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Effect of salinity on Growth and Secondary Metabolites of Sesbania Grandiflora seedlings: An Analytical Study

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Abstract— *Plants can undergo different types of stresses like biotic and abiotic stresses. Plants develop mechanisms to overcome these threats of biotic and abiotic stresses, which influence secondary metabolite production, enhancing the potential to overproduce useful phytochemicals for varied applications. Salt stress has a major impact on Agriculture. It results in losses of 806.4 billion rupees per year due to low production yield. Salinity affects plants in various aspects including water stress, ion toxicity, nutritional imbalance, oxidative stress, metabolic processes, membrane disorganization, cell division, and genotoxicity. Using in vitro plant cell culture to produce chemicals and pharmaceuticals has made great strides. A soft wooded tree, Sesbania grandiflora has many significant medicinal values that have been proven scientifically. It is known to work as an immune modulator along with the property of nitrogen fixation. A study was conducted to determine the effect of NaCl salinity on growth, morphological analysis, and phytochemical content in Sesbania grandiflora using in vitro method. Seeds of Sesbania grandiflora germinated on 10, 20, and 30mM NaCl salt concentrations for varying durations, and total flavonoid and phenolic content production was calculated to observe the correlation between salt stress and phenolic production. Thus, the salt stress effect and its effect on the phytochemicals were observed. The present study findings provide additional information for the accumulation of phytochemical compounds under salt stress in the plant which might help to explain the increased levels of secondary metabolites in other plant species.*
Keywords— ***Phenolics, Quantitative analysis, Salinity, Salt Stress, Sesbania grandiflora***

I. INTRODUCTION

Salinity is a major factor that affects crop production and agricultural sustainability in many regions of the world. It reduces the value and productivity of the affected land and has been seen for years. It has a major impact on crop production in arid and semi-arid regions and causes an agricultural problem under rain-fed conditions when annual precipitation is not enough to leach excessive salts and prevent salt accumulation in the root zone. (1) Cell and tissue culture systems have been considered relevant for the selection of plant species tolerant to salinity, drought, and other kinds of stresses (2)(3) A Salt environment leads to cellular dehydration which causes osmotic stress and removal of water from the cytoplasm resulting in a reduction of the cytosolic and vacuolar volumes. Salt stress often creates both ionic as well as osmotic stress in plants resulting in the accumulation or decrease of specific secondary metabolites in plants. (4) One of the most important factors affecting plant growth and the production of secondary metabolites is salt stress (5). Sesbania grandiflora, commonly known as Agastya or Heti, is a soft-wooded tree that belongs to the family Leguminosae [17][18]. It is a perennial tree and grows up to a height of 10 m. It is used as human food and animal fodder [19]. Although the exact origin of S. grandiflora is not known, it is widely distributed in parts of West Bengal, Assam, and Karnataka [20]. Since ancient times almost every single part of S. grandiflora has been used as traditional medicine to

treat an array of diseases such as dysentery, stomatitis, fever, smallpox, sore throat, and headache. This plant is also used in Indian traditional systems of medicine, Siddha, and Ayurveda, for the treatment of various acute and chronic diseases. Numerous reports have also mentioned the isolation of certain bioactive constituents (such as sterols, phenols, tannins, flavonoids, etc.) from the leaves, flowers, and aerial parts of the plant [21]. These chemical constituents are well known for their potential health benefits and have been reported to possess valuable biological activities such as antibacterial, antifungal, and antioxidant properties. Other than this,H it can fix atmospheric nitrogen and can be used as a green manure to improve soil conditions. So, the diversity of its use surely demands more research [22]. In various research, it was observed that the high levels of salinization induced a significant decrease in the contents of pigment fractions (chlorophyll a and b) and consequently of the total chlorophyll content as compared with control plants [23]. It appears at the beginning of growth that S. grandiflora seedlings are not deficient in carbohydrates and that the supply of carbon compounds is not limiting their growth. So, after prolonged periods of exposure to salinity, the levels of reserve carbohydrates increased particularly in the leaves [23] Antioxidants are the compounds that inhibit substrate oxidation possibly by acting as a free radical scavenger [24]. Free radicals are any species that contain one or more unpaired electrons that react with other molecules by taking or giving electrons [25]. When the amount of these highly unstable free radicals exceeds in the body, it damages the cells and may lead to several diseases. Thus, there is a need for antioxidants of natural origin to protect the human body from diseases caused by free radicals [25]. These natural antioxidants are present in plants [25] especially medicinally important plants. These plants produce bioactive secondary metabolites (such as phenols, flavonoids, etc.) naturally hence preventing several life-threatening degenerative diseases such as cancer caused by oxidative stress [24]. The oxidative stress leads to the excess production of highly reactive oxygen species (ROS) or reactive nitrogen species (RNS) that are extremely harmful to the cells causing cell death. Therefore, it is necessary to determine plant tissue containing many antioxidants for novel drug discovery purposes [24]. An increase in population and increasing demand for plant products along with illegal trades are causing the depletion of medicinal plants [26]. For this reason, the plant tissue culture technique has proved a potential alternative for the production and enhancement of the desirable bioactive components from the plants, to produce enough of the plant material that is needed and for the conservation of the threatened species [26]. Reduced rates of cell division and cell elongation due to stress are the main causes of reduced growth of plants under stress [27] Synthesis and accumulation of organic osmolytes, enhanced activities of antioxidant enzymes, and efficient compartmentalization of toxic ions into other cellular compartments like vacuoles help plants to avert stress-induced damage.[32] Hence, the present analytical study enlightens the effect of salinity on the growth and secondary metabolites of Sesbania Grandiflora seedlings.

II. MATERIALS AND METHODS

Collection of Seeds: Sesbania grandiflora seeds were procured from Numinous India, a seed distributor from Andhra Pradesh.

Surface Sterilization and Inoculation of seeds for germination: The seeds were surface sterilized to produce contamination-free plants that can be maintained under aseptic in vitro conditions. Seeds were checked for viability and were washed 2-3 times with tap water followed by washing 2 times consequently with distilled water and detergent. Seeds were then transferred to the sterilized laminar cabinet where $HgCl_2$ (0.1%) treatment was given to the seeds for 10 minutes with continuous stirring, which was then rinsed 2-3 times with distilled water followed by soaking it in a sterile tissue paper to remove excess water. Sterilized seeds were inoculated aseptically on Murashige and Skoog media (half strength,1L). 6 flasks were supplemented with different concentrations of salt. The inoculated flasks were then transferred to the culture room with controlled light and temperature.

Preparation of MS media (half strength): The medium was prepared such that for 1 L, 2.1 g of MS media (half strength) was dissolved in 1000 ml of distilled water with the addition of 8 gm agar per 1000 ml. Before autoclaving, the medium was buffered with 1N NaOH and 1N HCl to adjust its pH to 5.6–5.8 [8]. Preparation of MS media (half strength) for giving salt stress: A solution of NaCl was prepared by adding 58.44 gm salt per 1 liter. Salt stress of 10 mM,20 mM, and 30 mM for 15 Days,30 days, and 45 Days respectively was given. MS media solution for each concentration of NaCl i.e., 10 mM, 20mM, and 30 mM molar concentration on each solution was prepared. The dissolved medium was autoclaved at 15 lbs. pressure at 121°C for 20 minutes. Seeds were inoculated in the respective flasks along with control seeds for each treatment.

Determination of Toxicity: Toxicity was determined by visual screening of – In vitro-grown plantlets supplemented with different salt concentrations. Proliferation of seed growth based on salt concentration was observed. This was

determined based on seed germination rate, survival count of germinated plantlets, and plant biomass yield obtained (i. e. fresh weight and dry weight). Plantlets that grow with less proliferation, low survival count, and low biomass yield were referred to be toxic, As compared to control flasks (without salt concentration) germination of those with salt concentration was observed late, Germination of seeds was observed lowest in the highest concentration of salt, which is 30mM. 0 mM salt concentration> 10 mM Salt concentration> 20 mM salt concentration > 30 mM salt concentration. After respective periods of respective salt concentration plantlets were taken out from the growth room and morphological analysis was done. Stem length, root length, leaf lamina, No. of leaves were measured.

Preparation of extract: Enough plantlets (Normal, 10 mM,20 mM,30 mM) obtained were air dried and pulverized into powder using mortar and pestle separately such that each powder owns different concentrations of NaCl i. e. Normal, 10 mM, 20 mM, 30 mM. The drying process is important for the extraction of plant materials, the fresh plant materials have the active enzymes that produce the active constituents' intermediates and metabolic reactions in the plant materials [25]. The initial weight of powdered samples (0mM, 10mM, 20mM, 30mM) was measured and the pre-weight of the round water bottle was also measured. This process was carried out at 40-50°C adding 500mg per 10 mL of powder.

Hot water extraction process - Extraction solvent is taken in the round bottom flask and heated by using a heating mantle. After the Hot water extraction process the extracted material was concentrated by evaporating the solvent thereby reducing it into a semisolid mass [26].

The concentrated extract of different salt concentrations (0mM, 10mM, 20mM, 30mM) was stored at 4°C in the refrigerator until further use. These extracts were then subjected to phytochemical screening for the identification of different phytochemical analyses such as Quantitative and Qualitative analysis.

2.1 PHYTOCHEMICAL SCREENING

Three extracts (0mM, 10mM, 20mM, 30mM) of 15 days,30 days, and 45 days thus obtained were subjected to preliminary phytochemical screening following the standard protocols.

Phenols: 500µl of samples were added in 1 ml of 2% $FeCl_3$. The appearance of green, blue, or black color represents the presence of phenols. The absence of green, blue, or black color represents the absence of phenols

Saponins: 500 µl of the sample were added in 2 mL of Distilled water, after shaking vigorously, if stable soap forms it represents the presence of Saponins.

Steroids: 500 µl of sample added in 2 ml of chloroform then in this solution addition of conc. H_2SO_4 (drop by drop), the Appearance of Red color in the Chloroform layer represents the presence of Steroids.

Glycosides: SALKOWSKI'S TEST:500µl of sample and 1 ml of chloroform and the same volume of conc. H_2SO_4 was added in a test tube, the appearance of bluish-red to violet-red represents the presence of Glycosides.

Flavonoids: SHINODA TEST: 4 Pieces of Mg ribbon added into 500 µl of sample and conc.H_2SO_4 added drop by drop, reddish or orange color represents the presence of Flavonoids.

Terpenoids: 500µl of sample added in 1 ml of Chloroform. And left to evaporate to dryness, after chloroform is completely evaporated 2 ml conc. H_2SO_4 was added after heating the solution for 2 minutes. The appearance of gray color represents the presence of Terpenoids.

2.2 ANALYSIS OF ANTIOXIDANT ACTIVITY

TOTAL PHENOLIC CONTENT: The total phenolics of the samples i.e. 0mM, 10mM, 20mM, 30mM were determined using Folin and Ciocalteu reagent following the method of Shubhangi et.al [31] with some modifications.

Preparation of solutions:

1. Folin and Ciocalteu reagent- 1.5 ml of Folin and Ciocalteu's reagent (10-fold diluted) was prepared.
2. Sodium Carbonate- 50 ml of 7.5 % of Na_2CO_3 was prepared such that 3.75 g of Na_2CO_3 was dissolved in 50 ml of distilled water.
3. Gallic acid solution- the solution was prepared at a concentration of 20-100 µg/ml to obtain a standard curve at 760 nm.
4. Test Samples- 0mM, 10mM, 20mM, 30mM salt concentration sample.

After mixing, the solutions were further incubated for 30 minutes at room temperature. The absorbance of the tubes was measured at 760 nm. All the determination was performed in triplicate.

2.3 TOTAL FLAVONOID CONTENT- The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample i.e-0mM, 10mM, 20mM, 30mM following the method described by Shubhangi et.al [31] with slight modification.

III. RESULTS AND DISCUSSION

Morphological Analysis: The impact of Salt Stress on in vitro-grown plantlets was estimated by visual screening and *in vitro*-grown plantlets supplemented with different salt concentrations.

Fig.1. Systematic picture of grown plantlets in different concentrations.

Remarkable changes were observed in the leaves of plantlets, as compared to the plants of 0mM concentration color of the leaves was fading and they looked faint green, a reduction of chlorophyll is seen in plants with higher salt concentration. From the below data, we can interpret more about the germination rate which is reducing as we are increasing salt conc.

An inverse relationship is observed between salt concentration and seed germination rate when done Annova test with the germination data salt concentration %. With increasing salt concentration germination rate is decreasing. High salt levels in the media create osmotic stress which is making plants difficult to absorb water and nutrients essential for germination. As a result, higher salt concentrations can inhibit germination.

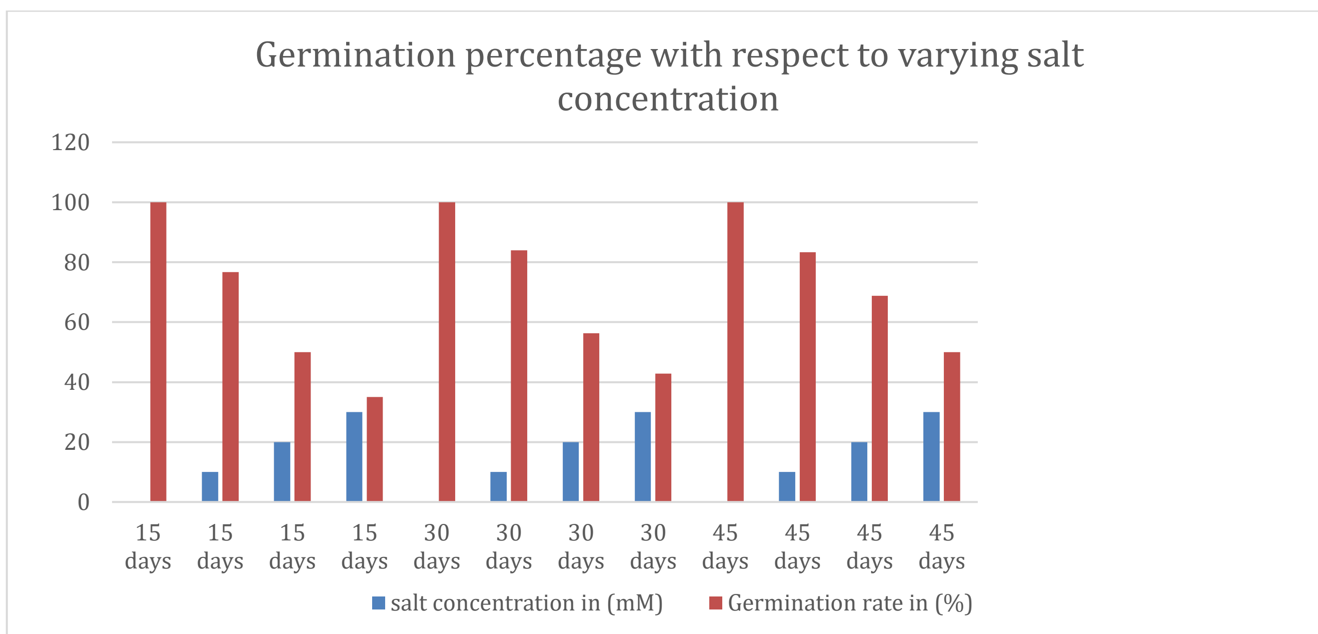


Fig.2: Effect on in vitro grown seedlings with and without Salt stress

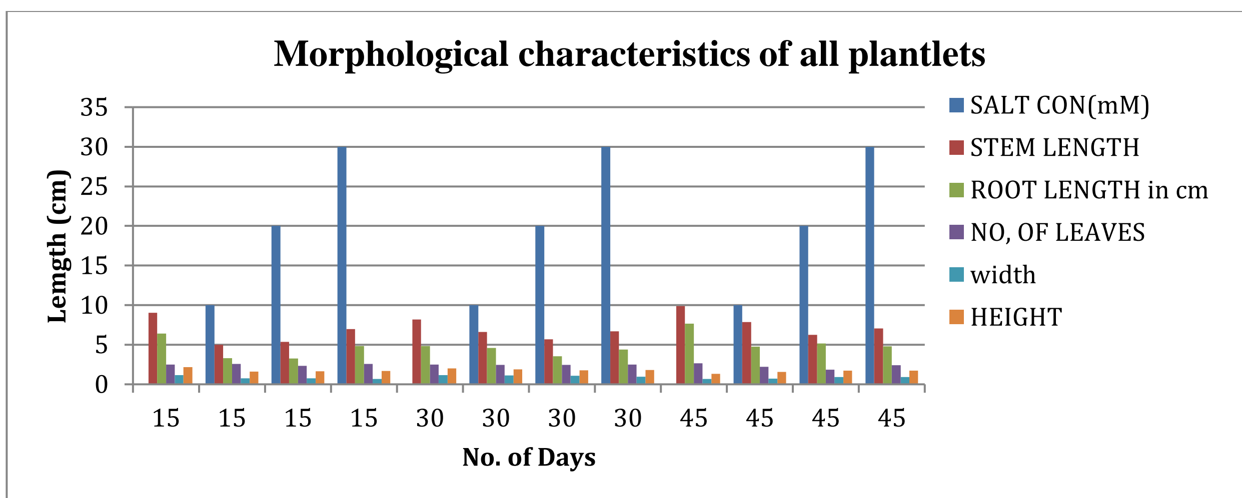


Fig.3. Morphological data of Plantlets in different concentration

It was observed that 0 mM concentration has a normal growth rate as compared to plantlets grown in (10, 20, and 30 mM) salt concentrations. High levels of salinization, (20mM and 30 mM) induce a significant decrease in Stem length, root length, and leaf lamina, It was also noticed that the root length in 45 days increased as compared to those in 30 days and 15 days, so the root length has shown significant growth with the increase in the duration. There is no change in no. of leaves in all concentrations. Overall morphological effect states that salt toxicity affects plants in such a way that they try to complete their life cycle earlier as compared to the control plant.**PHYTOCHEMICAL SCREENING**

Qualitative Analysis: Qualitative phytochemical analysis of plant extract of *Sesbania grandiflora* with concentrations 0mM,10mM,20mM, and 30mM represented below table which represents the presence of phytochemicals such as phenols, saponins, steroids, glycosides, terpenoids in plants with different salt concentration i.e. 10 mM, 20mM, 30mM kept for 15, 30 and 45 days respectively

Table 1. Phytochemical screening of Sesbania grandiflora with and without salt stress

Sr No,	Name of test	Plant Extract											
		15 days salt conc (in mM)				30 days salt conc (in mM)				45 days salt conc (in mM)			
		0	10	20	30	0	10	20	30	0	10	20	30
1	Phenolics	+	+	+	+	+	+	+	+	+	+	+	+
	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
	Steroids	+	+	+	+	+	+	+	+	+	+	+	+
	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+

(+) Indicates presence; (-) Indicates absence

The preliminary phytochemical analysis revealed the presence of Phenolics, Saponins, Terpenoids, Flavonoids Steroids, and glycosides in plant extract of *Sesbania grandiflora* with different salt concentrations. The addition of optimal concentration of salt did not result in any loss of medicinally active compounds found in the plant.

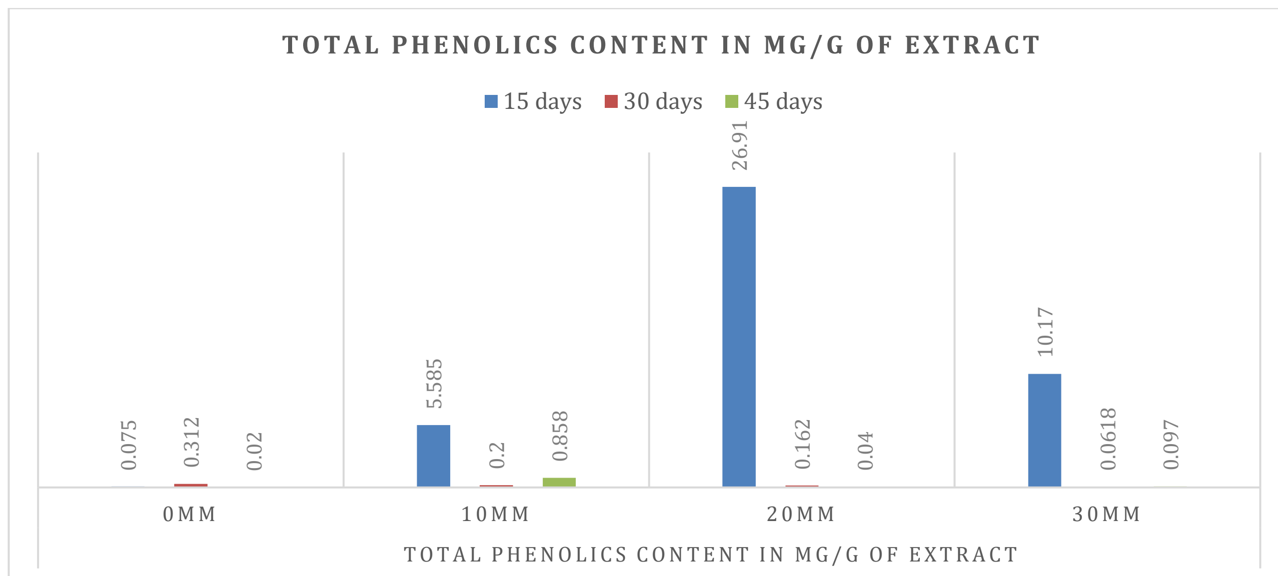
As we know the medicinal uses of secondary metabolites of this sesbania grandiflora plant, the above steroids are present in all salt concentrations, there is no remarkable changes are seen in qualitative analysis, Quantitative analysis of Phenols and Flavonoids is carried out to check the increment or reduction on the quantity of secondary

metabolites.

PHENOLICS CONTENT

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging [34]. As a basis, phenolic content was measured using Folin- Ciocalteu reagent in each extract C and was depicted in the table given below.

Table 2: Concentration of total phenolic content in the samples



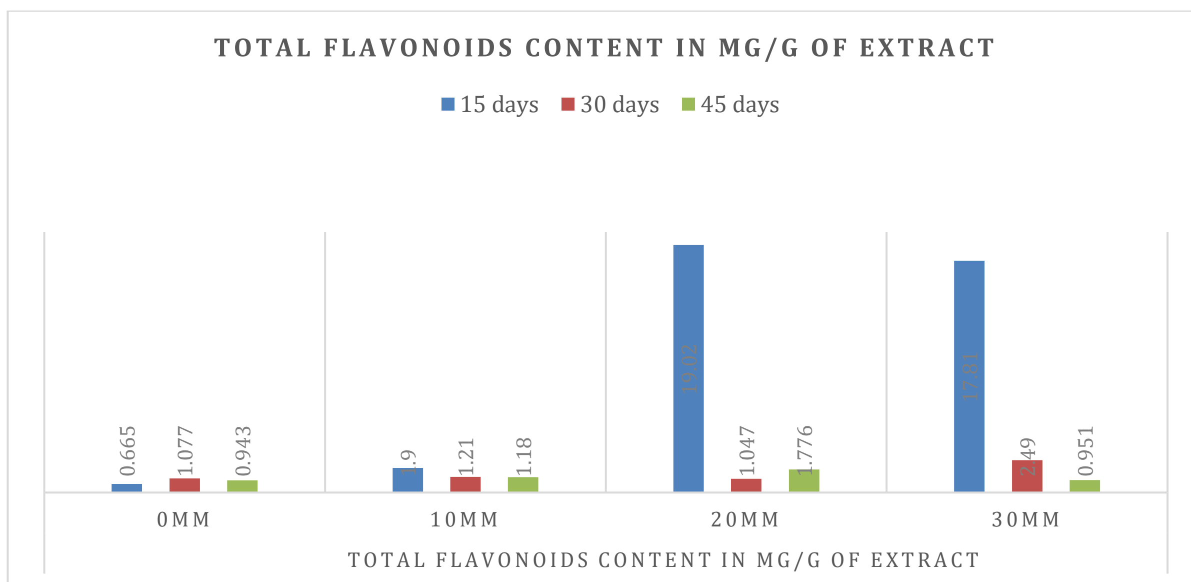
The results were derived from the calibration curve (Y=7.844x+0.046, R^2=0.988) of gallic acid(20-100 µg/ml) by measuring the absorbance of samples at 765 nm and was expressed in gallic acid equivalents (GAE) per gram dry extract weight.

TOTAL FLAVONOIDS CONTENT

Flavonoids, including flavones, and flavanols are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and act as antioxidants *in vivo* [35].

As a basis, Flavonoid content was determined using the aluminum chloride colorimetric method in each extract (0mM, 10mM, 20mM, 30mM) and was depicted in the table given below.

Table 3: Concentration of total flavonoid content in the samples



The Results were derived from a calibration plot (Y=1.6769x-0.0274, R^2=0.9930) of quercetine (50-500 µg/ml) by measuring the absorbance of samples at 415 nm and was expressed in quercetine equivalent (QE) per gram dry extract weight. Annova test conducted on the provided data suggests that there is a significant difference in the total flavonoid content among the various concentrations and periods

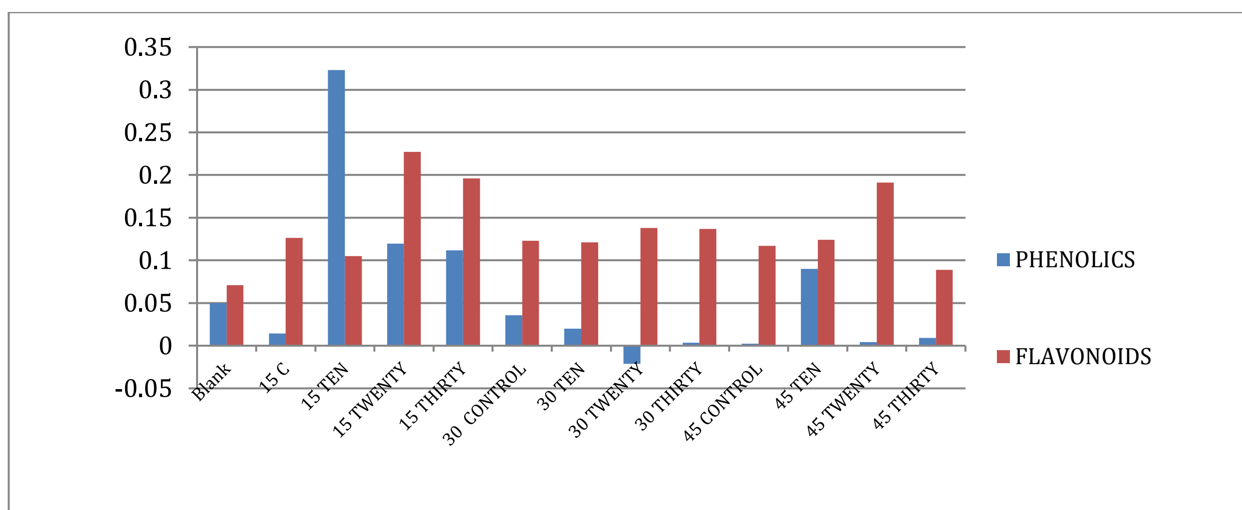


Fig.4. The total phenolic content and total flavonoid content

The table represents the impact of salt concentration on phenolic and flavonoid content in plantlets over different periods. Initially, no salt concentration control plantlets showed lower phenolic content when compared with 10 Mm salt concentration which shows an increase in phenolic levels. AT 20 Mm salt concentration, a decrease in phenolics is observed. Interestingly, a significant growth in phenolic content was noted in 10 Mm concentration after 45 days. Flavonoids showed higher levels of phenolic content in control plants at 15 days compared to 10Mm and 45 days.

Statistical Analysis - The experiment was performed in triplicates and results were represented as mean ± SE. The significance of the results of each experiment was checked by Student's t-test and ANOVAs using Microsoft, Excel. P < 0.05 was considered to suggest statistical significance.

The most significant salt level and duration of salt treatment for maximum phenolic production was found to be 10 Mm salt concentration after 45 days. The remarkable growth in phenolic content was observed at this specific salt level and time point. There is a noticeable increase in the phenolic content compared to other salt levels and time durations.

IV. CONCLUSION

10 Mm salt concentration for a longer duration is sufficient to trigger the secretion of secondary metabolites. This can be helpful for improved metabolite production from this important medicinal plant.

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REFERENCES

[1] Bolarin, M.C.; Perez-Alfocea, F.; Cano, E.A.; Estan, M.T.; Caro, M. "Growth, fruit yield and ion concentration in tomato genotypes after pre and post-emergence salt treatments". J. Am. Soc. Hort. Sci. 1993, 118, 655–660.T

[2] Lutts, S.; Kinet, J.M.; Bouthamont, J.M. Improvement of rice callus regeneration in the presence of NaCl. 1999, 57, 3–11.

[3] Rus, A.M.; Panoff, M.; Perez-Alfoceaf, F. Bolarin, M.C. NaCl responses in tomato calli and whole plants. J. Plant Physiol. 1999, 155, 727–733.

[4] Mahajan S, Tuteja N. Cold, salinity and drought stresses: An overview. Arch Biochem Biophys 2005; 444:139-58; PMID:16309626; DOI:10.1016/j. abb.2005.10.018

[5] Nikolova MT, Ivancheva SV. Quantitative flavonoid variations of Artemisia vulgaris L. and Veronica chamaedrys L. in relation to altitude and polluted environment. Acta Biol Szegediensis 2005; 49:29-32.

[6] Sreenivasulu N, Sopory SK, Kavi Kishor PB. Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 2007; 388(1-2):1-13.

[7] 19 Shalan MN, Abdel-Latif TAT, Ghadban EA E El. Effect of water salinity and some nutritional compounds of the growth and production of sweet marjoram plants (Majorana hortensis L.). Egypt J Agric Res 2006; 84(3):959.

[8] Abd El-Wahab MA. The efficiency of using saline and fresh water irrigation as alternating methods of irrigation on the

productivity of Foeniculum vulgare Mill subsp. vulgare var. vulgare under North Sinai conditions. Res J Agr Biol Sci 2006; 2(6):571-7.

[9] Aziz EE, Al-Amier H, Craker LE. Influence of salt stress on growth and essential oil production in peppermint, pennyroyal, and apple mint. J Herbs Spices Med Plants 2008; 14(1 & 2):77-87.

[10] Baghalian K, Haghiry A, Naghavi MR, Mohammadi A. Effect of saline irrigation water on agronomical and phytochemical characters of chamomile (Matricaria recutita L.). Scientia Hort 2008; 116:437-41.

[11] . Leithy S, Gaballah MS, Gomaa AM. Associative impact of bio-and organic fertilizers on geranium plants grown under saline conditions. Int J Acad Res 2009; 1(1):17-23.

[12] . Najafian S, Khoshkhui M, Tavallali V, Saharkhiz MJ. Effect of salicylic acid and salinity in thyme (Thymus vulgaris L.): investigation on changes in gas exchange, water relations, and membrane stabilization and biomass accumulation. Aust J Basic Appl Sci 2009; 3(3):2620-6.

[13] Zaki MF, Abou-Hussein SD, Abou El-Magd MM, El-Abagy HMH. Evaluation of some sweet fennel cultivars under saline irrigation water. Eur J Sci Res 2009; 30(1):67-78.

[14] Ben Taarit MK, Msaada K, Hosni K, M.Hammami M, Kchouk E, Marzouk B. Plant growth, essential oil yield and composition of sage (Salvia officinalis L.) fruits cultivated under salt stress conditions. Ind Crops Prod 2009; 30(3):333-7.

[15] Queslati S, Karray-Bouraoui N, Attia H, Rabhi M, Ksouri R, Lachaal M. Physiological and antioxidant responses of Mentha pulegium (Pennyroyal) to salt stress.Acta Physiol Plant 2010; 32(2):289-96.

[16] Tabatabaie SJ, Nazari J. Influence of nutrient concentration and NaCl salinity on growth, photosynthesis and essential oil content of peppermint and lemon verbena. Turk J Agric 2007; 31:245-53

[17]] Gowri, S. S., & Vasantha, K. (2010). Free radical scavenging and antioxidant activity of leaves from Agathi (Sesbania grandiflora)(L.) Pers. American-Eurasian Journal of Scientific Research, 5(2), 114-119.

[18] Vinothini, K., Devi, M. S., Shalini, V., Sekar, S., Semwal, R. B., Arjun, P., & Semwal, D. K. (2017). In vitro micropropagation, total phenolic content and comparative antioxidant activity of different extracts of Sesbania grandiflora (L.) Pers. Curr. Sci, 113(6), 1142-1147.

[19] Janani M. and Aruna A.(2017). A review on neutraceutical value of Sesbania grandiflora (Agati). World Journal of Pharmaceutical Research, 6(7) , 804-816.

[20] Abbs Fen Reji and N. Rexin Alphonse., (2013), Phytochemical study on Sesbania grandiflora. Journal of Chemical and Pharmaceutical Research, 5(2):196-201

[21] Arfan, N. B., Julie, A. S., Mohiuddin, A. K., Alam, S., & Labu, Z. K. (2016). Medicinal properties of the sesbania grandiflora leaves. Ibnosina Journal of Medicine and Biomedical Sciences, 8(6), 271-277

[22] Mohiuddin, A. K. (2019). Medicinal and Therapeutic values of Sesbania grandiflora. Medicine, 4, 18.

[23] S. Dhanapackiam and M.H. Muhammad Ilyas, Effect of salinity on chlorophyll and carbohydrate contents of Sesbania grandiflora seedlings , Indian Journal of Science and Technology Vol.3 No. 1 (Jan 2010)

[24] Manivannan, A., Soundararajan, P., Park, Y. G., & Jeong, B. R. (2015). In Vitro Propagation, Phytochemical Analysis, and Evaluation of Free Radical Scavenging Property of Scrophularia kakudensis Franch Tissue Extracts. BioMed research international, 2015, 480564.

[25] Bhalodia, N. R., Nariya, P. B., Acharya, R. N., & Shukla, V. J. (2013). In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. Ayu, 34(2), 209.

[26] Pant B. (2014). Application of plant cell and tissue culture for the production of phytochemicals in medicinal plants. Advances in experimental medicine and biology, 808, 25–39.

[27] Yeseen, B.T., Jurjees, J.A., Sofajy, S.A., 1987. Changes in some growth processes induced by NaCl in individual leaves of two barley cultivars. Indian J. Plant Physiol. 30, 1–6.

[28] Perveen, S., Shahbaz, M., Ashraf, M., 2011. Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (Triticum aestivum L.) plants raised from seed treated with triacontanol. Pak. J. Bot. 43, 2463–2468.

[29] Ahmad, P., Hakeem, K.R., Kumar, A., Ashraf, M., Akram, N.A., 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (Brassica juncea L.). Afr. J. Biotechnol. 11, 2694–2703. Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30, 161–175.

[30] Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

[31] Shah, K., Kumar, R.G., Verma, S., Dubey, R.S., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161, 1135–1144.

[32] Elsayed Fathi Abd_Allah a, *, Abeer Hashem b,1 , Abdulaziz Abdullah Alqarawi a , Ali Hassan Bahkali b , Mona S. Alwhibi, Enhancing growth performance and systemic acquired resistance of medicinal plant Sesbania sesban (L.) Merr using arbuscular mycorrhizal fungi under salt stress, Saudi Journal of Biological Sciences (2015) 22, 274–283

[33] Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western nepal. Plants, 8(4), 96.

[34] Klynger, Allan & Lobato, Silva & Juliane, Emily & Lima, Alvino & Silva, Elaine & Lobato, Guedes & Maciel, Gabriel & Marques, Douglas. (2016). Tolerance of Plants to Toxicity Induced by Micronutrients. 10.5772/62046.

[35] Shoib A. Baba, Shahid A. Malik, (2015), Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume, Journal of Taibah University for Science, 9(4), 449-454.

[36] Pant B. (2014). Application of plant cell and tissue culture for the production of phytochemicals in medicinal plants. Advances in experimental medicine and biology,808, 25–39

[37] Vinothini, K., Devi, M. S., Shalini, V., Sekar, S., Semwal, R. B., Arjun, P., & Semwal, D. K. (2017). In vitro micropropagation, total phenolic content and comparative antioxidant activity of different extracts of Sesbania grandiflora (L.) Pers. Curr. Sci, 113(6), 1142-1147.

[38] Janani M. and Aruna A.(2017). A review on neutraceutical value of Sesbania grandiflora (Agati). World Journal of Pharmaceutical Research, 6(7) , 804-816.

[39] Arfan, N. B., Julie, A. S., Mohiuddin, A. K., Alam, S., & Labu, Z. K. (2016). Medicinal properties of the sesbania grandiflora leaves. Ibnosina Journal of Medicine and Biomedical Sciences, 8(6), 271-277.

[40] Abbs Fen Reji and N. Rexin Alphonse., (2013), Phytochemical study on Sesbania grandiflora. Journal of Chemical and Pharmaceutical Research, 5(2):196-2016

[41] Mukul Bahera, Roop Karki, Chandru Shekar. (2012), Preliminary Phytochemical Analysis of Leaf and Bark methanolic extract of Sesbania grandiflora.The Journal of Phytopharmacology, 1(2), 10-22

[42] Yan-Xiu, Z., Dun-Yi, Y., & Harris, P. J. (1993). Plant regeneration from callus and explants of Sesbania spp. Plant cell, tissue and organ culture, 34(3), 253-260

[43] Gowri, S. S., & Vasantha, K. (2010). Free radical scavenging and antioxidant activity of leaves from Agathi (Sesbania grandiflora) (L.) Pers. American-Eurasian Journal of Scientific Research, 5(2), 114-119