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Anti-insect potential of lectins from *Arisaema species* towards *Bactrocera cucurbitae*

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Abstract: *Bactrocera cucurbitae (Coquillett), also known as melon fruit fly, is one of the major insect pests of cucurbits in several parts of Asia, Africa and Pacific. In the present investigation, effect of lectins from two sources i.e. Arisaema intermedium Blume and Arisaema wallichianum Hook f. (Family-Araceae) has been studied on the development of second instar larvae of melon fruit fly. The lectins were incorporated separately in artificial diet at a concentration of 10 to 160 µg ml^{-1} and fed adlibitum to the second instar larvae. Both the lectins were found to prolong the development period and significantly inhibited the pupation and emergence in a dose dependent manner. Total development period was found to be prolonged by 3.5 and 2.3 days in case of larvae fed on artificial diet containing A. intermedium (AIL) and A. wallichianum (AWL), respectively. LC_{50} values calculated on the basis of adult emergence came out to be 32.8 and 29 µg ml^{-1} for AIL and AWL, respectively. Both the lectins tested, were found to increase the activity of esterases as larvae proceeded from 24 to 72 hr of treatment. The activity of acid phosphatase decreased significantly in larvae reared on diet containing LC_{50} of AIL, while in case of AWL significant decrease was observed only at 72 hr of treatment. Alkaline phosphatase activity decreased significantly on treatment with both of these lectins. These results showed that AIL and AWL have promising anti-insect potential. So, lectin gene/s from either of these species can be cloned and subsequently can be employed to develop transgenics to control melon fruit flies specifically and insect pests in general. This approach could be used as a part of Integrated pest management (IPM) strategies.*

Key words: *Araceae, Arisaema, Bactrocera, Diptera, Esterases, Fruit fly, Lectin, Phosphatases*
PDF of Full length paper is available online

Introduction

Fruit flies (Diptera-Tephritidae) are the serious crop pests in most areas of the world. There are many species of fruit flies with different host plant preferences and geographic distributions. In order to avoid the indiscriminate use of insecticides to control this pest, it is important to develop alternative methods of controlling this pest, including host plant resistance through genetically transformed plants (Khanna *et al*., 2003; Sharma *et al*., 2004). The first report on the use of a plant-derived insect control protein gene in transgenic plants came with the expression of cowpea trypsin inhibitor gene in tobacco (Hilder *et al*., 1987). Plant lectins are another group of compounds, which are being exploited to impart resistance to insects in crop plants. Lectins constitute a heterogenous group of sugar-binding proteins, which 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 anti-insect activity of plant lectins can be economically of great potential in pest management because lectins being primary metabolic product, their genes can be good candidates to confer insect resistance to transgenic crops (Macedo and Damico, 2003). 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.

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A perusal of literature revealed *Arisaema* genus to be a rich source of lectins (Shangary *et al*., 1995). In previous studies (Kaur *et al*., 2005; Kaur *et al*., 2006a,b; Singh *et al*., 2008), it has been shown that these tetrameric araceous lectins have specificity for a disaccharide, N-acetyl-D-lactosamine (LacNAc) and a glycoprotein, asialofetuin. These lectins have been purified by affinity chromatography using asialofetuin as ligand (Shangary *et al*., 1995). Unlike various other monocot lectins reported in literature, these lectins did not show any specificity for mannose even at very high concentration. In case of monocot mannose lectins, one factor that may affect their anti-insect property is the number of subunits per molecules. As for instance, GNA, a tetramer, was found to be more toxic when fed to *Nilaparvata lugens* Stål than the trimeric or dimeric lectin from *Narcissus pseudonarcissus* L. and *Allium sativum* L., respectively (Powell *et al*., 1998a,b). Thus, these monocot lectins from genus *Arisaema* having similar structure (tetramers) but with different sugar specificity, needs to be investigated in this regard. Already the lectins from two species of genus *Arisaema* i.e. *A. helleborifolium* Schott (Kaur *et al*., 2006a) and *A. jacquemontii* Blume (Kaur *et al*., 2006b) having anti-insect activity against *Bactrocera cucurbitae* (Coquillett) have been reported. These studies support the need to explore lectins from other species of this genus to decide the most potent species for development of genetically engineered crops. The present communication reports the biological effect of lectins from two species of *Arisaema* on the growth and development of *B. cucurbitae*. Effects of these lectins on activity of some hydrolytic enzymes involved in metabolism

and detoxification *i.e.* esterases, acid phosphatases and alkaline phosphatases, were also studied.

Materials and Methods

Purification of phytolectins: Purification of phytolectins was performed by Affinity chromatography. For this, tubers of *A. intermedium* and *A. wallichianum* were collected from Shimla, Himachal Pradesh India, in the month of August. Lectins from tubers of *Arisaema* spp. were extracted with 10 mM phosphate buffered saline (PBS), pH 7.2. The mixture was allowed to stand overnight at 4°C followed by centrifugation at 20,000 xg for 30 min. Asialofetuin, found to inhibit lectin-induced hemagglutination in sugar-inhibition assay, was used as affinity ligand for the purification of these lectins. The column of asialofetuin-linked amino activated silica beads (1000 Å; pore size, 100 µ; diameter) was prepared as described by Shangary *et al*. (1995) and equilibrated with PBS. The bound lectin was eluted with 100 mM glycine-HCl buffer, pH 2.5 and the eluted fractions were neutralized immediately with 2 M Tris-HCl buffer, pH 8.3. Protein concentration was estimated by the method of Lowry *et al*. (1951). The protein rich lectin positive fractions were dialyzed against PBS and stored at 4°C for further analysis.

Hemagglutination assay: Hemagglutination assay was carried out to ascertain lectin activity. The assay was carried out in a microtitre plate following a two-fold serial dilution method (Shangary *et al.,* 1995). 30 µl of the lectin solution was incubated with 30 µl of 2% suspension of rabbit erythrocytes (3.5×10^8 cell ml^{-1}). After 1 hr of incubation at 37°C, the reaction was stabilized at 4°C for half an hour. The agglutination was checked with unaided eye and the agglutination activity unit (AU) or titre was defined as the reciprocal of the last dilution that showed agglutination.

Electrophoresis: Electrophoresis techniques such as SDS-PAGE at pH 8.3 and native PAGE at pH 4.5 were performed to check the purity of lectin samples. SDS-PAGE was carried out by the method of Laemelli (1970) using molecular mass markers in the range of 14.4-94 kDa. Native electrophoresis was carried out by the method of Reisfeld (1962) using 7.5% tube gels. The gels were stained with Coomassie brilliant blue followed by destaining to visualize the clear bands.

Artificial diet bioassay: Artificial diet assay was performed to evaluate anti-insect potential of affinity purified lectin preparations. Melon fruit flies, *B. cucurbitae*, were reared by the procedure described by Gupta *et al*. (1978). The cultures were maintained at 25±2°C, photoperiod (L10:D14) and 70-80% relative humidity. Diet, prepared according to the method described by Srivastava (1975), was dispensed in aliquots of 5 ml into glass vials (2.5 diameter × 10 cm length). Lectins were incorporated in the diet at five different concentrations, *i.e.* 10, 20, 40, 80 and 160 µg ml^{-1}. Ten second instar larvae (64-72 hr old), procured from charged pumpkin pieces, were released into each vial with the help of camel hair brush. There were seven replications for each treatment as well as control. The vials were kept in the insect rearing laboratory at the above mentioned conditions of relative humidity and temperature and photoperiod. Observations were recorded regarding pupation and adult emergence. LC_{50} values were calculated from adult emergence. Another experiment was set up to adjudge the influence of LC_{50} of these lectins on pupal weight. Pupae were weighed after one day of pupation and pupal weight was compared with that of control.

Biochemical analysis: To perform the biochemical analysis the second instar (64-72 hr) larvae were released on both the treated (containing LC_{50} of respective lectins) and control diet for periods of 24, 48 and 72 hr. The larvae were harvested after specified treatment period and were assayed for activity of three hydrolytic enzymes (involved in digestion, development, growth, metamorphosis and detoxification) *i.e*. esterases, acid phosphatases and alkaline phosphatases. There were six replications for each experiment. Esterases activity was estimated by the method of Katzenellenbogen and Kafatos (1971). Phosphatases activity (acid and alkaline) was determined by following the method given by Mc Intyre (1971).

Stastistical analysis: The data were subjected to statistical analysis with the help of SPSS computer program (Landau and Everitt, 2004). One-way analysis of varience (ANOVA) and Student's 't' test was applied to assess the significance of the effect of lectin on the developmental period, percentage pupation, percentage emergence and enzymatic activity. Probit analysis was employed to calculate LC_{50} value for the lectins under study.

Results and Discussion

The present work describes the anti-insect potential of two lectins from genus *Arisaema* i.e. *A. intermedium* and *A. wallichianum* towards melon fruit fly, *B. cucurbitae*. The purity of lectin preparations was ascertained by SDS-PAGE at pH 8.3 and native PAGE at pH 4.5 (Kaur *et al*., 2005 a,b). Both the lectins gave a single band in SDS-PAGE corresponding to subunit molecular mass 13.1 kDa (Fig.1A) and a single band in native PAGE (Fig. 1B).

The effect of these lectins was demonstrated in the form of significant prolongation ($p<0.01$) in the total development period of second instar larvae of *B. cucurbitae* and decrease in percentage pupation and emergence. In artificial diet bioassay containing *A. intermedium* lectin (AIL), at a concentration of 40 µg ml^{-1}, the larval period prolonged by 2.3 days and pupal period by 1.2 days as compared to control leading to a delay of 3.5 days in total development period (Table 1). In case of larvae reared on artificial diet containing 40 µg ml^{-1} of *A. wallichianum* lectin (AWL), pupation was prolonged by 2.29 days while pupal period by 0.94 days, thus increase of 2.3 days in total development period (Table 2). There was significant ($p<0.01$) reduction in percentage pupation and emergence. As is clear from Fig. 2, the percentage pupation and percentage emergence exhibit inverse relationship with concentrations of lectins tested, showing a significant decrease as the concentration of lectins increases. The pupation was only 50% relative to untreated diet at 40 µg ml^{-1} concentration of lectins. Percentage emergence of adult flies from these pupae was less than 50% at 80 µg ml^{-1} as compared to larvae reared on controlled diet *i.e.* 24% in AIL (Fig. 2A) and 35% in AWL

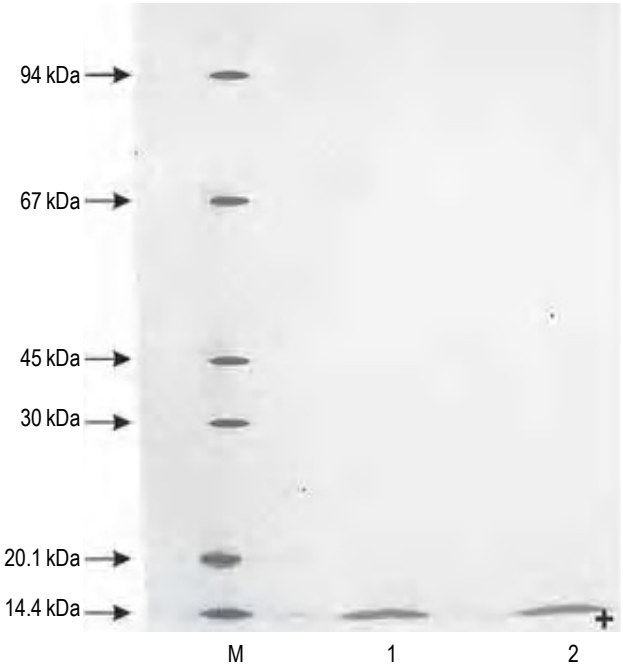

Fig. 1A: SDS-PAGE of standard molecular markers (M) and affinity purified lectin preparations (1-AIL, 2-AWL). Standard markers as well as lectins were loaded separately in separate wells. 20 µg of each lectin and 10 µg of standard proteins were used. Electrophoresis was performed at a constant 100V

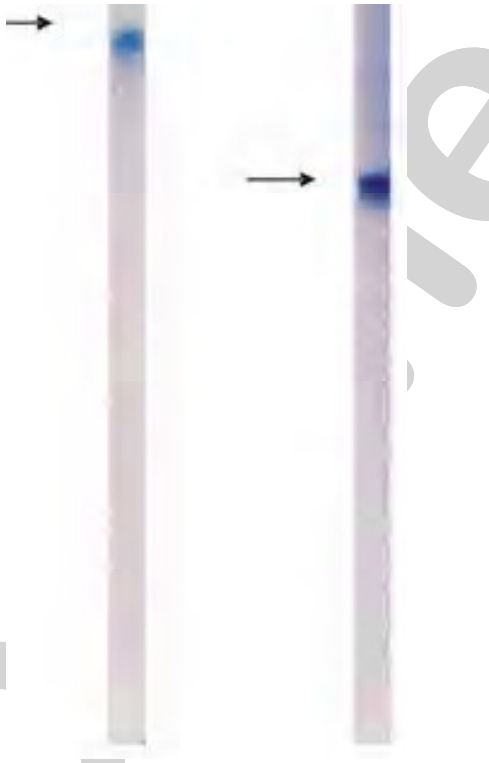

Fig. 1B: Native PAGE of affinity purified non-denatured lectins at pH 4.5 using 7.5% tube gels. 80 µg of each sample was loaded separately on each tube gel and electrophoresis was carried out using β-alanine-acetic acid buffer, pH 4.5 at a constant 150 V

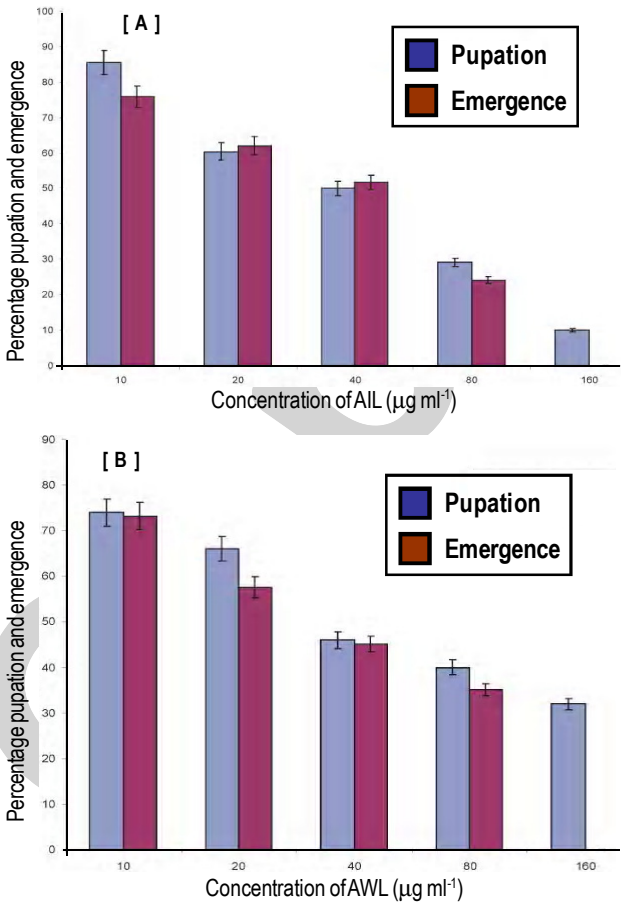

Fig. 2B: Percentage pupation and emergence of second instar larvae of *B. cucurbitae* reared under the influence of various concentrations (10-160 µg ml^{-1}) of *A. intermedium* (A) and *A. wallichianum* (B) lectin

(Fig. 2B). There was no emergence above 80 µg ml^{-1} in either of these lectins. The LC_{50} values of AIL and AWL, as calculated on the basis of adult emergence, were 32.8 and 29 µg ml^{-1}, respectively.

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 (Eisemann *et al*., 1994). This effect of lectins can 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 (Murdock and Shade, 2002). Another mechanism can be binding of lectins to the surface of the digestive epithelial cells in the insect midgut leading to ultrastructural changes in the gut epithelium (Harper *et al*., 1995; Powell *et al*., 1998a,b; Zhu-salzman *et al*., 1998) or increase in the production of some hydrolytic enzymes (Fitches and Gatehouse, 1998).

In the present study, the effect of AIL and AWL was also ascertained on the activity of two groups of hydrolases i.e. esterases and phosphatases. The activity of esterases, which are usually involved in metamorphosis, hydrolysis and detoxification, was increased significantly ($p<0.01$) and continually as 2nd instar larvae (64-72 hr old) proceed from 24 hr of treatment to 72 hr of treatment

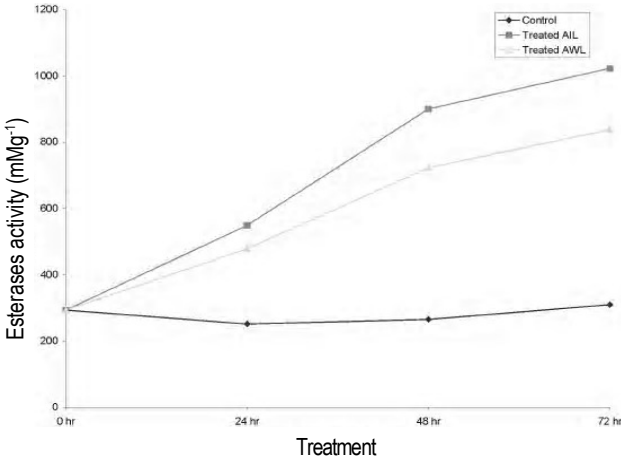

Fig. 3: Effect of *A. intermedium* and *A. wallichianum* lectin on the activity of esterases at various time intervals

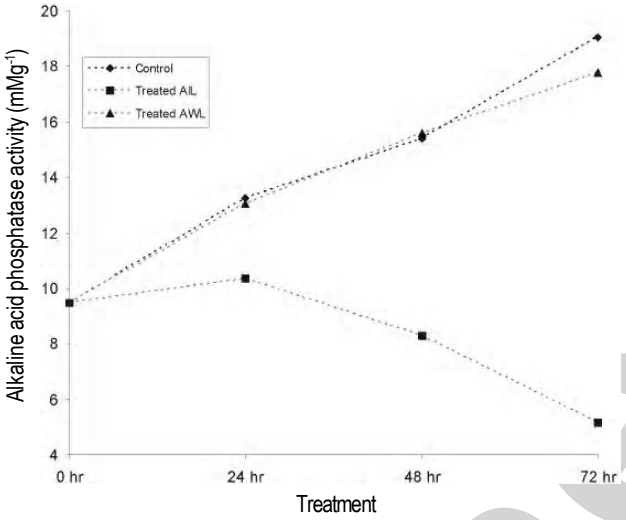

Fig. 4: Effect of *A. intermedium* and *A. wallichianum* lectin on the activity of acid phosphatases at various time intervals

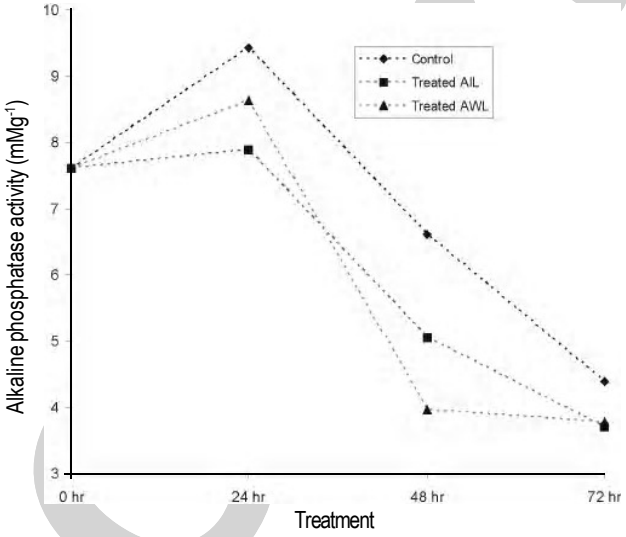

Fig. 5: Effect of *A. intermedium* and *A. wallichianum* lectin on the activity of alkaline phosphatases at various time intervals

Table - 1: Total developmental period of *B. cucurbitae* under the influence of *A. intermedium* lectin (AIL)

Concentration (µg ml^{-1})	Larval period in days (mean±SE)	Pupal period in days (mean±SE)	Total development period in days (mean±SE)
Control	8.6±0.3	10.2±0.3	18.8±0.14
10	9.6±0.2	10.4±0.2	20.0±0.2
20	10.2±0.3	10.8±0.4	21.0±0.30
40	10.9±0.2	11.4±0.4	22.3±0.3
80	11.2±0.4	11.7±0.4	22.9±0.3
160	11.7±0.4	00.0±0.0	00.0±0.0
F-value	*12.45	** 2.98	*32.26

* = Significant at 1% , ** = Significant at 5%

Table - 2: Total developmental period of *B. cucurbitae* under the influence of *A. wallichianum* lectin

Concentration (µg ml^{-1})	Larval period in days (mean±SE)	Pupal period in days (mean±SE)	Total development period in days (mean±SE)
Control	8.7±0.2	10.8±0.2	19.5±0.2
10	9.4±0.3	10.7±0.5	20.1±0.4
20	9.9±0.2	11.5±0.4	21.3±0.5
40	10.0±0.2	11.7±0.7	21.8±0.2
80	10.3±0.2	12.0±0.3	22.0±0.1
160	10.4±0.2	00.0±0.0	00.0±0.0
F-value	19.29*	1.3^{NS}	7.84*

* = Significant at 1% , ^{NS} = Non-significant

i.e. from the age of 72 hr to 144 hr. The levels are significantly high as compared to control of same age group (Fig. 3). In case of AIL and AWL the activity was 1022.6 and 837.7 mM g^{-1} respectively at 72 hr of treatment, respectively, while in control the activity was 309.4 mM g^{-1}. The increase in the level of esterase activity in treated larvae suggests that esterases might be playing a significant role in lectin detoxification and increased activity can be attributed to positive feedback response (Rup *et al*., 1999). It is reported in literature that the major insecticide resistance mechanism in the brown planthopper *Nilaparvata lugens* Stål. involves overproduction of esterase isoenzymes (Small and Hemingway, 2000). The role of esterases in insecticide-resistance is also documented in *Anopheles stephensi* Giles (Ganesh *et al*., 2002). The present study is in corroboration with these findings. In case of larvae fed on AIL incorporated diet, acid phosphatase activity showed a significant decrease at all the three time intervals assayed as compared to respective controls. The decrease in activity was maximum at 72 hr of treatment. In case of larvae treated with AWL, the acid phosphatase activity showed a significant decrease at 72 hr of treatment compared to control (Fig. 4). Alkaline phosphatase activity showed a significant decrease in larvae treated with either of these lectins at all the three time intervals as compared to respective controls (Fig. 5). These results indicate that phosphatases activity got suppressed significantly, which can be attributed to negative feedback response.

Further these enzymes were not seemed to involve in lectin detoxification in larvae fed on AIL/AWL incorporated diet.

It is evident from the above discussion that AIL and AWL have shown great potential as anti-insect compounds. The results obtained in the above mentioned study were compared with the anti-insect effect of already reported lectins from *A. helleborifolium* and *A. jacquemontii* (Kaur *et al*., 2006 a,b). However, there was no significant difference in LC_{50} being in the range of 29-39 µg ml^{-1}. Lectins from three of the four *Arisaema* species *i.e. A. helleborifolium, A. intermedium* and *A.jacquemontii* significantly suppressed acid phosphatase activity whereas *A. wallichianum* lectin has no influence on enzyme activity. All the lectins suppressed alkaline phosphatase activity, while esterases activity showed a significant increase in all the four lectins screened and increase was maximum in case of two lectins under present study. These findings indicated that lectin molecules present in the four species have almost similar anti-insect potential. So, lectin gene/s from either of these species can be cloned and subsequently can be employed to develop transgenics to control melon fruit flies specifically and insect pests in general. This approach could be used as a part of Integrated pest management strategies (IPM).

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