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Insecticidal Activity of *Arum maculatum* Tuber Lectin and Its Binding to the Glycosylated Insect Gut Receptors

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Mannose binding ~50 kDa homotetrameric lectin, purified from edible *Arum maculatum* tuber, was analyzed through SDS–PAGE and studied for its agglutination property using rabbit erythrocytes. Cross reactivity of the purified lectin was verified through western blot using *Colocasia esculantum* (Family, Araceae) tuber lectin antibody. The insecticidal activity of *Arum maculatum* tuber lectin (ATL) was tested against two economically important sucking pests, *Lipaphis erysimi* and *Aphis craccivora*, in an artificial diet. The LC_{50} values for *L. erysimi* and *A. craccivora* were determined to be 21 μg/mL and 16 μg/mL, respectively. Addition of α-D-mannose in ATL-supplemented diet reduced the aphid mortality. Two major receptor proteins of ATL (~40 kDa and ~35 kDa) were detected from the brush border membrane vesicle (BBMV) protein of *L. erysimi* and *A. craccivora* guts, respectively, using ligand-binding assay. α-D-Mannose was found to be a deterrent to such binding of ATL to the BBMV receptors.

KEYWORDS: ***Arum maculatum* tuber lectin (ATL); *Lipaphis erysimi*; *Aphis craccivora*; LC_{50}; BBMV; mannose inhibition.**

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INTRODUCTION

Increasing food production is highly desirable to meet the demand of the ever-growing population of the world. However, this production has to be achieved from the available cultivable land area, which is decreasing day by day. During the past decades, plant breeders have developed varieties with high-yield potential that have already reached a plateau of productivity. Additionally, much emphasis on the development of disease and insect resistance leads to a poor quality of produce. Development of resistance by conventional breeding techniques is limited due to the lack of sufficient and satisfactory levels of genetic variability within a cultivated gene pool. Moreover, conventional insect resistance is controlled by several loci; therefore, the progress is slow and difficult to achieve. One scientifically sound strategy to achieve increased crop productivity is to minimize pest-associated losses. Insect pests cause direct loss to agricultural production. Apart from the direct damage, they also act as vectors of various plant pathogens. *Lipaphis erysimi* and *Aphis craccivora* are two such pests, affecting the most important oil seed crop, the Indian mustard, and the leguminous crops, namely, chickpea and pigeon pea, respectively, by extracting plant nutrients and also by transmitting viral diseases.

Controlling insect pests through synthetic insecticides is costly and environmentally hazardous. The search for naturally occurring cheaper and more environment friendly biocontrol agents is going on throughout the world. The most popular biological control agent, *Bacillus thuringiensis* δ-endotoxin, though found to be very effective against many insects (*1,2*), is not effective against homopteran sucking pests. On the other hand, the insecticidal activity of some plant lectins against homopteran, lepidopteran, dipteran, and coleopteran insects has been well documented (*3*). More precisely, the efficacy of carbohydrate-binding plant lectins, like *Galanthus nivalis* agglutinin, *Phaseolus* haemagglutinin, and wheat germ agglutinin, has been reported against homopteran pests when fed through an artificial diet as well as with plant samples engineered with lectins (*4–17*). Lectins, especially mannose-binding ones from monocotyledonous plants, have been found to be effective against sucking pests (*4*, *14–16*) due to their interaction with a specific carbohydrate residue of the cell membrane. Biochemical characterization and cloning of gene sequences of several closely related members of the mannose-binding super family of lectins has been successfully studied in detail.

Very little emphasis has been given so far to the study of the mechanism of action of these lectins against insects; hence, the knowledge of this aspect is incomplete. However, it seems that resistance to proteolytic degradation by insect digestive enzymes and binding to the insect gut epithelial cells are absolute prerequisites for such control agents to act. The gut epithelial cells of insects are lined with glycoproteins, providing a number of choices for binding of the lectins.

Therefore, the present studies were undertaken to purify a lectin from the common edible tuber, *Arum maculatum,* investigate the antagonistic property, and unravel its possible mode of action against *L. erysimi* and *A. craccivora*.

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MATERIALS AND METHODS

Plant Materials and Insects Used. Fresh *A. maculatum* tubers were collected from a local garden. Nymphs and adults of mustard (*L. erysimi*) and chickpea (*A. craccivora*) aphids were collected from the experimental farm. Aphid cultures were maintained on mustard and chickpea plants at 25 °C and 70% relative humidity.

Extraction and Purification of Lectin through Chromatographic Technique. Twenty grams of fresh tuber tissue was used for the crude preparation as described previously by Van Damme et al. (18). This was further adsorbed over α-D-mannose on a 4% beaded agarose affinity chromatographic column (Sigma) equilibrated with 20 mM TrisCl, pH 7.2 (*4*). The column was washed with 20 mM TrisCl (pH 7.2) until the OD at 280 nm was below 0.01. The protein was desorbed with 20 mM unbuffered 1,3-diaminopropane (DAP) and checked in 15% SDS−PAGE. The fractions with lectin were pooled and dialyzed against 20 mM phosphate-buffered saline (PBS) (pH 7.2). The dialyzed sample was then loaded onto a DEAE-sephacel ion-exchange column equilibrated with 20 mM PBS (pH 7.2). The matrix was then washed with 20 mM PBS (pH 7.2), and a gradient of 0−500 mM NaCl in PBS (pH 7.2) was passed through. The eluted fractions, after verification in SDS−PAGE, were collected, pooled, lyophilized, and dialyzed against 20 mM TrisCl (pH 7.2) for further use. The purity of the purified lectin was monitored through SDS−PAGE analysis.

Characterization of ATL through Agglutination Assays. The purified lectin fractions were extensively dialyzed against PBS. The lectin solutions were dispensed into 10 wells of a microtiter plate at the concentrations of 1−10 μg/30 μL PBS. Erythrocytes were collected from rabbit blood by centrifugation. The erythrocytes were extensively washed with 0.9% saline solution and finally made up to 20% (v/v) with 0.9% saline. Erythrocyte suspension (20 μL) was then dispensed in each microtiter well and kept at 37 °C. The agglutination reaction was observed under a microscope after 1 h.

Western Blot Analysis of ATL. Anti-*Colocasia* IgG fraction was used to perform western blot analysis of the purified ATL to check the cross reactivity of ATL against Anti- *Colocasia* antibody (*15*).

Insect Bioassay in Artificial Diet. Insect bioassays were conducted using an artificial diet (*4*). Second instar nymphs of *A. craccivora* and *L. erysimi* were used in multiple sets. Polycarbonate petriplates, which were used as a bioassay cage, were perforated for air passage at the bottom. Nymphs were released into the plates, and the upper edges of the plates were covered with fully stretched Parafilm. A modified synthetic diet mixture (200 μL), supplemented with different concentrations of ATL (5, 10, 25 μg/mL), was dispensed on the stretched Parafilm membrane. Another Parafilm membrane was stretched onto this, to make a pouch. For control plates, 20 mM TrisCl, pH 7.4, was added to the artificial diet instead of ATL. For another set of control experiments, aphids were fed with only 20 mM TrisCl, pH 7.4, without the diet. Four replications for each dose were used. Data were recorded on the numbers of nymphs surviving at a 12 h interval for 72 h (**Figure 2**). The LC_{50} values for the toxin corresponding to each insect were determined by Probit analysis (*19*).

To check the effect of α-D-mannose on aphid mortality, different concentrations of α-D-mannose (0, 0.5, 1, and 2 M) were added to the artificial diet supplemented with respective LC_{50} doses of ATL (16 μg/mL for *A. craccivora* and 21 μg/mL for *L. erysimi*). Four replications of each set were used, and data were recorded on the percentage of aphid survival after 48 h.

Isolation of Brush Border Membrane Vesicles. For isolation of total brush border membrane vesicles (BBMV), approximately 1000 aphids were dissected under a simple microscope, and their midguts were isolated. Isolated midguts were stored at room temperature in insect ringer solution (0.65% NaCl, 0.25% KCl, 0.03% $CaCl_2$) until further study. The midguts were homogenized in a hand-held Potter homogenizer in a minimum volume of the isolation buffer (300 mM Mannitol, 5 mM EGTA, 12 mM TrisCl; pH 7.4). BBMV was isolated from total gut extract using the method described by Van Rie et al. (*20*) and Banerjee et al. (*21*).

Identification of the BBMV Receptor Protein of ATL. The BBMV pellet was resuspended in 20 mM TrisCl and electrophoresed in a 12% SDS−PAGE. After electrophoresis, they were transferred to a Hybond-C membrane (Amersham Biosciences). The membrane was blocked with 5% nonfat milk solution in PBS and incubated for 1 h. After a wash with PBS, the membrane was incubated with 10 nM of ATL for 24 h at 37 °C. The membrane was then washed with PBST and further incubated for 2 h with anti-*Colocasia* lectin antibody at 1:10 000 dilution. Anti-rabbit IgG secondary antibodies with HRP conjugate were used for probing the primary antibodies after washing the primary antibodies with PBST. Western blot was developed using the Amersham hybond-ECL kit on a hyperbond-ECL film.

The effect of mannose on ATL-receptor binding was studied using ligand blot analysis, where 1 M α-D-mannose was preincubated for 12 h along with 10 nM ATL before probing the membrane. The membrane was then developed as described above.

Glycoprotein Specific Staining of BBMV. BBMV proteins from both the aphids were run on 12% SDS−PAGE and stained specifically for glycoproteins using the method of Moller and Poulsen (22). The gel was then further stained with coomasie brilliant blue to see the total protein profile.

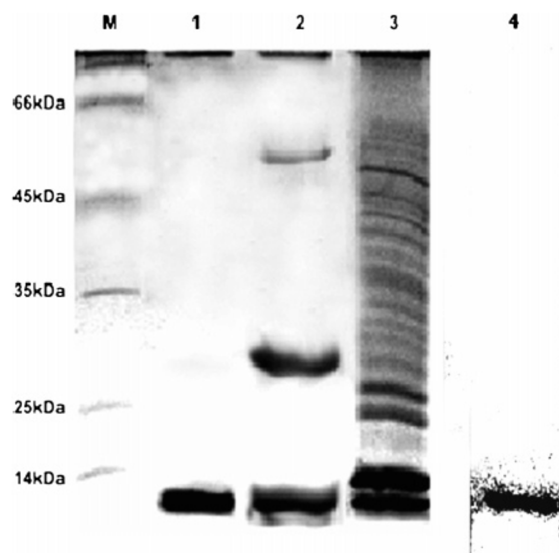
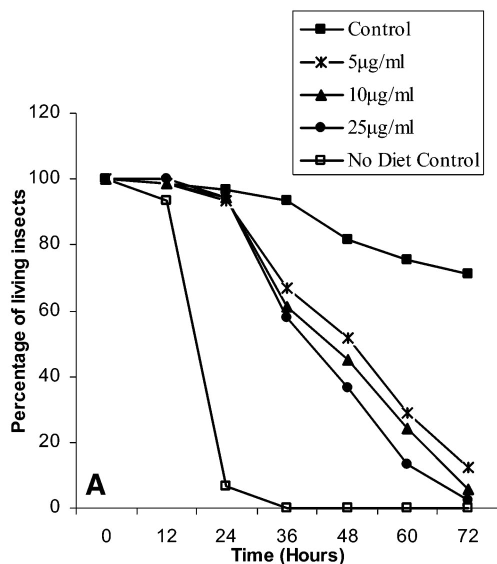


Figure 1. SDS–PAGE and western blot analyses showing ATL purification and its cross reactivity. Lane **M**: molecular weight marker. Lane **1**: purified fraction of ATL. Lane **2**: affinity chromatography purified sample showing contaminating bands along with desired ATL band. Lane **3**: crude protein profile of *A. maculatum* tuber. Lane **4**: western blot analysis of purified ATL showing cross reactive ∼12.5 kDa band recognized by anti *Colocasia* antibody.

RESULTS

Analysis of *A. maculatum* Tuber Lectin (ATL) through SDS−PAGE. The first affinity column-purified fractions of *A. maculatum* lectin analyzed using 15% SDS−PAGE, demonstrated that, in all fractions, there were a few bands of contaminating proteins (**Figure 1**, lane **2**). These pooled fractions, after passing through a DEAE-Sephacel column, were eluted at 450 mM NaCl (**Figure 1**, lane **1**). The purity of the lectin was assayed as ∼12.5 kDa monomer using SDS−PAGE. Western blot analyses carried out with anti-*Colocasia* (a close relative of *A. maculatum* of the Araceae family) antibody as the primary antibody established the cross reactivity of ATL (**Figure 1**, lane **4**). The molecular weight of purified ATL under native conditions was analyzed using gel filtration chromatography and was found to be ∼50 kDa which matches with the observation of Van Damme et al. (*18*).

Agglutination Assays. The agglutination assays performed with different concentrations of purified ATL, on rabbit erythrocytes, demonstrated that the erythrocytes agglutinated



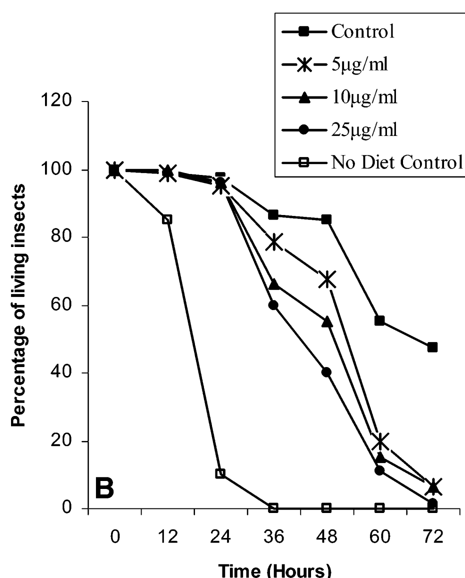


Figure 2. Bioassay of ATL against aphids. Second instar nymphs were used for the assay, 20 nymphs in the case of *L. erysimi* and 15 nymphs in the case of *A. craccivora* were used in 4 replicates for each bioassay. The graphs show the percentage survival of aphids plotted against time in hours, and each point indicates the percentage at a particular concentration of ATL. **A**: survival percentage graph of *L. erysimi*. **B**: survival percentage of *A. craccivora*.

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within 15 min of incubation even at a lowest concentration of 1 μg/30μl. Similar results were obtained in cases of previously reported lectins (*4*, *15*, *16*). However, α-D-mannose inhibited the agglutination reaction at 100 mM concentration when incubated with ATL before adding to erythrocytes.

Insect Bioassay with an Artificial Diet Supplemented with Different Concentrations of ATL. The LC_{50} values of ATL for *L. erysimi* and *A. craccivora* were calculated to be 21 μg/mL and 16 μg/mL, respectively (**Figure 2** and **Table 1**). However, for aphids fed with LC_{50} concentrations of ATL mixed with α-D-mannose, the rate of mortality due to ATL changed after 48 h. The presence of α-D-mannose at concentrations of 0.5, 1, and 2 M with 16 μg/mL and 21 μg/mL of ATL in artificial diet for *A. craccivora* and *L. erysimi,* respectively, exhibited a reduction in insect mortality (**Table 2**). Thus, it is evident from the present finding that α-D-mannose interferes with the insect mortality rendered by ATL.

Table 1. Susceptibility of Aphids to ATL

aphid	LC_{50}	fiducial limit (95%) lower	fiducial limit (95%) upper	regression equation	SE of slope	χ^2 value	d.f.
L. erysimi	21 μg/mL	11.35	51.08	$y = 3.339 + 1.286x$	0.37	0.029	1
A. craccivora	16 μg/mL	13.03	20.76	$y = 4.199 + 0.67x$	0.4	1.48	1

Table 2. Antagonistic Effect of α-D-Mannose on ATL Toxicity against Aphids after 48 Hours[a]

aphid	mortality percentage: diet + ATL	diet + ATL + 0.5 M mannose	diet + ATL + 1 M mannose	diet + ATL + 2 M mannose
L. erysimi	50	40	25	12.5
A. craccivora	55	30	20	7.5

[a] In all cases the respective LC_{50} values of ATL concentration were supplemented with diet. The data in each column represents the percentage mortality of aphids.

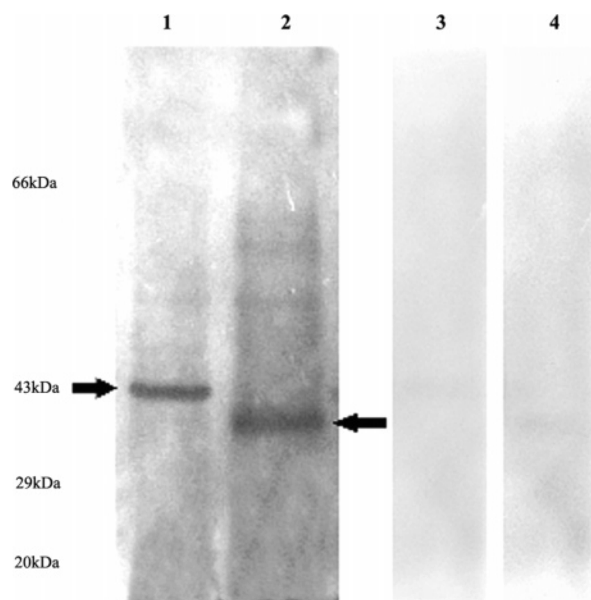

Figure 3. Ligand blot analysis of BBMV receptors of ATL. Lane **1**: ligand blot of *L. erysimi* BBMV proteins, with the arrow showing the ~40kDa receptor of ATL. Lane **2**: ligand blot profile of *A. craccivora* BBMV proteins, with the arrow showing the ~35kDa receptor of ATL. Lane **3**: ligand interaction inhibited by 1 M α-D-mannose of *L. erysimi* BBMV proteins. Lane **4**: ligand interaction inhibited by 1 M α-D-mannose of *A. craccivora* BBMV proteins.

Ligand Assay of BBMV Receptor Protein of ATL. BBMV preparations transferred in membrane after the SDS−PAGE run, when challenged with anti-Colocasia antisera, ~40 kDa protein (**Figure 3**, lane **1**) and ~35 kDa protein (**Figure 3**, lane **2**), respectively, of *L. erysimi* and *A. craccivora*, lighted up. Although a few other bands showed weak signals, the above-mentioned two bands were quite apparent indicating their strong specificity to ATL.

The competitive inhibitory effect of α-D-mannose at 1 M concentrations on the aphid BBMV receptor is shown by the lack of binding signal (**Figure 3**, lanes 3 and 4, for *L. erysimi* and *A. craccivora*, respectively). The results suggest that the binding of ATL to the receptor in the insect midgut takes place at the N-linked mannosylated oligosaccharide and is dependent upon the mannose-binding capacity of the lectin.

Glycoprotein Specific Staining of BBMV. The specific staining method used here usually stains all the proteins including glycoproteins under longer development period, while

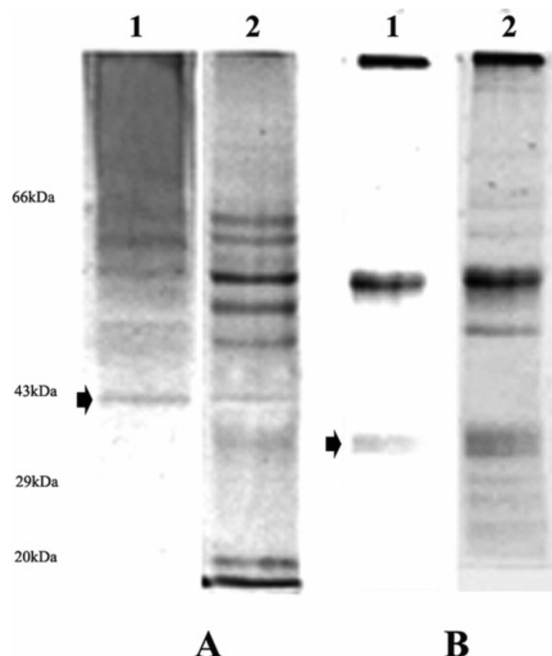

Figure 4. Monitoring of the glycosylated nature of BBMV receptors. Panel **A**: BBMV receptors of *L. erysimi.* Lane **1**: BBMV receptors after glycoprotein specific staining, with the arrow showing the ligand positive band. Lane **2**: the same, stained with coomassie brilliant blue. Panel **B**: BBMV receptors of *A. craccivora*. Lane **1**: BBMV receptors after glycoprotein specific staining, with the arrow showing the ligand positive band. Lane **2**: the same, stained with coomassie brilliant blue.

in a shorter period of development (30−45 s), only glycoproteins are stained (*22*). **Figure 4** demonstrates that respective ligand positive receptor proteins along with many other BBMV proteins of *L. erysimi* as well as *A. craccivora* are indeed glycoproteins which had been supported by the previously described mannose inhibited ligand binding assay (as shown in **Figure 3**).

DISCUSSION

The insecticidal activity of different plant lectins reported in recent years represent themselves as naturally occurring insecticides against the pests which restrain increased crop production. The efficacy of the mannose binding superfamily lectins, viz. *Galanthus nivalis* agglutinin (GNA), *Allium sativum* leaf agglutinin (ASAL), *Allium sativum* bulb lectin (ASA), *Dieffenbachia sequina* agglutinin (DEA), and *Colocasia esculantum* agglutinin (CEA) had been reported previously (*4, 15*−*17*). *Arum maculatum* tuber lectin (ATL), another member of above-mentioned superfamily, was purified earlier using asialofetuin affinity chromatography (*18*), but its insecticidal activity was not studied. In the present study, ATL was purified from the crude extract of arum tuber, using mannose agarose column and subsequently through DEAE sephacel column. The same procedure was followed earlier for purification of ASAL, ASA, CEA, and DEA lectins (*4*, *15*, *16*). The cross-reactivity of ATL was authenticated by western blot analysis challenging with anti-CEA antibody. This cross-reactive property was further exploited to detect aphid BBMV receptors of ATL using the above-mentioned CEA antibody. The insecticidal activity of ATL against two important sucking pests, *L. erysimi* and *A. craccivora*, was studied using an ATL-supplemented artificial diet. The LC_{50} values were calculated to be 16 μg/mL and 21 μg/mL for *A. craccivora* and *L. erysimi,* respectively. This value is comparable to earlier data obtained on the two above aphids treated with ASAL (*4*, *16*).

Earlier studies indicated that the presence of competitive sugars in the insect diet decreased the toxicity of lectins (*23*). In the present study as well addition of free α-D-mannose to the artificial diet supplemented with ATL reduced insect mortality (**Table 2**). This clearly indicates that the insecticidal activity of ATL depends on its underlying binding to the mannose residue of the insect gut receptor. Such a competitive inhibitory effect of lectin may also have occurred when plants are transformed with carbohydrate-binding lectins. But this possibility can be ruled out for the mannose specific lectin, namely ATL, due to the absence or very small amount of mannose present in the plant system.

Lectin binding to the insect gut and the subsequent toxic effects had been reported for a number of insects (*3*, *4*). Further support for the dependence of related insecticidal activity upon binding of lectins to the gut has also been provided by Zhu-Salzman (*24*). Ligand binding assay performed to investigate the molecular mechanism of the interaction between the aphid gut receptor and ATL detected the receptor bands in the form of a chemiluminescence signal. It has further been necessary to check whether the ATL specifically binds with the mannose residue of glycoproteins present in gut epithelium. This stimulated us to use α-D-mannose as a competitive inhibitor of ATL in ligand blot analysis. The results ascertained the role of α-D-mannose in the ATL−receptor binding reaction. The glycoprotein characteristic of these interacting receptor proteins was verified using glycoprotein specific staining.

Earlier workers were unable to establish the correlation between *in vitro* binding of lectin and BBMV and its insecticidal activity (*25*). The present investigation demonstrated that *in vitro* binding of ATL is related to its toxicity. This opens the possibility of using lectins as a natural insect control agent. The disadvantages of some plant lectins as potent control agents include the following: (a) the propensity to avoid proteolysis both in insects and in mammals, and (b) the ability to bind to glycoproteins possibly due to which mammals show a high rate of oral toxicity against them (*3*). The well studied mannose binding plant lectin, GNA exhibited very little detrimental effect on rats (*26*). The possible reason for this low activity on mammals may be due to the high specificity of GNA toward the mannose residue so that it hardly finds specific glycosylated receptors to bind, thus, showing minimal toxicity within a period of 10 days of experimentation. This holds good also for ATL, as this is a member of the mannose binding superfamily of lectins, which can be considered as a new component for insect control.

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LITERATURE CITED

(1) Barton, K.; Whiteley, H.; Yang, N. S. *Bacillus thuringiensis*-Endotoxin in Transgenic *Nicotiana tabacum* Provides Resistance to Lepidopteran Insects. *Plant Physiol.* **1987**, *85*, 1103−1109.

(2) Schuler, T. H.; Poppy, G. M.; Kerry, B. R.; Denholm, I. Insect Resistant Transgenic Plants. *Trends Biotechnol.* **1998**, *16*, 168−174.

(3) Vasconcelos, I. M.; Oliveira, J. T. A. Antinutritional Properties of Plant Lectins. *Toxicon* **2004**, *44* (4), 385−403.

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Publication Date (Web): July 26, 2005 | doi: 10.1021/jf051155z

(4) Bandyopadhyay, S.; Roy, A.; Das, S. Binding of Garlic (*Allium sativum*) Leaf Lectin to the Gut Receptors of Homopteran Pests is Correlated to Its Insecticidal Activity. *Plant Sci.* **2001**, *161*, 1025−1033.

(5) Couty, A.; Down, R. E.; Gatehouse, A. M. R.; Kaiser, L.; Pham, D. M. H.; Poppy, G. M. Effects of Artificial Diet Containing GNA and GNA-Expressing Potatoes on the Development of the Aphid Parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). *J. Insect Physiol.* **2001**, *47*, 1357−1366.

(6) Czapla, T. H. Plant Lectins as Insect Control Proteins in Transgenic Plants. In *Advances in Insect Control, the Role of Transgenic Plants*; Carozzi, N., Koziel, M., Eds.; Taylor and Francis: UK, 1997.

(7) Down, R. E.; Gatehouse, A. M. R.; Hamilton, W. D. O.; Gatehouse, J. A. Snowdrop Lectin Inhibits Development and Decreases Fecundity of the Glasshouse Potato Aphid (*Aulacorthum solani*) When Administered *in vivo* and via Transgenic Plants Both in Laboratory and Glasshouse Trials. *J. Insect Physiol.* **1996**, *42*, 1035−1045.

(8) Du, J.; Foissac, X.; Carss, A.; Gatehouse, A. M. R.; Gatehouse, J. A. Ferritin Acts as the Most Abundant Binding Protein for Snowdrop Lectin in the Midgut of Rice Brown Planthoppers (*Nilaparvata lugens*). *Insect Biochem. Mol. Biol.* **2000**, *30*, 297−305.

(9) Fitches, E. C.; Woodhouse, S. D.; Edwards, J. P.; Gatehouse, J. A. *In vitro* and *in vivo* Binding of Snowdrop (*Galanthus nivalis* agglutinin; GNA) and Jack Bean (*Canavalia ensiformis*; Con A) Lectins within Tomato Moth (*Lacanobia oleracea*) Larvae; Mechanisms of Insecticidal Action. *J. Insect Physiol.* **2001**, *47*, 777−787.

(10) Fitches, E. C.; Ilett, C.; Gatehouse, A. M. R.; Gatehouse, J. A.; Greene, R.; Edwards, J. P.; Gatehouse, L. N. The Effects of *Phaseolus vulgaris* Erythro- and Leucoagglutinating Isolectins (PHA-E and PHA-L) Delivered via Artificial Diet and Transgenic Plants on the Growth and Development of Tomato Moth (*Lacanobia oleracea*) Larvae; Lectin Binding to Gut Glycoproteins *in vitro* and *in vivo*. *J. Insect Physiol.* **2001**, *47*, 1389−1398.

(11) Hilder, V. A.; Powell, K. S.; Gatehouse, A. M. R.; Gatehouse, J. A.; Gatehouse, L. N.; Shi, Y.; Hamilton, W. D. O.; Merryweather, A.; Newell, C. A.; Timans, J. C. Expression of Snowdrop Lectin in Transgenic Tobacco Plants Resulted in Added Protection Against Aphids. *Transgenic Res.* **1995**, *4*, 18−25.

(12) Karnar, S.; Venkateswami, J.; Kirti, P. B.; Chopra, V. L. Transgenic Indian Mustard (*Brassica juncea*) with Resistance to the Mustard Aphid (*Lipaphis erysimi* Kalt.). *Plant Cell Rep.* **2002**, *20*, 976−981.

(13) Powell, K. S.; Gatehouse, A. M. R.; Hilder, V. A.; Gatehouse, J. A. Antifeedant Effects of Plant Lectins and an Enzyme on the Adult Stage of Rice Brown Plant Hopper, *Nilaparvatata lugens*. *Entomol. Exp. Appl.* **1995**, *75*, 51−59.

(14) Sauvion, N.; Rahbe′, Y.; Peumans, W. J.; Van Damme, E. J. M.; Gatehouse, J. A.; Gatehouse, A. M. R. Effects of GNA and Other Mannose Binding Lectins on Development and Fecundity of the Peach-Potato Aphid *Myzus persicae*. *Entomol. Exp. Appl.* **1996**, *79*, 285−293.

(15) Roy, A.; Banerjee, S.; Majumder, P.; Das, S. Efficiency of Mannose-Binding Plant Lectins in Controlling a Homopteran Insect, the Red Cotton Bug. *J. Agric. Food Chem.* **2002**, *50*, 6775−6779.

(16) Majumder, P.; Banerjee S.; Das, S. Identification of Receptors Responsible for Binding of the Mannose Specific Lectin to the Gut Epithelial Membrane of the Target Insects. *Glycoconjugate J.* **2004**, *20*, 525−530.

(17) Rao, K. V.; Rathore, K. S.; Hodges, T. K.; Fu, X.; Stoger, E.; Sudhakar, D.; Williams, S.; Christou, P.; Bharathi, M.; Bown, D. P.; Powell, K. S.; Spence, J.; Gatehouse, A. M. R.; Gatehouse, J. A. Expression of Snowdrop Lectin (GNA) in Transgenic Rice Plants Confers Resistance to Rice Brown Planthopper. *Plant J.* **1998**, *15*, 469−477.

(18) Van Damme, E. J. M.; Goossens, K.; Smeets, K.; Leuven, F. V.; Verhaert, P.; Peumans, W. J. The Major Tuber Storage Protein of Araceae Species Is a Lectin. *Plant Physiol.* **1995**, *107*, 1147−1158.

(19) Chi, H. *Computer Program for the Probit Analysis*; National Chung Hsing University: Taichung, Taiwan, 1997.

(20) Van Rie, J.; Jansens, S.; Hofte, H.; Degheele, D.; Mellaert, H. V. Receptors on the Brush Border Membrane of the Insect Midgut as Determinants of the Specificity of *Bacillus thuringiensis* δ-Endotoxins. *Appl. Environ. Microbiol*. **1990**, *56*, 1378−1385.

(21) Banerjee, S.; Hess, D.; Majumder, P.; Roy, D.; Das, S. The Interactions of *Allium sativum* Leaf Agglutinin with a Chaperonin Group of Unique Receptor Protein Isolated from a Bacterial Endosymbiont of the Mustard Aphid. *J. Biol. Chem.* **2004**, *279*, 23782−23789.

(22) Möller, H. J.; Poulsen, J. H. Staining of Glycoproteins/Proteoglycans on SDS-Gels. In *Protein Protocols Handbook*, Walker, J. M., Eds.; Humana Press: Totowa, NJ, 1996; pp 627−632.

(23) Triguéros, V.; Wang, M.; Père, D.; Paquereau, L.; Chavant, L.; Fourniey, D. Modulation of Lectin Insecticidal Activity by Carbohydrates. *Arch. Insect Biochem. Physiol.* **2000**, *45*, 175−179.

(24) Zhu-Salzman, K. J.; Shade, R. E.; Koiwa, H.; Salzman, R. A.; Narasimhan, M.; Bressam, R. A.; Hasegawa, P. M.; Murdock, L. L. Carbohydrate Binding and Resistance to Proteolysis Control Insecticidal Activity of *Griffonia simplicifolia* Lectin II. *Proc. Natl. Acad. Sci. USA*, **1996**, *95* (25), 15123−15128.

(25) Harper, S. M.; Crenshaw, R. W.; Mullins, M. A.; Privalle, L. S. Lectin Binding to Insect Brush-Border Membranes. *J. Econ. Entomol*. **1995**, *88* (5), 1197−1202.

(26) Pusztai, A.; Koninkx, J.; Hendriks, H.; Kok, W.; Hulscher, S.; Van Damme, E. J. M.; Peumans, W. J.; Grant, G.; Bardocz, S. Effect of the Insecticidal *Galanthus nivalis* Agglutinin on Metabolism and the Activities of Brush Border Enzymes in the Rat Small Intestine. *J. Nutr. Biochem*. **1996**, *7*, 677−682.

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