Biological activity of Citrullus colocynthis L extracts on Culex pipiens molethus and Musca domestica

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ABSTRACT

The two most hazardous vectors for humans are *Culex pipiens molethus* and *Musca domestica*. Several environmental and health issues have emerged because of the overuse of chemical pesticides. This study sought to determine the efficacy of aqueous, alcohol, and oil extracts of *Citrullus colocynthis* L. plant under in vitro conditions using different concentrations (0, 5, 10, and 20 mg/mL) on the various stages of *Culex pipiens molethus* and *Musca domestica*. It also sought to identify natural biological alternatives to synthetic insecticides. The current research revealed that the adult and juvenile stages of mosquitoes and house flies were considerably impacted by the extracts of bitter melon. Concentrations demonstrated a distinct effect and significantly outperformed the control treatment after 24, 48, and 72 hrs of exposure. Compared to other bitter melon extracts, the impact of the oil extract of *C. colocynthis* was superior. At a dosage of 20 mg/ml, oil extract treatment significantly reduced hatchability and increased mortality in the second and fourth larval stages of *C. pipiens molethus*. The outcomes also demonstrated the superiority of the oil extract of *C. colocynthis* in influencing the different stages of the house fly *M. domestica* with the same previous concentrations (20, 10,5 mg/mL) on egg productivity (22, 72, 89, 82%) and third larval instar (66, 79, 71, 94, 82.88%) mortality rates, as well as pupae weight rates (0.10, 0.11, 0.100 mg). With the help of the current study's findings, we may suggest using bitter melon extracts, particularly the oil extract, as a potentially effective control measure against mosquitoes and house flies. These naturally occurring extracts, which do not contain any industrial chemicals, are difficult for the insect they are intended to kill to withstand. For the aim of understanding the mechanism of action of these extracts on mosquitoes and house flies, as well as understanding the effect of bitter melon extracts on numerous therapeutic insects in the future, more research is necessary.

Keywords: Plant extracts, Bitter melon, Mosquitoes, House flies, Environmentally friendly pesticides.

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INTRODUCTION

All around the world, house flies are significant veterinary and medical insects. Several illnesses, including typhoid and paratyphoid of all types, summer diarrhea in children, eye disorders, TB, cholera, and anthrax, are mechanically transmitted to people and animals by this bug. Moreover, some germs reach the fly's digestive tract from the contaminated substances it consumes, and some of the microorganisms it carries enter human food or drink through its saliva or faeces (Wamaket *et al.*, 2018).

Mosquitoes are just as deadly and aggressive as house flies in terms of their ability to transmit a wide range of diseases to humans and their annual toll of fatalities, particularly in rural areas (Wamaket *et al.*, 2021). Several blood parasites are spread by such arthropods to vertebrates

(Rajesh *et al.,* 2015). Many viruses, including those that cause viral hepatitis C and dengue fever are also spread by mosquitoes (Sarwar, 2016). There are around 300 different types of mosquitoes, divided into 39 genera and 135 subspecies (Kiarie-Makara *et al.,* 2015). Iraq is home to a species called *C. pipiens* mole thus (Hamer *et al.,* 2008).

For a long time, there were numerous simple and conventional ways for controlling the *M. domestica* and *C. pipiens*, as well as synthetic chemical pesticides, but the two species of insects shown strong resistance to these pesticides (Sehgal *et al.,* 2002). Excessive use of chemical pesticides has many negatives, as they are expensive, polluting to the environment, and harmful to humans and animals, especially aquatic organisms, in addition to plants (Wang *et al.*, 2010). For decades, researchers have resorted to finding alternatives of plant origin. Plant extracts are characterized by being quick to decompose when exposed to environmental conditions. In addition to their good effectiveness in low concentrations, and thus they are of low toxicity to humans and animals and are very specialized (Scott *et al.*, 2003). Koul *et al.* (2002) stated that pesticides of plant origin have many characteristics that have attracted and attracted the interest of researchers in the long term. They are quick to decompose into non-toxic materials because of their high sensitivity to light, heat, and moisture, unlike chemical pesticides. Most of the plant pesticides affect the respiratory system or prevent the digestive system from performing its work well, as well as acting as anti-nutritional and insect repellents. Plants contain many chemical or semi-chemical compounds that the plant produces within cells during its growth and development.

The plant produces these compounds in small quantities, but their importance is great, as some of them are of great importance in the life of the plant for the purpose of growth and development, and others are considered secondary for the purpose of attracting pollinators and reproduction. They help the plant to adapt to environmental conditions and compete with other plants as well. They are defensive compounds that help the plant to resist the attack of many insects and expel them (Isman, 2014) or invoke natural enemies (Dicke *et al.*, 1990). The Iraqi environment contains a variety of plants rich in compounds of known medical and veterinary importance, and some of them contain toxic compounds (Chhabi *et al.*, 2017). *Citrullus colocynthis* is one of the Iraqi plants widely spread in the western and southern deserts. It contains important active compounds, including the alkaloids represented by citrulline, which is due to its bitter taste, resins, resins, glycosides, soaps and cucurbitacin (Al-Ghaithi *et al.,* 2004).

The possibility to use the two insects in place of chemical pesticides should be taken into account, as should the lack of study on the effects of the alcoholic and aqueous extract of *C. colocynthis* on specific stages of the *M. domestica* and *C. pipiens* mosquito life cycle. This study sought to ascertain the impact of bitter melon extracts on a few elements of *M. domestica* and *C. pipiens* mosquitoes' life performance.

Materials and Methods

Bitter apple collection and identification

Apples that are acrid, *C. colocynthis* were collected in March 2022, during the time of fruit ripening, from the Al-Salman location in the southern desert of Samawah city. With the aid of an electric grinder, the fruits were separately crushed.

Cold and hot water extraction

Aqueous extracts were produced both cold and hot as described in Al-Ghannoum and Karso (2015) and (Ali and Aljanabi, 2020). 20 g of powdered bitter apple fruit seeds were weighed out and added to a beaker with 200 mL of distilled water. The hot water extract was made by following the same previous procedures, where water was heated for two hrs at (60–70°C). To get adequate dry residue for the studies, the extraction procedure was carried out many times. Each (100 mL) of aqueous extract received (0.5 mL) of Fabco-surfix Jordan's (condensed ethylene phenol oxide) (cold and hot). Each aqueous extract was made in three concentrations (5,10 and 20%) in addition to the control treatment, which consisted just of distilled water.

Preparation of alcoholic and oil extract of bitter apple fruit C. *colocynthis*

The preparation of alcoholic and oil extract of bitter apple fruit extract was done as mentioned in (Ali and Aljanabi, 2020) with some modifications. The oil bitter apple fruit plant extract was prepared by taking (20 g) of the previously prepared bitter apple fruit seed powder and placed in a Soxhlet apparatus. The solvent n-hexane was added to the apparatus and extracted over a period of (24 hrs) and then the extract was filtered using a Whatman No. The extract was concentrated under vacuum pressure and at a temperature of (40-50 °C) using a rotary evaporator (Harborne, 1973). Three concentrations were prepared: 5% (5 mL oil + 5 ml Y-methyl sulfoxide DMSO + 95 mL distilled water), 10% (10 mL of bitter melon seed oil + 10 mL of methyl sulfocide + 90 mL of distilled water), 20% (20 mL of bitter melon seed oil + 20 mL of methyl sulfocide + 20 mL of distilled water) and surfix is added to it. The control treatment was prepared by using the same quantity of the solvent and the diffuser (surfix). A quantity (20 g) of *C. colocynthis* fruit powder was put into a beaker and (200) mL of ethanol alcohol (96%) was added to it, and the mixture was shaken well using a magnetic stirrer. The beaker was kept closed in the refrigerator for (24) and then filtered under reduced pressure twice by Whatman filter paper (No. 1). The solvent was separated using a rotary evaporator at 45 °C. The concentrate extract was placed in Petri dishes and left to dry at laboratory temperature overnight. Then the dry matter was weighed and kept in glass containers at temperatures (4°C) until use (Riose *et al.*, 1987). Ethanol alcohol (96%) was used as a polar solvent, then, 20 g of the pre-prepared dried bitter apple fruit powder was weighed and placed in the extraction apparatus (Soxhlet). The extracts were concentrated by a rotary evaporate device, and from the crude extract, concentrations (5,10,20%) were prepared as mentioned in the method of preparing aqueous extracts. The comparison treatment (control) was (1.5 mL) ethyl alcohol and the volume were completed to (100 mL) surfix adhesive was added to all the prepared concentrations.

Collection, identification, and breeding of *Musca domestica*

Using a tulle-made net, housefly adults were collected from a residential area in Al-Salman District, Al-Muthanna Governorate, and placed in rectangular breeding cages with dimensions of (70 x 70 x 70 cm). The cages' sides were covered with tulle and had a wooden foundation. Petri dishes with a capacity of 200 mL were set inside each cage. To feed the adults, milk and 15 g of sugar were added to it (Doucette and Bureall, 1972) In a lab setting with a temperature of (27±1°C), relative humidity of (70 5%), and a period of darkness, insects were grown and fed (14:10 hrs). Dough made of wheat bran (330 g), dry milk powder (26 g), yeast (17 g), and distilled water (300 ml) was used to make larvae food. The pupae that resulted from this process were retrieved and housed in the cages under identical rearing conditions. The age of the eggs was determined for use in later trials, and the adults were fed throughout the egg-laying time (Massoud *et al.*, 2001).

Collection and breeding of mosquitoes *Culex pipiens molethus*

C. pipiens molethus larvae were obtained from marshes in the Western Badia of the Al-Muthanna Governorate, about one kilometre from Sawa Lake. They were moved to the lab at Sawa Lake and the Badia Studies Center, where they were raised at a temperature of 30°C with a relative humidity of 65%. In large glass vats, the time from light to dark (10:14 hrs) was recorded (5 x 5 x 10 cm). The larvae in the glass vats were fed a paste made of (yellow maize flour, wheat, powdered milk, yeast, and protein) in a ratio of (1: 1:1:1:0.25) at an amount of (2 g) per glass vat, along with chlorine-free water. Tulle was used to cover the glass vats. Before they transformed into pupae, the larvae were fed every day. To obtain the adults, the pupae were moved to spherical plastic containers.

Bitter apple fruit seeds extract on *M. domestica* and *C. pipiens molethus*

The effect of the prepared extracts was tested on all stages of *M. domestica* and immature stages of mosquitoes, except for eggs due to the difficulty of counting eggs and controlling adults. 50 eggs were taken for the house fly at the age of one day for each replicate and three replicates for each concentration separately. The eggs were transferred with a fine brush to Petri dishes and treated with 1 mL of aqueous extract concentration (0,5,10,20 mg/mol) by spraying it with a small hand sprayer. The treated eggs were transferred to the incubator at a temperature of (27±1°C) and relative humidity (70 ± 5%) Light unit: darkness (14:10) hrs. Eggs hatch rate (viability of eggs) were calculated after 24,48,72 hrs from conducting the test, and the depreciation rates were corrected according to the Abbott equation (Abbott, 1925). The same treatments were repeated for the moving stages of the housefly. Larvae of the second and fourth stage of mosquitoes were treated with concentrations 0, 5, 10, 20 mg/mL after transferring 50 larvae from the colony to a test tube of 100 mL of chlorine-free water and adding to it the same previous feeding. Dead larvae were counted after 24, 48, 72 hrs from the start of the treatment with three replicates for each concentration. Pupae of *C. pipiens molethus* mosquitoes were treated with the same extracts and the same concentrations, where 50 fresh pupae were taken with three replicates for each concentration in addition to the control treatment. The pupae were transferred to test tubes as mentioned previously in the larval treatment and under the same conditions. Pupae were deemed dead if they failed to reach the adult stage or if they continued to float on the surface of the water in the container.

Statistical analysis

In accordance with the CRD design, the data were analysed using the Genstat12 programme, and the averages were compared after the results significance was confirmed using the least significant difference (LSD) test at the level of significance 0.05. The Abbott equation (Abbott, 1925) was used to adjust mortality percentages, and the angular transformation was used to the statistical analysis of mortality.

Results and Discussion

The current study's findings demonstrated that the different bitter apple fruit *C. colocynthis* extracts significantly affected some facets of the life performance of the housefly *M. domestica* and the mosquito *C. pipeins* melestus. The oil extract of the seeds and the aqueous and alcoholic extracts of bitter apple fruit were more effective at influencing mosquitoes at different stages. Egg viability, extract concentration, and exposure time all directly correlate with rising egg destruction rates (egg viability; see Table 1). At a dose of 20 mg/mL, the percentage of eggs that hatched was the lowest and reached 32.1% after 48 hrs of exposure to the oil from bitter melon. While 86.77% of the eggs hatched after being exposed to *C. colocynthis* extract in cold water for 72 hrs. But the highest rate of egg hatching was 86.77% 72 hrs following exposure to *C. colocynthis* cold water extract. The mortality rate (63.66%) at the concentration (20 mg/mL) and after treatment 72 hrs indicated that the oil bitter melon extract had a substantial impact on the proportion of eggs that hatched. Following a 72-hrs treatment period, statistical analysis revealed that alcohol and hot water bitter melon extract significantly reduced the percentage of eggs that died, reaching (58.1 and 44.75%), respectively, at concentrations of 20 mg/mL. In contrast to the other extracts, the cold aqueous extract had the lowest mortality rate, reaching 39.21% at the same concentration and exposure time. In comparison to the other extracts, the cold aqueous extract had the lowest death rate,

Table 1. Effect of (aqueous and alcoholic) extract of bitter melon pulp and oil extract of *C. colocynthis* on mortality rate of *C. pipien* mosquito pupae

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	0.00	0.00	0.00	0.00
	5	11.67	22.55	31.93	22.99
	10	23.89	37.05	46.68	36.96
	20	53.82	61.93	71.89	63.34
Hot aqueous extract	0	0.00	0.00	0.00	0.00
	5	15.64	36.95	34.75	29.11
	10	32.11	45.12	48.63	41.95
	20	71.09	72.51	79.06	74.38
Ethanolic Extract	0	0.00	0.00	0.00	0.00
	5	21.12	43.96	49.00	38.03
	10	49.86	57.56	58.74	55.39
	20	88.66	74.03	76.77	79.82
Oil extract	0	0.00	0.00	0.00	0.00
	5	23.65	45.46	53.94	41.02
	10	55.58	64.82	72.11	64.16
	20	95.63	98.50	100.00	97.79
	Average	33.92	41.27	45.22	

reaching (39.21%) at the same concentration and exposure time. The difference in the quality of the active chemicals isolated from the bitter apple fruit plant using various solvent materials may be the cause of this variation in the rates of killing mosquito eggs. It has been demonstrated that the oil bitter melon plant's seed extract is superior to the other extracts in terms of effectiveness and performance because it is thought to be more effective at penetrating the interior of the egg through the opening of (aeropyles) on the outer eggshell, which permits gas exchange between the embryo and the external environment (Smith and Salkeld, 1966). The bitter melon seed extract enters the egg and causes the foetus to suffocate and become poisoned, killing it. Alternatively, it may induce the oil extract to form an oily layer or cover over the egg's surface, causing the foetus to suffocate and die (Ibrahim and Sisay, 2011). The findings also demonstrated a significant impact of bitter apple fruit extracts on the rate of mortality of second and fourth stages of the *C. pipeins melestus* mosquito, with a direct correlation between the increase in extract concentration and the length of exposure and the increase in the rate of mortality of the second and fourth stages of mosquitoes, as shown in Tables 2, 3. After 72 hrs of exposure to the concentration 20 mg/mL of the oil extract, the death rates of the second and fourth larval stages of mosquitoes were 84.98 and 75.91%, respectively. The findings also demonstrated a significant impact of bitter apple fruit extracts on the rate of mortality of second and fourth stages of the *C. pipeins melestus* mosquito, with a direct correlation between the increase in extract concentration and the length of exposure and the increase in the rate of mortality of the second and fourth stages of mosquitoes, as shown in Tables 2, 3. After 72 hrs of exposure to the concentration 20 mg/mL of the oil extract, the death rates of the second and fourth larval stages of mosquitoes were 84.98 and 75.91%, respectively.

Table 2. Effect of aqueous and alcoholic extract of bitter melon pulp and oil extract of *C. colocynthis* on mortality rate of the second larval stage of *C. pipiens* mosquitoes

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	0.00	0.00	0.00	0.00
	5	15.64	23.88	27.17	23.06
	10	29.82	38.22	43.15	37.74
	20	55.66	66.22	69.57	64.70
Hot aqueous extract	0	0.00	0.00	0.00	0.00
	5	23.76	31.46	35.02	30.65
	10	35.29	34.34	37.88	35.88
	20	62.90	71.08	74.77	70.19
Ethanol Extract	0	0.00	0.00	0.00	0.00
	5	28.65	37.63	41.07	36.43
	10	39.39	47.88	51.64	46.96
	20	67.28	75.96	80.51	75.25
Oil extract	0	0.00	0.00	0.00	0.00
	5	33.14	42.45	47.04	41.58
	10	45.66	56.26	60.86	55.04
	20	71.57	80.36	84.98	79.64
	Average	31.80	37.86	40.85	

Least significant differences of means (5% level)- Extraction type = 1.240, Time in hour= 1.074, Concentration = 1.240, Extraction type * Time in hour * = 2.147, Extraction type * Concentration = 2.47

Table 3. Effect of aqueous and alcoholic extract of bitter melon pulp and oil extract of *C. colocynthis* seeds on the mortality rate of the fourth larval stage of *C. pipiens* mosquitoes.

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	0.00	0.00	0.00	0.00
	5	6.86	12.96	21.80	14.51
	10	15.83	28.06	36.99	27.97
	20	31.66	42.96	51.76	43.08
Hot aqueous extract	0	0.00	0.00	0.00	0.00
	5	17.66	22.90	25.82	22.53
	10	22.09	32.10	37.24	31.24
	20	48.03	52.03	60.77	54.12
Ethanol Extract	0	0.00	0.00	0.00	0.00
	5	21.84	27.00	29.90	26.32
	10	26.45	37.28	42.83	35.70
	20	50.64	58.08	67.96	58.81
Oil extract of bitter melon	0	0.00	0.00	0.00	0.00
	5	24.95	30.90	34.30	30.05
	10	31.62	43.00	52.89	42.50
	20	52.93	63.64	75.91	64.16
	Average	21.91	28.18	33.64	

Least significant differences of means (5% level) L.S.D, Extraction type = 1.654, Time in hour= 1.433, Concentration = 1.654, Extraction type * Time in hour = 2.866, Extraction type * Concentration= 3.309

Table 4. The effect of bitter apple fruit *C. colocynthis* on the weight of housefly *M. domestica* pupae

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	0.21	0.19	0.20	0.20
	5	0.20	0.18	0.16	0.18
	10	0.18	0.15	0.14	0.16
	20	0.16	0.14	0.12	0.14
Hot aqueous extract	0	0.21	0.20	0.20	0.20
	5	0.19	0.17	0.15	0.17
	10	0.17	0.13	0.13	0.14
	20	0.14	0.13	0.11	0.12
Ethanolic extract	0	0.21	0.19	0.19	0.20
	5	0.16	0.15	0.13	0.15
	10	0.14	0.11	0.10	0.12
	20	0.11	0.09	0.08	0.09
Oil extract	0	0.21	0.19	0.21	0.21
	5	0.14	0.13	0.11	0.11
	10	0.10	0.09	0.09	0.09
	20	0.09	0.07	0.06	0.06
	Average	0.164	0.144	0.136	

Least significant differences of means (5% level) L.S.D, Extraction type = 0.005, Time in hour= 0.004, Concentration (mg/mL) = 0.005, Extraction type *concentration= 0.010

Table 5. The effect of bitter apple fruit *C. colocynthis* on mortality of 3rd larval stage of the housefly *M. domestica*

Extraction type	Concentration mg/mL	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	0.00	0.00	0.00	0.00
	5	25.56	28.95	33.18	29.23
	10	37.31	39.12	44.66	40.37
	20	51.87	56.99	61.23	56.69
Hot aqueous extract	0	0.00	0.94	0.00	0.31
	5	33.73	44.02	47.97	41.91
	10	39.64	48.66	57.13	48.48
	20	55.59	60.90	72.11	62.87
Ethanol Extract	0	0.00	1.99	0.00	0.00
	5	41.93	52.15	59.91	59.91
	10	56.07	59.74	64.56	64.56
	20	60.63	64.92	75.75	75.75
Oil extract	0	0.00	0.00	0.00	0.00
	5	51.29	61.93	64.78	59.33
	10	58.97	65.08	76.46	66.83
	20	66.80	71.94	82.88	73.87
	Average	36.211	41.083	46.288	

Least significant differences of means (5% level) L.S.D, Extraction type= 1.409, Time in hour= 1.220, Extraction type * time in hour= 2.440, Extraction type* Concentration (mg/mL) = 2.818, Concentration (mg/mL) = 1.409

Table 6. The effect of bitter apple fruit *C. colocynthis* on mortality of 1st larval stage of the housefly *M. domestica*

Extraction type	Concentration mg/mL	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	3.07	2.38	0.00	1.81
	5	21.04	30.74	58.10	36.63
	10	30.95	39.17	60.19	43.43
	20	37.75	41.71	67.28	48.91
Hot aqueous extract	0	2.04	2.49	3.38	2.63
	5	28.21	39.65	66.28	44.71
	10	35.78	40.68	62.77	46.41
	20	41.88	46.86	71.65	53.46
Ethanol Extract	0	1.91	0.94	0.00	0.95
	5	46.86	57.60	75.49	59.98
	10	51.41	62.06	79.97	64.48
	20	46.85	66.52	84.82	66.06
Oil extract	0	1.65	0.00	1.88	1.18
	5	57.94	68.14	77.88	67.98
	10	60.00	69.94	83.32	71.09
	20	62.90	71.83	89.83	74.85
	Average	33.141	40.045	55.178	

Least significant differences of means (5% level) L.S.D, Extraction type = 2.981, Time in hour = 2.582, Extraction type* time in hour= 5.163, Extraction type * concentration (mg/mL) = 5.962, Concentration (mg/mL) = 2.981

making the oil extract of *C. colocynthis* superior to the other extracts utilized in this investigation. The findings also indicated a significant relationship between cold water, heated water extract, and alcoholic bitter apple fruit extracts on the mortality rate of the second and fourth larval instars. Second and fourth larval instar mortality rates in the alcoholic extract were 80.51 and 67.96%, respectively, as well as 74.77 and 60.76% in the hot water extract and cold-water extract. At a concentration of 20 mg/mL for both alcoholic and aqueous extracts, the mortality rate for the second and fourth instars was 69.5 and 58.6%, respectively. The presence of active compounds in the bitter apple fruit extract affects the metabolism process, resulting in a decrease in larval weight. These chemical compounds may also have an increased effect on moulting hormones, which could affect the moulting process and the development of immature instars, which is the cause of the increased mortality rate of the immature larval stage of *C. pipeins* melestus. This has been verified by (El-Sheikh, 2015). The findings of the present investigation are consistent with those of (Abutaha *et al.*, 2018), who discovered that the ethyl acetate (EtOAc) and chloroform ($CHCl_3$) extracts were the most efficient against the extract of Althaea ludwigii, which had a substantial impact on *C. pipiens* 4th instars The results showed a substantial association between a rise in pupae mortality rate, an increase in concentration and time of exposure to the extract, and an increase in pupae mortality rate caused by the various extracts of the bitter apple fruit plant (Table 4). The results of the current study, bitter melon oil extract increased pupae mortality rates by 98.04 percent at a concentration of 20 mg/mL after being exposed to it for 72 hrs. Other bitter apple fruit extracts had a significant impact on the results as well. At the same concentration, the alcoholic extract significantly increased pupae mortality, the hot aqueous extract caused pupae to die at a rate of about 74.21%, and the cold aqueous extract caused

Table 7. The effect of bitter apple fruit C. colocynthis extracts on female fecundity of the housefly *M. domestica*

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	223.75	222.22	190.94	212.30
	5	133.39	121.88	108.27	121.18
	10	86.28	83.33	81.58	83.73
	20	70.05	64.89	63.79	66.24
Hot aqueous extract	0	221.50	222.50	111.16	185.05
	5	99.67	97.15	93.95	96.92
	10	51.93	48.23	45.60	48.59
	20	50.32	48.34	42.89	47.18
Ethanol Extract	0	218.33	223.93	101.70	181.32
	5	84.87	77.43	71.99	78.10
	10	47.88	48.04	44.79	46.90
	20	39.40	35.32	31.88	35.53
Oil extract	0	220.94	222.88	219.50	221.11
	5	65.78	62.05	59.92	62.58
	10	52.79	53.17	41.00	48.99
	20	22.73	18.76	12.41	17.97
	Average	105.60	103.13	82.59	

Least significant differences of means (5% level) L.S.D, Extraction type = 0.994, Extraction type* Concentration (mg/mL) = 1.988, Time in hour = 0.861, Concentration (mg/mL) = 0.994, Extraction type*time in hour = 1.721.

pupae to die at a rate of 62.54%. The cause of this is a result of the active substances' buildup in the gut of the fourth instar larvae before they transition to the pupae stage, which is found in the investigated extracts. It comes from *Melia azedarach* L. and results in a flaw in the hormones that regulate moulting. It hinders the production and classification of ecdysteroids, which results in a defect in the full- or partial-molting of larvae.

The statistical analysis's findings revealed that there was no significant difference in the average number of eggs laid by adult females in the boiling water treatment after exposure to the extract for test periods, as well as in the treatment of the alcoholic extract following treatment with 24,48 hrs. Not substantially different in the treatment of the oily extract after 24 and 48 hrs of exposure, but significantly different in the two treatments of the alcoholic extract even after 72 hrs of exposure. The comparison treatment, however, significantly outperformed the other treatments in terms of the average number of eggs laid by females, with averages of 212.33, 221.06, 220.83, and 221.10 eggs/female for each of the examined treatments, according to the data. The results also showed that cold water treatments produced more eggs (121.3) per female than the remaining extracts did, while the oil extract treatment produced the fewest eggs (2.40 per female) after 72 hrs of treatment. The active ingredients in the extracts under study have a blatantly unfavorable impact on the digestion, metabolism, and rate of absorption of mosquito larvae, as well as the impact on the insect's life cycle, which may account for the variance in the average number of eggs laid by one female.

Although the concentration 20 mg/mL outperformed the effects of the other concentrations used in this study, which recorded the highest average mortality 74.83, 66.06, 53.46, 48.91% for each of cold water extract, boiling water, alcoholic extract, and oily extract,

Table 8. The effect of bitter apple fruit *C. colocynthis* extracts Egg hatch rate of the housefly *M. domestica*

Extraction type	Concentration (mg/mL)	Exposure time in hour			
		24	48	72	Average
Cold aqueous extract	0	80.88	91.93	100.00	90.93
	5	75.81	75.08	86.78	79.22
	10	70.79	72.63	70.59	71.34
	20	56.82	62.93	64.79	61.52
Hot aqueous extract	0	79.87	91.90	100.00	90.59
	5	73.90	70.20	74.02	72.70
	10	58.01	58.85	52.85	56.57
	20	41.98	47.96	45.00	44.98
Ethanol Extract	0	81.15	92.38	100.00	91.18
	5	69.42	66.90	66.86	67.73
	10	41.85	43.01	40.98	41.95
	20	30.88	33.48	30.93	31.77
Oil extract	0	79.85	90.90	100.00	90.25
	5	64.88	67.97	61.88	64.91
	10	45.97	38.12	34.51	39.53
	20	30.02	25.96	23.00	26.33
	Average	61.38	64.38	65.76	

Least significant differences of means (5% level) L.S.D, Extraction type= 1.053, Concentration (m/mL) = 1.053, Extraction type * Concentration (m/mL) = 2.107, Time in hour = 0.912, Extraction type *Time in hour = 1.825

respectively. The biological evaluation of aqueous (cold and hot), alcoholic, and extracts showed that all concentrations were very effective in killing the first-stage larvae of the house fly *M. domestica* (72 hrs). The outcomes also demonstrated that the concentration 5 mg/mL had a significant impact on the first instar larvae, recording a mortality rate of 67.98, 59.98, 44.71, and 36.62% after exposure to cold water, boiled water, alcoholic extract, and oil extract) of bitter apple fruit, respectively, after 72 hrs. The difference in mortality rates is attributable to a difference in the quality and concentrations of the active compounds.

In the third larval stage of the house fly *M. domestica*, there was a high significant difference between the treatments with bitter melon extracts (Table 7), with the concentration 20 mg/mL showing the highest mortality rate after 72 hrs, where it reached 88.9%. After 72 hrs, the alcoholic extract, boiling water, and cold water, respectively, reached 75.7, 72.10, and 61.22% for the bitter melon oil extract, which differed significantly from the other bitter melon extracts. The lowest average of the pupae weights (0.100 mg) was recorded at the concentration 20 mg/mL after 72 hrs of exposure to the extract, suggesting that the treatment with the oil extract of the rue plant was superior to the treatment with other extracts of the bitter melon plant in reducing the weights of the pupae. While the results revealed that after 48 hrs of treatment, there was no discernible difference between the effects of cold water and alcoholic bitter melon extract at lowest doses (10 and 20 mg/mL). Whatever the case, it was discovered that the weight of the virgins decreased with both the concentration and period of exposure to the extract. Additionally, when the extract concentrations increased, deformed virgins were produced as well and the virgins that managed to survive destruction for the first time experienced considerably delayed growth. This may be explained by the potency of poisonous substances produced from the bitter melon plant that prevent virgins from eating and interfere with the function of enzymes like the middle alimentary canal's protase enzyme. This study is also in agreement with the study (Ali and Aljanabi, 2020), where the findings demonstrated a substantial effect of the alcoholic and aqueous extract of Melia Azedarach on the juvenile stages of *Culex pepienis mosquitoes*.

According to the findings of this study, all of the extracts utilized in it for the fruits and seeds of the bitter melon plant have resulted in high rates of mortality in the various roles played by mosquitoes and house flies. After being exposed to different bitter melon extracts for 72 hrs, the concentration 20 mg/mL was exceeded. These findings highlight the need for additional research into the bitter melon plant's extracts and the potential for their use as plant-based, ecologically friendly, and highly effective natural insecticides against a variety of insect pests.

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