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Comparative changes in metabolism of *Vigna radiata* by foliar and root application of brassinolide at different concentrations

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The present study shows the effect of the foliar and root application of brassinolide on growth and metabolism of *Vigna radiata*. For this purpose, three concentrations of brassinolide (1, 0.01 and 0.0001 ppm) were applied on the plant. Brassinolide (BL) solutions were applied as foliar and root treatments for 21 days after sowing (DAS). Samples were collected after two and four weeks of treatments. Content of total sugars, reducing sugars, chlorophyll and proteins, activity of peroxidase (EC 1.11.1x), leaf area and height of plants were estimated. All these parameters increased significantly by 1 ppm foliar spray of BL as compared to the other concentrations. It was concluded that BL enhanced growth of *V. radiata*. L. under both root and foliar applications, but foliar treatment was more effective.

Key words: *Vigna radiata,* foliar spray, root irrigation, brassinolide, concentrations.

INTRODUCTION

Vigna radiata L. belongs to the pulse family. It is a rich source of protein, especially in under developed countries like Pakistan where proteinaceous diet is not accessible to every person. Its worth is very important. The total area under major pulse crops in Pakistan is about 1.5 m hectares. With the development of short duration and uniform maturing varieties, mung bean can be fitted in various cropping systems (Pakistan Agriculture Research Council).

Brassinolide is a compound of Brassinosteroids (BR) class, the sixth class of plant hormones, discovered in the 1970s. Brassinosteroids are involved in many physiological processes like stem elongation, xylem differentiation, root inhibition, pollen tube growth, ethylene biosynthesis, leaf epinasty, regulation of gene expression and photosynthesis (Sasse, 2003). A lot of work has done on the effects of this hormone on plants grown under different stress conditions and it is found to be effective in amelioration of stress. BRs can act efficiently in plants as immunomodulators when applied at the appropriate concentration and at the correct stage of plants' development (Bajguz and Shamsul, 2009). The foliar spray either with 24-epiBL or 28-homoBL signi-ficantly enhanced the growth, photosynthesis, and protein content in mung bean; they play a critical role in a range of developmental processes, for example stem and root

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growth, floral initiation, and the development of flowers and fruits (Shamsul and Ahmed, 2003). Kalinich et al. (1985) investigated *Phaseolus vulgaris* and *Phaseolus aureus* and elucidated that BRs had an impact on the transcription, leading to an increase in protein content. Exogenous application of BRs enhanced the prospective efficiency of crops by stimulating cell elongation, vascular differentiation and/or proton pump (Hayat and Ahmad, 2003).

In the present study, the effects of brassinolide on growth and metabolism of *Vigna radiata* L. were studied. Observation regarding total proteins, total carbohydrates, reducing sugars, photosynthetic pigments, peroxidase activity, and leaf area and plant heights were recorded.

MATERIALS AND METHODS

Field experiments were performed. For this purpose, healthy seeds were sterilized with 1% solution of mercuric chloride for 5 min and then washed with distilled water several times before sowing. Plastic pots of 6 inches diameter were used for the experiment. In each pot, equal quantity of soil was used. Brassinolide solution with concentrations of 1, 0.01 and 0.0001 ppm was applied to plants 21 days after sowing (DAS) at two leaf stage (as foliar and root treatments to separate set of plants and to compare results of both treatments). For each treatment, three replicates were used. For biochemical evaluations, samples were taken at 2nd and 4th week after treatment. Leaf area and height of plants were taken when plants reached their maximum heights.

Estimation of chlorophyll

Chlorophyll was estimated by the method of Maclachaln and Zalik 1963. 0.5 g of fresh leaves was taken and macerated in 7 ml of 80% acetone and centrifuged at 1000 rpm for 5 min. The debris was then washed 3 times using 3 ml of 80 % acetone each time. The supernatants were then pooled and made up to the final volume with 80 % acetone. Optical density of this solution was then recorded at 663 and 645 nm.

The chlorophyll content was calculated using the formula given below and expressed in milligram per gram fresh weight.

$$\text{Chlorophyll a (mg / g)} = \frac{12.3\ D663 - 0.861\ D645 \times V}{D \times 1000 \times W}$$

$$\text{Chlorophyll b (mg /g)} = \frac{19.3\ D645 - 3.6\ D663 \times V}{D \times 1000 \times W}$$

Total carbohydrates

0.3 g fresh leaves were taken and macerated in 7 ml of tris HCl buffer and then centrifuged at 2500 rpm for 20 min. Supernatant was collected for estimation. 1 ml of aliquots was taken and 4 ml distilled water was added to it. 10 ml anthrone reagent was used. The reaction mixture was incubated at boiling water bath for 16 min, and then cooled. Optical density was recorded at 680nm. For reagent blank, 1 ml buffer was used instead of leaf extract. Sucrose (1000 µg/ml) was used to prepare standard curve. Total carbohydrates were determined by the method of Hassid and Abraham (1957).

Estimation of reducing sugars

Determination of reducing sugars was done according to Bernfeld (1955). Leaf extracts were prepared by taking 0.3 g fresh leaves, macerated in 7 ml of tris HCl buffer and centrifuged at 2500 rpm for 20 min. 1 ml of this leaf extract was mixed with 2ml of DNS (3,5-dinitrosalicylic acid). It was prepared by dissolving 1g of DNS in 50ml water and then 30 g of potassium sodium tartarate was added slowly. Then, 20 ml 2N NaOH was added and diluted up to 100ml. Samples were incubated in boiling water bath for 15 min. Reaction was terminated in an ice bath and optical density was recorded at 540 nm. Maltose was used to prepare calibration curve in µg/ml.

Bradford protein assay

Protein standards

Protein standards were prepared in the same buffer as the samples to be assayed. A convenient standard curve was made using bovine serum albumin (BSA).

Assay reagent

Coomassie blue G250 (100 mg) is dissolved in 50 ml of methanol and filtered through a glass-fiber filter. The solution is added to 100 ml of 85% H_3PO_4 and diluted to 200 ml with water. The assay reagent is then diluted 4 folds.

Assay

0.04 ml of aliquots was added to 2 ml of assay reagent. After 30 min of incubation at room temperature, the optical density was recorded at 595 nm. The total protein was calculated in µg/ml by using standard curve.

Peroxidase activity

Peroxidase activity was analyzed by the method of Chance and Maehly (1955). 0.5 g sample was crushed in 5 ml of phosphate buffer of pH = 6. It was centrifuged at 1600 rpm for 10 min and then the supernatant used for the estimation of enzyme was collected. 0.1 ml enzyme extract of plant was mixed with 2.1 ml deionized water, 0.32 ml buffer and 0.16 ml hydrogen peroxide (5%) (freshly prepared). 0.32 ml pyrogallol solution (5 %) (freshly prepared) was added and the reaction mixture was chilled. The absorbance of the reaction maximum was measured at 420 nm with a double bean UV/ visible spectrophotometer. For reagent blank, buffer was used instead of enzyme extract. The calibration curve was prepared by using peroxidase solution. Enzyme activity was measured in µg/min/mg FW by the following formula:

$$\text{Enzyme activity} = \frac{\text{Reading from std. curve x Amount of extract}}{\text{Activity time x wt. of material x volume of extract used in test}}$$

RESULTS AND DISCUSSION

The aim of this study was to check whether brassinolide foliar spray or root irrigation is beneficial for plant growth and metabolism and to find which method and

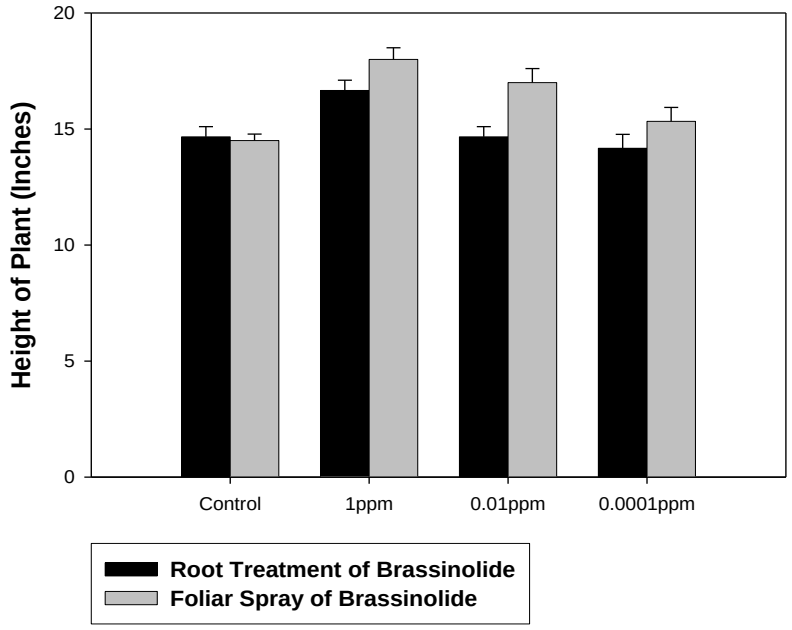

Figure 1. Effect of different concentrations of brassinolide on height (inches) of *Vigna radiate.* Bars show standard error, SE ± (n=3).

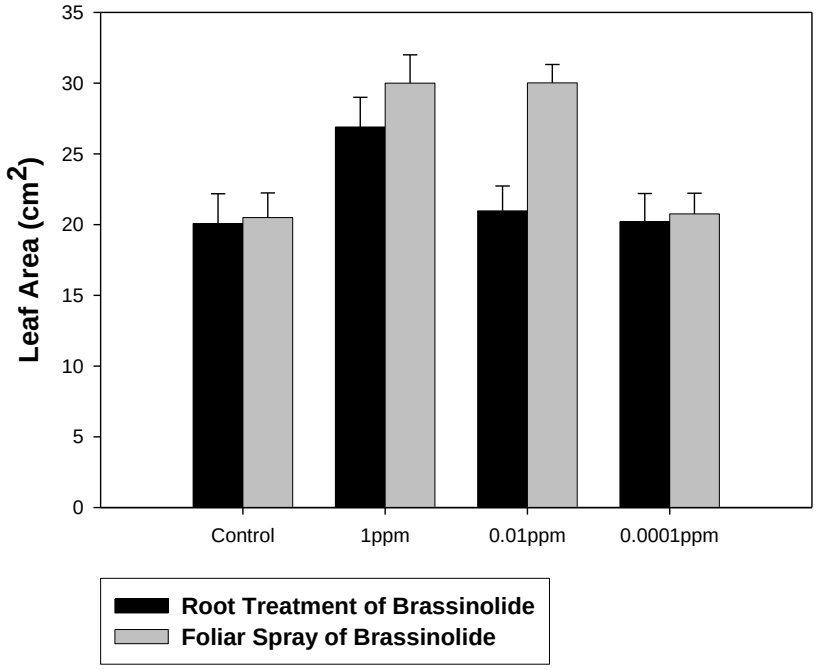

Figure 2. Effect of different concentrations of brassinolide on leaf area (cm^2) of *Vigna radiata*. Error Bars represent standard error of mean (n=3).

concentration of brassinolide application enhance the quality of crops under normal conditions.

The morphological parameters like plant height, leaf area increased significantly ($P < 0.05$) with sprays and dosage of brassinolide compared to control and root irrigation (Figures 1 and 2). Similar result was reported by Ramraj et al. (1997). Plants sprayed with brassinolide reached the reproductive stage earlier than plants which

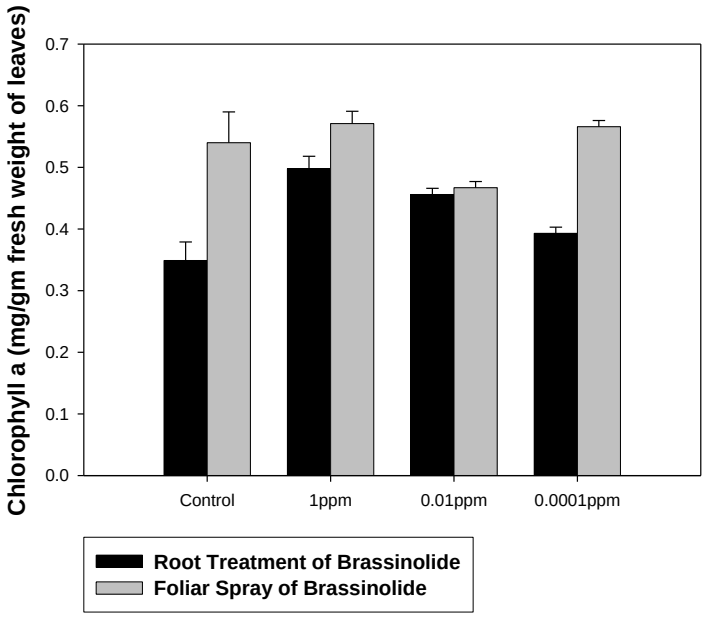

Figure 3. Changes in chlorophyll 'a' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 2nd week. Significantly different at ($p < 0.05$).

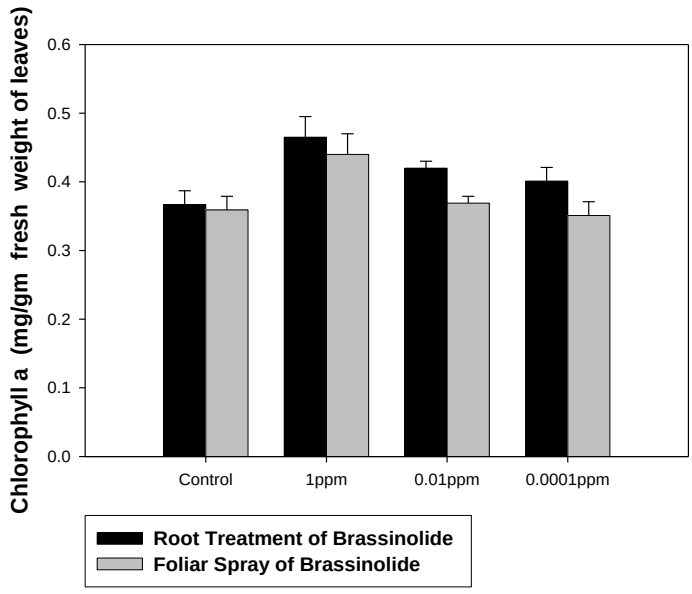

Figure 4. Changes in chlorophyll 'a' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 4th week. Significantly different at ($p < 0.05$).

were not sprayed with brassinolide. This increase in height and leaf area is due to the increase in cell division and cell elongation. This is supported by Rao et al. (2002).

Brassinolide affects the growth and quality of crop when treated with irrigation water or spray method. The amount of chlorophyll "a" and chlorophyll "b" increased significantly when plants were sprayed with solutions of BL (Figures 3 and 4). Chlorophyll content decreased with decrease in concentration (1ppm, 0.01ppm, 0.0001ppm)

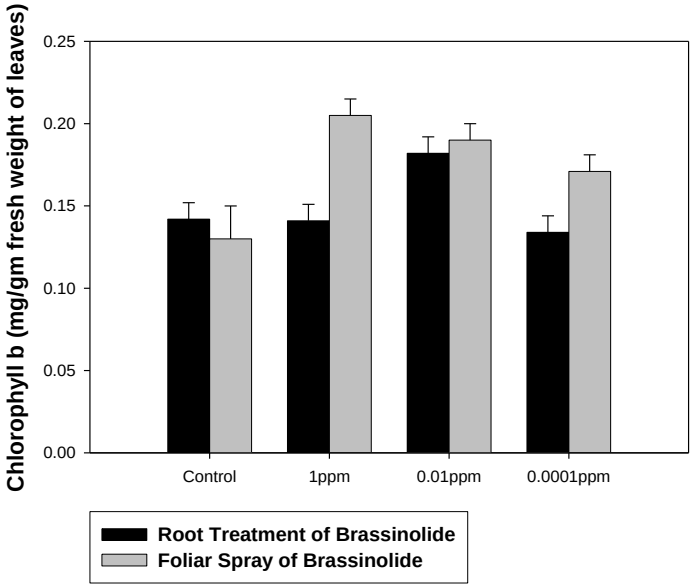

Figure 5. Changes in chlorophyll 'b' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 2nd week. Significantly different at (p< 0.05).

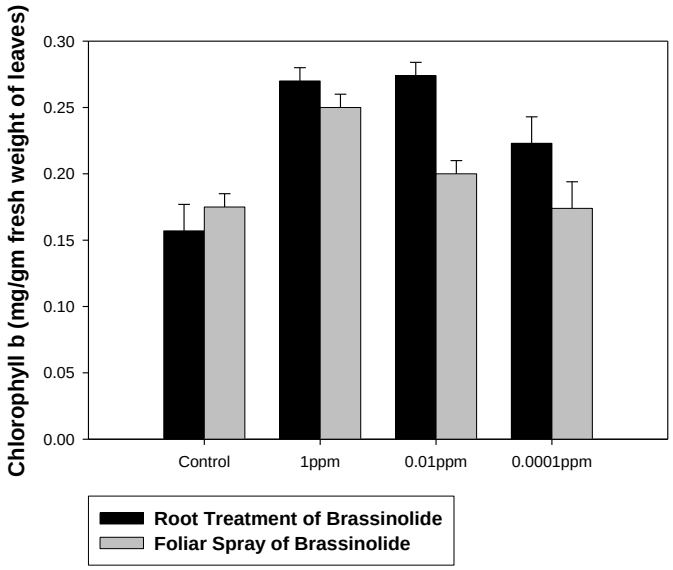

Figure 6. Changes in chlorophyll 'b' in *V. radiata* by foliar and root application brassinolide at different concentrations after 4th week. Significantly different at (p< 0.05).

but remained higher than control. These results were supported by the work of Sairam (1994). The interaction of BL and time period also showed significant (P< 0.05) results. In 2nd week, the amount of chlorophyll pigments was increased with foliar spray but later, after the 4th week, root application was effective (Figures 5 and 6).

Foliar spray of BL increases significantly (p<0.05) the amount of total sugars and reducing sugars as compared to root application after 2nd week of treatment (Figures 7 and 8). It was also revealed that the effect of BL increases with the time duration as the amount of total sugars is high in the samples taken at 4th week (Figures 9

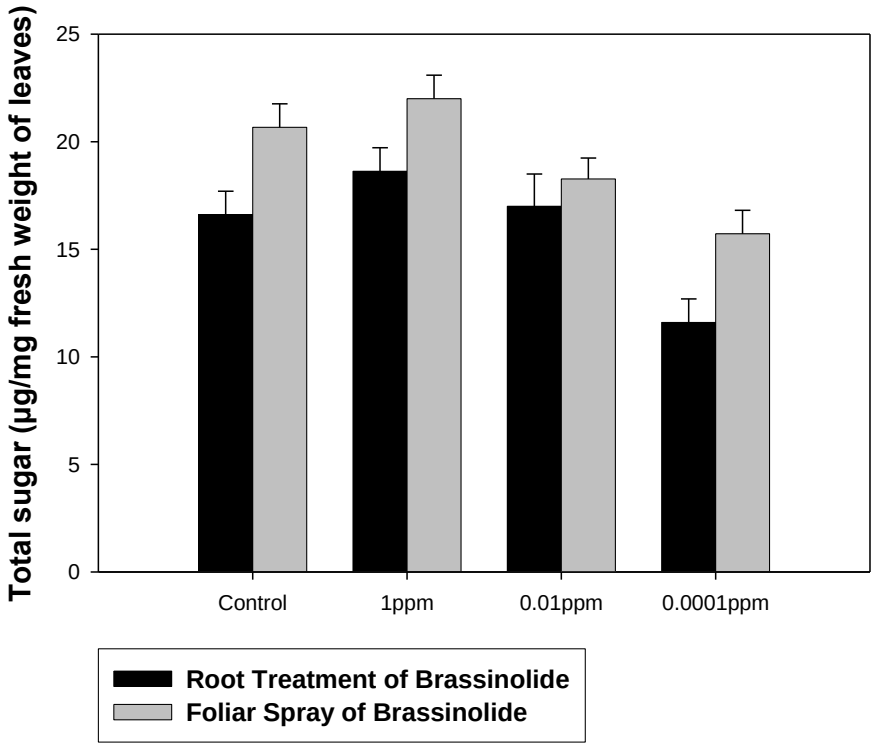

Figure 7. Effect on the amount of total sugar in *Vigna radiata* treated after 2nd week at different concentartions of brassinolide apply as foliar and root treatment.

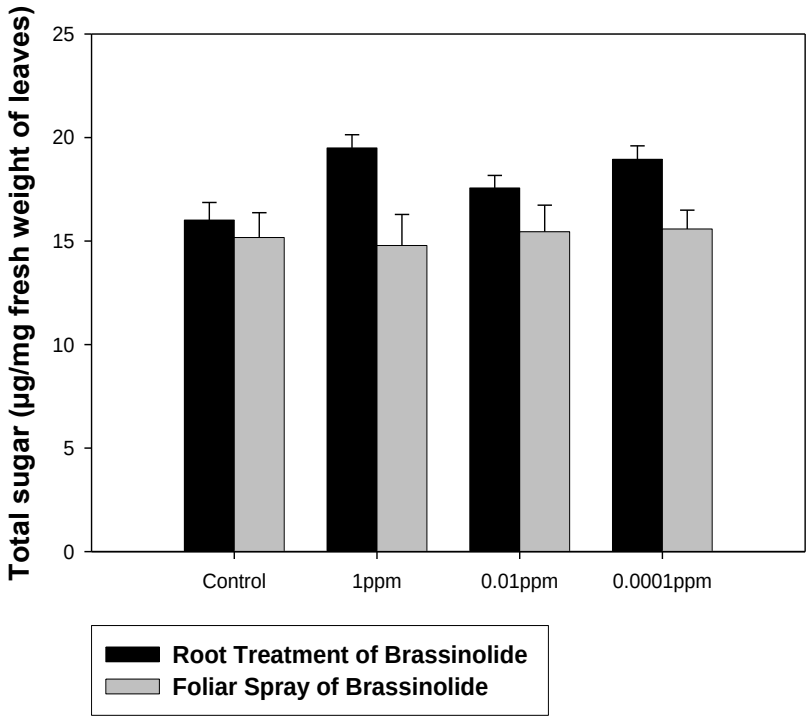

Figure 8. Effect on the amount of total sugar in *Vigna radiata* treated after 4th week at different concentartions of brassinolide apply as foliar and root treatment.

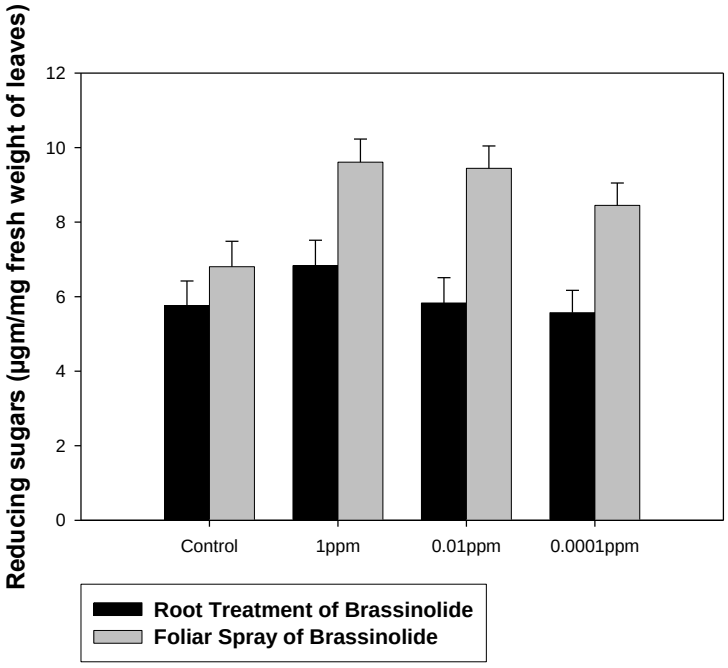

Figure 9. Effect of different concentrations of brassinolide on amount of reducing sugar in *Vigna radiata* after 2nd week of treatment. Significant results at ($p < 0.05$).

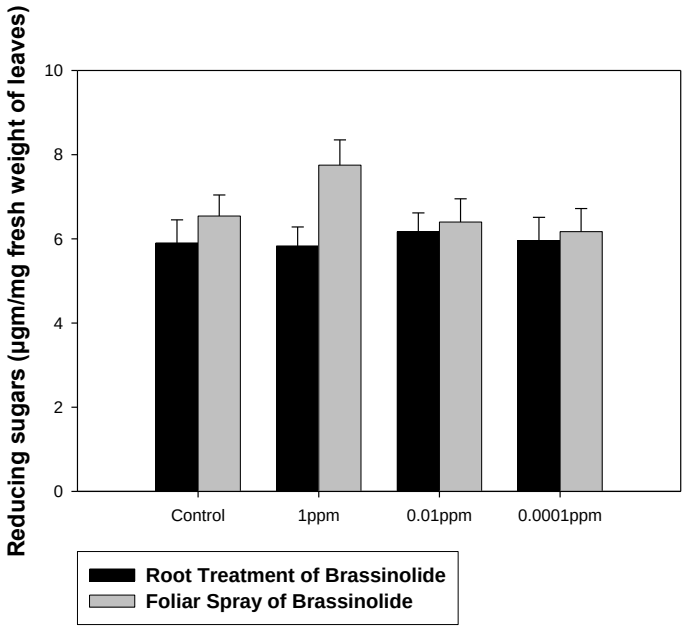

Figure 10. Effect of different concentrations of brassinolide on amount of reducing sugar in *Vigna radiata* after 4th week of treatment. Significant results at ($p < 0.05$).

and 10). The highest amount of total sugars was found in plants sprayed with 1 ppm of BL. This indicates that brassinolide increases the metabolic activities in mung beans (Vardhini and Rao, 1998). The increase in total

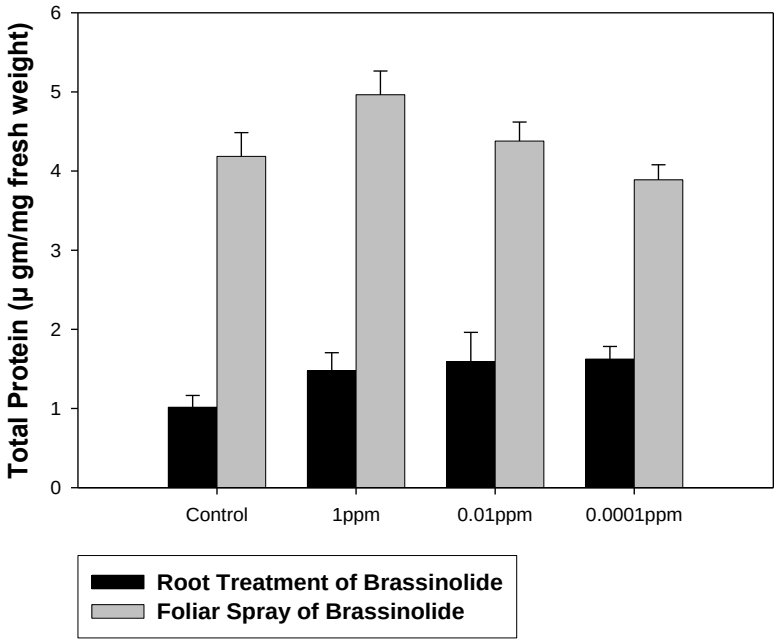

Figure 11. Changes in total protein (µg/mg fresh weight) in *Vigna radiata* by foliar and root application of brassinolide after 2nd week.

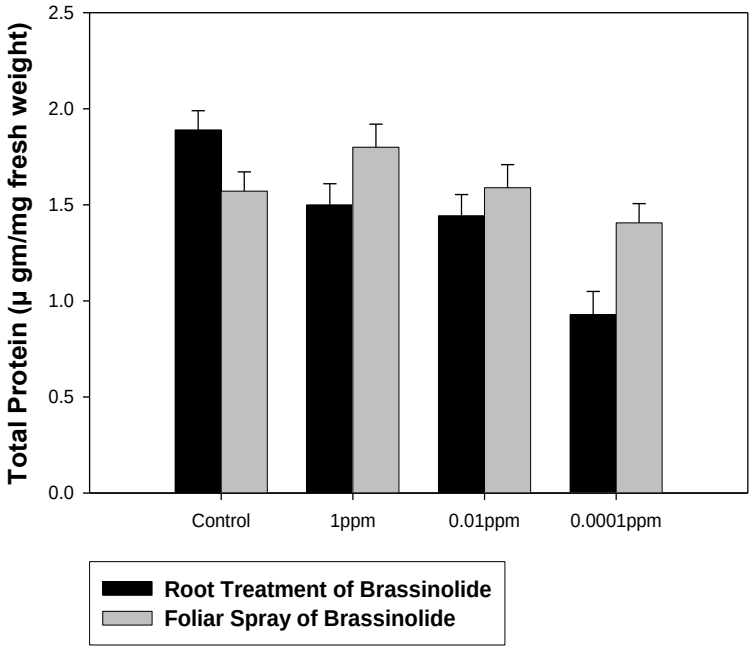

Figure 12. Changes in total protein (µg/mg fresh weight) in *Vigna radiata* by foliar and root application of brassinolide after 4th week.

sugar was noticed due to the rise in the rate of photosynthesis with increase in the amount of chlorophyll content (Braun and Wild, 1984).

Total proteins were significantly enhanced ($P< 0.05$) by spraying different concentrations of BLs solutions compared to root application (Figures 11 and 12). Result

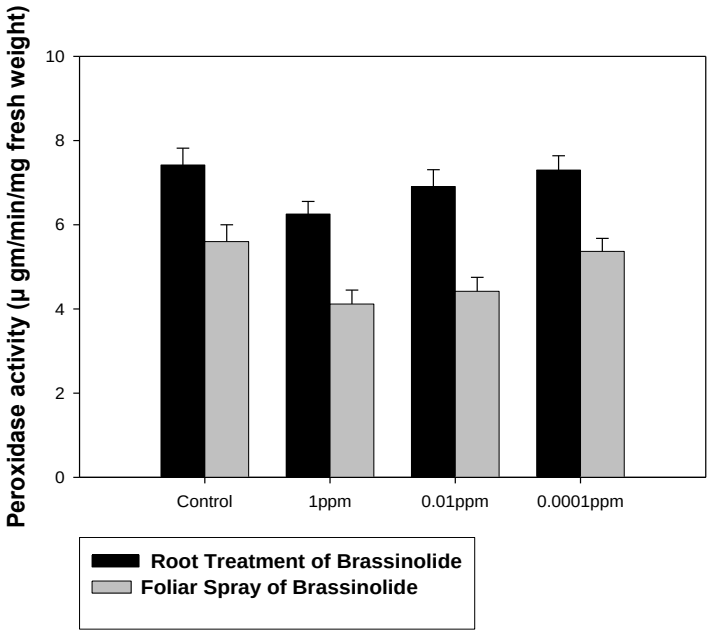

Figure 13. Peroxidase activity (µg/min/mg fresh weight) show changes in *Vigna radiata* after 2nd week of brassinolide application. Bars represent standard error of mean (n=3).

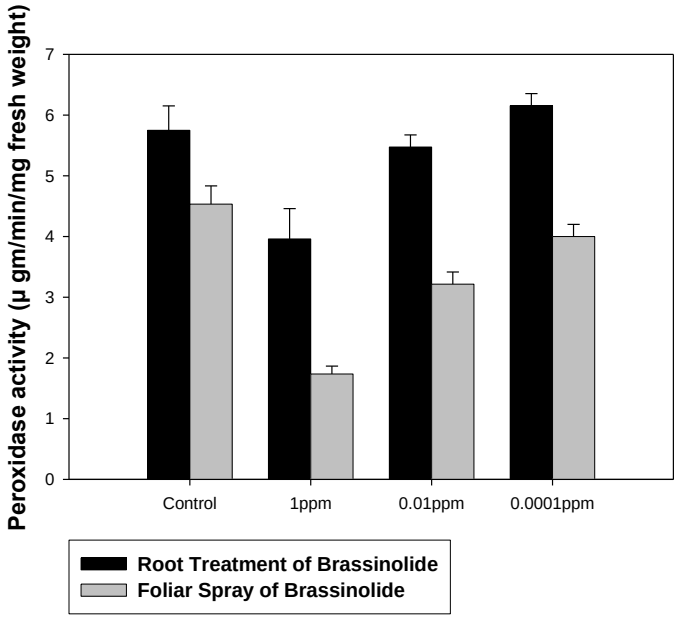

Figure 14. Peroxidase activity (µg/min/mg fresh weight) show changes in *Vigna radiata* after 4th week of brassinolide application. Bars represent standard error of mean (n=3).

analysis showed the direct relationship between amount of proteins and concentration of BL. Similar results were observed by many researchers and co-workers (Sharma et al., 2014; Bajguz, 2000).

The activity of peroxidase was decreased by the supplementation of BRs rather than sprays and root irrigation. It was higher in control samples (Figures 13 and 14). Result of peroxidase activity is supported by

Arora et al., 2008. Higher peroxidase activities are closely associated with growth of the plants (Zheng and van Huystee, 1992). BL suppressed the activity of peroxidase. It indicates advanced and healthier growth of the plant over the control sample because it was revealed that activity of peroxidase is responsible for the gluconeogenesis of lipids (Jones, 1972).

Conclusion

Our investigation demonstrated that both foliar and root application of brassinolide are effective. Initially, in 2nd week, effect of foliar treatment is significant because it is readily absorb in plant cell through stomata and enhanced metabolic activities. But as time passes, plants suck up the brassinolide by their roots and utilize it in their cells. This plays a constructive role in their growth and metabolism. The order of effective response to BL treatment was 1 > 0.01 > 0.0001 ppm > control.

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