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Some Natural Plant Extracts Having Biocide Activities against the American Bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

In the present research, the toxicity, antifeedant activity and biological effects of ethanolic leaves extract of four medicinal plants named *Eucalyptus rostrata*, *Dodonea viscosa*, *Rhyza stricta* and *Cymbopogon schoenanthus* were evaluated on 2nd, 3rd and 4th instar larvae of *H. armigera* under laboratory condition. The results showed that values of LC_{50} in mg of different plant extracts in mg/100ml of the larval diet can be arranged in an ascending order as follows: *Dodonea* 7.23 > *Cymbopogon* 12.59 > *Rhazya* 14.52 > *Eucalyptus* 29.42 mg/100ml diet (the least LC_{50} is more toxic than the higher one). All the tested extracts had antifeedant and starvation effects against the 2nd, 3rd, 4th instar larvae. *D. viscose* extract possesses the least antifeedant effect even of their higher toxicity. There was clear relation between the percent of starvation and antifeedant of the 2nd, 3rd, and 4th larval instar. All extracts were nearly the same in their effect on the biotic potential; of the insect, and possess latent effect when tested against 2nd instar larvae, the value of LC_{50} of the extract was added to the diet, extracts increased larval duration, deformation between pupae and adult stages, moths sterility, increased as decreasing in females egg production. Other effects were noticed, reduction in percentage of pupation and moths emergence. The plant extracts can be arranged ascending according to percentage of their sterility effects as follows: *C. schoenanthus* < *E. rostrata* < *R. stricta* < *D. viscose.* All extracts cause disruption on the biology and physiology of the insect, and all extract induced percentages of deformation between pupal and moth stages. The ethanolic extract of the plant leaves of the tested plans may be used for control *H. armigera* in combination with other methods in the integrated program in order to decrease the buildup of the resistance and protect the environment from chemical pollution.

Keywords

Helicoverpa armigera, Bioagent, Crude Plant Extract, Biocontrol Activity, Medicinal Plants

1. Introduction

The American bollworm or tomato fruit worm *Helicoverpa armigera* (Hubn.) is highly polyphagus feeding on about 200 plant species, mainly annuals, developing on a wide range of food, fiber, oil and fodder crops as well as on many wild plants and perennial horticultural crops. It has long been recorded as a pest of many crops in the Kingdom of Saudi Arabia, like Tomato, Maize, Zucchini, Green pepper, Gourd, Muskmelon, Okra, Cassia, Potato, Bean, Chickpea, Sunflower, Red beet, Tobacco, Cowpea, and Turnip. It also attacks the cotton plantation in many parts of the world [1] [2] [3], the larvae initially feed on leaves, later boring into flowers and, when flowers became bolls the small larvae break through the bolls fed on it, they move from boll to boll; the affected bolls fail to develop and the quality of the lint is severely spoilt. Slightly damaged bolls are also failed due to the invasion by rot microorganisms. In tomatoes, the larvae bore into immature, ripening and ripe fruit, preferring the latter, and cause rot. In maize, larvae bore into stems and can cause serious plant lodging [2]. Many problems have been encountered as a result of the extensive use of synthetic pesticides. Increasing problems concerning the application of such pesticides include pest resistance, residue, contamination of humans foods, mammalian toxicity and pollution of environments [4] [5]. Therefore, several insecticides occurring naturally in plants have been investigated as alternatives bioagents to the highly toxic synthetic chemical pesticides for pest control. Such natural products having pesticide activity are assumed to be environmentally more acceptable because of their low persistent nature and are less hazardous to humans [6] [7]. Bioactive compounds of plant origin are considered as ecologically safe alternative and the plant extracts with complex mixtures of compounds have been widely investigated for their insecticidal repellent, ovicidal, antifeedant and antioviposition properties [8]. There is an increasing interest in the role of the plant products in insect-plant interaction, particularly in host acceptance and rejection [9]. While plant chemicals may produce toxic effects when ingested by insects, antifeeding activity may determine the extent of insect herbivores. Several researches have recorded the entomotoxic properties of the plant extracts from different plant species [10] [11] [12]. The present research aimed to study the biological activity of four ethanolic leaves extract derived from medicinal plant named *Eucalyptus rostrata*, *Dodonea viscose*, *Rhyza stricta* and *Cymbopogon schoenanthus* in an effort to find out their biological activity against *H. armigera* as safe alternatives to chemical pesticides and their possible use in IPM programme for control, and protect the crops from infestation by *H. armigera*,

also to delay the buildup of resistance strain.

2. Materials and Methods

2.1. Insect Breeding Larvae

Larvae and adults of *H. armigera* were collected from Berseem, Okra, and tomato field in KSA during summer season. The larvae were reared on a synthetic artificial diet mentioned by [13] under laboratory condition at 27˚C, at 10 h light: 14 h dark (LD10:14) photoperiod. Pupae were sexed and kept in separate plastic boxes until adult moth emergence. Adults were transferred to rearing jars allowed to mate and lay egg, and fed with 10% honey solution, black cloth strips were hanged inside the jars as a site for egg laying. The eggs were collected daily in other boxes. After hatching, neonate larvae were transferred to the artificial diet in order to feeding and complete their life cycle. 3rd instar larvae were kept individually in plastic vials to avoid cannibalism habit, different instars larvae were chosen from the rearing diet for different bioassay tests.

2.2. Plant Material and Extract Preparation

Plant leaves of *E. rostrata* and *D. vescosa* were collected from the garden in Dammam, *R. stricta* and *C. schoenanthus* were collected from the desert around AL-Dowami governorate in KSA. The plant were identified according the description of [14]. The extracts were prepared as follows; the collected leaves from each plant were dried in a shad at room temperature (27˚C ± 2˚C) for some days, the dried leaves were pulverized in fine powder with electric grinder and sieved. For extraction, the procedure of [15] was adopted at 27˚C ± 2˚C as follows, 250 g. from the dried fine powder of each plant leaves were soaked in 750 ml. Ethanol 80% as solvent for 3 - 4 days. The mixtures were stirred for one hour, the solution was filtered through What man filter paper No.4. The extract were concentrated by Rotary evaporator until solvent was completely evaporated to get the solidify crude ethanol extract. The obtained crude extracts were stored in sterilized dark bottles kept at 4˚C in refrigerator. Different concentration were prepared by dissolving a known weight of the extract in 2ml. of ethanol 80% with two drops of tween 80, then will mixed with 100ml. of the artificial diet that recorded by [13].

2.3. Bioassay Tests

Bioassay tests included, the toxic effect of ethanolic plant leaves of the four tested plants against the 2nd, 3rd and 4th instar larvae of *H. armigera,* antifeedant and starvation effects, and effect of the toxic concentration LC_{50} on some biological parameters of the insect.

2.3.1. Toxic Effect of the Plant Extract on the Larval Stage

With seven different concentrations of ethanolic extract (5, 10, 15, 20, 25, 30 and 40 mg extract/100ml. diet) were prepared of each of the tested plant. Diet was

prepared with different serial concentrations of each extract. The prepared treated diet was cut into small pieces and placed in Petri dish. The diet prepared with the 2 ml. ethanol as a solvent with 2 drops of Tween 80 was used as positive control. One larva per Petri dish was released on the treated diet was left to fed for 7 days. Ten replicates were used each repeated five times for each concentration. Mortality counts were noted daily until 7 days. Moribund larvae were considered as dead.

$$\text{Percent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

Percent mortality corrected according to Abbot's formula (Abbot, 1925),

$$\text{Corrected mortality} = \frac{\%T - \%C}{100 - \%C}$$

T, % mortality in treatment—*C*, % mortality in control.

LC_{50}, and LC_{95} calculated for 2nd, 3rd, 4th instar larvae according to [16], and Probit regression (line Ldp-line) was used.

2.3.2. Antifeedant & Starvation Effect of Different Ethanolic Plant Extracts

Diet having LC_{50} value of extract of each plant was weighed and placed in Petri dishes for larval consumption. The diet prepared without extract was used as control. The tested instar larvae were starved for 8 h before the experiment. Starved larvae were released on treated and control diet in Petri dishes. Data was recorded after 24 h. Weight loss of diet due to water evaporation was quantified by establishing another control diet left without any larvae, then weighed at the end of the test. The percent diet consumed was calculated using feeding deterrence index (DI), [17]

$$\text{Deterrent: CCA} = \left(C - \frac{T}{C} + T \right) \times 100$$

where, CAA is corrected antifeedant or deterrent activity; *T* is diet consumed in treatment and *C* is diet consumed in control.

Percent starvation: Larvae that fed on LC_{50} treated or untreated diet and also another left without any food were weighted after 48 h. from the beginning of the experiment. The test was run on newly molted 2nd instar in three replicates each contained 10 larvae. % Starvation calculated according [18].

$$\text{Percent starvation} = \frac{C - E}{C - S} \times 100$$

where *E*, is mean weight of treated larvae; *C*, is mean weigh of untreated larvae ; *S* is mean weight of larvae left for natural starvation.

2.3.3. Development and Survival of *H. Armigera*

For studying the effect of LC_{50} values of the ethanolic extract of the different tested plant, method of [19] was used, 100 of second instar larvae were chosen from the artificial diet left to fed for 48 h. on diet having LC_{50} value that recorded

from the previous toxicity tests. Remained alive larvae were transferred on untreated diet to complete their development, for each test 100 larvae were used with 10 replicate. Different biological parameters were noticed like larval duration, larval weight, % pupation, pupal weight, pupal duration, % adult emergence, deformation between pupae & adults, sex ratio, egg production/female, egg hatchability and % sterility that recorded by [20].

All data were subjected to statistical analysis of variance (ANOVA) SPSS computer program.

3. Results and Discussion

Data obtained from the LdP line (Lethal Probit Regression Line) recorded the values of LC_{50} and LC_{95} of ethanolic extracts of the different plants against *H. armigera* 2nd instar larvae are summarized in (**Table 1**) all extracts induced toxic effect in variable values of LC_{50} as follows, *D. viscosa* 7.23 > *C. schionanthus* 12.29 > *R. stricta* 14.52 > *E. rosyrata* 29.42 mg/100ml (the least LC_{50} is more toxic than the higher one). Percentage of mortality was increased by increasing the extract concentration or the duration of feeding time.

The results agreed with that mentioned by [21] who found that *D. viscosa* extract at 5% concentration induced 75% death to *Spodoptera littoralis* 2nd instar larva fed for one day. The toxicity of *D. viscosa* because of the containment from sterols, viscosol, tannins, and high percentage of flavonoids, alkaloids saponins, coumarins and phenols [22] [23] [24], recorded *R. stricta* extract had toxic effect on *C. pipienes*, LC_{50} reached 190 ppm. After 10 day post treatment, also alchoholic extract gave larval mortality within two day and LC_{50} was 251 ppm. *R. stricta* contained high percentage of alkaloids [25] [26] [27], recorded that *C. schoenanthus* contained 11 terpines. [28], found that 2% *C. citratus* oil leading to high toxicity of 3rd instar larvae of *Spodoptera. exigua* within 2 h. from treatment LC_{50} reached 0.215% after 24 h. We could conclude that variation in LC_{50} and the different extracts may be related to different larval ability to analyze the components of each extract.

There was a clear relation between the four tested extracts (**Table 2**). All extracts may be inhibited the gustatory chemoreceptors on the mouth parts of the larvae leading to lost its response for feeding, the stop food consumption effect on the digestive and hormonal system [29]. The higher antifeedant and starvation percentage induced by *R. stricta* extract attributed to their high content of alkaloids.

Table 1. LC_{50} & LC_{95} values in mg of plant extract/100ml diet on the 2nd instar larvae of *H. armigera.*

Plant extract	LC_{50}	LC_{95}	Slope	Variance
E. rosyrata	29.42	278.88	1.68	0.047
D. vescosa	7.23	33.75	2.46	0.052
R. stricta	14.52	23.41	7.93	0.467
C. schoenanthus	12.59	22.50	6.53	0.401

Table 2. Effect of plant extracts on percentage of starvation & antifeeding on different larval instars of *H. armigera*.

Plant extract	Instar larvae	% Starvation	% Antifeeding
E. rostrata	2nd	82.58	61.12
D. viscosa		36	47.03
R. stricta		90.45	79.20
C. schoenanthus		82.85	67.58
E. rostrata	3rd	85.37	85.67
D. viscosa		53.77	67.53
R. stricta		99.88	97.24
C. schoenanthus		77.40	84.61
E. rostrata	4th	82.73	79.84
D. viscosa		33.14	58.61
R. stricta		93.51	98.39
C. schoenanthus		85.38	81.92

Data agreed with [30] recorded that highest level of alkaloids in methanolic and ethanolic extract of *R. stricta* leading to stop feeding of *H. brunneipennis* and *A. ipsilon.* Some alkaloids such as nicotin, strychnine, caffeine exhibited feeding of *Dysdercus spp*. [31] [32] [33] mentioned Melia extrac play as antifeedant on insects by their effect on juvenile hormone JH that reduced the insect growth and development and disrupted the metabolism. *D. viscosa* extract cleared the least antifeedant and starvation effect in spite of their higher toxicity, that was as result of consuming a large amount of the treated diet, it depends on the solvent used in the extraction process. [19], found chloroform was the most suitable solvent for extraction the antifeedant material from *D. viscosa* plant leaves.

Effect on biological parameters: Data in **Table 3** indicated that all tested extracts were nearly the same in their effect on the biotic potential and possess latent effect when the value of LC_{50} of the extract incorporated into the diet fed to 2^{nd} instar larvae comparing with the control. The extracts of different plants increase the larval duration, percentage of deformed pupae and adults and the percentage of moth sterility. Other effects include decrease in the larval weight, reduced percentage of survivor larvae, pupation and moths emergence. Life cycle bioassay using the extracts revealed that it inhibited larval growth and delayed mean time to pupation; extremely potent inhibitor the larvae to reach the pupal stage. The pupae showed an intermediate form between larvae and pupae, **Figure 1(b)** and finally the pupae died. The pupae were minute in size **Figure 1(a)**, with soft puparium and lost their body fluid. Appearance of deformed moths with twisted and shrinkage wings **Figure 1(c)**, **Figure 1(d)**, and intermediate form between pupae and moth were also observed.

The plant extracts can be arranged ascendingly according to the percentage of their sterility effects as follows: *C. shoenanthus* 6.62 < *E. rostrata* 39 < *R. stricta* 51.27 < *D. viscose* 68.8. It cleared that the plant extract cause disruption on the insect biology as a results of their effect on the insect hormone unbalance. The

Table 3. Biological aspects of *H. armigera* maintained on artificial diet treated with the concentration of LC_{50} values in mg. of different plant alcoholic extracts/100ml diet.

Biological aspects	Ethanolic extract of *E. rostrata D. viscosa R. stricta C. schoenanthus* Mean ± SE				Control
Larval duration in day	22.3 ± 0.41**	22.9 ± 0.31**	21.5 ± 0.25**	21.8 ± 0.3**	17.06 ± 0.19
Larval weight/mg	117 ± 1.62**	149 ± 5.70**	136.8 ± 3.89 **	136.3 ± 6.30**	239.7 ± 13.8
%Pupation	77	76	89	62	92
%Pupal deformation	7.79	5.26	4.49	6.45	3.26
Pupal weight/mg.	165.3 ± 5.3**	194 ± 5**	288.4 ± 5.7**	294.6 ± 5.3*	334.3 ± 4.45
Pupal duration in day.	12.4 ± 0.2	11.8 ± 0.11	12.9 ± 0.11*	10.83 ± 0.22	11.4 ± 0.10
%Moth emergence	63	70	84	59	89
%Moths deformation	15	10	5.7	8.47	0
Sex ratio ♂	46	52.8	54.76	62.71	52.60
♀	54	47.2	45.24	37.72	47.40
Moth longevity in day ♂	9 ± 1.1**	10.7 ± 1.6**	15.10 ± 1.04	15.6 ± 1.1	14.10 ± 1.15
♀	9.3 ± 0.8**	9.7 ± 1.3**	12 ± 0.97	12.3 ± 1.04	12.5 ± 0.90
Egg production/♀	269.3 ± 109.4**	128.8 ± 7**	228.8 ± 143.4**	377 ± 121.3	475.5 ± 108.9
%Egg hatching	27.79	35.3	58.64	75.01	98.17
% Sterility	73.13	64.70	41.36	24.99	1.83

P < 0.05 Significant* P < 0.01 Highly Significant**.

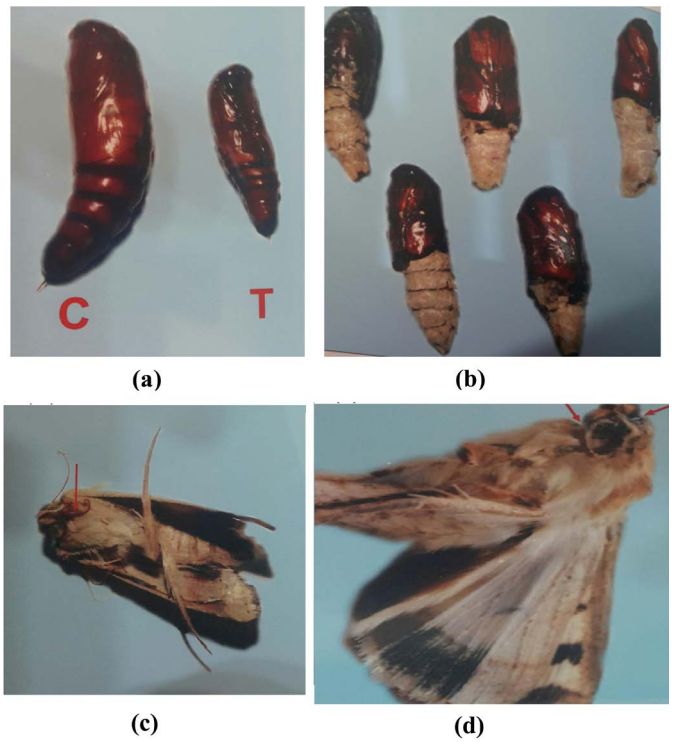

Figure 1. Showing different morphological deformation occurred between pupal and adult stages of *H. armigera* treated with the different tested. extracts, (a) Minute pupa, T, compared with the untreated one C; (b) Pupae in intermediate form between papa and adult; (c) Deformed moth with twisted legs and minute antennae; (d) Deformed moth with twisted wings, and mouth parts with shrinkage abdomen.

differential inter-specific insect response to the extracts and its constituents could be attributed to compound structure-activity relationships and physiolog-

ical-structural induced cellular changes resulting in poisoning of insects by blocking octopamine receptors [29] [34] [35] [36] suggested that the toxic effect may be attributed to a reversible competitive inhibition of acetylcholinesterase by site of the enzymes active center. It has been found that some plant that have insecticidal activity could be developed into product suitable for integrated pest management because they are selective to pests, have no or little harmful action against ecosystem. They act in many ways on various types of pests complex and may be applied to the plant in the same way as other agricultural chemicals [37] [38].

4. Conclusion

All extracts of the recorded plants induced different effects as toxicants and growth regulators; the most effective one was *Dodonea* LC_{50} value 7.23 mg/100ml on 2nd instar larvae followed by Cymbopogen 12.59 > Rhazya 14.52 > Eucalyptus 29.42 g/100ml diet (lees concentration gave more mortality). The diet that was treated with plan extract LC50 then ingested by the 2nd instar larvae induced latent biocidal activities as growth regulators, and percentage of moths sterilization occurred. Foregoing results may shed some light on the possibility of using the four tested plant extracts in field application as a mean for controlling *H. armigera* between other means of IPM programs.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publications

Not applicable.

Availability of Data and Materials

All dataset on which abstracted of the study have been drawn are presented in the main manuscript.

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Authors Contributions

The corresponding author suggested the idea and designed the research, was supervisor, and wrote the manuscript, contributed to the writing and approved the manuscript.

The another co-author analyzed the data, preparation of tables and figures, contributed to the writing and approved the manuscript

Significant Statement

This study is very important for the clean environment free of the pollution of

chemical insecticides, create new agents from local available plant materials safe, easy preparing, cheap, possible use in combination with other biological control method of IPM program, decrease build up resistance strains of the insect because of the extracts contained different constituents have different mode of actions on the insects.

Conflicts of Interest

The authors declare that they have no competing interests.

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