Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Effects of alcoholic and alkaloid extract of *Solanum nigrum* in some aspect's life cycle of the green bottle fly (*Lucilia sericata* (Diptera: Calliphoridae)

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Abstract

Research explains evaluating the efficiency the alcoholic and alkaloid extract of the leaves and fruits of *Solanum nigrum* and testing its effectiveness against the immature stages of the green bottle fly *Lucilia sericata* within 24 hours at concentrations (5, 10, 15, 20, 0.0) mg/ml, at a temperature of 30 ± 1 and a relative humidity of 60 ± 5%. The alcoholic extract of the plant's fruits had the highest effect on killing *Lucilia sericata* eggs in all the concentrations used, where the death rates reached (42.34 - 89.11) % at concentrations 5 and 20 mg/ml, respectively, compared to the control amounting to 9.27 % alkaloid extract, of leaves excelled in recording highest rates killing for green bottle fly eggs with a death rate of (35.58- 68.41) % in concentrations 5 and 20 mg/ml compared with the control of 10.40%. The first larval stage was more sensitive than the other larval stages to all extracts of the leaves and fruits in different used concentration, where the highest rates mortality of third larval stages when 20mg/ml of fruit alcoholic extract compared with the alcoholic extract of the plants leaves. The percentages mortality of the third larval stage with the highest concentration (53.19, 57.11)% for the extract of alcoholic for fruits and leaves respectively as for the extract of alkaloid, we find that the alkaloid extract of the leaves more efficient than the alkaloid extract of the fruits in recording the highest death rates for all larval stages with all the used concentrations, the mortality rates of the third larval stage with the highest concentration were (58.13 - 66.09%) for the alkaloids fruits and leaves respectively.

Keywords: *Solanum nigrum*, *Lucilia sericata*, alcoholic and alkaloid extract.

Introduction

Insects are very important organisms for humans, as different types cause multiple damages to humans, animals and plants many of them are directly harmed or their harm is indirect by transferring many pathogens to humans, animals and plants, leading to heavy losses (**1**). Order of Diptera includes the most important medically and veterinary insects, in addition to the fact that large numbers of them live on plants or live as shoots. The two wings transmit a number of pathogens to humans and animals a number of them infect different animals in their larval stage and parasitize their tissues, causing the myiasis of various kinds, are harmful to human and animals health, which negatively affects the general economy (**2**). *Lucilia sericata* belongs to the family Calli Phoridae spread everywhere where there is a lot of garbage, food waste and slaughterhouses, decomposing carcasses of animals and humans, the insects are one of medical veterinary and criminal importance (**3**). The success of modern pesticides has encouraged scientists to believe that there is ample scope for terminating insects and limiting the spread of many the diseases they transmit. Therefore, these pesticides revolutionized preventive medicine in the

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tropics, but this success did not last long, as scientists faced two problems that were not taken into account. One is the emergence of resistance in insects and the second is the pollution of the environment with these manufactured chemicals, the first problem has made it difficult to eliminate insects, The second problem has provoked outcry and disapproval, especially for those who love nature. one of the most important reasons that made many demand a return to the use pesticides of plant origin is their desirable qualities such as rapid decomposition, also the toxicity of animals and humans very low compared chemical pesticides that characterized by slow decomposition and toxicity to mammals is high (**4**), Where plants contain chemical compounds that they produce during their growth and development, some of these compounds are important in the life of plants, but another part of these as secondary products that are made inside the plant cell in small quantities. As plants are rich in anti-insect products and compounds, and their high efficacy and importance in maintaining the ecosystem, research on these compounds has increased. In different plant species, because they are secreted as repellents, preventers of feeding, or delaying molting in insects, where the role of plant extracts appears as food repellants for insects, laying eggs, exiting adults and molting (**5**). The plants of Iraqi environment rich in compounds that importance, and contain toxic compounds, one of these plants which S. *nigrum* widely spread in Iraq in orchards and public gardens and is a major source of many different alkaloid compounds, (**6**), (**7**). It follows the Solanaceae family. The plant contains asparagine solanine, lutein, solangiosteine tannin, palmitic acid and linoleic acid and used medicinally as a sedative, hypnotic and to soften the skin. Fruit juice has a mitigating effect for dental pain and a lot of eating the latter causes loss of memory and awareness and lead to poisoning then death because they contain steroidal alkaloids also harms vegetative total of livestock when grazing them, leaves and fruits contain toxic substances for sheep, cows, horses, goats and pigs (**8**). Due to the lack of studies on the effect of *S. nigrum* on some aspects of the life cycle of green bottle fly, so the current study was aimed to use the extract of ethyl alcohol and alkaloid of the leaves and fruits of this plant as alternatives to chemical pesticides, the goals

1-Preparation the extract of ethyl alcohol and alkaloid of the leaves and fruits of the plant and its effect on the immature stages of *Lucilia sericata*.

2-Determining the phenotypic distortions caused by the different concentrations of the organic and alkaloid extract of the insect.

Materials and Methods

Plant collection and identification

Fresh leaves and fruits of *S. nigrum* plant were collected during October 2020 from Al-Diwaniya province orchards, the plants were cleaned and dried in an oven at a temperature of 45°C until dryness. The dry parts of the plant (leaves and fruits) were crushed separately by a blender grinder. The ground forms were spun into sealed glass containers at a laboratory temperature (20-22) °C until use. The plant was classified according to the taxonomic keys as *Solanum nigrum* of the Solanaceae family.

Insect collection and diagnosis

Lucilia sericata were collected from one of the residential areas in Diwaniya province by means of a net made from tulle, and the whole populations placed in rectangular breeding cages of dimensions (60 x 60 x 60) cm, the base from solid nylon، the side faces of cages are covered with net. Inside the cage metal utensils open from the top containing fish of feeding and eggs laying, and a quantity of clay soil was placed inside the cage, containing short plants, and it was constantly sprayed with

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water for the purpose of providing adequate moisture for the insect's life.

Preparation of plant extracts

Preparation of the organic solvent extract of the plant

The organic solvent extract of plant was prepared according to the method of (**9**). The solvent of ethyl alcohol was chosen as a polar solvent. 20 g of dry powder of the leaves and fruits have been weight separate and placed in Sexhulate and 200 ml of ethyl alcohol added to it, and the extraction lasted 24 hours whit temperature 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. The sample dried in electric oven at 45°C. 2 g of dry each matter was extracted with ethyl alcohol and dissolved in 3 ml of ethyl alcohol, the size is completed to 100 ml with distilled water, the concentration of the Stock solution became 2% or equivalent to 20 mg /ml, and from it the concentrations (5, 10, 15 and 20,) mg / ml were prepared. The control treatment, was prepared as 3% ethel alcohol.

Preparation of the alkaloid extract of the plant

The method mentioned in (**10**), taking 20 gm from the powder (fruits and leaves) of plant sample and placing in the extraction paper container separately, then placed in extraction device with 200 ml 96% ethyl alcohol. The samples were extracted for 24 hours at 38 °C, then it was dried by a rotary evaporator. Resulting samples were dissolved in 5 ml alcohol, after which 30 ml was added of 2% sulfuric acid, and alcohol could be disposed by using rotary evaporator again keep the solution acidic. A quantity of 10% ammonium hydroxide solution was added until PH = 9, the solution extracted by funnel separation using 10 ml chloroform, step back several times and left the mixture to separate two layers, the lower layer containing alkaloids dissolved in chloroform. last step repeated three times and lower layer taken each time so that aggregated solution became 40 ml. Resulting sample was dried and weighed. The concentrations were prepared and the control treatment as mentioned in the above paragraph.

Effect of ethyl alcohol and alkaloid extract of *S. nigrum* on life cycle of *Lucilia sericata*

1- Effect on the eggs

50 eggs/replicators were harvested from the farm after 24 hours of laying and placed in petri dish. These eggs were treated with different concentrations of alcoholic extract. The alkaloid was applied to the leaves and fruits of the plant and sprayed the extract separately by hand sprayer at a rate of 3 ml for each repeater. the treatments of control, using distilled water with solvent used in extraction. each these dishes covered with a perforated Petri cover, eggs transferred to incubator at a

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www.qu.edu.iq/journalvm

temperature 30 ± 1 and humidity 65 ± 5%. The mortality rates of eggs after hatching recorded through 24 hours of treatment and mortality rates was adjusted according to (**11**).

2-The effect on the third larval instars

50 larvae/replicant was taken from the first instar within 24 hours of hatching , with 3 replicates for each concentration and , 5 ml of all concentrations of extracts were added for each plant and separately to 3 g of the nutrient medium, as for the control treatments distilled water used with the solvent used in the extraction, The plastic tubes transformed to the incubator under the same previous conditions, mortality rates recorded in the first larval stage after 24 hours of treatment and mortality rates adjusted according to (**11**). The same process repeated for second and third larval instars of the alcoholic and alkaloid extract of leaves and fruits for plant, each separately.

Statistical analysis

Results of the experiments with effect of extracts of organic solvents and extracts of secondary compounds of plants on the destruction of eggs analyzed according to a completely randomized design, while results of experiments with extracts on the destruction of different larval instars and adults analyzed according to the factorial System using a completely randomized design. Least significant difference test under 0.05 probability level to test the significance of results. The corrected death percentages corrected to modified Abbott formula (**11**), where the corrected death percentage was calculated according to the following:

$$\text{Percentage of death} = \frac{\text{\% Perish in the treatment - \% perish in comparison}}{\text{100 \% to be lost in comparison}} \text{ X 100 \%}$$

The corrected death percentages were converted into angular values to be included in statistical analysis (12).

Results and Discussion

Table (1) explain rates of egg mortality in extract of ethyl alcohol and alkaloid extract of leaves and fruits of currant plant *S. nigrum*, where we note the alcoholic extract of fruits have highest effect on killing *L. sericata* eggs at all concentrations used, where death rates reached (42.34 - 89.11) % in 5 and 20 mg /ml, respectively, compared with control amounting to 9.27 %. The results of the statistical analysis, the average effect of type of extract, confirmed the superiority of ethyl alcohol extract of fruits in recording highest rates of egg killing compared with alcoholic extract of leaves with a percentage of (55.50-46.64%), respectively. Alkaloid extract of leaves of plant outperformed in recording highest rates of killing blue fly eggs, with a death rate of (35.58-74.93) (28.34-68.41) % for the leaves and fruits of the plant at concentrations 5,20 mg/ml, respectively, compared to the control and the amount (10.40 - 11.28) %. The results of direct relationship between percentages mortality and the concentrations of extracts. The ability of the extracts to kill the eggs is explained by prevent development of larva formation, or penetration inside the egg and killing the larva (4), the cause of the destruction may be attributed to the effect of

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

plant toxic substances. In the extracts in vital systems of larva or as a result of its obstruction of gas exchange inside the egg, which leads to its death, or the failure of the egg to hatch is due to the hardening of the shell or the direct effect on the protoplasm, which causes the death of the embryo inside the egg (13); (14). Treating the outer surface of the egg with some hormonal analogues disrupts the embryonic growth and thus does not hatch the egg, which indicates the possibility that plant extracts contain such hormonal analogues (15). The present results are in agreement with the findings of the study (16) regarding the superiority of the alcoholic extract of *L.barbarum* at 20 mg/ml in the mortality rates for the eggs of the *Musca domesticae* followed by the bramble plant and then bitter melon with death rates of (67.45, 72.31, 90.00) mg/ml of the same extract and the same concentration compared to control, which amounted to 22.79%. Ethyl alcohol extract of Datura plant recorded highest mortality rates for *Culex quinqufasciatus* eggs, followed by *L.barbarum* , *Rubus sanctus*, and then Lalla Abbas plant, as it reached LC_{50} ppm (2199.9, 2580.6 and 3062.7) for plants, respectively (17). While (18) indicated the superiority of the alkaloid extract of *D.innoxia* over the alkaloid extract of *Citrullus colocynthis* in the events of highest death rates eggs of *Sesamia cretica*, reaching 95.29% and 100%, respectively, as indicated by (19) in the alkaloid extract of the flowers of the *Albizzia lebbeck* plant recorded the highest mortality rate for house fly eggs at 10 mg/ml, which amounted to 90.0%, then the alkaloid extract of leaves 83.6%, and then the alkaloid extract of the seeds 62%.

Table 1. Effect of the *S. nigrum* extract on the egg mortality of *Lucilia sericata*

Extract type		Concentration mg/ml					Average effect of extract type
		0	5	10	15	20	
Alcoholic	Leaves	8.13	42.21	46.90	46.90	66,09	42.05
	fruits	9.27	42.34	54.79	82.02	89.11	55.51
Alkaloids	Leaves	10.40	35.58	55.25	58.84	74.93	47.00
	fruits	11.28	28.34	39.67	55.13	68.41	40.57
Average effect of extract concentration		9.77	37.12	49.15	60.72	74..64	
LSD ($P \leq 0.05$)		for extract type = 5.04 For extract concentration = 5.63 intervention = 11.27					

Table (2) shows the rates of mortality of larval instar in the extract of ethyl alcohol and alkaloid extract of leaves and fruits of *S. nigrum* plant, where highest rates of mortality of the third larval stages were using the alcoholic extract of fruits with the highest concentration of 20 mg/ml compared with alcoholic extract the leaves of plant. The percentages of mortality of the third larval stage with the highest concentration (53.19, 57.11) % for the alcoholic extract of the fruits and leaves of the plant, respectively. As for the alkaloid extract, we find the alkaloid extract of leaves of *S. nigrum* plant was more efficient than the alkaloid extract of fruits in recording the highest death rates for all larval stages and in all concentrations used where mortality rates of third larval instar with highest concentration were (58.13 - 66.09), respectively.The results also indicate a different sensitivity of the larval stages to the extracts, as the first stage was the most sensitive compared with another larval instar. The superiority of the alkaloid extract of the leaves of the *Rubus sanctus* in recording

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

the highest mortality rates for all larval stages of *Musca domestica* at all the concentrations used, followed by *Lycium barbarum* and *Citrullus colocynthis*, as it was mentioned that the extract was ethyl alcohol (20). The highest effect on the destruction of the third larval instars was followed by hexane extract and then ethyl acetate. Also, the first larval stage was more sensitive than the rest of the other larval instar when treated with the concentrations of all extracts, while the third stage was the most resistant, as the resistance increases with the age of the stage. The reason for this may be that the last larval stages were able to converting the toxic compounds found in various plant extracts into non-toxic compounds through detoxification by an enzyme called mixed function oxidation M.F.O, while the first phase cannot do that due to its lack of this enzyme system (4).Some phenotypic abnormalities were recorded in the dead infected larvae, represented by the shrinkage of the body sometimes, blackening or the death of the larvae during their molting to the later larval stage or pupal stage, and their death after that without completing their life cycle or the occurrence of elongation in the larvae and its size more than normal limit for the control treatment or shortening in larva or abnormalities in the abdominal rings, and the reason for this is explained by the insect's sensitivity to toxic substances found in the tested plants, which indicates the inhibitory action of plants on larval growth of organic and secondary extracts, which is similar to the action of growth regulators (21). Also, most of the alkaloids and saponins vary in their quantities in the different parts of the same plant, but they may be made in the roots and transferred to other vegetative parts later. or stored in it (22).

Table 2. Effect of the of *S. nigrum* extract on the larval mortality of *Lucilia sericata*

Type of extract	Larval instar	Concentration mg/ml					The 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	68.64	40.53
	Third instar	9.27	18.39	42.78	55.72	57.11	36.65
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	74.79	49.84
	Third instar	8.13	43.47	44.32	63.15	66.09	45.03
Alkaloids fruits	First instar	9.27	47.03	52.03	69.77	88.97	53.41
	Second instar	11.54	45.67	46.61	59.22	67.84	46.18
	Third instar	9.27	36.37	36.38	50.98	58.13	38.23
Average effect of extract concentration		9.89	35.88	42.57	54.91	72.47	
LSD (P ≤ 0.05)	Larval stage = 2.65 For extract concentration = 1.71 intervention = 5.92						

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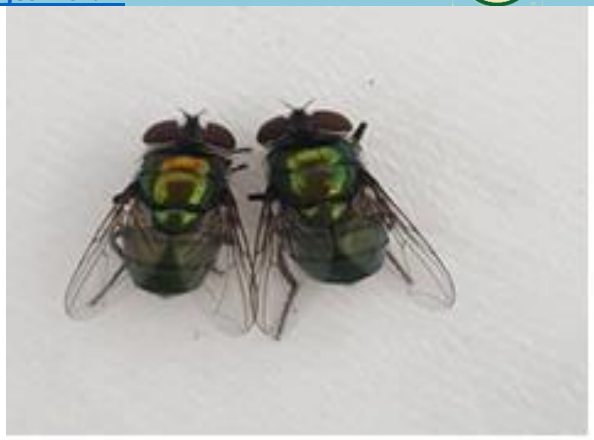
Control male



Control female

Control pupa

Culture of bottle fly

Alkaloids fruits extract

Eggs of bottle fly

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

Alkaloids leaves extracts

Alcoholic leaves extracts

A panel (1) showing the distortions that occurred when using different concentrations of alcoholic and alkaloid extract of fruits and leaves of plant in immature stages of bottle fly after 24 hours of treatment.

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Al-Qadisiyah Journal of Veterinary Medicine Sciences
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