



Assessment of Bitter Apple, *Citrullus colocynthis* Extracts on the Housefly, *Musca domestica* L.

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ABSTRACT

In this work, evaluated two concentrations (10 and 50%) of the bitter apple, *Citrullus colocynthis* extracts against field and laboratory strains of the 2nd larval instars of the housefly, *Musca domestica* using dipping and feeding application bioassay techniques under laboratory conditions. It was found that the laboratory strain was more sensitive to plant extract with two concentrations and in the two bioassay techniques (feeding and dipping) than field strain. The plant extract using a concentration 50% gave more effects on larval, pupal, adult mortality (%) and adult emergency inhibition than using 10% concentration on the laboratory strain comparing with field strain in the two bioassay techniques. On the other hand, dipping bioassay technique gave results better than feeding bioassay technique. Can be recommending for use the extract of the bitter apple, *C. colocynthis* with concentration 50% against the larval instars of the housefly, *M. domestica* under field conditions. Plant extract can play a good role as Integrated Pest Management (IPM) of the housefly and may be an effective alternative to conventional synthetic insecticides and also does not effect on the environment, human and animal.

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INTRODUCTION

The house fly represents a pest of great economic importance in livestock and poultry production, contaminating animal products and transmitting a variety of pathogens to animals, as well as causing problems by invading residential areas neighboring on livestock units, affecting the quality of life of these populations [1]. House flies feed by using sponging type mouthparts. As the fly moves about from one food source to another, it samples and eats its food by regurgitating liquid and dropping it on the food to liquefy it. Feeding and breeding habits along with its persistence for invading homes and feeding on human food enable the housefly to spread many intestinal diseases. They feed and breed on decaying matter, human waste and food, and therefore considered to be mechanical vectors of pathogens (bacteria, protozoa and viruses) to mankind and livestock [2, 3]. These pathogenic transmissions from housefly cause many contagious diseases such as diarrhea, dysentery (shigellosis), cholera, typhoid and paratyphoid (salmonellosis) and anthrax. Some may also be the vectors of variety eggs of worm parasites [4]. *M. domestica*, is actually a companion animal of livestock and humans and is not actually an ectoparasite, but it is far more important economically in many instances than any of the flies associated with livestock and poultry. The housefly becomes economically important to confined livestock by virtue of its biotic potential and synanthropic behaviour. These factors, coupled with the ability of houseflies to exploit different developmental habitats on feedlots and dairies, have made this pest economically important [5]. *C. colocynthis* (Cucurbitaceae), commonly known as the colocynth, bitter apple, bitter cucumber, desert gourd, egusi or vine of Sodom, is a desert vine plant native to the Mediterranean Basin and Asia. *C. colocynthis* has deterrent, antifeedant, growth-regulating and fertility-reducing properties on insects [6].

The objectives of this study aims to evaluate two concentrations (10 and 50%) the bitter apple, *C. colocynthis* extracts against field and laboratory strains of the 2nd larval instars of the housefly, *M. domestica* using dipping and feeding application bioassay techniques under laboratory conditions.

MATERIALS AND METHODS

Mass rearing of housefly, M. domestica:

The colonies of *M. domestica* originated from adults collected from Slaughterhouse sheep in Jeddah Governorate, using a sweeping net. They were transferred into a small cage (16×16×16 cm) and transported to the Laboratory of Public Health Pests, Jeddah Amana, Jeddah Governorate, KSA for identification and colonization.

M. domestica colonies established from Laboratory of Public Health Pests, Jeddah Amana, Jeddah Governorate, KSA (generation > F25) and maintained under a 14:10 (light:dark) and maintained at 268±2°C and 70–80% humidity were used. Houseflies were reared using standard conditions [7] to generate similar-sized individuals. Adult houseflies were reared in wire-frame cages covered with wire-gauze (75x75x75 cm), with mesh screening on top, provided a 10% sugar solution; adults were laid their eggs in artificial diet containing from 600 gm powdered milk, 1000 gm wheat bran and 30 gm yeast then mixed together with one liter of water and put in plastic tray (25x10X10 cm) also the artificial diet was developed according [8,9]

Extraction of bitter apple, C. colocynthis:

Sample preparation:

The leaves samples were leaves in healthy places to dry after that grinded to powder and kept in a clean container for further extract preparation.

Extract preparation:

The active ingredient extracts from leaves were prepared using the solvents water, ethanol and chloroform. In this process 10 g of the samples were taken and homogenized with 100 ml of the respective solvents (hydro-ethanol mixture (80/20, v/v) for 6 hours. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000 rpm for 20 mins. The supernatant containing the plant extract was then transferred to a beaker and the extract was concentrated by evaporating the solvent at 60°C. The crude extract was weighed and dissolved in a known volume of distal water to obtain a final concentration of 0.1 mg/ml [10].

Bioassays:

Dipping technique:

The dipping method was applied according to the method described by Sukontason *et al*. [11] with some modifications. All tests were run at 14:10 (light:dark) and maintained at 268±2°C and 70–80% humidity. The second instar larvae were used in the assays. Five replicates of twenty larvae each group were used at each concentration level. The experiments were repeated on subsequent days. The larvae of each group were gently dipped into insecticide solutions with a dip net, whereas those of the controls were dipped in tap water. After being dipped for exactly 30 sec, the larvae were transferred to the rearing jars containing food. After the larvae had been dipped, they were reared to determine the effect of different compounds of the life cycle also success of emergence on the other hand, the number of emerging flies was recorded.

Feeding technique:

Feeding technique was carried out according to the method described by Kristensen and Jespersen [12]. To test the toxicity of different compounds, portions of artificial larval rearing artificial diet containing (600 gm powdered milk, 1000 gm wheat bran and 30 gm yeast then mixed together with one liter of water) were treated with different concentrations of compounds. Fifty grams of artificial diet were put in a container and 2.5 ml of water containing different compounds or water alone was added and mixed into the medium. Twenty 2nd newly housefly larvae were putted on the surface of container and follow up daily. The number of emerging flies was recorded and the larvicidal activity was recorded as the percentage. All larvicidal assays were carried out at 14:10 (light:dark) and maintained at 268±2°C and 70–80% humidity in the laboratory. Five concentrations of compounds were used, plus a control. Four replicates of twenty larvae each were used for each treatment. The experiment was repeated three times on subsequent days.

Statistical analysis:

Mortality counts were made after 24 hours. Mortality percentages were corrected according to Abbott's [13].

RESULTS AND DISCUSSION

1. Efficiency of *C. colocynthis* extract (50%) against larvae of *M. domestica* by feeding technique:

The results presented in Table, 1 exhibit the toxicity of leaves extract of *C. colocynthis* (50%) with feeding bioassay technique against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) of the extract exhibited relatively lower percent mortality at field strain comparing with laboratory strain.

The extract was found to be quite effective against *M. domestica* larvae, which were 9 to 31% according to the concentrations from 100-1000 ppm against laboratory strain, respectively, while reached 6-23 against field strain with the same concentrations, respectively. Pupal mortality ranged between 8-33% when using the same concentrations (100-1000 ppm) against laboratory strain, on the other hand, reached 6-25% against field strain with the same concentrations, respectively. Results revealed that the adult mortality of the laboratory strain of the larvae of *M. domestica* was 7% at concentration of 100 ppm, while, reached 24% at 1000 ppm, whereas, reached 5-17% against field strain with the same concentrations, respectively. The survival rate% reached 76-12% according to 100-1000 ppm concentrations comparing with control (96%) on the laboratory strain, which ranged between 83-35% against field strain with the same series concentrations. Finally, adult emergency inhibition were 24 to 88% when treated with series concentrations ranged between 100-1000 ppm against laboratory strain, while ranged between 17-65% against field strain with the same series concentrations.

When comparing between the effectiveness of leaves extract of *C. colocynthis* using a concentration of 50% against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) on laboratory and field strains, it was found the extract gave more effects on larval, pupal, adult mortality (%) and adult emergency inhibition on the laboratory strain comparing with field strain, due to the laboratory strain was reared under laboratory conditions and found individual differences between them were very low, while in field strain will be found degrees of individual differences normally high according to exposure to different kinds of insecticides which would lead to selective pressure or the housefly lived under different weather conditions, which gave variation between individuals.

Our results agree with those obtained by El-Naggar *et al.* [14] they found *Hydrated colocynthin* (active ingredient) alcoholic extracts of *C. colocynthis* was toxic to the adult house fly. Plant extracts have played an important role in the management of housefly and may be an effective alternative to conventional synthetic insecticides. The data indicate that the mixed flower waste extract can be applied as an optional point source control of *M. domestica*.

Table 1: Biological effect of the bitter apple, *Citrullus colocynthis* (50%) against the housefly larvae, *Musca domestica* using feeding application technique

Conc. (ppm)	Larval mortality (%)	Pupal mortality (%)	Adult mortality (%)	Survival rate (%)	Adult emergence inhibition
Laboratory strain					
100	9	8	7	76	24
200	12	10	9	69	31
400	15	13	11	61	39
600	19	18	15	48	52
800	24	29	19	28	72
1000	31	33	24	12	88
Control	2	0	3	96	4
Field strain					
100	6	6	5	83	17
200	10	8	7	75	25
400	12	10	9	69	31
600	17	13	12	58	42
800	20	22	14	44	56
1000	23	25	17	35	65
Control	1	0	1	98	2

2. Efficiency of *C. colocynthis* extract (10%) against larvae of *M. domestica* by feeding technique:

The results presented in Table, 2 exhibit the toxicity of leaves extract of *C. colocynthis* using a concentration of 10% with feeding bioassay technique against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) of the extract exhibited relatively lower percent mortality at field strain comparing with laboratory strain.

The extract was found to be quite effective against *M. domestica* larvae, which were 5 to 19% according to the concentrations from 100-1000 ppm against laboratory strain, respectively, while reached to 3-15 against field strain with the same concentrations, respectively. Pupal mortality ranged between 3-18% when using the same

concentrations (100-1000 ppm) against laboratory strain, on the other hand, reached to 2-15% against field strain with the same concentrations, respectively. Results revealed that the adult mortality of the laboratory strain of the larvae of *M. domestica* was 4% at concentration of 100 ppm, while, reached 14% at 1000 ppm, whereas, reached 2-11% against field strain with the same concentrations, respectively. The survival rate% reached 88-49% according to 100-1000 ppm concentrations comparing with control (98%) on the laboratory strain, which ranged between 93-59% against field strain with the same series concentrations comparing with control (99%). Finally, adult emergency inhibition were 12 to 51% when treated with series concentrations ranged between 100-1000 ppm against laboratory strain, while ranged between 7-41% against field strain with the same series concentrations.

Table 2: Biological effect of the bitter apple, *Citrullus colocynthis* (10%) against the housefly larvae, *Musca domestica* using feeding application technique

Conc. (ppm)	Larval mortality (%)	Pupal mortality (%)	Adult mortality (%)	Survival rate (%)	Adult emergence inhibition
Laboratory strain					
100	5	3	4	88	12
200	7	6	5	82	18
400	9	8	6	77	23
600	13	10	8	69	31
800	16	13	11	60	40
1000	19	18	14	49	51
Control	1	1	0	98	2
Field strain					
100	3	2	2	93	7
200	6	4	3	87	13
400	8	5	5	82	18
600	11	7	7	75	25
800	12	11	9	68	32
1000	15	15	11	59	41
Control	0	1	0	99	1

Also as mentioned before, when comparing between the effectiveness of leaves extract of *C. colocynthis* (10%) against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) on laboratory and field strains, it was found the extract gave more effects on larval, pupal, adult mortality (%) and adult emergency inhibition on the laboratory strain comparing with field strain, and consequently, when testing effectiveness of leaves extract using two concentrations (10 and 50%), the highly percentage of extract gave more effects than low concentration, against laboratory and field strains with the same concentrations (100-1000 ppm).

The medical and veterinary pest *M. domestica* L. has developed resistance to most insecticides used against it. For this reason, there is a constant search for new alternative control tools [15,16].

Our results agree with those obtained by Al-Rabeae and Al Zobaidy [17] showed that the hot and cold aqueous extracts of *C. colocynthis* caused high effects on mortality of all larval instars of house fly, *M. domestica*. Highest mortality percentages was recorded in first larval instars when treated with both aqueous extracts compared with lower mortality percentage was recorded in other larval instars. In all larval instars the highest mortality percentages was recorded after 24 hours of treatment by both aqueous extracts.

3. Efficiency of *C. colocynthis* extract (50%) against larvae of *M. domestica* by dipping technique:

The results presented in Table, 3 exhibit the toxicity of leaves extract of *C. colocynthis* using a concentration 50% with dipping bioassay technique against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) of the extract exhibited relatively lower percept mortality at field strain comparing with laboratory strain.

The extract was found to be quite effective against *M. domestica* larvae, which were 11 to 34% according the concentrations from 100-1000 ppm against laboratory stain, respectively, while reached to 8-28 against field strain with the same concentrations, respectively. Pupal mortality ranged between 10-34% when using the same concentrations (100-1000 ppm) against laboratory strain, on the other hand, reached to 7-29% against field strain with the same concentrations, respectively. Results revealed that the adult mortality of the laboratory strain of the larvae of *M. domestica* was 9% at concentration of 100 ppm, while, reached 26% at 1000 ppm, respectively, whereas, reached 6-20% against field strain with the same concentrations, respectively. The survival rate% reached 70-6% according to 100-1000 ppm concentrations comparing with control (96%) on the laboratory strain, which ranged between 79-23% against field strain with the same series concentrations comparing with control (98%). Finally, adult emergency inhibition were 30 to 94% when treated with series concentrations ranged between 100-1000 ppm against laboratory strain, while ranged between 21-77% against field strain with the same series concentrations.

When comparing between the effectiveness of leaves extract of *C. colocynthis* (50%) against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) using dipping bioassay technique on laboratory and field strains.

Jesikha [18,19] mixed flower waste extract have been screened for their larvicidal activities against larval stages of *M. domestica*. Three instars larvae of *M. domestica* were treated with the different concentrations by dipping method for 24 and 48 h.

Table 3: Biological effect of the bitter apple, *Citrullus colocynthis* (50%) against the housefly larvae, *Musca domestica* using dipping technique.

Conc. (ppm)	Larval mortality (%)	Pupal mortality (%)	Adult mortality (%)	Survival rate (%)	Adult emergence inhibition
Laboratory strain					
100	11	10	9	70	30
200	16	14	11	59	41
400	18	17	16	49	51
600	22	21	19	38	62
800	28	32	23	17	83
1000	34	34	26	6	94
Control	1	1	2	96	4
Field strain					
100	8	7	6	79	21
200	12	11	8	69	31
400	15	15	12	58	42
600	19	18	14	49	51
800	24	26	17	33	67
1000	28	29	20	23	77
Control	0	1	1	98	2

4. Efficiency of *C. colocynthis* extract (10%) against larvae of *M. domestica* by dipping technique:

The results presented in Table, 4 exhibit the toxicity of leaves extract of *C. colocynthis* using a concentration of 10% against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) of the extract exhibited relatively lower percent mortality at field strain comparing with laboratory strain when using dipping technique.

The extract was found to be quite effective against *M. domestica* larvae, which were 8 to 22% according the concentrations from 100-1000 ppm against laboratory strain, respectively, while reached to 6-18 against field strain with the same concentrations, respectively. Pupal mortality ranged between 5-21% when using the same concentrations (100-1000 ppm) against laboratory strain, on the other hand, reached to 3-19% against field strain with the same concentrations, respectively. Results revealed that the adult mortality of the laboratory strain of the larvae of *M. domestica* was 6% at concentration of 100 ppm, while, reached 16% at 1000 ppm, whereas, reached 5-14% against field strain with the same concentrations, respectively. The survival rate% reached 81-41% according to 100-1000 ppm concentrations comparing with control (97%) on the laboratory strain, which ranged between 86-49% against field strain with the same series concentrations comparing with control (98%). Finally, adult emergency inhibition were 19 to 59% when treated with series concentrations ranged between 100-1000 ppm against laboratory strain, while ranged between 14-51% against field strain with the same series concentrations.

When comparing between the effectiveness of leaves extract of *C. colocynthis* (10 and 50%) against the 2nd larval instars of *M. domestica* with series concentrations (100-1000 ppm) on laboratory and field strains, it was found the extract gave more effects on larval, pupal, adult mortality (%) and adult emergency inhibition on the laboratory strain comparing with field strain. On the other hand, when comparing between feeding and dipping bioassay technique, it was found dipping technique gave better results than feeding technique.

We can say biological activities of *C. colocynthis* (the colocynthin and hydrated colocynthin) isolated from the alcoholic extract showed toxicity against cockroaches, adult honey bee, housefly, cotton leaf worm, bed bug, and mosquito [14]. The petroleum ether and ethyl acetate seed extracts showed antioviposition, F1 adult emergence, and ovicidal and repellent activity against several insects [6]; the crude extracts of *C. colocynthis* (70% ethanol) were tested against the housefly for their mortality, repellency, and the number of eggs laid [20].

From the previous results we can recommend for use the extract of the bitter apple, *C. colocynthis* with concentration 50% against the larval instars of the housefly, *M. domestica* under field conditions. Plant extract can play a good role as Integrated Pest Management (IPM) of the housefly and may be an effective alternative to conventional synthetic insecticides and also does not effect on the environment, human and animal.

Table 4: Biological effect of the bitter apple, *Citrullus colocynthis* (10%) against the housefly larvae, *Musca domestica* using dipping technique.

Conc. (ppm)	Larval mortality (%)	Pupal mortality (%)	Adult mortality (%)	Survival rate (%)	Adult emergence inhibition
Field strain					
100	8	5	6	81	19
200	10	7	7	76	24
400	13	9	9	69	31
600	16	11	11	62	38
800	19	15	13	53	47
1000	22	21	16	41	59
Control	1	1	1	97	3
Laboratory strain					
100	6	3	5	86	14
200	8	5	6	81	19
400	11	7	8	74	26
600	13	10	10	67	33
800	16	12	11	61	39
1000	18	19	14	49	51
Control	1	1	0	98	2

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