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Research Article

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Seasonal Variation in Morphological, Pigment and Nitrogen Metabolism of *Acalypha indica L.*

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ABSTRACT
The present study examines the influence of seasonal variations on the morphological, physiological and biochemical characteristics of *Acalypha indica L.,* a herbaceous plant with recognized therapeutic value. Comparative analysis was performed during winter and summer seasons to examine changes in plant morphology, photosynthetic pigment and nitrogen metabolism. Winter plants exhibited longer roots (15.7 ± 2.0 cm) and shoots (57.0 ± 8.0 cm), whereas summer plants had significantly higher leaf number (226.7 ± 9.3), biomass (34.86 ± 4.3 g), and larger leaf area (27.74 ± 3.2 cm²). Chlorophyll a, chlorophyll b, and carotenoids were higher in winter (1.24 ± 0.10, 1.06 ± 0.08, and 1.40 ± 0.05 mg/g FW, respectively) than in summer (1.02 ± 0.04, 0.88 ± 0.03, and 0.88 ± 0.02 mg/g FW), indicating enhanced pigment accumulation under cooler conditions. Conversely, nitrate reductase activity and total nitrogen content were higher in summer (0.835 ± 0.04 OD at 540 nm; 0.496 ± 0.02 OD at 440 nm) than in winter (0.414 ± 0.03; 0.316 ± 0.05), reflecting increased nitrogen assimilation during warmer conditions. These results indicate a clear seasonal variation in pigment synthesis and nitrogen assimilation, with cooler conditions favoring pigment accumulation and warmer conditions enhancing nitrogen metabolism. The findings provide preliminary insights into how environmental factors affect the physiological performance of *A. indica*, potentially influencing its medicinal properties.
Keywords: *Acalypha indica,* Morphological, Nitrogen Metabolism, Photosynthetic pigments

INTRODUCTION

Acalypha indica L., commonly referred to as Indian copperleaf, is widely occurring herbaceous plant species belonging to the family Euphorbiaceae. It has been traditionally used in Ayurveda, siddha and Unani medicine for treating various respiratory ailments, gastro intestinal disorders, skin infections, etc., throughout various regions of Asia and Africa (Ogbeide, 2025).

The pharmacological activities of *A. indica* are attributed to the presence of diverse secondary metabolites like alkaloids, tannins, flavonoids and terpenoids (Teli *et. al,* 2025; John and Rekha, 2011).

In addition to its pharmacological significance, the physiology of *A. indica* is strongly influenced by seasonal and environmental conditions. Changes in seasons can alter photosynthetic pigment levels, leaf morphology, and nitrogen metabolism in many tropical herbs (Singh and Verma, 2018). Chlorophylls and carotenoids play a pivotal role in photosynthesis, light harvesting and stress adaptation. Chlorophylls, particularly chlorophyll a and chlorophyll b are the primary pigments responsible for capturing light energy, whereas carotenoids play a protective role by quenching reactive oxygen species (ROS) and preventing damage to chlorophyll (Morais *et. al,* 2022; Taiz *et. al,* 2015). Their relative levels are strongly influenced by factors such as temperature, photoperiod, light intensity and water availability. Nitrogen metabolism also plays a vital role in determining the physiological status of the plant. Nitrate reductase (NR) activity and total nitrogen content of the plant indicates the efficiency of nitrogen assimilation which plays a vital role in protein synthesis and plant growth (Sinha *et. al,* 2016). Seasonal changes have a strong effect on plant's growth and function, due to which, it becomes important to study how *A. indica* modulates its growth and physiology in winter compared to summer.

Understanding how these environmental factors influence *A. indica's* morphology, physiology and biochemistry, is important for identifying optimal harvesting seasons, for medicinal and pharmacological purpose (Krishnan *et. al,* 2000).

MATERIALS AND METHODS

Sample collection

Acalypha indica plants were collected from Botanical Garden of DDU Gorakhpur University, Gorakhpur during winter (December) and Summer (June). Three independent plants per season were selected at a similar developmental stage, and all measurements were performed in triplicate (n = 3).

Root and shoot lengths were measured with the help of measuring scale, leaf number was counted manually and leaf area was determined by using a leaf area meter. Fresh biomass was determined immediately after uprooting the plant. Values are presented as mean ± SD and compared statistically using t-test ($p < 0.05$).

Fig. 1. Seasonal variation in Acalypha indica: (A) Winter specimen showing reduced shoot biomass, fewer leaves, and smaller leaf area; (B) Summer specimen exhibiting greater vegetative growth, larger leaves, and higher biomass.

Pigment Estimation:

Leaf pigments were estimated by Arnon (Arnon, 1949) method.

50 mg of fresh leaf samples were collected and homogenized in 80% acetone by using a motor and pestle.

The homogenate was centrifuged at 6000rpm for 10minutes, and the supernatant was collected. The absorbance was measured by using UV-Vis spectrophotometer available in the departmental laboratory at three different wavelengths:

663 nm for chlorophyll a

645 nm for chlorophyll b

440 nm for carotenoids

Pigment content was calculated using:

$$\text{Chla (mg/g FW)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645} \times V/W}{1000}$$

$$\text{Chl b (mg/g FW)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663} \times V/W}{1000}$$

$$\text{Carotenoids (mg/g FW)} = \frac{(1000 \times A_{440} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}) \times V/W}{198}$$

Where V = volume of extract (mL) and W = weight of leaf tissue (g).

Biochemical Assays:

Nitrate reductase activity:

Nitrate reductase activity was estimated by Jaworski (Jaworski, 1971) method.

200mg of fresh leaves were treated with KNO3 for about 30minutes.

Thin strips of leaves were dipped into the mixture (4 ml buffer, 0.5 ml KNO_3, and 0.5 ml of 5% n-propanol) for 30 minutes.

Took Optical Density (OD) at 540 nm of 1ml extract added in 1 mg of n-napthyl diamine hydrochloride and 1ml of sulphonyl amide.

Total Nitrogen Content:

Total nitrogen content was estimated by micro-Kjeldahl method (Kjeldahl, 1883). OD was measured at 440 nm.

RESULTS AND DISCUSSION

Morphological Comparison:

Winter plants exhibited longer roots (15.7 ± 2.0 cm) and shoots (57.0 ± 8.0 cm) as compared to summer plants (8.3 ± 1.5; 51.0 ± 2.5, respectively). On the other hand, summer plants developed significantly higher leaf number (226.7 ± 9.3*) and fresh biomass (34.86 ± 4.3) compared to winter (26.0 ± 2.0 leaves; 4.93 ± 0.8g). Summer leaves also displayed a wider range of leaf area than winter leaves.

Table 1. Root length, Shoot length, Number of leaves, Fresh weight, largest leaf area and smallest leaf area

	Winter plant	Summer plant
Root length (cm)	15.7 ± 2.0	8.3 ± 1.5*
Shoot length (cm)	57.0 ± 8.0	51.0 ± 2.5*
No. of leaves	26.0 ± 2.0	226.7 ± 9.3*
Fresh weight (g)	4.93 ± 0.8	34.86 ± 4.3*
Largest leaf area (cm^2)	12.0 ± 2.0	27.74 ± 3.2*
Smallest leaf area (cm^2)	1.8 ± 0.3	0.44 ± 0.08*

*Significant difference compared to winter, $p < 0.05$

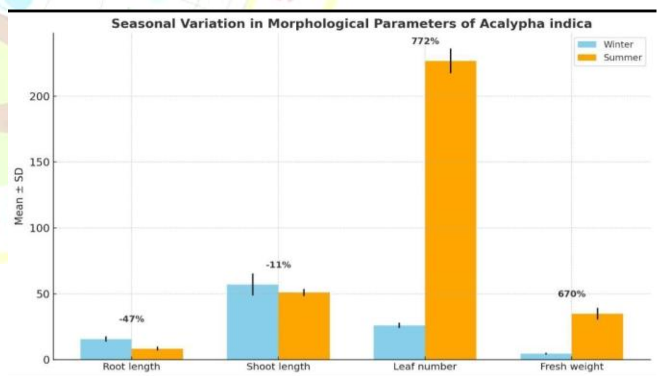

Fig. 2. Comparative Pigment and Biochemical Content of *Acalypha indica*

Pigment Estimation:

Pigment content was higher in winter compared to summer (Table 2).

Table 2. Pigment content of A. indica leaves (mg/g FW, mean ± SD, n = 3)

	Winter	Summer
Chlorophyll a (663 nm)	1.24 ± 0.10	1.02 ± 0.04*
Chlorophyll b (645 nm)	1.06 ± 0.08	0.88 ± 0.03*
Carotenoids (440 nm)	1.40 ± 0.05	0.88 ± 0.02*

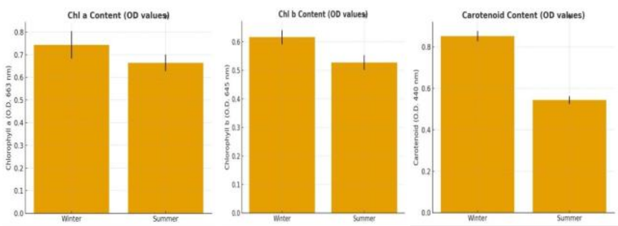

Fig. 3. Comparative Pigment Content in *Acalypha indica* (chlorophyll a, chlorophyll b, and carotenoid).

Biochemical Assays:

Nitrate Reductase Activity:

The Optical Density (OD) at 540 nm was significantly higher in summer plants (0.835 ± 0.04*) than in winter plants (0.414 ± 0.03), indicating enhanced nitrate reduction capacity during the summer season.

Total nitrogen content:

Total nitrogen content, estimated at 440 nm, also exhibited seasonal variation. Summer plants recorded a higher OD (0.496 ± 0.02*) compared to winter plants (0.316 ± 0.05), indicating greater nitrogen accumulation during the summer season.

Table 3. Biochemical parameters of A. indica (mean ± SD, n = 3)

Parameters	Winter	Summer
Nitrate reductase OD (540 nm)	0.414 ± 0.03	0.835 ± 0.04*
Total nitrogen OD (440 nm)	0.316 ± 0.05	0.496 ± 0.02*

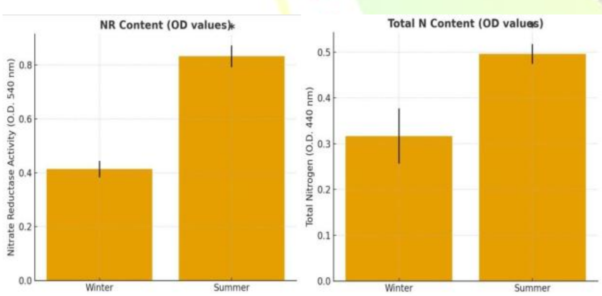

Fig. 4. Seasonal comparison of biochemical parameters in Acalypha indica showing nitrate reductase activity and total nitrogen content (OD values).

The results demonstrate clear seasonal plasticity in A. indica. Winter plants favored elongated root and shoot growth with higher pigment accumulation, which shows adaptations for efficient light harvesting under shorter day length and lower temperature (Sharma *et. al,* 2020). On the other hand, summer plants prioritized leaf proliferation and biomass accumulation, potentially as a strategy to maximize photosynthetic surface area under intense light and higher temperature (Singh and Verma, 2018). Higher chlorophyll and carotenoid levels in winter align with previous findings that cooler conditions stabilize pigment-protein complexes and enhance photoprotection (Taiz *et.al,* 2015). On the other hand, enhanced nitrate reductase activity and nitrogen content during summer suggest that nitrogen assimilation is more active under higher metabolic demand, supporting rapid biomass accumulation (Sinha *et.al,* 2016). Overall, winter growth supports pigment enrichment (quality), while summer growth supports biomass accumulation (quantity). These findings emphasize that the ability of A. indica to adjust growth and physiology with seasonal changes represents a key ecological adaptation contributing to its persistence as a tropical weed (Akiode *et. al,* 2021).

CONCLUSION

This comparative study shows significant seasonal differences in morphology, pigment composition, and nitrogen metabolism of A. indica. Winter plants showed higher pigment concentrations and elongated growth, while summer plants exhibited greater biomass, leaf number, and nitrogen assimilation. Such insights are valuable for guiding harvesting practices and understanding the ecological adaptability of this medicinal species.

CONFLICT OF INTEREST

The author here declares there is no conflict of interest in the publication of this article.

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