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DETERMINATION OF CONDENSED TANNIN, TOTAL GOSSYPOL CONCENTRATION AND POPULATION BUILD UP OF JASSID, *AMRASCA (DEVASTANS* DIST.*) BIGUTTULA BIGUTTULA* (ISHIDA) IN DIFFERENT COTTON LINES

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FMK designed the project, TMJ & SAT carried field work, RRHA & AMS carried lab. work & KAK & RP complied and revised the draft.

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Condensed tannin, total gossypol, gamma irradiation, jassid and resistance.

ABSTRACT

The M5 generations of three cotton lines St-7, BNT and B-3 were irradiated with different gamma ray doses, i.e. 150, 200 and 250 Gy. The experiment was conducted in cages for determination of condensed tannin, total gossypol and population buildup of jassid, *A.* (*devastans* Dist.) *biguttula biguttula* (Ishida) during 2009. The physiological characters of parent cotton lines like condensed tannin and total gossypol were changed after the application of gamma rays. The mutant line B-3 irradiated with 150 Gy found comparatively susceptible to jassid infestation. Whereas, the lowest jassid population was recorded in mutant line B-3 treated with 250 Gy. In the leaves of susceptible cotton lines to jassid infestation had moderate amount of condensed tannin and total gossypol compounds. The correlation coefficient showed positive relations with the condensed tannin and total gossypol substances in all parents and their gamma irradiated (150, 200, 250 Gy) cotton lines against *A.* (*devastans* Dist.) *biguttula biguttula* (Ishida) populations. The linear regression analysis indicated that the minimum condensed tannin and total gossypol concentration are the most important factors for increasing the resistance level against jassid population build up.

1. INTRODUCTION

Cotton crop is a most important fiber crop of Pakistan. It plays a vital role in the economy of the country and provides local employment. The cotton crop shares 1.4 percent in GDP and 6.7 percent of raw material to textile [1]. [2] reported that cotton crop provides 40% employment in different sectors of Pakistan.

Cotton crop, like other crops is attacked by various insects because of it has attractive food for insect pests during full growing season and about 96-148 insects and mite species are recorded [3, 4]. However, out of them sucking complex is responsible for 5-45% losses in yield [5, 6, 7]. The growers of cotton crop rely only on insecticides to control these insects in Sindh province as well as in Pakistan. The harmful side effects of insecticides are about 94% observed indirectly in the use of pesticides by the growers [8]. However, [9] reported that the misuses of pesticides are most serious problem of the world; it

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is creating significant environmental pollution and health hazards to human. The host plant resistance which is natural and environmental friendly being used as the major component of integrated pest management; the Physio-morphological attributes of plants contributing economical control of the insect pests. [10] reported that effects of tannin on herbivore depend on dosage and concentrations. However, [11] reports that tannin substances in cotton cultivars enhance resistance to insect pests. [12] recognized tannins are polyphenols that are the part of the plant defense mechanism. [13] reported that biochemical mechanisms of resistance against the herbivores are extensive highly active, and are associated with the direct and indirect defense. The [14] reported the gossypol is a phenolic compound present in pigment glands in different parts of cotton *Gossypium* spp. [15] worked on the cotton (*G. hirsutum* L.) terpenoid aldehyde (TA) compounds that produce toxic substance in epidermal glands. [16] reported that jassid population increased with higher amount of phenol in cotton leaves. [17] and [18] suggested that the gossypol and other related terpenoids induced defense mechanism in the plant against some insect herbivores. [19] reported that tannins and free gossypol concentration in the cotton leaves had significant and negative relations with leafhopper eggs. The purpose of this study is also to emphasize on developing alternative methods to pest control through radiation that regulating the biochemical function which is responsible to reduce the infestation of noxious cotton pest.

2. MATERIALS AND METHODS

2.1 Population of jassid in cage plants

The jassid population was recorded on three selected cotton parent lines as well the same treated with three different doses of gamma rays i-e 150, 200 and 250 Gy, seeds were sown separately in each pot for jassid population buildup. There were three replications of each line. All the potted plants in cages (1.5 m²) were covered with muslin cloth in the field to prevent the plants from the approach of non test insect pests. One pair of freshly emerged moths (Male and female) from a culture maintained in the laboratory was released in each cage. The jassid adults were collected from an okra field of Entomology Section, A.R.I., Tandojam. The jassid adults were released on most susceptible cotton line plant in field cages for their reproduction. The newly emerged jassid nymphs were collected in a patri dish for rearing adults in the Department of Entomology, Faculty of Crop Protection laboratory. The insect was reared at constant temperature and humidity 27±2 °C and 70±5% r.h; the fresh okra leaves were provided in each parti dish for jassid feeding. The freshly developed jassid adults were sexed (male and female) on the basis of well developed V-like structure at the end of ovipositer of female one adult male and female were released on potted plants in the cage. The observations were recorded on a weekly basis after one week of release in cages for their offspring development in caged plants. The potted plants were kept unsprayed against insect pests during the study period.

2.2 Determination of tannins

Tannin content in the leaves of different cotton lines was determined with butanol–HCl–iron method [20]. For the tannin assays, ground samples (0.2 g) were extracted in 10 ml aqueous acetone (acetone: methanol, 7:3) and shaken for 10 minutes in an ultrasonic water bath (105W). The extracted samples were centrifuged (3000×*g*, 10 minutes, 4 ^{o}C), and the supernatants were collectively used for tannin analysis on the same day [21]. The condensed tannins were determined by the butanol–HCl–iron method. The tannins were expressed as tannic acid equivalent and condensed tannins as anthocyanidins equivalent.

2.3 Procedure:

Leaves 200 mg powder was taken with 10 ml of acetone: methanol (70:30) and it was mixed occasionally by swirling, sonicated for 20 minutes and warmed in boiling water at the 95-100 ^{o}C in a water bath for one hour, then it was kept too cool for a half hour to attain the room temperature to be centrifuged for 10 minutes on 3000 rpm at 4 ^{o}C. The collected sample supernatant was filled in the spectrophotometer cavity to determine on lamda max 550 nm along with blank. The blank was prepared with the same sample procedure, except using plant materials. The tannin was determined from a standard curve (r^2 = 0.9945) of tannic acid from plant sample.

2.4 Total gossypol extraction and determination

The leaves sample was collected from all parents and gamma treated lines from experimental field of Cotton Research Section A.R.I., Tandojam, for total gossypol determination.

2.5 Sample Grinding Methods

For proper crushing and extraction, the cottonseed was carefully washed with water to remove all adhering impurities such as sticks, stones; insect parts and the remaining lint were removed properly. After drying up the seeds were ground in a pestle and mortar. Then solvent was added to iron mash, thereafter mash had pressed to get the extract which was a dark-brown in colour.

2.6 Total gossypol extraction and determination

Ground material of leaves sample (200 mg) was separately dissolved in 10 ml of isopropanol-n-hexane (60:40) after addition of 0.8 ml aniline, it was shaked in ultrasonic for 10 minutes at room temperature. The sample was warmed in tightly capped glass tubes in a water bath for 30 minutes at 95-100 ^{o}C. The samples were cooled for half an hour to attain the room temperature and centrifuged for 10 minutes at 3000 rpm at 4 ^{o}C. The sample supernatant was collected to fill spectrophotometer cavities 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 (r^2 = 0.9987) of gossypol from the determination of unknown concentration of plant samples.

2.7 Statistical Procedures

The recorded data were statistically analyzed on factorial design for significant difference in jassid population, tannin and gossypol contents in different cotton lines by Tukey HSD (P< 0.05; HSD) range test on computer software Statitix 8.1 (Analytical Software, USA).

3. RESULTS

3.1 Jassid population in caged plants

The population of jassid in caged plants was recorded after one week releasing of a pair of insect. The highly significant (F= 6.21; df= 2; P= < 0.0001) differences amongst the parent and gamma irradiated cotton lines were observed. The mean populations per leaf are presented in Fig. 1. The highest mean population of jassid/leaf was recorded in B-3 irradiated with 150 Gy (2.486/leaf), followed by BNT irradiated with 250 Gy (2.292/leaf) and St-7 treated with 150 Gy (2.236/plant) were highly susceptible cotton lines. Whereas, the moderately mean/leaf jassid population was recorded in St-7 irradiated with 250 Gy (1.306/plant), BNT irradiated with 150 Gy (1.153/plant) and parents BNT (1.139/plant) proved moderate susceptibility against jassid infestation respectively. While, the lowest mean population was observed significantly in B-3 irradiated with 250 Gy (0.208/plant) followed by B-3 irradiated with 200 Gy (0.264/plant), BNT treated with 200 Gy (0.278/plant), St-7 irradiated with 200 Gy (0.278/plant), parent line B-3 (0.319/plant), parent line St-7 (0.431/plant) was proved highly resistant against jassid damage respectively.

3.2 Condensed tannin concentration (mg/L) in leaves of parents and their gamma irradiated cotton lines

The results showed condensed tannin concentration in leaves of three parents and their gamma irradiated cotton lines varied significantly (F= 210894; df= 2; P= < 0.0001). The maximum condensed tannin concentration in leaves was obtained in mutant St-7 treated with 250 Gy (0.843 mg/L) and mutant B-3 treated with 150 Gy (0.714 mg/L). While the minimum condensed tannin content was determined from mutant B-3 treated with 200 Gy (0.029 mg/L) and mutant B-3 irradiated with 250 Gy (0.015 mg/L) (Table-2).

3.3 Total gossypol concentration (mg/g) in leaves of parents and their gamma irradiated cotton lines

The results showed that the total gossypol concentration in the leaves of three parents and their gamma irradiated cotton lines varied significantly (F= 1446.15; df= 2; P= < 0.0001). The maximum total gossypol concentration in leaves was obtained from mutant St-7 treated with 250 Gy (0.248 mg/gm) followed by mutant B-3 treated with 150 Gy (0.233 mg/gm). While minimum total gossypol content was determined from mutant B-3 treated with 200 Gy (0.034 mg/gm) and mutant B-3 irradiated with 250 Gy (0.026 mg/gm) (Table-3).

3.4 Correlation studies of jassid population with condensed tannin (mg/L) in leaves of parent and gamma irradiated cotton lines

Correlation coefficients and linear regression models worked out among the jassid population and condensed tannin concentration in leaves of the parent and their gamma irradiated 150, 200 and 250 Gy cotton lines presented in (Fig. 2). The results

revealed that (r) value of jassid population with condensed tannin concentration found positive and non-significant relations with parent lines (0.9864), and highly significant and positive relation with gamma irradiated 150 Gy (0.9918), significant in irradiated with dose 200 Gy lines (0.9991) and non-significant positive relations in gamma irradiated 250 Gy lines (0.6056) during 2009, respectively. Regression studies based on regression analysis by taking jassid pest population (y) as a dependent variable and condensed tannin in the leaves (x) as independent variables following equations were fitted for the year 2009. The regression equation indicated that an increase condensed tannin concentration and increased jassid population in parents, 150, 200 and 250 Gy cotton lines per leaves during 2009, respectively.

3.5 Correlation studies of jassid population with total gossypol (mg/g) in leaves of parent and gamma irradiated cotton lines

Correlation coefficients and linear regression models worked out among the jassid population and gossypol concentration mg/gm in the leaves of the parent and their gamma irradiated 150, 200 and 250 Gy cotton lines are presented in (Fig. 3). The results revealed that (r) value of jassid population with gossypol concentration in leaves found highly significant and positive relation with parent cotton lines. While positive and non-significant relation observed in 150, 200 and 250 Gy cotton lines during 2009, respectively. Regression studies based on regression analysis by taking jassid pest population (y) as a dependent variable and total gossypol in leaves (x) as independent variables following equations were fitted for year 2009. The regression equation indicated that with the increasing total gossypol concentration the jassid population was increased in parents, 150, 200 and 250 Gy cotton lines per leaves during 2009, respectively.

4. DISCUSSION

Present results for jassid food preferences on caged plant revealed moderate jassid population on cotton lines having maximum concentration of tannin and gossypol in the plant leaves of St-7 gamma irradiated 250 Gy cotton lines, whereas, moderate concentrated cotton lines were highly susceptible i.e., St-7 and B-3 treated with 150 Gy and BNT irradiated with 250 Gy cotton line. The present experimental results are in agreements with those of [22] reported that jassid prefer susceptible cotton varieties for oviposition compared to resistant. Further mentioned that antibiosis mechanism affected the survival of jassid nymph, jassid nymph survives 75% on moderately resistant varieties and 92-96% of susceptible cotton varieties. [23] studied on two cotton varieties Barac (67) B and Barakat 90 to assess economic threshold levels (ETL) of cotton jassid, *Jacobiasca lybica*, (Homoptera: Cicadellidae) infestation in the field and cage conditions, reported that Barac (67) B was more susceptible to jassid infestation in the field and caged conditions.

Present study of the determination of tannin and gossypol concentration in the leaves of the parent and their gamma irradiated cotton lines, showed that maximum tannin and gossypol concentration in the leaves of cotton lines was positively associated with the maximum jassid population. While, the lowest amount of plant chemicals tannin and gossypol in leaves had a minimum jassid infestation. The present results in agreement with those of Sharma and [24]; [25] who found out tannin positively correlated with jassid populations in cotton. [26] gossypol, phenol and tannin concentrations in the leaves were positive and significantly influenced jassid population in *Arboreum* and *Hirsutum* cotton varieties. Whereas, [27] reported that tannin was significantly and positively correlated with jassid population in different cotton genotypes. The results are in partial agreement with [28] who reported gossypol gland positively correlated with whitefly populations in cotton. . The present results did not agree with those of [29] reported a free gossypol cotton varieties were highly susceptible to jassid infestation. [24] and [25] found out gossypol was significantly and negatively correlated with jassid population in cotton. [30] reported maximum concentration of tannins and free gossypol in cotton leaves had significant and negative correlations with jassid infestation on different cotton genotypes. [19] reported egg laying preferences of cotton leafhopper *A. biguttula biguttula* (Ishida) on cotton and okra genotypes significantly and inversely correlated with tannin and free gossypol content in the leaves. [31] reported that the high tannin concentration caused significant and negative effects, while low level of tannin concentration was

positively correlated with piercing and sucking insect infestation in Egyptian cotton varieties. It was recorded that jassid population was significantly and negatively correlated with tannin and gossypol concentrations in different cotton genotypes [27, 32, 33].

5. CONCLUSION

During present study it was proved that condensed tannin and total gossypol contents in leaves are allelochemicals that are present naturally in the parts of the cotton plant. However, high ratio of these compounds in cotton leaves was moderately attracted to jassids populations. It is concluded that gamma irradiation doses 200 and 250 Gy was found best to decrease condensed tannin and total gossypol concentrations as compared to their parent cotton lines, that results unattractive for jassid feeding and reproduction.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding publication of this article.

7. ACKNOWLEDGMENT

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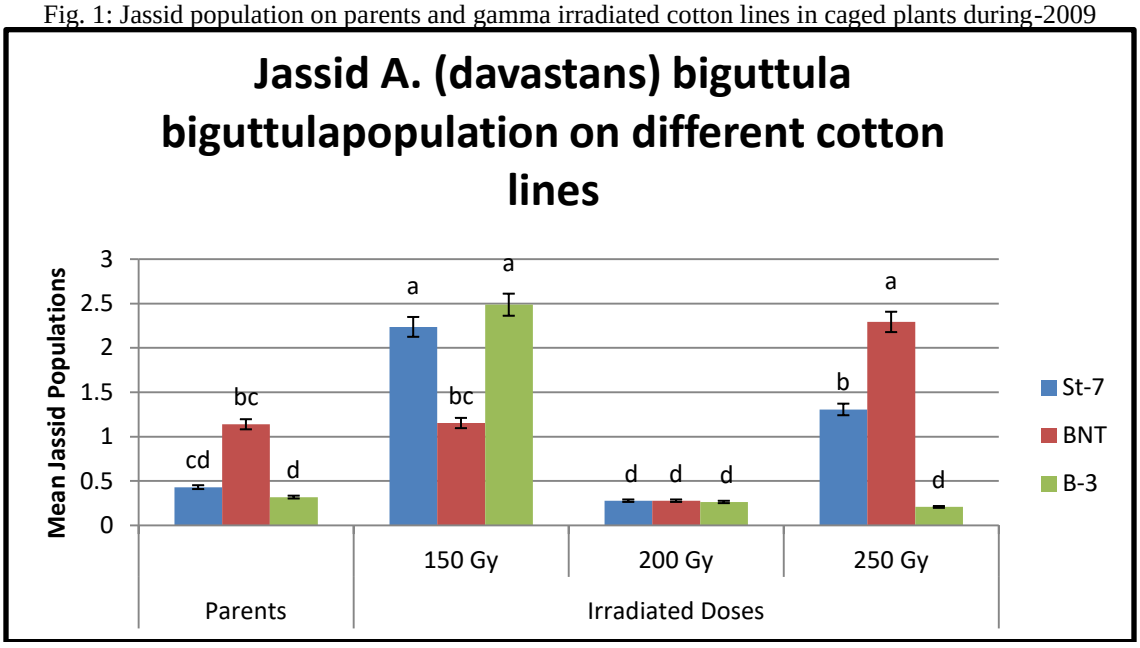
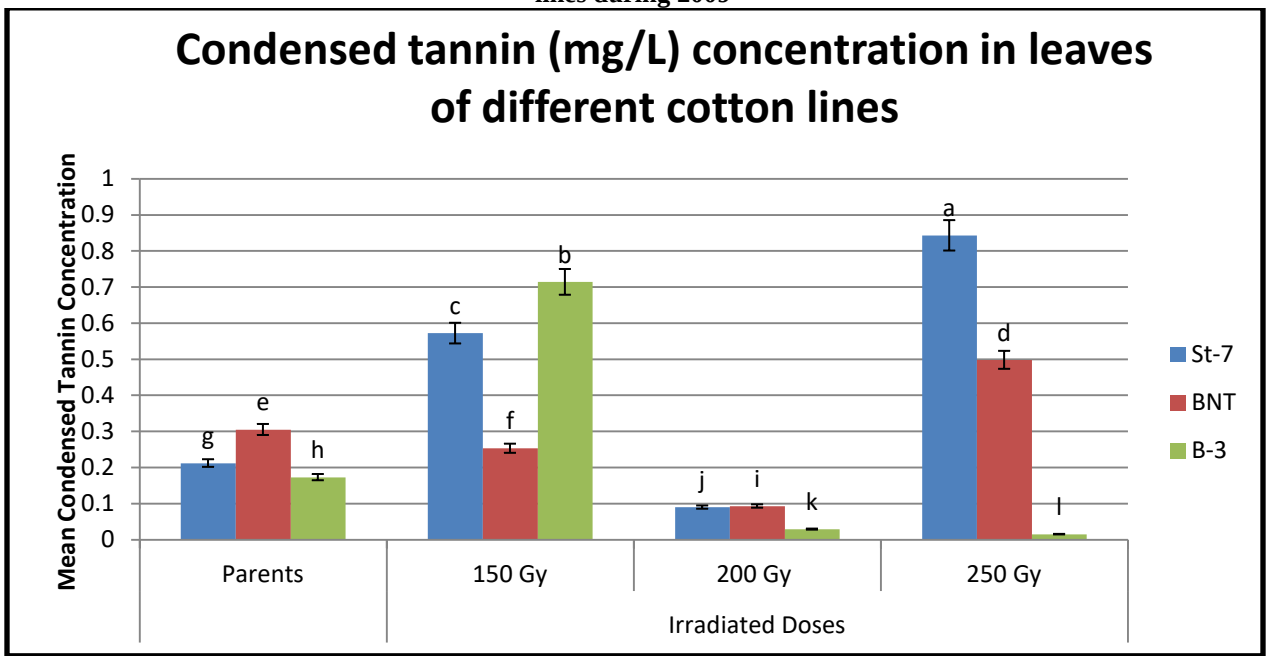
Fig. 1: Jassid population on parents and gamma irradiated cotton lines in caged plants during-2009



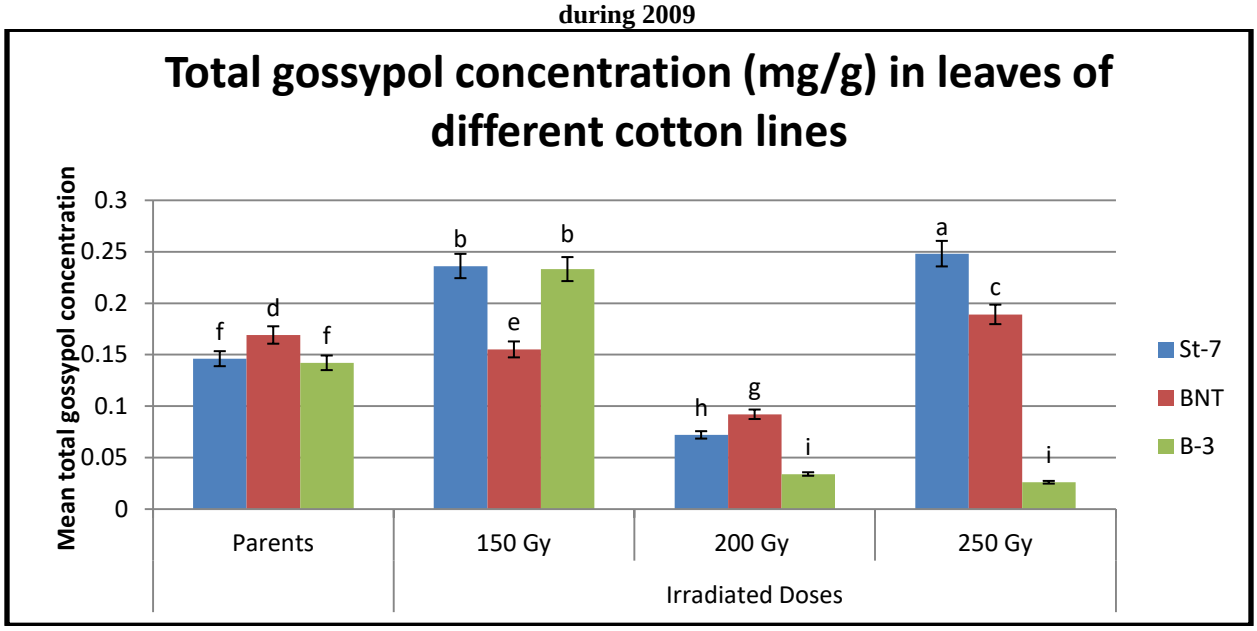
Mean±S.E followed by same letters are not significantly different from each other, (P< 0.05; HSD)

Fig. 2: Condensed tannin (mg/L) concentration in leaves of untreated (parent) and gamma irradiated cotton lines during 2009



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

Fig. 3: Total gossypol concentration (mg/g) in leaves of untreated and their gamma treated cotton lines during 2009



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

Table 1: Pearson's correlation coefficients and the Liner regression r^2 value, among condensed tannin mg/L in leaves with jassid population on parents and their three gammas irradiated cotton lines in caged during 2009

Parameter	r- Value	P- Value	Linear Regression		R^2
			y	x	
2009					
Condensed Tannin in leaves vs jassid Population in Parents	0.9864	0.1053	-0.8578	6.4671	0.9729
Condensed Tannin in leaves vs jassid Population in Dose-150	0.9918	0.0816	0.43137	2.97653	0.9837
Condensed Tannin in leaves vs jassid Population in Dose-200	0.9991	0.0264	0.25753	0.22361	0.9983
Condensed Tannin in leaves vs jassid Population in Dose-250	0.6056	0.5858	0.58252	1.51802	0.3668

Table 2: Pearson's correlation coefficients and the Liner regression r^2 value, among gossypol mg/gm in leaves with jassid population on parents and their three gammas irradiated cotton lines in field trial during 2008 and 2009

Parameter	r- Value	P- Value	Linear Regression		R^2
			y	x	
2009					
Gossypol in leaves vs jassid Population in Parents	0.9999	0.0073	-4.0183	30.5118	0.9999
Gossypol in leaves vs jassid Population in Dose-150	0.978	0.1337	-1.1804	15.0899	0.9565
Gossypol in leaves vs jassid Population in Dose-200	0.9406	0.2205	0.2563	0.2581	0.8848
Gossypol in leaves vs jassid Population in Dose-250	0.7303	0.4788	0.24682	6.62105	0.5333