CLIMATE CHANGE AND GLOBAL SUSTAINABILITY:

AN ECOCRITICAL PERSPECTIVE

Editors:

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Climate Change
and Global Sustainability:
An Ecocritical Perspective

(Collection of Articles)

Editors - Dr. Siny G Benjamin & Dr. Nandini N.J

First Edition - March 2023

Prepress & Publishing

Pravda Books, Kollam

Ph: 9400294893

Email: pravdabookskerala@gmail.com

www.pravdabookskerala.in

www.pravdabookskerala.com

Price : **300**/-

Cover Design - Pravda Books

ISBN 978-93-89446-92-0

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STRATEGIC APPROACH FOR THE CONSERVATION OF A POTENT MEDICINAL PLANT *BACOPA MONNIERI* (L.) WETTST. OCCURRING IN KERALA

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Bacopa monnieri (L.) Wettst. (Plantaginaceae) is a perennial herbaceous species distributed pantropically, and known as 'Brahmi' or 'Neerbrahmi' in Malayalam. This species is widely used in traditional systems of medicine such as Ayurveda, Siddha and Unani, and also in Homoeopathy (Singh and Singh 1980), for the treatment and management of a line of mental defects and as an energizer for the nervous system and heart (Mukherjee and Dey 1966). It also possesses an array of therapeutic properties such as anti-oxidant (Tripathi et al. 1996), anti-cancer (Elangovan et al. 1995), immuno-modulatory (Dahanukar and Thatte 1997), anti-stress (Chowdhuri et al. 2002) and adaptogenic activities (Rai et al. 2003). The principal active factors in Bacopa are steroidal saponins, known as 'memory

chemicals', that help repair damaged neurons by enhancing proteins involved in the regeneration of neural-cell synapses (Rastogi et al. 1994).

The Planning Commission of India Task Force on Conservation and Sustainable Use of Medicinal Plants identified B. monnieri as one of the core species in great demand in the pharmaceutical industry of Indian traditional systems of medicine (Planning Commission 2000). In the priority list of the most important medicinal plants of the country, The Export-Import Bank of India has placed the species in the second position based on the evaluation of its medicinal importance, commercial value and potential for further research and development. (Brinckmann 2008; Rajani 2008; Bammidi et al. 2011). B. monnieri is one of the 32 medicinal plants identified for cultivation and conservation by the National Medicinal Plants Board (NMPB 2008). It is estimated that about 10,000 to 12,000 tons of fresh biomass of the species are collected annually from the wild in India (Karthikeyan et al. 2011). Recently, there are many reports that B. monnieri has become a locally endangered species (Tripathi et al. 2012; Karthikeyan et al. 2011; Ramesh et al. 2011; Bansal et al. 2014), and its cultivation has already been undertaken in various parts of India (Sharma 2004). Due to the high demand for the species, many adulterants are being used in herbal preparations. Thus authentication of the species for herbal preparation is a necessity.

Nowadays B. monnieri is undergoing indiscriminate collection in Kerala from the wild due to its immense medicinal value and consequent high demand in the pharmaceutical industry, resulting in depletion of this herbal resource as well as genetic erosion in the species. Degradation and destruction of wetlands - the habitat of the species - is yet another reason for the extinction of its genotypes from the wild and dwindled supply of raw drugs to the pharmaceutical industry. Extinction of genotypes of the

species is an irreversible setback to the richness of its germplasm, since a genotype once lost is a loss forever. Loss of genotypes of a species and consequent shrinkage of the germplasm would detrimentally affect the prospects of its conservation, cultivation, genetic improvement, and utilization for the manufacture of herbal medicines. To achieve conservation and effective utilization of germplasm of the species in cultivation, crop improvement, and direct utilization of the wild genotypes in the pharmaceutical industry, an in-depth understanding of the genetic resource of B. monnieri, especially its primary gene pool which constitutes its intraspecific variants is a prerequisite.

It is estimated that about one million quintals of brahmi material are collected from the wild every year for commercial use (Ahmad, 1993; Mathur and Kumar, 2001). With increasing demands for herbal drugs, the natural populations of Bacopa monnieri are threatened with overexploitation leading to the depletion of valuable genetic resources. Little work has been done for the conservation of natural variability in the existing germplasm. Hence an attempt has been made by adopting a strategic approach which makes sure not only the biosystematic study but also the conservation of live accessions of the species in the field gene conservatory which make available for the gene pool of the species occurring in Kerala.

Against this backdrop, a biosystematic study on populations of B. monnieri occurring in the State is significant and assumes importance.

The major objectives of the study were twofold:

(i) Identification of genotypes of the species occurring in Kerala and their detailed biosystematic characterization

(ii) Assessment of intraspecific variation in the gene pool of

the species, in Kerala, based on diverse biosystematic parameters and the conservation of the species.

To achieve the objectives, field surveys were conducted throughout Kerala to locate and collect the accessions of the species. The collected species are conserved in the Field Gene Bank of JNTBGRI, Palode. An experimental plot having three replications of each accession was made giving uniform environmental conditions. a biosystematic study on 60 populations of the species occurring in Kerala has been envisaged involving diverse biological disciplines areas such as morphology, morphometrics, anatomy, cytology, palynology, phytochemistry and molecular biology.

In the present study sixty populations (Table I), from its distributional range in the State of Kerala were located, and found that it grows in shallow waters and mostly in marshy ecosystems. The habitat of most of the accessions is marshy and that of certain others aquatic. It is interesting to note that some of the accessions prefer brackish water ecosystems too. The accessions which were collected from the saline conditions were difficult to nurture in the Field Gene Bank at JNTBGRI. The accessions studied also include representatives from varying altitudinal ranges, near sea level (Karumadi, Alappuzha) to 1462 m (Munnar, Idukki). In this context, it may be noted that the maximum elevation of the place of occurrence of the species reported earlier is 1500 m (Lansdown et al. 2013). During the field survey it was observed that in Kerala, the habitat of the species is under great threat due to anthropogenic factors, leading to their destruction and consequent extinction of the genotypes. It is also noted that this species is a poor competitor and its growth is suppressed by grassy weeds. The inflow of saline water into the aquatic freshwater habitat of the species also leads to the destruction of the populations.

Table 1. Collection locations, Geographical Co-ordinates and Percentage content of bacoside A, bacopaside I, and total bacosides of the 60 accessions of Bacopa monnieri

Acc. No.	Collection locations	District	Longitude	Latitude	Altitude (m) (gm)	Extract weight %, w/w	Bacoside A %, w/w	Bacopaside I	Total bacosides %, w/w
Bm1	Viraly	Tvm	77° 05'E	8° 18'N	14	0.1133	2.82± 0.16	0.72± 0.07	3.54± 0.23
Bm2	Kadakkavoor	Tvm	76° 45'E	8° 40'N	14	0.0809	3.04± 0.07	0.30± 0.01	3.35± 0.08
Bm3	Karikkakom	Tvm	76° 54'E	8° 30'N	15	0.1242	2.45± 0.16	0.69± 0.04	3.14± 0.18
Bm4	Aakkulam	Tvm	76° 54'E	8° 31'N	16	0.0963	2.23± 0.12	0.83± 0.03	3.05± 0.12
Bm5	Vetta mukku	Klm	76° 33'E	9° 1'N	17	0.1095	1.54± 0.18	0.67± 0.03	2.21± 0.21
Bm6	Panayam	Klm	76° 37'E	8° 57'N	18	0.1008	2.75± 0.05	0.89± 0.03*	3.64± 0.07
Bm7	Kochupla moodu	Klm	76° 39'E	9° 0'N	9	0.1133	2.26± 0.20	1.07± 0.03**	3.33± 0.21
Bm8	Dalava puram	Klm	76° 33'E	8° 56'N	12	0.1103	3.28± 0.07*	0.74± 0.04	4.02± 0.05**
Bm9	Padukkottukala	Ptnta	76° 43'E	9° 11'N	34	0.0978	3.10± 0.16	1.46± 0.02**	4.56± 0.14**
Bm10	Thakazhi	Alpz	76° 24'E	9° 22'N	7	0.0954	2.35± 0.08	0.82± 0.04	3.17± 0.09
Bm11	Pattani yidukku	Alpz	76° 19'E	9° 30'N	10	0.1413	5.37± 0.19**	1.38± 0.16**	6.75± 0.25**
Bm12	Pathira pally	Alpz	76° 19'E	9° 32'N	9	0.0892	2.85± 0.06	0.79± 0.02	3.63± 0.05
Bm13	Vattayil	Alpz	76° 20'E	9° 28'N	10	0.1020	2.07± 0.07	0.79± 0.03	2.86± 0.08
Bm14	Nerekadavu	Ktym	76° 30'E	9° 38'N	48	0.1037	3.57± 0.34**	0.58± 0.06	4.14± 0.38**

Bm15	Kodu ppadom	Ktym	76° 23'E	9° 47'N	8	0.1179	4.00± 0.11**	1.05± 0.03**	5.05± 0.11**
Bm16	Kuma rakom	Ktym	76° 25'E	9° 36'N	9	0.1181	2.60± 0.07	0.86± 0.05	3.46± 0.06
Bm17	Vazhi kkadavu	Ktym	76° 53'E	9° 40'N	972	0.1137	4.42± 0.16**	0.86± 0.03	5.28± 0.17**
Bm18	Kumba lam	Ekm	76° 18'E	9° 54'N	10	0.0938	3.44± 0.17**	0.86± 0.06	4.31± 0.18**
Bm19	Thripu nithura	Ekm	76° 20'E	9° 57'N	11	0.9610	2.85± 0.18	1.08± 0.16**	3.93± 0.33*
Bm20	Charthe dam	Ekm	76° 13'E	10° 11'N	12	0.0860	3.64± 0.09**	0.36± 0.01	4.00± 0.10**
Bm21	Nr. Aarch dam	Idukki	76° 58'E	9° 50'N	562	0.1070	3.98± 0.25**	1.11± 0.03**	5.09± 0.24**
Bm22	Vellayam kudi	Idukki	77° 5'E	9° 46'N	907	0.1046	3.88± 0.17**	0.73± 0.04	4.61± 0.15**
Bm23	Munnar	Idukki	77° 3'E	10° 5'N	1462	0.1029	3.54± 0.19**	0.66± 0.02	4.20± 0.20**
Bm24	Chellar kovil	Idukki	77° 10'E	9° 40'N	1095	0.1098	2.17± 0.24	0.65± 0.12	2.82± 0.29
Bm25	Kumily	Idukki	77° 9'E	9° 37'N	892	0.0959	1.78± 0.12	0.39± 0.03	2.17± 0.11
Bm26	Chela kkara	Thrsr	76° 21'E	10° 40'N	54	0.1049	2.68± 0.07	0.79± 0.07	3.47± 0.08
Bm27	Karuppada nnapalam	Thrsr	76° 12'E	10° 15'N	12	0.1165	2.46± 0.09	1.20± 0.05**	3.66± 0.13
Bm28	SN beach	Thrsr	76° 10'E	10° 16'N	9	0.1196	1.72± 0.09	0.83± 0.03	2.55± 0.09
Bm29	Undai kadavu	Thrsr	76° 10'E	10° 13'N	13	0.1062	2.08± 0.06	0.80± 0.04	2.88± 0.08
Bm30	Koda kara	Thrsr	76° 18'E	10° 22'N	17	0.1054	2.18± 0.08	0.91± 0.04**	3.09± 0.12
Bm31	Pattambi	Plkd	76° 11'E	10° 48'N	35	0.1154	2.68± 0.07	0.73± 0.04	3.41± 0.11

Bm32	Kam bram	Plkd	76° 11'E	10° 53'N	97	0.1283	2.45± 0.11	0.77± 0.04	3.22± 0.13
Bm33	Pala kkad	Plkd	76° 39'E	10° 47'N	78	0.1238	2.00± 0.06	0.63± 0.06	2.63± 0.11
Bm34	Vara kulam	Mlpm	76° 17'E	10° 20'N	36	0.1159	3.33± 0.03**	1.23± 0.06**	4.56± 0.05**
Bm35	Para vanna	Mlpm	75° 53'E	10° 54'N	11	0.1212	2.17± 0.09	1.04± 0.06**	3.22± 0.14
Bm36	Kannoor kettu	Kzhd	75° 46'E	11° 27'N	15	0.1322	2.48± 0.14	0.89± 0.03*	3.36± 0.14
Bm37	Musan kandi	Kzhd	75° 43'E	11° 21'N	10	0.1097	2.78± 0.13	0.91± 0.08**	3.69± 0.14
Bm38	Kak koor	Kzhd	75° 49'E	11° 22'N	20	0.0910	2.72± 0.09	0.65± 0.05	3.38± 0.12
Bm39	11th mile	Kzhd	75° 50'E	11° 22'N	17	0.1956	2.65± 0.11	0.62± 0.01	3.26± 0.11
Bm40	Chela pram	Kzhd	75° 47'E	11° 20'N	10	0.1114	4.11± 0.08**	0.79± 0.07	4.91± 0.14**
Bm41	Mani cherryhills	Kzhd	75° 51'E	11° 30'N	478	0.1099	3.42± 0.14*	0.98± 0.04**	4.40± 0.18**
Bm42	Payya nnur	Kannur	75° 11'E	12° 5'N	10	0.1126	4.19± 0.14**	0.79± 0.10	4.98± 0.11**
Bm43	Muttu katti	Kannur	75° 15'E	12° 0'N	12	0.1112	3.68± 0.08**	0.86± 0.04	4.54± 0.10**
Bm44	Kakkad	Kannur	75° 23'E	11° 53'N	20	0.0890	3.51± 0.12**	0.83± 0.05	4.34± 0.10**
Bm45	Anda lloorkavu	Kannur	75° 28'E	11° 47'N	11	0.1112	3.37± 0.11**	0.76± 0.02	4.13± 0.11**
Bm46	Sasi mala	Wynd	76° 11'E	11° 48'N	761	0.1009	2.74± 0.06	0.31± 0.05	3.06± 0.07
Bm47	Channo thukolli	Wynd	76° 12'E	11° 49'N	728	0.0930	3.09± 0.15	0.71± 0.01	3.80± 0.15*
Bm48	Seetha mount	Wynd	76° 12'E	11° 50'N	752	0.1023	2.74± 0.06	0.48± 0.04	3.22± 0.04

Bm49	Nada vayal	Wynd	76° 7'E	11° 44'N	797	0.1014	2.68± 0.26	0.60± 0.02	3.28± 0.28
Bm50	Meppadi	Wynd	76° 13'E	11° 55'N	870	0.1170	4.69± 0.34**	0.89± 0.04*	5.58± 0.35**
Bm51	Padanna kkadu	Ksrgd	75° 6'E	12° 15'N	13	0.1251	2.85± 0.13	0.81± 0.04	3.66± 0.15
Bm52	Kumbla	Ksrgd	74° 56'E	12° 35'N	9	0.1148	5.57± 0.22**	1.17± 0.05**	6.74± 0.25**
Bm53	Edayi lekkadu	Ksrgd	75° 9'E	12° 8'N	10	0.1043	2.53± 0.17	0.83± 0.03	3.36± 0.20
Bm54	Mada kkara	Ksrgd	75° 7'E	12° 13'N	14	0.0911	1.31± 0.04	0.61± 0.03	1.92± 0.04
Bm55	Manakka kadavu	Ktym	76° 24'E	9° 48'N	8	0.0979	1.64± 0.08	0.63± 0.04	2.27± 0.11
Bm56	Channi kkadavu	Alpz	76° 18'E	9° 51'N	6	0.1177	2.29± 0.13	0.60± 0.03	2.89± 0.12
Bm57	Pedikattu thuruthu	Ekm	76° 20'E	10° 58'N	8	0.0855	2.23± 0.06	0.72± 0.04	2.95± 0.04
Bm58	Cheriya thuruthu	Ekm	76° 14'E	10° 3'N	10	0.0967	2.69± 0.14	0.70± 0.02	3.39± 0.15
Bm59	Chun gam	Alpz	76° 20'E	9° 29'N	8	0.0958	1.96± 0.06	0.74± 0.01	2.70± 0.07
Bm60	Kina noor	Ksrgd	75° 10'E	12° 16'N	15	0.1071	3.19± 0.09	0.86± 0.05	4.05± 0.09**

** Significant at $p < 0.01$; *Significant at $p < 0.05$

Morphological, Cytological, Palynological, Phytochemical, and Molecular characterization of the species revealed that the plant shows wide variations in the gene pool.

Morphology

Detailed morphological characterization, based on 15 qualitative and 25 quantitative characters was carried out.

Variations in the qualitative characters were assessed visually, and their character states were identified. Concerning the quantitative characters, the character states were delimited based on the property of normal distribution as Low, Medium, and High. The mean, Standard Deviation, Range, and Critical Difference of the characters were computed. Based on the disposition of primary branches, the accessions were grouped into three habit types – prostrate, sub-erect, and erect. The traits such as node-centric anthocyanin pigmentation, corolla throat colour, corolla base colour, and style colour showed four character states each. Eight characters showed three character states each, and the two traits (corolla lobe type and capsule shape) exhibited only two character states each. Among the 25 quantitative characters, except one (long stamen length) all the other characters showed considerable variation, and estimated the magnitude of their variation in the gene-pool of the species.

The genetic parameters such as the Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Correlation between characters, Heritability and Genetic gain of eight agro botanic characters, and Selection indices of the 60 genotypes were estimated. Estimation of GCV and PCV values revealed the considerable influence of the environment on most of the characters. Six pairs of traits showed a highly significant positive correlation (at 1% level) and one character pair (internode length and biomass yield) exhibited a negative correlation at 5% level. Heritability and Genetic gain studies showed that bacoside % is highly heritable and it possesses high genetic gain (Cinthya et al, 2016). Divergence analysis of the 60 accessions grouped them into 12 clusters (Cinthya et al, 2018). and hierarchical cluster analysis has grouped the accessions into 10 clusters at a 33.8% similarity level (Christopher et al, 2019).

Anatomy: The stem anatomical characteristics such as (i) Shape of CS of the stem (ii) No. of cortical layers in broad area and

short area (iii) Diameter of the broad area and short area (iv) Stelar diameter (v) Phloem thickness (vi) No. of xylem strands and (vii) No. of vessels/strands were studied in the sixty accessions and leaf anatomical characters analyzed were (i) stomatal types (ii) frequency of their occurrence, (iii) stomatal index, (iv) vein pattern, and (v) trichome types. The study revealed that the accessions varied from one another with regard to these features.

Cytology: Mitotic and Meiotic studies on 30 accessions were carried out and two cytotypes (2n= 64 and 2n= 68) were identified in the species. Meiosis was normal in all the accessions with regular bivalent formation and normal anaphase separation, which is suggestive of the allotetraploid origin of the species. In the absence of major chromosomal variations in the accessions, except the presence of two cytotypes, the intraspecific phenotypic differences of the accessions observed in the species, could be attributed to point mutations (Cinthya et al, 2014).

Palynology: LM studies were carried out in the 60 accessions and SEM in 8 accessions. The study showed consistency in pollen features, in general, but certain accessions exhibited considerable variation, especially concerning the shape of the grains and exine ornamentation pattern.

Phytochemistry

Medicinal plants show therapeutic properties due to the presence of active principles. Bacopa monnieri shows its nootropic activity due to the marker compounds like bacoside A and Bacopaside I. HPTLC densitometric analysis of the sixty accessions for screening the bioactive compounds revealed that, bacoside A ranged from 1.31 (Bm 54) to 5.57 %, w/w (Bm 52) and bacopaside I 0.30 (Bm 2) to 1.46 %, w/w (Bm 9). The total content of both the bioactive constituents (bacoside A + bacopaside

l) ranged from 1.92 (Bm 54) to 6.75 %, w/w (Bm 52). Three showed high total percentage content of bacosides (Bm 11 - 6.75 %, w/w; Bm 50 - 5.58 %, w/w; Bm 52 - 6.74 %, w/w), the same accessions also registered high percentage content of bacoside A. These accessions were those collected from Pattaniyidukku (Alappuzha), Meppadi (Wayand), and Kumbala (Kasaragode) respectively which were the most affected districts of Kerala in the flood calamity. These estimates of total bacosides content are the highest ever reported in the species. Considering the presence of high total bacoside content, these accessions can be considered elite genotypes, which merit prospects of their multiplication and cultivation on a large scale for pharmaceutical/ industrial purposes (Cinthya et al, 2017).

Molecular Taxonomy

Genetic diversity studies (ISSR) have been carried out in the 60 accessions of the species using ISSR. Sixteen primers were used for the analysis. The study showed that all the accessions vary from each other, and an estimated 31.81% polymorphism revealed moderate genetic diversity in the germplasm of B. monnieri occurring in Kerala. The sixty accessions were clustered into three major groups concerning their genetic similarity. This may be due to the vegetative mode of reproduction predominantly occurring in the species, alongside with sexual reproduction.

There is a possibility that the members of the population of this accession may be decreased or lost during the natural calamity which Kerala faced recently. But the collection and conservation of the accessions from the diverse agroclimatic conditions of Kerala and its proper maintenance in the Field Gene Bank of JNTBGRI, make sure that the genetic diversity of the species is protected.

Conclusion

The whole gamut of biosystematic studies undertaken in the work revealed the magnitude and pattern of variation of the characteristics in the 60 accessions of Bacopa monnieri. The information obtained through the present investigation will be useful as it reveals the patterns of gene flow within and between populations and its effects on reproductive and demographic processes, to assess its impact on population viability. The data generated on variability in the species enable a host of applications in the conservation, cultivation, and genetic improvement of the species and effective utilization of its germplasm in the pharmaceutical industry. Global environmental change leading to natural calamities poses a threat to most of the potential medicinal species of Kerala. The need of the time is to conduct this strategic method as a model for the conservation of genotypes of other potent medicinal plants.

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