Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Research Article

Cotton Host Plant Resistance against Spotted Bollworm, *Earias vittella* (Fab.)

Fateh Muhammad Kanhar[1], Hassan Zubair[2*], Misha Aslam[3], Tajwar Sultana Syed[4], Taj Muhammad Jahangir[5], Saeed Ahmed Tunio[1], Aslam Bukero[4], Rehana Panhwar[6], Khalil Ahmed Kanher[7], Fakhra Soomro[7]

[1] *Department of Agriculture Extension, Sindh.*
[2] *Department of Agronomy College of Agriculture, University of Sargodha Punjab, Pakistan.*
[3] *Department of Zoology, Government College University, Faisalabad, Punjab, Pakistan.*
[4] *Department of Entomology Sindh Agriculture University, Tandojam.*
[5] *Institute for Advanced Research Studies in Chemical Sciences, University of Sindh.*
[6] *Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam.*
[7] *Department of Zoology Shah Abdul Latif University, Khairpur.*

ABSTRACT

The seeds of three cotton lines were irradiated with gamma rays, i.e. 150, 200 and 250 Gy for phenotypic changes in plant for resistance development against spotted bollworm *Earias vittella*. After gamma irradiation plant Physio-morphological characters, i.e., gossypol gland density, gland size, the boll bract size and phenol concentration in floral square flowers and seeds were changed as compared to that of parents (non-irradiated) cotton lines. The field results of the parents and its gamma irradiated cotton lines in relation to resistance against *E. vittella* populations, the mutant line SP* (150 Gy) was found to be comparatively resistance with maximum number of gossypol density, minimum their size on bolls and higher phenol content in floral square flowers and seeds. Whereas, the mutant line SB* (250 Gy) was recorded highly susceptible to *E. vittella* with the lowest number of gossypol gland density, maximum their size and minimized amount of phenol concentration in floral square flowers and seeds. The phenol mixed artificial diet is a highly effective in larval growth and pupal weight loss. The gossypol density was showed non-significant and negative effects on spotted bollworm population in all parents and their gamma irradiated cotton lines. The gossypol gland size was observed non-significant and positive correlations with *E. vittella* in all parents and their irradiated cotton lines during 2008 and 2009, respectively. However, the bract size showed positive and significant relations, though, phenol concentrations in floral square, flowers and seeds showed non-significant and negative correlations in all parents and gamma irradiated cotton lines.

Keywords: Boll Bract, Gamma irradiation, Gossypol Gland, Resistance, Total Phenol.

OPEN ACCESS

Correspondence
Hassan Zubair
hassanzubair477@gmail.com

Article History
Received: December 11, 2024
Accepted: February 27, 2025
Published: March 17, 2025

INTRODUCTION

Executives at the company have selected the *Gossypium hirsutum* L. species to produce cotton fibers. The product functions critically in multiple production lines and workforce activities such as ginning textile processing oil extraction, representing forty percent of Pakistani industrial jobs (Javed et al., 2006). Insects responsible for pest infestations have decreased the current acreage of produced cotton seeds. Atwal (2002) established that 1,326 insect species damage cotton crops worldwide in research. Marquis (1992) describes *Earias vittella* (Fab.) as one of the most destructive cotton pests, indirectly leading to decreased yields. Research by Karar et al. (2013) and Kanher et al. (2014) discovered that *E. vittella* Pest damages crop by 19% to 26% in yield reduction. The unapproved use of insecticides is the leading cause behind insect population resistance formation. This unregulated pesticide application disturbs the environment and creates significant wellness dangers for every life form (Soerjani, 1988; Soomro et al., 2000; Dhawan et al., 2009; Azmi and Naqvi, 2011; Khan et al., 2013). Synthetic chemical insecticides that cotton growers in Sindh apply to their crops happen without pest density monitoring since they get used regardless of actual population levels (Khooharo et al., 2008). Scientists recognize plant resistance to hosts as the proven method that controls detrimental insect damage to cotton crops. According to Hagenbucher et al. (2013), insect dietary choices depend on feeding behaviours and plant physio-morphological factors. Plants use these defensive characteristics to prevent herbivores from attacking, according to the research findings by Elegbede et al. (2014). Insects experience favourable or resistant conditions for interaction with their host plants through secondary metabolite characteristics modifications (Elena et al., 2005; Cherry, 1983; Sunilkumar et al., 2006). The cotton variety displays resistance through the combination of trichomes with plant secondary metabolites, according to Price et al. (1980). The research from Mohan et al. (1994) showed that *E. vittella* chose to consume cotton genotypes, with the densest gossypol glands appearing on their bolls' outer surfaces. The content of pigments in gossypol glands causes harmful effects on insect specimens and proves valuable as a defense mechanism against pests (Wang et al., 2004; Yingfan et al., 2010). The weight substance displays two main functions impeding sperm growth and blocking normal organism development (Wang, 1997). Research by Río and associates (2004) proves that phenolic compounds among plant secondary metabolites significantly influence fungal spore development to protect plants against insects (Wuyts et al., 2006). The research published by Mazid and colleagues (2011) showed that these chemical substances protect against multiple insects that feed on plant samples. The research aims to investigate the defensive systems host plants use to prevent *E. vittella* from entering their tissues. A research project investigates plant gossypol gland, boll bract measurements, and floral square and seed phenolic content analysis to determine *E. vittella* deterrent effects in artificial dietary supplements to develop sustainable cotton crop pest controls.

MATERIALS AND METHODS

The insurance process involved exposing cotton seeds from lines SB, SP, and B-4 to different Gamma ray doses at 150, 200, and 250 grams (Gy). Nine new mutant cotton lines which researchers designated as SB* and SP* and B-4* joined the parent lines after mutation selection. Twelve plant specimens underwent evaluation at the Cotton Research Section of the Agriculture Research Institute in Tandojam, Sindh, Pakistan throughout the years 2008 and 2009. The seeds sent to the Nuclear Institute of Agriculture and Biology (NIAB) in Faisalabad for Cobalt-60 irradiation treatment. The investigators designed the study as a randomized complete block design (RCBD) with four replications. A total of four rows made up each replication with 30 cm spacing between plants and 75 cm space among rows which were planted on May 15th of both 2008 and 2009. Researchers tested gamma irradiation effects on both M4 and M5 generation samples for their resistance abilities against untreated parent specimens of spotted bollworm. The approved agricultural practices received proper and on-time execution.

Screening of Spotted Bollworm, *Earias spp.* Population

Researchers documented spotted bollworm presence throughout all the fruiting bodies of five tagged plants while examining the entire plant. Scientific studies by Dhawan et al. (2009) confirmed that cotton fruiting bodies with 5% damage from spotted bollworms exceeded the economic threshold level (ETL). The weekly insect pest population assessment included damage observations until the first harvest of the crop. Teams made precise observations that they recorded and analyzed the data during the morning time between 8 and 10 am.

Gossypol Pigment Analysis

Scientists investigated the gossypol pigment levels in immature bolls from three parental lines and the irradiated cotton lines across three gamma-dose levels consisting of 150, 200, and 250 Gy. Plant Breeding and Genetics at the Faculty of Crop Production, Sindh Agriculture University, Tandojam received the bolls for gossypol gland analysis using epidermal section material from a one cm^2 surface area. The Nikon Alpha picture photomicroscope (Ys, Japan) was used to observe 10 randomly selected bolls from each line at 4x magnification and 10x magnification. White paper was cut into 0.5cm square holes to position on decayed boll sections during the counting procedure for gossypol glands. The measurement from the half cm^2 section served as the basis to calculate the total number of gossypol glands in each cm^2 of surface area. An ocular micrometer in the microscope eyepiece measured the dimensions of gossypol glands in thirty immature bolls per cotton line per treatment while the values became millimeters (mm).

Spotted Bollworm Rearing on Artificial Diet

Larvae of spotted bollworms came from the field and researchers kept them in the Department of Entomology lab. at

Sindh Agriculture University Tandojam with 27±2 °C temperature and 70±5% relative humidity conditions. Raising *E. vittella* larvae occurred through bottle-stage feeding of immature cotton bolls and okra fingers inside glass jars which used muslin fabric for covering (Mehta, 1971). A sufficient knob-like structure located at the antero-dorsal end of the cocoon identified male pupae according to Gupta (1978). Thereafter, researchers placed the pupae into separate glass jars alongside wet sponges covered by filter paper. The adult pair received plastic jars reaching dimensions of 15 to 30 cm diameter and 7 to 10 cm depth. The adult insects received their liquid food from 10% honey solutions kept in the jars. The female oviposition site was established through black cloth wrapped in white tape secured inside a plastic jar until egg laying occurred. A female insect laid eggs on black fabric before lab personnel collected the fabric into Petri dishes with damp tissue paper that maintained proper humidity for monitoring the eggs until they hatched. After hatching the research subjects received transfer to jars containing prepared fake food with multiple phenol concentrations before being covered with muslin fabric throughout the subsequent developmental cycle. The artificial diet process was followed as described by Gupta et al. (2005).

Table 1. Composition of artificial diet for rearing *Earias vittella* larvae.

Groups	Ingredients	Quantity (g)
Fraction A (main ingredients)	Soybean (*Glycine max*) flour	60.0
Fraction A	Chickpea (*Cicer arietinum* var. kabuli) flour	58.0
Fraction A	Wheat germ (Xian Chono Chem Co., Ltd, China)	16.0
Fraction B	Dried yeast powder (United Trading Corporation Co. Ltd Pakistan)	16.0
Fraction B	Casein (Sigma-Aldrich)	8.0
Fraction B	L-Ascorbic acid (Sigma-Aldrich)	2.4
Fraction B	Cholesterol (Sigma-Aldrich)	0.4
Fraction C	Methyl-p-hydroxybenzoate (Sigma-Aldrich)	0.4
Fraction C	Sorbic acid (Sigma-Aldrich)	2.4
Fraction C	Streptomycin sulphate (Pharmax Pakistan Pvt. Ltd)	0.4
Fraction D	Agar–agar (Sigma-Aldrich)	16.0
Fraction D	Distilled water	820 ml

Larvae Rearing Procedure on Artificial Diet

Testing took place in the laboratory with 32.4±3 °C temperature measurement and 56.33% level of relative humidity. Each vial received one of the four prepared phenol amounts (0.1, 0.5, 1.0, or 2.0 ml) and contained tested fake food specimens which were placed in freezing conditions at -21 ºC. Pretreated two grams of artificial diet reached room temperature during 2.5 to 3.5 hours prior to locating them within plastic jars outfitted with muslin fabric for larval feeding. The experiment used twenty 0-24-hour-old emerging larvae in each plastic container where scientists provided prepared diet for feeding until pupation occurred. METU (2018, p.42-44) measured the weight decline of larvae and pupae that consumed meals with different phenol concentrations while rearing them separately. The antifeedant activities evaluation followed the method Bentley et al. (1984) established.

$$\text{Antifeedent activity} = \frac{\text{Consumption in control- Consumption in treated}}{\text{Consumption in control}} \times 100$$

Determination of Total Phenols Content

Total phenol content measurements were performed on a spectrophotometer after modifying the Folin-Ciocalteu test according to procedures by Makkar (2000) as well as Makkar et al. (2007). Researchers used four different volumes of phenolic solutions starting at 0.1 ml up to 2.0 ml to develop the calibration curve. A solution containing 200 mg of freeze-dried crude extract along with 2.0 ml Folin-Ciocalteu reagent and 1.25 ml 5% sodium carbonate solution was prepared. The required 10 ml volume of testing solution was measured through a volumetric flask which included a 7:3 mixture of acetone and methanol. The researcher maintained a one-hour dark period for the solution while mixing the substances at ambient temperature which remained at approximately 25°C. The UV/visible spectrophotometer (Perkin Elmer Lambda 35, USA) measured the absorbance values of samples at 750 nm against the blank reagent

https://doi.org/10.55627/agribiol.003.01.1226

(plant material-free) according to Waterhouse (2001). A Perkin Elmer Lambda 35 spectrophotometer model manufactured in USA measured phenol standards for calibration purpose with r^2 value at 0.9986. The entire phenolic content of extracted materials equated to pure phenolic concentration levels which reached 1 mg for every 100 g of drain-weighted substance.

Data Analysis

The comparison of means between cotton lines used the LSD test with statistical significance at P = 0.05. Researchers studied the association between basic associations and Pearson within spotted bollworm population samples along with various plant characteristics. The collected data processed statistical analysis through ANOVA which Statistical software 8.1 executed.

RESULTS

Spotted Bollworm *E. vittella* Population on Different Cotton Lines Under Field Condition

A significant statistical difference (F= 145.55; df= 2; P= < 0.0001) and (F= 78.19; df= 2; P= < 0.0001) emerged regarding the untreated (parent) and gamma-irradiated cotton lines. Table 1.0 shows that the mutant line SB (250 Gy) contained the maximum population numbers of 3.065/plant and 5.365/plant while exhibiting heavy susceptibility. The resistant response of the mutant line SP (150 Gy) against spotted bollworm resulted in the lowest recorded larval population which amounted to 0.932/plant in 2008 and 1.497/plant in 2009.

Gossypol Gland Density

The research data found in Table 2.0 shows that different cotton lines contain different numbers of gossypol glands on their immature green boll surfaces per square centimetre. The results showed significant variations of gossypol glands density (F= 319.13; df= 2; P= < 0.0001) between untreated (parent) and irradiated lines and revealed another significant result (F= 370.33; df= 2; P= < 0.0001). Rates of gossypol gland development reached 75.2/cm^2 and 74.8/cm^2 after 150 Gy gamma irradiation of the cotton line SP. The occurrence of gamma irradiation at 250 Gy in SB lines resulted in decreases in gossypol gland density to 30.2/cm^2 and 30.8/cm^2 when measured across 2008 and 2009.

Gossypol Gland Size

Table 3.0 shows how gamma irradiation of cotton lines alters gossypol gland size compared to untreated controls with strong significance (F= 36.72 and F= 33.6; df= 2; P= < 0.0001). The gossypol gland measurements from 250 Gy-treated SB mutant line achieved the highest level at 0.0949/gland and 0.0952/gland. The research showed that among all experimental samples the smallest gossypol glands appeared in the 150 Gy-treated mutant SP with 0.0496/gland in 2008 and 0.0495/gland in 2009.

Boll Bracts

Table 4.0 shows bract size has a significant statistical difference (F= 111.41; df= 2; P= < 0.0001) as well as (F= 50.19; df= 2; P= < 0.0001) compared to the parent cotton lines. The bract sizes of mutant line B-4 (150 Gy) reached their maximum at 4.555 then 4.435 as the Radiosensitive material evolved in the susceptible category in 2008 and 2009.

Total Phenol Concentration in Squares and Flowers of Parents and Gamma Irradiated Lines

Total phenol concentration levels differed significantly between dried cotton squares and flowers according to Table 5.0 data (F= 10162.4; df= 2; P= < 0.0001). Different levels of radiation effects surfaced when analyzing cotton lines next to their original genetic counterparts. The concentration of phenols rose to 25.175 mg/gm in mutant line SP when exposed to 150 Gy but SB showed reduced levels at 250 Gy.

Total Phenol Concentration in Seeds of Parents and Gamma Irradiated Lines

Total phenol concentration levels differed significantly between dried cotton squares and flowers according to Table 5.0 data (F= 10162.4; df= 2; P= < 0.0001). Different levels of radiation effects surfaced when analyzing cotton lines next to their original genetic counterparts. The concentration of phenols rose to 25.175 mg/gm in mutant line SP when exposed to 150 Gy but SB showed reduced levels at 250 Gy.

Effect of Different Phenol Concentrations Mixed with Artificial Diet on Larval and Pupal Weight (Mg) of Spotted Bollworm, *E. vittella*

The artificial diet weights of both larval and pupal stages were substantially affected by different phenol concentrations based on analysis of Table 7.0 (larval: F= 23744.4; df= 4; P= < 0.0001, pupal: F= 1332.47; df= 4; P= < 0.0001). The different levels of phenol exposure during the life cycle caused significant weight reduction in the spotted bollworm *E. vittella* larvae and pupate exceeds what was seen in untreated control populations. High concentrations of phenol in combination with artificial diet caused the maximum weight loss of 94.031% for larvae and 86.89% for pupae.

Table 2. Spotted Bollworm *E. vittella* population on parents and gamma irradiated cotton lines during 2008-2009.

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	1.979±0.23c	1.656±0.19de	2.294±0.29b	3.065±0.34a	3.744±0.45b	2.947±0.35d	3.977±0.54b	5.365±0.6a
SP	1.691±0.19de	0.932±0.11f	1.9±0.21cd	1.544±0.19e	3.0±0.36d	1.497±0.18e	3.6±0.46bc	3.129±0.35cd
B-4	3.085±0.38a	2.891±0.34a	1.682±0.19de	2.894±0.34a	5.027±0.57a	5.303±0.7a	3.038±0.36cd	5.194±0.65a

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)

Table 3. Gossypol Gland Density boll/cm² in Parents and Gamma Irradiated Cotton Lines during 2008 and 2009.

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	40.8±0.63fg	44.6±0.79cd	38.8±0.59gh	30.2±0.83j	40.6±0.85fg	44.2±0.48d	38.8±0.92g	30.8±0.77i
SP	43.2±0.66de	75.2±0.89a	41.2±0.66ef	46.8±0.81c	42.8±0.73de	74.8±0.55a	41.6±0.56ef	46.6±0.81c
B-4	34.8±0.92i	36.6±0.7hi	50.6±0.91b	38.2±0.83h	34.8±0.94h	34.4±0.69h	49.6±0.57b	38.6±0.8g

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)

Table 4. Gossypol Gland Size on bolls in (mm) in Parents and Gamma Irradiated Cotton Lines during 2008 and 2009.

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	0.076± 2.307E-03*cd	0.068± 4.297E-03de	0.0828± 3.658E-03bc	0.0949± 2.652E-03a	0.0759± 2.819E-03cd	0.0689± 2.817E-03de	0.0832± 3.017E-03bc	0.0952± 4.772E-03a
SP	0.0699± 3.385E-03de	0.0496± 2.632E-03g	0.0756± 2.105E-03cd	0.0651± 3.566E-03ef	0.0706± 2.463E-03de	0.0495± 2.305E-03g	0.0753± 2.764E-03cd	0.0652± 3.599E-03ef
B-4	0.0946± 4.180E-03a	0.0938± 3.353E-03a	0.0559± 2.031E-03fg	0.0918± 3.342E-03ab	0.0947± 3.161E-03a	0.0941± 3.528E-03a	0.0561± 2.349E-03fg	0.0874± 4.315E-03ab

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)
2.307E-03*
*2.307E-03=0.00307

Table 5. Boll Bract Size in (cm) in Parents and Gamma Irradiated Cotton Lines during 2008 and 2009.

Cotton Lines	2008				2009			
	Parents	Irradiated Doses			Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
SB	3.52±0.06f	3.915±0.06e	3.87±0.04e	4.21±0.03d	3.52±0.05e	3.92±0.12d	3.905±0.08d	4.135±0.06c
SP	3.205±0.03g	3.505±0.07f	4.48±0.06ab	3.575±0.06f	3.425±0.06e	3.575±0.06e	4.29±0.04abc	3.57±0.05e
B-4	4.405±0.04bc	4.555±0.06a	3.615±0.05f	4.33±0.04cd	4.385±0.04ab	4.435±0.04a	3.5±0.06e	4.25±0.03bc

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)

Table 6. Total phenol concentration (mg/g) in floral square and flowers of untreated (parent) and gamma irradiated cotton lines during 2009.

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
SB	13.368±0.03f	13.947±0.03d	13.158±0.03g	9.491±0.046k
SP	13.544±0.091e	25.175±0.018a	13.404±0.046f	14.667±0.046c
B-4	10.421±0.03j	12.158±0.03i	16.421±0.03b	12.860±0.046h

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)

Table 7. Total phenol concentration (mg/g) in seeds of untreated (parent) and gamma irradiated cotton lines during 2009.

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
SB	18.866±0.079g	19.991±0.022d	16.598±0.03h	11.995±0.044l
SP	19.622±0.046e	47.01±0.03a	19.313±0.069f	23.351±0.06c
B-4	13.163±0.035k	14.811±0.062j	26.804±0.03b	16.134±0.03i

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)

Table 8. Effect of different phenol concentrations mixed with artificial diet on larval and pupal weight (mg) of spotted bollworm, *E. vittella.*

Phenol Percentage in Diet	Larval and Pupal weight (gm)			
	Larval Weight (mg)	Larval Weight Decreased as Compared to Control	Pupal Weight (mg)	Pupal Weight Decreased as Compared to Control
Control	4.537±2.313E-03* a	-	0.671±0.0136 a	-
0.5	3.477±0.0203 b	23.36	0.291±1.815E-03 b	56.31
1.00	1.701±0.0216 c	62.51	0.227±1.742E-03 c	66.17
1.5	0.882±0.0182 d	80.56	0.181±2.730E-03 d	73.03
2.00	0.258±6.506E-03 e	94.031	0.088±1.996E-03 e	86.89

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)
2.313E-03*
*2.313E-03=0.00313

Table 9. Correlations (Pearson) spotted bollworm populations with different morphological characters of parent and gamma irradiated cotton lines during-2008 and 2009.

Plant Characters	Parents	2008			Parents	2009		
		Irradiated Doses				Irradiated Doses		
		150 Gy	200 Gy	250 Gy		150 Gy	200 Gy	250 Gy
Gossypol Gland Density	-0.997	-0.889	-0.88	-0.921	-0.995	-0.911	-0.988	-0.902
P-Value	0.054	0.303	0.315	0.255	0.065	0.271	0.101	0.285
Gossypol Gland Size	0.999	0.999	0.91	1.000	0.987	0.998	0.992	0.983
P-Value	0.027	0.034	0.272	0.005	0.102	0.039	0.078	0.117
Boll Bract Size	0.998	0.999	0.126	0.969	0.961	0.999	0.606	0.974
P-Value	0.038	0.015	0.919	0.159	0.179	0.015	0.585	0.145

Table 10. Correlations (Pearson) spotted bollworm populations with total phenol concentration in parent and gamma irradiated cotton lines during-2009.

Total Phenol in Square Flowers and Seeds	2009			
	Parents	Dose-150 Gy	Dose-200 Gy	Dose-250 Gy
Total Phenol in Square Flowers	-0.945	-0.861	-0.942	-0.809
P-Value	0.204	0.339	0.218	0.399
Total Phenol in Seeds	-0.965	-0.873	-0.989	-0.956
P-Value	0.168	0.325	0.096	0.191

Correlation of *E. vittella* populations with gossypol gland density, their size, bract size, phenol concentration in square flowers and seeds.

According to Tables 8.0 and 9.0 *E. vittella* populations did not show significant negative correlations with gossypol gland density in all parental lines and their gamma-irradiated cotton variants at 150, 200, and 250 Gy during the years 2008 and 2009. The gossypol gland size displayed non-significant positive correlations in 2008 200 Gy irradiated lines and across all parent lines and their respective 200 and 250 Gy gamma-irradiated progeny in 2009. The researcher documented a meaningful positive relationship between gossypol gland size and *E. vittella* population counts in parent line and both 150 Gy and 250 Gy cotton lines during 2008 and in 150 Gy lines from 2009. *E. vittella* populations in gamma-irradiated cotton lines at 200 and 250 Gy during the year 2008 displayed positive correlations with bract dimensions along with non-significant relation in 2008 and 2009 between *E. vittella* populations and bract dimensions across all parent lines and gamma-irradiated counterparts. The parent and 150 Gy cotton lines displayed important positive associations in both the year 2008 and the 2009 gamma-treated 150 Gy cotton lines. The total phenolic content measurements of floral squares and flowers and seeds from parental cotton lines and their gamma-irradiated counterparts did not show significant changes according to assessments with *E. vittella* populations

DISCUSSION

The reproductive capability of the spotted bollworm decreases because specific traits exist in cotton plants. Cotton mutant line SP (150 Gy) evolved the best density of gossypol glands on boll surfaces, due to which *E. vittella* displayed no interest in feeding from this cotton variety. Physical treatment of cotton at 250 Gy within the SB mutant line achieved maximum boll bract expansion while lowering the gossypol glands population to produce the densest *E. vittella* population.

The research showed that gamma irradiation applied to cotton parents directly affected spotted bollworm infestation rates on squares, flowers and green bolls. The population density of spotted bollworms showed minimum impact on the gamma-irradiated mutant line SP, which received 150 Gy. The exposure to 250 Gy radiation resulted in maximum damage to the mutant line SP, which became infested with *E. vittella*. The research data matches previous research indicating NIAB-78 high-yielding mutant cotton shows outstanding resistance against spotted bollworms, according to Rehman et al. (2001), Abro et al. (2004) and Pathan et al. (2007). When exposed to *E. vittella*, the fruiting bodies of the SP mutant line treated with 150 Gy experienced almost no infection level, according to Kanher et al. (2014).

Spotted bollworms preferred cotton plants with a low gossypol gland density (per cm^2) on green bolls, which caused less damage than plants with more gossypol glands on these bolls. Millions of gossypol glands formed on green bolls of SP mutant plants after treating them with 150 Gy, but SB mutant growers received the lowest quantity of these glands following exposure to 250 Gy. Research by Mohan et al. (1994) confirmed earlier findings since they monitored that *E. vittella* infestations reached minimum levels in NAS3, LD230, and Lohit as the number of gossypol glands peaked on green bolls.

According to Bastailal (2002), gossypol (a terpenoid aldehyde synthesizing pigment gland) exists in plant aerial parts and cotton seeds. Environmental toxins produced by the glands protect plants from particular insect predators. The low levels of gossypol glands at 2.5 and 4.6/cm and 20-25 mg/100 grammes of gossypol combined with 0.28-1.94% tannin content in Egyptian cotton bolls showed no impact on bollworm distribution according to Mansour et al. (2004). The reduction of gossypol and tannin content in Egyptian cotton cultivars failed to stop spotted bollworms from damaging cultivated cotton specimens. Gossypol works as a natural allelochemical found throughout different cotton plant components, according to the research by Stipanovic et al. (2006). The plant deployed gossypol alongside multiple terpenoids as a defence strategy against particular insect pests. The egg-laying abilities of *E. vittella* increased when trichome density increased yet gossypol glands decreased the number of surviving larvae (Keshav et al. 2013).

G. arboreum B11A line displayed exceptional resilience to insect egg placement because its gossypol glands developed optimally, while hair density decreased and bracts shortened.

The current research demonstrates a negative direct relationship between boll bracts and the spotted bollworm populations, affecting both initial cotton varieties and the three previously radiated cotton lines at 250 Gy. Through two years of study, the spotted bollworm population remained statistically independent from the population figures of cotton lines exposed to 150 and 200 Gy gamma irradiation. The research by Keshav et al. (2013) confirmed that cotton cultivars display bract dimensions between 4.68 to 6.41 cm^2, demonstrating lower susceptibility to *E. vittella* infestation. Stretchy bracts in cotton plants entice feeding bollworms, according to Baloch et al. (2001), and their research shows that shorter bracts make crops more vulnerable to bollworm damage. A research study by El-Gohary et al. (1995) proved that cotton plants' bract morphological characteristics enhance pest resistance according to environmental factors. Xia (1995) mentioned that cotton plants' frego bract trait protects them against the pink bollworm. The Frego bract showed no capability of providing resistance to the pink bollworm, according to the research by Wilson and George (1982).

The investigation analyzes phenolic compounds throughout different parent genetic lines and irradiated lines found within cotton squares, flowers, and seeds. The total phenol content reached its highest point in the mutant SP cotton plant sections, including squares, flowers, and seeds, after receiving a 150 Gy gamma-ray dose that caused minimal *E. vittella* damage. The field study identified that *E. vittella* suffered maximum losses from the cotton line SB treated with 250 Gy because this line contained minimal total phenolic content. Mutant irradiated cotton lines exposed to 250 Gy did not show the same correlation trend between phenolic acid content and *E. vittella* infestation. However, other mutant lines displayed highly significant negative associations between phenolic acids and *E. vittella* damage. The latest research about phenols in insect-resistant cotton matches the work done by Parveen et al. (2001). Many phenolic acids show the potential to slow down both the development and multiplication of *E. vittella* cotton bollworms.

A research study conducted by Balakrishnan (2006) demonstrated that increased phenolic compounds found in cotton bolls and squares create substantial population reduction in *E. vittella.* The combination of artificial meals containing different phenolic concentrations causes *E. vittella* larvae and pupae to lose their maximum weight. A solution with 0.1% phenol reduced bollworm larval weight between 61–96%, according to the study by Parveen et al. (2001). Daniel et al. (1990) established that phenolic compounds and phytochemicals in artificial feeding diets caused mortality of larval stages while also reducing pupil mass. According to Wang et al. (2006), the inclusion of pyro catechol, gramine and ferulic acid plant metabolites into hypothetical food affected the weight of Helicoverpa armigera (Hübner) cotton bollworm life stages.

CONCLUSIONS

The research showed that SP* cotton line (150 Gy) achieved the highest gossypol gland density together with phenol content thereby producing superior protection against *E. vittella* population attacks when compared to the parent cotton line. The high resistance of SP cotton after 150 Gy treatment resulted in maximum gossypol gland density on boll surfaces which made the material unpalatable for *E. vittella.* Spotted bollworm populations caused little damage to the SP mutant line when it received 150 Gy exposure. Mutant line SB yielded the most damage after receiving a treatment with 250 Gy. The combination of artificial food and different phenol concentration levels led to decreased weights for both larvae and pupae. The findings of this study displayed a major negative relationship between parent cotton and three gamma-irradiated lines at 250 Gy regarding the spotted bollworm population although the correlations for lines receiving 150 Gy and 200 Gy treatments showed no significance during both study years. This study examined the total phenol levels within the squares and flowers and seeds of gamma-irradiated as well as parental cotton lines. A mutant SP cotton exposure to 150 Gy irradiation led to the highest observed phenolic levels in its seeds together with flowers and squares even with limited *E. vittella* damage. The SB cotton line that received 250 Gy radiation showed the lowest total phenolic concentration which resulted in the highest *E. vittella* field damage level. Both larvae and pupae experienced maximum weight reduction when *E. vittella* ate artificial meals containing different phenol levels.

ACKNOWLEDGEMENT

The research article is a part of Ph.D thesis of the main author submitted to Sindh Agriculture University, Tandojam. The authors are highly thankful to Emeritus Professor Dr. Muhammad Yar Khuhawar, Institute of Advanced Research Studies in Chemical Sciences (IARSCS), University of Sindh, Jamshoro, Pakistan for encouraging, providing chemicals and required laboratory facilities

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

REFERENCES

Abro, G.H., Memon, A.J., Syed, T.S., et al 2004. Infestation of Earias spp. on cotton and okra grown as mono and mix crops. *Pak. J. Biol. Sci.* 7(6): 837-942.

Atwal, A.S. 2002. Agricultural pests of South Asia and their management. Kalyani Publ., Ludhiana, India. 221.

Azmi, M.A., Naqvi, S.N.H. 2011. Pesticide pollution, resistance and health hazards. *The Impacts of Pesticides Exposure*, Prof. Margarita Stoytcheva [Ed.], ISBN: 978-953-307-531-0. InTech, http://www.intechopen.com/books/pesticides-the-impacts-of-pesticides-exposure/pesticide-pollution-resistance-and-health-hazards. pp. 15.

Balakrishnan, N. 2006. Influence of allelochemical contents in plants on the incidence of major pests of cotton. *Ind. J. Plant Prot.* 34(2): 202-205.

Baloch, M.J., Lakho, A.R., Bhutto, H., et al 2001. Bract size in relation to bollworm damage in upland cotton varieties. *Pak. J. Biol. Sci.* 4(8): 986-987.

Bastailal, H. 2002. Introgression of the glanded plant and glandless seed trait to the cultivated cotton (G. hirsutum L.) varieties. *Ziraat Fak. Derg. Mustafa Kemal Univ.* 7(1/2): 45-50.

Bentley, M.D., Leonard, D.E., Stoddard, W.F., et al 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm Choristonewa fumiferana (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.* 7: 393-397.

Cherry, J.P. 1983. Cottonseed oil. *J. Am. Oil Chem. Soc.* 60(2): 360-367.

Daniel, J.G., Cothren, J.T., Phillips, J.R., et al 1990. Influence of selected phenolic compounds on development of bollworm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 83(5): 2115-2118.

Dhawan, A.K., Singh, S., Kumar, S., et al 2009. Integrated pest management (IPM) helps reduce pesticide load in cotton. *J. Agric. Sci. Tech.* 11: 599-611.

Elegbede, M.T., Glitho, I.A., Akogbeto, M., et al 2014. Influence of cotton plant on development of Aphis gossypii Glover (Homoptera: Aphididae). *Int. Res. J. Agric. Sci. Soil Sci.* 4(2): 40-46.

Lena, V.K., Vladimir, J.O., Svetlana, O., et al 2005. Delayed induced responses of birch glandular trichomes and leaf surface lipophilic compounds to mechanical defoliation and simulated winter browsing. *Oecologia.* 146(3): 385-393.

El-Gohary, A., Moawad, G.M., El-Moghazy, M., et al 1995. Current strategy for non-chemical control of cotton pests in Egypt. New sources of genetic resistance to cotton pests. *ICAC Manila, Philippines.* pp. 10-13.

Gupta, B.D. 1978. Differentiation of sex in pupae of the spotted bollworm, Earias fabia (Lepidoptera: Noctuidae-Erastriinae). *Curr. Sci.* 47: 642.

Gupta, G.P., Rani, S., Birah, A., et al 2005. Mass rearing of the spotted bollworm, Earias vittella (Lepidoptera: Noctuidae) on an artificial diet. *Int. J. Trop. Insect Sci.* 25(2): 134-137.

Hagenbucher, S., Olson, D.M., Ruberson, J.R., et al 2013. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Crit. Rev. Plant Sci.* 32: 458-482.

Javed, M.S., Hassan, S., Adil, S.A., et al 2006. Comparative advantage of cotton production and its policy implications in Pakistan. *Pak. J. Agric. Sci.* 43(3-4): 193-196.

Kanher, F.M., Syed, T.S., Jahanger, T.M., et al 2014. Evaluation of gamma irradiation on cotton lines fruiting parts and infestation of spotted bollworm, Earias vittella (Fab.). *Sindh Univ. Res. J. (Sci. Ser.).* 46(2): 231-238.

Karar, H., Babar, T.K., Shahazad, M.F., et al 2013. Performance of novel vs traditional insecticides for the control of Amrasca biguttula biguttula (Homoptera, Cicadellidae) on cotton. *Pak. J. Agric. Sci.* 50(2): 223-228.

Keshav, A., Shera, P.S., Singh, J., et al 2013. Morphological basis of resistance to spotted bollworm, Earias vittella (Fabricius) in Asiatic cotton. *Phytoparasitica.* 41(3): 235-240.

Khan, M.H., Ahmad, N., Rashdi, S.M.M., et al 2013. Management of sucking complex in BT cotton through the application of different plant products. *P. J. L. S.* 1(1): 42-48.

Khooharo, A.A., Memon, R.A., Mallah, M.U., et al 2008. An empirical analysis of pesticides marketing in Pakistan. *Pak. Econ. Soc. Rev.* 46(1): 57-74.

Lee, S.C., Kim, J.H., Jeong, S.M., et al 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. *J. Agric. Food Chem.* 51: 4400-4403.

Makkar, H.P.S., Siddhuraja, P., Becker, K., et al 2007. Plant secondary metabolites. Humana Press, Totowa, New Jersey.

Makkar, H.P.S., 2000. Quantification of tannins in tree foliage – A laboratory manual. Joint FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture. Working Document, IAEA, Vienna.

Mansour, M.H., Zohdy, N.M., Gengaihi, S.E., et al. 2004. The status of gossypol and tannin contents in some Egyptian cotton cultivars in correlation to bollworm infestation. *Bull. Natl. Res. Cent. (Cairo)* 29(1): 121-128.

Marquis, R.J. 1992. Selective impact of herbivores. pp. 301-325. In: Fritz, R.S., Simms, E.W. [eds.]. *Ecology and Plant Resistance to Herbivores and Pathogens.* Univ. Chicago Press.

Mazid, M., Khan, T.A., Mohammad, F. 2011. Role of secondary metabolites in defense mechanisms of plants. *Biolog. Medici.* 3(2) Special Issue: 232-249.

Mehta, R.C. 1971. Survival and egg production of cotton spotted bollworm, Earias fabia (Stoll) (Lepidoptera: Noctuidae) in relation to plant infestations. *Appl. Entomol. Zool.* 6: 206-209.

Mohan, P., Singh, P., Narayanan, S.S., et al. 1994. Relation of gossypol-gland density with bollworm incidence and yield in tree cotton (*Gossypium arboreum*). *Indian J. Agric. Sci.* 64(10): 691-696.

Parveen, S.S., Qaisrani, T.M., Bhutta, S., et al. 2001. HPLC analysis of cotton phenols and their contribution in bollworm resistance. *J. Biol. Sci.* 1(7): 587-590.

Parveen, S.S., Qaisrani, T.M., Amin, S., et al. 2001. Biochemical basis of insect resistance in cotton. *J. Biol. Sci.* 1(6): 496-500.

Pathan, A.K., Chohan, S., Laghari, M.A., et al. 2007. Comparative resistance of different cotton lines against insect pest complex of cotton. *Sarhad J. Agric.* 23(1): 141-143.

Price, P.W., Bouton, C.E., Gross, P., et al. 1980. Interaction between three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst.* 11(1): 41-65.

Rehman, M., Arif, M.J., Murtaza, M.A., et al. 2001. Comparative resistance and susceptibility in different lines of normal and stub cotton against bollworms. *Int. J. Agri. Biol.* 3(1): 129-130.

Río, D.J.A., Gómez, P., Báidez, A., et al. 2004. Phenolic compounds have a role in the defense mechanism protecting grapevine against the fungi involved in Petri disease. *Phytopathol. Mediterr.* 43: 87-94.

Scheffler, J.A., Romano, G.B., Blanco, C.A. 2012. Evaluating host plant resistance in cotton (*Gossypium hirsutum* L.) with varying gland densities to tobacco budworm (*Heliothis virescens* F.) and bollworm (*Helicoverpa zea* Boddie) in the field and laboratory. *Agri. Sci.* 3(1): 14-23.

Soerjani, M. 1988. Current trend in pesticide use in some Asian countries. *Envir. Implic. Res. Pestic., Intern. Atomic Energy Agency, Vienna, Austria.* 19(17): 219–234.

Soomro, A.R., Soomro, A.W., Soomro, K., et al. 2000. Jassid resistant variety CRIS-7A. Central Cotton Research Institute, Sakrand, Sindh, Pakistan. *Pak. J. Biolog. Sci.* 3(2): 332-334.

Stipanovic, R.D., Puckhaber, L.S., Bell, A.A. 2006. Ratios of (+)- and (-)-gossypol in leaves, stems, and roots of selected accessions of *Gossypium hirsutum* var. marie galante (Watt) Hutchinson. *J. Agri. Food Chem.* 54(5): 1633-1637.

Sunilkumar, G., Campbell, L.M., Puckhaber, L., et al., 2005. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *PNAS* 102(48): 18054-18059.

Wang, C.Z. 1997. Effects of gossypol and tannic acid on the growth and digestion physiology of cotton bollworm larvae. *Acta Phytoph. Sinica.* 24(1): 13-18.

Wang, J.Y., Cai, Y., Gou, J.Y., et al. 2004. VdNEP, an elicitor from *Verticillium dahliae*, induces cotton plant wilting. *Appl. Environ. Microb.* 70(8): 4989-4995.

Wang, Y., Cai, Q.N., Zhang, Q.W. et al. 2006. Effect of the secondary substances from wheat on the growth and digestive physiology of cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Eur. J. Entomol.* 103: 255-258.

Waterhouse, A.L. 2001. Determination of total phenolics. In: Wrolstad, R.E. (ed.) *Current Protocols in Food Analytical Chemistry.* Wiley, Weinheim, pp. I1.1.1–I1.1.8.

Wilson, F.D., George, B.W. 1982. Effects okra-leaf, frego-bract, and smooth-leaf mutants on pink bollworm damage and agronomic properties of cotton. *Crop Sci.* 22(4): 798-801.

Wuyts, N., De Waele, D., Swennen, R. 2006. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* grand naine) roots. *Plant Physiol. Biochem.* 44: 308-314.

Xia, J. 1995. Research in cotton resistance to insects in China. New sources of genetic resistance to cotton pests. *ICAC Manila, Philippines*, pp. 13-14.

Yingfanm, C., Youngfang, X., Jinggao, L. 2010. Glandless seed and glanded plant research in cotton: A review. *Agron. Sustain. Dev.* 30(1): 181-190.

https://doi.org/10.55627/agribiol.003.01.1226