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Effect of Organic Fertilizer on Growth and Physiology of *Brachychiton populneus* (Schott& Endl) Seedlings under Drought Conditions

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ABSTRACT

Drought stress negatively impacts plant growth and agricultural productivity. This study investigates whether organic fertilizer can mitigate these effects by improving the growth and physiological responses of *Brachychiton populneus* seedlings under drought conditions. Thus, local compost added to *Brachychiton populneus* (Schott & Endl) to determine whether it could aid the growth of the seedlings during drought conditions. Two drought stress levels (D1 and D2), or 60% and 30% of the soil water holding capacities (SWHC%), were applied to the seedlings compared to the control (C0). There were two additions of local compost (C1 and C2), 2000 and 4000 ppm, respectively, in comparison to the control treatments. D1C2 (soil water holding capacity of 60% and 4000 ppm compost) was the usual treatment combination. Which resulted in the longest shoot and root lengths. By registering the maximum dry matter content in the roots and shoots, the same combination also greatly increased the biomass of the seedlings. A general decline in the relative water content appears when biomass increases. The consequences of drought stress are lessened by the addition of compost, which also improves chlorophyll production and overall plant growth. Growth and physiological parameter correlations are shown by the component plot in rotated space. The highest variance is explained by component 1, and the second largest by component 2. A positive association was seen between root length, shoot height, and total dry weight, as they clustered together. Conversely, there is a negative association observed between the components of relative water content and shoot dry weight. However, component 2 and the leaf chlorophyll content (a, b, and total) showed a significant correlation. Compost provides a sustainable solution for improving drought resilience in *Brachychiton populneus*, making them crucial for forest nurseries and reforestation efforts in arid and semi-arid regions.

1.Introduction

Brachychiton populneus (Schott & Endl.) is a genus member of the Sterculiaceae family. It is native to Eastern Australia, ranging from Eastern Victoria to Townsville forests. The tree has a slow development pattern, and the height of the mature plant ranges between 15-20 meters (Anderson, 2016). It is commonly named; Kurrajong or Bottle Tree, because of its trunk's capacity for water storage. Additionally, it protrudes a strong root system that enables the plant to withstand drought conditions (Karim *et al*., 2020). In order to regulate the water use efficiency during seedling production, nurseries and greenhouses should optimize their irrigation patterns and fertilizing techniques. In addition to monitoring water uptake and fertilizer application, frequently utilized seedlings are used to maximize the growth of seedlings. (Beeson, R.C., Jr. 2006). Drought is one of the abiotic stress factors affecting vegetation growth worldwide within forests and agriculture sectors. Irregular precipitation patterns in arid and semi-arid regions and global warming due to climate change resulted in an extensive decline in agriculture and forests (Choat *et al*., 2012; Qadir et al., 2017). The plant is called to be stressed when not enough water is present in the soil for its metabolic function and physiological processes Choat *et al*., 2012. Although *Brachychiton populneus* are generally well known for their tolerance capability to reduce drought stress. But, still, the prolonged periods of water shortage pose a threat to its life longevity. Organic farming promotes beneficial interactions with the ecosystems to ensure soil fertility (Wander, 2004). Organic fertilizers are obtained either from plant or animal matter such as plant residues, compost, and animal manure (Han *et al*., 2016). Researchers found that organic fertilizers increase soil moisture and water use efficiency. Especially those with high organic matter because it improves the soil structure and water retention by the particles. Furthermore, because organic fertilizer decomposes slowly, nutrients are released into the soil gradually and continue to stimulate plant development even during periods of water scarcity (Ye et al., 2020). Baldi et al. (2010) found that frequent compost applications to a nectarine study orchard enhanced the growth of root length and overall growth compared to the control treatment. Furthermore, integrating organic fertilization and irrigation strategies studied by Dimkpa et al. (2020) showed that the best water-efficient practices were ensured by using organic fertilizer to mitigate drought impacts. Thus, the aim of the study focused on the ability of *Brachychiton populneus* (Schott & Endl.) to tolerate the limited water conditions by adding local organic fertilizers. And determining the physiological reactions of seedlings under water-limited conditions.

2. MATERIAL AND METHODS

Study layout and design: The study was conducted in a lath house of the College of Agricultural Engineering Sciences, Salahaddin University (Latitude: 36.1902° N, Longitude: 44.0104° E and Altitude: Approximately 426 meters above sea level) from the 1st of March 1st of November. One year old seedlings of *Brachychiton populneus* were purchased from a local nursery in Erbil city. They were planted in 4 kg pots containing loamy soil. The soil water holding capacity was calculated by saturating the soil pots with water and covered with aluminum foil to prevent the evaporation of the soil water and only the gravity water to be leached out from the soil. The pots daily weighted to the constant weight (Qadir et al., 2016). The study was laid as a factorial experiment with complete randomization of the treatments to the experimental units. The combined effect of three drought stress levels; D0; as control treatment, D1 and D2; 60 and 30 % of soil water holding capacity (SWHC). The pots weighted regularly to determine the current moisture content. If the soil loses water and the weight drops below the target, water was added to bring it back to its holding capacity. Two levels of local compost: C1; 2000 ppm and C2; 4000 ppm used compared to the control; C0. The compost was applied twice during the experiment: the first dose was applied in early March and the last in early June. Each treatment was replicated five times (3 drought stress levels * 3 levels of compost * 5 replications = 45 experimental units) as illustrated in table (1).

Table 1: Experimental treatment design matrix

D0C0R1	D0C1R1	D0C2R1	D1C0R1	D1C1R1	D1C2R1	D2C0R1	D2C2R1	D2C3R1
D0C0R2	D0C1R2	D0C2R2	D1C0R2	D1C1R2	D1C2R2	D2C0R2	D2C2R2	D2C3R2
D0C0R3	D0C1R3	D0C2R3	D1C0R3	D1C1R3	D1C2R3	D2C0R3	D2C2R3	D2C3R3
D0C0R4	D0C1R4	D0C2R4	D1C0R4	D1C1R4	D1C2R4	D2C0R4	D2C2R4	D2C3R4
D0C0R5	D0C1R5	D0C2R5	D1C0R5	D1C1R5	D1C2R5	D2C0R5	D2C2R5	D2C3R5

D: drought stress levels' C: compost application rates: and R: replicate numbers.

Local compost composition and preparation:
The local compost utilized in this study was prepared by the Agricultural Research Center, Ministry of Agriculture and Water Resources, Erbil, Kurdistan Region. The compost is composed of a mixture of 15% poultry waste and 85% wheat straw. The chemical analysis of the compost revealed that it contains 1.52 % total nitrogen (N), 0.42% phosphorus (as P_2O_5), and 0.55% potassium (as K_2O). The estimated carbon content is about 42 %. The compost has a pH of 8.3 and an electrical conductivity of 2.03 dS m^{-1}.

Data analysis: The data was analyzed using the statistical package for the social sciences (SPSS) version; 25. The analysis of variance (ANOVA) was calculated to find the general significant difference. Duncan's test was used to calculate the pair-wise comparison between each pair means of the studied characters at a significance level of 5% lath house-measured parameters and 1% for laboratory measurements (Statistics, 2013). Principal component analysis (PCA) was used to assort the similar and mostly correlated characters in identified components depending on their variance and similarity matrix (Khatun et al., 2022).

Experimental parameters: The parameters below were measured at the end of a 10-month experiment involving the watering and fertilizing of plants.

1. Morphological Characters: The shoots and root lengths (cm).
2. Biomass Characters: the seedlings were separated into shoots and roots. The dry weight was obtained by oven slow drying at 72°C until the constant weight (Paliwal et al., 1998 and Rashid et al., 2024). Shoot, root and total seedling dry weights (SDW, RDW, and TDW) in grams per plant were obtained. The roots-to-shoots ratio (R:S) is calculated by dividing the RDW/ SDW.
3. Leaf Chlorophyll Content: Chlorophyll content in the leaves extracted due to the method used by Horii et al. (2007) and Muhammad et al. (2023). 50 mg weighted from fresh leaves soaked in 30 ml of methanol (99.5 %) and kept in a dark condition for two hours. They were mixed thoroughly and centrifuged at 10000 rpm for 10 minutes to decant the precipitant and separate the supernatant. The absorbance of the supernatant was read at; 650 nm and 665 nm using a spectrophotometer (Genesys10 SUV-Vis spectrophotometer). Chlorophyll a, b and total content (µg/mL) were calculated by these equations: Chlorophyll a (µg/mL) = 16.5× A665– 8.3 × A650. Chlorophyll b (µg/mL) = 33.8 × A650– 12.5 × A665. Total chlorophyll (µg/mL) = 25.8 × A650+ 4.0 × A665.
4. Leaf Relative Water Content (LRWC): the water content in the leaves was obtained by taking mature and fully expanded leaves. The samples were kept in plastic bags in an ice box to remain fresh during transporting to the lab. Weighed immediately to obtain their fresh weight (FW). Then soaked in the water at room temperature for 24 hours to achieve turgid weight (TW). Then the dry weight (DW) of the leaves was obtained by drying the turgid leaves in oven at 70°C until constant weight (Smart and Bingham, 1974). LRWC was calculated using the formula: LRWC = [(FW - DW) / (TW - DW)].

3.RESULTS AND DISCUSSION:

The interaction of drought stress levels and compost application were significantly ($p < 0.05$)

affecting the seedling height and root length (cm) (Fig. 1). Adding 4000 ppm of compost under 60 % SWHC (D1C2) regarded as the most effective treatment resulted in recording the highest shoot; 50 cm, and longest root; 37.27 m. Studies indicate that soil amended with organic matter can retain up to 30 % more water compared to untreated soils (Kowaljow et al., 2017 and Zgallai et al., 2024). As a result, seedlings grown in compost-treated soils are better equipped to access water during prolonged dry periods. Organic fertilizers improve soil structure by increasing soil particle aggregation. That enhances the deeper penetration of roots into the soil and forms an extensive root system to ensure a high-water absorption rate. (Shaji et al., 2021). A larger and more dense root system allows the plant to contact a larger volume of the soil and uptake by the plant, which could minimize the effect of water shortage on the plant (da Silva et al., 2011). The increased plant height was observed with 4000 ppm compost application due to the high nitrogen content of compost. It plays a critical role in promoting cell division, elongation and overall shoot growth. Additionally, phosphorus primarily enhances root growth, healthy root systems can support taller plants by improving nutrient and water uptake. While, potassium, regulates various physiological processes, including water uptake and enzyme activation. It helps plants withstand stress and enhances overall growth (Taiz et al., 2015).

The same level of combination significantly affected the biomass accumulation in shoots, roots, and total seedling biomass. D1C2 led to a significant increase in dry weights of shoots, root, and total; 16.53, 16.56, and 33.09 g (Fig. 2). The increase trend in the overall growth of the plant through an increase in the length of root and shoot system as well as the biomass increase under drought conditions can be related to the high dose compost addition (4000 ppm). That directly affects the availability of nutrients in the soil, which stimulate root growth and development. That results in better behavior of the roots to form longer roots and more extensive branching systems. Better root performance ensures a better shoot length and storing most biomass in their tissues due to an increase in the photosynthesis rate. Compost application is attributed to the effects of key nutrients present in the compost. Nitrogen (N) promotes vigorous vegetative growth, leading to increased shoot biomass. Because it is a vital component of amino acids, proteins, and nucleic acids. It is crucial for cell division and elongation, which directly impacts shoot and root growth. Phosphorus is essential for energy transfer within the plant, as it is a key component of ATP (adenosine triphosphate). It also contributes to the formation of DNA, RNA, and phospholipids, which are crucial for cellular functions and development which supports both root and shoot biomass. Potassium (K) improves the efficiency of photosynthesis and enhances plant resilience to stress, leading to better growth and higher shoot biomass (Taiz et al., 2015). The balanced presence of these nutrients in the compost supports comprehensive growth, resulting in increased biomass accumulation in both shoots and roots.

The allocation of the biomass between the root and shoot system physiologically can be indicated by root-to-shoot ratio (R:S) which can be influenced by environmental stresses like the water deficiency in the soil. Depending on the data obtained the highest R:S (1:1) was recorded due to moderate water holding capacity subjected to the soil when 4000 ppm of compost (D1C2) (fig. 3). Plants distribute a greater part of their biomass to the roots than to shoots in response to drought stress. To maximize water uptake volume from the soil, this allocation approach helps the shoot system to remain hydrated and to keep optimum growth under the dry conditions in the soil (Dos Santos et al., 2022). Under normal conditions, the addition of compost may lead to a balanced growth between roots and shoots. The R:S trends may be increased when compost is added to obtain more substantial root growth to support the shoot growth and plant yield (Hale et al., 2021). The compost contains a high level of macronutrients, that supports equal growth of root and shoot. This balanced growth ratio reflects an optimal nutrient and water environment, leading to proportionate biomass accumulation in shoots and roots. This finding underscores the effectiveness of the 4000 ppm compost application in promoting balanced plant growth under controlled water conditions.

With an increase in biomass accumulation, there is a decrease in tissue water content. This is an indication of the complex relationship between plant development strategies and environmental settings. It also an inverse physiological relation between producing biomass and conserving water (Yan et al., 2023). Leaf relative water content decreased in all seedlings experiencing drought, but persisted to some extent depending on compost treatment; though relative water content was higher with 2000 and 4000 ppm compost under 90% soil water holding capacity (D0C1, D0C2), 6.6 and 6.3 respectively. The highest level of water retention was 6.9 under control (D0C0) as seen in Figure 4. The relationship is not always linear developments in relative water content resulting from increased biomass may relate to other influences (Przywara et al., 2023). There may also be a negative relationship between relative water content and biomass in the early stages of growth, as plants are quickly accumulating biomass. With increased biomass accumulation comes higher rates of transpiration and water uptake because metabolism is predominantly active in this stage of development (Yan et al., 2023). But at maturity generally, a more equilibrium relationship developed between biomass and relative water content. As drought stress or lack of water is imposed on a plant it seems that a plant may prioritize biomass production over water conservation, whereas under normal conditions, plants may choose to efficiently uptake and store water to bulk biomass (Enquist and Niklas, 2002).

The compost levels of 2000 and 4000 ppm significantly led to an increase in leaves' chlorophyll content (D2C1 and D2C2). But the highest levels of chlorophyll a, b, and total mean values were 0.24, 0.03, and 0.27 mg g-1 fresh weight, respectively under 30 % water holding capacity and 4000 ppm compost (D2C2) as shown in figure (5). Compost is a huge base of organic matter in the soil. Nitrogen as well as other nutrients; magnesium, iron, and sulfur are important for plant metabolism and chlorophyll synthesis in plants. These nutrients become most readily available (Ortiz, 2020). On the other hand, the application of compost lowers the pH of the soil which ensures nutrient uptake, supporting the plant metabolism even in stressful conditions (Rehman et al., 2023). Plants undergo various physiological responses under drought stress, such as adjustments in the hormone level, osmotic adjustment and the antioxidant defense systems activated to scavenge the ROS species. However, the added organic fertilizer increases drought tolerance in plants and might not undergo these physiological response changes, which will indirectly affect the amount of chlorophyll (Yang et al., 2021). The essential nutrients N, P and K are key elements for the chlorophyll content increase. Nitrogen is a main component of chlorophyll molecule increases the plant’s ability to capture more light. Phosphorus is a key component of ATP which is a carrier energy molecule in the light and dark reaction (Taiz et al., 20015). Composting can help reduce the effects of drought stress and enhance the growth and adaptability of *Brachychiton populneus* (Schott & Endl.) seedlings. This has been proven in several prior studies by Kammann et al. (2011), Tanure et al. (2019), Abdelrasheed et al. (2021), and Ullah et al. (2023). The result suggests that while 30 % SWHC is relatively low, the high nutrient availability from 4000 ppm compost allows plants to sustain higher chlorophyll levels, indicating effective management of both nutrients and water.

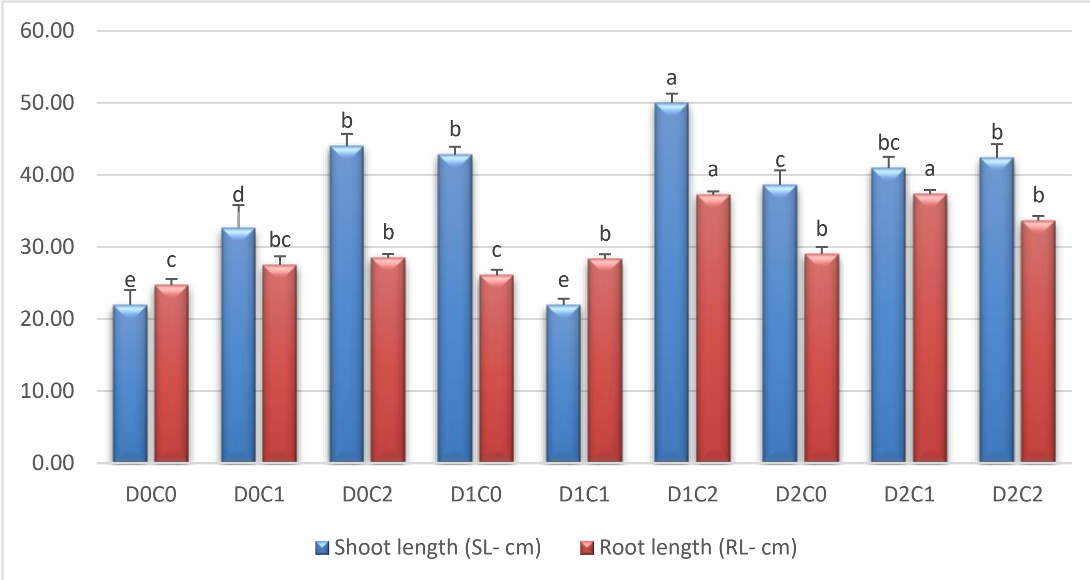

Fig. 1: Effect local compost levels on shoot and root length (cm) of *Brachychiton populneus* seedlings under drought conditions.

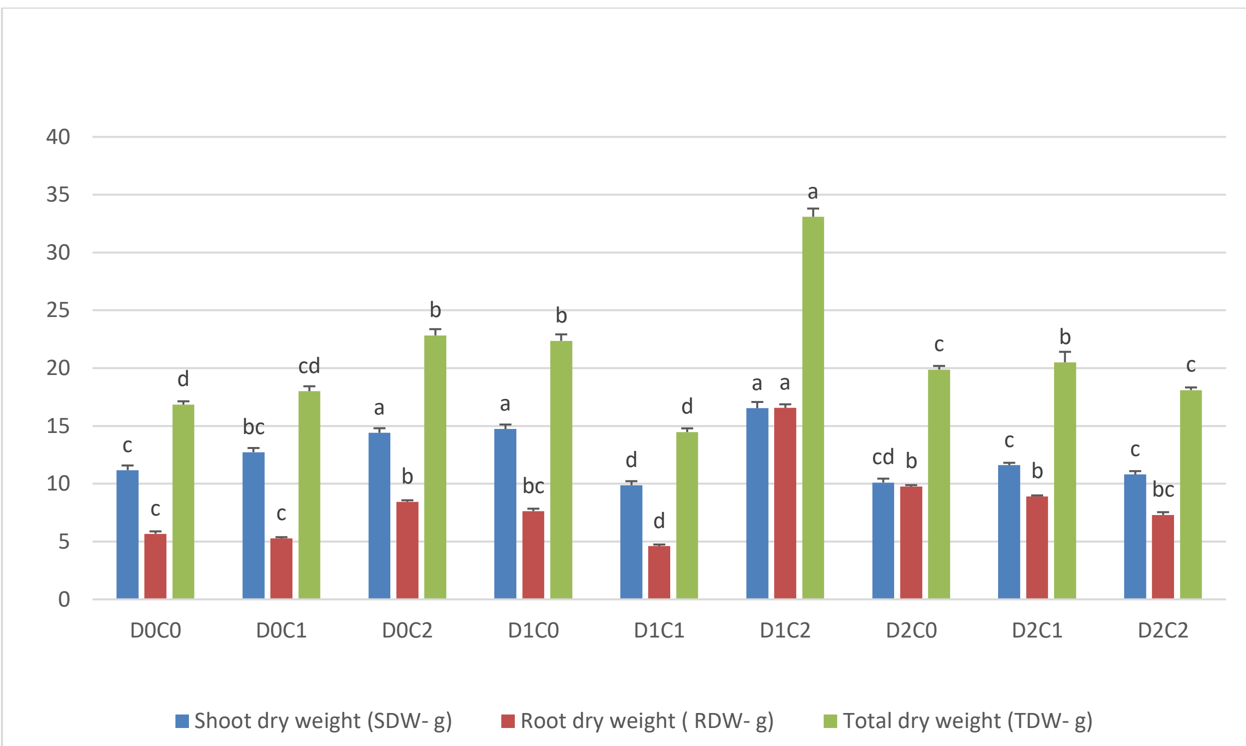

Fig. 2: Local compost effect on shoot, root and total dry weight (g) of *Brachychiton populneus* seedlings under drought conditions.

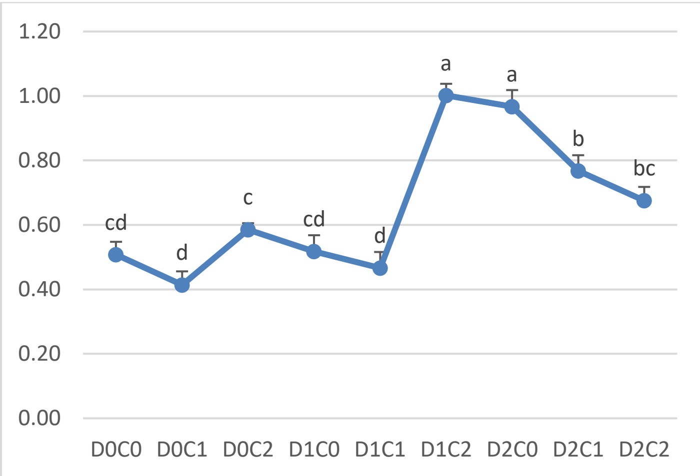

Fig. 3: Effect of local compost levels on root: shoot ratio (R:S) of *Brachychiton populneus* seedlings under drought conditions.

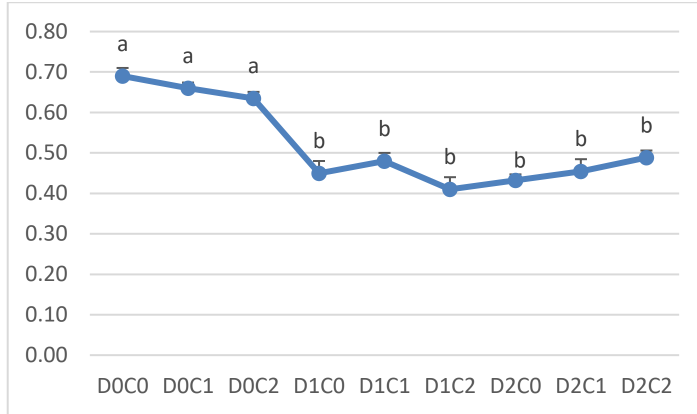

Fig. 4: Effect of local compost concentrations on relative water content (RWC) of *Brachychiton populneus* leaves under drought conditions.

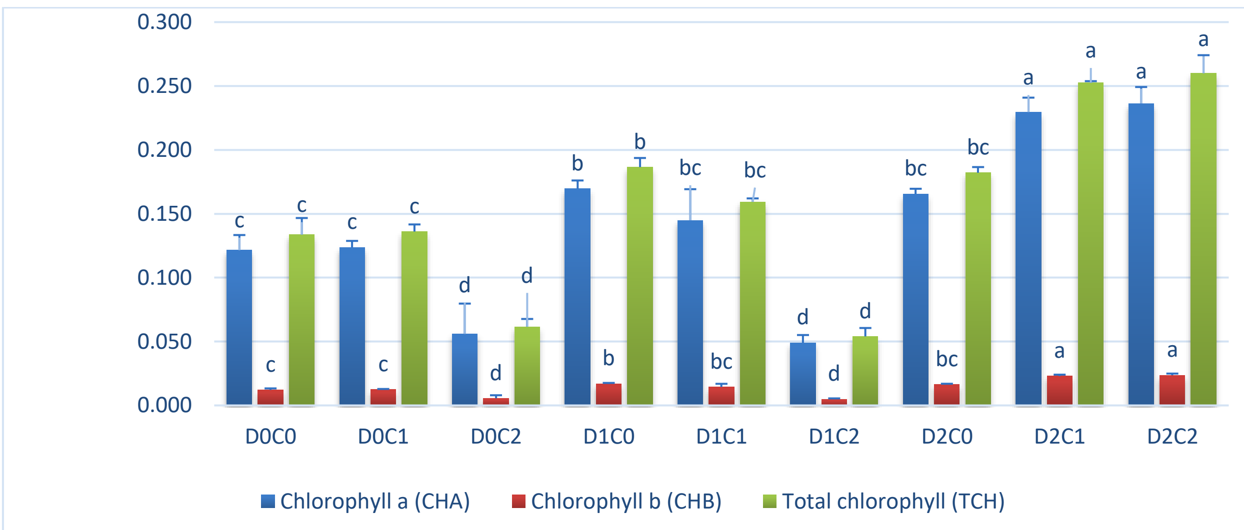

Fig. 5: The combined effect of local compost and drought stress levels on leaf chlorophyll content (mg g^{-1} fresh weight) of *Brachychiton populneus* leaves.

The principal component analysis (PCA) was used to analyze the correlation and similarity matrix among the ten morphological and physiological traits was measured in the study. Figure (6) shows that the first principal components located on the X-axis have the largest variance in the data. The second principal component is located on the Y-axis. Which has the second largest variance in the data. Relative Water Content (RWC) correlated negatively to the second component. It is a large distance from the origin, leading to its significant contribution to the component variance. Whether the Shoot dry weight (SDW) exhibits a negative correlation with the first component. Root and shoot length (RL and SL), root dry weight (RDW), total dry weight (TDW), and root-to-shoot ratio (R: S) are clustered together in the first component. That indicates their positive correlation with this component and possibly a positive correlation among them. They are regarded as essential indicators of plant growth and are positively correlated. Chlorophyll a, b and total content; (CHA, CHB and TCH), are positively correlated and clustered in the second component and strongly correlated with component 1. The positive correlation among RL, RS ratio, SL, RDW, and TDW due to the behavior of the plant that has longer roots generally have higher R:S, as well as longer shoots, more root dry weights, and higher total biomass. CHA, CHB and TCH are related measurements grouped together and positively correlated and significantly contribute to the second component. RWC and SDW are negatively correlated in their respective component. Which they have a different variance pattern compared to others. Relative water content (RWC) is an important indicator of plant hydration status, and its negative correlation with Component 2 suggests its variance is significant but in the opposite direction with chlorophyll content and accumulation biomass.

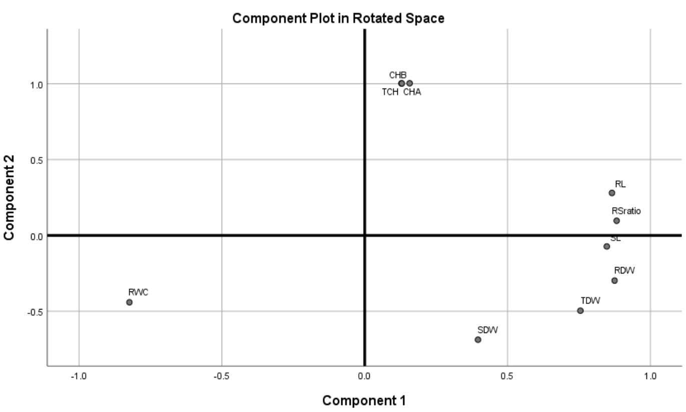

Figure 6: Principal components analysis of morphological and physiological responses of Brachychiton *populneus* seedlings under drought conditions.

4.CONCLUTION

This study concludes by highlighting the important advantages of compost for seedling growth of *Brachychiton populneus* (Schott & Endl.), particularly in drought-stressed environments. Compost applied at a rate of 4000 ppm demonstrated a significant increase in seedling height, root length, and biomass overall. Composting promotes the ideal root-to-shoot ratio, which is essential for more effective water intake and survival in drought-prone areas. Furthermore, the fact that large amounts of chlorophyll are maintained during extreme droughts emphasizes the significance of compost in maintaining vital physiological processes. These results support the practical application of composting in forest nurseries as a means of enhancing seedling resistance and growth in harsh environmental settings.

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