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# 黑杨水培中营养液抑菌处理研究

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**摘 要**：水培条件下进行快速繁殖是大量生产黑杨种苗的途径之一，营养液的抑菌处理是水培的一个重要方面。该研究进行了二硫氰甲烷、中生菌素、敌磺钠、链霉素、青霉素等五种杀细菌剂和多菌灵、福美双、三唑酮等三种杀真菌剂的抑菌试验，结果表明，杀细菌剂对微生物生长的抑制率要高于杀真菌剂，二硫氰甲烷和中生菌素能较好地抑制营养液中微生物的生长，浓度分别为 20 mg/L 和 15 mg/L 时，抑制率分别为 75.60% 和 78.94%。杀真菌剂和杀细菌剂复配对营养液中微生物生长的抑制表现出较好的加和作用，其中以中生菌素(15 mg/L)与福美双(5 mg/L)复配的效果最好，抑制率为 87.48%。

**关键词**：黑杨；营养液；杀细菌剂；杀真菌剂；抑菌处理

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# Antimicrobial treatment of nutrient solution in hydroponic cultivation of *Populus nigra*

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**Abstract**: Fast micro-propagation by hydroponic cultivation is one of the methods in large scale production of *Populus nigra* seedlings. Antimicrobial treatment of nutrient solution is an important aspect in hydroponic cultivation. Inhibition experiments on microbial growth were carried out by using five bactericides which were diisothiocyanatomethane, zhongshengmycin, fenaminosulf, streptomycin, penicillin, and three fungicides which were carbendazim, thiram and triadimefon. The results showed that the inhibition effects of bactericides against microorganisms in the nutrient solution were better than those of the fungicides. Of bactericides, both diisothiocyanatomethane and zhongshengmycin at their concentrations of 20 mg/L and 15 mg/L strongly inhibited the growth of the microorganisms which the corresponding inhibitory rate were 75.60% and 78.94% separately. It was found that microbial growth were inhibited to a great extent by the combination of one bactericide with one fungicide, and an additional effects of the agrochemicals were also observed. The inhibition rate of zhongshengmycin at 15 mg/L in combination with thiram at 5 mg/L was 87.48% which was the highest inhibition rate of all combination effects.

**Key words**: *Populus nigra* L.; nutrient solution; bactericide; fungicide; antimicrobial treatment

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# 1 Introduction

Poplar(*Populus* spp.)plants,which belongs to family Saliaceae,have many characters such as fast growth,drought resistance,easily hybrid and asexual cuttage propagation. They are suitable for short-rotation,intensive cultivation. Poplar plants are of considerable ecological value and capable of producing utilizable timber (Ceulemans *et al.*, 1999). There are more than 60 poplar species widely distributed in many parts of China(Delectis Florae Rerpublicae Popuparis Sinicae,1984). Black Poplar(*P. nigra* L.)was introduced from Europe to China in 1980s,and its farming has been established as this species has many good characters over other poplar species. It has become one of the first forest tree species to be cultivated in most parts of China(Shi *et al.*,2000).

Poplar asexual cuttage method has been used widely instead of traditional seed propagation(Kenny *et al.*,1990). As poplar seedlings are in great demand, tube micropropagation and hydroponic propagation which need a small amounts of plant materials have also been studied (Jafari *et al.*, 1995). In the process of hydroponic propagation, nutrient solution can be polluted easily by microorganisms. This would lead to fast replacement of nutrient solution, cast increasing, stem segment rot,and seedling disease occurring. In order to inhibit microbial growth in hydroponic system,some methods such as ultraviolet,ozone,surface activator, filtration, adsorption, osmotic pressure treatments have been studied (Zhang,1999). To the best of our knowledge,there is still no published report on antimicrobial treatment of nutrient solution with agrochemicals in hydroponic cultivation of *P. nigra*. In this study,appropriate antimicrobial agrochemicals were screened. The objective of our work was to lay a foundation for the hydroponic propagation of black poplar in large scale,and also to provide some evidences for other plants hydroponic propagation in order to prevent microorganism pollution.

# 2 Materials and Methods

## 2.1 Materials

The materials of Black Poplar(*P. nigra* L.) No. 110 was provided by Dadongliu Seedling Nursery of Beijing Forestry Bureau. The stems of healthy male poplar were selected and cut into segments as long as five to six centimeters. One bud was kept in morphological upper part on each segment. The morphological lower part would be kept in nutrient solution. All the segments were washed with tap water for 10 min,and then kept in 1% of fresh sodium hypochlorite solution for 10 min,then washed with tap water for 5 min. The sterilized segments would be utilized for further experiments.

## 2.2 Culture condition

The poplar stem segments were germinated and grown in a greenhouse with 12 h of light and 12 h of dark intervals,at 30～35 ℃ in daytime and 20～25 ℃ at night. Relative humidity was kept in 60%～80%. The light density was about 3 000 lx.

## 2.3 Plant nutrient solution

The compositions of Black Poplar nutrient solution was as follows. In one liter of nutrient solution,there were 500 mg of $Ca(NO_3)_2$, 125 mg of $MgSO_4 \cdot 7H_2O$, 125 mg of $KH_2PO_4$, 60 mg of KCl, 1.43 mg of $H_3BO_3$, 0.11 mg of $ZnCl_2$, 0.04 mg of $CuSO_4 \cdot 5H_2O$, 0.01 mg of $H_2MoO_4$, 0.04 mg of $MnCl_2$, 2.68 mg of $Na_2EDTA$, 2 mg of $FeSO_4 \cdot 7H_2O$, 2 mg of gibberellin, 0.2 mg of α-naphthalene acetic acid, 1.5 mg of benzyl adenine. The pH value of nutrient solution was adjusted to 5.8.

## 2.4 Agrochemicals and their inhibition on microorganisms

All the agrochemicals were kindly supplied by the Seed Coating Chemical Center of China Agricultural University. Agrochemicals included five bactericides which were diisothiocyanatomethone, zhongshengmycin, fenaminosulf, streptomycin and penicillin,and three fungicides which were carbendazim, thiram and triadimefon. When the poplar

segments were cultured in nutrient solution, the agrochemicals were added in at the same time. Ten replicates for each test nutrient solution were set up. After three weeks co-culture, the percentage growth inhibition was measured by following the absorbance at 655 nm and was defined as 100-times the ratio of the change in absorbance of the average growth in the control(△C) minus the change in absorbance in the test nutrient solution(△T), the difference then divided by △C; i. e. [(△C−△T)]/△C×100(Marcus *et al.*, 1997).

Table 1 Inhibition of agrochemicals on microbial growth in nutrient solution of *P. nigra*[1)]

| Agrochemicals | Concentration (mg/L) | Inhibition rate (%) | Agrochemicals | Concentration (mg/L) | Inhibition rate (%) |
|---|---|---|---|---|---|
| Diisothiocyanatomethone | 10 | 25.20 | Penicillin | 5 | 13.28 |
| | 20 | 75.60 | | 10 | 29.60 |
| | 40 | 73.47 | | 50 | 72.49 |
| | 80 | 74.21 | | 100 | 73.45 |
| Zhongsheng-mycin | 5 | 30.74 | Carbendazim | 0.5 | 0.00 |
| | 15 | 78.94 | | 5.0 | 3.97 |
| | 30 | 76.67 | | 50 | 10.53 |
| | 60 | 78.90 | | 100 | 9.78 |
| Fenaminosulf | 10 | 11.77 | Thiram | 0.5 | 1.84 |
| | 35 | 20.30 | | 5.0 | 6.70 |
| | 70 | 23.15 | | 50 | 7.91 |
| | 140 | 23.16 | | 100 | 10.34 |
| Streptomycin | 5 | 27.30 | Triadimefon | 0.5 | 0.00 |
| | 10 | 45.92 | | 5.0 | 3.42 |
| | 50 | 75.52 | | 50 | 9.11 |
| | 100 | 76.85 | | 100 | 10.82 |

[1)] There were both bacteria and fungi in the nutrient solution of *P. nigra*; Inhibition rate of the control was 0.

## 3 Results

### 3.1 Inhibition of agrochemicals on microbial growth in nutrient solution

It was found that all eight agrochemicals had no inhibitory effects on black poplar stem segments growth with the tested concentration in nutrient solution. Their inhibitory effects on microorganisms were shown in table 1. Appropriate concentration for agrochemicals to inhibit microbial growth were as follows: diisothiocyanatomethone 20 mg/L(inhibition rate 75.60%), zhongshengmycin 15 mg/L (inhibition rate 78.94%), fenaminosulf 35 mg/L(inhibition rate 20.30%), streptomycin 50 mg/L(inhibition rate 75.52%), penicillin 50 mg/L(inhibition rate 72.49%), carbendazim 50 mg/L(inhibition rate 10.53%), thiram 5 mg/L(inhibition rate 6.70%)and triadimefon 50 mg/L(inhibition rate 9.11%). Of five bactericides, diisothiocyanatomethone was the most effective one. Effects of bactericides were better than those of fungicides.

### 3.2 Inhibition of agrochemical combination on microbial growth in nutrient solution

In order to get a better inhibitory result, five bactericides with their appropriate concentration separately combined with three fungicides at their different concentrations. Inhibition effects of chemical complex on microbial growth of nutrient solution of black poplar was shown in fig. 1. Results showed that combination of one fungicide with a bactericide have an adding effects. Of them, 15 mg/L of zhongshengmycin in combination with 5 mg/L of thiram(Fig. 1:B), which inhibitory effect was 87.48%, had a good effect than any other combinations.

## 4 Discussion

The poplar stem segments(or called stem explants)were easily infected by microorganisms including pathogenic and non-pathogenic ones in *in vitro* culture. Otherwise, microorganisms also easily grew in nutrient solution with an open culture system. They can contaminate whole culture system in a short time. So it is very necessary to prevent poplar seedlings in nutrient solution from microorganism contamination. Main microorganisms in nutrient solution were bacteria, and fungi were also found. Separation, purification and identification of the microorganisms in poplar nutrient solution have not been finished. In this paper, we just reported the results of some bactericides and fungicides to inhibit microorganism growth in nutrient solution. Our results showed that appropriate agrochemicals with their combination in nutrient solution were very effective to inhibit microorganisms growth, and these agrochemicals have been proved

to have no inhibitory effects on black poplar seedling growing. Our results of the agrochemicals on poplar seedling growing are just primary, further investigation is needed. Otherwise, all the agrochemicals which we tested were low toxic to environment as well as to human. Combination of bactericides and fungicides at their appropriate concentrations i. e. 15 mg/L of zhongshengmycin in combination with 5 mg/L of thiram, had a good inhibitory effect on microorganism growth in nutrient solution. The nutrient solution could be replaced every 60 days with agrochemical treatment instead

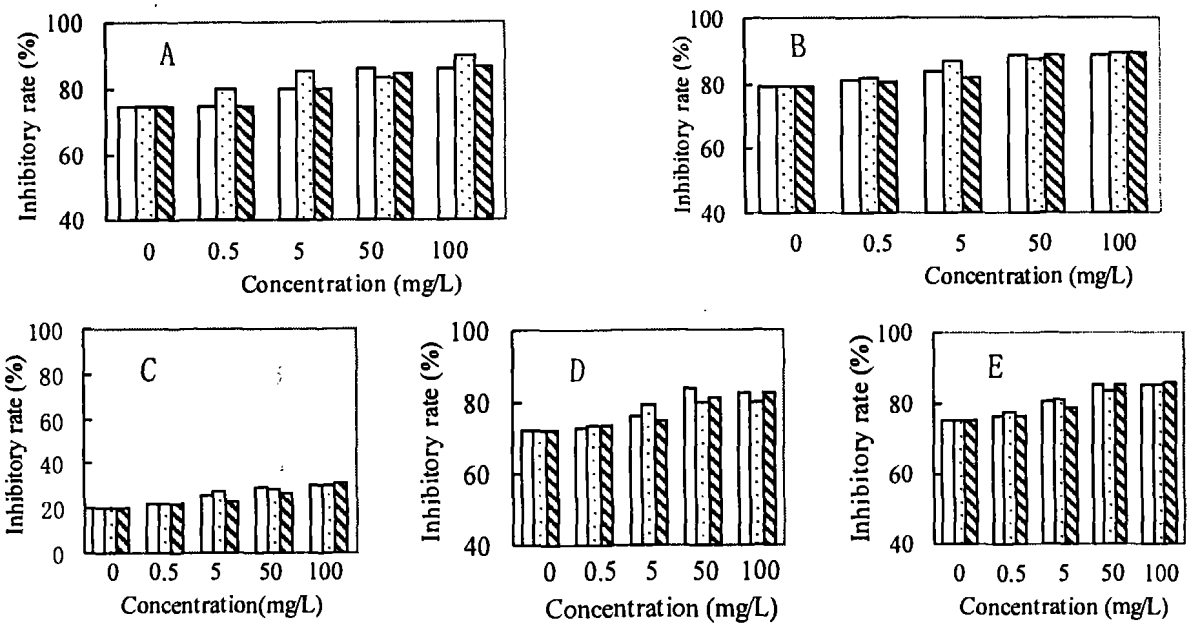


Fig. 1 Inhibition of agrochemical complex on microbial growth in nutrient solution of *P. nigra*

A was 20 mg/L of diisothiocyanatomethane combination with three fungicides separately; B was 15 mg/L of zhongshengmycin combination with three fungicides separately; C was 35 mg/L of fenaminosulf combination with three fungicides separately; D was 50 mg/L of streptomycin combination with three fungicides separately; E was 50 mg/L of penicillin combination with three fungicides separately. X-coordinate was the concentration of carbendazim, thiram and triadimefon; Y-coordinate was the inhibition rate of microbial growth; □was the carbendazim complex; ▨ was the thiram complex; ▧ was the triadimefon complex.

of previous 15 days without agrochemicals treatment(data not shown). This study will be benefit to hydroponics cultivation of black poplar as well as to other plant hydroponics culture. It will lay a foundation to decrease culture cost, provide healthy seedlings for poplar cultivation in future.

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