EFFECTS OF SEAWEED CONCENTRATE (Kelpak) ON NITROGEN FIXATION OF COWPEA (*Vigna ungulata* L. Walp.) AND SOYBEAN (*Glycine max* L. Merr.) AND ON THE GROWTH OF THEIR RHIZOBIAL SYMBIONTS (*Bradyrhizobium* STRAIN CB756 AND *Bradyrhizobium japonicum* STRAIN CB1809).

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ABSTRACT

Seaweed extracts are known to have a stimulatory effect on the growth and development of plants. This study investigated the effect of applying a commercial seaweed concentrate (kelpak) on rhizobia growth (*Bradyrhizobium* strain CB756 and *Bradyrhizobium japonicum* strain CB1809) and nitrogen fixation in cowpea (*Vigna ungulata* L. Walp.) and soybean (*Glycine max* L. Merr.) plants. Two concentrations of Kelpak (1:100 v/v and 1:500 v/v seaweed concentrate dilutions) were applied to pots with seeds or seedlings at sowing and after every 14 days (1:100A; 1:500A), at sowing and after every 7 days (1:100B; 1:500B) or after germination and after every 14 days (1:100C; 1:500C). From the first experiment, cowpea plants in the various treatments showed no change in shoot biomass. The root biomass was significantly inhibited in treatment 1:100B relative to the control. The nodule dry matter of cowpea was reduced in 1:100A, 1:100B and 1:100C Kelpak concentrations compared to control, with a significant increase only in 1:100B Kelpak concentration. As a result, cowpea plants showed the highest total biomass in 1:500B treatment. Although shoot N in cowpea plants remained unchanged under the various kelpak treatments, root N was significantly reduced. Soybean plants showed a significant decrease in shoot and root biomass compared to the control. Nodule dry matter was lowest for soybean plants in Kelpak treatments 1:500B, 1:100B and 1:100C. As a result, there was a decrease in soybean total growth in treatment 1:500B compared to the control. Total N in shoots and roots was highest in soybean plants growing in 1:500A relative to the control. Culturing cells of *Bradyrhizobium* strain CB756 with Kelpak showed a significant increase in growth at 1:100 and 1:500 dilutions compared to the control. However, over the 93 h period with sterile Kelpak culture there was an inhibition in growth of strain CB756 relative to the control. Beyond the 93 h there was a significant increase in growth of *Bradyrhizobium japonicum* strain CB1809 in all Kelpak treatments. The 1:100 concentration showed the highest bacterial growth compared to the control and the other treatments. These data suggests the presence of an active molecule in Kelpak that stimulates rhizobial growth and its symbiotic interaction with legumes.

CONTENTS

INTRODUCTION

The use of marine algae as manures and fertilizers for crops dates back to the Ancient Greeks. As early as the twelfth century, algae from the phylum Phaeophyta (brown seaweeds) were used as manure on the coastal lands of Europe (Crouch, 1990). Farmers in coastal regions mainly used seaweeds as fertilizers as the high cost involved in collection, drying and transportation precluded their use in inland areas. In the seventeenth century, the first seaweed industry was established. The wide diversity of commercial seaweed products available today is due not only to the use of different seaweed species, but also to the methods of preparation, which vary according to the manufacturer. The basic function of agriculture is provision of food and other organic materials to support and sustain certain life-styles (Crouch, 1990). Discontent with chemically based agriculture has led to increased interest in safer products such as seaweed extracts to help raise levels of grain yield, both in terms of quality and quantity, thus reducing the negative effects of artificial chemicals on the environment.

Exploitation of seaweed has been met with variable success owing to a number of factors: (1) the rising cost of collection and transportation of raw material, (2) the use of mineral fertilizers, and (3) the lack of published scientific information on the value of seaweeds. Results of some controlled laboratory and field experiments indicate that some seaweeds are beneficial to crop plants (Blunden 1991), while others are not (Temple and Bomke, 1988).

Marine algae contain all major and minor plant nutrients and trace elements (Booth, 1964). They are rich in carbohydrates, which act as chelating agents. Algenic acid, laminarin and mannitol present in commercial seaweed preparations represent nearly half of the total carbohydrate content. Seaweeds also contain a wide range of vitamins as well as over seventeen common amino acids (Crouch, 1990). So the possibility of their involvement in the observed growth responses cannot be ignored. Initially it was thought that certain constituents in seaweeds improve soil structure, making conditions more suitable for root growth (Milton, 1964). A better root system would enhance water and mineral uptake by the plants, resulting in improved growth. Additionally, the presence of endogenous trace elements in seaweeds was also thought to contribute to some of the beneficial effects of seaweed preparations (Francki, 1960).

Francki (1960) noted that leaves of tomato plants treated with seaweed extract contained more manganese than was present in the seaweed itself, hence suggesting that the seaweed had released previously unavailable manganese from the soil. By adding the seaweed extract to the mineral deficient solutions used on green peppers, Lynn (1972) showed improved utilization of boron, copper, iron, manganese and zinc. Crouch *et al.* (1990) have shown that the application of seaweed concentrate on lettuce plants receiving an adequate supply of nutrients enhanced the uptake of calcium and potassium but had little effect on nutrient stressed plants. It has however been argued that if the concentration at which these products are applied is taken into consideration then the level of mineral elements in the seaweed mixture would be too low to have any measurable effect on plant growth (Blunden, 1972). In view of the low rates of application necessary to elicit any physiological response it was suggested that organic compounds rather than mineral elements are responsible for yield increases (Crouch, 1990).

Recent research has shown that seaweed products contain certain plant growth regulators (Both, 1966; Blunden and Wildgoose, 1977; Featonby-Smith and van Staden 1983) and many of the observed effects are presently ascribed to these constituents. There has been a lot of speculation about the amounts and types of growth regulatory substances, especially plant growth hormones, which exist in seaweeds. Much of this speculation arose from the results of bioassays, performed to determine the presence of plant hormone-like substances. In recent years it has been postulated that the presence of plant growth regulators in commercial seaweed products plays a significant role in the expression of beneficial effects. Auxins or auxin-like compounds occur endogenously in many marine algae, but their activity in commercial seaweed products is regarded as low (Williams et al. 1976). Indole acetic acid (IAA) and the presence of gibberllin-like substances in seaweeds has been reported and documented (Wildgoose et al. 1978).

The involvement of plant hormones, and in particular cytokinins, was suggested by Booth (1966). This conclusion was reached as many of the responses obtained from seaweed applications were found to be similar to those from the application of cytokinins to plants (Featonby-Smith and van Staden, 1984; Crouch, 1990). Further evidence supporting this hypothesis came from the detection of cytokinins-like activity in a number of marine algae and later in commercial seaweed preparations (Mooney and van Staden, 1987).

Although cytokinins have been identified in seaweed products, it seems unlikely that they are the only beneficial growth substances involved, particularly in view of the wide range of physiological processes affected by seaweed application. This has led to an emphasis on research directed at identifying and isolating other plant regulators in seaweed extracts.

In the symbiosis between legumes and gram-negative soil bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*, root nodules are formed in which the bacteria actively fix atmospheric nitrogen (Dakora, 1994). This interaction involves physiological and biochemical processes of great significance to both agriculture and natural ecosystems. According to Dakora (1994), supplying supplemental amounts of nod gene inducers to legumes can enhance nodulation and nitrogen fixation. Plants and microbes can control elements of each other's growth and development. Leguminous plants secrete phenolic compounds (such as flavonoids) that induce nodulation (*nod*) genes in bacteria required in the formation of N_2-fixing root nodules. They are lipo-oligosaccharides (LCOs), which in legume-bacteria nitrogen-fixing symbiosis they are referred to as nod factor. Smith et al. (*Unpubl.*) have shown that if plant-to bacteria signal compounds are added to bacteria used as inocula on soybean plants, this accelerated the very early stages of nodulation, leading to earlier subsequent nodule development and the onset of nitrogen fixation. As a result, plants inoculated with bacteria that have been treated with plant-to-bacteria compounds become larger and with more nitrogen. Hence there is a potential control of plant growth by signal molecules of microbes. Hartwing *et al.* (1991) showed that flavonoids naturally released from Alfalfa seeds enhanced the growth rate of *Rhizobium meliloti*, hence they suggested that plants control growth of soil microbes with ecochemical zones created by releasing structurally different flavonoids from seeds and roots. Hence agronomists are able to increase legume yield by manipulating host-plant concentrations of these nod gene inducers through plant breeding, or by directly applying the inducer compounds to field molecules or even by inoculating with rhizobial strains that produce adequate quantity and quality of Nod factors.

The main objective of this study was to examine the effect of applying two seaweed concentrations of seaweed concentrate (Kelpak) on cowpea (*Vigna unguculata* [L.] Walp) and soybean (*Glycine max* [L.] Merr. cv Prima) on growth and their symbiotic performance. The extract was also tested for its effects on the growth of rhizobial cells.

MATERIALS AND METHODS

The two experiments included in this study were carried out at the Botany Department at the University of Cape Town, South Africa. The experiments involve the application of Kelpak concentrate on cowpea (*Vigna unguculata* [L.] Walp) and soybean (*Glycine max* [L.] Merr. cv Prima) growth (experiment 1) and *Bradyrhizobium* strain CB756 and *Bradyrhizobium japonicum* strain CB1809 growth (experiment 2).

Seaweed Concentrate (Kelpak)

Kelpak is a commercially available seaweed concentrate that was used in this study. Kelpak is manufactured by Kelp Products (Pty) Ltd., Simons Town, South Africa. The seaweed concentrate is manufactured from the stipes of the brown algae *Ecklonia maxima* (Osbeck) Papenfuss, using a cell burst process. This process involves the use of pressure on fresh material to compress the cells in the absence of air or water followed by a sudden release resulting in the rupture of the cell walls and release of the contents. Thus the seaweed is progressively reduced in particle size, and the particles passed under extremely high pressure into a low-pressure chamber where they disintegrate, resulting in the liquid extract. This process excludes the use of heat, chemicals or dehydration that could affect some of the organic components of the concentrate (Verkleij, 1992). In this study 1:100 v/v and 1:500 v/v seaweed concentrate dilutions (or concentrations) were used.

EXPERIMENT 1

Plant Culture and Treatments

The experiment was conducted in a glasshouse, were plants were grown conditions for 58 days between 9th April and 5th June 2001. Seeds of cowpea (*Vigna unguculata* [L.] Walp) and soybean (*Glycine max* [L.] Merr. cv Prima) were planted in per 30-cm diameter plastic pot containing sand at a depth of 5 cm and inoculated with *Bradyrhizobium* strain CB756 and *Bradyrhizobium japonicum* strain CB1809 respectively. The pots were watered twice a week with 100 mL N-free modified Hoagland nutrient solution (Appendix 1), which was adjusted to contain 1:100 or 1:500 concentration of Kelpak to water. The experiment consisted of seven treatments each with 4 replicates. Plants were treated with seaweed concentrate as indicated in Table 1. Three weeks after emergence the seedlings, plants were thinned out to three per pot.

Table 1. Outline of treatments applied to Cowpea and Soybean plants under glasshouse conditions.

Treatments	**Seaweed Concentrate Application**	
	Time of application	Number of applications
Control	None	0
1:500 A	Applied at sowing and after every 14 days	5
1:100 A	Applied at sowing and after every 14 days	5
1:500 B	Applied at sowing and after every 7 days	8
1:100 B	Applied at sowing and after every 7 days	8
1:500 C	Applied at germination and after every 14 days	4
1:100 C	Applied at germination and after every 14 days	4

Harvesting

Cowpea and soybean plants were harvested 58 days after planting. The numbers of nodules per plant were counted, and each plant separated into leaves, stems, roots and nodules.. These components were oven-dried at 80°C for 48 hours, weighed and finely ground for N analysis.

Nitrogen Analysis

Samples of cowpea and soybean shoots, roots and nodules from the various treatments were sent to the Soil Analysis Unit of the Department of Economic Affairs, Agriculture and Tourism in Elsenberg, Western Cape, Republic of South Africa for nitrogen analysis. The amount of N fixed per plant was calculated as the difference between plant total N and seed N.

Statistical Analysis

All data were subjected to analysis of variance, ANOVA, using the single factorial analysis package in STATISTICA. The least significance difference (LSD) test or planned comparison test was conducted at 95% level to distinguish significantly different results following the ANOVA test.

EXPERIMENT 2

Bacteria and Culture conditions

Eight liters of yeast-mannitol broth were prepared in two 4 L flasks (according to appendix 1) and the pH was adjusted to 6.8. The broth was then sterilized by autoclaving at 121°C for 60 minutes. *Bradyrhizobium* strain CB756 and *Bradyrhizobium japonicum* strain CB1809 were removed from two different glass vials using a sterile iron loop and 50 mL of glass-sterilized water. After cooling of the broth, *Bradyrhizobium* strain CB756 was added to the one flask and *Bradyrhizobium japonicum* strain CB1809 was added to the other flask. About 1000 mL from each 4 L flask were transferred into four sterilized 1 L flasks. Different concentrations of Kelpak were added to each of the four 1 L flasks. The treatments were as follows:

(1) **Control:** where no seaweed concentrate added;

(2) **1:100 K:** 10 ml of unsterilized seaweed concentrate per 1 L flasks;

(3) **1:500 K:** 2 ml of unsterilized seaweed concentrate per 1 L flask and

(4) **Sterile K:** 2 ml of sterilized seaweed concentrate per 1 L flask. (The seaweed concentrate was sterilized by autoclaving at 121°C for 60 minutes).

The 1 L volume of broth was then further divided aseptically into 4 replicate treatments each containing 250 ml. The bottles were shaken for 93 hours at 25°C. The optical density (OD at 600 nm) of each culture was measured every 9 hours through out the length of the experiment.

Statistical Analysis

Data was analyzed statistically using t-test for differences between two independent means, using STATISTICA package.

RESULTS

EXPERIMENT 1

Plant growth data for cowpea are shown in Figure 1. Relative to the control, shoot biomass did not change with the supply of different concentrations of seaweed concentrate to cowpea plants (Figure 1A). Applying 1:100 concentration at sowing and every 7 days (1:100B), significantly ($P<0.05$) decreased root growth in cowpea plants compared to the control and the other treatments (Figure 1B). Whether supplied at sowing and every 14 days (1:100A), at sowing and every 7 days (1:100B) or after germination and every 14 days (1:100C), the 1:100 concentrations significantly ($P<0.05$) reduced nodule dry mass in cowpea (Figure 1C) compared to the control and other treatments. However, nodule dry mass in cowpea increased significantly ($P<0.05$) under 1:500B treatment but not 1:500A or 1:500C (Figure 1C). Total biomass of cowpea was significantly increased ($P<0.05$) in treatment 1:500B compared to the control (Figure 1D). Although there was an increase in the total biomass of cowpea plants in treatments 1:500C, 1:100C and 1:500A, it was not significant ($P<0.05$) compared to the control (Figure 1D). There was reduced growth of cowpea in treatments 1:100A and 1:100B, although not statistically different ($P<0.05$) compared to the control. Only plants receiving treatment 1:500B showed a significant ($P<0.05$) increase compared to the control (Figure 1D).

Relative to the control, the nitrogen content of the shoots was not altered by the different Kelpak treatments (Figure 2A). However, treatment 1:100C significantly reduced the amount of nitrogen in roots of cowpea plants compared to the control and other treatments (Figure 2B). Even then, the total amount of N fixed per plant of cowpea was not significantly altered by different Kelpak treatments (Figure 2C).

The average length of cowpea plants under various Kelpak treatments is shown in Figure 3. Statistically there were no significant differences ($P<0.05$) in the shoot height, between all treatments and the control (Figure 3A). However, shoot height of cowpea plants was significantly ($P<0.05$) reduced in 1:100B compared to 1:500A, 1:100A and 1:500B treatments. Cowpea plants also showed significant ($P<0.05$) decrease in root length of plants treated with 1:500B and 1:100B, compared to the control and treatments 1:500A, 1:100A and 1:500C (Figure 3B). Cowpea growth, measured as combined shoot height and root length, was significantly ($P<0.05$) lower in 1:500B, 1:100B and 1:100C treatments compared to the control (Figure 3C).

The effects of different Kelpak treatments on the growth of soybean plants are shown in Figure 4. There was a significant ($P<0.05$) inhibition of shoot growth in soybean plants under treatment 1:500B relative to the control (Figure 4A). The same pattern was observed for root dry mass of soybean plants (Figure 4B). The nodule dry matter of soybean was highly reduced in plants grown with 1:500B, 1:100B and 1:100C treatments relative to the control (Figure 4C). However, only the 1:500B treatment significantly ($P<0.05$) decreased total dry matter of soybean plants relative to control (Figure 4D).

The amount of N in soybean plants receiving the various Kelpak treatments is represented in Figure 5. Soybean plants in treatment 1:500A had significantly more N in the shoot than the control and treatments 1:500B and 1:100C (Figure 5A). Similarly, treatment 1:500A significantly ($P<0.05$) increased the N in the roots of soybean plants compared to control and all other treatments except 1:100A and 1:500C (Figure 5B). As a result, the amount of fixed N in plants receiving 1:500A treatment was markedly higher relative to the control (Figure 5C).

The effect of various treatments on the height or length (cm/plant) of soybean plants is shown in Figure 6. Shoot height did not change with the supply of different concentrations of Kelpak to soybean plants relative to the control (Figure 6A) except in 1:100B where it was significantly lower than others. The root length of soybean in treatments 1:100A, 1:500B, 1:100B and 1:100C were significantly ($P<0.05$) shorter compared to the control (Figure 6B). The total length of soybean plants (shoot height and root length) in 1:100A, 1:500B and 1:100B were significantly ($P<0.05$) reduced relative to control and 1:500A (Figure 6C).

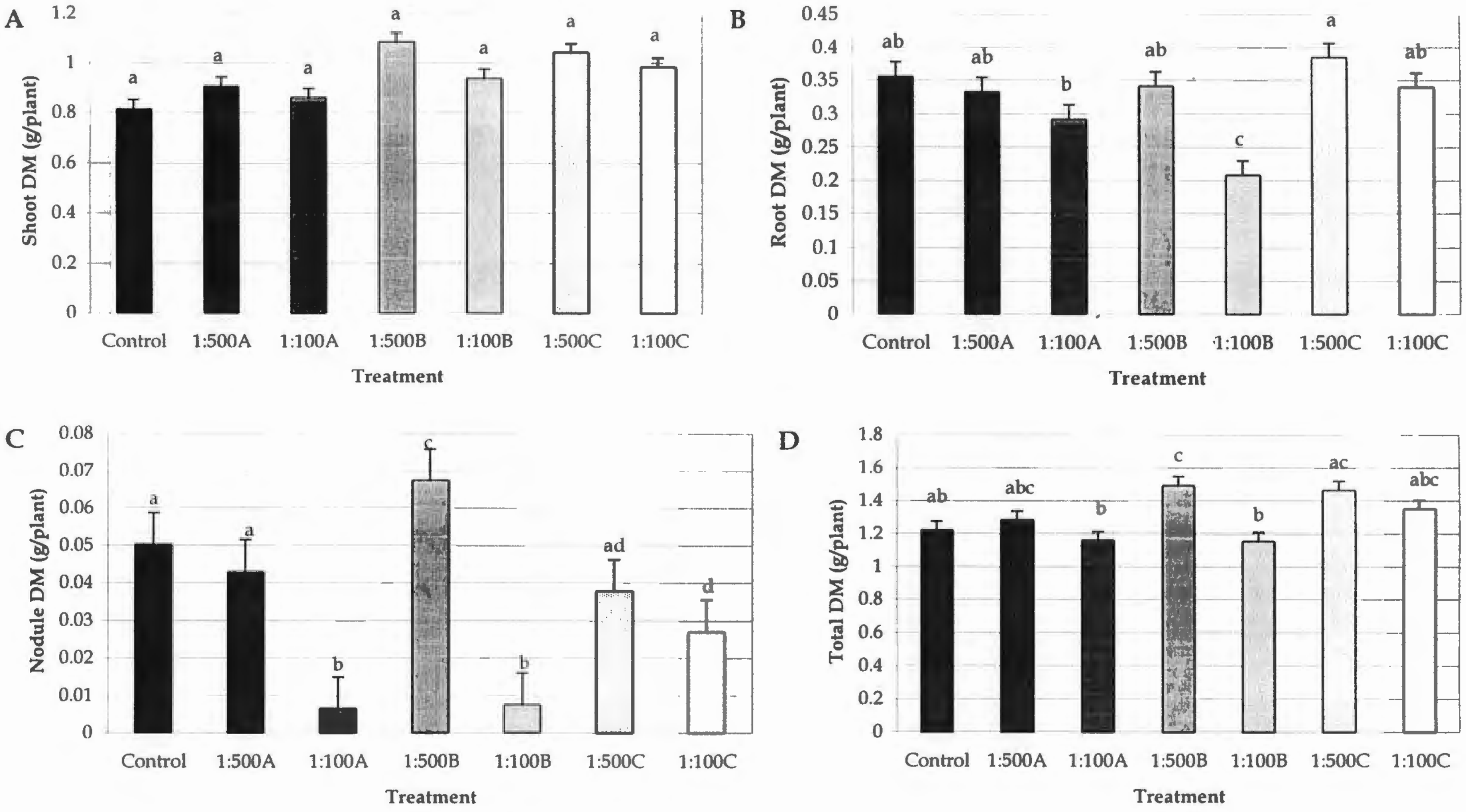

Figure 1. Effect of various treatments on cowpea plants dry matter **A.** Shoot dry matter **B.** Root dry matter, **C.** Nodule dry matter and **D.** Total dry matter. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

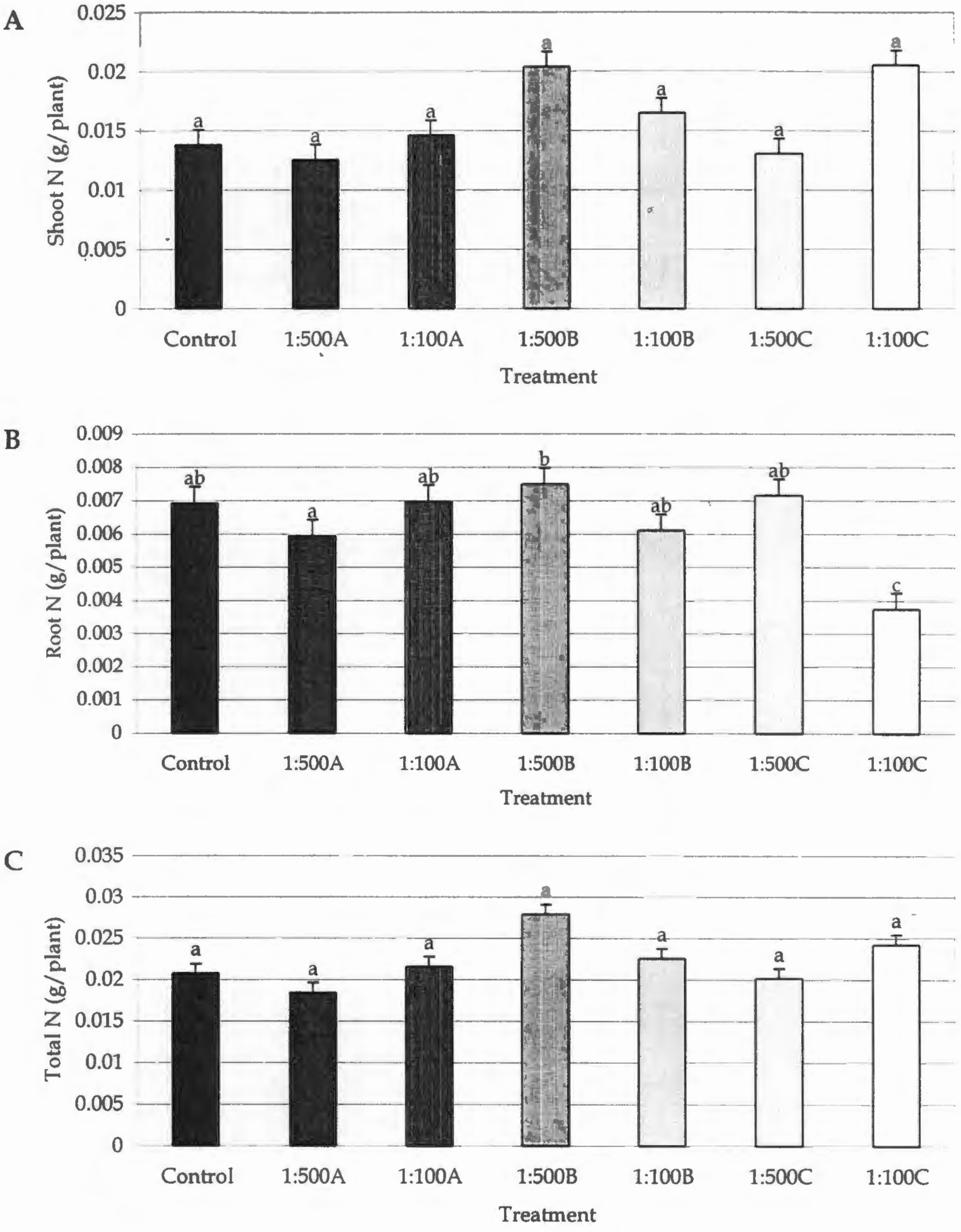

Figure 2. Effect of various treatments on amount of nitrogen fixed in cowpea **A.** Shoot Nitrogen, **B.** Root Nitrogen and **C.** Total Nitrogen. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

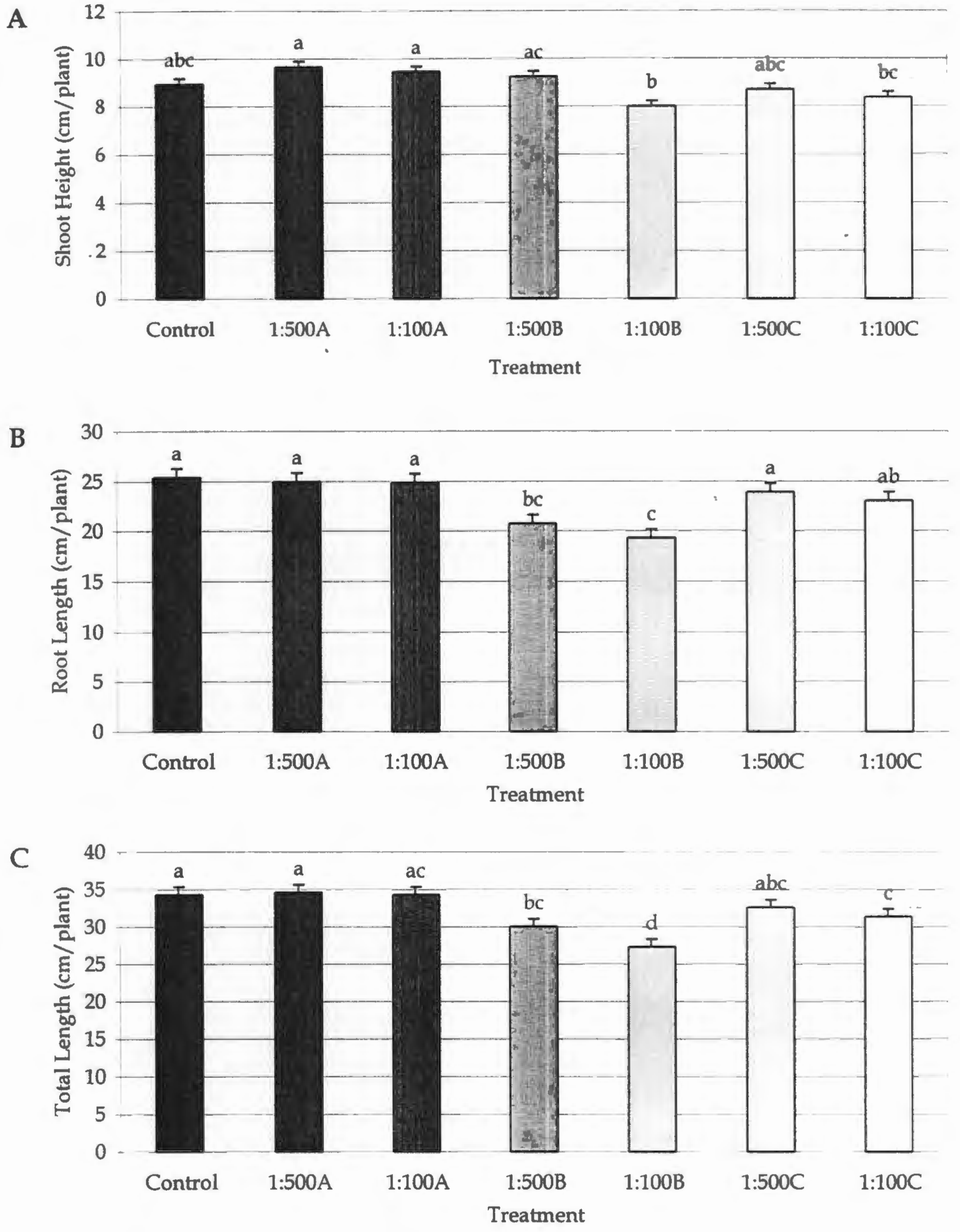

Figure 3. Effect of various treatments on cowpea **A.** Shoot height, **B.** Root length and **C.** Total length. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

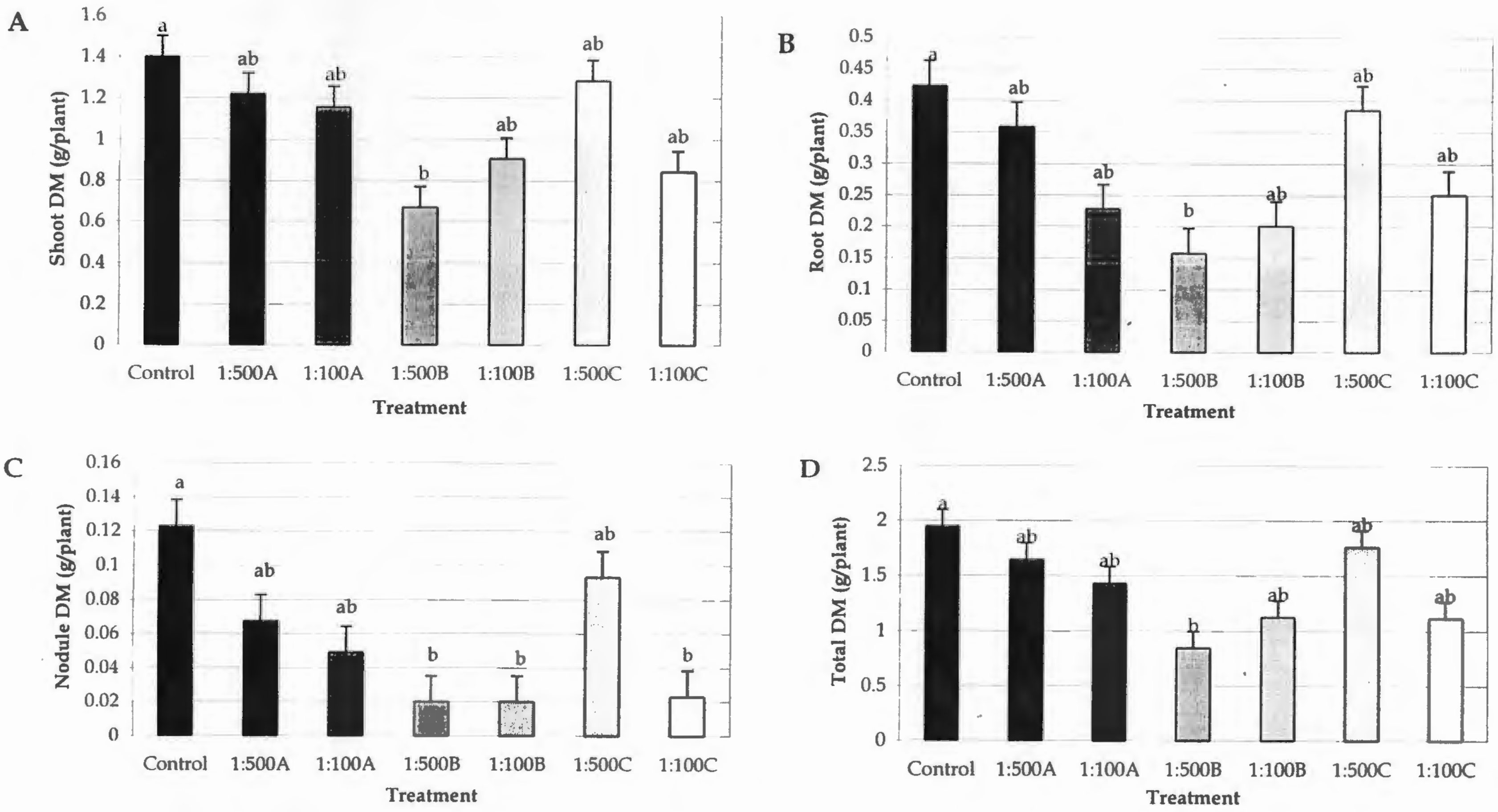

Figure 4. Effect of various treatments on soybean plants dry matter **A.** Shoot dry matter **B.** Root dry matter, **C.** Nodule dry matter and **D.** Total dry matter. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, $p = 0.05$.

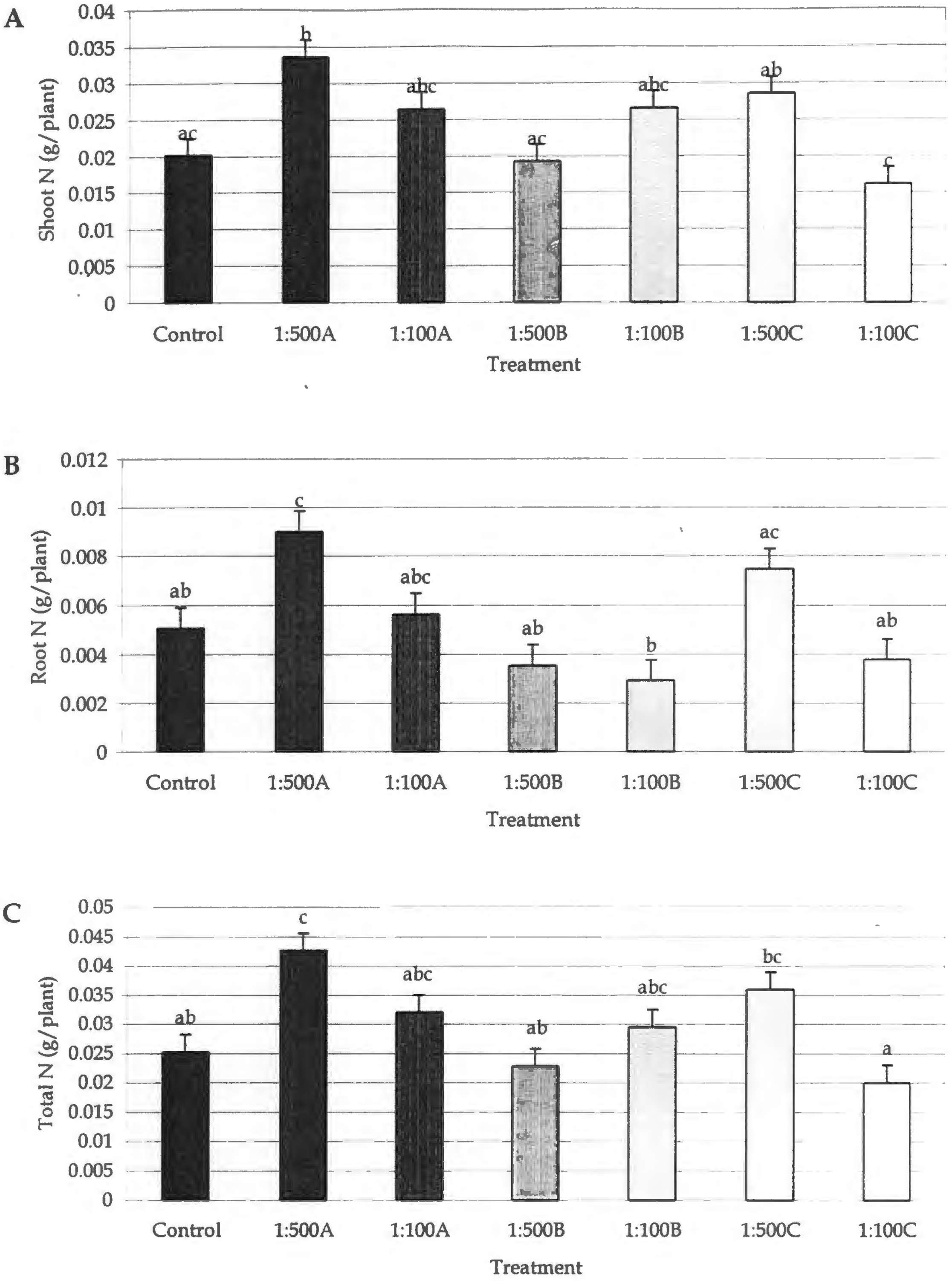

Figure 5. Effect of various treatments on amount of nitrogen fixed in soybean **A.** Shoot Nitrogen, **B.** Root Nitrogen and **C.** Total Nitrogen. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, $p = 0.05$.

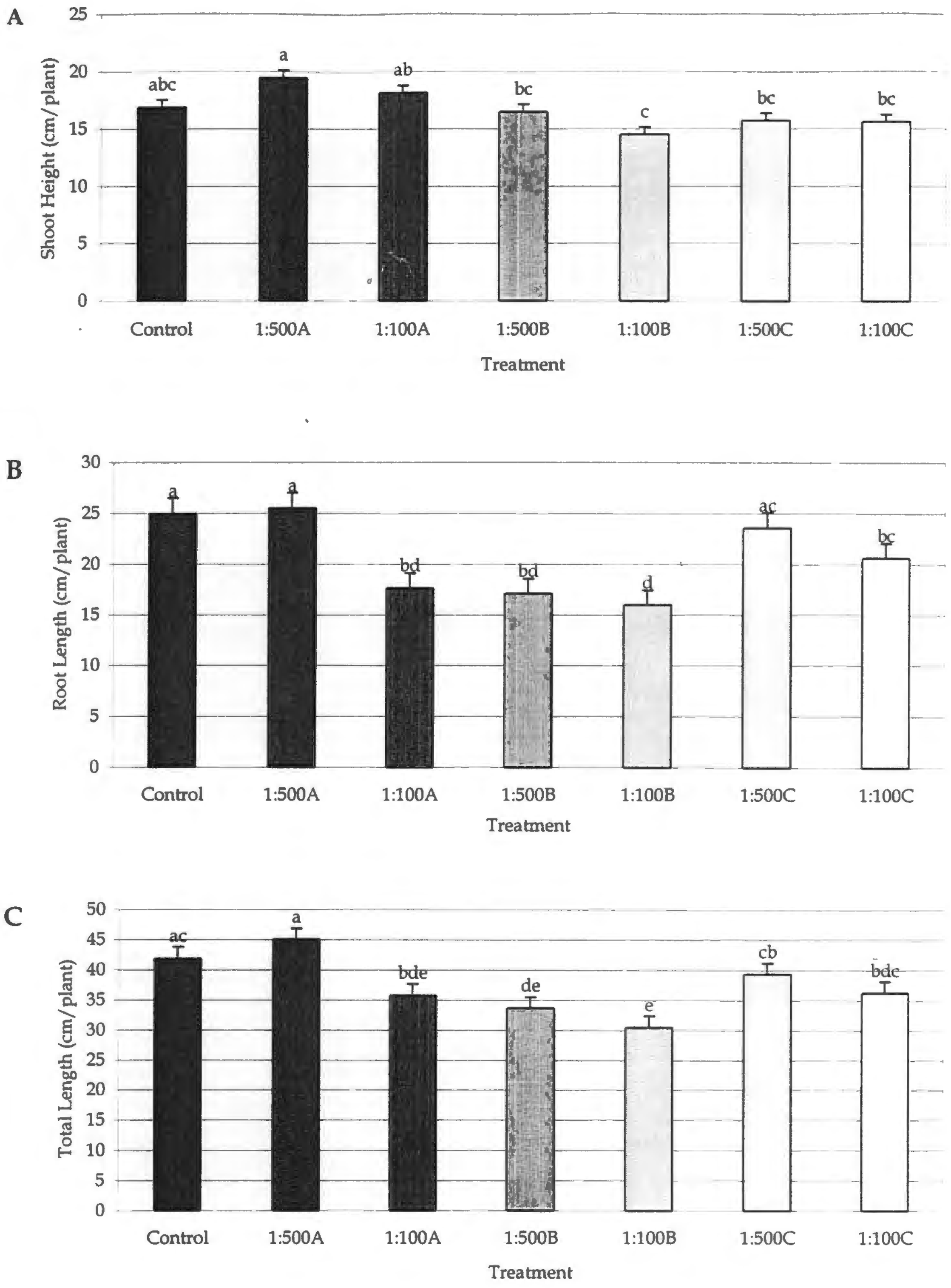

Figure 6. Effect of various treatments on soybean **A.** Shoot height, **B.** Root length and **C.** Total length. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, $p = 0.05$.

EXPERIMENT 2

Cell growth of *Bradyrhizobium* strain CB756, which nodulates cowpea, over a 93-hour period in different Kelpak concentrations is presented in Figure 7 and Figure 8. Figure 7, shows a gradual statistically unanalyzed increase in bacterial cell growth in the applied treatments with time. Compared to control, 1:100 Kelpak concentrate dilution promoted the highest bacterial cell growth, followed by 1:500 dilution. After 21 hours, there was a decrease in bacterial cell growth in the sterile 1:500 Kelpak concentrate compared to the control.

Figure 8 shows statistically analyzed data for *Bradyrhizobium* strain CB756 cell growth at each point in the time course. After 9 hours, there was a significant ($P<0.05$) increase in the bacterial cell growth in 1:100 and 1:500 Kelpak concentrations compared to the control and the sterile 1:500 Kelpak dilution (Figure 8). There was also a significant ($P<0.05$) increase in the bacterial growth in the sterile 1:500 seaweed concentrate dilution compared to the control. From 21 up to 93 the 1:100 Kelpak level markedly promoted rhizobial growth, compared to the control and the other treatments (Figure 8). The second highest growth ($P<0.05$) was observed in the 1:500 Kelpak dilution. From 45 to 93 hours, sterile 1:500 Kelpak dilution inhibited bacterial cell growth relative.

Bradyrhizobium japonicum strain CB1809, which nodulates soybean, was similarly studied for its growth response to Kelpak. The data in Figure 9 represents statistically unanalyzed growth patterns, which clearly show the superior performance of the 1:100 Kelpak level relative to control or the other treatments.

The statistically analyzed data for bacteria cell growth of *Bradyrhizobium japonicum* strain CB1809 are shown in Figure 10 for the entire time course of study. At 9 and 21 hours, bacteria cell growth was significantly inhibited by all Kelpak treatments compared to the control, and from 33 to 57 hours the Kelpak treatments increased bacteria cell growth to same level as the control. However, from 69 to 93 hours, the 1:100 Kelpak level significantly promoted bacterial growth compared to the control, followed by the other Kelpak treatments (Figure 10).

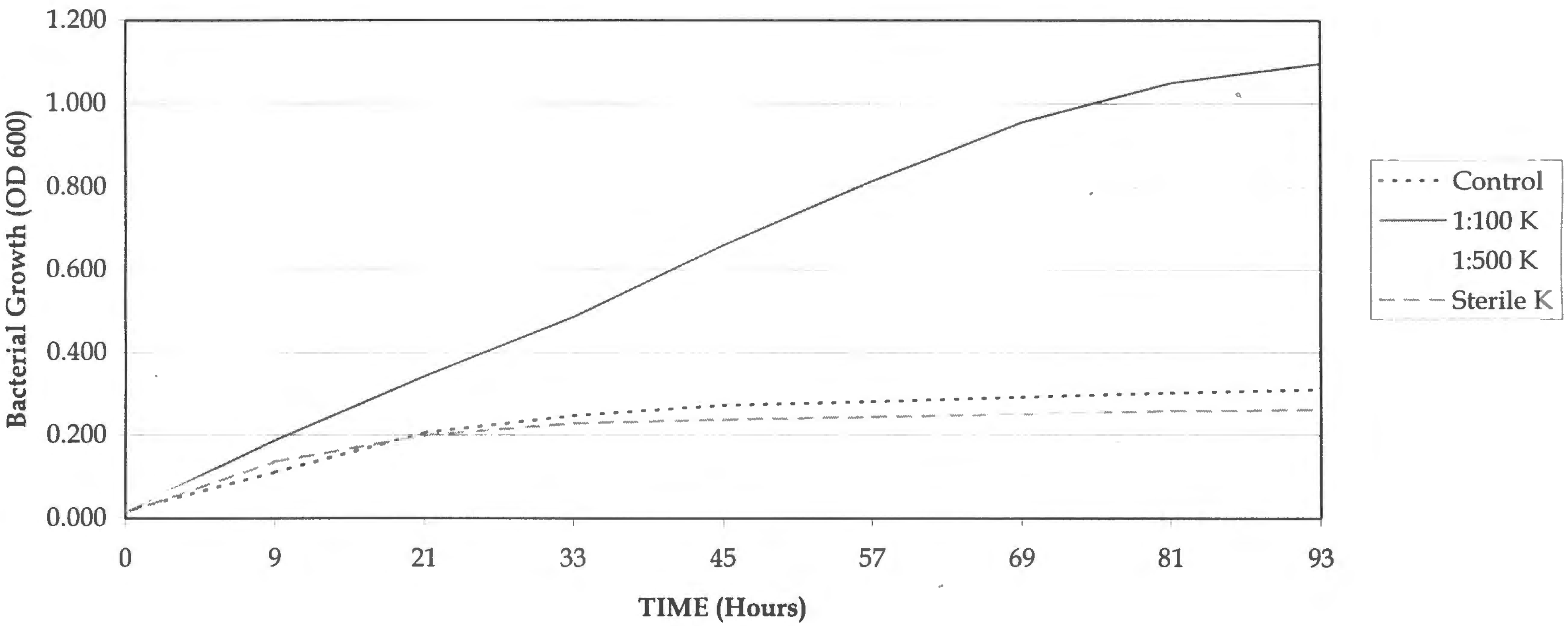

Figure 7. *Bradyrhizobium* strain CB756 bacterial growth under different treatments. Treatments are 1:100 K = 1:100 seaweed concentrate dilution; 1:500 K = 1:500 seaweed concentrate dilution; Sterile K = 1:500 sterile seaweed concentrate dilution.

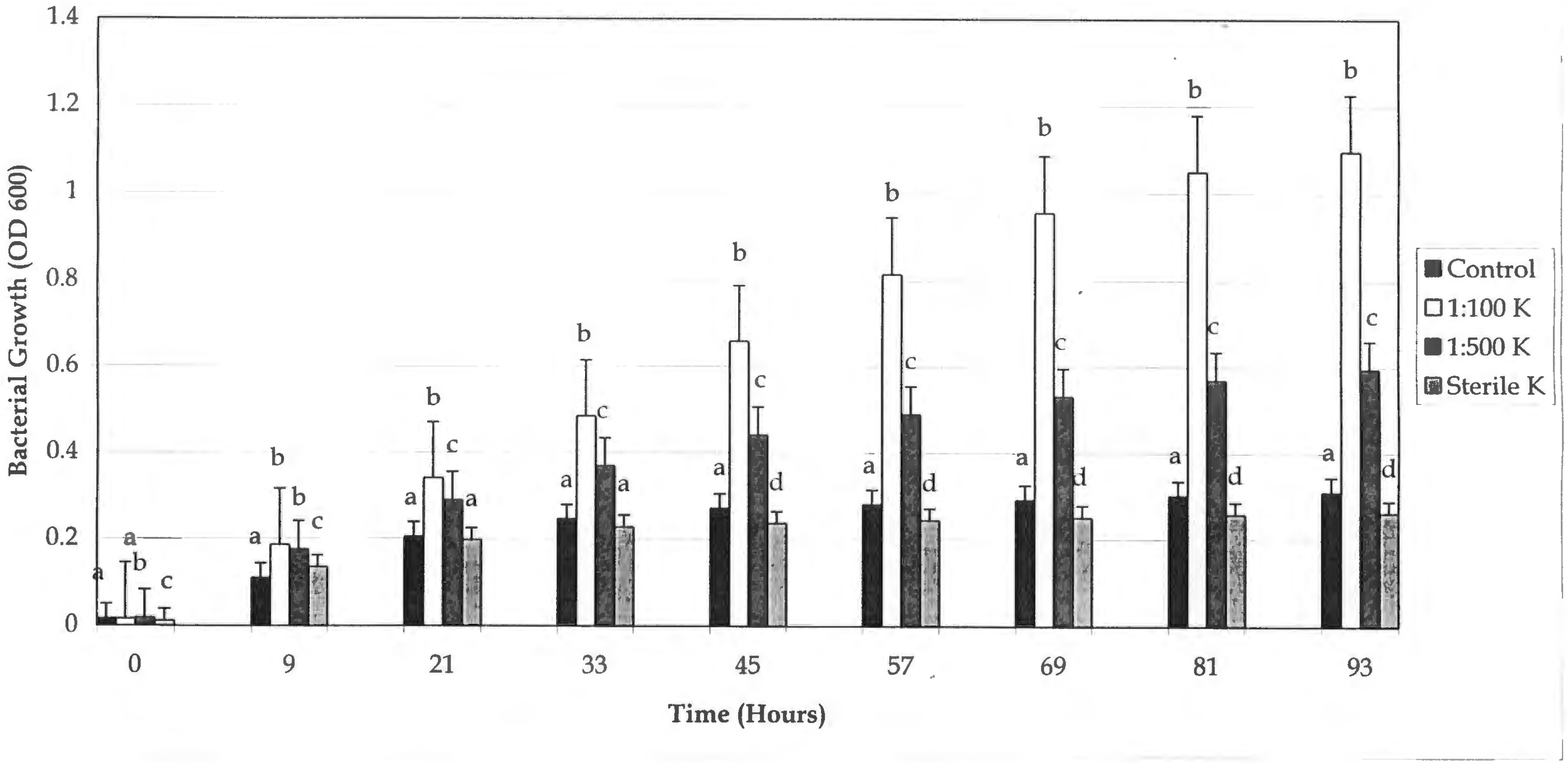

Figure 8. *Bradyrhizobium* strain CB756 bacterial growth under different treatments. Different letters indicate significant differences at 5 percent level (t-test for independent means). Vertical lines represent ± one Standard Error, p = 0.05. Treatments are 1:100 K = 1:100 seaweed concentrate dilution; 1:500 K = 1:500 seaweed concentrate dilution; Sterile K = 1:500 sterile seaweed concentrate dilution.

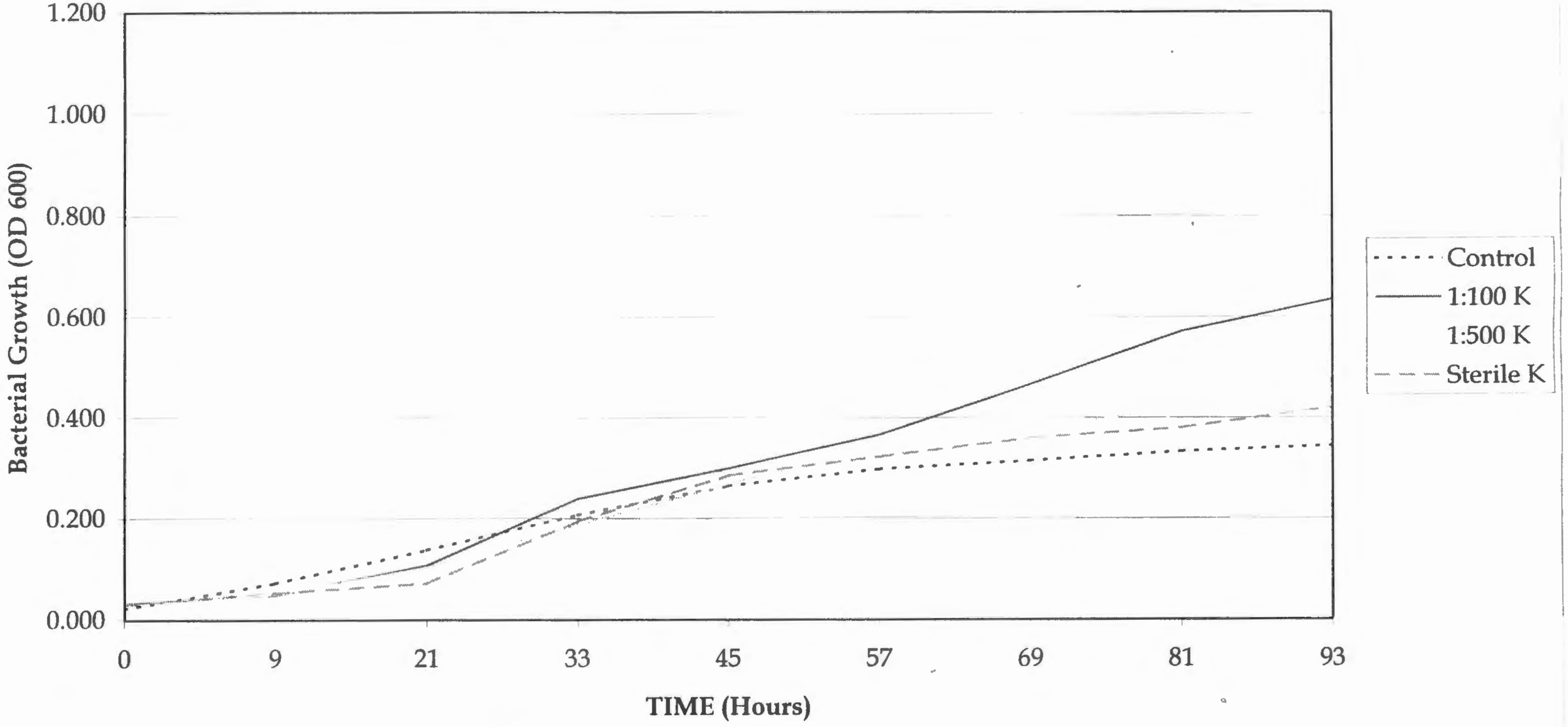

Figure 9. *Bradyrhizobium japonicum* strain CB1809 bacterial growth under different treatments. Treatments are 1:100 K = 1:100 seaweed concentrate dilution; 1:500 K = 1:500 seaweed concentrate dilution; Sterile K = 1:500 sterile seaweed concentrate dilution.

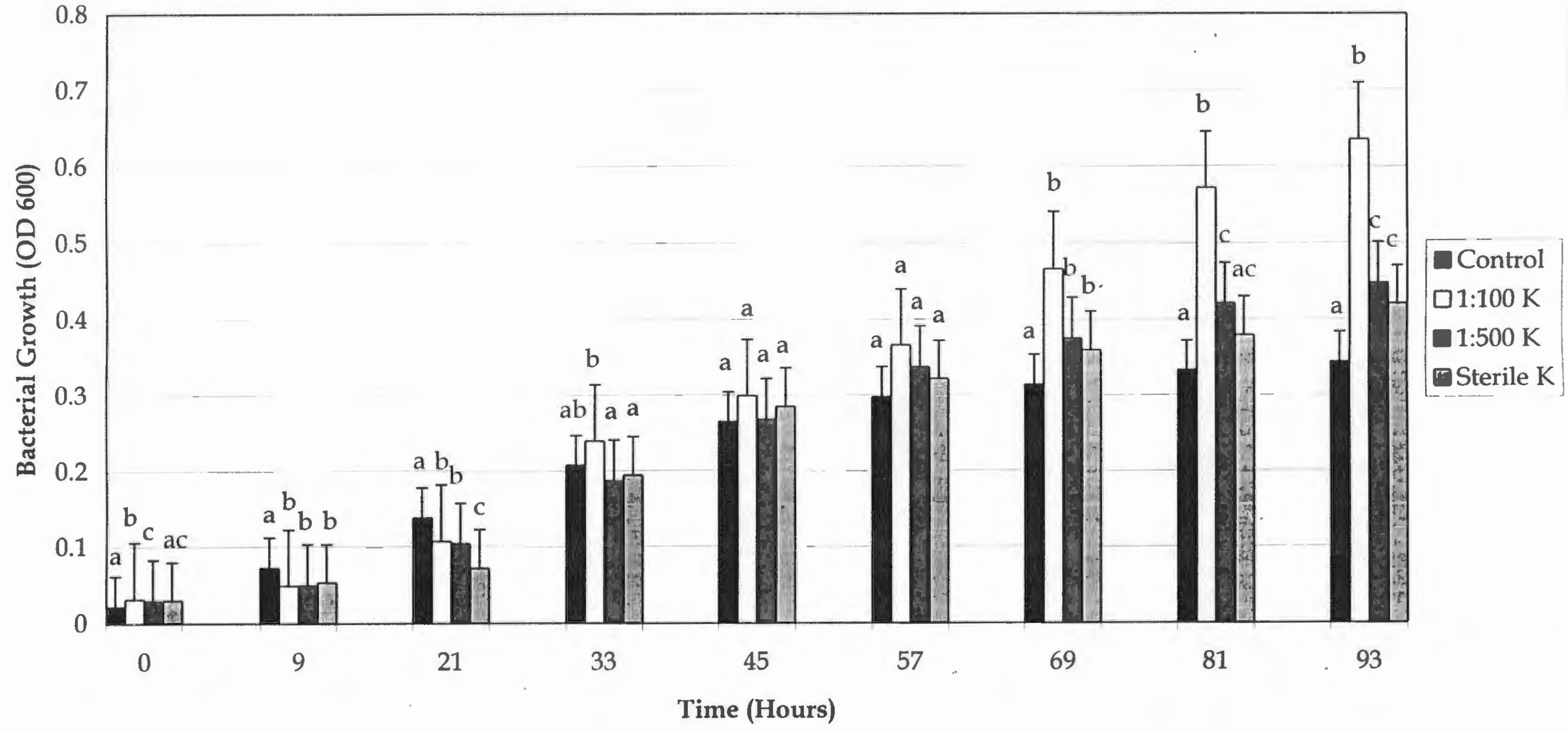

Figure 10. *Bradyrhizobium japonicum* strain CB1809 bacterial growth under different treatments. Different letters indicate significant differences at 5 percent level ((t-test for independent means). Vertical lines represent ± one Standard Error, $p = 0.05$. Treatments are 1:100 K = 1:100 seaweed concentrate dilution; 1:500 K = 1:500 seaweed concentrate dilution; Sterile K = 1:500 sterile seaweed concentrate dilution.

DISCUSSION

Kelpak Stimulation of Plant Growth

At harvest, total DM of cowpea was significantly increased in 1:500B compared to the control (Figure 1). This is consistent with the report of Crouch (1990) that seaweed concentrate (Kelpak) at dilution 1:500 applied regularly, improved the total DM of *Beta vulgaris* and *Phaseolus vulgaris*. However, the total DM of cowpea plants in the other treatments remained unchanged compared to the control. In contrast to cowpea, the total DM of soybean was significantly decreased in 1:500B treatment compared to the control. However, like cowpea, all the other treatments had no effect on soybean plants (Figure 4D). This result suggests species differences in plant growth responses to Kelpak, which supports the data by Temple and Bomke (1989) on *Phaseolus vulgaris*.

In a greenhouse study conducted by Nelson and van Staden (1984), they found that root growth of cucumbers was stimulated when plants were sprayed weekly with seaweed concentrate, leading to 56% increase in the total plant biomass. In that study, the seaweed treatment tended to increase the P content in the leaves and to decrease the N content. This led to the suggestion that the seaweed treatment had induced the uptake of 'unavailable' nutrients by cucumber roots or had improved the efficiency of utilization of 'available' nutrients. Similarly, the shoot and root dry mass of wheat, in a study conducted by Nelson and van Staden (1986), increased with the rate of application of seaweed concentrate. Interestingly, maximum yield was obtained at submaximal rates of seaweed concentration, suggesting that the seaweed did not have a direct effect on growth but acted as a stimulant. These increases in shoot and root dry mass contradict the data obtained in this experiment for both cowpea and soybean species. Whereas in this study, the shoot dry mass of cowpea was not affected by the different Kelpak treatments, soybean showed decreased shoot dry mass in 1:500B Kelpak treatment (Figure 1A, 4A).

Shariff and Dale (1980) found that under conditions of mineral nutrient stress assimilate supply to the shoot is reduced thus restricting the metabolites available for growth as well as the cytokinin supply from the roots. They also showed that tiller bud growth in barley was increased with the application of exogenous cytokinins to the roots of plants under nutrient stress and that this increase was greater when plants were supplied with both cytokinins and mineral nutrients. There are numerous reports in literature on the role of nutrients and in particular nitrogen in the cytokinin translocation (Featonby-Smith and van Staden, 1987, Shariff and Dale, 1980, Mooney and van Staden, 1984). Whether cytokinins present in Kelpak were responsible for the sometimes decreased or increased plant growth, is difficult to indicate.

Relative to the control, the amount of N fixed per plant of cowpea was not altered by the different Kelpak treatments (Figure 2A and 2C). However, the amount of N fixed in soybean plants was significantly higher in treatment 1:500A with greater N content in roots and shoots (Figure 5). These results contradict those of Temple et al. (1989), Featonby-Smith and van Staden, (1987) and Nelson and van Staden (1984) which showed that shoot N content of Kelpak-treated soybean plants were lower than the controls.

Cowpea plants did not show significant increase in length or height in the different treatments compared to the control. Rather, growth in length or height of cowpea and soybean were inhibited in 1:500B and 1:100B Kelpak treatments compared to the control (Figure 3C). Nevertheless, a number of investigators reported that application of seaweed extract did not affect growth and yield. The lack of growth obtained here agrees with the findings of McGeary and Birkenhead (1984) for onions, of Dwelle and Hurley (1984) for potatoes and of Miers and Perry (1986) for wheat (Cited in Verkleij, 1992).

In one study, the application of a water-soluble algal extract to groundnut plants produced a significant increase in growth and yield of one the cultivar tested but not the other (Ketring and Schubert 1981). This increase was attributed to the cytokinin content of the extract, a suggestion that was later repeated by Featonby-Smith and van Staden (1987). Perhaps, the only direct evidence of cytokinin promotion of plant growth is the study by Blunden and Wildgoose (1977), who showed aqueous seaweed extract of known cytokinin activity significantly increased the yield of potatoes. They found close correlations between the results obtained from the use of synthetic cytokinin, kinetin and seaweed extract of equivalent cytokinin activity. An investigation by Finnie and Van Staden (1985) showed that excised tomato roots exposed to low concentrations of seaweed concentrate (Kelpak) mimicked the effect of low levels of cytokinin. The stimulatory effect of the seaweed was lost if the material was ashed, indicating that the regulatory substance is associated with the organic rather than with the inorganic fraction. Because this study is very preliminary and used unfractioned concentrate, the data are inadequate to draw any conclusions.

Kelpak Stimulation of Bacterial Growth in Culture

To my knowledge, no one to date has ever tried to ascertain the effect of seaweed concentrate on bacterial growth, as done here. Growing rhizobia with Kelpak produced very interesting results. Culturing *Bradyrhizobium* strain CB756 in 1:100 Kelpak concentration dramatically increased bacterial cell growth by 3-fold compared to control (Figure 8). Even the weaker 1:500 concentration also increased growth of bacterial cells without sterilization. However, the sterile seaweed concentrate caused an inhibition in the growth of bacteria. This suggests that the seaweed concentrate either lost its growth-promoting properties when subjected to high temperature and pressure or produced degradation products that inhibited rhizobial growth. In contrast *Bradyrhizobium japonicum* strain CB1809, did not show any differences in bacterial cell growth until after 57 hours with the application of both dilutions. Thereafter, strain CB1809 grew significantly with 1:100 Kelpak concentration compared to control. However, strain CB756 had a generally higher growth response to all the treatments including the control compared to *Bradyrhizobium japonicum* strain CB1809. This clearly shows strain differences in bacterial growth response to Kelpak. This might suggest that *Bradyrhizobium japonicum* strain CB1809 is more sensitive to the Kelpak concentrate. Since the growth of these bacteria did not reach a stationary phase, this comparison of the two strains is probably not adequate. It should also be pointed out that the optical density often does not equal rhizobial growth as the bacteria produce different amounts of polysaccharide gum that can affect the OD readings. In further studies viable cell counts need to be done on the cultures to ascertain true bacterial numbers. Furthermore, future research should extend the study over a longer period of time. Since increasing the number of rhizobial cells in soil can potentially promote root nodule formation, knowing what molecule(s) in Kelpak enhance rhizobial growth has the chance of benefiting agriculture.

CONCLUSION

It is known that seed and roots of plants exudates molecular compounds such as flavanoids, aldonic acids that play a critical role in structuring microbial community around the developing root by promoting growth of nitrogen-fixing legume symbiont bacteria. Therefore there is a possibility that Kelpak do contain these molecular compounds that in turn are promoting the growth of the plants as well as their symbionts.

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APPENDIX 1

SOLUTIONS

MODIFIED HOAGLAND N-FREE NUTRIENT SOLUTION

(Hewitt, 1996)

Reagent	**Molecular Weight**	**Stock Solution (g/L)**	**$^1/_3$ Full Strength (m/20L)**
$MgSO_4.7H_2O$ (1M)	246.48	246.48	42.67
$CaCl_2$ (1M)	110.99	111.0	42.67
K_2SO_4 (0.5M)	174.27	87.14	42.67
KH_2PO_4 (1M)	136.09	68.0	21.33
K_2HPO_4 (1M)	174.18	87.1	21.33
SEQUESTRINE (138 Fe) Fe CHELATE		18.7	85.33
$MnCl_2.4H_2O$	197.91	0.724	21.33
$ZnCl_2$	136.28	0.11	21.33
$CuCl_2.2H_2O$	170.48	0.07	21.33
$Na_2MoO_4.2H_2O$	241.05	0.025	21.33
$CoCl_2.6H_2O$	237.95	0.06	21.33
H_2BO_3	61.87	5.72	21.33

Dissolve all the materials in 20-liter glass-distilled water.

YEAST-MANNITOL BROTH CULTURE SOLUTION

Reagent	
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
NaCl	0.1g
MANNITOL	10g
YEAST	0.4g

Dissolve all materials in 1000 ml glass-distilled water. Adjust the pH 6.8 and stir for 10 minutes. The medium was sterilized by autoclaving at 121°C for 60 minutes.