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The Effect of Cartoon Residue to Stimulate Wheat Growth

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Abstract: **The production of ethanol through fermentation of lignocellulosic materials by fungi offers a promising renewable alternative to fossil fuels. However, the breakdown process of raw materials during fermentation can lead to the formation of furan compounds, such as furfural, which can have detrimental effects on the process. In this study, we conducted in vitro spectro-photometric assays to investigate the formation of furfural during fungal metabolism. Our kinetic analysis revealed that furfural is indeed produced as a byproduct of fungal metabolism. Motivated by these findings, our study aimed to address the following objectives: (1) Characterize furfural production from cellulosic waste materials, and (2) Determine the optimal pH, duration, and sample type for furfural accumulation. Through our research, we aim to contribute to a better understanding of the factors influencing furfural formation during lignocellulosic fermentation, thereby facilitating the development of more efficient and sustainable biofuel production processes.**

Keywords: **Furfural, Fermentation, Cellulosic Garbage, Fungi.**

I. INTRODUCTION

The global reliance on fossil fuels for energy production has long been recognized as a significant contributor to environmental degradation. To mitigate these adverse effects and seek economically viable alternatives, there is a growing imperative to transition towards renewable energy sources. Among these alternatives, lignocellulose stands out as a promising resource due to its abundance and potential for sustainable energy production. Through fermentation processes, lignocellulosic biomass can be converted into ethanol, offering a renewable and environmentally friendly energy option [1]. Furthermore, the conversion of waste materials, such as garbage, into fuels utilizing non-edible cellulosic biomass as a feedstock, represents a crucial avenue for sustainable energy development [2]. This approach not only addresses waste management challenges but also harnesses the potential of abundant biomass resources for energy production.

However, the utilization of lignocellulosic biomass presents inherent challenges owing to its complex composition, which includes cellulose, hemicellulose, and lignin [3]. This complexity renders lignocellulosic biomass highly recalcitrant, necessitating pretreatment steps to make the sugar polymers accessible to fermenting microorganisms [4]. Various fuel molecules can be derived from lignocellulosic biomass, including ethanol, butanol, isobutanol, biofene, bisbolene, organic acids, long-chain alcohols, and furfural [5,6]. Furfural, in particular, holds promise as a feedstock for renewable fuel production due to its favorable carbon-to-heteroatom ratios[7]. Moreover, oleaginous microbes, characterized by their ability to accumulate more than 20% of their dry body mass as fuel molecules, play a crucial role in biofuel production [6,8]. Leveraging the capabilities of these microorganisms offers an opportunity for the sustainable production of renewable fuels, further advancing the transition towards a greener energy landscape.

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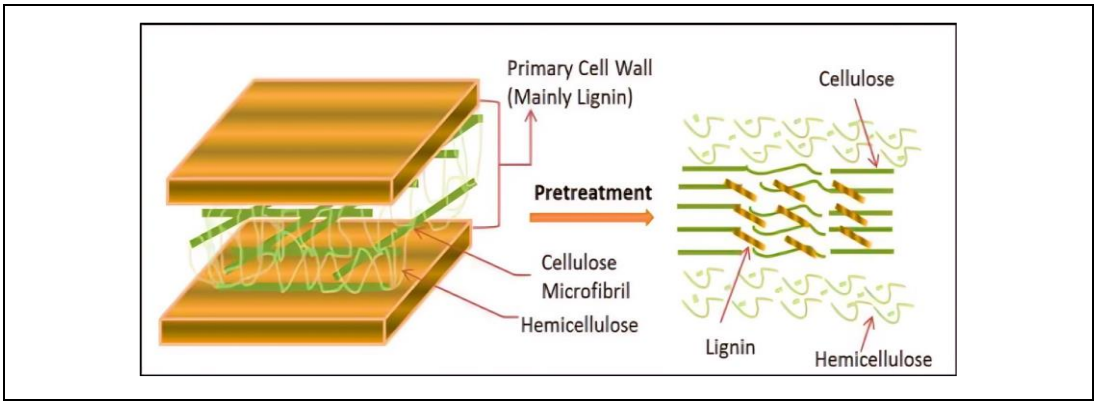

Fig. 1. Schematic diagram showing the effect of pretreatment on lignocellulosic biomass. The primary cell wall becomes compacted by a dense lignin network structure as the plant grows and ages.

A comprehensive literature survey conducted by Subramaniam et al. [9] delved into various microorganisms utilized for the production of single-cell oils (SCO). Among the microorganisms studied were microalgae, yeast, and molds, including Rhodosporidium toruloides, Cryptococcus curvatus, and Candida species. Additionally, the survey highlighted the bacterium Rhodococcus opacus, the mold Mortierella ramanniana, the yeast Trichosporon cutaneum, Trichosporon fermentans, and the mold Cunninghamella echinulata. These diverse microorganisms demonstrate the potential for SCO production across different biological kingdoms. While the literature showcases a plethora of microorganisms with SCO-producing capabilities, it is noteworthy that only a limited number of techno-economic studies have been conducted in this area [10]. Further research in this field is essential to assess the feasibility and scalability of SCO production using oleaginous microorganisms.

In addition to the mentioned studies, Candida curvata has emerged as a noteworthy candidate for SCO production, as evidenced by recent research efforts [11]. This yeast species presents promising characteristics for lipid accumulation and could contribute significantly to the diversification of SCO production platforms. Furthermore, recent advancements in genetic engineering and metabolic engineering techniques have opened up new avenues for enhancing lipid productivity and tailoring lipid profiles in oleaginous microorganisms [12]. These technologies offer opportunities to optimize SCO production processes and tailor lipid compositions to meet specific industrial and commercial requirements.

Expanding the literature survey to encompass a broader range of oleaginous microorganisms and exploring their potential for SCO production could provide valuable insights into the development of sustainable and economically viable biofuel production systems. This study aims to investigate the potential of using recycled cartoon waste as organic nutrients to enrich soils, assessing its impact on soil fertility, microbial activity, and plant growth. Additionally, it seeks to explore the environmental implications and feasibility of integrating cartoon waste utilization into sustainable soil management practices.

Efficiency Evaluation

The efficiency of biologics in the germination and growth of wheat seeds was evaluated in this study conducted in controlled conditions. Eighteen pots, each punctured at the base for drainage, were filled with soil obtained from the study area. Wheat seeds were meticulously prepared by cleaning them from impurities and subsequently sterilized with a 2% sodium hypochlorite solution for a duration of 5 minutes. Following sterilization, the seeds were thoroughly rinsed with sterile water to remove any residual bleach. The biologics were incorporated into the wheat seeds at varying concentrations of 1, 2, 4, 8, 10, and 15 grams per kilogram of seeds. For each concentration, approximately three pots were utilized for sowing the treated seeds, while an additional three pots were allocated for planting untreated (non-biologics) seeds, serving as controls.

Upon sowing the seeds, the pots were arranged in a randomized complete block design to minimize bias and ensure uniformity in environmental conditions. The experiment was conducted under controlled environmental conditions with regulated temperature, humidity, and light exposure.

Studied Attributes

Several attributes of wheat plants were studied throughout the experimental period, including the proportion of germination. The percentage of germination was calculated after 10 days of planting wheat seeds using the following equation:

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$$\text{Percentage of Germination} = \frac{\text{Total number of seeds}}{\text{Number of germinated seeds}} \times 100 \quad (1)$$

In addition to germination rate, other growth parameters such as seedling vigor, shoot height, root length, and biomass accumulation were measured at designated time intervals to assess the overall impact of biologics on wheat seedling growth and development. Overall, this experimental setup allowed for the systematic evaluation of biologics' effectiveness in promoting wheat seed germination and subsequent growth, providing valuable insights into their potential as eco-friendly alternatives for enhancing agricultural productivity.

Determination of Chlorophyll

The determination of chlorophyll content A and B in wheat leaves was conducted using the Harborne method. Leaves from wheat plants at 50 days of age were randomly sampled from each replicate. These leaves were then cut into small pieces, and 0.5 grams of leaf tissue was carefully weighed and placed into a mortar. To aid in extraction, a small amount of silica gel was added to the leaf tissue. Subsequently, 20 milliliters of 80% acetone solution were added to the mortar containing the leaf tissue and silica gel. The mixture was then crushed for 10 minutes to facilitate chlorophyll extraction. Following the crushing process, the mixture was filtered through a piece of gauze to separate the solvent from the solid residue.

The extraction process was repeated by adding the same volume of acetone to the residue in the mortar, and the aforementioned steps were repeated. The resulting extractions were combined, and the total volume of the extract was recorded. To estimate the chlorophyll content, spectrophotometric analysis was performed using a spectrophotometer apparatus. The absorbance of the extracted solution was measured at wavelengths of 663 and 646 nanometers, corresponding to the absorption peaks of chlorophyll A and B, respectively.

Chlorophyll content A and B were quantified using the following equations:

$$\text{Chlorophyll A (gm/l)} = (12.12 \times A_{663}) - (2.81 \times A_{646}) \quad (2)$$

$$\text{Chlorophyll B (gm/l)} = (20.13 \times A_{646}) - (5.03 \times A_{663}) \quad (3)$$

Where A_{663} and A_{646} represent the absorbance values at wavelengths of 663 nm and 646 nm, respectively. This method allowed for the accurate determination of chlorophyll content A and B in wheat leaves, providing valuable insights into the photosynthetic activity and physiological status of the plants. Then calculate the amount of chlorophyll per 100 grams of plant tissue, we can use the following equations:

$$\frac{Mg}{100gm} = \frac{100g}{1000ml} \times \text{Sample w. g} \quad (4)$$

Given that we've already calculated the concentrations of chlorophyll A and B in mg/g of leaf tissue, we can substitute these values into the equations to find the amount of chlorophyll per 100 grams of plant tissue.

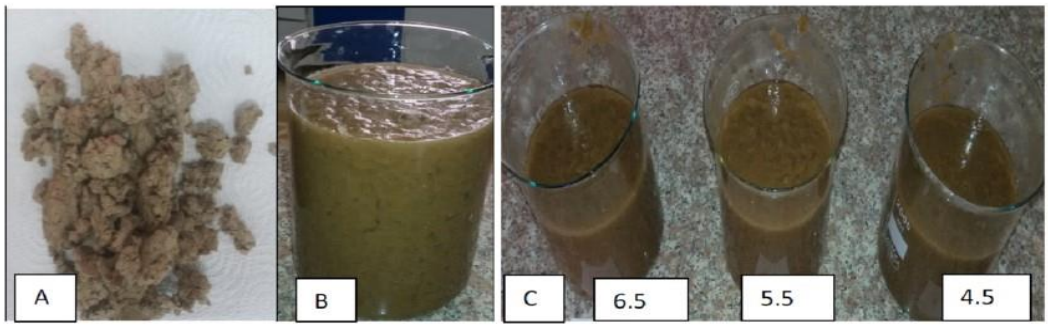

Fig. 2. Cellulosic garbage A-Grinding B- garbage with dilute acid after boiling C- PH value.

Measurements

Measurement of Fresh Total Vegetative Weight (g):

Three randomly selected plants, each 50 days old, were sampled from every experimental replicate and treatment. Using a sensitive balance, the fresh total vegetative weight of each plant was meticulously measured to ensure accuracy and consistency in data collection.

Measurement of Fresh Total Root Weight (g):

The roots of the sampled plants were carefully harvested to measure both the fresh weight of the vegetative mass and the fresh weight of the root system. Employing a sensitive balance, the fresh weight of the roots was determined to assess the overall biomass allocation and growth patterns of the plants under different treatments.

Total Carbohydrate Assay Kit:

For the quantification of total carbohydrates, a Total Carbohydrate Assay Kit was employed following standardized procedures. Glucose standards of known concentrations were utilized for colorimetric detection, with various dilutions prepared to establish a standard curve. Tissue samples (50 mg) were homogenized in ice-cold Assay Buffer, followed by centrifugation to remove insoluble material. The supernatant was then incubated with assay reagents, and the absorbance was measured at 490 nm using a spectrophotometer. The proline content was determined based on a previously established protocol[7].

Proline mg/g:

Proline content in the matured callus of each treatment was estimated using a rapid colorimetric method as suggested by Bates et al.[13]. The absorbance was measured at a specific wavelength using a spectrophotometer, and the proline concentration was calculated based on a standard curve generated using known proline concentrations.

Statistical Analysis:

Experimental data were analyzed using a complete random design, and the means were compared using the least significant difference (LSD) test at a probability level of 0.05. This rigorous statistical approach allowed for the robust assessment of treatment effects and the identification of significant differences among experimental groups.

II. RESULTS AND DISCUSSION

Effectiveness of biologics

The investigation into the effectiveness of biologics derived from cartoon residue on the germination percentage of wheat seeds yielded notable findings, indicating significant differences in germination rates across treatments. Statistical analysis revealed a clear impact of biologics application on wheat seed germination, with all concentrations showing significant differences compared to the control treatment. The highest germination percentage was recorded in the treatment with a concentration of 15 g/kg seed (see **Fig. 3**), reaching an impressive 98.9%. This substantial increase in germination rate highlights the potent efficacy of biologics derived from cartoon residue in promoting seed germination. Conversely, the control treatment exhibited a markedly lower germination rate of 71.8%, underscoring the importance of biologics application in enhancing wheat seed germination.

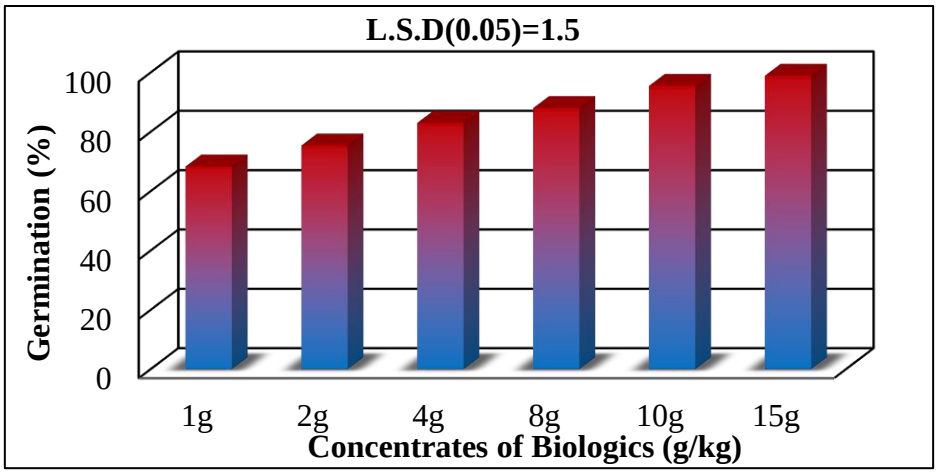

Fig. 3. Effect of different concentrations cellulose on the proportion of wheat seed. germination in pots. Estimation of chlorophyll (a and b) ratios in wheat leaf. The cellulose showed a significant increase in chlorophyll A and B rates.

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Estimating the Total Fresh Weights of the Root

The results of the statistical analysis presented in **Figure 4.** unveil a nuanced yet noteworthy trend in the efficacy of cellulose in augmenting the fresh root total of wheat plants. Interestingly, no significant differences were observed across all concentrations when compared to the control treatment. However, the concentration of 15g/kg seeds exhibited the highest rate, reaching 2.87g/plant, contrasting with the control treatment's rate of 1.27g/plant. Surprisingly, this concentration was surpassed by the 10g/kg seeds concentration, which registered a rate of 2.66g/plant.

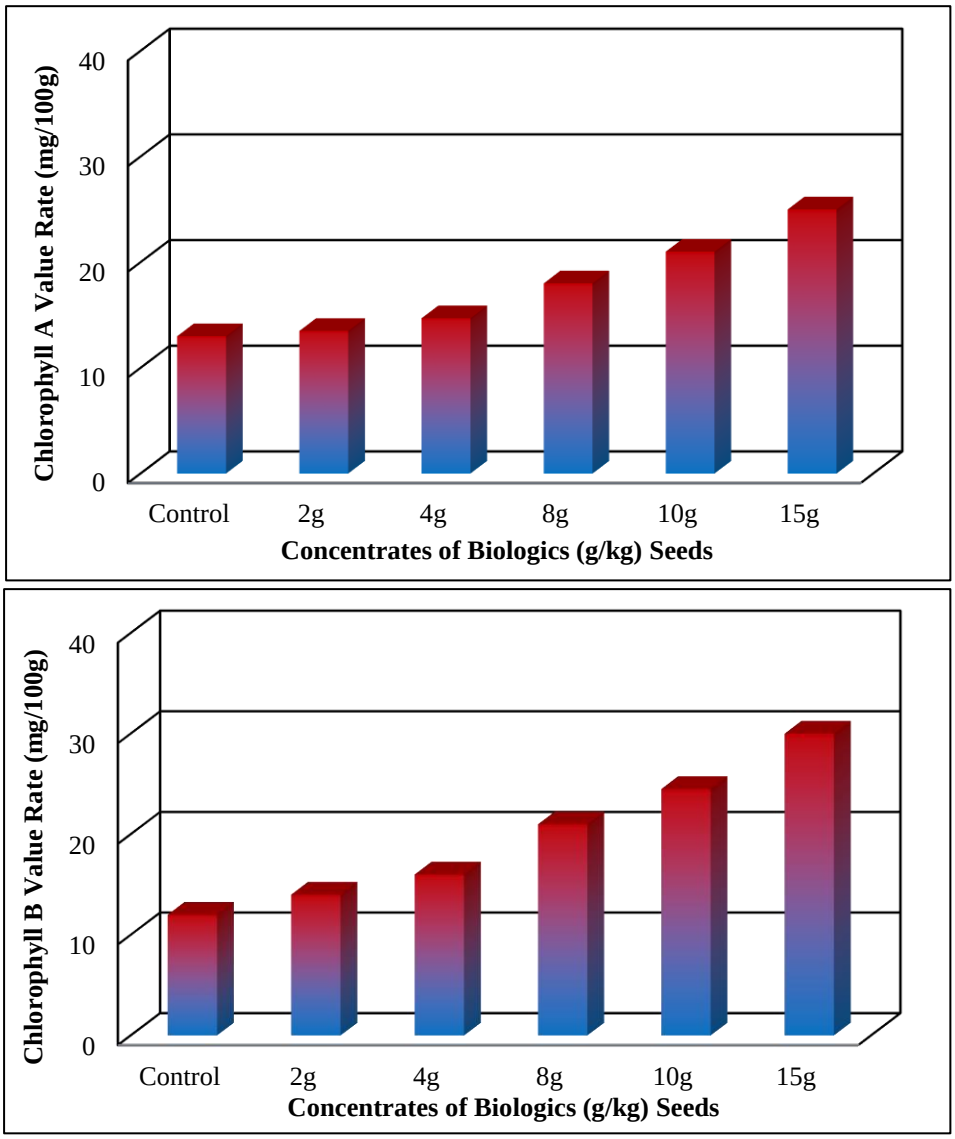

Fig. 4. Effect of different concentrations of cellulose on the Chlorophyll A and Chlorophyll B value biologics effect Concentrations in total vegetative Fresh Weight (FW).

Subsequently, there was a gradual decrease in fresh root total rates with decreasing concentrations, with values of 1.38g/plant, 1.74g/plant, and 2.5g/plant recorded for the concentrations of 2g/kg, 4g/kg, and 8g/kg seeds, respectively. This observed increase in root weight could potentially be attributed to several factors, including the water-absorbing properties of cellulose, which facilitate improved root development and vigor. Additionally, cellulose aids in enhancing soil porosity, thereby creating an optimal environment for robust root growth. It can also be inferred that a higher density of cellulose in the root zone correlates with more pronounced positive effects on root development and overall plant growth dynamics [1]. These findings underscore the potential of cellulose as a valuable soil amendment for promoting root health and overall plant performance in wheat cultivation. Further research is warranted to elucidate the underlying mechanisms driving these effects and to optimize cellulose application strategies for sustainable agricultural practices.

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Analysis of concentrations

All concentrations utilized in the experiment demonstrated a significant increase in the weight of the vegetative part of wheat plants compared to the treatment with cartoon residue. Notably, the highest weight was recorded at the concentration of 15g/kg seeds, where the rate reached an impressive 8.61g. In contrast, the control treatment exhibited a substantially lower weight of 1.37g. However, it's important to note a slight reduction in weight at the concentrations of 10g/kg and 8g/kg seeds, with average weights of 7.89g and 6.81g, respectively, albeit still significantly higher than the control treatment. Moreover, at the concentrations of 4g/kg and 2g/kg seeds, the average weights were 6.61g and 2.5g, respectively, both demonstrating a significant difference from the control treatment. These findings are illustrated in **Figure 5**. The observed increase in vegetative weight across all concentrations highlights the efficacy of the applied treatments in promoting wheat plant growth and development. Cellulose, derived from cartoon residue, likely plays a crucial role in enhancing nutrient uptake, root development, and overall plant vigor. The significant differences observed in vegetative weight underscore the potential of cellulose-based amendments in improving agricultural productivity and sustainability.

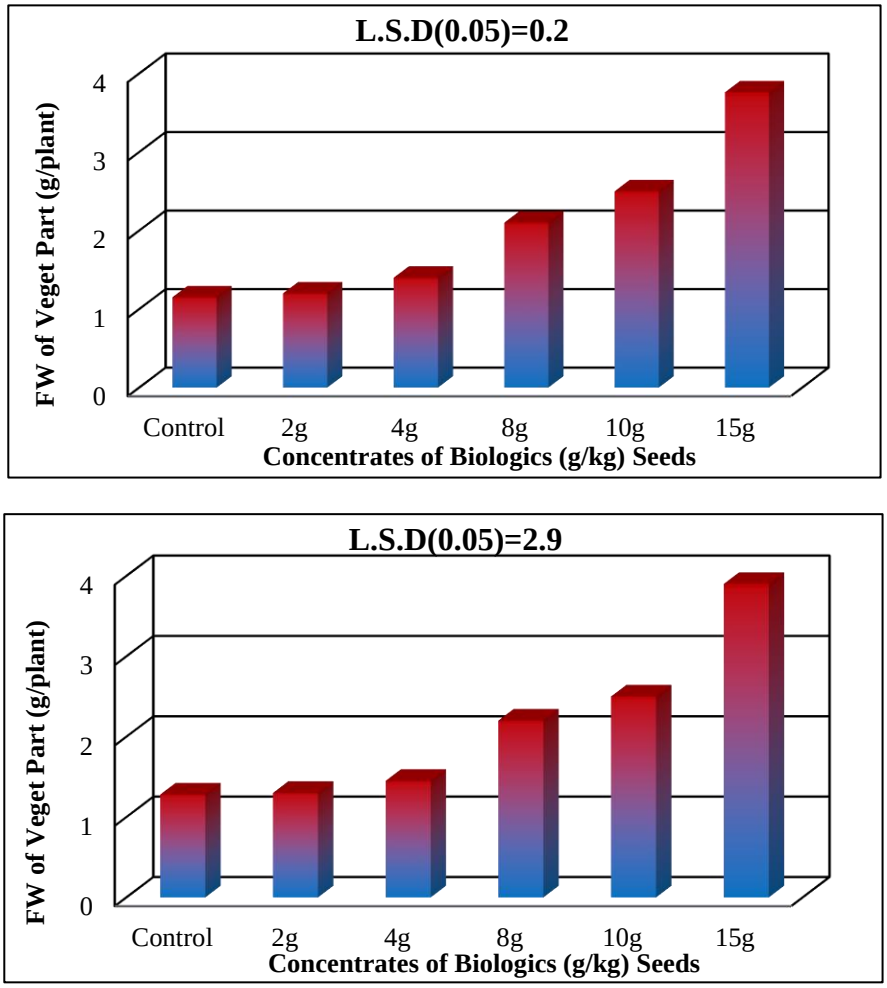

Fig. 5. The Effect of different concentration of cellulose on fresh weight of total vegetative part and total root part after 50 days.

Enhancement of Chlorophyll

The results depicted in **Figure 6** reveal a notable elevation in chlorophyll A levels across different concentrations. Remarkably, both the 15g/kg and 10g/kg seed concentrations exhibited the highest values, reaching 4.3mg/100g. Following closely, the 8g/kg seed concentration ranked second with a chlorophyll A level of 4.2mg/100g, while the concentration of 2g/kg seeds ranked third at 1.8mg/100g. In contrast, the concentration of 4g/kg seeds lagged behind, registering a chlorophyll A level of 1.6mg/100g.

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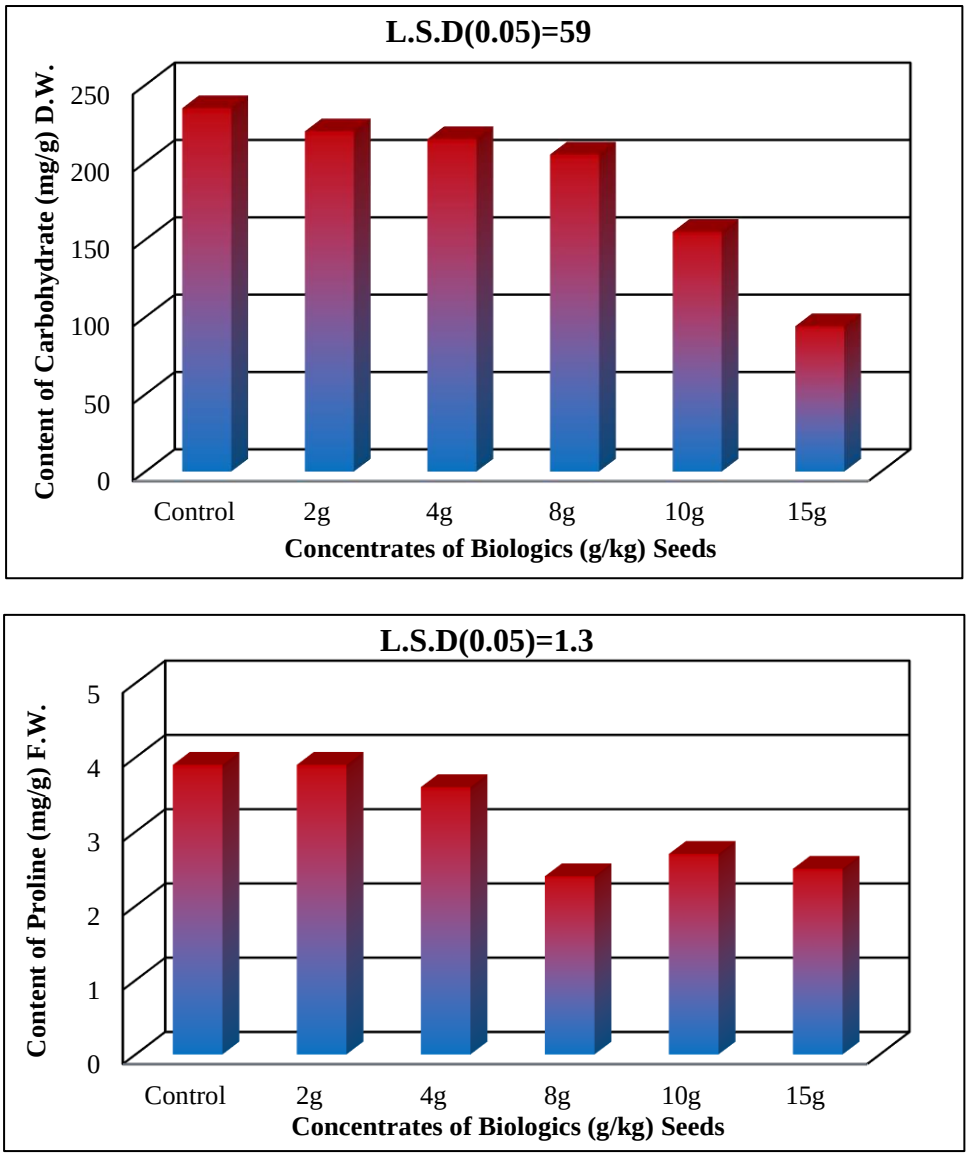

Fig. 6. The effect of cellulose concentration in wheat content of Carbohydrate (mg/g) D.W. and Proline (mg/g) F.W.

Significantly, all concentrations demonstrated substantial differences compared to the control treatment, which recorded a chlorophyll A level of 0.6mg/100g. This substantial disparity underscores the potent impact of the applied treatments in enhancing chlorophyll An accumulation in wheat leaves, indicative of improved photosynthetic activity and overall plant health. These findings highlight the efficacy of the applied treatments, particularly at higher concentrations, in promoting chlorophyll A synthesis in wheat leaves. The concentration-dependent response observed in this study underscores the importance of optimizing application rates to achieve maximal physiological benefits. Moreover, the significant differences observed compared to the control treatment underscore the potential of these treatments in enhancing crop productivity and sustainability.

Concentrations of cellulose

The effect of varying concentrations of cellulose on the content of total soluble carbohydrates and proline in wheat is depicted in **Figure 6**. The results revealed a significant decrease in soluble carbohydrates values with increasing biologics concentrations. Specifically, the highest content was observed in the control treatment, markedly differing from the other treatments. Conversely, the treatment with 15 g/kg of biologics exhibited the lowest soluble carbohydrate content. Additionally, the highest value of proline content (mg/g F.W.) was observed in the control treatment compared to the treatment with 15 g/kg of biologics, which demonstrated the lowest proline content. These findings suggest a discernible

impact of cellulose concentration on wheat biochemical composition, with implications for plant growth dynamics. This observed trend underscores the potential of cellulose, derived from cartoon residue, to modulate wheat biochemical pathways and influence physiological responses. The stimulation of wheat growth pathways by cartoon residue highlights its potential as a sustainable agricultural amendment, contributing to enhanced crop productivity and resilience.

III. CONCLUSION

Utilizing plant residues as supportive materials for soil substrates offers a promising avenue for enhancing soil fertility and nutrient availability. The incorporation of cellulose, derived from plant residues, demonstrates a notable positive impact on plant growth stimulation. This study underscores the potential of cellulose as a valuable soil amendment, capable of promoting robust plant growth and development. By harnessing the beneficial properties of cellulose, agricultural practitioners can optimize soil fertility and enhance crop productivity. Furthermore, the findings of this research highlight the importance of sustainable waste management practices in agriculture. Plant residues, often considered as wastes, can be repurposed as valuable resources for soil improvement and plant nutrition. Moving forward, future research endeavors should explore the synergistic effects of cellulose with other organic amendments and fertilizers to maximize agricultural outcomes. Additionally, elucidating the underlying mechanisms governing the interactions between cellulose and plant physiology can provide valuable insights for developing targeted strategies to enhance crop yields and promote environmental sustainability. In conclusion, the findings of this study emphasize the potential of plant residues, particularly cellulose, in advancing sustainable agriculture practices. By incorporating these materials into agricultural systems, we can foster soil health, optimize nutrient cycling, and ensure the long-term viability of food production systems.

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