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EFFECTS OF MICROALGAE FERTILIZER BASED ON CHLORELLA VULGARIS HM-3 ON WHEAT GROWTH AND THE NUTRITIONAL QUALITY OF ITS GREEN JUICE

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Abstract: Wheat is an important food crop in China, exerting a profound impact on agriculture and food products. Microalgae, characterized by rapid growth, short life cycles, and high carbon fixation capacity, hold great potential for promoting crop growth and improving quality. This study aims to investigate the effects of substituting 70% of chemical fertilizer with heavy-ion mutagenized Chlorella vulgaris (HM-3) during the topdressing stage on wheat seedling growth and the nutritional quality of green juice. A pot experiment was conducted to compare the effects of full chemical fertilizer (T2), 70% substitution of chemical fertilizer with Chlorella vulgarisHM-3 (T1), and a no-fertilizer control (T0) on wheat seedlings at 30 and 60 days. The results showed that, compared to the T2 treatment, the T1 treatment (substituting 30% of chemical fertilizer with Chlorella vulgaris HM-3) increased root length, plant height, fresh weight, and leaf number by 81.28%, 13.34%, 53.84%, and 26.34%, respectively, at 30 days under a 30% fertilizer reduction. Total photosynthetic pigments, chlorophyll a, and chlorophyll b were significantly increased by 9.11%, 9.16%, and 5.13%, respectively. Soluble protein, amino acids, total phenols, and flavonoids increased by 40.57%, 14.77%, 12.27%, and 12.90%, respectively, while calcium and magnesium content accumulated by 6.14% and 8.77%. Previous studies have mostly focused on substituting base fertilizers for wheat seedlings or investigating the entire growth stage of wheat. This study specifically concentrates on topdressing and the wheat seedling stage.
Keywords: HM-3 chlorella; Chemical fertilizer reduction; Wheat; Green juice; Nutritional quality

1 INTRODUCTION

The application of chemical fertilizers is of great significance for increasing crop yields and ensuring China's food security [1], However, in recent years, the excessive application of chemical fertilizers has been widespread in our country, resulting in a relatively low utilization rate of fertilizers [2], Not only did it fail to increase crop yields, but it also caused serious environmental pollution [3]. Microalgae bio-fertilizer, as a new type of fertilizer, provides theoretical support for reducing the use of chemical fertilizers in crops and achieving green and sustainable production [4], Microalgae are widely used in soil improvement, feed, bait and other fields because they contain nutrients and bioactive substances such as nitrogen, phosphorus, trace elements, proteins, polysaccharides, polyunsaturated fatty acids and plant hormones, which can promote the growth of animals and plants and improve the quality of agricultural products [5-7]. It is worth noting that current research on microalgae fertilizers mainly focuses on their application as base fertilizers or soil conditioners [4], In recent years, the potential of chlorella as a biological conditioner has been confirmed in many crops. Studies have shown that adding 2 to 3 g/kg of dry chlorella to the soil significantly increased the fresh weight and dry weight of lettuce seedlings [6], However, insufficient attention has been paid to its potential as top dressing. In fact, several characteristics of microalgae make them particularly suitable as top dressing: they release nutrients rapidly, which can meet the urgent needs during the critical growth period of crops; It has high application safety and avoids the risk of burning seedlings that may be caused by traditional chemical fertilizers as top dressing. It also has a biological stimulating effect, which can enhance the photosynthetic efficiency and nutrient absorption capacity of crops [6]. Wheat, as a major global food crop, has a long growth cycle and a clear nutrient requirement pattern, making it an ideal object for evaluating the effect of top dressing [8]. To systematically evaluate the potential of microalgae topdressing, this study adopted a progressive experimental design from "basic to application". Firstly, through germination experiments, the effects of microalgae extracts on the germination of wheat seeds and the growth of early seedlings were evaluated. This is not only the primary step in assessing the safety of fertilizers but also provides physiological baseline data for subsequent experiments [9]. On this basis, topdressing experiments at different growth stages were carried out to explore the comprehensive effects of microalgae replacing part of traditional chemical fertilizers on wheat growth, nutrient absorption and yield. Previous studies have also shown that when microalgae were applied to wheat, compared with the control group, the germination potential, germination rate, root length, bud length, fresh weight, dry weight and chlorophyll content of wheat seeds increased by 11.5%, 13.5%, 18.6%, 24.5%, 45.4%, 70.1% and 56.2% respectively[10], Chlorella can promote the growth of wheat, and at the same time increase the maximum light energy conversion efficiency per unit reaction center of wheat leaves, the energy of electron transfer and the photosynthetic performance index, which has a promoting effect on the accumulation of chlorophyll in wheat [9]. The chlorophyll a content, chlorophyll b content, root activity, water vapor pressure deficiency and intercellular CO2 in the treatment with Chloasma protein increased by 46.58%, 62.76%, 292.81%, 4.57% and 21% respectively[11]. Another study found that when applying base fertilizer to wheat, microalgae were used to partially replace chemical fertilizers to promote wheat growth. When the chlorella content was greater than 0.25g /L, it had a promoting effect on wheat growth, and the effect was most obvious when the application concentration was 2g /L. The plant height, fresh weight of the aboveground part, dry weight of the aboveground part, chlorophyll a and total chlorophyll content of wheat increased by 11.99%, 29.41%, 19.15%, 80.72% and 48.56% respectively. At the same time, the contents of soil organic matter, alkali-hydrolyzable nitrogen and other nutrients have all increased, and the soil pH has also slightly risen. This indicates that the application of chlorella as base fertilizer can not only promote the growth of wheat but also improve soil fertility[9].

2 MATERIALS AND METHODS

2.1 Test Materials

Shumai 26, potted soil from the Purple Soil Agricultural Ecological Experiment Station of Yanting, Chinese Academy of Sciences (31°16 'north latitude, 105°27' east longitude). Chlorella sp., Chlorella sp., Scenedesmus sp., and Chlamydomonas reinhardtii was provided by the Chengdu Institute of Biology, Chinese Academy of Sciences (30.415° N, 104.062° E). The algae solution was prepared in proportion with pure water and compared with pure water (T0) for experimental research.

2.2 Experimental Scheme

The experiment was conducted in the laboratory of Chengdu Institute of Biology, Chinese Academy of Sciences from September 2025 to November 2025. The method of potting soil was adopted. Polyethylene plastic POTS (with a diameter of 23cm and a height of 24cm) were selected for the experiment. Each pot can hold 4.65 kilograms of soil. Sowing will be carried out on September 15, 2025. The base fertilizer should be potassium sulfate compound fertilizer, with an application rate of 2g per pot. The top dressing should be urea, with an application rate of 1g per pot (set according to the field practice fertilization level). 100% potassium sulfate compound fertilizer should be applied as base fertilizer, and 70% common chlorella HM-3 and 30% urea should be applied as top dressing. Three treatments were set up, namely the control (CK): application of pure water treatment; T1: Treated with 0.7g of Chlorella HM-30.3 urea; T2: Treat with 1g of urea. Each treatment was repeated three times, totaling 18 POTS. Sampling was conducted on the 30th and 60th days respectively.

2.3 Screening of Microalgae

The experiment was divided into 4 experimental groups, with 3 replicates set in each group. The groups were compared with each other. Domestic sewage was simulated in the laboratory. The specific composition of the domestic sewage is as follows (mg /mL): sodium acetate 180, ammonium chloride 124.5, magnesium sulfate heptahydrate 33.2, potassium dihydrogen phosphate 7.2, and potassium dihydrogen phosphate 14.4. The determination range of water quality indicators for simulated domestic sewage is as follows: COD150-300 mg /L, ammonia nitrogen 20-40 mg /L, total phosphorus 4.5-6 mg /L, total nitrogen 20-50 mg /L, pH 6.5-8.0 mg /L. Before the experiment, the simulated domestic sewage was subjected to ultraviolet sterilization treatment in a high-pressure steam sterilizer for 30 minutes.

2.4 Wheat Germination Experiment

Firstly, the surface of wheat seeds was disinfected with 75% ethanol for 5 minutes. Then, they were disinfected with 2.5% NaClO solution containing 0.1% Tween-80 for 3 minutes. After rinsing with sterile water three times, they were placed in an incubator at a temperature of 25 ℃ and germinated in the dark for 24 hours. After the seeds germinated, select the seeds with consistent germination and place them in a petri dish. Add 20mL of sterile water to group T0, and add 20mL (common Chlorella HM-3, primordia Chlorella 34#, and Chlorella KXZ) in sequence to the other suspension group and the crushed liquid group. Then, place them in a 25 ℃ incubator for cultivation. During the cultivation period, continue to add clean water to the culture dish to keep the filter paper moist, which is used to promote the germination of wheat seeds.

2.5 Wheat Pot Experiment

2.5.1 Determination of soil physical and chemical indicators

Soil sampling was carried out by using the five-point sampling method to collect soil (excluding the rhizosphere) from a 5-7 cm layer in each pot and thoroughly mix it. The collected soil samples were air-dried and then sieved through a 100-mesh sieve. The contents of soil, total carbon, total nitrogen, total phosphorus, total potassium, alkali-hydrolyzable nitrogen, available potassium and available phosphorus were determined. The total carbon and total nitrogen contents in the soil were determined by an elemental analyzer [12], The total phosphorus was measured by the molybdenum-antimony colorimetric method [13], Total potassium was determined by flame atomic absorption spectrometry before melting with four acids [14], Alkali-hydrolyzed nitrogen was determined by the Kjeldahl nitrogen determination method before hydrolysis with 1.8mo1/1 sodium hydroxide [15], Available potassium was determined by flame atomic absorption spectrometry [16], The available phosphorus was determined by the HC1-H2SO4 pretreatment molybdenum-antimony colorimetric method [17].

2.5.2 Determination of growth indicators

Twelve days after all the treatments were completed, samples were taken. First, the sample wheat was rinsed three times with distilled water, then the residual water on the plant surface was dried with filter paper. Next, the wheat roots and stems were separated with scissors, and the root length and plant height were measured with a ruler. Then, the fresh weight was weighed with an analytical balance, and the number of blades was counted. The fresh weight is expressed as the average of three seedlings, with the unit being g.

2.5.3 Determination of nutritional indicators

Take healthy wheat plants aged 30 days and 60 days respectively. Wash the plants, drain them, cut the roots, homogenize and filter. The filtrate is the green wheat juice, which should be stored at -80℃ for future use. Take 1mL of the sample and determine the content of soluble protein by Coomassie brilliant blue method [18], The chlorophyll a, chlorophyll b, carotenoids and total photosynthetic pigments in green juice were determined by acetone extraction method [19]. The amino acids in green juice were determined by the triketone colorimetric method [20]. The mineral elements (Ca, Mg) in green juice were determined by the o-phenolphthalein complexone colorimetric method [21]. The total phenolic flavonoids in green juice were determined by the Forin-phenol method and the aluminum nitrate - sodium nitrite colorimetric method [22].

2.6 Data Analysis

All data are expressed as "mean ± standard deviation". One-way analysis of variance (ANOVA) was performed using SPSS software, and multiple comparisons were conducted using the Duncans new complex range method ($p<0.05$). The Pearson correlation coefficient was used to analyze the correlations among various indicators.

3 RESULTS AND ANALYSIS

3.1 Screening of Microalgae

3.1.1 Biomass of different microalgae in simulated domestic sewage cultivation

In this study, different biomass conditions over time were evaluated based on the increase of OD_{680} and the rise of chlorophyll a. Figure 1 shows the changes of OD_{680} and chlorophyll a over time of four microalgae in artificially simulated domestic sewage. In the first 0-5 days, the OD_{680} of Chlorella primordial 34#, Chlorella common HM-3, Chlorella KXZ and Chlamydomonas rheinalis increased by 150%, 213%, 153% and 139% respectively, and the chlorophyll a increased by 288%, 271%, 35.82% and 126% respectively. The growth of microalgae requires sufficient nitrogen and phosphorus elements. However, adding these elements in the absence of wastewater will significantly increase production costs [23]. Previous studies have shown that sewage is rich in nitrogen and phosphorus nutrients, which can provide energy sources for the growth of algal biomass. Therefore, microalgae can effectively remove a large amount of nutrients such as nitrogen and phosphorus [24]. The utilization degrees of nitrogen and phosphorus vary among different microalgae species [24-25]. The maximum OD_{680} and chlorophyll a ranges of the four microalgae cultivated in simulated domestic sewage in this study were 150% to 213% and 35.82% to 288%, respectively (Figure 1). Among them, the OD_{680} and chlorophyll a of chlorella HM-3 in the simulated wastewater reached 213% and 271% respectively, which were similar to the results of previous studies [26-27]. All four types of microalgae can successfully adapt to the simulated domestic sewage environment and achieve normal growth.

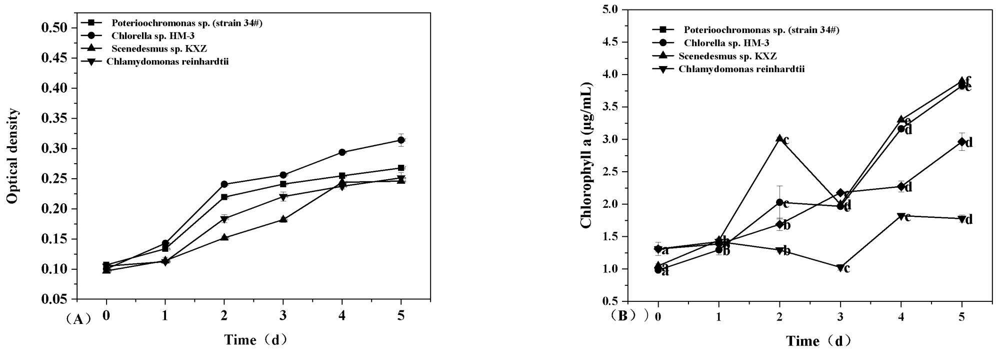
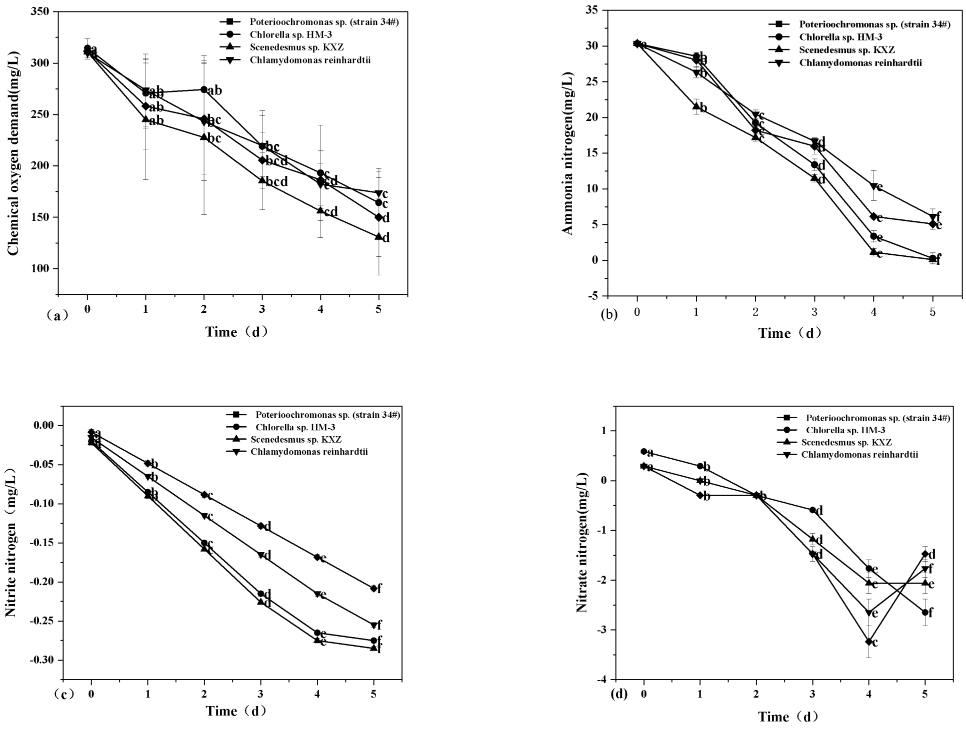


Figure 1 OD_{680} and Chlorophyll Growth of Primordial Chlorella 34#, Common Chlorella HM-3, Chlorella KXZ and Chlamydomonas Rheinalis During 5 Days of Cultivation in Simulated Domestic Sewage

3.1.2 Removal of pollutants from microalgae

In this study, the removal effects of different microalgae on wastewater were evaluated based on the reduction of chemical oxygen demand (COD) and the removal rates of total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH_4-N), and nitrite nitrogen (NO_2^--N). Figure 2 shows the pollutant removal of four types of algae in artificially simulated domestic sewage. The COD of the initial wastewater was approximately 311.33mg L^{-1}, and the initial concentration of total phosphorus was approximately 5.61 mg L^{-1}. The initial concentration of ammonia nitrogen was approximately 30.34 mg L^{-1}, and the initial concentration of total nitrogen was approximately 37.014mg·L^{-1}. After 5 days of cultivation of all microalgae, the removal rates of various pollutants in simulated domestic sewage by common Chlorella HM-3, Protochlorella 34#, Chlorella KXZ and Chlamydomonas rhinolalia, ammonia nitrogen: 99.63%, 99.06%, 79.74%, 83.15%, respectively; Total nitrogen: 87.46%, 89.42%, 61.63%, 65.54%; Total phosphorus: 65.84%, 93.28%, 65.07%, 69.93%. Except for Chlorella 34# and KXZ, the removal rates of COD by the other two microalgae both exceeded 50% (Table 1). At present, research on the use of microalgae to remove nitrogen from sewage has been widely carried out. According to Sydney et al, Chlorella was once cultivated in synthetic wastewater for nitrogen removal research [28]. After 14 days of cultivation, its nitrogen removal efficiency reached 73.77%. A recent study shows that the nitrogen removal rate of chlorella exceeds 98% after 25 days of cultivation [29]. To enhance the efficiency of nitrogen and phosphorus removal, it is usually necessary to extend the culture period of microalgae in experiments. Obviously, when the amount of sewage discharged is large, the ability of microalgae to remove pollutants will be limited. Microalgae tend to store excessive phosphorus to maintain growth and biosynthesis [30-31]. The experimental results of this study show that all microalgae species can effectively remove nutrients. Compared with the other three microalgae, common Chlorella HM-3 shows more significant denitrification activity, indicating that the denitrification capacity is species-specific [32]. The common Chlorella HM-3 can completely remove nitrate nitrogen from simulated domestic sewage on the fifth day, and its ammonia nitrogen removal effect on simulated domestic sewage is better than that of other algae. This means that when the sewage discharge volume is large, this microalgae can be selected for denitrification treatment, and it is also expected to shorten the cultivation period required for microalgae [33].



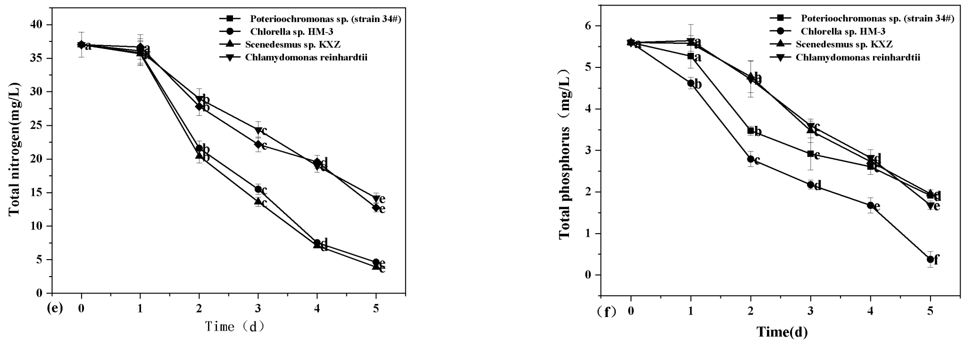


Figure 2 Nutrient Removal Performance of Four Microalgae Strains in Simulated Domestic Sewage Over a 5-Day Cultivation Period: (a) COD, (b) Ammonia Nitrogen, (c) Nitrite Nitrogen, (d) Nitrate Nitrogen, (e) Total Nitrogen, and (f) Total Phosphorus

Figure 2 shows the removal of chemical oxygen demand (COD), ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, total nitrogen and total phosphorus by Protoconchs 34#, Chlorella HM-3, Chlorella KXZ and Chlamydomonas reinhardtii during the 5-day cultivation period in simulated domestic sewage. In the figure, (a - f) respectively correspond to the changes in the removal rates of COD, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, total nitrogen and total phosphorus.
Table 1 shows the removal percentages of chemical oxygen demand (COD), nitrogen and phosphorus by five types of microalgae within a 5-day treatment period.

Table 1 Removal Percentages of COD, Nitrogen, and Phosphorus by Five Microalgae Species Over a 5-Day Treatment Period

Water quality indicator	Culture time	Protoconchs sp. 34#	Chlorella vulgaris HM-3	Scenedesmus sp. KXZ	Chlamydomonas reinhardtii
Chemical oxygen demand removal rate (%)	1	13.903	21.161	11.680	17.145
	2	12.789	26.726	21.555	21.099
	3	30.314	40.347	29.103	34.019
	4	38.588	49.793	41.213	40.136
	5	47.791	57.908	43.933	51.857
Ammonia nitrogen removal rate (%)	1	5.849	29.154	13.239	7.555
	2	36.544	43.365	32.565	39.954
	3	55.870	62.122	45.070	47.344
	4	88.838	96.227	65.533	79.743
	5	99.069	99.638	79.743	83.154
Total nitrogen removal rate (%)	1	3.288	3.680	2.504	0.937
	2	41.660	44.791	21.690	24.823
	3	58.105	63.195	34.219	40.093
	4	79.640	80.815	48.707	47.142
	5	87.470	89.428	61.628	65.543
Total phosphorus removal rate (%)	1	5.849	17.481	0.341	6.348
	2	37.945	50.127	14.725	15.805
	3	47.908	61.193	37.961	35.729
	4	53.441	70.047	51.240	49.564
	5	65.837	93.285	65.071	69.931

3.2 Effects of Microalgae on Wheat Germination

2.2.1 Effects on germination rate

The microalgae suspension treatment and the broken liquid treatment (T1, T2, T3) all achieved higher seed germination rates than the control group (T0). Among them, the germination rates of the suspended algae liquid treatment and the broken liquid treatment (T1) were the highest, at 96% and 76% respectively, and the germination potentials were the highest, at 71% and 51% respectively ($P < 0.05$) (Tables 1 and 2). In the T2 treatment group, the seed germination rate of wheat was significantly increased by 23.75% and 25.43% respectively, and the germination potential was significantly increased by 39.14% and 41.92% respectively. The T3 treatment group significantly increased by 19.93% and 21.06% respectively, and the germination potential significantly increased by 39.87% and 41.73% respectively. Compared with group T2, the germination rate of group T1 increased by 22.63% and 33.33% respectively, and the germination potential increased by 60.2% and 33.33% respectively. Compared with group T3, the germination rates increased by 20.99% and 30.63% respectively, and the germination potentials increased by 0.95% and 1.33% respectively ($P < 0.05$); Compared with group T3, the germination rate and germination potential of group T2 increased by 1.36% and 2% respectively. The germination rate of group T3 was the lowest. For the treatment of microalgae suspensions and crushed liquids, common Chlorella HM-3, primordial Chlorella 34#, Chlorella and Chlamydomonas rheinalis can all increase the germination rate and germination rate of wheat seeds. This might be attributed to the stimulating effect of protein and ash content in microalgae [34]. These greys are rich in essential trace elements such as Ca, magnesium, iron, copper, chromium and zinc [35]. Take the genus Alga as an example, there are also a large number of bioavailable silicon compounds [36]. These trace elements play a crucial role in tissue development and metabolic pathways, including activating enzymes in primary metabolism and the biosynthesis of plant hormones such as auxin [37]. In this study, the treatment with common Chlorella HM-3 (T1) in the microalgae suspension had a more significant effect on the germination rate and germination potential of wheat.

Table 2 Germination Parameters of Wheat Seeds Treated with Different Bio-Fertilizer Suspensions

Treatment method	Germination rate (%)	Germination potential (%)	Root length (cm)	Stem length (cm)
Control	63±8c	50±5c	3.53±0.50d	4.83±0.29d
T1	96±1a	76±8a	4.06±0.39a	6.42±0.11a
T2	78±3b	59±3bc	3.44±0.72c	5.46±0.16b
T3	79±2b	60±5b	3.70±0.51b	5.12±0.56c

Table 3 Germination Parameters of Wheat Seeds Treated with Different Bio-Fertilizer Crushing Liquids

Treatment method	Germination rate (%)	Germination potential (%)	Root length (cm)	Stem length (cm)
Control	38±8d	25±5c	3.77±0.25d	4.47±0.35d
T1	71±1a	51±8a	4.95±0.53a	6.58±0.40a
T2	53±3b	34±3b	3.89±0.24c	5.28±0.39c
T3	54±2c	35±5b	4.26±0.34b	5.85±0.40b

3.3 Effects of Chlorella HM-3 on Soil Physical and Chemical Properties

Different fertilization treatments have a significant impact on the physical and chemical properties of the soil (Figure 3). Compared with the control (T0), the combined application of common Chlorella HM-3 and chemical fertilizers significantly increased the contents of total nitrogen, total carbon, total phosphorus, total potassium, available nitrogen, available phosphorus and available potassium in the soil (Figure 3 (a-g)). At 30 days, it increased by 11.45%, 1.36%, 2.50%, 4.06%, 21.25%, 11.70%, and 5.65% respectively, and at 60 days, it increased by 2.80%, 2.87%, 1.53%, 8.68%, 9.59%, 7.71%, and 3.17% respectively ($p < 0.05$). In the 100% chemical fertilizer application (T2) treatment, the contents of total nitrogen, total carbon, total phosphorus, total potassium, available nitrogen, available phosphorus and available potassium in the soil also increased significantly. At 30 days, it increased by 5.49%, 0.34%, 1.37%, 3.16%, 18.88%, 1.44%, and 4.00%, and at 60 days, it increased by 1.65%, 0.85%, 0.43%, 5.74%, 3.95%, 5.95%, and 2.54% ($p < 0.05$). Among them, the group of common Chlorella HM-3 and chemical fertilizer (T1) had the best effect on improving the physical and chemical properties of the soil. The contents of total nitrogen, total carbon, total phosphorus, total potassium, available nitrogen, available phosphorus and available potassium in 30 days increased by 5.67%, 1.01%, 1.11%, 0.87%, 1.99%, 10.11% and 1.58% respectively compared with the group of all chemical fertilizers. The increase of 60d was 1.13%, 2.00%, 1.10%, 2.77%, 5.42%, 1.66%, and 0.62% ($p < 0.05$). Soil properties are important indicators for measuring soil quality and crop growth [38]. A deficiency of nitrogen and phosphorus can lead to a decline in soil fertility and affect the growth of crops. Chemical fertilizers, as fast-acting fertilizers, can provide nutrients quickly, while microalgae fertilizers, as slow-release bio-fertilizers, can maximize the utilization rate of fertilizer nutrients. They are suitable for providing nutrients for later plant growth and improving soil fertility [39]. Early studies have found that the combined application of organic and inorganic fertilizers has increased the content of soil organic matter and available nutrients, and improved soil quality [40], In this study, the application of common Chlorelma HM-3 and chemical fertilizer treatment (T1) significantly increased the contents of total nitrogen, total carbon, total phosphorus, total potassium, available nitrogen, available phosphorus and available potassium in the soil compared with the total chemical fertilizer treatment (T2).

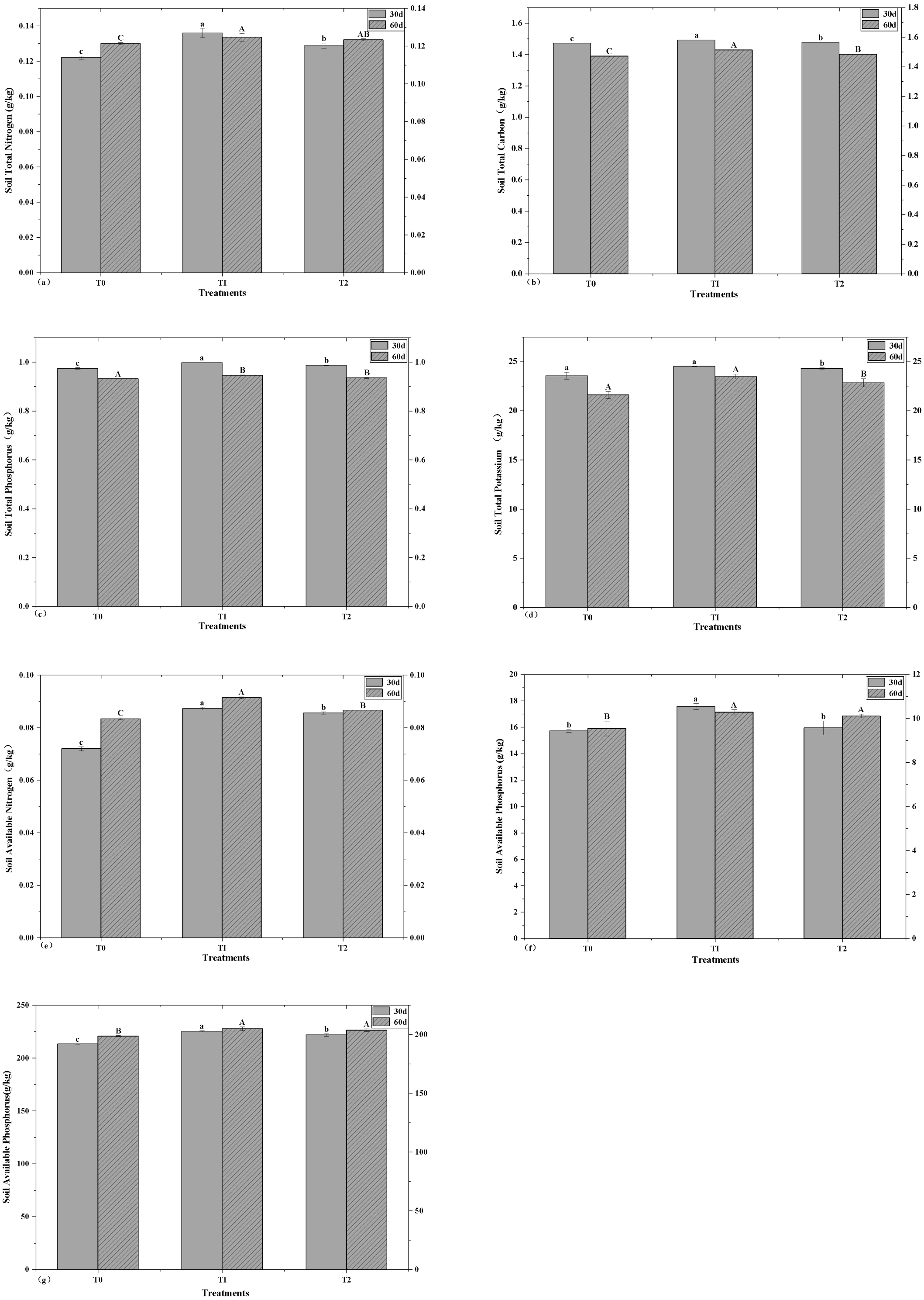


Figure 3 The Influence of Different Fertilization Conditions on the Physical and Chemical Properties of Soil: (a): Total nitrogen (b): Total carbon; (c): Total phosphorus; (d): Total potassium; (e): Available nitrogen; (f): Available phosphorus; (g): Available potassium.

3.4 Effects on Wheat Growth 2.4.1 Effects on Wheat Biomass

3.4.1 Effects on wheat biomass

In acidic purple loam soil, the application of chemical fertilizers and common Chlorella HM-3 can increase the growth parameters of wheat such as root length, plant height and leaf number (Figure 4). Compared with the control treatment, the group applying chemical fertilizers alone (T2 group) increased the root length of wheat by 56.55% and 30.17% respectively at 30 days and 60 days, the plant height by 8.22% and 6.20% respectively, and the number of leaves by 0% and 26.75% respectively. The simultaneous application of chemical fertilizers and common Chloreia HM-3 (Group T1) increased the root length of wheat by 81.28% and 53.27% respectively at 30 days and 60 days, the plant height by 13.34% and 14.99% respectively, and the number of leaves by 26.34% and 49.99% respectively. The combined effect of chemical fertilizers and common Chlorella HM-3 on promoting the root length, plant height and leaf number of wheat is more significant than that of applying chemical fertilizers alone. After 30 and 60 days of planting, the fresh weight of the plants in group T1 was higher than that in group T0. By the 60th day, the fresh weight of each wheat plant in group T1 reached 2.3367g·plant^{-1}, which was significantly increased by 115% compared with group T0 ($P < 0.05$) and 43.35% higher than that of group T2. In conclusion, the combined application of chemical fertilizers and common Chlorellae HM-3 is conducive to the accumulation of wheat biomass, and its effect is superior to that of applying chemical fertilizers alone. Previous studies have shown that the application of microalgae can improve the root length, plant height and stem thickness of wheat, rice, corn and turnip, verifying the positive role of microalgae in promoting plant growth and increasing biomass [41-42]. For instance, the polysaccharides contained in Chlorella can stimulate the germination of wheat seeds and enhance their growth indicators such as dry weight, fresh weight, leaf area, plant height and root length [43]. Some studies have also demonstrated that Chlorella can promote the growth of wheat and improve soil properties, increase the growth of wheat roots and fresh weight, and reduce the use of chemical fertilizers [44]. Microalgae biomass is a residue rich in carbon sources, containing carbohydrates, lipids, proteins and various active molecules. It has multiple benefits such as improving soil health, enhancing the stability of soil aggregates, increasing soil water retention, promoting carbon sequestration and preventing nutrient loss [45-47]; Studies have shown that microalgae can promote wheat growth and improve soil properties, highlighting the use of microalgae as biofertilizers to reduce the use of chemical fertilizers and promote wheat growth [44]. The synergistic effect of microalgae and chemical fertilizers to increase plant biomass is mainly achieved through two pathways: directly promoting plant nutrient absorption and indirectly enhancing soil fertility.

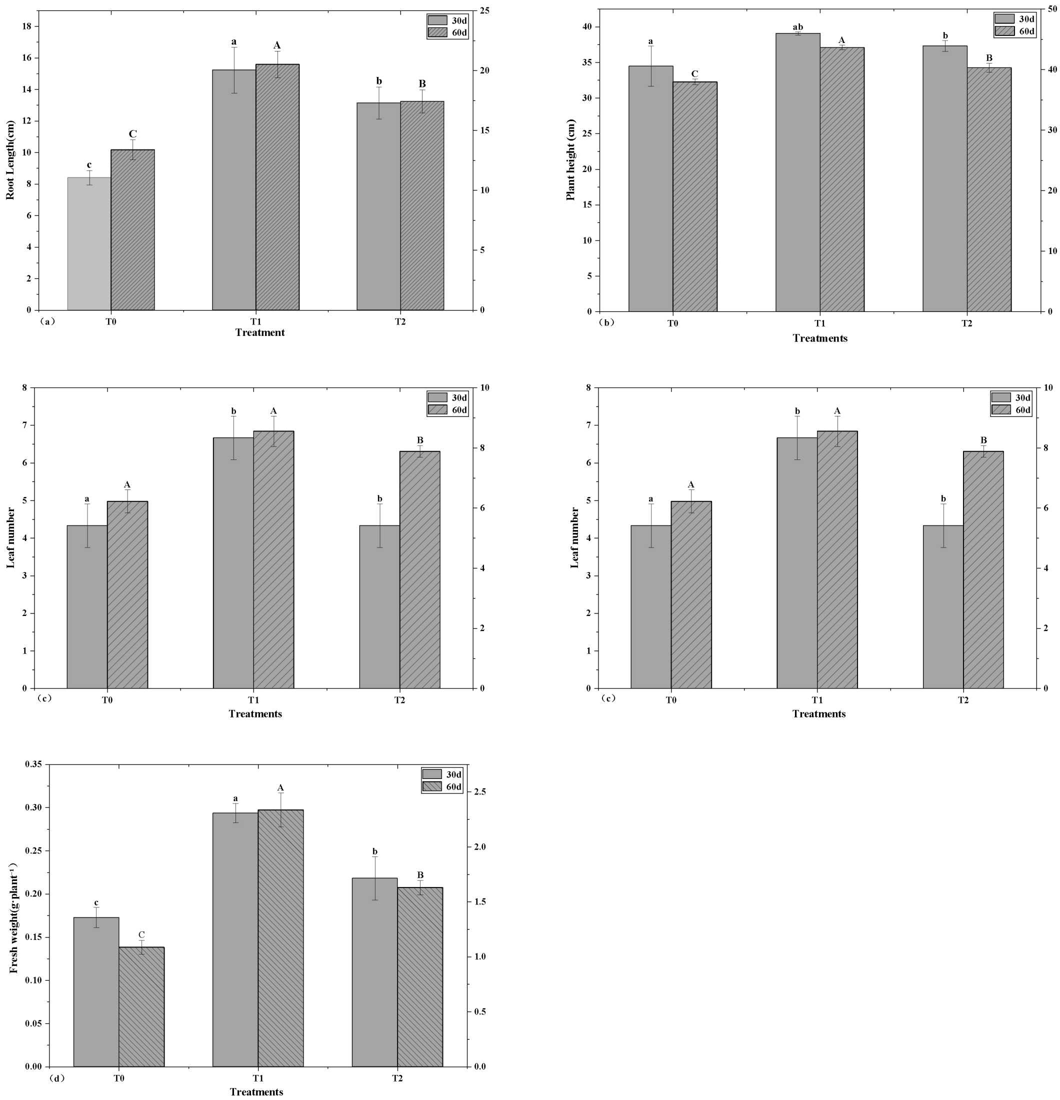


Figure 4 Effects of Different Fertilization Treatments on Plant Growth Indicators

Figure 4 shows the lengths of the roots and the aboveground parts of the plants after treatment (Figures (a) and (b)), as well as the number of leaves and the fresh weight biomass of the intact plants (Figures (c) and (d)). The control group was fertilized with pure water, the treatment group (T1) was fertilized with 50% of the algae and 50% of the chemical fertilizer, and the treatment group 2 (T2) was fertilized with 100% chemical fertilizer.

3.4.2 Effects of HM-3 chlorella on nutritional indicators of wheat

To deeply explore the effects of Chlorella HM-3 on the growth and nutrient accumulation of wheat seedlings, this study conducted juice analysis on wheat seedlings after 30 and 60 days of cultivation. Tables 4 and 5 show the quality indicators of the SAP of wheat seedlings obtained after full chemical fertilizer topdressing and topdressing with chemical fertilizer and common chlorella for 30 days and 60 days respectively. In terms of pigments, compared with the control group, T1 treatment significantly increased the contents of total photosynthetic pigments, chlorophyll a and chlorophyll b in green juice. The contents of total photosynthetic pigment, chlorophyll a and chlorophyll b in the juice of wheat seedlings after 30 days increased by 13.71%, 47.86% and 15% respectively, and those after 60 days increased by 9.79%, 11.16% and 10.08% respectively. The pigment levels in the T2 treatment group were all lower than those in the T1 treatment group. The contents of total photosynthetic pigment, chlorophyll a and chlorophyll b in 30 days were 9.11%, 35.46% and 9.4% respectively, and those in 60 days were 6.33%, 6.14% and 0% respectively. Compared with the total fertilizer treatment group, the T1 treatment significantly increased the contents of total photosynthetic pigments, chlorophyll a and chlorophyll b in green juice. The cumulative pigment contents of total photosynthetic pigment, chlorophyll a and chlorophyll b within 30 days were 9.11%, 9.16% and 5.13% respectively, and those within 60 days were 10.28%, 4.54% and 17.30% respectively ($p < 0.05$). In terms of protein concentration, compared with the control group, after the application of common Chlorellae HM-3 algal fertilizer in group T1, the protein content increased by 79.41% after 30 days and by 38.04% after 60 days. Compared with group T2, the protein content increased by 40.57% at 30 days and by 23.64% at 60 days. The pigment content of wheat planted for 30 days was higher than that of wheat planted for 60 days. The T1 treatment group was significantly higher than the T2 treatment group, especially the increase in chlorophyll a reached 47.86%. In the early growth stage, research has found that compared with full chemical fertilizer treatment, the application of microalgae fertilizer can promote the growth of wheat and increase the pigment content of wheat [44]. The fertilizer efficiency characteristics of green algae microalgae, such as Chlorella oblongata, Chlorella vulgaris, Chlorella tetraphylla, Chlamydomonas rheinalis, Chlorella soroigenia, Chlorella tetraphylla, Dunaliella salina and Chlorella ovale, on crops like wheat, corn, tomatoes, potatoes and lettuce, have gradually been studied [48-54]; These green algae biomass or extracts can have a positive impact on plant growth through pathways such as plant growth hormones, extracellular polysaccharides, and enhanced nutrient availability [50,55-56]. After adding Chlamydomonas powder of different concentrations, the content of soluble sugar and soluble protein increased [57]. The research findings of Chojnacka et al. (2015) and Fan et al. (2013) indicated that the application of commercial algal extracts to certain plants could increase the content of soluble total protein and amino acids, and these components were significantly positively correlated with the elevated transcriptional levels of enzymes related to nitrogen metabolism [58-59]. Microalgae can synthesize a rich variety of bioactive components, providing crops with abundant mineral nutrients and promoting their metabolic growth [60], The calcium content in the above-ground parts of sesame plants treated with spirulina extract and powdered Sargassum was significantly increased compared with the control group. It is worth noting that all microalgae extracts and powder treatments promoted the accumulation of magnesium in sesame seeds, among which the treatment group with added powdered Sargassum or spirulina extracts achieved the highest percentage of magnesium content [61]. In addition, the research observed that after the application of seaweed extract, the antioxidant defense system in plants was activated, and the synthesis of non-enzymatic antioxidant substances (such as carotenoids and phenolic compounds) was simultaneously enhanced. The contents of endogenous stress-related components in plants (including proline, cytokinin and antioxidant substances) all increased [59,62]. The application of microalgae increased the total flavonoid and phenolic content of broccoli plants and enhanced nutrient absorption [63]. This study indicates that Chlorelma HM-3 can enhance the pigment, soluble protein, amino acids, mineral elements (calcium, magnesium), plant flavonoids, and total plant phenols in wheat, which is similar to previous research.

Table 4 Quality Indicators of Wheat Seedling Juice Treated with Chlorella for 30 Days

All indicators	Control	T1	T2
Chlorophyll a (μ g/100 g FW)	0.92± 0.08b	1.36 ± 0.03a	1.25 ± 0.06a
Chlorophyll b (μ g/100 g FW)	27.59 ± 0.11c	31.73 ± 0.77b	30.18 ± 0.19a
Total photosynthetic pigments (μ g/mL FW)	19.99 ± 0.24c	22.73 ± 0.46b	20.83 ± 0.13a
Protein (μ g/ mL FW)	0.40 ± 0.01c	0.71 ± 0.00a	0.51 ± 0.01b
Amino acid (μ g/ mL FW)	6.81 ± 0.29c	8.69 ± 0.36a	7.57 ± 0.22b
Calcium (mmol/L FW)	2.82 ± 0.15b	3.27 ± 0.16a	3.08 ± 0.11ab
Magnesium (mmol/L FW)	0.59 ± 0.01b	0.61 ± 0.00a	0.61 ± 0.01a
Total phenols (mmol/L FW)	1.38 ± 0.03c	2.53 ± 0.08a	2.25 ± 0.00b
Total flavonoids (mmol/L FW)	5.06 ± 0.86b	6.94 ± 0.17a	6.15 ± 0.37ab

Table 5 Quality Indicators of Wheat Seedling Juice Treated with Chlorella for 60 Days

All indicators	Control	T1	T2
Chlorophyll a (μ g/100 g FW)	19.30± 0.49b	21.45± 0.97ab	20.52 ± 0.60a

Chlorophyll b (μ g/100 g FW)	51.16 ± 1.84b	56.32 ± 1.09a	48.01 ± 2.78a
Total photosynthetic pigments (μ g/mL FW)	60.22 ± 1.68a	66.12 ± 1.75a	59.95 ± 2.75b
Protein (μ g/ mL FW)	0.42 ± 0.00a	0.59 ± 0.00a	0.47 ± 0.00a
Amino acid (μ g/ mL FW)	12.27 ± 0.26c	14.43 ± 0.30a	13.35 ± 0.19b
Calcium (mmol/L FW)	2.83 ± 0.05c	3.37 ± 0.01a	3.10 ± 0.06b
Magnesium (mmol/L FW)	0.55 ± 0.00c	0.77 ± 0.01a	0.62 ± 0.00a
Total phenols (mmol/L FW)	1.54 ± 0.12c	2.71 ± 0.19a	2.42 ± 0.08b
Total flavonoids (mmol/L FW)	3.08 ± 0.00a	4.56 ± 0.86a	4.07 ± 1.49a

4 CONCLUSIONS

In conclusion, microalgae can recover nitrogen and phosphorus from simulated domestic sewage [33], Synthesize abundant biomass, provide rich mineral nutrition for crops, and promote the metabolic growth of crops [10]. This study reached the following conclusion: After treating the simulated domestic sewage with Chlorella-HM-3 for 5 days, more nitrogen and phosphorus were recovered from the simulated domestic sewage than other algal strains, with recovery rates of 99.63% and 65.84% respectively. In addition, replacing 30% of the chemical fertilizer with Chlorella HM-3 can promote the root growth, increase the plant height and the number of leaves of wheat plants compared with applying 100% of the chemical fertilizer. In addition, replacing 70% of the chemical fertilizer with Chlorella HP-3 can lead to the accumulation of pigments in wheat green juice, increase the content of soluble protein, raise the total amount of amino acids, increase the content of mineral elements such as calcium and magnesium, and significantly enhance the content of plant flavonoids and total plant phenols compared to applying 100% of the chemical fertilizer.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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