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Nutritional Indices and Efficacy of *Acokanthera spectabilis* (Hochst.) Extract and Chlorfluazuron against *Spodoptera littoralis* (Boisd.) Escorted by Mitigating Amendment to the Light Impact

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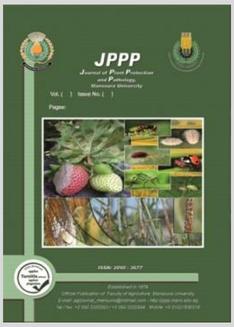

ABSTRACT

Counteractions of beta cyclodextrin (βCD) to the light intensity's impact were investigated on the nutritional indices of *Acokanthera spectabilis* (Hochst.) extract and chlorfluazuron 5% EC against the 4th instar larvae of *Spodoptera littoralis* (Boisd.). The LC_{10} value of chlorfluazuron 5% EC (0.92 mg L^{-1}) transcended *A. spectabilis* extract (0.56 mg L^{-1}) and vice versa for the LC_{90} values. The most abundant HPLC-compounds of the extract were ferulic acid (2082.49 µg g^{-1}), and ellagic acid (804.74 µg g^{-1}) as well as palmitic acid (39.89%), and cis-vaccenic acid (14.05%) in GC-MS analysis. There was relative advantage for βCD addition at 0.40 gm L^{-1} to the sub-lethal concentrations of the tested compounds, observed by their lowest relative potency and highest toxicity index. In darkness, 5000, and 10000 LUX, βCD-mixed extract affected all the larvae's nutritional indices, likewise βCD-mixed chlorfluazuron 5% EC at 10000 and 20000 LUX excluding dry gained weights, and relative growth rate. Equally highest alkaline phosphatase levels at 48 hrs of exposure to βCD-mixed extract and chlorfluazuron 5% EC were 125.73 and 131.00 U L^{-1}, respectively. Semi-field trials along two seasons showed equally high residual toxicities in net and βCD-mixed extract, as well as highest residual toxicity, and persistency in βCD-mixed chlorfluazuron 5% EC. Spray application of these compounds was safe for the chlorophyll-leaf content, with observed augmentation in all extract treatments. Ultimately, βCD-mixed chlorfluazuron 5% EC could enhance the controlling *S. littoralis*, while the *A. spectabilis* extract could be applied alone under light intensity.

Keywords: cotton leafworm, benzoylphenylurea, african wintersweet, alkaline phosphatase, residual toxicity, persistency

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most dominant polyphagous pests over 44 different families and 87 species of tropical and subtropical plants, for instance mallows, crucifers, legumes (European Public Prosecutor's Office (EPPO), 2019), and cotton plant (Lopez-Vaamonde, 2010). An approximated 4049 of egg-masses per acre of cotton field are the economic threshold for synthetic insecticides intervention (Hosny *et al.,* 1986). The feeding of its larval stage caused obvious deteriorations and defoliations on the leaves up to 70% of the invaded area that ultimately causes losses of about half the yield (Russel *et al.,* 1993; EPPO, 2023). According to the EPPO in 2022, *S. littoralis* (Boisd.) had been listed as a quarantine pest (EPPO, 2022; CABI, 2022). Recently, urgent calls have been raised to curb and rationalize the overuse of the insecticides that may cause population's resistance and environmental contamination (Saadati et al., 2012; Athukorala et al., 2023).

The leaf extract of *Acokanthera spectabilis* (Hochst.) (Gentianales: Apocynaceae) have been investigated for its insecticidal action in many researches (Abbassy et al., 1977; Benmerabet and Abed, 1973; Abdel-Aty et al., 2009). Plant of *A. spectabilis* is gradable in size, woody shrub featured by rigid, and dark green leaves (Taha and Sorour, 2019). Chlorfluazuron is an insect growth regulator belongs to benzoylphenylurea (BPU) that acts by stomach action and inhibits the chitin synthesis during the molting process on insect's early larval stage of several orders, leading to abnormal perception in the endo-cuticle, and futile molts (Hajjar and Casida, 1979; Yu, 2008; Umar and Ab Majid, 2020). Many investigations had been conducted on the insecticidal activity of some individual components, which were similarly detected in *A. spectabilis* extract. Where, the most potent phytochemical components, like gallic, ellagic, and caffeic acid possessed an important fatal role and growth inhibitor against the larval stage of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) (Punia et al., 2020; Punia et al., 2021). Likewise, p-coumaric acid, quercetin, and catechin were probably attained insecticidal activity against *S. frugiperda* (J. S. Smith) (Lepidoptera: Noctuidae) (Marques et al., 2016; Punia et al., 2023). Neophytadiene, quercetin, and double bond-free carboxylic or methylated fatty acids could realize larvacidal effect against *S. littoralis* (Boisd.) (Mesbah et al., 2007; Khamis et al., 2016; Saber et al., 2018; Eldesouky et al., 2019; Abdullah, 2019; Sung et al., 2023). On the other hand, several field experiments of chlorfluazuron showed a conspicuous growth inhibition in the early instars larvae and reduction population of many lepidoptrous pests, (Wang et al., 2021; Zhou et al., 2023; Eldessouky and Korish, 2023).

Antecedent investigations on the nutritional indices were achieved on some constitutes, which are relatively exhibited in *A. spectabilis* extract like; gallic acid (Punia et al.,

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2021), ellagic, and caffeic acid (Rani and Pratyusha, 2013; Punia et al., 2020; Punia et al., 2023) that realized clear retrogardation in the nutritional activity of *S. litura* (F.) larvae. Triterpenes, cardenolide glycosides, sterols, friedelin (Abbassy et al., 1977), and diterpene (Saber et al., 2018) could triggered an antifeedant activity against *S. littoralis* (Boisd.). Chlorfluazuron could also affect the feedant activity of the latest instar larvae of *Bradysia odoriphaga* (Diptera: Sciaridae) (Peng et al., 2017).

As one of the most important biochemical studies is the alkaline phosphatase (ALP) enzyme, which hasthe ability to induce the hydrolysis of the processes of phosphomonoesters and cytolysis. These bioprocesses could achieve obvious distributions in the membrane transport of specific tissue; intestinal epithelial cells, hemolymph, and salivary glands throughout the insect growth period (Dikbas et al., 2023). Hitherto, no available data have been established yet on the effect of *A. spectabilis* extract on the ALP enzyme in the lepidopterous insects. On the other hand, the increases of ALP activity were occurred by chlorfluazuron in the haemolymph of the larval stage of *S.Littoralis* (Boisd.) (Abdel Mageed et al., 2018).

In spite of the great importance of the photostability study on botanical extracts that may be unstable and degraded under visible light radiation, this issue is still not rolling enough in the researches of *A. spectabilis* extract (Tonnensen, 2001; Costa, 2001; Cristina de Morais et al., 2018). Meanwhile, photodegradation behavior of benzoylurea pesticides (BUPs) residues under visible light radiation may follow either one or more pathways of urea-bridge's cleavage, hydroxylation, and dehalogenation (Cristina de Morais et al., 2018; Zhu et al., 2021). Forasmuch, safe uses of the inclusion complexes of cyclodextrin isomers could reduce the photo-destruction on the phytocompounds, for instance quercetin that may originate many organic botanical extracts (Amiri and Amiri, 2017). Meanwhile, these inclusion complexes with benzoyl compound, as one of the common moieties of BFU, have been suggested as a photosensitivity agent for many pharmaceutical formulations (Sliwa and Girek, 2017).

In this respect, our study focused on the toxic effects, and nutritional indices of the sub-lethal concentrations of the extract of *A. spectabilis* versus chlorfluazuron 5% EC under different conditions of light intensities against the 4^{th} instar larvae of *Spodoptera littoralis* in laboratory conditions. The study tried to investigate the remedy role of beta cyclodextrin (βCD) as an anti-photodegradable compound to maintain a stable efficacy for these tested compounds under different light intensities. Investigations were conducted on the ALP activities of all tested compounds alone and in mixture with βCD against the 4^{th} instar larvae. Further supportive semi-field trials on cotton plants along two successive seasons, were accomplished on the efficacy of the net and βCD compounds.

MATERIALS AND METHODS

Tested compounds:

Crude ethanolic extract of the vegetative part of the African wintersweet, *Acokanthera spectabilis* (Hochst.) (Gentianales: Apocynaceae), obtained from Alexandria, Egypt. Chlorfluazuron (Tobron S 5% EC belongs to the benzoylphenylurea group and is manufactured by Agrochem for Fertilizers and Chemicals) was sprayed at a field dosage rate of 300-Liter $acre^{-1}$ that meets the prescriptions of the Agriculture Pesticides Committee in Egypt. Beta cyclodextrin hydrate (βCD, 99%, catalogue number 227281000, supplied by Acros Co., USA) was selected to evaluate its effect on the photo-stability of the tested compounds.

Procedure of *Acokanthera spectabilis* (Hochst.) extract

Acokanthera spectabilis leaves were dried at 25ºC for two weeks. Using a grinding mill, the dried leaves were ground into a superfine powder. One hundred grams of the obtained powder were immersed in 500 mL of 96% ethanol for a week. Then, the mixture was filtered, and the ethanol was discarded by using a rotary vacuum evaporator. The crude extract of *A. spectabilis* was well-sealed in a glass vial below 0ºC. Finally, concentrations of the crude extract were set and emulsified in dimethyl sulfoxide (DMSO) at the time of all required experiments.

Insect rearing:

Numerous egg-patches of *Spodoptera Littoralis* (Biosd.) were collected from various host crops and plants in different rural regions adjacent to Alexandria governorate, Egypt. The egg-patches were outgrew under standard conditioning into a bod incubator (27 ± 2°C, RH 60%, light at ~5000 Lux / dark duration automatically alternated every 12 hrs) (El-Defrawi *et al*., 1964). Larvae of *S. Littoralis* were fed on fresh leaves of the castor-oil plant, *Ricinus communis* (L.), for six generation, and followed by the last one generation on leaves of the cotton plant, *Gossypium barbadense* (L.). Eventually, a laboratory strain (LS) of *S. Littoralis* at the 7^{th} generation was ready to submit all the susceptibility tests of toxicity, nutritional indices, and semi-open field trials.

Toxicity bioassay:

A laboratory bioassay was conducted on the 4^{th} instar larvae of *S. littoralis* (Boisd.) (LS). Toxicity tests of the ethanolic extract of *A. spectabilis*, and chlorfluazuron 5% EC versus the control (distilled water) were implemented by the leaf-dipping technique using cotton leaves. Each tested compound had six gradual sub-lethal concentrations. The dipping period for treated leaves in each concentration was 30 seconds. The treated leaves were respited to dry at room temperature. Each concentration replicated four times. Twenty 4^{th}-instar larvae were used for each replicate. On the same trend, the toxicity of βCD was also investigated at different concentrations of 0.20, 0.40, and 0.60 gm L^{-1} at 48 hrs of exposure. Mortality percentages of each tested compound after 48 hrs of exposure underwent the probit analysis (Finney 1971) to assess their LC_{10} and LC_{90}.

Evaluation of relative potency and toxicity index of beta cyclodextrin combination with the tested compounds:

The lethal effects of the assigned LC_{10} and LC_{90} of the tested compounds alone and in combination with βCD at different concentrations of 0.20, 0.40, and 0.60 gm L^{-1} were evaluated on the 4^{th} instar larvae of *S. Littoralis* (LS). The lethality tests of the assigned concentrations of the tested compounds and the control (distilled water) were performed by the dipping method using cotton leaf disks. Dipping time for treated leaf disks took 20 seconds, and then they were left to dry at room temperature. Three treated leaf disks of each concentration were placed in glass cups (200 cm^3). Each cup was replicated three times. Ten identical sized larvae at the 4^{th} instar were inserted in each cup (replicate). Mortality percentages at 48 hrs of exposure were adjusted according to the control values by the formula of Abbott (1925). Finally, the mortality percentages were submitted to the calculations of LC alone / LC of the tested compound + βCD in order to estimate which concentration of βCD could fulfill the highest Relative potency (RP) when added to the tested compound. Furthermore, the concentrations of βCD could be verified for their highest leverage on the toxicity of the tested compounds by the calculations of the toxicity index (TI) based on the LC_{10} or LC_{90} of chlorfluazuron 5% EC as a reference insecticide.

Biochemical assay of alkaline phosphatase enzyme:

Biochemical tests were accomplished at 48 hrs post-treatment with the LC_{10} values of the tested compounds alone and their mixtures with βCD at 0.40 gm L^{-1} on the second day of 4^{th} instar larvae of *S. littoralis* (Boisd.). The colorimetrical detection of liberated phenol from phenyl phosphate substrate was an indicator for the ALP activity in the heamolemph samples from the healthy and treated *S. littoralis* (Boisd.) at 48 hrs. Five µl of hemolymph were added to 1 mL of 0.1 M ice-cold sodium phosphate buffer (containing 1.0% Triton X-100, pH 7.4) for each sample at 20ºC for 5 min in the dark. Meanwhile, the buffer was only used as a blank. The homogenate samples were centrifuged at 15,000 rpm for 15 min. The colorimetric measurements of ALP activities at 510 nm were performed after 1 hr using the technique of Belfield and Goldberg, (1971).

Evaluation on nutritional indices of cotton leafworm under different visible light intense:

A dipping technique a bioassay using a cotton leaf disc (diameter, 3 cm) was used to achieve the nutritional indices of the second day of the 4^{th} instar larvae of *S. Littoralis*. Parameters of anti-feedant activity and nutritional indices were detected under laboratory conditions (27 ± 2°C, RH 60 ±5%), at 48 hrs of darkness, and different visible light intensities at 500, 10000, and 20000 Lux. The constant artificial light intensity of an LED lamp (Bulb base, E27; 9 Watt; White light) was adjusted by the mean of a rheostat while controlling an appropriate distance from the tested object and measured by the light meter device (RS 180-7133). The adjusted light intensity was assigned to a transparent plastic container (diameter, 30cm; height, 35cm). Where the outer surface of the container is wrapped, and faced by the shiny, reflective side of aluminum foil. The purpose of this installation is to keep the light from being scattered and leaked while also keeping its intensity at a constant level. β-cyclodextrin at 0.40 gm L^{-1} (which achieved the highest RP and TI based on LC_{90}) was used with the LC_{10} values of the tested compounds and control (distilled water) as a convenient concentration of βCD in the subsequent field trials. Sufficient time (10 min.) was set aside for drying the treated leaf disc before using it in the exposure test on the larvae. Seven treated leaf discs (≡ 10.00 gm) were sufficient to feed a colony of 100 (24 hrs pre-starved) larvae for 48 hrs in a transparent container. The container was replicated three times (with a total of 300 larvae) for each treatment. Along the 48 hrs of exposure, the mean of dry weights of the survival larvae were calculated attributed to their fleshy weights using an oven at 50 ºC for 24 hrs (Dermott and Paterson, 1974). The initial fleshy weights of treated leaves were converted to dry weight ratios by using blank replicates (without larvae) to calculate the moisture content loss during the 48 hrs of exposure (Candy and Baker, 2002). The weight of the consumed leaf disc areas were calculated the anti-feedant activity percentage according to (Saleh et al. 1986).

$$\textbf{Anti-feedant activity percentage} = \left(1 - \frac{\text{percentage of the weight of treated eaten leaf}}{\text{percentage of the weight of untreated eaten leaf}}\right) \times 100$$

In addition, the feedant indices parameters were figured out by the equations of Waldbauer (1968), which could be illustrated as follows:

Relative consumption rate (RCR) = E / T A.
Relative growth rate (RGR) = P / T A.
Efficiency of conversion of ingested food (ECI) = (P x 100) / E.
Efficiency of conversion of digested food (ECD) = P ⁄ (E - F).
Approximate digestibility (AD) = (E - F) / E.
Consumption Index (CI) = E / A.

Where: E, dry weight of food eaten; T, duration of the experiment; A, mean of dry weight of larvae during T; P, dry weight gain of insect; F, dry weight feces of larvae during T.

Efficacy and persistency evaluation in field trials:

The semi-open field experiments were conducted in faculty of agriculture farm, Alexandria governorate, in two successive seasons of cotton crop, *Gossypium barbadense* (L.) (aged 30 days of plantation; variety, Giza 86) in 2022 and 2023. The rules of good crop management practices are closely adhered to in cotton crop plantations (Gibbs *et al*., 2005; Directorate Plant Production, 2016). Each treatment was allocated to four replicated micro-plots (12 m^2). All micro-plots over the assigned field area was sectioned according to the randomized complete block design. Sprays of the tested treatments were conducted in the early forenoon. An applicator of hand compression sprayer (5 L capacity) was used to accomplish the foliar spray of the assigned micro-plots. Each of *A. spectabilis* extract (≡ LC_{90} value), and chlorfluazuron 5% EC (recommended rate, 0.85 cm^3 micro-$plot^{-1}$) and their combination with βCD (≡ 0.40 gm L^{-1}, highest RS in table 1) were sprayed in a minimum total spray volume (1 L) that attained well coverage on the vegetative part of the cotton plants in each micro-plot (Hofman and Solseng, 2018). Meanwhile, the control micro-plots were sprayed only with water. Samples of 3 to 4 mid-aged leaves were pickedout from each treated micro-plot, and preserved in wide stitched sacks. Sampling were routinely scheduled every intervals of zero (2.5 hrs.), 2, 4, 6, and 8 DAT. Meantime, the light meter device (RS 180-7133) were used to measure three records (replicates) of light intensities for each selected time of 9 a.m., 12 a.m., and 3 p.m. throughout the foregoing intervals. The samples were delivered in the moment to the laboratory to evaluate the efficacy of the assigned treatments under the foregoing rearing conditions (27 ± 2°C, RH 60 ±5%, ~5000 Lux / dark duration every 12 hrs). Equally nourishing portions of treated cotton leaf were placed in glass cups (200 cm^3) to feed twenty (24 hrs pre-starved) 4^{th} instar larvae. Each cup was replicated four times for each treatment. The corrected mortality using Abbott's equation (1925) was recorded for each interval at 48 hrs of exposure to estimate the residual toxicity (total mean of mortality along the assigned intervals), and persistency, which expressed by the lethal time (days) needed to kill 50% (LT_{50}) of the 4^{th} instar larvae.

Chlorophyll content in fresh treated cotton leaves

Five mid-aged leaves were sampled from each treated micro-plot of the cotton field after two DAT. Each treatment had three evenly replicates of the micro-plot. The samples were transferred in wide stitched sacks the same day to the laboratory. The fresh samples were washed thoroughly with distilled water. A weight of 0.5 gm was collected from the soft tissue of each sample. Then the assigned weight of each sample was homogenized in a mortar. Quartz sand and 10 ml of 80% acetone were added to the homogenized tissue for 24 hrs. Thereafter, the solvent of each extract was filtered and preserved in a test tube. Each treatment had three replicated test tubes containing chlorophyll extract. The absorption detections of chlorophyll content were calculated at 662 and 644 nm by the equations performed by Ašimović et al., (2016):

Chlorophyll *a* (mg ml^{-1}) = 9.784 xA_{662} – 0.990 xA_{644}

Chlorophyll *b* (mg ml^{-1}) = 21.426 xA_{644} – 4.650 xA_{662}

Total chlorophyll (mg ml^{-1}) = Chlorophyll *a* + Chlorophyll *b*

Where:

A_{644} = absorbance at a wavelength of 644 nm.

A_{662} = absorbance at a wavelength of 662 nm.

High performance liquid chromatography (HPLC) analysis

Analysis of the crude ethanolic extract of *A. spectabilis* was accomplished by using an Agilent 1260 series. An Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) was used to achieve the separation step. The mobile phase was formed by water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) and adjusted at a flow rate of 0.9 ml min^{-1}. The program of the mobile phase was sequentially following a linear gradient of 0 min (82% A); 0 to 5 min (80% A); 5 to 8 min (60% A); 8 to 12 min (60% A); 12 to 15 min (82% A); 15 to 16 min (82% A) and 16 to 20 (82% A). The multi-wavelength detector was recorded at 280 nm. Each sample solution was injected at 5 µl. The column temperature was set at 40°C. The identified phyto-compounds emulate a list of 23 standard compounds.

Gas chromatography–mass spectrometry (GC–MS) Analysis

Analysis of the crude ethanolic extract was conducted using an Agilent 7000D GC–MS (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5% diphenyl / 95% dimethylpolysiloxane column and packed with an HP-5MS capillary column. The carrier gas of helium (99.99% purity) was adjusted at a flow rate of 1 mL min^{-1}. The ionization energy was regulated at 70 eV, and the scan time was 0.2 s. The fragment detection was ranged from 40 up to 600 m / z. Each injection of 1µL of the sample followed a split ratio of 10:1 at a constant temperature of 250ºC. The oven temperatures of the column were initiated at 50ºC for 3 min, then elevated gradually by 10ºC for each min up to 280ºC, and finally achieved 300ºC for 10 min. The recognition of phytochemical constituents was based on their retention time, peak area, and mass spectral that analogized the authentic compounds in Wiley registry 8E, replib, and mainlib libraries.

Statistical analysis

All the data from laboratory tests and field experiments were subjected to variance analysis (one-way ANOVA). Using software of Statistical Analysis System Institute (SAS) (2002), means were significantly differentiated at the LSD 0.05 test.

RESULTS AND DISCUSSION

Results

Toxicity of the tested compounds on cotton leafworm larvae:

The LC_{10} and LC_{90} values were calculated for each of *A. spectabilis* extract and chlorfluazuron 5% EC on the 4th instar larvae of *S. Littoralis* at 48 hrs of exposure under the foregoing rearing conditions (Table 1). The LC_{10} values of chlorfluazuron 5% EC (0.92 mg L^{-1}) were notably greater than those of *A. spectabilis* extract (0.56 mg L^{-1}). Contrariwise, the LC_{90} values of chlorfluazuron 5% EC (26.17 mg L^{-1}) were conspicuously lower than those of the plant extract (76601.76 mg L^{-1}). No observed mortalities were detected for all assigned concentrations of βCD treatment alone.

Table 1. Toxicity of the examined compounds on the 4th instar larvae of *Spodoptera Littoralis* (Biosd.) at 48 hrs of exposure

Tested compound	Instar	βCD* concentration (gm L^{-1})	Lethal concentration (mg L^{-1})		Confidence limits (mg L^{-1})	Slope ± SE**	χ^2***	*df*	N***
Acokanthera spectabilis (Hochst.) ethanolic extract	4th	-	LC_{10}	0.56	(0.18 - 1.70)	0.24 ±0.05	4.56	4	480
		-	LC_{90}	76601.76	(25938.26 - 226222.94)				
Chlorfluazuron 5% EC	4th	-	LC_{10}	0.92	(0.65 – 1.28)	0.97 ±0.05	2.08	4	480
		-	LC_{90}	26.17	(18.88 – 36.28)				
β-cyclodextrin alone	4th	0.2	N.D*****		-	-	-	-	80
		0.4	N.D		-	-	-	-	80
		0.6	N.D		-	-	-	-	80

***β-cyclodextrin, **Standard error, ***Chi square, ***Total numbers of larvae, Not detected mortality.**

Relative potency and toxicity index of β-cyclodextrin combination with the tested compounds:

Among the results of the lethal effects of all treatments at LC_{10} against the 4th instar larvae in table (2), the lowest values of RP were realized at 0.60 and 0.67 whenever adding βCD at 0.40 gm L^{-1} to *A. spectabilis* extract and chlorfluazuron 5% EC, respectively. Moreover, the highest TI occurred at 12.68 and 15.58 by adding the same concentration of βCD to *A. spectabilis* extract and chlorfluazuron 5% EC, respectively. Likewise, the lethal effects of all treatments at LC_{90} against the 4th instars in table (2) acquired the lowest RP at 0.94 and the highest TI (based on LC_{90} of chlorfluazuron 5% EC) at 1.06 whenever adding βCD at 0.40 gm L^{-1} to the tested compounds.

Table 2. Relative potency and toxicity index of the sub-lethal effects of the examined compounds alone and in combination with β-cyclodextrin

Tested compound	Instar larvae	+β-cyclodextrin (gm L^{-1})	Actual mortality of the lethal conc. (mg L^{-1})		Relative potency		Toxicity index	
			LC_{10}	LC_{90}	LC_{10} alone* / LC_{10} + βCD	LC_{90} alone* / LC_{90} + βCD	LC_{10}	LC_{90}
Acokanthera spectabilis (Hochst.) ethanolic extract	4th	0.00	7.00	88.67	1.00	1.00	7.61	1.00
		0.20	10.67	90.33	0.66	0.98	11.60	1.01
		0.40	11.67	94.33	0.60	0.94	12.68	1.06
		0.60	11.33	91.67	0.62	0.97	12.32	1.03
Chlorfluazuron 5% EC	4th	0.00	0.92	89.00	1.00	1.00	1.00	1.00
		0.20	11.00	94.00	0.88	0.95	11.96	1.06
		0.40	14.33	94.67	0.67	0.94	15.58	1.06
		0.60	13.00	94.67	0.74	0.94	14.13	1.06

***Values of LC_{10} and LC_{90} alone were mentioned in table 1.**

Alkaline phosphatase activity

Changes in ALP activity between the control and treated 4th instar larvae of *S. littoralis* (Boisd.) were determined at 48 hrs of exposure (Fig. 1). The result showed significant lowest ALP activities in the control (33.20 U L^{-1}) that came equally to the blank of βCD (34.78 U L^{-1}). All the ALP activities of the treated larvae significantly surpassed the corresponding activities in the control treatments. The highest ALP activities were equipollent in both βCD-binary mixtures of *A. spectabilis* (Hochst.) extract (125.73 U L^{-1}) and chlorfluazuron 5% EC (131.00 U L^{-1}). The βCD-binary mixture treatments excelled their corresponding net treatments. Likewise, ALP activities were equivalent in both net treatments of *A. spectabilis* extract (74.35 U L^{-1}) and chlorfluazuron 5% EC (66.80 U L^{-1}).

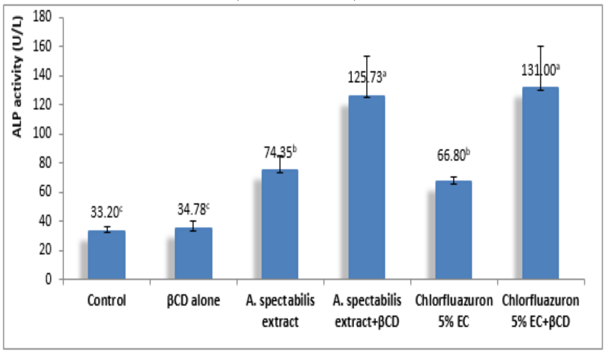

Fig. 1. Alkaline phosphatase activity in the 4th instar larvae of *Spodoptera littoralis* (Boisd.) at 48 hrs of exposure to the LC_{10} of the tested compounds alone and in combination with β-cyclodextrin at 0.40 gm L^{-1} compared to the control and β-cyclodextrin alone.

Nutritional indices under different visible light intensities

The effects of LC_{10}s of the tested compounds alone and in combination with βCD at 0.40 gm L^{-1} were evaluated on the nutritional indices of the survival 4th instar larvae of *S. Littoralis* (LS) after 48 hrs of exposure under dark and different light intensities (Table 3).

Eaten food percentages

The obtained results of eaten food percentages (E%) based on dried leaves' weight had the highest E% in all control treatments under all conditions. All the control treatments were on par with the βCD-controls under all conditions. In darkness, the E% in the colonies treated with βCD-mixed extract of *A. spectabilis* (76.54%) came on par with the net extract (78.48%), while βCD-mixed chlorfluazuron 5% EC (68.59%) was significantly lower than chlorfluazuron 5% EC (83.09%). In the 5000 LUX, βCD-mixed extract (49.21%) had the lowest E% than its net one (78.31%), while both net and βCD-mixed chlorfluazuron 5% EC had comparable E% of 71.28 and 73.74%, respectively. In the 10000 LUX, equipollent E% were realized in the colonies treated with βCD-mixed extract (64.12%) and βCD-mixed chlorfluazuron 5% EC (64.68%), which were significantly lower than the net ones. In the 20000 LUX, βCD-mixed chlorfluazuron 5% EC (59.68%) had a lower E% than its net one (65.25%), whereas βCD-mixed extracts (78.21%) were on par with its net extract (79.69%).

Anti-feedant activity percentages

The most potent anti-feedant activity percentages (AF%) attained by βCD-mixed extract (46.52%) was higher that the net extract (14.89%) at 5000 LUX, while βCD-mixed chlorfluazuron 5% EC (33.12%) excelled their corresponding net chlorfluazuron 5% EC (26.68%) at 20000 LUX.

Dry gained weights of larvae

The highest dry gained weights of larvae (P) was revealed in net and βCD-control under all conditions, likewise βCD-mixed extract (110.24%) at only 10000 LUX. The lowest P in βCD-mixed extract at 6.08 and 0.04 mg under darkness and 20000 LUX, respectively, as well as βCD-mixed chlorfluazuron 5% EC (0.01 mg) at 20000 LUX were equipollent to their corresponding net compounds.

Faeces

Under all lighting conditions, all the tested βCD-compound possessed significantly lower faeces excreted by larvae than their net compounds.

Consumption indexes

The lowest consumption indexes (CI) of βCD-mixed extract (42.36, and 54.06 at 5000, and 10000 LUX, respectively), and βCD-mixed chlorfluazuron 5% EC (58.84, 48.14, and 41.31 at 5000, 10000, and 20000 LUX, respectively) were significantly lower than their corresponding net ones. Where the net and βCD-control colonies had the highest CI.

Relative consumption rate

Perfect relative consumption rate (RCR) in *S. littoralis* (Boisd.) larvae was indicated by its lowest rates in all control colonies under all conditions. The βCD-mixed compounds were almost at lower rates than their corresponding net compounds under all tested conditions. In contrast, βCD-mixed extract at only 20000 LUX was distinguished by a higher RCR (87.68 mg (mg x day)$^{-1}$) than its net extract (69.43 mg (mg x day)$^{-1}$).

Relative growth rate

In darkness, the lowest relative growth rate (RGR) in βCD-mixed extract was 0.19 mg (mg x day)$^{-1}$, versus the net extract at 0.25 mg x (mg x day)$^{-1}$, and the whole control colonies (highest RGR).

Efficiency of conversion of ingested food percentages

In general, the efficiency of conversion of ingested food percentages (ECI%) in the net and βCD-tested compounds were significantly lower than their corresponding net and βCD-control colonies (highest ECI%). Only in dark conditions, βCD-mixed extract (0.36%) was significantly lower than its net extract (0.49%).

Approximate digestibility percentages

Generally, the approximate digestibility percentages (AD%) in the net and βCD-tested compounds were significantly lower than their corresponding net and βCD-control (highest AD%). The most potent effects of βCD mixtures on AD%, were found in βCD-mixed extract (9.09, and 10.00%), which was significantly lower than their net extract (16.67, and 15.00%) in dark conditions, and 10000 LUX, respectively. Moreover, βCD- chlorfluazuron 5% EC (9.09, and 16.67%), which was significantly lower than their net extract (16.67, and 23.08%) in dark condition, and 10000 LUX, respectively.

Efficiency of conversion of digested food percentages

Generally, the efficiency of conversion of digested food percentages (ECD%) in the net and βCD-tested compounds were significantly lower than their corresponding net and βCD-control (the highest ECD%). The most potent effects of βCD mixtures on ECD%, were found in darkness, where the lowest ECD% were comparable in βCD-mixed extract (3.91%) and the net extract (2.93%). At 10000 LUX, the lowest ECD% was exhibited in βCD-mixed chlorfluazuron 5% EC (0.46%), which was on par with its net one (0.05%).

Table 3. Effects of LC_{10}s of the tested compounds alone and in combination with β-cyclodextrin on the nutritional indices of the survival 4th instar larvae of *Spodoptera Littoralis* (Biosd.) at 48 hrs of exposure under different light intensities

Light intensity (LUX) / Treatment	+βCD[1] (gm L^{-1})	Nutritional indices parameters ±SD[2] E[3] %	AF %[4]	P[5] (mg)	F[6] (mg)	CI[7]	RCR[8] (mg(mg day)$^{-1}$)	RGR[9] (mg(mg day)$^{-1}$)	ECI %[10]	AD %[11]	ECD %[12]
Darkness											
Acokanthera spectabilis (Hochst.) extract	-	78.48	14.71	8.53	1463.04	69.92	52.36	0.25	0.49	16.67	2.93
		3.02	3.28	0.84	56.21	9.02	6.75	0.00	0.07	0.00	0.40
	0.40	76.54	16.81	6.08	1556.64	66.50	53.82	0.19	0.36	9.09	3.91
		0.63	0.69	0.27	12.91	3.57	2.88	0.00	0.02	0.00	0.20
Chlorfluazuron 5% EC	-	83.09	9.60	74.28	1548.99	84.09	20.13	0.76	4.01	16.67	24.06
		1.26	1.37	16.85	23.43	7.10	4.63	0.04	0.96	0.00	5.74
	0.40	68.59	25.45	87.49	1394.93	64.05	13.77	0.78	5.75	9.09	63.23
		5.84	6.35	2.91	118.84	8.53	1.40	0.01	0.58	0.00	6.34
Control	-	91.91	0.23	150.94	1370.73	77.38	11.59	0.85	7.34	33.33	22.03
		0.58	0.63	6.18	8.65	2.37	0.50	0.00	0.35	0.00	1.04
	0.40	92.01	0.02	121.48	1372.23	88.64	14.24	0.84	5.90	33.33	17.71
		0.14	0.15	6.30	2.07	3.92	0.63	0.01	0.31	0.00	0.92
5000 LUX											
Acokanthera spectabilis extract	-	78.31	14.89	8.39	1576.74	68.89	51.80	0.25	0.48	10.00	4.79
		0.89	0.97	0.45	17.91	0.24	0.93	0.01	0.03	0.00	0.31
	0.40	49.21	46.52	29.84	935.67	42.36	19.70	0.53	2.71	15.00	18.10
		3.28	3.57	1.66	62.43	2.31	0.70	0.01	0.08	0.00	0.57
Chlorfluazuron 5% EC	-	73.74	19.85	28.85	1374.73	63.33	30.08	0.52	1.75	16.67	10.51
		0.97	1.05	2.55	18.01	1.14	1.31	0.03	0.18	0.00	1.07
	0.40	71.28	23.34	55.98	1226.69	58.84	19.19	0.67	3.51	23.08	15.21
		0.33	0.36	1.67	5.74	3.39	0.50	0.01	0.09	0.00	0.41
Control	-	92.99	0.00	103.62	1485.93	77.37	15.97	0.79	4.98	28.57	17.43
		0.28	0.30	6.03	4.40	1.66	0.84	0.01	0.30	0.00	1.06
	0.40	92.01	1.06	121.48	1372.23	88.64	14.24	0.84	5.90	33.33	17.71
		0.14	0.15	6.30	2.07	3.92	0.63	0.01	0.31	0.00	0.92
10000 LUX											
Acokanthera spectabilis (Hochst.) extract	-	76.88	13.49	0.04	1461.83	86.34	86.18	0.00	0.00	15.00	0.01
		0.00	0.00	0.05	0.00	0.50	0.27	0.00	0.00	0.00	0.02
	0.40	64.12	27.85	110.24	1290.92	54.06	10.48	0.81	7.69	10.00	76.86
		0.38	0.42	1.04	7.55	2.28	0.08	0.01	0.08	0.00	0.81
Chlorfluazuron 5% EC	-	77.68	13.56	0.18	1336.81	54.53	54.21	0.01	0.01	23.08	0.05
		0.16	0.18	0.09	2.75	1.74	1.57	0.00	0.01	0.00	0.02
	0.40	64.68	27.22	1.10	1205.82	48.14	46.44	0.04	0.08	16.67	0.46
		0.17	0.19	0.38	3.17	0.77	0.32	0.01	0.03	0.00	0.16
Control	-	89.87	0.11	116.09	1436.14	73.87	14.07	0.81	5.77	28.57	20.18
		2.62	0.01	12.14	41.84	3.73	0.70	0.02	0.43	0.00	1.50
	0.40	88.87	1.12	122.37	1325.38	83.73	13.65	0.84	6.16	33.33	18.47
		0.19	0.21	9.82	2.79	3.57	0.99	0.01	0.51	0.00	1.52
20000 LUX											
Acokanthera spectabilis (Hochst.) extract	-	79.69	10.45	0.03	1604.58	69.53	69.43	0.00	0.00	10.00	0.02
		2.03	2.29	0.03	40.96	5.63	5.54	0.00	0.00	0.00	0.02
	0.40	78.21	12.12	0.04	1487.18	87.85	87.68	0.00	0.00	15.00	0.01
		1.89	2.12	0.05	35.86	2.64	2.39	0.00	0.00	0.00	0.02
Chlorfluazuron 5% EC	-	65.25	26.68	0.02	1216.48	85.43	85.32	0.00	0.00	16.67	0.01
		0.24	0.27	0.02	4.42	1.28	1.18	0.00	0.00	0.00	0.01
	0.40	59.68	33.12	0.01	1027.01	41.31	41.30	0.00	0.00	23.08	0.00
		4.26	4.77	0.01	73.28	2.27	2.28	0.00	0.00	0.00	0.00
Control	-	89.24	1.11	88.04	1426.00	63.58	16.86	0.73	4.41	28.57	15.44
		0.38	0.43	13.26	6.12	0.08	1.99	0.03	0.67	0.00	2.35
	0.40	88.99	0.28	85.86	1327.24	62.70	16.99	0.73	4.31	33.33	12.94
		0.38	0.43	11.16	5.71	3.97	1.41	0.03	0.57	0.00	1.70
$LSD_{0.05}$		3.83	4.10	11.40	72.89	7.43	4.48	0.03	0.63	452E-9	3.83

β-cyclodextrin[1], Standard error[2], Eaten food% based on dried weight[3]; Dry gained weight of larvae[4]; Faeces[5]; Consumption Index[6]; Relative consumption rate[7]; Relative growth rate[8]; Efficiency of conversion of ingested food%[9]; Approximate digestibility[10]; Efficiency of conversion of digested food %[11].

• Means evenly included in each column termed with limit values ≤ the $LSD_{0.05}$ are not significantly different.

Residual toxicity and persistency

Residual toxicity and persistency (LT_{50}) of the tested compounds alone and in combination with βCD at 0.40 gm L^{-1} were evaluated against the 4th instar larvae of *S. littoralis* (Boisd.) at 48 hrs of exposure along intervals of zero (2.5 hrs.), 2, 4, 6, and 8 DAT in seasons 2022 and 2023 (Table 4).

The results of the total mean of light intensities along the tested intervals of DAT were 42017.80 and 42993.87 LUX in seasons 2022 and 2023, respectively. The obtained data showed obvious superiority of chlorfluazuron 5% EC + βCD in total efficacy (65.50, and 65.75%), and persistency (5.76, and 6.56 days) in seasons 2022 and 2023, respectively. Oppositely, chlorfluazuron 5% EC alone had the lowest residual toxicity and persistency in both seasons. In the second rank, equally high residual toxicities were exhibited in *A. spectabilis* extract alone (56.25%) and βCD-mixed extract (56.00%) in 2022 and the same values for both of them (56.00%) in 2023. In the same trend, persistencies were apparently similar in *A. spectabilis* extract alone (3.15, and 3.06 days) and the βCD-mixed extract (3.17, and 3.14 days) in 2022 and 2023, respectively.

Table 4. Residual toxicity and persistency of the tested compounds alone and in combination with β-cyclodextrin against the 4th instar larvae of *Spodoptera littoralis* (Boisd.) at 48 hrs of exposure in two seasons of semi-field trials.

		Season 2022 (42017.80 LUX)[1]			Season 2023 (42993.87 LUX)		
Tested compounds	+βCD[3] (gm L^{-1})	Residual toxicity[3] ±SD[4]	Persistency[5] (day)	Confidence limit (day)	Residual toxicity ±SD	Persistency (day)	Confidence limit (day)
A. spectabilis	-	56.25[b] ±0.96	3.15	(1.99-4.99)	56.00[b] ±1.83	3.06	(1.93-4.83)
(Hochst.) extract	0.40	56.00[b] ±1.15	3.17	(2.20-4.57)	56.00[b] ±1.41	3.14	(2.19-4.50)
Chlorfluazuron	-	46.75[c] ±1.71	1.85	(1.31-2.60)	47.00[c] ±0.82	1.85	(1.31-2.60)
5% EC	0.40	65.50[a] ±1.00	5.76	(4.41-7.52)	65.75[a] ±1.89	6.56	(4.44-9.68)
Control	-	0.00[d] ±0.00	-	-	0.00[d] ±0.00	-	-

Total mean of light intensities (LUX) at 9 a.m., 12 a.m., and 3 p.m every interval of zero, 2, 4, 6, and 8 DAT[1], β-cyclodextrin[2], Express by the total mean of mortalities at the same intervals[3], Standard deviation[4], expressed by LT_{50} (days) on the 4th instar larvae[5].
• Means evenly included in each column termed with the identical characters are not significantly differentiated according to the $LSD_{0.05}$.

Chlorophyll content in treated cotton leaves

The results of chlorophyll content in treated cotton leaves by the tested compounds alone and in combination with βCD were accomplished at 48 hrs of exposure during the field trials in 2022 and 2023 on cotton plants (Table 5).

Table 5. Chlorophyll content in treated cotton leaves by the tested compounds alone and in combination with β-cyclodextrin at 48 hrs of exposure two seasons of semi-field trials.

Treatments	+βCD[1] (gm L^{-1})	Season 2022			Season 2023		
		Chlorophyll content (mg ml^{-1}) ±SD[2]					
		a	*b*	Total	*a*	*b*	Total
Control	-	19.07[bc] ±0.33	17.31[c] ±0.24	36.38[b] ±0.52	19.64[a] ±0.10	14.23[c] ±0.20	33.87[b] ±0.17
βCD only	0.40	19.32[bac] ±0.52	16.05[c] ±0.66	35.37[b] ±0.77	20.04[a] ±1.20	15.00[cb] ±1.71	35.04[b] ±2.42
Acokanthera spectabilis	-	20.28[a] ±0.48	33.34[b] ±0.69	53.61[a] ±0.81	20.34[a] ±0.78	34.11[a] ±1.14	54.45[a] ±1.08
(Hochst.) extract	0.40	19.91[ba] ±0.91	34.95[a] ±0.24	54.86[a] ±0.75	19.63[a] ±1.09	35.95[a] ±0.85	55.58[a] ±1.73
Chlorfluazuron	-	18.78[c] ±0.62	16.05[c] ±1.44	34.83[b] ±1.27	19.07[a] ±0.64	16.95[b] ±2.25	36.02[b] ±2.17
5% EC	0.40	18.47[c] ±0.44	16.44[c] ±1.31	34.92[b] ±1.30	18.70[a] ±1.26	16.75[b] ±0.60	35.46[b] ±0.70

β-cyclodextrin[1], Standard deviation[2]
• Means evenly included in each column termed with the identical characters are not significantly differentiated according to the $LSD_{0.05}$.

The data on chlorophyll a, b and total chlorophyll in the cotton leaves of the control plots were equivalent to their counterparts in the βCD plots in the two seasons. The content of chlorophyll *a* of *A. spectabilis* extract (20.28 mg ml^{-1}) surpassed the control in 2022, while there were no notably variations in the contents of chlorophyll *a* between all treatments in 2023. Chlorophyll *b* of *A. spectabilis* extract (33.34, and 34.11 mg ml^{-1}) and βCD-mixed extract (34.95, and 35.95 mg ml^{-1}) exceeded the control in 2022 and 2023, respectively. Chlorophyll *b* of chlorfluazuron 5% EC and its blend with βCD came on par with the control in 2022, while chlorfluazuron 5% EC (16.95 mg ml^{-1}) and its blend with βCD (16.75 mg ml^{-1}) exceeded the control in 2023. The total chlorophyll of *A. spectabilis* extract (53.61, and 54.45 mg ml^{-1}) and its βCD mixture (54.86, and 55.58 mg ml^{-1}) exceeded the control in 2022 and 2023, respectively. The total chlorophyll of chlorfluazuron 5% EC, and its blend with βCD came on the par with the control in both seasons.

HPLC analysis of *Acokanthera spectabilis* (Hochst.) extract

Phytochemical screening of total 17 phenolic and 6 flavonoid constituents was determined in *A. spectabilis* extract, and represented by their peak area, and concentration unit of µg g^{-1} (Table 6). The identified phenolic moieties in *A. spectabilis* extract, include gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, pro catechol, ellagic acid, coumaric acid, vanillin, ferulic acid, and cinnamic acid. The prevailing quantities of ferulic acid was exhibited at 2082.49 µg g^{-1}, and ellagic acid at 804.74 µg g^{-1}, then followed by chlorogenic acid at 290.43 µg g^{-1}, and methyl gallate at 265.52 µg g^{-1}. Notably, kaempferol in this extract could be detected in concentrations further lower than the detection limit of the present analysis. Additionally, the identified flavonoids in *A. spectabilis* extract contain rutin, naringenin, daidzein, quercetin, apigenin, and hesperetin. Quercetin was distinguished by its highest concentration at 1614.22 µg g^{-1}.

Table 6. Detection of phenolic and flavonoid compounds in *Acokanthera spectabilis* (Hochst.) extract using HPLC analysis.

Compounds	Peak area	Concentration (µg g^{-1})
Phenolic compounds:		
Gallic acid	9.08	55.26
Chlorogenic acid	31.48	290.43
Catechin	4.06	67.37
Methyl gallate	71.16	265.52
Caffeic acid	20.94	114.97
Syringic acid	12.32	57.88
Pyro catechol	0.00	0.00
Ellagic acid	63.41	804.74
Coumaric acid	54.20	115.46
Vanillin	31.17	91.04
Ferulic acid	483.08	2082.49
Cinnamic acid	78.85	98.61
Kaempferol	nd*	nd
Flavonoids:		
Rutin	146.98	1156.12
Naringenin	156.10	1134.92
Daidzein	237.95	974.22
Quercetin	178.38	1614.22
Apigenin	204.50	1024.45
Hesperetin	65.50	235.61

***nd= not detected**

GC-MS analysis of *Acokanthera spectabilis* (Hochst.) extract

The GC–MS chromatogram of *A. spectabilis* extract recorded a total of 19 peaks of phytochemical compounds which were identified by their retention time, relative abundance area (%), and compound class based on Wiley registry 8E, replib, and mainlib libraries (Table 7). The GC-MS phytochemical compounds of *A. spectabilis* extract belong to various classes, comprising saturated and unsaturated fatty acids and their derivatives of methyl and propyl ester, sesquiterpene, phylloquinones, diterpene, tetracyclic triterpenes, phytosterols, prenol lipids, and 1-pyrroline nitrones. The most bioactive compounds mentioned by their highest abundant area were palmitic acid (39.89%), and cis-vaccenic acid (14.05%) followed by octadecanoic acid (5.81%), oleic acid (5.03%), and á-Sitosterol (4.81%) (Table 7).

Table 7. Detection of phyto-compounds in *Acokanthera spectabilis* (Hochst.) extract using GC-MS analysis.

Retention time (min)	Relative abundance %	Compound	Compound Class
22.32	2.84	Tetradecanoic acid	Saturated fatty acid
24.01	1.03	2-Pentadecanone, 6,10,14-trimethyl-	Sesquiterpene
24.17	3.66	Neophytadiene	Sesquiterpenoids
24.99	1.18	Phytol acetate	Phylloquinones
25.62	1.32	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) meth yl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	Fatty acid methyl ester
26.36	39.89	Palmitic acid	Saturated fatty acid
28.15	1.85	Hexadecanoic acid, trimethylsilyl ester	Fatty acid derivative
29.12	2.57	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	Fatty acid derivative
29.31	4.27	9,12-Octadecadienoic acid (Z,Z)-	Polyunsaturated fatty Acyls
29.48	14.05	cis-vaccenic acid	Unsaturated fatty acids
29.57	5.03	Oleic Acid	Monounsaturated fatty acid
29.97	5.81	Octadecanoic acid	Polyunsaturated fatty acid
32.83	1.30	4,8,12,16-Tetramethylheptadecan-4-	Diterpene, lactones
41.01	1.38	3,5-Bis (P-dimethylaminostr Yl)-2,2-dimethyl-2h-pyrrol E 1-oxide	1-pyrroline nitrones
41.66	1.57	ç-Linolenic acid, TBDMS derivative	Polyunsaturated omega-6 fatty acid derivatives
42.25	1.75	9,12-Octadecadienoic acid (Z,Z)-, 2,3- bis[(trimethylsilyl)oxy]propyl ester	Fatty acid propyl ester
44.33	3.16	Stigmasterol	Tetracyclic triterpenes
45.18	4.81	á-Sitosterol	Phytosterols
45.49	3.95	á-Amyrin	Prenol lipids

Discussion

In the presence of visible light radiation, secondary photodegrading moieties of the botanical extracts (Tonnensen, 2001; Costa, 2001; Cristina de Morais et al., 2018) and chlorfuazuron, as one of the BUPs (Cristina de Morais et al., 2018), encounter probable dissipation and instability that may cause declines in their toxicity. Thus, our study was directed to evaluate the safely use of βCD inclusion complex, which may play an important role in enhancing the photostability and bioavailability of various chemical compounds (Deumié et al., 2000; Pouliquen et al., 2007; Garnero and Longhi, 2010; Zhang et al., 2011; Jin, 2013; Sliwa and Girek, 2017; Amiri and Amiri, 2017).

The obtained data of the LC_{10} and LC_{90} of the *A. spectabilis* extract and chlorfluazuron 5% EC in combination with 0.40 gm L^{-1} βCD on the 4th instar larvae of *S. littoralis* had the lowest RP and highest TI compared to their net compounds and the control. This finding may be interpreted by the capabilities of inclusion complexes of CDs that could obstruct the amount of light capable of reaching bioactive compounds in many organic botanical extracts, for instance quercetin (Amiri and Amiri, 2017) could reduce the photo-destruction, as well as benzoyl urease moieties (Sliwa and Girek, 2017) have been investigated to enhance the photosensitivity of many pharmaceutical formulations. Additionally, the βCD-mixed compound is also an augmentative agent for the guest molecules due to its photosensitive viscosity switches (Pouliquen et al., 2007; Garnero and Longhi, 2010).

Considering the vital role of the ALP enzyme in prompting the transphosphorylation influx in specific tissues in insects, ALP activity realized a proportional increase by the death time and a proportional decrease by the raise in the dead insects (Dikbaş et al., 2023). In this respect, our data on ALP activities of all tested compounds in the haemolymph of the 4th instar larvae at 48 hrs of exposure significantly surpassed the control treatment. The highest ALP activities were equivalent in all βCD-mixed compounds and excelled their corresponding net treatments. So far, the finding of the ALP activity of *A. spectabilis* extract alone and its mixture with βCD in this research has been considered novel and proactive among the biochemical studies. While in the haemolymph, decline in ALP activity of chlorfluazuron 5% EC was consistent with the results of Abdel Mageed et al., (2018) of the 4th instar larvae after 96 hrs of treatment.

Nutritional indices came as a supported indicator in which we may interpret the data of larval mortality that may be imputed to the cessation of feeding and thereby lead to conspicuous decreases in CI, ECI, ECD, RGR and AD rates (Ghoneim et al., 2020, Essa et al., 2022). The data on nutritional indices in βCD-mixed *A. spectabilis* extract fulfilled the lowest E% at 5000, and 10000 LUX and the most potent AF% at 5000 LUX. βCD-extract possessed the lowest P in darkness, and 20000 LUX. The lowest CI was realized at 5000, and 10000 LUX, whereas the lowest RGR, ECI, and ECD were fulfilled in darkness. These data met previous investigations that may have interpreted the decline in nutritional indices, like sesquiterpenoids, neophytadiene (Saber et al., 2018) exhibited antifeedant activity, whereas phylloquinones, and phytol (Anderson et al., 1993) had deterrent effects against the 4th instar larvae of *S. littoralis*. Likewise, the saturated fatty acid, palmitic acid attained antifeedant activity against *S. frugiperda* (J. S. Smith) (Sung et al., 2023). Additionally, some HPLC phyto-compounds of *A. spectabilis* extract, like gallic (Punia et al., 2021), ellagic, and caffeic acid (Rani and Pratyusha, 2013; Punia et al., 2020;

Punia et al., 2023) were potent compounds that significantly reduced RGR, RCR, ECI, ECD, and AD in the survival larvae of *S. litura* (F.). Otherwise, data on nutritional indices in βCD-mixed chlorfluazuron 5% EC showed the lowest E% in darkness, 10000, and 20000 LUX. The most potent AF% was attained at 20000 LUX. No effect was observed on the P rate. The lowest CI was realized at all light intensities. The lowest ECD% appeared at 10000 LUX, which was on par with their net compounds. These findings came par to the investigations that showed an inhibition by chlorfluazuron on the feeding activity of the latest instar larvae for a short transitory time in *Bradysia odoriphaga* (Diptera: Sciaridae) (Peng et al., 2017).

In the course of the total efficacy and persistency data against the 4th instar larvae of *S. littoralis*, *A. spectabilis* extract alone and in combination with βCD were equipollent in two running seasons of cotton crop. The exegesis of the toxic action of *A. spectabilis* extract may be attributed to the presence of specific HPLC phytochemical moieties. Where, antecedent studies showed that the increases in quercetin were correlated with the larvacidal effect on *S. littoralis* (Mesbah et al., 2007). The increases in apigenin concentration derived from alfalfa plant led to a significant declination in *S. littoralis*, pupae, and larvae populations (Rani and Pratyusha, 2013; Punia et al., 2023). Additionally, the increases in gallic, and ellagic acid concentrations raised the lethal effect on the six-day-old larvae of *S. litura* (Punia et al., 2020; Punia et al., 2021). The growth inhibition and mortality effects on the 2nd instar larvae of *S. litura* were exhibited through a wide range of sub-lethal concentrations of caffeic acid (Punia et al., 2023). The invasion of some instar larvae of *S. litura*, induced defense compounds of syringic, and vanillic acid in *Capsicum annuum* L. (Solanales: Solanaceae) plants (Movva et al., 2017). The compounds gallic, p-coumaric acid, and catechin in the extract of *Acerola bagasse* (Malpighiales: Malpighiaceae) flour increased the larvacidal effect on *S. frugiperda* (Marques et al., 2016; Punia et al., 2023). Isolated quercetin from *Solidago graminifolia* (Asterales: Asteraceae) extract (Herrera-Mayorga et al., 2022) and an extract of *Acerola bagasse* flour (Marques et al., 2016) had an observed toxicity against the larval stage of *S. frugiperda*. However, chlorogenic acid in *S. graminifolia* extract showed no larvacidal effect on *S. frugiperda*, but it could augment the antagonistic effect of quercetin (Herrera-Mayorga et al., 2022). Syringic, and ferulic acids in extracts of different cotton varieties resulted in complete mortality during the first week of treatment in *Helicoverpa armigera* (Hübner) (Perveen et al., 2001; Punia et al., 2023). Moreover, the presence of GC-MS free carboxylic fatty acids and their derivatives that have been detected in *A. spectabilis* extract, such as oleic, octadecanoic, ç-linolenic, palmitic, 9,12-octadecadienoic acid and, hexadecanoic methyl ester possessed double bonds in their chains that may be proportionally correlated with their insecticidal activity against the larvae of *S. littoralis* (Khamis et al., 2016; Eldesouky et al., 2019; Abdullah, 2019; Sung et al., 2023). In addition, diterpene compounds, and neophytadiene may be confirmed by the study of Saber et al., (2018) for their promising insecticidal activity and synergistic effects against *S. littoralis* larvae. On the other hand, the data of the field trials in both seasons showed obvious superiority in the total efficacy and persistency of βCD-mixed chlorfluazuron 5% EC to being alone against the 4th instar larvae of *S. littoralis*. Congruently, numerous field treatments of chlorfluazuron on the early instars of lepidoptrous larvae species belonging to the *Spodoptera* genus, resulted in significant inhibitory growth, and a reduction of population (Wang et al., 2021; Zhou et al., 2023; Eldessouky and Korish, 2023; Hasaneen and Attia, 2023).

Although a clear elevation occurred in the chlorophyll *a* content of the treated cotton leaves with the crude extract compared to the control in 2022, it seemed to be similar in all treatments in 2023. Chlorophyll *b* of the extract and its βCD mixture ascend the control in both seasons. Obvious increases in the total chlorophyll of the extract and its βCD blend transcended the other treatments in both seasons. Correlation between the increase of chlorophyll-leaf content and the phytochemical constitutes in the extract may be exhibited. Some of these phyto-compounds in the extract that may enhance chlorophyll-leaf content, were chlorogenic acid (Sheen et al., 1973), caffeic acid (Mehmood et al., 2021), rutin, gallic (singh et al., 2017), coumaric (Nkomo et al., 2019), ferulic (Zhu et al., 2018), Cinnamic acid (Araniti et al., 2018), Naringenin (Sharma 2021), Quercetin (Jańczak-Pieniążek et al., 2021), apigenin (Mekawy et al., 2018), and Kaempferol (Jan et al., 2022). On the other hand, chlorophyll *a*, *b* and total chlorophyll content in the treatment chlorfluazuron 5% EC and its βCD mixture were almost comparable to the control in both seasons. These data dovetailed with the demonstration of Na Zhu et al., (2021).

CONCLUSION

A potent declination in the nutritional indices of *S. littoralis* larvae in the laboratory was attained by adding 0.40 gm L^{-1} βCD to the crude extract under darkness and low light intensities (5000 and 10000 LUX). Likewise, βCD-mixed chlorfluazuron 5% EC did within relatively high light intensities (10000 and 20000 LUX). The leverages in toxicity and nutritional indices were directly proportional to the relatively high ALP levels in the βCD-mixed compounds. Along two sunny seasons on cotton plants, superiority in residual efficacy and persistency had been shown in βCD-mixed chlorfluazuron 5% EC, more than βCD-mixed extract that may prefer darkness and dim light, as previously realized in laboratory studies. Therefore, the addition of βCD had no observed changes in the residual toxicities and persistency of *A. spectabilis* extract. Not only was the spray application of the net and βCD-mixed extract safe, but also a conspicuous augmentation was recognized in the chlorophyll-leaf content. Meanwhile, the total contents of chlorophyll were not affected by net and βCD-mixed chlorfluazuron 5% EC. Ultimately, the recommendation of adding βCD to chlorfluazuron 5% EC could enhance the chemical control against *S. littoralis* and be more adequate throughout the relatively high intensity of light in the open field. Meanwhile, the application of *A. spectabilis* extract could be more adequate within low light intensity without the need to add βCD in the field. Thereby, the foliar application of *A. spectabilis* extract could be worthily applied within a few hours before the sunset.

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الشواهد التغذوية والكفاءة الإبادية لمستخلص الأكوكانثيرا ومبيدالكلورفلوازيرون ضد دودة ورق القطن برفقة تعديلات مخففة لتأثير شدة الضوء

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[2] قسم النبات الزراعي، كلية الزراعة (سبا باشا)، جامعة الإسكندرية، الإسكندرية ص. ب 21531، جمهورية مصر العربية

الملخص

تم دراسة الفعل المواجه للبيتاسيكلودكسترين لتأثيرات شدة الضوء على الشواهد التغذوية لمستخلص الأكوكانثيرا و الكلورفلوازيرون ضد العمر اليرقي الرابع لدودة ورق القطن. ووجد أن قيمة التركيز القاتل لـ10% للكلورفلوازيرون (0.92 مجم لتر$^{-1}$) قد تفوقت على مستخلص الأكوكانثيرا (0.56 مجم لتر$^{-1}$) وبالعكس فى التركيزات القاتلة لـ90%. وقد تبين أن المركبات المتوافر في تحليل الكروماتوجرافي السائل العالي الكفاءة في مستخلص الأكوكانثيرا هما حامضي الفيريليك (2082.49 ميكروجرام جرام$^{-1}$) والإلاجيك (804.74 ميكروجرام جرام$^{-1}$) أما في تحليل الكروماتوجرافي الغازي بمطياف الكتلة كان حامضي البالمتيك (39.89%) والسيز-فاكسينيك (14.05%). وهناك أفضلية نسبية لإضافة البيتاسيكلودكسترين بمقدار 0.40 جرام لتر$^{-1}$ إلى التركيزات تحت القاتلة للمركبات المختبرة بأقل فاعلية نسبية وأعلى دليل سمية. أظهرت شد الإضاءة عند الإظلام، 5000 و10000 لكس، أن مخلوط مستخلص الأكوكانثيرا-البيتاسيكلودكسترين قد أثر على الشواهد التغذوية لليرقات المعاملة وكذلك في مخلوط الكلورفلوازيرون-البيتاسيكلودكسترين عند شدة إضاءة 10000 و20000 لكس بإستثناء كلا من الوزن المكتسب الجاف ومعدل النمو النسبي. وأظهرت الدراسات الكيموحيوية أعلى نشاط لإنزيم الفوسفاتيز القلوي بشكل متكافئ عند تعرض اليرقات لـ48 ساعة لمخلوط البيتاسيكلودكسترين مع كلا من المستخلص والكلورفلوازيرون بقيم 125.73 و131.00 وحدة إنزيم لتر$^{-1}$، على التوالي. وجاءت التجارب النصف حقلية على مدار موسميين بأثر إبادي عالي ومتكافئ بين المستخلص منفردا ومخلوطه مع البيتاسيكلودكسترين مثلما في مخلوط الكلورفلوازيرون بأعلي آثر إبادي وفترة مثابرة معا. وقد تبين أن معاملات الرش الورقي لتلك المركبات كانت آمنة على محتوى الكلوروفيل فى الأوراق المعاملة فضلا عن ملاحظة تحسن ظاهري فيها بجميع معاملات مستخلص الأكوكانثيرا. وإجمالا، فإن إضافة البيتاسيكلودكسترين قد تكون محسنة لكفاءة الكلورفلوازيرون ضمن مكافحة دودة ورق القطن، بينما يفضل تطبيق مستخلص الأكوكانثيرا بمفرده تحت ظروف الإضاءة الطبيعية.