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Full Length Research Paper

Anti-insect potential of a lectin from the tuber, *Dioscorea mangenotiana* towards *Eldana saccharina* (Lepidoptera: Pyralidae)

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A lectin was purified from the tuber, *Dioscorea mangenotiana* in this study. The lectin agglutinated erythrocytes from human and rabbit, and the activity was inhibited by glucose and N-acetyl-D-glucosamine with minimum inhibitory concentration (MIC) of 50 and 25 mM, respectively. The lectin was composed of two isoforms, DML I and DML II. The apparent native molecular mass of DML I was estimated at 51 kDa and the subunit molecular mass at 25 kDa, suggesting a dimeric structure. The lectin was stable up to 90°C and within the physiological pH range. The lectin when incorporated in artificial diet at concentrations of 10 to 160 µg ml^{-1} and fed *ad libitum* to the second instar larvae of *Eldana saccharina*, prolonged the development period and significantly inhibited the pupation and emergence of the insect in a dose-dependent manner. The LC_{50} calculated based on mortality after 72 h of treatment was 66.6 mg/ml. The activities of hydrolytic enzymes - acid phosphatase, alkaline phosphatase and acetylcholine esterase in the larvae reared on diet containing lethal dose of the lectin were significantly affected as compared to those fed on diet without the lectin. The results showed that DML I has promising anti-insect potential and could be employed in a biotechnological strategy for the pest management.

Key words: Lectin, tuber, anti-insect, *Dioscorea mangenotiana,Eldana saccharina.*

INTRODUCTION

Insect pests are a major constraint affecting crop production in tropical and subtropical regions. Plant resistance to insect pests is mediated by a range of primary metabolites among which are lectins (Carlini and Grossi-de-Sa, 2002). Lectins constitute a heterogeneous group of carbohydrate- binding proteins that have been shown to affect the survival and development of insect pests belonging to different orders (Macedo and Damico, 2003; Vasconcelos and Oliveira, 2004; Coelho et al., 2007). The defensive role of plant lectins, such as

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anti-insect and anti-microbial, has been proposed on the basis of indirect and direct evidence (Sharma et al., 2004; Wong et al., 2010; Roy et al., 2014; Al Atalah et al., 2014). Thus, there is intensified research on the use of lectins in protecting crop plants from the attack of insects and fungal pathogens. *Eldana saccharina* (Lepidoptera; Pyralidae), the African sugarcane stalk and maize stem borer is widely distributed in sub-Saharan Africa and mainly infests mature sugarcane and maize plants. Infestation causes lodging of the plants due to tunneling and provides access for fungal diseases into stems and maize cobs. Adult exit holes can be seen in the stems and they are usually covered with fraises; the damage may reach up to 100% of crop loss (Wilson et al., 2015). In most countries where *E. saccharina* affects maize crops and sugar cane, farmers frequently use broad-spectrum insecticides to control the pest. The phytosensitive nature of this insect however defies chemical control, thus, this Lepidopteran has resisted conventional control measures with organic pesticides (Hogervorst et al., 2006; Fitches et al., 2010; Haase et al., 2015).

In the absence of more specific active ingredients for chemical control against the Lepidopteran, there is a need to find some suitable method to control these insect pests. A perusal of literature indicates that some plant lectins with varied sugar specificities such as mannose/ glucose specific, galactose specific and N-acetyl-D-galactosamine specific lectins have shown entomotoxic effects when fed to insects from Coleoptera, Homoptera and Lepidoptera. These plant lectins are being exploited to impart resistance to insect attacks in crop plants and this has, over the last few years, opened a promising field in the use of plant lectins in pest management strategies (Ma et al., 2010; Macedo et al., 2015). The most likely mechanisms underlying the entomotoxic activity of plant lectins involve interactions with different glycoproteins or glycans structures in insects, which may interfere with a number of physiological processes in the insects which include larval weight decrease, mortality, feeding inhibition, delay in total developmental duration, adult emergence and fecundity In the first and second generation (Jamal et al., 2014). Several plant lectins have also been shown to interfere with insect digestive enzyme activities, such as α-amylase, α- and β-glucosidases, trypsin-like enzymes and detoxification enzymes (acetylcholine esterase, acid and alkaline phosphatases) (Agra-Neto et al., 2014).

The anti- insect activity of plant lectins can thus have great economic potential in pest management because lectins being primary metabolic products, their genes can be good candidates to confer insect resistance to transgenic crops (Sharma et al., 2004). Therefore, the purification and characterization of lectins from new sources may reveal genes with the potential to be used in the genetic improvement of crops. In this study, a lectin from the yam tuber, *Dioscorea mangenotiana* was purified and characterized. The biological effect of the lectin on the growth and development of maize stem borer, *E. saccharia* was investigated as well as the effect of the lectin on the activity of some hydrolytic enzymes involved in the metabolism and detoxification in the insect.

MATERIALS AND METHODS

The tubers of *D. mangenotiana* were harvested from a farm in Iwo, Osun State, Nigeria authenticated at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife and stored at 25°C until use.

Sephadex G-100, Biogel P-100, Acrylamide, Methylene bisacrylamide, Coomassie blue, ammonium persulphate, N,N,N`,N`-tetramethylethylenediamine (TEMED), Folin-ciocalteau phenolic reagent, glycerol, sodium dodecyl sulphate (SDS), standard molecular weight markers and sugars are from Sigma-Aldrich, Inc. St. Louis, Mo, USA. All other chemicals and reagents used were of analytical grade.

Blood samples were obtained from healthy human volunteers and from rabbits (purchased from the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife) and fixed with glutaraldehyde according to the method of Bing et al. (1967) as modified by Kuku and Eretan (2004).

E. saccharina were reared at the Entomology Unit of the Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife by the procedure described by Gupta et al. (1978). The insects were provided with proteinex and 20% sugar solution as food with maize flour. Cultures of the insects were maintained at 25 ± 2°C.

Extraction and purification of lectin

The tubers of *D. mangenotiana* were peeled and homogenized in 0.15 M phosphate buffered saline (PBS), pH 7.2. The homogenate was allowed to stand overnight at 4°C followed by centrifugation at 3500 rpm for 30 min. The clear supernatant was collected as the crude extract and 10 ml (50 mg protein) was loaded on Sephadex G-100 column (40 × 2.5 cm) that has been previously equilibrated with PBS, pH 7.2. Fractions (4 ml) were collected at a flow rate of 20 ml/h. The absorbance was monitored at 280 nm and the fractions were assayed for haemagglutinating activity. The fractions in the active protein peak were pooled and dialyzed extensively against several changes of double distilled water. The dialyzate was lyophilized into powder form and re-suspended in phosphate buffered saline.

Protein concentration determination

The protein concentration was determined according to the method of Lowry et al. (1951) using Bovine serum albumin as standard.

Assay of hemagglutinating activity

Agglutination of red blood cells by the lectin was carried out as described by Kuku and Eretan (2004). PBS (100 µl) was delivered sequentially into wells arranged in rows (each row contained 12 wells) in a U – shaped microtitre plate. Lectin (100 µl) was added into the first well to obtain a two-fold dilution. A serial dilution was made by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS. Aliquots (50 µl) of the 2%

erythrocyte suspension were added to each well and the microtitre plates were left undisturbed for 1 h. The hemagglutination titre was taken as the reciprocal value of the highest dilution of the lectin causing visible haemagglutination of the erythrocytes. Specific activity of the lectin is the number of haemagglutination units per mg protein expressed as haemagglutination units (HU)/mg.

Effect of temperature, pH, carbohydrate and EDTA on hemagglutinating activity of D. mangenotiana lectin

In the heat stability test, aliquots of the protein sample were incubated in a water bath at different temperatures (20 - 100°C) for 30 min. The solution was cooled on ice immediately and then allowed to warm up to 25°C (room temperature). The hemagglutinating activity assay was performed. Percentage of hemagglutinating activity was calculated by dividing the activity of the sample by the activity of the control kept at 25°C (room temperature) multiplied by 100 (Sampaio et al., 1998).

In the test for pH stability, the lectin powder was dissolved in buffer solutions of different pH values: 2 – 6 (0.2 M Citrate buffer); 7 – 8 (0.2 M Tris-HCl buffer) and 9 – 11 (0.2 M glycine - NaOH buffer). Equal volumes of protein sample and buffer solution were mixed and incubated at room temperature for 30 min. The solution was neutralized and the hemagglutination assay was performed. The hemagglutination titre of the protein sample incubated in PBS, pH 7.2, served as the control. Percentage of hemagglutinating activity was calculated according to Nakagawa et al. (1996).

In the test for effect of carbohydrate, Hapten inhibition test was carried out using simple sugars; 0.2 M of each sugar in PBS was prepared. A serial dilution of the lectin sample was made in PBS until the end-point and hemagglutination was obtained. The sugar (50 µl) was added to each well and allowed to stand for 30 min at room temperature and then mixed with 50 µl of 2% erythrocyte suspension. The hemagglutination titres obtained were compared with a non-sugar containing blank. Reduction in hemagglutinating activity of lectin in the presence of any of the carbohydrates tested indicates its binding specificity towards the lectin. After identifying the carbohydrate specific for the lectin, a test was conducted to determine the minimal concentration of the carbohydrate for reduction of hemagglutinating activity of the lectin. The lectin was dissolved in solutions containing the specific carbohydrate at different concentrations in PBS. Assay of hemagglutinating activity was performed using the carbohydrate solutions of different concentrations for serial two-fold dilution instead of PBS (Koike et al., 1995).

Effect of detergent on lectin-induced haemagglutination

The effect of denaturing agents: urea and guanidine-HCl at a concentration range of 0.5 - 8.0 M in PBS was carried out on lectin activity by incubating 100 µl of each denaturant solution with an equal volume of lectin sample at 37°C for 1 h, after which hemagglutinating assay was carried out. Lectin sample in PBS served as control which was considered to be 100% activity (Paulova et al., 1971).

Effect of EDTA on lectin-induced hemagglutination

To examine divalent cation requirement of DML I for hemagglutinating activity, demetallization of the purified lectin using EDTA was carried out according to the method of Wang et al. (1996). The lectin sample was dialyzed against 100 mM EDTA for 24 h and the hemagglutinating activity of the lectin was determined (Wang et al., 1996).

Molecular weight determination

The apparent molecular weight of *Di. mangenotiana* lectin was estimated under non-denaturing conditions by gel filtration on Biogel P-100 (70 x 1.5 cm). A molecular calibration curve was prepared using the following protein markers: arginase (138 kDa), creatine phosphokinase (81 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa) and α- chymotrypsinogen (25 kDa).

SDS-PAGE was carried out by the method of Laemmli and Favre (1973) using molecular mass markers in the range of 14.2 – 66 kDa to determine the subunit molecular weight of the lectin. Non-detergent electrophoresis was performed to check the purity of the lectin sample. The gels were stained with Coomassie brilliant blue followed by destaining the background in 10% acetic acid to visualize the band.

Effect of chemical modification of amino acid residues of lectin on hemagglutinating activity

This was carried out by treating the lectin sample with specific modifying reagents. The residual hemagglutinating activity of the modified lectin was determined.

Modification of tryptophan residues was carried out according to the method of Spande and Witkop (1967) using tryptophan specific modifying reagent, N-bromosuccinimide (NBS). Lectin (1 mg/ml) in 10 mM sodium acetate buffer, pH 5.0 was titrated with 10 mM N-bromosuccinimide (NBS, 100 µL) at 20°C with rapid mixing. The reagent was added in five installments within 1 h, excess modifying reagent was removed by dialyzing the solution against distilled water after which the residual heamagglutinating activity was determined. The lectin sample in the absence of the modifying reagent served as the control. Percentage of residual heamagglutinating activity was calculated.

Serine residue modification was carried out by incubating the lectin in 100 µL of 50 mM Tris-HCl buffer of pH 7.4 with 5 mM phenylmethylsulfonyl fluoride (PMSF) at 27°C for 1 h. Excess reagent (PMSF) was removed by dialysing against distilled water. The residual activity was determined by determination of the heamagglutinating activity in the modified lectin. Unmodified lectin sample served as the control. Percentage of residual hemagglutinating activity was calculated (Riordan, 1979).

Arginine residues in the lectin were modified with phenylglyoxal by the method of Riordan (1979). Lectin sample (100 µL of 1 mg/ml in PBS, pH 7.5) was incubated with 10 µL of 10 mM Phenylglyoxal (in 0.1 M sodium carbonate, pH 8.0) at room temperature for 1 h. The reagent was added at 15 min interval. Heamagglutinating activity of the modified lectin was carried out to determine the residual activity with the unmodified lectin sample serving as the control. Percentage of residual hemagglutinating activity was calculated.

The cysteine residues in the lectin were modified as follows: The lectin sample (100 µL in 50 mM phosphate buffer. pH 8.0) was incubated with 10 µL of 0.1 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) at 27°C for 1 h. The modifying reagent (DTNB) was added at 15 min interval. The excess reagent was removed by dialysis against distilled water and thereafter determined the residual activity in the modified lectin using the unmodified lectin sample as control. Percentage of residual hemagglutinating activity was calculated.

Artificial diet bioassay

Artificial diet assay was performed to evaluate anti-insect potential of purified *D. mangenotiana* lectin (DML I). *E. saccharina* were reared by the procedure described by Gupta et al. (1978). The cultures of the insects were maintained at 25 ± 2°C photoperiod

(L10: D14) and 70 – 80% relative humidity. The insects were provided with proteinex and 20% sugar solution as food with maize flour. Lectin was incorporated in the artificial diet at concentrations of 10, 20, 40, 80 and 160 mg ml^{-1} prepared according to the method described by Srivastava (1975) as reported by Kaur et al. (2006b). Diet, was dispensed in aliquots into Petri dishes and fifteen second instar larvae were released into each Petri dish using three replicates for each concentration as well as for control and kept under conditions of relative humidity, temperature and photoperiod. Observations were recorded regarding larvae development, pupation, feeding rate, adult emergence and mortality. The lectin concentration that elicited 50% (LC_{50}) response with respect to mortality was determined. Another experiment was set up to adjudge the effect of the lethal dose of the lectin on the insects.

Biochemical analysis

Lethal concentration (LC_{50}) of the lectin calculated based on mortality recorded during the artificial diet experiment was used for these biochemical estimations. The second instar (64 – 72 h) larvae were released on both the treated (containing lethal dose of the lectin) and control diet for periods of 24, 48 and 72 h. The larva were harvested after specified treatment, homogenized in extraction buffer, mixed freshly in a 5:2 ratio and was assayed for activity of three hydrolytic enzymes - acetylcholine esterase, acid phosphatase and alkaline phosphatase. Three replications were used for each enzymatic assay.

Determination of acetylcholine esterase activity

The extract (1% w/v) was prepared by homogenizing 5 larvae in 12.5 ml chilled extraction buffer (0.1 M sodium phosphate buffer, pH 6.5). The homogenate was centrifuged at 2,500 g at 4°C for 20 min and the supernatant obtained was used for enzyme assay. The substrate was pre-incubated in a water bath at 30°C for 10 min. After adding 0.1 ml of homogenate, the enzyme-substrate complex was kept in water at a constant temperature of 30°C. The reaction was stopped by adding 1 ml post-coupling solution (4% sodium lauryl sulphate and 1% fast red TR salt solution prepared in extraction buffer, mixed freshly in a 5:2 ratio) and the absorbance of the mixture was recorded at 405 nm at intervals of 30 s for 3 min (Katzenellenbogen and Kafatos, 1971).

Determination of acid phosphatase activity

The extract (2% w/v) was prepared by homogenizing 5 larvae in 12.5 ml of chilled extraction buffer (0.05 M acetate buffer, pH 5.0). The homogenate was centrifuged at 2,500 g at 4°C for 20 min. The supernatant obtained was used for enzyme assay. The substrate was pre-incubated at 30°C for 30 min. After adding 1% of the homogenate, the mixture was kept in water bath at constant temperature of 30°C. The reaction was stopped by adding 2 ml of post-coupling solution (4% sodium lauryl sulphate and 0.2% fast red TR salt solutions prepared in extraction, mixed freshly in a 5:2 ratio) and the absorbance of the mixture was recorded at 405 nm at intervals of 30 s for 3 min (MacIntyre, 1971).

Determination of alkaline phosphatase activity

The extraction and estimation of alkaline phosphatases was done according to the method of MacIntyre (1971). The extract (2% w/v) was prepared by homogenizing 5 larvae in 12.5 ml of chilled extraction buffer (0.05 M Tris buffer buffer, pH 8.6). The homogenate was centrifuged at 2,500 g at 4°C for 20 min. The supernatant obtained was used for enzyme assay. The substrate was pre-incubated at 30°C for 30 min. After adding 2% of the homogenate, the mixture was kept in water bath at constant temperature of 30°C. The reaction was stopped by adding 2 ml of post-coupling solution (4% sodium lauryl sulphate and 0.2% fast red TR salt solutions prepared in extraction, mixed freshly in a 5:2 ratio) and the absorbance of the mixture was recorded at 405 nm at 30 s intervals for 3 min.

Statistical analyses

The data obtained was analyzed using one way analysis of variance (ANOVA) and student t-test. Probit analysis was used to determine the LC_{50}.

RESULTS AND DISCUSSION

The present work describes the anti-insect potential of *D. mangenotiana* lectin towards the maize stem borer or sugar cane stalkborer *E. saccharina*. The lectin from *D. mangenotiana* agglutinated non-specifically the red blood cells of human ABO system as well as rabbit erythrocytes and the hemagglutinating activity was inhibited by glucose and N-acetylglucosamine with minimum inhibitory concentration (MIC) of 50 and 25 mM, respectively. The sugar specificity exhibited by this lectin is contrary to the report on the lectin from *D. opposita* that was specific for galactose and *D. batatas* lectins that were maltose and mannose-specific. Purification studies revealed that *D. mangenotiana* lectin was composed of two isoforms (DML I and DML II) purified from phosphate buffered saline extract of *D. mangenotiana.* This is similar to the report on the lectin from the tuber, *D. batatas* that was composed of two isoforms, DB 1 and DB11 (Mariam et al., 2004).

A single-step purification procedure was used to purify the lectin. The chromatographic step on Sephadex G-100 (Figure 1) was able to remove most of the impurities in the crude extract with purification fold of 2.3 (Table 1). The native molecular weight of the purified lectin as determined by gel filtration on Biogel P-100 was 51 kDa while SDS-PAGE in the presence of mercaptoethanol revealed one single band with molecular weight of 25 kDa (Figure 2). This suggests a homodimeric structure for the lectin. A dimeric galactose-specific lectin was also reported to have been purified from the tubers of *D. opposita cv.nagaimo* with subunit molecular weight of 35 kDa (Chan and Ng, 2013) while a tetrameric mannose-binding lectin with subunit molecular weight of 31 kDa was purified from *D. batatas* (Mariam et al., 2004).

The thermostability of DML I showed that the lectin was heat stable up to 90°C as shown in Figure 3A. This is similar to the thermostability exhibited by lectin from *Indigofera heterantha* (Qadir et al., 2013). This finding, however, is contrary to the report on DB1 and DB3 from

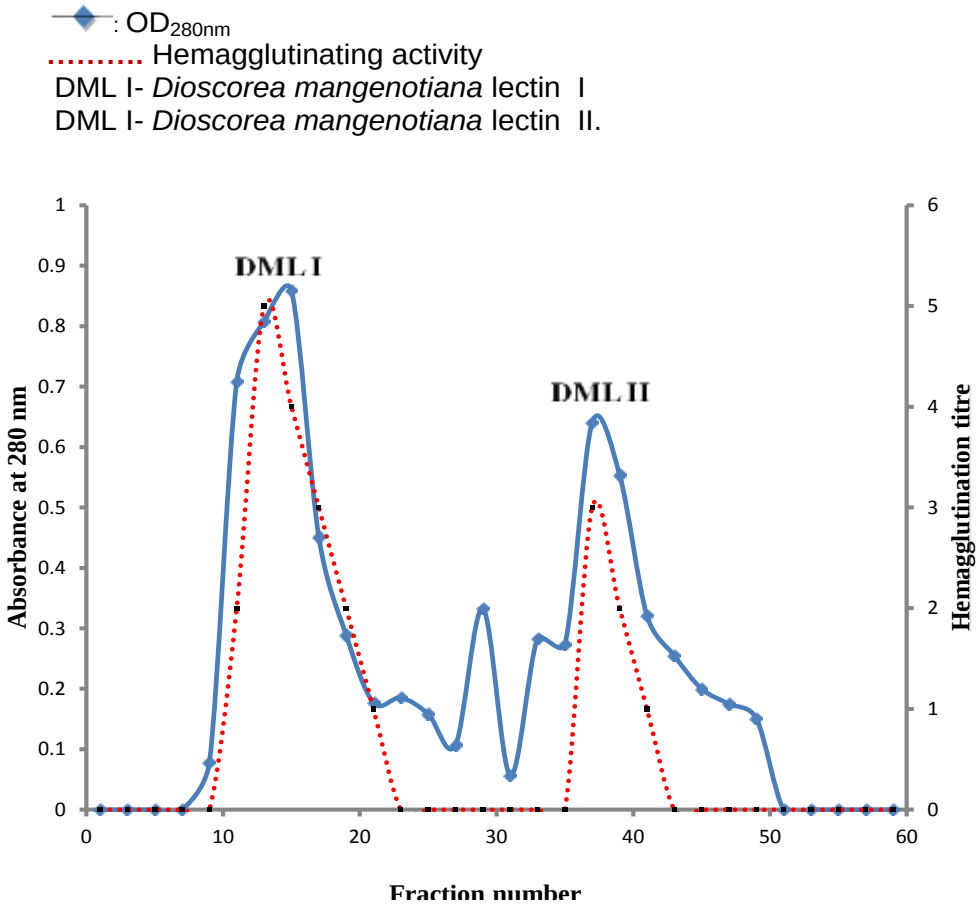

Figure 1. Elution profile of gel filtration of crude extract of *Dioscorea mangenotiana* on Sephadex G-100. The column (2.5 x 40 cm) packed with Sephadex G-100 was equilibrated with 10 mM phosphate buffered saline. 10 ml of crude extract (50 mg) was layered on the column and lectin was eluted with the same buffer at a flow rate of 18 ml/h and fractions of 4 ml were collected.

D. batatas which were reported to be heat stable up to 50°C and that on *Arisaema jacquemontii Blume* lectin that was stable up to 60°C (Kaur et al., 2006a). *D. mangenotiana* lectin was observed to be stable at both acidic and physiological pH (2 - 9) but activity was almost completely lost at alkaline pH (9 - 11) as shown in Figure 3B. This is similar to the report on the lectin from *D. batatas*, DB1 which was stable at pH 7 - 9 and DB3 at pH 3 - 9 (Mariam et al., 2004) but is contrary to the report on the lectin from *D. opposita* which displayed stability over a wider pH range (2 - 13) (Chan and Ng, 2013). The lectin from *Arisaema jacquemontii* Blume was reported to be stable at pH 2.5 (Kaur et al., 2006a).

EDTA treatment or addition of metal ions showed no effect on the heamagglutinating activity of DML I. This could suggest that either the lectin activity was not dependent on metal ions or that these metal ions were too strongly held in the lectin structure and could not be removed by dialysis with chelating agent. This finding is in agreement with the earlier findings on some other tuber lectins such as the lectins from *Arisaema jacquemontii* and *Arisaema helleborifolium* (Kaur et al., 2006a, b) and the lectin from the tubers of *Caladium bicolor* (Kaur et al., 2011).

Urea and guanidine- HCl at 5.0 M concentration reduced the lectin activity by 50%. The denaturation by these agents indicates the globular nature of lectins stabilized mainly by hydrogen bonding and hydrophobic interactions (Nelson and Cox, 2001).

Identification of specific amino acids involved in the biological activity of proteins elucidates the relationship between its structure and the role played by amino acid side chains in its activity. A common strategy for identifying the amino acid residues essential for the biological activity of any protein is to treat the protein with specific modifying agents; this provides clues on amino acids involved in the biological activity. Treatment of the lectin with phenylglyoxal and phenylmethane sulfonyl fluoride (PMSF) did not produce any significant alteration in the haemagglutinating activity of *D. mangenotiana* lectin meaning that arginine and serine did not play any important role in the activity of the lectin (Table 2), this

Table 1. Purification procedure for *Dioscorea mangenotiana* Lectin.

Fraction	Total Protein (mg)	Total activity (HU)	Specific activity (HU/mg)	Percentage Yield	Purification fold
Crude Extract	50	128	2.6	100	1
Gel filtration of crude extract on Sephadex G-100					
DML I	21.6	16	0.7	43.2	2.3
DML II	16	4	0.25	32	3.1

DML I- *Dioscorea mangenotiana* Lectin I; DML II- *Dioscorea mangenotiana* Lectin II.

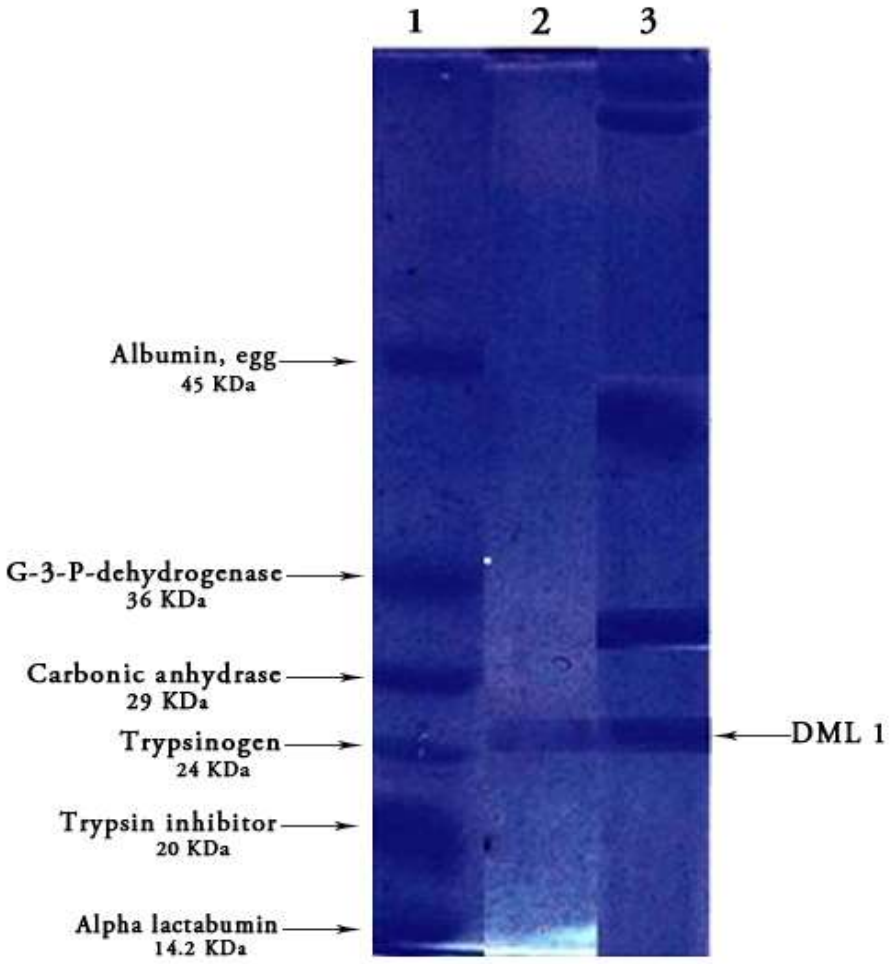

Figure 2. SDS-Polyacrylamide Gel Electrophoresis of *Dioscorea mangenotiana* Lectin and Molecular weight markers. 10 µl protein samples were loaded and the gel was stained with Coomassie Brilliant Blue. Lane 1: Standard proteins, Lane 2: Gel filtration pooled fraction, Lane 3: Crude Extract.

could be that arginine was not involved in direct interaction with sugar binding pocket and hence was not contributing to the hemagglutinating activity of the lectin. However, partial and total loss of activity was observed when *D. mangenotiana* lectin I was treated with 5,5`-dithiobis-(2-nitro benzoic acid) (DTNB) and N-bromosuccinimide (NBS), respectively. This result strongly suggests that cysteine and tryptophan were

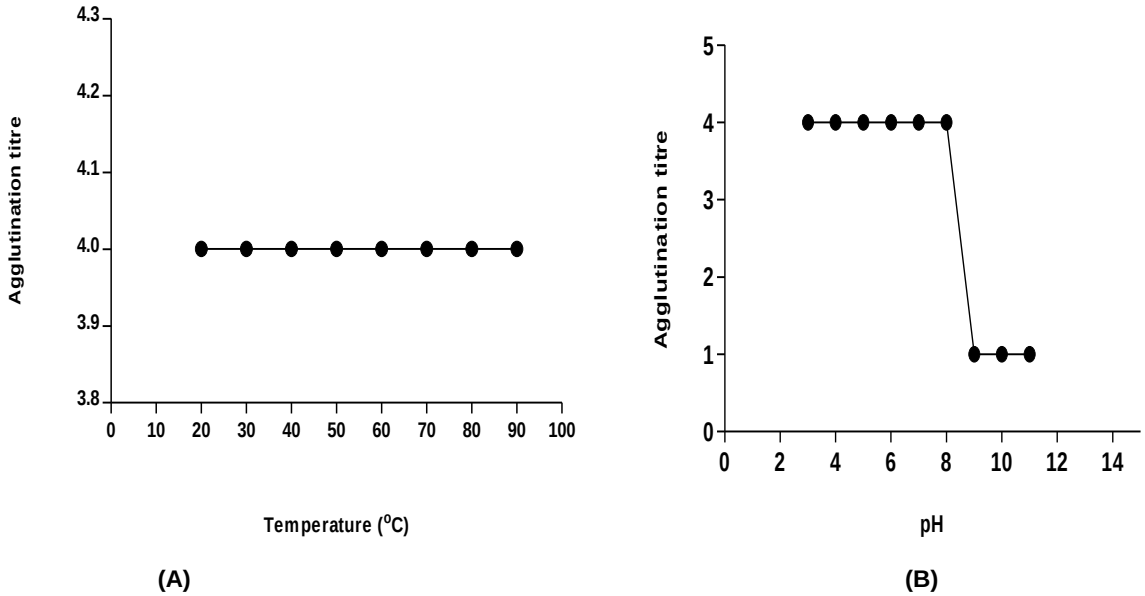

Figure 3. Effect of (A) temperature and (B) pH on the hemagggglutinating activity of *Dioscorea mangenotiana* lectin.

Table 2. Effect of chemical modification on haemagglutinating activity of *Dioscorea mangenotiana* Lectin.

Treatment	Modified Group/Amino acid	Residual of hemagglutinating activity (%)
Phenyl methyl sulfonyl fluoride (PMSF)	Serine	100
N-Bromosuccinimide (NBS)	Trptophan	0
5.5' Dithiobis-nitro benzoic acid (DTNB)	Cysteine	60
Phenyl glyoxal	Arginine	100

either located at the sugar binding site or involved in the maintenance of the lectin active conformation.

Artificial diet bioassay

The result obtained for the artificial diet bioassay, after the specified days of treatment, showed that the larval period, pupae period, total development of the insect was affected; even mortality was observed (Table 3). The percentage pupation and emergence were also significantly affected ($p < 0.01$) (Figure 4). The percentage of pupation declined to 50% at 80 mg/ml concentration of the lectin. The percentage emergence also showed a sharp decline in a dose-dependent manner; there was, however, no emergence at 160 mg/ml concentration of the lectin (Table 4). The significant increase in the total development period and drastic reduction in percentage pupation and percentage emergence of adults could be due to anti-feedant effect of the lectins. Furthermore, being resistant to digestion in insect digestive system, lectins might alter the intestinal protein content creating nutritional imbalance leading to detrimental effects on developmental parameters (Oliveira et al., 2011). This effect of lectins can also be attributed to binding of lectins to carbohydrate moieties associated with the membranes of the gustatory chemosensory sensillae, thus blocking access to food chemical signals (Sprawka et al., 2014). Another mechanism could be by binding of lectins to the surface of the digestive epithelial cells in the insect midgut leading to ultrastructural changes in the gut epithelium (Macedo et al., 2015) or increase in the production of some hydrolytic enzymes (Kaur et al., 2015). The LC_{50} calculated based on mortality after 72 h of treatment was 66.6 mg/ml. The significance of the present study lies on the fact that DML I had a marked effect on the growth and survival rate of *E. saccharina* at comparative lower concentrations indicating the possibility of using this lectin in a biotechnological strategy for insect pest management of *E. sachharina*.

The effect of the lethal concentration (LC_{50}) of DML I on *E. saccharina* was investigated by assessing the hydrolytic enzymes generally involved in digestion, development and metabolism of the insect-acetylcholine esterase, acid phosphatase and alkaline phosphatase (Figure 5). The result showed that activity of esterase

Table 3. Percentage mortality of *Eldana saccharina* larva fed with different concentrations of *D. mangenotiana* Lectin after 72 h.

Concentration of DML I (mg/ml)	No of larvae	No of death	Mortality (%)
Control	15	0	0
10	15	2	13
20	15	4	27
40	15	5	33.3
80	15	11	73.4
160	15	12	80

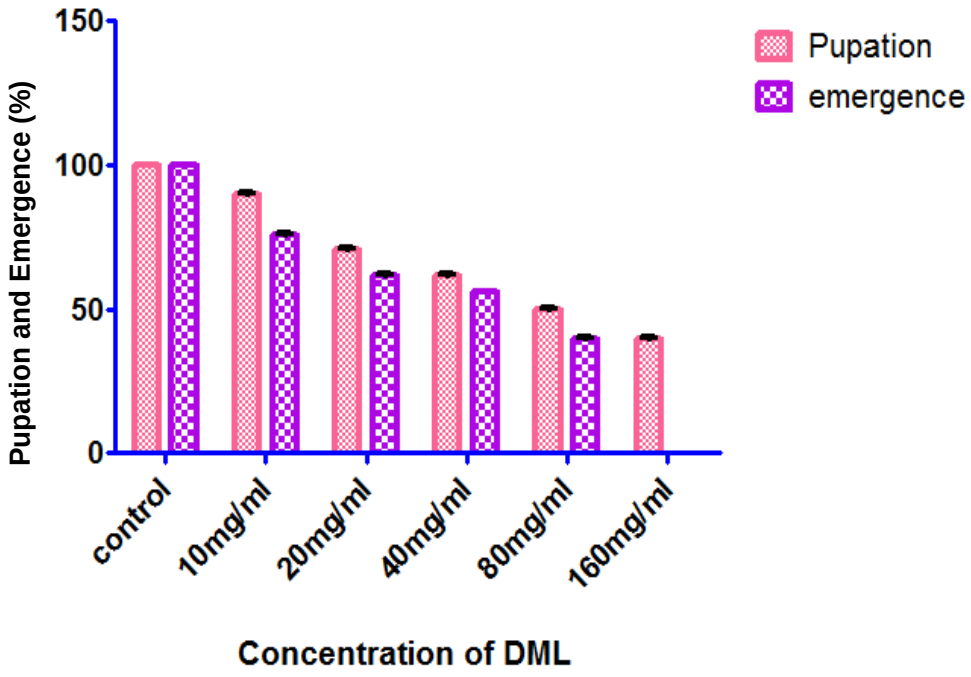

Figure 4. Effect of different lectin concentrations on the percentage pupation and emergence of *Eldana saccharina*.

decreased as the larvae developed from second instars and increased when they had reached the age of 72 h during the normal course of development. However, in the larvae treated with DML I, after 48 and 72 h of treatment, the esterase activity significantly increased ($p < 0.01$) as compared to the control group (Figure 5A). Acid phosphatase activity increased during the normal development of *E. saccharina* larvae whereas alkaline phosphatase showed a continuous decrease after 72 h. But the treatment of the larvae with DML I resulted in significant decrease in the activities of the two enzymes ($p < 0.05$) when compared with the control (Figure 5B and C). The role of esterases in development of resistance and in sequestration of xenobiotics has been established (Russel et al., 2011). The increase in the plateau of esterase activity in the lectin - treated larvae suggest that esterases might be playing a significant role in detoxification of *D. mangenotina* - lectin I and the increase in activity could be attributed to positive feedback response (Kaur and Arora, 2009). It was reported in literature that the major insecticide resistance mechanism in the brown plant hopper *Nilaparvata lugens* Stål. involved overproduction of esterase isoenzymes (Small and Hemingway, 2000). The role of esterases in insecticide-resistance was also documented in *Anopheles stephensi* (Ganesh et al., 2002). In supporting these previous reports, Shi et al. (2015) also reported that a high esterase activity is normally correlated with development of resistance in insects. This present study is in agreement with these findings. Phosphatases are reported to be biosensors as they are inhibited by organophosphorous compounds. They have been reported to be the detoxifying enzymes of insects. The alkaline phosphatases are involved in transport across

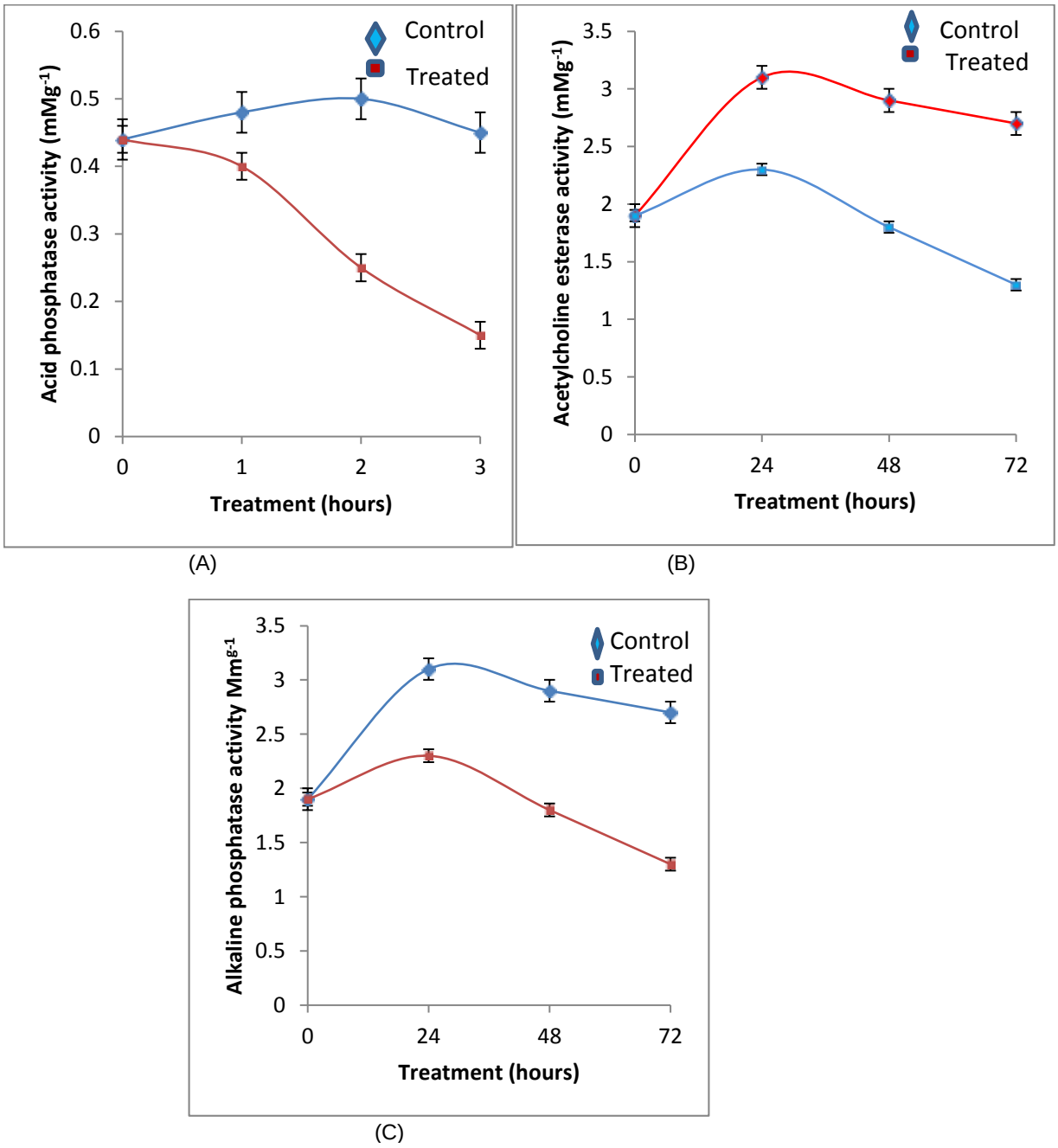

Figure 5. Effect of LC_{50} of lectin on the activity of various enzymes at various time intervals. The enzyme activity of the larva reared on Lectin-incorporated diet was compare with their respective control. (A) Effect of LC_{50} of lectin on the activity of acid phosphatase; (B) Effect of LC_{50} of lectin on the activity of acetylcholine esterase; (C) Effect of LC_{50} of lectin on the activity of alkaline phosphatase.

free cell borders. These hydrolytic enzymes have their roles in recycling of phosphate and energy transfer. Because of these roles, their expressions are expected to increase during high metabolic activity (Kaur et al., 2011). The suppression of these two hydrolases (acid and alkaline phosphatases) by *D. mangenotiana* lectin indicated that both acid and alkaline phosphatases played no role in the detoxification of the lectin in *E. saccharina* and that the lectin might be interfering with the feedback bio-mechanism of these enzymes during their synthesis. This is similar to the report on *Arisaema jacquemontii* Blume lectin when incorporated into the artificial diet of *Bactocera curcurbitae* (Kaur et al., 2006a) but is contrary to the report on *Caladium bicolor* lectin

that had no significant change in the activity of acid phosphatase but caused a decrease in alkaline phosphatase (Kaur et al., 2012). These results indicate that phosphatases activity was suppressed significantly, and this could be attributed to negative feedback response.

CONCLUSION AND RECOMMENDATION

The results of the study presented here indicate that the lectin from *D. mangenotiana* showed similar physicochemical properties with the lectins from other *Dioscorea* species and exhibited considerable anti-insect activity against the sugarcane (or maize stem borer) *E. saccharina* and thus may act as a more specific biodegradable active ingredient in the management of the pest. In general, the plants that are surrounding the main crops are the first barrier to insect attacks. The use of this plant, *D. mangenotiana*, in the surrounding of the sugarcane and maize crops, in what is known as intercropping, could be one of the potential ways of reducing the insect damage. It is also possible that the gene that encodes the anti-insect protein (lectin) in the tuber, *D. mangenotiana* can be engineered and expressed in these plants (maize and sugarcane) to protect against the phytophagous pest, *E. saccharina*. Further studies will however be required on the gene that codes for the protein and on the understanding of the protein-insect interaction. The potential of the incorporation of such resistance genes containing lectin domains in plants could provide a sustainable control strategy and should be further investigated. However, a series of factors must be considered such as the use of tissue-specific promoters for the genes and the adoption of refuge areas in the crops, as well as, the expression of the insecticide proteins in different tissues, especially those for consumption, the manner of preparation of transgenic food and biosafety of the transgenic crops.

Conflict of Interests

The authors have not declared any conflict of interests.

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