonoids, and glycosides were identified in the preliminary phytochemical analysis of *R. communis* (Abdul et al. 2018). Two alkaloids, ricinine and N-demethyl ricinine, as well as six flavones were detected in the dried leaves of *R. communis*. There are several types of quercetin-3-O- β -D-xylopyranoside and quercetin-3-O- β -D-glucopyranoside, including kaempferol-3-O- β -D-xylopyranoside, kaempferol-3-O- β -D-glucopyranoside, and quercetin-3-O- β -D-glucopyranoside (Saravana Kumar et al. 2022). Nour et al. (2023) worked on the chemical characterisation of *R. communis*. According to the GC-MS technique, six major compounds were identified in the chromatograms of the samples under study. The highest percentages of 9, 12, 15-Octadecatrienoic acid and n-Hexadecanoic acid were observed. *R. communis* showed the ability to grow in an adaptable manner, with coastal plants being sensitive to salt while inland plants were generally more drought-tolerant. The FTIR analysis also showed the presence of (N-H), a secondary amine, and liphatic fluoro compounds. Several explanations have been proposed for these effects. One such suggestion is that the longer durations of time that are typically observed after being exposed

to plant products suggest that these compounds disrupt the hormonal regulation of moulting (Diksha et al. 2023). The phytochemicals known as flavonoids make up 5–10% of all plant secondary metabolites. These have the ability to have detrimental impacts on insects, such as being an oviposition deterrent, insecticidal, an antifeedant, and having antibacterial activities. The flavonoids that were separated from *R. communis* have shown significant insecticidal properties against *Callosobruchus chinensis* (Shitu et al. 2020). The primary cause of the insecticidal activity is the blockage of specific critical enzymatic pathways. For example, flavonoids inhibit the hydroxylase enzyme through the action of cytochrome P450, which regulates the moulting process in insects (Jash 2023).

CONCLUSION

In the present study, a methanolic extract from *R. communis* showed the highest toxicity effect activity up to 72 h after the bioassay in the laboratory environment. This study provides valuable insights into the potential of *R. communis* as a source of natural insecticides against *B. cucurbitae*. The findings contribute to the development of environmentally friendly and sustainable pest management strategies, utilising invasive plant species as a bioresource for crop protection. However, before being incorporated into integrated pest management plans that are now being established, aqueous extracts of *R. communis* must be tested on non-target insects, including parasitoids. There is great potential for the plant to be taken up for development of bio pesticides in the near future. Future research directions may include elucidating the mode of action of specific bioactive compounds and assessing the broader ecological impact of utilising *R. communis* extracts in integrated pest management programmes.

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