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Laboratory Evaluation of the Effects of Colocynth (*Citrullus colocynthis*L.) Leaves Powder and Organic Extract on the House Fly (*Musca domestica* L.)

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***Abstract*— Laboratory experiments were carried out to evaluate the activity of Colocynth (cucurbitaceae) leaf powder and leaf chloroform extract against the house fly (*Musca domestica* L.) larvae and pupae. Addition of Colocynth leaf powder to the food of House fly larvae caused dose –dependant reduction in larvae weight, faster development to pupae with subsequent reduction in number of emerged adults especially at high dose. Further dipping of house fly pupae in leaf chloroform extracts, caused significant delay and reduction in number of emerged adults .**

Generally, the result indicated the presence of antifeedant and growth regulatory agent in the examined members of the family cucurbitaceae. Further lines of research were outlined.

***Index Terms*— Colocynth; House fly; cucurbitaceae; antifeedant.**

I. INTRODUCTION

The success of synthetic pesticide during past decades had led to their wide spread acceptant for use against various groups of agricultural and public health pests. However, the use of synthetic insecticides for controlling of insect pests globally may have problems such as their persistent toxicity in food, the subsequent development of resistance in insect populations, effects on non-target organisms and other adverse environmental impacts etc…… Insecticide resistance is now a major problem for the chemical control of a wide range of insect pests (Bisset *et al*., 1997; Liu and Yue, 2000) Approximately 500 agricultural pest insect species have developed resistance to various pesticides (Whalon *et al.*, 2008). Environmental and toxicological hazards of insecticides widespread use have spurred the search for alternatives (Munoza *et al*., 2013). In recent years, there has been a growing interest in the use of botanical pesticides as an alternative to synthetic pesticides, although botanical pesticides have been used for thousands of years against insect pests (Koul and Walia, 2009; Fetoh and Asiry, 2012). Botanical pesticides can be defined as the extraction of toxicant chemicals from plants by using a suitable solvent in which these chemicals occur naturally. Plants provide a valuable source of active chemicals such as alkaloids, terpenoids, cucurbitacin, glycosides, flavonoids and other compounds that have been used as toxins against several insect pests which affect commercial crops (Koul and Walia, 2009). Plant extracts have certain chemicals which can effectively substitute synthetic pesticides without phytotoxic properties (Schmutterer, 1990; Georges *et al.*, 2008; Munoza *et al*., 2013). These plant based pesticides have been used in agriculture since two millennia for their efficient pest management and safety to mankind (Bernays, 1983; Thacker, 2002). Member of the family cucurbitaceae have been the subject of intensive research work as promising source of insecticidal agents.

Bitter apple plants *Citrullus colocynthis* Linn., also known as bitter cucumber and colocynth or desert gourd, belong to the family of Cucurbitaceae and have a wide distribution, being commonly found in the sandy lands of India, Arabia, West Asia, and Tropical Africa and in the Mediterranean region (Pravin *et al.*, 2013). *C. colocynthis*, an annual herb, is found in warm regions of Pakistan, India, and Africa (Tallamy *et al*., 1997; Hussain *et al.*, 2014). It is an annual or perennial (in wild) herbaceous, native to dry areas of north Africa, being common throughout the Sahara, Areas of Egypt, Sudan, Eastwards through Iran to India, Pakistan, Afghanistan & other parts of tropical Asia. *C. colocynthis* Linn. is an important medicinal herb that traditionally have been used as a strong purgative, antitumor, (Especially of the Abdomen) Antidiabetic, Antioxidant, Antibacterial (Memon *et al*,2003; Al-Ghaithi *et al*,2004; Dehghani *et al*,2008; Kumar *et al*, 2008 and Dallak *et al*, 2009) . This plant is known to have a range of compounds, which show insecticidal, antibacterial, larvicidal, deterrent, antifeedant, growth regulating and antifertility effects (Mansoor *et al*., 2004; Seenivasan *et al*., 2004 Pravin *et al.*, 2013; Soam *et al*., 2013). Recently, an increased attention has been paid to the use of *C. colocynthis* as a natural insecticide and the biological activity of this plant has been investigated against many insect pests (Soam *et al.*, 2013). Cucurbit contain the chemical substance cucurbitacins which are used for defense

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against a variety of invertebrate and vertebrate herbivores (Metcalf, 1986; Salom *et al*, 1996; Brust and Barbercheck, 1992 and Achenbach and Horn,1993). Cucurbitacins is crystalline bitter substance related to tetracyclic triterpenes and occur in plants as monoglycosides of glycone or as oxygenated tetracyclic triterpenoides produced as secondary plant compounds by nearly all genera of cucurbitaceae (Enslin *et al*, 1957 and Metacalf, 1986). In facts, it`s member Yakteen (gourd tree)has been mentioned in the Holly Quran as possible insects repellent which help curing the prophit Yonis from the serious disease he had (Then we cast him from a desert shore while he was sick , and we caused a tree of ground to grow above him) Translation of Holly Quran, Muhammed, 1977). In the explanation books of the Holly Quran and Sonna, several authors have commend on gourd indicating that flies never come close to it (Ezamakhashari, 538 H). Based on the above facts, our current study was initiated to cast high light on the effect of colocynth leaf powder and organic extract on the development of house fly.

Materials and methods:

Insects rearing:

Copulating adult pairs (♀&♂) of *Musca domestica* L. were caught from restaurant in the college of Agricultural Studies –Sudan University of Science and Technology–Shambat. Caught insects were immobilized for about 5min. by chilling at (-5 C˚) (FAO,1979). They were then transferred to cylindrical plastic cages (30 cm long and 10 cm id.). The cages were covered with muslin cloth mixed with a rubber band. Cultured insects were allowed to lay their eggs on modified YMA medium coating the bottom of cage. The media consist of dried yeast, powdered milk and wheat bran as bulking agent –replacing agar in the original media (Spillar, 1963 and Elsayed, 1966). The cages were labeled (with species, place, origin and data of culture) and kept at 27±1 C˚ and 70 C˚±10% RH for about 12 hours for ovipositor. The newly emerged larvae were then transferred to fresh media and left to complete their cycle to the adult stage. Newly emerged adults were paired and left to lay eggs under the condition mentioned above. Newly emerged larvae, pupae, and adults were used in the bioassay test.

Source and preparing of colocynth products:

Seeds of colocynth (*Citrullus colocynthis*) were collected from wild species found near Khartoum- Algaili highway.The plant were grown in Shambat Research Station under recommended cultural practices during the period May- December 2017 and used as continuous sources of natural products used in the study. Preparation of powder extraction and bioasssay were carried out in the Medicinal and Aromatic Plant Institute- National Research Center and College of Agricultural Studies respectively. Colocynth leaves were left to dry at room temperature for three days. They were then thoroughly ground by electric mill sieved and kept in plastic bags until used in bioassay or extraction. Twenty five grams of colocynth leaves powder were placed in the soxhlet apparatus and extracted with 300 ml chloroform for 8 hours. The extraction procedure was repeated for a total 100 g powder. Excess solvent was stripped on under reduced pressure and remaining residue was weighted. Twenty mg of extracted residual were re- dissolved in 100ml ethanol and kept in a deep freezer (at -20 C˚) as stock solution (20%) till bioassay.

Bioassay:

- **Effect of colocynth leaves powder on larvae development of house fly larvae:**

Hundred grams of the YMA media of house fly larvae (described above) were separately placed in plastic cups (85 cm length and 6.5 cm i.d.). Different concentration (0.25%, 0.50 % and 1.00 % w/w.) of colocynth leaves powder were added separately each into the respective cup. The content of each cup were thoroughly mixed. Ten 3rd instars larvae of house fly *M. domestica* were introduced into each cup. Cups were covered with muslin cloth fitted by rubber band and kept in the laboratory at room temperature (28 C˚) and 70 R.H. Control cups containing untreated hundred grams YMA were included. Experimental unit were arranged in completely randomized design with four replicates. The weight of larvae and their development into pupae were observed daily for 7 days.

- **Effect of pupae dipping in chloroform leaves extract on adult emergency of house fly:**

Colocynth chloroform leaves extract was diluted to the desired concentration (0.1%, 1.0% and 10.0%) with ethanol (98%). Ten newly emerged pupae of house fly *Musca domestica* L. were dipped for one minute in the desired concentration (0.1%, 1.0% and 10.0%) and left to dry for a few minutes at room temperature (28 C˚). Similar number of pupae were dipped in ethanol (98%) and left to dry as above. Control set containing several numbers of untreated pupae was also included. Treated and untreated sets were arranged in a completely randomized design with four replicates. Emerged adults were recorded after 3, 4, 5 and 30 days.

Statistical analysis:

Data was subjected to ANOVA and means were separated by least significant different (LSD).

Result:

- Effect of addition of colocynth leaf powder to the food of house fly larvae were presented in the table (1). The results indicated that the average weight gain of 3rd instars' larvae of house fly was inversely proportional to the concentration of colocynth leaf powder in the diet throughout the study period . The highest weight gain was noticed in the larvae exposed to the lowest concentration (0.25%). All treatments were significantly different from control. The highest concentrations were significantly different from other treatments. All larvae exposed to treated food developed into pupae within 24hr., whereas the control counter parts developed within 48hr. Deformed pupae were noticed with dose related reduction in number of emerged adults .

Table 1: Effect of colocynth leaf powder as food additive on weight gain and development of house fly larvae 3rd instars:

Conc.%	No. of treated larvae	Larval wt.gain	Duration (days)	No. developed to pupae		No. developed to adult	
				Deformed	Normal	After 7 days	After 10 days
10	40	0.031 C	1	5	35	1	2
1	40	0.054 C	1	2	38	17	18
o.5	40	O.O60 C	1	1	39	19	27
0.25	40	0.065 AB	1	0	40	28	31
Control	40	0.78 A	2	0	40	33	40
LSD		0.019					
SE±		0.10					

Within each colun means followed by the same letter are not significantly different at p≥ 0.05 according to Duncan`s Multiple Range Test (DMRT).

Effect of colocynth chloroform leave extracts on adult emergence of house fly was given in (Table, 2). The results showed that the percentage of emerging adults from treated pupae was inversely proportional to the concentration of chloroform leave extracts and generally the number of emerging adults decreased as colocynth extract concentration increase, about 5% emergence were noticed after 3 days in the highest concentration (10%) compared to 83% and 85% in the solvent control and control respectively within the same period. Generally, the solvent control behaves similar to the and all treatments were significantly different from the two controls. No further development was noticed after 30 days (month).

Table 2: Emerging adults from pupae dipping in chloroform extracts of colocynth leaves:

Con.%	Time (days)			
	1	2	3	month
10	0.90(0) d	9.67 (5) c	0.67 (5) c	9.67 (5) c
1	9.67 (5) cd	24.16 (20) bc	31.02 (28) b	31.02 (28) b
0.5	9.97 (5) cd	27.70 (23) bc	32.90 (30) b	32.90 (30) b
0.1	17.75 (13) bc	36.00 (35) ab	40.61 (43) b	40.61 (43) b
Solvent control	32.0 (30) a	26.90 (53) a	68.67 (83) a	68.67 (83) a
control	27.70 (23) a	49.61 (58) a	70.17 (85) a	71.56 (90) a
LSD **	13.56	15.00	17.67	17.70
SE±	4.50	4.98	5.86	5.88

*Data transformed with Angular transformation (degree).
** Data in parenthesis is the actual data.
***within each column, means followed by the same letter are not significantly different at p≥0.05 according to Duncan`s Multiple Range Test (DMRT).

Discussion:

The current study was initiated for the investigation of the potential use of colocynth *Citrullus colocynthis* (wild ground) in the control of house fly *Musca domestica* . The results indicated that, colocynth leaves powder add to diet of house fly larvae caused dose dependant reduction in weight gain. These results generally indicated the possible presence of antifeedant materials to test insects. The feeding deterrence was argued based on the effect of reduction in weight gain among treated larvae as no measures for feeding rate were for some logistics reasons. Many authors noticed the feeding deterrence or stimulating effect in various insects. Tallamy and Gorski, (1997) reported that cucurbitacins are through to be potent feeding deterrent for all insects. Also they mentioned that cucurbitacin B extracted from *C. colocynthis* exhibit antifeedant activity for no adapted mandibulate insects and may be phagostimulate for phytophagous insect with hanstellate mouth parts. Baraercheck (1995), reported that leave of Diabrotica undecipunctate reared (for 10 days) on diet containing cucurbitacin D (0.6 mg/g) weighted less than those reared on diet contain no cucurbitacin D. All The bitter apple plant *C. colocynthis* has ntibacterial, larvicidal, deterrent, antifeedant, growthregulating ad antifertility components affecting the survivorship of insect pests (Pravin *et al.*, 2013; Soam *et al*., 2013). Such alkaloids, terpenoids, cucurbitacin, gycosides and flavonoids have insecticidal attributes of Ccolocynthis (Gurudeeban *et al.*, 2010). Treated larvae showed faster development to pupae with dose dependant increase in number of deformed pupae with consequently reduced number of emerged adults. On the other hand, dipping of house fly pupae in chloroform extracts of the colocynth leaves caused dose related delay and reduction in number of emerged adults ,about 83%and 85% of solvent and control were able to emerge after three days compared to range of 5%-43% emergence in treated sets at the same period. The rest of the treated pupae were completely unable to develop into adults, the quickness of the development of the pupae may be attributed to the presence of an inhibitory substance for the ecdyson hormone in the

colocynthis leaves. Rahuman *et al.* (2008) found out that the leaves extract of *C. colocynthis* had the oleic and linoleic acids, which showed toxic effects against mosquito larvae. These results could point to the possible presences of growth regulatory material in the extracts of the leaves as evident by the effects on weight gain, developmental period, deformation and emergence to adults. Previous authors reported different results of effects on growth of insects. The current result although it shed light on some aspects of a possible growth regulatory and /or antifeeding of colocynth leaves product. The insecticidal activity of *C.colocynthis* extracts might be justified to the presence of several compounds including: saponin, alkaloids and glycosides (Gurudeeban *et al*., 2010; Ali *et al.*, 2013). Yet many aspects need confirmation and more detailed, long-term studies on both the chemistry of *C. colocynthis and its* effects on the pests 'natural enemies are required before recommending its use as an alternative to synthetic pesticides.

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