Influences of application of slow-release Nano-fertilizer on green pepper growth, soil nutrients and enzyme activity

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Abstract. In order to examine the fertilizer effect of slow-release Nano-fertilizer and its impact on the ecological environment, a cultivation pot experiment was arranged to study the influences of application of slow-release Nano-fertilizer on green pepper growth, soil Nutrients, enzyme activity and microorganisms. The experiment was designed to have four treatments: treatment 1 (blank treatment), treatment 2 (slow-release Nano-fertilizer), treatment 3 (composite chemical fertilizer), treatment 4 (slow-release Nano-fertilizer + compound fertilizer), and each treatment was five parallels. The results were as follows: (1) The application of slow-release Nano-fertilizer can promote the growth of green pepper, but due to the slow effect of fertilizer, the growth of green pepper was not as good as that of chemical fertilizer. (2) Application of slow-release Nano-fertilizer significantly increased soil nutrients, especially alkaline nitrogen, with an increase of 100.0%. (3) Application of slow-release Nano-fertilizer significantly increased soil enzyme activity and soil microbial population. Compared with blank, soil dehydrogenase and catalase activities of treatment 2 increased by 37.4% and 21.3%, respectively, and soil bacteria, actinomycetes and fungi increased by 50%, 72% and 208%, respectively. Application of slow-release Nano-fertilizer can improved soil nutrient, soil enzyme activity and soil microorganism, reduce nutrient loss and improve soil ecological environment, with a good application value.

1. Introduction
At present, China's total cultivated land area is about 140 million ha, the per capita cultivated land area is only about 0.1 ha, far lower than the world's per capita cultivated area of 0.25 ha [1]. The per capita cultivated area was less than 1/6 of the United States and 1/15 of Canada [2]. Excessive application of chemical fertilizers in agricultural production was generally adopted for the purpose of increasing yield in China. The average fertilizer used was 1.46 kg ha⁻¹, which is far higher than the world level of 0.54 kg ha⁻¹, which was 2.5 times that of the EU and 2.6 times that of the United States. The total amount of chemical fertilizers used in China reached $5.84\times10^{10}$ kg in 2012, which accounting for 33.0% of the total amount of fertilizers in the world, and the amount of per unit area of cultivated land was three times higher than the world average [3]. The nitrogen fertilizer utilization rate was 30.0%~35.0%, and the utilization rate of phosphate fertilizer was only 10.0% - 20.0%, which means that most of the nitrogen and phosphorus applied into soil were lost to the environment through different ways, resulting in water
eutrophication [4]. In addition, long-term application of chemical fertilizers will cause soil compaction, reduce soil porosity, increase soil bulk density, and destroy soil structural stability [5].

Compared with chemical fertilizers, Nano-fertilizers has larger specific surface area, which makes nutrients more easily absorbed by plants, which significantly improves its fertilizer efficiency and has significant economic benefits. The application of Nano-fertilizer can improve the physical and chemical properties of soil and improve the ability of water and fertilizer conservation [6]. At the same time, it can be free from the influence of complex factors such as soil type, which can greatly reduce the pollution of soil and groundwater, and greatly improve the yield while reducing the pollution of crops. Therefore, it is called environmentally friendly fertilizer. Green pepper is a kind of four-season vegetable, which is grown in most parts of China and requires a large amount of fertilizer. In this paper, the effects of slow-release Nano-fertilizer application on green pepper growth, soil nutrient, enzyme activity and microbial content were studied by cultivation experiments. The fertilizer efficiency of slow-release Nano-fertilizer was investigated and the theoretical basis for the use of Nano-fertilizer was provided.

2. Material and methods

2.1. Experimental site
Maoming city has a tropical subtropical monsoon warm weather which located in the south of the Tropic of cancer, south of China. The annual average temperature of the city is 22.3 °C ~23.0 °C, the highest monthly average temperature of 26.5 °C to 28.7 °C (July), the lowest monthly average temperature of 14 °C - 16 °C (January), and the annual rainfall is 1500 - 1800 mm. The rainy day is between 100 and 170 days.

2.2. Material
Experimental flowerpots and green pepper seeds were purchased from the local flower market (pots can hold about 3 kg of soil); Slow release Nano fertilizer with the brand is Liulitian 2 (the contents of nitrogen, phosphorus, potassium, magnesium, calcium and humic acid are 26.0%, 17.0%, 13.0%, 0.2%, 0.9% and 3.0%, respectively); Compound fertilizer (the nitrogen, phosphorus and potassium contents are both 15.0%). The basic physical and chemical properties of the experimental soil were pH 6.7, the organic matter content was 25.1 g kg-1, and the nitrogen, phosphorus and potassium contents were 1.6 g kg-1, 1.45 g kg-1 and 0.83 g kg-1, respectively.

2.3. Experimental design
The experiment was designed to have four treatments: treatment 1 (blank treatment), treatment 2 (slow-release Nano-fertilizer), treatment 3 (composite chemical fertilizer), treatment 4 (slow-release Nano-fertilizer + compound fertilizer), and each treatment was five parallels. In order to study the effects of different fertilizers on the growth of green peppers, except for the treatment 1, the other treatments were applied with the same amount of nitrogen, 0.35 g kg-1 soils. In addition, in order to eliminate the effects of pesticides on the growth and ecology of green peppers, this experiment does not add pesticides and other herbicides during the green peppers growing season. The fertilization scheme was shown in Table 1.

| Treatments     | Base fertilizer (2017-11-18) | Topdressing 1 (2017-12-10) | Topdressing 2 (2017-12-24) |
|----------------|-----------------------------|---------------------------|---------------------------|
| Treatment 1    | 0                           | 0                         | 0                         |
| Treatment 2    | 2.63 g Nano-fertilizer      | 1.32 g Nano-fertilizer    | 0.66 g Nano-fertilizer    |
| Treatment 3    | 4.56 g chemical fertilizer  | 2.28 g chemical fertilizer| 1.14 g chemical fertilizer|
| Treatment 4    | 1.32 g Nano-fertilizer +    | 0.66 g Nano-fertilizer +   | 0.33 g Nano-fertilizer +   |
|                | 2.28 g chemical fertilizer  | 1.14 g chemical fertilizer| 0.57 g chemical fertilizer|
2.4. Sample collection and analyses
The green pepper seedlings were investigated from 4 to 5 leaves of the seedlings. Plant height, stem diameter and leaf age were recorded. In the middle of the growth of green pepper seedlings (December 27), five soil cores were randomly collected in a plot with a soil borer, and mixed to be a soil sample of the plot. Each soil sample was equally divided into two parts: One was kept fresh and stored at 4 °C for microbial and Soil enzyme activity testing; The other was air-dried and passed through 2-mm and 0.15-mm sieves consecutively for physical and chemical analyses.

Soil physical and chemical properties analysis [7]: Total N content in soil by the Kjeldahl method, alkali-hydrolyzable N by the alkali solution diffuse method, The nitrate nitrogen was determined by ultraviolet spectrophotometry; the citrate-soluble phosphorus was determined by molybdenum blue colorimetry.

Soil enzyme activity analysis [8]: Dehydrogenase activity was determined by thetriphenyl tetrazolium chlorine (TTC) reduction method, catalase activity by the titration of potassium permanganate, urease activity by the indophenol blue spectrophotometric method.

Soil microorganism analysis [9]: Bacteria in the soils were cultured by the beef-peptone agar medium, fungi by Charles Peter Kirschner (Czapek) medium, and actinomyces by ammonium starch agar medium. The numbers of these were counted by the dilution-plate method.

2.5. Statistical analyses
The statistical software package SPSS 19.0 was used for the analysis of variance (ANOVA), which was performed for data treatment using Duncan’s multiple range test at P = 0.05.

3. Results and discussion

3.1. Effect of different fertilization on the growth of green pepper
Fertilization can promote plant growth. The overall change trend of plant height and stem diameter of green pepper was consistent during the growth cycle of green pepper. Fertilization treatment was significantly higher than blank treatment (p<0.05) after December 12, and chemical fertilizer treatment is significantly higher than other treatments (p<0.05), while treatment 2 and treatment 3 were not significantly different (p>0.05) (Figure 1 and Figure 2). The leaf age difference of the four treatments was not obvious after fertilization. In the late growth stage of green pepper, the leaf age of chemical fertilizer treatment was higher than other treatments (Fig. 3). The use of compound fertilizer can increase the plant height, leaf age and stem diameter of green peppers by 31.7%, 23.5% and 17.7%, respectively. It indicates that after the application of the compound fertilizer, the chemical fertilizer efficiency was quickly released into the soil to supply the plants, so that the plants have sufficient nutrients during photosynthesis, and promoted the rapid growth of green peppers. The plant height and stem diameter of green pepper treated with slow release Nano-fertilizer were also significantly higher than treatment 1, with an increase of 12.2% and 12.3%, respectively. The effect of slow-release Nano-fertilizer on promoting green pepper growth was lower than that of compound chemical fertilizer. Compared with chemical fertilizer treatment, the fertilizer release of Nano-fertilizer was slow, and it can provide sustainable nutrients for plants. Related studies showed that: the roots of plants applied with slow-release Nano-fertilizer were developed, and the plant resistance to diseases, insect pests and diseases and fruit quality were significantly improved, which has a certain effect of increasing yield [10].
Figure 1. Variation in height of green pepper plants with different fertiliser treatments. Means in each column with the same letter are not significantly different at $P = 0.05$.

Figure 2. Variation in root size of green pepper plants with different fertiliser treatments. Means in each column with the same letter are not significantly different at $P = 0.05$. 
3.2. **Effect of different fertilization on soil nutrients**

After applying the base fertilizer to the topdressing, the difference of the alkali nitrogen, nitrate nitrogen and total nitrogen in the soils of treatment 2, treatment 3 and treatment 4 was not obvious, but significantly higher than the blank treatment (Figure 4). The slow release Nano-fertilizer was a high-nitrogen fertilizer, which can effectively increase the contents of total nitrogen, alkali nitrogen and nitrate nitrogen in the soils. Compared with the blank treatment, the total nitrogen, alkali nitrogen and the nitrate nitrogen in soils with slow release Nano-fertilizer treatment were increased by 60.0%, 100.0% and 27.8%, respectively.

Soil citric acid soluble phosphorus is the effective phosphorus in the artificial surface layer. The measurement of soil soluble phosphorus in lemon shows the status of soil fertilization and the supply of phosphorus in this region [11]. The application of fertilizer can increase the available phosphorus in the soil surface, while the content of dissolved phosphorus in treatment 4 was significantly higher than other treatments, indicating that the combination of chemical fertilizer and Nano-fertilizer promoted the simultaneous release of phosphorus, resulting in higher soluble phosphorus content in the soil. The difference of soluble phosphorus in treatment 2 and treatment 3 was not significant, indicating that the effective phosphorus content in the artificial surface layer promoted the growth of green pepper plants basically the same when using fertilizer and Nano-fertilizer respectively. The effective phosphorus content of the two fertilizers was about the same (Figure 4).
Figure 4. Variations of soil total nitrogen, alkaline nitrogen, nitrate nitrogen and citric acid soluble phosphorus in the different treatment. Means in each column with the same letter are not significantly different at $P = 0.05$. T1, treatment 1; T2, treatment 2; T3, treatment 3; T4, treatment 4.

3.3. Effect of different fertilization on soil enzyme activity

Soil enzymes are important participants in soil material circulation and energy flow. It is involved in the whole process of soil occurrence, development and soil fertility formation and evolution, and is one of the most active components in soil ecosystems [12-13]. Soil enzymes can characterize the soil comprehensive fertility characteristics and soil nutrient transformation processes. Soil enzyme activities are affected by the secretions of different plants in the growth and development process, mineralization and decomposition of dead root stubble, and different farming and management methods [14-15].

Compared with blank treatment, fertilization can significantly improve soil enzyme activity during the growth period of green pepper, (Figure 5, $p<0.05$), and the soil dehydrogenase and catalase activity treated with Nano-fertilizer were significantly higher than other treatments. Compared to the blank treatment, the soil dehydrogenase activities of treatment 2, treatment 3, and treatment 4 increased by 37.4%, 33.6%, and 22.9%, respectively. The soil urease activities of treatment 2, treatment 3, and treatment 4 increased by 26.8%, 12.1%, and 17.5%, respectively. The soil catalase activity of Treatment 2, Treatment 3, and Treatment 4 increased by 21.3%, 13.3%, and 19.3%, respectively (Figure 5). Consistent with previous studies, the application of Nano carbon can increase soil enzyme activity, and the higher the concentration of Nano carbon applied, the better the promotion effect [16]. Because the application of Nano carbon can change the structure and energy state of water molecules, increase their activity, and carry a large amount of nutrients into plants during the process of water being absorbed by plants. Nano-carbon can improve the water environment around the crops, make the nitrogen in the fertilizer more easily absorbed by the roots, increase the root activity of the crop, promote the increase of root exudates, and increase the microorganisms in the soil. Soil microbial activity increases, thereby increasing soil porosity, loose soil, and reduced soil bulk density [17]. Increasing soil urease activity
plays an important role in increasing nitrogen use efficiency and promoting soil nitrogen cycling, and promotes plant growth and development. Therefore, the increase in the number of soil microorganisms and the secretion of plant roots and the increase in activity intensity can promote the increase of soil enzyme activity.

Figure 5. Soil enzyme activities in the different treatment. Means in each column with the same letter are not significantly different at P = 0.05. T1, treatment 1; T2, treatment 2; T3, treatment 3; T4, treatment 4.

3.4. Effect of different fertilization on soil microorganism
Soil microorganisms is the most important and active components of soil organisms. Their dynamic changes are more significant and sensitive than other soil organisms in reflecting soil fertility and soil quality changes. Keeping a dominant role in maintaining ecosystem stability, soil nutrient decomposition, transformation and anti-interference, and control all key processes of soil ecosystem function [18].

The number of soil microorganisms was as follows: The population of bacteria was the biggest, followed by the actinomycetes and fungi. During the growth period of green pepper, the number of various fertilization treatment bacteria, actinomycetes and fungi was significantly higher than the blank treatment. The results showed that fertilization increased soil nutrients, improved soil ecological environment, and increased soil microbial quantity. Moreover, the number of soil microorganisms treated with Nano-fertilizer was significantly higher than that treated with fertilizer (Figure 6, p<0.05). Compared to the blank treatment, the number of bacteria in treatment 2, treatment 3, and treatment 4 increased by 50.0%, 40.0%, and 50.0%, respectively. The number of actinomycetes in treatment 2, treatment 3, and treatment 4 increased by 72.0%, 52.0%, and 60.0%, respectively. The number of fungi in treatment 2, treatment 3, and treatment 4 increased by 208.3%, 183.3%, and 204.2%, respectively (Figure 6). Slow-release Nano-fertilizer produces large amounts of humic acid during slow release. Humic acid is the core of soil fertility and provides carbon and nitrogen sources for soil microorganisms. In addition, humic acid can directly or indirectly improve soil temperature, moisture and gas permeability, regulate soil pH, promote soil microbial growth and reproduction, and increase its quantity and variety.
Figure 6. Numbers of bacteria, Actinomyces and fungi in the different fertiliser treatments. Means in each column with the same letter are not significantly different at $P = 0.05$. T1, treatment 1; T2, treatment 2; T3, treatment 3; T4, treatment 4

4. Conclusion
The use of chemical fertilizer can promote the growth and tillering of green pepper more effectively. The application of slow-release Nano-fertilizer can release nutrient slowly and lastingly, which was beneficial to increase soil microbial quantity and enzyme activity. Soil dehydrogenase and catalase activity at treatment 2 were 1.37 times and 1.21 times of treatment 1, 1.03 times and 1.07 times of treatment 3, and 1.12 times and 1.02 times of treatment 4, respectively. The amount of soil bacteria, actinomycetes and fungi treated by Nano-fertilizers were 1.07 times, 1.13 times and 1.09 times that of chemical fertilizer treatment. The production and application of slow-release Nano-fertilizer can improve the ecological environment of farmland soil and has good application value.

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