RESEARCH ARTICLE Legume Research- An International Journal, Volume 46 Issue 1: 56-61 (January 2023)

Effect of Salicylic Acid on Plant Physiological and Yield Traits of Soybean

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10.18805/LR-4527

ABSTRACT

Background: Salicylic acid (SA) is an endogenous plant growth regulator plays a vital role in plant growth, ion uptake, transport, interaction with other organisms and in the responses to environmental stress. The aim of the study was to find the effect of salicylic acid on chlorophyll content, superoxide dismutase and malondialdehyde level of leaves and seed yield parameters of soybean.

Methods: Field trials were conducted during *kharif* 2018 and 2019 at ICAR-Indian Institute of Soybean Research, Indore farm (22.78°N, 75.88°E), India. Salicylic acid applied as foliar spray with (50,100 and 200 ppm) concentrations at vegetative stage (22-25 days after sowing) and at pod filling stage (57-60 days after sowing). Chlorophyll content, lipid peroxidation activity and superoxide dismutase enzyme, plant height, number of pod per plant, seed yield and seed index were determined.

Result: Chlorophyll content, antioxidant enzyme were enhanced and the level of lipid peroxidation of leaves was reduced as compared to untreated plant when salicylic acid applied at critical stage of crop growth. Significant increase in soybean seed yield was observed both with concentration of 100 and 200 ppm salicylic acid. Foliar spray with salicylic acid @ 100 ppm at vegetative and at pod filling stage was very effective for better seed production programme to improve quantity as well as quality of soybean seeds.

Key words: Antioxidant enzyme, Salicylic acid, Soybean seed.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is the most important grain legume of the world, which contributes significantly to edible oil, protein concentrate for animal feed, food uses and various industrial products. Since 1970, the average annual increase in soybean area has been the highest among the major crops of the world (Hartman *et al.,* 2011). Within a short time of inception of commercial cultivation of soybean in India (in 1970's) it has occupied the first position among the oilseed crops in terms of acreage and production (Kuchlan *et al*., 2019). During previous years the degrees of climatic adversities added severe negative impact on soybean productivity and production of quality seeds. Soybean breeder seed is the most critical source of soybean seed production. During 2013 and 2019 seed production was severely affected due to excessive rainfall in the major seed production area of soybean in Madhya Pradesh and Maharashtra (AICRP's on Soybean Report 2013-19). Appearance of drought condition during 2014, 2015 and 2017 affected soybean production as well as soybean breeder seed production severely. The benefit cost ratio of soybean production and soybean seed production is marginal. During 2014 the all India soybean production was 10.37 million tonnes from 10.91 million hectare area with the productivity of 951 kg/h. In 2015 the production was 8.57 million tonnes from an area of 11.60 m ha with the productivity of 738 kg/h only. During 2017 the total soybean production in India was 10.93 mt from 10.33 m ha with the productivity of 1058 kg/h (DAC and FW, 2019). Data clearly indicates that climate variability influence production and productivity of soybean. The stress at vegetative phase has been reported to decline photosynthesis, leaf area and biomass and once the stress is over, plant can recover to a certain extent. However, stress at reproductive phase has often no chance of recovery and hence, results in severe loss of soybean productivity (Jumrani *et al*., 2017). Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature has been found to play a key role in the regulation of plant growth, development, interaction with other organisms and in the responses to environmental stresses (Raskin, 1992a, b; Yalpani *et al*., 1994; Senaratna *et al.,* 2000). Further, its role is evident in seed germination, fruit yield, glycolysis, flowering in thermogenic plants (Klessing and Malamy, 1994), photosynthetic rate, stomatal conductance and transpiration (Khan *et al*., 2003). Salicylic acid application which has multigenic effect on soybean growth and seed quality is an economical and environment friendly approach to improve plant productivity. Keeping in view the diverse physiological roles of salicylic acid in plants the present work was undertaken to improve our understanding on the effect of various concentrations of salicylic acid

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How to cite this article: Kuchlan, P. and Kuchlan, M.K. (2023). Effect of Salicylic Acid on Plant Physiological and Yield Traits of Soybean. Legume Research. 46(1): 56-61. doi: 10.18805/LR-4527.

Submitted: 10-10-2020 **Accepted:** 17-02-2021 **Online:** 09-03-2021

applied as foliar spray at different growth stages on the plant physiology and seed yield of soybean under natural climatic condition.

MATERIALS AND METHODS

Field trials were conducted during rainy season (June-October) of 2018 and 2019 at ICAR-Indian Institute of Soybean Research, Indore farm (22.78°N, 75.88°E), India. The experimental site has black (Vertisols) soils with high to moderate depth, high water holding capacity and medium fertility. Soybean variety JS 20-29 was planted in a randomized block design with three replications. Each plot consisted of 3 rows of 2 m length spaced at 0.45 m. Recommended dose of fertilizers was applied at the time of planting. Standard agronomic practices for weed and insect control were uniformly followed. SA 50,100 and 200 ppm were applied as foliar spray to the soybean plant twice at vegetative stage (22-25 days after sowing) and at pod filling (R5) stage (57-60 days after sowing). Chlorophyll content, lipid peroxidation and superoxide dismutase activity and height were determined after seven days of application of SA in each stage. Number of pod per plant was taken before harvest maturity, seed yield and seed index was noted after the harvest of crop.

Chlorophyll content estimation

Leaf samples were taken at vegetative and pod filling stage after 7 days of salicylic acid application for chlorophyll content estimation. 50 mg leaf material from fully emerged leaf was taken and placed in 10 ml of DMSO in a test tube and was kept in an oven at 65°C for about 4 hours. The extract was taken in a measuring cylinder and final volume was made up to 10 ml by adding DMSO. The absorbance of the solution was read at 663 nm and 645 nm using spectrophotometer against the DMSO as blank. The chlorophyll content was determined using the formula given by Arnon (1949) and expressed as mg/g of fresh leaf. Arnon's formulae to estimate chlorophyll a, chlorophyll b and total chlorophyll as follows:

Calculation

$$\text{Chlorophyll a} = [12.7(D_{663})-2.69(D_{645})] \times V / (1000 \times w)$$

$$\text{Chlorophyll b} = [22.9(D_{645})-4.68(D_{663})] \times V / (1000 \times w)$$

Where
D = Absorbance.
V = Final volume of DMSO (ml).
W = Weight of fresh leaf (g), Unit: mg /g of fresh leaf.

Superoxide dismutase (SOD) activity was assayed according to Bailly *et al*. (1996). Leaf samples were taken after seven days of foliar application of salicylic acid at both stages. Fifty-mg of fresh leaf tissue was crushed in 2 mL of 0.1 M EDTA-phosphate buffer, pH and centrifuged at 15000 × g for 10 min at 4°C. 12 µl of supernatant which acted as enzyme extract was added to a mixture of 1 mL of 50 mM potassium phosphate buffer (pH 7.5); 100 µl of 2.25 mM NBT, 100 µl of 3 mM EDTA, 200 µl of 200 mM L-methionine and 1.938mL distilled water and 150 µl of 0.075 mM riboflavin. The test tubes were placed at a distance of 30 cm away from15W fluorescent lamps for 6 min. Reaction mixture without enzyme developed maximum color and was used as control. The absorbance was recorded at 560 nm. A 50% inhibition in enzyme activity was calculated as unit of SOD.

The activity of enzymes was calculated using the following formula:

$$\frac{(\text{OD value of control - OD value of sample}) \times \text{dilution factor/time}}{(\text{OD value of sample - OD value of blank})}$$

Malondialdehyde content (MDA) was estimated following Cakmak and Horst (1991) protocol. After 7 days of foliar application at both the stage leaf samples were taken for MDA observation. Leaf samples (0.2 g) were homogenized in 3 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15000 g for 15 min. To 1.0 ml aliquot of the supernatant, 2.0 ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 95°C for 30 min in a water bath and then cooled in an ice bath. After centrifugation at 10000 × g for 10 minute, the supernatant was collected and the observance was measured at 535 nm and 600 nm. The value for nonspecific absorption of each sample was recorded at 600 nm and subtracted from the absorbance recorded at 532 nm. Difference in OD values measured at 535 nm and 600 nm was used to compare the effect of treatments.

Plant height (cm)

Randomly selected 20 plants of each plot were observed for plant height (cm) after 7 days of spray at vegetative stage and at pod filling stage. The mean of 20 random plants height (cm) was taken.

Seed yield

At maturity, seed yield was observed from each plot of all the treatments of every replication. Average weight was recorded of all the treatment in gram per plot.

Number of pod/plant

Randomly selected 20 plants of each plot were observed before harvest maturity. The mean of 20 random plants pod number were taken.

Seed index

Three replication of 100 seeds from each treatment were taken weight for seed index value.

Statistical analysis

Analysis of variance using two was carried out using MSTATC software and the treatment means were compared based on least significant differences (LSD) at $p<0.05$.

RESULTS AND DISCUSSION

Chlorophyll a and Chlorophyll b content

The application of salicylic acid at vegetative stage increased chlorophyll a content in leaves. The chlorophyll a content at this stage varied from 2.70 to 3.24 mg/g of fresh

leaves from different doses of SA as compared to control 2.49 mg/g in fresh leaves (Table 1). Significant increase in the chlorophyll a content was observed than control with the 200 and 100 ppm SA. The percent increase in chlorophyll a content at vegetative stage due to SA application with the doses (50, 100 and 200 ppm) over control was 6.8, 23.7 and 30.1 per cent respectively (Fig 1). Chlorophyll b at vegetative stage ranged from 1.01 to 1.18 mg/g due to SA application against the control 0.97 mg/g of leaves. With 200 ppm and 100ppm SA application there was significant increase in chlorophyll b content than control. The chlorophyll b content increased up to 4.62, 20.72 and 21.84 per cent with application of SA doses 50, 100 and 200 ppm respectively than the control (Fig 1). At pod filling stage (R5), the chlorophyll a content was varied from 3.79 to 4.03 mg/g of fresh leaves with SA application over the control 3.58 mg/g leaves. The per cent increase in chlorophyll a at this stage was 5.72, 10.32 and 12.41 per cent with the SA doses 50,100 and 200 ppm respectively. Chlorophyll b at R5 stage was ranged from 2.45 to 2.80 mg/g mg/g of leaves with the SA treatment over control (2.34 mg/g leaves). There was significant increase in chlorophyll b content with 200 and 100 ppm than control but non significant with 50 ppm SA application (Table 1). The percentage increase in chlorophyll b was 4.69, 18.34 and 19.62 per cent over the control with SA doses (50, 100 and 200 ppm) respectively (Fig 1). In the present study the photosynthetic pigments chlorophyll a and b increased significantly as a result of foliar application of salicylic acid especially with concentration of 200 and 100 ppm at both the vegetative and pod filling stage (Table 1). These findings are similar to those of Ghai and Setia (2002) who showed a considerable improvement in chlorophyll contents due to foliar applied SA (200 mg L^{-1}). Similar result were also found by the Khan *et al.* (2003) when the chlorophyll content of soybean leaves was increased due to foliar application of SA.

Superoxide dismutase activity and malondialdehyde content

Exogenous application of salicylic acid increased the antioxidant enzyme at both vegetative and pod filling stage

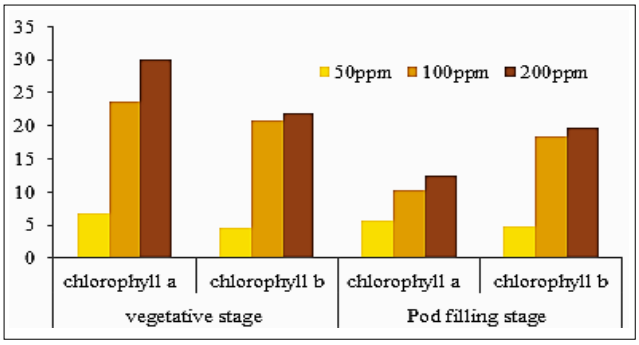


Fig 1: The per cent increase in chlorophyll content due to salicylic acid application over control.

Table 1: Effect of exogenous application of salicylic acid on plant physiological traits of soybean.

Plant physiological parameter	Stage of SA application	Year	SA 50 PPM	SA 100 PPM	SA 200 PPM	Control	LSD at 0.05%
Chlorophyll a (mg/g)	Vegetative stage	Year I	2.9	3.4	3.74	2.64	Y - 0.1465
		Year II	2.4	2.7	2.74	2.35	T - 0.2072
		Mean	2.7	3.1	3.24	2.49	Y×T - 0.293
Chlorophyll b	Vegetative stage	Year I	1.09	1.35	1.36	1.03	Y - 0.05538
		Year II	0.93	0.98	0.99	0.89	T - 0.07832
		Mean	1.01	1.17	1.18	0.97	Y×T - 0.1108
Chlorophyll a	R5 stage	Year I	3.98	4.16	4.25	3.76	Y - 0.4382
		Year II	3.6	3.75	3.81	3.41	T - 0.506
		Mean	3.79	3.95	4.03	3.58	Y×T - 0.7156
Chlorophyll b	R5 stage	Year I	1.449	1.656	1.665	1.386	Y - 0.1591
		Year II	1.352	1.496	1.528	1.288	T- 0.1837
		Mean	2.455	2.775	2.805	2.345	Y×T - 0.2597
SOD Activity	Vegetative stage	Year I	3.08	3.57	3.62	2.90	Y - 0.2605
		Year II	2.89	3.05	3.09	2.77	T - 0.3008
		Mean	2.99	3.31	3.36	2.83	Y×T - 0.4254
	R5 stage	Year I	4.15	4.63	4.8	3.75	Y - 0.1626
		Year II	3.95	4.22	4.43	3.69	T - 0.1878
		Mean	4.05	4.43	4.62	3.72	Y×T - 0.2656
MDAContent ($nmol.g^{-1}$)	Vegetative stage	Year I	0.595	0.565	0.545	0.659	Y -0.3391
		Year II	0.607	0.573	0.558	0.683	T - 0.3916
		Mean	0.601	0.569	0.551	0.671	Y×T - 0.05538
	R5 stage	Year I	0.826	0.758	0.749	0.889	Y - 0.2837
		Year II	0.839	0.796	0.781	0.9	T - 0.3276
		Mean	0.833	0.777	0.765	0.895	Y×T - 0.4633

of soybean. In the present study foliar application of salicylic acid with all the three concentrations (50, 100 and 200 ppm) caused a significant increase in SOD activity as compared to control (Table 1). At vegetative stage superoxide dismutase activity was ranged from 2.99 to 3.36 against control 2.83. The increase in SOD activity with SA (50 100 and 200 ppm doses at vegetative stage was 5.7, 17.0 and 18.7 per cent respectively over the control (Fig 2). At R5 stage the SOD activity due to SA treatment was ranged from 4.05 to 4.62 as compared to control 3.72. Significant higher SOD content was observed with 200 ppm followed by 100 ppm SA foliar spray as compared to control. The increase in SOD activity with SA (50 100 and 200 ppm doses at R5 stage was 8.9, 19.1 and 24.2 per cent respectively over the control (Fig 2). At both vegetative and pod filling stage the superoxide dismutase activity was increased significantly with 200 and 100 ppm of exogenous application of salicylic acid in soybean leaves (Table 1). Stressful environment induce the generation of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) *etc*, in plants thereby creating a state of oxidative stress in them (Panda *et al*., 2003a and b). This increased ROS level in plants cause oxidative damage to bio molecules such as lipids, proteins and nucleic acid, thus altering the redox homeostasis (Smirnoff 1993). The presence of MDA in leaves indicate the extent of membrane injury. In present study it was found that foliar application of salicylic acid influences the amount of production of malondialdehyde content. At vegetative stage the MDA was ranged from 0.551 to 0.601 nmol.g^{-1} with SA treatment as compared to control 0.671 nmol.g^{-1}. Minimum MDA production with 200 ppm foliar application of salicylic acid and maximum with control (Table 1). The reduction in MDA content at vegetative stage was -10.4, -15.2 and -17.9 per cent over control plots leaves with different doses of SA 50,100, 200 ppm respectively (Fig 2). At R5 stage the MDA was ranged from 0.765 to 0.833 nmol.g^{-1} with SA treatment as compared to control 0.895 nmol.g^{-1}. Significant reduction in MDA production at this stage also was with 200 ppm foliar application of salicylic acid. The reduction in MDA content at R5 stage was -6.9, -13.2 and -14.5 per cent over control plots leaves with different doses of SA 50, 100, 200 ppm respectively (Fig 2). In our study each concentration of salicylic acid reduced the production of malondialdehyde and enhances the efficiency of antioxidant system in plants when applied exogenously at vegetative and pod filling stage of crop growth The maximum SOD and minimum MDA was observed with 200 ppm foliar application of SA at both vegetative and pod filling stage which protects plants for oxidative damage which reflect as lower malondialdehyde content in the leaves (Table 1). Similar results were found by (Sadeghipour and Aghaei, 2012) where spray with SA decreases the level of MDA induced by water stress with increasing the production of antioxidant enzymes activities like SOD and APX. Khatun *et al*., (2016) also observed and suggested that spray with salicylic acid acts as one of antioxidant substances concentrated in the chloroplast and protect the photosynthetic apparatus when a plant is subjected to stress, by scavenging the excessively reactive oxygen species known as free radicals. Such effects might be due to protecting the endogenous anti-oxidant systems often correlated with increased resistance to oxidative stress and/or controlling the level of free radicals within plant tissues (Sreenivasulu *et al*., 2000).

Plant height

Plant height was increased with salicylic acid foliar application to soybean at both the stage as compared to control. At vegetative stage the plant height with SA treatment was ranged from 22.42-24.53 cm against the control 21.08 cm (Table 2). The increase in plant height at vegetative stage was 6.4, 11.1 and 16.4 per cent more than control by foliar application of salicylic acid @ 50, 100 and 200 ppm respectively. At pod filling stage the plant height was varied from 63.38-64.55 cm with SA treatment as compared to control 61.71 cm. There was no significant increase in plant height with SA treatment at R5 stage. At both the stage the plant height was observed maximum with 200 ppm SA followed by 100 ppm. Our result was also in agreement with the (Yildirim and Dursun, 2008) where with foliar SA application increase plant growth, early yield and total yield of tomato.

Pod number

The number of pod per plant was influenced by salicylic acid foliar application. Mean pod number was varied from 25.48-29.0 with SA treatment than control 25.11 (Table 2). Maximum number of pod per plant was observed with 200 ppm salicylic acid and minimum with untreated control.

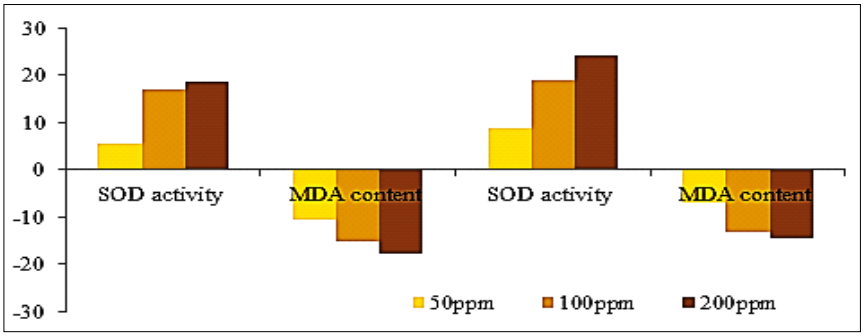


Fig 2: The change in SOD activity and MDA content due to SA application over control.

The increase in pod number at vegetative stage was 2.1, 14.4 and 16.1 per cent more than control by foliar application of salicylic acid @ 50,100 and 200 ppm respectively (Fig 3). SA can increase sink strength via cell division in the immature ovaries and conducts the metabolites stream to the developing grains which leads to reduce the abortion rate (Horvath *et al*., 2007). Our results are also in agreement with (Calvin and Siregar 2019) where the Salicylic acid (150ppm) enhanced the number of pod and seed for Burangrang soybean variety on waterlogged condition compared with normal condition.

Seed index

Seed index varied from 12.26 -12.66g with the SA treatment against control 12.19 g (Table 2). Non significant increase in seed index 0.5, 2.9 and 3.8 per cent than control was observed by foliar application of salicylic acid @ 50,100 and 200 ppm respectively (Fig 3).

Seed yield

It was found that exogenous application of salicylic acid treatment effect on soybean crop resulted with quantum increase in seed yield (Table 2). The mean yield from both the year was varied from 0.476-0.726 g/2.7 m^2 plot area, highest yield with 200 ppm SA and lowest from untreated control (Table 2). Seed yield was significantly improved by 200 followed by 100 ppm salicylic acid. The increase in seed yield due to different concentration of salicylic acid was 0.5, 17.6 and 20.4 per cent from 50, 100 and 200 ppm respectively (Fig 3). The increase in seed yield may be due to increase in the number of pod per plant and stimulant effect on chlorophyll content in soybean leaves as well as increased activity of antioxidant enzyme and reduced oxidative damage which reflects with lower MDA production due to treatment effect of SA. Photosynthetic pigments such as chlorophyll *a* and *b* are chief components of photo system driving the mechanism of photosynthesis and hence growth in terms of biomass production or seed yield (Hussein *et al*. 2007). Similar result was also found by Khatun *et al*., (2016) when SA improved soybean genotypes tolerance to water stress by limited lipid per oxidation, promote antioxidant enzymes activity and improvement yield components and grain yield particularly in Williams genotype. According to

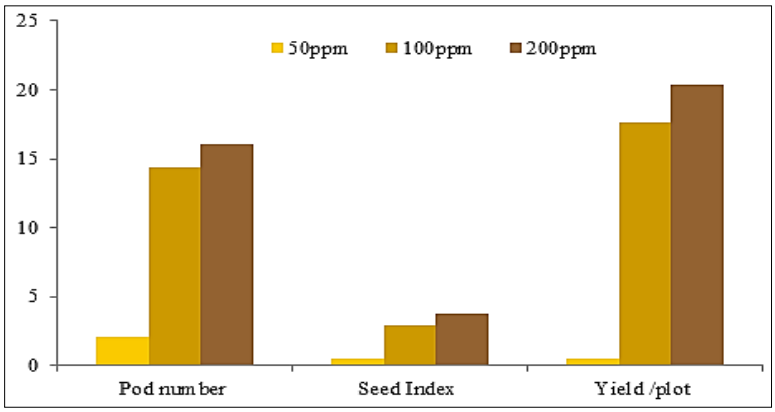


Fig 3: The increase in pod per plant, seed index and yield due to SA application over control.

Table 2: Effect of exogenous application of salicylic acid on plant growth and yield parameters of soybean.

Plant growth and Yield parameter	Stage of SA application	Year	SA 50 PPM	SA 100 PPM	SA 200 PPM	Control	LSD at 0.05%
Plant height (cm)	Vegetative stage	Year I	23.32	23.91	24.85	21.41	Y - 1.221
		Year II	21.52	23.04	24.22	20.75	T - 1.41
		Mean	22.42	23.42	24.53	21.08	Y×T - 1.994
	R5 stage	Year I	64.24	65.1	64.75	62.59	Y – 3.261
		Year II	62.53	63.73	64.36	60.83	T – 3.765
		Mean	63.38	64.41	64.55	61.71	Y×T - 5.325
Seed yield (g/plot)	Effect of SA application at VG and R5	Year I	0.626	0.717	0.736	0.615	Y - 0.0413
		Year II	0.605	0.702	0.717	0.591	T - 0.04633
		Mean	0.606	0.709	0.726	0.603	Y×T - 0.06552
Number of Pod/Pt.	Effect of SA application at VG and R5	Year I	25.91	28.51	29.02	25.46	Y - 1.417
		Year II	25.05	28.59	28.97	24.76	T - 1.636
		Mean	25.48	28.55	29.00	25.11	Y×T - 2.314
100 seed weight (g)	Effect of SA application at VG and R5	Year I	12.24	12.7	12.81	12.18	Y - 1.025
		Year II	12.28	12.40	12.50	12.21	T - 1.184
		Mean	12.26	12.55	12.66	12.195	Y×T - 1.674

Solamani *et al*., 2001, SA treatments were generally effective on vegetative growth, photosynthetic ability and thereby helping in effective flower formation and fruit development and ultimately enhance productivity of the crops. Our result was also in agreement with the (Yildirim and Dursun, 2008) where with Foliar SA application increase plant growth, early yield and total yield of tomato.

CONCLUSION

With the present study it can be concluded that exogenous application of salicylic acid is considered effective technique for improving the plant productivity. Foliar application of salicylic acid increased the chlorophyll content of soybean leaves also influenced the reduction in malonaldehyde content and enhances the efficiency of antioxidant enzyme (SOD) in plants when applied exogenously at suitable stage of crop growth. Although SA 200 ppm resulted with best among the treatment but foliar application with 100 ppm can also be very effective to apply at vegetative and pod filling stage in seed production programme to improve yield and quality of soybean seeds, as the yield with 200 ppm and 100 ppm was not significantly different.

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