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Efficacy of *Ricinus communis* L., *Cassia occidentalis* L. and *Bacillus thuringiensis* against *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*)

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Abstract

Helicoverpa armigera is one of the most destructive pests of field crops worldwide. The Study was designed to investigate through laboratory screening the insecticidal activity of *Ricinus communis*, *Cassia occidentalis* and *Bacillus thuringiensis var. Kurstaki (Btk)* and their combinations against 2nd larval instar of *H. armigera*. Five concentrations (4%, 6%, 8%, 10% and 12%) of each plant extract and (0.62, 1.25, 2.5, 5, and 10 mg/ml) of *Bt* were used in this experiment. Mortality (%) was recorded after 24,48,72 and 96 hrs post treatment. The results showed that *R. communis*, *C. occidentalis* and *Btk* have insecticidal activity against 2nd instar larvae of *H. armigera*. The highest concentration (12%) caused 80% and 70% larval mortality after 96 hrs for *R. communis* and *C. occidental* respectively.

The statistical analysis revealed that there is a significant differences between all treatments and control. Additionally, all combinations (plant/plant or plant/*Bt*) increased the mortality %. The results also clearly demonstrate that the *R. communis* are significantly more toxic than *C. occidental* where the LC_{50} values were 6.4 % for *R. communis* and 8.1% for *C. occidental*. On the other hand LC_{50} value for *Bt* was 0.41 mg/ml.

The binary mixture of *R. communis* and *C. occidental* have a potentiation effect after 48 hrs. Regarding the mixture of *Bt* and plant extracts the results revealed that *R. communis* and *Bt* mixture induced a potentiation effect whereas *C. occidental* and *Bt* mixture induced a an additive effects.

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Introduction

Helicoverpa armigera Hübner (*Lepidoptera: Noctuidae*) is one of the most destructive pests of field crops worldwide. It is a highly polyphagous multivoltine, and economically important pest of cotton and other crops and has developed resistance against most of the modern classes of synthetic insecticides[1]. In semiarid tropics the annual losses caused by this pest estimated by US$ 2 billion, even though US$ 500 million worth pesticides are applied to control this pest [2].

African bollworm has been reported on 35 crops and 25 wild host plants in eastern and southern Africa. In Sudan, it attack a wide range of host plants such as cotton, sunflower, french beans, dry beans, okra, peas, legumes, maize, sorghum, tobacco and tomato [3]. Also it was reported as the main insect pest on chickpea. Economics losses ascribed to direct yield reduction and cost of chemical applications to control this pest are considerable [4].

The problem of this pest is magnified due to its direct attack on fruiting structures, voracious feeding habits, high mobility, fecundity and multivoltine overlapping generations. Besides, it has developed resistance to broad spectrum of insecticides due to exposure of successive generations while moving from one crop to another, which made this pest highly resistant to many insecticides such as cyclodiene, pyrethroids, organophosphates, carbamates etc [5].

Some insecticides such as carbaryl, karate, cypermethrin, dimethoate and monocrotophos have been used to control this pest [6], cyprofen 220 ul [3], and kung fu 5% ec and karate zeon 10% were also used in sudan. [7]. however because of the health and environmental risks of synthetic insecticides, recently the pesticidal effects of botanical extracts have been studied by many researchers worldwide [8-10].

Castor bean (*Ricinus communis*) has traditionally been used in agriculture. In fact it is a unique species of the genus ricinus in the family euphorbiaceae as its seeds contain 2.8–3% toxic substances such as ricin, a potent inhibitor of protein synthesis, and agglutinin-1[11].

Cassia occidentalis are candidate plants and strongly suggests that they possess chemical compounds possibly oils with insecticidal properties[12].

Joint action among botanical extracts has been investigated by many researchers worldwide[13-15]. Also the combined effects between botanicals and *Bacillus thuringiensis (Bt)* have been explored by many researchers [16-18]. The purpose of this study were to: 1) evaluate the lethal effect of *Ricinus communis*, *Cassia occidentalis* and *Bacillus thuringiensis* against *H. armigera*, and to 2) investigate the joint action of *Bacillus thuringiensis* and tested plant extracts

Materials And Methods

Study Location

The experiments were conducted in the Research Laboratory, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during February - March, 2021. The average temperature is between 25-32°C.

Insect Collection and Rearing

Larval instars of *H. armigera* were collected from unsprayed tomato plants grown in Gamouaia Scheme Southern Khartoum and brought to the laboratory for mass rearing. Early instar were reared in groups of 100 larvae in plastic cages 19 cm in diameter covered with muslin cloth and fed on okra fruits, whereas 4th instars were reared separately in plastic cubs 5 cm in diameter and 7 cm in height to avoid cannibalism and the bottom of each cubs was filled with sand for pupations. Upon emergence the adults were transferred to plastic cages 31x20x19 cm covered with muslin cloth and fed on 10% sugar solution [19], cotton stripes were hung on the margins of the cages for eggs laying and were replaced daily with new stripes while newly hatching larvae were transferred to the larval rearing cages. The rearing process continued until a sufficient number of homogenous populations of larvae was collected for the experiments.

Plant Materials and Extraction Methods

Seeds of *R. communis* and *C. occidentalis* were collected from river bank, Omdurman area and brought to the laboratory for shade-drying. After complete dryness the plant samples were crushed by an electronic blender to obtain the powder. 120g of prepared seeds powder were extracted with absolute ethanol using Soxhlet apparatus for six hours, and rotary evaporator was used to remove the solvent[20].

Biotect® 9.4 % WP commercial formulation containing *Bacillus thuringiensis kurstaki* from(Organic Biotechnology Co., First industrial zone , El Noubareya, El Beheira, Egypt) were used in 5 concentrations (0.62, 1.25, 2.5, 5, and 10 mg/ml). For plant extracts also 5 concentrations (4%, 6%, 8%, 10% and 12%) were used.

Bioassay Tests

Second larval instar was used in this study. Fruits dipping method [21] was followed, small pieces of fresh okra fruits were dipped for 30 seconds in different concentrations and left to dry under room conditions for 10 minutes. Ten pre starved larvae (one hour) were used for each treatment and each treatment was replicated three times. Three replicates were also used as a control set. All treated larvae were kept in Petri-dishes 9 cm in diameter at temperature of 25±1 °c. Treated larvae were provided with fresh okra pieces till the end of experiment. The mortality % was recorded 24, 48, 72 and 96 hrs after application.

To evaluate the joint action of tested plant extracts and *Bt* the method of Mansour et al. [22] was adopted with some modifications. In which paired mixtures of plant extracts were prepared at concentration levels of their respective LC_{25} values at 1:1 ratio. Each mixture was tested in three replicates along with controls. Mortality percentages were determined after 24to 48 hrs and the combined action of the different mixtures was expressed as Co-toxicity factor. The following formula were used to determine potentiation, antagonism and additive effect:

Co - toxicity factor = (O – E) x 100/E; where:

O : is observed % mortality and E : is expected % mortality.

The co-toxicity factor differentiates the results into three categories. A positive factor of ≥ 20 indicates potentiation, a negative factor of ≤ -20 indicates antagonism, and the intermediate values of >-20 to < 20 indicates an additive effect [22]. The LC_{25} values of each extract and *Bt* were tested again against 2nd larval instar in order to determine the accurate expected mortality. The expected mortality of the combined pair is the sum of the mortalities of single compound at recorded LC_{25} and the observed mortality is the recorded mortality obtained after 24 – 48 hrs of exposure to the mixture.

Statistical Analysis

The obtained data were statistically analyzed according to analysis of variance (ANOVA); Duncan's Multiple Range Test was used for means separation using genstat version 12.1 Also the data were subjected to Probit analysis using SPSS 16.0 software.

Results

Effect of Pant Extracts

The results presented in (Table 1) clearly proved that all concentrations of the seeds ethanolic extract of *R. communis* L. And *C. occidentalis* gave significantly higher mortality percentage than the control throughout the experimental period. Additionally, the lethal effect of these extracts were dose and time dependant. The highest concentration (12 %) of *R. communis* induced 80% after 96 hrs which were significantly different and higher that caused by the same concentration of *C. occidental* which cause 70% larval mortality after the same period. Its observed from the results exhibited in (Table 2) that the seeds ethanolic extract of *R. communis* are significantly more toxic than its counterpart of *C. occidental* where the LC_{50} values were 6.4 % for *R. communis* and 8.1% for *C. occidental.*

Activity of Bacillus thuringiensis (Bt)

The results shown in (Figure 1) proved that all *Bt* concentrations generated a significantly ($p < .001$) higher mortality percentage than the control throughout the experimental period. It should be noted that the percent mortality increases with the increase of both concentration and exposure period. LC_{50} value of *Bt* was 0.41 mg/ml as shown in (Table 2).

Combinations (Joint Action) Activity

Paired mixtures of plant extracts and *Bt* were tested against 2nd larval instar of *H. armigera* as described in material and method section. The results shown in (Table 3) illustrated that the binary mixture of *R. communis* and *C. occidental* have an additive effect after 24 hrs whereas, after 48 hrs of application a potentiation effect (CTF = +23.5) was recorded. Regarding the binary mixture of *Bt* and plant extracts

Table 1. Lethal effect of *R. communis* and *C. occidental* against 2nd larval instar of *H. armigera*

Treatments	Concentra-tions(%)	Means mortality (%)			
		Exposure time (hrs.)			
		24	48	72	96
R. communis	4	26.7 (5.2)de	36.7 (6.1)cde	36.7 (6.1)def	43.3 (6.6)ef
	6	30.0 (5.5)de	40.0(6.3)cde	40.0(6.3)d	46.7 (6.9)de
	8	43.3(6.6)bc	46.7(6.9)bcd	53.3(7.3)bc	53.3(7.3)cd
	10	50.0 (7.1)ab	50.0(7.1)bc	56.7(7.6)bc	60.0(7.8)c
	12	56.7 (7.6)ab	56.7 (7.6)ab	73.3(8.6)a	80.0(8.9)a
C. occidentalis	4	16.7 (4.1)f	26.7(5.2)e	26.7(5.2)e	30.0 (5.5)g
	6	23.3 (4.9)ef	33.3(5.8)de	36.7(6.1)de	36.7(6.1)f
	8	30.0 (5.5)de	36.7 (6.1)cde	40.0(6.3)d	46.7(6.9)de
	10	36.7 (6.1)cd	40.0 (6.3)cde	46.7(6.9)cd	53.3(7.3)cd
	12	60.0(7.8)a	66.7 (8.2)a	66.7(8.2)ab	70.0(8.4)b
Control	-	0.0(0.7)g	0.0 (0.7)f	0.0(0.7)g	0.0(0.7)h
SE±	-	0.5	0.6	0.5	0.3

Means followed by the same letter (s) are not significantly different at ($p < .001$).

Means between brackets are transformed according to $\sqrt{(X+0.5)}$

SE = Standard Error

Table 2. LC values of tested plant extracts and *Bacillus thuringiensis* against 2nd larval instar of *H. armigera* after 96 hrs of exposure.

Treatments	LC* values and 95% Confidence limits (Lower – Upper)			
	LC_{50}	LC_{90}	Slope± SE*	Chi- square χ2
R. communis (%)	6.4 (2.6 – 8.3)	17.7 (13.6 – 35.9)	1.8±0.62	1.5
C.occidentalis (%)	8.1 (6.2 – 11.3)	18.7 (14.6 – 34.2)	2.0±0.64	1.11
Bt (mg/ml)	0.41(0.14 – 0.66)	1.9 (1.3 – 3.5)	1.9±0.5	1.5

* LC = lethal concentration * SE = standard error

Table 3. Joint action of tested plant extracts and *Bacillus thuringiensis* against 2nd larval instar of *H. armigera*

Combination	Mortality %		CTF	Joint action
	Expected	Observed		
	24 hrs			
Ricinus + Cassia	53.4	56.7	+6.2	Ad.
Ricinus + Btk	50.0	63.3	+26.7	Po.
Cassia + Btk	50.0	53.3	+6.7	Ad.
	48 hrs			
Ricinus + Cassia	56.7	70	+23.5	Po.
Ricinus + Btk	63.4	76.7	+21.0	Po.
Cassia + Btk	66.7	76.7	+14.9	Ad.

*Ad. = additive, po. = potentiation

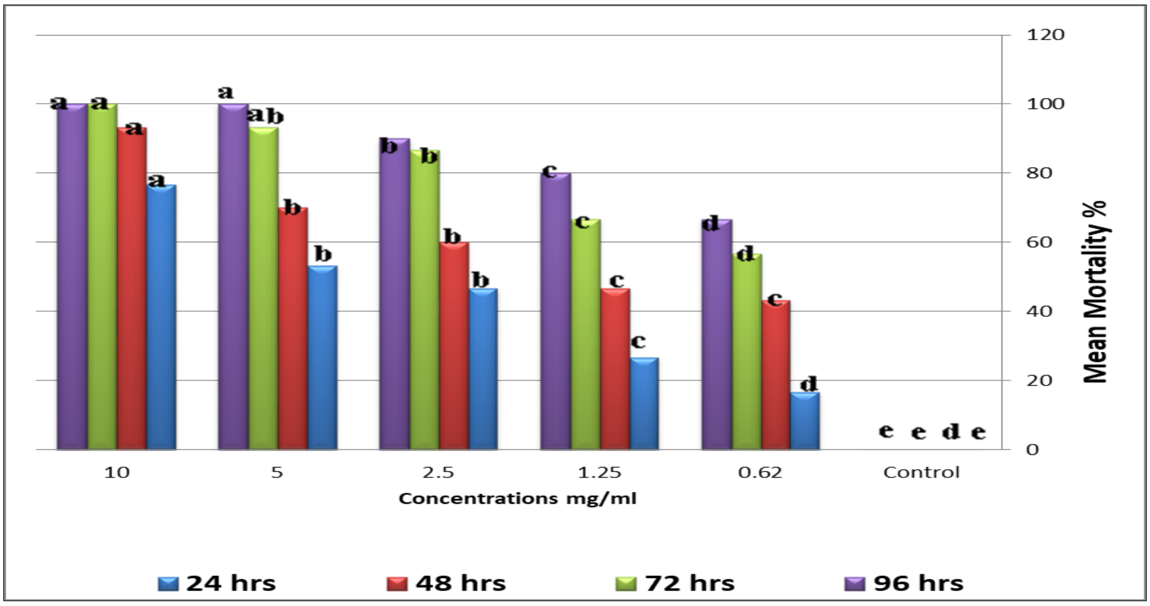

Figure 1. Lethal effect of *Bacillus thuringiensis* against 2nd larval instar of *H. armigera*.

Means within the same colored column followed by the same letter (s) are not significantly different at (p< .001).

the results revealed that the *R. communis* and *Bt* mixture induced a potentiation effect (CTF = +26.7), meanwhile, *C. occidental* and *Bt* mixture induced a an additive effect (CTF = +14.9).

Discussion

Botanicals have long been proposed as smart alternatives to synthetic insecticides for pest management because they are safe to the environment and human health. More than thousands species of plants have been reported to have chemicals in its various parts which have insecticidal properties. However, a few of them were used for insect control on a commercial scale [23]. The study findings clearly proved the efficacy of *R. communis* against 2nd larval instar o *H. armigera*. In fact its highest concentration (12%) gave 80% mortality of tested larvae after 96 hrs of application. Kodjo et al. [24] found that 5% oil emulsion of *R. communis* caused 89.58% mortality of the diamondback moth *Plutella xylostella* in ingestion toxicity test. Parajapati et al. [25] also recorded that the seed extracts of *R. communis* showed better insecticidal activity than the leaf extracts against *S. Frugiper* due to the active compounds such as castor oil and ricinine.

The mortality percent recorded after 48 hours of exposure by the lowest and highest concentrations (4% and 12%) of seeds ethanolic extract of *C. occidentalis* does not changed even after 72 hrs post treatment. This may indicate to an acute action of this plant extract. Similar results were recorded by Elnour [20] who found that the percent mortality caused by various concentrations of seeds ethanolic extract of *C. occidentalis* against African melon ladybird *Henosepilachna elaterii* after 24 hrs dose not changed after 48 hrs of exposure. Also Vashishta et al. [26] found that *C. occidentalis* generate an acute toxic effects in vertebrates and its toxins do not accumulate in body tissues.

The results also revealed that all *Bt* concentrations caused a significantly higher mortality percentage ($p<.001$) than control throughout the experimental period. The estimated LC_{50} value of *Bt* was 0.41 mg/ml in this study. Plata-Rueda et al. [27] found that LC_{50} of *Btk* on nettle caterpillar *Euprosterna elaeasa* was 1.25 mg/ ml.

The use of extract mixtures may increase the spectrum of activity of extract mixtures against target pests. In addition, if the extract mixture show synergistic effect, then low concentration is needed to control target pests. Further, low extract application rates might minimize the risk to non-target organisms as well as to the environment. Also the use of synergistic extract mixtures might delay the development of insecticide resistance [28].

Study findings illustrated that the binary mixture of *R. communis* and *C. occidental* have an additive effect after 24 hrs, whereas after 48 hrs of application a potentiation effect (CTF = +23.5) was recorded. Regarding the binary mixture of *Bt* and plant extracts the results revealed that the *R. communis* and *Bt* mixture induced a potentiation effect (CTF = +26.7), meanwhile, *C. occidental* and *Bt* mixture generated a an additive effect.

Reddy and Chowdary [29] noted that the compatibility of a plant extract for combination with microbial insecticides depends on qualitative and quantitative variations of secondary metabolites, which may affect the microbes. Many plants extract such as *Annona squamosa L., Datura stramonium L., Eucalyptus globules Labile, Ipomea carnea Jacq., Lantana camara L., Nicotiana tabacum L., and Pongamia pinnata L.* Showed a synergistic effect when mixed with *Btk*.

Conclusion

The obtained results clearly proved that the *R. communis*, *C. occidentalis* and *Bt* have insecticidal activity against 2nd instar larvae of *H. armigera*.

Its observed form the study finding that the efficacy of *R. communis* and *C. occidental* can be enhanced by mixing them together or can be used in a combination of *Bt* which may reduce the amount required for application to control this pest as well as to reduce the environmental hazards.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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