Insecticidal Activity, Growth Inhibitory and Biochemical Effects of Plant Lectins and *Bacillus thuringiensis var. kurstaki* against the Pink Bollworm, *Pectinophora gossypiella*

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ABSTRACT

Insecticidal activity and sublethal effects of white kidney bean and soybean lectins compared to *Bacillus thuringiensis var. kurstaki* were evaluated against pink bollworm (PBW), *Pectinophora gossypiella* (Saunders). The *in vivo* effects of these lectins on the total proteases and α-amylase activities of PBW were also carried out. Results revealed that, *B. thuringiensis* (LC_{50} = 61.7 and 36.1 µg / g diet) was approximately 4.7 and 4.8 times more toxic than kidney bean lectins (LC_{50} = 289.2 and 175.0 µg / g diet) and 2.2 times more toxic than soybean lectins (LC_{50} = 134.3 and 81.2 µg / g diet) after 5 and 6 days of treatment on the 2nd instar larvae. The LC_{25} equivalent concentration of *B. thuringiensis* and soybean lectins achieved the highest reduction of larval weight (11.9 and 13.0 mg / larva compared to 27.1 mg / larva in control) after 9 days of treatment. While kidney bean and soybean lectins at LC_{10} and LC_{25} increased the average time to pupation of PBW, *B. thuringiensis* at LC_{25} decreased the average time to pupation compared to control. All treatments significantly decreased the pupal mean weight and adult emergence rates compared to control. Kidney bean lectins, soybean lectins and *B. thuringiensis* at LC_{25} reduced the average number of eggs laid / female (fecundity) to 35.2, 31.5 and 34.5, respectively, compared to 89.0 in control. In addition, %egg hatch (fertility) significantly decreased to 53.8, 51.7 and 49.1% in kidney bean lectins, soybean lectins and *B. thuringiensis* LC_{25} treatments compared to 93.0% in control. Kidney bean and soybean lectins inhibited the activity of total proteases and α-amylase. Results of the present study suggest that kidney bean and soybean lectins can be used as suitable alternatives for *B. thuringiensis* in integrated management programs of pink bollworm.

Keywords: Pink bollworm; Plant lectins; *Bacillus thuringiensis*; sublethal effects.

INTRODUCTION

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), is a major pest of cotton in Egypt and worldwide. Larvae burrow into cotton bolls to feed on the seeds, cause serious boll damage resulting in great yield losses (Gutierrez *et al*, 2006; El-Aswad and Aly, 2007; Muhammad, 2017). The qualitatively and quantitatively cotton production depends mainly on the efficient control of PBW. The control of PBW relies mainly on chemical control (El-Feel *et al.*, 1993; Khan *et al*., 2007; Magdy *et al*., 2009; El-Deeb *et al.*, 2017) and recently *Bacillus thuringiensis*-transgenic cotton (Vaek *et al.*, 1987; Mohan *et al.*, 2016). However, chemical control is still adopted as the major tool for combating this serious insect pest.

The harmful effects of insecticides on the beneficial organisms, mammals and environment have been documented. In addition, the development of resistant strains of PBW against most of the commonly used insecticide groups (Mohamady, 2017) and *Bt*-transgenic cotton (Tabashnik *et al.*, 2009; Liu *et al.*, 2010) leads to the continuing need for new effective and economical insecticides for PBW management (Sexton *et al.*, 2007). During the last years a lot of attention was made in the study of entomotoxic proteins called plant lectins and their insecticidal properties (Chen, 2008; Vishwanathreddy *et al.*, 2014; Mohsen *et al.*, 2020) .

Plant lectins are a class of proteins of non-immune origin which used as a defense tool against pathogens which attack plants (Peumans and Van Damme, 1995). Lectins are carbohydrate-binding proteins, ubiquitous in nature, have been found to be promising against homopteran (Chakraborti *et al.*, 2009; Saha *et al.*, 2006), lepidopteran (Macedo *et al.*, 2007; Mohsen *et al.*, 2020), and coleopteran insects (Vandenborre *et al.*, 2011). Lectins are survival in the digestive system of herbivores under a wide range of pH that gives them a strong insecticidal potential (Vandenborre *et al.*, 2011). They act as anti-nutritive and/or toxic substances by binding to membrane glycosyl groups lining the digestive tract, leading to an array of harmful systemic reactions (Stoger *et al.*, 1999). Disruption of lipid, carbohydrate, and protein metabolism has negative effects on growth and development of insects (Dutta *et al.*, 2005).

Many plant and fungal lectins have been used as a part of integrated pest management systems for many agricultural pests. Lectins have been demonstrated to affect the insect survival, development and fecundity (Carlini and Grossi-de-Sá, 2002).

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In the present study, the insecticidal activity of white kidney bean and soybean lectins and *Bacillus thuringiensis var*. *kurstaki* against PBW was evaluated. The sublethal effects of these lectins on some biological parameters were also examined. The *in vivo* effects of lectins on the total proteases and α-amylase activities of PBW were also studied.

MATERIALS AND METHODS

Tested insect:

A laboratory strain of PBW was obtained from Plant Protection Research Institute, Cairo, Egypt and reared for several generations in the laboratory under conditions of 26 ± 2°C and 75 ± 5 RH as described by Rashad and Ammar (1985).

Tested *B. thuringiensis*:

Bacillus thuringiensis var. *kuristaki* as an active ingredient (1×10^7 spore / mg) was obtained from Organic for Biotechnology Company, Nubaria City, Egypt.

Extraction of lectins from kidney bean and soybean seeds:

Tested lectins were extracted from kidney bean and soybean seeds as described by Varrot *et al.*, (2013). Seed coats of kidney bean and soybean seeds were removed, 100 g of uncoated seeds were taken and soaked in phosphate buffer saline for 24 hrs. The uncoated seeds were grinded with a minimum volume of phosphate buffer saline and centrifuged at 5000 rpm for 10 min and the supernatant was used for lectins extraction. The supernatant was fractionally precipitated with ammonium sulfate at 60% saturation and centrifuged again. The pellets were dissolved in a minimal volume of water and dialyzed in glass distilled water at 4°C, and then lyophilized. Hemagglutinating activity test was used for detection of lectins in the extracted samples. Hemagglutination was performed on microtiter plate with the extracted lectins from white kidney bean and soybean using blood groups O. The hemagglutination happens when the lectins bind to carbohydrate from erythrocyte surface (Varrot *et al.*, 2013).

Bioassay and determination of sublethal concentrations:

A series of concentrations of lectin products and *B. thuringiensis* were prepared in water and mixed with the semi artificial diet (Rashad and Ammar, 1985). For each treatment, 30 newly molted 2nd instar larvae were divided on 15 glass cups (28 ml capacity). Each glass cup was provided by 5 g agar based PBW semi-artificial diet mixed with the tested lectins or *B. thuringiensis*. Control received semi-artificial diet only. Cups were covered with lids and maintained at 26 ± 2 °C. Cups were examined for larval mortality after 5 and 6 days of treatment and the larval mortality percentages were calculated. Mortality percentages were corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The LC_{50}, LC_{25} and LC_{10} concentrations, confidence limits and the slopes were calculated.

Sublethal effects of tested lectins and *B. thuringiensis* on some biological parameters of pink bollworm:

The semi- artificial diet was mixed with the determined LC_{10} and LC_{25} equivalent concentrations for tested lectins and *B. thuringiensis*. Eighty 2nd instar larvae in 4 replicates (2 larvae in each tube; 10 tubes per replicate) were used in each treatment and provided with the treated diet. Larvae were observed daily for pupation and emergence. Larval, pupal and adult durations were determined. Also, larval and pupal weights and percentages of adult emergence were recorded. Adults were sexed and placed in glass cups provided with a folded sheet paper as oviposition site. Two adult males were kept with one adult female to maximize the probability of successful mating. Laid eggs were counted and followed until hatching. The sublethal effects of tested lectins and *B. thuringiensis* on fecundity (total number of eggs / female) and fertility (hatchability percentages of eggs) were determined. Initially, 12 mating were planned for each insecticide treatment as well as control. The mating cups were checked daily and eggs were removed until female death. The total number of eggs /female for each mating and hatched eggs percentages were evaluated.

Effect of tested lectins and *B. thuringiensis* on the total proteases activity:

Newly molted 2nd instar larvae were fed diet containing the LC_{25} and LC_{10} equivalent concentrations of kidney bean lectins, soybean lectins and *B. thuringiensis* and surviving larvae after 6 days were collected. Untreated larvae were used as control. One gram of total larvae was homogenized in 5 ml 100 mM Tris-HCl buffer pH 7 using Polytron Kinemetica on ice. The homogenate was centrifuged at 4000 rpm for 15 min at 4ºC using IEC-CRU 5000 cooling centrifuge. The supernatant was used for total proteolytic activity estimation. Total proteolytic activity was measured using asocasein as a substrate according to (Olga *et al.*, 2002; Mohen and Gujar, 2003). The homogenate was incubated in a total volume 60 µl of assay buffer (100 mM Tris-HCl pH 8) for 20 min at 37 ºC before addition of 200 µl of 2% azocasein (w/v in assay buffer). The reaction was allowed to proceed for 180 min at 37 ºC, and then stopped by addition of 300 µl cold 10% trichloroacetic acid (TCA). The reaction mixture was centrifuged at 3000 rpm for 10 min IEC-CRU 5000 cooling centrifuge. Excess acidity was neutralized by

adding 10 µl NaOH (10 N) to the reaction mixture and absorbance was measured at 440 nm using Sequoia-Turner Model 340 spectrophotometer. An assay mixture without enzyme was used as the blank.

Effect of tested lectins and *B. thuringiensis* on **the amylase activity:**

Surviving larvae after 6 days of treatment with LC_{25} and LC_{10} equivalent concentrations of kidney bean lectins, soybean lectins and *B. thuringiensis* were collected. Untreated larvae were used as control. One gram of total larvae was homogenized in 5 ml glass distilled water using Polytron Kinemetica on ice. The homogenate was centrifuged at 15000 rpm for 15 min at 4ºC using IEC-CRU 5000 cooling centrifuge. The supernatant was used for α-amylase activity assay. Alpha-amylase activity was assayed by the dinitrosalicilic acid (DNS) according to Bernfeld, (1955), using 1% soluble starch solution as substrate. An assay mixture without enzyme was used as the blank. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35ºC.

Protein measurements:

Lowry *et al*. (1951) method was used to determine protein content in the supernatant comparing to the standard curve of BSA.

Statistical analysis:

Estimates of LC_{50} and their 95% confidence limits were obtained using the POLO program (Russell *et al.*, 1977) based on Finney (1971). The criterion used to estimate the differences between LC_{50} values was non-overlap of their 95% confidence intervals. All other quantitative estimations were replicated four times and the values are expressed as mean ± standard error. The SAS 8.0 software was used for analysis of the data obtained from each experiment and the means were tested for significant differences using analysis of variance (ANOVA) test (LSD at $P < 0.05$) (SAS Statistical software, 1999).

RESULTS

Toxicity of tested lectins and *B. thuringiensis* against pink bollworm larvae:

The median lethal concentration, LC_{10}, LC_{25} and their confidence limits of tested lectins and *B. thuringiensis* against the 2nd instar larvae of pink bollworm are presented in Table 1. *Bacillus thuringiensis* (LC_{50} = 61.7 and 36.1 µg / g diet) was approximately 4.7 and 4.8 times more toxic than kidney bean lectins (LC_{50} = 289.2 and 175.0 µg / g diet) after 5 and 6 days of treatment, respectively. *Bacillus thuringiensis* was approximately 2.2 times more toxic than soybean lectins (LC_{50} = 134.3 and 81.2 µg / g diet) after 5 and 6 days of treatment, respectively. Soybean lectins was approximately 2.2 times more toxic than kidney bean lectins after 5 and 6 days of treatment, respectively. The LC_{10} and LC_{25} values after 6 days of treatment were 4.9 and 12.6 µg / g diet for *B. thuringiensis*, 13.0 and 31.0 µg / g diet for soybean lectins, and 31.6 and 71.1 µg / g diet for kidney bean lectins (Table 1).

Table 1. Toxicity of kidney bean lectins, soybean lectins and *B. thuringiensis* against 2nd instar larvae of pink bollworm

Treatment	Time after exposure (days)	LC_{50} (µg/g diet) (95% CL)	LC_{25} (µg/g diet) (95% CL)	LC_{10} (µg/g diet) (95% CL)	Slope ± SE
Kidney bean lectins	5	289.2 (240.0 – 353.2)	101.6 (77.0 – 126.5)	39.6 (25.3 – 54.9)	1.49 ± 0.14
	6	175.0 (147.7 – 205.8)	71.1 (54.0 – 88.0)	31.6 (20.8 – 43.0)	1.73 ± 0.15
Soybean lectins	5	134.3 (112.1 – 163.1)	48.7 (37.1 – 60.2)	19.5 (12.6 – 26.9)	1.53 ± 0.14
	6	81.2 (67.7 – 96.5)	31.0 (22.8 – 39.2)	13.0 (8.1 – 18.3)	1.61 ± 0.15
B. thuringiensis	5	61.7 (50.9 – 75.8)	20.6 (15.1– 26.1)	7.7 (4.6 – 11.1)	1.42 ± 0.14
	6	36.1 (29.5 – 43.5)	12.6 (8.8 – 16.4)	4.9 (2.8 – 7.2)	1.47 ± 0.14

Sublethal effects of tested lectins and *B. thuringiensis* on some biological parameters of pink bollworm:

Sublethal effects of tested lectins and *B. thuringiensis* on some biological parameters of pink bollworm are presented in Tables 2, 3 and 4. All treatments significantly decreased the average weight of treated pink bollworm larvae compared to control after 6 and 9 days of treatment. *B. thuringiensis* and soybean lectins at LC_{25} (12.6 and 31.0 mg / g diet) achieved the highest reduction of larval weight where the larval weight averages were 11.9 and 13.0 mg / larva compared to 27.1 mg / larva in control after 9 days of treatment. The larval weight averages were 17.8, 17.5 and 18.3 mg / larva when larvae were treated with LC_{10} of *B. thuringiensis*, soybean lectins and kidney bean lectins after 9 days of treatment, respectively (Table 2).

Moreover, kidney bean and soybean lectins at LC_{10} and LC_{25} increased the average time to pupation of pink bollworm. However, *B. thuringiensis* at LC_{25} decreased the average time to pupation compared to control. These times were 17.7 and 19.5 days for kidney bean lectins and 18.0 and 20.1 days for soybean lectins at LC_{10} and LC_{25}, respectively, compared to 13.5 days in control. On the other hand, *B. thuringiensis* at LC_{25} reduced the time to pupation of pink bollworm to 11.3 days (Table 2). Sublethal concentrations of tested lectins and *B. thuringiensis* had a considerable effect on pupation percentage of pink bollworm. Kidney bean and soybean lectins at LC_{25} gave the lowest pupation percentages 34.7 and 37.2% compared to 93.4% in control. Also, significant decrease in pupation percentage was achieved with the LC_{10} of tested lectins and *B. thuringiensis* at LC_{10} and LC_{25} (Table 2).

As shown in Table 3, all treatments significantly reduced the pupal mean weight compared to control treatment. Soybean lectins and *B. thuringiensis* at LC_{25} were the most effective where the pupal mean weight was 14.5 and 13.2 mg / pupa compared to 27.2 mg / pupa in control. Kidney bean lectins, soybean lectins and *B. thuringiensis* at LC_{10} achieved less effect with pupal weight averages 19.3, 18.7 and 17.8 mg / pupa, respectively. However, pupal duration did not changed significantly in all treatments compared to control (Table 3).

Table 2. Effect of kidney bean lectins, soybean lectins and *B. thuringiensis* when applied to the 2nd instar larvae of pink bollworm on the larval weight, larval duration and %pupation

Treatment	Conc*. (µg/g diet)	Mean weight (mg/larva) (± SE) after different days of treatment			Larval duration (days) ± SE	Pupation (%) ± SE
		3	6	9		
Control	-	6.7 ± 0.5a	18.4 ± 1.1a	27.1 ± 1.5a	13.5 ± 1.2c	93.4 ± 4.2a
Kidney bean lectins	31.6	4.9 ± 0.6ab	13.4 ± 1.0b	18.3 ± 0.8b	17.7 ± 0.7b	63.2 ± 2.5c
	71.1	4.3 ± 0.3b	10.5 ± 0.6c	14.5± 0.7c	19.5 ± 1.5a	34.7 ± 2.3e
Soybean lectins	13.0	5.0 ± 0.4ab	14.2 ± 0.7b	17.5 ± 0.9b	18.0 ± 1.6b	69.1 ± 3.7b
	31.0	4.7 ± 0.2ab	10.3 ± 0.3c	13.0 ± 0.8cd	20.1 ± 0.8a	37.2 ± 1.9e
B. thuringiensis	4.9	5.2 ± 0.6ab	13.2 ± 0.3b	17.8 ± 0.5b	13.1 ± 0.5c	68.1 ± 2.4b
	12.6	4.9 ± 0.2ab	9.8 ± 0.4c	11.9 ± 0.7d	11.3 ± 1.8d	44.8 ± 2.5d

*These concentrations are equivalent to the LC_{10} and LC_{25} of tested lectins and *B. thuringiensis*. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Table 3. Effect of kidney bean lectins, soybean lectins and *B. thuringiensis* when applied to the 2nd instar larvae of pink bollworm on the pupal weight, pupal duration and %adult emergence

Treatment	Conc*. (µg/g diet)	Pupal mean weight (mg/pupa) ± SE	Pupal duration (days) ± SE	%Adult emergence ± SE
Control	-	27.2 ± 1.4a	6.4 ± 0.5a	91.0 ± 3.5a
Kidney bean lectins	31.6	19.3 ± 0.9b	7.3 ± 0.4a	55.4 ± 2.1b
	71.1	15.4 ± 0.6c	6.9 ± 0.3a	37.2 ± 1.5d
Soybean lectins	13.0	18.7 ± 0.5b	7.0 ± 0.4a	46.3 ± 2.1c
	31.0	14.5 ± 0.4cd	6.7 ± 0.5a	29.4 ± 1.7e
B. thuringiensis	4.9	17.8 ± 0.3b	6.8 ± 0.3a	53.2 ± 2.4b
	12.6	13.2 ± 0.6d	6.3 ± 0.2a	30.1 ± 1.4e

*These concentrations are equivalent to the LC_{10} and LC_{25} of tested lectins and *B. thuringiensis*. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Reduction in adult emergence rates were significantly achieved by all treatments. Soybean lectins and *B. thuringiensis* at LC_{25} achieved the higher reduction in the adult emergence rates, where it was 29.4 and 30.1%, respectively, compared to 91.0% in control. The adult emergence rate was 55.4 and 53.2% at the LC_{10} kidney bean lectins and *B. thuringiensis* treatments, respectively (Table 3).

Effect of kidney bean lectins, soybean lectins and *B. thuringiensis* when applied on the 2nd instar larvae of pink bollworm on adult fecundity, fertility and longevity is presented in Table 4. Kidney bean lectins, soybean lectins and *B. thuringiensis* at LC_{25} had the highest effect on the adult fecundity where the average number of eggs laid / female were 35.2, 31.5 and 34.5, respectively, compared to 89.0 in control. Also, tested lectins and *B. thuringiensis* at the LC_{10} significantly decreased the adult fecundity where the average number of eggs laid / female were 58.7, 57.4 and 56.6, respectively. Fertility (percentages of egg hatch) was significantly decreased as a result of all treatments. The LC_{25} values of all treatments were the most effective compared to the LC_{10} values. The % egg hatch was 53.8, 51.7 and 49.1% in kidney bean lectins, soybean lectins and *B. thuringiensis* LC_{25} treatments compared to 93.0% in control. Adult longevity was significantly decreased to 7.1 and 6.2 days in LC_{10} and LC_{25} kidney bean lectins treatments, 6.8 and 6.4 days in LC_{10} and LC_{25} soybean lectins treatments and 7.2 and 6.8 days in LC_{10} and LC_{25} *B. thuringiensis* treatments compared to 9.5 days in control (Table 4).

Effect of kidney bean lectins, soybean lectins and *B. thuringiensis* on the pink bollworm total proteases and α-amylase activity:

The specific activity of total proteases (OD_{440} / mg protein / hr) in pink bollworm 2nd instar larvae after 6 days of treatment with LC_{10} and LC_{25} of kidney bean and soybean lectins is significantly decreased compared to control (Table 5). The highest enzyme activity inhibition was occurred by kidney bean and soybean lectins at LC_{25}, where the inhibition percentage was 54.0 and 51.6%, respectively.

Table 4. Effect of kidney bean lectins, soybean lectins and *B. thuringiensis* when applied to the 2nd instar larvae of pink bollworm on adult fecundity, fertility and longevity

Treatment	Conc*. (µg/g diet)	Fecundity (No. eggs laid / female) ± SE	fertility (%egg hatch) ± SE	Adult longevity (days) ± SE
Control	-	89.0 ± 2.5a	93.0 ± 3.1a	9.5 ± 0.3a
Kidney bean lectins	31.6	58.7 ± 2.2b	62.5 ± 3.5b	7.1 ± 0.2b
	71.1	35.2 ± 1.5c	53.8 ± 2.2c	6.2 ± 0.4b
Soybean lectins	13.0	57.4 ± 2.1b	64.6 ± 3.8b	6.8 ± 0.5b
	31.0	31.5 ± 1.9c	51.7 ± 2.6c	6.4 ± 0.2b
B. thuringiensis	4.9	56.6 ± 2.7b	66.4 ± 2.9b	7.2 ± 0.5b
	12.6	34.5 ± 1.3c	49.1 ± 1.2c	6.8 ± 0.3b

*These concentrations are equivalent to the LC_{10} and LC_{25} of tested lectins and *B. thuringiensis*. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Table 5. *In vivo* effect of kidney bean lectins, soybean lectins and *B. thuringiensis* on the 2nd instar larvae of pink bollworm total proteases activity after 6 days of treatment

Treatment	Conc*. (µg/g diet)	Specific activity (OD_{440}/mg protein/hr) ± SE	Activity (%control)	%Inhibition
Control	-	0.213 ± 0.004a	100 ± 1.9	0.0
Kidney bean lectins	31.6	0.127 ± 0.003c	59.6 ± 1.4	40.4
	71.1	0.098 ± 0.004d	46.0 ± 1.9	54.0
Soybean lectins	13.0	0.149 ± 0.006b	70.0 ± 2.8	30.0
	31.0	0.103 ± 0.004d	48.4 ± 1.9	51.6
B. thuringiensis	4.9	0.203 ± 0.007a	95.3 ± 3.3	4.7
	12.6	0.205 ± 0.003a	96.2 ± 1.4	3.8

*These concentrations are equivalent to the LC_{10} and LC_{25} of tested lectins and *B. thuringiensis*. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

On the other hand, total proteases activity did not differ significantly in *B. thuringiensis* treatments compared to control (Table 5). In addition, tested lectins and *B. thuringiensis* at LC_{10} and LC_{25} exhibited remarkable inhibitory effect on α-amylase enzyme activity of pink bollworm larvae (Table 6). Kidney bean lectins at LC_{10} and LC_{25} (achieved 55.9 and 61.9% inhibition) was significantly the most effective inhibitor of α-amylase activity, followed by soybean lectins and *B. thuringiensis* (Table 6).

DISCUSSION

The production of cotton fibers depends mainly upon the efficient control of pink bollworm because it is the most destructive insect pests infested cotton. The evolution of resistance in this insect to insecticides (Kristensen, 2005; Mohamady, 2017) and *B. thuringiensis* transgenic cotton (Dhurua and Gujar, 2011; Wang *et al.*, 2016; Akhtar *et al.*, 2018) requires the use of different strategies for management of this insect. In the present study toxicity of lectins from kidney bean and soybean compared to *B. thuringiensis* against pink bollworm larvae were tested. Results revealed that, *Bacillus thuringiensis* is more toxic than soybean lectins which is more toxic than kidney bean lectins. Lectins from different sources exerted an insecticidal activity against many phytophagous insects such as caterpillars, tobacco hornworm, cotton leaf worm and beetles (Vandenborre *et al.*, 2011). Sadeghi *et al.*, (2009) reported a significant larval mortality of *S. littoralis* fed on the transgenic plants expressing *Allium porrum* L. lectins. In addition, soybean and kidney been lectins achieved high mortality of the 2nd instar larvae of *S. littoralis* (Mohsen *et al.*, 2020).

Annona coriacea lectins showed high insecticidal activity against *Anagast kuehniella* and *Corcyra cephalonica* larvae when it was incorporated into an artificial diet (Coelho *et al*., 2007). Both the heterodimeric and homodimeric garlic lectins exhibited high mortality when fed to pea aphids in artificial diet (Fitches *et al*., 2008). Sá *et al*., (2009) and Napoleão *et al*., (2012) recorded a high insecticidal activity of lectins isolated from *Myracrodruon urundeuva* bark, heartwood and *Myracrodruon urundeuva* against *Aedes aegypti* larvae. Furthermore, *Sclerotium rolfsii* lectins showed a remarkable mortality rate of *Spodoptera litura* larvae (Vishwanathreddy *et al.*, 2014).

Results of the present study also revealed that, kidney bean lectins, soybean lectins and *B. thuringiensis* significantly decreased the average weight of pink bollworm larvae and pupae. In addition, the percent pupation, adult emergence, fecundity and fertility were significantly suppressed in pink bollworm larvae fed diet contains kidney bean lectins, soybean lectins and *B. thuringiensis*. In previous studies, bioassays using detached leaves from transgenic tobacco plants expressing the *Allium porrum* L. lectins reduced the weight gain of 2nd instar larvae of *S. littoralis*. In addition the lectins retarded the development of the larvae and metamorphosis, reduced pupal weight and increased mortality rate (Sadeghi *et al.*, 2009). Mohsen *et al.*, (2020) also reported a significant decrease in the *S. littoralis* larval and pupal weights, percent pupation, adult emergence, fecundity and fertility. Similarly, snowdrop lectin affected the growth and development of beet armyworm, *Spodoptera exigua* (Naghdi and Bandani, 2013), while it reduced survival, biomass and caused longer instar durations in *Lacanobia oleracea* (Fitches *et al.*, 1997; Wakefield *et al.*, 2006).

Table 6. *In vivo* effect of kidney bean lectins, soybean lectins and *B. thuringiensis* on the 2nd instar larvae of pink bollworm α-amylase activity after 6 days of treatment

Treatment	Conc*. (µg/g diet)	Specific activity (µmol maltose/min/mg protein) ± SE	Activity (%control)	% Inhibition
Control	-	1.37 ± 0.03a	100 ± 2.2	0.0
Kidney bean lectins	31.6	0.604 ± 0.02e	44.1 ± 1.5	55.9
	71.1	0.522 ± 0.02g	38.1 ± 1.5	61.9
Soybean lectins	13.0	0.798 ± 0.01d	58.2 ± 0.7	41.8
	31.0	0.582 ± 0.02f	42.5 ± 1.5	57.5
B. thuringiensis	4.9	0.945 ± 0.03b	69.0 ± 2.2	31.0
	12.6	0.872 ± 0.01c	63.6 ± 0.7	36.4

*These concentrations are equivalent to the LC_{10} and LC_{25} of tested lectins and *B. thuringiensis*. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

The remarkable inhibitory effects of kidney bean and soybean lectins on total proteases and α-amylase activities of pink bollworm larvae in the present study also explain their negative effects on the growth parameters. Plant lectins may be interact with glycosylated moieties or other regions of the enzyme molecules (Agra-Neto *et al.*, 2014). It is documented that, many plant lectins have been shown to interact with α-amylase (Santos *et al.*, 2020), α-and β-glucosidases (Lagarda-Diaz *et al.*, 2017) and trypsin-like enzymes (Oliveira *et al.*, 2020).

Histological analysis of lectin-treated midgut revealed disrupted and diffused secretory cells surrounding the gut lumen in larvae of *Hyblaea puera* and *Probergrothius sanguinolens.* The damage noticed in the gut of both insects suggests the interference of lectins with the secretory layer of the gut lumen, thereby revealing that this lectins probably affected the insect secretory mechanism and hampered food uptake in larvae of the tested insect pests (George *et al.*, 2018). Findings in the present study suggest that kidney bean and soybean lectins might be suitable protein for integrating into plant genomes for the controlling pink bollworm.

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الملخص العربي

النشاط الإبادى على الحشرات والتأثيرات المثبطة للنمو والتأثيرات البيوكيميائية للكتينات النباتية وبكتيريا الباسيلس ثيورينجينزز ضد دودة اللوز القرنفلية

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تم دراسة السمية الحشرية والتأثيرات التحت مميتة للكتينات المستخلصة من الفاصوليا البيضاء وفول الصويا مقارنة ببكتيريا الباسيلس ثيورينجينزز على دودة اللوز القرنفلية. كذلك تم دراسة تأثير اللكتينات على نشاط الإنزيمات المحللة للبروتينات وإنزيمات الألفا–أميليز. أظهرت النتائج أن سمية بكتيريا الباسيلس ثيورينجينزز (التركيز اللازم لقتل ٥٠٪ من الحشرات المعاملة = ٦١.٧ و٣٦.١ ميكروجرام/ جرام بيئة) تقريبا ٤.٧ و٤.٨ مره ضعف سمية اللكتين المستخلص من الفاصوليا البيضاء (التركيز اللازم لقتل٥٠٪ من الحشرات المعاملة= ٢٨٩.٢ و١٧٥ ميكروجرام / جرام بيئة) و٢.٢ مرة ضعف سمية اللكتين المستخلص من فول الصويا (التركيز اللازم لقتل ٥٠٪ من الحشرات المعاملة= ١٣٤.٣ و٨١.٢ ميكروجرام / جرام بيئة) بعد ٥ و٦ أيام من المعاملة. التركيز المكافئ للتركيز اللازم لموت ٢٥٪ من الحشرات المعاملة لكل من بكتيريا الباسيلس ثيورينجينزز واللكتين المستخلص من فول الصويا كانت الأعلى فى خفض أوزان اليرقات المعاملة (١١.٩ و١٣ مجم/ يرقة مقارنة ب ٢٧.١ مجم / يرقة فى الكنترول) بعد ٩ أيام من المعاملة. بينما اللكتينات المستخلصة من الفاصوليا البيضاء وفول الصويا أدت إلى زيادة الوقت اللازم لتحول اليرقات إلى عذارى لدودة اللوز القرنفلية فإن بكتيريا الباسيلس ثيورينجينزز أدت إلى خفض هذا الوقت. كل المعاملات المختبرة أدت إلى خفض متوسط وزن العذارى ونسبة خروج الحشرات الكاملة مقارنة بالكنترول. اللكتينات المستخلصة من الفاصوليا البيضاء وفول الصويا وبكتيريا الباسيلس ثيورينجينزز خفضت متوسط عدد البيض / أنثى إلى ٣٥.٢ و٣١.٥ و٣٤.٥ على الترتيب مقارنة ب ٨٩ فى الكنترول. كما أن هذه المعاملات خفضت نسبة خصوبة البيض إلى ٥٣.٨ و٥١.٧ و٤٩.١٪ على الترتيب مقارنة ب ٩٣٪ فى الكنترول. كما وجد أن اللكتينات المستخلصة من الفاصوليا البيضاء وفول الصويا أدت إلى تثبيط نشاط الإنزيمات المحللة للبروتينات وإنزيمات الألفا–أميليز. من هذه النتائج يمكن القول أن اللكتينات المستخلصة من الفاصوليا البيضاء وفول الصويا يمكن أن تكون بديل مناسب لبكتيريا الباسيلس ثيورينجينزز من خلال إدخالها فى جينات نباتات القطن لمكافحة دودة اللوز القرنفلية.