EFFECT OF 2-(PHENYLSULFONYL) HYDRAZINE CARBOTHIOAMIDE ON PHOTOSYNTHETIC PIGMENTS IN WHEAT (*Triticum aestivum* L.) VARIETIES

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Abstract

Many antibiotics such as sulfonamides are widely used to protect against diseases. Interestingly, these antibiotics are released into the environment via animal manure and polluted the environment. These antibiotics have negative effects on crops and other plants also. We studied the potential effect of new derived sulfonamide 2-(phenylsulfonyl) hydrazine carbothioamide (TSBS-1) on photosynthetic pigments (chlorophyll and carotenoids contents). Two wheat varieties (Chakwal-50 and Faisalabad-2008) are selected for pot experiment. Plants were treated with four treatment levels (2.25 mg/mL, 2.5 mg/mL, 3 mg/mL and 4 mg/mL) of antibiotic and one control treatment (distilled water). Furthermore, the effects of microbial inoculation (*Actinobacter* spp, RS-3a, RS-4a, RS-5a and RS-7a) were also examined for their level of tolerance and susceptibility to sulfonamide. Sulfonamides alter many physiological activities of plant. Our study demonstrated that high concentration of 2-(phenylsulfonyl) hydrazine carbothioamide leads to significantly decrease in photosynthetic pigments of both wheat varieties. The most conspicuous results were remarkable increase in chlorophyll and carotenoids contents for most of the inoculated plants. Both wheat varieties Chakwal-50 and Faisalabad-2008 showed high chlorophyll content when treated with RS-5a (4.390 µg/g FW) and RS-4a (4.3006 µg/g FW) respectively. Effect of TSBS-1 on both varieties showed that carotenoids contents decrease significantly at 4mg/ml. Faisalabad-2008 showed maximum carotenoids content with RS-5a (600 µg/g FW) while it decreased significantly when treated with 4 mg/mL (150 µg/g FW). These results are valuable in understanding how both wheat varieties respond to 2-(phenylsulfonyl) hydrazine carbothioamide.

Keywords: 2-(phenylsulfonyl) hydrazine carbothioamide, Photosynthetic pigments, Rhizospheric bacteria, Tolerance, Toxicity, *Triticum aestivum* L.

Introduction

Antibiotics have been widely and effectively used in human and veterinary medicines. Large number of these antibiotics are released into the environment in an unaltered form after use (Sarmah *et al*., 2006) and have been observed in soil, manure, sediment, industrial wastewater, groundwater, surface water, and drinking water also (Hou, *et al*., 2015). After this these antibiotics are mixed with our agricultural soils due to improper management and have adverse effects on our environment and plants (Kim *et al*., 2010). Antibiotic contamination of soil has harmful effects on bacterial strains present in agricultural soil (Baguer *et al*., 2000).

Wheat (*Triticum aestivum* L.) is first important staple food. Chen *et al*., 2011 observed the single and joint toxicity of Chloramphenicol with Hg on wheat crop. Sulfonamides delay the production of dihydropteroate of folic acid, which reduce bacterial reproduction (Demoling *et al*., 2009). Some of these considerations with the environmental presence and exposure of antibiotics consist of stunned plant growth and abnormal physiology (Phillips *et al*., 2004; Kaniou *et al*., 2005). Accumulation of sulfonamides in different tissues of plants observed when plants grown in soil with contaminated manure. Liu *et al*. (2012) analyzed the effect of various antibiotics on the growth of rice, turmeric and sweet oats. There are numerous reports on photosynthetic characteristics under stress (Wei *et al*., 2006). Generally, photosynthesis is inhibited by stress (Koyro, 2006).

The main aim of this was to determine the effect of sulfonamide 2-(phenylsulfonyl) hydrazine carbothioamide on photosynthesis pigments (chlorophyll and carotenoids) of two wheat varieties.

Materials and methods

Selected species: Two wheat varieties Chakwal-50 and Faisalabad- 2008 were selected and the seeds of these plants were taken from seed center, University of the Punjab, Lahore.

Selected Antibiotics: The new sulfonamides (SAs) derivative; 2-(phenylsulfonyl) hydrazine carbothioamide ($TSBS^{-1}$)

Selection of Bacteria: Five rhizospheric bacterial strains i.e. AC (*Actinobacter* spp), RS-3a (*Bacillus* spp.), RS-7a (*Bacillus subtilis*), RS-4a (*Enterobacter* spp.) and RS-5a (*Enterobacter* spp.) used.

Plant material and Experimental conditions: Plant Growth experiment was conducted in wire house of

Botanical Garden, University of the Punjab, Lahore. Plants were grown in plastic pots (according to design expert) under natural conditions of humidity, temperature and light. All dilutions of each antibiotic were added directly into the form of solution in the soil (Baguer *et al*., 2000). Furthermore, the plants were inoculated with rhizospheric bacterial strains that are described above after the 15 days of growth.

Determination of photosynthetic pigments

Chlorophyll Content: For the estimation of Chlorophyll contents 0.05 gm of fresh leaves of all plants treated with different treatment levels of 2-(phenylsulfonyl) hydrazine carbothioamide (TSBS-1) and bacterial strains were dissolved in 0.5 mL of Acetone. The tubes were placed in dark for 48 hours. All the extracts were assayed in UV 1800 spectrophotometer for absorbance at 645 and 663nm. Chlorophyll content was determined by using the formula described by Arnon (1949).

Chlorophyll (ug/gFW) = [(0.00802) (D-663) + (0.0202) (D-645) (ml of solvent)/ gram fresh weight of plant]

Carotenoids Content: For carotenoids test first take 0.05 gram of fresh leaves of all plants treated with different treatment levels of 2-(phenylsulfonyl) hydrazine carbothioamide and bacterial strains. Then leaves were dissolved in 0.5ml of Acetone in corning tubes. The tubes were kept under dark condition. After 48 hours absorbance was taken with UV 1800 spectrophotometer for absorbance at 480 nm. Carotenoids content was determined according to Wellburn, (1994).

Carotenoids Content (ug/gFW) = (1000 x 480A-1.29 x chlorophyll a (D-663) – 53.78 x chlorophyll b (D-645) / 220.

Statistical Analysis: The statistical analysis was done by using Design Expert Software 12. All data were analyzed by the ANOVA multiple comparison statistics under three factor factorial designs. The experiments were replicated four times. The values are means of four replications ± standard error (SE) of each treatment. Differences between values at $p< 0.05$ were considered statistically significant.

Results and Discussion

The experimental data under three factor factorial designs was analyzed for photosynthetic response of two wheat varieties. The analysis includes the ANOVA table under selected design, the diagnostic graphs/plots based on residual analysis, comparison of estimated and observed values, interaction graphs are also used to check individual and joint effect of each factor on biochemical characteristics of wheat plant.

The ANOVA table using above discussed three factors (TSBS-1, bacterial strains and wheat varieties) interaction is given below.

Chlorophyll Content

Table 1: ANOVA under three factor factorial

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Block	1.34	3	0.4476			
Model	106.11	39	2.72	26.74	< 0.0001	significant
A-Varieties	1.75	1	1.75	17.24	< 0.0001	
B-TSBS-1	89.76	4	22.44	220.54	< 0.0001	
C-Bacteria	7.81	5	1.56	15.36	< 0.0001	
AB	1.48	4	0.3706	3.64	0.0069	
AC	2.11	5	0.4219	4.15	0.0013	
BC	3.20	20	0.1598	1.57	0.0629	

The Model F-value of 26.74 implies the model is significant. There is only a 0.01 % chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicated that model terms are significant. In this case A, B, C, AB, and AC are significant model terms.

All the diagnostic figures including; Normal Probability, Box-Cox Plot for Power Transforms, Residuals vs. Predicted, Residuals vs. Run, Predicted vs. Actual are followed.

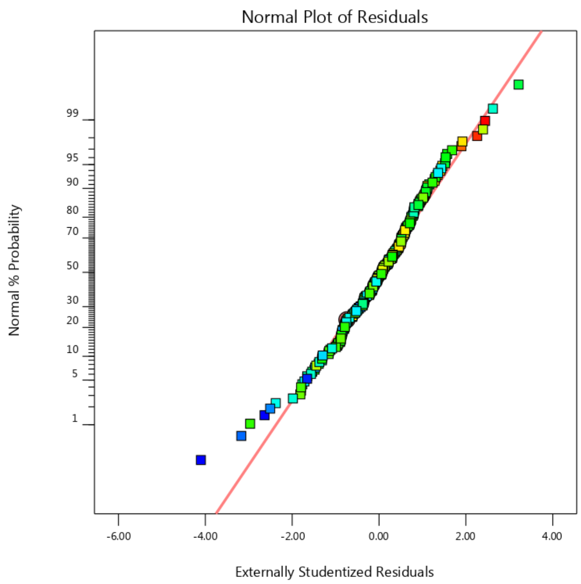


Fig.1: Normal plot of residuals showing normal % probability as dependent variable and externally studentized residuals as independent variable and points on the line representing the normal distribution of the data.

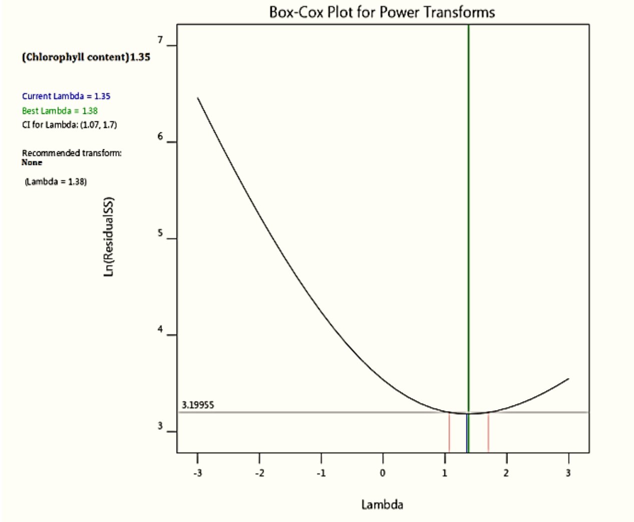
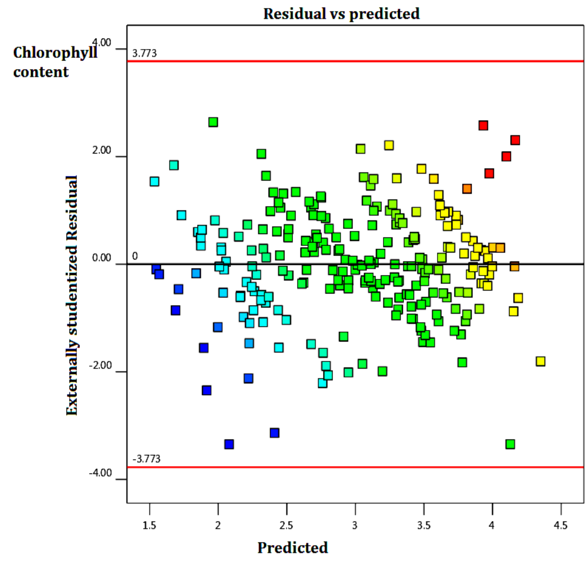
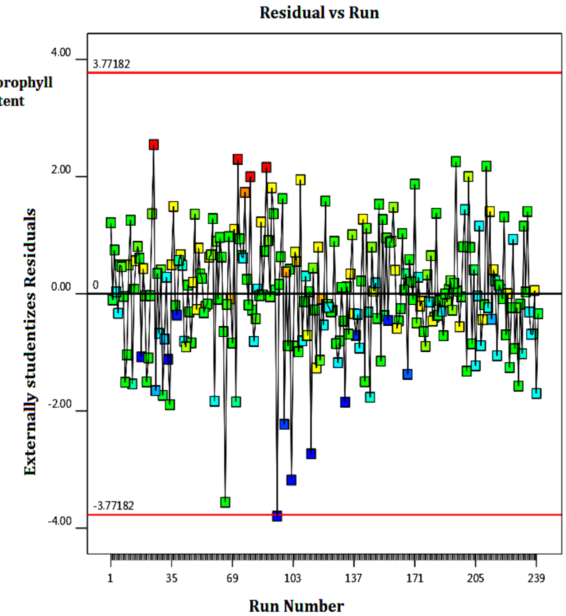


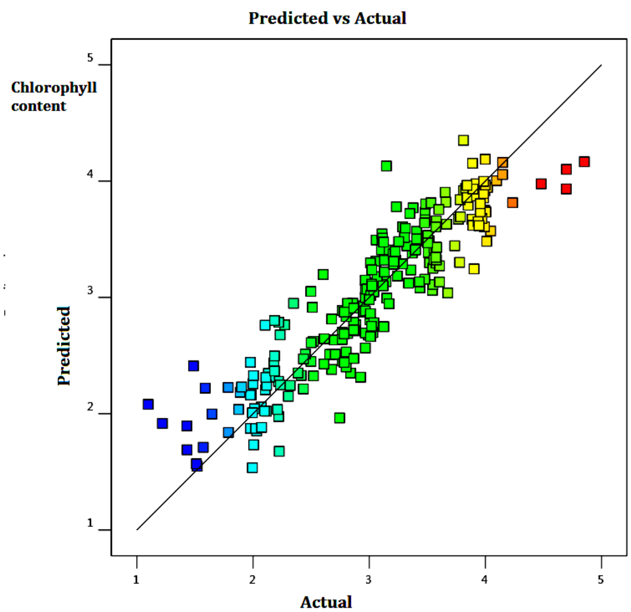
Fig. 2: The Box-Cox normality plot shows that the maximum value of the correlation coefficient is at λ = 1.38 which is best lambda and histogram of the data after applying the Box-Cox transformation with λ = 1.35 (current lambda) shows normality assumption is reasonable.



(A)



(B)



(C)

Fig. 3: Plot of (A) Residual predicted (B) Residual vs Run and (C) predicted vs. actual for 239 experimental runs according to three factor factorial when using antibiotic and rhizospheric bacteria with two wheat varieties. It shows lurking variables having influenced the response during the experiment. These assumptions plots show a random scatter.

These values provide measures of the influence, potential or actual, of individual runs. The graphical plots provide a better perspective and show regression.

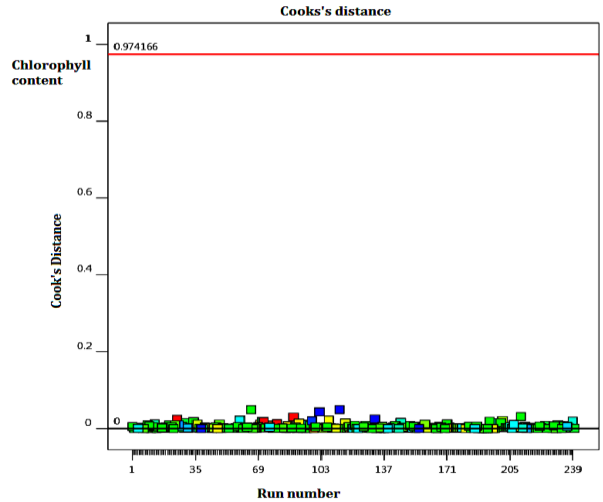


Fig.4: This plot is showing observation in a linear regression to find outlier in a set of predictor variables and identifying outliers in the X values (observations for predictor variables). An observation with Cook's distance larger than three times the mean Cook's distance might be an outlier.

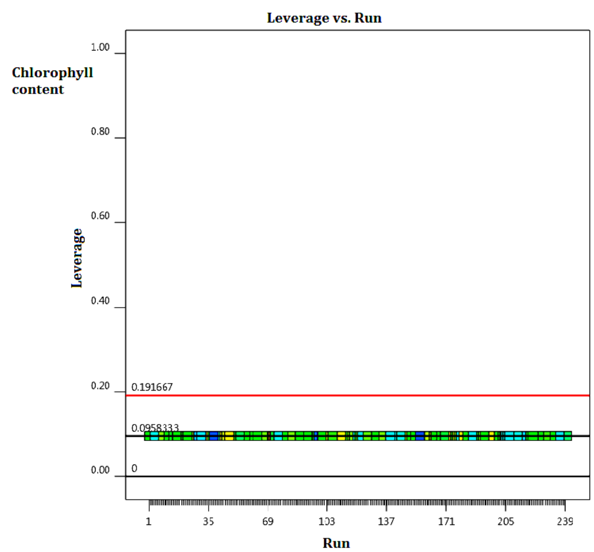


Fig.5: The Leverage vs. Run plots showing influential data points on our model. Outliers can be influential; the model exactly fits the observation at that point

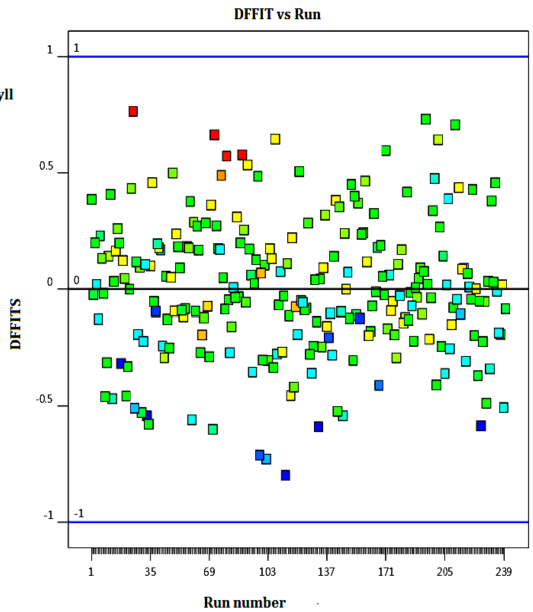


Fig. 6: DFFIT - difference in fits, is showing influential data points and prediction changes. It quantifies the number of standard deviations that the fitted value changes when the data point is omitted

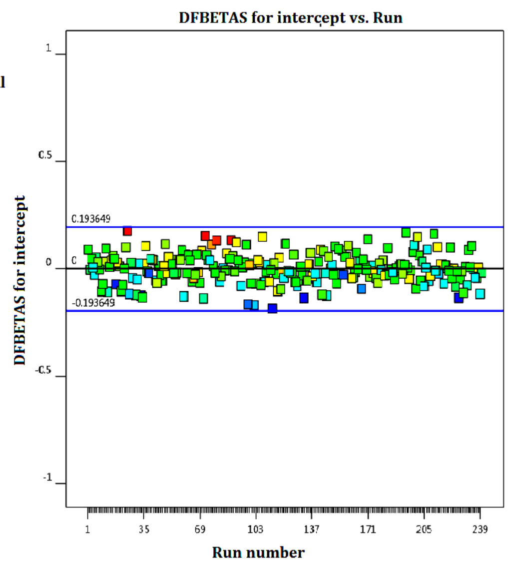


Fig.7: DFBETAS for intercept vs. run in which the DFBETAS for intercept as dependent on Y axis while Run Number on X axis as independent variable which indicate the difference between the regression coefficient calculated for all of the data.

An interaction occurs when the response (chlorophyll content) is depending on two factors which were antibiotic and rhizospheric bacterial strains. Non-parallel lines in figures indicating the effect of factor (chlorophyll and carotenoids) depends on the level of the TSBS-1 and different colors are showing different bacteria used in inoculation.

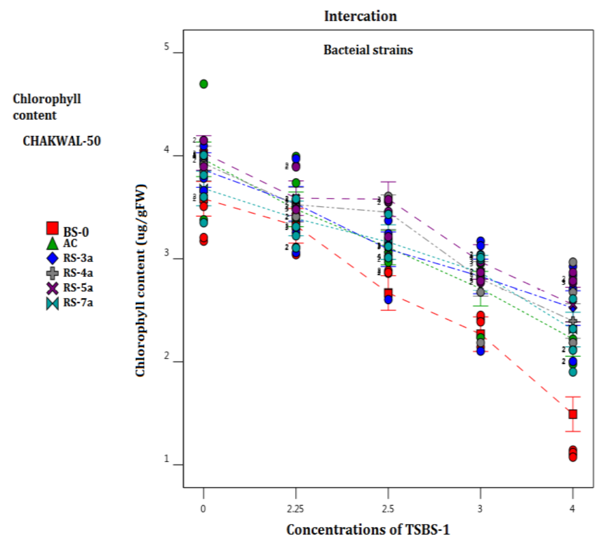


Fig. 8: Effect of TSBS-1 on Chakwal-50 indicating that by increasing treatment level the chlorophyll content decreased significantly. The inoculation of rhizopheric bacterial strains in comparison of control the chlorophyll contents was high. The bacterial strain RS-5a had maximum chlorophyll contents.

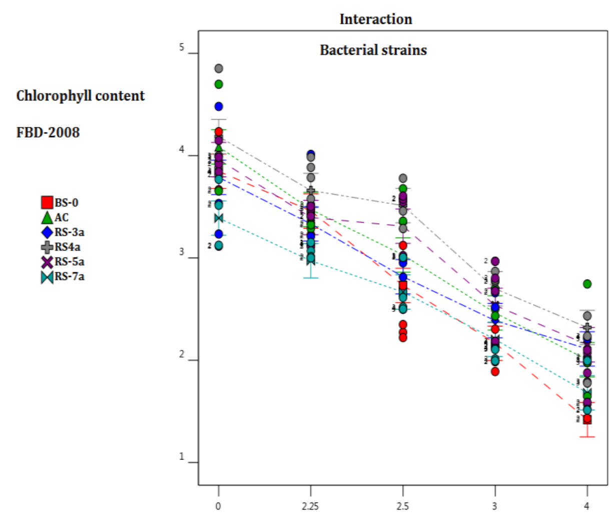


Fig. 9: Interaction of TSBS-1 on FBD-2008indicating that by increasing treatment level the chlorophyll content decreased significantly. In the cae of faisalabad varietey RS-4a showed maximum content.

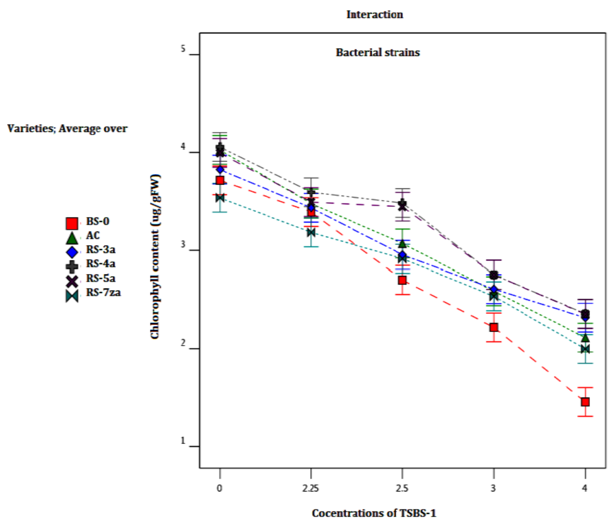


Fig. 10: Interaction of TSBS-1 and bacterial strains with both varieties indicating that at lower treatment level (0, 2.25 mg/mL) the bacterial strains RS-4a showed maximum contents while at the other concentration levels (2.5 mg/mL, 3 mg/mL and 4 mg/mL) the chlorophyll content was high in RS-5a inoculated plants.

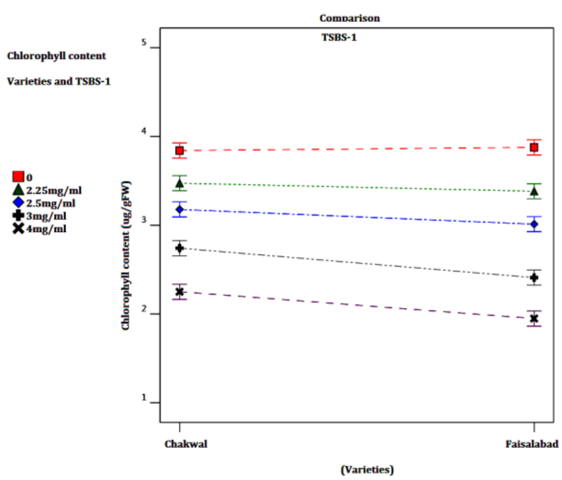


Fig. 11: Comparison between both varieties indicating that the chlorophyll content was maximum in faisalabad-2008 at 0 while at other concentration levels (2.25 mg/mL, 2.5 mg/mL, 3 mg/mL and 4 mg/mL) chakwal-50 had high chlorophyll content.

Application of antibiotic solution with different treatment levels (2.25 mg/mL, 2.5 mg/mL, 3 mg/mL and 4 mg/mL) and their interaction with varieties significantly affected the total chlorophyll content even at the lowest concentration level i.e. 2.25 mg/mL. The chlorophyll contents decreased significantly by increasing the concentrations of TSBS-1 as shown in interaction graphs. Decline in photosynthesis exhibited in other plants under stress condition Opriş *et al*. (2013).

In the Chakwal-50 variety, significant decrease ($P<0.05$) in chlorophyll content was observed with the addition of different concentration levels of TSBS-1(2.25 mg/mL, 2.5 mg/mL, 3 mg/mL and 4 mg/mL) and rhizospheric bacterial strains as shown in fig. 8. Maximum increase in chlorophyll content with the mean value 4.390 (ug/g FW) was observed RS-5a inoculated plants without any addition of TSBS-1 and this value was greater than control has value of 3.6 (ug/g FW). Plants treated with the concentration level of 2.25 mg/mL and RS-5a showed an increase in content 3.6 (ug/g FW) compared to control showed 3.4 (ug/g FW). However, a significant decrease in chlorophyll pigment was observed at concentrations of 2.5 mg/mL, 3mg/mL and 4mg/mL.

The significant ($P<0.005$) decline in chlorophyll was observed when plants were treated with high concentration level of 4mg/ml and maximum content was observed with the inoculation of RS-5a (ug/g FW) as compared to non-inoculated plant with mean value of 1.4 (ug/g FW). The study on phytotoxicity of sulfonamide by Jjemba, (2000) showed that sulfonamide exposure to the environment produce alterations in physiological activities of many plants.

In Faisalabad-2008 variety, maximum increase in chlorophyll content with mean value 4.3006 (ug/g FW) was observed when inoculated with RS-4a only. The chlorophyll value in control leaves was 3.8 (ug/g FW) as in Fig; 9. The significant reduction in chlorophyll contents was observed in RS-7a inoculated plants even at lowest concentration (0, 2.25 mg/mL). At the concentration levels of 2.5mg/ml and 3mg/ml the chlorophyll content was almost equal to those found in control leaves. The minimum value of chlorophyll content was evaluated in non-inoculated plants (BS-0) at the concentration of 4 mg/mL.

The interaction graph (fig. 10) of TSBS-1 and bacterial strains shows that maximum chlorophyll content was found in RS-4a inoculated plants at low concentration (0, 2.25 mg/mL). At other treatment levels (2.5 mg/mL, 3 mg/mL and 4 mg/mL) RS-4a and RS-5a inoculated plants showed maximum chlorophyll values.

In comparison of both wheat varieties Faisalabad-

2008 showed better results without any antibiotic treatment. However, it was found that Faisalabad-2008 variety was more susceptible than chakwal-50 at all concentrations as shown in fig. 11.

Carotenoids Content

Table 2: ANOVA table when response variable is 'carotenoids content' in wheat plant leaves

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Block	1876.70	3	625.57			
Model	4.761E+06	39	1.221E+05	263.12	< 0.0001	significant
A-Varieties	7.768E+05	1	7.768E+05	1674.35	< 0.0001	
B-TSBS-1	2.648E+06	4	6.620E+05	1426.93	< 0.0001	
C-Bacteria	1.002E+06	5	2.005E+05	432.13	< 0.0001	
AB	65248.20	4	16312.05	35.16	< 0.0001	
AC	1.845E+05	5	36900.34	79.54	< 0.0001	
BC	83787.43	20	4189.37	9.03	< 0.0001	

The Model F-value of 625.57 implies the model is significant. There is only a 0.01 % chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicated that model terms are significant. In this case A, B, C, AB, AC was significant model terms.

The relation between rhizospheric bacterial strains and antibiotic (TSBS-1) and then effect of both on wheat varieties (Chakwal-50 and Faisalabad-2008) was determined by interaction graphs which are given below.

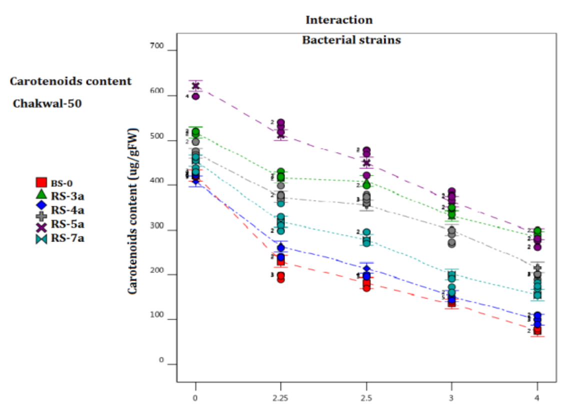


Fig. 12: Effect of TSBS-1 on Chakwal-50 indicating that by increasing treatment level the carotenoids content decreased significantly. The inoculation of rhizopheric bacterial strains in comparison of control the contents was high. The bacterial strain RS-5a had maximum carotenoids contents.

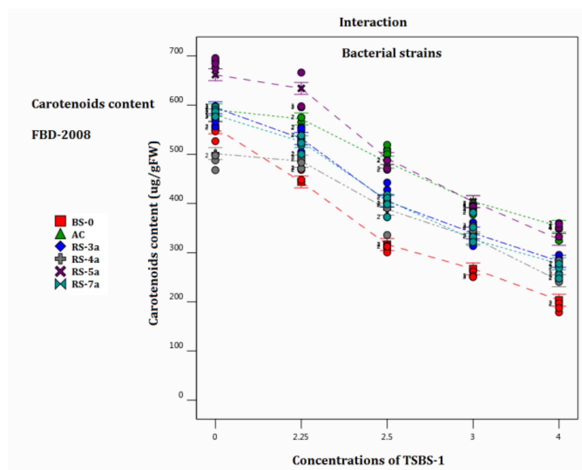


Fig. 13: Effect of TSBS-1 on faisalabad-2008 indicating that by increasing treatment level the carotenoids content decreased significantly and RS-5a inoculated plants showed maximum content as described in fig. 11.

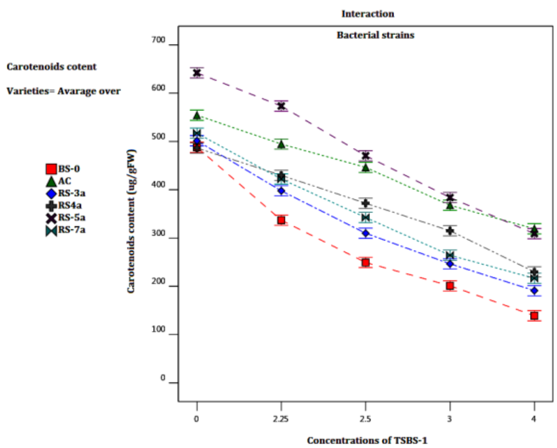


Fig. 14: Interaction of TSBS-1 and bacterial strains with both varieties indicating that at each treatment level (0, 2.25 mg/mL, 2.5 mg/mL, 3 mg/mL and 4 mg/mL) the chlorophyll content was high in RS-5a inoculated plants.

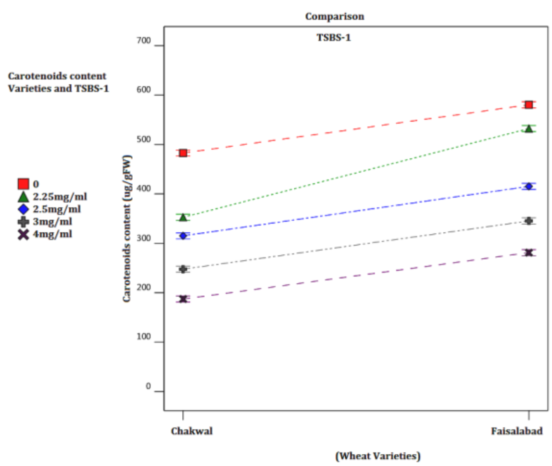


Fig. 15: Comparison between both varieties indicating that the carotenoids content was maximum in faisalabad-2008 at each treatment level than chakwal-50.

In the Chakwal-50 variety, significant ($P<0.05$) decline in Carotenoids contents was observed at different concentration levels of TSBS-1 (2.25 mg/mL, 2.5 mg/mL. 3 mg/mL and 4 mg/mL). Maximum increase in carotenoids content 600 (ug/g FW) was observed in the plants which were inoculated with RS-5a without any addition of TSBS-1 while control show the value 410 (ug/g FW). Plants treated with the concentration level of 2.25mg/ml and RS-5a showed significant ($P<0.0001$) increase in content 542 (ug/g FW) as compared to control 196 (ug/g FW).

The minimum contents were observed at the concentration level of 3mg/mL and 4 mg/mL. RS-5a inoculated plants showed the value 376 (ug/g FW) at 3 mg/mL which was highest than non-inoculated plants 148 (ug/g FW) as shown in fig. 12. Many author as Moorthy *et al*. (2010) have reported similar decrease in photosynthetic pigments (chlorophyll and carotenoids) in many cultivars under different stress conditions, as in case of Cr contamination in soil.

In the Fasisalabad-50 variety, significant decrease ($P<0.05$) in Carotenoids was observed with the addition of different concentration levels of TSBS-1 (2.25 mg/mL, 2.5 mg/mL. 3 mg/mL and 4 mg/mL) and rhizospheric bacterial strains as shown in fig. 13. Maximum increase carotenoids content 686 (ug/g FW) was observed in the plants inoculated with RS-5a without any addition of TSBS-1, compared to the other plants and control 535 (ug/g FW). The significant decrease ($P<0.0001$) in carotenoids was observed when plants treated with high concentration level (4 mg/mL) and maximum height was observed with the inoculation of RS-4a 417 (ug/g FW) as compared to non-inoculating plant with mean value of 287 (ug/g FW).

The interaction of TSBS-1 and bacterial strains with average over varieties is shown in fig. 14. RS-5a inoculated plants showed high chlorophyll content in both varieties. Comparison of Chakwal-50 and Faisalabad-2008 is shown in fig. 15. The maximum Chlorophyll content was recorded for Faisalabad-2008. Similar findings have been reported by Ashraf *et al*. (2010), they report decline in photosynthetic contents is due to stress which cause disturbance in the supply of certain ions.

Conclusion

Release of antibiotics from animal manure to the environment can take place by application of animal manure to agricultural land as organic fertilizer. Our study demonstrated the effect of new antibiotics (sulfonamides) on physiology of wheat varieties. In each case antibiotic had toxic effect even at small concentration (2.25 mg/mL). In comparison with the controls tested TSBS-1 inhibited photosynthetic pigments (chlorophyll and carotenoids). The results of this study can be applied to the environmental risk assessment of antibiotics including estimation of the determination of sulfonamide (TSBS-1) in the environment.

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