Journal of Entomology and Zoology Studies 2015; 3(4): 296-302

Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(4): 296-302

Received: 02-06-2015
Accepted: 04-07-2015

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Effect of total Gossypol Concentration on Spotted Bollworm *Earias Spp.* in different Gamma Irradiated Cotton Lines

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Abstract
The gossypol content of cotton *Gossypium spp.* has been of interest to plant breeders because those that have high levels often carry resistance to the spotted bollworm. However, its presence has economic disadvantages to the seed and oil processing industry. The cage experiment was evaluated on no food choices of *Earias insulana* (Boisd.) and *Earias vittella* (Fab.) and their relation with gossypol concentration in floral squares, flowers and green bolls during 2009. The result showed that highly susceptible parent line St-7 and B-3 irradiated with 250 Gy and highly resistance St-7 and SP cotton lines treated with 150 Gy to both *Earias spp.* A correlation showed significant and non-significant negative correlations with gossypol concentration in all parent and their gamma irradiated (150, 200, 250 Gy) cotton lines against both *Earias* spp. Correlation coefficient results revealed that the maximum gossypol concentration was most important factor to develop resistance against both spotted bollworm species.

Keywords: Cotton, Gossypol, Spotted Bollworm, Resistance, Gamma irradiation.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is a prominent fiber crop of Pakistan beside cultivated throughout the world. The yield per hectare is not as much of many other cotton producing countries. The low yield are poor agronomical practices, and damage caused by sucking and bollworm complex from germination to maturity. The damage of insect pests of cotton crop was estimated by several earlier workers about 30-50% [1-2]. However, [3] reported that the *E. vittella* caused singly 19-20% cotton crop yield losses. Whereas, [4] in previous studies was estimated maximum damage percent of *E. vittella* approximately 18% on square and flowers; while, 17% damage on green bolls of gamma irradiated cotton lines. The majority of growers confidently rely on synthetic chemicals to minimizing damage of insect pest on cotton crop. The excess usage of non-selective insecticides caused insect pest resistance, regular pest out breaks and imbalance natural agro-ecosystem. The different control measure practices against insect pest in cotton crops were decreased damage percentage. The host plant resistance and application of insecticides are trusted approaches against *E. vittella* in cotton crop [5]. Host plant resistance is major integrated pest management (IPM) component to decreasing insect pest infestation as an inexpensive and safe environmentally [6].
Cotton plant created physiological and morphological characters for self-defense from insect pests. Gossypols, and other allelochemicals are essential compounds of cotton for their resistance mechanism; these compounds defend from insect pests and other few diseases [7]. The [8] was reported that the gossypol and other secondary plant chemical substances are present in cotton squares and bolls. [9] Reported cotton plant allelochemicals gossypol glands act as anti-feeding to first instar larvae of *Heliothis virescens* (F.). However, [10] reported mostly pigments and other plant secondary compounds are toxic terpenoids and flavonoids, and these glands protect plant against insect's herbivore and rodents. The [11] reported gossypol concentration 0.5% elongated significantly *Helicoverpa armigera* larvae and declined the pupation period. Whereas, [12] showed gossypol complex assists to defend the plant from pests. The cotton plant chemical gossypol concentrations build resistance against insects and diseases [13]. The hemi gossypolone and heliocides provides host plant resistance against (*H. zea* Boddie) and (*Heliothis virescens* F.) [14]. While, [15] reported gossypol and other associated aldehydes have created resistance against insect pest in cotton. The aim of this study were find

out host plant resistance in gamma irradiated cotton lines compare to their parents and determination of gossypol concentration in floral squares, flowers and seeds in relation to *E. insulana* and *E. vittella* under experimental caged plants.

2. Material and Methods

2.1. *Infestation of Spotted Bollworm spp. in caged plants*

The seed of six parents St-7, BNT, B-3, SB, SP, B-4 and their gamma irradiated 150, 200 and 250 Gy cotton lines was sown in separate pots during 2009. The feeding of spotted bollworm was recorded in cages. For recording population buildup on the six selected parent cotton lines and their three dosages of gamma irradiated 150, 200 and 250 Gy lines, the seeds were sown in separate pots; one plant in a pot. There were three replications of each line. All the potted plants were screened with muslin cloth cages (1.5 m^2) in the field to prevent the plants from non-test insect pests. One pairs of freshly emerged moths (Male and female) from a culture maintained in the laboratory were released in these cages. The spotted bollworm culture was maintained from the field collected larvae in the laboratory, at 27±2 °C and 70±5% r.h. The larvae *E. insullana* were collected from cotton and okra farmer fields of District Sukkur and *E. vittella* were collected from cotton and okra field of Cotton Research Section, A.R.I, Tandojam; then they reared until pupation in glass jars (15×20 cm2) covered with muslin cloth on immature bolls of cotton and okra, the preferred natural food [16]. The pupae were sexed (male and female) on the basis of well-developed knob-like structure at the antero-dorsal end of the male cocoon [17] and placed in jars having a moist sponge at the bottom covered with filter paper. The freshly emerged moths were used for the present study. The fruiting bodies damage was recorded one week after each of the release of moths and the muslin cloth cover on cages were removed eight weeks after the release. The potted plants were kept unsprayed against insect pests during study period.

3. Determination of Total Gossypol

3.1. *Seed, Squares and flowers sample processing*

The collected sample of seed, squares and flowers was dried and ground in cold temperature in grinding mill, finally ground material sieved through 2 mm screen (Ogawa Seiki Co, Ltd. Tokyo Japan). Finally the material was stored in air tight plastic bags until it was chemically analyzed in the laboratory Institute of Advanced Research Studies in Chemical Sciences (IARSCS), University of Sindh, Pakistan.

3.2. *Total gossypol extraction and determination*

Ground material of different parts of cotton lines such as floral squares, flowers and seeds sample (200 mg) were separately dissolved in 10 ml of isopropanol-n-hexane (60:40) after addition of 0.8 ml aniline, it was shacked in ultrasonic for 10 minutes at room temperature. The sample was warmed in tightly caped glass tubes in water bath for 30 minutes at 95-100 °C. The samples were cooled for half an hour to attain the room temperature and centrifuged for 10 minute on 3000 rpm at 4 °C. The sample supernatant was collected to fill spectrophotometerm cavetti to determine on lamda max 440 nm along with blank. The blank was prepared with the same sampling procedure except of using plant material. The pure gossypol acetic acid 95% was used as standard obtained from Sigma Chemical Co. (St. Louis, Mo.). A standard curve ($_{r2}$ = 0.9987) of gossypol from the determination of unknown concentration of plant samples. The analysis of the sample, the above explained method was slightly modified according to developed by [18].

3.3. *Statistical Procedures*

The statistical analysis using general ANOVA was followed by least significant difference (LSD) at 0.05% probability level. The data were subjected for significant difference of E. insulana and E. vittella infestation in squares, flowers and green bolls. The gossypol concentration in different cotton lines was identified with simple correlation and linear regression analyses were carried out using Statistix software 8.1 (Analytical Software, USA).

4. Results

4.1. *Damage percent of Spotted Bollworm E. insulana on floral squares, flowers and green bolls in caged plants*

The damage caused by spotted bollworm E. *insulana* on floral squares, flowers and green bolls in caged plants of different untreated (parent) and gamma irradiated cotton lines was observed significant ($P< 0.05$) differences. The result on damage percent by spotted bollworm E. *insulana* in caged plants presented in Table 1.0 indicated that damage percent significantly higher was observed in untreated parent St-7 line (7.373±1.161/plant) followed by B-3 treated 250 Gy (6.799±0.891/plant) and B-3 treated with 200 Gy (5.999±0.912/plant), whereas SP treated with 150 Gy significantly calculated lowest damage percent (0.911±0.24/plant) followed by St-7 treated with 150 Gy (1.341±0.239/plant) and BNT treated with 250 Gy (1.779±0.507/plant). The spotted bollworm E. *insulana* damage was recorded significantly difference amongst untreated and treated with gamma radiation 150, 200 and 250 Gy cotton lines.

4.2. *Damage percent of spotted bollworm E. vittella on floral squares, flowers and green bolls in cage plants*

The damage percent of spotted bollworm E. *vittella* on floral squares, flowers and green bolls was estimated significant ($P< 0.05$) different variability amongst untreated (parent) and each gamma irradiated cotton lines. The data presented in Table 2.0 showed significantly maximum damage percent in B-3 treated with 250 Gy (8.406±1.254/plant) followed by untreated St-7 (7.538±0.975/plant) and B-3 treated with 200 Gy (6.565±0.957/plant) observed highly susceptible, whereas significantly minimum percent damage observed in SP treated with 150 Gy (1.613±0.296/plant), St-7 irradiated with 150 Gy (2.803±0.39/plant) and B-3 treated with 150 Gy (3.015±0.4/plant) was found highly resistant cotton lines under no food choice. Further it was observed that *E. vittella* prefer more on same cotton lines in field and caged plant due to plant morphological and chemical characteristics.

4.3. *Total gossypol concentration (mg/g) in squares and flower of parents and their gamma irradiated cotton lines*

The total gossypol concentration in squares and flowers of six parent and their gamma irradiated cotton lines differed significantly ($P<0.05$). The maximum total gossypol concentration in squares and flowers was found in mutant SP treated with 150 Gy (0.272 mg/g) followed by mutant BNT treated with 250 Gy (0.232 mg/g). While minimum total gossypol content in squares and flowers was recorded from parent St-7 (0.026 mg/g) and mutant B-3 irradiated with 250 Gy (0.02 mg/g) (Table 3.0).

4.4. *Total gossypol concentration (mg/g) in seeds of parents and their gamma irradiated cotton lines*

The total gossypol concentration in the seeds of parents and their gamma irradiated cotton lines varied significantly ($P< 0.05$). The maximum total gossypol concentration in seeds was found in mutant SP treated with 150 Gy (17.659 mg/g) followed by mutant BNT treated with 250 Gy (16.333 mg/g). While minimum total gossypol content in seed was recorded from parent St-7 (1.265 mg/g) and mutant B-3 irradiated with 250 Gy (0.988 mg/g) (Table 4.0).

Table 1: Damage percent of spotted bollworm *E. insulana* on square, flowers and green bolls in cage plants

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
St-7	7.373±1.161 a	1.341±0.239 ij	5.892±0.85 cd	2.32±0.418 gh
BNT	5.796±0.903 cde	5.001±0.756 ef	5.335±0.739 cde	1.779±0.304 hi
B-3	5.11±0.814 def	3.063±0.467 g	5.999±0.912 bc	6.799±0.891 ab
SB	5.404±0.825 cde	5.368±0.848 cde	5.248±0.848 cdef	5.581±0.951 cde
SP	4.468±0.784 f	0.911±0.24 j	5.118±0.785 def	2.312±0.263 gh
B-4	5.427±0.857 cde	5.588±0.847 cde	2.448±0.435 gh	5.138±0.796 def

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

Table 2: Damage percent of spotted bollworm *E. vittella* on floral squares, flowers and green bolls in caged plants

Cotton Lines	Parents	Gamma Rays doses (Gy)		
		150 Gy	200 Gy	250 Gy
St-7	7.538±0.975 a	2.803±0.39 f	5.939±0.865 bcde	3.107±0.436 f
BNT	5.169±0.705 de	5.533±0.797 cde	5.624±0.933 cde	3.099±0.507 f
B-3	5.505±0.718 cde	3.015±0.4 f	6.565±0.957 b	8.406±1.254 a
SB	5.797±0.843 bcde	5.163±0.829 de	5.489±0.89 cde	6.04±1.002 bcd
SP	5.042±0.661 e	1.613±0.296 g	5.136±0.754 e	3.124±0.413 f
B-4	6.239±0.899 bc	5.26±0.796 de	3.158±0.464 f	5.393±0.88 cde

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

Table 3: Total gossypol concentration (mg/g) in squares and flowers of untreated and their gamma treated cotton lines

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
St-7	0.026±1.654E-04*p	0.203±1.654E-03 d	0.066±1.654E-03 n	0.173±2.526E-03 e
BNT	0.117±2.525E-03 k	0.138±1.654E-03 i	0.086±1.653E-03 m	0.232±1.653E-03 b
B-3	0.115±1.653E-03 k	0.226±1.653E-03 c	0.042±2.525E-03 o	0.02±1.654E-04 q
SB	0.144±2.526E-03 h	0.161±2.525E-03 f	0.129±1.654E-03 j	0.069±1.653E-03 n
SP	0.152±1.653E-03 g	0.272±1.653E-03 a	0.147±2.525E-03gh	0.201±2.526E-03 d
B-4	0.109±1.654E-03 l	0.115±1.653E-03 k	0.206±1.653E-03 d	0.126±1.653E-03 j

Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; LSD)
*1.654E-04= 0.000654

Table 4: Total gossypol concentration (mg/g) in seeds of untreated (parent) and gamma irradiated cotton lines

Cotton Lines	Parents	Irradiated Doses		
		150 Gy	200 Gy	250 Gy
St-7	1.265±0.017st	15.946±0.013 d	1.861±0.083 r	15.578±8.265E-03e
BNT	9.689±0.208 l	12.027±0.165 i	3.436±0.083 p	16.333±0.021 b
B-3	6.443±0.165 n	16.275±0.013 bc	1.418±0.058 s	0.988±0.017 t
SB	13.221±0.126h	15.559±0.048 e	11.502±0.126 j	3.007±0.082 q
SP	14.528±0.031f	17.659±0.126 a	13.793±0.126 g	15.788±0.013 de
B-4	5.536±0.126 o	7.827±0.126 m	16.022±8.268E-03 cd	10.357±0.126 k

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

4.5. Correlation of *E. insulana* and *E. vittella* infestation with gossypol concentration in squares, flowers and seeds

Correlation studies was conducted to determine the relationship between *E. insulana* and *E. vittella* larval infestation with gossypol concentration in squares, flowers and seeds of different parent and their gamma irradiated cotton lines. Table 3.0 indicate that *E. insulana* and *E. vittella* infestation with total gossypol concentration in floral squares and flowers was found highly significant and negatively correlated with *E. insulana*, significant and negatively correlations in parent lines respectively; whereas, significant and negatively correlated with *E. insulana* in irradiated with 150 and 200 Gy cotton lines, while *E. vittella* was highly significant and negatively correlated in gamma irradiated 150 and 200 Gy cotton lines. The *E. insulana* and *E. vittella* was found highly significant and negatively correlated in irradiated with 250 Gy cotton lines respectively, during 2009. The results in Table 4.0 revealed that (r) value of *E. insulana* and *E. vittella* infestation with total gossypol concentration in seeds was found non-significant and negative correlations in parent, 150 and 200 Gy cotton lines with *E. insulana*, whereas, non-significant and negatively in parent and 150 Gy cotton lines with *E. vittella*, while, significant and negatively correlated with *E. vittella* in irradiated with 200 Gy cotton lines. The response of *E. insulana* and *E. vittella* with gossypol concentration in seeds was found highly significant and negatively correlated in gamma irradiated 250 Gy cotton lines during 2009, respectively. Further results of correlation coefficients were indicates that an increased total gossypol concentration in squares, flowers and seeds resulted in decreased *E. insulana* and *E. vittella* per plant infestation in all parent and their gamma irradiated 150, 200 and 250 Gy cotton lines (Fig. 1.0-4.0).

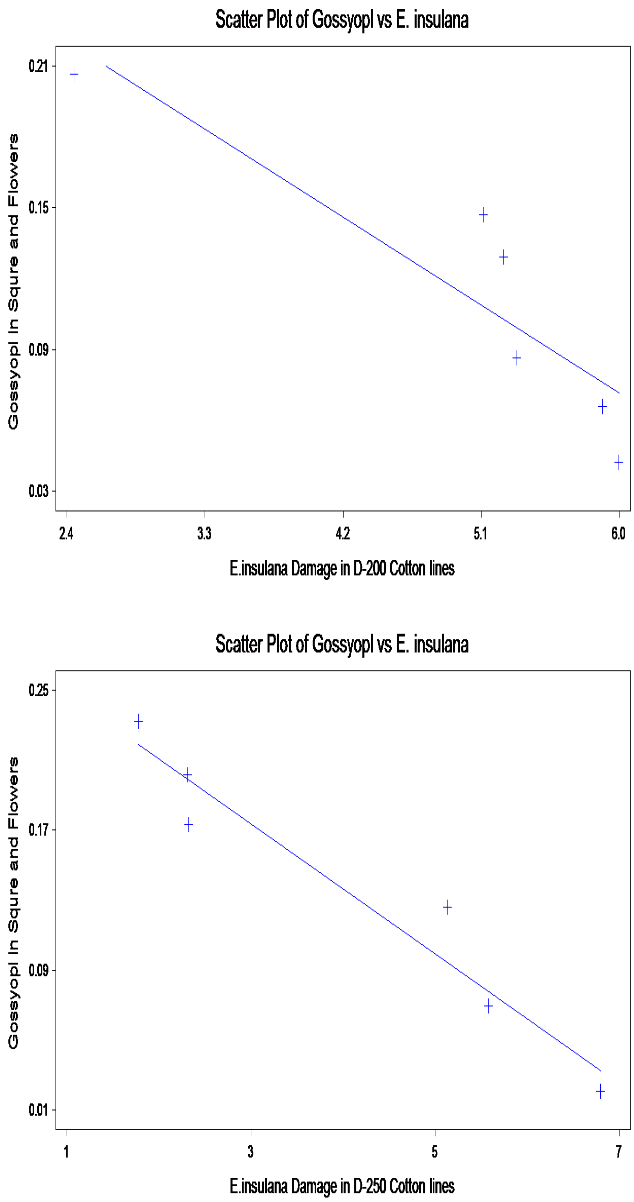
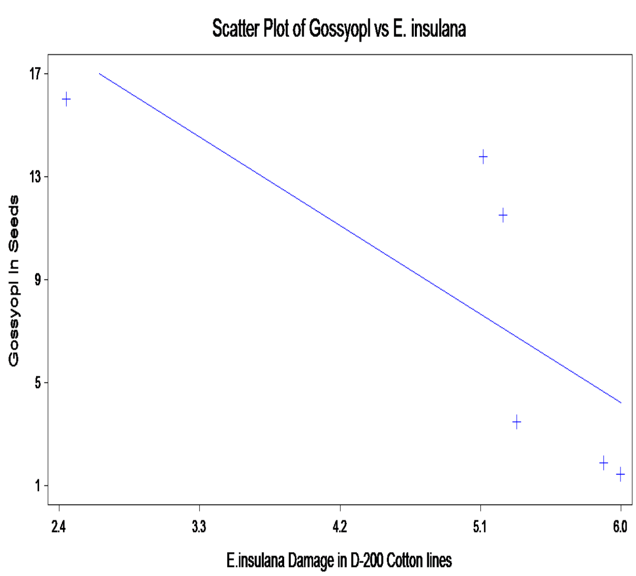


Fig 1: Correlation coefficient and scatter diagram of gossypol concentration in square and flowers, and *E. insulana* infestation under caged plants.



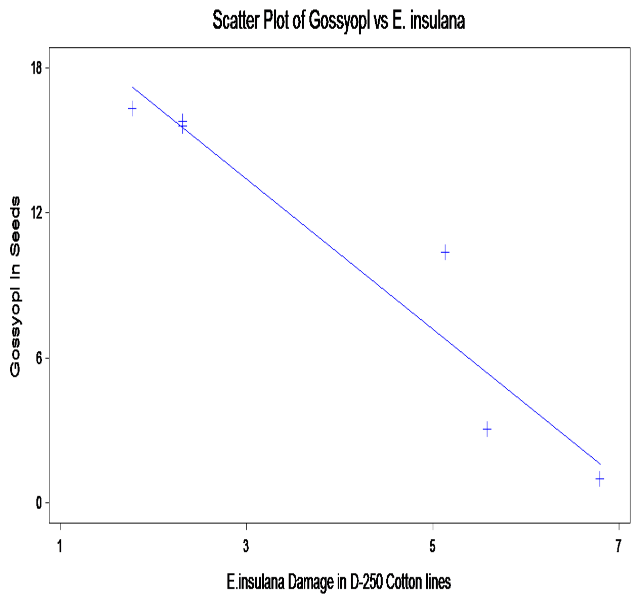
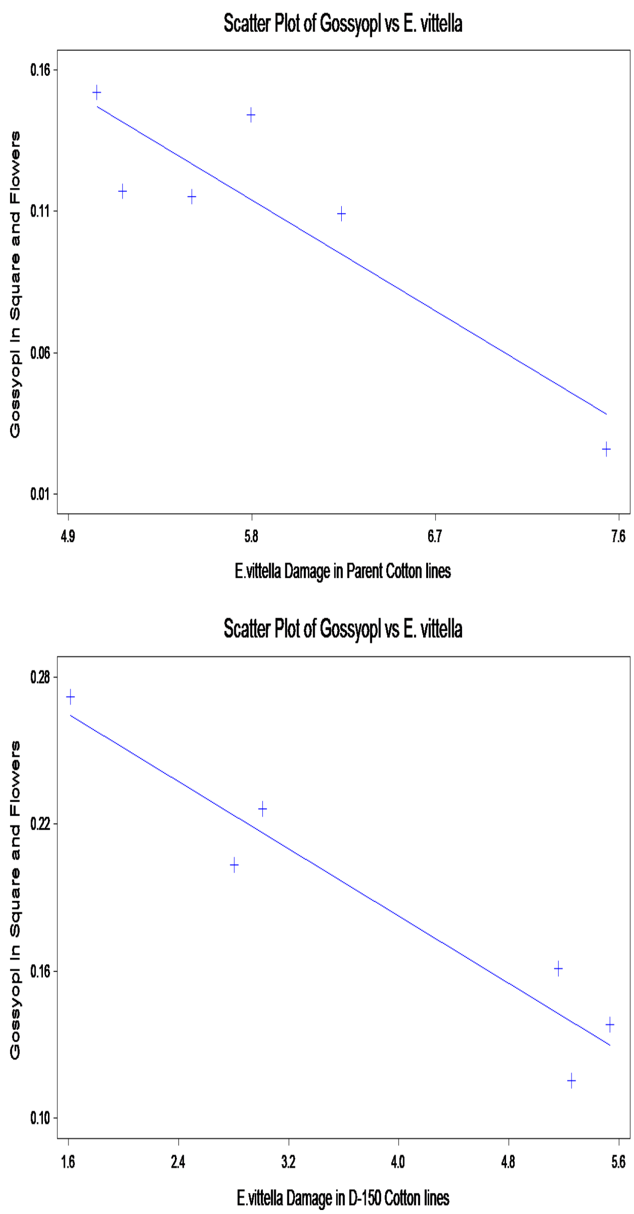
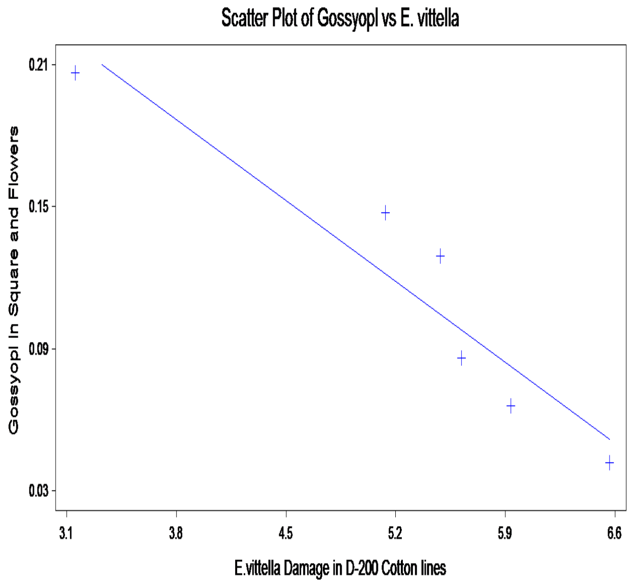


Fig 2: Correlation coefficient and scatter diagram of gossypol concentration in seeds, and *E. insulana* infestation under caged plants.





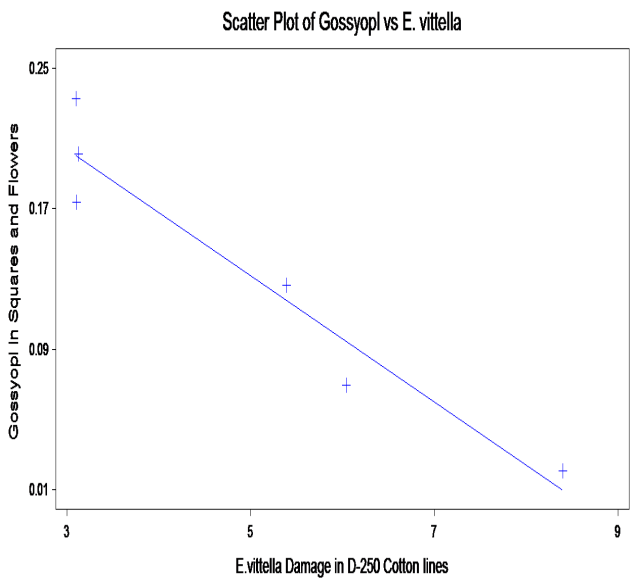
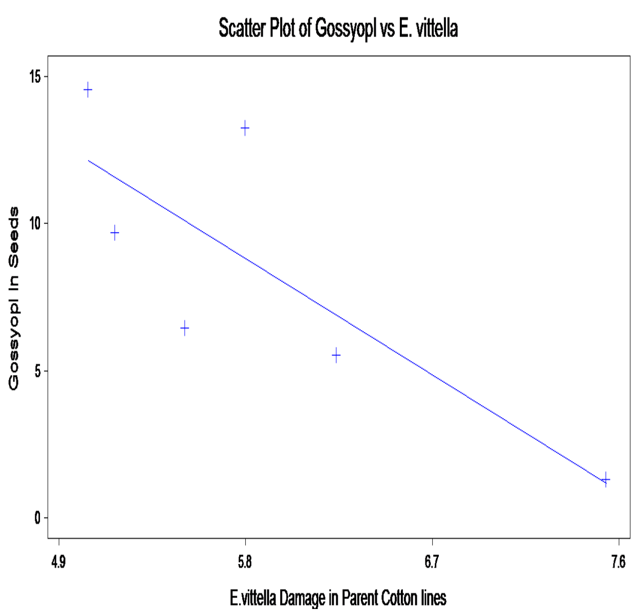
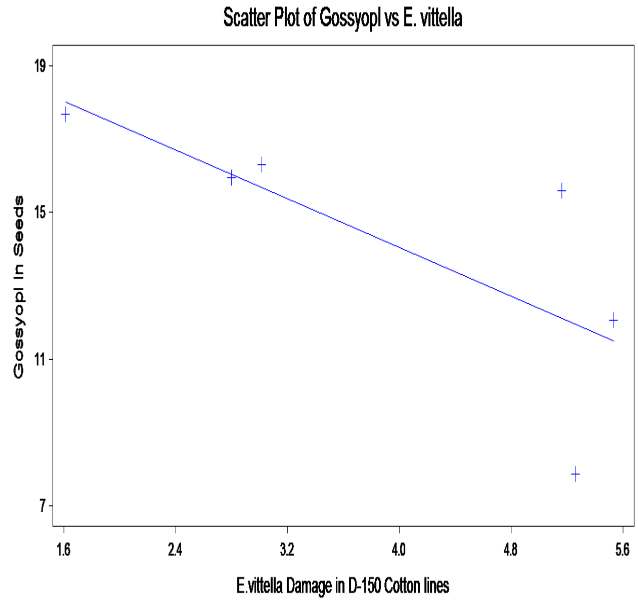
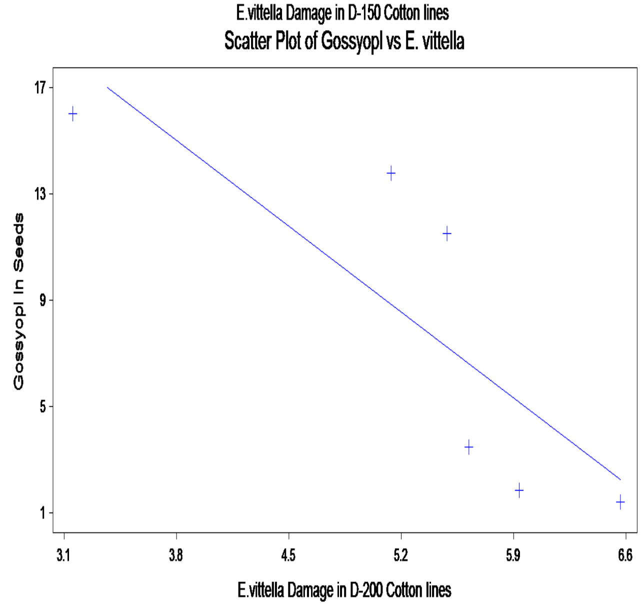


Fig 3: Correlation coefficient and scatter diagram of gossypol concentration in square and flowers, and *E. vittella* infestation under caged plants.







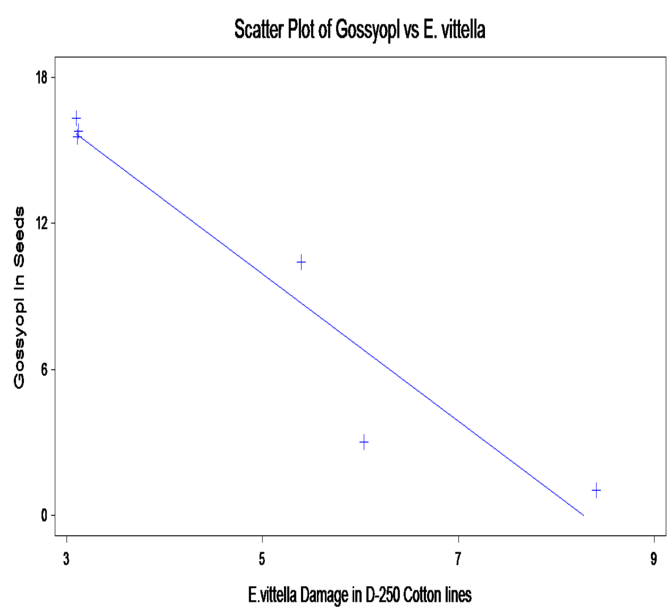


Fig 4: Correlation coefficient and scatter diagram of gossypol concentration in seeds, and *E. vittella* infestation under caged plants.

5. Discussion

The present study on parent (untreated) cotton and gamma irradiated lines against spotted bollworm spp. infestation on squares, flowers and green bolls, clearly showed significant effect of gamma irradiation on bollworm infestation. Spotted bollworm caused minimum damage in mutant lines, SP and St-7 treated with 150 Gy and maximum in B-3 irradiated with 250 Gy and parent St-7 cotton lines. Whereas, gamma irradiated cotton line treated with 150 Gy were found to improve resistance against bollworms. The results are in strongly agree with those of Rehman *et al.*, [19], Abro *et al*., [20] and Pathan *et al.*, [21] who reported that high yielding mutant cotton variety NIAB-78 proved moderately resistant against spotted bollworm. Similarly Kanher *et al*., [4] reported that *E. vittella* infestation was lowest on squares and flowers and green bolls in mutant line SP treated with 150 Gy. Present study on determination of total gossypol concentration in squares, flowers and seeds of different parent and their gamma irradiated cotton lines; it attained maximum total gossypol concentration in squares, flower and seeds of mutant SP irradiated with 150 Gy cotton line with minimum damage of *E. insulana* and *E. vittella*. While minimum concentration of total gossypol content in cotton line B-3 treated with 250 Gy had found highest damage of *E. insulana* and *E. vittella* in the caged experiment. These results are in agreements with those of Dongre and Rahalkar [22] reported that *E. vittella* larvae reared in laboratory on 1.0% of gossypol treated leaves; found decreasing larval survival rate, pupation time and in their weight, and increasing in pupation time period. Baloch *et al*., [23] stated that a gossypol free variety was highly susceptible to bollworms. Sharma *et al*., [24] reported that cotton plant chemical gossypol concentration was negatively correlated with spotted bollworm infestation. Sharma and Agarwal [25] reported gossypol and other cotton plant secondary substances in squares and bolls acted as a biochemical resistance against *E. vittella*. Klein *et al*., [26] reported that bearing high gossypol BR-8 cotton line bolls were resistant to larval infestation. McCarty *et al.*, [27] worked on cotton gossypol contents in different parts and found out 0.07 mg/g to 0.79 mg/g in square of cotton plants. Chakrabartyi *et al*., [28] reported that gossypol was decreased in amount in susceptible cotton lines compared to resistant ones. Cai, *et al*., [29] recorded gossypol in the top leave of florescence ranged from 0.218 mg/g to 0.384 mg/gm. The present results are in partial conformity with those of Rahim *et al*., [30] who reported that gossypol concentration was significant and negative correlated with pink bollworm infestations. Stipanovic *et al*., [31] reported those different types of (+)-gossypol and their dosages affect *Heliothis virescens* larvae; when larvae were reared on diets 0.16, 0.24 and 0.32% containing gossypol. However larvae reared on 0.24% gossypol containing diet significantly affected survival of larvae compare to all other dosages. Scheffler *et al*., [32] worked on terpenoid aldehydes (TAs) in field and laboratory for estimating cotton plant resistance against bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*) TAs, confered host plant resistance against *H. zea* and *H. virescens* in field and labouratory. Abdo and Fadni [33] worked on rearing of African bollworm, *H. armigera* on artificial diet containing gossypol 0.5, 1.0, 1.5 and 2.00g. Their results indicated that higher concentration of gossypol delayed growth of larvae and pupation periods.

6. Conclusion

It is concluded that St-7 and SP treated with 150 Gy cotton lines were found to improve resistance against *Earias* spp. compare to their control (parent) cotton lines under caged experiment, during 2009.

7. Acknowledgement

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 providing required chemicals.

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