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Analysis of Growth and Resistance to Different Population of Fusarium Solani in Soybean Legume Plant

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ABSTRACT
Experiment was conducted to study the effect of different concentrations (10,000, 100,000 and 1000, 000 cfu) of *Fusariumsolani* on growth and resistance to soybean (*Glycine max* (L.) Merr) leguminous plant. Sterilized seeds of *Glycine max* were sown in 350g of acid washed sand. The plants were regularly watered with complete Nutrient Hoagland solution. Leaves samples were weekly collected for analysis of biochemical tests. The growth and morphology of *G. max* were adversely affected with *F. solani* which show damping off seedling root rot. The symptom was first appearing in root. Infected seedling of *G. max* showed a marked decreased in root, shoot length and discoloration and decay in roots. Stem diameter was also decreased in infected plants as compared with the control plants. There were not marked differences occurring in leaf area but the color of leaves turn yellowish green in infected plants. The infected tissues of soybean with different colonies of *F. solani* showed the highest level of total phenolic content as compared to healthy tissues.

1. INTRODUCTION
Sudden death syndrome (SDS) is the common name for a root-rot of soybean caused by the fungus *Fusariumsolani* f. sp. *glycine*. From many years the disease is of minor importance. The "sudden death" refers to the early defoliation and death of the soybean plant. Sudden death syndrome is caused by the soil-inhabiting fungus *Fusariumsolani* f. sp. *glycine* The fungus survives in the soil for many years. Researchers have suggested that the foliar symptoms are the caused of toxin produced by the fungus. The symptoms of the SDS foliar diseases more likely a response of the plant to stress at pod filling[1].

Fusariumoxysporum and *F. solani* were isolated with high frequency hypocotyls of soyabean. *F. oxysporum* and *F. solani* isolates were delayed seedling emergence and cause significant reduction in stem length and plant fresh weight[2]. There is no chemical control or cultural method has been neither effective nor any cultivars immune to the disease, so the use of more resistant cultivars is the best hope for SDS control or plant cohabitation fungus[3].

The Biochemical defense mechanism in the host plant is based on the group of chemical substances which interfere with the invasion, growth and development of the pathogen. These metabolites may exist in the plant even before the invasion of the pathogen or may appear after the infection. A different variety of phytotoxins are produced by fungal pathogens and a variety of methods have been used to assay fungal toxins. It has been reported that 20 to 46% of soybean yield losses by *F. solani*[4].

The phenol system operates in diseased plant is that after infection by pathogen the plant releases various phenolic compounds Some workers reported that inhibition of growth of the pathogen by phenolic compound may be part of the allelopatheic bio control mechanism[5]. Reduction in disease incidence was associated with increased levels of polyphenol oxidase (PPO), peroxidase (PO) and total phenols[6]. It was reported that there was some sort of relationship between phenolic concentration and degree of resistance[7]. Present research was carried out to observe the growth and resistance to different population of *F. solani* in soybean plant.

2. MATERIALS AND METHOD
Commercial variety of soyabean (Glycine *max*) was used for investigation. The plant inoculated with different concentration (10,000,100,000 and 1000,000 cfu) of *Fusariumsolani* separately. Seeds surface were sterilized with 0.2% Sodium hypochlorite for 5 mints and then washed and soaked for 4 hours in distilled water.10 seeds were sown in each polythene bag and later were transplanted in pots containing 350gm sand. Plants were inoculated after 15 days by *Fusariumsolani.* The soil in the pots was directly in touch with the roots of the plant inoculated. Leaf samples of both control (uninoculated) and treated (inoculated) plants were collected after 7 days interval up to 3rd weeks. Plants were analyzed for changes in Growth, free and bound Phenolic acid.

2.1 *Phenolic Acid*
Phenolic acid were extracted by the method of Hahn *et.al* (1983).

2.2 *Extraction of Free Phenolic acid*
1 gm of dried sample was grinded and extracted by vigorous shaking for 30 mints in 10 ml of 100% methanol. The tissues were removed by centrifugation and the supernatant was reduced to near dryness under vacuum and the residue was suspended in diethyl ether dried under methanolic extract was used for the estimation of free phenolic acid[7].

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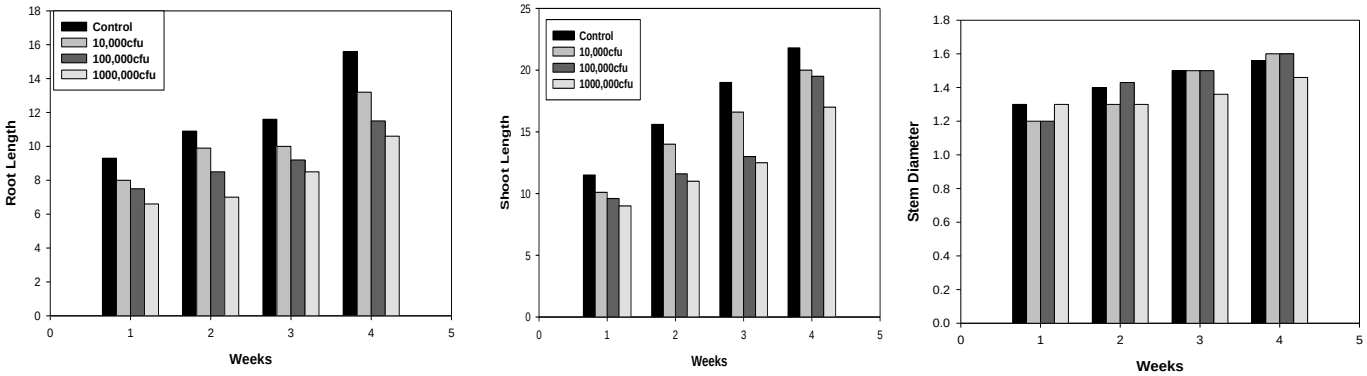

Fig-1: The growth parameters of G*lycine max* to different population of *Fussariumsolani* ($p<0.001$)

2.3 *Extraction of Bound Phenolic acid*

The tissues from free phenolic acid extraction were hydrolyzed for 30 mints with 10 ml of 2N HCL in boiling water. The Hydrolyzed was allowed to cool and then centrifuge. The supernatant was extracted trice with 10 ml of diethyl ether and then extract were pooled. The extracts were vacuum evaporated to dryness and dissolve in 100% methanol. The methanolic extract was used for the estimation of bound phenolic acid[7].

Table-1: The Growth Parameters of *Glycine max* to different population of *Fussariumsolani*

Weeks	Treatment	Root length	Shoot length	Stem Diameter	Leaf Area
		cm	cm	cm	
	Control	10.9	11.5	1.3	29.2
1st	10,000cfu	10	10.1	1.2	29
	100,000cfu	8.5	9.7	1.1	28.7
	1000,000cfu	6.8	9.5	1	22.9
	Control	9.3	15.6	1.4	27.1
2nd	10,000cfu	8	14	1.3	22
	100,000cfu	7.5	11.5	1.29	21.3
	1000,000cfu	6.5	11	1.2	19.4
	Control	11.8	19	1.5	21.3
3rd	10,000cfu	9.8	16.6	1.45	20
	100,000cfu	8.9	13.1	1.4	11.6
	1000,000cfu	8.4	12	1.3	11.2
	Control	15.6	21.8	1.56	18.4
4th	10,000cfu	13	20	1.5	16.6
	100,000cfu	11.6	19.5	1.46	15.8
	1000,000cfu	10.5	17.1	1.43	15.5

2.4 Estimation

Take 1 ml of methanolic extract and add 5 ml of Folin reagent after 3 mints. Saturated sodium carbonate was added in the tubes were incubated for 30 mints at 25C and the absorbance was recorded at 660nm on spectrophotometer[7].

3. RESULTS

3.1 *Morphology of soybean with inoculation of Fusariumsolani*

Initially both root and shoot length increase in 10,000 cfu and 100,000 cfu inoculated plants over control but root length is more as compare to shoot length but latter on the length of both root and shoot decrease over control. Although length of root is less as compare to shoot length throughout the experiment. There is no marked increase occurs in leaf area (Table-1). In 1000,000 cfu inoculated plant both root and shoot length decrease as compare to control and root is larger over shoot. Leaves turn yellowish green in inoculated plant (Fig. 1).

3.2 *Free Phenolic acid*

The free phenolic acid content in leaf sample of inoculated with 10,000 cfu plants showed a marked increase ($p<0.001$) over the control throughout the experiment. In first week, soybean inoculated with 100,00cfu*F.solani* showed a little increase in free phenol over control and a significant increase were observed in 2nd week after inoculation. In the 3rd week free phenol shows a decrease ($p<0.001$) but remain higher as compare to control. According to the result obtained, the free phenolic acid show a significant increase ($p<0.001$) in all inoculated sample concentration (10,000,100,000 & 1000, 000) cfu over control (Fig. 2).

3.3 *Bound Phenolic acid*

The bound phenols in leaf sample of 10,000 cfu inoculated plants showed an increase ($p<0.001$) as compare to control throughout the experiment. Inoculation of 100,0 00cfu plant showed an increase in bound phenol over control, this significant increase is followed up to 3rd week. Plants inoculated with 1000, 000cfu F. *solani* also represent the remarkably increase ($p<0.001$) in bound phenol (Fig. 2).

4. DISCUSSION:

The morphology of *G. max*plants was also been observed in this investigation after infection with pathogen *F. solani.* During early stages, the length of root is higher than the shoot but with the age of seedling a gradual decrease occur in

Table-2: The Weekly changes of Free Phenolic content of Glycine max to different population of Fusariumsolani ($p<0.001$)

Weeks	Treatment	µg/mg Free Phenol
	Control	0.826±0.001
1st	10,000cfu	1.08±0.001
	100,000cfu	1.633±0.001
	1000,000cfu	2.28±0.003
	Control	1.346±0.001
2nd	10,000cfu	2.4±0.001
	100,000cfu	2.25±0.001
	1000,000cfu	2.56±0.003
	Control	1.65±0.001
3rd	10,000cfu	1.89±0.002
	100,000cfu	1.94±0.001
	1000,000cfu	2.13±0.003
	Control	1.77±0.001
4th	10,000cfu	1.92±0.002
	100,000cfu	2.26±0.002
	1000,000cfu	2.18±0.002

Table-2: The Weekly changes of Bound Phenolic content of Glycine max to different population of Fusariumsolani ($p<0.001$)

Weeks	Treatment	µg/mg Bound Phenol
	Control	0.361±0.001
1st	10,000cfu	1.36±0.001
	100,000cfu	1.6±0.001
	1000,000cfu	1.76±0.003
	Control	0.92±0.001
2nd	10,000cfu	1.08±0.001
	100,000cfu	1.24±0.001
	1000,000cfu	1.49±0.003
	Control	0.96±0.001
3rd	10,000cfu	1.56±0.002
	100,000cfu	1.76±0.001
	1000,000cfu	2.05±0.003
	Control	1.32±0.001
4th	10,000cfu	1.48±0.003
	100,000cfu	1.69±0.003
	1000,000cfu	2.00 ±0.001

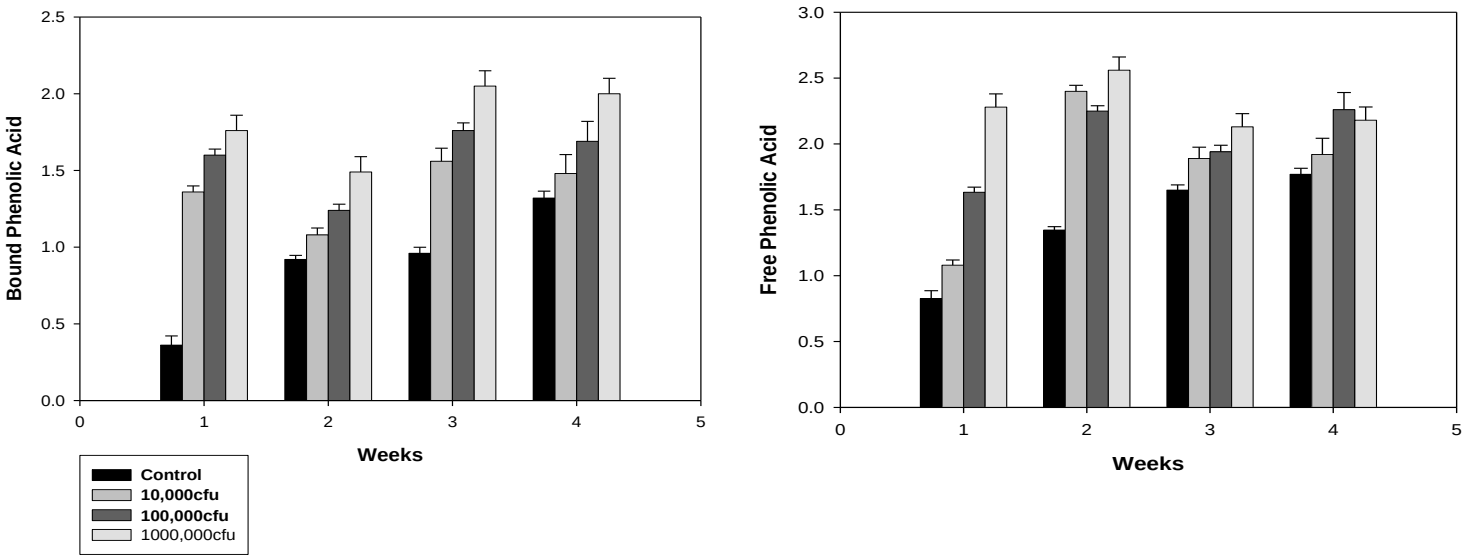

Fig-2: The weekly changes of bound and free phenolic content of *Glycine max* to different population of *F. solani* ($p<0.001$)

root length of inoculated plants. The plant infected with different colonies of F. *solani,* showing the yellowing of the leaves starting at the edges, the veins remaining green for a short time period. *F. solani* transmit a disease to roots of various plant species.*F. solani f. sp*. glycines, the causal organism of SDS of soybean, colonize soybean roots and produces phytotoxins. A variety of phytotoxins which are produce by colonization of *F. solani* in soybean roots that are translocated and cause chlorosis and necrosis in the leaves (Hartman *et al.,* 2004).According to Harborne[8] the acceleration of phenol biosynthesis in plant after inoculation with plant pathogen has been known for a long time. There is often a great increase in phenol biosynthesis in resistant rather than in susceptible host. In the present studies the both free and bound Phenolic acid showed an increase in all plants inoculated with 10,000 cfu, 100,000 cfu and 1000,00 cfu *F. solani.* However increase in biosynthesis of free and bound phenolic acid were more pronounced in 1000,000 cfu inoculated sample and least in 10,000 cfu inoculated plant. The quantities of bound phenolic acid remain much lower than that of free phenolic acid. A noticeable increase in free phenolic acid may be either due to net synthesis of phenols as a response to infection or due to conversion of bound phenolic acid into free phenolic acid by the activity of certain enzyme[9]. The phenol content noticeable increases might be due to accumulation of phenols from surrounding healthy tissues, net synthesis of phenols as a reply to infection or release of bound phenolic acid by the enzymatic activity of the fungus[5]. It was found that decline in disease was related with increase level of polyphenols oxidase (PPO) and peroxidase (PO) and total phenols. PO activity more evaluated to PPO specific activity and increases clearly after infection of solani fungus[6]. The phenolic content (bound &free) help in disease plant to resists by inhibiting the growth of the pathogen. It was reported that phenolic acid happen in bound form but due to fungal attack it was converted in to free form[7]. The phenolic contents are accumulated in disease plants can possibly be endorsed the resistance of plant against pathogen[10].

5. CONCLUSION

In the present investigation, it was observed that with the increase in concentration of pathogen, both free and bound phenolic acid content increased which showed that the plant resist to the pathogen *F. solani* by build up defense mechanism.

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