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Effects of Microbial Biochar-Based Fertilizer on Yield and Quality of Rice in Cadmium-Contaminated Paddy Fields

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Abstract: A randomized block design was used to set up three treatments: "biochar-based fertilizer + microbial inoculant + silicon foliar fertilizer" (microbial biochar-silicon fertilizer), "biochar-based fertilizer + microbial inoculant" (microbial biochar-based fertilizer), and regular compound fertilizer (control). A large-scale demonstration of the field effectiveness of microbial biochar-silicon fertilizer was also conducted. The study investigated its effects on the growth physiology and grain cadmium content of rice variety Longjing Youdi in cadmium-contaminated soil. The results showed that the net photosynthetic rate of rice flag leaves during the grain-filling stage in the microbial biochar-silicon fertilizer and microbial biochar-based fertilizer treatments was significantly different from the control, with the order being microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer. Microbial biochar-based fertilizer can enhance the rice's physiological resistance, improve the photosynthetic capacity during the booting, heading, and grain-filling stages, and increase the plant height and relative yield at harvest. The rice in the microbial biochar-silicon fertilizer demonstration fields grew uniformly and had full grains. Field measurement results showed that the actual yield of the microbial biochar-silicon fertilizer demonstration model was 558.6 kg/667m2, an increase of 9.2% compared to the control model. The grain cadmium content in the microbial biochar-silicon fertilizer demonstration model was 0.225 (mg/kg), a reduction of 79.0% compared to the control model. The effects of increasing yield and reducing grain cadmium content in the microbial biochar-silicon fertilizer demonstration model were significant, thereby improving grain quality.

1. Introduction

In recent years, with the rapid development of agricultural science and technology and the intensification of agricultural production, significant changes have occurred in the methods of agricultural production. Under these circumstances, the role of chemical fertilizers in agricultural production has become increasingly important. To improve yields, the use of chemical fertilizers has been continuously increasing. However, this approach has also led to serious problems such as agricultural soil environmental pollution. Relevant studies indicate that the proportion of agricultural soil pollution in global water pollution is increasing year by year, with about 30-50% of surface water globally affected by agricultural soil pollution [1-3].

Cadmium is a non-essential element for plant growth, and cadmium present in nature is one of the highly pathogenic elements for humans [4-6]. Rice is one of the world's major food crops [7]. Cadmium enters rice mainly through roots and leaves, accumulates in the grains, and eventually enters the human body through the food chain, thus posing a risk to human health [8]. Therefore, technical measures to reduce the availability of cadmium in arable soil and the cadmium content in rice grains have attracted attention. Rational use of soil conditioners can improve soil structure, regulate soil pH, and ameliorate or repair heavy metal-contaminated soils, etc. [9]. In recent years, new materials such as biochar and microbial inoculants, which are interdisciplinary materials, have received close attention. Biochar is a porous carbonaceous material produced by the high-temperature pyrolysis of biomass under limited or no oxygen conditions, and it is recognized as a good adsorbent material for slow-release fertilizers, soil improvement, and environmental restoration, etc. [10]. Rice husk carbon, produced by the pyrolysis of rice husks at 500 ℃ using tap water as a catalyst under anoxic conditions, contains high silicon and low cadmium [11]. Microbial inoculants containing bacteria such as Bacillus mucilaginosus, Bacillus megaterium, Bacillus subtilis, Trichoderma, photosynthetic bacteria, and mycorrhizae, when used in combination with fertilizers, can improve the photosynthetic rate, root vitality, water use efficiency, and fertilizer utilization rate of crops such as wheat, potatoes, and melons, achieving the purpose of increasing yield [12-14]. Silicon, a beneficial element required in large amounts by most plants [15], can improve the photosynthetic rate and dry matter accumulation in plants, improve water metabolism and water use efficiency within the plant, and mitigate both biotic and abiotic stresses in rice [16-18], which is a silicon-loving plant [19]. The combination of biochar with mineral fertilizers, and the interaction between mineral elements and biochar, can enhance the tolerance of rice under different types of abiotic stress [20-21]. The application of interdisciplinary soil conditioner technology research materials such as rice husk biochar, active silicon, and microbial inoculants to prepare a multifunctional material "microbial biochar-based fertilizer" and explore its effect mechanism on the growth physiology and cadmium absorption of rice in the cadmium-contaminated soil-rice ecosystem has not been reported. Therefore, when applying rice husk biochar, it is also necessary to use foliar active silicon fertilizer and functional microbial inoculants together. Through synergistic and complementary effects, they enhance the soil's acid buffering capacity and improve the physiological and biochemical resistance of rice roots to cadmium, thereby achieving the purpose of improving rice yield and quality.

2. Materials and Methods

2.1 Experimental Materials

The late rice variety used in the experiment is a high-quality, high-yielding new three-line hybrid rice variety called Longjing Youdi. The nutrient content of the biochar-based compound fertilizer (N+P2O5+K2O) = 25%. The nutrient content of the regular compound fertilizer (N+P2O5+K2O) = 40%. The total nitrogen (N) content of urea = 46.4%. The basic physicochemical properties of the soil are as follows: The soil type is gleyic paddy soil, pH = 6.00, organic matter content 41.37 g kg^{-1}, total nitrogen content 2.58 g kg^{-1}, total phosphorus content 1.26 g kg^{-1}, total potassium content 15.27 g kg^{-1}, hydrolyzable nitrogen content 264.25 g kg^{-1}, available phosphorus content 33.50 g kg^{-1}, available potassium content 143.01 g kg^{-1}, cadmium content 1.84 g kg^{-1}. Rice husk biochar is produced by biomass multi-energy production technology of rice husks, with a pyrolysis temperature under limited oxygen conditions of 500 ℃. The basic physicochemical properties of rice husk biochar are as follows: pH = 9.97, total specific surface area = 33.70 m^2/g, carbon content = 75.00 g/kg, phosphorus content = 0.19 g/kg, potassium content = 0.96 g/kg, silicon content = 501 mg/kg, cadmium content = 0.29 g ·kg^{-1}. The microbial inoculant is a solid and inactivated self-produced mixed inoculant, containing Bacillus mucilaginosus, Bacillus megaterium, Bacillus subtilis, Trichoderma, photosynthetic bacteria, and mycorrhizae. When activated for use, the number of viable cells is not less than 2 billion CFU/g, with a total effective viable count of ≥ 100 billion CFU/g. The active silicon is a cadmium-reducing silicon fertilizer produced by a certain environmental technology company, with a silicon content of 85 g/L.

2.2 Field Experiment Design and Rice Cultivation Management

The mid-season rice field experiment was conducted in 2022 in a rice-growing area of a certain county in Hunan Province. The field experiment adopted a randomized block design. The experimental field included experimental plots, isolation rows, drainage ditches, and protective rows. Each experimental plot was 30 m^2 (6m in length and 5m in width). Isolation rows (30cm in width) were set between the plots, covered with black polyethylene plastic film extending 30 cm below the field surface. The drainage ditches were 0.5m wide. Protective rows were more than 1.5m wide. Each experimental plot implemented a single irrigation and single drainage water irrigation method. Three treatments were set up: "biochar-based fertilizer + microbial inoculant + silicon foliar fertilizer" (microbial biochar-silicon fertilizer), "biochar-based fertilizer + microbial inoculant" (microbial biochar-based fertilizer), and regular compound fertilizer (control), with each treatment replicated three times. For the microbial biochar-silicon fertilizer and biochar-based fertilizer treatments, 900 kg/hm^2 of rice husk biochar was first applied as a soil conditioner, incubated in the soil for 7 days in a one-time application. For the fertilization treatments, 900 kg/hm^2 of biochar-based fertilizer and 600 kg/hm^2 of regular fertilizer were applied as base fertilizers in a one-time application, with the applied amounts of biochar-based and regular fertilizers having equivalent effective nutrient contents (N+P2O5+K2O). A top-dressing of urea at 60 kg/hm^2 was applied during the mid-tillering stage of rice. At the initial stage of rice tillering, 60 kg/hm^2 of microbial inoculant was added to the field. Silicon foliar fertilizer was applied during the rice booting stage and flowering stage, spraying 100ml of cadmium-reducing silicon fertilizer concentrate mixed with 15kg of tap water per 667m^2 of rice to the leaves. Additionally, a large-scale demonstration area of "biochar-based fertilizer + microbial inoculant + silicon foliar fertilizer" (microbial biochar-silicon fertilizer) covered 3 hm^2. During the growth of rice, field management followed conventional practices, with all plots receiving the same management measures such as irrigation, weeding, and pest control, apart from the fertilization treatments.

2.3 Sample Collection and Measurement Methods

2.3.1 Measurement of Soil Electrical Conductivity

During the rice booting stage, heading stage, and grain-filling stage, the soil electrical conductivity in the rice field plots is measured using a WET-2 sensor (Delta-T Devices, Cambridge, UK).

2.3.2 Measurement Methods for Leaf Chlorophyll Relative Content and Photosynthesis Parameters

During the rice booting stage, heading stage, and grain-filling stage, the relative chlorophyll content (SPAD value) of rice leaves is measured using a handheld SPAD502. Photosynthesis parameters of the flag leaf are measured using a LI-6400 portable photosynthesis system, with a photon flux density of 1000 μmolCO2·m^{-2} s^{-1}. Measurements are taken on clear, windless days, between 9:00-11:00 am.

2.3.3 Measurement of Plant Height and Relative Yield

Plant height is measured with a tape measure from the base of the stem to the tip of the panicle at harvest. After harvesting, the thousand-grain weight and relative yield are measured.

2.3.4 Measurement of Grain Heavy Metal Cadmium Content

Randomly select rice fields for harvesting with a combine harvester. The moisture content of the rice grains (seeds) is determined using a moisture meter after threshing and cleaning, and converted to actual grain yield (moisture content 13.5%). The grain heavy metal cadmium content is determined on-site using an X-ray fluorescence heavy metal analyzer, utilizing high precision X-ray fluorescence (HDXRF) technology.

2.4 Data Processing

Data calculation, charting, and statistical analysis are performed using Microsoft Excel 2010 and the Data Processing System analysis software (IBM SPSS v20). Statistical analyses are conducted, and Duncan's multiple range test is used for multiple comparison analyses.

3. Results and Analysis

3.1 The Effect of Biochar-Based Fertilizer on Soil Electrical Conductivity

During the booting stage, heading stage, and grain-filling stage of rice, compared to the control, the application of biochar-based fertilizer treatment had a significant impact on the soil electrical conductivity of the rice field (Table 1). The addition of foliar silicon fertilizer, as observed from the average values of soil electrical conductivity, indicates that there were significant differences in soil electrical conductivity between the microbial biochar-silicon fertilizer and microbial biochar-based fertilizer treatments compared to the control. Soil electrical conductivity was not affected by the rice growth stage.

Table 1: The effect of microbial biochar-based fertilizer on soil electrical conductivity in rice fields

Treatment	Microbial Biochar-Silicon Fertilizer	Microbial Biochar-Based Fertilizer	Regular Fertilizer
Soil Electrical Conductivity during Booting Stage (μs/cm)	0.686±0.012a	0.708±0.011a	0.624±0.012b
Soil Electrical Conductivity during Heading Stage (μs/cm)	0.711±0.013a	0.705±0.012a	0.607±0.011b

Note: The different small letters within the same level in the table indicate siganificant differences between treatments at $P<0.05$ level according to Duncan’s multiple range test. The table below is the same.

3.2 The Effect of Biochar-Based Fertilizer on Leaf Chlorophyll

During the booting stage, heading stage, and grain-filling stage of rice, compared to the regular fertilizer treatment, the application of microbial biochar-based fertilizer treatment had a significant difference in the relative chlorophyll content (SPAD value) of rice leaves (Table 2). Compared to the microbial biochar-based fertilizer treatment and control, the application of foliar silicon fertilizer in the microbial biochar-silicon fertilizer treatment could increase the relative chlorophyll content of rice leaves. However, there was only a slight increase in the relative chlorophyll content of leaves between the microbial biochar-silicon fertilizer and microbial biochar-based fertilizer treatments, with no significant difference.

Table 2: The effect of microbial biochar-based fertilizer on the relative chlorophyll content of rice leaves

Treatment	Microbial Biochar-Silicon Fertilizer	Microbial Biochar-Based Fertilizer	Regular Fertilizer
SPAD Value during Booting Stage	42.5±0.2a	42.3±0.1a	38.8±0.2b
SPAD Value during Heading Stage	43.9±0.3a	42.6±0.2a	39.1±0.1b
SPAD Value during Grain-Filling Stage	44.1±0.1a	43.2±0.2a	37.9±0.2b

3.3 The Effect of Microbial Biochar-Based Fertilizer on the Photosynthetic Parameters of Flag Leaves during the Grain-Filling Stage

As shown in Table 3, during the grain-filling stage, the net photosynthesis rate of rice flag leaves was the lowest in the control, showing significant differences between microbial biochar-silicon fertilizer, microbial biochar-based fertilizer, and the control. The order of net photosynthesis rates was microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer, with the net photosynthesis rate for microbial biochar-silicon fertilizer being 19.07μmolCO_2 m^{-2} s^{-1}, an increase of 25.46% compared to the control. The order for transpiration rate, stomatal conductance, and intercellular CO_2 concentration was also microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer. This indicates that the treatment pattern of "biochar-based fertilizer + microbial inoculant + silicon foliar fertilizer" can significantly improve the photosynthetic capacity of rice during the grain-filling stage.

Table 3: The effect of microbial biochar-based fertilizer on the photosynthetic parameters of rice flag leaves during the grain-filling stage

Treatment	Microbial Biochar-Silicon Fertilizer	Microbial Biochar-Based Fertilizer	Regular Fertilizer
Net Photosynthesis Rate/μmolCO_2 m^{-2} s^{-1}	(19.07±0.21)a	(18.77±0.78)a	(15.20±1.51)b
Stomatal Conductance/ mmol m^{-2} s^{-1}	(0.79±0.11)a	0.80±0.06)a	(0.75±0.02)a
Transpiration Rate/ g ·m^{-2} h^{-1}	(12.83±0.15)a	(12.80±0.25)a	(11.67±0.15)a
Intercellular CO2 Concentration/μmolCO_2 mol^{-1}	(331.00±1.00)a	(330.33±1.53)a	(320.67±3.06)b

3.4 The Effect of Microbial Biochar-Based Fertilizer on Plant Height and Yield Components at Harvest

As shown in Table 4, the order of rice plant height at harvest was microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer, with a significant difference between microbial biochar-silicon fertilizer and regular fertilizer treatments. The order of thousand-grain weight among treatments was microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer, with the smallest value for regular fertilizer treatment being 18.04g, and the largest value for microbial biochar-silicon fertilizer treatment being 19.62g. There was no significant difference in the thousand-grain weight between microbial biochar-silicon fertilizer, microbial biochar-based fertilizer, and regular fertilizer treatments. The relative yield of the microbial biochar-silicon fertilizer treatment increased by 9.37% compared to the regular fertilizer treatment, and the microbial biochar-based fertilizer treatment increased by 8.94% compared to the regular fertilizer treatment. The order of relative yield was microbial biochar-silicon fertilizer > microbial biochar-based fertilizer > regular fertilizer, with both microbial biochar-silicon and microbial biochar-based fertilizer treatments showing a significant difference compared to the regular fertilizer treatment. The results indicate that microbial biochar-based fertilizer can increase plant height at harvest and significantly increase relative yield.

Table 4: Effect of biochar based fertilizer on plant height and yield composition at harvest

Treatment	Microbial Biochar-Silicon Fertilizer	Microbial Biochar-Based Fertilizer	Regular Fertilizer
Plant Height/cm	(114.00 ±3.05)a	(113.97 ±3.30)ab	(108.27 ±9.71)b
Thousand-Grain Weight/g	(19.62 ±0.20)a	(18.43 ±0.34)ab	(18.04 ±0.78)b
Yield/g	(58.48 ±3.49)a	(58.25 ±3.52)a	(53.47 ±3.52)b
Increase Rate/%	9.37	8.94	/

3.5 The Effect of Microbial Biochar-Based Fertilizer on Field Measured Yield and Grain Cadmium Content

The rice in the microbial biochar-silicon fertilizer demonstration field grew uniformly and well-colored, with full grains and no obvious pest damage. As shown in Table 5, field measurement results indicated that the actual yield of the microbial biochar-silicon fertilizer demonstration model was 558.6 kg/667m^2, an increase of 9.2% compared to the control model. The grain cadmium content in the microbial biochar-silicon fertilizer demonstration model was 0.225 (mg/kg), a reduction of 79.0% compared to the control model. The microbial biochar-silicon fertilizer demonstration model significantly increased yield and reduced grain cadmium content.

Table 5: The effect of microbial biochar-based fertilizer on field measured yield and grain cadmium content

Application Model	Harvest Area(m^2)	Fresh Grain Yield(kg)	Moisture Content(%)	Actual Yield (kg/667 m^2)	Grain Cadmium Content (mg/kg)
Microbial Biochar-Silicon Fertilizer	450.4	413.0	21.0	558.6	0.225
Control	735.5	616.1	20.8	511.6	1.048

4. Discussion

4.1 Microbial Biochar-Based Fertilizer Enhances the Physiological Resistance of Rice

The physiological resistance of rice to stress is typically indicated by the content of malondialdehyde, a product of lipid peroxidation in leaves during stress and natural senescence, antioxidant enzyme activity, and electrical conductivity. Peroxidase, catalase, and superoxide dismutase are three key enzymes in the plant antioxidant enzyme system, which can eliminate free radical damage to plant cells under stress conditions, promoting plant growth [22-24]. Rice husk biochar, with its porous structure and active silicon content, possesses strong adsorption properties. Beneficial microbes can promote plant growth and enhance the ability to resist stress conditions [25]. Silicon can increase the activity of superoxide dismutase; the application of silicon fertilizer can promote the growth and development of the aerial parts of rice, significantly increasing the accumulation of dry matter in the aerial parts [26-28]. Microbes active around the root system can promote plant growth and enhance the ability to resist stress conditions [29]. This study also indicates that the use of microbial biochar-based fertilizer improves soil electrical conductivity and enhances the physiological resistance of rice to stress.

4.2 Microbial Biochar-Based Fertilizer Can Improve the Photosynthetic Capacity of Rice, Increase Plant Height and Relative Yield at Harvest

Biochar can improve the net photosynthesis rate of crops [30]. Silicon fertilizer can convert the free iron and aluminum in phosphate fertilizers into insoluble forms, thereby reducing the fixation of phosphorus; dissolved silicic acid entering the lattice of soil clay minerals can reduce soil adsorption of phosphorus, thus improving crop fertilizer utilization. This study shows that rice husk biochar, microbes, and active silicon synergistically promote the growth physiology of rice, consistent with the findings of Xu Yi et al. [31]. Treatments with microbial biochar-silicon fertilizer and microbial biochar-based fertilizer resulted in increased plant height compared to the control, directly related to improved soil nutrient elements and root system plant hormone homeostasis. Additionally, the application of microbial biochar-based fertilizer can improve the relative yield of rice, closely related to the combined application of carbon-silicon-microbes improving rice yield. In summary, microbial biochar-based fertilizer can improve the photosynthetic capacity of rice, increase plant height, enhance plant biomass, thereby increasing yield.

4.3 Microbial Biochar-Based Fertilizer Can Reduce Cadmium Content in Grains, Improving Grain Quality

Rice husk biochar, with its porous structure, has strong physical adsorption properties. Additionally, rice husk biochar is alkaline, has a low surface positive charge density, contains many alkaline salts, and exposes a large number of adsorption groups such as amino, phosphate, carboxyl, and organic hydroxyl groups, which helps to enhance the precipitation and adsorption capacity of rice husk biochar for cadmium ions [32]. Therefore, the cadmium content in grains treated with microbial biochar-based fertilizer was significantly lower than in the control in this study. Silicon in potassium silicate mainly forms Si-Cd coprecipitates with cadmium, which are poorly soluble and immobile; silicon is a beneficial element for plant growth and helps plants overcome biotic and abiotic stress caused by diseases [33]. The porous structure of rice husk biochar can provide habitats for microbes, some of which can block the movement of cadmium in the soil. Therefore, microbial biochar-based fertilizer can reduce the cadmium translocation coefficient from stem to grain, thereby reducing cadmium content in grains and improving grain quality.

5. Conclusions

In conclusion, the findings of this study underscore the multifaceted benefits of applying microbial biochar-silicon fertilizer in rice cultivation, particularly in cadmium-contaminated soils. By enhancing plant growth and physiological resilience, increasing yield, and significantly reducing grain cadmium content, this treatment represents a promising approach to sustainable agriculture. It offers a viable solution for improving crop productivity and safety in polluted environments, thus contributing to the overarching goals of environmental protection and public health promotion. These results advocate for the broader adoption and further research into biochar-based fertilizers combined with microbial inoculants and silicon, aiming at optimizing their formulation and application methods for wider agricultural and environmental benefits.

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