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INOCULATION OF *Rhizobium japonicum* AND β-SITOSTEROL EFFECT ON GROWTH, YIELD AND SOME BIOCHEMICAL CONSTITUENTS OF SOYBEAN PLANT

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ABSTRACT

The study was conducted to determine the effect of using *Rhizobium* inoculation (0, 20, 40 g kg^{-1} seeds) and β-sitosterol (0, 10, 20, 40 mgL^{-1}) on nodes number/plant, root and shoot mass, biochemical contents of roots, leaves and seeds of soybean (*Glycine max* L.). These treatments or their interaction revealed significant improvement on the studied characters. Node number per plant significantly increased by *Rhizobium* inoculation (20 gkg^{-1}) at flowering stage or β-sitosterol (40 mgL^{-1}) at vegetative stage. A combination of *Rhizobium* inoculation (20 gkg^{-1}) and β-sitosterol (20 mgL^{-1}) resulted in significant increases in node number per plant at vegetative and flowering stages. *Rhizobium* inoculation (20 gkg^{-1}) or β-sitosterol (40 mgL^{-1}) caused the highest effect on fresh and dry mass for root and shoot per plant. However, the maximal seed yield per plant was obtained in response to 10 mgL^{-1} β-sitosterol. Also, the interaction between *Rhizobium* inoculation (40 g kg^{-1}) and β-sitosterol (40 mgL^{-1}) resulted in the maximal values of root and shoot mass at seed filling stage, which led to significant increase in pod number per plant. Total sugar content in the roots and leaves were increased in response to *Rhizobium* inoculation (40 g kg^{-1}) or β-sitosterol (40 mgL^{-1}) as well as when both treatments used together. However, biochemical contents such as free amino acids, protein phenols and indoles were floaculated within and between roots and leaves of soybean, which led to varied seed biochemical contents.
Keywords: Soybean, Inoculation, β-sitosterol, Growth, Biochemicals.

INTRODUCTION

The economic and environmental importance of legume crops is largely due to their ability to fix atmospheric nitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). A plant mediated, feedback-regulated process termed autoregulation generally restricted the extent of nodulation upon successful inoculation. This process involves suppression of nodule emergence from ontogenetically younger root tissues by previously formed nodules on older parts of the root system (Kosslak & BoMool, 1984, Pierce & Bauer, 1993). It is proposed that once a critical number of subepidermal cell divisions in the root cortex are initiated, a precursor molecule from the root was transported to the shoot where it was converted into the shoot derived inhibitor, which in turn is transported back to the root and suppressed the later formed subepidermal cell divisions from developing into emergent nodules (Caetano & Gresslioff, 1991). Root nodule formation and symbiotic nitrogen fixation are associated with an exchange of chemical signals and coordinated expression of specific genes of both plant and bacterium (Schultze & Kondorosi, 1998 ; Gualtieri & Bisseling, 2000).

Nodule development is also regulated by plant hormones and biomass (Ferguson & Mathesius, 2003; Wall *et al.*, 2003).

Sterols are major constituent of different membranes and regulate membrane properties and functions (Goss, 1972; Bloch, 1983). Brassinosteroids have been found to evoke cell elongation, cell division, swelling, curvature and splitting of the internode leaf bending, vascular differentiation, proton pump-mediated membrane polarization and sink/source regulation (Mandava, 1988; Clouse & Sasse, 1998; Sasse, 1999). Sterols have been reported to enhance growth characters and yield of maize (Abd El-Wahed *et al.*, 1999; Abd El-Wahed 2001). Additionaly, seedling growth of rice plants was improved by 24-epibrassinolide treatment (Őzdemir *et al.*, 2004) and sitosterol application overcome herbicide application effects on rice (Abd El-Wahed *et al.*, 2003). This evidence indicates that sterols could be essential for normal plant growth and development. The objective of the present investigation was to examine the putative role of β-sitosterol in enhancing nodule development in soybean through inoculation of *Rhizobium japonicum*. This interaction effect was addressed by determining growth, yield and biochemical constituents of soybean plant at vegetative, flowering and seed filling stages.

MATERIALS AND METHODS

Bacteria inoculation treatments. Soybean seeds (Glycine max L., Cultivar G-35) were inoculated with two levels (20 and 40 g/kg) of active strain *Rhizobium japonicum* before sowing. Control seeds received no inoculation. *Rhizobium Japonicum* was obtained from Agricultural Unit, Soil Department, Agricultural Research Center, Giza, Egypt.

Sitosterol treatments. The treatments were consisted of three concentrations (10, 20 and 40 mg/L) of β-sitosterol (Stigmasta-5-en-3 β-ol: (24R)-24-ethylcholest-5-en-3 β-ol). Soybean seeds were soaked in the previous concentrations of β-sitosterol for one hour before sowing under laboratory conditions. Control seeds soaked in distilled water for the same period. Next, soybean seeds were grown in earthern pots (30 cm diameter) filled with loamy soil at April 9 and 15, 2002 and 2003, respectively. The plants were grown for 5 months at greenhouse under the following growth conditions: 15 h photoperiod 32.2°/22.5° day/night temperature and 58% relative humidity. The experiments were arranged as a complete randomized blocks design with six replications. Each replicate represented by 2 pots with 4 plants per each pot. Phosphorus fertilizer as calcium superphosphate (15.5% P_2O_5) was presowing added at the rate of 6 g/pot. Nitrogen fertilizer as urea (46.5% N) and potassium as potassium sulphate (48% K_2O) at rate of 6 g/pot were applied after 21 days of sowing.

Growth And Yield Measurement. Fresh mass and dry mass of root and shoot were determined at vegetative stage (30 days after sowing), flowering stage (60 days after sowing) and seed filling stage (150 days after sowing). Yield and its components as pods number/plant, seed number/pod, seed yield/plant and 100-seed weight were taken at seed filling stage.

Biochemical Constituents Determination. Root, leaves and seeds were dried in a ventilated oven at 70°C and then finally ground in stainless steel mill for determination of total sugars (Dubois *et al.*, 1956), free amino acids (Plumer 1978), protein percentage (A.O.A.C., 1975), total phenols (Danial & George, 1972) and total indole compounds (Glickman & Dessaux, 1995). Oil percentage in seeds was determined according to A.O.C.S. (1964).

Statistical Analysis. Analysis of variance of the two year data was carried out as described by Snedecor and Cochran (1980). LSD at 5% level for significant F values was calculated to compare between means of different treatments.

RESULTS AND DISCUSSION

Effect of *Rhizobium* inoculation or β-sitosterol and their interaction on node number, root, shoot and yield components: Data presented in Table (1) show that inoculation of soybean seeds by *Rhizobium* or soaking it in β-sitosterol solution caused significant increase in the node number, fresh and dry mass of root and shoot, yield and its components per plant at vegetative, flowering and seed filling stage. Maximal value of the node number was obtained by 20 gkg^{-1} or 20 mgL^{-1} of both treatments at flowering stage. While, *Rhizobium* inoculation (40 gkg^{-1}) or β-sitosterol (40 mgL^{-1}) gave the highest values of root and shoot mass at seed filling stages. However, seed yield per plant and 100-seed mass were more affected by *Rhizobium* inoculation (20 gkg^{-1}) or β-sitosterol (10 mgL^{-1}) as a result to fixed nitrogen and β-sitosterol necessarily to flower pollination and fertilization.

Regarding to *Rhizobium* inoculation and β-sitosterol interaction in Table (2), 20 gkg^{-1} of *Rhizobium* and β-sitosterol (20 mgL^{-1}) significantly increased node number at vegetative and flowering stage. The maximum values of root and shoot fresh and dry mass were obtained by *Rhizobium* inoculation (40 gkg^{-1}) and β-sitosterol (40 mgL^{-1}) at seed filling stage, Table (2). Whereas, the organs mass increased with plant development as a result to stimulation of physiological processes, which eventually led to improve growth and yield of soybean plant. This effect was clear in seed yield/plant by *Rhizobium* inoculation (20 gkg^{-1}) and β-sitosterol (40 mgL^{-1}). However, the highest yield/plant and 100-seed weight were obtained by 10 mgL^{-1} of β-sitosterol. This might be due to plant mediated, feed back regulated process termed autoregulation generally restricted the younger root tissues by previously formed nodules on older parts of root system (Valverde & Wall, 1999) that reflected on growth and yield. On the other hand, the node factor might act in legumes by changing the internal plant hormones balance (Ferguson & Mathesius, 2003), which was found to increase seed yield of inoculated faba bean relative to uninoculated with bacteria (Al-Kahal *et al.*, 2001). It is suggested that nodal roots enhanced shoot branch development through photosynthate supplying (Thomas *et al.*, 2003). Vardhini and Seeta (1998) similarly reported that brassinosteroids increased the growth and yield of plant. Other studies indicate that sterols application improved root and shoot growth and yield of corn (Abdel El-Wahed, 2001) and rice (Abd El-Wahed *et al.*, 2003).

Table I. Effect of inoculation or β-sitosterol on node number, root and shoot growth and yield components of soybean.

Treatments	Node Number		Root (g/plant)						Shoot (g//plant)						Yield components			
			Fresh mass			Dry mass			Fresh mass			Dry mass			Pod number Per plant	Seed number per pod	Seed yield per plant (g)	100-seed mass (g)
	Veg.	Flow.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.				
A. *Rhizobium* inoculation (g kg^{-1})																		
0	11.1	16.6	0.51	5.3	35.0	0.12	0.9	2.3	3.6	28.6	94.6	0.70	2.6	13.3	24.3	2.2	6.8	15.1
20	15.2	21.3	0.60	4.8	24.8	0.15	1.0	2.7	5.0	32.3	92.1	0.91	3.2	15.2	23.1	2.4	6.1	13.9
40	16.9	13.8	0.47	3.6	31.4	0.14	0.8	5.3	3.8	29.9	96.5	0.60	2.9	13.2	27.4	2.6	4.4	10.48
LSD at 5%	0.8	1.4	0.04	0.7	1.7	0.02	0.1	0.7	0.7	1.7	NS	0.04	0.04	1.5	NS	0.2	0.5	0.5
B. β-sitosterol (mg L^{-1})																		
0	12.8	21.3	0.53	4.1	26.9	0.13	0.86	2.2	4.1	30.2	71.0	0.76	3.0	11.4	16.0	2.2	5.8	13.8
10	11.7	12.4	0.50	4.2	28.9	0.12	0.78	2.3	4.1	28.5	79.2	0.69	2.7	14.5	25.5	2.4	7.5	13.5
20	16.4	19.4	0.57	4.8	24.8	0.15	0.94	3.7	4.2	33.9	90.7	0.71	3.2	12.2	27.6	2.6	5.0	12.7
40	16.7	15.7	0.51	5.1	40.9	0.14	0.94	5.3	4.2	28.5	136.7	0.79	2.6	17.5	30.4	2.4	4.8	12.9
LSD at 5%	0.9	1.6	0.07	NS	1.9	NS	0.10	0.8	NS	2.0	8.4	0.04	NS	1.7	4.1	0.2	0.6	0.6

Veg.= Vegetative stage Flow.= flowering stage Fill.= Seed filling stage.

Table 2. Effect of interaction between inoculation and β-sitosterol on node number, root and shoot growth, and yield components of soybean.

Treatments		Node number		Root (g/plant)						Shoot (g/plant)						Pod Number Per plant	Seed number per pod	Seed yield per plant (g)	100-seed mass (g)
				Fresh mass			Dry mass			Fresh mass			Dry mass						
Inoculation (gkg^{-1})	β-sitosterol (mgL^{-1})	Veg.	Flow.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg	Flow.	Fill.				
0	0	8.0	22.7	0.51	3.1	27.1	0.11	0.68	1.5	3.4	24.6	77.8	0.7	2.4	11.1	12.6	2.1	6.4	14.7
	10	12.7	15.7	0.58	4.8	27.3	0.12	0.76	2.4	4.0	25.8	100.3	0.7	2.5	17.0	35.2	2.1	11.1	17.1
	20	16.7	18.0	0.51	6.1	29.4	0.12	1.08	2.3	3.2	35.7	84.6	0.7	2.9	9.2	30.5	2.2	6.0	14.4
	40	7.0	10.0	0.46	7.1	56.1	0.12	0.91	2.9	3.8	28.4	115.6	0.8	2.6	15.7	18.8	2.2	3.7	14.5
20	0	7.0	21.3	0.43	4.6	18.5	0.12	0.93	2.4	5.4	33.0	70.7	1.0	3.4	13.9	17.1	2.1	4.3	15.9
	10	7.3	12.3	0.57	4.7	30.7	0.14	0.95	2.4	5.1	28.7	84.5	0.8	3.1	14.7	16.3	2.4	5.6	11.7
	20	23.7	30.7	0.78	4.3	22.5	0.18	0.82	3.4	4.9	33.8	85.6	0.9	3.3	15.9	24.7	2.7	6.0	13.5
	40	22.7	20.7	0.61	5.6	27.4	0.14	1.30	2.5	4.5	33.6	127.7	0.9	3.0	16.3	34.2	2.3	8.5	14.5
40	0	23.3	20.0	0.65	4.5	35.0	0.16	0.98	2.6	3.5	33.2	64.5	0.6	3.3	9.2	18.5	2.4	6.8	10.7
	10	15.0	9.3	0.35	3.2	28.7	0.11	0.61	2.0	3.1	30.9	52.9	0.6	2.5	11.6	25.0	2.5	5.9	11.6
	20	9.0	9.7	0.42	4.0	22.4	0.13	0.93	5.3	4.4	32.1	102.0	0.6	3.4	11.6	27.8	2.8	3.0	10.1
	40	20.3	16.3	0.47	2.6	39.3	0.14	0.63	10.5	3.6	23.5	166.7	0.7	2.3	20.5	38.2	2.7	2.1	9.5
LSD at 5%		1.9	3.1	0.10	1.6	3.7	0.06	0.2	1.6	1.94	3.9	16.7	0.1	1.1	3.4	8.3	0.5	1.1	1.2

Veg.= Vegetative stage Flow. = flowering stage Fill. = Seed filling stage.

Effect of *Rhizobim* inoculation or β-sitosterol and their interaction on root biochemical constituents of soybean plant: *Rhizobium* inoculation, β-sitosterol and their combination significantly increased total sugar content in the root at vegetative stage Table (3) and (Figs. 1,2) especially *Rhizobium* inoculation (40 gkg^{-1}) and β-sitosterol (40 mgL^{-1}) were more effective. While, this content was decreased at the other stages (Figs. 1,2). Free amino acid and protein contents were contrasted within the root by *Rhizobium* inoculation (20gkg^{-1}) that was more effective on root free amino acids content at vegetative stage. This could be supported by the fact that invading bacteria provide reduced nitrogen to the plant in exchange for carbohydrates to the bacteriods (Crespi & Galvez, 2000; Poole & Allaway, 2000; Day *et al.*, 2001). In this trend, root protein and phenol contents at seed filling stage were more responsive as a result to *Rhizobium* inoculation (40 gkg^{-1}). Increasing *Rhizobium* dose led to increment root indole content in the root with plant development. Additionally, β-sitosterol (40 mgL^{-1}) was more effective on free amino acid at seed filling stage. So, the maximal values of protein and phenol content at seed filling stage were obtained by application 10 mgL^{-1} of β-sitosterol, while maximum indole content was obtained at vegetative stage with β-sitosterol (20 mgL^{-1}).

Treatments application together reflected their effect in root biochemical contents (Table 3). Whereas, the maximum values of total sugar at vegetative stage and free amino acid content at seed filling stage were obtained by *Rhizobium* inoculation (40 gkg^{-1}) and β-sitosterol (40 mgL^{-1}) combination. Protein at seed filling tended to show the highest content by *Rhizobium* inoculation (20gkg^{-1}) and β-sitosterol (10mgL^{-1}) interaction. So, phenol content was significantly affected by *Rhizobium* inoculation (zero or 40 gkg^{-1}) and β-sitosterol (10 mgL^{-1}) at seed filling stage as well as indole content at vegetative stage by 20 gkg^{-1} and 20 mgL^{-1} of the previous treatments, respectively (Table 3). It seemed that a shift between the previous compounds has been done within and between the plant organs at different stages.

This metabolic shift may involved translocation and reconstructed of amino acids (Osaki *et al.*, 1991), activation of dehydrogenase activity (Wang & Wang, 1`997) or stimulation of DNA and RNA replication (Mandava, 1988; Szekers & Konez, 1998). This might lead to the observed obtained changes in the biochemical contents in the plant. The published data are in agreement with present results, e.g. Charitha and Reddy, (2002) reported that groundnut inoculation increases the quantities of phenolic compounds. Additionally, Abd El-Wahed (2001) and Abd El-Wahed *et al.* (2003) found that sterol application to corn and rice increased root sugar and phenol contents and decreased free amino acids.

Effect of *Rhizobium* inoculation or β-sitosterol and their interaction on leaf biochemical constituents of soybean plant: *Rhizobium* inoculation, β-sitosterol and their interaction resulted in significant increases in the biochemical contents of soybean leaves as compared with control.

Table 3. Effect of interaction between inoculation and β-sitosterol on root biochemical constituents of soybean at different growth stages.

Treatments		Total sugars (%)			Free amino acids (mg/g)			Protein (%)			Phenols (mg/g)			Indoles (mg/g)		
	β-sitosterol (mgL^{-1})	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.
0	0	33.1	34.5	14.4	8.4	4.9	8.3	3.5	2.4	8.2	8.8	4.5	9.8	7.4	4.4	2.5
	10	25.3	30.8	13.4	7.4	5.0	5.9	3.1	3.3	7.9	4.4	5.8	10.9	6.4	4.6	2.3
	20	30.9	38.0	15.3	7.6	4.0	7.4	3.7	4.8	7.4	4.8	3.8	8.4	6.7	4.7	2.5
	40	33.8	31.0	16.0	11.2	4.6	8.2	2.3	4.7	7.4	4.6	4.2	11.5	6.2	4.6	2.4
20	0	42.4	33.0	14.5	9.7	4.9	8.1	2.8	2.8	8.2	6.5	4.4	9.5	5.8	4.4	2.4
	10	38.5	25.5	14.3	9.3	3.3	5.3	3.1	2.9	8.7	5.1	4.5	9.0	6.4	4.2	2.5
	20	26.6	35.6	13.4	8.7	2.2	5.6	3.0	2.9	5.6	5.2	5.6	10.1	6.4	4.5	2.4
	40	26.4	28.1	7.7	8.4	6.8	8.2	3.5	3.1	6.5	4.7	3.8	8.0	6.0	4.2	2.3
40	0	28.2	30.9	12.7	9.5	5.1	8.5	2.9	2.8	8.0	5.3	4.7	10.4	5.9	4.2	2.5
	10	40.3	27.5	5.7	8.7	4.3	7.1	3.5	2.7	8.0	5.4	5.3	10.8	5.8	4.3	2.3
	20	43.8	27.4	8.4	4.3	4.2	7.6	3.4	2.8	6.4	4.6	6.6	7.5	6.0	4.5	2.2
	40	47.2	30.5	11.7	3.9	4.4	8.8	3.2	2.6	7.6	5.8	4.8	10.4	6.1	4.1	2.4
LSD at 5%		1.1	1.9	1.8	1.5	1.2	0.9	0.4	0.3	0.6	1.4	0.6	1.3	1.0	0.6	0.1

Veg.= Vegetative stage **Flow. = flowering stage** **Fill.=Seed filling stage.**

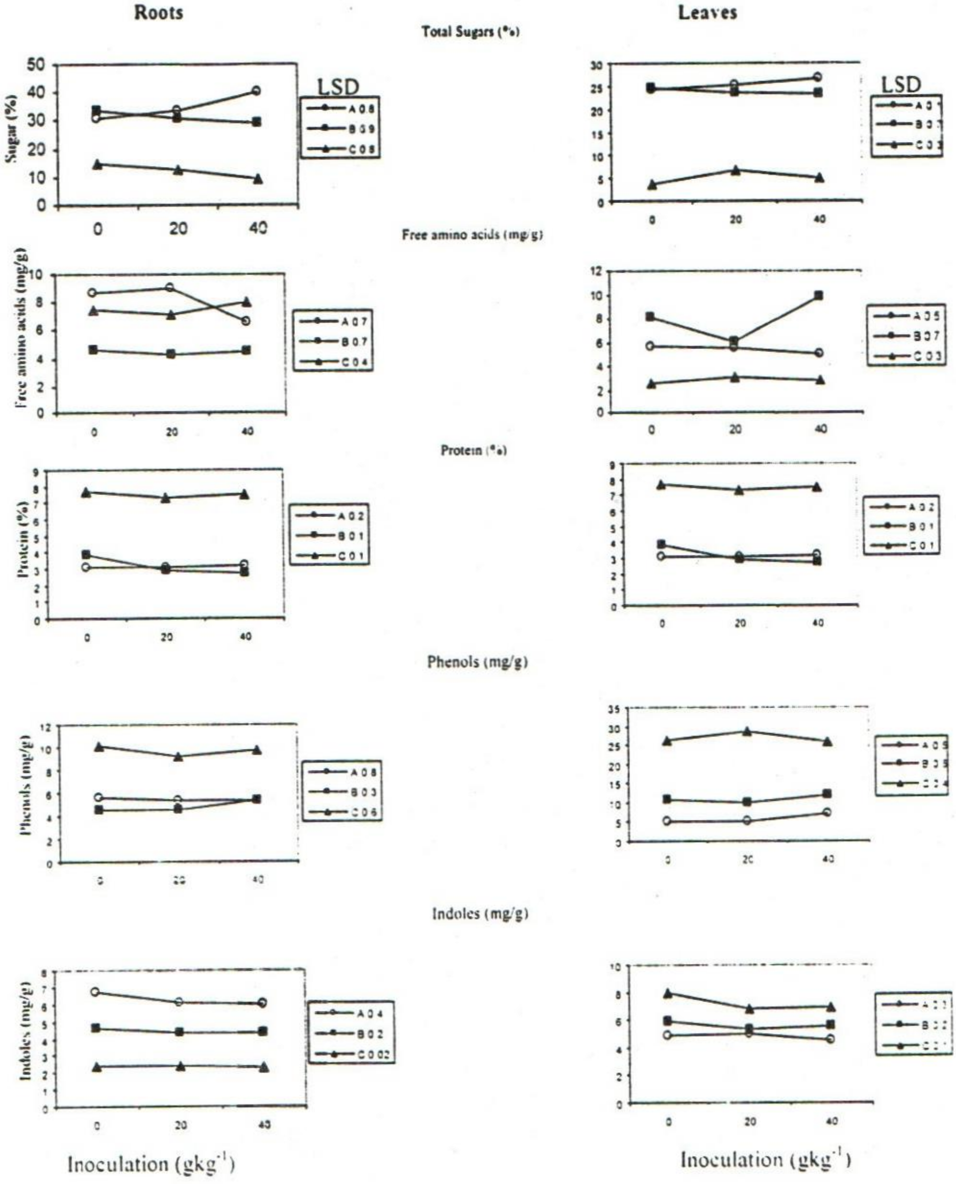

Fig. 1. Effect of inoculation on root and leaf biochemical constituents of soybean at vegetative (A), flowering (B) and seed filling (C) stages.

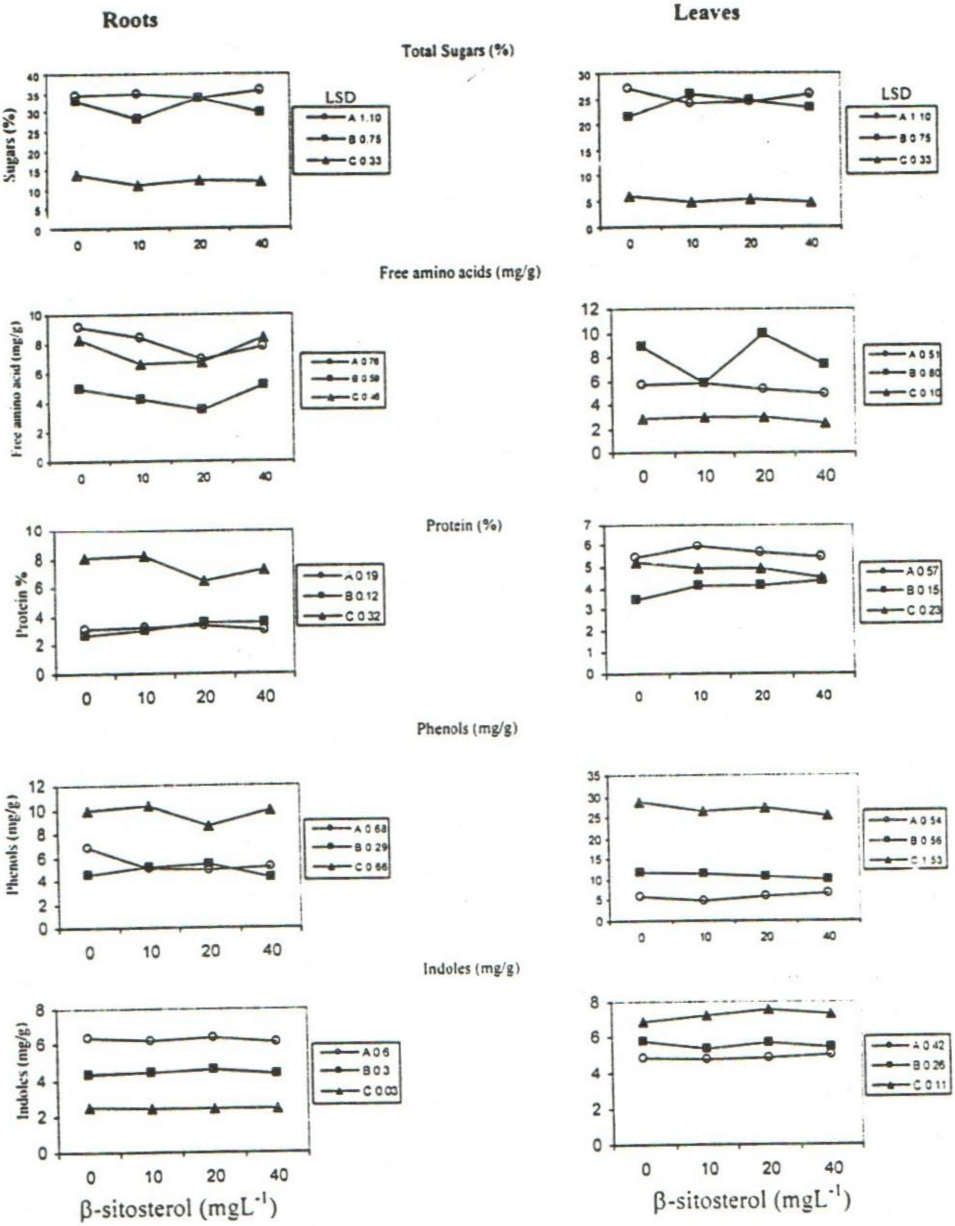

Fig. 2. Effect of β-sitosterol on root and leaf biochemical constituents of soybean at vegetative (A), flowering (B) and seed filling (C) stages.

Fig. 2. Effect of β-sitosterol on root and leaf bic chemical constituents of soybean at vegetative (A), flowering (B) and seed filling (C) stages.

Maximum values of total sugars at vegetative, free amino acid at flowering and protein at filling stage were recorded by *Rhizobium* inoculation (40gkg^{-1}) as shown in Fig. (1). While phenol content was more affective at seed filling stage by *Rhizobium* inoculation (20gkg^{-1}).

Increasing indole content at seed filling although decreasing, it significantly with increment *Rhizobium* level. It appears from the results that invading *Rhizobium* led to change biochemical constituents in soybean leaves. In addition, β-sitosterol (40 mgL^{-1}) significantly increased leaf total sugar content at vegetative stage. Maximum values of free amino acid at flowering, phenol and indole at seed filling stage were obtained by β-sitosterol (20 mgL^{-1}). which was contrasted with protein content that was more affective by β-sitosterol (10 mgL^{-1}) at vegetative stage (Fig. 2).

Regarding to *Rhizobium* inoculation and β-sitosterol interaction (Table 4), leaf content of total sugar at vegetative by *Rhizobium* inoculation (20 gkg^{-1}) and β-sitosterol (40 mgl^{-1}) combination, free amino acid at flowering and indole at seed filling stage were significantly increased as well as Protein at vegetative and phenol at seed filling stage by *Rhizobium* inoculation (40gkg^{-1}) and β-sitosterol (20 mgL^{-1}) combination by *Rhizobium* inoculation (40gkg^{-1}) and β-sitosterol (40 mgL^{-1}) combination Table (4). From the results, it appears that the treatments combination has been an associated effect on the biochemical synthesis in soybean leaves and their change between plant organs at various development stages.

The results indicate that decline in total sugar, free amino acids and protein leaves content occurred, that return to increases in phenols and indoles content in the leaves with advancing soybean plant development. This effect might be due that photosynthesis depend on nitrogen assimilation to make essential molecules (e.g. amino acids and protein) that is directly related to fixing carbon dioxide in the leaves (Amphell, 1985). Charitha and Reddy (2002) reported that accumulation of phenolics and the activation of oxidative enzymes were more in the dual inoculated plants. Moreover, Cook *et al.*, (1993) suggested that the influence of sterols on the specific activity of H^+-ATPase in plasma membrane vesicles from oat, rye and rice shoots is a master or key enzyme in plant. That led to increase dehydrogenase activity (Wang & Wang, 1997) and stimulate DNA and RNA (Mandava, 1988; Szekers & Konez, 1998). Our previous interpretation is supported by the fact that biochemical change is the end result of physiological interactions between *Rhizobium* and β-sitosterol on soybean plant at various physiological stages.

Effect of *Rhizobium* inoculation and β-sitosterol and their interaction on seed biochemical contents: The data given in Table (5) and illustrated in Fig. (3) show that *Rhizobium* inoculation (20gkg^{-1}) significantly increased total sugars and free amino acid contents in soybean seed. While, the maximum value of protein and phenol contents were obtained by *Rhizobium* inoculation (40 gkg^{-1}). Seed oil content significantly decreased by *Rhizobium* inoculation. Additionally, β-sistosterol (40 mgL^{-1}) significantly increased oil and phenol contents in soybean seeds.

Table 4. Effect of interaction between inoculation and β-sitosterol on leaf biochemical contents of soybean at different growth stages.

Treatments		Total sugars (%)			Free amino acids (mg/g)			Protein (%)			Phenols (mg/g)			Indoles (mg/g)		
Inoculation (gkg^{-1})	β-sitosterol (mgL^{-1})	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.	Veg.	Flow.	Fill.
0	0	24.3	24.6	5.0	5.2	6.1	2.5	4.8	4.0	4.3	4.3	11.9	29.7	4.9	5.9	7.4
	10	21.9	25.2	2.2	5.5	10.3	2.7	5.4	4.4	5.3	4.7	10.7	26.2	4.9	6.3	8.2
	20	24.4	25.1	2.7	6.2	9.1	2.6	5.8	4.2	4.3	5.0	10.5	25.2	4.8	5.6	8.2
	40	26.7	23.0	5.2	6.0	6.9	2.5	4.8	4.5	5.1	5.8	10.5	24.4	4.8	5.6	7.9
20	0	25.5	16.5	8.4	6.5	7.6	2.8	6.2	3.9	5.7	5.8	10.8	35.0	5.0	5.6	6.7
	10	22.3	27.0	5.2	5.8	3.3	3.3	5.6	4.2	4.0	4.8	11.0	27.8	4.6	4.6	6.2
	20	25.0	26.2	7.3	5.2	8.0	3.8	5.1	4.4	5.5	4.8	9.8	28.7	5.0	5.8	7.2
	40	27.9	25.1	6.7	4.8	5.4	2.6	4.8	4.4	3.6	5.8	7.9	23.7	5.5	5.0	7.2
40	0	31.3	23.2	4.7	5.4	13.9	3.6	5.2	2.7	5.5	8.0	12.3	21.1	4.4	5.8	6.5
	10	28.5	25.4	6.9	6.2	3.9	2.9	6.6	3.7	5.4	5.3	13.1	26.0	4.7	5.1	7.1
	20	24.1	22.8	5.7	4.4	12.5	2.6	5.7	3.7	4.9	7.7	11.6	27.3	4.6	5.8	7.3
	40	22.7	21.6	2.5	3.9	9.8	2.1	6.7	3.9	4.8	7.8	11.3	28.7	4.7	5.7	6.9
LSD at 5%		2.2	1.5	0.7	1.0	1.6	0.2	0.8	0.3	0.4	1.1	1.3	3.1	0.7	0.5	0.2

Veg.= Vegetative stage   Flow.= flowering stage   Fill.= Seed filling stage.

Maximum value of free amino acid and protein contents were obtained by 20 or 10 mgL^{-1} of β-sitoserol. However, total sugar content was significantly decreased at 40 mgL^{-1} β-sitosterol.

Table 5.Effect of interaction between inoculation and β-sitosterol on seed biochemical contents of soybean.

Ttreatments		Total sugars (%)	Oil (%)	Free amino acids (mg/g)	Protein (%)	Phenols (mg/g)	Indoles (mg/g)
Inoculation (gkg^{-1})	β-Sitosterol (mgL^{-1})						
0	0	19.9	28.3	4.5	33.0	2.1	2.8
	10	19.0	26.5	6.2	32.0	2.8	2.5
	20	19.0	28.1	5.3	32.4	2.8	2.5
	40	18.1	27.1	4.0	28.2	2.8	2.5
20	0	21.6	26.6	4.9	30.8	2.6	2.6
	10	23.3	27.5	4.7	34.6	2.9	2.5
	20	20.7	26.3	7.5	31.8	3.2	2.6
	40	18.3	26.4	5.3	30.2	3.4	2.7
40	0	21.3	25.6	5.0	32.4	2.6	2.5
	10	16.3	25.0	2.3	32.4	3.8	2.6
	20	19.7	26.5	2.2	31.4	3.0	2.6
	40	19.1	26.4	2.4	30.4	3.6	2.7
LSD at 5%		1.1	1.8	0.8	0.7	0.8	0.1

In Table (5), *Rhizobium* inoculation ($20gkg^{-1}$) and β-sitosterol (10 mgL^{-1}) combination significantly increased total sugar, oil and protein content in soybean seed. While, the interaction between *Rhizobium* inoculation ($20gkg^{-1}$) and β-sitosterol (20 mgL^{-1}) significantly increased free amino acid content. In addition, *Rhizobium* inoculation ($40gkg^{-1}$) and β-sitosterol (10 mgL^{-1}) gave the highest seed content of phenol. However, indole content significantly decreased compared with control treatment.

These results are in agreement with Tzen & Huang (1992) who reported that oil bodies accumulated mostly acyl lipids surrounded by a monolayer of phospholipids containing basic proteins, which might be related to a high nitrogen demond for high quality legumes seed production when nitrogen fixation was shut off rather than to high production potential and rearrangement in macromolcules (Pozo *et al.*, 2000).

Additionally, the translocation efficiency for fixed nitrogen was great during the seed filling period (Koutroubas *et al.*, 1998) That led to be the seed contained more of the biochemical contents than the other organs. This might be due to regulation transport of N and C compounds (Persson & Nasholm, 2003). However, sterols application significantly increased free amino acids, protein and indoles contents of rice grains (Abd El-Wahed *et al.*, 2003). On the other hand, total sugars of corn grains signficiantly decreased by sitosterol application (Abd El-Wahed, 2001). Gene encoding started to accumulate and the signal was spread systemically after inoculation (Yoo *et al.*, 2004) and sterols application (He *et al.*, 2003).

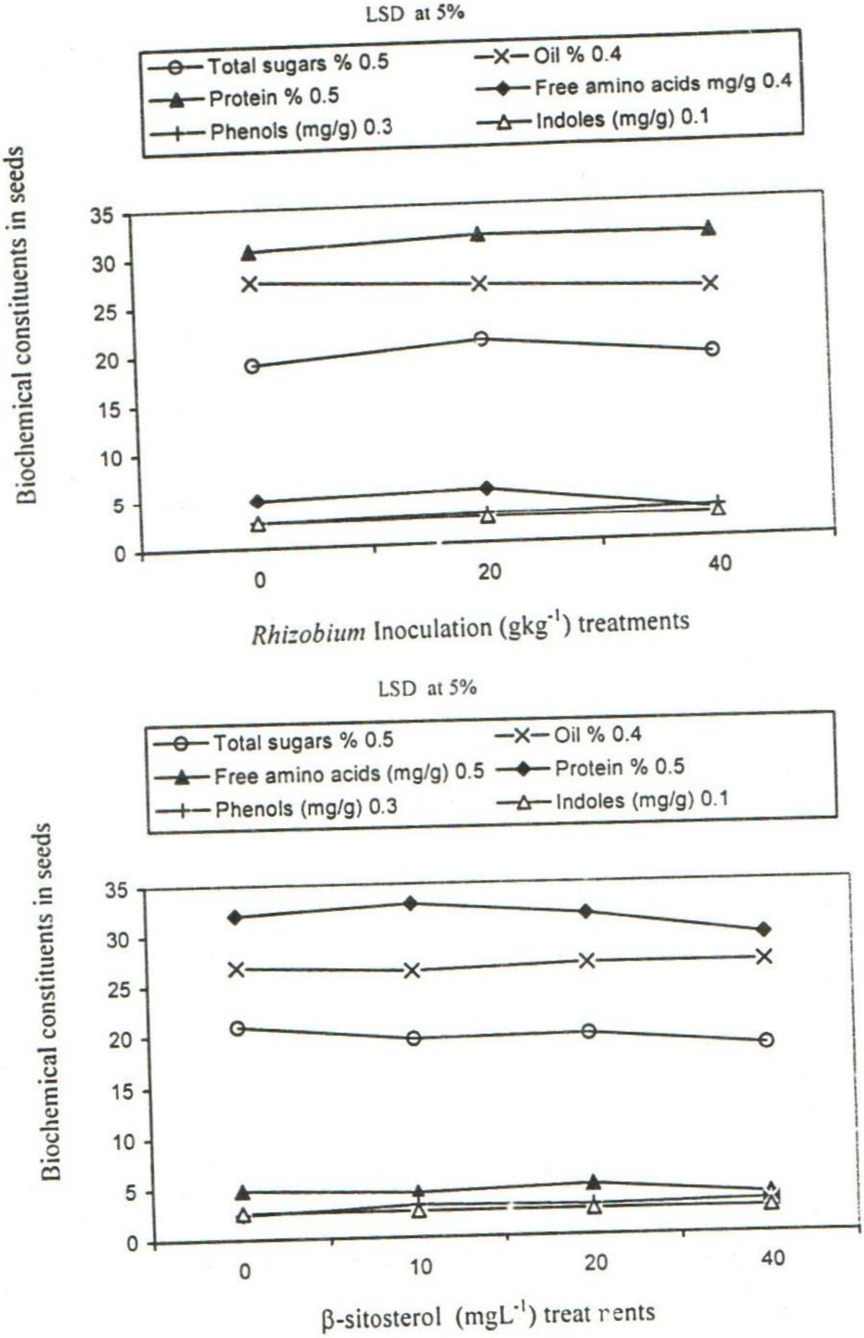

Fig. 3. Effect of inoculation or β-sitosterol on biochemical constituents of soybean seeds LS D are given in bracelets.

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تأثير التلقيح البكتيرى والبيتا سيتو ستيرول على النمــو والمحصــول والتركيــب الكيماوى لنبات فول الصويا

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أجريت هذه الدراسة بصوبة المركز القومى للبحوث فى موسمى ٢٠٠٢-٢٠٠٣ بهدف دراسة تأثير إضافة التلقيح البكتيرى والبيتاسيتوستيرول وتفاعلاتهمـا علـى النمـو والمحصـول والتركيب الكيماوى لنبات فول الصويا (جيزة ٣٥).

وقد أستخدم التلقيح البكتيرى بثلاثة تركيزات (صفر، ٢٠، ٤٠ جم/كجم بـذرة) مـن بكتيريا فول الصويا والبيتاسيتوستيرول بأربعة تركيزات (صفر، ١٠، ٢٠، ٤٠مجم/لتـر) كانـت تضاف منفردة أو فى توافيق فيما بينهما. وقد تم تصميم التجارب فى نظـام القطاعـات الكاملـة العشوائية. وقد أخذت العينات فى ثلاث مراحل فسيولوجية (مرحلة النمـو الخضـرى – مرحلـة التزهير – مرحلة أمتلاء البذور) لتقدير صفات النمو والمكونـات الكيماويـة للجـذور والأوراق والبذور تحت تأثير هذه المعاملات . وكانت النتائج كالآتى:

(١) تأثير التلقيح البكتيرى:

- أدت إضافة البكتريا إلى زيادة العقد البكتيرية معنويا/ نبات.
- زاد الوزن الغض والجاف للجذور/نبات معنويا فى مراحل النمو المختلفة بزيادة معدل التلقـيح البكتيرى. كان لإضافة التلقيح البكتيرى أثر فى زيادة النمو الخضرى للنبات معنويا أثناء مراحل النمو المختلفة.
- تحسن محصول نبات فول الصويا معنوياً بإضافة التلقيح البكتيرى خاصة عند ٢٠جم/كجم مـن بذور فول الصويا.

- زادت المكونات الكيماوية للجذور من السكريات الكلية، الأحماض الأمينية والفينولات أثناء مرحلة النمو الخضرى بينما كان محتوى الجذور من البروتين والفينولات أعلى فى مرحلة أمتلاء البذور عن المرحلة الأخرى.
- ازدادت المكونات الكيماوية للأوراق كالسكريات الكلية فى مرحلة النمو الخضرى والأحماض الأمينية فى مرحلة التزهير وكمية البروتين والفينولات والإندولات فى مرحلة أمتلاء البذور بإضافة التلقيح البكتيرى.
- أدى التلقيح البكتيرى إلى زيادة مكونات البذور من السكريات الكلية والبروتين والأحماض الأمينية الحرة والفينولات والإندولات مقارنة بالكنترول.

ثانياً: تأثير البيتاسيتوستيرول:

- أدت إضافة البيتاسيتوستيرول إلى زيادة تكوين العقد البكتيرية/ نبات معنوياً أثناء مرحلة النمو الخضرى. زاد الوزن الغض والجاف للجذور والمجموع الخضرى/ نبات معنوياً فى مراحل النمو المختلفة.
- تحسنت عدد القرون/ نبات وعدد البذور للقرن ووزن البذور/ نبات معنوياً بإضافة البيتاسيتوستيرول.
- أدت إضافة البيتاسيتوستيرول إلى زيادة المكونات البيوكيميائية للجذور من السكريات الكلية والأحماض الأمينية والإندولات فى مرحلة النمو الخضرى والبروتين والفينولات فى مرحلة أمتلاء البذور.
- أزدادت محتويات الأوراق معنوياً من السكريات الكلية والبروتين فى مرحلة النمو الخضرى والأندولات فى مرحلة امتلاء البذور بينما كان محتوى الأوراق من الأحماض الأمينية أعلى فى مرحلة الإزهار.
- أدت إضافة البيتاسيتوستيرول إلى زيادة المحتويات الكيماوية معنوياً فى البذور ماعدا السكريات الكلية.

تأثير تفاعل التلقيح البكتيرى و البيتاسيتوستيرول:

- أدت إضافة البيتاسيتوستيرول والتلقيح البكتيرى إلى زيادة تكوين العقد البكتيرية على جذور نبات فول الصويا فى مرحلتى النمو الخضرى والأزهار وكانت أفضل معاملة هى ٢٠جم من البكتريا/كجم من البذور + ٢-مجم/لتر بيتاسيتوستيرول.
- أختلف تأثير التفاعل على نمو نبات فول الصويا طبقاً لمرحلة النمو ولكن كان تأثير إضافة ٤٠جم من البكتريا/كجم من البذور + ٤٠مجم/لتر من البيتاسيتوستيرول تأثيرا معنوياً على نمو الجذور والنمو الخضرى بينما أعطى التفاعل ٤٠جم من البكتريا/كجم من البذور+ ٢٠مجم/لتر من البيتاسيتوستيرول أفضل محصول من البذور/نبات.
- أختلفت محتويات الجذور والأوراق الكيماوية فى جميع مراحل النمو طبقاً للحالة الفسيولوجية للنبات التى أنعكست على محتويات البذور من هذه المكونات.

يتضح من النتائج السابقة بأن المعاملة ١٠مجم/لتر بيتاسيتوستيرول فقط هى أفضل المعاملات فى إنتاج أعلى محصول لنبات فول الصويا.