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Bacopa monniera L.: Pharmacological Aspects and Case Study Involving *Piriformospora indica*

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Abstract

Medicinal plants are in great demand in modern civilization to extract various drugs for human welfare. Natural resource is getting scarce. It is possible to increase the biomass and active ingredients of *Bacopa monniera*. It's product is used as a traditional ayurvedic medicine, for memory enhancing, anti-inflammatory, anti-oxidant, analgesic, antipyretic, sedative, cardiotonic and antiepileptic drug. With the increasing population in the modern world there is corresponding increase in the disease possibilities. In the last years, urbanization and changes in early habits have caused a shift towards the consumption of herbal products. This has been followed by a growing commercialization of medicinal plants. These products come from a labor- and capital-intensive activity, where chemical input plays an essential role, but also brings up a set of problems linked with the degradation of natural environment and resource base. Therefore, awareness in this direction for curing by using medicinal plants is one of the main objectives of the present article. Also in the present scenario, herbal medicines are gaining popularity in day-to-day life because they are cheap, easily available and have little or no side effects.

Keywords: *Bacopa monniera*; *Piriformospora indica*; Brahmi; nootropic; Saponins; Bacosides; Arbuscular Mycorrhizal Fungus (AMF).

INTRODUCTION

During the course of our study on medicinal plants, *Bacopa monniera* L. (Scrophulariaceae), a widely distributed herb in the warmer parts of Asia, Australia and America, commonly known as 'Brahmi' in India or Indian water hyssop has been investigated (Kapoor, 1990). Brahmi is an important ancient medicinal plant. The name Brahmi is derived from the word "Brahma", the mythical "Creator" in the Hindu pantheon. Because the brain is the center for creative activity, any compound that improves the brain health is called Brahmi. Other Sanskrit (ancient Indian language) names for this plant are "Bahuphene", Ahiphena" and "Phenavati". The word "Phena" means "foaming property". When mixed with water, *Bacopa* plant parts produce a stable froth that is attributed to the saponins present in the plant. The plants have been selected based on their use in traditional systems of medicine, and research has identified a number of natural compounds that could act as nootropic agents (Russo and Borrelli, 2005). Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in curing mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy. Bacosides, Brahmi's active principles responsible for improving memory related functions, are attributed with the capability to enhance the efficiency of transmission of nerve impulses, thereby strengthening memory and cognition (Anon, 2004). Most of this research is based on plants used in the ancient systems of medicine to promote brain health.

Today's medicinal plants are important to the global economy, as approximately 80% of traditional medicine preparations involve the use

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of plants or plant extracts (Dhyani and Kala, 2005). The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. An efficient and most suited alternative solution to the problems faced by the phytopharmaceutical industry is development of *in vitro* systems for the production of medicinal plants and their extracts (Banerjee and Shrivastava, 2008).

The aim of this article is to present new developments in the potentials use of biological tools such as the symbiotic fungus *Piriformospora indica*, which should ensure adequate levels of herbal drug production. Our experiments have indicated strong and viable interaction between *Bacopa* root system and fungal hyphae. They were able to colonize the root at inter- and intraradical region leading to profuse sporulation. The hyphae also proliferate extra radically into the substratum. The potential of this symbiotic fungus as biofertilizers and bioprotectors to enhance crop production is well recognized, but not well exploited because of the current agronomic practices, with the implications for the environment.

Characteristic features of *Bacopa monniera* L.

The plant source for Brahmi is *B. monniera*, a small creeping herb with numerous branches, small oblong leaves, and light purple flowers. It grows in wet and sandy areas and near streams in tropical regions. Flowers and fruits appear in summer and the whole plant is medicinally useful (Bone, 1996; Chopra, 1958; Anon, 2004).

The Mechanism for the Sustainable Memory

Russo *et al.* (2003a) suggested that because of its ability to reduce NO-induced cellular alterations, brahmi has a therapeutic potential in treatment or prevention of neurological diseases. It was also reported that extract of brahmi rich in saponins, is able to induce a dose-related increase in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in rat frontal cortex, striatum and hippocampus (Bhattacharya *et al.*, 2000).

The hippocampus, the brain's seat of memory, is located in the temporal (left and right) sides of the brain. It processes signals sent to the brain by the senses into the templates of memory, which are then stored in other parts of the brain, creating a long-term memory. Signals are converted into electrical impulses in the nerve cells due to a rapid change in protein composition. These impulses are then conducted across neurons and through synapses, which connect nerve cells. This process continues until the bonds between the nerve cells strengthen and memory is created.

Normal synaptic activity is a process mediated by neurotransmitters. Each neuron is a single nerve cell. It has one or more arms called axons that send signals (impulses) and one or more arms called dendrites that receive signals. When a signal is transmitted through an axon terminal, spherical bodies called vesicles fuse with its membrane. Neurotransmitters are released when the vesicles burst open into the synaptic space, the minute space between the sending and receiving cells used to discharge neurotransmitters ("passengers"). To end the signal, the axons reabsorb some neurotransmitters; and the enzymes in the synapse neutralize the other neurotransmitters. It is evident that a disruption in any part of normal synaptic activity would affect memory. This normally occurs with advanced age and continuous electrical activity, which wears out the synapses. As a result, new memory creation is impaired and memory loss occurs.

Chemical substances and plant extracts that are known to restore the memory work in different ways. The bacosides are the memory chemicals in Brahmi (Rastogi *et al.*, 1994; Sivaramakrishna *et al.*, 2005). The active ingredients vary from 0.9 to 1.8 percent (Mathur *et al.*, 2002). According to scientists at the Central Drug Research Institute (CDRI) located in Lucknow, India, the bacosides help to repair damaged neurons by adding muscle to kinase, the protein involved in the synthesis of new neurons to replace the old ones. Depleted synaptic activity is thus restored, leading to augmented memory functions.

Mechanisms of Action

Since Bacopa's primary therapeutic use is to enhance cognitive function and mostly research has been focused on the mechanism of action.

The triterpenoid saponins and their bacosides are responsible for Bacopa's ability to enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission and boosting the synthesis of new protein in the brain (Singh and Dhawan, 1997).

Loss of cholinergic neuronal activity in the hippocampus is the primary feature of Alzheimer's disease. It is indicated by depleted concentrations of the neurotransmitter, acetylcholine, a reduction in choline esterase activity and decreased muscarinic cholinergic receptor binding in the frontal cortex and hippocampus in rats (Enz *et al.*, 1993). Based on animal study results, bacosides appear to have antioxidant activity in the hippocampus, frontal cortex, and striatum (Bhattacharya *et al.*, 2000). Animal research has shown *Bacopa* extracts modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain (Chowdhuri *et al.*, 2002). *In vitro* research has shown Bacopa exerts a protective effect against DNA damage in astrocytes and human fibroblasts (Russo *et al.*, 2003a,b).

In animals, *Bacopa* has a relaxant effect on pulmonary arteries, aorta, trachea, and ileal and bronchial tissue, possibly mediated by inhibition of calcium-ion influx into cell membranes (Channa *et al.*, 2003). *Bacopa* appears to stabilize most cells *in vitro*, (Samiulla *et al.*, 2001) and possesses anti-inflammatory activity *via* inhibition of prostaglandin synthesis and lysosomal membrane stabilization (Jain *et al.*, 1994). *In vitro* research suggests an anticancer effect for *Bacopa* extracts, possibly due to inhibition of DNA replication in cancer cell lines (Elangovan *et al.*, 1995)

Chemical Constituents and Pharmacokinetics

The major chemical constituents isolated and characterized from *B. monniera* are dammarane type triterpenoid saponins with jujubogenin or psuedojujubogenin moieties as aglycones. The pharmacological effects of *B. monniera* are attributed to the presence of a number of biologically active compounds, including alkaloids, flavonoids, saponins and sterols. These saponins especially bacoside A and B, which are considered as bioactive marker compounds for this species and also have the same sugar moiety but differs only in optical rotation (Deepak and Amit, 2004). Bacoside A is considered the major active component, first identified by Chatterji *et al.* in 1963, while bacoside B being an optical isomer of A (Singh *et al.*, 1988). The composition of bacoside A and B has been established as a mixture of four triglycosidic and four diglycosidic saponins, respectively (Bacoside A as a mixture of Bacoside A_3, Bacopaside II,3-O-[α-L-arabinofuranosyl-(1→2)-{ß-D-glucopyranosyl-(1→3)-}-α-L-arabinopyranosyl] jujubogenin and Bacopasaponin C and bacoside B as a mixture of Bacopaside N1, Bacopaside N2, Bacopaside-IV and Bacopaside-V) (Deepak *et al.*, 2005; Sivaramakrishna *et al.*, 2005). Many active constituents- the alkaloids brahmine, herpestine, saponins d-mannitol, hersaponin acid A, and monnierin-were isolated in India over 40 years ago. Other active constituents have since been identified, including betulinic acid, stigmastarol, *beta*-sitosterol, as well as numerous bacosides and bacopasaponins. The constituents responsible for Bacopa's cognitive effects are bacosides A and B. The compounds responsible for the memory enhancing effects of *B. monniera* are triterpenoid saponins called "bacosides" (Mahato *et al.*, 2000; Chakravarty *et al.*, 2001; 2003; Hou *et al.*, 2002; Russo and Borrelli, 2005).

It is significant that dammarane-type saponins are also the primary active constituents in Panax ginseng (Blumenthal, 2003). They have been shown to promote memory acquisition, retention and retrieval in rats and survival of experimentally damaged neurones in chicken and rat cerebral cortex (Ma and Yu, 1993; Ma *et al.*; 1991; Himi *et al.*, 1989). Pharmacological studies showed that this plant can be used as a memory-enhancing, anti-inflammatory, analgesic, and antidepressant agent (Bhattacharya *et al.*, 2000; Sairam *et al.*, 2002).

Clinical and Preclinical Studies

An alcoholic extract of *Bacopa* showed anticancer activity against walker carcinosarcoma 256 (intramuscular) in rat and significantly inhibited Sarcoma 180 cell culture growth, possible by affecting DNA replication (Bhakuni *et al.*, 1969; Elangovan *et al.*, 1995). It also improved the performance of rats in several learning tests which measured the acquisition, consolidation and retention of learnt responses

Table 1. Clinical studies of *Bacopa monniera*

Treatments/ Dosages	Results	References
12g/day dried Bacopa in syrup form 4-weeks	Decreased anxiety, Enhancing of concentration and memory span. No side-effects reported.	Singh and Singh, 1980
350mg three times daily in syrup form 12-weeks	Enhancing Learning, memory, perception and reaction times. No side-effects reported.	Sharma et al., 1987
Bacopa standardized extract	Effective in enhancing learning and in controlling abnormal behaviour	Dave et al., 1993
100 mg/day 12-weeks	Improvements in a range of cognitive assessments. No side effects reported.	Negi et al., 2000
300 mg/d (standardized to min 55% bacosides) 2-hours post administration	No effect on various measures of memory performance found with acute administration of Bacopa.	Nathan et al., 2001
300 mg/d (standardized to min 55% bacosides) 12-weeks	Significantly improving early information processing, verbal learning rate and memory consolidation and reduced state anxiety. Adverse reactions reported: nausea, dry mouth and fatigue.	Stough et al., 2001
300 mg/d (standardised to min 55% bacosides) 12-weeks	Significant effect on retention of new information. Task assessing attention, verbal and visual short term memory and the retrieval of pre-experimental knowledge and anxiety measures unaffected.	Roodenrys et al., 2002
Combination of standardized extract of Bacopa 300 mg/day and *Ginko biloba* 120 mg/day 4-weeks	Measurements at baseline, 2 and 4-weeks. No significant effect found on cognition and memory. No difference in side effects compared to placebo.	Nathan et al., 2004
BacoMind™ capsule oral dose of 300 mg once a day for first 15 days and 450 mg once a day for next 15 days	Optimal health and mental functioning and is safe for oral consumption and tolerable. Minor gastrointestinal side effects.	Pravina et al., 2007

(Singh *et al.*, 1988). The preclinical studies supported the two open clinical studies reported enhancing memory and learning effects with *B. monniera* in children and patients with anxiety state (Sharma *et al.*, 1987; Singh and Singh, 1980). The clinical investigations are summarized in Table 1.

In pre-clinical and clinical studies, *B. monniera* has been shown to contribute to enhanced exploratory behavior and greater desire to seek out novelty, attention, concentration, sharpened memory and increased learning and retention. Scientific studies have also reported its use as anticancer, antihypertensive, tranquilizer and sedatives. This concurs with Singh and Dhawan (1997) findings regarding the effects of isolated *Bacopa* saponins on memory. Administration of bacosides to mice attenuated experimentally induced anterograde amnesia and improved memory as measured by a well validated learning task- the Morris Water maze test (Kishore and Singh, 2005).

In rat models of Alzheimer's disease, *Bacopa* was shown to significantly promote memory as well as reversing induced reductions of acetylcholine in the frontal cortex and hippocampus regions. The activity of choline acetyltransferase and muscarinic receptor binding of acetylcholine were also improved (Bhattacharya *et al.*, 1999). Acetylcholine is a neurotransmitter which plays an important role in memory and learning functions in the cerebral cortex and the hippocampus (Carlson, 2002). Furthermore, depletion of choline

acetyltransferase and hence acetylcholine, is one of the central neuropathological features of Alzheimer's disease (WHO, 1992).

B. monniera has been studied clinically for its acute and chronic effects on cognitive function. In children and adults, it appears chronic administration is associated with cognitive enhancing effects (Russo and Borrelli, 2005). This plant has been introduced onto the market in India and other countries, alone or in association with other phytocomplexes, and utilized in the treatment of memory and attention disorders (Shukia *et al.*, 1987). The commercial preparation has shown a remarkable nootropic activity, above all in very young subjects (Dave *et al.*, 1993). After clinical trials on human volunteers, a standardized extract *of B. monniera* is available for clinical use by the Central Drug Research Institute, Lucknow, India (Dhawan and Singh, 1996).

Traditional uses of *Bacopa monniera* L.

B. monniera often called "Brahmi" or "The thinking person's herb," is widely used in traditional Indian Ayurvedic System of medicine as a potent nervine tonic to enhance memory function, improve intellectual and cognitive functions (Rastogi *et al.*, 1994). It is also used in treatment of asthma, leprosy, hoarseness, water retention and blood cleaning (Singh and Dhawan, 1982).

B. monniera has been used in Ayurvedic formulations for conditions ranging from catarrhal complaints, gastrointestinal disturbances due to excessive tobacco use, habitual abortions and high blood sugar due to anxiety disorders, hysteria, epilepsy etc. (Chopra *et al.*, 1956; Nadkarni, 1976). In certain parts of India, Brahmi is believed to be an aphrodisiac. In Sri Lanka, under the name of Loonooweela, Brahmi is prescribed for fevers. In the Phillipines, it is used as a diuretic (Uphof, 1968).

The traditional use of the plant, of particular relevance to contemporary medicine, is its validated efficacy in promoting memory functions and providing relief to patients with anxiety neurosis. In Ayurveda, Brahmi is described as "medhya rasayana" or brain tonic with the ability to promote mental functioning along with providing general rejuvenative effects (Singh and Singh, 1980).

Brahmi has a bitter taste. Traditionally, the fleshy leaves and stems were made into a paste or pressed for juice extraction. Sugar, jaggery or honey was added to make it more palatable. Some of the known preparations with Brahmi are Brahmi Ghrita (in clarified butter), Sarasvatarishta (a decoction used as a brain tonic), Brahmi Rasayana (a rejuvenating formulation with other herbs), Brahmi Taila (medicated oil), and Brahmi Sarbat (a cooling drink).

In recent years, "Memory Plus", a product that contains the standardized extract of bacosides from Brahmi, has been marketed in India. Under the definition of herbal drugs in the guidelines of herbal medicines issued by the world health organization (WHO) in 1991, an herbal product that has been used traditionally without demonstrable harm does not require specific regulatory action unless new evidence demands a revised risk/benefit analysis. Subsequently, several formulations containing *B. monniera* extracts standardized for bacosides content, have appeared in the global market place.
The evaluation of BacoMind^{TM} in healthy adult volunteers revealed that, at the given oral dose of 300 mg once a day for first 15 days and 450 mg once a day for next 15 days, the herbal supplement was found to be safe and tolerable. Though minor gastrointestinal side effects were reported in 3 out of 23 volunteers, the general physical, systemic, hematological, biochemical and electrocardiographic parameters were within the normal limits (Pravina *et al.*, 2007). It is also reported that BacoMind^{TM} can be used as a natural dietary supplement for supporting optimal health and mental functioning and is safe for oral consumption. It also has the capacity to be used as an anti-oxidant and an anti-carcinogenic agent particularly against those carcinogens that mediate their effects via formation of oxygen free radicals (Deb *et al.*, 2008).

Therefore, ayurvedic medicines for traditional treatment of a number of disorders, particularly those involving anxiety, intellect and poor memory (Singh and Dhawan, 1997) epilepsy, insanity and retardation (Mathur *et al.*, 2002) and to counteract the effects of mental stress and neurosis, revitalization of sensory organs (Sivarajan and Balachandran, 1994). The well-known nootropic plant reported to possess sedative (Malhotra and Das, 1959), cardiotonic

(Mathur *et al.*, 2002), cognitive enhancer (Nathan *et al.*, 2001; Roodernrys *et al.*, 2002), broncho-vasodilatory (Channa *et al.*, 2003), hepatoprotective (Sumathy *et al.*, 2001; Sumathi and Nongbri, 2008), antidepressant (Sairam *et al.*, 2002), calcium antagonistic (Dar and Channa, 1999), smooth muscle relaxant (Dar and Channa, 1997), neuropharmacological (Russo and Borrelli, 2005) cell stabilizing (Samiulla *et al.*, 2001) and antiulcer (Sairam *et al.*, 2001) properties. The said plant also showed antistress effect, via modulation of Hsp 70 expression, superoxide dismutase and cytochrome p450 inhibitory activity in rat brain (Chowdhuri *et al.*, 2002) and antioxidant activity (Tripathi *et al.*, 1996). *B. monniera* is reported to play a protective role on morphine-induced brain mitochondrial enzyme status in rats (Sumathy *et al.*, 2002). It is also active against leishmaniasis (Sinha *et al.*, 2002).

It helps to regain general mental health through its rejuvenative effect. It is currently being marketed in Western countries as a memory enhancing agent. Studies have shown that the herb contains many active constituents, including a number of alkaloids and saponins, however, the major constituents are the steroidal saponins, Bacosides A and its optical isomer bacosides B, several other chemicals like stigmastanol, *beta*-sitosterol and stigmasterol is found in all parts of the plant (Mathur *et al.*, 2002). The recent discovery of the memory-enhancing property of the bacosides has enhanced the demand of this plant and resulted in its extensive use in several commercial preparations. Therefore, mass cultivation of this plant in consideration with its quantitative and qualitative improvement is the prime interest of herbal research.

A growing interest is centered on the protection of host organism by means of antioxidants because of their putative role in the modulation of smoking induced stress protein expression and apoptosis (Pinot *et al.*, 1997; Aoshiba *et al.*, 2001). Besides, the plant extract has been shown as potent free radical scavenger, antilipid peroxidative agent and antioxidant (Tripathi *et al.*, 1996), antiaging potential (Jyoti and Sharma, 2006). Preliminary studies indicated that bacosides, the major saponins are responsible for the facilitatory and modulatory effects of *B. monniera* (Singh *et al.*, 1988).

Anbarasi *et al.*, (2005a,b, 2006) have reported the protective effect of bacoside A on cigarette smoking-induced brain damage. The role of bacoside A against chronic cigarette smoking-induced hsp70 expression and apoptosis in rat brain cerebral cortex. Apoptosis was monitored by DNA fragmentation and terminal deoxynucleotidyl transferase-mediated deoxy uridine triphosphate nick end labeling (TUNEL) staining, and further confirmed by electron microscopy (Anbarasi *et al.*, 2005c).

The studies have shown that it improves the rate of learning in a brightness discrimination task and a conditioned avoidance task, that it improves retention, as demonstrated by savings in relearning, and that it attenuates amnesia induced by immobilization, electroconvulsive shock and scopolamine (Singh and Dhawan, 1997). This latter finding involved administration of the extracted bacosides A and B, and suggests that they influence cholinergic systems. Bacosides, Brahmi's active principles responsible for improving memory related functions, are attributed with the capability to enhance the efficiency of transmission of nerve impulses, thereby strengthening memory and cognition.

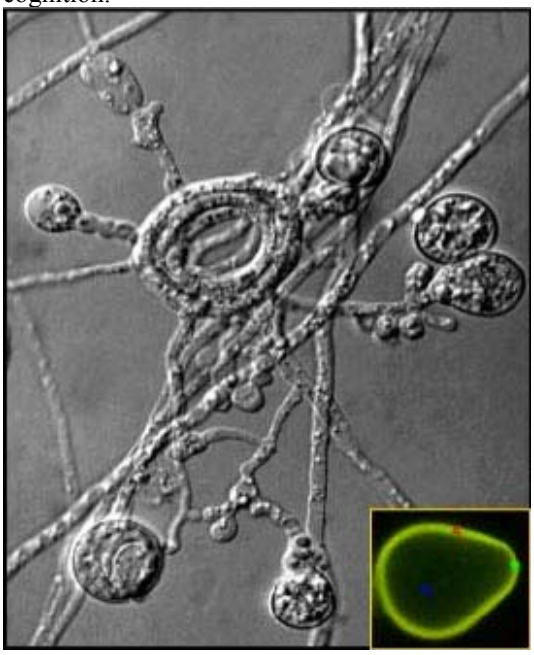

Figure 1: *Piriformospora indica*: A Model Organism. An overall view of the typical growth and differentiation. The inset showing fluorescent chlamydospore.

A Case Study

Active Symbiotic-Mycobiont

We have screened a novel symbiotic fungus *Piriformospora indica*, which mimics the capabilities of a typical, Arbuscular Mycorrhizal fungus (AMF)**.** It is related to the Hymenomycetes of the Basidiomycota (Verma *et al.*, 1998; Weiß *et al.*, 2004). It possesses highly coiled hyphae with pear shaped chlamydospores (Fig. 1). In contrast with AM fungi, it can be easily cultivated in axenic conditions. The fungus associates with the roots of various plant species in a manner similar to mycorrhiza and promotes their growth (Varma *et al.*, 1999; Pešken-Berghöfer *et al.*, 2004; Pham *et al.*, 2004a; Oelmüller *et al.*, 2005) also the rooting of tobacco calli and cuttings of ornamental plants was promoted (Varma *et al.*, 1999; Dru¨ge *et al.*, 2007).

The fungus acts as plant promoter (auxin), biofertilizer (solid and liquid engineering), bioregulator, bio-herbicide, immunomodulator, phytoremediator, biological hardening agent for micro-propagated plantlets; imparts resistance against heavy metals, biocontrol against insects and pathogens, stress tolerance – both temperature and salt; works as stimulator with PGPRs, antioxidant and drug enhancer, anti-ageing agent; promotes synthesis of anti-cancer drugs and possesses potential for its use in bionanomedicines (Varma *et al.*, 1999; Rai *et al.*, 2004; Waller *et al.*, 2005; Deshmukh *et al.*, 2006; Shahollari *et al.*, 2005, 2007; Serfling *et al.*, 2007; Sherameti *et al.*, 2008a,b).

Cultivation of *Piriformospora indica*

The fungus *P. indica* cultivated on Hill and Kafer medium (Hill and Kafer, 2001; Pham *et al.*, 2004b; Prasad *et al.*, 2005). Fungal discs are made by using bottom of the sterile Pasteur's pipette measuring about 4mm in diameter. One disc is placed in the centre of the medium containing 1% agar. Inoculum contained spores and actively growing hyphae. Petri plates were incubated in dark an inverted position in specially designed culture room and/or incubated (Memmert type) for 7-14d at 28±2°C.

Co-cultivation of *P. indica* and *B. Monniera*

The aseptic nodal segments (about 2 cm) were implanted on the sterile Hill and Kafer media. The culture jars were incubated in tissue culture laboratory maintained at temperature (25±2°C) and 16h photoperiod (fluorescence light 1000 Lux). Fungus treated plants were compared with untreated control plants in terms of morphological and anatomical distinctiveness.

Micropropagation of *B. monniera* is used to produce its mass culture, that improves its commercial availability and to increase the overall plant performance, at quantitative as well as qualitative levels. Pre-establishment of mycorrhiza in the host roots helps in the development of the synergistic effect with other rhizosphere microflora (PGPRs) that competes in the ecosystem for successful survival. Direct shoot bud regeneration in *B. monniera* cultures provides the basis for commercial cultivation, germplasm conservation and for exploring somaclonal variants with high medicinal value (Tiwari *et al.*, 2006).

Control **Treated**

Figure 2: Significant growth promotional effect of *P. indica* on *Bacopa monniera* L. in tissue culture medium. (a) Control; (b) co cultivated with *P. indica*. One plantlet 2 - 2.5 cm in height containing one node was placed in the middle of the Jar. The Incubation was done in tissue culture raised laboratory with temperature (25±2°C) and 16h photoperiod (fluorescence light 1000 Lux). Once the roots were initiated one agar disc infested with spores and hyphae placed next to plantlet. The experiment were continued for 12 weeks.

The fungus treated plants showed enhanced growth in comparison to the control (Fig. 2). Extensive root colonization was also observed. Hyphae were present on the surface and occupied the root cortex at inter-and-intracellular levels. Characteristic pear shaped chlamydospores in single, diploid, tetrad and in long chains were observed (Prasad *et al.*, 2008). In general, this fungus treated plant produced several fold more anti-oxidant activity, Bacosides and plant biomass (Unpublished data).

CONCLUSION

Due to over-exploitation of medicinal plants, their conservation is of paramount importance that can be made *in situ* and *ex situ*. For the purposes of quantitative and qualitative increments of the medicinal plants, various strategies have been employed that enhance the plant yield in terms of its total biomass and perfect survival, of which the use of chemical fertilizers play a pivotal role.

The estimated annual requirement of *Bacopa* is around 12,700 tons of dry biomass valued at Rs 1.5 billion, and is met solely from the natural populations, leading to their gradual depletion. Tissue culture techniques could play an important role in the clonal propagation, germplasm conservation and improvement of *B. monniera* (Prasad *et al.*, 2004). Rahman *et al.* (2002) first time reported that the potential of the cell suspension cultures of *B. monniera* for bacoside production. The first reported broth culture media used in rooting forms the highly significant observations found to be great importance in maintaining efficiency in multiplication, shortened time span, simplicity and benefit of genetic stability. This protocol is novel because of its minimal requirements and cost effectiveness for propagation (Banerjee and Shrivastava, 2008).

Micropropagation is widely used technique for mass production of important plants of economic importance, especially medicinal plants. Commercialization of micropropagated plants is the theme of the day in developed and developing countries. Unfortunately, the acclimatization phase raises problems concerning the survival rate and initial transplant shock on the transfer of the tissue culture raised plantlets to the field (Prasad *et al.*, 2004).

The present study represents a classical example of herb biomass production and the synthesis of excessive active ingredients by the early intervention of eco-friendly fungus, here *P. indica in vitro* experiment to lead a positive direction even trial in field condition. The association of the useful microorganism also promise the protection of the plant against pathogens.

Future Outlook

Isolation and derivatization or value added compounds from the bacosides isolated from *Bacopa monniera*. These derivatized compounds should be tested for its activity through biochemical analysis, *in vivo* and *in vitro* testing. Formulations needs to be prepared from the new compounds formed and commercialized. The other aspect would be selecting few other medicinal plants from traditional knowledge, having same chemical behaviour as Bacosides. Drug-drug interaction should be carried out from the lead compounds obtained and a similar study regarding the formulation would be carried out making use of human cell lines. The selected may be interacted with *P. indica* before carrying out further analysis. This work would not only lead to commercialization but also lead to patent of the formulations obtained.

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