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Effect of Preparations of Bamboo Vinegar and Copper Sulfate on Green Algae and Tobacco Seedling Growth

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Abstract To provide theoretical and technical basis for the application of preparations of bamboo vinegar and copper sulfate in the prevention of green algae in floating system for tobacco seeding, the soaking and spraying methods were employed to study the effect of different concentrations of preparations of bamboo vinegar and copper sulfate on green algae and tobacco seedling growth. The results showed that the preparations of bamboo vinegar and copper sulfate of different concentrations played a strong role in inhibiting green algae growth and had better effect than copper sulfate. Spraying 1:200 preparations of bamboo vinegar and copper sulfate could significantly promote tobacco seedling growth, and the leaf fresh weight increased by 15.80% compared with CK, with significant difference; spraying 1:50 preparations was not conducive to tobacco seedling growth. It was recommended to spray 1:200 preparations of bamboo vinegar and copper sulfate in floating system for tobacco seeding, which could promote tobacco seedling growth and reduce green algae to varying degrees.

Key words Preparations of bamboo vinegar and copper sulfate, Green algae, Tobacco seedlings, Agronomic traits, Fresh matter

1 Introduction

The floating system of flue-cured seedlings, as a mature technology, has been widely used in China^[1], which promotes the standardized management, specialized production, commercialized seedling supply and intensified operation of flue-cured seedlings^[2]. However, due to special ecological environment and natural conditions in part of China's tobacco-growing areas, it is easy to produce green algae in the nursery pond and on the seedling tray surface, thereby affecting the normal growth of tobacco seedlings. Green algae belong to the photosynthetic prokaryotes that can release oxygen, with strong ability to adapt and wide distribution^[3]. Green algae compete for nutrients with tobacco seedlings in the process of floating nursery, and can secrete algal toxins to harm tobacco seedlings^[4]. The studies of Liu Guohun *et al.*^[1] and Yao Yuanyuan *et al.*^[2] showed that adding copper sulfate solution with concentration of less than 250 mg/L to the nutrient solution or spraying 500 mg/L copper sulfate solution on the nursery tray surface could reduce the of harm of green algae. The studies of Huang Guangrong *et al.*^[6] also pointed out that 20 d after sowing flue-cured tobacco, applying 50–100 mg/L copper sulfate in the nursery pond could control the occurrence and spread of blue-green algae. Peng Gang *et al.*^[7] also proved that copper sulfate had strong inhibitory effect on green algae growth. However, in 2015, Li Najia *et al.*^[7] found that using 90–250 mg/L copper sulfate could cause yellowing of leaves and block root development of tobacco seedlings. The test tried to find a synergistic agent to enhance its inhibitory effect on green algae while holding the application rate of copper sulfate without damaging roots. Data^[9–11] have shown that bamboo vinegar as pollution-free natural prepara-

tion can not only promote tobacco seedling growth, but also have bactericidal and insecticidal and pest control effect. The studies of Sun Jianhua *et al.*^[12] showed that on the basis of 1:600 pepper leaf pesticide solution, adding 1:400 bamboo vinegar could improve control efficiency by about 30%; Hu Chunshui *et al.*^[13] found that the spider mite control efficiency of 1:1000 40% dimethoate + 3% bamboo vinegar could reach 99.5%; Wang Wenjie *et al.*^[14] pointed out that adding bamboo vinegar to metalaxyl mancozeb could help to achieve significant control effect. Currently, there is no report about the complex preparations of bamboo vinegar and copper sulfate used for prevention and control of green algae. Therefore, in 2016, we used soaking and spraying method to study the control effect of preparations of bamboo vinegar and copper sulfate of different concentrations on green algae as well as the effect on tobacco seedling growth, in order to determine the suitable range of concentration and provide theoretical and technical basis for green algae prevention and control in floating system for tobacco seeding.

2 Materials and methods

2.1 Materials The flue-cured tobacco variety was Yunyan 87, provided by Tobacco Production and Management Department of Qiandongnan Tobacco Company; bamboo vinegar was purchased from Zhejiang Suichang Wenzhao Charcoal Co., Ltd. (density of 1.006 g/mL, pH of 2.62, acetic acid content of 4.54%); copper sulfate was purchased from Sinopharm Chemical Reagent Co., Ltd. (AR); preparations of bamboo vinegar and copper sulfate were made by Organic Technology Research Center of China Agricultural University, with the inorganic copper ion concentration of 0.1 mol/L.

2.2 Experimental methods

2.2.1 Effect of preparations of bamboo vinegar and copper sul-

fate on green algae growth. The test was done in nursery greenhouse in Baiyanping Village, Tianma Town, Cengong County, Guizhou Province ($108^{\circ}43'3''E$, $27^{\circ}22'7''N$, altitude of 740 m) from March 1 to March 5, 2016. The test had 6 treatments, and $T_1 - T_4$ meant first applying preparations of bamboo vinegar and copper sulfate with Cu^{2+} concentration of 128, 64, 32 and 16 mg/L, respectively, and then applying water (CK_1) and 128 mg/L copper sulfate solution (CK_2) as blank control and conventional control, repeated five times. In each treatment, 0.5 g green algae were weighed and placed in a transparent container, then 20 mL treatment fluid was added for soaking, and the green algae changes were observed and recorded after 120 h.

2.2.2 Effect of preparations of bamboo vinegar and copper sulfate on tobacco seedling growth. The test was carried out in the nursery greenhouse from March 10 to April 20, 2016. The test had 5 treatments, and $T_1 - T_4$ meant first applying preparations of bamboo vinegar and copper sulfate with Cu^{2+} concentration of 128, 64, 32 and 16 mg/L, respectively, with water in equal amount (CK) as a control, repeated five times, placed randomly in the same breeding pool. Different treatments were separated by partition board to avoid interference during spraying. After thinning of tobacco seedlings, spraying was performed every 10 d, a total of three times. Spraying was done on sunny afternoon, and the liquid was sprayed evenly onto leaves until liquid dripped up. 10 d after the third time spraying, 5-point method was used to take 15 tobacco seedlings from the seedling tray. Based on YC/T142 - 1998 method, we determined the stem height, stem diameter, maximum leaf length, maximum leaf width and effective leaf number of tobacco seedlings. The tobacco seedlings were weighed after being washed and dried, and the fresh matter weight was calculated. The 15 tobacco seedlings were averaged as the data results.

2.3 Statistical analysis Microsoft Excel 2003 and SPSS 17.0 were used for the statistical analysis of test data.

3 Results and analysis

3.1 Effect of preparations of bamboo vinegar and copper sulfate on green algae growth As shown in Fig. 1, compared with CK_1 , the preparations of bamboo vinegar and copper sulfate of different concentrations had obvious inhibitory effect on green algae growth, and the effect was enhanced with increasing concen-

tration. Under T_1 , the level of yellowing was highest for green algae, basically losing growth trend. Under CK_2 , the green algae were still green and tended to grow, indicating that T_1 had more significant inhibitory effect on green algae growth. Meanwhile, the green algae under T_2 and T_3 were yellower than under CK_2 , indicating that the two had stronger suppression effect, and the color was not significantly different from that under T_4 .

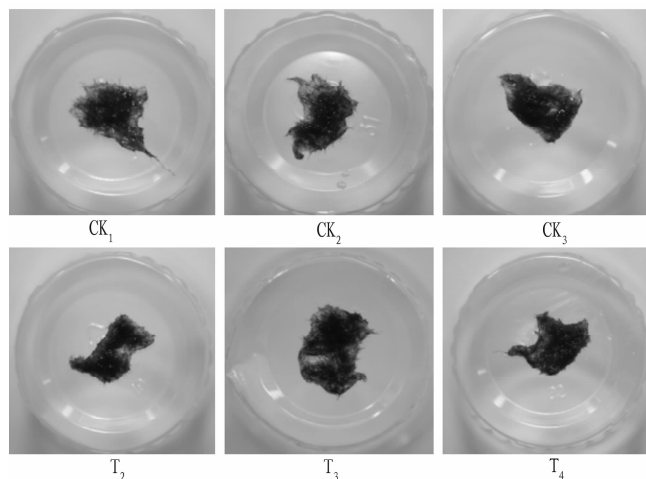


Fig. 1 Effect of preparations of bamboo vinegar and copper sulfate on green algae growth

3.2 Effect of preparations of bamboo vinegar and copper sulfate on tobacco seedling growth

3.2.1 Tobacco seedling growth under treatment of preparations of bamboo vinegar and copper sulfate. From Table 1, it was found that compared with CK, tobacco seedling growth was good under T_3 , followed by T_2 and T_4 , and tobacco seedling growth was inhibited under T_1 . Compared with CK, stem girth and maximum leaf width under T_3 increased by 15.00% and 15.39%, respectively, with significant differences; the stem girth under T_3 was 14.18% significantly larger than under T_4 , not significantly different from that under T_2 ; there was no significant difference between CK and T_2 or T_4 . Compared with CK, the stem height and maximum leaf width under T_1 decreased by 46.96% and 17.08%, respectively, with a significant difference. There was no significant difference in maximum leaf length and effective leaf number among treatments.

Table 1 Tobacco seedling growth under different treatments

Treatment	Stem height//mm	Stem girth//mm	Maximum leaf length//mm	Maximum leaf width//mm	Effective leaf number
T_1	24.70 ± 2.09 b	2.64 ± 0.10 c	90.29 ± 3.05 a	43.78 ± 2.92 c	3.00 ± 0.00 a
T_2	34.82 ± 1.86 a	3.11 ± 0.10 ab	133.82 ± 2.64 a	53.62 ± 2.04 ab	3.20 ± 0.20 a
T_3	38.54 ± 1.54 a	3.22 ± 0.03 a	143.49 ± 2.51 a	59.15 ± 0.97 a	3.40 ± 0.25 a
T_4	36.23 ± 3.44 a	2.82 ± 0.07 bc	136.36 ± 2.78 a	53.04 ± 0.78 ab	3.40 ± 0.25 a
CK	36.30 ± 1.37 a	2.80 ± 0.11 bc	132.70 ± 1.96 a	51.26 ± 0.28 b	3.20 ± 0.20 a

Note: Different lowercase letters in the same row represent significant difference at 5% level, the same in the following table.

3.2.2 Fresh matter accumulation of tobacco seedlings under treatment of preparations of bamboo vinegar and copper sulfate. As apparent from Table 2, the tobacco seedling fresh matter had the

highest content under T_3 , and the leaf fresh weight increased by 15.80% compared with CK, with significant difference; it was not significantly different from T_2 and T_4 , but the leaf, stem and root

fresh weight increased by 106.31%, 134.76% and 126.47%, respectively, compared with T₁, with significant differences. There were no significant differences in leaf, stem and root between CK and T₂ or T₄, and the leaf, stem and root fresh weight under T₂,

T₄ and CK increased by (73.79%, 79.27%, 50%), (85.44%, 84.15%, 38.24%), (78.16%, 76.83%, 38.24%), respectively, compared with T₁.

Table 2 Fresh matter accumulation of tobacco seedlings under treatment of preparations of bamboo vinegar and copper sulfate

Treatment	Leaf//g	Stem//g	Root//g
T ₁	0.412 ± 0.042 c	0.164 ± 0.006 b	0.068 ± 0.005 b
T ₂	0.716 ± 0.014 b	0.294 ± 0.027 a	0.102 ± 0.026 a
T ₃	0.850 ± 0.019 a	0.358 ± 0.012 a	0.154 ± 0.014 a
T ₄	0.764 ± 0.017 ab	0.302 ± 0.014 a	0.122 ± 0.011 a
CK	0.734 ± 0.025 b	0.290 ± 0.022 a	0.094 ± 0.010 a

4 Conclusions and discussions

1:50 – 1:400 preparations of bamboo vinegar and copper sulfate had inhibitory effect on green algae growth, and as the concentration increased, the inhibitory effect increased, and 1:50 solution had significantly stronger inhibitory effect than the copper sulfate of the same concentration. The preparations of bamboo vinegar and copper sulfate had two-way regulation effect on tobacco seedling growth. The tobacco seedling growth by spraying 1:200 solution was better than under CK, and the stem girth, maximum leaf width and leaf fresh weight significantly increased by 15.00%, 15.39% and 15.80%, respectively; the treatment of 1:50 solution inhibited tobacco seedling growth, and the leaf, stem and root fresh weight significantly decreased by 78.16%, 76.83% and 38.24%, respectively, compared with CK. Therefore, in the future floating system for tobacco seeding, we can try to apply preparations of bamboo vinegar and copper sulfate to control the harm of green algae, which can not only reduce the use of pesticides, but also improve the tobacco leaf quality and promote the healthy development of green tobacco. Xu Lingjie *et al.* [15] pointed out that the bamboo vinegar combined with onion and cordate houttuynia extract had significantly stronger bacterial wilt inhibitory effect than bamboo vinegar. The studies of Ma Liangjin *et al.* [16] and Luo Min *et al.* [17] indicated that the bamboo vinegar combined with carbendazim and triazole had better inhibitory effect on *Fusarium* than the bamboo vinegar and the mixture of bamboo vinegar, berberine and *Magnolia officinalis* could have significant synergy effect. This was consistent with the experimental results, that is, in the same copper ion concentration, the preparations of bamboo vinegar and copper sulfate had stronger inhibitory effect on green algae than copper sulfate, which might be related to characteristics of bamboo vinegar. The studies of Wu Xuan *et al.* [18] indicated that applying 1:100 – 1:300 bamboo vinegar could promote the growth of cucumber, while 1:50 solution would inhibit its growth. However, in this experiment, the 1:100 – 1:200 preparations of bamboo vinegar and copper sulfate had significantly stronger inhibitory effect on green algae than 1:50 copper sulfate, and the reason was not clear. Meanwhile, in the breeding process, spraying 1:50 preparations of bamboo vinegar and copper sulfate had inhibitory effect on tobacco seedling growth, possibly because the high concentration of copper ions destroyed the crop tissue structure [19], resulting in hazard, unsuitable for production. It was recommended to spray 1:200 preparations of bamboo vinegar and copper sulfate in floating sys-

tem for tobacco seeding, which could promote tobacco seedling growth and reduce green algae to varying degrees.

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transformation efficiency, and changes in properties of microbial population), and establish standardized parameter system of agricultural waste, which is key to the improvement, popularization and application of formulas for culture materials.

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