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Full Length Research Paper

Effect of chitosan on common bean (*Phaseolus vulgaris* L.) plants grown under water stress conditions

Sharifa S. Abu-Muriefah

Faculty Science, Princess Norah Bint Abdulrahman University

E-mail: ansary80@yahoo.com

Abstract

The present study showed that water stress impaired the growth of common bean (Phaseolus vulgaris L) plants and decreased the content of nutrient elements and photosynthetic pigments as well as carbohydrate concentration in the shoots. Also water stress affect the yield and its quality represented by nutrient elements, protein and carbohydrate concentrations. Foliar-applied chitosan, in particular 200 mg/l, increased plant growth, yield and its quality as well as physiological constituents in plant shoot under stressed or non-stressed conditions as compared to chitosan-untreated plants. It is suggested that chitosan could be a promising material used to reduce the harmful effect of water stress on the growth and yield of *phaseolus vulgaris* plants.

Keywords: Water stress, chitosan, bean, growth, chemical contents.

INTRODUCTION

Plants under water stress can avoid the harmful of drought throw several ways among them stomatal closure, leaf rolling, osmotic adjustments, reductions and consequently decreases in cellular expansion, and alterations of various essential physiological and biochemical processes that can affect growth, productivity and yield quality (Hefny, 2011; Farouk and Amany, 2012). In this respect, Bittelli *et al.* (2001) reported that occasional or episodic drought events can be counteracted through the use of anti-transpirants, compounds applied to foliage to limit the water loss. These compounds are able to increase leaf resistance to water vapor loss, thus improving plant water use and increasing biomass or yield (Tambussi and Bort, 2007).

Chitosan is an anti-transpirant compound that has proved to be effective in many crops (Khan *et al*., 2002; Karimi *et al*., 2012) and wasw used to protect plants against oxidative stress (Guan *et al.,* 2009) and to stimulate plant growth (Farouk *et al.,* 2008, 2011). Chitosan is a natural, low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture.

It was found that foliar applications with chitosan resulted in higher vegetative growth and improvement in fruit quality of pepper, radish and cucumber (Farouk *et al.,* 2008; Ghoname *et al.,* 2010,). Bittelli *et al.* (2001) reported that foliar application of chitosan decreased transpiration in pepper plants, and reduced water use while maintaining biomass production and yield. Recently, Sheikha and AL-Malki (2011) indicated that chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root and leaf area as well as the level of chlorophylls.

Common bean (*Phaseolus vulgaris* L) is one of the most favorable grain legumes valued for its nutritional value, especially its high protein content (20%) (Ogbonnaya *et al.,* 2003). The crop also has ability to maintain soil fertility through its excellent capacity to fix atmospheric nitrogen, and thus does not require very fertile land for growth (Lobato *et al.,* 2006). *Phaseolus* forms an integral part of sustainable agriculture and land use (Ogbonnaya *et al.,* 2003). Improvements in water economy probably will help water-stressed *Phaseolus* plants in maintaining their physiological and biochemical processes. To the best of our knowledge there has been no previous report regarding the effects of foliar application of chitosan on *Phaseolus* plant growth and yield. Therefore, the purpose of this study was to determine the ability of chitosan to alleviate the deleterious effects of water stress on common bean (*Phaseolus vulgaris* L.) plants grown in the Kingdom of Saudi Arabia.

MATERIALS AND METHODS

Experiments

Pot experiments were conducted in the Faculty of Science, Princess Norah Bint Abdulrahman University, Kingdom of Saudi Arabia during the two successive summer seasons of 2011 and 2012. Common bean (*Phaseolus vulgaris* L.) seeds were sterilized with 1.5% chlorox, washed three times with distilled water, and coated with N-fixer (rhizobia). Seeds were then sown in plastic pots (30 cm inner diameter) filled with 10 kg sandy soil. After sowing, irrigation was applied to supply seedlings with 100% available water, at two-day intervals until the seedlings reached the fourth leaf stage. Plants were fertilized with Sangeral complete fertilizer in two equal portions; the first during the seedling stage and the second at the start of flowering. After that the pots were divided into four groups for water-stress treatments, with each group divided into four subgroups for chitosan foliar application. The soil moisture for all pots was kept at 80% field capacity until 15 days after sowing. After that, the water stress treatments were initiated.

Pots were subjected to one of the 3 water-stress treatments: 80% (FC1), 60% (FC2) and 40% (FC3) of field capacity. All pots were weighed every two days. Losses in pot weight represent transpiration and evaporation. Cumulative water losses were added to each pot to compensate for transpiration and evaporation. Accumulated water loss was calculated as the differences in pot weight between successive weightings. At 40, 50 and 60 days from sowing, the plants were sprayed with either tap water or chitosan at 100 (CH1), 200 (CH2) or 400 (CH3) mg/l until dripping, using a small pressure pump after adding Tween 20 (0.5%) as a wetting agent.

Vegetative measurements

Three uniform plants were uprooted from each pot at the full blooming stage (80 days from sowing) to measure morphological and physiological characteristics. The plants were cleaned and plant height, number of branches, number of leaves and leaf area (using a leaf-area meter) were determined. Fresh and dry weights were estimated by drying each plant at 70°C to a constant weight.

Chemical measurements

Chlorophyll a and b were determined in 80% acetone extracts according to Saric *et al*. (1967), total carbohydrate content was estimated using the anthrone method as described by Sadasivam and Manickam (1996). In a mixture of sulfuric and perchloric acid total nitrogen was determined by the micro-Kjeldahl method, Potassium was determined by flame-photometry (Kalra, 1998) and Phosphorous was estimated using ammonium molybdate and ascorbic acid (Cooper, 1977).

Yield and its quality

At harvest time (120 days from sowing) the total yield per plant was recorded. Seed quality was represented by the concentrations of nitrogen, phosphorous, potassium, protein and carbohydrates determined in the dry seeds as previously described in shoots. The protein percentage in dry seeds was measured by multiplying nitrogen content by 6.25.

Statistical analysis

All data were analyzed statistically using one-way ANOVA, followed by Duncan's Multiple Range Test using COSTAT software. The values presented are all mean for three samples in each group.

RESULTS AND DISCUSSION

1- Vegetative measurements

Registered data indicate in general that, severe reductions in plant growth, manifested by vegetative parameters were recorded due to water stress. All plant growth characters including plant height, number of branches, number of leaves, leaf area per plant (Table 1), shoot fresh and dry weights (Figure 1), significantly decreased due to water stress. The largest reduction in growth parameters was observed under severe water stress (FC3). On the other side, foliar application of chitosan, and especially at 100 (CH1) and 200 (CH2) mg l^{-1}, improved all plant growth parameters compared to untreated control plants. Regarding the interaction effects, application of chitosan CH1 and CH2 significantly increased all growth features of *Phaseolus* with an increasing effect with greater stress. However, while application CH1 and CH2 counteracted the harmful effect of water stress on plant growth, application of chitosan at 400 mg l-1 (CH3) significantly decreased all the tested parameters.

The inhibiting effects of water stress on plant growth have previously been reported for soybean (Abdalla, 2011) and white lupins (Hefny, 2011). It is well known that water stress conditions cause a multitude of molecular, biochemical and physiological changes, thereby affecting plant growth and development (Boutraa, 2010). A decline in plant growth in response to water stress might be due either to decreases in cell elongation resulting from the inhibiting effect of water shortage on

Table 1. Effect of water stress and chitosan on plant height, number of branches, number of leaves and leaf area of *Phaseolus vulgaris* plants.

Treatment	Plant ht (cm)	Branch No./plant	Leaves No./plant	Leaves area (cm^2)
FC1	40.5	6.6	18.6	440
FC2	38.6	6	16.5	375
FC3	30.3	3.5	10.6	320
FC1+CH1	42.3	7.3	22.4	625
FC2+CH1	40.2	6.8	20.6	560
FC3+CH1	33.5	4.6	16.6	520
FC1+CH2	45.2	9.5	24.1	755
FC2+CH2	43.3	8.6	22.7	640
FC3+CH2	35.8	6.5	17.5	510
FC1+CH3	35.4	6	17.4	415
FC2+CH3	32.3	5.4	15.2	350
FC3+CH3	28.6	3.2	9.5	300
LSD 5%	4.3	2.2	3.1	98

Each value is the mean of 3 replicates. FC1 = 80%, FC2 = 60%, FC3 = 40% of Field capacity; CH1 = chitosan 100 mg l^{-1}, CH2 = 200 mg l^{-1}, CH3 = 400 mg l^{-1}.

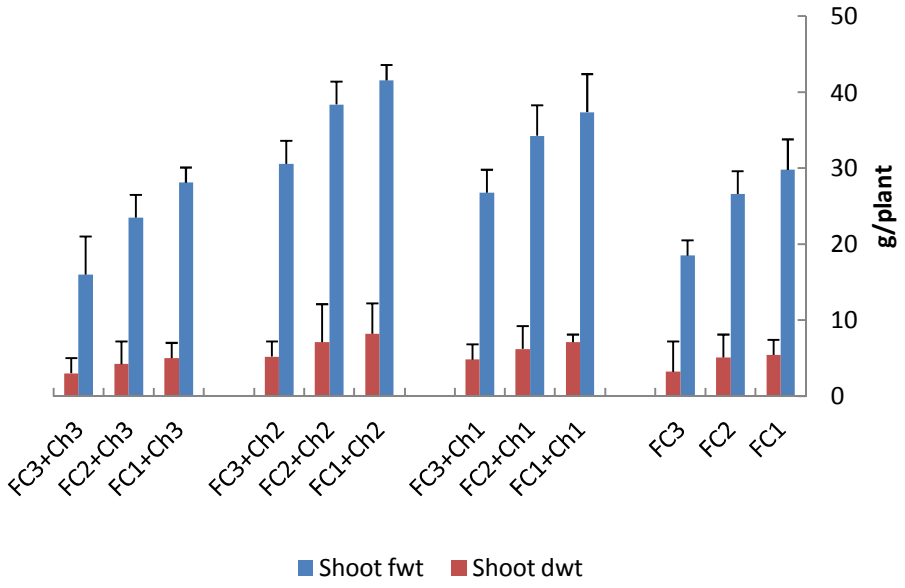

Figure 1. Effect of water stress and chitosan on fresh and dry weights of *phaseolus vulgaris* shoots. Vertical dashs indicate SE values.

growth-promoting hormones which, in turn, lead to decreases in cell turgor, volume and eventually growth (Banon *et al.,* 2006). Water-stress conditions cause a marked suppression in plant photosynthetic efficiency, mainly due to the closing of stomata and inhibition of (Rubisco) enzyme (Lawlor and Cornic, 2002). The depressive effect of water stress on growth parameters may also be attributed to a drop in leaf water content and a reduction in the assimilation of nitrogen compounds (Reddy *et al.,* 2003), affecting the rate of cell division and enlargement. Drought stress also reduced the uptake of essential elements and photosynthetic capacity (Kandil *et al.,* 2001) as well as the excessive accumulation of intermediate compounds such as reactive oxygen species (Yazdanpanah *et al.,* 2011) which cause oxidative damage to DNA, lipid and proteins and conse-

quently a decrease in plant growth. Finally, water stress leads to increases in abscisic acid which cause an inhibition of the growth (Abdalla, 2011).

Foliar spraying of chitosan in most cases resulted in a significant increase in *Phaseolus* plant growth parameters under normal or stressed conditions, more pronounced at the intermediate concentration (200 mg l^{-1}) of chitosan (CH2). This result is similar to that in sweet pepper (Ghoname *et al.,* 2010) and cucumber and radish plants (Farouk *et al.,* 2008, 2011). The stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals (ORS) by increasing antioxidants and enzyme activities (Guan *et al.,* 2009). In addition, the positive effect of chitosan on plant growth may be due to its effect on increasing nutrient uptake in increase elements content such as nitrogen, phosphorous, and potassium, as found in the present study. Phosphorous and potassium is an essential nutrient playing an important role in the biosynthesis and translocation of carbohydrates, and necessary for stimulating cell division, cell turger and forming DNA and RNA (Farouk and Amany, 2012). The mechanisms of chitosan in counteracting the harmful effect of water stress are not well understood and there are a few reports in the literature. Transcriptional activation, induced by chitosan and jasmonate, of genes encoding phenylalanine ammonia lyase and protease inhibitors, suggests that chitosan may influence pathways involving jasmonic acid (Doares *et al.,* 1995) which plays a key role in the regulation of water use by plants. The reported effects of chitosan on stomatal aperture suggest the possibility that it might be a valuable anti-transpirant with useful agricultural applications.

Chemical measurements

It is obvious that chl a, chl b and the total content of chlorophylls (Figure 2), nutrient concentrations as well as total carbohydrates (Table 2) significantly decreased under water stress as compared with the control plants. Foliar application of chitosan, especially CH2, significantly increased both chlorophylls, nutrient elements and carbohydrates within plant tissues, compared with chitosan-untreated plants under water stress. As for chitosan interactions with water stress, it was clear that the effect of chitosan in counteracting the water stress decreased with increasing water stress, therefore the effect of chitosan at FC3 was lower than that of chetosan at FC1 or FC2.

It is a fact that water stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in deficiency of some nutrients. In this respect, water stress decreased significantly the percentages of nitrogen, phosphorus and potassium (Table 2) in plant tissues. The largest reduction occurred under severe water stress (FC3). However, statistical analysis showed highly significant increases in all these nutrients occurred after exogenous application of chitosan, especially at CH2. As with other measures, application of chitosan at 400 mg l^{-1} (CH3) reduced this positive effect. Again, the impact of chitson was greater in stressed than unstressed plants.

The present investigation showed that water stress decreased chlorophylls and total carbohydrates concentrations. The decrease in chlorophyll content under drought is a commonly observed phenomenon (Nikolaeva *et al.,* 2010, Kumar *et al.,* 2011). The decrease in chlorophyll under water stress might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (Allakhverdiev *et al.,* 2003)., or to destruction of the pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids and proteins (Lai *et al.,* 2007). Similarly, the reduction in total carbohydrates induced by water stress treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations (Figure 2) or the activity of Rubisco enzyme leading to decreases in all sugar fractions (Stibrova *et al.,* 1986). The effects of chitosan, especially CH2 treatment, in increasing chlorophylls and total carbohydrate contents were confirmed in cucumber, radish and cowpea (Farouk *et al.,* 2008; 2011; 2012). Results in the present study indicate significant increases in both nitrogen and potassium content in plant shoots (Table 2), which may be play an important role in increasing the number of chloroplasts per cell, cell size and number per unit area, as well as increased synthesis of chlorophyll (Possingham, 1980).

Water stress affects the availability of nutrients in the soil by its effects on the solubility and precipitation of salt, and alters physiological processes within the plant, including nutrient uptake and translocation (Power, 1990). The decrease in N content due to water stress has been reported in various crops including wheat (Singh and Usha, 2003) and soybean (Tanguilig *et al.,* 1987). Phosphorus is one of the most important nutrients in the growth and development of plants. It plays a key role in cellular energy transfer, respiration, photosynthesis. Phosphorus uptake decreases with decreasing soil moisture in various crops such as pepper (Turner, 1985) and wheat (Ashraf *et al.,* 1998). The role of chitosan in increasing ionic content may be due to its effects on stabilizing cellular membranes through increasing antioxidants substances, saving cell membranes from oxidative stress and hence improving plant cell permeability. This observation is supported by the results of Guan *et al.* (2009), where the application of chitosan significantly decreased lipid peroxidation by stimulating antioxidant enzymes, leading to decreased membrane

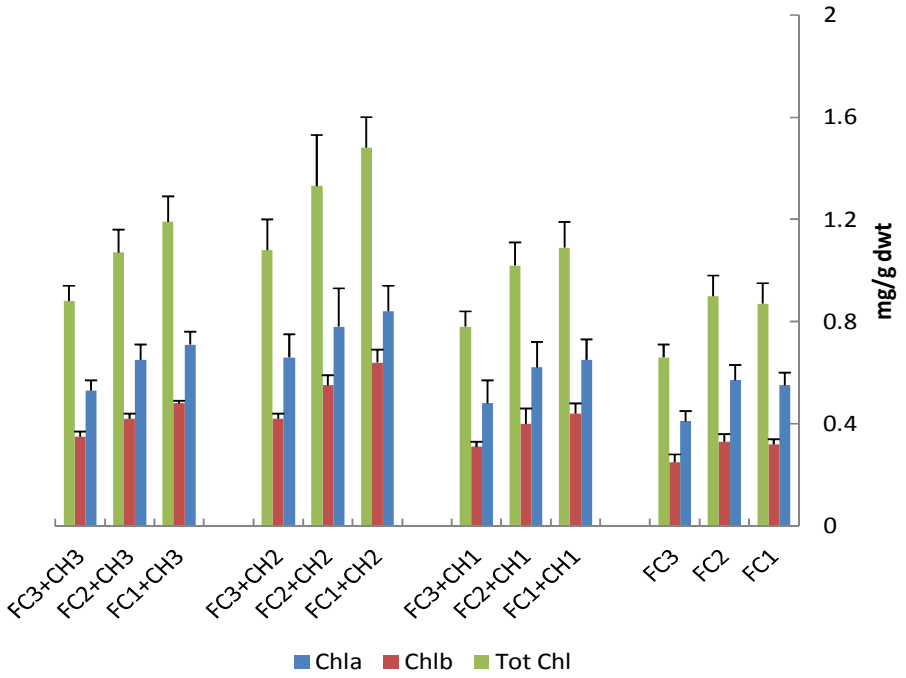

Figure 2. Effect of water stress and chitosan on chl a, chl b and total chlorophyll in *Phaseolus vulgaris* leaves. Vertical dashs indicate SE values.

Table 2. Effect of water stress and chitosan on nutrient concentrations and total carbohydrate of *Phaseolus vulgaris* plants.

Treatment	N%	P%	K%	Total carboh (mg/g dwt)
FC1	3.64	0.64	1.18	29.3
FC2	3.22	0.58	0.88	27.4
FC3	2.56	0.42	0.64	19.9
FC1+CH1	4.72	0.75	1.36	32.3
FC2+CH1	4.33	0.66	1.12	30.2
FC3+CH1	3.64	0.54	0.95	24.5
FC1+CH2	5.11	0.84	1.87	38.4
FC2+CH2	4.86	0.72	1.36	34.5
FC3+CH2	4.14	0.62	1.07	30.2
FC1+CH3	3.43	0.56	1.03	27.2
FC2+CH3	3.15	0.46	0.75	25.4
FC3+CH3	2.12	0.38	0.52	16.8
LSD 5%	1.04	0.17	0.34	9.8

Each value is the mean of 3 replicates. FC1 = 80%, FC2 = 60%, FC3 = 40% of Field capacity; CH1 = chitosan 100 mg l^{-1}, CH2 = 200 mg l^{-1}, CH3 = 400 mg l^{-1}.

permeability and improved function. Other reports have confirmed these results (Farouk *et al.,* 2008, 2011).

Yield and its quality

It is well known that water deficit affects all stages of growth and development, therefore, grain yield was significantly depressed. Pod yield per plant and seed quality significantly decreased with increasing water deficit (Table 3), while foliar application of chitosan, especially the treatment CH2, tended to reverse this negative effect and increased the yield and improved seed quality as assigned by the concentration of nutrient

Table 3. Effect of water stress and chitosan on pods yield/plant and nutrient concentrations in seeds of *Phaseolus vulgaris* plants.

Treatment	Pods/plant	N%	P%	K%
FC1	18.5	14.2	0.58	1.68
FC2	16.5	13.7	0.50	1.60
FC3	14.4	11.6	0.42	1.48
FC1+CH1	20.3	16.4	0.72	1.84
FC2+CH1	18.4	15.2	0.67	1.73
FC3+CH1	16.6	13.3	0.60	1.66
FC1+CH2	24.4	19.3	0.88	1.95
FC2+CH2	20.2	17.5	0.81	1.82
FC3+CH2	18.8	15.2	0.74	1.71
FC1+CH3	17.7	12.8	0.45	1.55
FC2+CH3	15.3	11.5	0.42	1.42
FC3+CH3	12.7	10.2	0.38	1.37
LSD 5%	3.8	3.5	0.19	0.12

Each value is the mean of 3 replicates. FC1 = 80%, FC2 = 60%, FC3 = 40% of Field capacity; CH1 = chitosan 100 mg l^{-1}, CH2 = 200 mg l^{-1}, CH3 = 400 mg l^{-1}.

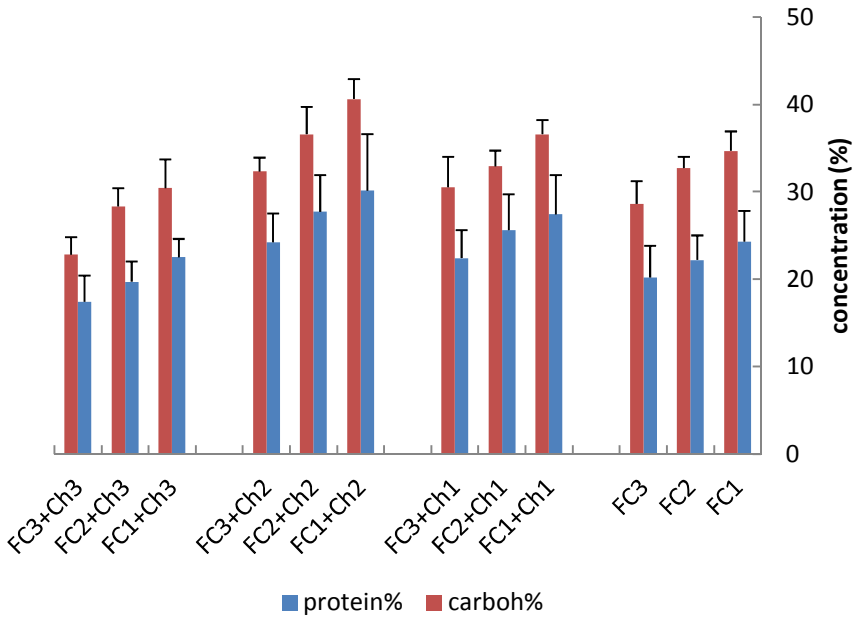

Figure 3. Effect of water stress and chitosan on protein and carbohydrate percentages in seeds of *phaseolus vulgaris*. Vertical dashs indicate SE values.

elements in the seeds (Table 3) or protein and carbohydrate contents of the seeds (Figure 3). The interactions treatment indicated that application of CH2 (200 mg/l^{-1}) under moderate and severe water deficit significantly increased the yield and its quality. Meanwhile the low and high concentration of chitosan under moderate and severe water stress counteract the harmful effect of water deficit in this respect.

Previous studies reported that water stress reduced the yield of many crops (Costa *et al.,* 2008; Vurayai *et al.,* 2011; Hefny, 2011; Farouk and Amany, 2012). In legume plants such as *Phaseolus*, seed yield is determined by several factors, the number of pods is one of these factors. The reduced yield may by due to the negative effect of water stress on the number of branches and leaves per plant as well as leaf area (Table 1), resulting

in a reduction in the supply of carbon assimilate and photosynthetic rate by plants and consequently less biomass produced as well as decreased translocation of assimilates towards the developing fruits (Kumar *et al.,* 1994). In addition, yield may be reduced under drought conditions due to increasing the rate of flower abscission and pod abortion (Liu *et al.,* 2003). A decreased rate of carbohydrate flux from leaves to reproductive structures has been reported to control pod set in well-watered plants (Kokubun *et al.,* 2001, Setter *et al.,* 2001). On the other side, the increase in *Phaseolus* yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photoassimilates from source to sink tissues. The increases in plant biomass may be due to improving photosynthetic machinery (Khan *et al.,* 2002). Ghoname *et al.* (2010) also observed that foliar application of chitosan on sweet pepper increased significantly the number of fruits per plant and the mean weight of fruit, as well as quality characteristics of the fruit. The role of chitosan in alleviating the harmful effect of water stress on yield may be due to an increase in stomatal conductance and net photosynthetic CO2-fixation activity under water stress (Khan *et al.,* 2002), and to its role in reducing transpiration to save water.

CONCLUSION

It can be concluded that chitosan may play an important role in the growth and productivity of *Phaseolus vulgaris* plants grown under water stress conditions, perhaps because they can produce various metabolites which cause a reduction in transpiration and thus more water become available to plants for better growth and production.

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