Effects of Different Application Methods of a Bacteria-enzyme Compound Preparation on Rice Yield, Soil Nutrients, and Economic Benefits

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Abstract [**Objectives**] This study was conducted to clarify the effects of bacteria-enzyme compound preparations and their application methods on rice yield, yield components, growth at the maturation stage, soil nutrients, and economic benefits. [**Methods**] The medium-late maturing japonica rice variety Runnong 303 was used as the test material, and a field experiment was conducted in Laixi City, Shandong Province. Based on equal amounts of conventional fertilization, three treatments were set up: conventional fertilization (CK), conventional fertilization + one application of compound enzyme preparation at the sowing stage (base fertilizer) (T_1), and conventional fertilization + one application of compound enzyme preparation during the basal fertilization + seven foliar sprays of microbial agent at 10 – 15 d intervals during the growth period (T_2). [**Results**] The application of the bacteria-enzyme compound preparation significantly increased rice yield, and the effect was enhanced with the application frequency of the microbial agent increasing. The actual yields of T_1 and T_2 were significantly increased by 11.61% and 27.42%, respectively, compared with the CK, and the yield increase was mainly attributed to the significant increase in the number of grains per panicle. At the maturation stage, the plant height, basal stem diameter, leaf width, and leaf length of T_1 and T_2 were all significantly greater than those of the CK. At harvest, soil nutrients were significantly improved with the application frequency of the microbial agent increasing. Based on the assumed prices, the net economic benefits of T_1 and T_2 were increased by 2 626.5 and 4 057.5 yuan/hm^2, respectively, compared with the CK, and the marginal output-input ratios were 8.96 and 2.54, respectively. In summary, the application of the bacteria-enzyme compound preparation can significantly increase rice yield, improve soil nutrients, and enhance planting economic benefits. Multiple applications of the microbial agent throughout the entire growth period (T_2) achieved the greatest total increases in yield, soil fertility, and income, while a single application at the sowing stage (T_1) exhibited the highest input-output efficiency. The corresponding application method can be selected according to production objectives. [**Conclusions**] This study provides a theoretical basis for the scientific and efficient application of bacteria-enzyme compound preparations.

Key words Bacteria-enzyme compound preparation; Microbial agent; Application method; Rice; Yield
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Rice is one of the most important food crops in China, and its stable and high yield is directly related to national food security. For a long time, the production-increasing model characterized by high chemical fertilizer input has increased rice yield, while also causing problems such as declining fertilizer use efficiency, soil degradation, and agricultural non-point source pollution[1]. Under the national strategy of promoting chemical fertilizer reduction, efficiency enhancement, and green agricultural development, how to maintain and improve rice yield while reducing chemical inputs has become an urgent issue in current production[2].

Microbial agents are live microbial preparations made by industrial-scale propagation and processing of one or more functional microorganisms. They can promote crop growth through multiple pathways, including activating immobilized nitrogen, phosphorus, and potassium nutrients in soil, secreting plant hormones and growth-promoting substances, regulating rhizosphere microbial communities, inhibiting soil-borne pathogens, and enhancing plant stress resistance[3 – 4]. However, the actual efficacy of microbial agents is closely related to their application methods and application rates. Li *et al.*[5] pointed out that different application methods of biofertilizers can significantly alter their regulatory effects on nitrogen accumulation dynamics and nitrogen use efficiency in rice. Zeng *et al.*[6] further found that the application rate of microbial agents has significant effects on soil chemical properties, enzyme activity, and bacterial communities. It indicates that the time, frequency, and dosage of microbial agent application are key factors determining their field effectiveness.

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However, there is still a lack of systematic exploration for this approach in practical production, particularly regarding multiple applications throughout the entire growth period in combination with rice growth characteristics. Therefore, in this study, a field experiment was conducted in Laixi, Shandong, to systematically analyze the effects of different application methods of a bacteria-enzyme compound preparation on rice yield, yield components, growth at the maturation stage, and soil nutrients, and to evaluate its economic benefits. This study will provide a basis for the scientific and efficient application of bacteria-enzyme compound preparations.

Materials and Methods

General situation of the experimental site

The experiment was conducted in 2025 in Xinglongzhai Village, Yuanshang Town, Laixi City, Qingdao, Shandong Province (36°48′N, 120°17′E). The experimental site is located at an altitude of 48 m, and has an average annual precipitation of 717 mm and an average annual temperature of 14.5 ℃. The soil type is loam, and the planting mode was winter wheat-rice rotation. After the previous wheat crop was harvested, the straw was crushed and returned to the field, and water and fertilizers were managed uniformly. The physicochemical properties of the 0 – 20 cm soil layer before sowing are shown in Table 1.

Table 1 Basic information of the experimental site

Experimental site	pH	Organic matter // g/kg	Total nitrogen // g/kg
Xinglongzhai Village, Yuanshang Town	7.84	12.24	1.22

Experimental site	Hydrolysable nitrogen mg/kg	Available phosphorus mg/kg	Available potassium mg/kg
Xinglongzhai Village, Yuanshang Town	76.15	13.92	93.38

Test materials

The test variety was the medium-late maturing japonica rice Runnong 303, which was approved by Shandong Province in 2019 with a growth period of approximately 158 d. The bacteria-enzyme compound preparation used in this study included a compound enzyme preparation (powder) and a microbial agent (liquid), both provided by the Shandong Academy of Agricultural Sciences. The compound enzyme preparation contained protease > 180 IU, lipase > 80 IU, amylase > 280 IU, and cellulase > 5 IU. The microbial agent contained effective viable bacterial count $\geq 2.0 \times 10^8$ CFU/g (Bacillus amyloliquefaciens and Bacillus licheniformis).

Experimental design

The experiment adopted a randomized complete block design with three treatments in three replicates, totaling nine plots. Each plot had an area of 15 m × 2 m = 30 m^2, with a planting density of 210 000 holes per hectare and a row spacing of 30 cm × 13 cm. Field management was consistent in all treatments. In specific, land preparation and base fertilizer application were carried out in mid-June after wheat harvest. Sowing was conducted on June 20, and water and fertilizer management as well as pest, disease, and weed control were carried out according to local conventional practice throughout the entire growth period. Harvesting was conducted in late October. Basal fertilization (identical for all treatments): Organic fertilizer was applied at 7 500 kg/hm^2 on June 14; 15-15-15 compound fertilizer was applied at 160 kg/hm^2 by unmanned aerial vehicle (UAV) on July 19; and compound fertilizer was applied at 320 kg/hm^2 on August 30. On the basis of equal basal fertilization, the compound enzyme preparation and the microbial agent were applied according to the experimental design. Specifically, the CK received no microbial agent throughout the entire growth period; T_1 received only one application of the compound enzyme preparation (9 kg/hm^2, diluted 500-fold and sprayed onto the soil surface by UAV) together with the base fertilizer on June 14; and T_2 received, on the basis of the basal application, foliar sprays of the microbial agent (4.5 L/hm^2 each time, diluted 5-fold) on July 19, July 25, August 7, August 19, September 4, September 15, and September 26, at 10 – 15 d intervals according to rice growth characteristics, totaling eight applications throughout the entire growth period (one basal application of the compound enzyme preparation + seven foliar applications of the microbial agent). The spray volume for foliar application was in accordance with local UAV operational parameters.

Measurement items and methods

Yield Yield and yield components were measured. At the yellow ripening to maturation stage, plot-based yield measurement was conducted. Specifically, three 1 m^2 quadrats were randomly selected in each plot to investigate the number of effective panicles, and representative plants were selected to determine the number of grains per panicle, seed setting rate (filled grains/total grains × 100%), and 1 000-grain weight (1 000 filled grains were dried at 80 ℃ to constant weight). In addition, a representative area of 2 m^2 in the middle of each plot was harvested and threshed, and the grains were air-dried to a moisture content of 13.5% and weighed to calculate the actual yield. Theoretical yield was calculated based on the product of the number of effective panicles, the number of grains per panicle, seed setting rate, and 1 000-grain weight. Plant height, basal stem diameter, leaf width, and leaf length were also measured. Each treatment had three replicates.

Soil physicochemical properties Soil samples were collected at the rice harvest stage. In each experimental plot, a five-point sampling method was adopted with an "S"-shaped pattern, and soil was collected from the 0 – 20 cm tillage layer while avoiding field ridges and fertilization furrows. Fresh soil samples were air-dried in a cool and ventilated place, and then sequentially ground and sieved through 2, 1, and 0.25 mm nylon sieves. The sieved samples were separately sealed in bags and stored for subsequent physicochemical property analysis.

The determination of soil physicochemical properties was carried out strictly in accordance with the *Methods of Soil Agrochemical*

Analysis and relevant national standards. pH was determined by the electrode potential method (soil ∶ water ratio of 2.5 ∶ 1). The organic matter content was determined according to the potassium dichromate external heating oxidation method (volumetric method). The total nitrogen content was determined by the semi-micro Kjeldahl method. The hydrolysable nitrogen content was determined by the alkaline hydrolysis diffusion method. The available phosphorus content was determined by the sodium bicarbonate extraction - molybdenum antimony colorimetric method. And for the available potassium content, the ammonium acetate extraction - flame photometric method was adopted.

Data processing

Data were processed using Excel, and statistical analyses were performed using SPSS 26.0 software.

Results and Analysis

Yield

As shown in Table 2, compared with the conventional fertilization treatment (CK), the application of the bacteria-enzyme compound preparation significantly increased rice yield, and the yield-increasing effect was enhanced with the application frequency of the microbial agent increasing. The actual yields ranked as $T_2 > T_1 > CK$, and there were significant differences among all three treatments. Compared with the CK, T_1 and T_2 increased yield by 11.61% and 27.42%, respectively. The theoretical yields showed a consistent trend, with T_1 and T_2 increasing by 9.27% and 27.41%, respectively, compared with the CK. In terms of yield components, the number of grains per panicle was the main driving factor for yield increase, with significant differences among the three treatments. Compared with the CK, T_1 and T_2 increased the number of grains per panicle by 4.97% and 16.64%, respectively. No significant differences were observed among treatments in the number of effective panicles, seed setting rate, and 1 000-grain weight. These results indicated that bacteria-enzyme compound preparation could promote rice yield increase by significantly increasing the number of grains per panicle, and increasing application frequency within a certain range was conducive to further releasing the yield-increasing potential.

Table 2 Yield and yield components

Index	Number of effective panicles // $\times 10^4$/hm²	Number of grains per panicle // grain	Seed setting rate // %	1 000-grain weight // g	Theoretical yield // kg/hm²	Actual yield // kg/hm²
CK	316.67 a	91.96 c	82.65 a	28.38 a	6 830.04 c	7 048.80 c
T_1	322.22 a	96.53 b	83.73 a	28.66 a	7 462.93 b	7 867.44 b
T_2	331.11 a	107.26 a	83.56 a	29.32 a	8 702.01 a	8 981.40 a

Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$, $n = 3$). The same applies below.

Rice growth parameters at the maturation stage

The bacteria-enzyme compound preparation significantly promoted the vegetative growth of rice (Table 3). Compared with the CK, the plant height, basal stem diameter, leaf width, and leaf length of T_1 and T_2 were all significantly increased, and most indices increased with the application frequency increasing. Compared with the CK, the basal stem diameter, leaf width, and leaf length of T_2 were increased by 28.8%, 20.2%, and 12.5%, respectively, and the plant height was increased by 9.6%, with T_2 being the highest and the CK being the lowest. Split application of the microbial agent provided a better vegetative growth foundation for yield formation, particularly by promoting stem thickening and leaf expansion, which enhanced photosynthetic material production and translocation capacity, thereby providing structural support for the improvement of grains per panicle and 1 000-grain weight. It indicated that the split application strategy could more effectively coordinate the relationship between vegetative growth and reproductive growth, and served as a key link in realizing the yield-increasing potential of the bacteria-enzyme compound preparation.

Soil physicochemical properties

Soil measurements at harvest showed that the application of the bacteria-enzyme compound preparation significantly improved soil nutrient status, and the degree of improvement increased with the application frequency increasing (Table 4). Compared with the CK, the contents of soil organic matter, total nitrogen, hydrolysable nitrogen, available phosphorus, and available potassium in T_1 and T_2 were all significantly increased, and most indices showed an increasing trend of $CK < T_1 < T_2$. Among the various treatments, T_2 exhibited increases of 56.1%, 16.91%, 21.34%, 79.44%, and 33.24%, respectively, compared with the CK. Available phosphorus and organic matter presented the most pronounced improvements. Soil pH was decreased slightly from 7.53 in the CK to 7.16 and 7.08 in T_1 and T_2, respectively. These results indicated that the bacteria-enzyme compound preparation could promote nutrient transformation and accumulation, contributing to soil fertility improvement, and T_2 achieved the most significant improvement effects.

Table 3 Growth indices of rice at the maturation stage under different treatments

Treatment	Plant height // cm	Basal stem diameter // mm	Leaf width // cm	Leaf length // cm
CK	84.42 b	11.95 c	1.14 c	25.37 c
T_1	90.74 ab	13.81 b	1.23 b	27.31 b
T_2	92.55 a	15.39 a	1.37 a	28.55 a

Economic benefits

Economic benefits were estimated using the incremental method relative to the CK (Table 5). The rice price was set at

3.0 yuan/kg (the national minimum purchase price for japonica rice in 2025 was 2.62 yuan/kg, and the market price ranged from approximately 3.0 to 3.5 yuan/kg). In the bacteria-enzyme compound preparation, the price for the compound enzyme preparation was 20 yuan/kg, and that for the microbial agent was 40 yuan/L. The price for UAV foliar spray operation was 150 yuan/(hm^2 · time). All the prices were estimated values. Since the basal fertilization was identical for the three treatments, the incremental costs consisted solely of the bacteria-enzyme compound preparation and additional UAV operation costs. The results showed that compared with the CK, T_1 and T_2 increased the output value by 2 956.5 and 6 697.5 yuan/hm^2, respectively. After deducting the additional inputs of 330 and 2 640 yuan/hm^2, the net economic benefits of T_1 and T_2 were 2 626.5 and 4 057.5 yuan/hm^2, respectively. The marginal output-input ratios were 8.96 for T_1 and 2.54 for T_2. The results indicated that the single basal application of the compound enzyme preparation at the sowing stage (T_1) exhibited the highest input-output efficiency, while multiple applications of the microbial agent throughout the entire growth period (T_2) achieved the greatest total net economic benefit.

Within the rice price range of 2.6 – 3.5 yuan/kg, the net economic benefit of T_2 remained consistently positive, and the break-even price of the bacteria-enzyme compound preparation for T_2 relative to CK was far higher than the actual market price, indicating that the conclusion of income increase was robust to price assumptions.

Table 4 Soil nutrients at harvest under different treatments

Treatment	pH	Organic matter // g/kg	Total nitrogen // g/kg	Hydrolysable nitrogen // mg/kg	Available phosphorus // mg/kg	Available potassium // mg/kg
CK	7.53 a	15.26 c	1.36 c	80.23 b	15.81 c	101.52 c
T_1	7.16 b	18.79 b	1.43 b	93.29 a	21.74 b	124.93 b
T_2	7.08 b	23.82 a	1.59 a	97.35 a	28.37 a	135.27 a

Table 5 Economic benefits under different treatments (estimated increments relative to the CK, yuan/hm^2)

Treatment	Yield kg/hm^2	Output value // yuan/hm^2	Yield increase compared with the CK // kg/hm^2	Increased output value // yuan/hm^2	Increased input cost // yuan/hm^2	Increased net income // yuan/hm^2
CK	6 816.0	20 446.5	–	–	–	–
T_1	7 801.5	23 403.0	985.5	2 956.5	330	2 626.5
T_2	9 048.0	27 144.0	2 232.0	6 697.5	2 640	4 057.5

The marginal output-input ratios (additional output value/additional input) were 8.96 for T_1 and 2.54 for T_2. The estimation was based on rice at 3.0 yuan/kg, compound enzyme preparation at 20 yuan/kg, microbial agent at 40 yuan/L, and UAV operation at 150 yuan/(hm^2 · time).

Conclusions and Discussion

The results of this study demonstrated that the yield-increasing and soil fertility-enhancing effects of the bacteria-enzyme compound preparation both increased with the application frequency increasing. Multiple applications of the microbial agent throughout the entire growth period (T_2) generally outperformed the single basal application of the compound enzyme preparation at the sowing stage (T_1) in terms of yield, grains per panicle, growth parameters, and soil nutrients. The microbial agent improved plant morphology and vegetative growth, resulting in more robust plants at the maturation stage, which provided a better morphological foundation for yield formation. In terms of yield components, the yield increase was mainly attributed to the significant increase in the number of grains per panicle, while the number of effective panicles, seed setting rate, and 1 000-grain weight were not significantly altered. This may be because the basal application of the bacteria-enzyme compound preparation only functioned once in the early growth stage, whereas continuous foliar supplementation of the microbial agent from tillering to grain filling stages, in accordance with rice growth characteristics, could sustain the activity of functional microbial populations and nutrient supply during critical periods.

The application of the bacteria-enzyme compound preparation significantly improved soil nutrients at harvest, with an increasing trend as the application regimen intensified. The contents of soil organic matter, total nitrogen, hydrolysable nitrogen, available phosphorus, and available potassium in T_1 and T_2 were all significantly increased, with available phosphorus and organic matter showing the most prominent improvements. The underlying mechanisms may be as follows: the tested bacteria-enzyme compound preparation contained *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, and various hydrolases. *Bacillus* species can secrete growth-promoting substances and participate in soil nutrient transformation, while the multiple enzymes facilitate organic matter decomposition and nutrient release, thereby improving the rhizosphere nutritional environment[7]. Furthermore, they promote the mineralization and activation of immobilized phosphorus and potassium through phosphate-solubilizing, potassium-solubilizing, and nitrogen mineralization activities, enhancing the supply of available nutrients[8]. Soil pH decreased from 7.53 in the CK to 7.08 in T_2, indicating a shift of the slightly alkaline soil toward neutrality, which may be related to microbial metabolic acid production and organic matter transformation[9].

In summary, on the basis of equal conventional fertilization, the application of the bacteria-enzyme compound preparation could significantly increase rice yield, improve soil nutrients, and enhance planting economic benefits, and the effects were enhanced

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was evaluated *in vitro*. At a concentration of 2.0 mg/ml, the inhibition rate reached 58.2%, which was higher than that of the positive control arbutin at the same concentration, indicating significant inhibitory potential. In B16-F10 melanoma cell assays, the flavonoids exhibited no cytotoxicity within the tested concentration range (0.031 25 – 4.0 mg/ml) and showed a concentration-dependent inhibition of melanin synthesis, with the strongest effect observed at 1.0 mg/ml (melanin content reduced to 76.30% of the control). Notably, a promoting effect on melanin synthesis was observed at the lowest concentration (0.125 mg/ml), suggesting a biphasic regulatory profile that warrants further investigation.

Statistical analyses were performed using SPSS software. Collectively, these findings demonstrate that *H. mollis* total flavonoids possess notable tyrosinase-inhibitory and melanin-reducing activities, with favorable safety profiles in B16 cells. This work provides valuable experimental data and a technical foundation for the potential application of *H. mollis* as a natural whitening ingredient in cosmetic formulations, and supports its further development toward industrial utilization.

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with the frequency of microbial agent application increasing. From an economic perspective, multiple applications of the microbial agent throughout the entire growth period (T_2) achieved the greatest total increases in yield, soil fertility, and income, while a single basal application of the compound enzyme preparation at the sowing stage (T_1) exhibited the highest input-output efficiency. The corresponding application method can be selected and promoted according to production objectives.

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