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EVALUATION OF INFLUENCE OF SALICYLIC ACID AND EPSOM SALT STRESS ON PLANT GROWTH (*Beta vulgaris* L.).

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ABSTRACT

Beta vulgaris L. is an edible plant of the Chenopodiaceae family. Economically, it is the most important crop consumed as vegetable, processed juice and food colour. The presence of nitrogen-containing water-soluble pigments betalains gives red beet its characteristic colour. The effects of salicylic acid Epsom salt treatments on beet germination and growth were investigated in the current study. The various concentrations (2.5,5.0,7.5,10.0,12.5,15.0,17.5 and 20.0 mScm^{-1}) of Epsom salt were used for soil application and for foliar application the various concentrations of salicylic acid (0.4,0.8,1.2,1.6,2.0,2.4,2.8 and 3.2 mM) was used. In this experiment, germination percentage and the growth parameters like plant height, number of leaves, and leaf area were studied to evaluate the effect of salicylic acid (SA) and Epsom salt (ES) on beet plants. Among all these treatments, a significant increase in height was seen specifically at 1.2 mM of SA at 120 DAG (days after germination) and a linear increase in height was observed at ECe 5, 7.5, 10 mSm^{-1} of ES at 120-days. as well as the Number of petioles increased significantly.

Keywords: *Beta vulgaris,* salicylic acid, Epsom salt, foliar spray, growth parameters.

INTRODUCTION:

Beta vulgaris L. commonly called beet belongs to the family Chenopodiaceae. It contains carbohydrates, proteins, and a major source of vitamins, iron, and other minerals. It is mainly used for food, sugar, and food color production. A dark red variety of beetroot is commonly cultivated in Ahmednagar and nearby regions. Indian soils have become deficient not only in major plant nutrients like phosphorus, nitrogen, and potash, but also in secondary nutrients, like magnesium, sulfur (Ministry of Agriculture, 2012), and an epsom salt, which is also called magnesium sulfate can be useful in the deficient soils. In the case of Salicylic acid, it naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plants such as stomatal closure, organic synthesis, nutrient uptake, chlorophyll synthesis, protein synthesis. Foliar application of Salicylic acid to the plants greater influential in comparison to soil application (Kazemi, 2013), and Plant growth regulators can enhance the physiological performance consisting of photosynthetic potential and thereby assist in effective flower formation, fruit and seed development and in the long run, enhance the productivity of the plants (Solamani et al. 2001).

Magnesium is an essential plant nutrient required for chlorophyll formation and Sulfur is an essential component of proteins. Magnesium deficiency can cause by low-level magnesium in the soil, misuse or overuse of fertilizers, and imbalance/interfere with other nutrients in the soil can affect root uptake (Bir et al., 1988; Finér 1992; Fuksman et al., 1998, Boynton and Erickson 1954; Constable 1954; Drosdoff and Kenworthy 1944; Weber 1955; Williams et al., 1945). The deficiency of Magnesium is most commonly found in sandy, acidic soils

(Heymann-Herschberg 1951; Wallace 1939). In view of this background, the present studies were undertaken to evaluate the effect of salicylic acid and epsom salt on growth parameters of *beta vulgaris*.

MATERIAL AND METHODOLOGY:

The present investigation is aimed to carry out the study of the effects of different concentrations of Salicylic Acid and Epsom Salt treatments on *Beta vulgaris* L. (Beet). The seed of a variety of dark red beetroot selected for the present study was obtained from local seed vendor Ahmednagar, Maharashtra. The pilot experiments have been carried out to determine the optimum concentration for application for further studies. All the experiments were done with replicates. The Heavy-duty plastic pots (34 cm diameter top, 30 cm depth.) were filled with 14 kg of sundried garden soil and a 3:1 ratio of well-rotten compost.

Germination percentage and seedling height: The percentage of germination is calculated from the number of normal seedlings from the total number of seeds evaluated (showing emergence of the radical) kept in germination paper by the paper towel method. During its early stage, which is up to 10 days seedling development was studied. For raising the seedlings, the method suggested by Myhill and Konzak (1967) was used.

Salicylic acid treatment: Plants were treated with various concentrations of Salicylic acid (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, and 3.2 mM), which were sprayed manually using a 1000 mL hand sprayer twice a day for 3 days of every week.

Epsom salt treatment: Fifteen days after thinning, seedlings were treated with epsom salt (Magnesium sulfate heptahydrate) solutions according to the recommendations of U.S.D.A. Handbook No.60 (1954). The salt treatment includes series of increasing ECe (Electrical Conductivity of Soil Saturation Extract) 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 $mScm^{-1}$ of epsom salt maintained throughout the experiment with the help of a pH meter (Hanna, Portable High Range EC/TDS Meter - HI99301P).

RESULTS AND DISCUSSION:

In the present study, the increased levels of salicylic acid and epsom salt shows the effects on the height of plants have been depicted (table1). From the results, it is clear that under application of salicylic acid at 0.4, 0.8- and 1.2 Mm SA significantly increase in germination, while the significantly decrease in germination percentage at a higher level of concentrations of salicylic acid 2.4, 2.8- and 3.2 mM SA over the control at $P<0.05$ level. According to Dotto et al. (2017) pre-treatment with 1-2 mM salicylic acid is one of the most effective techniques to promote germination and growth of sugar beet seedlings. Seed germination and vigor potential are altered by the physiological priming of beet seeds, and the response differs depending on the cultivar and form of conditioning used. Physiological measurements combined with global expression profiling were used to evaluate the effect of salicylic acid (SA) on the elicitation of defense mechanisms in Arabidopsis (Arabidopsis thaliana) seeds and seedlings. The proteomic data revealed a close interplay between abscisic signaling, the observed effects are likely to improve seed vigor and germination under salt stress (Rajjou et al., 2006).

However, in a previous study by Soliman et al., (2016) reported germination decreased as well as seedling growth reduced compared to control in the seed of *Vicia faba* L. by increasing salicylic acid up to three millimolar. Table 1 results was recorded that pretreatment of beet seeds with salicylic acid increased the seedling length of beet seeds compared with higher concentrations and control. Whereas the minimum seedling length was recorded at the highest concentrations 2.4, 2.8-, and 3.2 mM SA which was 1.9, 1.2, 0.9 cm, respectively while the significant increase in seedling length was seen at 0.4, 0.8- and 1.2 mM SA (2.8, 3.5, 4.1 cm). As the concentration of salicylic acid increased, seedling length decreased. The findings of this study clearly show that salicylic acid has a variable effect on seed germination and seedling growth in beet seed, the effect of SA depends upon its concentration.

In the case of Epsom salt, High concentrations of $MgSO_4$ (Epsom salt) decrease germinated seeds of *Oryza sativa* L. cv. MR219 and high salinity delayed seed germination. As a result, it was discovered that at higher salt concentrations, seeds took longer to germinate and the number of germinated seeds was also decreased (Nahid Kalhori, et al., 2018). *Kalidium caspicum* (Chenopodiaceae) seeds were incubated in $MgSO_4$ and other salts showed Mg^{2+} had toxic effects on the radicles and a high proportion of Mg^{2+} with the lower concentrations of Ca^{2+} cations risk the survival of *Kalidium caspicum* radicles (Tobe et al., 2002). Seeds are unable to germinate after prolonged exposure to extreme salinity and temperature stress (Zehra et al., 2013). The inhibitory effect of chloride and sulfate salts of Na^+, K^+, and Mg^{2+} on reduced seed germination of a halophytic grass *Phragmites karka* can be explained by osmotic and ionic effects (Afzali et al., 2011). Rajput et al. (2014) studied in 15 provenances of India, Salts including epsom salt with five level of salinity concentration, which have EC of 3, 6, 9, 12, and 15 dS m^{-1}, sequentially. Electrical conductivity 15 dS m^{-1} was found most deleterious to the seed germination. In our study (Table 1), the significant stimulation of germination over control was observed up to ECe 7.5 mSm^{-1} ES (4.5cm). while higher levels of treatment recorded significant decreased germination percentage and also decreased seedling length.

Growth observations on plant height: For each treatment of salicylic acid (Table 2.) and Epsom salt (Table 3.), the plant growth parameters were measured at maturity. Under the foliar application of salicylic acid, significant increase in plant height and number of leaves over the control at $P<0.05$ level was noticed up to 1.2 Mm SA at 120 DAG (days after germination). Results in this study are in agreement with several reports related to applications of SA increased plant growth and those parameters that were reduced by drought, salinity, and water stress (Souri, M.K., 2019, Mahdi et al., 2013, Sadeghipour and Aghaei, 2012). Salicylic acid is a phenolic endogenous plant growth regulator that is important in the regulation of plant growth, development, and responses to environmental stresses. Exogenous application of S.A. significantly increased nutrient uptake and leaf concentrations of K, Ca, Zn and Fe, as it significantly reduced leaf Na concentration under salinity (Gunes A. et al., 2007).

On the other hand, in plant biology, the role of SA in plant growth and development is still a contentious issue. Salicylic acid dose-dependently plays important role in plant growth under normal and saline conditions. In our study, while low concentrations of exogenous salicylic acid show a positive effect on height and number of leaves, its high concentrations have the opposite effect on these parameters of beet like plant height and leaf area were significantly reduced by 2 - 3.2 mM SA treatment compared to control plants. No or less effect on the number of senescent leaves compared to control.

Foliar application of one millimolar SA significantly increased the Leaf area of coriander was observed over the control (Yeganehpoor, F., et al. 2016). The additive effect of 0.1 mM SA on leaf area under water stress was also reported in *Coriandrum sativum* L., Delavari et al. (2010). In our study, we found at higher concentrations 1.6 - 3.2 mM SA recorded a significant decreased leaf area at maturity. Also, the thickness of leaves at harvest was increased significantly from 1.2 to 3.2 mM SA treatment.

As was pointed out previously by Hernández-Medina, E., (1961), Whether the nutrient was applied to the roots or the foliage of the plants, magnesium was effective in improving pineapple yields significantly. The study by Melanie Hauer-Jkli and Merle Trnkner (2019) confirmed that Plants, regardless of species, respond to adequate Mg availability by reducing oxidative stress and increasing net CO2 assimilation rates. Akter et al. (2020) stated that in aquaponics, epsom salt (magnesium sulfate) plays an important role in reducing harvesting time and improving beetroot yield. In our study compare to control and SA treatment epsom salt shows a significantly positive effect on plant height, leaf area, and the number of leaves at lower concentrations of 2.5 to 7.5 mSm^{-1} ES. There was no or less effect of the number of senescent leaves up to 12.5 mSm^{-1} ES (table 3).

However, at the high concentration of magnesium sulfate, the effects are on the negative side as warned by Nagai, K., 1966, the overuse of magnesium sulfate can be damaging to plants. Magnesium sulfate is a salt, which produces salinity stress to the plant and excessive levels can cause salt injury to plants (Abid et al. 2008, Vijayata et al., 2019). Magnesium excess has resulted in various toxicity symptoms on leaves, veins, and slightly on of region midrib of the tea plant. After the treatments, the plant that received the highest Mg^{2+} died on the 20th day (Venkatesan and Jayaganesh, 2010). In our study (Table 3), higher the electrical conductivity of magnesium sulfate negatively affects the plant growth senescence of leaves increased from EC 15 mSm^{-1}-20 mSm^{-1} ES. At maturity, leaf thickness increased also parameters like plant height, leaf area, and the number of leaves affected negatively as the EC increased in the soil.

Dry Matter Production: At the time of harvesting two sets of plants were used to make determinations for the dry matter production study. A study of senescent was conducted after the initiation of the senescence.

Unless there are general stress circumstances, exogenous plant growth regulators aren't required. However, there are conflicting data on the potential benefits of salicylic acids on plant growth under normal conditions. In comparison to salinity alone, particularly pretreatment of foliar SA increased root fresh and dry weights, leaf proline, and soluble sugars concentrations (Souri, M.K.,2019). Foliar salicylic acid treatment significantly reduced the harmful effect of salinity and improved the dry matter and other parameters of the tomato plant (Naeem et al., 2020).

The observations of the increasing levels of foliar salicylic acid with their effects on the dry weight of plants have been depicted in table 5. From the results, it is clear that under foliar application of the salicylic acid significant increase in dry weight of root is noticed on the concentration of 0.4, 0.8, and 1.2 over the control at $P<0.05$ level, and a significant decrease was noticed from 1.6 mM, followed by a 2, 2.4, 2.8- and 3.2 Mm SA. A significant increase in leaf dry matter was noticed up to 2 mM SA treatment and the decrease in the dry matter can be seen at 2.8- and 3.2 mM SA. There was no increase in the senescent leaves but the significant decrease can be seen at 1.6 to 3.2 mM of salicylic acid treatment. Total dry matter can be seen decreasing as the level of concentration of the treatment is increasing from 0.4 mM to 3.2 mM SA.

Treatment 0.4 to 1.2 mM SA is showing a positive effect on dry matter content of root and leaf of the beetroot. Negative effect on the dry matter at higher levels.

Application of magnesium sulfate and supplemental L-ascorbic acid on timothy grass increased the dry matter yield and the content of organic and mineral components (Radkowski A. et al.,2017). Magnesium deficiency has a big impact on how dry matter and carbohydrates are distributed between shoots and roots. An increase in photosynthesis may have occurred as a result of $MgSO_4$ application, resulting in increased dry matter production in *Carthamus tinctorious*. Magnesium fertilizer has influenced plant growth by increasing the amount of foliage and dry mass-produced (S.V. Khadtare et al., 2017). Magnesium treatment increased tomato yield significantly over the control. Also, a similar trend was observed in dry matter correlated with the fruit yield. On the other hand, Plant height and the number of branches per plant were both reduced at a higher level of mg application (Kasinath et al., 2015).

The observations of the increasing levels of Epsom salt with their effects on the dry weight of plants have been depicted in table 5. From the results, it is clear that under application of the Epsom salt significant increase in dry weight of root is noticed on the concentration of ECe 5.0, to ECe 12.5 mSm^{-1} over the control at $P<0.05$ level, and a significant decrease was noticed from ECe 15.0, 17.5, and 20.0 mSm^{-1}. A significant increase in leaf dry matter was noticed up to ECe 12.5 mSm^{-1} treatment and the significant decrease in the dry matter can be seen at ECe 15, 17.5, and 20.0 mSm^{-1}. There was no significant increase in the senescent leaves but the significant decrease can be seen at ECe 12.5 to 0.0 mSm^{-1} of Epsom salt treatment. Total dry matter can be seen decreasing as the level of concentration of the treatment is increasing from ECe 2.5 to 20.0 mSm^{-1}.

ECe 5.0, to ECe 12.5 mSm^{-1} treatment is showing a positive effect on dry matter content of root and leaf of the beetroot. negative effect on the dry matter at higher levels of EC.

CONCLUSION:

In conclusion, our results indicate that in response to the application of Salicylic acid and Epsom salt treatments increased productivity leading to increased biomass is possible. Epsom salt may improve the photosynthetic activity leading to enhancement in leaf surface area, accumulation of biomass, increase in root size. The SA was found to be effective in the different forms of application foliar spray, the low concentration of SA showed advantageous effects in tolerance of plants. In contrast, the high concentration of SA showed toxic effects. Thus, both the concentration and application method of SA are critical to obtaining its best effect on beetroot.

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Table 1: Study on Germination Percentage and Seedling Length in Beta Vulgaris L. Under Increasing Concentrations of Salicylic Acid.

	Treatments	**Germination Percentage (%)**	**Seedling Length (cm)**
	Control	68	2.8 ± 0.4
Salicylic Acid	0.4 mM	60	3.5 ± 0.6*
	0.8 mM	55	4.1 ± 0.5*
	1.2 mM	60	4.1 ± 0.6*
	1.6 mM	73	3.3 ± 0.4
	2.0 mM	75	2.7 ± 0.6
	2.4 mM	62	1.9 ± 0.5#
	2.8 mM	69	1.2 ± 0.3#
	3.2 mM	56	0.9 ± 0.1#
Epsom Salt	2.5 mSm^{-1}	71	4.4 ± 0.2*
	5.0 mSm^{-1}	65	4.6 ± 0.2*
	7.5 mSm^{-1}	70	4.5 ± 0.6*
	10.0 mSm^{-1}	56	3.5 ± 0.3
	12.5 mSm^{-1}	52	2.3 ± 0.7
	15.0 mSm^{-1}	56	2.1 ± 0.7
	17.5 mSm^{-1}	54	1.5 ± 0.9#
	20.0 mSm^{-1}	41	1.2 ± 1.0#

*Significantly higher than control at probabilities of 0.05. # Significantly lower than control at probabilities of 0.05.

Fig 1: Progressive height in *beta vulgaris* l. at 120 days under application of salicylic acid.

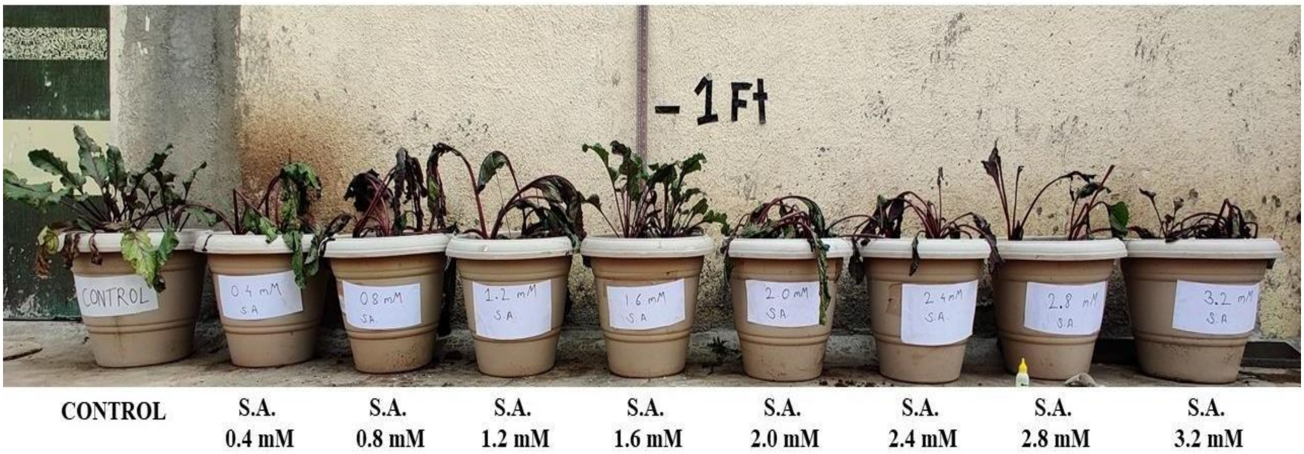

Fig 2: Progressive height in *beta vulgaris* l. at 120 days under application of epsom salt.

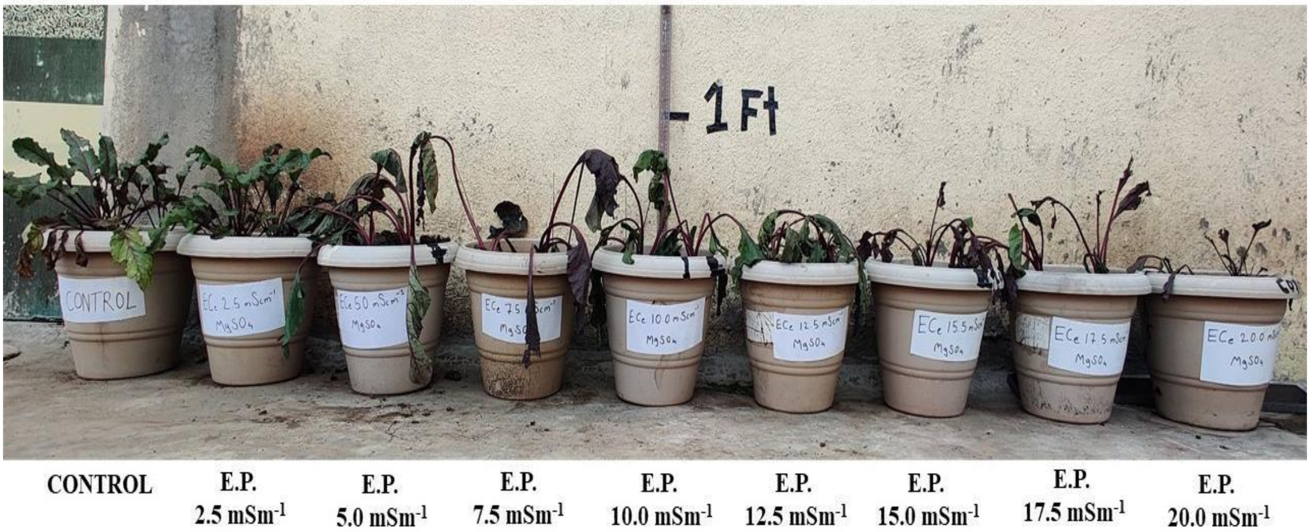

Table 2: Study on Mean Progressive Growth in Beta Vulgaris L. Under Increasing Concentrations of Salicylic Acid.

Treatments	**Plant Height (cm)**	**Leaf area (cm^2)**	**Leaf Thickness (µm)**	**No. of green Leaves**	**No. of Senescent leaves**
Control	24.38 ± 0.54	302 ± 4.4	169 ± 7.0	10 ± 0.6	8 ± 1.0
0.4 mM SA	25.3 ± 0.58	291 ± 4.6	225 ± 6.0	13 ± 1.1*	7 ± 1.0
0.8 mM SA	25.0 ± 0.50	282 ± 7.8	277 ± 6.6	12 ± 0.9	6 ± 1.7
1.2 mM SA	26.5 ± 0.72*	293 ± 6.9	297 ± 7.0*	14 ± 1.1*	8 ± 1.7
1.6 mM SA	23.50 ± 0.52	242 ± 7.9#	317 ± 5.3*	12 ± 1.8	9 ± 1.0
2.0 mM SA	23.0 ± 0.50 #	181 ± 7.5#	365 ± 6.0*	10 ± 0.8	7 ± 1.7
2.4 mM SA	23.4 ± 0.72 #	119 ± 7.5#	437 ± 3.6*	9 ± 1.0	8 ± 1.0
2.8 mM SA	23.0 ± 0.52 #	119 ± 6.2#	485 ± 5.6*	8 ± 0.8	6 ± 1.7
3.2 mM SA	20.0 ± 0.4 #	116 ± 3.6#	533 ± 6.9*	8 ± 1.8	6 ± 1.0

*Significantly higher than control at probabilities of 0.05. # Significantly lower than control at probabilities of 0.05.

Table 3: Study on Mean Progressive Growth in Beta Vulgaris L. Under Increasing Concentrations of Epsom Salt.

Treatments	Plant Height (cm)	Leaf area (cm^2)	Leaf Thickness (µm)	No. of green Leaves	No. of Senescent leaves
Control	24.38 ± 0.54	302 ± 4.4	169 ± 7	10 ± 0.6	8 ± 1.0
2.5 mSm^{-1}	24.6 ± 0.33	322 ± 7.2*	205 ± 7.0	12 ± 1.0*	8 ± 1.0
5.0 mSm^{-1}	27 ± 0.75 *	296 ± 6.2	277 ± 5.0*	12 ± 0.6*	9 ± 1.7
7.5 mSm^{-1}	28 ± 0.6 *	306 ± 6.9	285 ± 5.3*	14 ± 0.8*	7 ± 1.0
10.0 mSm^{-1}	29.3 ± 0.38 *	231 ± 7.5#	309 ± 5.3*	13 ± 1.3*	8 ± 1.0
12.5 mSm^{-1}	24.6 ± 0.36	243 ± 5.6#	349 ± 7.0*	11 ± 0.3	10 ± 1.7
15.0 mSm^{-1}	23 ± 0.26#	119 ± 7.2#	413 ± 7.2*	8 ± 0.9#	11 ± 1.0*
17.5 mSm^{-1}	18 ± 0.26 #	127 ± 7.0#	445 ± 6.0*	6 ± 0.8#	12 ± 1.0*
20.0 mSm^{-1}	16 ± 1.00 #	129 ± 2.6#	473 ± 5.0*	6 ± 1.1#	11 ± 1.7*

*Significantly higher than control at probabilities of 0.05. # Significantly lower than control at probabilities of 0.05.

Table 4: Study on Dry Weight and Total Dry Matter in Beta Vulgaris L. Under Increasing Concentration of Salicylic Acid (SA).

Treatments	**Root dry weight (gm)**	**Leaf Dry weight (gm)**	**Weight of Senescent Leaves (gm).**	**Total Dry Matter (gm)**
Control	16.03 ± 0.2	4.84 ± 0.2	3.67 ± 0.1	24.54 ± 6.8
0.4 mM SA	18.61 ± 0.1*	5.98 ± 0.1*	3.61 ± 0.2	28.2 ± 8.1
0.8 mM SA	17.32 ± 0.2*	5.58 ± 0.2*	3.64 ± 0.2	26.54 ± 7.4
1.2 mM SA	20.98 ± 0.1*	7.16 ± 0.2*	3.02 ± 0.1	31.16 ± 9.4
1.6 mM SA	15.01 ± 0.2#	5.39 ± 0.1*	2.24 ± 0.2#	22.64 ± 6.7
2.0 mM SA	13.62 ± 0.4#	5.13 ± 0.2*	2.23 ± 0.2#	20.98 ± 5.9
2.4 mM SA	13.03 ± 0.2#	4.74 ± 0.2	2.12 ± 0.1#	19.89 ± 5.7
2.8 mM SA	11.76 ± 0.2#	3.06 ± 0.1#	1.53 ± 0.2#	16.35 ± 5.5
3.2 mM SA	7.16 ± 0.1#	2.03 ± 0.1#	1.02 ± 0.2#	10.21 ± 3.3

*Significantly higher than control at probabilities of 0.05. # Significantly lower than control at probabilities of 0.05.

Table 5: Study on Dry Weight and Total Dry Matter in Beta Vulgaris L. Under Increasing Concentration of Epsom Salt.

Treatments	Root dry weight in (gm)	Leaf Dry weight (gm)	Weight of Senescent Leaves (gm)	Total Dry Matter (gm)
Control	16.03 ± 0.2	4.84 ± 0.2	3.67 ± 0.1	24.54 ± 6.8
2.5 mSm^{-1}	15.02 ± 0.1	5.81±0.2*	3.23 ± 0.2	24.06 ± 6.2
5.0 mSm^{-1}	18.83 ± 0.3*	6.91±0.1*	3.18 ± 0.1	28.92 ± 8.2
7.5 mSm^{-1}	22.16 ± 0.1*	7.43±0.2*	2.22 ± 0.2	31.81 ± 10.3
10.0 mSm^{-1}	20.53 ± 0.2*	7.51±0.2*	2.32 ± 0.1	30.36 ± 9.4
12.5 mSm^{-1}	16.6 ± 0.2*	5.66±0.1*	1.98 ± 0.1#	24.24 ± 7.6
15.0 mSm^{-1}	13.41 ± 0.1#	4.44±0.2#	1.57 ± 0.2#	19.42 ± 6.2
17.5 mSm^{-1}	10.1 ± 0.1#	2.93±0.1#	0.93 ± 0.1#	13.96 ± 4.8
20.0 mSm^{-1}	8.92 ± 0.1#	1.81±0.2#	0.86 ± 0.1#	11.59 ± 4.4

*Significantly higher than control at probabilities of 0.05. # Significantly lower than control at probabilities of 0.05.