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Original Article

Biological Activity of Extract *Solanum nigrum* on some Biological Aspects of the Blue Fly *Chrysomya albiceps* (Diptera: Calliphoridae)

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Abstract

The current research evaluated the efficiency of alcoholic and alkaloid extracts of the leaves and fruits of the *Solanum nigrum* and investigated their effectiveness against the immature stages of the blue fly with 24-hour age at concentrations of 5, 10, 15, 20 mg/ml at the temperature of 30±1°C and relative humidity of 60±5%. It was revealed that the alcoholic extract of the fruits had the highest effect on killing the eggs of blue fly at all concentrations; accordingly, the death rates were estimated at 89.11% and 42.43% at the concentrations of 5 and 20 mg/ml, respectively, compared to that in the control, accounting for 9.27% mortality. It was also found that the alkaloid extract of the leaves of the plant outperformed in recording the highest rates of killing the eggs of blue fly, with death rates of 88.83% and 31.14% in the concentrations of 5 and 20 mg/ml, compared to the control group, amounting to 10.40%. Regarding the larval stages, the first stage was more sensitive than the other larval stages to all extracts of leaves and fruits of the plant. The highest mortality rates of the three larval stages were achieved by using the alcoholic extract of the fruits with the highest concentration of 20 mg/ml, compared to the alcoholic extract of the leaves; accordingly, the death rates of the third larval stage reached the highest rates of 57.11%, 68.20%, and 88.69% for the alcoholic extract of fruits and 53.19%, 68.64%, and 89.11% for that of leaves. The recorded data showed that the alkaloid extract of the *S. nigrum* leaves led to the highest mortality rates, compared to the alkaloid extract of the fruits, for all larval instars and at all concentrations. The mortality rates of the third larval stage with the highest concentration were 58.13% and 67.64%, respectively. The results of gas chromatography-mass spectrometry to investigate the chemical compounds in the alcoholic and alkaloid extracts of the leaves and fruits showed the presence of chemical compounds of varying numbers. The numbers of chemical compounds in the alcoholic extract of the leaves and fruits were 10 and 9, respectively, while it reached 17 and 23 for the alkaloid extract of the leaves and fruits of the plant, respectively.

Keywords: *Solanum nigrum*, Blue fly, Alcoholic extract, Alkaloid extract, GCM

1. Introduction

Insects are highly important organisms for humans as different types of them cause multiple damages to humans, animals, and plants. Many insects directly infect the products or materials used by humans or indirectly transfer numerous pathogens to humans, animals, and plants, leading to serious disorders. Order Diptera includes the most important insects in case of medical and veterinary issues. Insects transmit several pathogens to humans and animals. A number of them infect different animals in their larval stage and parasitize their tissues, causing various kinds of myiasis which are harmful to human health and animals and negatively affect the general economy (1).

Species *Chrysomya albiceps*, belonging to the family Calliphoridae, spreads everywhere where there is a lot

of garbage, food waste and slaughterhouses, decomposing carcasses of animals and humans, and fish and meat exposed to the sun to dry.

The success of modern pesticides has encouraged scientists to believe that there is a wide scope for exterminating insects and limiting the spread of various diseases transmitted by them. Therefore, these pesticides revolutionized preventive medicine in the tropics; however, this success lasted no long as scientists faced two problems that were not taken into account, firstly, the emergence of resistance in insects, and secondly, the pollution of the environment with these manufactured chemicals. The first problem has made it difficult to eliminate insects and the second one has provoked outcry and disapproval, especially among those who care about nature and the Earth. Therefore, the best solution to these two problems is to resort to other alternatives. Among the most important reasons that made demands to return to the use of pesticides with plant origins are their desirable qualities (e.g., rapid decomposition) and very low toxicity to humans and animals, unlike chemical pesticides, which are characterized by slow decomposition and high toxicity to mammals (2). Plants contain chemical compounds that produce during their growth and development, some of which are important in the life of plants. Another part of these plant compositions, such as secondary products that are produced inside the plant cell in small quantities, are highly important in helping plants adapt to environmental conditions and compete with other plants. As plants are rich in anti-insect products and compounds and are highly efficient and important in maintaining the ecosystem, research on these compounds has increased. Different plant species secret special chemicals as repellents, preventers of feeding, or delaying molting in insects. Due to this fact, the role of plant extracts has been demonstrated as repellants of insects, preventing them from laying eggs, exiting adults, and molting (3).

The Iraqi environment includes diverse plants rich in compounds of known importance, some of which are known to contain toxic compounds (4). One of these plants is *Solanum nigrum*, widely spread in Iraq in orchards and public gardens.

Therefore, the current study was designed due to the lack of studies on the effect of *S. nigrum* on some aspects of the life of blue flies and aimed to investigate the possibility of its application as an alternative to chemical pesticides. The research goals were 1) preparing ethyl alcohol and alkaloid extracts of the leaves and fruits of the plant and identifying their effects on the immature stages of the blue fly, 2) determining the phenotypic distortions caused by different concentrations of the organic and alkaloid extract of the plant, which prevent the insect from being able to survive normally, leading to its death, and 3) investigating the active compounds of the ethyl alcohol and alkaloid extracts of the plant using gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

2.1. Plant Collection and Identification

Leaf and fruit samples of *S. nigrum* were collected in October 2019 from one of the orchards in the Mrammd village, district of Daghara, Iraq. The plant samples were dried separately in laboratory conditions, crushed to obtain a fine vegetable powder, kept in a tightly closed bottle, and placed in the refrigerator until further use. The plant was classified according to the taxonomic keys as *Solanum nigrum* of the Solanaceae family.

2.2. Insect Collection and Diagnosis

Chrysomya albiceps samples were collected from the residential area in the Mrammad village utilizing a net made of tulle, and the whole population was placed in a rectangular breeding cage with the dimensions of 90×90×90 cm^3. The base of the cage was wooden and its side faces were covered with tulle. It was decomposed for the purpose of feeding and laying eggs, and a quantity of clay soil was placed inside the cage, containing short plants. The cage was constantly sprayed with water to provide adequate moisture for the insect's life.

2.3. Preparation of Plant Extracts

2.3.1. Preparation of the Organic Solvent Extract of the Plant

The organic solvent extract of the plant was prepared according to the method proposed by Ladd Jr, Jacobson (5). The solvent of ethyl alcohol was chosen as a polar solvent. Dry powders of the leaves and fruits of the plant were weighed separately (20 g each). It was placed in a Soxhlet extractor and 200 ml of ethyl alcohol was added to it, the extraction lasted 24 h at 45°C. The process was repeated several times to obtain the required quantity for the experiment. After that, the extract was concentrated by a rotary evaporator at 45°C. Subsequently, the sample was dried in an electric oven at 45°C. To estimate the biological activity of the extract of ethyl alcohol, 2 g of dry matter extracted with ethyl alcohol from each of the leaves and fruits of the plant were weighed separately and dissolved in 3 ml of ethyl alcohol. The volume was completed to 100 ml with distilled water; consequently, the concentration of the stock solution became 2% or equivalent to 20 mg/ml, and the concentrations (5, 10, 15, and 20 mg/ml) were prepared using this solution. For the control treatment, 3 ml of ethyl alcohol was taken and completed by adding 100 ml of distilled water.

2.3.2. Preparation of the Alkaloid Extract of the Plant

The method mentioned by Harborne (6) was used to get alkaloid extract. To this end, 20 g of the powder of each plant sample was weighed and placed in the paper extraction container separately. Afterward, it was placed in the extraction device with 200 ml of 95% ethyl alcohol. The substance was extracted for 24 h at 40°C, and then the extract was dried by a rotary evaporator. The resulting substance was taken and dissolved in 5 ml of ethyl alcohol and added 30 ml of 2% sulfuric acid. The alcohol was disposed of by reusing the rotary evaporator to keep the solution acidic. A quantity of 10% ammonium hydroxide solution was added until the pH was raised to 9. Subsequently, the solution was extracted by funnel separation using 10 ml of chloroform, shaken several times, and left to be separated into two phases. The lower layer containing the dissolved alkaloids in chloroform was taken. The last step was repeated three times and the lower layer was taken each time; the aggregated solution condensed to approximately 40 ml. The resulting sample was dried and weighed.

2.4. Study Design

A total of 50 eggs were taken from the farm within 24 h of age and placed in a petri dish containing filter paper with 3 replicates for each concentration. The eggs were subjected to treatments with different concentrations of alcoholic extract. The alkaloid was applied to the leaves and fruits of the plant, and the extract was sprayed separately by a hand sprayer at a rate of 3 ml for each repeater. As for the control treatments, distilled water was employed with the solvent used in the extraction. After that, each of these dishes was covered with a perforated Petri cover, and then the eggs were transferred to the incubator at a temperature of 30±1°C and relative humidity of 65±5%. The mortality rates of eggs after hatching were recorded 24 h after the treatment.

A total of 50 larvae/replicate were taken from the first instar larvae within 24 h of age, with 3 replicates for each concentration separately. Volumes of 5 ml of all concentrations of the extracts were added to each plant and separately to 3 g of the nutrient medium. Regarding the control treatments, distilled water was employed with the solvent used in the extraction. The plastic tubes were transferred to the incubator, and under the same previous conditions, the mortality rates were recorded in the first larval stage after 24 h of treatment. The same process was repeated for the second and third larval instars of the alcoholic and alkaloid extracts of the leaves and fruits of the plant, each separately.

2.5. Identification of Active Compounds in Ethyl Alcohol and Alkaloid Extracts of *Solanum nigrum* Leaves and Fruits Using Gas Chromatography-Mass Spectrometry

Dry extracts of the leaves and fruits of the plant were taken separately (1 g each) and used to investigate the active compounds using the GC-MS technique:

2.5.1. Method of Analysis

The analysis was carried out utilizing the GCMS-QP2010 Plus GC-MS system, which includes an autosampler unit for vehicles and a gas chromatograph connected to a mass spectrometer instrument according to the following conditions:

An Elite-Fused silica column with the dimensions of 25 mn ID (30 mm) working in the EV 70 effect mode:

1- Helium gas (999-99%) was used as a carrier gas at a constant flow rate of 1 ml min -1.

2- The volume of the injected liquid was 0.5 µl and it worked in a split ratio (1:10)

3- The temperature of the injector was 260°C

4- The temperature of the ionic source was 200°C

5- The oven temperature was programmed automatically at 60°C (equal temperature for 2 min) with an increase of 10°C/min to 270°C, then 5°C until 290°C, after which it settled at 210°C for 9 min.

6- The mass spectra were taken based on EV 70 with a scan-interval of 0.5 sec and a splitting rate of 40-450 Daltons.

7- The total start-to-finish period of the chromatograph was 60 min.

The determination of the components was carried out according to the interpretation of the MS-GC mass spectrometry using the database of the National Institute of Measurement and Technology (NIST). This test was conducted in the Environment and Water Laboratories of the Ministry of Science and Technology.

2.6. Statistical Analysis

The results of the experiments on the effect of organic solvent extracts and plant secondary compound extracts on eggs were analyzed according to a completely randomized design. The results of the experiments of the extracts in the decimation of the different larval instars were analyzed according to the factorial experiments with a completely randomized design. The least significant difference test was used below the 0.05 probability level for the significance of the results. The mortality percentages were corrected according to Abbott (7).

3. Results and Discussion

Table 1 presents the rates of egg mortality in the ethyl alcohol and alkaloid extracts of the leaves and fruits of the *S. nigrum*, the results of which showed that the alcoholic extract of the fruits had the highest effect. The treatment of blue fly eggs with all the concentrations showed that the mortality rates reached 89.11% and 42.43% in the 5 mg/ml and 20 mg/ml concentrations, respectively, compared to the 9.27% mortality rate in the control group. The alkaloid extract of the leaves of the plant outperformed in recording the highest mortality rates of blue fly eggs, resulting in 88.83% and 31.14% at 5 and 20 mg/ml concentrations, compared to the 10.40% mortality rate in the control group.

Table 1. Effect of the type and concentration of plant extract on the egg mortality

Extract type		Concentration mg/ml					Average effect of extract type
		0	5	10	15	20	
Alcoholic	Leaves	8.13	42.21	46.86	46.90	89.11	46.64
	Fruits	9.27	42.34	54.79	82.02	89.11	55.50
Alkaloids	Leaves	10.40	31.14	55.25	58.84	88.83	48.89
	Fruits	11.28	28.34	39.67	55.13	68.41	40.56
Average effect of extract concentration		9.77	36.01	49.14	60.72	83.86	
LSD ($P \leq 0.05$)		For extract type=4.36; For extract concentration=4.88; Intervention=9.75					

LSD: Least significant difference

The results of the statistical analysis through the average effect of the extract type confirmed the superiority of the ethyl alcohol extract of the fruits. The findings showed that the highest rates of egg mortality were achieved using the alcoholic extract of the leaves, accounting for 55.50% and 46.64% at 5 and 20 mg/ml concentrations, respectively.

Considering the alkaloid extract, it was revealed that the alkaloid extract of the leaves had superiority over the alkaloid extract of the fruits. Accordingly, the mortality rates were obtained at 31.14% and 88.83% for the leaves and 28.34% and 68.41% and fruits at the concentrations of 5 and 20 mg/ml, respectively, while they were estimated at 10.40% and 11.28% in the control group, respectively. It was found that there was a direct relationship between the percentages of mortality and concentrations of extracts.

The susceptibility of extracts to the destruction of eggs is explained by their effect on the fetal movement during its formation or their penetration into the eggs and killing the fetuses (2), as well as the failure of eggs to hatch due to the hardening of the shell or the direct effect on the protoplasm, which causes the death of the embryo inside the egg, indicating that the treatment of the outer surface of the egg disrupts embryonic growth, and therefore, the egg does not hatch. These results indicate that the plant extracts possibly contain some chemicals with harmful effects on the outer surface of eggs (8).

Based on the findings of a study by Al-Zubaidi (9), the alkaloid extract of *Datura innoxia* had superiority over the alkaloid extract of *Citrullus colocynthis*. Accordingly, the highest mortality rates of eggs of the corn stem borer *Sesamia cretica* were 95.29% and 100%, respectively. As indicated by Al-Zubaidi (9), the alkaloid extract of *Albizzia lebbeck* flowers recorded the highest mortality rate for housefly eggs at the concentration of 10 mg/ml (up to 90.0%), followed by the alkaloid extracts of leaves (83.6%) and seeds (62%).

Table 2 summarizes the mortality rate of larval stages in the ethyl alcohol and alkaloid extracts of the leaves and fruits of *S. nigrum.* The results showed the superiority of the ethyl alcohol extract of the leaves in the destruction of the larval stages, compared with the alcoholic extract of the fruits of the plant, at a rate that reached the death of the third larval stage with 11.57%, 68.20%, and 88.69% for the alcoholic extract of the fruits and 53.19%, 68.64%, and 89.11% for the alcoholic extract of the leaves.

Table 2. Effect of plant extract type and concentration on the percentage of larval mortality

Type of extract	Larval instar	Concentration mg/ml					Average effect of the extract type on the larval stage
		0	5	10	15	20	
Alcoholic leaves	First instar	8.13	37.63	43.89	47.69	89.11	45.29
	Second instar	9.27	17.08	30.73	39.36	68.64	33.01
	Third instar	8.13	17.31	20.04	31.66	53.19	26.07
Alcoholic fruits	First instar	11.54	39.12	46.13	48.84	88.69	46.86
	Second instar	9.27	29.08	43.19	52.50	68.20	40.45
	Third instar	9.27	27.62	42.78	55.72	57.11	38.50
Alkaloids leaves	First instar	13.30	53.21	55.85	72.22	88.46	56.61
	Second instar	11.54	46.13	48.88	67.84	81.74	51.23
	Third instar	8.13	43.47	44.32	63.15	67.64	45.34
Alkaloids fruits	First instar	9.27	47.03	53.85	69.37	88.97	53.70
	Second instar	11.54	45.67	46.61	59.22	72.05	47.02
	Third instar	9.27	36.37	36.38	51.43	58.13	38.32
Average effect of extract concentration		9.89	36.64	42.72	54.92	73.49	
LSD ($P \leq 0.05$)	Larval stage=2.85; For extract concentration=1.84; Intervention=6.36						

LSD: Least significant difference

The alkaloid extract of the leaves of *S. nigrum* was more efficient than that of the fruits. The results also indicated a different sensitivity of the larval stages to the extracts, as the first stage was more sensitive than the rest larval stages. The findings of another study by Alhuraysi, Elsheikh (10) reported the superiority of the alkaloid extract leaves of *Artemia absinthium*. Regarding, the resistance increased as the age of the instar increased, and a direct relationship was observed between concentrations and mortality rates. The reason for this may be explained by the ability of the last larval stages to convert toxic compounds (2).

Some phenotypic abnormalities were recorded in the dead larvae, such as shrinkage of the body, blackening, or death of the larvae during its molting into the subsequent larval stage (11). Kristensen and Jespersen (12) explained that some chemical compounds affected the epithelial cells of the digestive canal of insects and led to the decay of the membrane lining, and consequently, impeded the process of digestion and absorption.

Table 3 presents the results of GC-MS technology for alcoholic and alkaloid extracts of the leaves and fruits of *S. nigrum* (Figures 1-4). It should be noted that some of the compounds in table 3 have insecticidal activity, such as n- Hexadecanoic acid and Oleic acid, cyclopropaneoctanal, 2-octy and 9-octadecenoic acid which have larvicide activity. Moreover, there are some compounds with different properties, such as anti-oxidant and anti-inflammatory activities.

Table 3. Identification of the active compounds in plant extracts using GC-MS

	Active Compounds of Ethyl Alcohol Extract of *Solanum nigrum* Leaves	**Molecular weight**	**Formula**	**Area**	**RT standard material**	**RT laboratory material**	**Activity**
1	9-Octadecenoic acid	282.46	$C_{18}H_{34}O_2$	257	21.66	21.065	Larvicidal
2	Linoelaidic acid	280.45	$C_{18}H_{32}O_2$	20.92	30.070	30.072	Antibacterial
3	Glycidyl acid	312.5	$C_{19}H_{36}O_3$	24.72	31.730	31.729	No activity
4	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	16.35	34.844	34.84	Insecticidal
5	Oleic acid	284	$C_{18}H_{32}O_2$	9.57	35.064	35.62	Insecticidal
	Active compounds of ethyl alcohol extract of *Solanum nigrum* fruits	**Molecular weight**	**Formula**	**Area**	**RT standard material**	**RT laboratory material**	**Activity**
1	Propanoic acid	74.08	$C_3H_6O_2$	0.29	5.075	5.073	Antibacterial
2	1,22-docosandiol	326.6	$C_{22}H_{46}O$	8.49	21.073	21.070	Antimicrobial
3	1-Heptadecane	545	$C_{37}H_{72}O$	2.29	28.160	28.157	Antibacterial
4	Linoelaidic acid	280.45	$C_{18}H_{32}O_2$	17.97	30.079	30.078	Antibacterial
5	Glycidyl palmitate	312.5	$C_{19}H_{36}O$	11.85	31.691	31.689	No activity
6	Oleic acid	284	$C_{18}H_{32}O_2$	3.87	33.715	33.712	Insecticidal
7	n-Hexadexanoic acid	256	$C_{16}H_{32}O_2$	10.87	34.846	34.848	Insecticidal
	Active compounds of the alkaloid extract of the leaves of *Solanum nigrum*	**Molecular weight**	**Formula**	**Area**	**RT stander material**	**RT laboratory material**	**Activity**
1	Propanoic acid	74.08	$C_3H_6O_2$	0.36	5.075	5.073	Antibacterial
2	Tetradecane	198	$C_{14}H_{30}$	1.53	22.163	22.162	Antioxidant
3	Cyclpropaneoctanal,2-octy	154.29	$C_{11}H_{22}$	2.02	28.174	28.174	Insecticidal
4	Glycidyl palmitate	312.5	$C_{19}H_{36}O_3$	13.34	31.74	31.71	No activity
5	Nonadecane	284.52	$C_{19}H_{40}O$	2.79	33.668	33.667	Antimicrobial
6	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	14.54	34.847	34.844	Insecticidal
7	Oleic acid	284	$C_{18}H_{32}O_2$	5.82	35.065	35.67	Insecticidal
	Active compounds of the alkaloid extract of the fruits of *Solanum nigrum*	**Molecular weight**	**Formula**	**Area**	**RT stander material**	**RT laboratory material**	**Activity**
1	Heptanal	809	$C_7H_{14}O$	0.75	9.837	9.840	Antimicrobial
2	2-Decanal	154.25	$C_{10}H_{18}O$	1.41	18.494	18.493	Antioxidant
3	Pentadecane	49.396	$C_{15}H_{32}$	0.63	24.713	24.711	Antioxidant
4	5-Dodecanoic acid	186	$C_{12}H_{26}O$	1.41	30.080	30.087	Antibacterial
5	Glycidal palmitate	312.5	$C_{19}H_{36}O_3$	14.48	31.694	31.695	No activity
6	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	23.73	34.855	34.850	Insecticidal

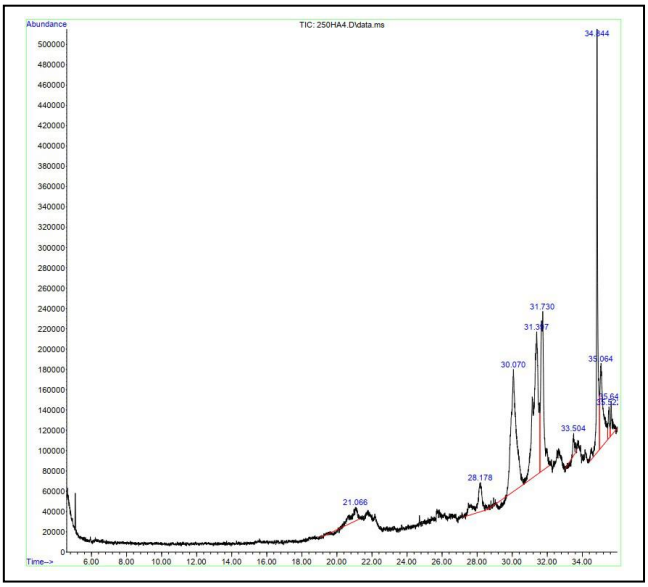

Figure 1. GC-MS data for the alcoholic extract of *Solanum nigrum* leaves

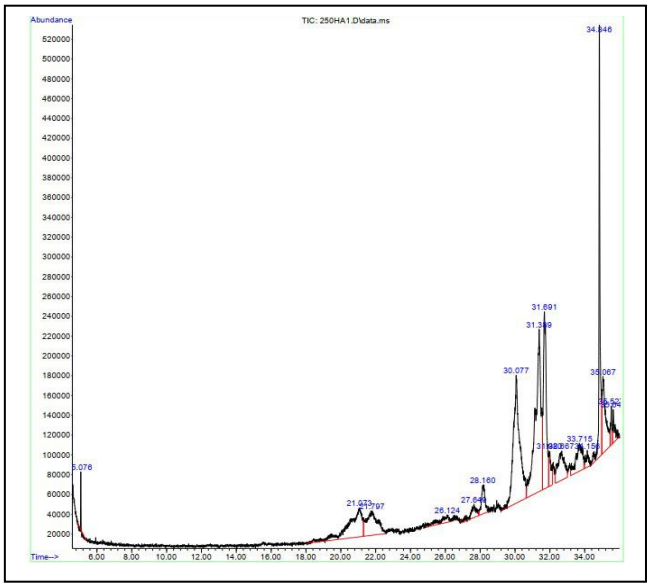

Figure 2. GC-MS data for the alcoholic extract of *Solanum nigrum* fruits

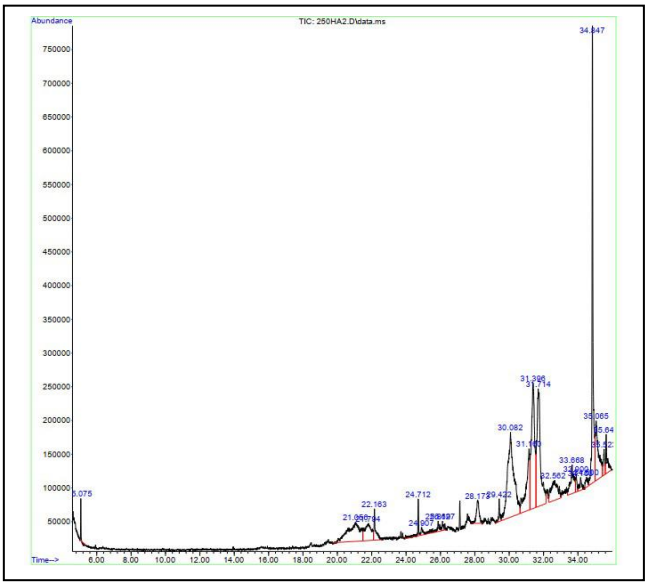

Figure 3. GC-MS data of the alkaloid extract of *Solanum nigrum* leaves

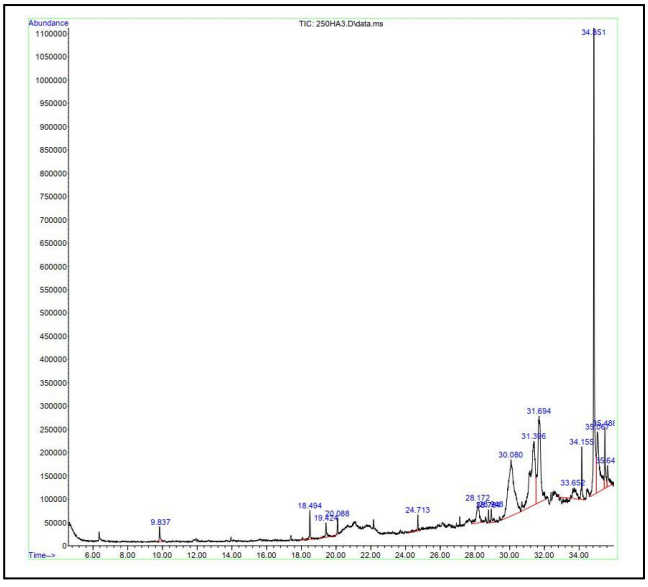

Figure 4. GC-MS data for alkaloid extract of *Solanum nigrum* fruits

In another research, Falodun, Siraj (13) analyzed the leaf extract of *Pyenacantha staudtii* by GC-MS, the results of which demonstrated its effectiveness against *Tribolium castaneum*. Ramos-López, González-Chávez (14) found four chemical compounds from *Ricinus comminis* leaf extract and reported their effectiveness in controlling *Spodoptera frugiperda* larvae.

The alcoholic extract of *Solanum lycocarpum* fruit showed that the most important chemicals of this fruit were Linoleic acid and Oleic acid, which can be used as insecticides against mosquitoes. Thenmozhi and Rajan (15) concluded through GC-MS analysis that there were four chemical compounds in the ethanolic extract of the *Psidium guajava* leaves having

insecticide effects.

Zhang, Chen (16) performed a study to detect the components of *Vernicia fordii* seed extract using GC-MS technology and found 20 compounds, some of which had insecticidal activity against the insect *Odontotermes formosanus*.

It is concluded that the alcoholic and alkaloid extracts of *S. nigrum* fruit and leaves can be beneficial in controlling *C. albiceps* that reduce economic burden since this plant contains certain active components causing larval mortalities, such as n-hexadecenoic acid, Oleic acid, and palmitic acid.

Authors' Contribution

Study concept and design: H. R. L.
Acquisition of data: H. R. L.
Analysis and interpretation of data: H. R. L.
Drafting of the manuscript: H. R. L.
Critical revision of the manuscript for important intellectual content: H. R. L.
Statistical analysis: H. R. L.
Administrative, technical, and material support: H. R. L.

Conflict of Interest

The authors declare that they have no conflict of interest.

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