Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2018; SP1: 1386-1389

**Amol D Sable**
Assistant Professor, Plant biotechnology, College of Agricultural Biotechnology Georai Tanda, Paithan Road, Aurangabad (M.S.), India.

**Prashant B Kardile**
Assistant Professor, Agril. Botany, Dadasaheb Patil college of Agriculture, Dahegaon, Auranagabad (M.S.), India.

**Asmita D Sable**
Assistant Professor, MGM's Institute of bioscience & technology, Auranagabad (M.S.) India

**Ashok V Kharde**
Assistant Professor, MGM's College of Agricultural Biotechnology, Auranagabad (M.S.), India

**Correspondence**
**Amol D Sable**
Assistant Professor, Plant biotechnology, College of Agricultural Biotechnology Georai Tanda, Paithan Road, Aurangabad (M.S.), India.

# Studies on effect of different concentration of Nacl on bacoside production from brahmi (*bacopa monnieri*) under *in vitro* condition

**Amol D Sable, Prashant B Kardile, Asmita D Sable and Ashok V Kharde**

**Abstract**
*Bacopa monnieri* has been chosen as a model medicinal plant to study the Abiotic stress (salinity) under in vitro system. In the present investigation effect of incrasing concentration of NaCl on Bacoside production of Brahmi (Bacopa monnieri (L.) Pennell) was studies under in vitro condition. Nodal segments of Brahmi were inoculated on the MS media supplemented with BA(1.1µM) and IBA(0.30µM). The salt stress was given to these plants by incorporating increasing amount of NaCl (00, 05, 10, 15, 20, 25 and 30 mM) in seven different treatements with three replication. After 4 weeks of inoculation, Bacoside content (4.13 %), Number of shoots (5.33/ explant), were produced in the treatement without NaCl. The Bacoside content was produced up to (0.70 %) in 30 mM NaCl at 30 DAI. The overall growth is reduced in proportion to the increase in NaCl concentration. Brahmi tolerates salt stress up to two levels of NaCl concentration i.e. 05 and 10 mM with minimum reduction in Bacoside production up to 25 and 54% respectively above reduction i.e. up to 94 % in 30 mM NaCl.

**Keywords:** Brahmi, Abiotic stress, bacoside content, NaCl.

## Introduction
Brahmi *(Bacopa monnieri)* belongs to family *Scrophulariaceae*. It is an important medicinal herb which is endangered. It is found in humid and warmer parts as well as in damp or marshy area streams or on the border of ponds, throughout India and the world. The genus Bacopa includes over 100 species of aquatic herbs distributed throughout the warmer regions of the world and India, Nepal, Sri Lanka, China, Taiwan and Vietnam, and is also found in Florida and other southern states of the USA where it is recognized as weeds in rice fields (Barrett and Strother, 1978; Russo and Borrelli, 2005). The plant grows well in poorly drained soils. The plant prefers the soil of acidic nature for its congenial growth. Growth is faster at high temperature range of 33-40°c with relative humidity of 60-80 %. Pragyashakti and Subodhak are two varieties of Brahmi yielding high herb and higher content of Bacoside A developed by Lucknow are widely cultivated. It is used in traditional Indian medicine in Ayurveda, It is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to posse's anti-inflammatory, antiallergic, antipyretic, antiviral, antibacterial, sedative, free radicalscavenging and anti-lipid peroxidative activities. By considering the need to improve bacoside production in *Bacopa monnieri* the present experiment is entitled on "Studies on effect of different concentration on NaCl on Bacoside production in Brahmi (*Bacopa monnieri*) under *in vitro* condition." is carried out with two objectives, i) study the effect of Nacl on growth of Brahmi under *in vitro* condition and ii) study the Bacoside production of Brahmi using different concentration of Nacl.

## Material and Methods
Present investigation entitled "Studies on effectof Different Concentration of NaCl on Bacoside production from brahmi (*Bacopa monnieri*) under *in vitro* condition." was carried out *in-vitro* conditions during December 2014 –April 2015 in Department of Plant Biotechnology, MGM College of Agricultural Biotechnology, Aurangabad (M.S). Experiment was laid out in Completely Randomized Design with Seven treatments of Nacl (00, 05, 10, 15, 20, 25, and 30 mM) and three replications. Plants of Brahmi (*Bacopa monnieri*) were collected from river side areas of Paithan, Maharashtra**.** The MS medium was prepared by adding required amounts of sucrose 3% +0.65% agar + Nacl (00, 05, 10, 15, 20, 25, and 30 mM) and plant hormone such as cytokinin (BA 1.1µM), auxin (IBA 0.30 µM) The pH was adjusted to 5.8 and agar was used for solidifying the medium. 20 ml media was poured into test tube.

Then autoclaved at 121 °C for 20 minutes at 15 psi pressure and transferred the media to the storage room and they kept under aseptic conditions for their further use (Murashige and Skoog, 1962).

After that explants sterilization with 70% ethanol for few seconds follow by 2-3 washing with sterilize double distilled water. Further the explants were treated with 0.01% $HgCl_2$ for 10 min. Finally the explants were washed with sterile double distilled water.

The cut ends of explants were kept in such a way so as to have maximum contact with the medium (Patni, *et al.*, 2010). Explant was observed periodically, a) Number of shoots per explants (After 4 weeks)b) Fresh weight of shoot. (After 4 weeks)c) Dry weight of shoot. (After 4 weeks)d) Bacoside content in per gram of Dry sample.

Explants were removed from MS media and washed with water to remove debris of media. The fresh plant was oven dried at 60 ºc for 12 hours. The Dried explants were crushed with mortal and pistal. Uniform dry weight was selected for extraction of Bacoside. 500 mg of coarsely powdered Brahmi samples was extracted and dissolved in 3 ml absolute ethanol and kept for 24 hrs. The extracts then were filtered by Whatmann's no. 42 filter. (Sharma *et al.*, 2011).The ethanol extract was used for quantitative detection of Bacoside content in Brahmi. 40µl of ehanolic extract was taken from 3ml and final volume was made up to 4ml by using 95% ethanol and compared with standard Bacoside concentration. The analysis was carried out by using UV Spectrophotometer at 278 nm. Standard graph of Bacoside was prepared by using Bacoside mixture purchased from Sigma Aldrich. The reading of test sample was compared with the standard Bacoside (Sahani, *et al*., 2012). The data obtained on various Biometric and analytical observation is analyzed by "Analysis of variance" method by using Completely Randomized Design (panse and sukhatme, 1967).

**Experimental results**

The results obtained in the present investigation on "Studies on effect of different concentration of Nacl on bacoside production from barhmi (*bacopa monnieri*) under *in vitro* condition*."* are presented under the following headings.

**Bacoside content (%)**

Data onmean Bacoside concentration recorded at 30 DAI are presented in table no 1. and depicted in fig. 1. Data presented in Table 1 revealed that mean content of Bacoside per explants of *bacopa monnieri* was influence significantly due to different levels of NaCl at 30 DAI. The treatment T0 (control) was significantly superior over rest of the treatment and recorded highest Bacoside content (4.13%). Similarly the treatment T1 (05mM) and T2(10 mM) where on at par with each other and significantly superior over all treatment except control and recorded the bacoside content (2 and 1.90 %) respectively.

Treatment T3 (15mM) and T4 (20mM) did not differ significantly with each other and superior over concentration of NaCl 25 mM and 30 mM. Treatment T5 and T6 where at par which each other and showed decreased content of bacoside i.e 0.60% and 0.70.

**Table 1:** Response of various concentrations of NaCl on Bacoside production 30 (DAI).

| Treatments (NaCl mM) | Percent Bacoside |
|---|---|
| $T_0$(00) | 4.30 |
| $T_1$(05 ) | 2.00 |
| $T_2$(10) | 1.90 |
| $T_3$(15) | 1.40 |
| $T_4$(20) | 1.13 |
| $T_5$ (25) | 0.60 |
| $T_6$ (30) | 0.70 |
| Mean | **1.71** |
| **S.E** | **0.12** |
| **C.D** | **0.54** |

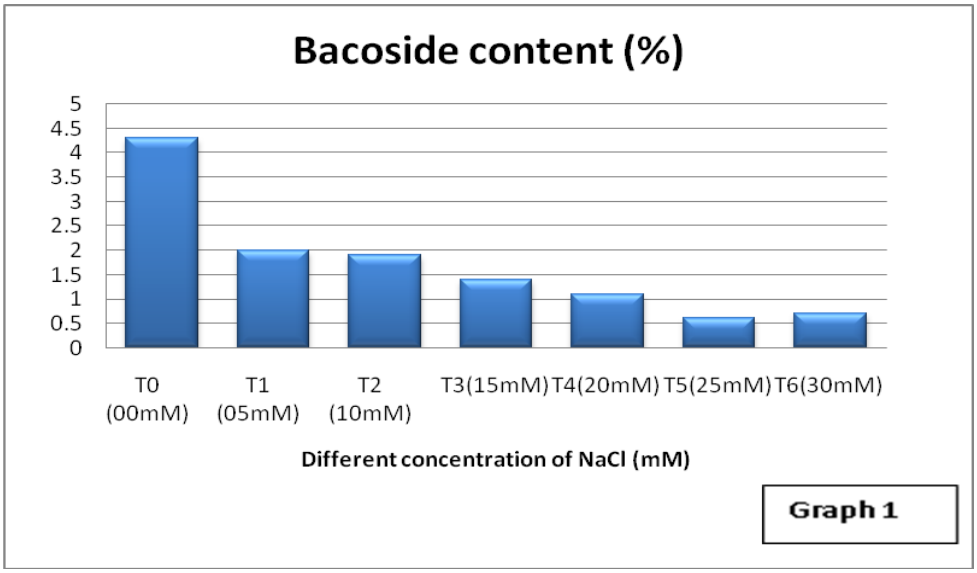


**Fig 1:** Effect of different conc. of NaCl on Bacoside

**Production in brahmi.**

**Shoot proliferation**

Mean number of shoots per explants at 30 DAI was 2.80. Data presented in table (2) indicated that shoot proliferation was influenced significantly due to various concentration of NaCl at 30 DAI. With increasing NaCl concentration decresed shoot number of regenerates declined as compaired to T0 (control). The treatment T0 (control) produced maximum no. of shoot (5.33) as compaired to other treatment.

Treatment T1 (5mM) significantly superior over concentration of Nacl 10mM, 20mM, 25mM, 30mM except control. Treatment T2 (10mM) significantly superior over concentration of NaCl 15mM, 20mM, 25mM & 30mM.

Treatment T3 (15mM), T4 (20mM), T5 (25mM), T6 (30mM), were on at par with each other & recorded least number of shoot i.e (1.66,1.33,1.33,1.00) respectively.

**Mean fresh weight and dry weight of explants (gm)**

Treatment $T_0$(control) recorded significantly superior fresh and dry w. (1.40 and 0.14 respectively ) of explants over rest of treatment. Similarly, treatment T1 (05mM) proved significantly superior over concentration of NaCl 10nM, 15mM, 20mM, 20 mM, 25mM, and 30mM exvept the control. Treatment T2 (10mM) significantly superior over conc. of NaCl 15mM, 20mM and 30mM.Treatment t3 (15mM) significantly superior over concentration of NaCl 20mM, 25mM and 30mM.

Treatment t4 (20mM), T5 (25mM) and T6 (30mM) were on at par with each other and recorded decreased fresh weight of explants i.e (0.11, 0.09 and 0.05 gm)respectively.

**Table 2:** Mean number of shoots per explants as different concentration of Nacl.

| Treatments (NaCl mM) | No. of Shoot |
|---|---|
| $T_0$(00) | 5.33 |
| $T_1$(05) | 4.33 |
| $T_2$(10) | 3.32 |
| $T_3$(15) | 1.66 |
| $T_4$(20) | 1.33 |
| $T_5$(25) | 1.33 |
| $T_6$(30) | 1.02 |
| Mean | **2.61** |
| **S.E.** | **0.30** |
| **C.D** | **1.29** |

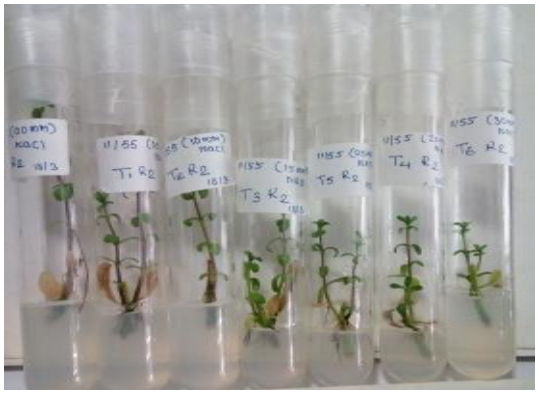

**Plate 1:** Number of shoot per explants

Treatment T1 (05mM), T2 (10mM), T3 (15mM) were on at par with each other and superior over rest of the treatment except control.

Treatment T4 (20mM), T5 (25mM) and T6 (30mM) did not differ significantly with each other and recorded decreased dry wt. of explants i.e (0.05, 0.04, 0.03 gm) respectively.

The maximum fresh wt. (1.40gm) and dry wt. (0.14 gm) was produced in treatment without NaCl. This was due to higher no. of shoots in T0 (control) and contribute to increase in biomass.

**Table 3:** Mean number of fresh weight and dry weight of explant

| Treatments (NaCl mM) | Fresh Wt.(gm) | Dry Wt.(gm) |
|---|---|---|
| $T_0$(00) | 1.40 | 0.14 |
| $T_1$(05) | 0.61 | 0.11 |
| $T_2$(10) | 0.30 | 0.10 |
| $T_3$(15) | 0.21 | 0.09 |
| $T_4$(20) | 0.11 | 0.05 |
| $T_5$(25) | 0.09 | 0.04 |
| $T_6$(30) | 0.05 | 0.03 |
| **Mean** | **0.39** | **0.08** |
| **S.E.** | **0.04** | **0.01** |
| **C.D** | **0.12** | **0.03** |

The healthy, disease free, young nodal segments (3rd and 4 th node from top) of the explants were selected for experimentation (Binita, *et al.*, 2005). Sterilization of explants was done with tween 20 solution, 70% ethanol, 0.01% mercuric chloride and double distilled water. Microprapogation of explants was carried out after sterilization. The explants were trimmed and inoculated on MS media. containing MS basal medium + sucrose 3% +0.65% agar and different concentrations of NaCl (00, 05, 10, 15, 20, 25, and 30 mM) supplemented with 1.1 µM BA and 0.30 µM IBA. The pH of media was adjusted to 5.6 - 5.8 and autoclaved the media at 121°C for 20 minutes.

The microprapogation was carried out for 4 weeks. Then, the explants were dried in hot air oven for 12 hrs at 60 ºc and powdered out with the help of mortal and pistal. The uniform powder 500 mg was used for ethanolic extraction of Bacoside. The analysis was carried out by using UV Spectrophotometer at 278 nm. Standard graph of Bacoside was prepared by using Bacoside mixture purchased from Sigma Aldrich. The reading of test sample was compared with the standard Bacoside.

**Conclusion**

Based on finding present investigations following conclusions were drawned.

1. The increasing concentration of NaCl cause inhibitory effect on the bacoside content and number of shoot *bacopa monnieri.*
2. The NaCl concentration 05mM and 10mM tolerates salt stress with decrease in bacoside content, shoot multiplication, fresh weight and dry weight upto 25% and 54 % respectively. On the basis of results of present investivation there is need, to study the effect of Nacl on Bacoside production at Field level and to investigate the interactive effect of physiochemical condition with NaCl to enhance the Bacoside production.

**References**


1. Binita BC, Dave MA, Jasraj YT. *B. monnieri*: rapi deficient and cost effective micro propogation. Plant Tissue Culture and Biotech 2005; 15(2):167-175.
2. Barrett SCH, Strother JL. Taxonomy and natural history of Bacopa in California. Syst. Bot. 1978; 5:408-19.
3. Murasige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum 1962; 15:473-497.
4. Panse VM, Sukhatme PV. Statistical Methods for Agricultural Workers. ICAR, New Delhi. 1967; 2:381.

5. Sharma R, Khan M. *Bacopa monnieri L.* (highly endangered species): an improved micropropagation protocol for germplasm preservation. Indian J Applied & Pure Bio. 2011; 26(2):361-370.
6. Sahani N, Mathure R, Agrawal SS. Qualitative and Quantitative Assessment of four marketed formulation of Brahmi. Indian J Pharmacitical Science. 2012, 24-28.