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Impact of Seabuckthorn Pomace Fermentation Fertilizer Coupled with *Bacillus* sp. T28 on Soil Properties and Chinese Cabbage Growth

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Abstract: To study the effect of a microbial compound fertilizer on the soil properties and growth of Chinese cabbage, providing a basis for its application in the cultivation and production of Chinese cabbage. The seabuckthorn pomace fertilizer (JS) was prepared by fermentation and further, it was coupled with *Bacillus* sp. T28 (JSB). Four treatment experiments in pots were established, including the treatment group fertilized with JS (JS1), fertilized with JSB (JS1+ M), fertilized with *Bacillus* sp. T28 (M) and fertilized with water (CK). The effects of the compound microbial fertilizer on the pH, Organic Matter (OM), alkali-hydrolyzed Nitrogen (N), available Phosphorus (P), available potassium (K) of the soil and on the dry weight, nitrogen, phosphorus, potassium of Chinese cabbage were studied. The compound microbial fertilizer of JSB had a better positive effect on both soil and Chinese cabbage than other treatments ($p<0.01$). The use of *Bacillus* sp. T28 alone could also improve OM, N, P, and K of the soil and the dry weight, nitrogen, phosphorus, and potassium of Chinese cabbage. The use of JS alone could improve OM, N, P, and K of the soil, but decrease the dry weight, nitrogen, phosphorus, and potassium of Chinese cabbage. It was related to the fact that the JS contained a lot of organic acids, which reduced the soil pH which couldn't meet Chinese cabbage. It is concluded that the seabuckthorn pomace fertilizer coupled with *Bacillus* sp. T28 had a synergistic effect, which had a potential application in agriculture.

Keywords: Seabuckthorn Pomace, Fermentation, Fertilizer, Soil, Cabbage

Introduction

Seabuckthorn is a characteristic economic and ecological crop in West China, with a planting area of 19 provinces, accounting for 95% of the world's total resources. Seabuckthorn processing will leave a large amount of pomace every year. If not used comprehensively, it will cause continuous resource waste and environmental pollution. The full utilization of seabuckthorn pomace has become a research hotspot. Developing seabuckthorn pomace into an ecological organic fertilizers or a microbial fertilizers can realize the full utilization of seabuckthorn resources, achieve zero industrial emissions, improve the utilization level of agricultural waste resources, and improve the ecological environment in the production area.

In recent years, the negative impact of long application of Chemical Fertilizer (CF) has increased. Excessive use of CF damages the environment and agricultural sustainable development. For example, the content of organic matter in the soil continues to decline, the pH is unbalanced, the nutrients are unbalanced and soil compaction aggravates (Lou *et al*., 2021). As a special organic fertilizer, fruit and vegetable waste contain extremely rich organic matter, active substances, and trace elements, which may greatly promote the soil quality, improve the growth of crops, optimize the micro-environment of crop roots (Ma *et al.*, 2020; Qaswar *et al*., 2020; Ye *et al*., 2020). Plant Growth-Promoting Rhizobacteria (PGPR), including nitrogen-fixing bacteria, can improve the micro-environment of crop rhizosphere, release soil trace elements, and convert difficult utilizable substances to plant nutrients. It also can absorb pesticide residues, repair soil, and inhibit pathogenic

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bacteria. The combination of PGPR and organic fertilizer can fix nitrogen and phosphorus in the soil, promote crop growth, help plants resist pathogens, and tolerate abiotic stress, which can reduce pesticide use and have advantages for crops and the environment (Benaissa, 2019; Koul *et al.*, 2019). Therefore, the exploration and expansion of organic fertilizer sources and the study of PGPR-coupled organic fertilizers will improve soil productivity, reduce chemical fertilizers and pesticide use, and reduce agricultural pollution.

The seabuckthorn pomce not only contains protein, amino acids, soluble dietary fiber, vitamin C, and other nutrients but also is rich in flavonoids, polyphenols, anthocyanins, and other active substances. Compared with other plants, seabuckthorn has a better environmental protection effect during its growth. The roots can accumulate PGPR, which plays a role in nitrogen fixation, growth promotion, resistance to stress, and prevention of harmful bacteria infection (Chen *et al.*, 2022). Seabuckthorn ingredients may contain active substances for plant-beneficial bacteria growth. Returning it directly to the field or as a carrier of PGPR, will it promote a sustainable cycle of crop root microenvironment? Will it have great potential application value for crop growth? There are few reports of such studies and even fewer applications.

According to few researches of microorganism fertilizer on seabuckthorn pomace. The effect of seabuckthorn pomace fermentation fertilizer coupled with *Bacillus* sp. T28 on soil properties and Chinese Cabbage growth was studied here. It would provide a basis for its application in the cultivation and production of Chinese cabbage.

Materials and Methods

Soil Sampling

Soil was collected from Hengxi County, Ningbo City, Zhejiang Province, China. Random collection of 0-20 cm depth of soil and pretreated it: Pick out small stones, plant roots, plastic debris, and other impurities, the soil was mixed and air dry treatment, and then the soil was sieved in a 20 mesh sieve.

Seabuckthorn Pomace and Others

The C, N, H, and S of Seabuckthorn Pomace (SP) used in this experiment were determined by an elemental analyzer (Vario EL cube, Elementar, Hanau, Germany). The total nitrogen content was 1.2499%, the total carbon content was 53.4838%, the total hydrogen content was 5.2661%, and the total sulfur content was 0.0534%.

L. Paracasei, S. cerevisiae and Sucrose were purchased from Xi'an Jushengyuan Biotechnology Co., Ltd. (Xi'an, China), Angelyeast Inc. (Yichang, China) and the local market, respectively.

The crop used in the pot experiment was Chinese cabbage. The plastic pot used in the experiment had a diameter of 20 cm and a height of 14 cm. The *Bacillus* sp. T28 is a novel nitrogen-fixing bacteria that was reported previously by Yang *et al.* (2023), it combined SP significantly changed soil microbial community structure, indirectly affected crucial environmental factors, such as pH, TC, and the recruitment of beneficial functional microorganisms; at the same time, it enhanced the abundance of *nif*H, *nar*G, *nir*S, *nir*K and *nos*Z genes and could reduce the environmental risks caused by N_2O emission and NO_3^- leaching.

Preparation of JS

The JS was prepared according to the reported method by Wu *et al.* (2022) with modifications. 300 g SP and 300 g sterile water was taken into the homogenizer (DS-1, shanghai specimen and model factory, China) to mix. Then added 0.075% (w/w) *S. cerevisiae*, 0.25% (w/w) *L. paracasi,* and 10% sucrose, mixed again. The mixture was fermented at 37°C in an incubator for 48 h and then filtered by 400 mesh filter cloth. The JS was prepared.

Preparation of JSB

By weight percentage, 60% JS, 2% urea, 0.5% calcium dihydrogen phosphate, and water were mixed evenly to form the JS mixture.

The JSB was prepared according to the liquid biofertilizer preparation method reported by Zhang *et al.* (2010) with modification. The *Bacillus* sp. T28 stored in freezing was activated inoculated in Luria-Bertani (LB) medium, 160 r/min at 28°C for about 7 days (Yang *et al.*, 2023). when *Bacillus* sp. T28 was in the logarithmic stage (CFU $\geq 1\times10^8$ cells/mL), taking 1 ml of bacterial solution into 200 mL JS-mixture. Stir and mix for 25 min, aerobic inoculated until the number of viable bacteria of *Bacillus* sp. T28 no longer increased significantly, and the JSB was prepared.

Pot Experiment

Four fertilization treatments were done, three repeated experiments for each treatment:

Treatment JS1:	Fertilize with JS
Treatment JS1+ B:	Fertilize with JSB
Treatment B:	Fertilize with *Bacillus* sp. T28
Treatment CK:	Fertilize with water

Weighed 2.0 kg of air-dried soil into the pot, adjusted its moisture to 60% of the measured field water capacity, and pre-cultured the soil for a week. As a base fertilizer, 0.15 g urea per pot was applied. Planted 20 seeds of high-quality and evenly sized cabbage seeds into each pot. After sprouting, each bottle kept 5 cabbages with basically the same growth condition. The cabbage was fertilized every 7 days during growth. The root soil was irrigated with 50 times dilution of JS, JSB, and *Bacillus* sp. T28, 50 mL each time. In addition, water each pot 20-30 mL

daily to ensure it grows normally. After 30 days, the cabbages were harvested.

The soil samples collected after the completion of the pre-culture experiment and the pot experiment were air-dried for analysis. After the cabbage was ripe, the above-ground parts were collected and dried for analysis.

Soil Analysis

The air-dried soil samples were ground with a mortar and passed through a 100-mesh sieve.

The soil pH was detected according to the standard "NY/T 1377-2007 Determination of pH in soil (Ministry of Agriculture of the PRC, 2007)". The Organic Matter (OM) was detected according to the standard "NY/T 1121.6-2006 Soil testing part 6: Method for determination of soil organic matter (Ministry of Agriculture of the PRC, 2006)". The Alkali-hydrolyzed Nitrogen (AN) was detected by the alkaline hydrolysis diffusion method (Zhang *et al*., 2024; Bao, 2000). The available Phosphorus (P) and available potassium (K) were carried out according to the following standards: HJ 704-2014 Soil quality-determination of available Phosphorus-Sodium hydrogen carbonate Solution-Mo-Sb anti spectrophotometric method (Ministry of Environmental Protection of the PRC, 2014) and NY/T 889-2004 Determination of exchangeable potassium and non-exchangeable potassium content in soil (Du *et al*., 2004).

Plant Analysis

The dried Chinese cabbages of each treatment were weighed, respectively. Then, they were ground into powder to determine the nitrogen (NC), phosphorus (PC), and potassium (KC) by the method "NY/T 2017-2011 Determination of nitrogen, phosphorus, and potassium in the plant (Ministry of Agriculture of the PRC, 2014)".

Statistical Analysis

All experiments were conducted in triplicate and the results were expressed as mean ± standard deviation. The figure was obtained using WPS 2019 (Beijing Kingsoft Office Software, Inc. Beijing, China). Variance analysis was performed using Data Processing System (DPS) software v20.05 (Tang and Zhang, 2013). Correlation analysis was performed using the Metware Cloud, a free online platform for data analysis (https://cloud.metware.cn).

Results and Discussion

The Parameters of Soil Samples

The soil samples were collected after the completion of the pre-cultured experiment. Before planting, the parameters of the soil were detected, as shown in Table (1).

According to soil evaluation standards (Table 2). The initial soil before being planted was rich in organic matter, lacking in alkali-hydrolyzed nitrogen, average in available phosphorus, and lacking in available potassium. According to the soil pH value in Table (1), it belonged to acid soil (Shi *et al*., 2023).

Table 1: The initial parameters of soil samples

Materials	pH	OM g/kg	AN mg/kg	P mg/kg	K mg/kg
Soil	4.79	20.20	42.00	6.33	49.95

Table 2: Soil evaluation standard (Shi *et al*., 2023)

Organic matter g/kg	Alkali-hydrolyzed nitrogen mg/kg	Available phosphorus mg/kg	Available potassium mg/kg	Level
≤6	≤30	≤3	≤30	very lack
>6,≤10	>30,≤60	>3,≤5	>30,≤50	lack
>10,≤20	>60,≤90	>5,≤10	>50,≤100	average
>20,≤30	>90,≤120	>10, ≤20	>100,≤150	rich
>30	>120	>20	>150	Very rich

Table 3: Analysis of variance for effects on soil pH

Source of variation	Sum of square	Degrees of freedom	Mean Square	F	*P*
Treats	2.989	3	0.996	568.347	<0.0001
Error	0.011	6	0.002		
Total	3.015	11			

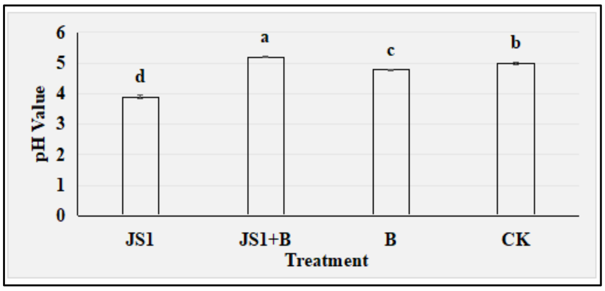

Fig. 1: Effects of fertilization on soil pH. Different letters marked indicate statistically significant differences in the results ($p<0.01$)

Effects on Soil pH

After the pot experiment, soil was collected for analysis. The results are shown in Fig. (1) and Table (3). In general, soil of pH<4.5 was strongly acidic soil, pH 4.5~5.5 acid, pH 5.5~6.5 slightly acidic (Ning *et al*., 2022). So the soil of initial and treatment of JS1+ B, B, and CK were acidic, and the soil of treatment JS1 was strongly acidic. Table (3) showed that different treatments had significant effects on soil pH ($p<0.01$). The pH value from low to high was as follows: JS1<B<CK<JS1+ B. JS was a fermented liquid fertilizer, that contained a lot of organic acids (Tkacz *et al*., 2019), which might reduce the soil pH. Its effect on soil pH

was contrary to the unfermented seabuckthorn pomace solid fertilizer (Wan *et al.*, 2022). The effects of microbial fertilizer on soil pH were different, some reports showed not significant, and some were first decreased and then increased (Wang *et al.*, 2023; Xu *et al.*, 2024). This might be related to the characteristics of different microorganisms in different growth periods, as well as plant root exudates; Microbial fertilizer keeps soil pH at a level suitable for plant root growth and microbial growth (Samanhudi *et al.*, 2018; Xu *et al.*, 2024). *Bacillus* sp. T28 used here was isolated from acidic soil, it decreased the soil pH value, and the result was similar to the report (Yang *et al.*, 2023). JS1+ B treatment had a higher pH than CK, it was suggested that TS28, JS, and soil may had a synergistic effect, which was different from the single component. This phenomenon was consistent with the fact that solid seabuckthorn microbial fertilizer increased the pH value of paddy soil, and the more seabuckthorn content, the more significant the increase (Yang *et al.*, 2023). The mechanism of treatment JS1+ B to increase soil pH must be further studied.

Effects on Soil OM, AN, and PK

The effects of fertilization on soil OM, AN, and PK are shown in Fig. (2) and Table (4). Table (4) showed that different treatments had significant effects on soil OM, AN, P, K (p<0.01). Among the all treatments, the JS1+ B had the best effect on soil, with OM 2.08 times than CK, 1.10 times than JS1, 1.31 times than B; AN was 2.82 times than CK, 1.75 times than JS1, 1.24 times than B; P was 2.51 times than CK, 1.57 times than JS1, 1.21 times than B; and K was 2.82 times than CK, 1.66 times than JS1, 1.23 times than B. The content of soil OM from high to low was as follows: JS1+ B>JS1>B>CK (Fig. 2 (1)). The content of soil AN, P, and K from high to low were as follows: JS1+ B>B>JS1>CK (Fig. 2(1-4)).

Table 4: Analysis of variance for effects on soil OM, AN, and PK

Source of variation	Sum of square	Degrees of freedom	Mean square	F	*P*
OM					
Treats	4.711	3	1.570	474.683	<0.0001
Error	0.020	6	0.003		
Total	5.197	11			
AN					
Treats	1437	3	4789.333	1121.440	<0.0001
Error	25.624	6	4.271		
Total	15164.917	11			
P					
Treats	54.812	3	18.271	61.091	0.0001
Error	1.794	6	0.299		
Total	64.856	11			
K					
Treats	7714.737	3	2571.579	85.847	<0.0001
Error	179.733	6	29.956		
Total	7906.768	11			

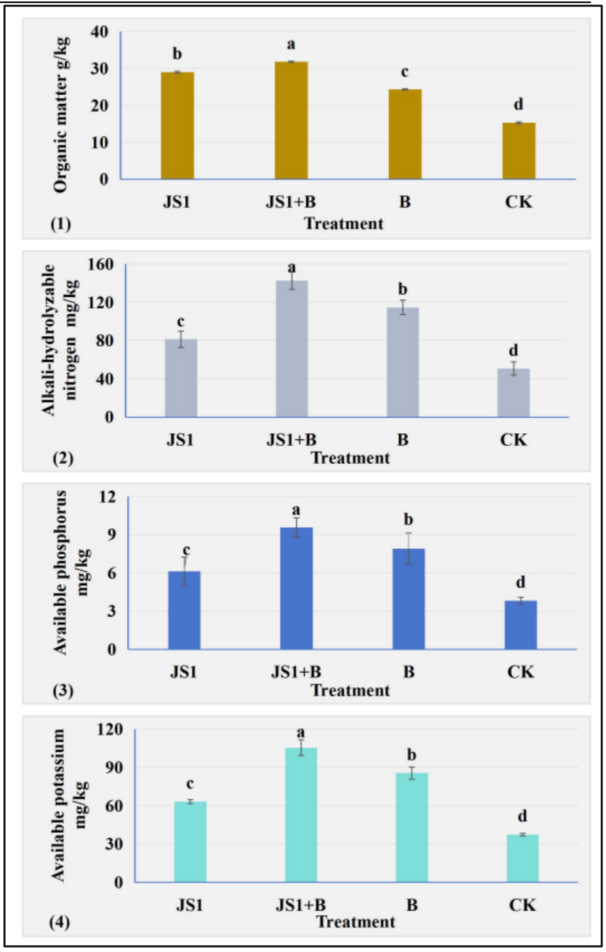

Fig. 2: Effects of fertilization on soil (1) OM; (2) N; (3) P; (4) K. Different letters marked indicate statistically significant differences in the results (p<0.01)

According to Table (2) and Fig. (2), the OM content of CK treatment was at an average level, JS1 and B were at an abundant level, and JS1+ B was at an extremely abundant level; the AN content of CK treatment was at in lack level, JS1 was in average level, B was in rich level, and JS1+ B was in very rich level. The fertilization had a positive effect on soil AN; the P content of CK treatment was in lack level, JS1, B, and JS1+ B were the average level; the K content of CK treatment was in lack level, JS1 and B were in average level, JS1+ B reached to the rich level.

Besides AN, the OM, P, and K contents of soil for CK treatments were lower than the initial value in Table (1), it seemed that if no fertilization was applied, the soil OM, P, and K would decrease with the crop growth. Therefore, fertilization kept the soil OM, P, and K stable or increased, which met crop growth needs. The cultivation process without fertilization might lead to the continuous decline of soil OM, P, and K. Soil alkali-hydrolyzed nitrogen includes inorganic nitrogen (ammonium nitrogen, and nitrate nitrogen) and easily hydrolyzed organic nitrogen (amino acids, acylammonium, and easily hydrolyzed proteins). Its content was affected by soil pH, organic matter, and conversion of related components (Jin *et al*., 2024). The initial AN content of the soil in Table (1) was lower than CK treatment, the changes are different from the OM, P, and K indicators, watering might promote the release of AN-related components, further exploration was needed.

In summary, the coupling of *Bacillus* sp. T28 and JS had the best effect on the soil ecological environment, compared to that of any single fertilizer. They had a synergistic effect on the improvement of soil ecology. As an azotobacter, *Bacillus* sp. T28 could produce azotase, which catalyzed nitrogen in the atmosphere to nitrogen compounds that were easily absorbed by plants (Shameem *et al*., 2023; Huang *et al*., 2022). The microbial organic fertilizer had a better effect than the organic fertilizer on soil fertility, they increased the microbial diversity and abundance, which also activated soil nitrogen, phosphorus, and potassium nutrients (Pu *et al*., 2022, Jia *et al*., 2020). Yang *et al*. (2023) used gene technology to explore the effects of *Bacillus* sp. T28 combined seabuckthorn pomace on the abundance of *nifH, narG, nirS, nirK, and nosZ* genes involved in paddy soil nitrogen cycling, it showed that the biofertilizer increased the abundance of these soil N functional genes; by using C-PLFA analysis, it showed that the biofertilizer altered soil pH and total carbon, which improved the soil nutrient turnover rate, the microbial community diversity, and ecosystem stability; and at the same time, this biofertilizer reduced the soil nitrite content and the release rate of nitrous oxide, but increased the ammonium nitrogen content in the soil, that increased the available nitrogen of crops. The mechanism of action of JSB fertilizer on soil might be similar to the research of Yang *et al.* (2023).

Effects on Cabbage Dry Weight (DW), Nitrogen (NC), Phosphorus (PC) and Potassium (KC)

The Chinese cabbage in the mid-growth stage is shown in Fig. (3). When the cabbage was mature, the above-ground parts of the cabbage were collected, dried, and weighed. The dry weight, nitrogen, phosphorus, and potassium were detected and the results are shown in Fig. (4). There was good consistency between Figs. (3-4). Figure (3) shows the growth trends of Chinese cabbage from good to poor as follows: JS1+ B>B>CK>JS1 and the dry weight, the nitrogen, phosphorus, and potassium contents of cabbage in each treatment from high to low were JS1+ B>B>CK>JS1. Among the all treatments, the JS1+ B had the best effect on cabbage, which dry weight 2.13 times than CK, 2.52 times than JS1, 1.56 times than B; nitrogen was 1.50 times than CK, 1.64 times than JS1, 1.28 times than B; phosphorus was 1.32 times than CK, 1.48 times than JS1, 1.14 times than B; and potassium was 1.30 times than CK, 1.34 times than JS1, 1.02 times than B.

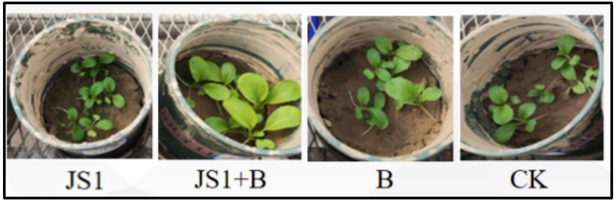

Fig. 3: Chinese cabbage in the middle growth stage

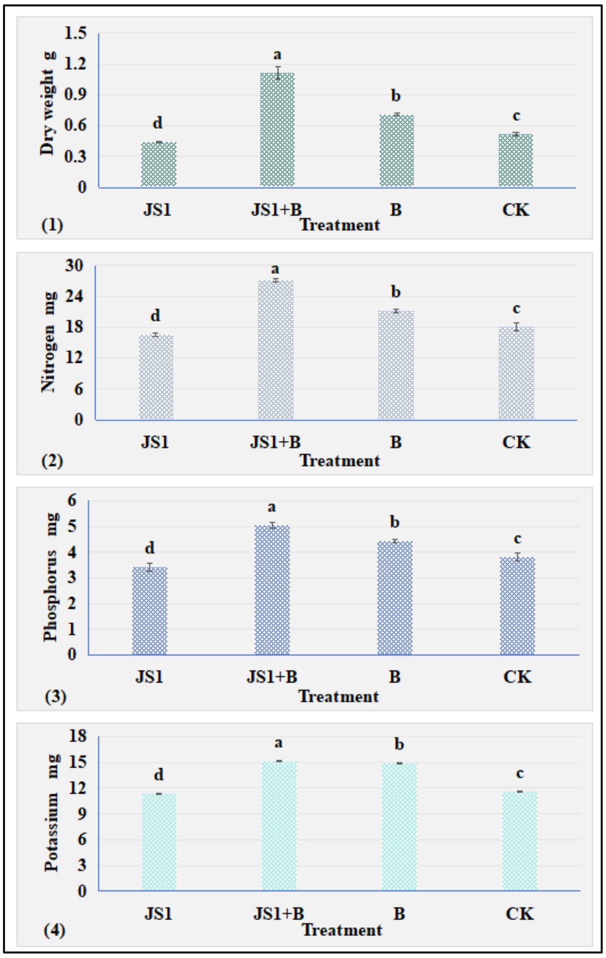

Fig. 4: Effects of fertilization on cabbage for (1) Dry weight; (2) Nitrogen; (3) Phosphorus; (4) Potassium. Different letters marked indicate statistically significant differences in the results ($p<0.01$)

Table (5) showed that different treatments had significant effects on Chinese cabbage dry weight, nitrogen, phosphorus, and potassium ($p<0.01$). Compared with CK treatment, B and JS1+ B fertilization treatments had positive effects on the yield of Chinese cabbage, including dry weight, nitrogen, phosphorus, and potassium, which the JS1+ B treatment had the best effect. While JS1 treatment had a negative effect on cabbage. Its growth trend and physical-chemical indicators detected were inferior to those of the CK treatment, which might due to the strongly acidic soil of JS1treatment. Chen (2022) reported that the pH of the soil would affect the growth of Chinese cabbage, the more acidic of soil, the more severely inhibited the cabbage grew.

There was a good synergistic effect between fertilizer and soil, the couple of *Bacillus* sp. T28 and JS might promote soil nutrient release and plant nutrient absorption and utilization. Liang *et al*. (2021) reported that microbial organic fertilizer significantly increased the dry weights of crops, it could improve crop growth by providing appropriate nutrients and regulating the rhizosphere microbial community. Wang *et al*. (2023) reported that the application of microbial organic fertilizer effectively stimulated the colonization of beneficial microbial communities, improved the soil available potassium and pH value, and boosted the yield of crops. Microbial organic fertilizer played an important role in reducing the total acidity and the accumulation of nitrate, increasing the content of vitamin C and soluble sugar in lettuce, celery, and other vegetables (Stamford *et al*., 2019; Wang *et al.,* 2023).

The correlation between the soil's chemical properties and the yield factors of Chinese cabbage was analyzed in Fig. (5) and Table (6). The higher the correlation, the redder the circle in Fig. (5), and the larger the correlation coefficient r in Table (6). The results showed that soil AN, P, K had highly correlated with the cabbage Dry Weight (DW), Nitrogen (NC), Phosphorus (PC) and potassium (KC), with correlation coefficient r≥0.8; the soil OM had moderately correlated with cabbage DW and NC (0.5≤r<0.8), it had weakly correlated with PC and KC (0.3≤r<0.5); the soil pH had moderately correlated with cabbage DW, NC, PC and KC (0.5≤r<0.8). On the other hand, for soil alone, pH and OM were negatively correlated Fig. (5), which was related to the fact that the SP contained a large amount of organic acids (Tkacz *et al*., 2019). The higher SP content would decrease soil pH, but the increase of organic matter.

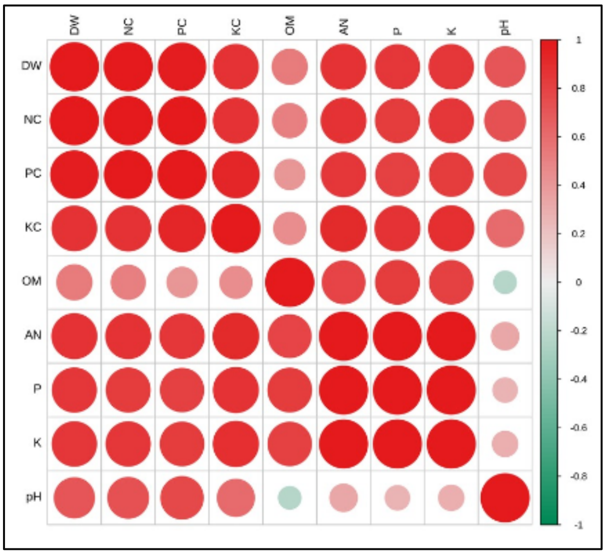

Fig. 5: Correlation heat map between soil chemical properties and cabbage properties

Table 5: Analysis of variance for effects on cabbage for dry weight, nitrogen, phosphorus, and potassium

Source of variation	Sum of square	Degrees of freedom	Mean square	F	*P*
Dry weight					
Treats	0.8129	3	0.271	257.393	<0.0001
Error	0.0063	6	0.0011		
Total	0.8259	11			
Nitrogen					
Treats	195.92	3	65.3067	695.163	<0.0001
Error	0.5637	6	0.0939		
Total	198.8826	11			
Phosphorus					
Treats	4.5950	3	1.5317	495.4200	<0.0001
Error	0.0185	6	0.0031		
Total	4.7887	11			
Potassium					
Treats	38.0765	3	12.6922	15436.405	<0.0001
Error	0.0049	6	0.0008		
Total	38.1326	11			

Table 6: Correlation coefficients between soil chemical properties and cabbage properties

Index	DW	NC	PC	KC
OM	0.54	0.51	0.40	0.45
AN	0.87	0.87	0.85	0.90
P	0.84	0.84	0.81	0.87

K	0.85	0.85	0.82	0.88
pH	0.70	0.72	0.78	0.60

Conclusion

The effect of seabuckthorn pomace fermentation fertilizer coupled with *Bacillus* sp. T28 on soil properties and Chinese Cabbage growth was studied here. The result showed that seabuckthorn pomace fermentation fertilizer coupled with *Bacillus* sp. T28 had positive effects on soil and cabbage. For soil, the organic matter was 2.08 times more than the control group, the alkali-hydrolyzed nitrogen was 2.82 times, the available phosphorus was 2.51 times, and the available potassium was 2.82 times. For cabbage, the dry weight was 2.13 times more than the control group, the nitrogen was 1.50 times, the phosphorus was 1.32 times, and the potassium was 1.30 times. It can become a new type of microbial organic fertilizer that has the potential to be applied to agriculture.

Seabuckthorn pomace fermentation fertilizer alone showed a promotion effect on the content of soil nutrients, but it showed a negative effect on cabbage. The decreasing soil pH caused by seabuckthorn pomace fermentation couldn’t meet the growth of Chinese cabbage. In the potential application of seabuckthorn pomace fermentation fertilizer, the pH of the soil should be considered.

Different soil types, crops, fertilizer forms, and other factors can significantly affect the growth promotion effect of seabuckthorn pomace fermentation fertilizer coupled with *Bacillus* sp. T28 on host crops. The practical application effect of field experiments needs further study.

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Author’s Contributions

Dan Wu: Wrote the manuscript.
Huang Mengyun: Data-analysis.
Zhang Qichun: Grammar checking and modification.

Ethics

This article is original and contains unpublished material. The authors declare no conflict of interest.

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