Pak. J. Chem. 4(2): 92-95, 2014 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2014.v04.i02.p07

Biochemical Response of Glycine Max after Inoculation of Different Concentrations of Fusarium Solani

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ABSTRACT
Diseases of agricultural crops are old as agriculture itself. It has been estimated that fungal diseases are responsible for an annual reduction for more than 20% of total potential world food production from crop plants. Randomized complete block design experiments were conducted to study the effect of different colonies of *Fusarium solani* on some biochemical and physiological parameters of *Glycine max*. Sterilized seeds of *Glycine max* (L.) Merr was artificially inoculated with conidia of 3 different concentrations (10,000, 100,000 and 1000, 000 cfu). Seedlings of *Glycine max* was grown in 350g acid washed sand. Complete Nutrient Hoagland solution was regularly used to irrigate the plants. Leaves samples were weekly collected for analysis of biochemical tests. It was observed that inoculation of *Fusarium solani* significantly increase the concentration of total soluble carbohydrate and reducing sugar in inoculated plants over healthy tissues. Protein analysis revealed a significant reduction with the development of disease. Activity of invertase illustrate a variable change initially it was increased than afterward declined.

Keywords: biochemical response, *glycine max*, inoculation, different concentrations, fusarium solani

1. INTRODUCTION
Glycine max (L.) Merr is economically important oil crop as a fodder.[1, 2] Seeds of Glycine max are commonly used for different purpose in many countries of Asia.[3, 4] Sudden death syndrome of soybean has severe and potentially restraint which reduces the marketable yield of crop and can be controlled by host plant resistance.[5] Fusarium solani is soil-inhabiting fungus it stay alive in decomposed plant tissue and soil. High F.solani inoculums rate were expected to cause more severe SDS leaf symptom. Soil moisture enhanced the infection of F.solani[6]. By the invasion of the pathogen different phytotoxins are synthesized in plant and about 20-46% of soybean yield losses by F. solani[7-8] The biochemical changes in carbohydrate, protein, reducing sugar and invertase activity was correlated by the infection of the fungal disease which ultimately attributed for the reduction of crop production.[9] Seed dressing with Fusarium solani has provided significant reduction in soybean protein. The penetration of the fungus in the host cell alters the protein content of the plant[9-10]. It was reported that the infection of pathogen is accountable to increase in the content of reducing sugars[11]. The reducing sugars possibly increased due to microbial enzymatic degradation of complex sugars in the host cell wall into simple sugars[9-12]. Analysis of soluble carbohydrate by Morkunas[13] showed reduction in activity of invertase enzyme after drastic infection of pathogenic fungus.

The aim of this research was to investigate the biochemical changes in the metabolism of Glycine max after the inoculation of different colonies of Fusarium solani.

2. MATERIALS AND METHODS
In order to investigate the effect of different concentrations (10,000, 100,000 and 1000,000 cfu) of *Fusarium solani*, the pot experiment was designed using Randomized complete block design. The healthy seeds of *Glycine max* were obtained from the local market. Seeds were surface sterilized with 0.2% sodium hypochlorite for 5 mints and soaked in distilled water. Ten seeds were sown in each polythene bag and later were transplanted in pots containing 350g sand. Plants were subjected to inoculate with *F. solani* after 15 days sowing. There were 3 replicates of each sample. Leaf samples of both control (uninoculated) and treated (inoculated) plants were collected after 7 days interval up to 4th weeks. Plants were analyzed to examine the changes in carbohydrate, protein, invertase enzyme activity and reducing sugar.

2.1 *Estimation of carbohydrate content*
The total carbohydrate was estimation on fresh wt. basis by Yemm & Willis (1956) method using Anthrone reagent[14]. Absorbance was determined at 620 nm. The carbohydrate content was calculated by µg/mg fresh weight.

2.2 *Protein estimation*
Protein was estimated in control and treated leaf by Lowry *et al.*, (1951)[15]. The total protein content was calculated from a standard curve of bovine serum albumin.

2.3 *Reducing sugar*
The leaf samples used to investigate the changes in reducing sugar by the colorimetric procedure of Nelson (1944)[16] and Inman (1965)[17].

*Corresponding Author Received 3rd April 2014, Accepted 23rd July 2014

2.4 *Invertase activity*

Invertase enzyme activity estimated by Bergmeyer (1974)[18]. Method of Enzymatic analysis, (1): 450-451

The data of *G. max* inoculated with different concentration (10,000, 100,000 and 1000,000 cfu) of *F. solani* was analyzed using the "SPSS" statistical 15.0 program by one-way analysis of variance (ANOVA) to compare the means of different treatment. "SIGMA PLOT" program (2000) was used for graphical representation of data.

3. RESULTS AND DISCUSSION

Highly significant result ($p<0.01$) was obtained during the present investigation on the effect of *F. solani* on soybean protein. In the initial stage such as up to 1st week, protein content increases in 10,000 cfu inoculated plant and afterward it was decrease (Fig.2). These results are also supported by Kiran (2013)[19] who found that initially proteolytic enzymes like protease and peptidase which degrade proteins into amino acids then after severity of infection that may be decrease due to their utilization by the developing of plant.

Similarly, the same pattern of changes in protein content occur in this study when plant inoculated with 10,000 cfu *F. solani* that is the protein content initially increase and later on it was decrease, which illustrates that in earlier stage the disease intensity was very low and the effect on protein content was not prominent (Fig.2).

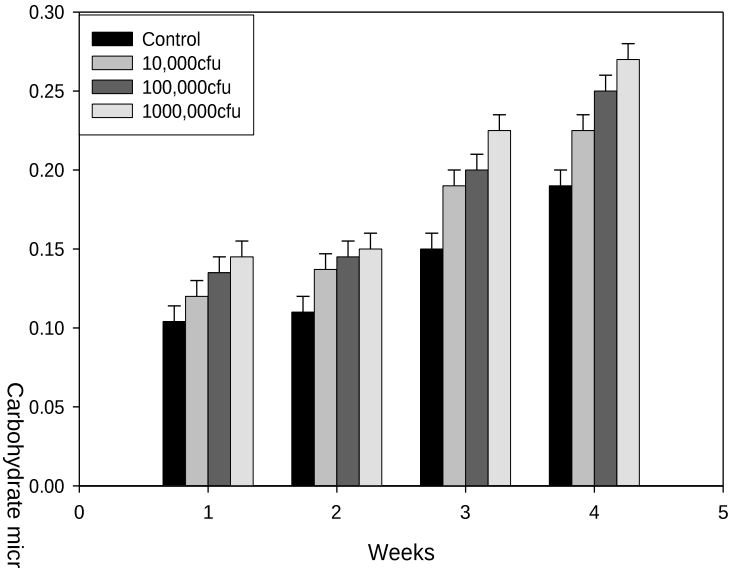

Fig-1: Changes in Total carbohydrate of *Glycine max* inoculated with different colonies of *F.solani*. Error Bars represent standard error of mean (n=3)

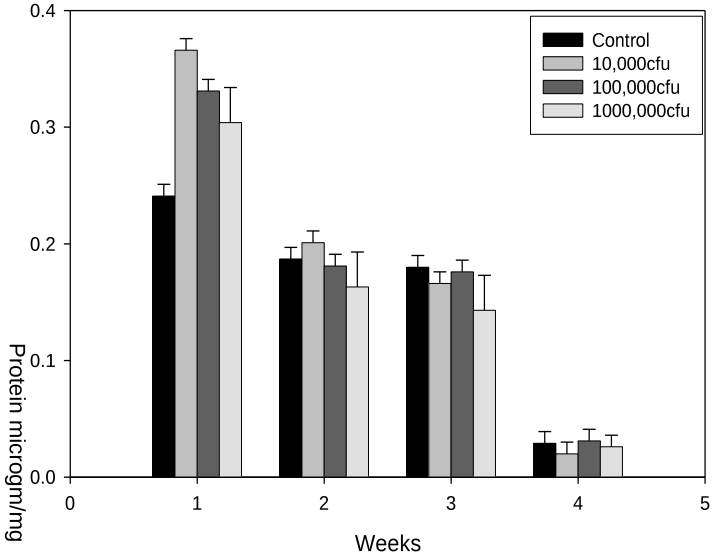

Fig-2: Changes in Protein content of *Glycine max* inoculated with different colonies of *F.solani*. Error Bars represent standard error of mean (n=3).

Several researchers finding demonstrated that total content of protein usually increase during the early stage of infection, when the contribution of pathogen is reported change in plant protein has been found in most diseased tissues.[10] Soon after the disease intensity increase, it effect on protein content. Total proteins declined drastically in infected tissue during disease development, and free amino acids accumulate.[20] The plant inoculated with high concentration of pathogen, the protein content showed a gradually reduction in infected plants.[21]

Analysis of carbohydrate show appreciable difference between healthy and diseased plant.[21] By recent results, the carbohydrate content in inoculated plant higher as compared to control (Fig.1). Decrease in starch content is due to inhibition of phosphorylase activity but in high inoculums concentrations (10,000, 100,000 and 1000, 000 cfu), the carbohydrate content was notably increase ($p<0.01$) in infected tissues as compared to control one (Fig.1). The amount of reducing sugar was striking increased ($p<0.01$) in infected plants with increase the concentration of pathogen (Fig.3). Khan & Kalim [9, 11] revealed that infection caused by *F.solani* in soybean evaluates an increase in the content of reducing sugar and total soluble sugar and declined the non reducing sugar in diseased plant tissues.

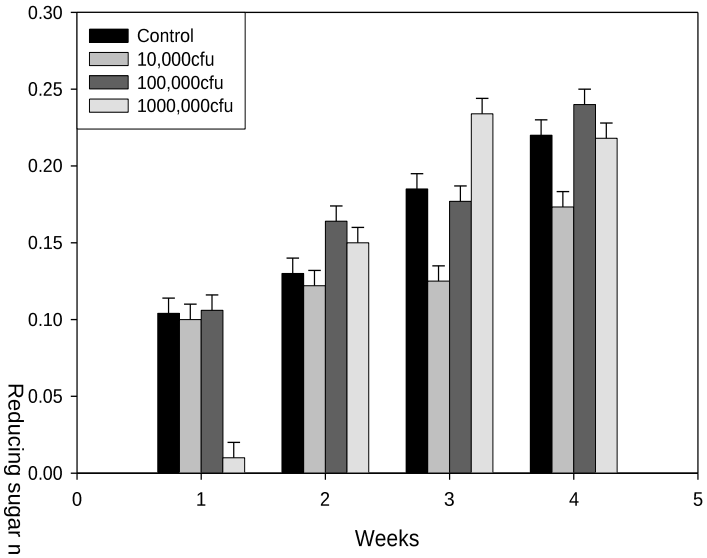

Fig-3: Changes in reducing sugar of *Glycine max* inoculated with different colonies of *F.solani*. Error Bars represent standard error of mean (n=3).

Changes in invertase activity of *Glycine max* inoculated with *F.solani*

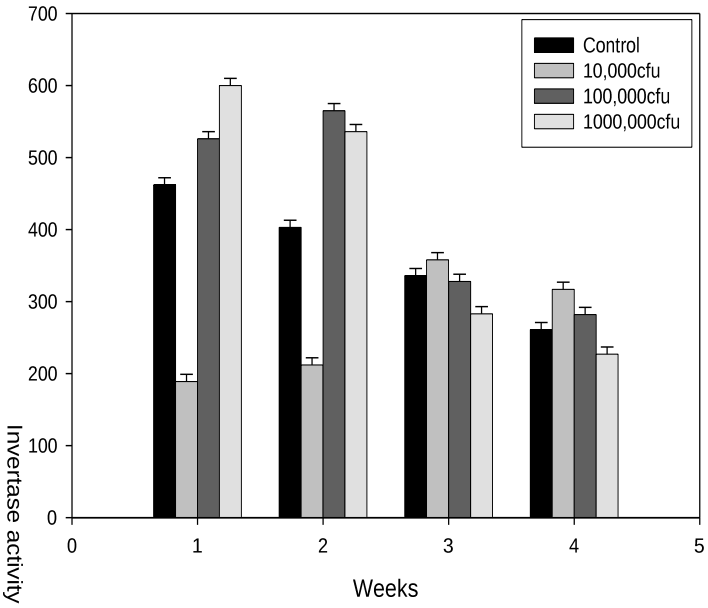

Fig-4: Changes in Invertase activity of *Glycine max* inoculated with different colonies of *F.solani*. Error Bars represent standard error of mean (n=3).

The activity of invertase is significantly high ($p<0.01$) and later it was decreased (Fig.4). It could be due to that during the early stages, the plants enable to perform photosynthesis and the starch stored in the seeds convert into most soluble form glucose. After the development of leaves the plant now able to perform photosynthesis so the invertase

activity becomes slow down. This result is well defined by Morkunas[13] that activity of invertase, i.e. enzyme which hydrolyze sucrose enhanced after inoculation of *F. solani*, which provided hexose sugar for secondary metabolism of plant.

4. CONCLUSION

Results of this research supported that the different concentrations (10,000, 100,000 and 1000, 000 cfu) of *F. solani* effect the biochemical metabolites of *G. max*. These biochemical changes gradually arise due to the severity of infection of *F. solani* on *G. max* that's mean high level inoculation more adversely affect the plant growth.

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