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Short Communication

Enhanced metabolite yield with compensatory biomass reduction revealed by moisture stress induction in *Centella asiatica* (L.)

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ABSTRACT

The exposure to any kind of stress tends to accelerate the secondary metabolism in medicinal plants increasing the production of secondary metabolites. The present investigation was undertaken to study the effect of moisture stress (100, 75, 50, 25 and 10% pot capacity) and control (as without plant) on growth, yield and metabolite content of *Centella asiatica* var. 'Arka Prabhavi', for two growing seasons under polyhouse conditions. Results revealed that moisture stress treatments had a significant effect on all observed growth and yield traits. Plants maintained at 100% PC exhibited luxurious vegetative growth with maximum leaf length (6.28 cm), leaf breadth (8.14 cm), petiole length (22.32 cm) and fresh biomass yield (164 g/pot). Cumulative water transpired and water use efficiency of the plants was also observed to be maximum at 100% PC. In contrary to biomass yield, increased asiaticoside (1.864%, 1.892%), madecassoside (2.856%, 3.382%) and total triterpenoid content (5.356%, 5.578%) at higher moisture stress levels of 75% and 50% PC, respectively, was observed. Hence, it is appropriate to grow *Centella* either at 100% or 75% PC to get optimum biomass and metabolite yield on a commercial scale.

Keywords: *Centella asiatica*, moisture stress, water use efficiency, triterpenoid content

INTRODUCTION

Centella asiatica (L.) Urban, commonly known as *Gotu Kola*, is a prominent herb in traditional and modern systems of medicine used for its memory boosting, antioxidant, anti-ageing, and skin care properties. Pharmacological activities of *Centella asiatica* are attributed to the presence of a wide array of bioactive compounds namely asiaticoside, madecassoside, asiatic acid and madecassic acid (Puttarak & Panichayupakaranant, 2012). Plants grown under water limiting environments experience stress which will aggravate the secondary metabolism leading to accumulation of more active substances for combating the stress and its related effects (Albergaria et al., 2020).

Centella asiatica is presently in great demand, farmers and industries are interested to cultivate it under resource limiting environments to get the best quality raw material with a higher bioactive yield. The present study was carried out to investigate the impact of moisture stress induction on the biomass and metabolite yield of *Centella asiatica* var. 'Arka Prabhavi'. This will help to understand the stress-induced metabolic responses in *Centella asiatica* which can have implications for sustainable cultivation practices offering insights into optimizing its bioactive compound yield while conserving resources.

The experiment was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, India during November, 2021 to November, 2022 covering two growing seasons *viz.*, November, 2021 to May, 2022 and from June, 2022 to November, 2022. Two weeks old rooted cuttings of *Centella asiatica* var. Arka Prabhavi were transplanted into 12-inch pots filled with soil and FYM in 2:1 ratio, during November, 2021. Different levels of moisture stress *viz.*, 100%, 75%, 50%, 25%, and 10% of pot capacity were applied in randomized block design, inside the polyhouse, wherein ten plants for each treatment were planted. In each treatment, a set of control pots without plants was maintained to assess direct soil evaporation. The whole experiment was repeated in the second growing season (June, 2022 to November, 2022) and average values from both seasons were analyzed and interpreted.

To impose moisture stress, initially, dry weight of the pot and soil was taken, then all the pots were set to 100% pot capacity by saturating the pot soil with excess water and top of the pot was covered to avoid evaporation. When the gravitational water seized, the net weight of the pot and soil was taken again to calculate the weight at 100% PC. Accordingly, the weight of pots at 75%, 50%, 25% and 10% pot capacity were calculated using the formula:

Weight of pots at specific pot capacity= [(Weight at 100% PC- Dry weight of pot and soil) x required pot capacity/100] + Dry weight of pot and soil

Moisture stress was imposed by gradually decreasing the water content in the pots by regular weighing and replenishing the amount water lost by the pots irrespective of evapo-transpiration to arrive to desired pot capacity of soil. The pots were monitored and watered daily to maintain the specific pot water capacity. The position of the pots was changed weekly on rotation basis to remove any effects of pot position on the treatments. Once, the pots attained to the required levels of pot capacity, they were maintained in that pot capacity for a period of four months. At 120 days after transplanting, observations were recorded on morphological traits, biomass yield and secondary metabolite content. Morphological traits like leaf length, leaf breadth and petiole length were recorded on five mature leaves from each plant. Fresh and dry biomass yield was calculated on per plant basis and expressed as grams/pot. Dry biomass yield was recorded after shade drying the herbage to 10% moisture content.

Total water added to each pot to attain the specific pot capacity over the entire experimental period was added to calculate the cumulative water added. Cumulative water transpired (CWT) from each pot was calculated by subtracting the total water added to the pots without plants from the cumulative water added to pots with plants. Water use efficiency (WUE) is the amount of dry biomass produced by plant per unit amount of water consumed. WUE is calculated as per the below formula:

WUE= Total dry matter (TDM) accumulated / CWT during the experimental period

Secondary metabolite analysis for the bioactives under different moisture stress conditions was estimated using HPLC technique standardized for *Centella asiatica* (Rohini & Smitha, 2022). The standards for analysis were procured from Natural Remedies, Bengaluru. The leaves were sampled from individual pots from all the treatments for the analysis.

The experimental data was analyzed using ANOVA by using statistical software WASP 2.0. The results were presented at 5% level of significance (P = 0.05) and the critical difference (CD) values were calculated to compare the various treatment means.

The results showed that there is a significant difference in the morphological traits or vegetative traits of *Centella asiatica* when it is exposed to different moisture stress conditions. Leaf length and leaf width showed maximum values at 100% pot capacity (6.28 cm and 8.14 cm, respectively), followed by 75% and 50% pot capacity. It was interesting to note that plants didn't survive beyond 3 days at 25% PC and 10% PC indicating that the plants are water loving and can't survive under moisture limiting environments. Hence, all the observed parameters had "null values" under 25% PC and 10% PC (Fig. 1).

Fresh biomass yield varied significantly between different moisture stress levels with the highest yield under 100% PC (164 g/pot) and least yield under 50% PC (55.6 g/pot). Similarly, dry biomass yield was also highest under 100% PC (32.34 g/pot) and least under 50% PC (11.18 g/pot) (Table 1). The results have shown that plants maintained at 100% PC has maximum CWT that means over the period of four months the plant has transpired 13.47 litres which was

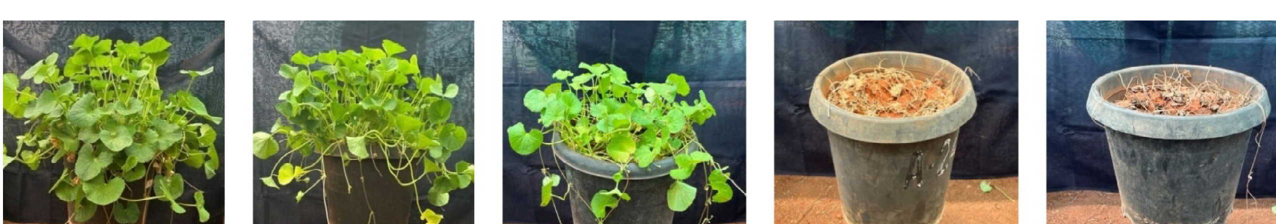

Fig. 1 : Growth of *Centella asiatica* plants 120 days after transplanting at different pot capacities (PCs)

Table 1 : Growth and yield traits of *Centella asiatica* under different moisture stress levels

Pot capacity	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Fresh Biomass (g/pot)	Dry biomass (g/pot)	CWT (litre)	WUE (g/l)
100%	6.28[a]	8.14[a]	22.32[a]	164.00[a]	32.34[a]	13.47[a]	2.41[a]
75%	4.86[b]	6.90[b]	21.78[a]	114.20[b]	24.74[b]	11.87[a]	2.07[ab]
50%	4.48[c]	2.70[c]	18.56[b]	55.60[c]	11.18[c]	6.81[b]	1.65[b]
25%	0.00[d]	0.00[d]	0.00[c]	0.00[d]	0.00[d]	0.00[c]	0.00[c]
10%	0.00[d]	0.00[d]	0.00[c]	0.00[d]	0.00[d]	0.00[c]	0.00[c]
CD @ 0.05	0.308	0.443	1.276	25.890	5.944	2.064	0.445
CV (%)	7.466	9.458	7.718	14.250	14.900	8.920	8.880

Significant difference between treatments indicated by different alphabets (P = 0.05); CWT: cumulative water transpired; WUE: water use efficiency

significantly on par with plants maintained at 75% PC. Plants maintained at 100% PC has reported the highest water use efficiency (2.410 g/l) in biomass production whereas the plant at 50% PC has shown the least water use efficiency (1.650 g/l) in biomass production. Comparison of all the morphological and yield traits showed that at 100% pot capacity, *Centella asiatica* has performed well (Table 1).

Analysis of the secondary metabolites showed significant difference between the moisture stress treatments. The highest values for madecassoside (3.382%), asiaticoside (1.892%) and total bioactive content (5.578%) were obtained at 50% PC. Plants maintained at 100% PC showed the least values for all the active components except madecassic acid (Table 2). The observations on metabolite yield contrasted with biomass yield at different water stress levels showing an inverse relation between the bioactive component and biomass yield. HPLC chromatogram of the standards and the sample Arka Prabhavi at various pot capacities is shown in the Fig. 2. The yield of total triterpenoids per unit area was calculated and it was found that maximum yield of bioactives with 100% PC (1.318 g/plant) and 75% PC (1.218 g/plant) which were significantly on par with each other.

Exposure to any kind of stress (biotic or abiotic) tends to accelerate the secondary metabolism in medicinal plants altering the physiological and biochemical characteristics (Anjum et al., 2011), hence, increasing the production of secondary metabolites (Ramakrishna & Ravishankar, 2011). The results shown that plants maintained below 50% PC has not survived beyond a period of 3 days indicating that it must be cultivated with assured irrigation. The plants exposed to 100% PC exhibited a luxurious vegetative growth with enhanced leaf traits and biomass yield. Devkota & Jha (2011) reported that *Centella* plants maintained at

Table 2 : Secondary metabolite yield in *Centella asiatica* under different moisture stress levels

Pot capacity	Madecassoside (%)	Asiaticoside (%)	Madecassic acid (%)	Asiatic acid (%)	Total terpenoid (% w/w)	Yield of total terpenoids (g/plant)
100%	2.050[b]	1.202[b]	0.338[a]	0.158[ab]	3.746[b]	1.218[a]
75%	2.856[ab]	1.864[a]	0.400[a]	0.234[a]	5.356[ab]	1.318[a]
50%	3.382[a]	1.892[a]	0.206[ab]	0.100[bc]	5.578[a]	0.564[b]
25%	0.000[c]	0.000[c]	0.000[b]	0.000[c]	0.000[c]	0.000[c]
10%	0.000[c]	0.000c	0.000[b]	0.000[c]	0.000[c]	0.000[c]
CD @ 0.05	0.291	0.500	0.126	0.065	1.718	0.325
CV (%)	16.06	10.26	11.48	6.33	15.25	9.25

Significant difference between treatments indicated by different alphabets (P = 0.05)

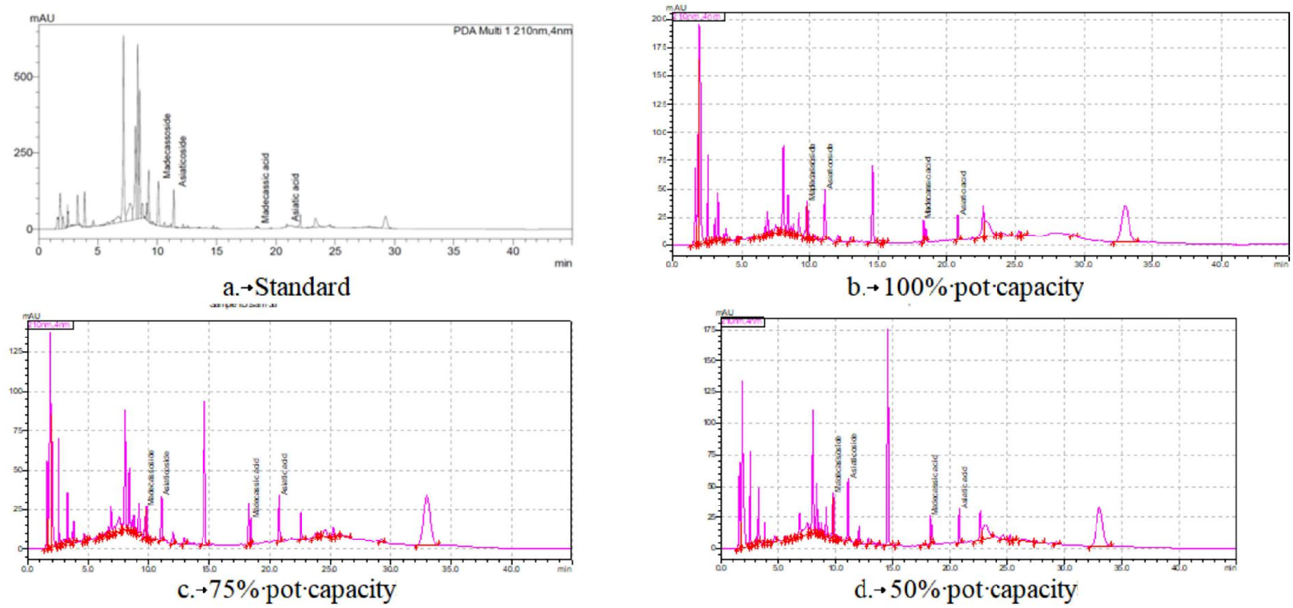

Fig. 2 : HPLC Chromatogram of *Centella asiatica* samples

100% pot water capacity exhibited maximum growth and yield. Further reduction in water availability induced destructive effect on plant growth and biomass production as it is evident from the longer and broader leaves with long petiole at 100% PC, whereas, smaller leaves with short petiole at 50% PC. Water stress significantly reduced the plant growth development as measured by biomass reduction, plant height and branching in Japanese mint (Misra & Srivastava, 2000). Reduction in dry biomass yield due to drought stress was also reported in *Oscimum* species and *Origanum vulgare (*Khalid, 2006; Khalil et al., 2010; Said-Al Ahl et al., 2009).

The present study showed that plants maintained at 100% PC had maximum water use efficiency indicating that *Centella* is a highly water loving crops ensuring the highest yield under 100% PC and optimum yield under 75% PC.

Further, exposing *Centella asiatica* to moisture stress has enhanced the yield of most of the active metabolites. Thus, *Centella asiatica* needs to be cultivated under assured irrigated condition in order to get the optimum yield of biomass as well as secondary metabolites.

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