Effects of Culture Filtrates of Trichoderma Harzianum on Maize Seed Germination and Seedling Vigor

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Abstract

Trichoderma species are commonly used as biological control agents against phytopathogenic fungi and some of their isolates are able to improve plant growth. In the current study, we evaluated the effect of cultural filtrates and its dilution solution of Trichoderma harzianum GIM3.442 on seed germination and seedling vigor of maize. Results revealed that the 50-fold culture filtrate was found effective to enhance the germination percentage, length of coleoptile, Length of radicle and the number of radicles compared to control ; The 10-fold diluted filtrate had the best promoting effect on shoot length, fresh weight of roots, fresh weight of shoot, and leaf area of maize, and the 10-fold filtrate was found effective to enhance the fresh root weight and fresh shoot weight compared to control, the 50-fold diluted filtrate can increase the chlorophyll content of maize leaves and promote the growth of maize seedlings in advance.

Keywords

Trichoderma harzianum , Cultural filtrates , maize , Seed germination, Seedling vigor.

1. Introduction

Trichoderma spp.,which are common saprophytic filamentous fungi in almost any soil and rhizosphere microflora, are well recognized as biocontrol agents against various plant pathogenic fungi[1]. Trichoderma spp. has been used to control a broad range of phytopathogenic fungi, such as Rhizoctonia solani, Sclerotinia sclerotiorum, and Botrytis cinerea[2]. In addition to biocontrol ability, some Trichoderma species are able to promote plant growth, This is because of their ability in beneficial interactions with plants (maize, cotton, cucumber) through production of auxin like compounds and secondary metabolites[3,4]. Trichoderma derived volatile organic compounds are reportedly promoting plant growth[5,6]. It was observed that the fertility of soils treated with some Trichoderma strains could be significantly improved beyond disease control, which increased the attractiveness of these fungi for general use in crop production[7].

Maize is an important grain crop for human food security, fodder, and biofuel production. However, inadequate studies are available on maize growth stimulants produced by Trichoderma species. Therefore, The aim of this study is to evaluate different dilution culture filtrate of Trichoderma harzianum GIM3.442 on maize seed germination and seedling vigor through the analysis of some indicating factors such as germination rate, root elongation, plant height and fresh weight of the seedlings as well as chlorophyll content and leaves area.

2. Materials and methods

2.1 Materials

Trichoderma harzianum GIM3.442 was provided by the Guangdong culture collection center; mazie seed (Zhengdan 958) was purchased from Henan Faculty of Agricultural Sciences Seeds Industry Co., Ltd.other analytical grade were provided by Aladdin (Shanghai, China). Ultrapure water (18.2 MW ·cm) was obtained from Millipore (Bedford, MA, USA), and used throughout the experiments. The T. harzianum strains was grown on potato dextrose agar(PDA) medium, maintained on PDA medium and stored at 4 ℃ for further use.

2.2 Preparation of culture filtrates of Trichoderma harzianum

The T. harzianum strains was grown on PDA medium at 28 ℃ for senev days for activation, then gently rinse mycelium surface with PBS(Gibco PH 7.4) to collect spores. The concentration of Trichoderma spores in pbs was counted using haemocytometer and adjusted to 10^5 spores per ml. 100 µl suspension of Trichoderma spores were inoculated into Erlenmeyer flasks (150 cc) containing 60 ml of potato dextrose broth. The flasks were incubated at 28 ℃ on a rotary shaker set at 150 rpm for 7 days. After incubation, the culture was filtered through millipore filter in order to remove mycelia mats and then sterilized by passing through 0.22µm pore biological membrane filter get culture filtrates. The culture filtrate was diluted 10 times, 50 times, and 100 times respectively to obtain different dilutions of the culture filtrate, and the filtrate and its dilution solution were stored at 4 ℃ for further use.

2.3 Effects of culture filtrates on maize seed germination and seedling vigor

Seed treatment: Seeds with no cracks or other visible deformations were selected and surface sterilized for 15 minutes with 0.1 % mercury chloride solution. Seeds were then rinsed three times with sterilized distilled water and air dried.

Seed soaking: The test consists of 5 treatments: sterile water, culture filtrate, 10-fold diluted filtrate, 50-fold diluted filtrate, and 100-fold diluted filtrate. The surface-sterilized seeds are immersed in a beaker(50ml) containing 30 ml of treatment liquid, and each beaker is placed in 50 seeds. Sterile water treatment group as control group.

Effect of filtrate on seed vigor: After soaking for 8 hours, eleven seeds from each treatment were placed on filter paper moistened with 5 ml of distilled sterile water in 90-mm-diameter Petri plates, three parallel groups per treatment. Experimental plates were incubated at 28 ℃ in growth chamber,

Timely replenishment of sterile water in petri dishes. Percentage of seed germination was counted 48h after incubation, the number of radicles was counted 96h after incubation as well as the radicle length and coleoptile length was measured.

Effect of filtrate on growth of seedlings: In field experiment, the seeds were sown in a sterilized nutrient soil after soaking for 8 hours, three seeds were sown in each pot, water was replenished periodically, and cultivation was performed under natural conditions, three parallel groups per treatment. After 7 days of culture, the seedling height, root length, fresh weights of the root and shoot, leaf area, chlorophyll content were measured. The chlorophyll content was measured according to the method of Qiu, N[8].

2.4 Statistical analyses

The average mean of growth parameters from three lab experiments (each experiment was carried out in three replicates) were subjected to analysis of variance and treatment means were computed by Duncan's multiple range test (DMRT) at P = 0.05.

3. Results and discussion

3.1 Effect of Filtrate on Maize Seed vigor

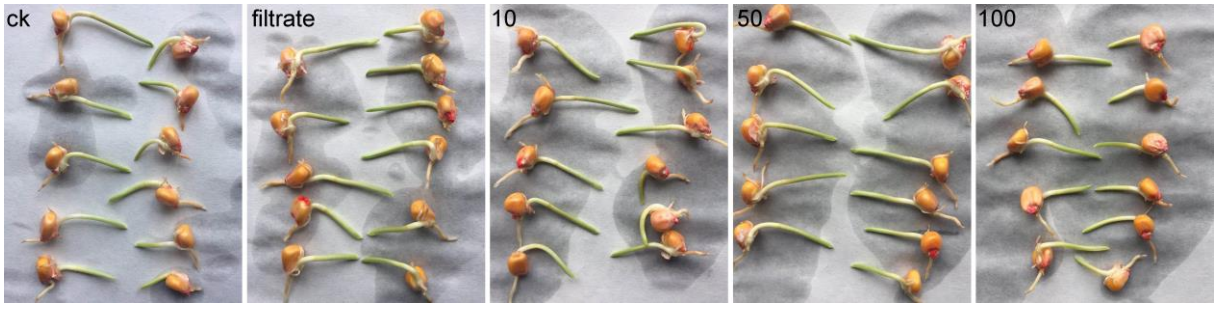

Fig. 1 Mazie seed germination after 96 hours of filtrate treatment

The effect of culture filtrates and its dilution of Trichoderm strain on seed germination of maize are presented in Fig. 1 and table 1. Statistical analysis of figure showed significant differences in treatments at $P < 0.05$ levels. Results showed that 50-fold filtrate was found effective to enhance the germination percentage and length of coleoptile compared to control, there was a slight increase in other treatment groups compared to control. Both Trichoderma filtrate and its dilutions significantly promoted the growth of secondary radicles in maize, and 10-fold and 50-fold filtrates significantly increased the length of corn radicles. Through comprehensive analysis and evaluation, the effect of 50 times filtrate on the growth of corn seed is the best.

Table 1 Effect of Trichoderma cultural filtrate on maize seed germination

Treatment	%Seed germination after 48h	Length of coleoptile (mm after 96 h)	Length of radicle (mm after 96 h)	Number of radicles after 96 h
Ck	81.9±5.3 b	25.7±2.1 b	11.1±0.9 b	2.8±0.3 b
filtrate	81.9±5.3 b	29.0±1.7 ab	12.9±0.9 b	4.1±0.2 a
10-fold diluted filtrate	86.2±2.6 ab	30.0±2.3 ab	15.8±1.2 a	3.8±0.2 a
50-fold diluted filtrate	100.0±0 a	34.2±1.6 a	17.6±0.5 a	3.9±0.2 a
100-fold diluted filtrate	90.9±5.3 ab	27.0±1.9 b	12.5±0.7 b	3.6±0.2 a

Values with the same letter within the column are not significantly different ($P<0.05$) according to Fischer's protected LSD test; results are means of three replicates for each treatment; the value in parentheses is the standard error of the mean

3.2 Effect of filtrate on growth of seedlings

After the corn seed was treated with the filtrate and its dilution solution, the seedling growth status as shown in Fig. 2, and the effect of culture filtrates on seedling growth parameters as shown in Table 2.

The results showed that the root length, shoot height, and fresh root weight of the treated group were higher than those of the control group. The filtrate had the best effect on the elongation of the maize root, and with the continuous dilution of the filtrate, the length of the root was decreasing. The 10-fold diluted filtrate had the best promoting effect on shoot length, fresh weight of roots, fresh weight of shoot, and leaf area of corn, and the 10-fold filtrate was found effective to enhance the fresh root weight and fresh shoot weight compared to control. Based on this, it is inferred that the culture filtrate of Trichoderma contain plant growth stimulating factors and phytohormones such as indol acetic acid (IAA) and their analogs, and vitamins. Production of some organic acids such as gluconic citric and/or fumaric acidsreduces the soil pH resulting in solubilization of phosphates.

Fig. 2 Effect of filtrate on growth of seedlings

Table 2 Effect of culture filtrates on seedling growth parameters

Treatment	Root length (cm)	Shoot length (cm)	Fresh root weight (mg)	Fresh shoot weight (mg)	Leaf area (cm^2)

Ck	18.3±0.9 a	5.3±0.1 a	29.7±7.9 b	498.0±26.1 ab	24.2±1.0 a
filtrate	20.5±1.5 a	5.7±0.1 a	47.4±9.6 a	571.2±44.4 ab	26.9±2.2 a
10-fold diluted filtrate	19.9±1.0 a	5.8±0.1 a	51.4±16.3 a	616.7±40.6 a	29.3±2.3 a
50-fold diluted filtrate	19.2±1.8 a	5.5±0.2 a	48.8±8.3 a	472.8±42.9 b	23.5±1.3 a
100-fold diluted filtrate	18.9±0.9 a	5.7±0.1 a	32.8±2.6 b	471.0±59.6 b	23.2±3.4 a

Values with the same letter within the column are not significantly different ($P<0.05$) according to Fischer's protected LSD test; results are means of three replicates for each treatment; the value in parentheses is the standard error of the mean

3.3 Effects of Filtrate on Chlorophyll Content of Maize Leaves

Chlorophyll is one of the important indicators of plant growth.The chlorophyll content of maize leaves after different treatments is shown in Figure 3. Results showed that the 50-fold diluted filtrate had the most obvious increase in chlorophyll content in maize leaves,and the 100-fold diluted filtrate chlorophyll content also increased significantly. Fome that we get the larger diluted filtrate can promote the growth of maize seedlings in advance compare with other treaments.

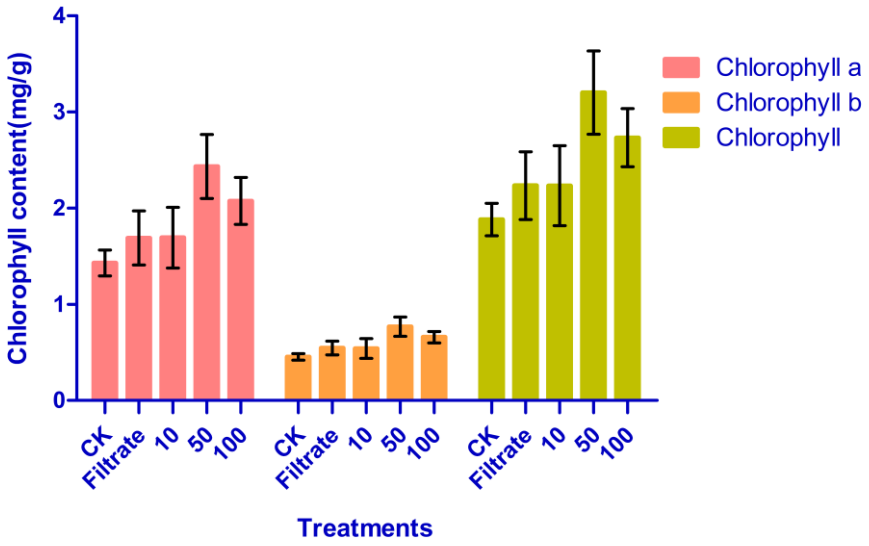

Fig. 3 Chlorophyll content after different treatments

4. Conclusion

The potential of using culture filtrates of Trichoderma isolates as enhancers of seed germination and plant growth and development could have important economic implications such as shortening the plant growth period and time, as well as improving plant vigor to overcome biotic and/or abiotic stresses, resulting in increase plant productivity and yields .The present study concludes that culture filtrates and its dilution of Trichoderm strain GIM3.442 have a significant effect on the seed vigor of maize and the growth of maize seedlings, among them, the 50-fold diluted filtrate had the best promoting effect on the corn seed vigor, and the 10-fold dilution filtrate had the best effect on the growth of maize seedlings. Further investigation can be carried out to determine the potential of Trichoderma spp., on the growth promotion of other crops.

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