

Evaluation of *Eclipta alba* L. selections for higher biomass and bioactive compound

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Abstract

Known by several names, including Bhringaraj and False Daisy, *Eclipta alba* (L.) is a highly valued and well-known medicinal plant in India. It is thought to be the best liver disease medicine and is an annual herb that belongs to the *Asteraceae* family. This herb has a long history of use in ethnomedicine. An estimated 2000–5000 tons are traded annually. On the other hand, overexploitation of this species' wild populations is the result of strong domestic demand. In order to cultivate *E. alba* and lessen the strain on the wild population, the current study aims to identify elite germplasm from several places in India through an analysis of growth, yield, and content variation of primary active constituents. HPLC (High-Performance Liquid Chromatography) was used to analyze the wedelolactone content of 142 wild accessions of *E. alba*. Six lines of analysis showed a significant variance in wedelolactone throughout the entire plant, ranging from 0.09 to 0.53% on a dry weight basis. These lines were evaluated for two years for growth, yield, and active principle content. One elite accession (IIHR Sel EA 43) with 0.5% of the wedelolactone was identified with higher growth and yield attributes. This line is found to be suitable for large-scale commercial cultivation of *E. alba*.

1. Introduction

Eclipta alba (L.) is an annual herbaceous plant that is a member of the *Asteraceae* family. It is also referred to as Bhangara in Hindi, Bhiringaraj in Sanskrit, and False daisy in English (Puri, 2003). It is extensively available in Brazil, Thailand, China, and India (Soni and Soni, 2017). It can also be found in the southeast, where it thrives in low-lying and high-lying regions of Indonesia, Sri Lanka, the Philippines, Nepal, and Malaysia, as well as in clay and moist ground bunds, rice fields, water courses, and tanks. The therapeutic plant is found growing in marshes, rivers, lakes, and the foothills of the Himalayas across India. It can also be found growing up to 2000 meters above sea level in the hills. It is prevalent along roadsides, in waste areas, marshy areas, hedges, and especially in the country's tropical regions (Sharma *et al.*, 2017).

Because herbal medications have fewer negative effects, demand and usage have skyrocketed in recent years. The *E. alba* is widely utilized in Ayurveda and has a bitter, hot, harsh and dry flavor. The secondary metabolites found in *E. alba* are useful medicinally. Pharmacological activities have been reported for *E. alba* extracts and metabolites (Mithun *et al.*, 2011). Wedelolactone and dimethyl wedelolactone are the primary active ingredients in *E. alba* and have anti-hepatotoxic properties (Wagner *et al.*, 1986 and Franca *et al.*, 1995). It has long been believed to be the best medication for treating liver conditions (Khare, 2007). It is also used to treat headaches, night blindness, and conditions affecting the growth and development of hair (Datta *et al.*, 2009). *E. alba* has yielded a diverse array of chemical substances, such as coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes, and their glycosides. The herb's anti-inflammatory, anti-mutotoxic, analgesic, anti-hepatotoxic, anti-oxidant, anti-bacterial, anti-hyperglycemic, anti-hemorrhagic, & immunomodulatory qualities have all been used (Leal *et al.*, 2000; Kartik *et al.*, 2007) (Jayathirtha and Mishra, 2004). It is regarded as a potent rejuvenator and is well-known for its anti-aging and UV protection properties (Chan *et al.*, 2014). Recent studies showed an antivenom property of bioactive compounds of *E. alba* (Diogo *et al.*, 2009).

E. alba is one of the top ten traded medicinal plants in India. The estimated annual trade is 2000–5000 tonnes (<https://nmpb.nic.in/>). The increased demand for this herb is leading to the destruction of the natural habitat since the plant material is gathered from natural & wild settings. The indiscriminate harvesting of plants as a source of drugs is endangering these plants, hence large-scale, systematic plant cultivation is needed. For cultivation, superior and elite varieties are essential and there are no commercial varieties in *E. alba*. At ICAR-Indian Institute of Horticultural Research, about 142 wild collections from different parts of the country are maintained and evaluated for yield and bioactive compounds to identify elite lines in *E. alba* for commercial cultivation. The present study's main objective was to identify elite lines of *E. alba* with high biomass and wedelolactone content from the selected accessions with higher content.

2. Materials & methods

2.1. Study site details

The experimental farm of the ICAR-Indian Institute of Horticultural Research (IIHR) in Bengaluru, which is situated 890 meters above mean sea level at 13°7' North latitude and 77° 29' East longitude, is where the current study was carried out between 2020 and 2022. The mean minimum temperature fluctuated between 13.2°C and 21.3°C during the course of the experiment, whereas the mean maximum temperature varied between 26.2°C and 34.9°C. The total amount of rainfall during the study ranged from 302 to 311 mm. At the experimental site, the red sandy loam soil exhibits a stable level of fertility. The pre-experimental soil characteristics showed that the pH, EC, and SOC were, respectively, 7.26, 0.24 dS m⁻¹, and 0.65%.

2.2. Experimental details

About 142 wild collections from different parts of the country are maintained and evaluated for yield and wedelolactone content. Five lines showed a significant variance in wedelolactone throughout the entire plant, ranging from 0.09 to 0.53% on a dry weight basis. These lines were evaluated for two years for growth, yield and active principle content. The field trial was carried out with five selected lines from germplasm along with one local type as a check (IIHR EA 4). The studies were set up in a roughly 30-m² area using an RBD: Randomized Block Design having 4 replications. After two ploughings, raised beds measuring six meters in length and eighty meters in breadth were constructed. There was enough room between treatments and duplicates—30 cm. For planting material production of different lines, well-matured selfed seeds from selected lines were collected previously. The seeds were sown in 50 cells trays filled with coir pith (@ approximately 2 kg/tray). The trays were irrigated immediately with a rose can and regular irrigation was given. 25–30 days old healthy and uniform seedlings were transplanted to the main field.

One-month old healthy seedlings, which were raised in the nursery, were planted on raised beds at a spacing of 20 cm x 20 cm in triangular pattern in gross plot area of 1.2 m² area for each treatment and the net plot area with 21 plants were considered for all estimations. All treatments were produced using the same standard procedures. During the land preparation process, 15 t ha⁻¹ of farm yard manure was applied and thoroughly mixed with the soil. The complete P & K dose was utilized as a basis, as per the recommended amount of fertilizers (30: 40: 20 kg N, P₂O₅, & K₂O ha⁻¹) administered. 30 days after transplanting, around 75% of the N was administered, and 50 days after transplanting, the remaining 25% of the N was applied. For N, P, and K, the fertilizers utilized were urea, single super phosphate & muriate of potash, in that order. Weeding was done on a regular basis and irrigation was provided daily through a drip system during the moisture stress period. The diseased downy mildew caused by *Plasmoparas phagneticolae* was first reported in India and spraying of Dimethomorph fungicide @ 1% (Acrobat) was taken up to manage the disease.

2.3 Morphological traits

Data pertaining to growth traits viz., plant height, plant spread, internodal length, stem circumference, leaf length, leaf width, number of primary and secondary branches, flower head diameter, and pedicel length were recorded in five randomly tagged plants at harvest. Qualitative data was taken using the RHS (Royal Horticulture Society) color chart.

2.4 Biomass and yield estimation

Plant samples were harvested at 90 days after planting and the fresh weight was recorded immediately after harvest in five tagged plants. The entire plant was then shade-dried, sealed in brown paper bags, and oven dried at 50°C until its weight remained constant (ref), yielding its dry weight. The fresh & dry herbage yield per hectare was estimated based on the herbage yield per plot. The samples were broken up and kept in butter-filled paper bags so that the biologically active substances could be examined later.

2.5. Reagents & chemicals used to estimate bioactive compounds

The “chemicals and reagents used for the estimation of bioactive compounds through HPLC analysis are methanol (CH₃OH, Merck 99.9% purity), acetonitrile (C₂H₃N, Merck 99.9% purity), orthophosphoric acid (H₃PO₄, Sisco Research Laboratories, 99% purity), and” anhydrous potassium dihydrogen phosphate (KH₂PO₄). The HPLC analysis uses wedelolactone (Natural remedies with 99.9% purity) as the reference standard.

2.6. Estimation of bioactive compounds

The concentration of active chemicals in *E. alba* was estimated using standard procedures. About 250 g of the dried leaf sample from each line were crushed using a matrix mixer. After that, the powder was collected and stored in butter paper bags. 40 milliliters of HPLC-grade methanol were added to a 100-milliliter beaker that held five grams of powdered leaf sample. Following twenty minutes of constant stirring at 90°C in a water bath, the mixture was collected in a volumetric flask to collect the supernatant. The supernatant was collected after the residue was extracted again for ten minutes with thirty milliliters of HPLC-grade methanol. After adding 20 milliliters of methanol of HPLC grade once more, the mixture was extracted for 10 minutes. Until a clear solution was reached, the same process was repeated. The material was extracted three times in total. After cooling the extract, a volumetric flask containing HPLC-grade methanol was filled to the final volume of 50 ml. For HPLC analysis, a Millipore filter was used to filter the extract with a 0.2 mm mesh size.

Wedelolactone, an active molecule, was subjected to phytochemical examination using the HPLC approach (Shimadzu LC model Nexera X2 HPLC/UPLC fitted with LC-30 CE pump with SPD-M20A photodiode array detector PDA), in accordance with the earlier published protocol by Anil et al. (2021). Version 5.54 SP 5 of the Lab Solutions Chromatography software was used to process data and control the instruments. Chromatographic separation was accomplished at 40°C using a 5 µm particle size column on a 250 mm length by 4.6 mm ID CAPCELL PAK C18 (SHISEIDO) column. The mobile phase in terms of chromatographic conditions was composed of acetonitrile (Solvent B) & buffer (1 mM KH₂PO₄), which were pumped at a flow rate of 1.5 ml/min. Before being injected into the system, the mobile phases

were filtered via a 0.45 µm nylon membrane filter and degassed using a sonicator. To achieve ideal separation and acceptable peak shape, gradient elution was used. Since there was little to no solvent influence on the target compounds' absorbance maxima, the detector wavelength was set at 351 nm because this is the wavelength at which the target compounds showed their maximum absorbance. A prepared sample of approximately 20 microliters (µL) was injected, and a 45-minute run time was used to record the chromatogram at 351 nm for each injection. The chromatogram and calibration curve with the peaks of the reference standards visible are shown in Fig. 1.

2.7. Screening for Downy mildew disease

Selected lines along with check were evaluated against downy mildew caused by *Plasmoparas phagneticolae* under field conditions for two consecutive years. One infected leaf was collected from each of the three plants for microscopic examination and pathogen identification. For the assessment of sporulation intensity on a leaf, a scale ranging from 0–5 is used and modified from Lebeda (1986a, 1987).

2.8. Statistical analysis

Statistical software such as SPSS and Microsoft Excel was used to analyze the experimental data, and tables containing the standard error of the mean (S.Em.)—a measure of the deviation from the mean value were produced. At the 0.05 probability level, crucial difference (CD) values were used to compare the significance of the method of treatment.

3. Results & discussion

3.1. Growthperformance of different E. alba lines

Table 1 displays the data on growth factors, such as plant height and plant spread, for various years. Both years saw roughly identical plant development in terms of height and dispersion along distinct lines.However, the plant height and spreadvaried significantly among different lines in both years. The plant vigor in terms of height (82.9 cm) and spread (63.85 cm in NS and 69.1 cm in EW) was significantly higher in accession IIHR EA Sel 43 in 1st yr. A comparable result was obtained in 2nd yr. IIHR EA Sel 43 registered maximum plant height and spread (74.38 cm, and 63.85 cm in NS and 65.6 cm in EW, respectively) followed by IIHR EA 58 – 1. The plant height and spread were significantly lower in controllIIHR EA-4 (30.5 cm of height, 29.25 cm spread in NS, 30.4 cm in EW). The branching pattern in terms of internodal length, stem circumference, and number of primary and secondary branches at harvest in different years(Table 2) indicatedsignificant variations among different accessions. The accession IIHR EA Sel 43 was significantly superior in terms of intermodal length, stem diameter, and number of secondary branches over other accessions in both years.

Table 1
Plant growth in different lines of *E. alba*

Line	2020-21		2021-22			
	Plant height (cm)	Plant spread (cm)		Plant height (cm)	Plant spread (cm)	
		NS	EW		NS	EW
IIHR EA 58 – 1	57.6	51.8	47.8	55.2	50.9	53.5
IIHR EA 59 – 2	54.6	65.0	58.9	48.1	53.8	56.9
IIHR EA-4 (C)	30.5	29.3	30.4	45.7	27.6	30.4
IIHR EA-6	32.2	30.4	29.2	41.0	28.3	38.2
IIHR EA Sel 43	82.9	63.9	69.1	74.4	63.8	65.6
S.Em±	1.43	2.42	0.92	1.17	2.14	0.93
C.D. (<i>P</i> = 0.05)	4.40	7.44	2.82	3.61	6.59	2.87

Table 2
Branching parameters in different lines of *E. alba*

Line	2020-21				2021-22			
	Internodal length (cm)	Stem diameter (cm)	No. of primary branches/plant	No. of secondary branches/plant	Internodal length (cm)	Stem diameter (cm)	No. of primary branches/plant	No. of secondary branches/plant
IIHR EA 58 – 1	5.69	3.31	1.65	15.45	5.59	3.38	1.72	15.23
IIHR EA 59 – 2	5.75	2.2	2.58	16.85	5.36	2.29	2.70	14.70
IIHR EA-4 (C)	2.95	2.71	1.49	15.1	3.64	2.84	1.58	16.10
IIHR EA-6	2.53	2.33	1.44	16.3	2.66	2.33	1.45	16.30
IIHR EA Sel 43	7.94	3.66	1.47	17.7	6.33	3.79	1.52	17.70
S.Em±	0.1	0.07	0.04	0.47	0.07	0.09	0.07	0.63
C.D. ($P = 0.05$)	0.31	0.21	0.14	1.45	0.21	0.28	0.23	1.95

The data on leaf and flower parameters viz., leaf length, leaf width, flower head diameter, and peduncle length (Table 3a) indicated significant variability among accessions in both years without any definite trend among identified lines. The accession IIHR EA Sel 43 was superior in leaf length and peduncle length. The accession IIHR EA-4 (control) registered minimum values for leaf and flower parameters. The erect plant habit in IIHR EA Sel 43 (Table 3b) has an added advantage for easy harvesting and profuse flowering contributed to higher seed yield. In flowering plants, intraspecific differences in morphological features are not uncommon. *Eclipta* genotypes differed in morphology, as Gupta (1977) has previously reported. Since variations within a species have the same number of chromosomes, the variability can be explained by genetic factors. According to Atlay *et al.* (2019), there could be a genetic makeup difference, external climate conditions, or a combination of the two reasons for the discrepancies between the accessions.

Table 3
a Leaf and flower quality parameters in different lines of *E. alba*

Line	2020-21				2021-22			
	Leaf length (cm)	Leaf width (cm)	Flower head diameter (cm)	Peduncle length (cm)	Leaf length (cm)	Leaf width (cm)	Flower head diameter (cm)	Peduncle length (cm)
IIHR EA 58 – 1	6.98	1.77	0.96	2.20	6.16	1.80	0.99	2.33
IIHR EA 59 – 2	4.42	1.01	0.86	1.69	4.26	0.97	0.88	1.61
IIHR EA-4 (C)	3.30	0.70	0.57	0.22	3.53	0.76	0.57	0.22
IIHR EA-6	7.30	1.39	0.63	0.23	6.70	1.48	0.68	0.24
IIHR EA Sel 43	8.03	1.55	0.86	3.05	8.08	1.59	0.84	3.03
S.Em±	0.17	0.06	0.04	0.08	0.13	0.03	0.02	0.04
C.D. ($P = 0.05$)	0.52	0.19	0.11	0.25	0.39	0.08	0.06	0.11

Table 3
b Qualitative traits in different lines of *E. alba*

Lines	Plant habit	Leaf colour	Terminal shoot colour	Basal stem colour	Leaf pubescence	Leaf thickness	Leaf margin serration	No. of flowers/plant	No. of seeds/capitulum	Flowering habit
IIHR EA 58 – 1	Semi-erect	Green (N 137 A)	Purple (N 77 A)	Purple	High	Thick	Highly serrated	Moderate	High	Moderate
IIHR EA 59 – 2	Prostrate	Green (138 A)	Grey brown (199 A)	Greenish white	Medium	Thin	Moderately serrated	Less	Moderate	Moderate
IIHR EA 4	Prostrate	Green (137 C)	Brown (200 D)	Greenish white	Medium	Thin	Moderately serrated	Moderate	Moderate	Moderate
IIHR EA 6	Semi erect	Green (137 B)	Grey Brown (N199 B)	Brown	Medium	Thin	Highly serrated	Moderate	Moderate	Sparse
IIHR Sel 43	Erect	Yellow Green (146 A)	Grey Brown (N199 A)	Green	Medium-high	Thick	Highly serrated	High	High	Profuse

3.2. Fresh and dry biomass accumulation

Accessions had a significant effect on fresh as well as dry biomass production per plant during 2020-21 and 2021-22 (Table 4). The accession IIHR EA Sel 43 accumulated higher fresh biomass as well as dry biomass per plant. This can be ascribed to this accession's improved growth performance in terms of height, spread, leaf length, and quantity of secondary branches. With respect to per-hectare yield levels (Table 5), a similar trend was observed as that of per-plant yield level. The accession IIHR EA Sel 43 produced 6.25 t ha⁻¹ more dry biomass in the first year than the control IIHR EA-4 (2.5 t ha⁻¹). Similar biomass production trends were seen in the second year in relation to the lines (6.39 t ha⁻¹ in IIHR EA Sel 43 and 2.31 t ha⁻¹ in IIHR EA-4).

Table 4
Biomass yield as influenced by different lines in both years

Line	Fresh biomass (g plant ⁻¹)			Dry biomass (g plant ⁻¹)		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
IIHR EA58-1	198.0	208.0	203.0	32.50	34.00	33.25
IIHR EA59-2	161.0	156.3	158.6	26.00	27.50	26.75
IIHR EA-4 (C)	130.0	124.0	127.0	23.00	20.75	21.88
IIHR EA-6	180.0	185.0	182.5	29.50	30.25	29.88
IIHR EA Sel 43	268.0	275.8	271.9	56.25	57.50	56.88
S.Em±	2.82	2.57	2.35	0.98	1.52	0.92
C.D. (<i>P</i> = 0.05)	8.79	7.92	7.26	3.03	4.7	2.84

Table 5
Dry biomass yield (t ha⁻¹) as influenced by different lines

Line	2020-21	2021-22	Pooled
IIHR EA58-1	3.61	3.78	3.69
IIHR EA59-2	2.89	3.06	2.98
IIHR EA-4 (C)	2.56	2.31	2.44
IIHR EA-6	3.28	3.36	3.32
IIHR EASel 43	6.25	6.39	6.32
S.Em±	0.107	0.159	0.129
C.D. (<i>P</i> = 0.05)	0.334	0.490	0.397

The pooled data of 2-yr dry biomass yield showed that the yield increase in IIHR EA Sel 43 accession was 2.63–3.88 t ha⁻¹ over other accessions (Table 5). It implies that there was a considerable yield increase in IIHR EA Sel 43 accession by 1.7 to 2.6 times over other accessions clearly indicating its superiority over other lines. This is mainly due to higher fresh and dry biomass yield per plant in accession IIHR EA Sel43 and particularly due to superior control in terms of plant height, branch count, spread, internodal length, and breadth and length of leaves. Interactions between a genotype and the environment have a significant impact on the biomass production in medicinal plants. These findings are consistent with the reports of Ravi (2019), Rohini and Smitha (2022) on *Centella asiatica*; Venugopal et al. (2021) in ashwagandha; Ibrahim et al. (2013) in *Ocimum* spp. & Aharizadet al. (2012) in patchouli.

3.3. Bioactive compound

The quantitative estimation of wedelolactone concentration by HPLC revealed significant variation in germplasm lines (Table 6). Wedelolactone content was significantly higher in IIHR EA Sel 43 (0.5%) followed by IIHR EA 58 – 1 (0.25%). This is the highest content reported so far. The lines IIHR EA58-1 (0.25%) and IIHR EA59-2 (0.22%) registered higher wedelolactone concentration compared to IIHA EA-4 (0.14%) and IIHA EA-6 (0.11%). Kaur (2011) investigated wedelolactone variation in *Eclipta alba* accessions and found that the mean value of the accumulated wedelolactone varied quantitatively between different accessions, ranging from as low as 0.111 mg g⁻¹–1.43 mg g⁻¹ in dry weight. EPBG2 has the highest wedelolactone concentration (0.0143%). Differences in the genetic composition of the components utilized, the cropping season, the time of harvest, and location can all contribute to variations in the content of different accessions (Sharma et al., 2009). Pandey and Mandal (2010) reported variability in andrographolide content in dried leaves of kalmegh collected from five locations. Variations in active principles content among the accessions were additionally revealed by Archana et al. (2013) & Pandey et al. (2019) for andrographolide content in kalmegh; Suganthi et al. (2018) for total alkaloid content in *mako*; Gupta et al. (2017) and Othman et al. (2018) for stevia. Genotype-to-genotype variation for root yield and alkaloid content was observed in Ashwagandha by Venugopal et al. (2022).

Table 6
Concentration and yield of wedelolactone as influenced by different lines

Line	Wedelolactone content (%)			Wedelolactone yield (kg ha ⁻¹)		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
IIHR EA58-1	0.233	0.259	0.25	8.43	9.805	9.12
IIHR EA59-2	0.211	0.226	0.22	6.26	6.883	6.57
IIHR EA-4 (C)	0.122	0.156	0.14	3.13	3.586	3.36
IIHR EA-6	0.090	0.133	0.11	2.94	4.476	3.71
IIHR EA Sel 43	0.530	0.501	0.52	33.14	31.980	32.56
S.Em±	0.007	0.003	0.004	0.447	0.502	0.452
C.D. (<i>P</i> = 0.05)	0.021	0.01	0.0124	1.392	1.564	1.401

3.4. Yield of bioactive compound

The yields of bioactive compounds varied significantly. Different lines of *E. alba* had a significant effect on wedelolactone yield (Table 6). The wedelolactone yield was significantly higher in IIHR EA Sel 43 during 2020-21 and 2021-22 (33.1 and 31.9 kg ha⁻¹, respectively) compared to control IIHA EA-4 (3.1 and 3.5 kg ha⁻¹, respectively). As in IIHR EA Sel 43, this is caused by increased content and biomass accumulation. In therapeutic plants, quantitative variation in a variety of phytoconstituents has frequently been observed. This diversity might be continuous or discrete, coded by many or few genes in conjunction with inputs from the environment, and result from the synthesis and accumulation of different biochemicals (Mallet, 1996). Owing to the fact that these accessions were cultivated in identical climatic and edaphic circumstances, the observed diversity may have a genetic basis.

3.5.Screening for downy mildew disease

Microscopic observation revealed downy growth on the abaxial surface of leaves with numerous sporangia. The mycelium was hyaline, aseptate, and branched, 5–15 µm in diameter. Sporangiophores were slender with monopodial branching at right angles and with a slightly bulbous base. The sporangia were hyaline, smooth, globose to ellipsoidal with apical papilla and basal hilum on three sterigma, measuring 18–30 × 14–20 µm (Fig. 2). Based on these characteristics, the pathogen was identified as *Plasmoparas phagneticolae* (Davis et al., 2020; Wang et al., 2021). This is the first report of *P. sphagneticolae* in India and the first record of this pathogen causing downy mildew on bhringraj (Sonavane et al., 2023)

Screening of lines for downy mildew incidence showed only IIHR EA Sel 43 exhibited resistance and IIHR EA58-1 exhibited moderate resistance in both consecutive years. The percent disease incidence (PDI) and disease reaction of the lines are given in Table 7. This confirms the resistance reaction shown by the line IIHR EA Sel 43.

Table 7
Evaluation of bhringraj germplasm against downy mildew caused by *Plasmoparas phagneticolae* under field conditions

Lines	PDI 2021	PDI 2022	Disease reaction
IIHR EA 58 – 1	34.15 (35.76)	39.62 (39.01)	MS
IIHR EA 59 – 2	58.46 (49.90)	53.00 (46.74)	S
IIHR EA 4 (C)	86.49 (68.47)	79.33 (62.99)	HS
IIHR EA 6	83.14 (65.79)	75.33 (60.25)	HS
IIHR EA Sel 43	07.44 (15.84)	9.00 (17.47)	R
Figures in parenthesis are Arcsine transformed values			
0 = no visible Sporulation - Immune			
1 = ≤ 10% of leaf surface is covered with sporangiophores with spores; Resistant			
2 = ≤ 25% of leaf surface is covered with sporangiophores with spores; moderately Resistant			
3 = > 25 - ≤ 50% of leaf surface is covered with sporangiophores with spores; Moderately Susceptible			
4 = > 50 - ≤ 75% of leaf surface is covered with sporangiophores with spores; Susceptible			
5 = > 5 % of leaf surface is covered with sporangiophores with spores; Highly Susceptible			

4. Conclusion

Wedelolactone yield is a critical characteristic of *Eclipta alba* for farmers. It is necessary, therefore, to suggest to farmers that improved cultivars with increased wedelolactone yield and adaptable to different settings. The wedelolactone yield trait includes fresh and dried leaf weight as well as growth characteristics. In IIHR, Bengaluru, we assessed the biomass yield and active content in five distinct genotypes for our study. To get a variety of origins, these genotypes were collected from several sites. The superiority of IIHR EA Sel 43 in terms of yield, bioactive chemicals, and disease resistance in both years makes it evident from the current study that it is an exceptional accession. Hence this accession can be recommended for cultivation since there were no identified/released varieties in this crop.

Declarations

Declarations of interest

None

Conflict of interests

No conflicts of interest are disclosed by the writers.

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Author Contribution

K. Hima Bindu: Funding acquisition, Project administration, Conceptualization, design of the work, supervision, data analysis, Critical review and editing V. Keshav Rao: Methodology and analysis of bioactive compound Shivakumar: Experimentation, data acquisition, Project administration Anil Kumar: Methodology, bioactive analysis and data acquisition R. M. Shalini: investigation, Writing and editing Prithi Sonavane: Screening for disease reaction, data acquisition, editing, K. S. Divya: Methodology, chemical analysis and data acquisition S. Sujatha: Review & editing

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Figures

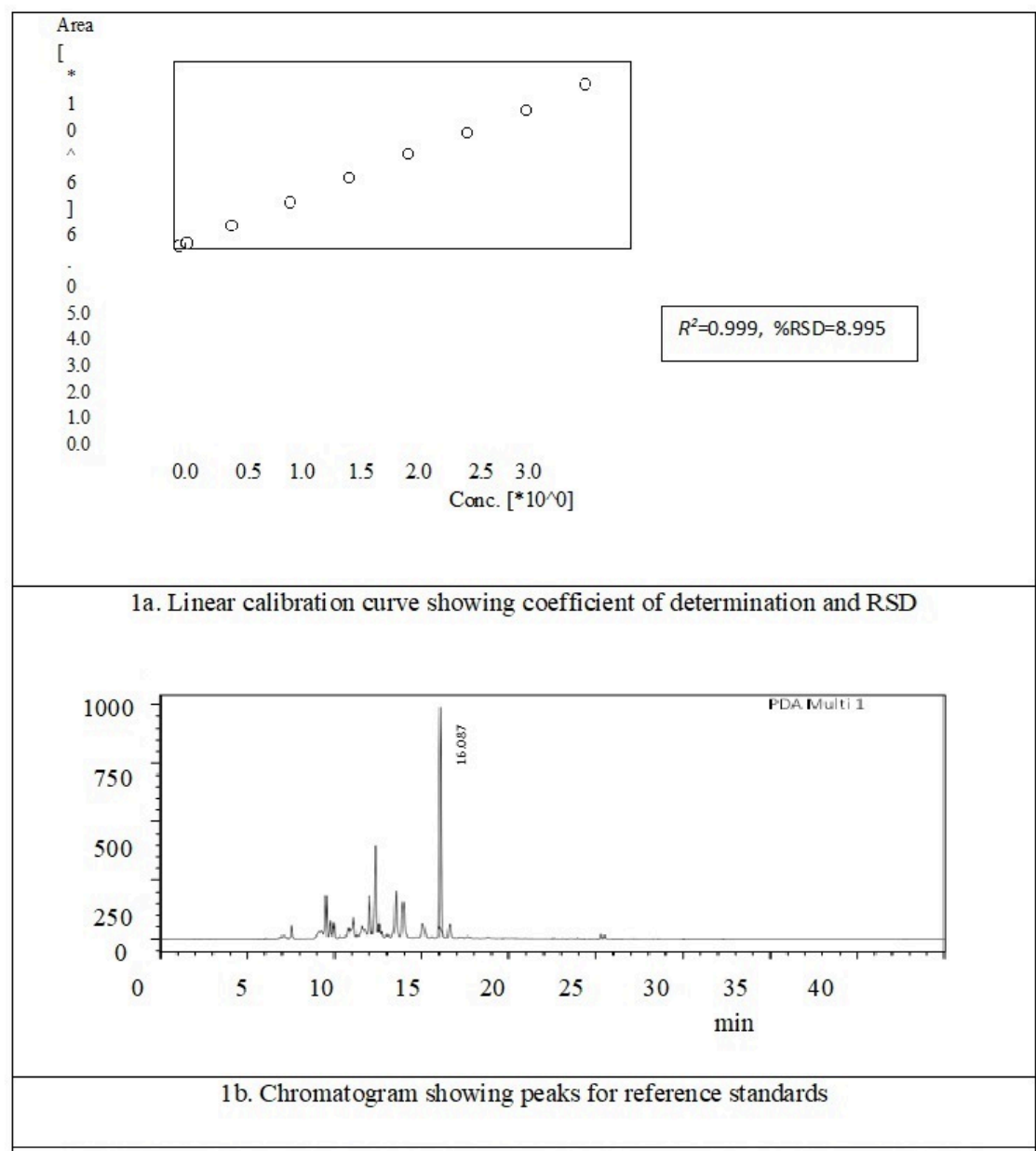


Figure 1

Calibration curve and chromatogram for the reference standard in *Eclipta alba*

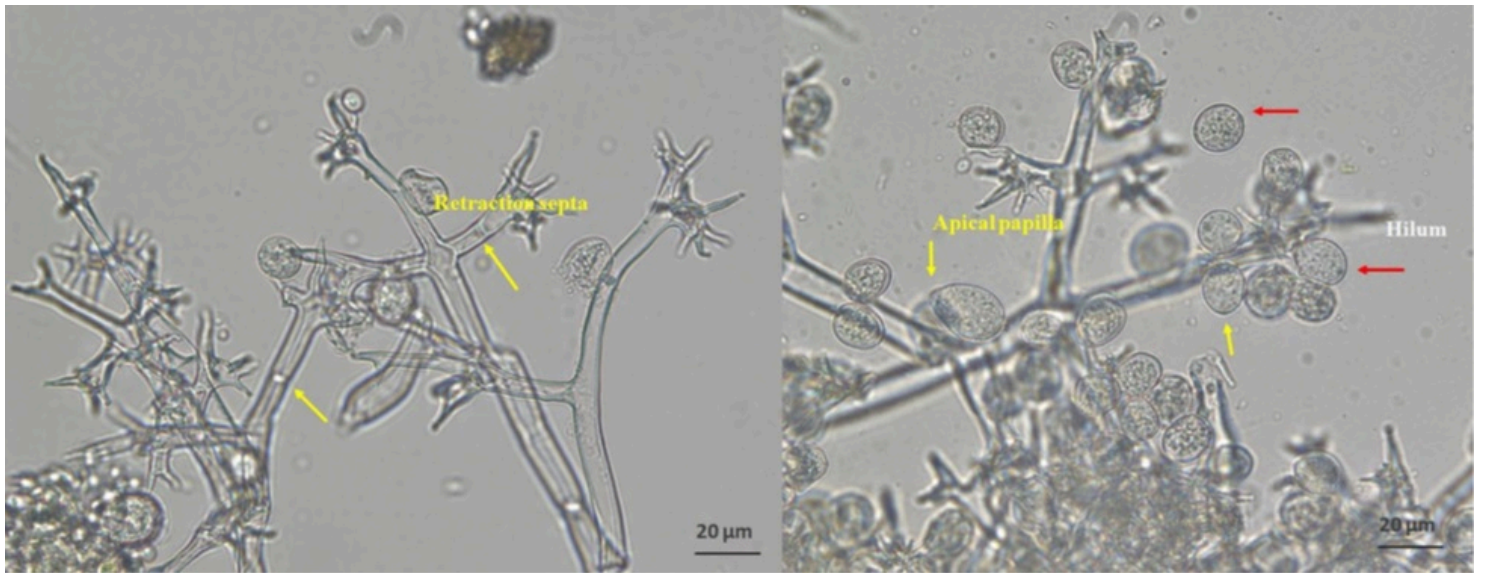


Figure 2

Sporangia and sporangiophores of *Plasmopara sphagneticolae* on bhringraj