Anti-insect activity of ethyl acetate extract of *Citrullus colocynthis* (L.) Schrad fruit against *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera)

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ABSTRACT

The effect of ethyl acetate extract of bitter apple, *Citrullus colocynthis* fruit was studied against *S. litura* using poison food bioassay experiments. Extraction of toxic compounds from the fruits of *C. colocynthis* with Soxhlet and purification of extracts with column chromatography were done. In this study, there were seven treatments – five elutions, control and absolute control replicated thrice. The results showed that among the five elutions, ethyl acetate elution 3 had registered 80.00 and 83.33 per cent adult malformation, followed by ethyl acetate elution 1, 2 and 4 compared to control and absolute control in preliminary and confirmation screenings, respectively. The determination of promising dose exhibited that, ethyl acetate elution 3 exhibited the LC_{50}value of 29.62 per cent concentration. In ethyl acetate elutions, it was found that among the elutions, the lowest mean faecal and pupal weight were registered in ethyl acetate elution 3 compared to control and absolute control. Similar trend of results were also observed in confirmation and dose determination experiments.

Keywords: Bitter apple Fruits, *Spodoptera litura,* Bioefficacy, Poison Food Bioassay, Growth Parameters

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INTRODUCTION

The bitter apple, *Citrullus colocynthis* (Linn.) Schrad (Cucurbitaceae) is a relative of watermelon (Asyaz *et al.,* 2010). The leaves, fruits, vine and roots of *C. coloncynthis* contain many phytochemicals (Sharafzadeh and Alizadeh, 2012). The methanol extract of *C. colocynthis* fruits were identified the cucurbitacins (Sturm and Stupper, 2000), 9, 12-Octadeca dienoic acid (Z, Z)-, methyl ester, Cannabinol, Pentafluoropropinonate, Germanicol, Hexadecanoic acid, Methyl ester, Squalene, Azulene, Octadecanoic acid and Linoleic acid ethyl ester which had potent insecticidal, ovicidal and repellent activities (Selvaraj and Sosses, 2016). The biological activity of *C. colocynthis* as a natural insecticide was investigated against many insect pests like *Aphis craccivora*, *Schistocerca gregaria*, *Spodoptera litura, Tetranychus urticae, Sitophilus oryzae, S. zeamais*, and *Rhopalosiphum padi* (Khalid and Asiry, 2015). *C. colocynthis* had deterrent, antifeedant, growth-regulating and fertility-reducing properties on insects like *S. gregaria*, *S. litura* and *A. craccivora* (Seenivasan *et al.*, 2004; Torkey *et al.*, 2009).

The tobacco leaf caterpillar, *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) is the most dreaded insect pest of 112 host plant species belonging to over 40 plant families, making the species highly polyphagous. Some common host plants include tobacco, cotton, soybean, chickpea, black gram, green gram, sorghum, maize, sunflower, ground nut, castor, gingelly, bhendi, tomato, cabbage, cauliflower *etc*. (Cabi, 2017). The pest developed resistance to all insecticides, which are available in the market, due to the suppression of natural enemy population. Effective botanical usage is essential for modern agriculture to manage *S. litura* by avoiding public health hazards (Chandrayudu *et al.,* 2015). Botanicals namely neem, pungam, calotrophis, rotenone, ryania, *Thevetia nerifolia*, *Acorus calamus*, *Chrysanthemum coccineum*, *Allium sativam* etc., performed well against *S. litura* at different level of efficacy (David and Ramamurthy, 2011). Antifeedant property of leaf components of *Curculigo orchioides* Gaetrn, *Evolvulus alsinoides* Linn, *Phyllanthus deblis* Klein. *ex.* wild, *Swertia corymbosa* Griseb and *Zanthoxylum limonella* Alston (Arivoli and Tennyson (2012), *P. murex* root (Sahayaraj *et al.* (2008), *Azadirachta indica* parts (Shu *et al.,*2018) on *S. litura* was studied by various authors.

Botanical pesticides have long been touted as attractive alternatives to synthetic chemical pesticides for pest management, because botanicals reputedly pose little threat to the environment or to human health (Nathan and Kalaivani, 2005). Since there is no attack on the leaves, fruits, vines and roots of *C. colocynthis* by sucking insects, defoliators and root feeders, this plant has variety of biological compounds to keep all the group of insects away from it. So, this plant was selected to conduct a preliminary study in order to know the insecticidal activity against *S. litura*. The objective of this study was to extract the toxic compounds from the fruits of *C. colocynthis* by ethyl acetate solvent and to evaluate the insecticidal effect of ethyl acetate based fruit extracts of *C. colocynthis* against *S.litura*.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The fruits of *C. colocynthis* were collected in the month of July and August 2017 from the coastal region of Karaikal district (10º95'N latitude and 79º78'E longitude with an altitude of 4 m above MSL), Pondicherry, India. The fruits were brought to the laboratory, washed with dechlorinated water, shade dried under room temperature. The dried fruit material was pulverized into fine powder using mixer-grinder. About ten gram of powdered fruit material was weighed and packed in whatman filter paper no.40 as thimbles using stapler and soaked in ethyl acetate solvent in a 500 ml conical flask for overnight with occasional shaking. Then these were extracted with ethyl acetate solvent in Soxhlet apparatus and again the extract was purified by column chromatography then different elutions collected at different times were used for experiment.

Collection and raring of *Spodoptera litura*

The initial culture (adults and larvae) was collected from the field of PAJANCOA and maintained in the insect rearing laboratory under controlled conditions (temperature, 25 ± 2° C, humidity 70 %, L 12 hrs :D 12 hrs). The larvae were fed with castor leaves till pupation. After the completion of feeding the larvae were took pupation in the soil. Pupae were washed in 10 per cent formaldehyde solution and then pupae were washed in tap water. The pupae were placed on the layer of absorbent cotton kept in the petriplate (9.3 cm dia x 1.4 cm ht.) which was placed in the cage for emergence of moth. In the cage, a 5 ml penicillin vial (2.1 cm dia x 3.2 cm ht) containing 10 per cent honey solution (Dabur) with a few drops of multivitamin (Health OK™) syrup for adult food was kept. After the completion of mating by moths a tender shoot of neerium was kept to lay eggs. The eggs were incubated at high relative humidity of about 80 per cent and maintained in plastic trays with tender castor leaves (*Ricinis communis*) to provide food for newly emerged neonates **(**Jeyasankar *et al.*, 2016).

Growth Inhibitory Activity

The five elutions of ethyl acetate namely ethyl acetate elution (EAE) 1, EAE 2, EAE 3, EAE 4 and EAE 5 were diluted to 50 per cent concentration by adding one ml of elution of each fraction to one ml of distilled water in a centrifuge tube. Poison food bioassay was carried out with seven treatments *viz.*, five elutions, a control and an absolute control, replicated thrice. In each replication two third instar larvae were released. The bioassay experiments were carried out during evening hours only, since larvae are nocturnal in habit (Ray *et al.*, 2009). The 3 cm^2 diameter of castor leaf disc was taken and 10 µL of the diluted elution was pipetted out with the micropipette and smeared on both adaxial and abaxial surface and allow to dried. The larvae were pre starved for 3 hours in petridish (9.3 cm dia x 1.4 cm ht.) plates with filter paper before the experiment. The treated leaf discs were place over the filter paper in the petriplates and then the prestarved larvae were released and allowed to feed for 24 hrs. From the next day of treatment fresh leaves were supplied for the treatments and control daily until the larvae reached pupation. Growth regulatory activities *viz.*, larval malformation, larval mortality, pupal malformation, pupal mortality and adult malformation and the phyto insecticidal activity, larval and pupal mortality were also recorded (Ray *et al*., 2009).

Statistical Analysis

The data obtained from laboratory experiment were analyzed in a Completely Randomized Block Design by "F" test for significance. Standard Error of difference (S.E(d)) and Critical difference values were calculated at 5 per cent probability level and the treatment mean values of the experiments were compared using Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The toxic effect of *C. colocynthis* fruit extract by ethyl acetate solvent with five elutions against the third instar larvae of *S. litura* revealed that ethyl acetate elutions caused adult malformation ranged from 34.33 to 80.00 per cent (Table 1). Among the five elutions, ethyl acetate elution 3 caused 80.00 per cent adult malformation followed by ethyl acetate elution 4 compared to control and absolute control. The ethyl acetate elutions 1, 2 and 5 had recorded the adult malformation of 50.00, 50.00 and 34.33 per cent, respectively. Haung *et al.* (2016) stated that azadirachtin is a predominant IGR compound, The present findings are in consonance with the above studies. The toxic compounds of fruit extract of *C. colocynthis* in ethyl acetate elution 3 would have inhibited the digestion and absorption of ingested food and had insect growth regulatory activity rather than phytoinsecticidal action. Therefore, the larvae exposed in ethyl acetate elutions produced malformed adults. interacts with the neuro endocrine control of metamorphosis. The effect of azadirachtin ranged from mortality, notable at moult into morphogenic defects such as production of malformed wings in adults.

Table 1. Effect of *C. colocynthis* fruit extract (ethyl acetate) on the abnormality of *S. litura*

Ethyl acetate Elutions (EAE)	Preliminary screening	Confirmatory screening
	Per cent adult Malformation	Per cent adult Malformation
EAE 1	50.04^{b}	50.00^{b}
EAE 2	50.00^{b}	50.00^{b}
EAE 3	83.33^{a}	80.00^{a}
EAE 4	50.00^{b}	55.33^{b}
EAE 5	33.38^{c}	34.33^{c}
Control	0.00^{d}	0.00^{d}
Absolute control	0.00^{d}	0.00^{d}
S.E(d)	1.75	2.31
F – test	*	*
CD (P = 0.05)	3.75	4.96

In column mean followed by the common letter are not significantly different by DMRT ($P<0.05$) by one way ANOVA # Mean of three replications * - Significant at P= 0.05

The determination of promising dose in the fruit extract by ethyl acetate solvent showed that, ethyl acetate elution 3 exhibited the LC_{50} value of 29.62 per cent. The present results are in line with the findings of Govindarajan *et al.* (2012) who studied the larvicidal activity of essential oil from *Metha spicata* against three species of mosquito and found that LC_{50} value of carvone, cis–carveol and limonene appeared to be most effective against *A. stephensi* (LC_{50} = 19.33, 28.50 and 8.33 ppm) followed by *A. aegypti* LC_{50} 23.69, 32.88 and 12.01 ppm) and *C. quinquefasciatus* (LC_{50} = 25.47, 35.20 and 14.07 ppm).

The effect of ethyl acetate extract of *C. colocynthis* fruit on the basis of faecal weight showed that ethyl acetate elution 3 recorded the lowest mean faecal weight of 0.10 ± 0.01 and 0.06 ± 0.01 g per two larvae in preliminary and confirmation study, respectively, compared to control and absolute control (Table 2). The effect on the pupal weight exhibited that the among the five elutions, ethyl acetate elution 3 recorded the lowest mean pupal weight of 0.15 ± 0.01 g

Table 2. Effect of fruit extract (ethyl acetate) of *C. colocynthis* against *S. litura* on the basis of faecal and pupal weight

Ethyl acetate Elutions (EAE)	Preliminary screening		Confirmation screening	
	Mean faecal weight (g) ± standard error	Mean pupal weight (g) ± standard error	Mean faecal weight (g) ± standard error	Mean pupal weight (g) ± standard error
EAE 1	0.14^{b}± 0.01	0.23^{b} ± 0.01	0.09^{b} ± 0.01	0.22^{b} ± 0.003
EAE 2	0.14^{b}± 0.01	0.25^{b} ± 0.01	0.08^{b} ± 0.01	0.25^{b} ± 0.01
EAE 3	0.10^{a}± 0.01	0.15^{a} ± 0.01	0.06^{a} ± 0.01	0.14^{a} ± 0.01
EAE 4	0.14^{c}± 0.01	0.29^{c} ± 0.03	0.11^{c} ± 0.01	0.29^{c} ± 0.04
EAE 5	0.13^{bc}± 0.01	0.26^{bc} ± 0.003	0.10^{bc} ± 0.01	0.26^{bc} ± 0.01
Control	0.35^{d}± 0.03	0.56^{d} ± 0.03	0.33^{d}± 0.02	0.55^{d} ± 0.03
Absolute control	0.35^{d}± 0.03	0.54^{d} ± 0.03	0.33^{d}± 0.03	0.56^{d} ± 0.03
S.E(d)	0.08	0.02	0.01	0.02
F – test	*	*	NS	*
CD (P = 0.05)	0.16	0.04	0.03	0.04

In a column mean followed by a common letter are not significantly different by DMRT (P=0.05) by one way ANOVA; * Significance at P=0.05 #- Mean of 3 replications ± standard error

and 0.14 ± 0.01 in preliminary and confirmation screenings, respectively, compared to control and absolute control. The present findings are in confirmation with the results of Almeida *et al.* (2014) who stated that NSKE was efficient in controlling *A. gemmatalis* and exhibited increasing mortality, decrease in food consumption, larval and pupal weight. The reason for the production of lowest faecal material and pupal weight in ethyl acetate elution 3 was found that toxic compounds present in the ethyl extract of *C. colocynthis* fruit reduced the ingestion rate of food after the treatment.

The effect of promising dose showed that, the lowest mean faecal weight of 0.08± 0.01 g per two larvae was recorded in ethyl acetate elution 3 at 50 per cent concentration, compared to control (Table 3).

Table 3. Effect of fruit extract of *C. colocynthis* by ethyl acetate solvent against *S. litura* on the basis of faecal and pupal weight (Promising dose)

Ethyl acetate Elutions (EAE)	Dose	Mean faecal weight (g) # ± S.E	Mean pupal weight (g) # ± S.E
EAE 3	10	0.22^{e}± 0.01	0.25^{e} ± 0.01
EAE 3	15	0.21^{e} ± 0.01	0.25^{e} ± 0.003
EAE 3	20	0.20^{e} ± 0.01	0.24^{e} ± 0.01
EAE 3	25	0.19^{d} ± 0.01	0.21^{d} ± 0.01
EAE 3	30	0.17^{d}± 0.01	0.20^{d}± 0.003
EAE 3	35	0.17^{d}± 0.01	0.19^{cd} ± 0.01
EAE 3	40	0.13^{c}± 0.01	0.18^{bc} ± 0.01
EAE 3	45	0.09^{b} ± 0.01	0.16^{ab} ± 0.003
EAE 3	50	0.08^{a}± 0.01	0.14^{a} ± 0.01
Control	-	0.37^{f} ± 0.03	0.55^{f} ± 0.01
S.E(d)		0.10	0.01
F – test		NS	*
CD		0.17	0.02

In a column mean followed by a common letter are not significantly different by DMRT (P=0.05) by one way ANOVA. * Significance at P=0.05 #- Mean of 3 replications ± standard error (S.E)

The effect on the pupal weight exhibited that the lowest mean pupal weight of 0.14 ± 0.01g per two larvae was recorded in ethyl acetate elution 3 at 50 per cent concentration, compared to control. In this study it was inferred that after completion of initial feeding, the high dose of ethyl acetate elution 3 at 50 per cent would have interfered the intake of food further and digestion of ingested food. Hence, the treated larvae had produced very low faecal materials and pupal weight, compared to control. when the concentration increased in promising dose, the faecal production was very low in the treated larvae.

It was concluded that ethyl acetate elution 3 was found to be more effective in causing the highest per cent adult malformation of 80 and 83.33 per cent, compared to control (0.00) and absolute control. The study indicated that the toxic compounds present in the ethyl acetate fruit extract of *C.colocynthis* inhibited the digestion and absorption of ingested food in the treated larvae. Therefore, the treated larvae consumed very less quantity of untreated leaves throughout larval period and produced malformed adults.

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