Preliminary Screening of Agents for the Management of Root Rot in Tobacco Floating Seedlings

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Abstract [**Objectives**] To screen effective agents for the management of root rot in tobacco floating seedlings. [**Methods**] A total of eight treatments were implemented in a large-scale field trial, which included three replications. Each treatment was administered at the recommended dosages. [**Results**] The application of Metalaxyl-M · Fludioxonil + amino acids demonstrated a control efficacy of 93.65% against root rot in tobacco floating seedlings. In comparison, the application of Metalaxyl-M · Fludioxonil alone exhibited a control efficacy of 88.42%. The most effective treatment for promoting root growth in tobacco seedlings was Metalaxyl-M · Fludioxonil + amino acids, which resulted in a 54.65% increase in root growth compared to the blank control. This was followed by the application of Metalaxyl-M · Fludioxonil alone, which yielded a 39.53% increase in root growth relative to the blank control. [**Conclusions**] The application of Metalaxyl-M · Fludioxonil + amino acids is recommended for use in the seedling pools of tobacco seedling cultivation. Specifically, it is advised to incorporate 1 mL of Metalaxyl-M · Fludioxonil and 2 mL of amino acids into each seedling tray prior to their introduction into the seedling pool. This treatment has demonstrated significant efficacy in preventing and controlling root rot in tobacco floating seedlings, while also promoting the health of the root system and ensuring the quality of transplanted tobacco seedlings.

Key words Tobacco seedlings, Seedbed, Disinfection, Fungicide

1 Introduction

Tobacco serves as a significant cash crop in China, primarily cultivated for the production of leaves that are processed into various cigarette products to satisfy consumer demand. The quality of tobacco leaves is contingent not only upon effective management practices in the field[1], but also on the health of tobacco seedlings grown in seedbeds, which significantly influences the plants' resistance to diseases in the field[2]. Research indicates that the implementation of scientific seedling cultivation methods can improve the agronomic characteristics and disease resistance of tobacco seedlings[3-4].

Tobacco seedling root diseases encompass various conditions, including damping off, blight, and anthracnose[5-9]. Among these, tobacco seedling root rot is a particularly detrimental disease[5]. The floating seedling technology for tobacco, which has recently been popularized in China's tobacco-growing regions, represents an innovative approach to seedling cultivation. This method has the potential to enhance the overall production levels of tobacco, improve its quality, and address the limitations associated with previous soilless cultivation techniques, such as high costs, challenges in large-scale implementation, and stringent management requirements. Since the initiation of research by Fang Dunhuang *et al.*[10] in 1996, extensive experimental demonstrations were conducted in various locations throughout Yunnan Province in 1998, subsequently leading to nationwide promotion[11]. The promotion and implementation of tobacco floating seedling technology over the years, coupled with inconsistent seedbed management practices, have led to a gradual increase in reported cases of root rot in tobacco seedlings[12-14]. In 2012, Lao Chao *et al.*[15] first reported root rot in tobacco floating seedlings caused by *Pythium diclinum* Tokunaga in Ito & Tokunaga. Subsequently, in 2017, Li Xiaojie *et al.*[16] provided the initial report of root rot in tobacco floating seedlings attributed to *Aphanomyces iridis*. At the beginning of 2024, during the cultivation of tobacco floating seedlings in Xingyi City, located within the Qianxinan Buyi and Miao Autonomous Prefecture of Guizhou Province, the symptoms of slow growth, stunted plant height, and gray-brown, soft necrotic root system were observed. These symptoms can result in the mortality of tobacco seedlings in severe instances. Through an analysis of the disease's pathogenesis characteristics, it was preliminarily concluded that the observed condition was attributable to root rot in tobacco floating seedlings, caused by the pathogen *A. iridis*.

Aphanomyces belongs to Saprolegniaceae, Saprolegniales, Oomycetes, and Mastigomycotina. This pathogen is known to induce diseases in rice seedlings, pea plants, and tobacco seedlings[16-19]. Tobacco floating seedling root rot, caused by *A. iridis*, can significantly impact the quality of tobacco seedlings. This disease leads to a reduced number of transplanted seedlings, increases the cost of seedling cultivation, and can adversely affect the growth of seedlings post-transplanting, as well as the quality of the leaves after harvesting. According to the *China Statistical Yearbook 2024*, the area of tobacco cultivated in Guizhou in 2023 totaled 142.2 thousand hm^2[20], generating significant direct and indirect economic value. Current research on the management of tobacco root and stem diseases includes several notable studies.

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Chang Jianbo *et al.* [21] identified chemicals that exhibit significant efficacy against tobacco black shank. Yao Chenxiao *et al.* [22] assessed the indoor activity of 10 systemic chemical fungicides against *Fusarium oxysporum*, the principal causative agent of tobacco root rot. Liu Tiebin *et al.* [23] demonstrated that Hymexazol possesses strong inhibitory effects against a range of pathogens, including *Fusarium* spp., *Pythium* spp., *Aphanomyces* spp., and other pathogenic bacteria. The findings of the inoculation test conducted by Miao Pu *et al.* [24] indicated that 30% Propamocarb · Azoxystrobin demonstrated significant indoor efficacy against root rot in tobacco seedlings caused by *A. iridis*, achieving a control effect exceeding 86%. Despite the significance of addressing root rot in tobacco floating seedlings caused by *A. iridis*, research in this area remains limited. Consequently, this study was undertaken to conduct preliminary screening of potential control agents for root rot in tobacco floating seedlings. The findings aim to provide a foundational reference for relevant agricultural practices in the field.

2 Materials and methods

2.1 Materials

2.1.1 Location and time. The test was carried out in Louna Village, located on Dingxiao Street in Xingyi City, within the Qianxinan Buyi and Miao Autonomous Prefecture of Guizhou Province. The fungicide application occurred on July 8, 2024, and the subsequent investigation took place on September 12, 2024.

2.1.2 Tobacco varieties and test agents. Tobacco seeds that had been germinated and coated (Yunyun 87) were supplied by the Guizhou Flue-Cured Tobacco Fine Seed Breeding Base of the Guizhou Academy of Tobacco Science. The fundamental information regarding the test agents is presented in Table 1.

Table 1 Information on test agents

Trade name	Active ingredient	Content	Dosage form	Manufacturer
Kangyou	Trichloroisocyanuric acid, Dichloroisocyanuric acid	Available chlorine 50%	Tablet	Shandong Jiajie Water Treatment Technology Co., Ltd.
Panlijie	Jingfukang AB agent (In terms of CHD-7 reactive oxides produced)	≥12%	Tablet	Zhengzhou Huinong Pest Control Co., Ltd.
Luxiang	Chlorobromoisocyanuric acid	50%	Soluble powder	Henan Yintian Fine Chemical Co., Ltd.
Huawei	Chlorine dioxide	10%	Tablet	Linqu Huawei Biotechnology Co., Ltd.
Liangdun	Metalaxyl-M, Fludioxonil	25 g/L; 37.5 g/L	Flowable suspension for seed treatment	Syngenta (Shanghai) Crop Protection Technology Co., Ltd.
Jinapulong	Metalaxyl-M	350 g/L	Seed treatment emulsion	Syngenta (Shanghai) Crop Protection Technology Co., Ltd.
Yishikang	Amino acids + Manganese zinc	110 g/L + 24 g/L	Aqueous solution	Syngenta (Shanghai) Crop Protection Technology Co., Ltd.

2.2 Methods This trial comprised 8 distinct treatments, with the medication regimen and time for each treatment detailed in Table 2. A field block trial was conducted, incorporating three replications and utilizing 8 seedling trays for each replication. In addition to the treatments outlined in the experimental design, all other aspects of water and fertilizer management, subterranean pest control, and foliar disease and pest management were standardized across the trial. The dosage administered for each treatment adhered to the recommended guidelines. The specifications for the seedling ponds were as follows: length of 1.48 m, width of 1.16 m, and a water surface height of 0.015 m.

Table 2 Basic information of treatments

Treatment	Agent	Dosage	Application period	Application method
1	Trichloroisocyanuric acid · Dichloroisocyanuric acid	0.05 g/pool	Seedling cultivation after medication	Medication in the pool
2	Jingfukang AB agent	0.26 g/pool	Seedling cultivation after medication for 24 h	Medication in the pool
3	Chlorobromoisocyanuric acid	0.64 g/pool	Seedling cultivation after medication	Medication in the pool
4	Metalaxyl-M · Fludioxonil	8 mL/pool	Seedling cultivation after medication	Medication in the pool
5	Metalaxyl-M	1 mL/pool	Seedling cultivation after medication	Medication in the pool
6	Chlorine dioxide	0.625 g/pool	Seedling cultivation after medication	Medication in the pool
7	Metalaxyl-M · Fludioxonil + amino acids	8 mL/pool + 16 mL/pool	Seedling cultivation after medication	Medication in the pool
8	Blank control	Water	Seedling cultivation after medication	Medication in the pool

2.3 Data survey and analysis A comprehensive survey of the plants within the designated plot was conducted to quantify the incidence of root rot among the plants.

Diseased plant rate (%) = (Number of diseased plants/Total number of plants) × 100

Control effect (%) = (Diseased plant rate in control group − Diseased plant rate in treatment group)/Diseased plant rate in control group) × 100

An agronomic traits survey was conducted in which all plants within the plot were assessed and scored based on their root systems. Root growth was classified into three categories: poor (not enveloped by substrate) received a score of 3 points, average (enveloped by substrate) received 6 points, and good (enveloped by substrate with a well-developed root system) received 9 points.

3 Results and analysis

3.1 Occurrence of root rot As illustrated in Table 3, the most effective treatment for controlling root rot in tobacco floating seedlings was Metalaxyl-M · Fludioxonil + amino acids, achieving a control efficacy of 93.65%. The subsequent most effective treatment was Metalaxyl-M · Fludioxonil alone, which demonstrated an efficacy of 88.42% against root rot in tobacco floating seedlings. Chlorine dioxide followed, exhibiting a control efficacy of 47.82% for the same condition. The treatments were ranked in descending order of their efficacy in controlling root rot as follows: Metalaxyl-M · Fludioxonil + amino acids, Metalaxyl-M · Fludioxonil, Chlorine dioxide, Jingfukang AB agent, Trichloroisocyanuric acid · Dichloroisocyanuric acid, Metalaxyl-M, and Chlorobromoisocyanuric acid.

Table 3 Control effects of preliminary screening agents in controlling root rot in tobacco floating seedlings %

Treatment	Agent	Diseased plant rate				Control effect
		Repetition 1	Repetition 2	Repetition 3	Average	
1	Trichloroisocyanuric acid · Dichloroisocyanuric acid	18.52	96.88	100.00	71.80	-8.97
2	Jingfukang AB agent	12.00	100.00	100.00	70.67	-7.25
3	Chlorobromoisocyanuric acid	100.00	100.00	100.00	100.00	-51.77
4	Metalaxyl-M · Fludioxonil	8.57	12.5	1.82	7.63	88.42
5	Metalaxyl-M	97.50	98.21	100.00	98.57	-49.60
6	Chlorine dioxide	7.32	0	95.83	34.38	47.82
7	Metalaxyl-M · Fludioxonil + amino acids	7.14	0	5.41	4.18	93.65
8	Blank control	97.67	0	100.00	65.89	-

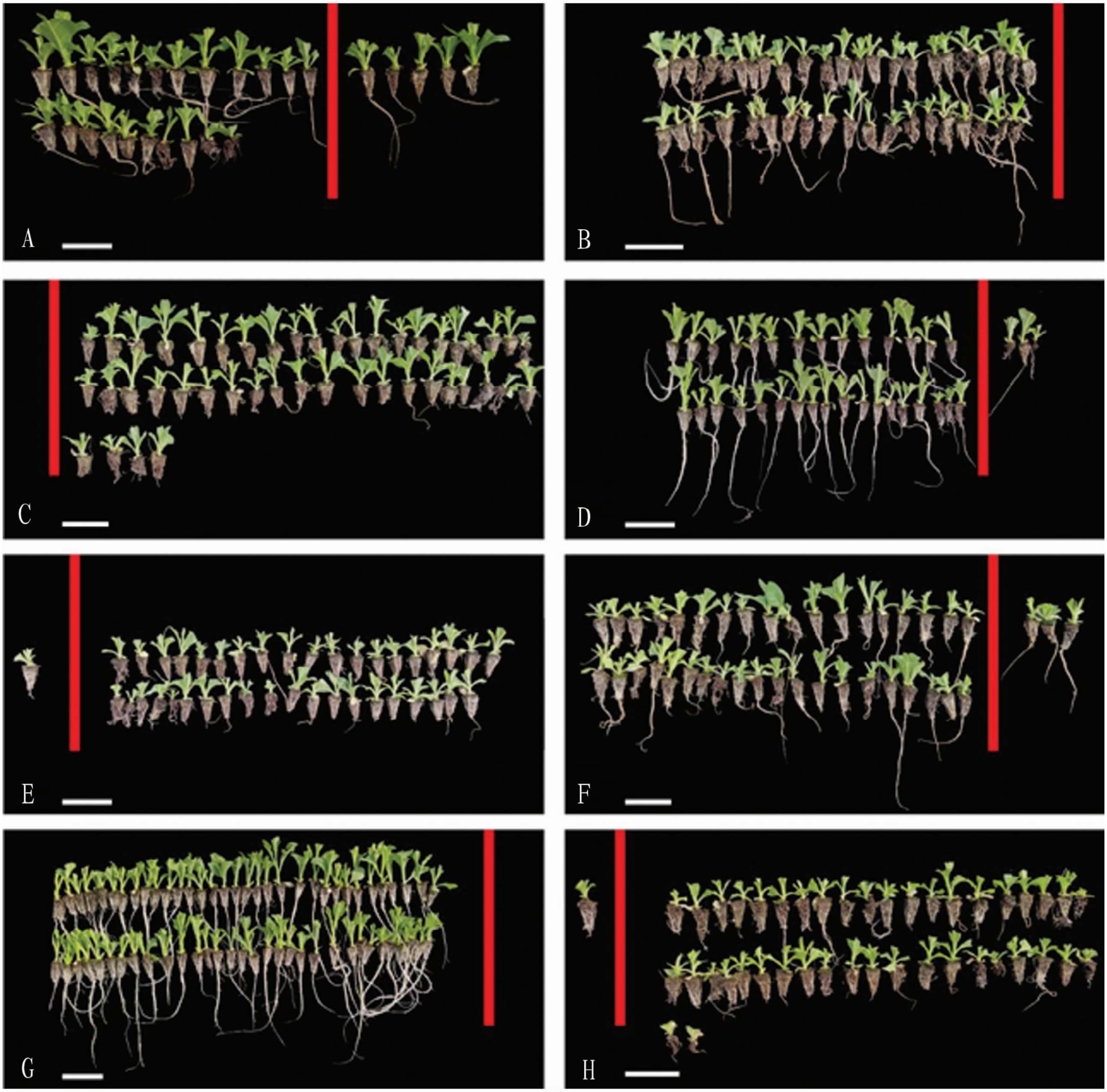
3.2 Root growth of tobacco seedlings As illustrated in Table 4, the most effective treatment for promoting root growth in tobacco seedlings was Metalaxyl-M · Fludioxonil + amino acids, which resulted in a 54.65% increase in root growth compared to the blank control. This was followed by the application of Metalaxyl-M · Fludioxonil alone, which yielded a 39.53% increase in root growth relative to the blank control. Chlorine dioxide was the less effective treatment, producing a 9.68% increase in root growth when compared to the blank control. The growth status of tobacco seedling roots treated with various agents is illustrated in Fig. 1. The treatments, ranked in descending order based on their efficacy in promoting root growth, were as follows: Metalaxyl-M · Fludioxonil + amino acids, Metalaxyl-M · Fludioxonil, Chlorine dioxide, Trichloroisocyanuric acid · Dichloroisocyanuric acid, Jingfukang AB agent, Metalaxyl-M, and Chlorobromoisocyanuric acid. A healthy and robust root system can significantly influence the survival rate of transplanted tobacco seedlings, as well as the overall vigor of the plants in later growth stages. Tobacco plants possessing a strong root system exhibit greater resistance to diseases and environmental stresses compared to those with a weak root system.

Table 4 Root growth of tobacco floating seedlings

Treatment	Agent	Average score for root growth//point				Growth rate//%
		Repetition 1	Repetition 2	Repetition 3	Average	
1	Trichloroisocyanuric acid · Dichloroisocyanuric acid	6.96	5.00	4.68	5.55	5.25
2	Jingfukang AB agent	4.44	4.40	5.51	4.78	-9.23
3	Chlorobromoisocyanuric acid	4.89	4.64	3.77	4.43	-15.88
4	Metalaxyl-M · Fludioxonil	7.18	6.75	8.13	7.35	39.53
5	Metalaxyl-M	5.10	5.28	3.75	4.71	-10.63
6	Chlorine dioxide	5.36	7.48	4.50	5.78	9.68
7	Metalaxyl-M · Fludioxonil + amino acids	7.29	8.87	8.29	8.15	54.65
8	Blank control	4.56	6.16	5.08	5.27	-

4 Discussion and conclusions

4.1 Discussion In the preliminary screening test conducted to evaluate agents for the control of root rot in tobacco floating seedlings, the treatments were ranked in descending order of their efficacy in controlling root rot as follows: Metalaxyl-M · fludioxonil + amino acids, Metalaxyl-M · Fludioxonil, Chlorine dioxide, Jingfukang AB agent, Trichloroisocyanuric acid · Dichloroisocyanuric acid, Metalaxyl-M, and Chlorobromoisocyanuric acid. The utilization of Trichloroisocyanuric acid · Dichloroisocyanuric acid, Chlorine dioxide, and Jingfukang AB agent, while effective in certain treatments, exhibits instability when employed. This instability may lead to a significantly elevated incidence of adverse effects in some applications. Consequently, the application of these agents for the management of root rot in tobacco floating seedlings is not advisable and poses inherent risks.



NOTE A. Trichloroisocyanuric acid · Dichloroisocyanuric acid; B. Jingfukang AB agent; C. Chlorobromoisocyanuric acid; D. Metalaxyl-M · Fludioxonil; E. Metalaxyl-M; F. Chlorine dioxide; G. Metalaxyl-M · Fludioxonil + amino acids; H. Blank control. Healthy tobacco seedlings are positioned to the left of the red vertical line, while diseased seedlings are located to the right. The scale bar is a ratio of 1 : 10 cm.

Fig. 1 Growth status of tobacco seedling roots treated with various agents

The treatments, ranked in descending order based on their efficacy in promoting root growth, were as follows: Metalaxyl-M · Fludioxonil + amino acids, Metalaxyl-M · Fludioxonil, Chlorine dioxide, Trichloroisocyanuric acid · Dichloroisocyanuric acid, Jingfukang AB agent, Metalaxyl-M, and Chlorobromoisocyanuric acid. Metalaxyl-M · Fludioxonil emerged as the most effective treatment for controlling root rot in tobacco floating seedlings. Furthermore, the incorporation of amino acids enhanced the prevention of root rot by fostering healthy root development, thereby contributing to the overall robustness of the tobacco seedlings. Tobacco seedlings that possess robust root systems are more likely to acclimate to their new environment swiftly following transplantation. This adaptability is crucial as it establishes a solid foundation for a successful final tobacco harvest and enhances the overall quality of the tobacco produced.

The efficacy of disease prevention and the capacity to promote root growth of Metalaxyl-M · Fludioxonil + amino acids, Metalaxyl-M · Fludioxonil, and chlorine dioxide were all ranked among the highest. However, prolonged use of chlorine dioxide over many years may lead to the development of resistance in tobacco plants, resulting in a slightly diminished disease prevention capability compared to that of Metalaxyl-M · Fludioxonil. According to the manufacturer, Yishikang, an amino acid water-soluble fertilizer, functions as a bio-activator that significantly enhances root development and resilience to adverse conditions. Furthermore, a robust root system contributes to improved disease resistance. Consequently, the application of Metalaxyl-M · Fludioxonil + amino acids demonstrates superior disease prevention and rooting capabilities compared to the use of Metalaxyl-M · Fludioxonil alone.

In the study conducted by Miao Pu *et al.* [24], the efficacy of 48% Propamocarb · Cupric ammonium and 30% Propamocarb · Azoxystrobin against root rot in tobacco seedlings caused by *A. iridis* was observed to range from 86.88% to 94.61%. However, it was noted that the leaves of the tobacco seedlings began to exhibit water-soaked necrotic spots one day after treatment with 48% Propamocarb · Cupric ammonium, and the severity of the water-soaked necrosis increased over time. 30% Propamocarb · Azoxystrobin treatment did not yield any significant effects on the growth of tobacco seedlings, which was consistent with the results observed in the sterile water control group. In contrast, the Metalaxyl-M · Fludioxonil treatment demonstrated not only a remarkable preventive effect in field conditions but also facilitated root growth. Furthermore, the enhancement of the root system was notably more pronounced when amino acids were incorporated in the treatment.

4.2 Conclusions In summary, prior to the cultivation of tobacco seedlings, the application of Metalaxyl-M · Fludioxonil + amino acids is recommended for use in the seedling pool. Each tray should contain 1 mL of Metalaxyl-M · Fludioxonil and 2 mL of amino acids. This combination has been shown to effectively prevent and control root rot in tobacco floating seedlings caused by *A. iridis*. Furthermore, it contributes to the enhancement of root system health and improves the quality of transplanted tobacco seedlings, thereby ensuring a safe environment for the healthy growth of tobacco.

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