

## Snowdrop Lectin (GNA) Affects Growth and Development of *Spodoptera exigua* (Hubner)

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### ABSTRACT

Beet armyworm (*Spodoptera exigua* (Hubner)) (Lepidoptera: Noctuidae) is the most economically important sugar beet (*Beta vulgaris*) pest worldwide. The main control method of this pest is insecticides use. Thus, it is important to develop alternative means of controlling this pest, including host plant resistance using plant lectins. In the current study, the effects of GNA (*Galanthus nivalis* agglutinin) on the growth and development of beet armyworm were investigated using artificial diet. The presence of GNA in the diet at a level of 0.1, 0.25, 0.5 and 1.0% of the total dietary protein significantly reduced larval and pupal survivability compared with the control insects ( $P < 0.001$ ). When high doses of GNA (0.5 and 1.0% of dietary protein) were incorporated into the diet, no larvae reached the fourth stadium. Only the lowest dose (0.1% GNA) allowed for larval and pupal development to continue to adult. The lectin retarded larval development in a dose dependent manner. For example, developmental time of the first instar larva in the control was 2.3 days, while in 0.1, 0.25, 0.5, and 1.0% GNA treatments this value increased to 2.6, 2.7, 2.8, and 2.9 days, respectively. Larval developmental time (time taken from neonate first instar larvae to pupation) in the control and lectin treatment (0.1% GNA) were 14.1 and 17.2 days, respectively. GNA also affected duration of pupal period, adult longevity, and adult emergence. In conclusion, it should be said that GNA has a good potential to be used in IPM program in order to combat this insect species.

**Keywords:** Beet armyworm, *Galanthus nivalis*, Growth.

### INTRODUCITON

Beet armyworm (*Spodoptera exigua* (Hubner)) (Lepidoptera: Noctuidae) is the most economically important sugar beet (*Beta vulgaris*) pest worldwide. Being polyphagous, it is a major pest of many field crops such as alfalfa, corn, cotton, sorghum, soybean, tobacco and many vegetable crops such as bean, cabbage, chickpea, and cowpea (Cartwright *et al.*, 1987; Ruberson *et al.*, 1994). Due to indiscriminate use of insecticide against this pest, it has developed a high level of resistance to many conventional insecticides. Therefore, it is important to develop alternative methods of controlling this pest, exploiting factors such

as host plant resistance (Gatehouse and Hilder, 1994; Shukla *et al.*, 2005). However, the levels of resistance in the cultivated species are quite low and there is a need to explore the possibility of using novel gene/s expressed in genetically modified plants to control these pests (Murdock and Shade, 2002). In recent years, considerable progress has been made in developing transgenic plants with expressing toxin genes from *Bacillus thuringiensis* (Bt) in different crops, against many different insect pests, especially lepidopteran and coleopteran pests (Tabashnik *et al.*, 1997). However, problem associated with development of insect resistance to Bt toxins in genetically engineered crops has resulted in interest in

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exploiting plant defensive proteins such as lectins for deployment through transgenic crops to control insect pests (Fitches *et al.*, 1997). Lectins are a class of proteins, isolated from a diverse array of organisms including bacteria, fungi, higher plants, and vertebrate and invertebrate animals that bind carbohydrates (Harper *et al.*, 1995; Peumans and Van Damme, 1995). In plants, lectins are present in many tissues, but they primarily occur in seeds where they may constitute a large portion of the total seed protein (Czapla and Lang, 1990). It has been suggested that plant lectins function as a defense mechanism against a variety of fungal, bacterial, and viral pathogens as well as animal herbivores including insects (Peumans and Van Damme, 1995; Fitches and Gatehouse, 1998). Lectin toxicity to higher animals is thought to be mediated by lectin binding to glycoproteins on the inner surface of the gut (Pusztai, 1991). GNA (*Galanthus nivalis* agglutinin, snowdrop lectin) was first isolated from snowdrop bulbs by performing affinity chromatography on immobilized mannose. This lectin exhibits strict specificity for  $\alpha$ -D-mannose and is thus considered as part of the monocot mannose-binding family of lectins (Chrispeels and Raikhel, 1991; Peumans and Van Damme, 1995). GNA is non-toxic to mammals but is toxic to several economically important insect pests of the Homoptera, Coleoptera and Lepidoptera in both *in vitro* and transgenic plants (Czapla and Lang, 1990; Harper *et al.*, 1995; Fitches *et al.*, 1997; Setamou *et al.*, 2003; Shukla *et al.*, 2005). However, the effects of GNA in insects vary and its toxicity against insects is species-specific (Harper *et al.*, 1995). When incorporated into artificial diet at 0.1% (W/V) concentration, GNA significantly affected both the development and survival rates of *Empoasca fabae* H. (Powell *et al.*, 1993). Incorporation of snowdrop lectin at three concentrations of 0.5, 1.0, and 2.0% of total dietary protein in the artificial diet of *Eoreuma loftini* Dyar significantly decreased larval survivability and adult emergence (Setamou *et al.*, 2003). Several plant lectins

have been found to have adverse effects on the larval development and survival of different species including the tomato moth (*Lacanobia oleracea*) (Fitches *et al.*, 1997), *Helicoverpa armigera* (Shukla *et al.*, 2005), *Ostrinia nubilalis* and *Diabrotica undecimpunctata* (Czapla and Lang, 1990), *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Osborn *et al.*, 1988), and *Aulacorthum solani* (Down *et al.*, 1996). The present study was undertaken to explore the effects of different concentrations of GNA on beet armyworm larval and pupal mortality, larval and pupal development time, larval weight, pupal and adult emergence and longevity.

## MATERIALS AND METHODS

### Insect Culture

A culture of insect was originally collected from a sugar beet farm in Mashad Province, Iran. The insects were maintained at  $25\pm 2^{\circ}\text{C}$  and a relative humidity of  $55\pm 5\%$  under a 16:8 (L:D) photoperiod. Insects were reared on artificial diet based on that developed for the Beet armyworm (Merx-Jacques and Bede, 2005). Paper strips inserted in the insects reared containers served as oviposition substrate. Wild individuals were regularly incorporated into these colonies to maintain colony vigor.

### Insect Bio-assays on Artificial Diet

GNA was obtained from Sigma (Sigma Company, USA). Bioassays were conducted on artificial diet supplemented with four concentrations of GNA: 0.1, 0.2, 0.5 and 1.0% of total dietary protein ( $0.02\text{ mg ml}^{-1}$ ). Also, one control diet was included that consisted of the artificial diet supplemented with an equivalent weight of casein to account for the extra protein (i.e. GNA) added to the experimental diet. GNA was added to the artificial diet only when the diet temperature fell below  $40^{\circ}\text{C}$ . For each

treatment, 30 first instar larvae was used. Using a fine hair brush, newly emerged larvae (0-24 hour old) were individually placed in 40 ml plastic airtight cups containing a small piece of diet. Seven days after larvae were transferred to the cups, small air-holes were made in the cup lids. The cups were placed in trays, one tray per treatment, and kept in an incubator in aforementioned conditions.

Fresh diet was prepared every week and stored at 4°C in an airtight container. Fresh diet, depending on the larval size, was provided to the larvae daily. Larval mortality, larval weight (0-24 hour post emergence), pupal weight (0-24 hour post emergence), pupal mortality, larval development time (time taken for each larval instar), pupal development time (time taken between pupation and adult emergence) and the percentage of insects that reached the pupal and adult stages were monitored and recorded.

#### Determination of Protein Content

Protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

#### Data Analysis

All data analysis was carried out using

SAS (SAS institute, 1996). One way ANOVA (Analysis of Variance) was conducted to evaluate treatment effects on larval and pupal weights, percent of pupation, adult emergence, larval and pupal developmental time and mortality. Mean comparisons were made using Duncan's multiple range test.

## RESULTS

### The Effect of GNA on Larval and Pupal Survivability

The effect of GNA on larval and pupal development is shown in Table 1. As seen in the Table, the presence of GNA in the diet significantly increased larval and pupal mortality compared with the controls ( $P < 0.001$ ). Also, there were significant differences between the treatments. When high doses of GNA (0.5 and 1.0% of dietary protein) were incorporated into the diet, none of the larvae reached the fourth stadium (Table 1). For example, in 1.0% GNA, percentage of mortality as first, second, and third instar larvae was 36, 33, and 30, respectively. Only at the lowest dose (0.1% GNA) did larval and pupal development continued to adult stage. However, only 36.7% of larvae reached adult stage and 63.3% insects died either in the larval (50%) or pupal (13.3%) stages.

**Table 1.** The effect of GNA incorporated into the artificial diet on different larval instars and pupae survivability (%) of beet armyworm.

Dose*	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Prepupa	Pupa
Control	93.3±2.1a**	92.8±2.2a	96.1±1.8a	100.0±0.01a	100.0±0.01a	96±3.2a	91.66±1.5a
0.1	83.3±1.7b	84.0±1.9b	85.7±1.5b	88.8±1.9b	93.7±2.1b	86.66±1.6b	76.92±2.9b
0.25	70.0±1.8c	52.3±1.4c	36.3±0.95c	-	-	-	-
0.5	66.6±1.1d	50.0±0.99d	-	-	-	-	-
1	63.3±1.2e	47.3±1.1e	-	-	-	-	-
F	19.41	10.36	36.14	25.73	21.34	11.14	10.12
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
df	4	4	4	1	1	1	1

\* GNA incorporated into the artificial diet at a level of 0.1, 0.25, 0.5 and 1% of total protein.

\*\*Means±SE followed by different letters are significantly different using Duncan test.



### The Effects of Lectin on the Insect Developmental Time and Longevity

The lectin significantly increased larval developmental time (Table 2). In other words, dietary GNA retarded larval development in a dose dependent manner i.e. the lectin dose increased in approximately the same proportion as the larval developmental time. For example, developmental time of the first instar larva in the control was 2.3 days, while in 0.1, 0.25, 0.5 and 1.0% GNA treatments, development took 2.6, 2.7, 2.8, and 2.9 days respectively (Table 2). The time taken for the second instar larvae to go to the next stage in the control and GNA treatments was 2.1, 3.2, 3.6, 3.7 and 3.8 days, respectively. Developmental time retardation in the first instar larvae was 0.28–0.6 days and in the second instar larvae were 1.4-1.9 days in different treatments. Only at the lowest dose (0.1% GNA) could larvae complete their growth and development and reached adult stage. Larval developmental time (time taken from the first instar larva to pupa) in the control and in the 0.1% GNA treatment was 14.1 and 17.2 days, respectively (Table 2). The corresponding pupal developmental time (prepupa and pupa) in the control and in 0.1% GNA treatment was 10.5 and 11.2 days. Therefore, retardation of development time of the larva and pupa in 0.1% GNA treatment was 3.1 and 0.7 days, respectively. Adult longevity decreased significantly with lectin treatment ( $F= 5.19$ ,  $df= 1$ ,  $P< 0.001$ ). For example, adult longevity in the control was 10.3 days while for the 0.1% GNA treatment it was 6.1 days (Table 2).

### The Effect of GNA on Larval and Pupal Weight

The effect of GNA on larval and pupal weight was determined using two treatments (0.1 and 0.25% GNA). Larvae were given GNA from the first stadium but weight determination was undertaken from the third stadium. As seen in Table 3, there were

Table 2. The effects of GNA on larval and pupal developmental time (day) and adult longevity (day) of beet armyworm.

Dose	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Prepupa	Pupa	Adult
Control	2.33±0.03 d**	2.13±0.03 d	2.63±0.08 c	2.26±0.03 b	4.76±0.06 b	1.93±0.06 b	8.56±0.1 b	10.33±0.3a
0.1	2.61±0.02 c	3.21±0.01 c	3.70±0.05 b	2.72±0.01 a	5.00±0.05 a	2.23±0.03 a	8.96±0.3 a	6.10±0.1b
0.25	2.74±0.06bc	3.63±0.01 b	4.28±0.03 a	-	-	-	-	-
0.5	2.85±0.02d	3.72±0.05 ab	4.52±0.02 a	-	-	-	-	-
1	2.93±0.06 a	3.81±0.05 a	4.98±0.02a	-	-	-	-	-
F	2.11	2.24	4.18	0.98	4.02	1.93	5.41	5.19
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
df	4	4	4	1	1	1	1	1

\* GNA incorporated into the artificial diet at a level of 0.1, 0.25, 0.5 and 1% of total protein.

\*\*Means±SE followed by different letters are significantly different using Duncan test.

**Table 3.** The effects of GNA incorporated into the artificial diet on different larval instars and pupal weight (g).

Dose*	Second instar	Third instar	Fourth instar	Fifth instar	Pupae
Control	0.0073±0.0005a**	0.026±0.005a	0.087±0.006a	0.205±0.001a	0.188±0.006a
0.1	0.0035±0.0002b	0.017±0.005c	0.074±0.005c	0.194±0.005b	0.156±0.005b
0.25	0.0030±0.0002bc	0.015±0.005c	0.077±0.001b	-	-
0.5	0.0029±0.0002c	0.020±0.001b	-	-	-
1	0.029±0.0001c	0.021±0.001b	-	-	-
F	8.54	11.58	16.73	21.97	10.21
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
df	4	4	2	1	1

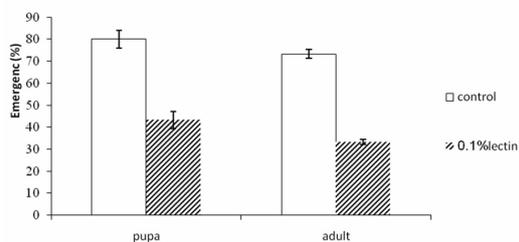
\*GNA incorporated into the artificial diet at a level of 0.1, 0.25, 0.5 and 1% of total protein.

\*\*Means±SE followed by different letters are significantly different using Duncan test.

significant differences in larval and pupal weight between the treatments ( $P < 0.001$ ). For example, the third instar larval weight in the control and in the two treatments (0.1 and 0.25% GNA) was 0.03, 0.02 and 0.02 mg, respectively. Almost the same results were obtained when the weight of the fourth and fifth instar larvae was taken (Table 3). There were also significant differences in pupal weights between the lectin treatments and the control. Pupal weights in the control and the GNA treatment were 0.06 and 0.03 mg, respectively.

### The Effect of Lectin on Percentage of Pupal and Adult Emergence

GNA had a serious effect on the emergence of pupal and adult insects. As seen in Figure 1, the percentages of pupal emergence in the control treatment was



**Figure 1.** The effects of GNA incorporated into the artificial diet on percentage of pupal and adult emergence.

about 80%, whereas in GNA-treated insects, it fell to 43.3% ( $F = 0.92$ ,  $df = 1$ ,  $P = 0.83$ ) (Figure 1).

Also, difference in the percentage of adult emergence between control and treatment was significant. For example, adult emergence in the control and GNA treatment were 73.3% and 33.3%, respectively ( $F = 1.42$ ,  $df = 1$ ,  $P = 0.83$ ) (Figure 1).

## DISCUSSION

The current study provides new data on the effects of pure GNA delivered through artificial diet on beet army-worm. The results shows that the effect of GNA on beet army worm is dose dependent i.e. higher GNA levels in artificial diet lead to stronger deleterious effects on the insects. In this study, it was found that larval survivability significantly decreased when they were reared on artificial diet containing higher dose of GNA. Larval survival varied from zero, in larvae reared on diet containing high doses of GNA (0.25, 0.5 and 1.0% of dietary protein), to about 30%, in larvae reared on diet containing only 0.1% GNA. The effects of GNA on insect survival in this study were similar to those found for other insects (Shukla *et al.*, 2005; Setamou *et al.*, 2003; Czaplá and Lang, 1990; Fitches *et al.*, 1997). Czaplá and Lang (1990) found that lectins from wheat (*Triticum vulgare* L.), castor



beans (*Ricinus communis* L.), and camels foot tree (*Bauhinia purpurea* L.) were lethal to the neonate larvae of *Ostrinia nubilalis* (Hubner) when these lectins were topically applied to the diet surface as a 2.0% solution. Shukla *et al.* (2005) found that larval survival was significantly affected when artificial diet was impregnated with snowdrop lectin and chickpea lectin. Incorporation of snowdrop lectin, chickpea lectin, and soybean trypsin inhibitor into the artificial diet of *Helicoverpa armigera* lowered survivability of larvae to 64%, 56% and 49%, respectively (Shukla *et al.*, 2005). Impregnation of GNA at three concentrations (0.5, 1.0, and 2.0% of total dietary protein) in the larval diet of Mexican rice borer [*Eoreuma loftini* (Dayer)] significantly decreased larval and pupal survival (Setamou *et al.*, 2003). Setamou *et al.* (2003) found that the effects of GNA on larval and pupal mortality were not dose-dependent. In the current study, it was found that the effect of GNA on larval mortality was dose-dependent, similar to findings of Czaplá and Lang (1990). Fitches *et al.* (1997) examined the effects of GNA on tomato moth (*Lacanobia oleracea*) larval survival, growth, and development. Their study included three different assays including artificial diet containing GNA at 2.0% (w/w) dietary protein, excised leaves of transgenic potato expressing GNA at approximately 0.07% of total soluble proteins, and on transgenic potato plants expressing GNA at approximately 0.6% of total soluble proteins in glasshouse trials. They found that GNA did not affect larval survival when tested in the artificial diet and detached leaf bioassays, whereas survival decreased to about 40% in the glasshouse bioassay. They also found that larval weight was reduced by approximately 35% and larval survival decreased significantly. In the present study, it is also found that GNA significantly reduced larval weight and development time. Setamou *et al.* (2003) showed that GNA significantly reduced larval and pupal size of *Eoreuma loftini*, but did not affect larval and pupal development

periods and longevity. GNA is also toxic to sucking insects such as aphids (*Acyrtosiphon pisum*, *Myzus persicae*, and *Sitobion avenae*, *Aulacorthum solani*), plant- and leaf-hoppers e.g. rice brown plant hoppers (*Nilaparvata lugens*) and rice green leaf hoppers (*Nephotettix ciniteps*) (Powell *et al.*, 1998; Down *et al.*, 1996; Rahbe *et al.*, 1995; Murdock and Shade, 2002). GNA is the most extensively studied as anti-insect lectin because of its effects on diverse insect species including lepidopteron, coleopteran and homopteran insects (Murdock and Shade, 2002; Chrispeels and Raikhel, 1991; Van Damme *et al.*, 1998) while exhibiting low to zero toxicity to mammals (Van Damme *et al.*, 1998). Therefore, the anti-insect activity of GNA and the negligible toxicity of GNA to mammals in comparison with the other lectins make it a leading candidate for transfer and expression in crop plants (Chrispeels and Raikhel, 1991; Fitches and Gatehouse, 1998; Van Damme *et al.*, 1998; Murdock and Shade, 2002). So far, many plant species have been transformed with GNA gene including potato (*Solanum tuberosum*) (Gatehouse *et al.*, 1996), rice (*Oryza sativa*) (Rao *et al.*, 1998), wheat (*Triticum aestivum*) (Stoger *et al.*, 1999), and tobacco (*Nicotiana tabacum*) (Wang *et al.*, 1999).

In this study, it was found that GNA caused 45% and a 6% reduction in beet army worm pupal and larval weight, and prolonged larval and pupal development by 3.0 and 1.0 days, respectively. Although in this study anti-feedant effects of GNA were not studied, some reports exist regarding lectin anti-feedant effects. Also, it has been reported that lectins have deterrent effects on some insects. Effects of plant lectins on insect food behavior have specially been observed in several studies (Powell *et al.*, 1995; Fitches *et al.*, 1997; Sauvion *et al.*, 2004). A sharp drop in feeding activity of *N. lugens* adults and *L. oleracea* larvae have been reported when fed on artificial diet containing GNA (Powell *et al.*, 1995; Fitches *et al.*, 1997). When larvae of *L. oleracea* were put on transgenic plants

expressing GNA, decrease in food consumption persisted. However, in an assay using excised leaf material, larvae tended to eat more of the GNA-containing leaf. Since lectin concentrations in the excised leaves in this study were rather low (0.07% of total soluble protein), larvae might still be able to counteract any adverse effect of the lectin on their growth or development through compensatory feeding (Fitches *et al.*, 1997). Significant differences in honeydew production of *N. virescens* (rice green leaf hopper) nor of *N. lugens* (brown plant hopper) was not observed when fed GNA-containing transgenic rice as compared to wild type control plants. However, in a choice assay, *N. lugens* showed some preference for the control plants (Foissac *et al.*, 2000). Preference for wild type control plants over GNA-containing leaves also have been reported for potato aphid (*Aulacorthum solani*) as well as the onion thrips (*Thrips tabaci*) and the red spider mite (*Tetranychus urticae*) (Rovenska and Zemek, 2006).

In the present study, it was found that GNA has anti-beet armyworm property even in low doses (0.1% of dietary protein); therefore, it could be a good candidate for use in IPM program in order to combat this insect species.

#### ACKNOWLEDGEMENTS

This work was funded by a grant from the vice-chancellor for research of the University of Tehran, Iran.

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## لکتین گالانتوس نیوالیس رشد و نمو کرم برگخوار چغندرقد (*Spodoptera exigua* Hubner) را تحت تأثیر قرار می‌دهد

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### چکیده

کرم برگخوار چغندرقد (*Spodoptera exigua* Hubner) (Lepidoptera: Noctuidae) مهم‌ترین آفت چغندرقد (*Beta vulgaris*) در دنیا می‌باشد. روش اصلی کنترل این آفت استفاده از حشره کشها می‌باشد. بنابراین مهم است تا راههای جدید مبارزه جهت کنترل این حشره مانند تولید گیاهان مقاوم با استفاده از لکتین‌ها توسعه یابد. در این مطالعه اثرات GNA روی رشد و نمو کرم برگخوار چغندرقد با استفاده از رژیم غذایی مصنوعی بررسی شدند. وجود لکتین در جیره غذایی به نسبت ۰/۱، ۰/۲۵، ۰/۵ و ۱/۰ درصد کل پروتئین جیره باعث کاهش معنی دار زنده مانی لاروها و شفیره‌ها در تیمارها نسبت به کنترل گردید ( $P < 0.001$ ). وقتی که دز بالای لکتین (۰/۵ و ۱/۰ درصد پروتئین جیره) در جیره غذایی استفاده شدند هیچکدام از لاروها به سن چهارم لاروی نرسیدند. فقط پائین ترین دز (۰/۱ درصد لکتین) باعث شد که لاروها و شفیره‌ها به حشرات کامل برسند. لکتین باعث تاخیر در رشد لاروها به صورت وابسته به دز شد. برای مثال طول دوره رشدی در لارو سن یک در کنترل ۲/۳ روز بود در حالیکه در تیمارهای ۰/۱، ۰/۲۵، ۰/۵ و یک درصد لکتین طول دوره رشد لاروی به ترتیب ۲/۶، ۲/۷، ۲/۸، ۲/۹ روز بود. کل دوره رشد لاروی (زمانی که طول میکشد لارو سن یک به شفیره تشکیل شود) در کنترل و در تیمار ۰/۱ درصد لکتین به ترتیب ۱۴/۱ و ۱۷/۲ روز بود. GNA همچنین طول دوره شفیرگی، طول عمر حشرات کامل و درصد ظهور آنها را تحت تأثیر قرار داد. نتیجه گیری اینکه GNA دارای پتانسیل خوبی در جهت کنترل این آفت در برنامه‌های مدیریت انبوهی دارد.