ECO-FRIENDLY FOLIAR TREATMENT ENHANCES GROWTH AND BIOCHEMICAL TRAITS IN SUNFLOWER

Muhammad Asad[1*], Afsheen Khan[1,2,3*], Saira Qadir[1], Muniza Riaz[1], Bushreen Jahan[1] and Ijaz Ahmed Qureshi[4]

[1]*Department of Botany, Federal Urdu University of Arts, Science and Technology Karachi, Pakistan. 73500*
[2]*Dr. Moinuddin Ahmed Research Laboratory of Dendrochronology and Plant Ecology. Department of Botany, Federal Urdu University Gulshan Iqbal campus Karachi, Pakistan-75300.*
[3]*Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Québec G7H 2B1 Canada*
Plants Breeder's Right Registry, Ministry of Food Security and Research, Govt. of Pakistan
[*]Corresponding author's email: asadmughal127@gmail.com, khanafsheen913@ymail.com

Received: September 04, 2025; Accepted: October 15, 2025

ABSTRACT

In natural environments, various medicinal plants grow that possess significant ethnobotanical importance and a high potential for containing therapeutic compounds. The present study investigates the effects of foliar application of lemon grass (*Cymbopogon citratus* L.) leaf extract on the biochemical parameters of sunflower (*Helianthus annuus* L.). Physiological traits such as carbohydrate, protein, and chlorophyll contents are extracted and analyzed from the roots, stems, and leaves. Different concentrations of lemon grass extract are applied as a foliar spray, with the 1% concentration proving to be the most effective in enhancing physiological performance. Among the plant parts examined, the leaves exhibit the highest adaptive response, synthesizing greater amounts of total chlorophyll (0.043 ± 0.03 mg/g), carbohydrates (2.99 ± 0.003 mg/g), and proteins (2.11 ± 0.05 mg/g) in plants treated with 1% extract. The findings support the use of natural biostimulants to promote crop growth. Foliar spraying with aqueous lemon grass extract is a low-cost and effective method for improving sunflower cultivation and contributes to increased essential oil production in agribusiness. Additionally, higher concentrations of the extract may offer potential for weed control. While lemon grass has traditional medicinal applications, it should be used with caution, as excessive usage may cause adverse effects, including cellular damage.

Key words: *Helianthus annuus*, *Cymbopogon citratus*, Protein, Carbohydrate, Pigments, Medicinal

INTRODUCTION

The use of medicinal plants in human civilization dates back thousands of years. Approximately 15,000 plant species have been studied, many of which are known to contain therapeutic biochemicals used in traditional and modern medicine (Negrelle and Gomes, 2007). These plants often produce a wide array of secondary metabolites that serve defensive functions against pathogens, herbivores, and insect attacks. Such compounds include phytochemicals—chemically active substances produced by plants and allelochemicals, which are a subclass of secondary metabolites known to influence the growth and development of surrounding plants (Stamp, 2003).

Allelochemicals are present in various plant organs including roots, leaves, flowers, fruits, and buds. Compounds such as sorgoleone, phenolics, and cyanogenic glycosides like dhurrin have been shown to exhibit weed-suppressing abilities (Weston *et al*., 2020; Ashrafi *et al*., 2007). These biochemicals may be released into the surrounding environment via root exudates or leachates from rain-washed plant parts, and they can significantly affect germination, growth, and other physiological processes of neighboring plant species (Hussain *et al*., 2007; Iqbal *et al*., 2010). While such interactions can sometimes be beneficial, they may also negatively impact adjacent plants, especially in agricultural ecosystems.

Cymbopogon citratus, commonly known as lemon grass or West Indian lemon grass, is one of the most widely recognized aromatic and medicinal plants. It is referred to by more than 28 vernacular names globally (Jayasinha, 1999). Lemon grass is rich in bioactive compounds with documented ethnopharmacological uses across cultures (Kumar *et al*., 2010). Its essential oils and extracts possess a broad spectrum of biological activities and have been traditionally used to treat fever, flu, leprosy, malaria, digestive problems, and respiratory ailments (Garg *et al*., 2012; Mirghani *et al*., 2012; Kumari *et al*., 2009; Oloyede, 2009). In addition to its antimicrobial and antioxidant properties, lemon grass has been reported to have hypoglycemic, hypocholesterolemic, and hypolipidemic effects, potentially reducing plasma cholesterol and low-density lipoproteins (Agbafar and Akubugwo, 2007; de Melo *et al*.,

2002). Its phytochemicals have also demonstrated activity against bacteria, fungi, and protozoa (Trindade *et al*., 2015).

Helianthus annuus L., commonly known as sunflower, is an economically significant oilseed crop cultivated worldwide. It ranks as the fourth most important source of edible oil globally (Anonymous, 2016). In Pakistan, sunflower is primarily grown for oil production but also has applications in the production of biodegradable products such as bio-plastics and eco-friendly paints derived from its vegetative biomass (Hussain, 2018; Fernandez *et al*., 2019). Its adaptability to subtropical and temperate climates, high oil content, and growing market demand make it an agriculturally valuable species.

Chemical ecology investigates the interactions among organisms mediated by natural biochemicals. Within this context, plant to plant interactions whether beneficial or detrimental are of particular interest in agriculture. Certain plants, through their secondary metabolites, may promote growth in neighboring species, while others may inhibit it. Understanding such interactions can lead to sustainable and eco-friendly agricultural practices.

Therefore, to obtain eco-friendly resolutions in agricultural practices, current study is designed. Our aim is to investigate the biochemical interaction between *Cymbopogon citratus* (lemon grass) and *Helianthus annuus* (sunflower) by evaluating the effects of lemon grass aqueous leaf extract on the early growth parameters of sunflower plants. We hypothesize that foliar application of lemon grass leaf extract at low concentrations promotes physiological growth in sunflower by enhancing chlorophyll, protein, and carbohydrate content, while higher concentrations may exhibit allelopathic suppression due to the presence of potent secondary metabolites.

MATERIAL AND METHODS

Lemon grass plants were grown in normal conditions in the month of February 2018 and fresh leaves were collected from the Botany Department of Federal Urdu University of Arts, Science and Technology, Gulshan Campus, Karachi. Four concentrations of aqueous extracts were prepared from the leaves (A = 0.5%, B= 1%, C=2% and D = 3%). After 24 h, at room temperature, the extracts were filtered then used as spray to treat sunflower plants.

Sunflower seeds were sown in plastic bags in late October 2018 which were germinated in garden supported by loam soil with natural organic fertilizer in 3:1 ratio. Seeds of similar sizes were sown in the superficial layer of soil not more than 2 cm deep. After the development of seedlings, they were shifted in large pots having 24kg soil mixed with natural fertilizer, diameter of pot was 34 inches & depth of pot was 29 inches. After establishment of plants (at third whorl of leaves) the extracts were sprayed in foliar form on the whole plant at alternate days and explore pigment analysis and biochemical analysis after every 10 times treatments till 40 times treatments of lemon grass leaf extract.

Pigment analysis

To determine the amount of pigment, the third whorl of leaf from the top of plant from each pot of the three replicates from each treatment was selected at particular time period. The total chlorophyll was estimated according to Arnon, (1949).

Soluble sugars

For soluble sugars the extracts were made in ethanol and observed by the method of Yemm & Willis, (1954).

Protein content

Protein content was estimated according to Bradford method (1976).

Statistical analysis

Data were subjected to Sigma Plot version 12.5 for the analysis of variance by Tukey's method for the significance level of physiological aspects at $p < 0.05$.

RESULTS

Under different exposures of treatment (0.5%, 1%, 2% and 3% lemon grass extract), sunflower plants showed a variety of responses throughout their consecutive physiological stages.

Carbohydrates (soluble sugar)

Aqueous extract of lemon grass (leaves) at 0.5%, 1%, 2% and 3% concentration with different time of exposures was given to the leaf, stem and roots of sunflower. It was observed that carbohydrate concentration of leaf

was enhanced at first 10 times spray of 0.5% (0.83 ± 0.012 mg/g) and 1% (0.88 ± 0.003 mg/g). However, a significant reduction in carbohydrates content was noted in 3% (0.419 ± 0.010 mg/g) and 2% (0.572 ± 0.006 mg/g). Whereas, in case of stem, it was seen that stem tissues preserved a considerably higher concentration in 0.5% and 1% treatments *i.e.*, 0.65 ± 0.034 mg/g and 0.71 ± 0.035 mg/g, respectively. 2% achieved similar level (0.55 ± 0.01 mg/g) as that of control. However, in roots of 1% treatments, carbohydrate concentration was recorded as 0.637 ± 0.032 mg/g which was the highest concentration of all other treated roots samples.

At 20 times spray, carbohydrate synthesis in leaves, roots and shoots found to be greatly productive as greater amount of carbohydrates were synthesized in all parts of treatments than control. The highest value of carbohydrates evaluated from leaves of 1% treatment (2.43 ± 0.03 mg/g) than from stems treated with 1% (1.107 ± 0.07mg/g) and from roots treated with 2% (0.69 ± 0.03 mg/g). Despite that, lower carbohydrate production was observed in roots treated with 3% *i.e.*, 0.52 ± 0.03 mg/g which was quite higher than control (0.51 ± 0.006 mg/g). A consistent increment in concentration of soluble sugars was observed in roots from 0.5% to 2% *i.e.*, 0.66 ± 0.01 mg/g, 0.67 ± 0.01 mg/g and 0.69 ± 0.03 mg/g, respectively while 3% treatments showed a sharp decrease (0.52 ± 0.03 mg/g) which was approximately equal to the synthetic level of control (0.51±0.006 mg/g) in roots.

At 30 times spray, the synthetic level of carbohydrate showed a clear decrease from 0.5% to 1% in leaves. Control leaves synthesized 1.65 ± 0.04 mg/g which was the highest concentration recorded at 30 times spray however, 2% (1.47 ± 0.03 mg/g) than in 3% (1.00 ± 0.007 mg/g) competed well to resist against the exceeding exposures but remained below the control tolerance range. Similarly, roots of 2% (0.69 ± 0.01 mg/g) and 3% (0.81 ± 0.08 mg/g) competed against control (1.001 ± 0.06 mg/g) but failed to achieve greater levels of carbohydrates than control while the rest of the treatments *i.e.*, 0.5% and 1% (1.21 ± 0.05 mg/g and 1.61 ± 0.05 mg/g) slightly raised their carbohydrate contents after 30 times spray than control. In stem 1% treatments showed highest concentration of soluble sugars *i.e.*, 2.99 ± 0.003 mg/g, although, the carbohydrate synthesis was declined in shoots in 2% and 3% treated treatments (0.64 ± 0.03 mg/g and 0.30 ± 0.001 mg/g). While the control sample plants showed an acceptable range *i.e.*, 1.501 ± 0.01 mg/g, the treated sample plants seemingly produced imbalanced responses towards higher exposures of aqueous extract.

At 40 times spray period, leaves responded with lower carbohydrate synthesis than 10th and 20th spray exposures, even in the control samples of leaves *i.e.*, 1.13 ± 0.12 mg/g. Leaves of 1% samples gave a dramatic increase in synthesis of carbohydrates *i.e.*, 1.702 ± 0.05 mg/g. Stems of 1% samples showed greater values of sugars *i.e.*, 3.173 ± 0.16 mg/g which was highest among all treatments. Roots synthesized relatively greater sugars in 0.5% and 1% samples (0.98 ± 0.01 mg/g and 1.13 ± 0.08 mg/g, respectively) than control roots (0.90 ± 0.06 mg/g) (Fig.1, 2, 3 and 4).

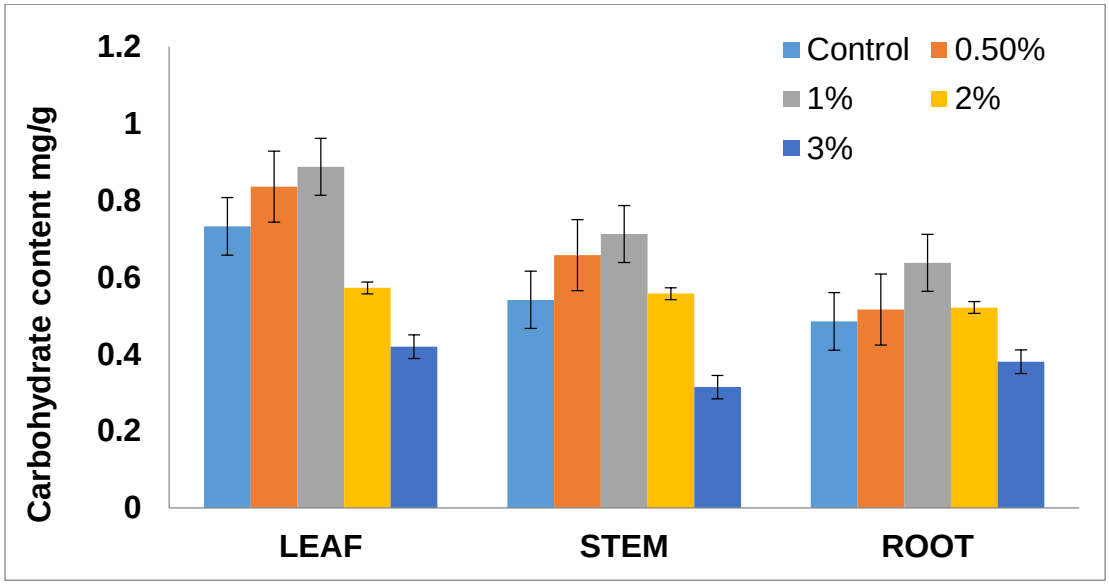

Fig. 1. Carbohydrate content in leaf, stem and roots of sunflower seedling at 10 times spray.

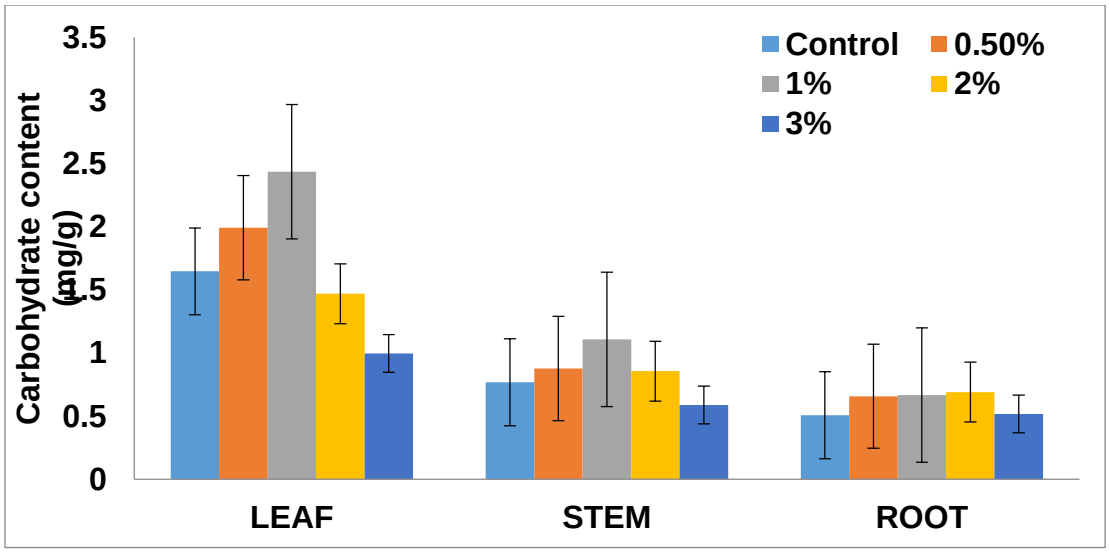

Fig. 2. Carbohydrate content in leaf, stem and roots of sunflower seedling at 20 times spray.

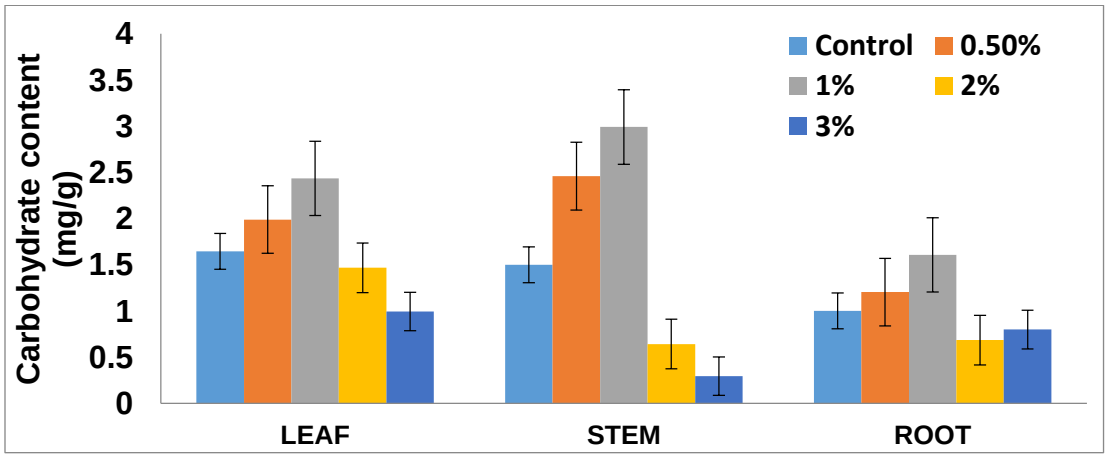

Fig. 3. Carbohydrate content in leaf, stem and roots of sunflower seedling at 30 times spray.

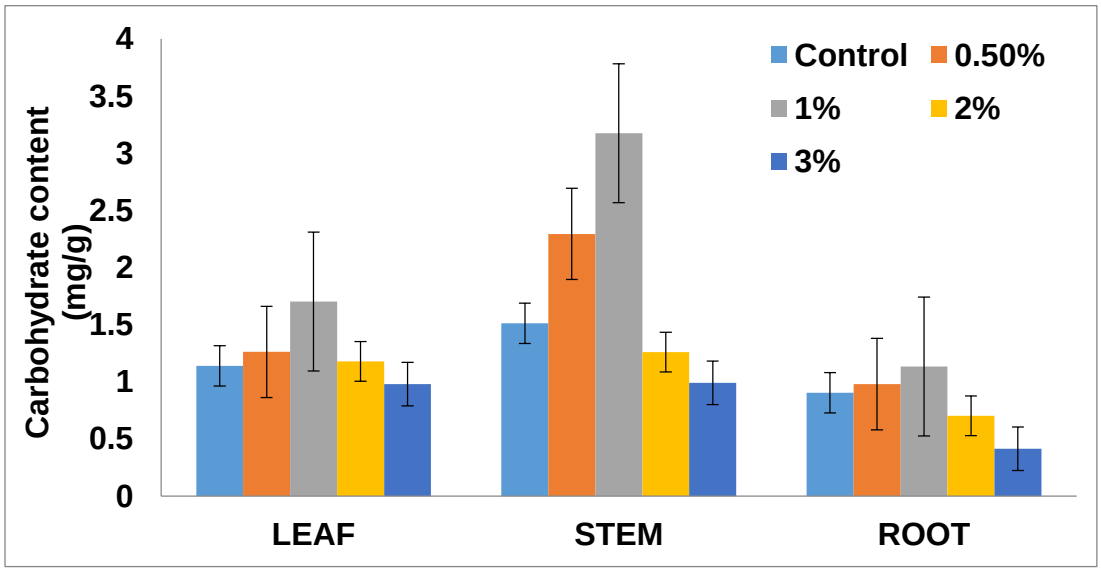

Fig. 4. Carbohydrate content in leaf, stem and roots of sunflower seedling at 40 times spray.

Total chlorophyll

Chlorophyll analysis showed 1% treatments coping up with the consecutive exposure of aqueous extract on plants. As the spray time increased, chlorophyll synthesis fluctuated randomly in treatment samples as well as in control. 20th time spray and 30th time sprays were significantly effective for increased synthesis of chlorophyll in leaves of control samples *i.e.*, 3.29 ± 0.15 mg/g and 3.73 ± 0.1 mg/g, respectively. 1% show highest gain in chlorophyll content at 10th time spray *i.e.*, 4.05 ± 0.03 mg/g and at 30th time spray *i.e.*, 4.04 ± 0.03 mg/g while decreased chlorophyll was recorded at 40th time spray (2.42 ± 0.17 mg/g). Although the predefined values were greater than control and other treatments at their respective spray time phases, but they were even lower than the actual production level recorded at the initial 10th time spray except that of 30th time spray phase. Surprisingly, 3% treatment samples produced lowest level of chlorophyll (1.17 ± 0.12 mg/g) at 10th time spray (Fig.5).

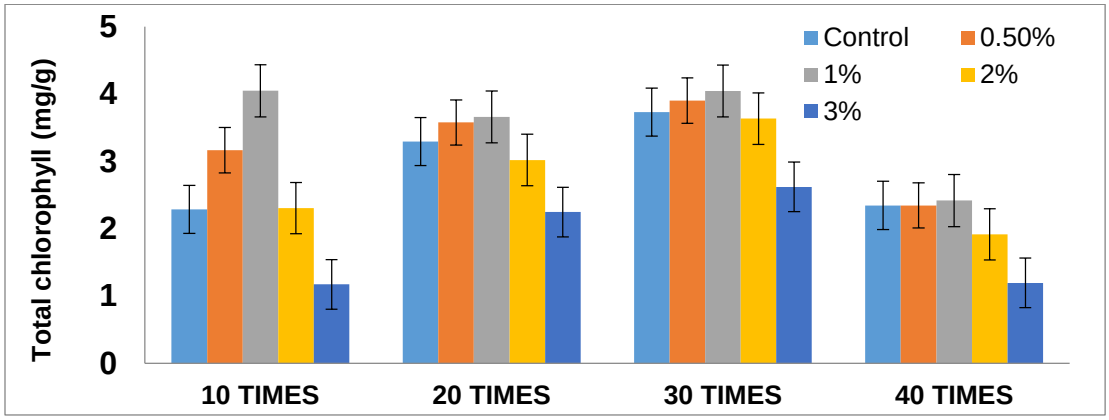

Fig. 5. Total chlorophyll content in leaves at 10, 20, 30 and 40 times spray of sunflower seedling.

Protein

Protein extraction from roots, shoots and leaves revealed random results from all treatments after 10, 20, 30 and 40 times stress exposures (Fig. 6, 7, 8 and 9.) Leaf of control sample provided with the highest concentration of proteins which was 1.93 ± 0.1 mg/g among all control samples of plants at different exposure period. In leaf samples of 1% treatment superseded at all exposure periods by conserving 2.11 ± 0.05 mg/g, 1.37 ± 0.02 mg/g, 1.53 ± 0.06 mg/g and 2.0 ± 0.005 mg/g at 10th, 20th, 30th, and 40th times spray. while rest of the samples govern relatively lower amounts of protein in them Lowest protein content attained in 3% treatment at 30th time spray was 0.82 ± 0.06 mg/g. A random distribution of proteins appeared in stem samples that showed greater protein content in 3% treatment samples at 10th time spray (2.13 ± 0.13 mg/g) which is the highest value among all samples in the stem. Surprisingly, none of the samples exceeded their protein content greater than control at 30 times spray. Seemingly 30 times spray did not favor protein production. Contradictory results were obtained at the very next level (40 times spray) in which a gradual increase was observed in samples of 0.5% and 1% treatments (2.0 ± 0.002 mg/g and 2.5 ± 0.03 mg/g, respectively). In roots, greater values were attained by 3% treatment samples at 10, 30 and 40 times exposure periods by gaining 2.04 ± 0.03 mg/g, 1.43 ± 0.23 mg/g and 2.03 ± 0.04 mg/g, respectively. While at 20th times spray, 0.5% treatment samples attained greater protein content *i.e.*, 1.93 ± 0.03 mg/g (Fig. 6).

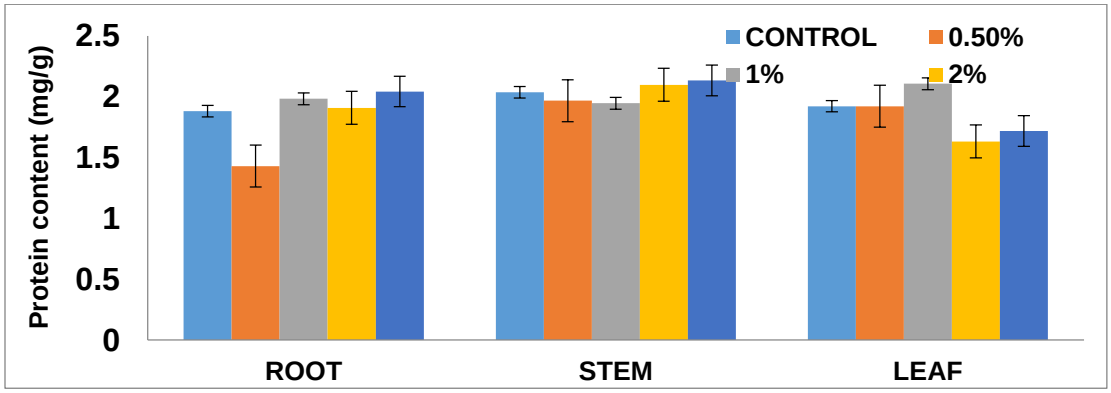

Fig. 6. Protein content in leaf, stem and roots at 10 times spray of sunflower seedling.

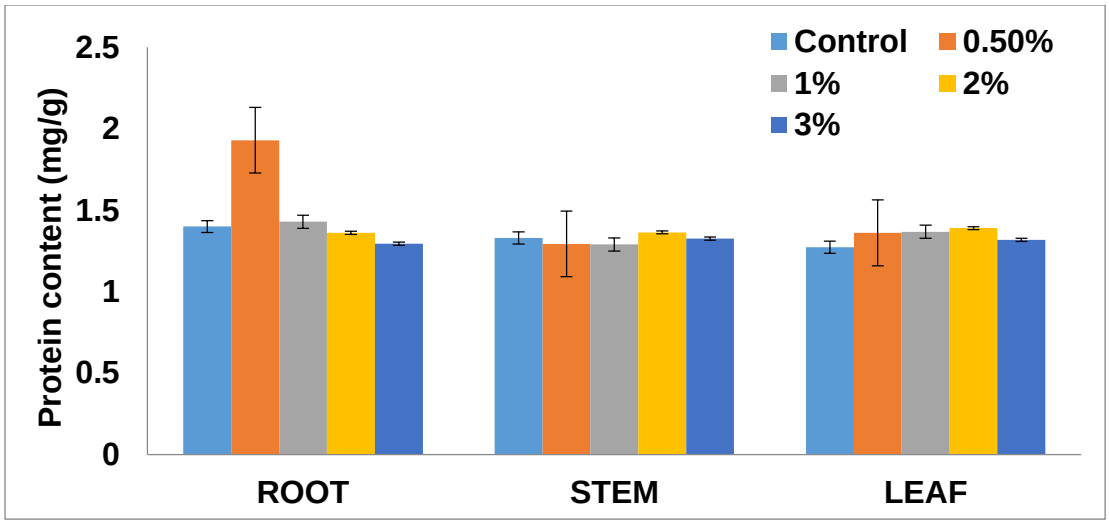

Fig. 7. Protein content in leaf, stem and roots at 20 times spray of sunflower seedling.

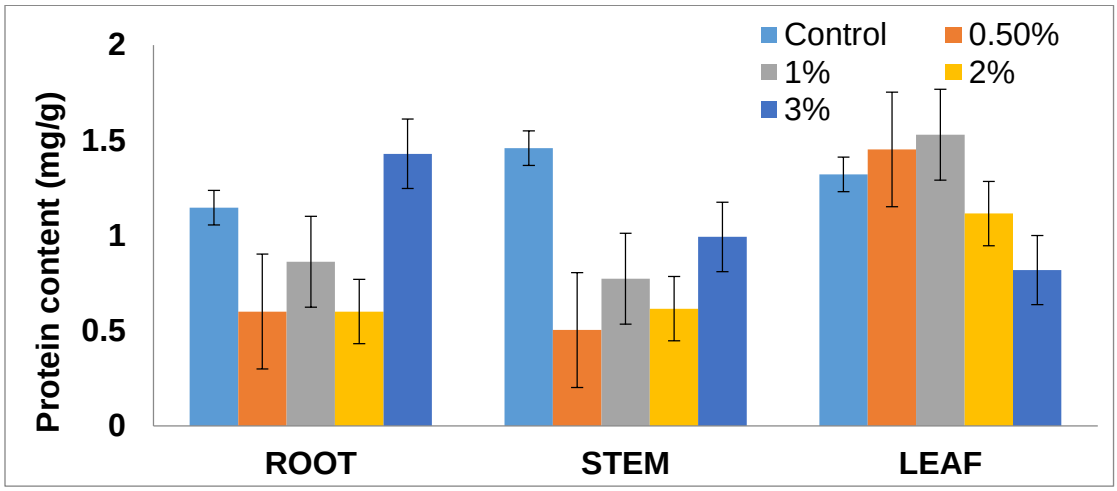

Fig. 8. Protein content in leaf, stem and roots at 30 times spray of sunflower seedling.

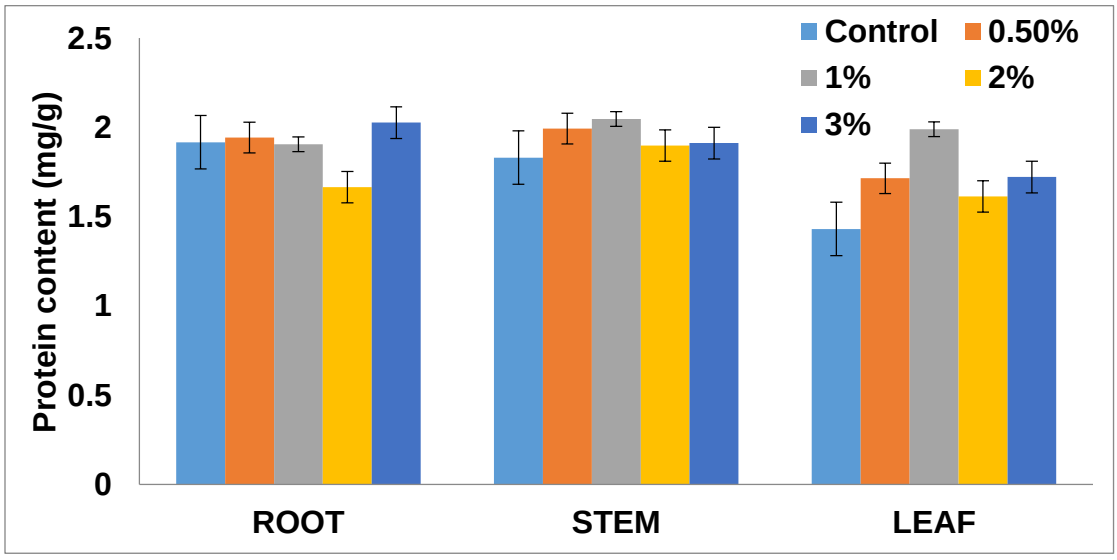

Fig. 9. Protein content in leaf, stem and roots at 40 times spray of sunflower seedling.

The biochemical concentrations of proteins, carbohydrates, and chlorophyll varied among different plant parts in control and treated plants (Fig. 10 and 11). Protein content was relatively consistent across leaves, stems, and roots, with median concentrations ranging between 1.4 and 1.7 mg/g in control while the range exceeded to 2 mg/g in treatments. The stem exhibited slightly higher protein levels than the other parts, and minor outliers were observed, particularly in the roots. Carbohydrate concentrations showed a decreasing trend from leaves to roots in control plants. Leaves exhibited the highest median carbohydrate content, while roots had the lowest. Notably, stems displayed the greatest variability, with several high outliers extending up to approximately 3.5 mg/g in treated samples (Fig. 11). Chlorophyll concentration was recorded exclusively in the leaves and was considerably higher than protein and carbohydrate levels, with values reaching up to 3.5 mg/g and minimal variability in control while treatments seems to exhibit higher concentration of chlorophyll with maximum range to 3.75 mg/g (Fig. 10 and 11). These results suggest a differential distribution of biochemical constituents across plant parts, with chlorophyll being restricted to leaves, while proteins and carbohydrates are more evenly distributed but exhibit tissue-specific variations in concentration and variability. However, leaves and stem are found more actively functioning sites in treatments plants than control plants, hence, producing a significant relation ($p < 0.05$) between control and treated plants while producing major nutritive biochemicals.

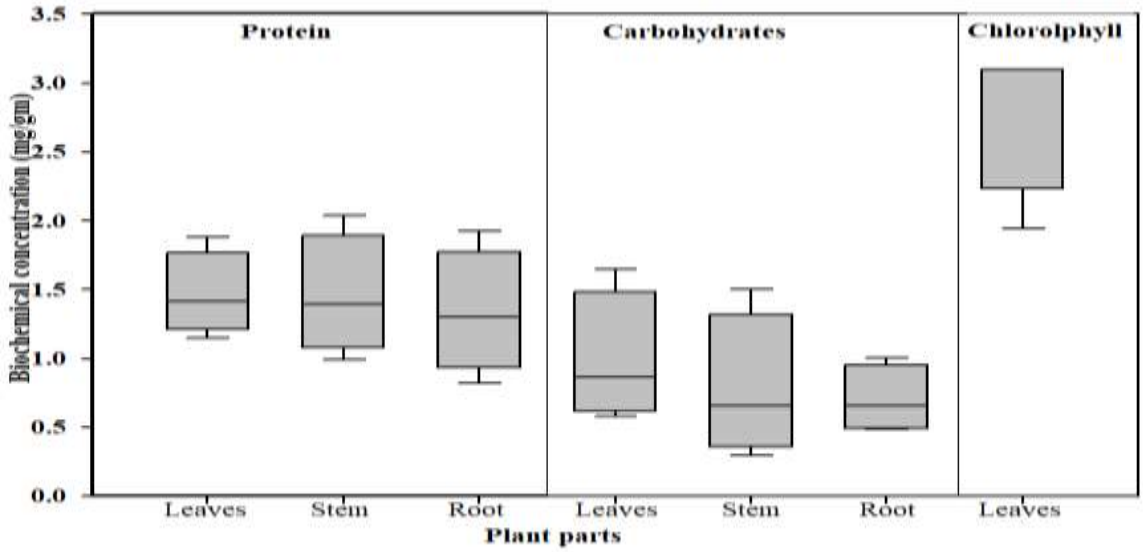

Fig. 10. Elaborates ANOVA among biochemicals (proteins, carbohydrates and chlorophyll) in plant parts (leaves, stem and roots) in control plants.

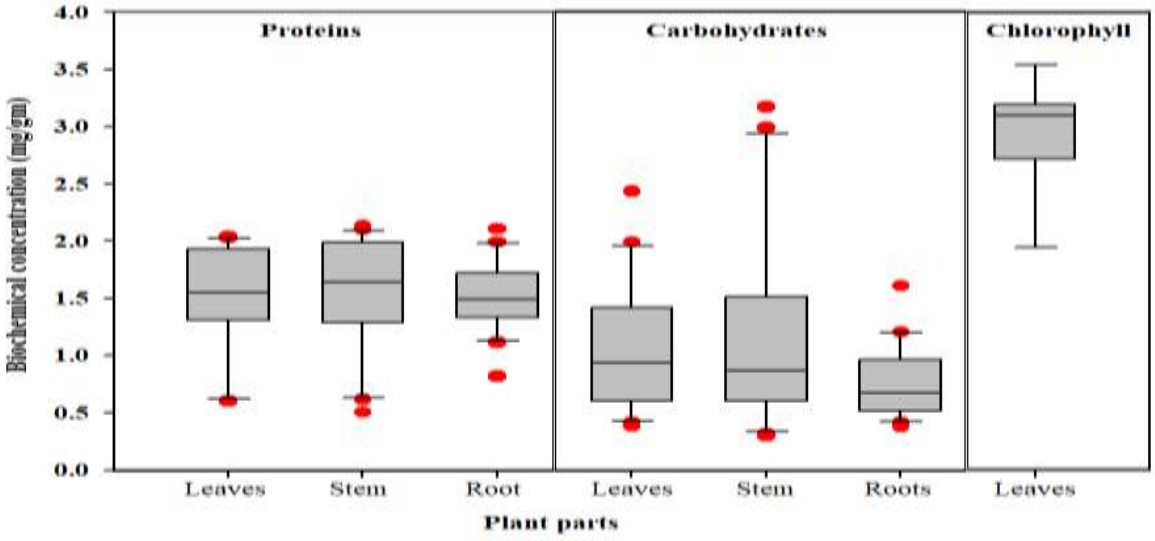

Fig. 11. Elaborates ANOVA among biochemicals (proteins, carbohydrates and chlorophyll) in plant parts (leaves, stem and roots) in control plants.

DISCUSSION

Chlorophyll content

Chlorophyll is widely recognized as an indicator of plant health and, indirectly, a contributor to human health through its role in crop nutritional quality. Pigment synthesis, particularly chlorophyll production, plays a crucial role in determining the nutritional value of crops, with chlorophyll content being a major component (Sheoran *et al*., 2021). In the present study, a clear enhancement in chlorophyll content was observed at lower extract concentrations, while higher concentrations led to a reduction in chlorophyll levels. These findings align with previous reports on treated plants and suggest a potential mechanism regulating pigment synthesis in the samples. Kamble *et al*. (2015) reported lower chlorophyll synthesis in young plants compared to mature ones and provided data illustrating differences in chlorophyll content between developmental stages. Their findings are generally consistent with our observations, with a slight variation: in mature leaves of *Ficus benjamina* and *Datura metel*, chlorophyll *b* content was found to be slightly higher than chlorophyll *a*.

Carbohydrate content

Carbohydrates are essential primary metabolites produced by plants, serving as both simple and complex sources of energy. According to Trouvelot *et al*. (2014), carbohydrates not only function as vital energy reserves but also contribute significantly to the pool of organic compounds and structural components within plant tissues. Numerous studies have assessed carbohydrate levels to evaluate plant health, nutritional status, defense mechanisms, and immune responses (Koch, 1996, 2004; Sheen *et al*., 1999; Rolland *et al*., 2006; Smeekens *et al*., 2010). Birnbaum *et al*. (1977) reported a deficiency of carbohydrates in various nutrient-deficient crops, while Han *et al*. (2008) suggested that such deficiencies could lead to chloroplast disruption or impaired sugar transport due to molecular damage. Under stress conditions, plants utilize significant amounts of carbohydrates to counteract high osmotic flux, a mechanism critical for their survival. Trouvelot *et al*. (2014) further emphasized the role of carbohydrates in strengthening plant immune systems and acting as protective agents. In the present study, the application of lemon grass extract at higher concentrations resulted in a reduction of sugar levels in sunflower plants. Conversely, lower concentrations of the extract appeared to promote carbohydrate synthesis. These findings are consistent with those of Khan *et al*. (2018), who proposed that increased osmotic pressure triggers enhanced photosynthetic activity due to the accumulation of stress-related exudates in the leaves, thereby representing a heightened stress response.

Protein content

Proteins are among the primary constituents of plant cells, playing essential roles in basic cellular functions. As amino acids serve as the building blocks of proteins, their increased presence within plant tissues directly correlates with elevated protein synthesis. Protein production involves three key stages initiation, elongation, and termination each of which can be disrupted by external factors, potentially inhibiting protein formation. Several studies have reported that the application of GA_3 can promote protein synthesis in plants such as soybean (El-Hadary and Chung, 2013; Taiz and Zeiger, 1991; Salem, 1989). Similarly, Lemma *et al*. (2009) observed an increase in soil nitrogen content following the application of low concentrations of foliar extracts from *Malva parviflora* and *Artemesia ludia*, whereas higher concentrations had a detrimental effect. Since nitrogen is a key element in amino acid composition, fluctuations in nitrogen availability directly influence protein synthesis.

The present study demonstrated that lemon grass extract positively affected protein synthesis in sunflower leaves at a low concentration (1%), while higher concentrations led to a decrease. However, at elevated extract levels (3%), protein levels increased again, particularly in the roots, followed by stems. This trend suggests a differential tissue-specific response to extract concentration. These findings are consistent with Sobhanian *et al*. (2011) and Bray (2000), who proposed that increased intracellular nitrogen levels may lead to the formation of stress-related or false proteins, which are stored or transported to aerial parts. Under elevated stress, plants may initiate the synthesis of specific stress proteins as an adaptive response.

Noshad and Khan (2019) also reported minimal degradation of nutritional compounds in plants irrigated with food waste water, along with an increase in protein levels in *Solanum melongena* treated with *Catapa* leaf extract. Several other studies have documented the beneficial impact of plant extracts and organic amendments on protein and nitrogen-based molecule synthesis. However, such effects can be species-specific and may inhibit growth in certain crops (El-Hadary and Chung, 2013). These results support the findings of El-Hadary and Chung (2013) and Khan *et al*. (2018), who listed a range of botanical herbicides known to alter nitrogen metabolism in key crops such as wheat, sorghum, and soybean. These alterations influence the synthesis of proteins and nucleic acids, and affect vital cellular processes including division, elongation, and maturation.

Overall, the present study demonstrates that eco-friendly foliar application of lemon grass leaf extract can positively influence growth and biochemical attributes in sunflower, particularly at lower concentrations. By enhancing protein synthesis, chlorophyll content, and carbohydrate accumulation, this natural treatment offers a sustainable alternative to synthetic inputs. These findings highlight the potential of botanical extracts as effective biostimulants for improving crop health and productivity, while promoting environmentally responsible agricultural practices. This approach aligns with the global shift toward sustainable agriculture and supports the integration of organic treatments for enhancing physiological resilience and biochemical efficiency in economically important crops like sunflower.

Conclusion

Foliar application of lemon grass leaf extract at varying concentrations demonstrated that a 1% solution was most effective in enhancing key physiological and biochemical traits in sunflower. Among the plant parts examined roots, shoots, and leaves the leaves showed the greatest adaptive response, producing the highest levels of total chlorophyll (0.043 ± 0.03 mg/g) and carbohydrates (2.99 ± 0.003 mg/g) under the 1% treatment. These results underscore the potential of eco-friendly foliar treatments using natural plant extracts to promote plant growth and improve metabolic functions. The study supports the broader conclusion that such sustainable, organic approaches can play a significant role in improving crop performance while reducing reliance on synthetic agrochemicals.

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