

Salicylic Acid Induced Early Responses on Growth, Biochemical Composition and Metabolite Contents in *Vigna mungo* 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 in *Vigna mungo* (L.) Hepper. SA was applied at five different concentrations (50ppm, 100ppm, 150ppm, 200ppm and 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 mungo*.

Keywords— *Vigna mungo*, salicylic acid, vegetative parameters, physiological and biochemical constituents.

I. INTRODUCTION

Salicylic acid is new class of phytohormone and applies multiple purposes in plants growth, development, seed germination, mass production and pathogen resistances. (SA) or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics. Salicylates from plant sources have been used in medicines since antiquity. In 1828 in Munich was isolated for the first time a small amount of salicin, the glucoside of salicyl alcohol, from willow bar. Ten years latter Raffaele Piria named it SA, from the Latin word Salix for willow tree. The first commercial production of synthetic SA began in Germany in 1874 [1, 2]. Aspirin, a close analog of salicylic acid, was introduced by the Bayer Company in 1898 and rapidly became one of the most popular pharmaceutical preparations in the world. During the 19th century many compounds belonging to the group of salicylates were isolated from a variety of plants.

Using current analytical techniques it was found that salicylates are distributed in many important agricultural plant species. In many plants, such as rice, crabgrass, barley, soybean, the levels of SA has been found to be approximately $1\mu\text{g g}^{-1}$ fresh weight. A survey of SA in leaves and reproductive structures of non-thermogenic angiosperms confirmed the ubiquitous distribution of this compound in plants. Levels of SA varied substantially in the floral parts of seven non-thermogenic species and in the leaves of 27 non-thermogenic plants [3]. The highest levels of SA were determined in the inflorescence with necrotizing pathogens [1].

II. MATERIAL AND METHODS

A. Plant material

Healthy and uniform seeds of *Vigna mungo* were purchased from Agricultural Research Station, Kovilpatti and surface sterilized with 0.1% HgCl washed repeatedly in 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 h. The percentage of seed germination was nearly 80%. Soon after emergence, the seedlings were shifted to daylight conditions. After (7 and 15 days) growth of seedlings, the seedlings were sprayed with different concentrations of SA (50ppm, 100ppm, 150ppm, 200ppm, 250ppm) using an atomic sprayer. The seedlings were sprayed with solutions until dripping. Each plant required about 10ml of spray solution. Salicylic acid (SA-2-hydroxybenzoic acid) was obtained from Sigma Chemical Co. (St. Louis, U.S.A), SA was initially dissolved in 100 μl of dimethyl sulfoxide (or) ethanol and concentrations of $5 \times 10^{-6}\text{M}$ to $100 \times 10^{-6}\text{M}$ (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 [4].

B. Determination of vegetative growth, photosynthetic pigments 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 Chl and carotenoid content was calculated using the formula of Wellburn and Lichtenthaler [5].

$$\text{Chlorophyll } a \text{ (mg/L)} = (11.75 \times A_{662}) - (2.35 \times A_{645})$$

$$\text{Chlorophyll } b \text{ (mg/L)} = (18.61 \times A_{645}) - (3.96 \times A_{662})$$

$$\text{Chlorophyll } a+b \text{ (mg/L)} = (7.79 \times A_{662}) + (16.26 \times A_{645})$$

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

$$(Ca = \text{Chlorophyll } a; Cb = \text{Chlorophyll } b)$$

C. Estimation of total soluble sugar

Total soluble sugar was estimated by anthrone method [6]. 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. To the supernatant was added with 2ml of 10% TCA was added and kept in the ice cold condition for 10mins 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.

D. Estimation of free amino acid

Free amino acid was estimated by ninhydrin assay method [6]. 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 and 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.

E. Estimation of total phenols

Total phenols were estimated by folin – ciocalteu method [7]. 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 bath 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.

F. 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.

III. RESULTS AND DISCUSSION

The results of SA responses on *Vigna mungo* morphology are shown in Figure.1(a-f) respectively. On the basis of shoot length, root length, fresh weight and dry weight, SA treatment induced positive changes on plant morphology of *Vigna mungo*. The concentrations of SA applied as foliar spray were 50, 100, 150, 200 and 250ppm. Among the concentrations, 100ppm of SA was found to increase the overall growth in *Vigna mungo* whereas both 100 and 150ppm of SA was found to cause maximum growth in *Vigna mungo* (Fig.1). Regarding shoot length, 100ppm of SA induced an increase up to 4 to 17% and 3 to 16% in *Vigna mungo* at different stages of growth. (Fig.1a). The root length increased at 100ppm of SA with maximum increase to about 5 to 20% in *Vigna mungo* seedlings (Fig1.b) after 7 and 15 days of growth. Application of SA at 100ppm and 150ppm to *Vigna mungo* caused an increased in shoot fresh weight to a tune of 2 to 8% and 1 to 6% (Fig.1c). With regard to shoot dry weight, a significant increase upto a level of 6 to 23% was observed at 100ppm of SA in *Vigna mungo* (Fig.1d). High concentration of SA was found to have less impact on promotion of growth. Root fresh weight was increased to 13-32% (Fig.1e) in 7 and 15 days old *Vigna* seedlings respectively. Similar changes in root dry weight at 100ppm and 150ppm were 12 - 50% and 6 - 40%, in *Vigna mungo* respectively (Fig.1f).

Exogenous application of SA enhanced shoot, root and total plant dry weight in *C. officinalis*, besides promoting early flowering and high number of floral buds [8]. These results corroborate the findings of [4], whereas increase of leaf area and shoot dry weight of soybean and corn occurred with 10^{-5} M of SA application. On the contrary, high concentration of SA caused a decreased in growth parameters. Similarly [9] 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. According to [10] 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. In this respect, many investigators found that low concentrations of salicylic acid enhanced growth of soybean [11]; maize [12] and wheat plants [13], whereas high concentrations caused an inhibitory effect on growth of tomato, lupine, wheat and maize plants [14]. Low concentration salicylic acid foliar application also promote and influence the growth, development, differentiation of cells, and tissues of plants and enhanced growth parameters [15].

With regard 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. Chl content at 100 and 150 ppm of SA got increased from 10 to 12 and 7 to 10% in *Vigna mungo*. SA treatment significantly increased the chlorophyll *b* content at 100ppm to about 8 to 12% in *Vigna mungo*. Maximum increase in total chlorophyll content was observed at 100ppm of SA concentration, nearly 10 to 14% and 7 to 9% at 100 & 150ppm in *Vigna mungo*, shown in (Fig. 2c). Application of SA at 100ppm to *V.mungo* caused an increased in carotenoids to a tune of 7 to 22% in 7 and 15 days of growth respectively. (Fig.2d). Almost all the SA concentrations favored the pigment increase (Fig.2 a-d). Foliar spray of SA caused an increase of Rubisco and PEP carboxylase activity [12]. The improvement of all these characteristics ultimately increased P_n (net photosynthetic rate). Increased photosynthetic rate naturally increases dry mass per plant. Application of SA in *Zea mays* [16], barely [17], wheat [18] and *Brassica napus* [19] increased Chl *a* content. Chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA [20]. Thus, low dose (100ppm) foliar application of salicylic acid is effective in improving growth parameters of *Vigna mungo* than higher doses. Respect to photosynthetic pigment composition [Figure 2 (a-d)] foliar application of salicylic acid at 100ppm enhanced accumulation of chlorophyll *a* *b*, total chlorophyll and carotenoids compared to control.

Biochemical constituents like total soluble sugar, free amino acid, total phenols were analysed in both control and hormone treated seedlings. Foliar application of SA improved the soluble sugar content of *Vigna mungo* at about 15% and 25% in 7 and 15 days old *Vigna* seedlings respectively as shown in Fig.3a. SA application on tomato increased polysaccharide level of soluble sugars and activate d the metabolism and consumption of soluble sugar by increasing osmotic pressure [21]. 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 [22].

Foliar application of SA improved the free amino acid content of *Vigna mungo* seedlings at almost all concentrations (Fig.3 b). The levels of total phenol was found to be increased in SA concentrations in 7 and 15 days. SA plays in regulating PAL activity and phenolic compound biosynthesis and brings about a relationship among SA, PAL, and phenolic compounds in biosynthesis [23]. Higher free amino acid content in tomato plants treated with elicitors *viz.*, jasmonic acid and SA as compared to infected control plants [24].

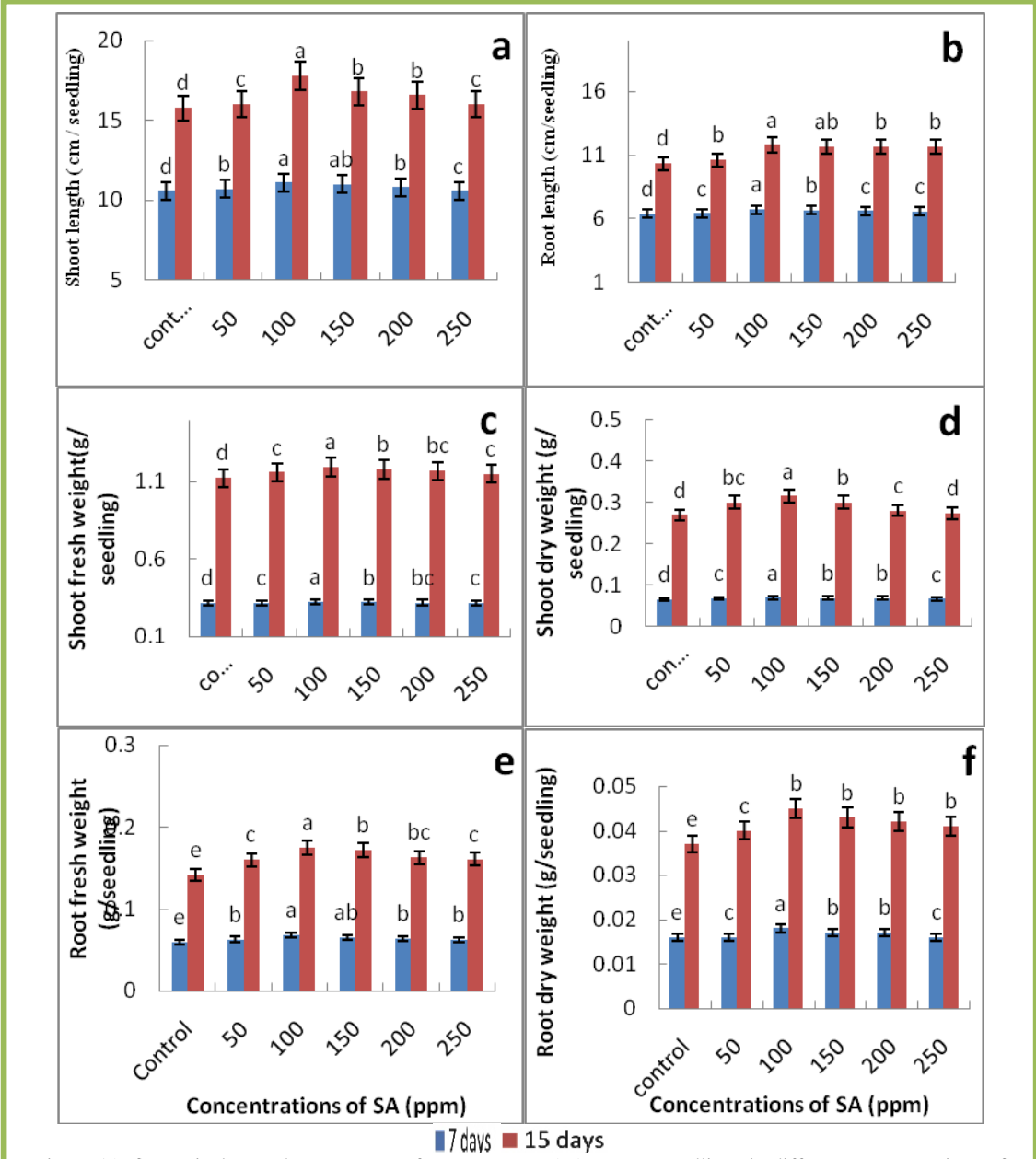
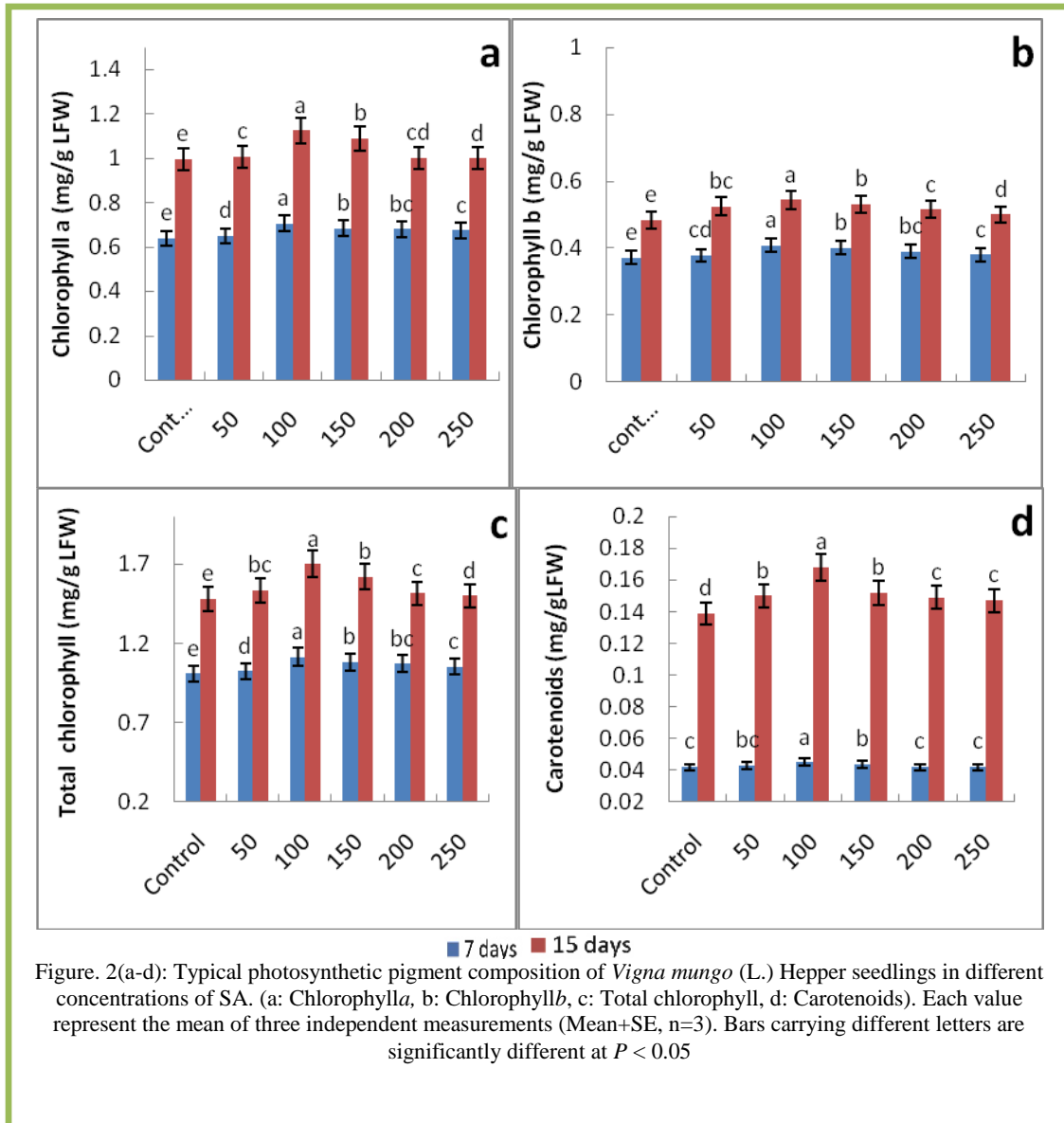


Figure 1(a-f): Typical growth parameters of *Vigna mungo* (L.) Hepper seedlings in different concentrations of SA. (a:shoot length, b:root length, c:shoot fresh weight, d:root fresh weight, e:shoot dry weight, f:root fresh weight.) Each value represent the mean of six independent measurements (Mean+SE,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



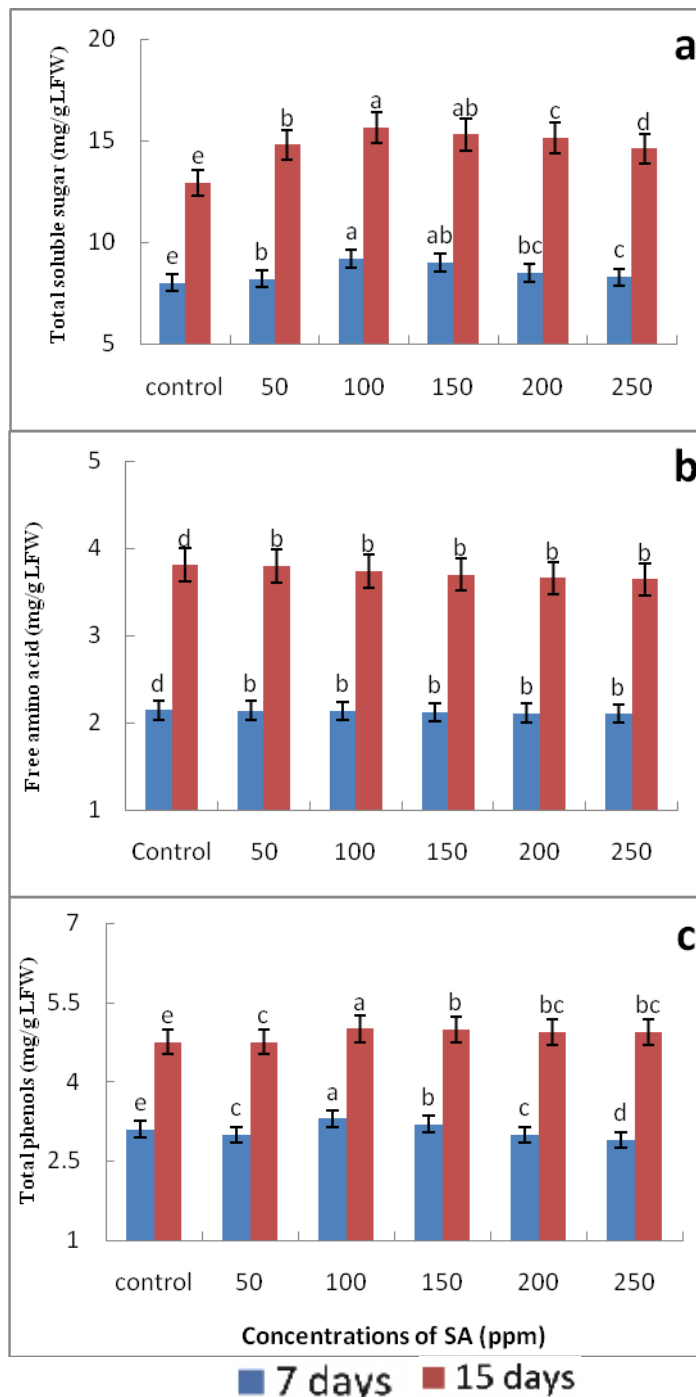


Figure 3(a-c): Biochemical composition of *Vigna mungo* (L.) Hepper seedlings indifferent concentrations of SA. (a:soluble glucose, b:free aminoacid, c:total phenols). Each value represent the mean of three independent measurements (Mean+SE, n=3). Bars carrying different letters are significantly different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



IV. CONCLUSION

In conclusion, the results obtained in this study suggest that the foliar application of SA can significantly regulate the plant growth parameters, pigment synthesis as well as bioactive compounds in *Vigna mungo*. From the preceding results and discussion, it can be concluded that foliar application on *Vigna mungo* 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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