Physiological Studies for Different Concentration from Biochikol 020 PC (Chitosan) on Bean Plant

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Abstract

The experiment was conducted in successive seasons of 2009 in AL-Khober(Saudi Arabia). The aim of the experiment was to investigate the effect of the Biochikol 020 PC Chitosan on the growth of bean plants and the effect of Biochikol 020 PC Chitosan on the level of chlorophyll in the beans leaves too. The plants were watered with the Biochikol 020 PC Chitosan solution once at the beginning of the experiment at different concentrations as follow: 0 (control), 0.5, 1, 1.5, 2, 2.5 & 3% until saturation. The results show that Biochikol 020 PC Chitosan works as a positive factor in enhancing shoot and root length, fresh & dry weights of shoots & roots, leaves area and total soluble sugars and sucrose.

Introduction

Beans plant known as (*Phaseolus vulgaris* super stryke) belgons to the leguminosae family, which is considered one of the most important plants after the gramineae in the beans category because of its economic value *(Saad, 2000)*. Water and nitrogenic fertilizer are among the factors that effect its growth and productivity. *(liebman and corson, 1995)*.

Bean considered to be one of the most important legume yields around the world as it contains all the important nutritious elements for human being, and it has been used in different industries such as the conditioned beans canes or in frozen packages.

The importance of bean comes from the fact that is cheap and rich with vegetal protein in human food. The research indicates that Biochikol 020 PC (Chitosan) can be used as an enhancer for plant's growth and also has multiple usages in the agricultural field.

The literature provides information about the possibility of using Biochikol 020 PC Chitosan to protect plants against different diseases.*(LafontaineandBenhamou,1996;Mazur et al.,2003;Orlikowskiand Skrzypczak,2003 ;Pieta and Pastucha,2002; Pospieszny,1997and Wojdyla,2001), Biochikol 020 PC (2% of Chitosan).*

Bean belongs to the inducers of plants resistance and has demonstrated its antiviral, antibacterial and antifungal effect. (Benhamou *et al*.,1994;Orlikowski *et al*.,2002;Patkowska and Pieta,2004;Pieta *et al*.,2006;Pospieszny,1995 and Pospieszny *et al*.,1995).

This research aims to enhance bean plant growth with the use of Biochikol 020 PC (Chitosan) soluble in the irrigation water.

Material and Methods

(1) Pre Testing

- Choosing the appropriate time for soaking bean seeds, at the following times: (1, 2, 3, 4 & 6) hours respectively.

- Choosing the appropriate period for bean seeds incubation, at the following times: (24, 48 & 72) hours respectively.

(2)Main experiments

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Bean (Phaseolus vulgaris super stryke) seeds sterilized with a solution of sodium hypochlorite at the concentration rate of 5% for three minutes, and soaked repeatedly in distilled water for an hour. Subsequently placed on filter paper moistened with distilled water in Petri dishes and left in the chamber for 24 hours at the temperature of 25c (± 1). Beans were transferred afterwards into pots filled with sandy soil and treated with the Biochikol 020 PC Chitosan substance at different concentrations as follow: (0.5, 1, 1.5, 2, 2.5 and 3 %). Each concentration was represented by three pots - in addition to the control group- and each pot contained 15 plants. Plants left in natural conditions and samples were taken on the seventh day to set the standards growth of (root & shoot length - the number of roots). On the fourteenth day samples were also taken to set the standards growth of (root & shoot length, area of leaf, fresh weight for shoot & root, dry weight for shoot & root and chlorophyll level, *(Inskeep and Bloom, 1985),* total soluble sugars(Handel,1968and Yemm and Wills,1954)and sucrose(Handel,1968).

Results

Pre Testing results:

Choosing the appropriate time for bean seed soaking

Table (A) and Chart (A) Show the appropriate time for bean seed soaking

Table (A) Shows the appropriate time for bean seed soaking (%)

Soaking Period / **Day by hours**	**1**	**2**	**3**	**4**	**6**
24	6.67	13.33	16.67	16.67	3.33
48	83.33	80	60	50	23.33
72	100	93.33	66.67	60	26.67
96	100	93.33	70	66.67	33.33
120	100	96.67	70	70	40

Table (A)

Chart (A) Shows the appropriate time for bean seed soaking

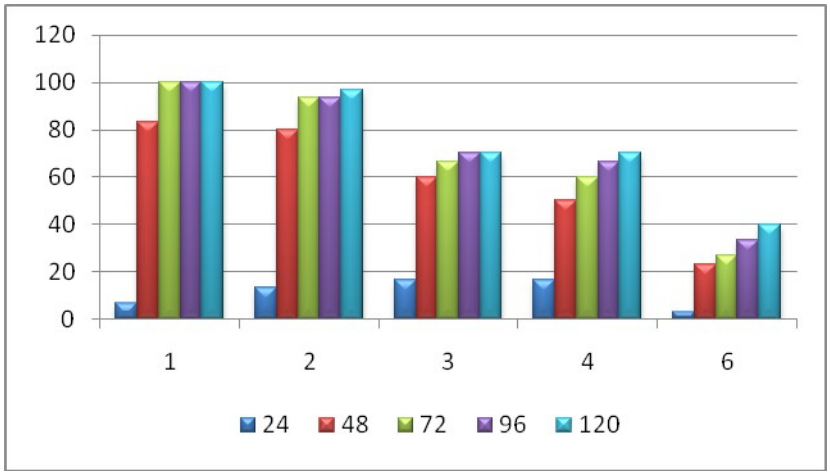

Chart (A)

Choosing the appropriate period for bean seeds incubation

Table (B) and chart (B) Show the most appropriate period for bean seeds incubation.

Table (B) shows the appropriate period for bean seeds incubation (cm).

	The average of stem length upon		
Incubation Period	**5 days**	**7 days**	**10 days**
24 hours	**13.3**	**23**	**27**
48 hours	**14.9**	**23.9**	**26.1**
72 hours	**8.5**	**16.8**	**20.1**

Table (B):

Chart (B) shows the appropriate period for bean seeds incubation

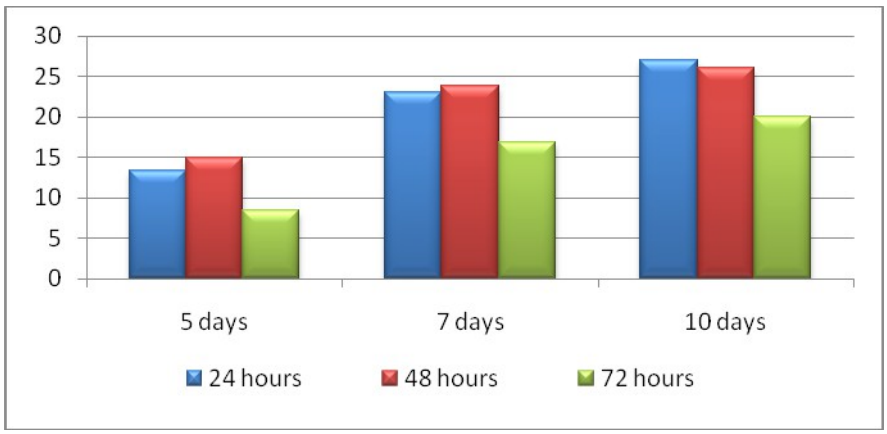

Chart (B)

Main experiments results

Results recorded for bean plants on the seventh day

Effect of different concentrations of Biochikol 020 PC chitosan treatments on the vegetative growth.
Table (1) and chart (1) Show the response of shoot and root length of bean plants to different concentrations of Biochikol 020 PC Chitosan applications. The highest length of the shoot was at 2.5%, however, the highest length of the root was at 3%.
Table (1) shows the effect of different concentrations of Biochikol 020 PC chitosan applications on the shoot & root of bean plants.

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Treatments	The average length of shoot ((cm	The average length of root ((cm
Control	13.8	6.1
(Bio (0.5	12.9	6.6
(Bio (1	13	6.3
(Bio (1.5	13.4	5.8
(Bio (2	13.5	6.3
(Bio (2.5	14.2	6.5
(Bio (3	14	6.9

Table1: Shows various figures of shoot & root length as per concentration.

Chart (1) shows length shoot and root of bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

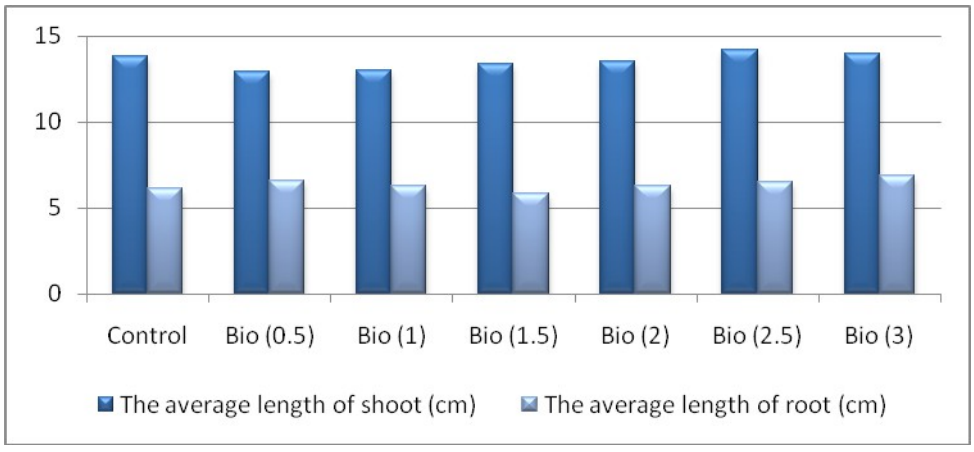

Chart1: Shows the effect of different concentrations of the Biochikol 020 PC Chitosan on shoot & roots

Table (2) & chart (2) Show the number of roots of bean plants responded to the application of different concentrations of the Biochikol 020 PC Chitosan.

The highest number of roots was at control.

Table (2) shows the number of roots of bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

Treatments	The average of number of root
Control	13
(Bio (0.5	9
(Bio (1	9
(Bio (1.5	8
(Bio (2	11
(Bio (2.5	9
(Bio (3	8

Table2: Shows various figures of root length as per concentration.

Chart (2) shows the number of bean plants roots affected by the application of different concentrations of the Chito Biochikol 020 PC san.

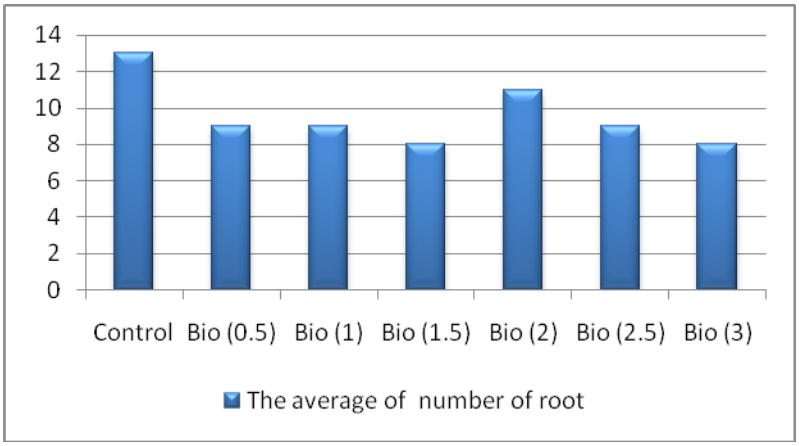

Chart 2: Number of roots affected for each concentration

Results recorded for bean plants on the fourteen days

Showing the effect of different concentrations of Biochikol 020 PC Chitosan and its application on vegetative growth.

Table (3) and chart (3) show the response of the bean plants shoots and roots to the application of different concentrations of the Biochikol 020 PC Chitosan.

The highest length of the shoots was at 1.5% and the highest length of the roots was at 0.5% & 2%.

Table (3) shows the length figures of shoots and roots of the bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

Treatments	The average length of shoot ((cm	The average length of root ((cm
Control	18.4	6.6
(Bio (0.5	18.3	6.9
(Bio (1	18.7	6.7
(Bio (1.5	19.2	6.7
(Bio (2	18.6	6.9
(Bio (2.5	18.9	6.3
(Bio (3	18.3	6.8

Table3: Shows various figures of shoot & root length as per concentration.

Chart (3) shows the length of shoots and roots of the bean plants affected by the application different concentrations of the Biochikol 020 PC Chitosan.

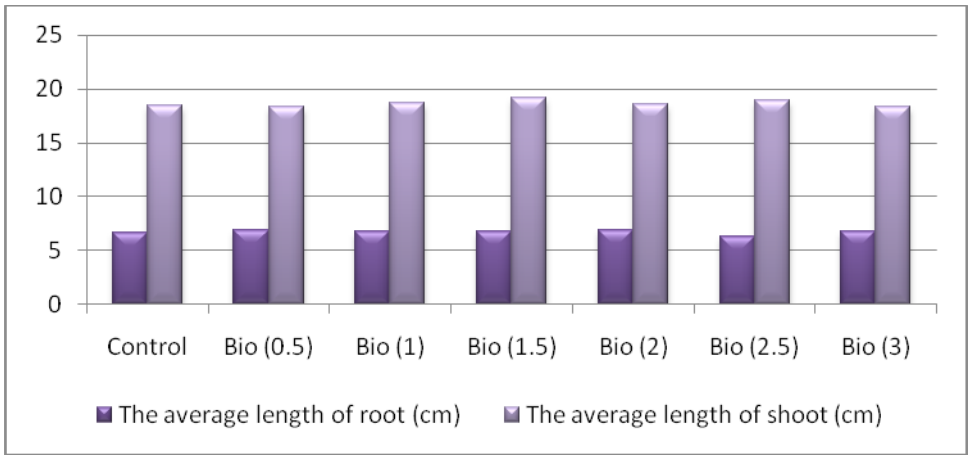

Chart 3: Shows the effect of different concentrations of the Biochikol 020 PC Chitosan on the shoots & roots of plants.

Table (4) & chart (4) show the response of fresh & dry weight of shoots and fresh & dry weight of roots of the bean plants to the application of different concentrations of the Biochikol 020 PC Chitosan.

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The highest figure of fresh shoots weight was at 3%, and the highest figure of dry shoots weight was at 1%, however, the highest figure of fresh & dry roots weight was at 2%.

Table (4) shows the weight figures of fresh & dry shoots and fresh & dry roots of the bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

Treatments	The average fresh (weight of shoot(gm	The average fresh (weight of root(gm	The average dry weight of (shoot(gm	The average dry (weight of root(gm
Control	1.183	0.2886	0.086	0.034
(Bio (0.5	1.220	0.230	0.0856	0.038
(Bio (1	1.100	0.282	0.087	0.050
(Bio (1.5	1.352	0.276	0.080	0.052
(Bio (2	1.146	0.339	0.081	0.065
(Bio (2.5	1.188	0.291	0.084	0.062
(Bio (3	1.432	0.284	0.0796	0.047

Table 4: shows various figures of shoot & root weight as per concentration.

Chart (4) shows the weight of fresh & dry shoots and fresh & dry roots of the bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

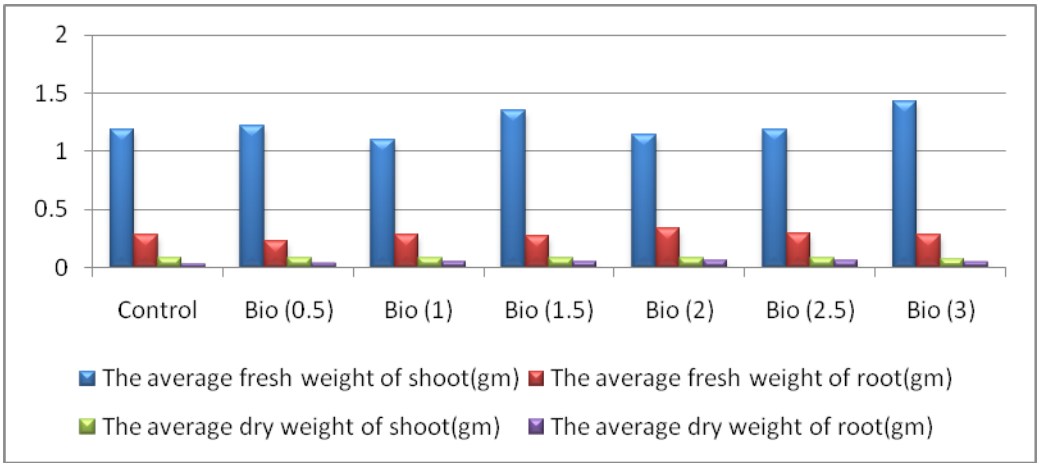

Chart (4) shows the weight of fresh& dry shoots and fresh& dry affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

Table (5) and chart (5) show the response of the bean leaf area to the application of different concentrations of the Biochikol 020 PC Chitosan. The highest leaf area was at 1%.

Table (5) shows leaf area of the bean plants affected by the applications of different concentrations of the Biochikol 020 PC Chitosan.

Treatments	(The average leaf area (cm^2
Control	**9.247**
(Bio (0.5	**9.966**
(Bio (1	**7.479**
(Bio (1.5	**6.719**
(Bio (2	**7.808**
(Bio (2.5	**7.808**
(Bio (3	**10.325**

Table 5: shows various figures of leaf area as per concentration

Chart (5) shows the leaf area of the bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

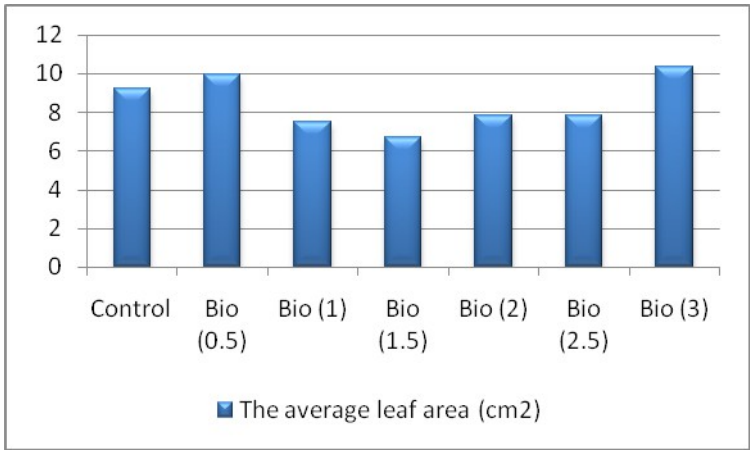

Chart 5: the effect of different concentrations of the Biochikol 020 PC Chitosan on plants leaf

The effect of the Biochikol 020 PC Chitosan on the chlorophyll content:

Table (6) and chart (6) show the response of chlorophyll content of bean plants to the application of different concentrations of the Biochikol 020 PC Chitosan.

The highest concentration of chlorophyll (A) & total chlorophyll was at 0.5%, however, the highest concentration of chlorophyll (B) was at 2.5%.

Table (6) shows chlorophyll (A) content, chlorophyll (B) content and total chlorophyll content of bean plants affected by the application of different concentrations of the Biochikol 020 PC Chitosan.

Treatments	The average chlorophyll (a (mg\l	The average chlorophyll b ((mg\l	The average of total (chlorophyll (mg\l
Control	**7.9284**	**1.4781**	**8.5661**
(Bio (0.5	**8.7852**	**1.5460**	**10.3288**
(Bio (1	**6.8934**	**1.4209**	**8.3114**
(Bio (1.5	**6.7205**	**1.5272**	**8.2470**
(Bio (2	**6.1744**	**1.3598**	**7.6051**
(Bio (2.5	**6.3357**	**1.6804**	**9.4266**
(Bio (3	**6.2805**	**1.5014**	**7.7766**

Table 6: shows various figures of chlorophyll content as per concentration

Figure (6): chlorophyll A content, chlorophyll B content and total chlorophyll content of bean plants affected by different concentration of Biochikol 020 PC (Chitosan) applications.

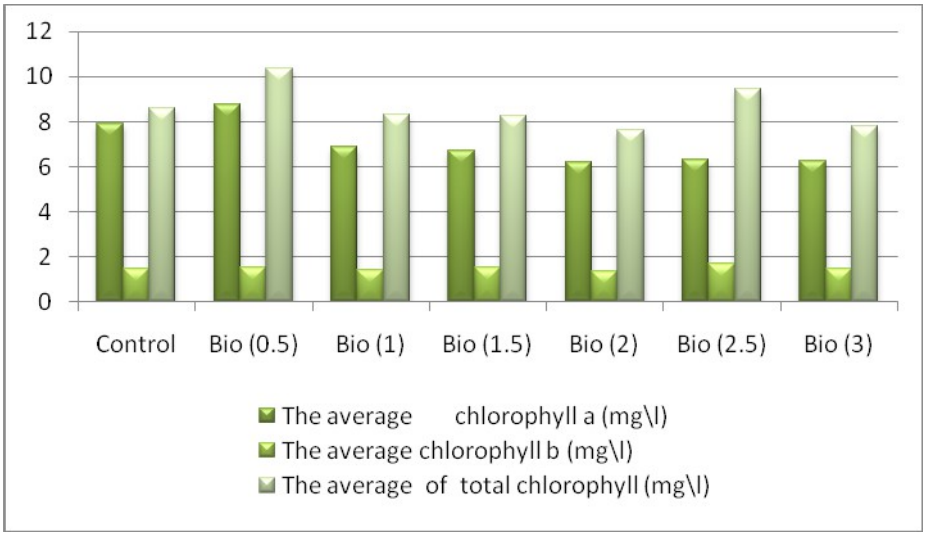

Chart 6: The effect of the Biochikol 020 PC Chitosan on the chlorophyll content

Table (7) and chart (7) show the response of total soluble sugars content and sucrose content of bean plant to different concentration of Biochikol 020 PC (Chitosan) application.

The highest concentration of total soluble sugars in shoot was at 1.5%, however, the highest concentration of total soluble sugars in root at 0.5%.And the highest concentration of sucrose in shoot was at 0.5%, however, the highest concentration of sucrose in root at 3%.

Table (7) total soluble sugars content and sucrose content of bean plants as affected by different concentration of Biochikol 020 PC (Chitosan) applications.

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Treatments	The average of total soluble (sugars (nm		The average of sucrose ((nm	
	Shoot	Root	Shoot	Root
Control	0.052	0.006	0.014	0.006
(Bio (0.5	0.118	0.044	0.088	0.009
(Bio (1	0.063	0.006	0.060	0.009
(Bio (1.5	0.179	0.018	0.063	0.014
(Bio (2	0.106	0.031	0.005	0.026
(Bio (2.5	0.090	0.031	0.023	0.024
(Bio (3	0.072	0.034	0.013	0.034

Table (7): Shows various figures of total soluble sugars content and sucrose content

Chart (7): total soluble sugars content and sucrose content of bean plants as affected by different concentration of Biochikol 020 PC (Chitosan) applications.

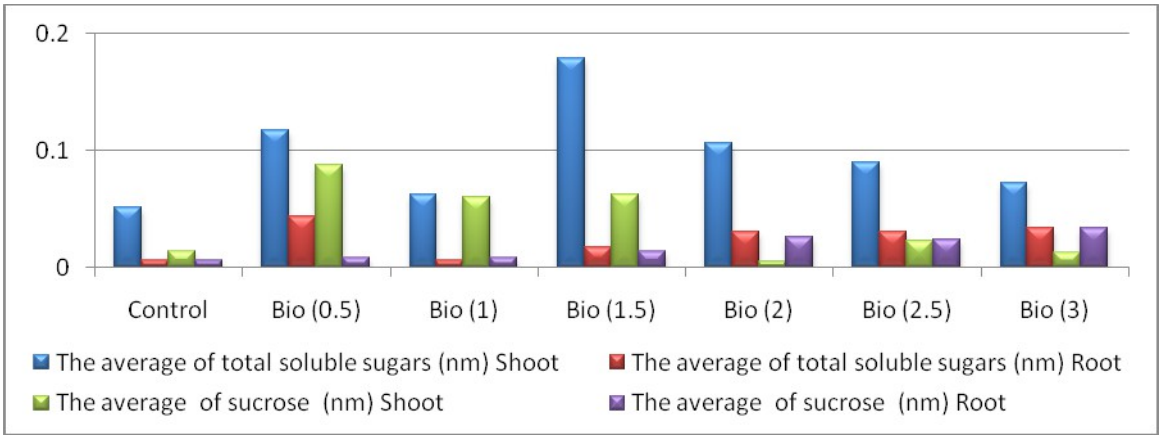

Chart (7): The effect of the Biochikol 020 PC Chitosan on total soluble sugars content and sucrose content

Discussion:

It can be concluded from the results of this study that Biochikol 020 PC (Chitosan) application to bean plants improves the plant's growth, chlorophyll content, total soluble sugar and sucrose content. However, more studies are needed to determine the exact mechanism(s) that lead to those improvements.

The Chitin is a natural polysaccharide, which consists of a copolymer of N-acetyl-D-glucosamine residues, linked by B-1, 4 glycosidic bonds that can be found in various species such as shells of crustaceans, insects' cuticles and cell wall of fungi and some algae. Chitosan is the deacetylated form of Chitin.

- The Chitosan biopolymer is used to treat recycled water, pulp & paper, medical & cosmetic products, biotechnology, food & feed and membranes (*Hirano, 1997*).
- In the agriculture domain, Chitosan is used

1. To improve seed, leaf, fruit and vegetable coating (Devlieghe+re *et al*, 2004). Also used as fertilizer (Sukwattanasinitt*etal*, 2001). To increase plants production (*Wanichpongpan et at, 2001; chandrkrachang, 2002 and New at al, 2004*).

2. To stimulate the plants immunity and growth (*Hadwiger et al, 2002*).

3. To protect plants against microorganisms (*Pospieszny etal,1991;Struszczyk and Pospieszny,1997 and Bautista-Banos etal,2003*).

The idea behind this study is to see the responses of bean plants when exposed to the Biochikol 020 PC Chitosan - specially the growth of shoot & roots and the chlorophyll content.

Throughout the whole study, both recorded growth parameters and chlorophyll content responded positively to the application of Chitosan. Scientists (*Cuibu and Shiayama, 2001*) reported positive effects of Chitosan incorporated into the soil on early growth stages of soyabean, mini-tomato, upland rice and lettuce. These improvements include plant height, leaf area, and dry weight of plants.

Observations conducted during the whole study, (*Cuibu and Shiayama, 2001*) indicate the presence of higher chlorophyll content in plants treated with Chitosan. Both factors (higher area of leaves and chlorophyll content) has contributed into the increase of the photosynthesize production which lead to the increase of plants dry weight and productivity.

It is also reported that Chitosan increased the growth rates of roots and shoots of daikon radish. *(Raphanus sativus L.) (Tsugita et al, 1993), (Utsunomiya and Kinai, 1994*).

The application of (Chitosan – Oligosaccharides) to the soil gave better results for cultivating passion fruit *(Passiflora edulis Sims)*, it showed that (Chitosan – Oligosaccharides) can increase the flowering time and flowers number. *(Utsunomiya and kinai1994)*. Another study been conducted to see the effect of Chitosan on the growth of gerbera plants showing that Chitosan significantly enhanced the growing factors and improved the average values of flower-stem length and the number of growing leaves (including the width & length of leaves as well as the flowers number per bush). *(Wanichpongpan el at.2000)*. Chitosan also promoted the growth of various crops such as cabbage *(Brassica oleracea L. var. Capitata) (Hirano1988),* soya bean sprouts *(Lee el at. 2005)* and sweet basil *(kim2005)*. Chitogel is a derivative of Chitosan which was discovered to improve vegetative growth of grapevine plantlets. *(Ait Barka el at.2004)*.

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