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Salicylic Acid Induced Early Responses on Growth, Pigment Composition and Metabolite Content in *Vigna radiata* seedlings

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ABSTRACT

This study was conducted to determine the effect of foliar salicylic acid (SA) applications on vegetative growth, physiological and biochemical constituents of *Vigna radiata* (L.)Wilczek. SA was applied at five different concentrations (50ppm, 100ppm, 150ppm, 200ppm 250ppm) during the growth period of 7 and 15 days old *Vigna* seedlings. Growth parameters such as shoot and root lengths, shoot and root fresh weights and dry weights and physiological and biochemical constituents such as photosynthetic pigment composition, total soluble sugar, free amino acid and total phenols were recorded from treated and control plants on 10 and 18 days after sowing. From the experiment done all of five doses of SA application produce considerable increase in plant growth, physiological and biochemical constituents compared to the control. The optimal concentration for increased overall plant growth was found to be at 100ppm. As compared to Chlorophyll*a*, Chlorophyll*b* was found to synthesize more which could be ascribed to change in the stoichiometry of PSII to PS I. Thus, the present work confirms the promotory effects of SA on overall growth, pigment synthesis and other biochemical constituents in *Vigna radiata.*

Keywords: *Vigna radiata*, salicylic acid, vegetative parameters, physiological and biochemical constituents.

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INTRODUCTION

Salicylic acid (SA) named after Salix plant (Willow), was first discovered as a major component in the extracts of willow tree bark that had been used as a natural anti-inflammatory drug from the ancient times to the 18th century[1]. Salicylic acid naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plant such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, inhibition of ethylene biosynthesis, transpiration and photosynthesis[2, 3]. It has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack[4]. Salicylic acid, jasmonic acid and ethylene-dependent signaling pathways regulate plant responses to both abiotic and biotic stress factors[5]. SA has a direct physiological effect through the alteration of antioxidant enzyme activities, induces flowering, increase flower life, retards senescence and increases cell metabolic rate. The sustained level of salicylic acid may be a prerequisite for the synthesis of auxin and/or cytokinin[6]. Salicylic acid promotes as well as inhibits some physiological processes depending on its concentration, plant species, development stages and environmental conditions[7]. SA at high doses inhibited plant growth and chlorophyll contents in tomato[8] and wheat plants[9]. Salicylates are known to affect various physiological and biochemical activities of plants and by playing a key role in regulating growth and productivity[10]. SA and its close analogues enhanced the leaf area and dry mass production in corn and soybean[11]. The dry matter accumulation was significantly enhanced in *Brassica juncea*, after spraying with low concentrations of SA[12]. Effect of SA on the leaf number, fresh and dry mass per plant of wheat seedlings increased significantly treated at 10 µM of SA[13]. Similar growth promoting response was observed in barley seedlings sprayed with SA[14]. Thus SA could be influence the growth, physiological and biochemical constituents of *Vigna radiata* plant. The mung bean (*Vigna radiata*), alternatively known as the moong bean, green gram, and golden gram, is a plant species in the legume family. Native to the Indian subcontinent, the mung bean is mainly cultivated today in India, China, and Southeast Asia. Mung bean is a warm-season crop and grows mainly within a mean temperature range of 20–40°C, the optimum being 28–30°C. Mungbean is a pulse crop of special importance and provides an inexpensive source of vegetable dietary protein. It is popular for its nutritive value and digestibility, containing higher protein contents (28%), fat (1.3%),carbohydrates (60.4%) and reasonable amount of, vitamins and essential micronutrients[15]. Therefore, the objective of this research is to investigate the effects of foliar salicylic acid

applications on vegetative growth, physiological and biochemical constituents at early stages of growth of *Vigna radiata* (L.) Wilczek.

MATERIALS AND METHODS

Plant material

Healthy and uniform dry seeds of *Vigna radiata* (L.) Wilczek were procured from Agricultural Research Station, Kovilpatti, Tamilnadu, India and surface sterilized with 0.1% $HgCl_2$ for one minute and washed repeatedly with distilled water. Healthy seeds were selected and sown in pots containing mixture of red soil, black soil, and sand mixed in the ratio of 2:2:1. The seeds were allowed to germinate in dark for 48 hours. The percentage of seed germination was nearly 80%. Soon after emergence, the seedlings were shifted to daylight conditions. Since the ambient climate was too hot for the seedlings, a 40% cut off mesh filter was used to surround the pots for an initial period of 2-3 days.

Salicylic acid Foliar Spray

After (7and 15 days) initial growth of *vigna radiata* seedlings, the seedlings were sprayed with five different concentrations of SA (50ppm, 100ppm, 150ppm, 200ppm, 250ppm) using an atomic sprayer. The seedlings were sprayed with SA until dropping. Each plant required about 10ml of spray solution. Salicylic acid (o-hydroxybenzoic acid) was purchased from Sigma Chemical Co., Tamil Nadu, India. SA was initially dissolved in 100µl of dimethyl sulfoxide (DMSO) and concentrations of 5 x 10^{-6}M to 100 x 10^{-6}M (pH 6.5) were made up with distilled water containing 0.02% Tween-20 (Polyoxyethylene sorbitan monolaurate). Plants sprayed with 0.02% Tween-20 served as the control. The plants were arranged in a completely randomized design with three replicates [8]. The foliar spray was given for three days early in the morning and growth analyses were done after 7 and 15 days old seedlings.

Determination of vegetative growth, physiological and biochemical constituents

After 7 and 15 days of plant growth, the morphological measurements such as, shoot and root length of treated and control plants was measured with the help of meter scale. Fresh and dry weight of shoot and root were determined by using electronic balance. 50mg of fresh leaf tissues of control and treated plants were ground in 10ml of 100% acetone and the extract was centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was measured at 662nm, 645nm and 470nm for Chl *a*, Chl *b* and carotenoids respectively using an ELICO SL-171 Spectrophotometer. The amount of Chl *a*, Chl *b*, total Ch l, and carotenoid content was calculated using the formula of Wellburn and Lichtenthaler[17].

Chlorophyll *a* (mg/L) = (11.75 x A_{662}) – (2.35 x A_{645})

Chlorophyll *b* (mg/L) = (18.61 x A_{645}) – (3.96 x A_{662})

Chlorophyll *a*+*b* (mg/L) = (7.79 x A_{662}) + (16.26 x A_{645})

$$\text{Carotenoids} = \frac{1000 \times A\ 470 - 2.27 \times Ca - 81.4 \times Cb}{227}$$

Ca=Chlorophyll a; Cb= Chlorophyll b Total soluble sugar was estimated by anthrone method[18]. Leaves of (100 mg) both control and treated plants were ground in 10ml of distilled water and the extract was centrifuged at 3000 rpm for 5min. The supernatant was added with 2ml of 10% TCA and kept in the ice cold condition for 10min and again centrifuged at 5000 rpm for 5min. The supernatant was used as test solution. 0.1 ml of test solution was added with 0.9ml of distilled water and 4ml of anthrone reagent (0.2%) the test tubes were boiled in water both for 10 min. After cooling, the absorbance was measured at 620nm; total soluble sugar content was measured using standard curve. Free aminoacid was estimated by ninhydrin assay method[18]. Leaves of (100mg) both control and treated plants were ground in 10ml of ethanol and the extract was centrifuged at 5000 rpm for 3min. The supernatant was used as the test solution. To 1ml of the test solution, 3ml of distilled water and 1ml of ninhydrin reagent were added and mixed thoroughly then the test tubes were kept in boiling water bath for 10min. Then the tube was cooled down to room temperature and 1ml of 50% ethanol was added. The absorbance was measured at 550nm; the amino acid content was estimated from standard curve prepared with glycine as amino acid source. Total phenols were estimated by folin – ciocalteu method[19]. To 1ml of the alcohol extract taken in a test tube, 1ml of folin – ciocalteu reagent and 2ml of sodium carbonate solutions were added. The tubes were shaken well and kept in boiling water both for 1minute. Then the tubes were cooled to obtain blue colour. The absorbance was recorded at 650nm using a proper blank. The amount of phenol was calculated from catechol standard curve.

Statistical Analysis

The experiments were performed in a randomized order. Data were expressed as means of three replicates with standard error. Statistical assays were carried out by one-way ANOVA using Tukey's test to evaluate whether the means were significantly different, taking $p<0.05$ as significant.

RESULTS AND DISCUSSION

Data presented in [Figure 1(a-f)] show that foliar application of salicylic acid at 50ppm, 100ppm, 150ppm, 200ppm and 250ppm promoted growth criteria of *Vigna radiata's*shoot and root length,

shoot and root fresh weight, shoot and root dry weight compared to corresponding control plants at the end of 7 and 15 days. In all cases the increments in growth parameters were often highly significant at 100ppm in comparison with untreated plants. Growth characters of *Vigna radiate* plants decreased by increasing salicylic acid concentration at 250ppm. Similarly Kord and Hathout (1992) found that foliar application of salicylaldehyde at 10^{-5} M stimulated different morphological and growth criteria of tomato plants but reduced effects were observed at 10^{-3}M[8]. According to Gharib, application SA at low concentration increased photosynthetic activity in basil and marjoram which enhanced their plant height, number of internodes, number of branches and leaves as well as leaf area, fresh and dry weights[20]. In this respect, many investigators found that low concentrations of salicylic acid enhanced growth of soybean[21], maize[22]and wheat plants[3], whereas high concentrations caused an inhibitory effect on growth of tomato, lupine, wheat and maize plants[23, 24]. Low concentration salicylic acid foliar application also promote and influence the growth, development, differentiation of cells, and tissues of plants and enhanced growth parameters[25]. Thus, low dose (100ppm) foliar application of salicylic acid is effective in improving growth parameters of *Vigna radiata* than higher doses. Respect to photosynthetic pigment composition [Figure 2 (a-d)] foliar application of salicylic acid at 100ppm enhanced accumulation of chlorophyll *a* and *b*, total chlorophyll and carotenoids compared to control. Maximum increase in total chlorophyll content was observed at 100ppm of SA concentration, nearly 16% and 7% increase was noticed in 7and 15 days' old *Vigna radiata* seedlings (Figure 2 c). The percentage of increase was much apparent in chl *a* content than chl *b* (Figure 2 a, b). The changes in carotenoids level under SA treatment at 100ppm was significantly increased to 41%, 17% was noticed at 7, 15 days *Vigna* seedlings respectively (Figure 2 d). Almost all the SA concentrations favored the pigment increase (Figure 2 a-d). Salicylic acid enhanced the photosynthetic ability of apple leaves[26]. In soybean plants, salicylic acid treatment increased pigment content as well as the rate of photosynthesis[27]. Chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA[28]. Biochemical constituents like total soluble sugar, free amino acid, total phenols were analyzed in both control and hormone treated seedlings. From [Figure 3 (a-c)] foliar application of SA improved the soluble sugar content of *Vigna radiata* 10% and12% in 7and 15 days old seedlings respectively (Figure 3 a). Salicylic acid application resulted in a significant increase in total soluble carbohydrate content in leaves of tomato and sunflower, thus maintaining the carbohydrate pool in the chloroplasts at a high level[29, 30].Foliar application of SA improved the free amino acid content of *Vigna radiata* seedlings at almost all concentrations (Figure 3 b).The levels of total phenol was found to decrease with increased in SA concentrations in 7, 15 days old

seedlings (Figure 3 c). Thus, SA proved to be potent growth regulator by inducing overall growth, pigment synthesis and other biochemical constituents in crops like *Vigna*.

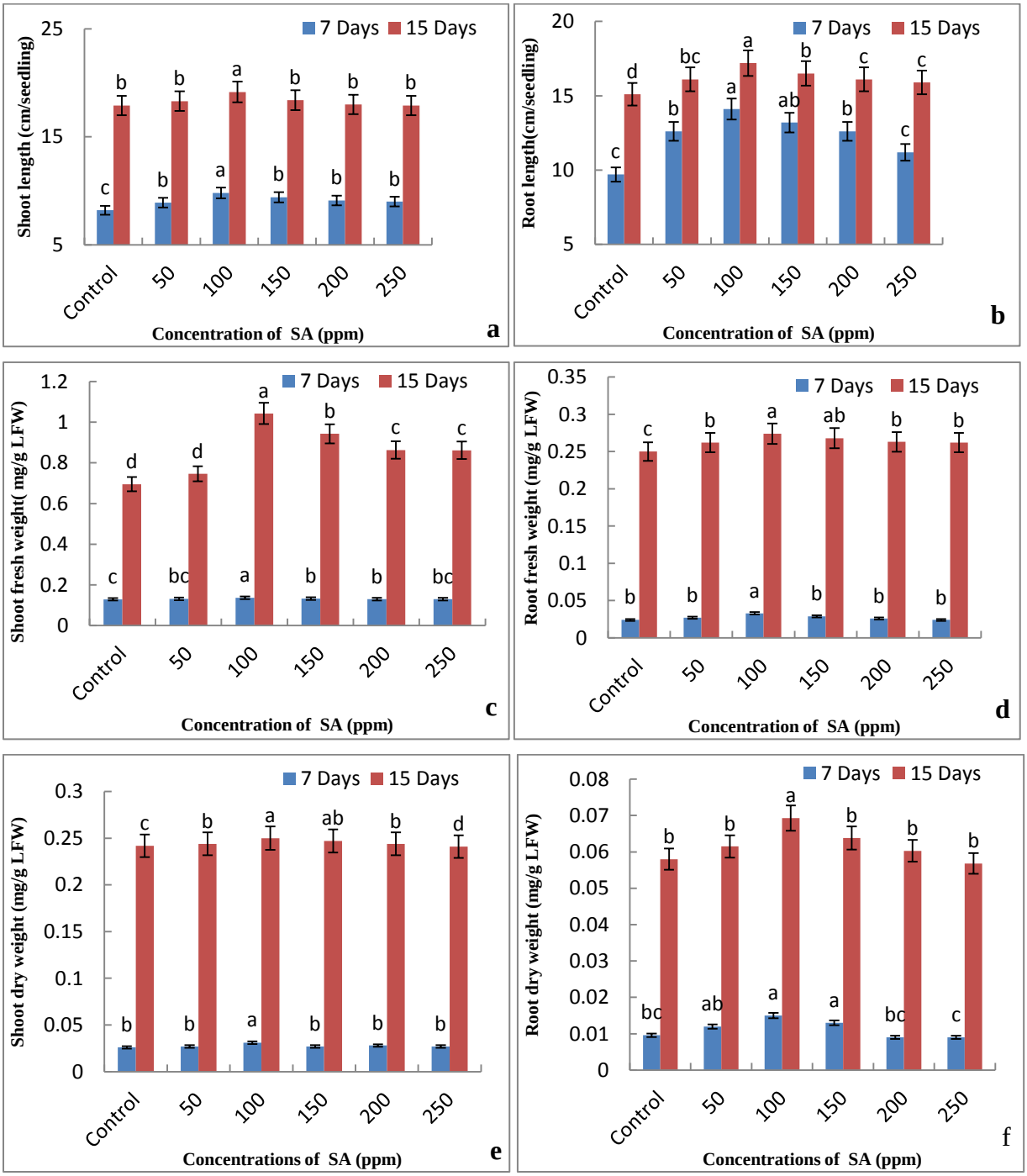

Figure 1(a-f): Typical growth parameters of *Vigna radiata* (L.) Wilczek seedlings in different concentrations of SA. (a:shoot length, b:root length, c:shoot fresh weight, d:root fresh weight, e:shoot dry weight, f:root fresh weight.) Each value represent the mean of six independent measurements (Mean±SE, n=6). Bars carrying different letters are significantly different at $P < 0.05$

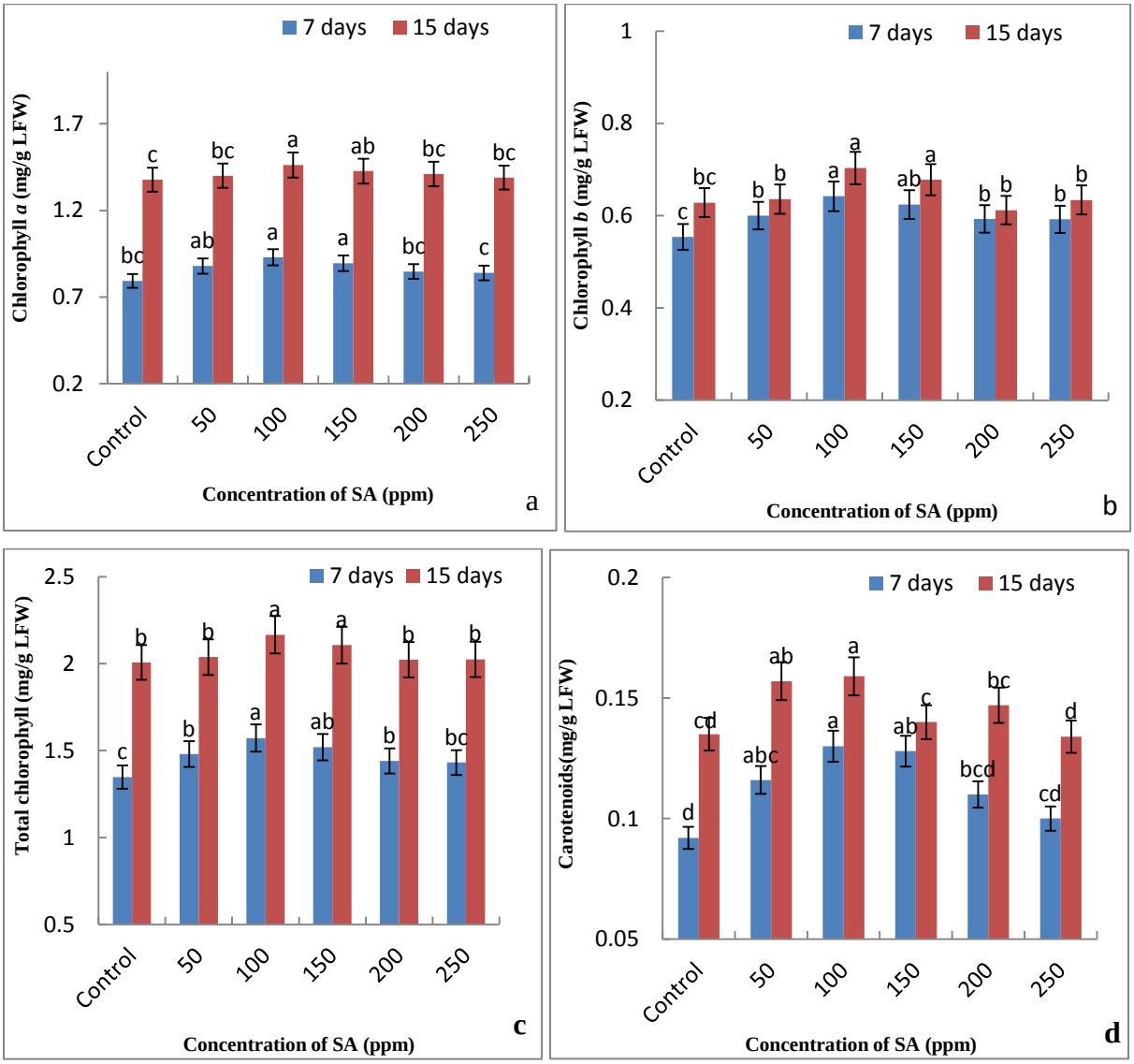

Figure 2(a-d): Typical photosynthetic pigment composition of *Vigna radiata* (L.)Wilczek seedlings in different concentrations of SA. (a: Chlorophyll*a*, b: Chlorophyll*b*, c: Total chlorophyll, d: Carotenoids).Each value represent the mean of three independent measurements (Mean±SE, n=3). Bars carrying different letters are significantly different at *P* < 0.05

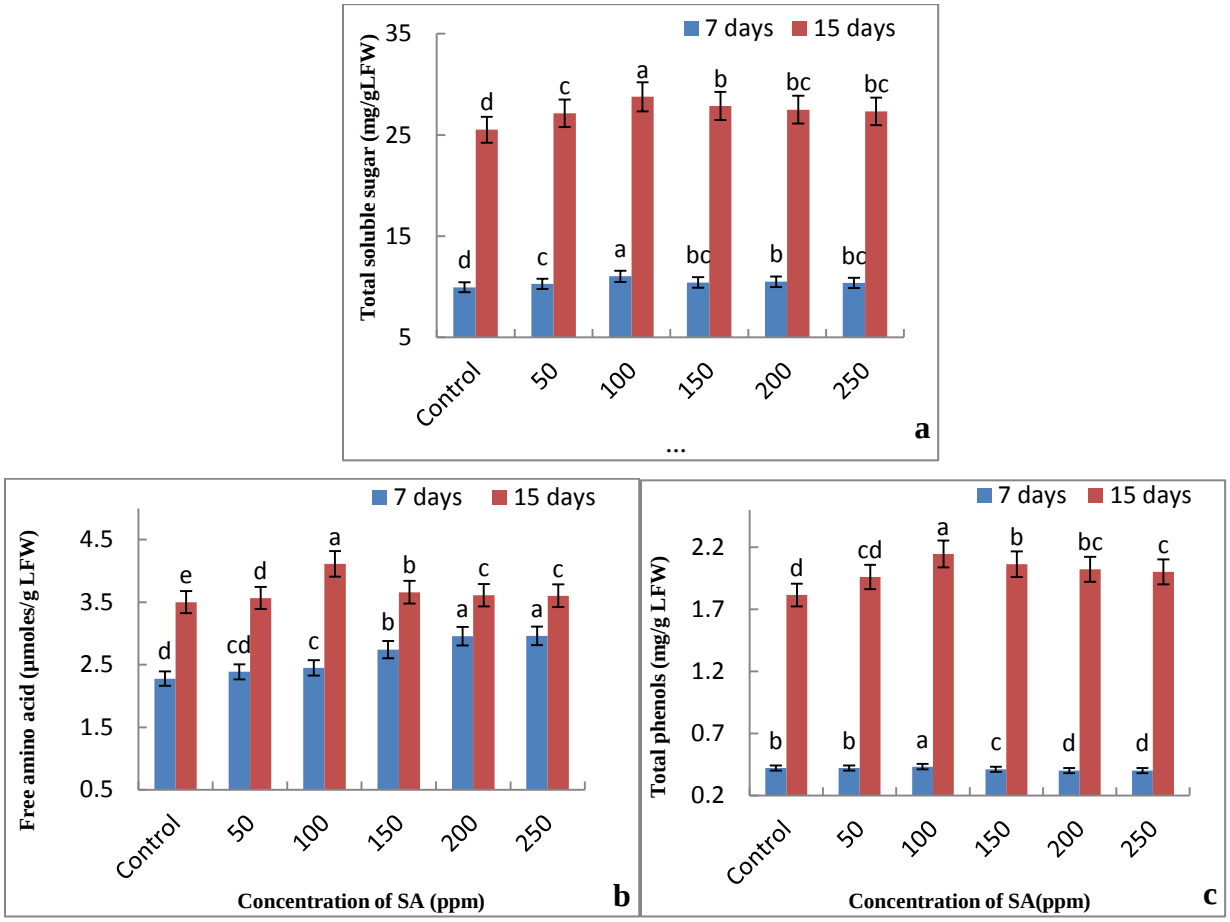

Figure 3(a-c): Biochemical composition of *Vigna radiata* (L.) seedlings indifferent concentrations of SA. (a:soluble glucose, b:free aminoacid, c:total phenols). Each value represent the mean of three independent measurements (Mean±SE, n=3). Bars carrying different letters are significantly different at *P* < 0.05

CONCLUSION

In conclusion, the results obtained in this study suggest that the foliar application of SA can significantly regulate the plant growth parameters, pigment synthesisas well as bioactive compounds in *Vigna radiata*. From the preceding results and discussion, it can be concluded that foliar application on *Vigna radiata* with salicylic acid at 100ppm dose enhance physiological and biochemical constituents such as photosynthetic pigment composition, total soluble sugar, free amino acid and total phenols.

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