Seasonal variations in harvest index and bacoside A contents amongst accessions of *Bacopa monnieri* (L.) Wettst. collected from wild populations

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Abstract Bacoside A, a major active principle of *Bacopa monnieri* known for its cognitive effects is a mixture of saponins like bacoside A3, bacoside II, isomer of bacosaponin C and bacosaponin C. Seasonal changes in biomass and bacoside A levels in fourteen accessions of *B. monnieri* were evaluated after maintaining these at a common site at Thapar University campus, Patiala (30°19'36.12"N and 76°24'1.08"E) for 1 year. Harvestable biomass and total bacoside A contents varied significantly between the accessions and also in a particular accession during different seasons of the year. The maximum dry weight of plant (biomass 1.64 g) and bacoside A levels (6.82 mg/plant) were recorded in accession BM1. Harvestable biomass was highest during summer in accessions BM1 and BM7 (FW 4.2 g/plant), whereas bacoside A levels were also highest during summer and in accession BM1 (6.82 mg/plant). The lowest bacoside A level (0.06 mg/plant) was recorded in accession BM14 during winter. Principal component analysis showed that samples of summer were positively correlated with both the components suggesting an appropriate time for the harvest.

Keywords Bacoside A · Harvest index · Memory enhancer · Relative growth rate

Introduction

Bacopa monnieri (L.) Wettst. family Scrophulariaceae (common name 'Brahmi'), is an important source of bacosides, which possess a legendary reputation as a memory vitalizer with a proven nootropic action (Anonymous 1988). It has been a focus of many studies due to its medicinal uses (Russo and Borrelli 2005). Brahmi-based herbal drugs like 'Mentat', 'Memory Plus' and 'Memory Perfect' rich in bacoside A are gaining popularity in both developed and developing countries (Pase et al. 2012). Recent studies also reported anti-cancer activity of the extracts of *B. monnieri* (Sharma et al. 2012). Many bioactive molecules have been reported from *B. monnieri* (Bammidi et al. 2011) and a major chemical entity responsible for its well-known memory-facilitating action is bacoside A (Chatterji et al. 1965). Due to these reasons *B. monnieri* has been placed second in a priority list of most important medicinal plants (Rajani 2008) and is one among 32 medicinal plant species identified for conservation and cultivation (National Medicinal Plants Board 2004). It is estimated that the yearly market demand for its raw material is about 1000 tons, which is increasing at a steady pace (National Medicinal Plants Board and Department of Science and Technology, Government of India 2007). This heavy demand of raw material is entirely met from wild populations, and levels of active principle in the raw material collected from the wild populations have been reported to show considerable variations (Nadeem et al. 2007), which could be due to genotypic variations (Bansal et al. 2014) and also the season of harvest (Walker et al. 2001). Because all the above ground parts of *B. monnieri* have been reported to contain active principle and therefore, total harvestable biomass along with levels of bacoside A are important for the yield of active principle.

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There are some studies investigating variations in the levels of bacoside A in *B. monnieri* samples collected from limited geographical area (Naik et al. 2012; Mathur et al. 2003) and plants of a particular population have also been evaluated for the bacoside A content during different seasons (Sharma et al. 2013; Phrompittayarat et al. 2011). These populations were evaluated from the samples collected from different regions thus, the observed results could also be due to environmental and edaphic factors (Rawat et al. 2016; Cirak et al. 2007). A close perusal of literature revealed no report studying the variations in harvestable biomass and levels of bacoside A during different seasons in various populations of *B. monnieri* collected from different agro climatic zones after these has been maintained at a common location for 1 year. Such an investigation will be useful for selection of superior genotypes with higher growth rate and potential of accumulating higher levels of bacoside A. Further, identification of the appropriate season when bacoside A level is higher coupled with maximum biomass will also help in developing the harvest strategy. Therefore, the present study was taken up with the objectives to study variations in the levels of bacoside A and harvestable biomass during different seasons in fourteen accessions of *B. monnieri* (collected from various agro climatic zones of India) after growing at a common place at Thapar University campus for at least 1 year

Materials and methods

Plant material

Fourteen accessions of *B. monnieri* (L.) Wettst. (BM1–BM14) were collected from different locations of India and brought to the Thapar University and grown in the nursery. The herbarium vouchers of these accessions were deposited in Department of Botany, Punjabi University, Patiala, India under the accession numbers mentioned below in parenthesis

BM1-Kolkata (58597); BM2-Solan (58598); BM3-Delhi (58599); BM4-Yamunanagar (58600); BM5-Chandigarh (58601); BM6-Haridwar (58602); BM7-Dehradun I (58603); BM8-Dehradun II (58604); BM9-Manakpur (58605); BM10-Ambala (58606); BM11-Varanasi (58607); BM12-Shaaranpur (58608); BM13-Rohtak (58609); BM14-Jogindernagar (58610).

Sample collection

The collected accessions were planted in the experimental field at Thapar University Patiala (30°35'N, 76°36'E) in 2 × 2 m plots in a random block design (RBD) and

acclimatized for 1 year and all sampling was carried out in the second year from these accessions. Plants (above ground biomass) were harvested individually (at random three plants from each plot) from all the accessions at the end of each season (spring: 15th March; summer: 15th June; autumn: 15th September and winter: 15th December) and were dried in shade. The total dry above ground biomass (AGB) of each plant was recorded and average weight was calculated. These were ground to fine powder using a blender and stored in sealed polypropylene bags till use.

Extraction of bacoside A

Bacoside A was extracted from the samples and purified following the procedure described earlier (Bansal et al. 2014). Briefly, samples (1.0 g dried powder in triplicate) were soaked in 10.0 ml water for 24 h, filtered through glass wool and filtrates were discarded. The residues were extracted with 20.0 ml of aqueous ethanol (95 %, v/v) for 3 days and then filtered. The extraction was repeated 3 × (×20 ml) with aqueous ethanol (95 %, v/v). Filtrates from three extractions were pooled and dried in vacuo. Residues were reconstituted in 1.0 ml methanol and filtered through 0.45 µm pore size filters (Millipore–Carrigtwohill, Ireland) prior to quantification using high performance liquid chromatography (HPLC).

Estimation of bacoside A

Quantification of bacoside A content in purified extracts was carried out using reverse phase HPLC (Waters Corporation, USA) equipped with high-pressure binary pump system (515), diode array detector (2998) and Rheodyne injector with 20 µl sample loop. Samples (20 µl) were injected through the injector into Sunfire™ C18 column, 250 mm × 4.6 mm i.d. particle size 5.0 µm (Waters, Ireland) and elution was carried out in an isocratic mode with a mobile phase consisting of aqueous acetonitrile (65:35 v/v) containing phosphoric acid (0.2 %, v/v; pH 3.0) at a flow rate of 1.0 ml min^{−1}. Column eluates were monitored with online photo diode array (PDA) detector set at 205 nm. Quantifications were carried out using external standard curves plotted by taking known quantities of standard compounds (individually bacoside A3, bacoside II and bacosaponin C) (Sigma Chemical Co., St Louis, MO). The levels of various components of bacoside A (mg/g dry weight) were also converted to mg per plant AGB by multiplying the calculated values (mg/g dry weight) with average dry AGB of a plant as obtained in “[Sample collection](#)” section. During initial analyses, the authenticity of the compounds eluting from peaks was established by thin layer chromatography (data not shown). The average level

of bacoside A components during different seasons were then used for grouping the accessions by principal component analysis (PCA) using loading plots (SPSS 16, IBM, Chicago, USA).

Determination of harvest index and relative growth rate

The above ground biomass of individual plant was harvested in each season and fresh weight (FW) per plant was recorded. The dry AGB for each plant was determined by drying the samples at 80 °C till a constant weight was obtained. The bacoside A content in these plants was estimated and harvest index (HI) was calculated by dividing the total bacoside A content per plant with dry AGB per plant.

The relative growth rate (RGR) was calculated using below mentioned equation describe by Evans (1972)

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_2 and W_1 represent mean dry weights at harvest (T_2) and initial (T_1) times respectively. In the beginning W_1 (initial weight for spring season) was taken on Dec. 15 (T_1) when the plant has minimum biomass and W_2 (final weight for spring season) was taken as on March 15 (T_2). These W_2 and T_2 values of spring will then be considered as W_1 and T_1 for summer season. Similarly the cycle was completed for all the four seasons.

Three plants from each plot corresponding to each accession were taken to measure HI and RGR and average of these values were taken.

Statistical analysis

All experiments were performed in triplicates and repeated twice and data were analyzed by two-way analysis of variance (ANOVA) using GraphPad Prism Version 5.0 (GraphPad Software Inc., San Diego CA) and means were compared with Duncan's multiple range test at $P \leq 0.05$.

Results and discussion

The bacoside A contents varied among the accessions and also within the accessions during different seasons (Table 1). A 10–15-fold variation in the levels of bacoside A were recorded among accessions in a particular season and 1–3-fold variation were recorded during different seasons in a particular accession. The maximum level of bacoside A was recorded in accession BM1 (6.82 mg/plant) during summer, whereas the minimum level of bacoside A was recorded in accession BM14 (0.34 mg/plant) during winter. Amongst seasons,

maximum level of bacoside A was recorded in summer when the average temperature was higher (Ca. 40 °C) and minimum level of bacoside A was recorded during winter when the average temperature was lower (Ca. 5.0 °C) (Fig. 1a). In this study, growth and bacoside A levels in these accessions were determined after growing in a uniform environment at Thapar University, Patiala at least for 1 year. Thus the observed variations could only be due to genotypic differences of these accessions. Genetic variation in these accessions were reported earlier (Bansal et al. 2014).

Components of bacoside A also varied during different seasons (Table 1). Three major components of bacoside A namely bacoside A3, bacopaside II and bacopasaponin C were also maximum during summer and minimum during winter. Levels of bacoside A3 during all sampling dates were maximum in accessions BM1 and BM7 and minimum levels were detected in the accession BM14 (Table 1). However, maximum 'bacopaside II' contents in every season were recorded for accession BM4 and the lowest level of bacopaside II was recorded in samples of accession BM13. Bacopasaponin C content of either season were higher in accession BM1 and lowest in accession BM14 (Table 1). Overall, all the components of bacoside A were higher during summer. This is the first report investigating the changes in the components of bacoside A in accessions as well as during different seasons.

Variation in the levels of active principle amongst the populations collected from different locations have been reported earlier in many plant species (Bagdonaite et al. 2010; Chaves et al. 2013; Filippini et al. 2010; Nadeem et al. 2000, 2007; Soni et al. 2015; Rawat et al. 2016) including *B. monnieri* (Naik et al. 2012). Naik et al. (2012) have studied variations in bacoside A content in the samples collected from different populations growing in a limited area of the homogenous environment from the state of Karnataka in India. In the present study, the accessions were collected from a much broader region and were growing in diverse environmental conditions ranging from about 300 to 1500 m amsl. Therefore there is a possibility of higher levels of variations in such diverse populations. Such populations collected from different climatic zones could also be genetically diverse. Till date, no report could be traced regarding the variation in the components of bacoside A within accessions during different seasons. This work was helpful in identifying the accession rich in the particular component of bacoside A and also the season when that component is higher.

Among all the four sampling seasons, maximum levels of bacoside A were detected during summer. Earlier, there are few reports pertaining to the effect of different season on bacoside A content (Phrompittayarat et al. 2011; Mathur et al. 2002) and these authors also reported summer as an

Table 1 The levels of ‘bacoside A’ and its various components (mg/plant) in the accessions of *B. monnieri* during different seasons

Seasons	Accessions													
	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8	BM9	BM10	BM11	BM12	BM13	BM14
Bacoside A3														
Spring	1.33 ^{bA}	0.64 ^{bF}	0.45 ^{aG}	0.74 ^{aE}	0.88 ^{aD}	0.61 ^{aF}	1.22 ^{bB}	0.15 ^{aI}	0.60 ^{aF}	0.58 ^{bF}	1.01 ^{bC}	0.23 ^{bH}	0.23 ^{bH}	0.02 ^{bJ}
Summer	1.44 ^{aA}	0.74 ^{aE}	0.49 ^{aG}	0.78 ^{aE}	0.91 ^{aD}	0.63 ^{aF}	1.33 ^{aB}	0.18 ^{aI}	0.56 ^{aF}	0.72 ^{aE}	1.11 ^{aC}	0.36 ^{aH}	0.31 ^{aH}	0.09 ^{aJ}
Autumn	1.11 ^{cA}	0.52 ^{cF}	0.36 ^{bG}	0.68 ^{bE}	0.74 ^{bD}	0.51 ^{bF}	1.02 ^{cB}	0.12 ^{bI}	0.55 ^{aF}	0.50 ^{cF}	0.91 ^{cC}	0.19 ^{cH}	0.15 ^{cH}	0.01 ^{bJ}
Winter	0.61 ^{dA}	0.31 ^{dC}	0.15 ^{cE}	0.38 ^{cC}	0.43 ^{cB}	0.28 ^{cD}	0.56 ^{dA}	0.04 ^{cF}	0.25 ^{bD}	0.21 ^{dD}	0.55 ^{dA}	0.02 ^{dF}	0.08 ^{dF}	0.00 ^{bG}
Bacopaside II														
Spring	1.05 ^{bA}	0.71 ^{bD}	0.54 ^{aF}	0.80 ^{aC}	0.66 ^{bE}	0.62 ^{aE}	0.96 ^{bB}	0.25 ^{aH}	0.57 ^{aF}	0.10 ^{bI}	0.81 ^{aC}	0.39 ^{bG}	0.03 ^{bJ}	0.14 ^{bI}
Summer	1.18 ^{aA}	0.82 ^{aC}	0.51 ^{aF}	0.86 ^{aC}	0.73 ^{aD}	0.65 ^{aE}	1.06 ^{aB}	0.32 ^{aH}	0.54 ^{aF}	0.17 ^{aJ}	0.84 ^{aC}	0.47 ^{aG}	0.11 ^{aJ}	0.26 ^{aI}
Autumn	0.80 ^{cA}	0.51 ^{dD}	0.38 ^{bF}	0.61 ^{bC}	0.48 ^{bE}	0.46 ^{bE}	0.71 ^{cB}	0.19 ^{bH}	0.46 ^{bE}	0.04 ^{cI}	0.59 ^{bC}	0.29 ^{cG}	0.05 ^{bI}	0.07 ^{cI}
Winter	0.44 ^{dA}	0.32 ^{dB}	0.18 ^{cD}	0.37 ^{cB}	0.29 ^{cC}	0.27 ^{cC}	0.43 ^{dA}	0.09 ^{cE}	0.22 ^{cC}	0.01 ^{cE}	0.37 ^{cB}	0.12 ^{dD}	0.00 ^{bF}	0.04 ^{cE}
Bacosaponin C														
Spring	3.94 ^{bA}	1.48 ^{bF}	1.30 ^{bG}	1.62 ^{aE}	2.80 ^{aD}	1.31 ^{aG}	3.61 ^{bB}	0.52 ^{bJ}	1.29 ^{aG}	1.19 ^{bH}	3.17 ^{aC}	0.96 ^{bI}	0.52 ^{bJ}	0.17 ^{aK}
Summer	4.19 ^{aA}	1.66 ^{aE}	1.47 ^{aF}	1.68 ^{aE}	2.87 ^{aD}	1.33 ^{aG}	3.84 ^{aB}	0.63 ^{aJ}	1.19 ^{bH}	1.47 ^{aF}	3.21 ^{aC}	1.08 ^{aI}	0.60 ^{aJ}	0.22 ^{aK}
Autumn	3.66 ^{cA}	1.18 ^{cF}	1.07 ^{cG}	1.37 ^{bE}	2.39 ^{bD}	1.08 ^{bG}	3.14 ^{cB}	0.43 ^{cI}	1.17 ^{bF}	1.06 ^{cG}	2.80 ^{bC}	0.79 ^{cH}	0.29 ^{cJ}	0.06 ^{bK}
Winter	2.03 ^{dA}	0.74 ^{dF}	0.51 ^{dH}	0.80 ^{cE}	1.52 ^{cD}	0.65 ^{cG}	1.94 ^{dB}	0.21 ^{dJ}	0.58 ^{cH}	0.39 ^{dI}	1.81 ^{cC}	0.34 ^{dI}	0.17 ^{dJ}	0.02 ^{bK}
Total bacoside A														
Spring	6.32 ^{bA}	2.84 ^{bF}	2.30 ^{bH}	3.16 ^{bE}	4.35 ^{bD}	2.55 ^{bG}	5.80 ^{bB}	0.92 ^{bK}	2.47 ^{aG}	1.89 ^{bI}	5.01 ^{bC}	1.58 ^{bJ}	0.79 ^{bL}	0.34 ^{bM}
Summer	6.82 ^{aA}	3.22 ^{aF}	2.48 ^{aH}	3.33 ^{aE}	4.50 ^{aD}	2.61 ^{aG}	6.24 ^{aB}	1.14 ^{aK}	2.31 ^{bI}	2.36 ^{aI}	5.16 ^{aC}	1.67 ^{aJ}	1.02 ^{aL}	0.57 ^{aM}
Autumn	5.36 ^{cA}	2.22 ^{cF}	1.82 ^{cH}	2.65 ^{cE}	3.60 ^{cD}	2.06 ^{cG}	4.88 ^{cB}	0.74 ^{cK}	2.19 ^{cF}	1.61 ^{cI}	4.31 ^{cC}	1.28 ^{cJ}	0.45 ^{cL}	0.14 ^{cM}
Winter	2.64 ^{dC}	1.37 ^{dF}	0.85 ^{dI}	1.55 ^{dE}	2.24 ^{dD}	1.19 ^{dG}	2.94 ^{dA}	0.36 ^{dL}	1.07 ^{dH}	0.61 ^{dJ}	2.73 ^{dB}	0.49 ^{dK}	0.26 ^{dM}	0.06 ^{dN}

All the collected accessions were maintained at a common place at Thapar University campus for 1 year before collecting the samples. The samples (in triplicate) were collected during different seasons. Data were analysed by ANOVA separately for each component and means were compared using DMRT; values sharing common lower case letters (within columns) and upper case letters (within rows) are not significantly different at $P < 0.05$

appropriate season for the harvest. Further, Soni et al. (2015) reported that secondary metabolites accumulate during the growing seasons in medicinal plants, which is also in line with the present observations in *B. monnieri* accessions. Moreover, in this study accession BM1 collected from Kolkata (where the average temperature is higher) recorded maximum levels of bacoside A, whereas accession BM14 recorded lowest levels of bacoside A. This accession was collected from Jogindernagar, Himachal Pradesh (1500 m amsl) where temperate conditions prevail and the average temperature is lower. These results suggest that accessions collected from the warmer region have a higher potential of bacoside A production as compared to accession collected from the colder area. However, Sharma et al. (2013) investigated the variations in the levels of only bacoside A2 and A3 in one accession of *B. monnieri* (collected from Regional research Laboratory, Jammu) and reported the maximum levels of these compounds in October. This could be due to the genetic and/or agro climatic variations. The effect of the season on the active principle has also been studied in other medicinal plants such as *Hypericum perforatum* (Southwell and Bourke 2001; Bagdonaite et al. 2012). Such studies are important

to find suitable season of harvest, which is a pre-requirement for cultivation of a species.

The two-dimensional scatter plot by PCA analysis performed using components of bacoside A (bacoside A3, bacopaside II and bacopasaponin C) (Table 1) during different seasons grouped the samples according to seasons. The two principal components reported 99.99 % of the variation in the entire data set (Fig. 2). The sample of different seasons clearly clustered separately and exhibited a significant difference in the levels of various components of bacoside A throughout the year (Table 1). The samples of summer were only positively correlated on both axes again suggesting an appropriate time for the harvest. Similar findings with respect to secondary metabolite variability have been earlier reported in many other medicinal plants (Scognamiglio et al. 2014; Bagdonaite et al. 2010, 2012; Filippini et al. 2010). These authors also observed clustering of accessions based on secondary metabolite contents according to the season.

All the accessions were also grouped by hierarchical Cluster analysis (CA) based on levels of components of bacoside A. The general structure of dendrogram confirms the existence of five clusters based on the components of

Fig. 1 **a** Average monthly temperature and rainfall during period of study at the nursery at Thapar University Campus **b** Above ground biomass (g/plant, FW) and harvest index of all accessions of *B. monnieri* during different seasons. The values are average of all the 14 accessions

Fig. 2 The PCA scatter plot of different components of 'bacoside A' of accessions of *B. monnieri* during various seasons. S Spring, Su Summer, A Autumn, W Winter, BA Bacoside A3, BI Bacopasaponin B, BC Bacopasaponin C

bacoside A (Fig. 3). The accessions BM1 and BM7 were grouped together also showed maximum biomass and highest levels of bacoside A (Tables 1, 2). Earlier, accessions were grouped on the basis of their active principle content in *Hypericum perforatum* (Bagdonaite et al. 2012).

Variations in the harvestable biomass and harvest index (HI) of these accessions were recorded during the different seasons (Fig. 1b). Maximum average biomass (2.67) and HI (0.0032) of all accessions was recorded during summer; whereas minimum average biomass (1.27) and HI (0.0021) was recorded in the winter season (Fig. 1b). Nearly a 1.5-fold variation in the HI was observed during winter to summer season (Fig. 1b). The average HI of all the accessions was maximum in summer, thus suggesting the maximum economic yield during this period. Biomass and HI of these accessions also varied significantly (Table 2). Maximum biomass accumulation (4.2 g/plant) and HI (0.0041) were also observed in the case of accession BM1 and BM7 and minimum biomass accumulation (1.26 g/plant) and HI (0.0016) were recorded in accession BM14. As mentioned above, accession BM14 was collected from high altitude where the average temperature is lower. On the other hand, accession BM1 was collected from Kolkata (Lower altitude) where tropical conditions prevail and temperature is generally higher.

This study clearly established better performance of BM1 accession for high growth and bacoside A. Identification of such high yielding accession is important for

Fig. 3 The hierarchical CA on the basis of 'bacoside A' content in various accessions of *B. monnieri* during summer

Table 2 Variation in biomass and harvest index (HI) of various accessions of *B. monnieri* during summer

Accession	Biomass (g/plant)		Harvest index
	Fresh weight (FW)	Dry weight (DW)	
BM1	4.2 ^a	1.64 ^a	0.0041 ^a
BM2	2.73 ^e	1.07 ^e	0.0030 ^e
BM3	2.26 ^h	1.16 ^h	0.0028 ^f
BM4	2.86 ^d	1.09 ^d	0.0030 ^d
BM5	3.2 ^c	1.26 ^c	0.0038 ^c
BM6	2.51 ^f	1.31 ^f	0.0029 ^f
BM7	4.2 ^a	1.52 ^a	0.0041 ^a
BM8	1.78 ^k	0.64 ^k	0.0023 ^j
BM9	2.34 ^g	1.02 ^g	0.0028 ^g
BM10	2.06 ⁱ	0.87 ⁱ	0.0027 ^h
BM11	3.7 ^b	0.82 ^b	0.0041 ^b
BM12	1.86 ^j	0.76 ^j	0.0026 ⁱ
BM13	1.38 ^l	0.52 ^l	0.0022 ^k
BM14	1.26 ^m	0.32 ^m	0.0016 ^l

All the collected accessions were maintained at a common place at Thapar University campus for 1 year before collecting the samples. Data were in triplicate and analysed by ANOVA. The means were compared using DMRT; values sharing common lower case letters within columns are not significantly different at $P < 0.05$

cultivation and production of bacoside A. The variation in the biomass and HI has been reported earlier in many crops (Gilbert et al. 2006; White and Wilson 2006; Pilbeam 1996; Scully and Wallace 1990). These authors reported that increase in harvest index is due to the increase in biomass of the plant. Pandey et al. (2007) reported variation in the harvest index in plants of different ages of *P. hexandrum* and reported maximum HI in plants of 5 year age, which produce higher biomass.

The relative growth rate (RGR) also varied considerably amongst accessions of *B. monnieri* and also during different seasons (Table 3). The RGRs were higher during spring, whereas it was minimum during winter. During autumn and winter the RGR was found to be negative (Table 3) indicating negative growth. Earlier, there are few reports regarding the variations in RGR during different growing seasons of the crop species (Barradas and Lopez-Bellido 2009; Karimi and Siddique 1991). These authors also reported that relative growth rate was higher during early growth periods, decreasing deeply thereafter until the end of the growing season. The negative RGR observed from autumn to winter can be explained by an increase in the number of senesced leaves as reported earlier (Davidson and Campbell 1984). Amongst the accessions, RGR was higher in BM14 in all the seasons and was found minimum in accession BM1 and BM7 during summer. Pandey et al. (2007) reported the RGR as an index of biomass production in *P. hexandrum*. In this study, comparison of harvest index (HI) and relative growth rate was

Table 3 The relative growth rate of various accessions of *B. monnieri* during different seasons

Accession	Relative growth rate (RGR)			
	Spring	Summer	Autumn	Winter
BM1	0.0041 ^{dA}	0.0008 ^{dB}	−0.0013 ^{aC}	−0.0036 ^{cD}
BM2	0.0037 ^{eA}	0.0006 ^{dB}	−0.0013 ^{aC}	−0.0031 ^{bD}
BM3	0.0043 ^{dA}	0.0004 ^{dB}	−0.0013 ^{aC}	−0.0034 ^{cD}
BM4	0.0033 ^{eA}	0.0007 ^{dB}	−0.0014 ^{aC}	−0.0026 ^{aD}
BM5	0.0028 ^{fA}	0.0017 ^{bB}	−0.0022 ^{cC}	−0.0024 ^{aD}
BM6	0.003 ^{fA}	0.0007 ^{dB}	−0.0014 ^{aC}	−0.0023 ^{aD}
BM7	0.0044 ^{dA}	0.0008 ^{dB}	−0.0013 ^{aC}	−0.004 ^{dD}
BM8	0.003 ^{cA}	0.0016 ^{bB}	−0.0028 ^{dC}	−0.004 ^{dD}
BM9	0.0037 ^{eA}	0.0008 ^{dB}	−0.0017 ^{bC}	−0.0028 ^{bD}
BM10	0.0042 ^{dA}	0.0007 ^{dB}	−0.0015 ^{aC}	−0.0034 ^{cD}
BM11	0.0034 ^{eA}	0.0024 ^{aB}	−0.0035 ^{eC}	−0.0023 ^{aD}
BM12	0.0051 ^{cA}	0.0015 ^{bB}	−0.0017 ^{bC}	−0.0053 ^{eD}
BM13	0.0057 ^{bA}	0.0013 ^{cB}	−0.0026 ^{dC}	−0.0035 ^{cD}
BM14	0.0181 ^{aA}	0.0016 ^{bB}	−0.0028 ^{dC}	−0.0063 ^{fD}

All the collected accessions were maintained at a common place at Thapar University campus for 1 year before collecting the samples. Data were scored in triplicate and analysed by ANOVA. The means were compared using DMRT; values sharing common lower case letters within column and upper case letters within rows are not significantly different at $P < 0.05$

helpful for the selection of elite accessions (BM1 and BM7) and this study helped in identification of summer season when bacoside A levels are higher, which will help in developing management and conservation strategy for this important medicinal herb.

In the present study, both biomass and bacoside A contents were higher during summer and minimum during winter. Among the accessions, BM1 and BM7 recorded higher biomass accumulation with maximum levels of bacoside A in all the seasons. Thus, the observed higher level of bacoside A in these accessions could be due to genetic variations. It is also concluded that the summer is the best time for the harvest of *B. monnieri* for the production of bacoside A when maximum biomass can also be harvested.

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