Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 3311-3313
Received: 18-06-2020
Accepted: 22-08-2020

Misal DR
MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Kharde AV
MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Deshmukh VD
MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Talekar BK
MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Corresponding Author:
Kharde AV
MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Elicitation of bacoside content using *Aspergillus niger* filtrate in *Bacopa monnieri* (Brahmi)

Misal DR, Kharde AV, Deshmukh VD and Talekar BK

Abstract
Micropropagation technique is used for the elicitation of bacoside content in *Bacopa monnieri*. *In vitro* propagation was carried out on Murashige and Skoogs medium supplemented with 6-benzylaminopurine (1.1 µM) and Indole-3- butyric acid (0.30 µM) with 3% sucrose for four weeks. For elicitation variable concentrations of *Aspergillus niger* filtrate (0, 0.5, 1.0, 1.5, 2.0 ml/L) were incorporated in MS basal medium for 7 days. Present investigation clearly indicated that the *Aspergillus niger* filtrate significantly influenced the Bacoside production in Brahmi. After seven days elicitation, maximum enhancement in Bacoside (1.62% DW) was reported at lower concentrations of *Aspergillus niger* filtrate (0.5 ml/L) over control.

Keywords: *Bacopa monnieri*, micropropagation, *Aspergillus niger*, elicitation, bacoside

Introduction
Bacopa monnieri is a medicinal plant belongs to *Plantaginaceae* family commonly known as Brahmi, found throughout the Indian subcontinent in wet, damp and marshy areas. It is used in traditional Indian medicine and Ayurveda for the treatment of anxiety and improving intellect memory in several countries. In addition to memory boosting activity, 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 possess anti-inflammatory, analgesic, antipyretic, sedative free radical scavenging and anti-lipid peroxidative activities. The use of whole plant system for medicine, poor replenishment efforts and untrained plucking of the plant material leads this medicinal plant towards endangered. The major chemical entity shown to be responsible for neuropharmacological effects and the nootropic action or antiamnesic effect of *Bacopa monnieri* is *Bacopa* saponins A, B, and C which are dammarane-type triterpenoid saponins. Since the supply is limited and faces constraints in meeting the increasing demand of these biochemical.
Pharmacological properties of *Bacopa monnieri* were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as Bacosides. Bacoside have been indicated for memory-enhancing properties while Bacoside A assists in release of nitric oxide that allows the relaxation of the aorta and veins, to allow the blood to flow more freely through the body making this exceptional plant a nootropic drug. According to Central Drug Research Institute (CDRI) situated in Lucknow, the saponins, Bacosides A and B are responsible for repairing damaged neurons; furthermore *Bacopa monnieri* has been studied clinically for its acute and chronic effects on cognitive function.
Plants have been found to elicit the same response as the pathogen itself when challenged by compounds of pathogenic origin (elicitors). Biotic elicitors have biological origin derived from the pathogen or from the plant itself. Biotic elicitors were first discovered by Radman *et al.* (2003) [1]. *Aspergillus niger* is common soil born pathogenic fungus which is industrially important and utilized in the field of biotechnology and food microbiology. *Aspergillus niger* widely used as elicitor in tissue culture for the improvement of secondary metabolites *in vitro* condition [2]. Considering the above points, the present investigation was carried out with objective to study the effect of elicitation of bacoside content in Brahmi under *in vitro* condition.

Materials and Methods
Bacopa monnieri plants were collected from Paithan (19º28´33.9852´´N and 75º22´45.0948´´E) near Aurangabad (M.S.) and planted in the college nursery. Explants of 3-4 nodal segments were selected as explants [3] were selected from the healthy plantlets grown in the nursery for the experiment. Excised explants were washed and surface sterilized with 70% ethanol for fraction of seconds, then rinsed for thrice with sterile distilled water under aseptic

condition. Further antifungal treatment of 0.1% (w/v) mercuric chloride was given for 5 min followed by 4-5 repeated washing with sterile distilled water. Surface sterilized nodal explants were trimmed using sterile surgical blades and used for the further experiment. Treated explants were inoculated on Murashige and Skoogs (MS) media supplemented with 6-benzylaminopurine (1.1 µM) and Indole-3- butyric acid (0.30 µM) with 3% sucrose [4]. The pH of culture media was maintained in between 5.6-5.8. The inoculated culture tubes were incubated in culture room at 25 ± 2 °C for 16/8hrs photoperiod. Cut ends of explants were kept in such a way so as to have maximum contact with the medium [5]. After 4 week of inoculation, micro propagated plant was transfer on elicitation medium and incubated for 7 days in growth room at 25±2 ºC temperature with 16 hours photoperiod.

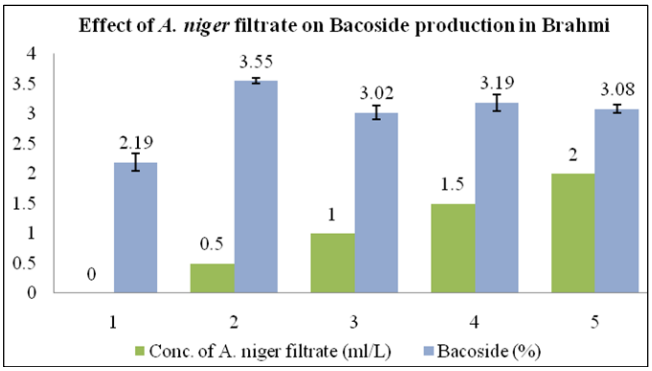
Fungal elicitor was prepared by using *Aspergillus niger* filtrate developed by inoculating loop full culture in 250 ml flasks containing potato dextrose broth. The flasks were incubating in shaking incubator for 37 ºC for 21 days at 80 rpm. The flasks will be autoclaved and the fungal mat separated from the culture medium/filtrate. The culture filtrate was filter through Whatman No. 1 filter paper and made up to a known volume. This volume was be again autoclaved and stored at 4 °C and designed as culture media filtrate. Elicitor was added to MS medium directly before planting to a new flask [6]. Elicitation of experiment was laid in completely randomized block design. Five different treatments of *Aspergillus niger* filtrate with four replication each. *Aspergillus niger* filtrate (0, 0.5, 1.0, 1.5, 2.0 ml/L) were incorporated in MS basal medium for 7 days.

Biochemical analysis of Brahmi was carried samples after one week elicitation. Elicited explants were removed from elicitation media and washed with water to remove debris of explants. Fresh elicited plant was oven dried at 60 ºC for 12 hours. Dried explants were crushed with mortal and pastel. Uniform dry weight was selected for extraction of Bacoside. Coarse powder (500 mg) of elicited samples was extracted and dissolved in 3 ml absolute ethanol and kept for 24 hrs. The extracts then were filtered through Whatman no. 42 filter paper [7]. Ethanolic extracts were used for quantitative detection of Bacoside content in Brahmi. Ethanolic extract 40µl were diluted by using 95% ethanol and made up to 4 ml final volume and compared with standard Bacoside concentration procured from Sigma Aldrich. Analysis was carried out by using UV Spectrophotometer at 278 nm [8]. Data obtained from various biometric and biochemical observation was analyzed by "Analysis of variance" method by using Completely Randomized Design [9].

Results and Discussion

Shoot initiation was observed from the auxiliary buds of nodal segments in second week after inoculation and in third week from the callus initiated at the base of the nodal segments. The shoot proliferation was due to the synergistic effects of cytokinin and auxin supplemented in the media. Similar results were reported by Ikeuchi *et al.* (2016) [10], the pericycle cells and the neighboring vascular parenchyma and/or procambium cells together serve as a primary source for shoot regeneration. Sarkar and Jha (2017) [11] were observed that, shoot initiation from internodal segments after second week of inoculation in *Bacopa monnieri* (L) Wettst.

Present investigation clearly indicated that the *Aspergillus niger* filtrate significantly influenced the Bacoside production in Brahmi. After seven days elicitation, maximum enhancement in Bacoside (1.62% DW) was reported at lower concentrations of *Aspergillus niger* filtrate (0.5 ml/L) over control. Higher concentrations of *Aspergillus niger* filtrate also showed increasing but undulating results in Bacoside production over the control. Elicitation effects were might be due to the fungal cell wall works as a polysaccharide elicitor, which induces calcium concentration in the cell and activates various defense responsive pathways leading to the accumulation of phytoalexins and low molecular weight antimicrobial compounds [12]. Earlier researchers reported similar type of results in different plants. Such as, Devi and Srinivasan (2011) [13] studied on stimulation of gymnemic acid production by using fungal elicitor in cell suspension cultures in *Gymnema sylvestre.* Manjula and Mythili (2012) [14] suggested that the improved phytochemical production by using biotic and abiotic elicitors in *Marsilea quadrifolia.* Vakil and Mendhulkar (2013) [2] worked on synthesis of andrographolide by *Aspergillus niger* and *Penicillium expansum* elicitors in cell suspension culture of *Andrographis paniculata*.



Effect of *A. niger* filtrate on bacoside production in Brahmi

Conclusion

On the basis of the results obtained from the present investigation, *Aspergillus niger* filtrate was proved to be a potential elicitor to enhance the bacoside production in *Bacopa monnieri*.

References

1. Radman R, Sacz T, Bucke C, Keshvartz T. Elicitation of plants and microbial cell systems. Bioteh. Appl. Biochem 2003;37:91-10.
2. Vakil MM, Mendhulkar VD. Synthesis of andrographolide through *Aspergillus niger* and *Penicillium expansum* elicitors in cell suspension culture of *Andrographis paniculata*. Bot Stud 2013;54:49.
3. Binita BC, Dave MA, Jasraj YT. Bacopamonnieri rapid efficient and cost effective micropropagation. Pl. Tissue cul. Biotech 2005;15(2):167-175.
4. Kharde AV, Chavan NS, Chandre MA, Autade RH, Khetmalas MB. *In vitro* enhancement of bacoside in Brahmi (*Bacopa monnieri*) using colchicine. J Pl. Biochem. Physiol 2017;5:1-6.
5. Patni S, Yaseer Z, Suhail A. Shamsuddin. *In vitro* propagation and callus formation of *Bacopamonnieri* (L) Penn. Pl. Tissue Cul. Biotech 2010;20(2):119-125.
6. Staniszewska I, Krolicka A, Malinski E, Jojkowska E, Szafranek J. Elicitation of secondary metabolites in *in vitro* cultures of *Ammi majus* L., Enz. Microbial. Technol 2003;33:565-568.
7. Pawar RK, Sharma SR, Singh KC, Sharma KR. Development and validation of HPTLC method for the

determination of andrographolide from *Andrographis paniculata* (whole plant). Int. Res. J Chem 2010;1(2):15-19.
8. Saini N, Mathur R, Agrawal S. Qualitative and Quantitative Assessment of four marketed formulation of Brahmi, Ind. J Pharm. Sci 2012; 74(1):24-28.
9. Panse VG, Sukhathme PV. Statistical Method for Agricultural workers, ICAR Publication, New Delhi 1967.
10. Ikeuchi M, Sugimoto K, Iwase A. Plant Callus: Mechanisms of induction and Repression. The Pl. Cell 2013;25:3159-3173.
11. Sarkar S, Jha S. Morpho-histological characterization and direct shoot organogenesis in two types of explants from Bacopa monnieri on unsupplemented basal medium. Pl. Cell, Tissue and Organ Culture. 2017; 130(2):435-441. https://doi.org/10.1007/s11240-017-1231-6.
12. Cordell GA. The alkaloids chemistry and biology. Academic press, New York 1997.
13. Devi SC, Srinivasan VM. *In vitro* studies on stimulation of gymnemic acid production by using fungal elicitor in suspension and bioreactor based cell cultures of *Gymnema sylvestre*. Rec. Res. Sci. Tech 2011;4:101-104.
14. Manjula R, Mythili T. Improved phytochemical production by using biotic and abiotic elicitors in *Marsilea quadrifolia*. Ind. J Cur. Sci 2012, 98-101.