# Effect of Vermicompost on the Regeneration of Medicinal Plant Bacopa Monnieri (Linn)

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**Abstract:** ***The conventional sources of Murashige and Skoog (MS) salts were substituted with vermicompost for the micro propagation studies. Vermicompost (1%- 30%) supplemented with 8 g/L agar as the experimental media and the conventional MS medium supplemented with 30 g/L of sucrose and 8 g/L of agar was used as the control. The study was initiated to find out the effects of humic acids (HA) from vermicompost on in situ development of medicinal plant Bacopa monnieri nodal tissue on the early stages of plant growth. It was found that the different fractions of vermicompost can bring about growth promotion in different somatic tissues. Plant growth increased with increasing concentrations of vermicompost media. Hardening of Tissue cultured plantlets to plants was taken up using vermicompost and its spray in the medium of cocopeat. The study has indicated that by standardizing the technique, it is possible to develop the plants through micropropogation in an economical way. Statistically Mann-Whitney 'U' test was considered for analyses of significance of various parameters. The mean number of nodes, leaves, roots and shoots were determined and significant differences were detected between the two media. Of the different fractions of vermicompost studied, 30% of vermicompost have given the best result. Thus the economical and the innovative technique implemented in this study enhance the production of the medicinal plant.***

**Keywords:** Economical Medium, In Vitro Plant Regeneration, *B.monneira*, vermicompost Media

## 1. Introduction

Tissue culture has enabled mass multiplication of plants. In the past maximum research has been directed to develop tissue culture protocols for floriculture and fruit plants. Recently, research is directed to carryout in developing tissue culture protocols for medicinal plants. *Bacopa monniera* and *Ginkgo biloba* are well-known cognitive enhancers in Indian and Chinese traditional medicine systems. Standardized extracts of these plants were used to evaluate the antidementic and anticholinesterase activities in adult male Swiss mice [21]. *Bacopa monnieri* is traditionally used as a brain tonic to enhance memory development, learning, and concentration and for centuries as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent [48] [3].

Earthworms promote the production of plant hormones-auxins, gibberellins and cytokinins from organic waste. Auxins are responsible for cell elongation, cytokinins for promoting cell division and gibberellins for stem elongation. These hormones are dose significant and play a fundamental role in plant metabolism. They can influence plant growth and development as well as crop quality significantly when present at very low concentrations [5].

Tissue culture has many advantages such as production of disease-free planting materials in large numbers. It permits rapid dissemination of healthy and improved plants that grow uniformly. These advantages attract their market value [58]

Tissue culture technology is cost effective for adoption and only few rich institutions and rich farmers have adopted this technology. The cost of the culture nutrient medium requires expensive chemicals [50]. In order to increase application of tissue culture technology in producing endangered medicinal plants, it is essential to lower the cost of micropropagule production.

The objective of this study was to find a way for an efficient and affordable protocol for the micropropagation. The investigations were carried out to study the effects of humic acids (HAs) from vermicompost on in situ development of *Bacopa monnieri* nodal tissue to promote vermicompost as an alternative nutrient media in plant tissue culture.

## 2. Materials and Methods

### 2.1 Preparation of Media for tissue culture

MS Media is the usual conventional medium used in plant tissue culture. Experiments were maintained to compare the response of *Bacopa monnieri* to different concentrations of vermicompost as nutrient growth inducer in micropropagation studies.

#### 2.1.1 Use of Vermicompost as nutrient media

Different fractions of vermicompost (1% - 30%) which contains humic and fulvic acids was used to make medium. This was supplemented with 8 g/L of agar.

### 2.2 Preparation of Explant

Explants from certified disease-free plants of *B. Monniera* were obtained from GKVK, Bangalore and planted in potted soil at the institution. Low cost medium using vermicompost was developed and used alongside the conventional MS medium to regenerate medicinal plant *Bacopa monnieri* from nodal cuttings.

### 2.3 Sterilization and Initiation of the Cultures

Healthy plantlets were collected from the net house and leaves were excised. The stem pieces were then cut into 2 cm long nodal cuttings, each having a bud. The nodal cuttings were washed in running tap water and then surface sterilized with tween 20 for 10 minutes. The explants were treated with 70% (v/v) ethanol and 0.1% $Hgcl_2$ and then rinsed four times with sterile distilled water. The healthy explants were introduced into the nutrient media.
The cultures were incubated at 25±2$^{o}$ C and a photoperiod of 16 hours light and 8 hours darkness at a light intensity of 2000 lux. The number of leaves, nodes, roots and plant height were determined and recorded every week. This experiment was repeated five times to test reproducibility of the results.

### 2.4 Acclimatization

After 4 weeks plantlets with well-developed root and leaf systems were washed with tap water to remove adhering media. They were then transplanted onto a mixture of garden soil, vermicompost and cocapeat in the ratio 2:1:1 and dispensed in rectangular trays. The number of surviving plantlets was recorded and the plantlets transplanted onto the soil

### 2.5 Experimental Data Analysis

The Mann-Whitney 'U' test was considered for analyses of significance of various parameters and was carried to compare the mean number of nodes, leaves, roots and shoots formed in the conventional MS medium and the alternative vermicompost medium.

## 3. Results and Discussion

Present study provides information on the response of *Bacopa monnieri* (nodal tissue) to different concentrations of vermicompost (Fig 1) in the culture tubes mixed with agar.

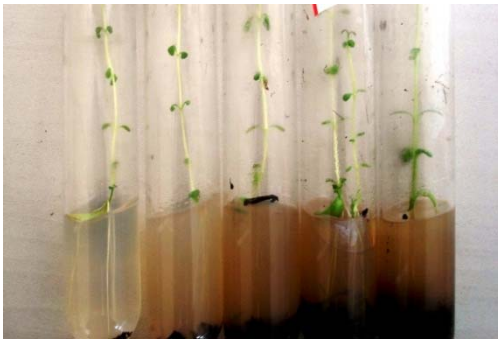

**Figure 1** Effect of increasing concentration of vermicompost on the micropropagation of *Bacopa monnieri* (nodal tissue)

There is a clear indication of short duration taken for growth induction in vermicompost used tubes compared to the tubes having regular MS medium. significant values were recorded (p=0.023), as per Table 1.
A significant difference was recorded between the control (MS Medium) and experimental samples (different concentration of Vermicompost) in various assessable parameters like number of nodes (Fig 2), leaves (Fig 3), shoots (Fig 4) and length (Fig 5) by the end of 3rd week (Table 2) 6th week (Table 3) and 10th week (Table 4).

**Table 1:** Comparative analysis on time taken for sprouting by the *B.monneira* when cultured on vermicompost (Study) and MS medium (control).

| TIME | | | |
|---|---|---|---|
| GROUP | Mean | Std. Deviation | Z |
| Study | 10.2857 | .48795 | 2.26800 |
| Control | 21.0000 | .00000 | p=.023 sig |

**Table 2:** Effect of Vermicompost (1% - 30%) on the microprapagation of *B.monneira* at 3rd week on comparing with MS media.

| | | Mean | Std. Deviation | Z |
|---|---|---|---|---|
| NODES3 | Control | 2.3333 | .57735 | 2.12100 |
| | study | 4.0000 | .00000 | p=.034 |
| LEAVES3 | Control | 4.6667 | 1.52753 | 1.5490 |
| | study | 6.0000 | .00000 | p=.121 |
| SHOOTS3 | Control | 1.0000 | .00000 | .00000 |
| | study | 1.0000 | .00000 | p=1 |
| ROOTS3 | Control | 2.0000 | .00000 | 2.23600 |
| | study | 4.0000 | .00000 | p=.025 |
| SIZE3 | Control | .9667 | 1.32791 | 1.55000 |
| | study | 2.6000 | .95394 | p=.121 |

**Table 3:** Growth Rate of Plantlets were analysed by differential statistical analyses between the study and the control at 6th week.

| | | Mean | Std. Deviation | Z |
|---|---|---|---|---|
| NODE6 | Control | 3.6667 | 2.08167 | 1.52800 |
| | study | 7.3333 | 3.05505 | p=.127 |
| LEAVE6 | Control | 8.0000 | 1.73205 | 1.15800 |
| | study | 15.3333 | 7.23418 | p=.246 |
| SHOOT6 | Control | 1.3333 | .57735 | 1.15800 |
| | study | 3.3333 | 2.08167 | p=.246 |
| ROOT6 | Control | 4.0000 | 1.00000 | 1.13800 |
| | study | 5.3333 | 1.15470 | p=.178 |
| SIZE6 | Control | 1.4000 | 1.44222 | 1.55000 |
| | study | 3.3333 | 1.15470 | p=.121 |

**Table 4:** Growth Rate of Plantlets were analysed by differential statistical analyses between the study and the control at 10th week.

**Group Statistics**

| | GROUP | Median | Std. Deviatio | Z |
|---|---|---|---|---|
| WT | study | 279.0000 | 34.35598 | 2.05000 |
| | Control | .0000 | .00000 | p=.046 |
| NODES10 | study | 17.0000 | 7.02377 | 1.73200 |
| | Control | 4.0000 | 2.82843 | p=.083 |
| LEAVES10 | study | 30.0000 | 5.29150 | 1.73200 |
| | Control | 8.5000 | 2.12132 | p=.083 |
| SHOOTS10 | study | 7.0000 | 1.41421 | 1.54900 |
| | Control | 1.5000 | .70711 | p=.121 |
| LEAV10 | study | 10.0000 | 5.50757 | 1.48100 |
| | Control | 4.0000 | 1.41421 | p=.139 |
| LENTH1 0 | study | 19.2000 | 2.31589 | 1.73200 |
| | Control | .9000 | .14142 | p=.083 |

**Table 5:** Effect of vermicompost (1%-30%) media on the growth rate of the plantlets were compared with MS media during 3rd, 6th and 10th week of invitro micropropagation of *B.monneira*.

**Paired Samples Test**

| | | Paired Differences | | | |
|---|---|---|---|---|---|
| GROUP | | Mean | Std. Deviation | Z | p |
| Control | NODES3 - NODES6 | -2.0000 | 2.82843 | 1.000 | .317 |
| | LEAVES3 - LEAV6 | -4.5000 | 3.53553 | 1.342 | .18 |
| | SHOOTS3 - SHOOT6 | -.5000 | .70711 | 1.000 | .317 |
| | ROOTS3 - ROOT6 | -2.0000 | 1.41421 | 1.342 | .18 |
| | LENGTH3 - LENGTH6 | -.7000 | .14142 | 1.342 | .18 |
| | NODES3 - NODES10 | -2.0000 | 2.82843 | 1.000 | .317 |
| | LEAVES3 - LEAVES10 | -4.5000 | 3.53553 | 1.342 | .18 |
| | SHOOTS3- SHOOTS10 | -.5000 | .70711 | 1.000 | .317 |
| | ROOTS3 - Roots10 | -2.0000 | 1.41421 | 1.342 | .18 |
| | LENGTH3 - LENTH10 | -.7000 | .14142 | 1.342 | .18 |
| Case | NODES3 - NODES6 | -3.3333 | 3.05505 | 1.342 | .18 |
| | LEAVES3 - LEAV6 | -9.3333 | 7.23418 | 1.604 | .109 |
| | SHOOTS3 - SHOOT6 | -2.3333 | 2.08167 | -1.941 | .192 |
| | ROOTS3 - ROOT6 | -1.3333 | 1.15470 | 1.342 | .18 |
| | LENGTH3 - LENGTH6 | -.7333 | .20817 | 1.414 | .157 |
| | NODES3 - NODES10 | -13.6667 | 7.02377 | 1.604 | .109 |
| | LEAVES3 - LEAVES10 | -22.0000 | 5.29150 | 1.604 | .109 |
| | SHOOTS3- SHOOTS10 | -6.0000 | 1.41421 | 1.604 | .109 |
| | ROOTS3 - Roots10 | -6.3333 | 5.50757 | 1.342 | .18 |
| | LENGTH3 - LENTH10 | -15.9667 | 2.66333 | 1.604 | .109 |

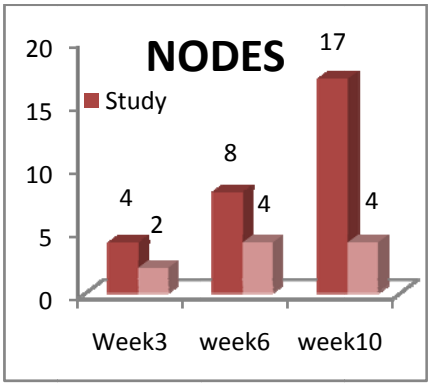


**Figure 2:** Effect of Vermicompost (Study) on growth of number of nodes of Bacopa monnieri

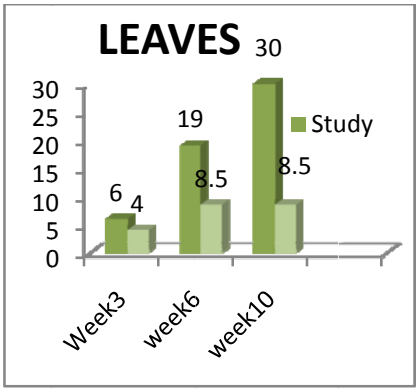


**Figure 3:** Effect of Vermicompost (Study) on invitro growth of number of leaves of Bacopa monnieri

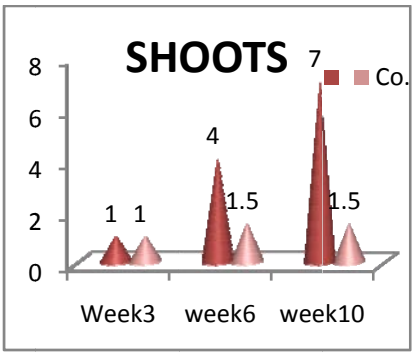


**Figure 4:** Effect of Vermicompost (Study) on invitro growth of Bacopa monnieri in terms of number of Shoots

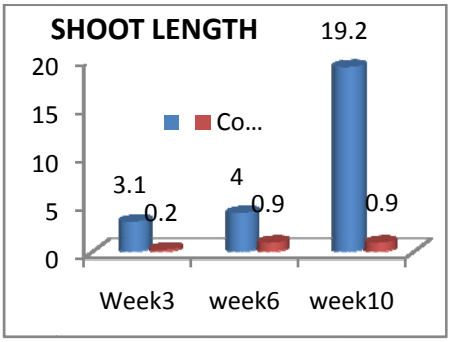


**Figure 5:** Response of B. monneira on vermicompost media (study) and MS media (control) in terms of shoot length

In case of roots and length of plantlets (Fig 4), when the study was compared with the control, statistically significant difference was observed for the group study and the same holds good for the individual study. This clearly indicates the continuous and slow release of nutrients from Vermicompost that prolongs the plant growth and development in *in vitro* when compared with the MS media. There was growth of plantlets in the culture tubes up to 10th week till they were taken for transfer for primary hardening. In control (MS Medium) tubes, the growth was impaired after 4th week and no significant difference was observed between 6th and 10th week.

Vermicompost is considered to enhance the rate of germination of seeds and establishment of seedlings. Early growth of seedlings was found to be better in vermicompost amended soils than the soil receiving normal compost [24]. The lower doses of vermicompost were preferred to 100% vermicompost to achieve significant effect on plant growth [53] [27] [44]. Studies have suggested that the plant growth regulator like substances may be released into the medium that was exposed to earthworm activity. Induction of profuse rooting and early flowering was reported by many scientists on using vermicompost as soil amendment. It is of the opinion that the kind of stimulation for establishment of crops is not merely due to the nutrient content in vermicompost [55] [30] [32]. High levels of humic substances are reported in the vermicompost derived from different organic wastes which have showed presence of hormone like properties [6] [41]. Development of innovative plant tissue culture system is pertinent to environmental sustainability. However for appropriate use it requires sufficient research to ensure the reliability.

The present investigation reveals that the varying response of plant tissue (*Bacopa monnieri*) to vermicompost media can be due to various factors. However, Plant growth (*Bacopa monnieri*) was reported to be better with vermicompost. Most trials of this present study have demonstrated the increased growth rate of the plants grown on vermicompost media. Of the different fractions of vermicompost studied, 30% of vermicompost have given the best result.

It has been shown that earthworms decompose the organic residue and their castings can be used as plant growth medium as an alternative for Soilless Media [37]. Humus is a complicated material formed during the breakdown of organic matter. One of its components, humic acid, provides many binding sites for plant nutrients, such as calcium, iron, potassium, sulfur and phosphorus. These nutrients are stored in the humic acid molecule in a form readily available to plants, and are released when the plants require them [4]. Humic acids (HAs) comprise one of the major fractions of humic substances. They are characterized by dark-colored, alkali-soluble, acid-insoluble organic matter [51]. These humic substances are endowed with hormone-like activity that improves plant nutrition and growth [57] [18]. Humus helps soil particles form into clusters, which create channels for the passage of air and improve its capacity to hold water. Humus is believed to aid in the prevention of harmful plant pathogens, fungi, nematodes and bacteria.

The previous reports have shown that the incorporation of humic acids extracted from pig manure vermicompost using the classic alkali/acid fractionation and humates extracted from pig manure and food wastes vermicompost were mixed with vermiculite along with soilless plant growth media, increased the growth of tomato and cucumber plants significantly, in terms of plant heights, leaf areas, shoot and root dry weights [6]. Humic acids isolated from earthworm compost has increased the root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots [16].The humic substances at the concentration of 1 mg C l-1 produced increased number of leaves in explants of Nicotiana plumbaginifolia than those grown in the control. Quantitative differences were also observed in the esterase and peroxidase activity during growth [40]. Use of pig manure vermicompost as a component of a horticultural bedding plant medium had resulted in improved physicochemical properties and plant growth [5].

In most cases innovations have received less attention. This study assessed the likelihood of biological importance of plant tissue culture using the source of organic manure.

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