Entomotoxic Properties of White Kidney Bean and Soybean Lectins and their Effects against Two Digestive Enzymes of the Spiny Bollworm, *Earias insulana* (Boisd.)

Mervat H. A. Metayi[1], Hend S. El-Tahawe[2], Alaa M. Khorchid[2]

ABSTRACT

Plant lectin, a heterogeneous group of carbohydrate-binding proteins, is a direct defensive mechanism in plants against the attacking insects. Lectins from the leguminous plants *Glycine max* (GML) and *Phaseoulus vulgaries* (PhVL) were tested for their entomotoxic and growth inhibitory effects against the spiny bollworm (SBW), *Earias insulana* Boisd. The impact of the examined lectins on the two digestive enzymes of SBW, α-amylase and total proteases, was also investigated. Bioassay studies conducted on second-instar larvae over five and six days showed that GML (LC_{50} values of 72.22 and 33.45 µg/gm diet) was more hazardous than PhVL (LC_{50} = 299.05 and 182.91 µg/gm diet). GML and PhVL at LC_{25} equivalent concentrations (8.97 and 34.43 µg/gm diet) significantly ($p < 0.05$) reduced the larval weight to 24.9 and 27.4 mg / larva compared with 55.3 mg / larva in control after 9 days of treatment. The average time for SBW larvae to pupate increased when GML and PhVL were added at LC_{10} and LC_{25} in comparison to the control. In addition, the tested lectins significantly ($p < 0.05$) reduced pupal mean weight, pupation, adult emergence, fecundity and fertility. Tested lectins demonstrated significant inhibition of α-amylase and total protease enzyme activity in larvae of SBWs fed on diets containing concentrations comparable to LC_{10} and LC_{25}. These findings imply that GML and PhVL are appropriate proteins to add to the cotton plant's DNA in order to control SBW.

Keywords: Spiny bollworm; Plant lectins; Fertility; Entomotoxic; Sublethal effects.

INTRODUCTION

Spiny bollworm (SBW), *Earias insulana* Boisd. (Noctuidae: Lepidoptera) is a very destructive insect causing great losses of cotton in Egypt and worldwide (Khurana & Verma, 1990 and Mansour, 2004). Larvae attack terminals of young cotton resulting in death of the growing buds and lead to development of lateral shoots and branches. With the progress of cotton growth, larvae of SBW feed on the squares and the bolls, destroying fiber and consuming seeds causing significant damage in cotton crop (Cayrol, 1972 and El-Deeb *et al.*, 2017). Cotton fiber production depends significantly on the efficient control of SBW in many areas.

Pyrethroids (Ramesh .*et al* 2010), organophosphorus, carbamate (Abou-Taleb and El-Aswad, 2010) and other insecticide groups (Jadhav *et al.*, 2009) are commonly used for chemical control of SBW. The environmental pollution (El-Sebae *et al.*, 1993) and development of insecticide resistance in SBW against these insecticide groups (Osman *et al.*, 1991 and Al-Beltagy *et al.*, 2001) as a result, there is a continual demand for effective and cost-effective pesticides for crop protection strategies. Lectins, widely-distributed plant proteins, have been studied because of their entomotoxic and growth inhibitory properties against number of insect pests (Afolabi-Balogun *et al.*, 2012).

Lectins attach to membrane glycosyl groups lining the digestive tract, causing a range of detrimental systemic responses that lead to anti-nutritive and toxic consequences (Stoger *et al.*, 1999). Insect growth is negatively impacted by disruptions in the metabolism of fat, carbohydrates, and proteins (Dutta *et al.*, 2005).

Lectins belong to a heterogeneous group of highly specific and reversible carbohydrate-binding proteins, with non-enzymatic and non-immune origin (Lis and Sharon, 2003). They exist in many plants, animals, microbes and fungi (Peumans *et al.*, 2001). Lectins have been shown to have entomotoxic effects on homopteran species (Saha *et al.*, 2006 and Chakraborti *et al.*, 2009), dipteran (Kaur *et al.*, 2006), lepidopteran (Macedo *et al.*, 2007; Hamshou *et al.*, 2010 and Mohsen *et al.*, 2020), and coleopteran insects (Macedo *et al.*, 2002 and Vandenborre *et al.*, 2011). Furthermore, it has been established that lectins influence the fecundity, fertility, development, and survival of insects (Carlini and Grossi-de-Sá, 2002).

Legume seeds contain high levels of lectins. So, they attracted many plant protection researchers and were known to be one of the best studied groups of lectins (Mohsen *et al.*, 2020; Cavada *et al.*, 2020 and Cavada *et al.*, 2021). White kidney bean, *Phaseoulus vulgaries*

DOI: 10.21608/asejaiqjsae.2024.337001

[1]Plant Protection Research Institute, ARC, Sabahia Station, Alexandria, Egypt;

[2]Plant Protection Research Institute, ARC, Giza, Egypt.

*Email of corresponding author: aboelhamedmervat@gmail.com

Received, December 15, 2023, Accepted, January 17, 2024.

lectins (PhVL), and soybean, *Glycine max* lectins (GML) were known to have fungicidal (Mohsen *et al.*, 2018) and insecticidal activities (Mohsen *et al.*, 2020 and El-Deeb, 2021). The present study investigated the entomotoxic and insect developmental effects of PhVL and GML and their effects on two digestive enzymes of SBW.

MATERIALS AND METHODS

Tested insect:

A lab strain of SBW was acquired from the Plant Protection Research Institute (PPRI), Egypt. Several generations of the tested insect were reared in the lab at 27 ± 2 °C and a humidity of 70 ± 5. According to Rashad and Ammar's description (1985) and modified by Metayi *et al.* (2016) where white kidney bean was replaced by broad bean. Larvae were fed semi-artificial diet containing agar (12 gm), yeast (30 gm), broad bean (150 gm), ascorbic acid (3 gm), methyl-*p*-hydroxy benzoate (nipagin) (3 gm), formaldehyde 40% (1 ml) and water (700 ml), while Adults were fed 10% sugar solution.

Extraction and purification of PhVL and GML:

Seeds of soybean and white kidney bean as lectin sources were obtained from Crop Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Seed coats were removed and 100 g of uncoated seeds were soaked in 10 mM sodium phosphate buffer, pH 7 containing 150 mM sodium chloride for 24 hours. The soaked seeds were ground with a minimal volume of buffer, centrifuged for 10 minutes at 5000 rpm, and the supernatant was utilized to extract lectins. Once more, centrifugation was performed after the supernatant was fractionally precipitated using 60% ammonium sulfate. The pellets dissolved in a small amount of glass distilled water. The pellets were dialyzed at 4 °C in glass distilled water, and then they were lyophilized. GML and PhVL detection was performed using a blood group O hemagglutination test in microtiter plate (Varrot *et al.*, 2013). Hemagglutination occurs when lectins attach themselves to the surface of erythrocytes that contain carbohydrates.

Toxicity of tested lectins and estimation of sub-lethal dose:

A range of lectin concentrations were created in water and used with the semi-artificial diet for rearing. For each treatment, forty freshly second instar larvae were distributed into 20 glass cups (28 ml size). Each cup contained two grams of agar-based SBW semi-artificial diet supplemented with the tested lectins. The control group only received a semi-artificial diet. Cups with lids were kept at 27 ± 2 °C and observed after five and six days. The mortality percentages were computed and adjusted in accordance with Abbott (1925), and then probit analysis was performed (Finney, 1971). Calculations were made for the LC_{10}, LC_{25}, and LC_{50} concentrations.

Developmental effects of tested lectins:

The LC_{10} and LC_{25} corresponding concentrations for the investigated lectins were combined with a semi-artificial food for spiny bollworms. In each treatment, two hundred larvae in their second instar (four repetitions; two larvae per tube; 25 tubes per replicate) were fed the treated diet. The durations of larvae, pupae, and adults were established. Furthermore, percentages of pupation and adult emergence, as well as mean weights of larvae and pupae, were noted. Adults were sexed; one male was kept with one female in glass cups and provided with a folded sheet of paper serving as the oviposition site and counting and monitoring of laid eggs till they hatch. The sublethal effects of the investigated lectins on fecundity and fertility were assessed. Ten matings were planned for each lectin treatment and control. Every day, the mating cups were inspected, and the eggs were taken out until the female died. The total number of eggs produced by each female during each mating, as well as the percentage of hatched eggs, were assessed.

Impact of examined lectins on total proteases activity *in vivo*:

The LC_{10} and LC_{25} equivalent concentrations of GML and PhVL were given to freshly second instar larvae of SBW, and after six days, the surviving larvae were collected. The control group consisted of untreated larvae. The whole larvae was homogenized in 100 mM Tris-HCl buffer pH 7 (1:5 w/v) using homogenizer (Polytron Kinematica) on ice. The cooling centrifuge (IEC-CRU 5000) was used to centrifuge the homogenate for 15 minutes at 4 °C at 4000 rpm. The total proteolytic activity in the supernatant was determined using azocasein as a substrate, as stated by Olga *et al.* (2002) and Mohen and Gujar (2003). After incubating the homogenate in 60 µl of assay buffer (100 mM Tris-HCl pH 8) for 20 minutes at 37 ºC, 200 µl of 2% azocasein (w/v in assay buffer) were added. The mixture was kept at 37°C for 180 minutes, then 300 µl of cold 10% trichloroacetic acid (TCA) was added to halt the reaction. The mixture was subjected to a 10-minute centrifugation at 3000 rpm. 10 µl of NaOH (10 N) was added to the reaction mixture to neutralize the excess acidity. With a spectrophotometer (Sequoia-Turner, Model 340), absorbance was measured at 440 nm. The blank sample included the assay mixture in the absence of the enzyme.

Impact of examined lectins on the activity of α-amylase *in vivo*:

The larvae that survived treatment for six days with LC_{10} and LC_{25} concentrations of GML and PhVL were collected. The control group consisted of untreated larvae. Using Polytron Kinemetica homogenizer on ice, the whole larval population was homogenized in distilled water (1:5 w/v). Centrifuging the homogenate for 20 minutes at 4 ºC at 15,000 rpm was done. Alpha-amylase activity was measured in supernatant using soluble starch as a substrate according to Bernfeld (1955). An assay mixture 20 µL of the enzyme source, 80 µL of phosphate buffer (20 mM, pH 7.1) and 40 µL of soluble starch (1%) was incubated at 35 °C for 30 min. The reaction was stopped by addition of 100 µL dinitro salicylic acid. Reaction mixture has been set in boiling water for 10 min and then absorbance was measured at 545 nm. The blank sample was containing the assay mixture without the enzyme. Alpha-amylase activity (One unit) is the quantity of enzyme needed to generate one milligram of maltose at 35 degrees Celsius in thirty minutes.

Measurements of protein:

The protein content of the supernatant was calculated by comparing it to the BSA standard curve (Lowery *et al.*, 1951).

Statistical analysis:

The POLO software (Russell *et al.*, 1977) was used to determine median lethal concentration values (LC_{50}) and associated 95% confidence limits, which were based on Finney (1971). The difference between LC_{50} values was estimated using the non-overlap of their 95% confidence limits criteria. All quantitative estimations were duplicated four times, with results represented as mean ± standard error. SAS 8.0 software was used for statistical analysis of data from each experiment. Means were examined for significant differences using the analysis of variance (ANOVA) test (LSD at $P < 0.05$) (SAS Statistical Software, 1999).

RESULTS

Toxicity of GML and PhVL against SBW 2nd larvae:

Toxicity of GML and PhVL against SBW 2nd instar larvae after 5 and 6 days of treatment present in Table (1). GML (LC_{50} = 72.22 and 33.45 µg/gm diet) was roughly 4.14 and 5.47 times more toxic than PhVL (LC_{50} = 299.05 and 182.91 µg/gm diet) following exposure for five and six days, respectively. The LC_{10} and LC_{25} values are 2.74 and 8.97 µg/gm diet for GML, 7.66 and 34.43 µg/gm diet for PhVL after 6 days of treatment.

2. Effect of GML and PhVL on development of SBW:

Effects of LC_{10} and LC_{25} equivalent concentrations of GML and PhVL on larval mean weight, larval duration and %pupation of SBW are presented in Table (2).

The average weight of treated larvae was decreased significantly ($P < 0.05$) compared to the control during the observation period. The concentration equivalent to LC_{25} of both lectins showed the highest negative effect on the larval weight. When the larvae were treated with the LC_{25} of GML (8.97 µg/gm diet), the larval weight decreased to 6.3, 17.3 and 24.9 mg/larva compared with 15.6, 31.9 and 55.3 mg / larva in the control after 3, 6 and 9 days of treatment, respectively, while the larval weight averages were 7.1, 18.3 and 27.4 mg/larva when larvae were treated with LC_{25} of PhVL (34.43 µg/gm diet), after 3, 6 and 9 days of treatment, respectively. The average pupation period of SBW were significantly longer in the larvae treated with LC_{10} and LC_{25} concentrations of GML (18.5 and 20.4 days) and PhVL (17.2 and 19.4 days), respectively than those in the control treatment (14.3) days (Table 2).

Table 1. Toxicity of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) against 2nd instar larvae of spiny 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)	(χ^2)
GML	5	72.22 (55.92 – 92.69)	15.65 (10.24 – 21.78)	3.95 (2.04 – 6.44)	1.02 ± 0.09	6.84
	6	33.45 (25.77 – 42.27)	8.97 (5.51 – 12.70)	2.74 (1.29 – 4.60)	1.18 ± 0.12	1.50
PhVL	5	299.05 (232.04 – 389.65)	62.66 (42.65 – 85.27)	15.35 (8.30 –24.28)	0.99 ± 0.08	0.11
	6	182.91 (138.59 – 239.30)	34.43 (21.33 – 49.69)	7.66 (3.60 – 13.27)	0.93 ± 0.08	0.30

Sublethal concentrations of the tested lectins had a considerable effect on pupation, The LC_{25} of GML treatments showed the lowest pupation percentage 45.5% compared with 91.7% in the control. Also, a significant ($P < 0.05$) decrease in pupation percentage was observed with LC_{25} of PhVL (55.6%) and the LC_{10} of both GML and PhVL (67.5 and 72.0%, respectively) (Table 2).

The pupal mean weight was considerably lower with all treatments, compared to the control treatment (Table 3).

The LC_{25} for both GML and PhVL had the greatest impact, with pupal mean weights of 31.0 and 30.6 mg/pupa, respectively, followed by the LC_{10} of both lectins, with 38.2 and 36.7 mg/pupa, respectively, compared to 53.5 mg/pupa in the control group. However, pupal duration did not vary significantly in all treatments compared with control. Adult emergence rates were considerably lowered by all treatments; GML and PhVL at LC_{25} had the greatest reduction in adult emergence rates, at 57.5 and 52.8%, respectively, compared to 92.2% in the control group. At the LC_{10} of both lectins, the adult emergence rates were 75.9 and 68.5%, respectively (Table 3).

GML and PhVL at concentrations equivalent to LC_{10} and LC_{25} significantly ($P < 0.05$) reduced the SBW fecundity (No. eggs laid/female) and fertility (%egg hatch) (Table 4).

The insects fed PhVL at 34.43 µg/gm diet showed a significantly low fecundity of 53.5 eggs laid/female, while this was 142.0 eggs laid/female in control. Fecundity was significantly reduced to 65.2 eggs laid/female in insects fed GML at 8.97 µg/gm diet. Fertility was the lowest in insects fed PhVL at 34.43 µg/gm diet flowed by GML at 8.97 µg/gm diet, where it was 41.7 and 48.8% compared with 94.0% in the control. Adult longevity was considerably ($P < 0.05$) reduced to 8.4 and 6.5 days in LC_{10} and LC_{25} of GML treatments, 7.8 and 7.4 days in LC_{10} and LC_{25} of PhVL treatments, and 10.5 days in the control, as shown in Table (4).

Table 2. Effect of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) when applied to the 2nd instar larvae of spiny 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	-	15.6a±0.4	31.9a±1.5	55.3a±2.1	14.3c ± 0.9	91.7a ± 5.2
GML	2.74	9.4b ± 0.6	23.7b±0.8	34.7b±1.2	18.5b ± 1.4	67.5c ± 2.5
	8.97	6.3c ± 0.3	17.3c±0.7	24.9c±1.3	20.4a ± 1.7	45.5e ± 2.3
PhVL	7.66	10.1b ± 0.4	22.9b±1.2	35.2b±1.0	17.2b ± 1.6	72.0b ± 1.9
	34.43	7.1c ± 0.2	18.3c±0.4	27.4c±0.9	19.4a ± 0.8	55.6d ± 1.5

Table 3. Effect of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) when applied to the 2nd instar larvae of spiny 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	-	53.5a ± 2.4	8.4a ± 0.3	92.2a ± 3.5
GML	2.74	38.2b ± 1.6	8.0a ± 0.5	75.9b ± 2.9
	8.97	31.0c ± 1.2	8.6a ± 0.1	57.5c ± 3.1
PhVL	7.66	36.7b ± 1.5	7.9a ± 0.2	68.5b ± 2.7
	34.43	30.6c ± 0.4	8.3a ± 0.4	52.8c ± 2.1

Table 4. Effect of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) when applied to the 2nd instar larvae of spiny 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	-	142.0a ± 8.5	94.0a ± 3.1	10.5a ± 0.5
GML	2.74	98.5b ± 3.2	67.5b±2.5	8.4b ± 0.4
	8.97	65.2d±2.5	48.8d ± 3.2	6.5b ± 0.4
PhVL	7.66	87.4c± 4.1	57.6c±3.2	7.8b±0.5
	34.43	53.5e ± 2.9	41.7d±2.1	7.4b ± 0.3

3. Effect of GML and PhVL on SBW total proteases and α-amylase activity:

The highest inhibition of total proteases activity was occurred in SBW larvae fed diet contains 8.97 and 34.43 µg/gm diet of GML and PhVL with inhibition percentages of 63.8 and 63.4, respectively. In addition, treatment with GML and PhVL at 2.74 and 7.66 µg/gm diet (equivalent to LC_{10}) induced a significant ($P < 0.05$) reduction (45.6 and 42.1%) in total proteases activity (Table 5).

Tested lectins at LC_{10} and LC_{25} concentrations significantly inhibited SBW larvae's α-amylase enzyme activity (Table 6).

GML and PhVL at 8.97 and 34.43 µg/gm diet inhibited α-amylase activity by 66.0 and 64.4%, respectively, whereas GML and PhVL at 2.74 and 7.66 µg/gm diet inhibited it by 59.8 and 58.8% (Table 6).

DISCUSSION

The present study provides comparison between toxicity, developmental and biochemical effects of GML and PhVL against SBW larvae. Both GML and PhVL showed potent insecticidal activity against SBW 2nd instar larvae. GML was 5.47 times more toxic than PhVL after 6 days of exposure. In previous studies several legume lectins were reported to have entomotoxic effects (Melander *et al.*, 2003 and Wang *et al.*, 2003). *Spodoptera littoralis* larvae died significantly when fed on transgenic plants expressing *Allium porrum* L. lectins (Sadeghi *et al.*, 2009). *Sclerotium rolfsii* lectins were shown to have a notable mortality rate for *Spodoptera litura* larvae (Vishwanathreddy *et al.*, 2014). Moreover, high mortality of *S. littoralis* and pink bollworm larvae treated with GML and PhVL were reported (Mohsen *et al.*, 2020 and El-Deeb, 2021).

Most lectins did not display strong acute toxicities against insect pests instead they are known to have distinct negative effects on insect development and reproductive (Caccia *et al.*, 2012; Mohsen *et al.*, 2020 and El-Deeb, 2021). From a practical point of view these negative effects on insect development are suitable for IPM programs as the insect population is negatively affected. The present data showed high negative effects of GML and PhVL on the SBW larval and pupal weights, and significantly prolonged larval duration. In addition, pupation percentage and adult emergence rates of SBW were significantly reduced as a result of treatment by GML and PhVL. Previous research verified that S. littoralis 2nd instar larvae fed leaves on transgenic tobacco plants expressing *Allium porrum* L. lectins had lower larval and pupal weights (Sadeghi *et al.*, 2009) or diet contains *Rhizoctonia solani* lectins (Hamshou *et al.*, 2010) or diet contains PhVL and GML (Mohsen *et al.*, 2020). The growth and development of *Spodoptera exigua* was shown to be adversely affected by the snowdrop lectin (Naghdi and Bandani, 2013). Furthermore, in *Lacanobia oleracea*, snowdrop lectin delayed the instar durations and decreased survival (Fitches *et al.*, 1997 and Wakefield *et al.*, 2006).

Table 5. *In vivo* effect of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) on the 2nd instar larvae of spiny bollworm total proteases activity after 6 days of treatment.

Treatment	Conc. (µg/g diet)	Specific activity (OD440/mg protein/hr) ± SE	Activity (%control)	%Inhibition
Control	-	0.309a± 0.006	100±1.9	0.0
GML	2.74	0.168b± 0.005	54.4±1.6	45.6
	8.97	0.112c± 0.004	36.2±1.3	63.8
PhVL	7.66	0.179b± 0.008	57.9±2.6	42.1
	34.43	0.113c± 0.004	36.6±1.3	63.4

Table 6. *In vivo* effect of *Glycine max* lectins (GML) and *Phaseoulus vulgaries* lectins (PhVL) on the 2nd instar larvae of spiny 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	0	1.77a ± 0.05	100 ± 2.8	0.0
GmL	2.74	0.712b ± 0.06	40.2 ± 3.4	59.8
	8.97	0.601d ± 0.03	34.0 ± 1.7	66.0
PhVL	7.66	0.729b ± 0.05	41.2 ± 2.8	58.8
	34.43	0.631c ± 0.04	35.6 ± 2.3	64.4

GML and PhVL significantly reduced the SBW fecundity and fertility when the 2nd instar larvae fed diet contains concentrations equivalent to LC_{10} and LC_{25}. Reduction of reproductive parameters (fecundity and fertility) of SBW caused by PhVL and GML has been recorded in other insect pests such as *S. littoralis* (Mohsen *et al.*, 2020) and pink bollworm (El-Deeb, 2021). The investigation reveals that GML and PhVL have detrimental impacts on growth and reproductive parameters, which are partially explained by the evident inhibitory effects of total proteases and α-amylase activity in SBW. Incorporating the coagulant *Moringa oleifera* lectin (cMoL) in an artificial diet of moth flour resulted in decreased average larval weight, a series of nutritional disturbances and increased the total development time (De Oliveira *et al.*, 2011). In addition, *Hippeastrum* hybrid (*Amaryllis*) (HHA) bullbs lectin binds to the membranes of columnar cells of *S. littoralis* larval midgut, then cross the gut epithelial barrier and pass into the insect hemolymph resulting in its toxic effect (Caccia *et al.*, 2012).

GML and PhVL proved to have toxic and negative effects on the growth and development of SBW. In addition, in previous studies GML and PhVL had the same effects against *S. littoralis* (Mohsen *et al.*, 2020) and pink bollworm larvae (El-Deeb, 2021). These insects are the main cotton pests causing great reduction of production. Therefore, we suggest GML and PhVL to be included in main cotton insect pests IPM programs and genes that encode GML and PhVL can be incorporated into cotton for this purpose.

CONCLUSION

Lectin extracted from *Glycine max* (GML) and *Phaseoulus vulgaries* (PhVL) showed entomotoxic and growth inhibition effects against the spiny bollworm (SBW), *Earias insulana* Boisd. The GML lectin was more toxic than the PhVL one. They also negatively affected the development and protease α-amylase activity of SBW. Therefore, these lectins can be included in IPM programs to control the main lepidopteran insect pest. As lectins are considered environmentally friendly nature chemicals, they may provide an alternative for the traditional insecticides. However, further studies are suggested to adjust the filed rats and the proper formulations as well toxicity to the beneficial nature enemies.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abou-Taleb, H.K. and A.F. El-Aswad. 2010. Efficacy of selected insecticide rotations on cotton infestation by two bollworms and their glutathione S-transferase activity in cotton field in Egypt. J. Egypt. Soc. Toxicol. 41: 73-80.

Afolabi-Balogun, N.B., H.M. Inuwa, M.F. Ishiyaku, M.T. Bakare-Odunoola and A.J. Nok. 2012. Isolation and characterization of a mannose-binding insecticidal lectin gene from *Allium sativum* (garlic) and its putative role in insect resistance using bioinformatics tools. Infect. Genet. Evol. 12: 1508-1512.

Al-Beltagy, A.A.M., H.S. Radwan, Z.A. El-Bermawy, M.E. Nassar, A.G. Yousef and M.M. Shekeban. 2001. Monitoring for insecticide resistance in bollworms field populations using vial residue assay technique. Egypt. J. Agric. Res. 79: 935-948.

Bernfeld, P. 1955. Amylases, α and β. Meth. Enzymol. 1: 149-158.

Caccia, S., E.J. Van Damme, W.H. De Vos and G. Smagghe. 2012. Mechanism of entomotoxicity of the plant lectin from *Hippeastrum* hybrid (*Amaryllis*) in *Spodoptera littoralis* larvae. J. Insect Physiol. 58: 1177-1183.

Carlini, C.R. and M.F. Grossi-de-Sá. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40: 1515-1539.

Cavada, B.S., V.J.S. Osterne, M.V. Oliveira, V.R. Pinto-Junior, M.T.L. Silva, A.U. Bari, L.D. Lima, C.F. Lossio and K.S. Nascimento. 2020. Reviewing Mimosoideae lectins: A group of under explored legume lectins. Int. J. Biol. Macromol. 154: 159-165.

Cavada, B.S., V.R. Pinto-Junior, M.V. Oliveira, V.J.S. Osterne, C.F. Lossio and K.S. Nascimento. 2021. A review of Vicieae lectins studies: End of the book or a story in the writing?. Int. J. Biol. Macromol. 181: 1104-1123.

Cayrol, R.A. 1972. Famille des Noctuidae. In: Balachowsky, A.S. (Ed.), Entomologie Appliquée à Lágriculture. Tome II. Lépidoptères. Masson et Cie Èditeurs, Paris, 1255-1530.

Chakraborti, D., A. Sarkar, H.A. Mondal and S. Das. 2009. Tissue specific expression of potent insecticidal, *Allium sativum* leaf agglutinin (ASAL) in important pulse crop, chickpea (*Cicer arietinum* L.) to resist the phloem feeding *Aphis craccivora*. Transgenic Res. 18: 529-544.

De Oliveira, C.F.R., L.A. Luz, P.M.G. Paiva, L.C.B.B. Coelho, S. Marangoni and M.L.R. Macedo. 2011. Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control of insects. Process Biochem. 46: 498-504.

Dutta, I., P. Saha, P. Majumder, A. Sarkar, D. Chakraborti, S. Banerjee and S. Das. 2005. The efficacy of a novel insecticidal protein, *Allium sativum* leaf lectin (ASAL), against homopteran insects monitored in transgenic tobacco. Plant Biotechnol. J. 3: 601-611.

El-Deeb, D.A. 2021. Insecticidal activity, growth inhibitory and biochemical effects of plant lectins and *Bacillus thuringiensis var. kurstaki* against the pink bollworm, *Pectinophora gossypiella*. Alex. Sci. Exch. J. 42: 11-20.

El-Deeb, D.A., M.H. Metayi, D.A. Awad and A.F. Bedair. 2017. Field evaluation of selected insecticide sequences against two cotton bollworms with reference to side effects on *Coccinella undecimpunctata* L. J. Plant Prot. Pathol. 8: 305-309.

El-Sebae, A.H., M. Abou Zeid and M.A. Saleh. 1993. Status and environmental impact of toxaphene in the Third World—a case study of African agriculture. Chemosphere 27: 2063-2072.

Finney, D.J. 1971. Probit analysis, Cambridge Univ. Press. Cambridge, UK.

Fitches, E., A.M. Gatehouse and J.A. Gatehouse. 1997. Effects of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on the development of tomato moth (*Lacanobia oleracea*) larvae in laboratory and glasshouse trials. J. Insect Physiol. 43: 727-739.

Hamshou, M., E.J.M. Van Damme and G. Smagghe. 2010. Entomotoxic effects of fungal lectin from *Rhizoctonia solani* towards *Spodoptera littoralis*. Fungal Biol. 114: 34-40.

Jadhav, R.P., D.R. Mundhe, B.B. Bhosle and G.A. Yadav. 2009. Bioefficacy of new insecticide acetamaprid 20 SP and indoxacarb 14.5 SC against bollworm complex of cotton. Pestic. Res. J. 21: 150-154.

Kaur, M., K. Singh, P.J. Rup, A.K. Saxena, R.H. Khan, M.T. Ashraf, S.S. Kamboj and J. Singh. 2006. A tuber lectin from *Arisaema helleborifolium* Schott with anti-insect activity against melon fruit fly, *Bactrocera cucurbitae* (Coquillett) and anti-cancer effect on human cancer cell lines. Arch. Biochem. Biophys. 445: 156–165.

Khurana, A.D. and A.N. Verma. 1990. Comparative damage caused by bollworms and yield of seed-cotton during a dry and wet year in Haryana. J. Insect Sci. 3: 180-182.

Lis, H. and N. Sharon. 2003. The biochemistry of plant lectins (phytohemagglutinins). Annu. Rev. Biochem. 42: 541-574.

Loseva, O., M. Ibrahim, M. Candas, C.N. Koller, L.S. Bauer and L.A. Bulla Jr. 2002. Changes in protease activity and Cry3Aa toxin binding in the Colorado potato beetle: implications for insect resistance to *Bacillus thuringiensis* toxins. Insect Biochem. Mol. Biol. 32: 567-577.

Lowery, O.H., N.J. Rosenbourgh and R.J. FarrRondall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265-275.

Macedo, M.L.R., M.D.G.M. Freire, J.C. Novello and S. Marangoni. 2002. Talisia esculenta lectin and larval development of *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae). Biochim. Biophys. Acta, Gen. Subj. 1571: 83-88.

Macedo, M.L.R., M.D.G.M. Freire, M.B.R. da Silva and L.C.B.B. Coelho. 2007. Insecticidal action of *Bauhinia monandra* leaf lectin (BmoLL) against *Anagasta kuehniella* (Lepidoptera: Pyralidae), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 146: 486-498.

Mansour, E.S. 2004. Effectiveness of *Trichogramma evanescens* Westwood, bacterial insecticide and their combination on the cotton bollworms in comparison with chemical insecticides. Egypt. J. Biol. Pest Control. 14: 339-343.

Melander, M., I. Åhman, I. Kamnert and A.C. Strömdahl. 2003. Pea lectin expressed transgenically in oilseed rape reduces growth rate of pollen beetle larvae. Transgenic Res. 12: 555-567.

Metayi, M.H.A., M.M.K. Shekeban and A.S. El-Deeb. 2016. Evaluation of three semi-artificial diets for cotton leafworm mass rearing and their effects on some biological parameters. Alex. J. Agric. Sci. 61: 237-241.

Mohan, M. and G.T. Gujar. 2003. Characterization and comparison of midgut proteases of *Bacillus thuringiensis* susceptible and resistant diamondback moth (Plutellidae: Lepidoptera). J. Invertebr. Pathol. 82: 1-11.

Mohsen, S.F.E., M.A. Abbassy, E.I. Rabea and H.K. Abou-Taleb. 2018. Isolation and antifungal activity of plant lectins against some plant pathogenic fungi. Alex. Sci. Exch. J. 39: 161-167.

Mohsen, S.F.E., M.A. Abbassy, H.K. Abou-Taleb and E.I. Rabea. 2020. Plant-lectins as insecticidal agents against cotton leaf worm *Spodoptera littoralis* and their potential applications in crop protection. Nat. Prod. J. 10: 1-14.

Naghdi, M. and A.R. Bandani. 2013. The effect of GNA lectin on the α-amylase activity of the beet armyworm, *Spodoptera exigua* Hb. (Lepidoptera: Noctuidae). Arch. Phytopathol. Plant Prot. 46: 1270-1277.

Osman, A.A., T.F. Watson and S. Sivasupramaniam. 1991. Susceptibility of field populations of pink bollworm (Lepidoptera: Gelechiidae) to azinphosmethyl and permethrin and synergism of permethrin. J. Econ. Entomol. 84: 358- 362.

Peumans, W.J., J.M. Van Damme, A. Barre and P. Rougé. 2001. Classification of plant lectins in families of structurally and evolutionary related proteins. In: Wu, A.M. (eds) The Molecular Immunology of Complex Carbohydrates —2. Adv. Exp. Med. Biol. 491: 27– 54.

Ramesh., A. Pandey and K. Kashyap. 2010. Effectiveness of various insecticides against spotted bollworms (*Earias* spp.) at Shahjahanpur, U.P., India. J. Phytol. 2: 1-4.

Rashad, A.M. and E.D. Ammar. 1985. Mass rearing of the spiny bollworm, *Earias insulana* (Boisd.) on semi artificial diet. Bull. Soc. Ent. Egypt. 65: 239-244.

Russell, R.M., J.L. Robertson and N.E. Savin. 1977. POLO: A new computer program for Probit analysis. Bull. Entomol. Soc. Amer. 23: 209-213.

Sadeghi, A., G. Smagghe, E. Jurado-Jacome, W. Peumans and E. Van Damme. 2009. Laboratory study of the effects of leek lectin (APA) in transgenic tobacco plants on the development of cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). Eur. J. Entomol. 106: 21-28.

Saha, P., P. Majumder, I. Dutta, T. Ray, S.C. Roy and S. Das. 2006. Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap-sucking insect pests. Planta 223: 1329-1343.

SAS Institute, Inc. 1999. PC—SAS users guide, Version 8. North Carolina statistical analysis system Institute, Inc.

Stoger, E., S. Williams, P. Christou, R.E. Down and J.A. Gatehouse. 1999. Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis agglutinin*; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. Mol. Breed. 5: 65-73.

Vandenborre, G., G. Smagghe and E.J. Van Damme. 2011. Plant lectins as defense proteins against phytophagous insects. Phytochem. 72: 1538-1550.

Varrot, A., S.M. Basheer and A. Imberty. 2013. Fungal lectins: structure, function and potential applications. Curr. Opin. Struct. Biol. 23: 678-685.

Vishwanathreddy, H., G.G. Bhat, S.R. Inamdar, R.K. Gudihal and B.M. Swamy. 2014. *Sclerotium rolfsii* lectin exerts insecticidal activity on *Spodoptera litura* larvae by binding to membrane proteins of midgut epithelial cells and triggering caspase-3-dependent apoptosis. Toxicon 78: 47-57.

Wakefield, M.E., H.A. Bell, E.C. Fitches, J.P. Edwards and A.M.R. Gatehouse. 2006. Effects of *Galanthus nivalis* agglutinin (GNA) expressed in tomato leaves on larvae of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae) and the effect of GNA on the development of the endoparasitoid *Meteorus gyrator* (Hymenoptera: Braconidae). Bull. Entomol. Res. 96: 43-52.

Wang, W., B. Hause, W.J. Peumans, G. Smagghe, A. Mackie, R. Fraser and E.J.M. Van Damme. 2003. The Tn antigen-specific lectin from ground ivy is an insecticidal protein with an unusual physiology. Plant Physiol. 132: 1322-1334.

الملخص العربي

الخواص السمية الحشرية للفاصوليا البيضاء وفول الصويا وتأثيرها ضد إنزيمين هضميين لدودة اللوز الشوكية *Earias insulana* (Boisd.)

مرفت حسنين أبو الحمد، هند سعد الطهاوي، علاء خورشيد

الليكتينات النباتية، وهي مجموعة غير متجانسة من البروتينات المرتبطة بالكربوهيدرات، هي آلية دفاعية مباشرة في النباتات ضد الحشرات المهاجمة. تم اختبار الليكتينات من النباتات البقولية *Glycine max* (GML) و *haseoulus vulgaries* (PhVL)، لمعرفة آثارها السامة للحشرات ومثبطة للنمو ضد دودة اللوز الشوكية *Earias insulana* (SBW)، (Boisd). تمت أيضًا دراسة تأثيرات الليكتينات المختبرة على إنزيمين هضميين، البروتياز الكلي وألفا الأميليز، من SBW. أظهر اختبار المقايسة الحيوية أن GML (قيم LC_{50} هي ٧٢,٢٢ و٣٣,٤٥ ميكروجرام/جرام في الغذاء) كان أكثر سمية من PhVL (قيم LC_{50} هى ٢٩٩,٠٥ و١٨٢,٩١ ميكروجرام/جرام في الغذاء) بعد ٥ و٦ أيام من العلاج على يرقات العمر الثاني. GML و PhVL بتركيزات LC_{25} المكافئة (٨,٩٧ و٣٤,٤٣ ميكروجرام/جرام في الغذاء) خفضت بشكل ملحوظ ($P < 0.05$) وزن اليرقات إلى ٢٤,٩ و ٢٧,٤ ملجم / يرقة مقارنة بـ ٥٥,٣ ملجم / يرقة في السيطرة بعد ٩ أيام من العلاج. أدى كل من GML و PhVL عند LC_{10} وLC_{25} إلى زيادة متوسط الوقت اللازم لتشرنق يرقات SBW، مقارنة بالتحكم. بالإضافة إلى ذلك، أدت الليكتينات المختبرة إلى انخفاض ملحوظ ($P < 0.05$) في متوسط وزن العذراء، والتشرنق، وظهور البالغين، والخصوبة (متوسط عدد البيض الموضوع/ الأنثى) والخصوبة (فقس البيض). أظهرت الليكتينات المختبرة تأثيرات مثبطة ملحوظة على إجمالي البروتياز ونشاط إنزيم ألفا–الأميلاز في يرقات SBW التي تتغذى على نظام غذائي يحتوي على تركيزات مكافئة لـ LC_{10} وLC_{25}. تشير هذه النتائج إلى أن GML و PhVL هما بروتين مناسب لدمجهما في جينوم نبات القطن للتحكم في SBW.