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Genetically Modified Potatoes against the Black Cutworm *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) under Laboratory Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Some physiological and biological aspects of black cutworm *Agrotis ipsilon* feed on genetically modified potatoes, Newleaf Superior expressing Cry3Aa and Desiree GNA were examined using bioassay experiments under laboratory conditions. The results showed that significant different in body weight has been found between N.L. Superior, Desiree GNA and control with feeding larvae since 3rd larval instar. The larvae showed a highly significant prolongation in the larval duration in comparison with control and with N.L. Superior respectively. The leaves ingested and assimilated had a positive effect and significantly reduced in the larvae appeared the most food utilization parameters were less than those of the control larvae. The food consumed by the larvae was 37.36±2.87 and 30.80±1.31 mg for N.L. Superior and GNA respectively, and increased to 87.86±1.48 and 90.22±3.12 mg for the control of N.L. Superior and GNA respectively, while it reached to the maximum value with the larvae feed on non-transgenic potatoes leaves (94.16±2.48 mg). On the other hand the conversion of digested leaves to biomass was higher in the larvae feed on N.L. Superior concluded that the presence of Cry3Aa in N.L. Superior leaves and Desiree GNA

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reduced biomass increment reducing food intake and decreased digestibility but increased the ratio of digested food (ECD) converted to biomass.

Keywords: Agrotis ipsilon; Desiree GNA; genetically modified potatoes; Newleaf Superior expressing Cry3Aa.

1. INTRODUCTION

Bacillus thuringiensis (*Bt*) is a gram-positive, rod-shaped, spore-forming bacterium produces crystal (Cry) proteins that are toxic to insects [1,2]. *Bt*-based biopesticides have been used for several decades worldwide due to their larvicidal activity against target pests and environmentally friendly characteristics. The toxicity of *Bt* towards a wide range of insects, is attributed largely to the Cry proteins produced during the *Bt* growth cycle [3,4].

The proteins derived from *Bt* are called δ (delta)-endotoxins, or insecticidal crystal proteins and sometimes protoxins [5], while the genes that code for these proteins in transgenic plants are called cry genes [6]. The spores allow ion leakage that leads to cell lysis and disrupts pH gradient between the gut lumen and the haemolymph; the nutrient uptake is impaired, microorganisms invade the haemocoel, and the disturbance of homeostasis adversely affects the function of various organs. This scenario takes place in hosts that have receptors for the respective type of Cry toxin.

More than 150 Cry proteins are produced by different *Bt* strains [7]. Most act on a narrow range of insect hosts [8] and 40% are inactive on any insect [9].

Some literatures refer to these proteins as cry proteins [10]. These cry proteins bind to specific receptors in the midgut after ingestion, causing the death of the insect larva. Plants that express these cry genes are therefore protected from insects that are affected by these proteins.

It was evaluated the larvicidal activities of binary combinations (1:1 ratios) of six Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, Cry2Aa and Cry9Aa) against *Helicoverpa armigera* neonate larvae using droplet feeding bioassays, the results suggest that products containing mixtures of Cry1Ac and Cry2Aa or Cry1Ac and Cry1Ca may be useful components of *H. armigera* pest management programs [11]. The effects of corn MON810 was tested on *Spodoptera frugiperda* (J.E. Smith) under field conditions in Brazil. Results from MON810 corn fields were compared with those fields of conventional corn with and without the application of insecticides in four harvests in the region of Barretos, SP [12]. It was assessed the damage to *S. frugiperda* via direct counts of the number of fall armyworms on corn plants. The rate of *S. frugiperda* damage and the average numbers of larvae (large and small) were lower in the MON810 corn field relative to the control plot. The second crop season (“safrinha”) showed the greatest extent of *S. frugiperda* damage. MON810 was effective in controlling *S. frugiperda*.

The Cry3A proteins are selectively toxic for the beetles however it was found that Cry3Aa can cause some effects on the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) [13].

Lectins from different plants have been isolated and characterized. They are a complex and heterogeneous group of proteins that differ in the carbohydrate-binding specificity and exerted effects. Some lectins, such as the wheat germ agglutinin (WGA), are highly toxic to mammals [14] whereas others, such as the pea lectin act rather specifically on the insects. Several studies demonstrated that the pea lectin could be used as transgenic resistance factors against various insect pests. Transgenic tobacco expressing pea lectin has shown adverse effects against Cotton budworm *Heliothes virescens* [15].

The black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) is one of the most destructive insect pest attacking different field crops, such as cotton, soybean, corn, potatoes and tomatoes not only in Egypt but also in many different countries. Great losses occurred in yield due to *A. ipsilon* infestation especially at seedling stage [16].

The objective of this study was to evaluate the effect of the genetically modified potatoes, Newleaf Superior expressing Cry3Aa, and Desiree GNA on different biological and physiological aspects of the black cutworm *A. ipsilon* under laboratory conditions, and to evaluate their role that can minimize or even exclude the use of toxic insecticides in pest control, it may be suitable means for use in programs of integrated pest management (IPM).

2. MATERIALS AND METHODS

2.1 Insect Cultures

The stock of the black cutworm, *A. ipsilon*, was established from a collection in Egypt and kept in laboratory of National Research Centre for several generations at 24±2℃ and 65±5% R.H and 16:8 h light:dark photoperiod. *A. ipsilon* culture was reared on potato cv. Superior and Desiree control for more than five generations before experiments.

2.2 Potatoes

The cv. Superior and Desiree and their derived, genetically modified cv. NewLeaf Superior, which expresses the Cry3Aa endotoxin of *B. thuringiensis*, and GNA *Galanthus nivalis* agglutinin, which express lectin, were obtained from Institute of Entomology, Czech Republic while Monsanto Co. (USA) is the source of these potato plant varieties.

The potatoes were propagated as described by [13]. Leaves from well grown but non-flowering plants were used to feed *A. ipsilon* larvae.

2.3 The Bioassay Experiments

Genetically modified potatoes, Newleaf Superior expressing Cry3Aa, and Desiree GNA on the development and survival of the immature stages of *A. ipsilon*, 3^{rd} instar larvae, were determined. Stock culture of *A. ipsilon* was reared in the laboratory at 22±2℃ and 65±5% R.H. 3^{rd} instar larvae with weight range of 13-20 mg were chosen from the stock culture for bioassay study, they were starved for four h before being transferred to Petri dish .Discs of leaves (2.5 cm) of each potato type were placed separately in Petri-dishes (10x2.5 cm^2.) and offered to chosen larvae to eat daily till prepupae. Ten Petri-dishes were used per each type of potatoes; the same number of larvae placed individually on discs of normal leaves of potato as control. The larvae fed on transgenic and non-transgenic were checked daily to estimate the survival and larval development till pupation and pupal weight.

To determine the effect of transgenic leaves on the ingestibility, digestibility and other nutritional processes, in this respect newly ecdyzed caterpillars of the 3^{rd} larval instar of *A. ipsilon* have been chosen for this experiment. Larvae were distributed individually in Petri dishes and fed on either potato leaf discs of (Superior control, Newleaf Superior expressing Cry3Aa, Desiree control and Desiree GNA which expressing *Galanthus nivalis* agglutinin). Ten replicates with ten larvae were used in each type of potato leaves and other discs of normal leaves of potato used as control. The insect body weight, the food consumption and excrement production were measured daily. The parameters of food conversion efficiency were calculated from the fresh weight values according to [17]. The biomass increment, *B*, was measured as the difference between the initial and final fresh larval weight.

All calculations were based on the (dry / fresh) weight of different data in mg.

(1) Approximate digestibility (AD) which measures the digestion of food ingested

$$AD = a\text{-}b/a \times 100$$

(2) Efficiency of conversion of digested food (ECD) which measures the assimilation of digested food into body tissues.

$$ECD= c/a\text{-}b \times 100$$

(3) Efficiency of ingested food (ECI) which measures the overall ability of insect to convert ingested food body tissues.

$$ECI= c/a \times 100$$

(4) Relative growth rate (RGR)= c / TA
(5) Consumption index (CI)= a/ TA

Where:

a=dry weight of food consumed
b=dry wt. of feces
c=dry wt. gain
T= feeding period in days
A= mean dry body weight of larvae during feeding period

Feeding deterrence index (%FDI) = $(C\text{-}T)/(C + T) \times 100$

Where:

C=food consumed by control larvae
T=food consumed by treated larvae

and (6) Feeding deterrence index (%FDI) = (*C*-*T*)/ (*C* + *T*) x 100

Where:

C=food consumed by control larvae
T=food consumed by treated larvae

2.4 Statistical Analyses

Data were analyzed using one way ANOVA. Significant differences between treatments were determined using Duncan's test ($P<0.05$).

3. RESULTS

3.1 Effect of Genetically Modified Potatoes, Newleaf Superior Expressing Cry3Aa and Desiree GNA on Some Biological Aspects of the 3rd Larval Instar of *A. ipsilon*

The effect of genetically modified potatoes, Newleaf Superior expressing Cry3Aa and Desiree GNA on the body weight increment was indicated in Table 1. The results showed that significant difference in body weight has been found between N.L. Superior, Desiree GNA and control with feeding larvae since 3rd larval instar of *A. ipsilon*, the final larval weight was 186.7±1.86 mg, 169.2±2.40 mg for Larvae feed on Cry3Aa and Desiree GNA respectively compared with the control larvae which fed on non-transgenic potatoes leaves (585.8±2.03 mg). Marginally significant differences have been found in body increment between larvae fed on Cry3Aa and those fed on Desiree GNA (Table 1).

The pupae that resulted from larvae fed on leaf discs of genetically modified potato, N.L. Superior and GNA showed a significant low weight (151.1±2.41 and115.5±3.10 mg) respectively, compared with the control pupae (488.9±2.68 mg) (Fig. 1).

The 3rd larval instar of *A. ipsilon* larvae fed on GNA potatoes leaves showed a highly significant prolongation in the larval duration in comparison with control and with N.L superior respectively. The duration of larval stage since 3rd instar fed on Newleaf Superior and Desiree GNA were recorded 18.60±0.24 and 22.4±0.24 days respectively compared with the control larvae (13.2±0.20 days).

3.2 Effect of Newleaf Superior Expressing Cry3Aa, and Desiree GNA on Food Consumption and Nutritional Indices of the 3rd Larval Instar of *A. ipsilon*

The effect of two types of transgenic potatoes (Newleaf Superior expressing Cry3Aa, Desiree GNA and its control) and the non-transgenic potato leaves (as a control) on food consumption and utilization of the 3rd larval instars of *A. ipsilon* could be evaluated in Tables (2, 3 and 4). Data obtained indicated that the leaves ingested and assimilated had a positive effect and significantly reduced in larvae of *A. ipsilon* appeared the most food utilization parameters were less than those of the control larvae. The food consumed by the larvae was 37.36±2.87 and 30.80±1.31 mg for N.L. Superior and GNA respectively, and increased to 87.86±1.48 and 90.22±3.12 mg for the control of N.L. Superior and GNA respectively, while it reached to the maximum value with the larvae feed on non-transgenic potatoes leaves (94.16±2.48 mg).

The larvae fed on transgenic leaves excreted higher percentage of feces with respect to food consumption in comparison to that of control (53.73±1.82, 58.15±1.01, 38.29±1.27 and 36.33±0.53) for the N.L. Superior, GNA and its control respectively. In the case of control larvae excreted 43.30±2.83 of feces with respect to food consumed.

The treated larvae failed to assimilate the food in their body led to a reduction in the final body weight, the larvae feed on both transgenic potatoes type gained only 8.06±0.38 mg and 5.52±0 mg comparing to 32.00±1.32 mg and 37.9±1.35 mg for its control while in those larvae feed on non-transgenic potatoes leave the weight gain was 45.98±2.86 mg.

The larvae fed on N.L. Superior and GNA and its control showed always differences in weight increment compared with those larvae fed on non-transgenic potatoes leaves. The results indicated that the differences in biomass increment, consumption rate and excrement output were all significant with larvae fed on N.L. Superior and GNA (Tables 2,3). The biomass increment was differed between larvae consumed N.L. Superior and those fed on Superior control it was 167.6±1.88 mg and 287.00±2.31 mg respectively, while between GNA and Desiree control it was 150.5±2.53 mg and 432.7±4.73 mg respectively, and increased significantly in larvae fed on non-transgenic potatoes leave (567.8±2.26 mg).

The percentage of approximate digestibility (%AD), showed no significant difference on both cultivars indicating that, when consumed, the transgenic leaves were digested slightly more efficiently. By reducing feeding, Cry3Aa possibly slowed down the food passage through the gut, thereby allowing better digestion. The conversion of digested leaves to biomass was higher in larvae of *A. ipsilon* fed on Newleaf Superior (%ECD values in Table 2). Although the higher %AD values with N.L. Superior 46.25±1.82 and 42.81±1.03 with GNA, the better digestibility and lower metabolic losses caused significantly higher food conversion efficiency to biomass. %ECI and %ECD with N.L. Superior were 22.22±2.26 and 47.92±4.46%, while it was with larvae fed on Superior control 36.42±1.48 and 59.17±2.92. The same differences between those larvae fed on GNA and Desiree control has been observed (Table 3). We can conclude that the presence of Cry3Aa in N.L. Superior leaves and *Galanthus nivalis* agglutinin in Desiree GNA reduced biomass increment reducing food intake.

Table 1. Effect of genetically modified potatoes, Newleaf Superior expressing Cry3Aa and Desiree GNA on some biological aspects of 3rd larval instar of *Agrotis ipsilon*

Biological aspects	Newleaf Superior Cry3Aa	Desiree GNA	Control (non transgenic)	F-value
Initial larval wt/mg	19.18±0.44 a	18.72±0.29 ab	18.02±0.27 b	2.850*
Final larval wt/mg	186.7±1.86 b	169.2±2.40 c	585.8±2.03 a	12422.22**
Body increment	167.6±1.88 b	150.5±2.53 c	567.8±2.26 a	11031.009**
Larval duration/day	18.60±0.24 b	22.4±0.24 a	13.2±0.20 c	400.750**
Pupal wt/mg	151.1±2.41 b	115.5±3.10 c	488.9±2.68 a	6045.525**

*Mean (±SE) values with different letters within the same row are significantly different (P<0.05) (ANOVA) (Duncan test). ** Highly significant.* Significant*

Table 2. Effect of Newleaf Superior and its control on the food consumption and nutritional indices of 3rd larval instar of *Agrotis ipsilon*

Different parameters	Newleaf Superior (Cry3Aa)	Newleaf Superior control	Control (Non-transgenic leaves)	F-value
Dry wt. of food consumed/ larvae (mg)	37.36±2.87 b	87.86±1.48 a	94.16±2.48 a	174.745**
Dry wt. of feces (mg)	20.22±2.08 c	33.62±1.02 b	40.52±1.64 a	39.440**
%dry wt. of feces with respect to food consumed	53.73±1.82 a	38.29±1.27 b	43.30±2.83 b	14.311*
Dry wt. gain (mg)	8.06±0.38 c	32.00±1.32 b	45.98±2.86 a	109.088**
%AD	46.25±1.82 b	61.69±1.27 a	56.68±2.83 a	14.314*
%ECD	47.92±4.46 c	59.17±2.92 b	86.10±2.55 a	32.966**
%ECI	22.22±2.26 c	36.42±1.48 b	48.68±2.21 a	42.980**
CI	2.28±0.17 c	4.17±0.09 a	2.73±0.06 b	65.377**
RGR	0.48±0.02 c	1.51±0.05 a	1.33±0.08 b	87.856**
%FDI (with respect to its control)	40.32%			
%FDI (with respect to normal control)	43.18%			
Body increment (mg)	167.6±1.88 c	287.00±2.31 b	567.8±2.26 a	8984.369**

*Mean (±SE) values with different letters within the same row are significantly different (P<0.05) (ANOVA) (Duncan test). AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food ; CI, consumption index; RGR, relative growth rate; FDI, feeding deterrence index. ** Highly significant,* Significant*

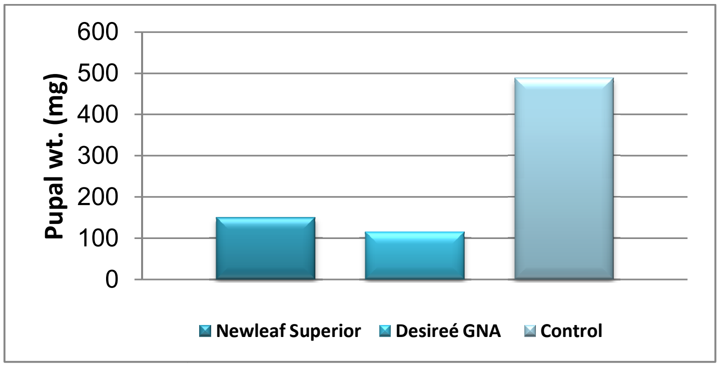

Fig. 1. Pupal weight resulted from larvae feed GM potato leaves

Table 3. Effect of GNA and its control on the food consumption and nutritional indices of 3rd larval instar of *Agrotis ipsilon*

Different parameters	Desiree GNA	GNA control	Control (Non-transgenic leaves)	F-value
Dry wt. of food consumed/ larvae (mg)	30.80±1.31 b	90.22±3.12 a	94.16±2.48 a	213.786**
Dry wt. of feces (mg)	17.56±0.47 c	32.76±1.09 b	40.52±1.64 a	98.900**
%dry wt. of feces with respect to food consumed	58.15±1.01 a	36.33±0.53 c	43.30±2.83 b	39.882**
Dry wt. gain (mg)	5.52±0.14 c	37.9±1.35 b	45.98±2.86 a	136.800**
%AD	42.81±1.03 c	63.66±0.53 a	56.68±2.83 b	35.993**
%ECD	42.37±2.32 c	66.008±1.08 b	86.10±2.55 a	109.672**
%ECI	18.01±0.64 c	42.03±0.86 b	48.68±2.21 a	128.633**
CI	1.43±0.05 c	3.21±0.11 a	2.73±0.06 b	115.108**
RGR	0.25±0.008 b	1.34±0.04 a	1.33±0.08 a	126.834**
%FDI (with respect to its control)	49.09%			
%FDI (with respect to normal control)	50.70%			
Body increment (mg)	150.5±2.53 c	432.7±4.73 b	567.8±2.26 a	4002.948**

*Mean (±SE) values with different letters within the same row are significantly different ($P<0.05$) (ANOVA) (Duncan test). AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food ; CI, consumption index; RGR, relative growth rate; FDI, feeding deterrence index. ** Highly significant,* Significant*

The %AD, %ECI, and %ECD values were not significantly different from those measured on control varieties of potato, but the slight decrease of all of them suggests that the lower body weight was a consequence of reduced nutrient assimilation. The presence of Cry3Aa and *Galanthus nivalis* agglutinin decreased digestibility but increased the ratio of digested food (ECD) converted to biomass.

The consumption index (CI) was significantly reduced with both type of transgenic potatoes compared with control, while the relation between the duration of feeding period and the weight of body gain (RGR) was significantly decreased in larvae fed on leaves of GNA transgenic potato. The feeding deterrence index (FDI) of the 3rd instar larvae showed low effect at both N.L. Superior and GNA. The antifeeding activity (FDI) was less than 50% with N.L. Superior while it recorded 50.70% with GNA feeding (Table 4).

Table 4. Comparison between the two types of transgenic and non-transgenic potatoes on some physiological parameters of 3rd larval instar of *Agrotis ipsilon*

Different parameters	Newleaf Superior (Cry3Aa)	Desiree GNA	Control (Non-transgenic leaves)	F-value
Dry wt. of food consumed/ larvae (mg)	37.36±2.87 b	30.80±1.31 b	94.16±2.48 a	225.278**
Dry wt. of feces (mg)	20.22±2.08 b	17.56±0.47 b	40.52±1.64 a	65.072**
%dry wt. of feces with respect to food consumed	53.73±1.82 a	58.15±1.01 a	43.30±2.83 b	14.061**
Dry wt. gain (mg)	8.06±0.38 b	5.52±0.14 b	45.98±2.86 a	184.001**
%AD	46.25±1.82 b	42.81±1.03 b	56.68±2.83 a	12.580*
%ECD	47.92±4.46 b	42.37±2.32 b	86.10±2.55 a	53.311**
%ECI	22.22±2.26 b	18.01±0.64 b	48.68±2.21 a	79.465**
CI	2.28±0.17 b	1.43±0.05 c	2.73±0.06 a	32.766**
RGR	0.48±0.02 b	0.25±0.008 c	1.33±0.08 a	131.296**
%FDI	43.18%	50.70%		
Body increment (mg)	167.6±1.88 b	150.5±2.53 c	567.8±2.26 a	11031.009**

*Mean (±SE) values with different letters within the same row are significantly different (P<0.05) (ANOVA) (Duncan test). AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food ; CI, consumption index; RGR, relative growth rate; FDI, feeding deterrence index. ** Highly significant, * Significant*

4. DISCUSSION

Transgenic plants provide many benefits; it is increasing the flexibility in crop management, decreasing the depending on chemical pesticides. Also it is enhancing yields and better crop qualities.

The present investigation was conducted to study the effect of the leaves of potato cultivar Newleaf Superior which expresses Cry3Aa and Desiree (GNA) and compared with non-transgenic potato leaves (as control). The data obtained in the present work show that the feeding of larvae on the two cultivars affect larvae by being toxic causing a delay in larval growth and can act as antifeedant this explains the growth inhibition resulted from larval feeding on our selected two cultivars which caused a reduction in the pupal weight of the pest, similarly [13] showed that the Cry3Aa expressed in potatoes or added to an agar-wheat germ diet reduced the final body weight accordingly causes a reduction in larval growth of *Spodoptera littoralis*, this attributed to the presence of Cry3Aa gene, certain parts of this Cry proteins are particularly well conserved and interact in discriminately with diverse cell membrane of the midgut. The *Bt* protein was present in all of the transgenic lines and that plants expressing Cry2Ab gene could be used for the management of the target lepidopteran insect pests [18].

Lepidopteran larvae presumably do not possess specific midgut receptors for Cry3Aa, but this does not fully exclude toxin interaction with the Egyptian cotton leafworm cells [13]. Several **α** -helices that form the first of the three domains of the Cry proteins are conserved between the moth- and the beetle-specific toxins [19,20]. Helix 5 from Cry3Aa was shown to insert readily in diverse planar lipid bilayers, to bind to the surface of midgut cells in the fall armyworm *Spodoptera frugiperda* (J.E. Smith), and to kill a cell line derived from the latter species [21]. From this standpoint, a reasonable explanation for the inhibitory Cry3Aa action on *Agrotis ipsilon* is that the toxin binds to and hinders the digestive functions of midgut cells, thereby reducing food intake accordingly larval body growth. Conclude that food digestibility was not affected significantly, but the metabolic losses were lower than on control in general. The decrease in metabolic costs could be linked to smaller food consumption, but other factors such as reduced mobility might have played a role with changes in enzymes, also the presence of Cry3Aa and *G. nivalis* agglutinin decreased digestibility but increased the ratio of digested food converted to biomass.

The obtained results agreed with [13,21,22]. They indicated that the *Bt* potatoes curb the growth and reproduction of the adults of *S. littoralis.*

5. CONCLUSION

The data obtained in the present work concluded that that the feeding larvae on the two different leaves of potato cultivar Newleaf Superior which expresses Cry3Aa, Desiree (GNA) affect larvae by being toxic and *Bt* potatoes curb the growth and reproduction of the adults of *A. ipsilon.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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