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The Effect of Salinity on Solamargine and Solasonine Contents of *Solanum incanum* Plants Grown in Oman

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ABSTRACT: In addition to its traditional medicinal importance, *Solanum incanum* (thorn apple) is also a rich source of important cytotoxic glycoalkaloids such as solamargine and solasonine. The effect of salinity stress on solamargine and solasonine production by *Solanum incanum* plants grown in soil has been investigated. Salinity stress has been applied by adding NaCl to the soil, in concentrations: 0.0 (control), 75, 150, and 225 mM for 8 weeks. HPTLC method was used for analysis of solamargine and solasonine in leaves, stem and roots. A positive correlation was observed between 150 mM NaCl salinity and production of solamargine and solasonine in leaves. In roots, solamargine content was not affected by NaCl treatment, whereas solasonine content increased with a short-term salinity treatment. However, salinity seems to reduce the production of solamargine and solasonine in the roots of *Solanum incanum*. The possibility of using NaCl as an efficient and economical elicitor of glycoalkaloid production in *Solanum incanum* plants is rejected on the basis of the results obtained.

Keywords: Salinity; Glycoalkaloids; Solamargine;Solasonine; HPTLC.

تأثير درجة الملوحة على إنتاج السولامارجين والسولاسونين بواسطة نبات السولانام إنكانام في سلطنة عمان

سناء بنت سالم السنانية والصادق عبدالله الطيب

ملخص: تعتبر نباتات السولانام إنكانام مصدراً هاماً لإنتاج الألكالويدات السكرية مثل السولامارجين والسولاسونين بالإضافة إلى إستخدامها في الطب الشعبي. تمت دراسة إنتاج السولامارجين والسولاسونين تحت تأثير الملوحة باستخدام محلول كلوريد الصوديوم عند درجات تركيز تتراوح بين صفر (المجموعة الضابطة)، 75، 150 و225 ميلي مول لمدة 8 أسابيع. استخدمت طريقة HPTLC لفصل المركبات وتحديد كمياتها في جذور وأوراق وسيقان النباتات. وقد توصلت الدراسة إلى أن هناك ارتباط موجب بين تركيز الملوحة عند 150 ميلي مول وإنتاج الألكالويدات في أوراق النباتات. أما في الجذور فلم تجد الدراسة تأثيراً يذكر لإنتاج السولامارجين في حين أن إنتاج السولاسونين ارتفع عند استخدام الملوحة لفترة قصيرة. عموما فإنه يبدو أن الملوحة تقلل إنتاج الألكالويدات في جذور النباتات. وتثبت هذه الدراسة أن إستخدام الملوحة لا يعتبر وسيلة فعالة لزيادة إنتاج الألكالويدات.

مفتاح الكلمات: الملوحة، الألكالويدات السكرية، السولاسونين، السولامارجين، HPTLC

1. Introduction

Salinity is the major environmental factor limiting plant growth and productivity [1,2]. It has been estimated that about 940 million hectares of land around the world are already salinized [3]. An excess of ions in the root medium often causes osmotic strain, ion specificity/ toxicity, nutritional imbalances, changes in cell metabolite levels and diminished growth and yield [4]. Salinity is one of the major factors that can reduce substrate water potential, thereby restricting the water-nutrient uptake by plants [5]. Plants have evolved a variety of physiological and biochemical processes as responses to stress conditions, e.g. solute accumulation and the development of enzymatic antioxidant systems [6]. Short-term effects include reduction in plant growth due to the osmotic effects of the accumulation of salts near the root zone, which reduces cell expansion. Long-term effects include excessive salt absorption, which causes plants to suffer ionic stress, leading to premature leaf aging following a reduction in the available photosynthetic area [1,3,7]. Accumulation of Na^+ in the tissues of plants growing in saline media restricts the uptake of essential nutrients (mainly N, P, K^+ and Ca^{2+}), thereby reducing the plant biomass production [8]. Thus, salinity affects growth and can alter leaf water potential, stomatal conductance, and transpiration [9].

In addition to osmotic stress and ion toxicity, plants subjected to salinity experience oxidative stress and lipid peroxidation, which can cause a loss of membrane integrity [7]. Salinity has been reported to disturb the integrity of cell membranes by inducing structural changes and replacing Ca with Na on the plasma membrane, thus altering K/Na ratio [10]. On the other hand, proline accumulation in plant cells exposed to NaCl-stress is a widespread phenomenon. Proline accumulation is correlated to growth inhibition induced by NaCl [11].

The negative effect of soil salinity depends on plant tolerance aptitude and salinity level [8]. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells [9]. Salt tolerance in plants is a complex phenomenon that may involve developmental changes as well as physiological and biochemical processes [3].

The genus *Solanum* (Family: Solanaceae), with more than 1700 species, is widespread in the temperate and tropical regions of the world [12,13] and is characterized by the presence of steroidal glycoalkaloids (SGAs) of great interest from the ecological view point and human health aspects [14-17]. About 20 *Solanum* species have been reported from Arabia. In Oman, seven species of *Solanum*, viz. *S. cordatum*, *S. incanum*, *S. melongena*, *S. nigrum*, *S. surattense*, *S. tuberosum*, and *S. villosum* are found [18]. *Solanum incanum*, commonly known as thorn apple, is an important medicinal plant. In Oman its leaves, fruits (berries) and roots are used as a traditional medicine [19]. In most of the plants containing glycoalkaloids, the main aglycone is solasodine [20]. It occurs in about 200 species of *Solanum* in the form of the water soluble triglycosides, solasonine (SN) and solamargine (SM) [21]. These two compounds bear the same aglycone, solasodine, and differ from each other only in the nature of the trioses involved, namely, solatriose for solasonine and chacotriose for solamargine (Figure 1) [15,22]. Solamargine and solasonine stand out economically because their chemical structures are very similar to steroidal hormones, and are therefore used as an important source of medicines, such as contraceptives and steroidal anti-inflammatory drugs [23]. These glycoalkaloids have been studied for their antidiabetic, antifungal, antiparasitic, antibiotic, antimicrobial, antiviral and most extensively, for anticancer properties [13,23,24].

Various biotic and abiotic factors, used as elicitors, have been reported to increase secondary-metabolite yield; application of NaCl enhances alkaloid production [25]. In a study on the effect of salinity on growth, tuberization and chemical composition with special reference to the total glycoalkaloid (TGA) content of seven potato varieties, salinity reduced the TGA levels of tubers mostly during the exponential phase of growth with further reduction at maturity [26].

Although much of the recent research focuses on physiological and metabolic processes under salt-stress, few data are available on glycoalkaloid accumulation. Most of the previous studies have attempted to enhance the production of solasodine by *S. nigrum* using NaCl-stress in the culture medium. The present study was undertaken with the objective of exploring the possibility of enhancing glycoalkaloid production in S. incanum plants under field conditions by using NaCl as an efficient and economical elicitor.

2. Experimental

2.1 Plant material

Solanum incanum L. seeds obtained from plants grown in Al Jabal Al Akhdar, were grown in the green house at Sultan Qaboos University. Germination (on damp filter paper in Petri dishes at 21-25 ˚C, 14 hr light, 70% RH) occurred within 3 weeks, and at one month, seedlings were transplanted to 15 cm diameter pots containing acid washed sand soil: clay soil: compost (1:1:1) and kept in University's green house at 21-25 °C, 14 hr light, 70% RH. The plants were irrigated with irrigation water 2 times a week until germination had completed.

2.2 NaCl treatment

Twelve-week old plants were divided into four groups and subjected to different NaCl treatments in order to study the effect of salinity-stress on GA production in *S. incanum* plants. Control plants were irrigated two times a week. The other three groups were treated separately with 75 mM, 150 mM, and 225 mM NaCl. The plants were irrigated with saline water two times a week. Sampling was done after 2, 4, and 6 weeks for each treatment, where five plants were randomly selected for analysis of the GAs. Each plant was divided into leaves, stem, and roots, weighed fresh, and dried in an oven at 60 °C to constant wt. (ca 3 days) and then reweighed.

2.3 Chemicals and reference compounds

Solamargine and solasonine obtained from Glycomix (Whiteknights Road, United Kingdom) were used as reference compounds. Distilled water was deionized before use. HPLC grade methanol and chloroform were obtained from Sigma Aldrich (Germany) and ammonia solution from Merck (Darmstadt, Germany).

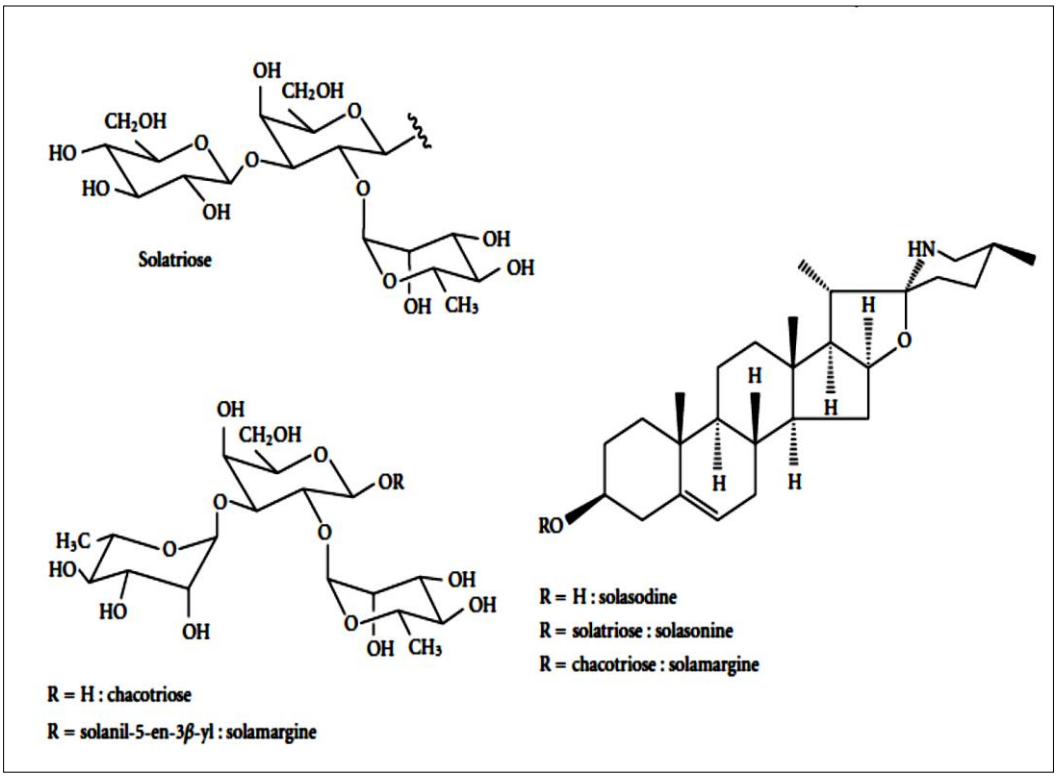

Figure 1. Chemical structure of solasodine and its respective glycoalkaloids.

2.4 HPTLC Instrumentation and conditions

The samples were spotted in the form of bands (4 mm width), with a CAMAG microlitre syringe on precoated HPTLC silica gel glass plates 60F-254 (20 cm x 10 cm, Merck KGaA, Germany) using a CAMAG Linomat V (Muttenz, Switzerland) and were controlled by WinCATS software (CAMAG). A constant application rate of 120 nL/s was employed and space between two bands was 6.2 mm. The slit dimension was kept at 5.00 mm×0.30 mm, and a 20 mm/s scanning speed was employed. The mobile phase of the GA consisted of chloroform: methanol: 5% ammonia, (7:3:0.50, v/v/v). Linear ascending development was carried out in a 20×10 cm twin trough glass chamber, saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 15 min at room temperature. The length of the chromatogram run was 90 mm. Subsequent to development, the HPTLC plates were dried in an oven at 60 °C for 5 min. Densitometric scanning was performed on a CAMAG TLC scanner IV (absorbance mode 530 nm) with WinCATS software after spraying the developed plate with anisaldehyde sulphuric acid reagent and heating on a hot plate at 110 °C for 5 minutes.

2.5 Sample preparation

In a simple method of extraction, 0.5 g of dried and powdered *S. incanum* organs (leaves, stems, and roots) were extracted using 30 mL methanol: chloroform (2:1) by sonication at 40 °C for 45 minutes. The mixture was filtered using Whatman No. 1 filter paper and washed with methanol. The filtrate was evaporated to dryness in a water bath at 40 °C. Dried extracts were dissolved in methanol and filtered using a 0.2 µm syringe filter. The final volume was made to 5 mL with methanol and stored at 4 °C for application onto HPTLC plate for quantification.

2.6 Standard solamargine and solasonine

One mg/mL (1000 µg/mL) solutions of the standard solamargine and solasonine were prepared in methanol. A mixture of the glycoalkaloids solamargine and solasonine was prepared by mixing 0.5 mL of each so that the concentration of each compound in the mixture was 0.5 mg/mL (500 µg/ mL). These solutions were used for application on HPTLC plate for preparation of a standard plot.

2.7 Calibration curve of solamargine and solasonine

Different volumes of standard solution of the mixture of GAs 0.1, 0.2, 0.4, 0.5, 1.0, 2.0, 4.0, 5.0, 10.0 µL of 500 µg/mL were spotted in triplicate on the HPTLC plate to obtain 50, 100, 200, 250, 500, 1000, 2000, 2500, 5000 ng/spot of each GA, respectively. The data in peak area vs. solamargine and solasonine concentration were treated with the linear least square regression and the regression equation thus obtained from the standard curve was used to estimate both the GAs in different samples.

2.8 Quantification of solamargine and solasonine

The same method was applied for the analysis of solamargine and solasonine contents in leaves, stem and root of *S. incanum* plants grown at different salinity concentrations (at intervals of 2, 4, and 6 weeks). Samples of 10 µL each were applied in quadruplicate on the HPTLC plate. The GA yield was quantified using the regression equation of the calibration curve.

3. Results and Discussion

3.1 GAs in *S. incanum* after salinity treatment

Solamargine and solasonine produced well separated compact bands on silica gel HPTLC plate with chloroform: methanol: 5% ammonia (7: 3: 0.5) after visualization using anisaldehyde sulphuric acid reagent (Figures not shown). The R_f values for solamargine and solasonine were 0.26±0.02 and 0.14±0.02 respectively. Chromatograms were scanned at 530 nm in absorbance mode (Figures 2 and 3). The identity of the GA band from the sample solution was confirmed by comparison of its R_f and spectra with those from the standard GAs.

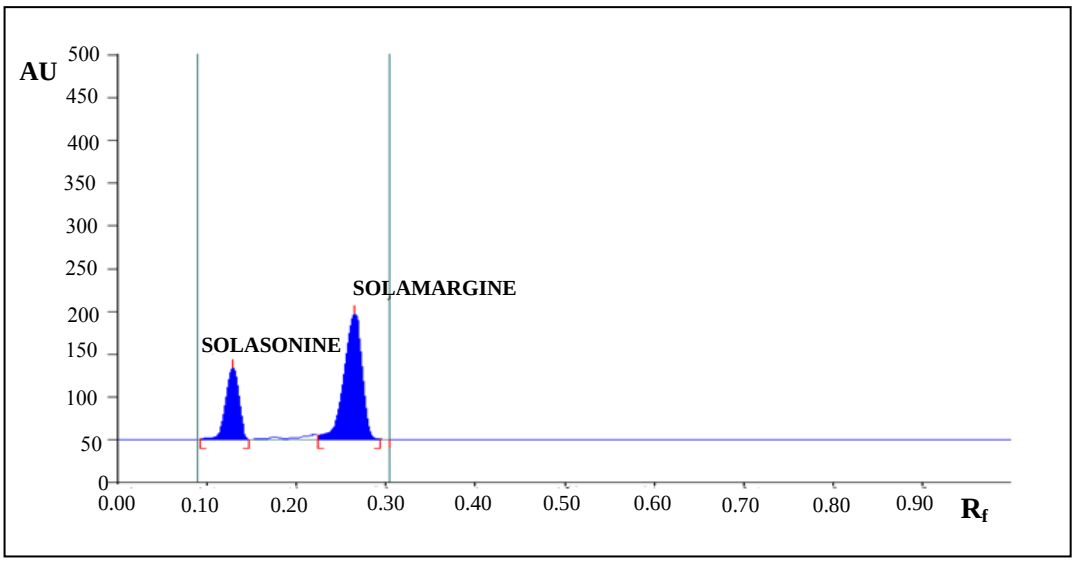

Figure 2. HPTLC chromatogram of solasonine (R_f 0.13) and solamargine (R_f 0.27) 1000 ng/spot each at 530 nm.

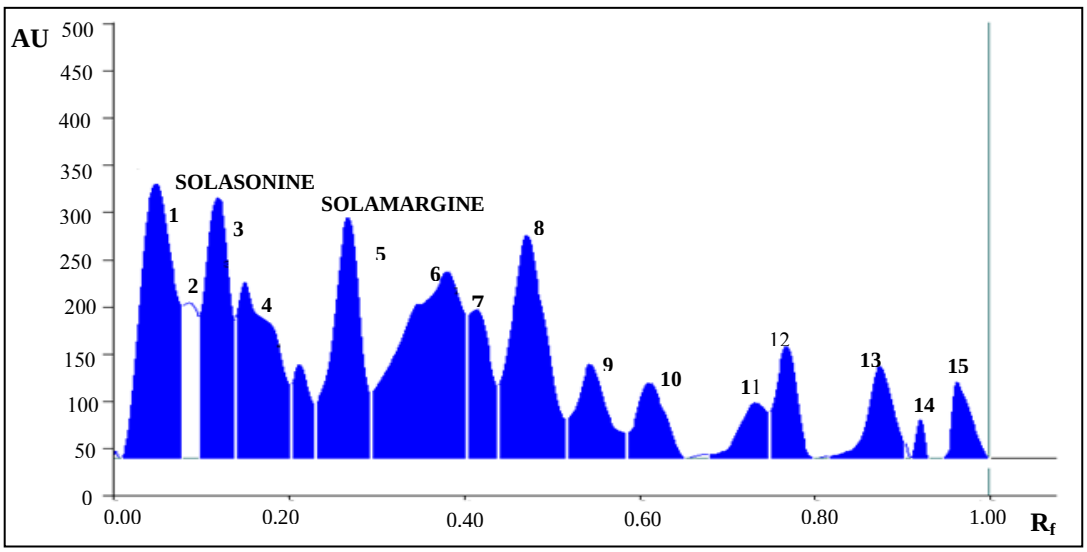

Figure 3. HPTLC chromatogram of Sample at 530 nm, using solvent system Chloroform: Methanol: 5% Ammonia (7: 3: 0.5) showing the presence of solasonine and solamargine.

Linear regression calibration curves were plotted using peak area against concentration, and found to be linear in the range of 50 to 2000 ng/mL for both solamargine and solasonine, with good linear relationships of 0.9987 and 0.9962, respectively.

The accuracy of the method was tested by the standard addition method at 3 concentrations (0, 50, 100, 150), which showed recovery within the range of 99.1%-101.7%. The method's precision was analysed for repeatability and reproducibility by analysing 3 concentrations of standard solution six times a day and on six different days, which showed a %RSD < 2%. The method was applied for the analysis of solasonine and solamargine content in leaf, stem and root samples of *Solanum incanum*, which had been subjected to the salinity treatment.

Solamargine and solasonine concentrations in different samples were analysed from the regression equation using values of area obtained from WinCATS software. The mean values of GAs in samples are shown in Figures 4 & 5.

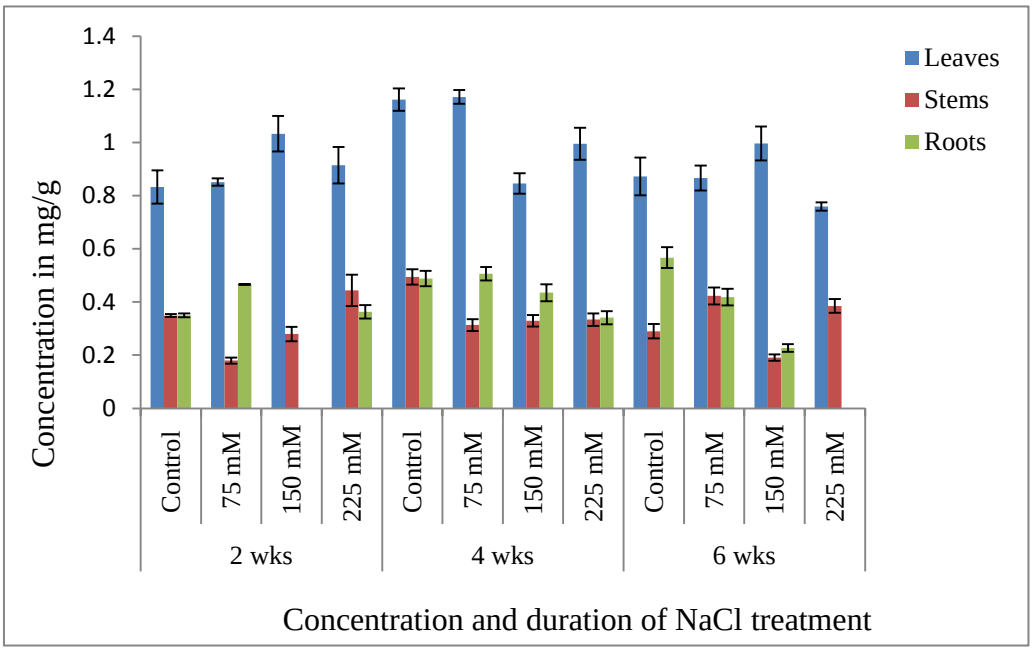

Figure 4. Effect of different concentrations of NaCl (untreated (control), 75 mM, 150 mM, 225 mM) on content of solamargine in leaves, stems and roots of *S. incanum* after 2, 4, and 6 weeks analyzed by HPTLC.

The study revealed that no significant difference or regular change occurred in solamargine content after salinity-stress among same samples of leaves collected at different time intervals or among treatments of up to 6 weeks. The control leaves as well as those treated with salt concentration of 75 mM NaCl showed a similar pattern of solamargine content whereas medium treatment (150 mM NaCl) increased the solamargine content in the 2 and 6 week old leaf samples. There was no regular pattern of change in solamargine concentration in the stem and root samples.

Among leaf samples, solamargine content was highest in samples at salinity-stress (75 mM NaCl) at the 4th week. The salinity stressed samples showed a good and constant amount of solamargine irrespective of treatment at different dose levels. Among stem samples, solamargine content was not affected by salinity stress, whereas it was reduced in roots.

With regard to solasonine content, again no regular pattern of change was observed among samples stressed with salinity. On the whole, root samples showed solasonine levels that are significantly greater quantity than stem and leaf samples. Salinity-stress did not produce any regular pattern in solasonine content of plant organs, but an increase in salinity above the minimal level increased the solasonine content after the second week of salinity treatment. In the stem also, an increase in the salinity significantly increased solasonine content as compared to the control. Equivalent levels of solasonine could be achieved in leaves and stems of untreated plants after 12 weeks of plant growth. After four weeks of salt-stress, solasonine content decreased in leaves and stem, but increased in roots at all the three NaCl concentrations, with the highest production being at 125 mM. Analysis of root samples showed that salinity-stress significantly increased solasonine content in the 4th week and caused no effect later on. These increased contents of solasonine in roots could be achieved in untreated plants at the 14th week of plant growth. Salinity treatments of eight weeks reduced the solasonine production in roots (at 75 and 125 mM NaCl concentrations), but had no significant effect in leaves and stems. This could be due to heavy disruption in physiological processes under high salinity levels.

Some of our results corroborate the earlier reports in certain respects, showing stimulated alkaloid production by salinity-stress [11,12,25]. Jasmin [11] investigated the effect of 8 weeks of salinity-stress on

in vitro solasodine production by *Solanum nigrum*, and reported that NaCl-stress stimulated solasodine accumulation. The response to NaCl-stress was more prominent after 4 and 6 weeks. However, differences were non-insignificant between 150 mM and 200 mM of NaCl [11]. Bhat *et. al.* [12] also noted enhanced solasodine production with increased NaCl level in *S. nigrum*. Ahmad and Abdullah [26] observed a decrease in total glycoalkaloid (TGA) level in potato tubers due to salinity but the differences between different concentrations of salt and the control were small.

Biosynthesis of solasodine starts from acetyl coenzyme A, which later converts to mevalonic acid, *via* the mevalonic acid pathway, in which cholesterol, a key intermediate of solasodine, is synthesized. High salinity seems to enhance *in vitro* cholesterol production, which in turn increases solasodine in tissues, or alternatively, the enhanced yield could be due to the over expression of the genes concerned. This can explain the short-term effect of salinity. Long-term effects include excessive salt absorption, which causes plants to suffer ionic stress, leading to premature leaf aging, following a reduction in the available photosynthetic area to maintain growth [1,3,7]. This may lead to a reduction in the production of sugars needed for the glycosylation of solasodine to produce glycoalkaloids.

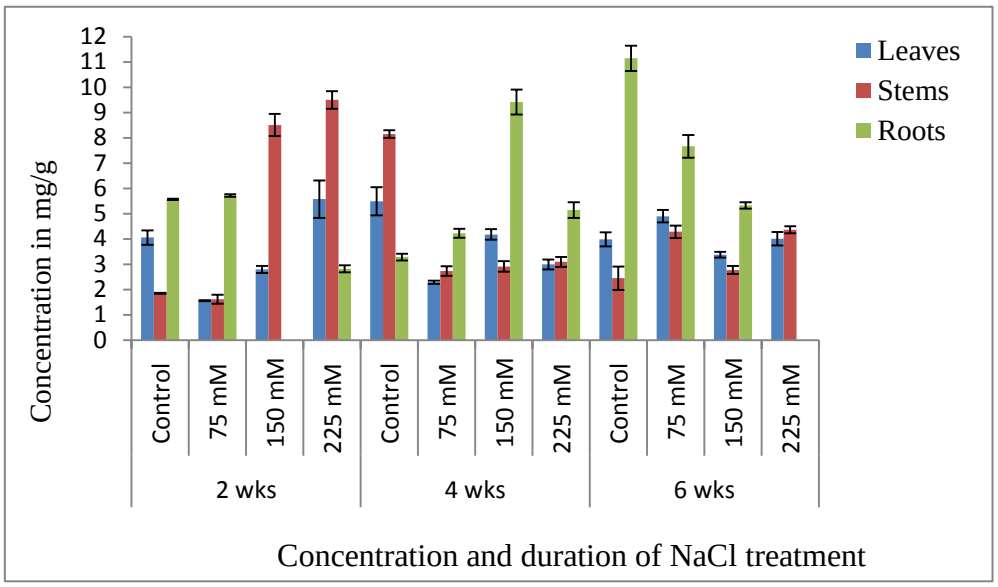

Figure 5. Effect of different concentrations of NaCl (untreated (control), 75 mM, 150 mM, 225 mM) on content of solasonine in leaves, stems and roots of *S. incanum* after 2, 4, and 6 weeks analyzed by HPTLC.

Although salinity seems to enhance solamargine production in leaves of *Solanum incanum*, using 150 mM NaCl, has no effect on solamargine production in stem and reduces the production of glycoalkaloid in roots. However, the enhanced contents of solamargine in leaves can be achieved without any salt treatment at 12 weeks of growth.

The salt concentrations that can give higher production in solasonine are 150 mM and 225 mM for leaves and stems but for a short term of two weeks of salinity. On the other hand, salinity decreased the solasonine contents in roots, which seem to be the higher source of the compound in *S. incanum* plants, for short and long terms of salinity.

As for solamargine, the untreated leaves, stems and roots can produce contents equal to those formed after salinity at 12 weeks of plant growth for leaves and stems and after 14 weeks of plant growth for roots. However, it should be mentioned that the plants were negatively affected by the salinity treatments (data are not shown). Our results do not support the claim that it is possible to enhance production of the glycoalkaloids solamargine and solasonine in field grown *Solanum incanum* plants using NaCl as an efficient and economical elicitor.

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