

DEPLETION IN THE BIOTIC POTENTIAL OF *SPODOPTERA LITURA* (FAB.) UNDER THE EFFECTS OF TRANSGENIC AND CONVENTIONAL COTTON VARIETIES

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ABSTRACT

Spodoptera litura (Fabricius) is a polyphagous pest, surviving on more than 120 plant species belonging to 44 families including cotton, groundnuts, jute, maize, paddy, soybeans, tea, tobacco and vegetables. Broad host range indicates its digestive capabilities for adaption of a variety of food. Keeping in view, the damage potential of *Spodoptera litura*, studies were conducted to determine the effects of transgenic cotton CEMB-33, Bt-906 and conventional FH-142, CIM-496 cotton varieties on biotic potential of *Spodoptera litura* under laboratory conditions, 27±5°C and R. H. 65±5%, at M. A. H. Qadri, Biological Research Centre, University of Karachi, Karachi. Transgenic cotton varieties were proved to be significantly effective and reduced pupation 10.80%, pupal weight 5.45% prolonged pupal duration 16.90% reduced adult emergence 29.41% as compared to the control.

Key words: *S. litura*, pupation, pupal weight, adult emergence, female/male ratio and cotton varieties.

INTRODUCTION

Cotton, *Gossypium hirsutum* (Linn.) family Malvaceae is the main cash crop of Pakistan. Agriculture industry and cotton related services play the foremost role in Pakistan's economy and contributes significantly in foreign exchange earnings. It is second in terms of area to wheat, which is the country's staple food (Naveed and Anwar, 2016). Cotton the most important cash crop in over 60 countries around the world is often called as "White gold" and provides the most versatile fiber which is even now considered as the "King of apparel fiber" (Ijaz, 2016). According to an estimate, an increase of one million bales in cotton production will mean 0.5 percent increase in the GDP (Naveed and Anwar, 2016). Among various cardinal factors responsible for poor yield of cotton, damage caused by the insect pests is one of the major causes for reduced yield. In the early stage, sucking pests like cotton aphid, *Aphis gossypii* Glover, leaf hopper, *Amrasca biguttula biguttula* (Ishida), whitefly, *Bemisia tabaci* (Gennadius) and thrips *Thrips tabaci* (Lind.) and in the late stage bollworm complex cause significant damage to the crop. The yield losses in cotton due to sucking pests alone were 46.5 percent (Panchabhavi *et al.*, 1990) and the bollworm complex accounted for 44.5 percent (Dhawan *et al.*, 1988).

Tobacco caterpillar, *Spodoptera litura* (Fab.) Lepidoptera: Noctuidae is a ubiquitous, polyphagous and multivoltine pest that feeds on 112 cultivated crops all over the world (Moussa *et al.*, 1960). On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, flower buds and occasionally also on bolls. In the mid-nineties, conventional cotton production posed a serious threat to the environment, farmers' health and economy. Cotton accounted for the use of 15% of the world's pesticides and 25 percent of the world's insecticides. This tackled two major responses one response was the development of genetically modified (GM) insect resistant cotton cultivars, which was rapidly adopted by many countries since its first commercial introduction in 1996. Approximately; the world's 90% cotton growing areas grown under genetically modified cotton in 2016 (ISAAA, 2016). The other response was the adoption of organic methods of cotton production by farmers who believed that holistic earth friendly responses, optimized crop rotation and organic fertilizer could reverse the trend of the soaring use of chemical pesticides. The rapid spread of GM seeds in cotton has resulted the problems for the organic cotton sector, a few of which can be briefly examined taking India and Burkina Faso as examples (Klaiss *et al.*, 2012). Toxins from a soil bacterium *Bacillus thuringiensis* are widely employed in control of many insect pests from decades. Earlier sprays containing Bt toxins was used to control agricultural pests. Since, 1996 (Qaim and Zilberman, 2003), plants engineered to produce these toxins showed great potential in pest management by reducing reliance on insecticides. However, continuous exposure to these toxins imposes a selection pressure on insect populations favoring evolution of resistance to Bt toxins expressed in transgenic plants (Tabashnik, 1994; Shelton *et*

al., 2002). Introduction of Bt cotton has revolutionized the cotton production in India as the cotton production prior to 2002 suffered huge losses due to its susceptibility to insect pests (Karihaloo and Kumar, 2009). Transgenics of cotton with *Bacillus thuringiensis* kurstaki genes encoding Cry1Ac proteins have been first introduced with the expectations of increase in natural enemies, reduction in pesticide applications and therefore, reduction in farmer's exposure to pesticides ultimately (Gianessi and Carpenter, 1999). The Cry1Ac provides control of the American bollworm, *Helicoverpa armigera* pink bollworm, *Pectinophora gossypiella* and spotted bollworm, *Earias vitella*. However, it is less effective for the control of beet armyworm, *Spodoptera exigua* and fall armyworm, *Spodoptera frugiperda* (MacIntosh *et al.*, 1990; Adamczyk *et al.*, 1998). The present work was conducted with a postulation that the transgenic cotton varieties exert some effects on biotic potential of this Lepidopteran pest.

MATERIALS AND METHODS

The present research investigations were conducted under laboratory conditions at Dr. Mohammad Afzaal Hussain Qadri (M. A. H. Q.) Biological Research Centre (BRC), University of Karachi to study the effects transgenic and conventional cotton varieties on cotton pest, *Spodoptera litura* (Fab.) during August, 2015 to October, 2016.

Collection and rearing of *Spodoptera litura*

Larvae of *Spodoptera litura* were collected from cauliflower, *Brassica oleracea* fields of Malir and brought under laboratory conditions which was identified (CABI, 2009) by using key at the Department of Zoology, University of Karachi. Larvae were kept in plastic jars providing fresh *Brassica oleracea* leaves on daily basis. The moist soil was also provided in plastic jars for pupation. When moths emerged from pupae, they were kept in insect rearing cage (30x30x45) cm. The frame and bottom of the cage were made of wood having sliding glasses at the sides that facilitate for removing eggs and feeding material inside the cage. The top of the cage was made of the fiber mesh for the movement of air. Five pairs of male and female moths were released in each breeding cage and fed with 10% honey solution which was replaced on daily basis. Moths were also provided with the fresh leaves of the cauliflower collected from pesticide free field. These leaves were inserted in water in a glass bottle to keep them fresh and turgid for rest and oviposition sites. The eggs were found laid on leaves, blotting papers and parts of the muslin cloth from where they were collected and placed in Petri dishes (10cm dia.) for hatching. The newly hatched larvae were transferred into the cages (60x120x60 cm) to avoid crowding. Every day the fresh cauliflower leaves were provided to feed them and left over food and feces were removed from the cages. In such a way a desirable number of uniform larvae of fifth instar were made available.

Transgenic and conventional cotton

Identified seeds of transgenic cotton CEMB-33, Bt-906 and conventional cotton FH-142, CIM-496 varieties were obtained from Central Cotton Research Institute (CCRI), Sakrand and plots were maintained besides cauliflower plots in the experimental fields. Common cultural practices were adopted and no any kind of the pesticides were applied against insect pests in the field.

BIOASSAY

Newly molted 60 (fifth instar) larvae of *Spodoptera litura* were selected for treatment. Petri dishes of (10 cm dia.) with a piece of blotting paper in the base were labeled. Early molted 5 larvae were placed in each Petri dish. Sixty larvae in separate (twelve Petri dishes) were used for each under test variety. Fresh leaves of 40 days aged plant of respective cotton variety were provided to feed the larvae in the Petri dishes labeled accordingly.

Pupation and pupal duration

After 72h the rest of live larvae counted and were transferred in to wide mouth labeled plastic jars provided with moist soil and covered with muslin cloth for pupation accordingly. Only ten larvae were placed in each jar to avoid suffocation and they were provided fresh *B. oleracea* leaves to feed daily until pupation. Pupal weight, pupal duration, percentage and adult emergence were recorded.

STATISTICAL ANALYSIS

All the tables contain mean (Effects) \pm standard error and 95% confidence limit was calculated through Tukey's test by using (SPSS, 2013) software to analyze the data.

RESULTS

Depletion in pupation of *Spodoptera litura* under the effect of cotton varieties

Results of effects of cotton varieties on pupation of *S. litura* are shown in the (Table 2) and reveal that transgenic cotton CEMB-33 found to be highly effective, 47.50 larvae become pupae followed by Bt-906, FH-142, CIM-496 and control 48.75, 49.75, 50.25, 53.25, respectively when fifth instar *Spodoptera litura* larvae fed with fresh leaves of cotton varieties. Further, the analysis of variance shows the significant difference in pupation of *Spodoptera litura* under the effect of cotton varieties at ($P<0.05$).

Table 1. Depletion in pupation of *Spodoptera litura* under the effect of cotton varieties.

Cotton varieties	No. of pupae Mean \pm SE	Confidence interval of mean at 95%	
		Lower bound	Upper bound
CEMB-33	47.50 \pm 0.65 ^b	45.45	49.56
Bt-906	48.75 \pm 0.85 ^{ab}	46.03	51.47
FH-142	49.75 \pm 0.63 ^{ab}	47.75	51.75
CIM-496	50.25 \pm 2.32 ^{ab}	42.86	57.64
Control	53.25 \pm 0.95 ^a	50.24	56.26

Depletion in pupal weight of *Spodoptera litura* under the effect of cotton varieties under laboratory conditions

Transgenic cotton CEMB-33 found to be highly effective with 314.26mg pupal weight followed by Bt-906, FH-142, CIM-496 and control 315.34, 319.41, 322.24, 332.39mg, respectively when sixty fifth instar of *S. litura* larvae fed with fresh leaves of cotton varieties (Table 2). The analysis of variance shows the strong significant difference in pupal weight of *Spodoptera litura* under the effect of cotton varieties at ($P<0.000$).

Table 2. Depletion in pupal weight of *Spodoptera litura* under the effect of cotton varieties.

Cotton varieties	Mean mg pupal weight \pm Std. Error	95% Confidence interval of mean	
		Lower Bound	Upper Bound
CEMB-33	314.26 \pm 2.08 ^c	307.645	320.880
Bt-906	315.34 \pm 1.21 ^c	311.500	319.175
FH-142	319.41 \pm 1.19 ^{bc}	315.633	323.192
CIM-496	322.24 \pm 1.01 ^b	319.036	325.439
Control	332.39 \pm 1.74 ^a	326.847	337.928

Depletion in pupal duration of *Spodoptera litura* under the effect of cotton varieties

Prolonged pupal duration was observed in transgenic cotton CEMB-33 15.93 days followed by Bt-906, FH-142, CIM-496 and control, 14.43, 13.69, 14.04 and 13.24 days, respectively (Table 3). The analysis of variance shows the strong significant difference in pupal duration of *S. litura* under the effect of different cotton varieties at ($P<0.05$).

Deletion in adult emergence of *S. litura* under the effect of cotton varieties under laboratory conditions

The lowest numbers of adults were emerged in CEMB-33, followed by Bt-906, FH-142, CIM-496 and control, 30.00, 34.25, 36.25, 37.25 and 42.50 respectively, when sixty fifth instar *Spodoptera litura* larvae feed with fresh leaves of cotton varieties (Table 4). The analysis of variance shows the strong significant difference in adult emergence of *Spodoptera litura* under the effect of cotton varieties at ($P<0.000$).

Table 3. Depletion in pupal duration of *Spodoptera litura* under the effect of cotton varieties

Cotton varieties	Overall days Mean + SE	Confidence interval of mean at 95%	
		Lower bound	Upper bound
CEMB-33	15.93 \pm 0.21 ^a	15.27	16.60
Bt-906	14.43 \pm 0.20 ^b	13.78	15.08
FH-142	13.69 \pm 0.21 ^{bc}	13.04	14.34
CIM-496	14.04 \pm 0.20 ^{bc}	13.39	14.69
Control	13.24 \pm 0.20 ^c	12.60	13.88

Table 4. Depletion in adult emergence of *Spodoptera litura* under the effect of cotton varieties.

Cotton varieties	No. of adults Mean + SE	Confidence interval of mean at 95%	
		Lower bound	Upper bound
CEMB-33	30.00 \pm 1.23 ^c	26.10	33.90
Bt-906	34.25 \pm 1.11 ^b	30.72	37.78
FH-142	36.25 \pm 0.75 ^b	33.86	38.64
CIM-496	37.25 \pm 0.85 ^b	34.53	39.97
Control	42.50 \pm 0.29 ^a	41.58	43.42

DISCUSSION

The results of the research study indicated that when early molted fifth instar larvae of *S. litura* were treated with the leaves of transgenic and conventional cotton varieties, little reduction in pupation was observed but the pupal weight and the adult emergence were affected remarkably. Lower pupation and adult emergence were observed and prolonged pupal duration was recorded when larvae were treated with the transgenic cotton varieties as compared to conventional varieties because of the presence of toxic protein (Table 1, 2, 3 and 4). Transgenic cotton CEMB-33 was found to be the most effective, than Bt-906, FH-142 and CIM-496 were found at a lesser level. The transgenic cotton CEMB-33 exerts the effects on pupation, pupal weight and adult emergence with significant margins as compared to conventional cotton varieties and the control. The effectiveness of Bt protein inoculated in transgenic cotton through genetic engineering is clearly reflecting from the results. Although in recent the past, several scientists have reported poor performance of transgenic crops against, *Spodoptera litura*. This is because of its broader host range means broader digestibility of a variety of biochemical present in different host plants i.e., trees crop plants and weeds, occasional pest status. These results are closely resemble with the finding of (Govindan *et al.*, 2012) they evaluated the toxicity of middle leaves of Bt and non Bt cotton hybrids and noticed mortality from 8.33% to 13.50 % at 168 h. after treatment in different transgenic cotton varieties against fourth and early fifth instar of *Spodoptera litura* larvae. The food is an important factor in variable insect population (Umbanhowar and Hastings, 2002) as the life cycle characteristics of herbivores may be affected by variation in host plant traits, for example the life history parameters pupation, pupal weight, pupal duration, adult longevity and survival may be influenced by the variation in host plant quality (Awmack and Leather, 2002). A finding of this research is also a reflection of the previous reports. (Ahmad and Ansari, 2012) studied the effects of neemarin at 20mg/l-1 on the life table indices of *Plutella xylostella* (L.) on cauliflower and reported total of 69% eggs hatched at 20mg/l-1 compared 85% in the control, reduced life expectancy, prolonged immature development times to 32 days as compared to 18.6 days in the untreated control, adults emergence was significantly reduced. The longevity of the insects treated with sublethal doses of insecticides may increase, decrease or remain unchanged (Kumar and Chapman, 1984). In the previous studies, toxicity of transgenic and conventional cotton was measured only in respect of mortality, none of evidence of other effects were found on further life stages of *Spodoptera litura* like have been reported pupation adult emergence % pupal weight. The present study also proving the findings of (Sangode and Rathod, 2015) studied on the effects of transgenic Bt cotton and conventional non Bt cotton against the armyworm, *Spodoptera litura* while rearing on leaves of Bt cotton, non Bt cotton and castor from first instar up

to pupation. They found 16.7% to 66.7% larval mortality, prolonged larval and pupal developmental duration. The present results are also matching with the evaluations of (Shahout *et al.*, 2011) they observed that the insects ovarian weight was significantly influenced by the different host plants as well as the male accessory gland length was significantly increased to when larvae fed on cabbage while it was significantly reduced to when larvae fed on cowpea. This is the reason that when larvae treated with transgenic either conventional cotton varieties, pupal weight was found to be affected by Bt protein inoculated in transgenic cotton and antifeedant effect of conventional cotton varieties.

CONCLUSION

It is therefore, concluded that the transgenic cotton varieties do not cause significant effects on the *Spodoptera litura* larval mortality, however; they exert effects on biotic potential of the under test pest, in this connection CEMB- 33 was found to be the most effective, than Bt-906, FH-142 and CIM-496 found to be at the lesser level. The transgenic cotton CEMB-33 exerts the effects on pupation, pupal weight and adult emergence with significant margins as compared to conventional cotton varieties and the control. The effectiveness of Bt protein inoculated in transgenic cotton through genetic engineering is clearly reflecting from the results. Although in recent past, several scientists have reported poor performance of transgenic crops against *Spodoptera litura*. This is because of transgenic cotton varieties do not cause significant effects on the larval mortality of *Spodoptera litura*, however; they exert effects on biotic potential, therefore an apparent failure is observed however; if the transgenic cotton varieties could be combined with the other pest controlling factors promising control results are expected.

REFERENCES

- Adamczyk, J. J. J., V. J. Mascarenhas, G. E. Church, B. R. Leonard and J. B. Graves (1998). Susceptibility of conventional and transgenic cotton bolls expressing the *Bacillus thuringiensis* CryIA (c) delta-endotoxin to fall armyworm (Lepidoptera: Noctuidae) and beet armyworm (Lepidoptera: Noctuidae) injury. *Journal of Agricultural Entomology (USA)*, 15: 163-171.
- Ahmad, N. and M. S. Ansari (2012). Effect of neemarin on life table indices of *Plutella xylostella* (L.). *Crop Protection*, 38: 7-14.
- Awmack, C. S. and S. R. Leather (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, 47: 817-844.
- CABI (2009). *Crop protection compendium: global modules*. Common wealth Agricultural Bureau International, Wallingford, UK. <http://www.cabi.org/compendia/cpc/>.
- Dhawan, A. K., A. S. Sidhu and G. S. Simwat (1988). Assessment of avoidable loss in cotton (*Gossypium hirsutum* and *G. arboreum*) due to sucking pests and bollworms. *Indian Journal of Agricultural Sciences*, 58: 290-292.
- Gianessi, L. P. and J. E. Carpenter (1999). *Agricultural biotechnology: Insect control benefits*. National Center for Food and Agricultural Policy Washington, DC.
- Govindan, K., K. Gunasekaran and S. Kuttalam (2012). Evaluation of Indian transgenic Bt cotton and non Bt cotton against *Spodoptera litura* Fab.(Noctuidae: Lepidoptera) fourth and fifth instar larvae. *Journal of Biopesticides*, 5: 171.
- Ijaz, A. R. (2016). Issues, Bt cotton in butcher's hand. <http://www.pakissan.com/english/issues/bt.cotton.in.butcher.hand.shtml>.
- ISAAA (2016). Global Status of Commercialized Biotech/GM Crops: 2016. ISAAA Brief No. 52. ISAAA: Ithaca, New York. http://www.isaaa.org/resources/publications/biotech_country_facts_and_trends/download/Country%20Facts%20and%20Trends_Complete.pdf.
- Karihaloo, J. L. and P. A. Kumar (2009). *Bt Cotton in India*. A Status Report.
- Klaiss, M., M. Messmer, D. Forster, R. Verma, R. Baruah, V. Rawal, L. S. Mandloi and Y. Shrivastava (2012). Influence of the fast spread of bt cotton on organic cotton production: examples from India and Burkina Faso. *Scientific Conference 2012 Advancing the Understanding of Biosafety, GMO Risk Assessment, Independent Biosafety Research and Holistic Analysis*, European Network of Scientists for Social and Environmental Responsibility (ENSSER), Tara Foundation and Third World Network (TWN).

- Kumar, K. and R. B. Chapman (1984). Sublethal effects of insecticides on the diamondback moth *Plutella xylostella* (L.). *Pest Management Science*, 15: 344-252.
- MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff and R. L. Fuchs (1990). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *Journal of Invertebrate Pathology*, 56: 258-266.
- Moussa, M. A., E.-S. A. Nasr and A. S. Hassan (1960). Factors affecting longevity and reproductive potentials of moths of the cotton leaf worm, *Prodenia litura* (Lepidoptera: Noctuidae). *Bulletin de la Societe Entomologique d'Egypte*, 44.
- Naveed, M. A. and M. A. Anwar (2016). Understanding Cotton Growers' Information Needs from Rural Bahawalpur. *Pakistan Journal of Information Management & Libraries (PJIM&L)*, 16.
- Panchabhavi, K. S., K. A. Kulkarni, G. K. Veeresh, P. C. Hiremath and R. K. Hegde (1990). Comparative efficiency of techniques for assessing loss due to insect pests in upland cotton (*Gossypium hirsutum*). *Indian Journal of Agricultural Sciences*, 60: 252-254.
- Qaim, M. and D. Zilberman (2003). Yield effects of genetically modified crops in developing countries. *Science*, 299: 900-902.
- Sangode, V. K. and M. K. Rathod (2015). Effect of Bt cotton on *Spodoptera litura* Fabricius: (Lepidoptera: Noctuidae). *International Journal of Pharmacology and Biological Sciences*, 9: 21.
- Shahout, H. A., J. X. Xu, X. M. Yao and Q. D. Jia (2011). Influence and mechanism of different host plants on the growth, development and, fecundity of reproductive system of common cutworm *Spodoptera litura* (Fabricius)(Lepidoptera: Noctuidae). *Asian Journal of Agricultural Sciences*, 3: 291-300.
- Shelton, A. M., J. Z. Zhao and R. T. Roush (2002). Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology*, 47: 845-881.
- SPSS (2013). IBM SPSS statistics version 22. *Boston, Mass: International Business Machines Corp*: 126.
- Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, 39: 47-79.
- Umbanhowar, J. and A. Hastings (2002). The impact of resource limitation and the phenology of parasitoid attack on the duration of insect herbivore outbreaks. *Theoretical Population Biology*, 62: 259-269.

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