J. Agr. Sci. Tech. (2021) Vol. 23(4): 943-954

Compound Effective Microorganisms Treatment Increases Growth and Yield of Vegetables

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ABSTRACT

Misuse or excessive use of chemical fertilizers cause non-point source pollution in the vegetable production system. Fertilizer is a key factor affecting nutrient absorption and biomass production of vegetables. However, there is little information on the role of Compound Effective Microorganisms (CEM) in vegetable production. In field trials, three fertilizer treatments were used to study their effects on the growth characteristics and quality of lettuce, spinach, and pakchoi. There were five treatments in the experiment including Control Treatment (CK), 0.3% (Mass volume ratio: w/v) of urea: Water (T_1), 0.3% (w/v) of compound fertilizer: Water (T_2), CEM fertilizer was diluted to 1: 1,000 (CEM fertilizer: Water, Volume ratio: v/v) before application (T_3), 1: 500 (CEM fertilizer: Water, v/v) (T_4), 1: 100 (CEM fertilizer: Water, v/v) (T_5). Ten representative plants were selected for each treatment to measure plant growth performance. Compared with the control, the plant height gradually increased after applying fertilizer during the entire growth period of the plant. Total leaf chlorophyll concentration of the CEM treatment was also significantly higher than the control treatments. CEM led to an increase in leaf area and leaf number. The effect of EM fertilizer on vegetable growth and yield was studied. In T_4 treatment, spinach had the highest plant fresh yield, which was 15.60 g plant^{-1}. Under different fertilizer treatments, the yield of pakchoi varied greatly, while under T_5 treatment, the yield of pakchoi increased significantly. The maximum yield of pakchoi in T_5 treatment was 30.66 g plant^{-1}. The yield of lettuce in T_3 treatment was the highest, 12.32 g plant^{-1}. CEM could maintain productivity of green vegetable and contained a variety of beneficial bacteria. The cultivation of EM increased the yield of plants and increased the growth of vegetables. In conclusion, these results showed that vegetables could produce high yield and high quality through CEM management.

Keywords: Biofertilizers, Chlorophyll meter, Lettuce, Pakchoi, Spinach.

INTRODUCTION

Fertilization is considered a fundamental method of increasing soil nutrient availability for plants (Abd El-Mageed *et al*., 2020; Rahbar *et al*., 2018). That may consequently influence plant growth, maturity time, yield, and phytochemical content. In the last few decades, the use of chemical fertilizers has increased constantly; however, the continued and over application of chemical fertilizers has generally caused many adverse effects on the environment and soil microorganisms (Souri and Hatamian, 2019). Many studies have shown that application of chemical fertilizers will drastically reduce soil enzyme activity and microbial biomass. Therefore, frequent use of chemical fertilizers will seriously affect soil quality (Franczuk *et al.*, 2019; Merajipoor *et al.,* 2020). In addition, excessive application of chemical fertilizers can results in N leaching and soil salinity

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and, consequently, environmental hazards (Dos Santos *et al.*, 2020; Hatamian *et al.*, 2020; Ahmadi and Souri, 2019; Aghaye Noroozlo *et al.*, 2019). In order to ensure China's food security and meet the demand of food grain production, it is necessary to optimize and rationally apply fertilizers, protect the environment, and improve soil fertility (Tao *et al.*, 2017; Dursun *et al.*, 2019).

Excessive use of N fertilizers and the loss of chemical fertilizers leads to groundwater nitrate pollution (Iriti *et al.*, 2019). Many studies have reported nitrogen accumulation and leaching in soil profiles under different irrigation schedules. Usually, fertilizers or crop residues are used to maintain soil nutrient utilization. Fertilization is a common method of increasing soil fertility, which may affect the growth of crops (Bonanomi *et al.*, 2017; Souri *et al.*, 2019).

In order to improve the efficiency of fertilizer use, many researchers have focused on finding solutions to reduce and optimize the use of agricultural fertilizers (Souri, 2016; Souri *et al.*, 2018; Riddech *et al.*, 2019). This is possible by developing a more effective fertilization system. In recent years, due to the positive effects of beneficial microorganisms on photosynthesis and crop yields, proposals have been made to use beneficial microorganism fertilizers in agriculture. Previous studies have shown that improving soil microorganisms can improve crop nutrient utilization, increase effective absorption of minerals and water, improve disease resistance, reduce heavy metal toxicity, improve soil structure, and achieve sustainable agriculture (Marschner, 2011; Naiji and Souri, 2018).

The Compound Effective Microorganisms (CEM) are composed of natural beneficial microorganisms, such as lactic acid bacteria, photosynthetic bacteria, yeast, fermenting fungi and actinomycetes, and are an example of microbial fertilizers (Rajper *et al.*, 2016). Many studies have shown that effective microorganisms can accelerate the growth and yield of plants by increasing photosynthesis, producing bioactive substances such as enzymes and hormones, promoting the decomposition of lignin substances and reducing diseases in the soil (Wang *et al*. 2019). Some studies have shown that the effects of Effective Microorganisms (EM) on plant growth, quality and yield are usually not obvious (Talaat, 2019).

Vegetables such as lettuce (*Lactuca sativa L. var. ramosa Hort.*), spinach (*Spinacia oleracea L.*) and pakchoi (*Brassica chinensis L.*) are the important green leafy vegetables in China. Over the past decade, the consumption of green leafy vegetables has gradually increased (Sripontan *et al.*, 2014). The fertilizer supply is an important factor in the quality and productivity of green leafy vegetables. Many research has reported that green leafy vegetables are highly sensitive to nitrogen fertilizer input, and excessive nitrogen input will reduce the quality. In addition, due to high nitrogen input, relatively short vegetable growth period and frequent cultivation, the vegetable production system will cause nitrogen leaching. However, it is possible to minimize the impact of agriculture on groundwater quality during the growing season through precisely scheduled fertilization measures and increase yields (Radkowski and Radkowska, 2018).

In the current situation of sustainable agricultural development, due to various disadvantages of chemical fertilizers, microbial fertilizers have attracted more and more attention to partly replace chemical fertilizers (Ratajkiewicz *et al*., 2017). Some studies have shown that when microbial agents are used in combination with organic fertilizers and chemical fertilizers, the fertilizer use of corn, wheat and other crops can be reduced by 25-30%, and the fertilizer use of potatoes and tomatoes can be reduced by 30-45%. In addition, research shows that, compared with the traditional single application of chemical fertilizers, the combination of composite microbial agents and chemical fertilizers can increase the yield of crops, improve the quality of crops,

[DOR: 20.1001.1.16807073.2021.23.4.15.4]

and increase yield by 5-10%. At present, the compound microbial agents are mainly used in yield-increasing field experiments, and the applied microbial agents are mostly single microbial agents (Vohra *et al*. 2016). To this end, this study explored the effects of compound bacterial agents and compound fertilizers on the growth and development of vegetable seedlings, providing a theoretical basis for crop planting and the promotion of new fertilizers.

MATERIALS AND METHODS

Experimental Site

This study was conducted in the research area of the experimental department, Qingdao, Shan Dong Province (120.2° E、36.04° N), China for growing season. The soil at the experimental site was sandy clay loam. The properties of the soil are listed in Table 1. The meteorological data of the experimental site of Qingdao during the period of study are listed in Table 2. The experiment had a randomized complete block design with three replications. Three plant species, namely, spinach (*Spinacia oleracea L.*), lettuce (*Lactuca sativa L. var. ramosa Hort.*), and pakchoi (*Brassica chinensis L.*) were used in the experiment. The seeds were pre-germinated in a tray, and twenty seedlings were transplanted in each pot. The seedlings were planted in the soil and watered daily.

Compound Effective Microorganisms (CEM) fertilizer was produced by Qingdao Haojitai Biotechnology Co., Ltd. Compound beneficial microorganisms mainly include photosynthetic bacteria, lactic acid bacteria, actinomycetes, bacillus, etc. The microbial fertilizers used in this study all improve the ability of crops to absorb nutrients and minerals (Li *et al*., 2017). The fertilizers were dissolved in water and then applied to the soil. The leaf characteristics of spinach, green vegetables and lettuce were analyzed. Leaf area, plant height, leaf number and total dry biomass were used to measure plant growth performance. The fresh weights of roots, stems and leaves were determined at the end of the experiment. Ten representative plants were selected for each treatment to measure dry matter accumulation. The plant was divided into roots, stems, and leaves, and dried in an oven at 70ºC for 48 hours. Then, the dry biomass was measured.

Experimental Design

The experiment was arranged based on randomized complete block design of fertilizer treatments. Fertilizer treatments included Control Treatment (CK), 0.3% (Mass volume ratio: w/v) of urea: Water (T_1), 0.3% (w/v) of compound fertilizer: Water (T_2), CEM fertilizer was diluted to 1: 1,000 (CEM fertilizer: Water, Volume ratio: v/v) before application (T_3), 1: 500 (CEM fertilizer: Water, v/v) (T_4), 1: 100 (CEM fertilizer: Water, v/v) (T_5). Urea and compound effective fertilizer were produced by Stanley Fertilizer Co., Ltd. CEM fertilizer was produced by Qingdao Haojitai

Table 1. Weather parameters of the experimental field in 2017.

Months	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)
September	31.8	15.9	70.6
October	23.0	6.4	64.6

Table 2. Physical and chemical properties of the field soil used in the research.

Soil organic matter (g kg^{-1})	pH	EC (μS cm^{-1})	Total N (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
26.87	7.74	547	1.02	16.33	125.75

[DOR: 20.1001.1.16807073.2021.23.4.15.4]

Biotechnology Co., Ltd. The pot size was 59 cm (Length)×20 cm (Width)×14 cm (Depth). The fertilizers were applied when the vegetable had five main leaves.

Measurements

The plant samples were divided into leaf, stem, and root. Leaf area and root parameters of vegetables were determined by using LA-S series plant image analysis system. Soil and Plant Analyzer Development (SPAD) was measured by a Minolta SPAD-520 chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan). 20 SPAD readings were collected in each treatment and the mean value was recorded. Then, the plant parts were dried for two days at 80ºC. The dry weight of vegetables was measured. The vegetables were harvested for determining yield.

Statistical Analysis

Yield, soil, and growth parameters of plants were recorded. One-way analysis of variance and Tukey's multiple-range test were used to compare the effects of different fertilizers on the growth of vegetables.

RESULTS

Plant Height and Leaf of Vegetables in Different Treatments

The plant height of spinach, pakchoi, and lettuce under different fertilizer treatments are summarized in Figure 1. Plant height is an important indicator reflecting the growth conditions of plants. The result indicated that CEM had a significant effect on the height of the three vegetables. The maximum height of spinach was 17.5 cm in T_4 treatment, which was 20% higher than the control treatment. Therefore, a higher dose of CEM fertilizer significantly increased the height of vegetables.

Different fertilizer treatments had significant effects on plant chlorophyll content. The SPAD meter is commonly used to determine nitrogen demand to optimize plant yield and nitrogen use efficiency. The results showed that there were significant differences in the SPAD values of all fertilizer treatments (Figure 2). Beneficial microorganisms could increase the chlorophyll content in vegetable leaves. The application of microbial fertilizer could significantly increase the chlorophyll content of vegetable leaves, delay the decomposition of chlorophyll, and maintain photosynthetic capacity.

CEM led to an increase in leaf area and leaf number (Figure 3). The highest leaf area in T_4 treatment had a greater contribution to the leaf expansion of spinach. The maximum leaf area of pakchoi obtained in T_5 treatment was 352.59 cm^2. However, in the T_3 treatment, the maximum leaf area of lettuce reached 267.25 cm^2. The leaf number had a similar trend. The results indicated that CEM treatment could effectively increase the growth of vegetable leaves. It has a beneficial effect on the photosynthesis and yield of vegetables.

Root and Plant Biomass of Vegetables in Different Treatments

The effects of different fertilizer treatments on root length are shown in Figure 4. The root length of the vegetables treated with T_3 and T_4 were longer than those treated with conventional fertilizer and T_5. After spraying CEM, the root of vegetables grew larger than other treatments.

Data analysis showed that fertilizer treatment had a great impact on dry matter. The root dry biomass of spinach and pakchoi in T_3 treatment was significantly higher than other treatments. This showed that microbial fertilizer had a significant effect on the growth of vegetables. The microorganisms and effective nutrients in microbial fertilizer had a good effect on the growth of the root system. In fertilizer applications, the

[DOR: 20.1001.1.16807073.2021.23.4.15.4]

maximum total dry biomass of spinach and pakchoi were observed in T_3 treatment. T_5 treatment obtained the maximum total dry biomass of lettuce (Table3).

Yield of Vegetables in Different Treatments

The plant yield of three vegetables are shown in Figure 5. Fertilizer treatments had

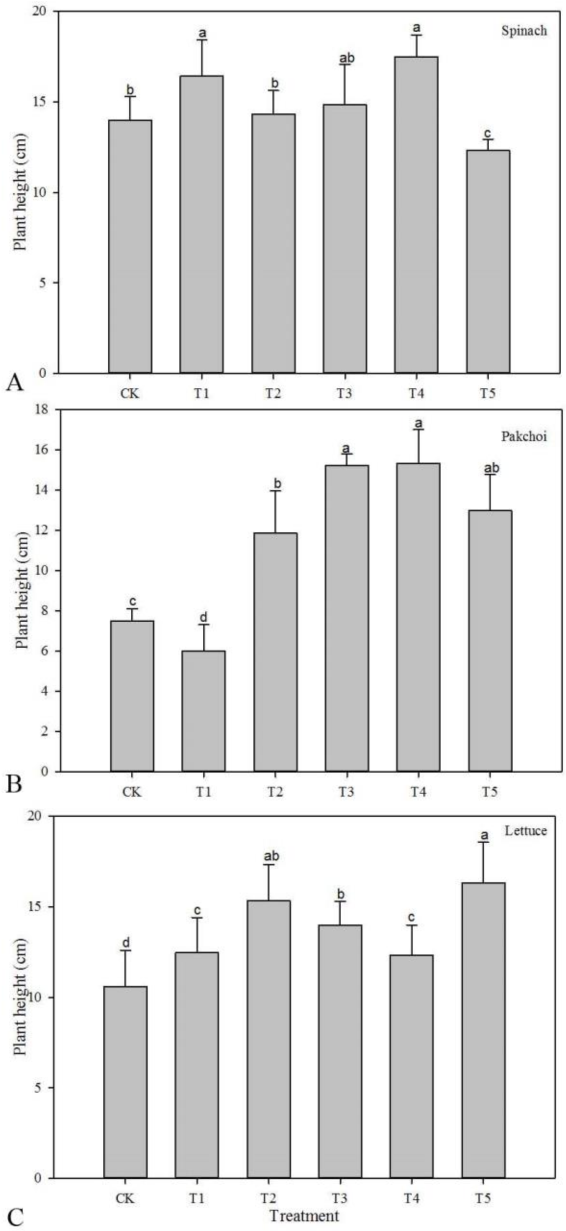

Figure 1. Plant height of spinach, pakchoi, and lettuce in the different fertilizer treatments. In each plant, values accompanied by different letters differ significantly at P< 0.05.

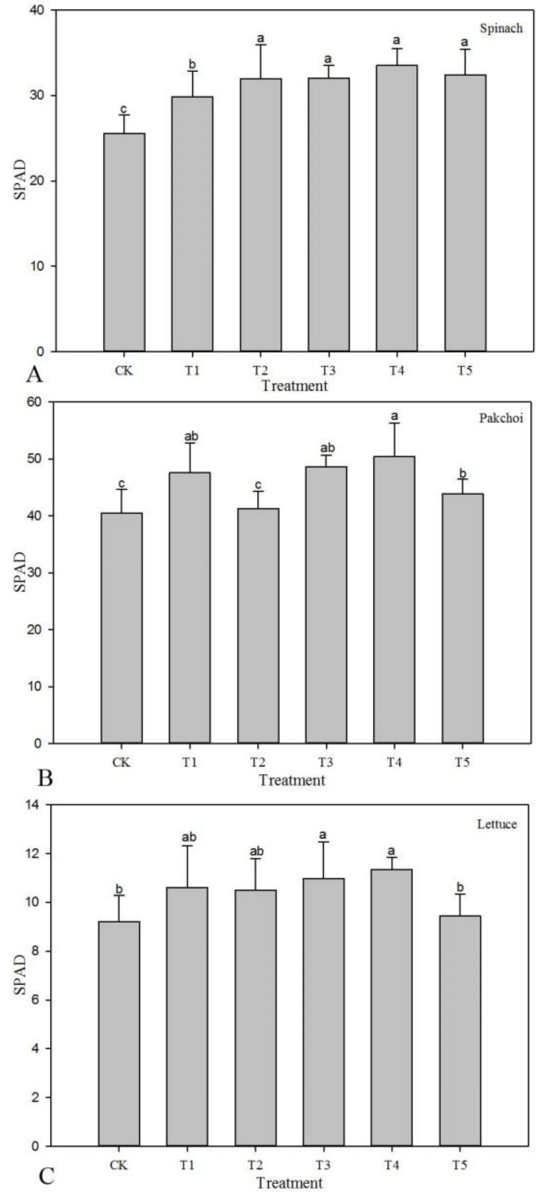

Figure 2. SPAD readings of spinach, pakchoi, and lettuce in the different fertilizer treatments. In each plant, values accompanied by different letters differ significantly at P< 0.05.

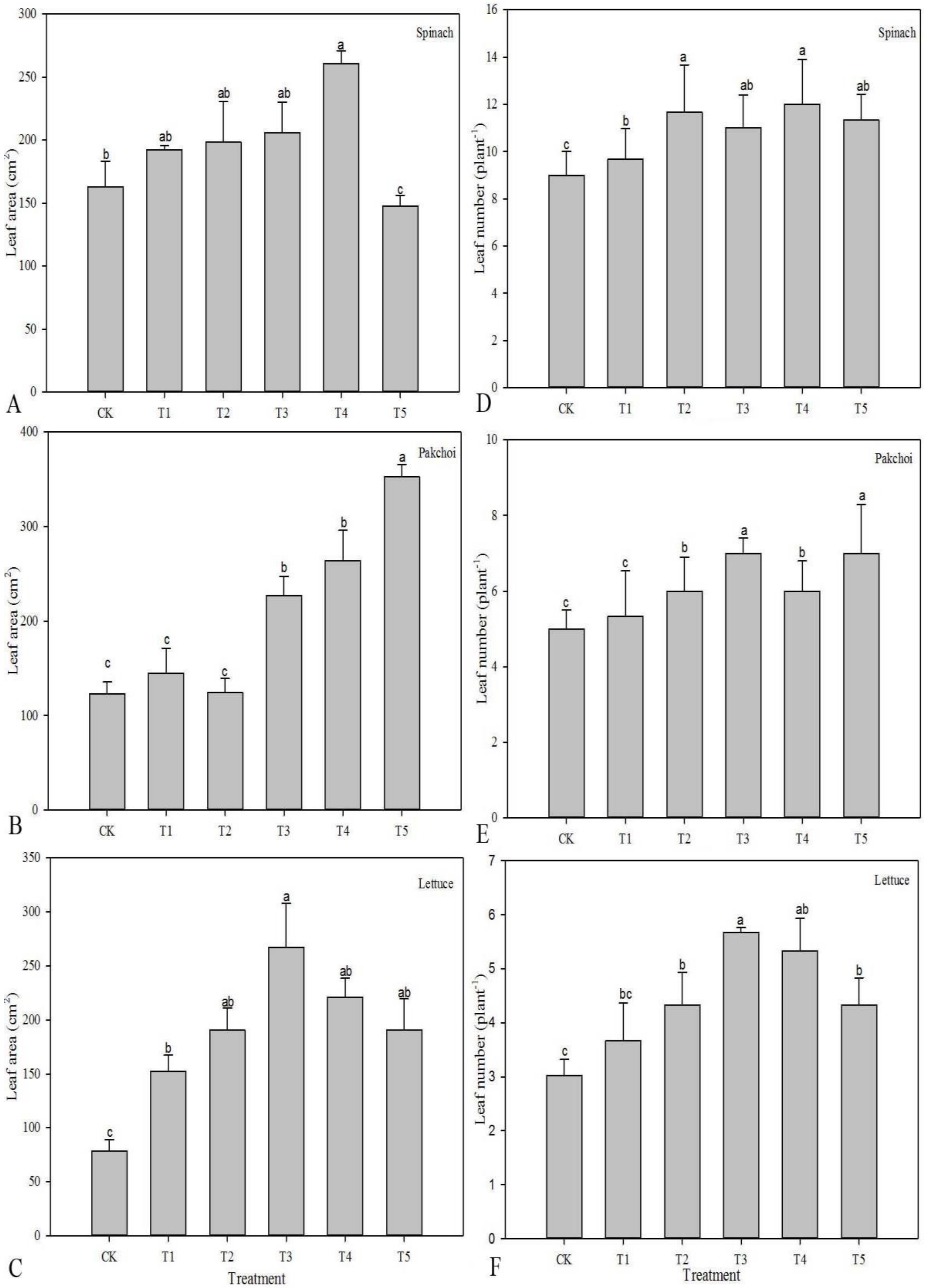

Figure 3. Leaf area and leaf number of spinach, pakchoi, and lettuce in the different fertilizer treatments. In each plant, values accompanied by different letters differ significantly at $P< 0.05$.

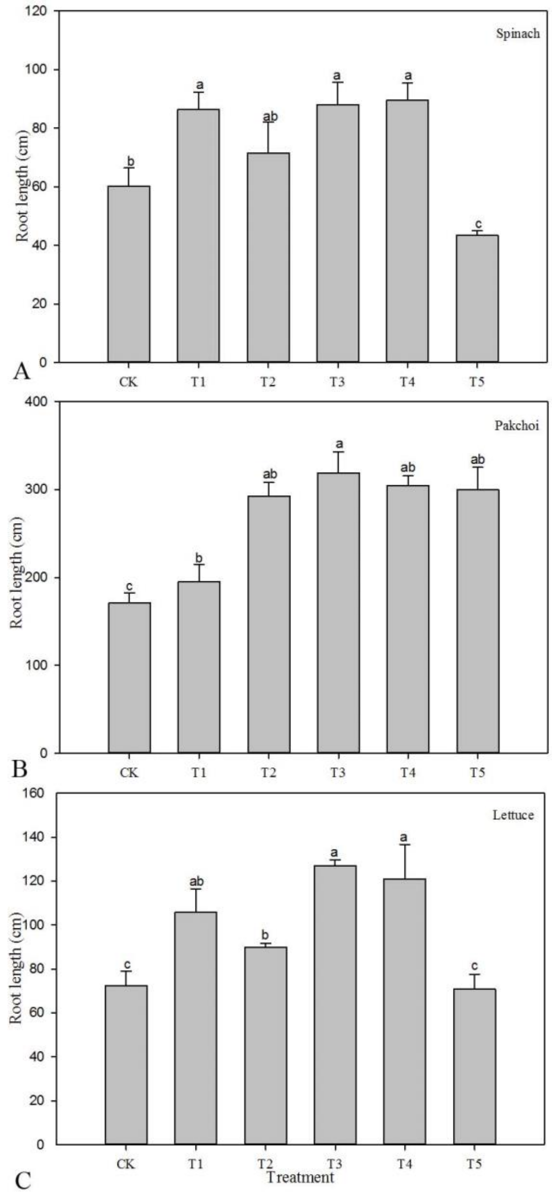

Figure 4. Root length of spinach, pakchoi, and lettuce in the different fertilizer treatments. In each plant, values accompanied by different letters differ significantly at P< 0.05.

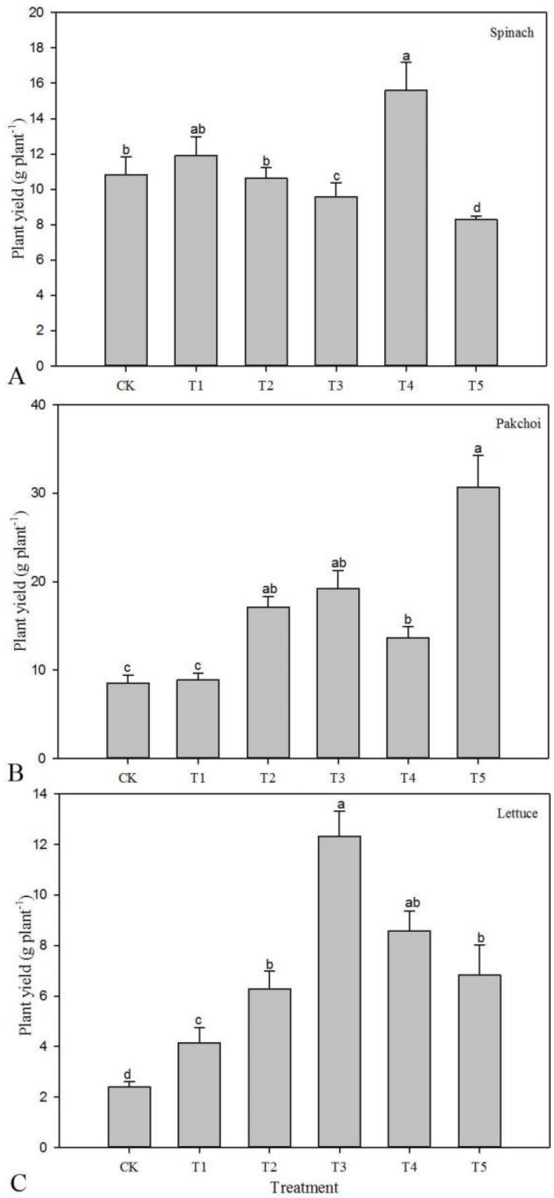

Figure 5. Plant yield of spinach, pakchoi, and lettuce in the different fertilizer treatments. In each plant, values accompanied by different letters differ significantly at P<0.05.

a significant effect on plants yield. In T_4 treatment, spinach had the highest plant yield, which was 15.60 g plant^{-1}. Without fertilization, the minimum yield was 2.7 g plant^{-1}. Under different fertilizer treatments, the yield of pakchoi varied greatly and increased significantly under T_5 treatment. The greatest yield of pakchoi in T_5 was 30.66 g plant^{-1}. The yield of lettuce in T_3 was the highest, 12.32 g plant^{-1}. Fertilizer inputs are usually used in conventional agriculture to maintain high yields. However, there are adverse impacts on the environment, such as water and soil

Table 3. Plant biomass of vegetables in the different fertilizer treatments.

Treatment	Root dry biomass (g)			Stem and leaf dry biomass (g)			Total dry biomass (g)		
	Spinach	Pakchoi	Lettuce	Spinach	Pakchoi	Lettuce	Spinach	Pakchoi	Lettuce
CK	0.03b	0.01c	0.04c	0.77b	0.13c	0.60c	0.80b	0.14c	0.64c
T_1	0.03b	0.05b	0.05c	0.75b	0.62b	0.66c	0.78b	0.67b	0.71c
T_2	0.03b	0.03c	0.06b	0.87ab	0.32c	0.80c	0.90ab	0.35c	0.86c
T_3	0.04a	0.07a	0.10a	1.07a	1.29a	1.20b	1.11a	1.36a	1.30b
T_4	0.04a	0.04b	0.06b	1.09a	0.41c	1.16b	1.13a	0.45c	1.22b
T_5	0.02c	0.07a	0.13a	0.60c	0.29c	2.33a	0.62c	0.36c	2.46a

[a] (a-c) Different letters indicate significant differences (P< 0.05) among the different treatments according to LSD multiple comparison.

pollution. CEM contains a variety of beneficial bacteria and could maintain the productivity of green vegetable.

DISCUSSION

Organic materials such as plant residues and fertilizers are usually used to maintain soil fertility. Due to the rapid economic development, mineral fertilizers have been widely used. Therefore, more mineral fertilizer and less organic fertilizer are now applied. In order to maximize crop yields, excessive and unreasonable application of mineral fertilizers not only wastes resources but also pollutes the environment. Attempt must be made to meet the needs for food production, ensure food security, optimize fertilization, maintain or improve soil fertility, and protect the environment.

Plant height is one of the most important agronomic characters in production and is related to the structure and yield of crops. Plant height is generally regarded as one of the indicators of plant vitality and a measure of plant respiratory and photosynthetic capacity, and it depends on the growth habit and vitality of plants. Soil nutrients are also essential for plant height. Field experiments showed that the growth characteristics of vegetable plants, such as plant height, leaf number and root length, were significantly improved. In this research, plant height was taken from the ground level to the maximum length of the leaf, and the maximum root length was also measured. The height of plants was analyzed in field studies conducted during the growing season. Fertilization could increase plant height. During the experiment, the plant heights of different fertilizer treatments differed greatly, and T_4 treatment showed the largest plant height. A similar research showed that the application of microbial fertilizer significantly increased plant height, weight, and yield (Aleksander *et al.*, 2018). Chlorophyll is an important factor for assessing plant nitrogen status and leaf senescence. Chloroplast pigment is the main substance for the photosynthetic photoenergy absorption, transmission, and transformation. It is closely related to photosynthesis, plant biomass and crop yield. The SPAD meter is the most commonly used instrument for measuring plant chlorophyll and nitrogen concentration. In most cases, fertilizer stress has reduced chlorophyll concentration and net photosynthesis. The study also found that under the fertilizer treatments that promoted plant growth, the level of SPAD was significantly higher than that of the control. This research found that higher SPAD combined with higher leaf number increased photosynthetic activity and improved vegetable growth and yield. Taia *et al.* (2020) studied the effect of effective

[DOR: 20.1001.1.16807073.2021.23.4.15.4]

microorganisms on the vegetable. Their research showed that effective microorganisms increased the SPAD chlorophyll of vegetable.

Leaf area is an important physiological indicator of vegetables. Previous studies have shown that increased application of nitrogen fertilizer leads to larger leaf area. The yield of crops increases with the increase in leaf area. When the leaf area increases to a certain limit, the yield will decrease because the field is closed, the light is insufficient and the photosynthetic efficiency decreases. Fertilizer stress reduced leaf area and SPAD, resulting in a decline in yield. Neveen (2014) found a similar trend. The application of microbial fertilizer increased the leaf area of vegetables.

Root is the most active nutrient absorption organ; root vitality can show the root absorption ability and the synthesis metabolism. The root has a direct beneficial effect on crop yield. Phosphorus is an important nutrient element for root growth and plays a vital role in the metabolism of crops. According to the study of Jusoh *et al.* (2013), application of microbial fertilizer can increase the nitrogen, phosphorus and potassium content of the soil. The results showed that CEM greatly promoted the growth of vegetables. Fertilizer could stimulate cell division and enlargement, causing tissue expansion, thereby improving the plant height and root length of vegetables. The microbial fertilizer was applied to increase the number of beneficial bacteria in the soil. It promoted the more efficient use of fertilizers, accelerated the circulation of soil materials and energy, activated soil nutrients, and promoted root growth of vegetables. At the same time, the activity of functional microorganism was enhanced through the interaction between microorganisms. The microbial fertilizer greatly improved the resistance of vegetables and promoted the growth of plants.

Generally, available nitrogen, phosphorus and potassium are essential nutrients for vegetable growth, and requirements for these nutrients are different at different growth stages. Because the yield of plants using microbial fertilizer was higher than the treatment without microbial fertilizer, it could be concluded that the microbial fertilizer had an effect on the growth of vegetables. Microbial fertilizers enhanced the use efficiency of available nitrogen, phosphorus and potassium during the vegetable growth phase. Therefore, vegetables could use more nitrogen, phosphorus and potassium in the soil, greatly increasing vegetable yields. Microbial fertilizer is a specific product containing specific living bacteria, which can increase the supply of plant nutrients and increase the yield of crops through the life activities of the contained microorganisms. Similar studies have shown that microbial fertilizer have greatly increased the production of Chinese cabbage (Souri, *et al*., 2019). This study evaluated the ability of the compound effective microorganisms to increase vegetable yield.

CONCLUSIONS

Proper fertilization methods could reduce fertilizer loss and increase vegetable growth and yield by affecting the fertilizer utilization rate of vegetables. Plant hormones, acids, and vitamins produced by microbial activities stimulate plant growth to varying degrees. Compared with the control treatment, the plant height gradually increased after applying fertilizer throughout the growth period of the vegetables. The total chlorophyll concentration of vegetables treated with the Compound Effective Microorganisms (CEM) was also significantly higher than that of the control treatment. Microbial fertilizers are widely used in ecological agriculture and it may be a viable alternative for organic agriculture. The application technology of biological fertilizer and organic fertilizer is an important way to improve the yield and quality of crop, reduce environmental

[DOR: 20.1001.1.16807073.2021.23.4.15.4]

pollution, and develop modern ecological agriculture.

ACKNOWLEDGEMENTS

This study was financially supported by the Open Fund of the Ministry of Education Key Laboratory of Modern Agricultural Equipment and Technology & High-tech Key Laboratory of Agricultural Equipment and Intelligentization of Jiangsu Province (No. JNZ201915) and Qingdao science and technology benefit the people key project (No. 19-6-1-81-nsh).

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کاربرد ترکیب میکروارگانیسم های موثر باعث افزایش رشد وعملکرد سبزیجات می شود

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چکیده

استفاده نادرست یا بی رویه از کودهای شیمیایی منجر به ایجاد منبع آلودگی غیرنقطه ای در سامانه های تولید سبزیجات می شود. کود شیمیایی عاملی کلیدی است که بر جذب عناصر غذایی و تولید زیست توده سبزیجات اثرمیگذارد. با اینهمه، اطلاعات در باره نقش میکروارگانیسم های موثر ترکیبی (CEM) در تولید سبزیجات کم است. در این پژوهش، برای بررسی اثر آنها روی ویژگی های رشد و کیفیت کاهو، اسفناج، و کرفس چینی (pakchoi) آزمایشی مزرعه ای با سه تیمار کودی (از این کود) و تیمارهای دیگر اجرا شد. تیمارهای آزمایش عبارت بود از تیمار شاهد (CK)، تیمارT_1 شامل کود اوره با نسبت جرم به حجم (w/v) اوره: آب برابر ۰/۳٪ ، تیمارT_2 شامل کودمرکب: آب با نسبت جرم به حجم (w/v) برابر ۰/۳٪ ، تیمار T_3شامل کاربرد CEM رقیق شده 1:1000 (کودCEM: آب با نسبت حجمی: v/v) قبل از مصرف، تیمار T_4 شامل کاربرد CEM رقیق شده 1:500 (کودCEM : آب با نسبت حجمی: v/v)، و تیمار T_5 مانندT_4 ولی با رقت 1:100. از میان گیاهان کاشته شده، ده بوته درهر تیمار برای انجام اندازه گیری های رشد انتخاب شد . بعد از افزودن کود و در طی دوره رشد، در مقایسه با تیمار شاهد، ارتفاع و بلندی گیاهان به تدریج افزایش یافت. غلظت کل کلروفیل در تیمارهای CEM به طور معناداری بیشتر از تیمار شاهد بود. کاربرد CEM منجر به افزایش سطح برگ و تعداد برگ شد. اثر کود میکروارگانیسم های موثر(EM) روی رشدو عملکردسبزیجات بررسی شد. در تیمار T_4، اسفناج بیشترین عملکرد را داشت که برابر ۱۵/۶ گرم در بوته بود. در تیمارهای مختلف کودی، عملکرد کرفس چینی تغییرات زیادی داشت و در تیمارT_5 عملکرد گیاه دارای افزایش معناداری بود. بیشترین عملکرد کرفس چینی در تیمارT_5 برابر ۳۰/۶۶ گرم در بوته بود. عملکرد کاهو در تیمار T_3 بیشتر از همه و برابر۱۲/۳۲ گرم در بوته بود. کود CEM می تواند بهره وری سبزیجات را پایدار کند و دارای باکتری های مفیدی است. کاربرد میکروارگانیسم های موثر باعث افزایش عملکرد و رشد گیاهان شد. این نتایج نشان می دهد که با مدیریت مصرف CEM، سبزیجات عملکرد و کیفیت بالایی خواهند داشت.

[DOR: 20.1001.1.16807073.2021.23.4.15.4]