

# *Effects of Bacillus Mucilaginosus with Different Carriers on Tobacco Growth*

Nan Zhao<sup>1,2</sup>, Zhen Ju<sup>1,2</sup>, Ke Fang<sup>1,2</sup>, Junwei Yang<sup>3</sup>, Tianhui Ye<sup>3</sup>, Ping Zhou<sup>1,2,\*</sup>

<sup>1</sup>Key Laboratory of Mountain Surface Processes and Ecological Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu, 610041, China

<sup>2</sup>University of Chinese Academy of Sciences, Beijing, 100049, China

<sup>3</sup>Panzhuhua Company of Sichuan Tobacco Company, Panzhuhua, 617061, China

\*Corresponding author

**Keywords:** Bacillus mucilaginosus, Carriers, Tobacco Growth

**Abstract:** The application of Bacillus mucilaginosus and carriers has positive effects on tobacco growth and soil microbial activity. In this paper, we investigated the characteristics of changes in tobacco growth under Bacillus mucilaginosus and carrier dosing, as well as under the effects of different fungicide additions and different carriers. The results of the study showed that (1) The addition of carriers has a good improvement effect on indicators such as plant height, maximum leaf length, maximum leaf width, and stem thickness of field tobacco. The four indicators of the biochar group were 21.32%, 5.80%, 10.39%, and 8.07% higher than those of the control group, respectively. The four indicators of the bacterial bran group were 19.10%, 7.30%, 11.45%, and 7.96% higher than those of the control group, respectively. These two carriers have the best performance. (2) The addition of Bacillus mucilaginosus has a good promoting effect on the height, maximum leaf length, maximum leaf width, and stem thickness of tobacco plants in the field, and this promoting effect generally shows a trend of "increasing first and then decreasing" with the increase of bacterial dosage. The four indicators of the moderate addition group were 26.76%, 15.24%, 19.37%, and 18.08% higher than the control group, respectively, and were higher than those in the other groups ( $p < 0.05$ ). (3) Overall, S2 (biochar+100g Bacillus mucilaginosus) and J2 (mycorrhizal bran+100g Bacillus mucilaginosus) treatments have the best promoting effect on the growth of tobacco in the field stage. This study can provide theoretical basis and data support for improving the quality of roasted tobacco and the microbial activity of the red soil in the southwest tobacco area.

## 1. Introduction

Microbial fertilizer is a product containing specific living microorganisms, which, through the activities of the microorganisms contained therein, can significantly contribute to the increase of plant nutrients, the improvement of the quality of agricultural products as well as the improvement of the agro-ecological environment[1]. Bacillus mucilaginosus, as a common soil microorganism, has received widespread attention due to its properties of promoting plant growth, improving soil structure and increasing soil phosphorus and potassium effectiveness[2,3]. As an important

component of microbial fertilizer, carriers have a significant impact on the survival, propagation and functioning of microorganisms in soil. Different carrier materials have different effects on the fixation, protection and release of microorganisms, and the selection of appropriate carriers can significantly promote the improvement of microbial fertilizers, so the selection of carriers is also the key to improve the effect of microbial fertilizers[4,5].

Tobacco as one of the important economic crops in China, its yield and quality directly affect the economic income of farmers. However, the traditional way of tobacco cultivation often has problems such as excessive use of chemical fertilizers and soil degradation, which not only affect the growth of tobacco, but also may lead to soil microbial ecological imbalance[6]. Therefore, this study took *Bacillus mucilaginosus* and its carrier as the research object, and compared and analysed the agronomic traits of tobacco with different amounts of *Bacillus mucilaginosus* and different carriers, with a view to providing theoretical basis and data support for the effective promotion of the enhancement of the quality of roasted tobacco production in the southwestern tobacco area and the maintenance of microbial activity of tobacco-planting soil.

## 2. Materials and Methods

### 2.1. Overview of the Study Area

The study area is located in Miyi County, Panzhihua City, Sichuan Province, Southwest China (26°52'13"N, 102°10'28"E). The area has a southern subtropical dry and hot river valley climate with abundant sunshine and strong solar radiation. The average annual sunshine hours are 2381.5 h, the average annual sunshine percentage is 54%, the average annual total solar radiation is 68.140.5 kcal/cm<sup>2</sup>-year, the average annual cumulative temperature of >0°C is 7208.2°C, the daily change of temperature is large, the annual change is small, the average temperature of many years is 19.7°C, the average annual rainfall is 1094.2 mm, the average annual wind speed is 2.1 m/s, and the average annual frost-free period is 308 days.

### 2.2. Experimental Design

#### 2.2.1. Experimental Deployment

Before the beginning of the experiment, *Bacillus mucilaginosus* fungicide was firstly prepared into microbial fertilizer with biochar, mycorrhizal bran and potassium xanthate respectively using the blending method, and the preparation dosage was as shown in Table 1, and each treatment was repeated 20 times. Among them, the amount of fungicide added was 50g/hole was regarded as low additive amount, 100g/hole was regarded as medium additive amount, and 150g/hole was regarded as high additive amount. Two rows of untreated tobacco were planted between each experimental plot as a separation.

When planting tobacco in the field, the spacing between rows was 120 cm, the spacing between plants was 50 cm, and the depth of the planting pit was about 5 cm. 225 g/square metre of organic fertilizer and 45 g/square metre of special fertilizer for tobacco were applied when transplanting the tobacco, and homemade microbial fertilizer was applied to the field simultaneously by hole application, and the seedlings were planted in the wall of the planting pit at a distance of 15 cm from the bottom of the pit and covered with a film on the surface of the ground. The follow-up fertilizer was 15 g/square metre of special tobacco fertilizer, 30 g/square metre of potassium sulphate and 18 g/square metre of seedling lifting fertilizer. A replanting was carried out 30d after transplanting, and the mulch was removed 60d after transplanting.

Table 1: Experimental deployment

Treatments	Carrier	Carrier application /g	Bacillus mucilaginosus application /g
00	None	150	0
S0	Biochar	150	0
J0	Mycorrhizal Bran	150	0
H0	Potassium Xanthate	150	0
01	None	150	50
S1	Biochar	150	50
J1	Mycorrhizal Bran	150	50
H1	Potassium Xanthate	150	50
02	None	150	100
S2	Biochar	150	100
J2	Mycorrhizal Bran	150	100
H2	Potassium Xanthate	150	100
03	None	150	150
S3	Biochar	150	150
J3	Mycorrhizal Bran	150	150
H3	Potassium Xanthate	150	150

### 2.2.2. Tobacco Growth Measurement and Data Processing

Starting from 30d after transplanting, plant height, maximum leaf length, maximum leaf width and other indexes were measured for each tobacco plant every 30d until the tobacco harvest for a total of four measurements. When the experiment was completed, the data were organised and pre-processed using Microsoft Excel 2021 and MySQL 8.0; descriptive statistics of the data were analysed using pandas 1.5.3; ANOVA and posthoc tests were performed using IBM SPSS Statistics 25 and SigmaPlot 14.0 (LSD,  $p < 0.05$ ); plots were performed using Origin 2021.

## 3. Results and Analysis

### 3.1. Effect of Bacillus mucilaginosus Dosing Carriers on Tobacco Growth

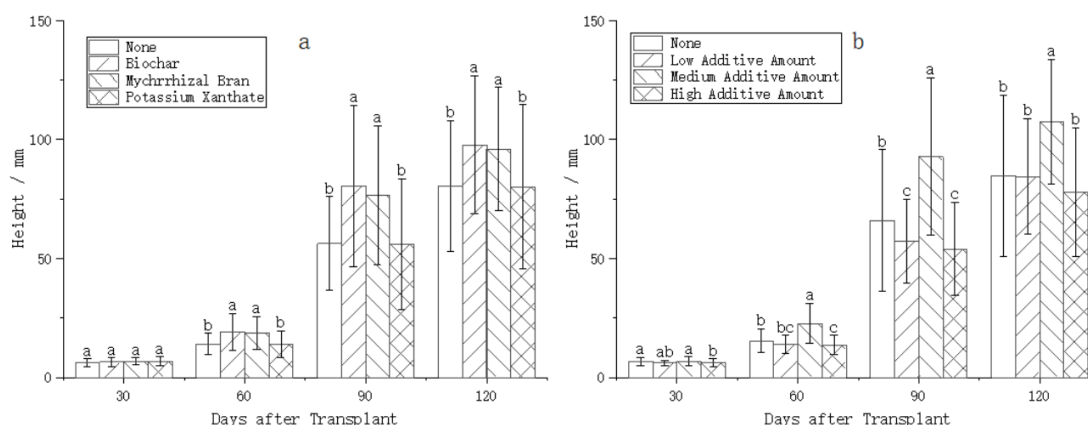
The effect of Bacillus mucilaginosus with carriers application on tobacco growth is shown in Table 2. At the early stage of transplanting, the addition of carriers promoted the agronomic traits of field tobacco more obviously, and the combination of Bacillus mucilaginosus and carriers strengthened this promotion to a certain extent. With the increase of transplanting time, Bacillus mucilaginosus's growth-promoting effect became more and more obvious, and the promotion effect on various agronomic traits of field tobacco was higher than that of the carrier. At harvest time, the synergistic effect of Bacillus mucilaginosus and the carrier became more and more significant, and the agronomic traits of the carrier group were better than those of the other experimental groups.

Table 2: Effect of *Bacillus mucilaginosus* dosed carriers on tobacco growth

Days after Transplant	Indicators	Carriers Free + <i>Bacillus mucilaginosus</i> Free	Carriers + <i>Bacillus mucilaginosus</i> Free	Carriers Free + <i>Bacillus mucilaginosus</i>	Carriers+ <i>Bacillus mucilaginosus</i>
30d	Height	5.73 ±1.45 <sup>b</sup>	7.14 ±1.58 <sup>a</sup>	6.25 ±1.68 <sup>b</sup>	6.47 ±1.70 <sup>b</sup>
	Maximum Leaf Length	10.39 ±2.12 <sup>a</sup>	10.95 ±2.84 <sup>a</sup>	10.09 ±1.7 <sup>a</sup>	11.33 ±3.37 <sup>a</sup>
	Maximum Leaf Width	5.39 ±1.06 <sup>a</sup>	5.19 ±1.39 <sup>a</sup>	4.56 ±0.95 <sup>b</sup>	5.27 ±1.68 <sup>a</sup>
	Stem Thickness	4.33 ±0.76 <sup>b</sup>	5.06 ±1.15 <sup>a</sup>	4.68 ±0.64 <sup>b</sup>	5.05 ±0.98 <sup>a</sup>
60d	Height	14.65 ±5.49 <sup>b</sup>	15.70 ±4.85 <sup>b</sup>	13.79 ±4.16 <sup>b</sup>	17.99 ±7.84 <sup>a</sup>
	Maximum Leaf Length	35.66 ±7.52 <sup>ab</sup>	34.73 ±8.05 <sup>b</sup>	31.78 ±7.89 <sup>b</sup>	38.34 ±10.35 <sup>a</sup>
	Maximum Leaf Width	18.64 ±5.66 <sup>ab</sup>	18.85 ±7.83 <sup>ab</sup>	16.93 ±6.54 <sup>b</sup>	20.40 ±7.81 <sup>a</sup>
	Stem Thickness	12.97 ±3.84 <sup>ab</sup>	12.68 ±3.41 <sup>ab</sup>	11.30 ±2.93 <sup>b</sup>	13.44 ±3.86 <sup>a</sup>
90d	Height	51.69 ±22.71 <sup>b</sup>	70.66 ±30.55 <sup>a</sup>	57.77 ±18.59 <sup>b</sup>	72.14 ±32.72 <sup>a</sup>
	Maximum Leaf Length	62.48 ±7.91 <sup>b</sup>	66.83 ±11.05 <sup>b</sup>	70.93 ±10.39 <sup>a</sup>	73.27 ±11.51 <sup>a</sup>
	Maximum Leaf Width	25.29 ±4.85 <sup>b</sup>	26.74 ±6.08 <sup>b</sup>	30.2 ±6.95 <sup>a</sup>	30.81 ±7.65 <sup>a</sup>
	Stem Thickness	21.40 ±5.14 <sup>b</sup>	22.29 ±5.35 <sup>b</sup>	25.86 ±5.68 <sup>a</sup>	27.05 ±6.32 <sup>a</sup>
120d	Height	65.17 ±25.3 <sup>b</sup>	91.48 ±33.93 <sup>a</sup>	86.09 ±26.25 <sup>a</sup>	91.76 ±29.68 <sup>a</sup>
	Maximum Leaf Length	68.95 ±12.01 <sup>c</sup>	74.71 ±9.17 <sup>b</sup>	77.35 ±10.26 <sup>b</sup>	82.87 ±9.87 <sup>a</sup>
	Maximum Leaf Width	22.29 ±5.14 <sup>c</sup>	26.89 ±4.86 <sup>b</sup>	27.26 ±5.03 <sup>b</sup>	29.25 ±6.03 <sup>a</sup>
	Stem Thickness	28.41 ±3.62 <sup>b</sup>	29.41 ±5.30 <sup>b</sup>	31.08 ±5.95 <sup>b</sup>	33.38 ±6.26 <sup>a</sup>

### 3.2. Effect of *Bacillus mucilaginosus* and Carriers on the Height of Tobacco Plants

The effect of *Bacillus mucilaginosus* with carriers application on the height of tobacco plants is shown in Figure 1. Differences existed between the effects of different carriers and different additions of *Bacillus mucilaginosus* on the presence of field tobacco plant height. Biochar and mycorrhizal bran as carriers had a better effect on the height of field tobacco plants from 60d after transplanting, while the effect of potassium xanthate group was not significantly different from that of the control group; *Bacillus mucilaginosus* at the right amount of addition to promote the height of field tobacco plants from 60d after transplanting and achieved the best effect at the harvesting stage. *Bacillus mucilaginosus* at low and high dosage did not perform well throughout the field period.

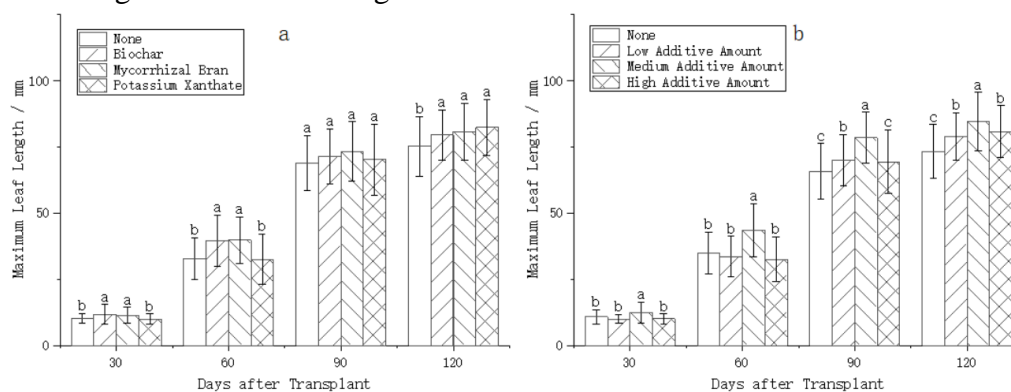


Note: Different lower case letters indicate significant differences ( $p < 0.05$ ) between different treatment groups under the same treatment conditions

Figure 1: Effect of *Bacillus mucilaginosus* and carrier dosing on the height of tobacco plants in large fields (a. The effect of different carriers on the height of tobacco plants in large fields; b. The effect of different fungicide additions on the height of tobacco plants in large fields)

### 3.3. Effect of *Bacillus mucilaginosus* and Carriers on Maximum Leaf Length in Tobacco

The effect of *Bacillus mucilaginosus* with carriers application on the maximum leaf length of tobacco plants is shown in Figure 2. There were some differences between the effects of different carriers and different *Bacillus mucilaginosus* additions on the maximum leaf length of field tobacco. Biochar and mycorrhizal bran as carriers had a greater effect on the maximum leaf length of field tobacco within 60 d after transplanting, while potassium xanthate as a carrier showed a stronger effect on the maximum leaf length of field tobacco starting from 90 d after transplanting; at harvest, the biochar and mycorrhizal bran groups were not as good as that of the potassium xanthate group, but were significantly higher than that of the control group. The *Bacillus mucilaginosus* group with medium dosage showed the best performance in all periods of field tobacco, and the *Bacillus mucilaginosus* with low and medium dosage also showed better growth-promoting effects in the middle and late stages of field tobacco growth.

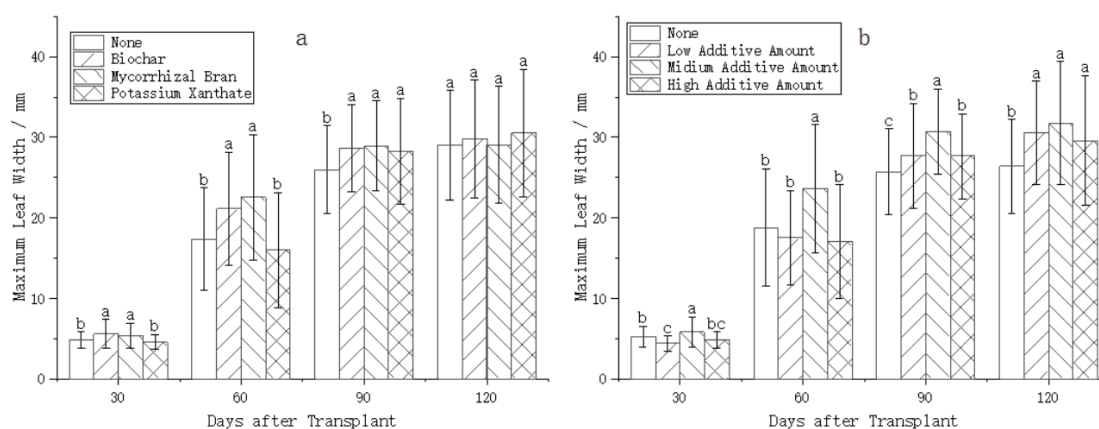


Note: Different lower case letters indicate significant differences ( $p < 0.05$ ) between different treatment groups under the same treatment conditions.

Figure 2: Effect of *Bacillus mucilaginosus* and carrier dosing on maximum leaf length of tobacco in large fields (a. The effect of different carriers on maximum leaf length of field tobacco; b. The effect of different fungicide additions on maximum leaf length of field tobacco)

### 3.4. Effect of *Bacillus mucilaginosus* and Carriers on Maximum Leaf Width in Tobacco

The effect of *Bacillus mucilaginosus* with carriers application on the maximum leaf width of tobacco plants is shown in Figure 3. The effects of different *Bacillus mucilaginosus* additions and different carriers on the maximum leaf width of field tobacco showed some differences. Both the biochar and bacillus groups had relatively strong positive effects on maximum leaf width of field tobacco at 90 d after transplanting, while the potassium xanthate group showed a promotion effect on maximum leaf width at 90 d after transplanting, but the carrier addition did not have a significant effect on maximum leaf width at harvest time. *Bacillus mucilaginosus* at medium additions always had the highest promotional effect on maximum leaf width in field tobacco, but the promotional effect of *Bacillus mucilaginosus* at low and high additions was demonstrated after 90 d after transplanting.



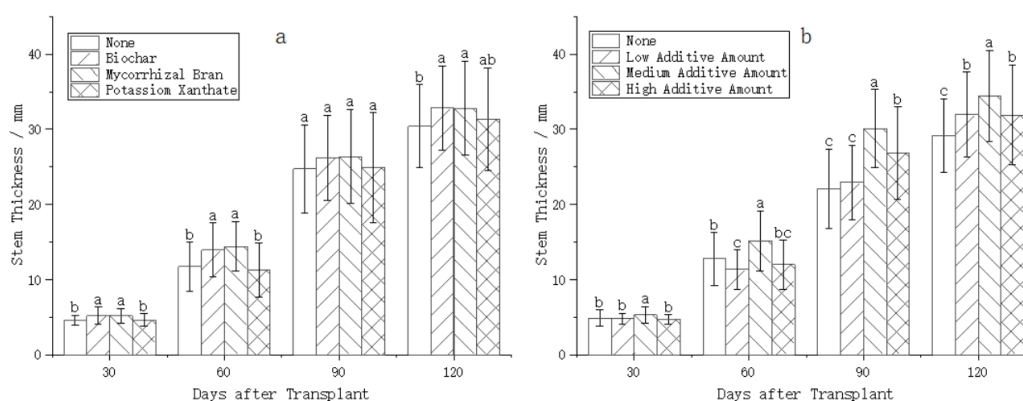
Note: Different lower case letters indicate significant differences ( $p < 0.05$ ) between different treatment groups under the same treatment conditions.

Figure 3: Effect of *Bacillus mucilaginosus* and carrier dosing on maximum leaf width of tobacco in large fields (a. The effect of different carriers on maximum leaf width of field tobacco; b. The effect of different fungicide additions on maximum leaf width of field tobacco)

### 3.5. Effect of *Bacillus mucilaginosus* and Carriers on Tobacco Stem Thickness

The effect of *Bacillus mucilaginosus* with carriers application on the stem thickness of tobacco plants is shown in Figure 4. The effects of different carriers and different *Bacillus mucilaginosus* additions on stem thickness of field tobacco varied. Biochar and chaff, as the two carrier materials, had a good effect on stem thickness at all stages of field tobacco growth, and the degree of their effects was similar; the potassium picolinate group began to show a certain positive effect on the improvement of stem thickness only at 90d after transplanting, and the effect was slightly weaker than that of biochar and chaff. *Bacillus mucilaginosus* at the medium dosage always had a positive effect on stem thickness in field tobacco, while *Bacillus mucilaginosus* at the low and medium dosages showed a positive effect on stem thickness only at the harvesting stage and 90 d after transplanting, and the effect was inferior to that of the medium dosage.





Note: Different lower case letters indicate significant differences ( $p < 0.05$ ) between different treatment groups under the same treatment conditions.

Figure 4: Effect of *Bacillus mucilaginosus* and carrier dosing on tobacco stem thickness in the field (a. Effects of different carriers on stem thickness of field tobacco; b. Effects of different fungicide additions on stem thickness of field tobacco)

## 4. Discussion

### 4.1. Effect of *Bacillus mucilaginosus* Application on Tobacco Growth

The results of this study showed that the addition of *Bacillus mucilaginosus* had a significant effect on the agronomic traits such as plant height, maximum leaf length, maximum leaf width, etc., but the above indexes generally appeared to "increase first, then decrease" with the increase in the dose of *Bacillus mucilaginosus*, which was similar to the results of the previous study[7]. The addition of microbial agents can not only improve the agronomic traits of tobacco, but also play a role in regulating the stomatal conductance of tobacco, increasing the photosynthetic rate and promoting the root growth and the chemical quality of tobacco, thus improving the organoleptic quality and yield of tobacco[8,9]. On the one hand, the addition of microbial fungicides has a direct effect on the soil microbial community[9]; on the other hand, after microbial fungicides are applied to the soil, their growth and reproduction, and other life activities can produce various secondary metabolites such as growth hormone, erythromycin, and cytokinin, which Thus, it can play a role in promoting tobacco growth and maintaining inter-root microbial equilibrium, and it can also enhance the resistance and disease resistance of tobacco through the interaction between microorganisms[8,10,11]. Therefore, the promotional effect of microbial agents on the growth of roasted tobacco is mainly reflected in the late stage[12].

### 4.2. The Effect of Different Carriers on Tobacco Growth

The effect of carriers on field tobacco should not be ignored. The results of this study showed that the addition of carriers had a significant effect on the agronomic traits of field tobacco, but the promotional effect of different carriers on the agronomic traits of tobacco showed variability, which is similar to the results of previous studies[10,12]. Firstly, the carriers themselves contain a certain amount of organic matter and trace elements, which can not only provide nutritional guarantee for the development of tobacco plants, but also become a source of energy necessary for the growth of the contained microorganisms[13]. Secondly, the presence of the carriers can provide a good habitat for the growth and reproduction of the contained microorganisms, and the pores on the surface of the carriers can provide a good basis for the attachment of microorganisms[10]. Finally, The synergistic effect of carriers and microorganisms has a strong regulatory effect on soil microbial

structure, which in turn affects soil nutrient content and tobacco plant growth[13]. Different carriers have different structural and nutritional characteristics, which also leads to the differences in the growth promotion effect of different carriers on field tobacco.

## 5. Conclusions

(1) The addition of carriers has a good improvement effect on indicators such as plant height, maximum leaf length, maximum leaf width, and stem thickness of field tobacco. The four indicators of the biochar group were 21.32%, 5.80%, 10.39%, and 8.07% higher than those of the control group, respectively. The four indicators of the bacterial bran group were 19.10%, 7.30%, 11.45%, and 7.96% higher than those of the control group, respectively. These two carriers have the best performance.

(2) The addition of *Bacillus mucilaginosus* has a good promoting effect on the height, maximum leaf length, maximum leaf width, and stem thickness of tobacco plants in the field, and this promoting effect generally shows a trend of "increasing first and then decreasing" with the increase of bacterial dosage. The four indicators of the moderate addition group were 26.76%, 15.24%, 19.37%, and 18.08% higher than the control group, respectively, and were higher than those in the other groups( $p < 0.05$ ).

(3) Overall, S2 (biochar+100g *Bacillus mucilaginosus*) and J2 (mycorrhizal bran+100g *Bacillus mucilaginosus*) treatments have the best promoting effect on the growth of tobacco in the field stage.

## References

- [1] Zheng Jianchao. Effects of fertilizer reduction combined with biologic fertilizer and potassium fulvate on tomato growth and fertilizer utilization ratio [J]. *Heilongjiang Agricultural Sciences*, 2022(8):106-110.
- [2] Wu Zuowei, Wu Yingyun, Li Xin, et al. Physiological characteristics of *Bacillus mucilaginosus* D4B1 [J]. *Soil and Fertilizer*, 2004(2):40-43.
- [3] Liu Wuxing, Xu Xushi, Yang Qiyin, et al. Study on decomposition of soil minerals by *Bacillus glia* [J]. *Soil Science*, 2004(5):547-550.
- [4] Malusa E, Sas-Paszt L, Ciesielska J. Technologies for Beneficial Microorganisms Inocula Used as Biofertilizers [J]. *The Scientific World Journal*, 2012, 2012: 1-12.
- [5] Chaudhary T, Dixit M, Gera R, et al. Techniques for improving formulations of bioinoculants [J]. *3 Biotech*, 2020, 10 (5): 199.
- [6] Ku Yongli, Xu Guoyi, Zhao Hua, et al. Effects of humic acid compound microbial fertilizer on soil improvement and fruit quality of aged kiwifruit orchard [J]. *Journal of North China Agricultural Science*, 2018, 33(3):167-175.
- [7] Gao Fangfang. Effect of increased application of microbial agent on yield and quality of tobacco [D]. *Shanxi Normal University*, 2021.
- [8] Wu Deyang, Zhang Zaigang, Peng Wenyong, et al. Effects of growth promoting bacteria on tobacco growth, soil microbial quantity and nutrient [J]. *Sichuan Agricultural Science and Technology*, 2021(12):76-80.
- [9] Li Yuefei. Application of PGPR in flue-cured tobacco cultivation [D]. *Sichuan Agricultural University*, 2022.
- [10] Li Xiangying, Zhang Yinghua, Xu Lina, et al. Effects of *Bacillus amylolyticus* on photosynthetic characteristics of tobacco leaves [J]. *Journal of Shandong Agricultural University (Natural Science Edition)*, 2018, 49(3):518-522.
- [11] Tang Xingui, Lu Xingli, Lei Ting, et al. Effect of microbial agent application on microflora in rhizosphere soil of tobacco field [J]. *Modern Agricultural Science and Technology*, 2020(22):86-88.
- [12] Wei Wei, Zheng Jinyuan, Wang Baoyi, et al. Effect of soil improvement Spore agent on agronomic characters of tobacco [J]. *Journal of Agricultural Science and Technology Communication*, 2019(9):123-126.
- [13] Li Diqin, Ren Zheng, Zhu Li, et al. Effects of combined application of soil conditioner and *Bacillus subtilis* on growth, development and disease of tobacco [J]. *Jiangsu Agricultural Sciences*, 2019, 50(10):88-94.