Original Research Paper

Volume - 11 | Issue - 07 | July - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

# PHYTOCHEMICAL AND ANTIOXIDANT STUDIES OF *BACOPA MONNIERI* TREATED WITH ARBUSCULAR MYCORRHIZAE FUNGI *GLOMUS FASCICULATUM* AND *GLOMUS MOSSEAE*.

**Veena Adishesha*** Research Scholar, Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore – 560 056, Karnataka, India. *Corresponding Author

**Dr. K.M. Srinivasa Murthy** Associate Professor, Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bangalore-560-056, Karnataka, India.

**ABSTRACT** Vesicular arbuscular mycorrhizae (VAM) play a crucial role in enhancing the growth of plant by increasing the uptake of nutrients from soil and also is known to enhance the secondary metabolites of plants. The response of VAM *Glomus fasciculatum*and *Glomus mosseae*on the growth, phytochemical constituents and antioxidant properties of*Bacopa monnieri*commonly known as Brahmi was carried out.*B.monnieri*was treated with both pure cultures individually and mixed culture in (1:1) ratio along with uninoculated sample as control and gown in polyhouse condition for varying intervals. The physiological parameters, phytochemical analysis and antioxidant scavenging activities were analysed after 30, 60 and 90 days of VAM inoculation, respectively. Colonization of VAM was seen in the plant samples signifying the symbiotic association. Physiological analysis showed an increase in shoot, root and wet weight of *B. monnieri*with increase in time. The preliminary phytochemical analysis inferred the methanolic leaf extracts of *B.monnieri*treated with *Gf, Gm and Gf+Gm*contained alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids and carbohydrates which increased quantitatively with time.DPPH free radical scavenging activity showed escalation in result with increase in time of treatment with *G. fasciculatum*showinghigher scavenging activity compared to *Glomus mosseae* and the mixed cultures.

**KEYWORDS :** Vesicular arbuscular mycorrhizae (VAM), *Bacopa monnieri, Glomus fasciculatum, Glomus mosseae,* Phytochemical and Antioxidant.

## 1. INTRODUCTION

Phytochemicals are chemical constituents of the plants. These components are naturally occurring and can play an important role in prevention and protection against diseases in humans.Data from the World Health Organisation(WHO) suggests that close to 80% of people in developing and underdeveloped countries rely on plants and plant-basedproductsfor their health care (WHO, 2019). According to WHO medicinal plants are the finest source to obtain active components for therapeutic use. The ease of availability, minimal extraction costs, low toxic profile, with high antioxidant and antimicrobial activity of phytochemical has led to plants becoming an efficient alternative source for medicinal use(Nyamai*et al*., 2016).The use of herbal medicine in the past decades has received considerable attention. The beneficial properties of healing and curing of these medicinal plants are associated with phytochemicals, which are broadly classified into phenolic compound, phenolic acids, stilbenes,lignins, saponins and tannins (Nyamai*et al*., 2016), These bioactive compounds have become a reliable basis for leads in exploring new compounds for pharmaceuticals against various diseases (Palombo, 2006).

Antioxidants are substances that delay or inhibit damage to cells via oxidation. Oxidation leads to formation of free radicals such as reactive nitrogen species (RNS) and reactive oxygen species (ROS)(Senguttuvan*et al*., 2014). Phytochemicals are known to scavenge the free radicals, acting as highly effective primary antioxidants (Anokwuru, 2011). The antioxidant action of phytochemicals is also attributed to their properties of acting as lipid peroxidation inhibitors and chelating agent (Nyamai*et al*., 2016; Volluri*et al*., 2011).

*Bacopa monnieri*(L.), family Scrophulariaceae, also known as 'Brahmi', is one of the traditional medicinal plants of Ayurveda. It is a creeping herb that grows in wetlands and sandy areas in the tropical regions of India (Rajani., 2008; Jain *et al*., 2017). It is best known for its nootropic herbal properties (Jeyasri*et al*., 2020), as it is used as a memory enhancer and intellect, anti-analgesic, dementia, brain aging, restoration of synaptic activity, digestive and skin disorders (Vishwakarma *et al.,* 2017). Brahmi contains large amounts of active chemical constituents helping individual and synergistic action of Phyto-molecules in antimicrobial and antioxidant properties (Jeyasri*et al.,* 2020).

*B. monnieri* has medicinally active compounds such as alkaloids (brahmine, nicotine and herpestine), glycosides (asiaticosides and thankunicide) and saponins. It also has becoside A (saponins), one of the active moleculesinmemory assisting actions (Vishwakarma *et al*., 2017). Phytochemical constituents such as proteins and polysaccharides (Ramadas*et al*., 2016) and natural occurring antioxidants such as phenols, flavonoids and tannins are also studied (Pawar *et al.,* 2016). These compounds mediate their effect on the human body through similar mechanism as that of conventional drugs.

Vesicular arbuscular mycorrhizae (VAM) establish a symbiotic association with plants and enhances the phytochemical constituents of the host (Gupta *et al*., 2001). They play a crucial role in improving the growth of the plants by escalating nutrient uptake, predominantly phosphorous (Vani *et al*., 2018). It preserves the biological conditions of plant during adverse climatic change, such as increased heat, water scarcity and soil with decreased nutrients (Chen, 2006). The symbiotic relationship of medicinal plants with VAM has showed enhanced growth as well as improved functionality of medicinal components (Karthikeyan, 2009). Among the known VAM fungi, Glomus is commonly used mycorrhizae in agriculture and horticulture sectors, particularly *Glomus fasciculatum and Glomus mosseae*. There are reports suggesting that *Glomus fasciculatum*(GF) helps in activating immune system, helping them to overcome pathogenic infections and amplifying the yield of agricultural important crops (Math *et al*., 2019).Studies have shown that *Glomus mosseae*facilitates the nutrient absorption by boosting the root system architecture, therefore increasing the photosynthesis efficiency and bioactive components (Chen *et al*., 2017).

*B. monnieri*has been found to have exhibited root colonization by VAM fungi and the presence of both vesicles and arbuscules in the roots has been reported by various authors. The alleviation of antioxidant activity of methanolic extract of *B. monnieri*leaves after association with VAM fungi has also been proved (Sainthiya*et al.,* 2018).

This current study is focused on the significance of *Glomus fasiculatum* and *Glomus mosseae*on the improvement of phytochemicals and antioxidant properties in *B. monnieri*.

## 2. MATERIALS AND METHODOLOGY

### 2.1 VAM and plant collection

For the present investigation, VAM culture (*Glomus fasciculatum*and *Glomus mosseae)* were obtained from Centre for Natural Biological Resources and Community Development (NBRCD), Bengaluru. *Bacopa monnieri*(Brahmi) was collected from Indian Institute of Horticultural Research (IIHR), Bengaluru.

Potting mixture was made with 600g sterilized soil and 200g sterilized farmyard manure (cattle dung) 3:1 w/w and were mixed thoroughly.

The mixture was filled into pots, in triplicates (Karthikeyan *et al.*,2009). The *Glomus fasciculatum*(Gf), *Glomus mosseae*(Gm) *and Glomus fasciculatum*along with *Glomus mosseae*(Gf+Gm) (1:1) culture of VAM fungi were used for pot inoculation of *Bacopa monnieri*. 10g of the fresh inoculum was added to the substrate mixture (approximately 1200 spores) in the pot at 15cm depth, prior to planting the host plant (Ramakrishnan, 2012) in triplicates and a control set without the VAM culture was kept for comparative studies. Plants were grown in polyhouse conditions and were watered regularly. The plant samples were harvested after 30, 60 and 90 days of transplanting and growth parameters were measured and calculated.

**2.2 Measuring the physiological parameters of VAM inoculated plants**

Plants were harvested after 30, 60 and 90 days of transplanting. Allgrowth parameters were measured and calculated. The length of the longest individual root, number of roots and weight of the plants were recorded for each plant for each treatment. Shoots and roots were excised and dried.

**2.3 Extract preparation**

The plant leaves were collected after 30days of VAM inoculation and were shade dried and powdered. The sample was cold extracted in 1:10 ratio successively using methanol as the solventto extract polar and non-polar compounds. The extract was reduced using rotary evaporator and concentrated to 50mg/ml.The above procedure was followed for 60days and 90days of VAM inoculated plant samples for each treatment and also the uninoculated control sample.

**2.4 Qualitative phytochemical analysis**

Qualitative phytochemical analysis was carried outfor 30, 60 and 90days of VAM inoculated plant samples to check the presence of major constituents namely Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins, Cardiac glycosides, Terpenoids and Carbohydrate. (Solomon Charles Ugochukwu *et al*., 2013)using the methanolic extract of leaf parts of*Bacopa monnieri.*

**2.5 Quantitative estimation of phytochemicals**

Based on the preliminaryqualitative results the quantitative assay was carried out for Alkaloids, Tannins, Phenols, Flavonoids, Proteins, and Carbohydrates.

**2.5.1 Total Tannin Content Determination**

The tannins were determined by slightly modified Folin and Ciocalteu method. To 1 ml of sample extract 7.5 ml of distilled water was addedwith 0.5 ml of Folin's-Phenol reagent and 1 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract. *(*Md. Sazzad Hossain*, et al., 2013*).

**2.5.2 Total Phenol content Determination**

To 200µl of the sample extract 800µl of Folin's-phenol reagent mixture was added and mixed. Then 2ml of 7.5% sodium carbonate was added and the volume was made up to 7ml using distilled water. The tubes were incubated for 2hrs in dark. The absorbance was measured at 765 nm. Gallic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of phenols were expressed in terms of Gallic acid in mg/ml of extract. (P. K. Ramamoorthy and A. Bono, 2007).

**2.5.3 Total Protein content determination**

The total proteins content was determined by using Bradford's method. To 100µl of the sample extract 3ml of Bradford's reagent was added and incubated in dark for 5min. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml to 0.5mg/ml) are used as standard solutions.

**2.5.4 Determination of saponins**

2 g of each sample was dispersed in 20ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered, and the residue re-extracted with another 20ml of 20 % ethanol. The combined extracts were reduced over a water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 2ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, and the purification process was repeated. 6ml of n-butanol was added. The combined n-butanol extracts were washed twice with 1ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. (Obadoni and Ochuko, 2001).

**2.5.5 Total Alkaloid content determination**

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle; collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed (Gracelline*t al*., 2013).

**2.5.6 Total Flavonoid Content Estimation**

5ml of 2% AlCl3 prepared in methanol was mixedwith same volume of extract solution. After 10 min, absorbance was taken at 415nm against blank. Blank was prepared as 5 ml of extract mixed with 5 ml of methanol without AlC3. Catechin was used as standard. (P. K. Ramamoorthy and A. Bono, 2007).

**2.5.7 Total Carbohydrate determination**

For estimating the polysaccharide content, 1ml of 5% phenol was added to1ml of sample solution, and then 5ml of concentrated $H_2SO_4$was added.The absorbance was measured after 10 minutes at 488nm against blank. Glucose was used as standard. To prepare Blank, 1ml of distilled water added to1ml of 5% phenol followed by 5ml of Concentrated $H_2SO_4$.

**2.5.8 Total Terpenoid determination**

Add 1.5 ml Chloroform in each tube and add 200µl sample, mix thoroughly & place for 3 minutes to settle. After 3 minutes add 100µl of cold Concentrated Sulphuric acid. Place in dark for incubation upto1.5-2hrs. After incubation gently decant all supernatant reaction mixture without disturbing reddish-brown precipitate. Add 1.5 ml of Methanol vortex until all the precipitate dissolves completely. Take OD at absorbance 538nm. Calculate total terpenoids content of unknown sample by Linalool equivalent (I R Suica-Bunghez, S Teodorescu2016, Ghorai*et al*., 2012).

**2.5.9 Quantitative estimation of Cardiac glycosides**

To 300µl of sample 2700µl of Buljet'sReagent (El-Olemyet al., 1994)was added and (Containing 95ml 1% aqueous picric acid + 5ml of 10% aqueous NaOH) placed for the 20 min incubation. The ODwas taken at 495 nm.

**2.6 *In vitro* Antioxidant assay**

**2.6.1 DPPH radical scavenging Assay (1,1-Diphenyl- 2- picryl hydrazyl)**

The free radical scavenging activity of host plant treated with VAM was carried out on 30, 60 and 90days and the uninoculated control sample. The solvent extracts of the sample were taken in triplicates in the following concentration range (100, 200, 400, 600, 800 and 1000µl) in each test tubes and make up the volume up to 1ml. 3ml of 0.1mM DPPH is added to each test tube. The mixture was shaken and incubated for 30mins in dark and the absorbance measured at 517nm using a Anatech UV-Vis Spectrophotometer. The free radical scavenging activity was calculated by the formula

$$\text{Scavenging activity}\,(\%) = \{(Ac - As)/Ac\} \times 100,$$

where Ac is the absorbance of the control and As is the absorbance of the sample (Hemalatha *et al.,* 2015).

**3. RESULTS**

**3.1 Physiological parameters of VAM inoculated plants**

**a**

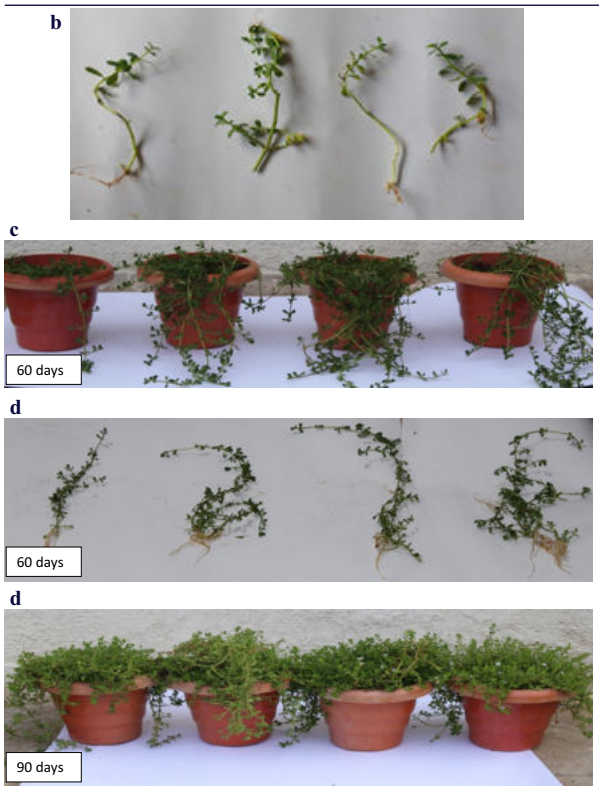



**Figure 1:** a. Pot with *Bacopa monnieri*inoculated with VAM after 30 days. b. uprooted plants. c. Pot with *Bacopa monnieri*inoculated with VAM after 60 days. d. uprooted plants. e. Pot with *Bacopa monnieri*inoculated with VAM after 90 days.

**Table 1:**Physiological parameters of VAM inoculated plant *Bacopa monnieri*.I.C-individual culture (Gm and Gf), M.C- Mixed cultureGm+Gf.

| Sl. No. | Sample | Average Shoot length(cm) | Average Root length(cm) | Average Wet weight(gm) |
|---|---|---|---|---|
| 1. | **Control** | 10.1 | 4.3 | 0.15 |
| 2. | **Gm** | 12.3 | 5.20 | 0.35 |
| 3. | **Gf** | 11.5 | 4.8 | 0.30 |
| 4. | **Gm+Gf** | 17.95 | 8.5 | 0.61 |

The symbiotic association of VAM with the host plant have showed direct impact with better shoot and root physiological parameters compared to the control. According to the study it was evident that the mixed VAM culture treatment showed greater shoot length, root length and wet weight (Table 1) of *B.monnieri*followed by *Gm* and *Gf* individual treatment respectively.

### 3.2 Qualitative phytochemical analysis

**Table 2:**Preliminary qualitative analysis of methanolic extracts of leaf of *B.monnieri*

| | | Alkaloids | Flavonoids | Saponins | Tannins | Phenols | Proteins | Glycosides | Terpenoids | Carbohydrates | Quinones |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **30 Days** | **C** | + | + | + | + | + | - | + | + | + | - |
| | ***Gm*** | + | + | + | + | + | - | + | + | + | - |
| | ***Gf*** | + | + | + | + | + | - | + | + | + | - |
| | ***Gm+Gf*** | + | + | + | + | + | - | + | + | + | - |
| **60 Days** | **C** | + | + | + | + | + | - | + | + | + | - |
| | ***Gm*** | + | + | + | + | + | - | + | + | + | - |
| | ***Gf*** | + | + | + | + | + | - | + | + | + | - |
| | ***Gm+Gf*** | + | + | + | + | + | - | + | + | + | - |
| **90 Days** | **C** | + | + | + | + | + | + | + | + | + | - |
| | ***Gm*** | + | + | + | + | + | + | + | + | + | - |
| | ***Gf*** | + | + | + | + | + | + | + | + | + | - |
| | ***Gm+Gf*** | + | + | + | + | + | + | + | + | + | - |

Qualitative phytochemical analysis of *B.monnieri*leaf extract study revealed that samples treated with Gf,Gm and Gf+Gmand the uninoculated control contained most of the phytochemical constituents like, alkaloids, flavonoids, saponins, phenols, tannins, glycosides, terpenoids and carbohydrates. However, protein was only detected in 90days of VAM treated plants and quinones were absent in all the samples (Table 2).

### 3.3 Quantitative estimation of phytochemicals

#### 3.3.1 Total Tannin content determination

**Table 3:**Total tannin content of methanolic extract of leaf of *B.monnieri*after VAM treatment.

| Samples | 30days Concentration (mg/ml) | 60days Concentration (mg/ml) | 90days Concentration (mg/ml) |
|---|---|---|---|
| **C** | 0.560 | 1.193 | 1.548 |
| ***Gm*** | 1.176 | 2.436 | 2.779 |
| ***Gf*** | 1.030 | 1.699 | 1.816 |
| ***Gm+Gf*** | 1.455 | 2.308 | 2.389 |

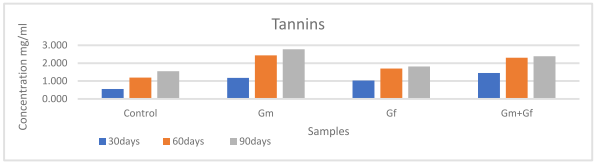



**Figure 2:**Quantitative analysis of Tannin (mg/ml)

The tannin content of VAM treated methanolic leaf extract of *B.monnieri*was determined, it was found to be in the range of 0.560mg/ml and 1.455mg/ml for 30 days of VAM treatment and between 1.193mg/ml and 2.436mg/ml after 60 days of VAM inoculation.

However, for after 90 days growth the tannin content were found to be higher in *Gm* treated *B. monnieri* with about 2.779mg/ml followed by coculture inoculum with 2.389mg/ml and least tannin was found in *Gf* treated plant with1.816mg/ml (Table 3).

#### 3.3.2 Total phenol content determination

**Table 4:**Total phenol content of methanolic extract of leaf of *B.monnieri.*

| Samples | 30days Concentration mg/ml | 60days Concentration mg/ml | 90days Concentration mg/ml |
|---|---|---|---|
| **C** | 0.801 | 3.778 | 7.338 |
| ***Gm*** | 7.799 | 13.149 | 14.660 |
| ***Gf*** | 6.372 | 12.058 | 13.084 |
| ***Gm+Gf*** | 2.344 | 4.802 | 9.880 |

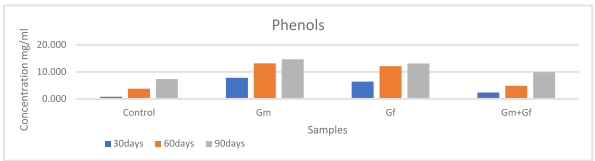



**Figure 3:**Quantitative analysis of phenol (mg/ml).

The phenol content of various VAM treated methanolic leaf extract of *B.monnieri*varied widely between 0.801mg/ml to 7.799mg/ml after 30 days of treatment whereas with 60d days treatment it was about 3.778mg/ml to 13.149mg/ml and after 90days it was around 7.338mg/ml to 14.660mg/ml.

The methanolic extract of *Gm* treated plants showed higher phenolic content of 14.660mg/ml compared to other samples (Table 4).

#### 2.3.3 Total protein content determination

**Table 5:**Total protein content of methanolic extract of leaf of *B.monnieri.* after VAM treatment.

| Samples | 30days Saponins mg/mL | 60days Saponins mg/mL | 90days Saponins mg/mL |
|---|---|---|---|
| **C** | 0.03 | 0.06 | 0.08 |
| **Gm** | 0.11 | 0.205 | 0.229 |
| **Gf** | 0.07 | 0.1461 | 0.150 |
| **Gm+Gf** | 0.1 | 0.184 | 0.206 |

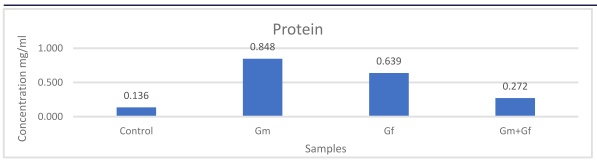



**Figure 4:** Quantitative analysis of protein (mg/ml).

The extract of *B.monnieri*no protein content on 30 and 60 days of VAM treatment. However, after 90 days of VAM inoculation the presence of protein was detected. The pure culture of Gmshowed 0.848mg/ml protein concentration, Gfshowed 0.639mg/ml protein concentration followed by mixed culture VAM inoculum with 0.272mg/ml and the least with the uninoculated control with 0.136mg/ml (Table 5).

### 2.3.4 Determination of Saponins

**Table 6:** Total saponin content of methanolic extract of leaf of *B.monnieri*treated with VAM fungi.

| Sample | Concentration (mg/ml) |
|---|---|
| **C** | 0.136 |
| ***Gm*** | 0.848 |
| ***Gf*** | 0.639 |
| ***Gm+Gf*** | 0.272 |

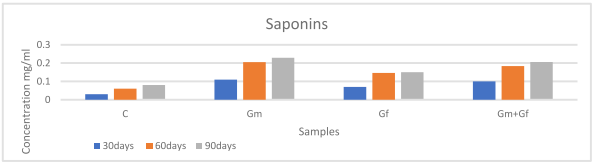



**Figure 5:**Quantitative analysis of saponins (mg/ml)

The saponin content of the VAM treated and uninoculated control methanolic leaf extract of *B.monnieri*after 30days of VAM treatment was determined to be in the range of 0.03mg/ml and 0.11mg/ml. There was an increase in saponin content after 60days and 90days of VAM treatment. Among the samples, plants treated with VAM *Gm* showed higher saponin content of 0.229mg/ml and the least were found in *Gf* treated plant with0.150mg/mlafter 90days (Table 6).

### 2.3.5 Total alkaloid content determination

**Table 7:** Total alkaloid content of methanolic extract of leaf of *B.monnieri*after VAM treatment.

| Samples | 30days Alkaloid content mg/ml | 60days Alkaloid content mg/ml | 90days Alkaloid content mg/ml |
|---|---|---|---|
| **C** | 0.01 | 0.012 | 0.019 |
| ***Gf*** | 0.014 | 0.021 | 0.04 |
| ***Gm*** | 0.047 | 0.05 | 0.05 |
| ***Gm+Gf*** | 0.021 | 0.024 | 0.03 |

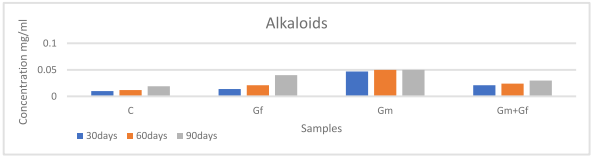



**Figure 6:** Quantitative analysis of alkaloids (mg/ml).

*B.monnieri*exhibited higher alkaloid content in *Gm* treated leaves with 0.05mg/ml compared to that of other VAM treatment after 60 days of treatment (Table 7). Similar alkaloid content was seen after 90days of VAM inoculation. The least alkaloid was seen in the uninoculated control plant samples.

### 2.3.6 Total Flavonoid content determination

**Table 8:** Total flavonoid content of methanolic extract of leaf of *B.monnieri* after VAM treatment.

| Samples | 30days Concentration (mg/ml) | 60days Concentration (mg/ml) | 90days Concentration (mg/ml) |
|---|---|---|---|
| **C** | 0.0330 | 0.092 | 0.135 |
| ***Gm*** | 0.0934 | 0.187 | 0.337 |
| ***Gf*** | 0.0451 | 0.092 | 0.177 |
| ***Gm+Gf*** | 0.0733 | 0.158 | 0.312 |

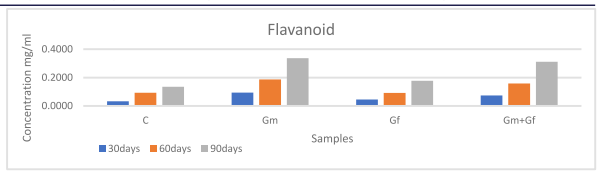



**Figure 7:** Quantitative analysis of Flavonoids (mg/ml)

An increase in flavonoid content was seen after 30, 60 and 90 days of treatment, respectively. The total flavonoid content was found to be higher in *Gm* at 0.337mg/ml followed by Gm+Gf0.312mg/ml and the least was seen inG.ftreated plants0.177 and 0.135 mg/ml in control uninoculated extract of *B.monnieri*after 90days of treatment(Table 8).

### 2.3.7 Total carbohydrate content determination

**Table 9:**Total carbohydrate content of methanolic extract of leaf part of *B.monnieri*after VAM treatment.

| Samples | 30day Concentration (mg/ml) | 60days Concentration (mg/ml) | 90days Concentration (mg/ml) |
|---|---|---|---|
| **C** | 0.060 | 0.061 | 0.090 |
| **Gm** | 0.053 | 0.126 | 0.155 |
| **Gf** | 0.046 | 0.052 | 0.102 |
| **Gm+Gf** | 0.125 | 0.149 | 0.155 |

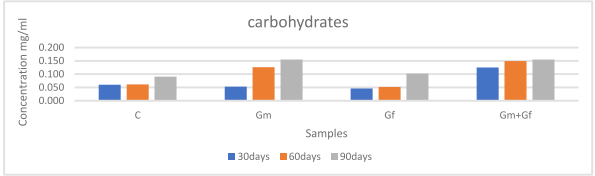



**Figure 8:** Quantitative analysis of Carbohydrate (mg/ml).

The carbohydrate content was found to be the highest in the mixed VAM culture of *B. monnieri*after 30, 60 and 90 with 0.125mg/ml, 0.149mg/ml and 0.155mg/ml respectively. The least was found in Gf0.046mg/ml, 0.052mg/ml and 0.102mg/ml after every 30 days of interval (Table 9). The 90 days mixed culture and *Gm* value in carbohydrate content was found to be similar at 0.155mg/ml (Table 9).

### 2.3.8 Total Terpenoid content determination

**Table 10:**Total terpenoid content of methanolic extract of leaf part of *B.monnieri*after VAM treatment.

| Samples | 30days Terpenoids (mg/mL) | 60days Terpenoids (mg/mL) | 90days Terpenoids (mg/mL) |
|---|---|---|---|
| **C** | 0.464 | 0.57 | 0.65 |
| **Gm** | 2.96 | 2.99 | 3.4 |
| **Gf** | 1.69 | 1.78 | 2.5 |
| **Gm+Gf** | 2.83 | 2.86 | 3.25 |

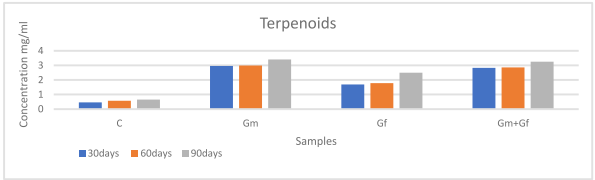



**Figure 9:**Quantitative analysis of Terpenoids (mg/ml).

The terpenoid content of various VAM treated methanolic leaf extract of *B.monnieri*after 30 days of VAM treatment was ranged between 1.69mg/ml and 2.96mg/ml. The terpenoid content was found to be higher in *Gm* of about 3.4mg/ml followed by Gm+Gfabout 3.25mg/ml and Gfwith 2.5mg/ml after 90days. The least terpenoid content was seen in the control sample for all the duration of growth (Table 10).

### 2.3.9 Quantitative estimation of cardiac glycoside

**Table 11:** Total cardiac glycoside content of methanolic extract of leaf part of *B.monnieri*after VAM treatment.

| Samples | 30days glycosides (mg/mL) | 60days glycosides (mg/mL) | 90days glycosides (mg/mL) |
|---|---|---|---|
| **C** | 0.62 | 0.8008 | 0.91 |

| | | | |
|---|---|---|---|
| ***Gm*** | 1.08 | 1.1 | 1.25 |
| ***Gf*** | 0.78 | 0.95 | 1.08 |
| ***Gm+Gf*** | 0.952 | 1.09 | 1.24 |

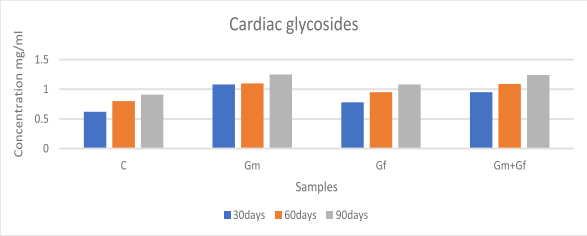



**Figure 10:**Quantitative analysis of cardiac glycosides (mg/ml).

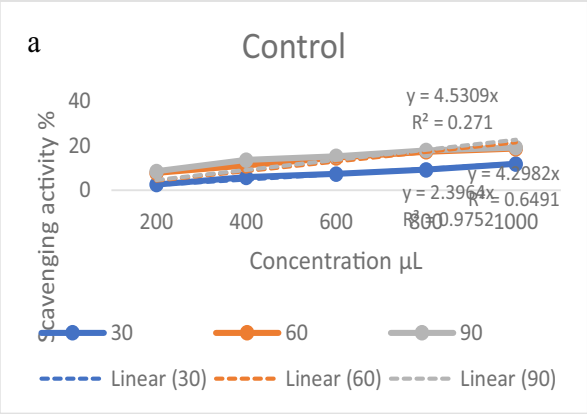
The cardiac glycoside content of VAM treated extract of *B.monnieri*varied widely between 0.78mg/ml to 1.08mg/ml after 30 days of treatment whereas after 60days it was about 0.95mg/ml to 1.1mg/ml.

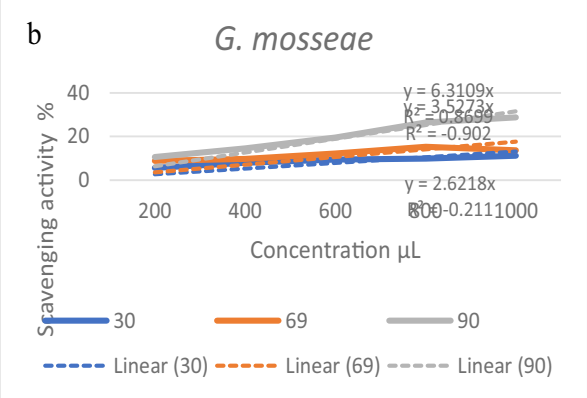
The cardiac glycoside content was found to be higher in Gminoculated plant with 1.25mg/ml followed by Gf 1.08mg/ml and withGm+Gfit was found to be 1.08mg/ml after 90days of VAM treatment. The least content was found in the control (Table 11).

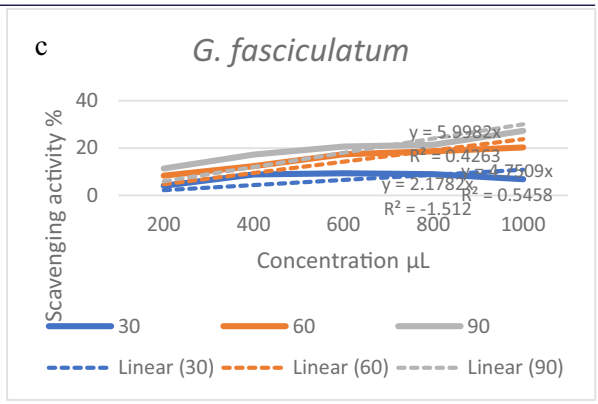
### 2.4 DPPH radical scavenging Assay

**Table 10:** DPPH radical scavenging activity of leaf methanolic extract of *B.monnieri.*

| | **Control** | | | ***G. mosseae*** | | | ***G. fasciculatum*** | | | **Gm+Gf** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dilution | 30 | 60 | 90 | 30 | 60 | 90 | 30 | 60 | 90 | 30 | 60 | 90 |
| 200 µL | 2.5 | 7.7 | 8.5 | 5.5 | 8.8 | 10.5 | 4.1 | 8.4 | 11.4 | 5.8 | 7.8 | 9.2 |
| 400 µL | 5.8 | 11.1 | 13.6 | 7.9 | 9.7 | 14.5 | 8.9 | 12.4 | 17.3 | 7.1 | 9.1 | 12.4 |
| 600 µL | 7.3 | 14.6 | 15.3 | 9.4 | 12.2 | 19.5 | 9.3 | 17.4 | 20.6 | 7.8 | 9.9 | 12.9 |
| 800 µL | 9.2 | 17.3 | 17.9 | 9.8 | 15.3 | 26.4 | 9 | 18.6 | 21.4 | 8.7 | 12.5 | 15.3 |
| 1000 µL | 11.8 | 18.7 | 19.2 | 11.1 | 13.6 | 28.7 | 6.8 | 20.3 | 27.3 | 9.4 | 14.2 | 18.78 |
| $IC_{50}$ | 20.86 | 11.63 | 11.04 | 19.08 | 14.18 | 7.92 | 22.95 | 10.52 | 8.34 | 21.96 | 15.56 | 12.07 |



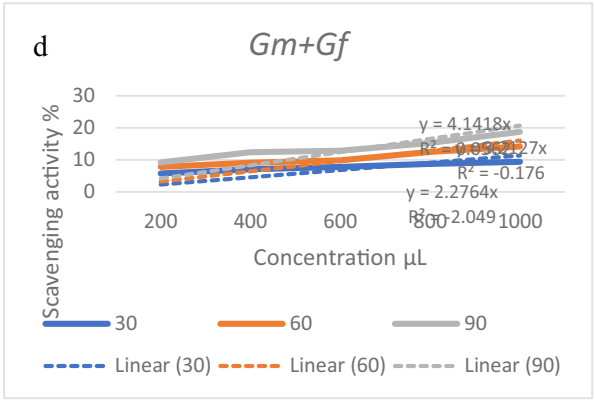




**Figure 11:a,b,c,d shows** DPPH radical scavenging activity of leaf methanolic extract of *B.monnieri.*

The DPPH radical scavenging assay revealed that *B.monnieri*leaf extract of *G. fasciculatum*VAM treatment after 30 days showed the highest $IC_{50}$ value at 22.95 µg/ml followed by mixed inoculum of(Gf+Gm)with $IC_{50}$ value at 21.96 µg/ml and uninoculated control at 20.86 and the least at 30days by*G. mosseae*$IC_{50}$ value as 19.08 µg/ml. The Scavenging activity decreased for 60 and 90 days, but in a similartrend as that of 30days for all the samples.Mixed culture (Gm+Gf)exhibited the highest scavenging activity of 12.07µg/ml than that of individual inoculated samples after 90days. It was observed that there was a considerable decrease in the scavenging activity with time in all the inoculated and uninoculated control samples.

### 4. DISCUSSION

AM are root-symbionts which uptake nutrient from host plant and in exchange they provide essential nutrients to host such as nitrogen, potassium, phosphorus, zinc, sulphur and calcium (Abbasi et al., 2015) thereby improving the nutrient intake of plant, increasing the secreting growth enhancing substances and creating synergetic interaction with beneficial microorganisms (Sreenivasa and Bagyaraj, 1989). It is apparent from the present study that there is evident association of VAM withthe roots of*B. monnieri.* The data from Table 1 show clear physiological changes with associated samples like shoot, root, and wet weight measurement after 30days of VAM treatment. It was evident from the data that there was a pronounced effect on shoot length of about 18.2cm and 17.7cm in host plant inoculated with mixed culture of VAM than that of non-inoculated plant which was about 10.1cm. The same trend was seen in root length of about 10.6cm and 6.4cm with mixed culture treatment and 4.3cm without VAM treatment. The plant association with single VAM inoculum of *G. fasciculatum*and *G. mosseae*showed significantly better results than that of the uninoculated control plant.

The preliminary phytochemical analysis of the methanolic leaf extract of *B. monnieri*showed the presence of major secondary metabolites such as tannins, terpenoids, saponins, alkaloids, cardiac glycoside, flavonoids, phenols and carbohydrates. The solvent has high polarity which is capable of extractingboth polar and non-polar plant constituents. However, proteins were not detected in non-inoculated plant samples for 30 and 60 days.However, after 90 days of VAM inoculation there was presence of protein in the plant extract (Table 2). These phytochemical constituents are reported to have therapeutic properties (Senguttuvan, 2014; Mathew *et al*., 2010; Nyamai*et al*.,

2015). They coverabroad range medicinal property with a pronounced variety of chemical structures (Singh and Dhawan, 1997).

Quantitative phytochemical analysis was carried out on different intervals of VAM treatment. According to the data it was observed that there was anincrease in tannin content from 0.560mg/ml to 2.779mg/ml from non-inoculated to 90 days of treatment respectively. *G.mosseae.G. fasciculatum*and mixed culture of Gm+Gfof *B. monnieri*showed higher tannin content compared to the control plant sample with 0.560mg/ml. Similarly,thephenolic content for both non-inoculated and VAM inoculated *B. monnieri* increased over time. It was found that for *G. mosseae*treated plant phenolic content was the highest compared to other samples. It was observed that an increase in excretion of tannin and phenolic compound is directly associated with escalation in antioxidant properties of the plant (Foti, 2007).

According to a study conducted by Saheli 2010 it was apparent that VAM association not only enhances the nutrient uptake or growth of the plant but also improves the secondary metabolite production. In the present investigation presence of protein was seen only after 90days of treatment. This could be due to protein enhancement by VAM treatment in the plant. *G. mosseae*showed greater protein content of 0.848mg/ml followed by 0.639mg/ml by *G. fasciculatum*followed by mixed inoculum with 0.272mg/ml and least was seen in the control at 0.136mg/ml. The increase in saponin content was observed to be 0.11mg/ml to 0.229mg/ml on 90 days of *B. monnieri* treated with *G. mosseae*fungi. The total alkaloid content was higher in *Gm* treated leaves with 0.05mg/ml of the plant after 60days of VAM treatment compared to others. Similar alkaloid content was seen after 90days of VAM inoculation. An increased pattern in flavonoid content was seen after 30, 60 and 90 days of treatment, respectively. The total flavonoid content was found to be greater in *B.monnieri treated* with *G. mosseae*with 0.337mg/ml followed by mixed culture treatment 0.312mg/ml followed by *G. fasciculatum*at0.177mg/ml and least by non-inoculated sample 0.135mg/ml. Similarly, the increase in carbohydrate (0.155mg/ml), terpenoids (3.4mg/ml) and cardiac glycoside (1.25mg/ml)were seen in *B.monnieri*treated with *G. mosseae*than that of *G. fasciculatum,* mixed culture(Gm+Gf)and non-inoculated treatment.

The potential Free radical scavenging activity of *B.monnieri*has been studied extensively. It was observed that methanolic extract exhibited maximal antioxidant activity (Lal and Baraik, 2019). The decrease in DPPH radical by antioxidant compound present in plant, by $IC_{50}$ is a parameter commonly used to evaluate the antioxidant activity (Krishna et al., 2013; Senguttuvan*et al*., 2014). In the present investigation the methanolic leaf extract of *B.monnieri*clearly demonstrates that *G. fasciculatum*VAM treatment after 30 days showed higher $IC_{50}$ value at 22.95 µg/ml followed by mixed culture (Gf+Gm) inoculum$IC_{50}$ value at 21.96 µg/ml followed by *G. mosseae*at 19.08 µg/ml and the least by non-inoculated plant sample at 20.86 µg/ml. The 60 and 90 days showed the same trend as that of 30days. Mixed culture (Gm+Gf)showed higher scavenging activity $IC_{50}$ of 12.07 µg/ml than that of pure culture and control after 90days of treatment (Table 10). Sainthiya*et al*., 2018 conducted similar studies and foundan elevation of antioxidant property on the medicinal plant after mycorrhizal treatment compared to the non-treated host plant.

## 5. CONCLUSION

*Bacopa monnieri* also known as Brahmi belonging to Scrophulariaceae family is known for its therapeutic value, is the native of wetland parts of India. The objective of the study was to understand the symbiotic association of VAM on *B. monnieri*on its physiological parameters, phytochemical constituents and antioxidant scavenging activity. *B. monnieri*showed better root and shoot growth compared to non-inoculated one after 30days of harvesting. For preliminary studies methanolic extract of leaf was used at different time intervals of 30, 60 and 90 days which showed the presence of major secondary metabolites. *B. monnieri*treated with *G. mosseae*showed better results for quantitative estimation of Tannins, phenols, proteins, Saponins, alkaloids, flavonoid, terpenoids and cardiac glycosides than that of *G. fasciculatum*, mixed culture (Gm+Gf)and non-inoculated (control). Whereas mixed culture showed better results for carbohydrates and DPPH antioxidant scavenging activity for 30, 60 and 90days, respectively. According to the data B.*monnieri*treated with VAM fungi revealed better results than that of untreated control plant sample.

## REFERENCES


1. Abbasi, Hisamuddin, Ambreen Akhtar, and RushdaSharf. (2015), "Vesicular arbuscular mycorrhizal (VAM) fungi: a tool for sustainable agriculture." American journal of plant nutrition and fertilization technology 5.2, 40-49.
2. Anokwuru, Chinedu Prosper, *et al*.,(2011),"Polyphenolic content and antioxidant activity of Hibiscus sabdariffa calyx." Research Journal of Medicinal Plant, 5.5, 557-566.
3. Belay, Kassa, and M. J. C. M. R. Sisay. (2014),"Phytochemical constituents and physicochemical properties of medicinal plant *(Moringa oleifera*) around Bule Hora". Chemistry and Materials Research, 6(7), 61-72.
4. Chen, Jen-Hshuan. (2006), "The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility." International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use.. Land Development Department Bangkok Thailand.Vol. 16. No. 20.
5. Chen, Meilan., *et al*., (2017). *Glomus mosseae* inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of liquorice under nutrient stress. Frontiers in plant science 8, 1-10.
6. El-Olemy, M.M., Al-Muhtadi, F.J. & Afifi, A.F.A. 1994. Experimental phytochemistry: A laboratory manual, pp 21-27. King Saud Univ. Press, Saudi Arabia.
7. Egamberdieva, Dilfuza, *et al*.,(2017), "Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: A review." Journal of Medicinally Active Plants 5.2, 59-75.
8. Foti, Mario C. (2007), "Antioxidant properties of phenols." Journal of Pharmacy and Pharmacology. 59.12, 1673-1685.
9. Gupta, M.L., *et al.*(2001). Effect of the vesicular–arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*Mentha arvensis*) under field conditions. Bioresource technology, 81, 77-79.
10. Jain, Paras, *et al.* (2017). "Phytochemical analysis of *Bacopa monnieri* (L.) Wettst. and their anti-fungal activities."
11. RajendranJeyasri, *et al.* (2020), "*Bacopa monnieri* and their bioactive compounds inferred multi-target treatment strategy for neurological diseases: a cheminformatics and system pharmacology approach." Biomolecules, 10(4):536.
12. Karthikeyan, B., M. M. Joe, and A. J. Cheruth.(2009), "Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations." Journal of Scientific Research, 1.2, 381-386.
13. Krishna, T. Murali, *et al*. (2013)"*In vitro* evaluation of antioxidant properties of *Cucumis melo* L. extracts of leaves and fruit." Int J Pharm Bio Sci, 4.1, 705-712.
14. Lal, Shalini, and Binit Baraik. (2019),"Phytochemical and pharmacological profile of *Bacopa monnieri*-an ethnomedicinal plant." Int J Pharm Sci & Res, 10.3, 1001-13.
15. Math, Siddharam., *et al*., (2019). Arbuscular mycorrhizal (*Glomus fasciculatum*) fungi as a plant immunity booster against fungal pathogen. Current Agriculture Research Journal, 7, 99-107.
16. Mathew, Jobin, *et al*. (2010),"*Bacopa monnieri* and Bacoside-A for ameliorating epilepsy associated behavioral deficits." Fitoterapia, 81.5, 315-322.
17. Nyamai, D. W., *et al*. (2015), "Phytochemical profile of *Prunus africana* stem bark from Kenya." Journal of Pharmacognosy and Natural Products, 1:110.
18. Nyamai, D. W., *et al*.(2016), "Medicinally important phytochemicals: an untapped research avenue." Journal of Pharmacognosy and phytochemistry, 4.4, 2321-6182.
19. Okwu, D. E.(2001), "Evaluation of chemical composition of indeginous species and flavouring agents." Global Journal of Pure and Applied Sciences, 7.3, 455-460.
20. Palombo, Enzo A. (2006), "Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function." Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,20.9, 717-724.
21. Pawar, S. S., M. G. Jadhav, and T. G. Deokar. (2016), "Study of Phytochemical Screening, Physicochemical Analysis and Antimicrobial Activity of *Bacopa monnieri* (L.) Extracts. International Journal of Pharmaceutical and Clinical Research, 8.8, 1222-1229.
22. Rajani, M. (2008), "*Bacopa monnieri*, a nootropic drug." Bioactive molecules and medicinal plants,175-195.
23. Ramadas, Dinesha, *et al*. (2016), "Phytochemical studies and antioxidant activity of *Bacopa monnieri* plant leaves proteins." Indo American Journal of Pharmaceutical Research, 6.1, 4302-4307.
24. Saheli, Chatterjee, *et al*. (2010), "A survey on VAM association in three different species of Cassia and determination of antimicrobial property of these phytoextracts." Journal of Medicinal Plants Research, 4.4, 286-292.
25. Sainthiya, Varsha, *et al*. (2018), "Vesicular–Arbuscular Mycorrhiza Association Alleviates Antioxidant Activity of *Bacopa monnieri*." International Journal of Plant Biotechnology, 4.1, 24-28.
26. Sainthiya, Varsha, *et al*.(2018), "Vesicular–Arbuscular Mycorrhiza Association Alleviates Antioxidant Activity of *Bacopa monnieri*." International Journal of Plant Biotechnology, 4.1, 24-28.
27. Senguttuvan, Jamuna, Subramaniam Paulsamy, and Krishnamoorthy Karthika.(2014), "Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaerisradicata* L. for in vitro antioxidant activities." Asian Pacific journal of tropical biomedicine, 4, S359-S367.
28. Singh, H. K., and B. N. Dhawan. (1997),"Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniei* Linn.(Brahmi)." Indian Journal of Pharmacology,29(5), 359-365.
29. Sreenivasa, M. N., and D. J. Bagyaraj. (1989),"Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum." Plant and soil, 119.1, 127-132.
30. Vani, M., *et al*,(2018). Beneficial effect of arbuscular mycorrhizal fungus, *Glomus fasciculatum* on plant growth and nutrient uptake in tomato. Indian Phytopathology, 71, 115-122.
31. Vishwakarma, Rishi K., Uma Kumari, and Bashir M. Khan. 2016."Memory booster plant *Bacopa monniera* (Brahmi): Biotechnology and molecular aspects of bacoside biosynthesis." Medicinal Plants-Recent Advances in Research and Development. Springer, Singapore, 167-189.
32. Volluri, Sharan Suresh, *et al*. (2011), "*In vitro* antioxidant activity and estimation of total phenolic content in methanolic extract of *Bacopa monniera*." Rasayan J. chem., 4.2, 381-386.
33. World Health Organization. *WHO global report on traditional and complementary medicine 2019*. World Health Organization, 2019.