Impact of partial shades on *Bacopa monnieri* L. for nootropic parameters

ABSTRACT

Indian traditional and ayurvedic medicine is used to relieve various complex diseases and health issues. *Bacopa monnieri* is a potent medicinal herb used for multiple disease remedies but is significantly known as a nootropic or brain tonic. Impact of different shade net intensity (SNIs) on biomass yield and bioactive compound on *Bacopa monnieri* analyze using the most efficient UPLC-MS/MS methods. The different shade net intensity viz., 0% SNI, 30% SNI, 40% SNI, 50% SNI, 75% SNI, and 90% SNI were used. Maximum total fresh herbage weight was obtained from the 75% SNI. The maximum content of bioactive compounds, viz., bacopaside I (0.287 % g-1), bacopaside II (0.236 % g-1), bacoside A (0.296 % g-1), and total bacoside content (0.582% g-1) were found under 75% SNI, whereas, maximum bacoside A3 was obtained in 50% SNI. However, maximum dry herbage weight (630 g), was obtained from 0% SNI. Therefore, the selected elite 'DBM-4' could be cultivated under partial shade conditions for harvesting maximum yield from nootropic parameters.

Keywords: *Bacopa monnieri* L.; bacoside content; carotenoid; herbage yield; shade net intensity.

1. INTRODUCTION

India has a very huge diversity of herbs and among the twelve biodiversity centers of the world, it comprised 45,000 different plant species. Out of these, 15,000-20,000 plants have medicinal properties and 7,000-7,500 species are utilized daily by the community for the presence of medicinal value. It has been reputed that area of medicinal plants increased 3 folds from 2006 to 2016 which was numerically 2, 62,000 ha to 6, 33,900 ha, respectively [1].

At present time, all over the world is reliant on medicines under the covid-19 pandemic era. Therefore, the demand for various products from medicinal plants and various medicines enlarging daily throughout the globe. Among various medicinal plants, 25 medicinal plants have always high demand as per Ayurvedic medicinal systems.

Among them, Jal Brahmi (*Bacopa monnieri* L.), is an important medicinal nootropic herb [2]. It is also known as, *Herpestis monnieri*, water hyssop, and generally known as Brahmi or Jalanimba in India. The Ayurveda, an Indian-origin holistic system of medicines is used for centuries. It is categorized as a medhyarasayana, which means to improve memory and intellect (medhya). Jal Brahmi is reported in 'Charaka Samhita' since the sixth century AD and in several other Ayurvedic treatises [3].

This plant grows in marshy soils and sandy areas up to the height of 1300m. It is a water-loving plant. It is found in wetlands and near streams in tropical regions. Therefore, it can be easily grown in the area in which water logging is the main problem [2]. It grows up to a height of 20-30 cm and it has 10-35 cm long branches. Among all the herbs which are investigated for their memory enhancement properties, Brahmi is the most preferable one from ancient times. Many eminently researched the chemical properties

of Jal Brahmi ensured that it enhanced the protein synthesis in the brain and nerve cells, which has a significant effect on the mental stamina of the brain [4]. It is directly correlated with human behaviour and health. When it is used as a drug it can make a person think clearer and the memory becomes more brilliant [2]. In our country, Brahmi is used in a variety of forms to improve the mental ability of school-going children. It also increases the grasping power of the brain as a result ability of people to understand and digest information easily. Therefore, Jal Brahmi is well known as an important anti-depressant and anti-anxiety agent. It is used frequently for head massage in India [5]. All these properties of Jal Brahmi are due to the presence of pharmacological secondary metabolites like alkaloids and saponins. It has many alkaloids like bacosides A and B, herpestine, nicotinine, and brahmine. Many saponins are present in Bacopa like triterpenoid saponins, saponins A, B, and C, stigmastanol, ß-sitosterol, D-mannitol, α-alanine, glutamic acid, aspartic acid, serine, and pseudojujubogenin glycoside [6]. Among them, Bacoside A and B are vital compounds mostly found together [7]. These compounds have neuropharmacological effects and nootropic activity [2].

For the last two decades in India, farmers are diverted towards protected cultivation. Protected cultivation comprised of various structures like a net house, poly house, and glasshouses. Medicinal plants like Jal Brahmi are naturally grown in their ordinary habitats. In the modern era, the transformation of wild crops to cultivation even under controlled conditions [8]. To achieve proper growth of the plants, there must be a control condition created which is likely to be similar to the natural ones as per the variety or type of the plant. As per the crop is concerned, there must be a specific amount of shade is required for its development. Here, shading nets are best for providing partial shade conditions for the plants [8]. Shading nets are developed to protect plants or crops from UV radiation, as well as protect from adverse climatic conditions. Favorable growth conditions for the crop can be gained due to the controlled conditions "created" in the selected area, with shade netting, for better herbage and chemical yield with better quality content. Different shade net intensity (SNI) has specific light quality with high tear resistance, low weight, easy and quick installation with different 30-90% shade nets [8]. High-yielding chemotype 'DBM-4' is used under different shade net intensity to study their impact on crop growth and bacoside content.

2. MATERIALS AND METHODS

2.1 Experimental site and plant materials: The experiment was conducted at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India during 2019-20 and 2020-21. It is located at N 22° 55', E 72° 66' E, and 45.1 m above mean sea level.

Initially, general Brahmi crop trials were conducted under the poly house (200-gauge), net house (50% SNI), intercrop with Aonla (25% SNI) as well as in open field conditions (0% SNI) to know the effect of different SNI on herbage yield and bacoside content during 2019-20. During the year 2020-21, Jal Brahmi elite 'DBM-4' was selected purposively as experimental material and multiplied at the Directorate nursery [2]. The method of nursery raising was through stolen/stem cuttings. After 3 months, elite were harvested and planted in the experimental plot on the first week of August 2020. The three-month-old runners are planted at 20 cm × 20 cm spacing with five replications in 3 × 2 m size individual plots. The crop was grown using the standard package of practices [9]. The crop was supplied with well-rotted 15 t ha^{-1} Farm Yard Manures (FYM) without any chemical fertilizers. The plots are raised under shade net with a different intensity viz., 0% (control), 30%, 40%, 50%, 75% and 90%, where 0% SNI refers open natural field conditions. Commercially available green colored agro shade net with average fabric weight 69, 75, 84, 95 and 130 GSM (g m^2) in 30%, 40%, 50%, 75% and 90% SNI, respectively [8]. The experiment was harvested in November 2020, March, and July 2021. The herbage yield was taken manually at the full bloom stage by cutting from ground level. The morphological observations like stolon thickness and flower pedicel length were taken using the Vernier caliper. The number of nodes and number of leaves were counted per stolon. Internodal length and stolen length were measured whereas leaf area, leaf length, and leaf width were taken with randomly selected 10 leaves, and leaf area was measured by Leaf Area Meter (LAI 3000). The fresh herbage yield was taken from a 100 cm^2 area in each replication and weight was measured using a weighing machine [2]. After harvesting, fresh herbage was dried under room temperature till 8-10% moisture content and reduced weight was weighed out.

2.2 Quality Parameters: Chlorophyll and carotenoid analysis: Leaf pigments like chlorophyll and carotenoid were extracted using 80% acetone. The chlorophyll and carotenoid content in fresh leaves were calculated as per the standard method [2, 8].

Bacosides analysis: The certified reference materials viz. Bacopaside I, Bacoside A3, and Bacopaside II with purity ≥99.9% were obtained from Sigma-Aldrich Pvt. Ltd., India. The MS grade reagents [Formic acid (99.5%) and ammonium formate] and organic solvent [methanol] were procured from Fisher Chemical (Fair lawn, NJ, USA) and Merck (Darmstadt, Germany), respectively. Ultrapure water was obtained from the Milli-Q® IQ Element purification system. The stock and working solutions were prepared in a binary organic solvent mixture [Methanol: water (9:1, v/v)]

Sample preparation: A 10 mL of 70% methanol was poured into the test tube containing 100 mg dried powder and was subjected to a sonication bath for 50 minutes. After that, the samples were centrifuged at 5000 rpm for 12 minutes. The supernatant (1.0 mL) was subjected to chromatographic separation and quantification after proper filtration using PTFE filters (0.22 micron) in a 2 mL vial (3,6).

Chromatographic and mass spectrometry conditions: All analytical procedures were performed on Thermo Scientific TSQ Quantis LC-QnQ MS/MS (Thermo Scientific, USA). For the separation of analytes, Acclaim TM 120 C18 column (120A^{o} 3.0 × 100 mm, 3µm) was used at 25^{o}C. The mobile phase consists of Water with 5mM ammonium formate, 0.1% formic acid (A), and methanol with 5mM ammonium formate, 0.1% formic acid (B). The gradient profile started at 65 % (v/v) A, held for three minutes and changed to 95% (v/v) at 10 minutes, held for 10 minutes, and changed to 65 % (v/v) at 23 minutes followed by a post-run of 2 minutes.

Analysis of Bacopaside I, Bacoside A3, and Bacopaside II were performed under positive ionization with capillary voltage 4500 V, vaporizer temperature was 350^{o}C, and sheath gas (N2) 50 arbitrary unit, auxiliary gas (N2) 10 arbitrary unit and ion transfer capillary temperature was 300^{o}C. Parent and productions were similar for Bacoside A3 and Bacopaside II, however, both were separated based on retention time (RT). The masses which were monitored and optimized are mentioned in Table 1. For the interpretation of the chromatograph, Trace finder 5.1 software (Thermo Scientific, USA) was used.

Analytical method validation: The method's performance was developed and validated by SANTE guidelines [10] by examining parameters such as linearity, the limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. Using seven calibration standards ranging from 1-100 µg/kg (1, 2.5, 5, 10, 25, 50, and 100 µg/kg), the linearities of bacopaside I, bacoside A3, and bacopaside II in their respective pure solvents were determined. The lowest addition level of the analysis method was designated as the limit of quantification (LOQ) of both bacopasides and bacoside throughout the experiment, and the limit of detection (LOD) was estimated as three times the signal-to-noise ratio (S/N). For LOD and LOQ calculation of all three compounds were calculated by using the following formulas: (a) LOD (µg/kg) = (mean of standard deviation/Slope) × 3; (b) LOQ (µg/kg) = (mean of standard deviation/Slope) × 10 [11,12]. A recovery study was used to assess the accuracy and precision. With seven replications, three concentration levels (0.005, 0.025, 0.05µg/kg) were employed for the fortification of two bacopasides and bacoside to determine percent recovery and relative standard deviation (RSD%).

2.3 Statistical analysis

In Randomized Block Design (RBD) analysis of variance for various observations recorded in the experiment by using SAS 9.2 (SAS, 2008) statistical software [8]. The different treatment means were compared with the DMRT procedure. The results were calculated based on a 5% level of significance (P= 0.05). Various treatment means were calculated by Critical Difference (CD) values.

3. RESULT AND DISCUSSION

3.1 Initial screening for quantitive and qualitative parameters

Initially, comparative analysis of herbage yield and phytochemical content in different environmental conditions was performed by utilizing poly house, net house, and intercrop with Aonla as well as in open field conditions (Fig. 1). The study revealed that among different environmental conditions, maximum fresh and dry herbage yield (q ha^{-1}) were obtained in the net house (50% SNI), followed by a poly house (fresh herbage yield) and in intercrop with Aonla (dry herbage yield) compared to open field condition. However, the higher bacoside-II and Bacoside-A3 (mg g^{-1}) content was also reported in the net house (50% SNI), followed by poly house condition. However, the open field condition (control) has reported the least bacoside-II and Bacoside-A3 content. Therefore, the significantly highest herbage yield combined with maximum phytochemical content was obtained from a net house with 50% SNI.

3.2 Morphological parameters

As per the results of initial screening for herbage yield and bacosides content, the crop favor partial shade for its growth and development. Intensely, Jal Brahmi elite 'DBM-4' was chosen for the experiment favorably due to being rich in bacoside A. Further, different treatments of green shade nets viz., 30, 40, 50, 75, and 90 % SNIs were used for three consecutive crop harvesting seasons. Significant differences for all six treatments were observed in all three harvest seasons for distinct morphological parameters of Jal Brahmi. Maximum plant height (24.67 cm) was reported in 40% SNI as compared to all shade net intensities of the third harvest, whereas, the least was in 0% SNI (6.37 cm) during the first harvest. The stolon length (98.67 cm) was significantly higher with 75% SNI in the third harvest, however least (34.43 cm) with 50% SNI during the first harvest. The maximum and a minimum number of nodes per stolon (44.67 and 13.50) were recorded in 30% SNI during the third and first harvest, respectively. The highest numbers of leaves (286.89, 219.88, and 216.07) were observed in 0% SNI as compared to all treatments in the third, second and first harvesting seasons, respectively. However, the least number of leaves (70.90) were found in 75% of SNI of the first harvest (Table 3). A maximum number of branches (21.53) was recorded in the control (0% SNI) as compared to all shade net intensities in the first harvesting seasons. The highest and lowest stolon thickness (2.50 mm and 1.55 mm) was recorded in 75% SNI of the second and third harvest, respectively, While the highest internodal length (3.77 cm) was observed under 30% SNI treatment of the first harvest. A maximum number of stolon (4068.50) in 100 m^2 and pedicel length (3.49 cm) was recorded for the second and third harvesting season in 40% SNI treatment compared to all shade net intensities (Table 3). The leaf parameters, viz., leaf length (19.03 mm) and leaf width (6.02 mm) were reported maximum with 75 % shade net intensity in second harvesting; however, the least values of leaf length (12.0 mm) and leaf width (3.87 mm) were recorded in control (0% SNI) condition. Maximum leaf area (9.20 cm^2) was recorded with 40% SNI during the third harvest, while the least (5.47 cm^2) was in 0% SNI compared to all treatments (Table 3).

Among different shade net intensity levels, the highest total fresh herbage weight (5330 g) was obtained during third harvesting in 75% SNI followed by 40% SNI (5250 g). Maximum total dry herbage weight (630 g) was also obtained in 0% SNI, followed by 30% SNI (604 g) (Table 3).

The impact of different SNIs on Jal Brahmi herbage growth and nootropic chemical parameters were observed. Under different treatments or environmental conditions, maximum herbage yield combined with maximum phytochemical content was obtained from a net house (50% SNI) cultivation. This is due to medicinal plants generally growing in wild habitats under partial shade, moist soil, high humidity, and mild temperature conditions [13]. The south Asian countries also have a tradition of mixed farming and multistory cultivation systems [8]. The morphological observations like stolon thickness, flower pedicel length, number of nodes, number of leaves, internodal length, stolen length, leaf area, leaf length, leaf width, and fresh herbage yield were reported maximum with different shade net intensity and

the least values were recorded in control (0% SNI) condition. The leaves under shade conditions made efficient use of the less intense irradiation reaching up to them like rauwolfia, patchouli, turmeric, Shatavari, and Brahmi [4,8]. Partially shade tolerates plant-like turmeric was reported higher shoot biomass under 48% relative light intensity (RLI) as compared to 100% RLI (Hossain et al. 2009) [14]. However, maximum dry herbage was observed under 0% SNI as compared to protected structures [4]. Light or solar radiation is the most important environmental factor that influences the plant growth parameters [15,16]. Similarly, maximum herbage and root yield in *Asparagus racemosus* were reported under open field (0% SNI) as compared to 50, 75, and 90% SNI [8]. The reduction in light interception leads to a reduction in the total dry matter [17].

3.3 Quality parameters

Method verification: After injecting a working solution into UHPLC, the retention times (RT) for bacopaside I, bacoside A3, and bacopaside were 7.41, 7.71, and 8.05 minutes, respectively. The efficiency of the analytical procedure was verified by the following method validation parameters: linearity, sensitivity, accuracy, and precision. First, the linearity study showed a linear response, and the coefficient of determination (R2) values for all bacopasides and bacoside were ≤ 0.99 (Fig.2) (Table 2). Third, the recovery studies were obtained in the range of 95.80%-104.05% for bacopaside I, 88.47%-89.13% for bacopaside II and 85.82%-88.47% for bacoside A3. Eventually, precision (RSD %) for all bacopasides and bacoside ranged from 11.94 % to 16.67% (Table 2).

Biochemical parameters: The effect of different shade net intensities on quality parameters were analyzed (Table 4). Significant differences were observed for different quality parameters of Jal Brahmi. Among different shade net intensity, maximum and minimum Chlorophyll a (0.739 and 0.553 mg g^{-1}), chlorophyll a: b (3.150 and 2.580 mg g^{-1}), and total chlorophyll (0.975 and 0.782 mg g^{-1}) were obtained with 0 % SNI and 90% SNI, respectively. For the rest of the components, viz., the highest chlorophyll b content was (0.263 mg g^{-1}) obtained in 75% SNI, whereas, the lowest (0.207 mg g^{-1}) was with 40% SNI. However, maximum carotenoid content (2.593 mg g^{-1}) was reported in 0% SNI (Control) and the least estimates (2.420 mg g^{-1}) were found in 50% SNI.

First-time UPLC-MS/MS used for estimation of bacosides content from dry herbage of Jal Brahmi (Table 4). Among different bioactive compound, maximum bacopaside I (0.287 % g^{-1}) and total bacoside content (0.582 mg g^{-1}) was recorded in 75% SNI, whereas, minimum bacopaside I (0.159 % g^{-1}) and total bacoside content (0.366 mg g^{-1}) was in 30% SNI. However, the highest and least estimates of bacopaside II (0.236 mg g^{-1} and 0.130 mg g^{-1}) and bacoside A (0.296 % g-1 and 0.171% g^{-1}) were reported in 75% SNI and 0% SNI, respectively (Fig. 3). Among different environmental conditions, maximum bacoside A3 (0.064 % g^{-1}) was found in 50% SNI, whereas, minimum (0.041 % g^{-1}) was observed in 0% SNI. Overall, results show that treatment with 75% SNI observed maximum bacosides but it was non-significant with 40% SNI. The method verification for retention times (RT) for bacopasides and efficiency of the analytical procedure (linearity, sensitivity, accuracy, and precision) were fitting for analysis. Second, the analytical procedure's LOD and LOQ were calculated using the signal-to-noise ratios obtained [8]. Maximum total bacoside content (0.582 mg g^{-1}) was recorded in 75% SNI, whereas, the minimum total bacoside content was in 0 to 30% SNI. By nature, medicinal plants like Shatavari, Brahmi, etc., prefer partial shade [8,13]. Due to preferences for partial shade, the curcumin, oleoresin, and essential oil content increased in turmeric under shaded conditions [18]. Similarly, maximum shatavarin IV was harnessed in *A. racemosus* under 25% SNI as compared to 0% and 50 to 90% SNI [8]. Plant under partial shade sustained their growth due to an increase in leaf area and efficient photosynthesis [19]. Under stress or partial shade leaves made efficient use of available irradiation [8]. The result indicated that the selected elite 'DBM-4' could be cultivated under partial shade (75% SNI) conditions and as an intercrop for fetching higher total bacoside yield as compared to open field conditions.

4. CONCLUSION

Maximum fresh herbage yield and bacosides content were found in 75% SNI. However, the maximum total dry herbage weight was obtained under 0% SNI. The elite, DBM-4 is suitably cultivated under 75% SNI for getting maximum secondary phytochemicals yield for efficient industrial use. At the same time, this crop could be cultivated as an intercrop or under partial shade conditions by stakeholders.

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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Table 1. Optimization of different parameters for Bacopaside I, Bacoside A3 and Bacopaside II on MS.

Parameters	Name of compounds		
	Bacopaside I	**Bacoside A3**	**Bacopaside II**
Parent ion (m/z)	979.540	951.492	
Product ion (m/z)	473.405	497.208 and 869.387	
Collision energy (eV)	19.7	55	
Tube lens (V)	294	299	

Table 2. Method validation parameters of Bacopaside I, Bacopaside II and Bacoside A3 from *Bacopa monnieri.*

Sr No.	Parameters	Particular	Bacopaside I	Bacopaside II	Bacoside A3
1.	Linearity (n=5)	Calibration concentration range	1-100 µg/kg		
		Regression equation	y = 131.07x + 674.34	y = 79.725x - 191.66	y = 1961.1x - 2972.2
		R^2 {$R^2 \geq 0.99$}	0.99	0.99	0.99
2.	Sensitivity (n=5)	LOD (µg/kg)	0.01	2.5	2.5
		LOQ (µg/kg)	1	10	10
3.	Accuracy (n=7)	% Recovery [70-120%] *F level [0.005, 0.025, 0.05µg/kg]	95.80±4.44 to 104.05±6.26	88.47±7.89 to 89.13±6.78	85.82±6.16 to 88.47±7.89
4.	Precision (n=7)	% RSD [≤ 20%]	13.41 to 15.99	16.53 to 16.67	11.94 to 16.57

R^2: correlation coefficient; LOQ:Limit of quantification; LOD:Limit of detection;*F level: Fortification levels; ±SD: Standard deviation; RSD: Relative standard deviatio; Figures in parenthesis are accpetable criteria of SANTE (2019)

Table 3. Effect of different SNIs on growth and herbage yield parameters in jal brahmi elite 'DBM-4'.

	Plant height (cm)			Stolen length (cm)			Number of nodes/stolons			Number of leaves		
Treatments	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest
0 % SNI	6.37^{c}	11.86^{c}	21.78^{ab}	36.40^{bc}	36.52^{c}	84.78^{bc}	15.93^{bc}	23.78^{a}	36.89^{c}	216.07^{a}	219.88^{a}	286.89^{a}
30% SNI	13.40^{b}	20.82^{ab}	24.44^{a}	43.20^{abc}	41.14^{b}	90.11^{ab}	13.50^{c}	23.00^{ab}	44.67^{a}	140.33^{b}	162.78^{b}	236.78^{b}
40% SNI	18.10^{a}	21.91^{a}	24.67^{a}	42.57^{abc}	41.40^{b}	89.67^{b}	20.80^{a}	20.00^{ab}	40.22^{bc}	122.50^{b}	95.00^{d}	168.67^{cd}
50% SNI	14.20^{b}	20.17^{ab}	22.22^{ab}	34.43^{c}	41.88^{b}	81.00^{c}	18.70^{ab}	19.00^{b}	42.67^{ab}	117.77^{b}	128.78^{c}	143.11^{cd}
75% SNI	18.43^{a}	19.07^{b}	23.33^{ab}	43.53^{ab}	50.84^{a}	98.67^{a}	19.77^{a}	23.78^{a}	38.44^{c}	70.90^{c}	119.78^{c}	133.89^{d}

90% SNI	18.53^{a}	21.19^{ab}	20.78^{b}	50.10^{a}	42.01^{b}	86.00^{bc}	19.57^{a}	19.00^{b}	36.11^{c}	147.70^{b}	123.78^{c}	177.56^{c}

Cont.

	Number of branches			**Stolon thickness (mm)**			**Internodal length (cm)**			**Total number of stolon**			**Pedicel length (cm)**		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Treatments	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest
0 % SNI	21.53^{a}	16.00^{a}	13.22^{b}	2.17^{ab}	2.03^{ab}	1.55^{b}	2.43^{bc}	1.67^{c}	1.83^{b}	1944.00^{c}	2766.00^{c}	1686.33^{c}	2.03^{ab}	2.26^{c}	2.73^{c}
30% SNI	15.00^{b}	12.78	12.44^{c}	2.23^{a}	1.97^{b}	1.80^{a}	3.77^{a}	2.00^{bc}	2.28^{a}	2776.23^{b}	3829.50^{a}	1865.44^{bc}	1.97^{b}	2.47^{b}	2.93^{bc}
40% SNI	14.90^{b}	10.78	12.78^{bc}	2.10^{ab}	2.40^{ab}	1.74^{ab}	2.30^{c}	2.15^{ab}	1.84^{b}	3154.57^{a}	4068.50^{a}	2015.67^{b}	2.40^{ab}	2.87^{a}	3.49^{a}
50% SNI	13.50^{b}	11.33	12.00^{c}	2.17^{ab}	1.97^{b}	1.64^{ab}	2.60^{bc}	2.50^{a}	2.06^{ab}	2726.00^{b}	3447.00^{b}	3155.67^{a}	1.97^{b}	2.61^{ab}	3.17^{b}
75% SNI	11.43^{c}	15.00	15.22^{a}	2.33^{a}	2.50^{a}	1.56^{b}	2.33^{c}	2.11^{b}	2.29^{a}	1952.77^{c}	2719.00^{c}	1901.22^{bc}	2.50^{a}	2.48^{b}	3.11^{b}
90% SNI	13.90^{b}	13.22	10.22^{d}	2.00^{B}	2.10^{ab}	1.61^{b}	2.70^{b}	2.25^{ab}	2.17^{a}	1672.10^{d}	1886.50^{d}	1712.78^{c}	2.10^{ab}	2.41^{bc}	2.72^{c}

Cont.

	Leaf length (mm)			**Leaf width(mm)**			**Leaf area (cm^2)**			**Total fresh weight (g)**			**Total dry weight (g)**		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Treatments	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest
0 % SNI	12.30^{C}	14.01^{d}	14.76^{d}	4.23^{c}	4.81^{c}	3.87^{c}	5.47^{b}	5.80^{d}	7.32^{c}	1330^{c}	1690^{c}	4660^{b}	204^{bc}	201^{cd}	630^{a}
30% SNI	15.40^{ab}	15.91^{bc}	15.56^{cd}	4.83^{bc}	4.77^{c}	4.24^{bc}	7.10^{ab}	6.78^{c}	8.31^{b}	2000^{ab}	2390^{bc}	5100^{a}	207^{bc}	263^{ab}	604^{b}
40% SNI	15.90^{ab}	15.10^{cd}	17.45^{ab}	5.13^{ab}	4.62^{c}	4.76^{a}	7.03^{ab}	8.15^{b}	9.20^{a}	2170^{a}	2780^{a}	5250^{a}	300^{a}	292^{ab}	565^{bc}
50% SNI	14.17^{bc}	16.81^{b}	18.36^{a}	4.47^{bc}	5.53^{ab}	4.30^{b}	7.40^{a}	7.74^{b}	7.80^{bc}	1870^{ab}	2550^{ab}	4680^{b}	234^{b}	310^{a}	533^{bcd}
75% SNI	17.53^{a}	19.03^{a}	15.78^{bc}	5.63^{a}	6.02^{a}	4.26^{bc}	7.17^{ab}	8.91^{a}	7.57^{bc}	1700^{bc}	2400^{ab}	5330^{a}	203^{bc}	243^{bc}	512^{cd}
90% SNI	15.93^{ab}	15.54^{bc}	16.75^{abc}	4.87^{bc}	5.08^{bc}	4.77^{a}	7.17^{ab}	7.66^{b}	7.98^{bc}	1770^{abc}	1690^{c}	4330^{c}	201^{bc}	160^{d}	480^{d}

Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) – (Duncan Multiple Range Test).

Table 4. Effect of different SNI on quality parameters in jal brahmi 'DBM-4'.

Treatment	Chlorophyll A	Chlorophyll B	Chlorophyll A: B	Total chlorophyll	Carotenoid	Bacopaside I	Bacopaside II	Bacoside A3	Bacoside A	Total Bacoside
	(mg g^{-1})			(mg g^{-1})				(% g^{-1})		
0 % SNI	0.739^{a}	0.236^{ab}	3.150^{a}	0.975^{a}	2.593^{a}	0.240^{ab}	0.130^{c}	0.041^{d}	0.171^{bc}	0.411^{bc}
30% SNI	0.702^{a}	0.229^{b}	3.100^{a}	0.931^{b}	2.543^{ab}	0.159^{c}	0.162^{bc}	0.044^{cd}	0.207^{b}	0.366^{c}
40% SNI	0.599^{c}	0.207^{b}	2.940^{a}	0.806^{de}	2.440^{b}	0.210^{b}	0.200^{b}	0.058^{ab}	0.258^{ab}	0.468^{abc}
50% SNI	0.606^{bc}	0.218^{b}	2.913^{a}	0.824^{d}	2.420^{b}	0.216^{b}	0.224^{ab}	0.064^{a}	0.289^{a}	0.504^{ab}
75% SNI	0.615^{b}	0.263^{a}	2.650^{b}	0.878^{c}	2.550^{a}	0.287^{a}	0.236^{a}	0.060^{ab}	0.296^{a}	0.582^{a}
90% SNI	0.553^{c}	0.228^{b}	2.580^{b}	0.782^{e}	2.480^{b}	0.206^{b}	0.204^{b}	0.052^{bc}	0.257^{ab}	0.462^{abc}

Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) – (Duncan Multiple Range Test).

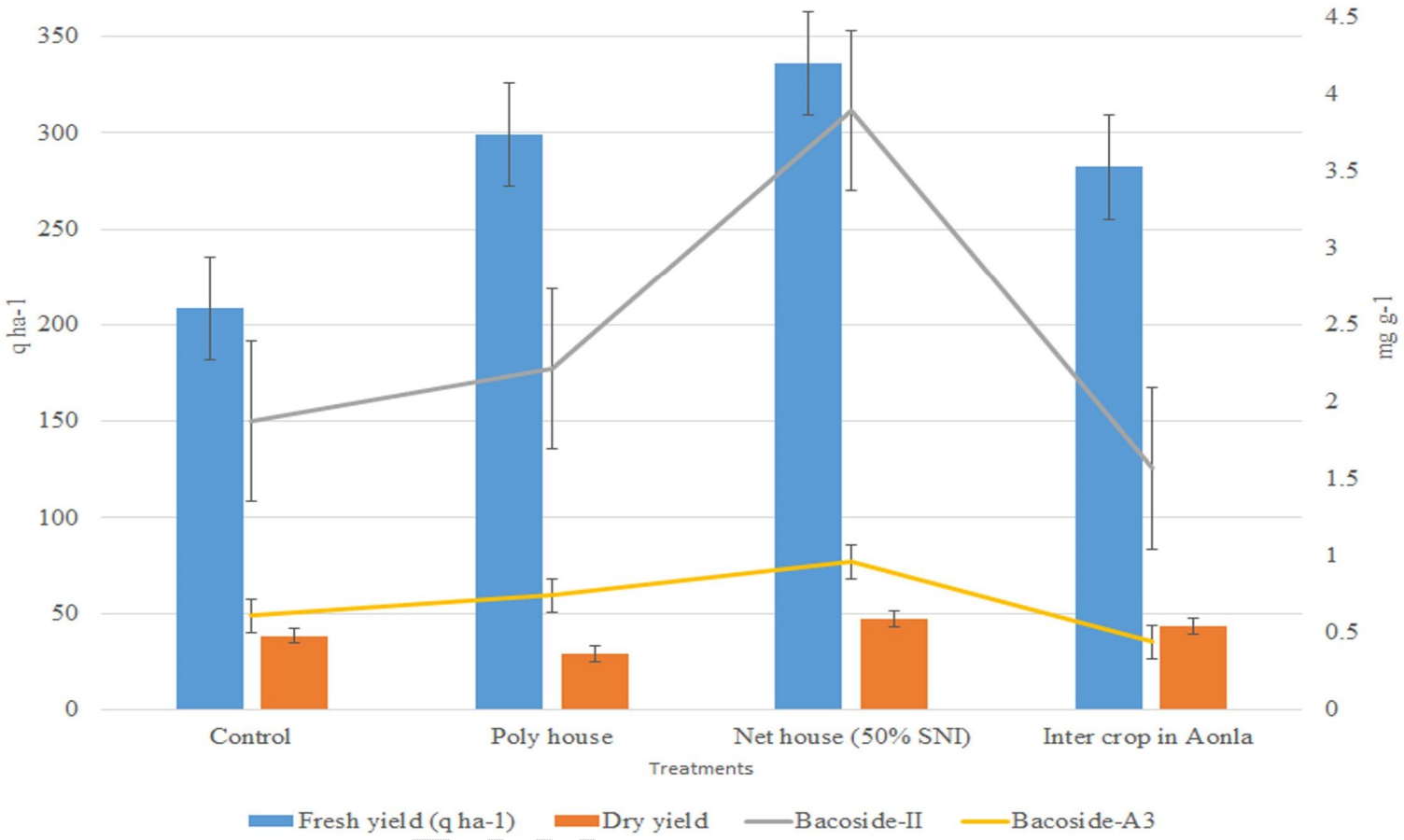

Figure 1. Effect of environmental conditions on nootropic parameters in Jal Brahmi.

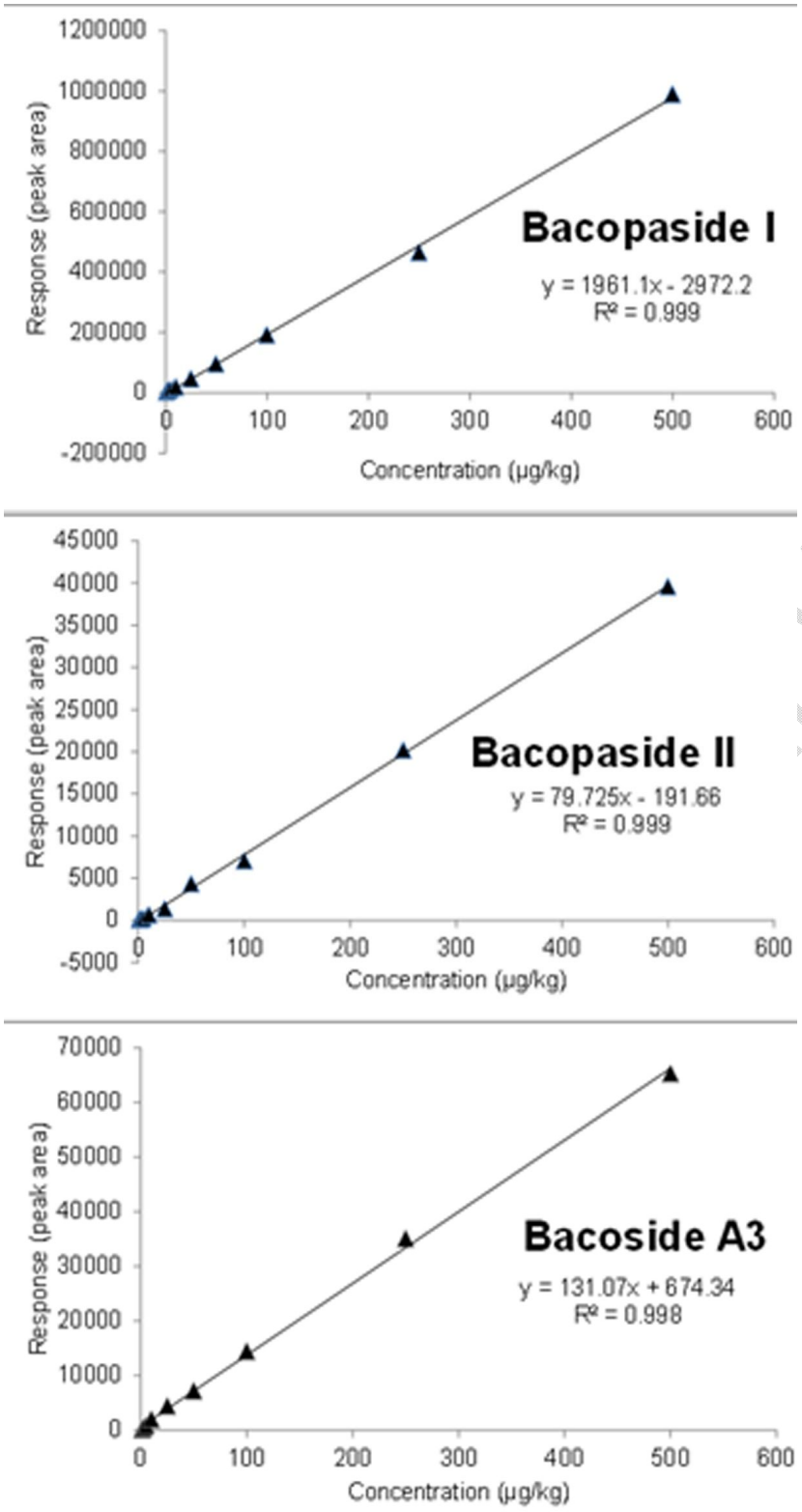

Figure 2. Linearity of (a) Bacopaside I (b) Bacopaside II and (c) Bacoside A3.

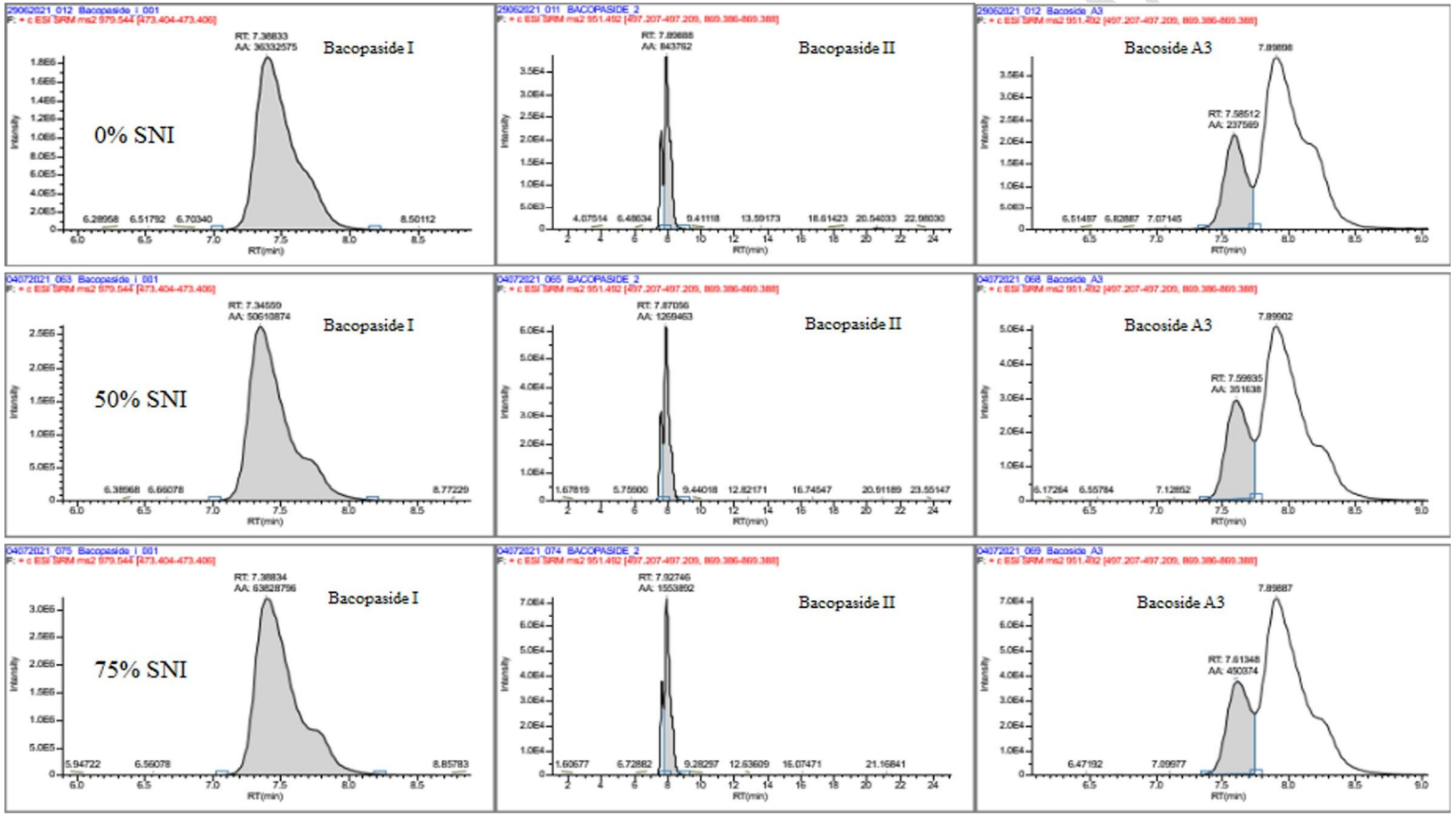

Figure 3. Chromatograph of different shade net intensity treatments.