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Research Article

Improvement of biocontrol efficacy of *Trichoderma harzianum* Tr6 vs. *Phytophthora drechsleri*, the causal agent of damping-off disease in *Cucumis sativus*

Zhila Delkhah and Keivan Behboudi*

Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Abstract: Biocontrol agents have different growth and biocontrol responses under the influence of physicochemical parameters. The culture medium is one of these parameters. Therefore, in this study, the effects of eight liquid media were investigated on the growth and antagonistic efficacy of *Trichoderma harzianum* Tr6 against *Phytophthora drechsleri*. Tr6 at 10^8 spores per ml was grown in these media. All media pH was set at 5.5. Treatments were maintained under a light intensity of 206 Lux and 130 rpm at 28 °C for 10 days. Maximum sporulation (2.5×10^{10} spore/g.dw) occurred in Mol. C. (Sugar beet molasses and Corn steep liquor) medium. The most inhibition against pathogen was related to Mol. M. (Sugar beet molasses and Malt extract) medium. Mol. M. and Mol. B. (Sugar beet molasses and Baker yeast) had the most effect on disease reduction (58.33%). Mol. M. had not superlative effect on plant growth factors but had a better effect compared with other treatments except Mol. Y. (Sugar beet molasses and Yeast extract). The following assays were done to simultaneously select the optimum medium for high sporulation, effective control of disease, and plant growth promotion. Therefore, Mol. M., Mol. B., Mol. C., and Mol. C. B. M. media were used. Mol. C., Mol. C. B. M. and Mol. M. had the highest spore production with 2.35×10^{10}, 1.83×10^{10}, and 1.65×10^{10} spore/gdw, respectively. There was no significant difference between the Mol. C. B. M. and Mol. M. Furthermore, Mol. C. B. M. reduced disease by 62.5%, but this reduction was not significantly different from Mol. M. and Mol. B. Therefore, Mol. M. medium had the most influence on growth and biocontrol performance of Tr6.

Keywords: antagonist, biocontrol, *Phytophthora drechsleri,* mass production, *Trichoderma*

Introduction

Phytophthora drechsleri damping-off is one of the most important diseases of cucumber *Cucumis sativus* (Sadeghi *et al.,* 2017). Control of this disease is complicated, and no single method is available (Maleki *et al.,* 2011). Resistant cucumber varieties to *P. drechsleri* are not available, and resistance to systemic fungicides has developed rapidly in this pathogen after their application in the field (Lamour and Hausbeck, 2000 and 2001). The use of microorganisms that reduce the amount of plant pathogens inoculums or their activity is called a biological control phenomenon (Tsegaye *et al.,* 2018). Biological control by an antagonistic microorganism is a potential

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* Corresponding author: behbodi@ut.ac.ir
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eco-friendly approach for controlling plant diseases (Bailey *et al.,* 2004). Biological control of *P. drechsleri* by fungal antagonists can be essential for controlling the disease (Soheiliara *et al.,* 2020). *Trichoderma* spp. are large microorganisms that play an important role in the environment and use different mechanisms to colonize various ecological niches. Several *Trichoderma* species are non-pathogenic and useful symbionts that are well suited against most of the fungal pathogens through the mechanisms of competition, mycroparasitism, antibiotic and enzyme production, stimulation of plant growth, and induction of defense responses in different plants (Soheiliara *et al.,* 2020). *Trichoderma* spp. are fast-growing fungi, widely used as biocontrol agents for controlling soil-borne diseases of plants, as well as growth promoters (Hewavitharana *et al.,* 2018). It is a prerequisite for biological control application to develop mass production, formulation, and delivery systems for microorganisms that control plants' diseases (Bhat *et al.,* 2009). Biocontrol agents have different growth and biocontrol responses under the influence of physicochemical parameters. Medium is one of these parameters. There is abundant literature on conventional synthetic media like glucose, cellulose, soluble starch, and molasses to produce *Trichoderma* spp. (Khan *et al.,* 2011). The use of inexpensive substrates such as molasses, yeast extract, and corn steep helps reduce the cost of biomass production. Mass production of *Trichoderma* has become a focus of research in the study for alternatives to pesticides and chemical fertilizers for plant disease control and plant growth promoters (Parkash and Saikia, 2015). Micropropagules of *Trichoderma* in the form of conidia is preferred over chlamydospores and mycelial biomass because of the viability and stability in field application (Hewavitharana *et al.,* 2018). The main aim of the mass-production technology of biocontrol agents is the maximum yield of spores while maintaining biocontrol efficacy and minimizing the cost of a medium using inexpensive materials. Solid and liquid state fermentation are two major methods for the production of *Trichoderma* spp. In a liquid fermentation system, *Trichoderma* is grown in cheap liquid media like molasses and yeast in stationary/ shaker/fermenter cultures and formulated on a commercial scale and used for seed dressing either by treating dry seeds or by seed biopriming to control some soil-borne diseases (Ramanujam *et al.,* 2010). However, solid-state fermentation is suitable for small-scale production and is used for direct soil application (Ramanujam *et al.,* 2010). Some of the disadvantages of this method are the high volume of the substrate required, contamination during fermentation, and the long time required for fermentation. However, liquid fermentation will help the mass production of microorganisms under axenic conditions within a short time (Khan *et al.,* 2011). In this study, *T. harzianum* Tr6 was chosen because this isolate has been successful in previous plant pathogen biocontrol and plant growth development projects. For instance, this isolate has shown the most impact on reducing fusarium stem and root rot disease of cucumber (Alizadeh *et al.,* 2013). Tr6 induces resistance more effectively than popular isolates T22 and T39 (Alizadeh *et al.,* 2013). T22 has reduced anthracnose symptoms caused by *Colletotrichum graminicola* through induction of systemic resistance in maize (Harman *et al.,* 2004), also induced systemic resistance in tomato infected by cucumber mosaic virus (Vitti *et al.,* 2016). T39 induced resistance against *Botrytis cinerea* (De Meyer *et al.,* 1998) and downy mildew by priming for defense without costs for grapevine (Perazzolli *et al.,* 2011). When Tr6 and *Pseudomonas* sp. P14 was used together. They had the highest colonization rate and increased the weight of the cucumber roots and shoots (Alizadeh *et al.,* 2013). Delkhah and Behboudi (2015 and 2016) investigated the growth and biocontrol response of Tr6 on the solid culture, and they reported that the culture medium had a positive effect on the improvement of biocontrol properties of Tr6 against *P.*

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drechsleri and *B. cinerea.* This isolate showed good antibiosis potential against these pathogens (Delkhah and Behboudi, 2015 and 2016). Therefore, the isolate was chosen for our study. The objective is the mass production of Tr6 using inexpensive and available liquid growth substrate for controlling damping-off disease caused by *P. drechsleri.*

Materials and Methods

Microorganisms and inoculum preparation

P. drechsleri was obtained from the Iranian Research Institute of Plant Protection, Evin, Tehran, and Tr6 were received from the Plant Protection Department of Tehran University. Tr6 had been isolated from the cucumber rhizosphere (Alizadeh *et al.,* 2013). The pathogen was incubated at 28 °C for five days on a P.D.A. medium for pathogen inoculum preparation. 50 g of millet seed was added to 25 ml of water in a 250 ml Erlenmeyer flask and autoclaved for 20 min 121 ^{o}C in 2 successive days. The flask was inoculated with three mycelial plugs from a 5-days-old culture of *P. drechsleri*, incubated at 26 ± 1 ^{o}C for two weeks. Also, the Tr6 was maintained at a light density of 206 Lux and 28 ^{o}C for five days on P.D.A. medium. After growth and sporulation, the spore suspension was obtained in distilled water as the antagonist inoculum.

Growth media and cultivation of Tr6

The following media were prepared.

1) Mol. C. N. G: Sugar beet molasses (50 ml/l), corn steep liquor (5 ml/l), NaCl (25 g/l) and glucose (25 g/l) (Lewis and Papavizas, 1983)

2) Mol. C. G: Sugar beet molasses (50 ml/l), corn steep liquor (5 ml/l), and glucose (25 g/l)

3) Mol. C: Sugar beet molasses (50 ml/l) and corn steep liquor (5 ml/l)

4) Mol. Y: Sugar beet molasses (50 ml/l) and yeast extract (10 g/l)

5) Mol. S: Sugar beet molasses (50 ml/l) and soybean extract (10 ml/l)

6) Mol. B: Sugar beet molasses (50 ml/l) and baker's yeast (10 g/l)

7) Mol. M: Sugar beet molasses (50 ml/l) and malt extract (10 g/l)

8) Mol: Sugar beet molasses (50 ml/l)

Media were apportioned in 50 ml portions into 250 ml Erlenmeyer flasks and autoclaved at 121 °C for 20 min. One ml of suspension containing 10^8 spores of the antagonist isolate, counted with a hemocytometer, was added to each flask, and treatments were maintained in a shaker incubator at a light density of 206 Lux and 130 rpm at 28 °C for 10 days.

Harvesting of Tr6 biomass and calculation of spore numbers

According to Lewis and Papavizas (1983) and Georgakopoulos et al. (2002), the flask contents were centrifuged and dried at 37 °C in an Oven for 72 h. Afterward, the dried mass was ground, and 0.1 g of the powder was suspended in 25 ml distilled water and blended in a Vortex for 3 min at a high rate. The material was settled, additional dilutions were made, and spores were counted by a hemocytometer. The spore numbers per gram dry weight of biomass were calculated (Lewis and Papavizas, 1983).

Antagonistic activity of Tr6 biomass against *P. drechsleri in vitro*

The antagonistic effect of Tr6 grown in various media against *P. drechsleri* was evaluated using the dual culture. P. D. A. plates were inoculated, on one side, with 1 cm mycelial plugs of the pathogen and maintained at 26 ± 1 °C. After 24 h, plugs of Tr6 were placed on the opposite side. Then, all treatments were incubated under the light intensity of 206 Lux at 26 ± 1 °C. Radial growth of the pathogen, Tr6, and colonization of the pathogen colony by Tr6 was measured at 24 h intervals for three days. Then inhibition percentage was determined by the following formula (Dennis and Webster, 1971):

$$I = \frac{C - T}{C} \times 100$$

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I = Inhibition percentage of the pathogen growth
C = Radial growth of the pathogen in control
T = Radial growth of the pathogen in the presence of the antagonist

Plant material

Cucumber seeds (*Cucumis sativus* L., cv. Soltan) were obtained from Flat Agriculture Co. (Tehran, Iran). The seeds were surface-sterilized for 3 min in 1% sodium hypochlorite and then rinsed three times with sterile distilled water (Shirzad *et al.,* 2012). Treated seeds were incubated at 28 °C for 36 h between layers of sterile wet filter paper.

Greenhouse assay on disease control by Tr6 biomass

The ability of each Tr6 biomass to repress cucumber phytophthora damping-off was tested on the *Cucumis sativus* L. Soltan cultivar. The pots were filled with 1 kg of the autoclaved potting mixture. One day before seeding, 2 g *P. drechsleri* inoculum was blended with potting mixture. Then, 3-days-old germinated seeds were dipped into a suspension containing 10^7 spores of Tr6 produced on each biomass mixed with 2 g gum per 1 liter of suspension for 30 min. Four seeds were planted in each plastic pot filled with a 1:1:2 mixture of peat, sand, and potting soil that had been inoculated with 2 g *P. drechsleri*. Plants were cultivated in a greenhouse (16 h light and 25-28 ^{o}C). The pots were watered once every third day for 15 days. The following were the different treatments.

1) Control^{+} (No treatment)
2) Control^{-} (Pathogen alone)
3) Pathogen + Tr6 obtained from culture on Mol. C. N. G.
4) Pathogen + Tr6 obtained from culture on Mol. C. G.
5) Pathogen + Tr6 obtained from culture on Mol. C.
6) Pathogen + Tr6 obtained from culture on Mol. Y.
7) Pathogen + Tr6 obtained from culture on Mol. S.
8) Pathogen + Tr6 obtained from culture on Mol. B.
9) Pathogen + Tr6 obtained from culture on Mol. M.
10) Pathogen + Tr6 obtained from culture on Mol.

Greenhouse assay on plant growth promotion by Tr6 biomass

The final product (biomass) of each flask was tested for its ability to promote plant growth factors. This assay was performed according to the previous experiment. However, an inoculum of the pathogen was not added to the treatments. The pots were watered once at a two days' interval for 1 month. The following were the different treatments.

1) Control (No treatment)
2) Tr6 obtained from culture on Mol. C. N. G.
3) Tr6 obtained from culture on Mol. C. G.
4) Tr6 obtained from culture on Mol. C.
5) Tr6 obtained from culture on Mol. Y.
6) Tr6 obtained from culture on Mol. S.
7) Tr6 obtained from culture on Mol. B.
8) Tr6 obtained from culture on Mol. M.
9) Tr6 obtained from culture on Mol.

Statistics

The experiment was performed in a completely randomized design (C. R. D.). The experiments were repeated two times. Also, each treatment was tested in four replicates for all trials. Analyses of variance were done using SPSS 16.0, and the mean values were compared by Duncan's multiple range tests at $P \leq 0.05$.

Results

Spore production

Tr6 produced a substantial number of spores per gram dry weight (gdw) of biomass in all media at the end of the incubation period. There was a significant difference between them ($P \leq 0.05$), except between Mol. M. and Mol. media (Table 1). The maximum number of spores (2.50×1^{10}/gdw) was produced in the Mol. C.

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medium and the minimum (0.96×10^{10}/gdw) in Mol. C. G. medium (Table 1).

Table 1 Spore production by *Trichoderma harzianum* Tr6 in various liquid media.

Name of media	Spore ($\times 10^{10}$ /gdw)[1]
Mol. C. N. G.	1.62 ± 0.01 b
Mol. C. G.	0.96 ± 0.06 g
Mol. C.	2.50 ± 0.10 a
Mol. Y.	1.27 ± 0.01 e
Mol. S.	1.40 ± 0.10 d
Mol. B.	1.10 ± 0.01 f
Mol. M.	1.51 ± 0.03 c
Mol.	1.49 ± 0.02 cd

Mol = Sugar beet molasses; C = Corn steep liquor; N = NaCl; G = Glucose; Y = Yeast extract; S = Soybean extract; B = Baker's yeast and M = Malt extract.
[1] Values followed by the same letters are not significantly different (Duncan test, $P \leq 0.05$).

In vitro antagonistic activity surveys

The results showed that all the media affected the mycelial growth and antagonistic efficacy (inhibition and colonization) of Tr6 against *P. drechsleri,* as one of the most damaging pathogens on cucumber, in different scales. In dual culture experiments on P. D. A., all of the treatments could inhibit the pathogen's growth within 72 h. Mol. M. medium with 68.43% had the most effect on inhibition (Fig. 1) but was not different from other treatments in terms of colonization and growth at 5% level. The final product from Mol. S. medium showed the most radial growth rate (Fig. 2) and colonization on the colony of *P. drechsleri* (Fig. 3).

Greenhouse surveys

Data of this investigation indicated that biomass of Tr6 obtained from various media had positive effects on controlling cucumber damping-off caused by *P. drechsleri*. However, they could not completely control it. Mol. M. and Mol. B. media with 58.33% disease reduction were the most effective, although there was no significant difference between them with other treatments except for the negative control (Fig. 4). According to the presented results in table 2, Mol. Y. medium had the most effect on plant growth factors. The average shoot length and root height in plants treated with Tr6 grown in Mol. Y.medium were 34.67 cm and 4.6 cm, respectively, while for control treatment, these values were 14.9 cm and 1.77 cm.

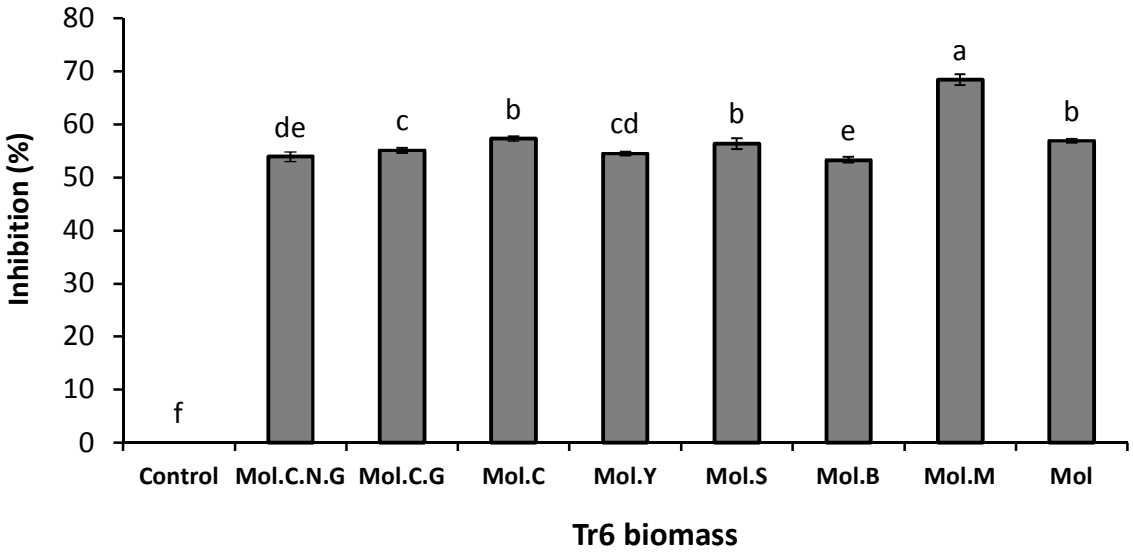

Figure 1 The effect of culture media on the inhibitory potential of *Trichoderma harzianum* TR6 against *Phytophthora drechsleri* in dual culture, after 72 h. Bars with the same letter are not significantly different (Duncan test, $P \leq 0.05$).

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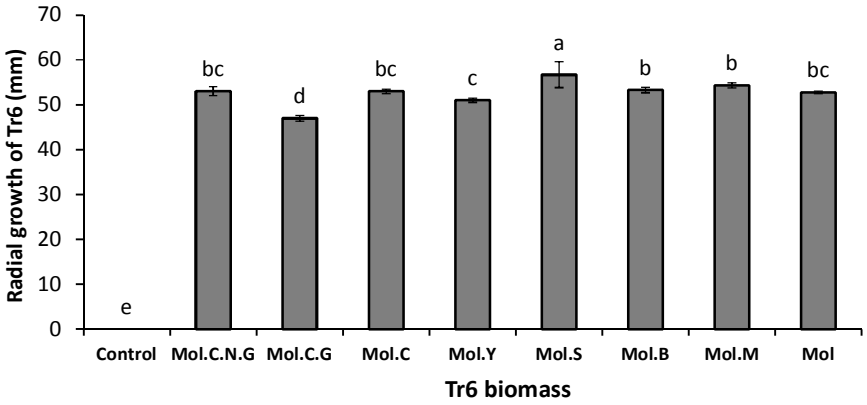

Figure 2 Effect of media on radial growth of *Trichoderma harzianum* Tr6 in dual culture, after 72 h. Bars with the same letters are not significantly different (Duncan test, $P \leq 0.05$).

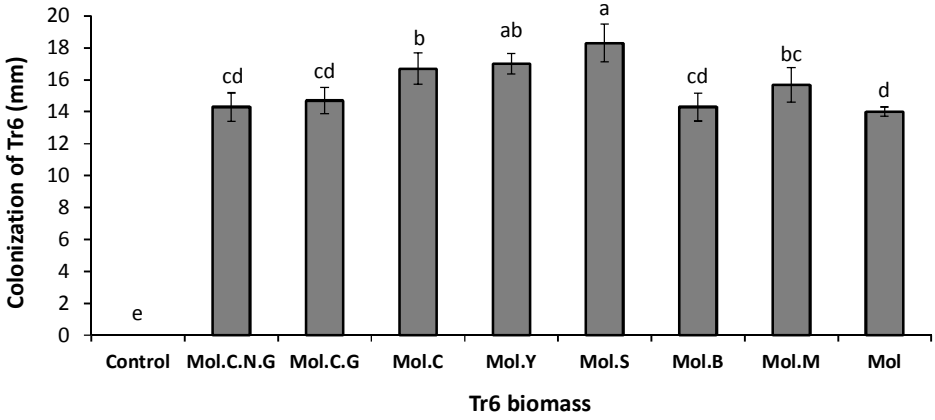

Figure 3 Effect of media on the colonization of *Trichoderma harzianum* Tr6 in dual culture, after 72 h. Bars with the same letters are not significantly different (Duncan test, $P \leq 0.05$).

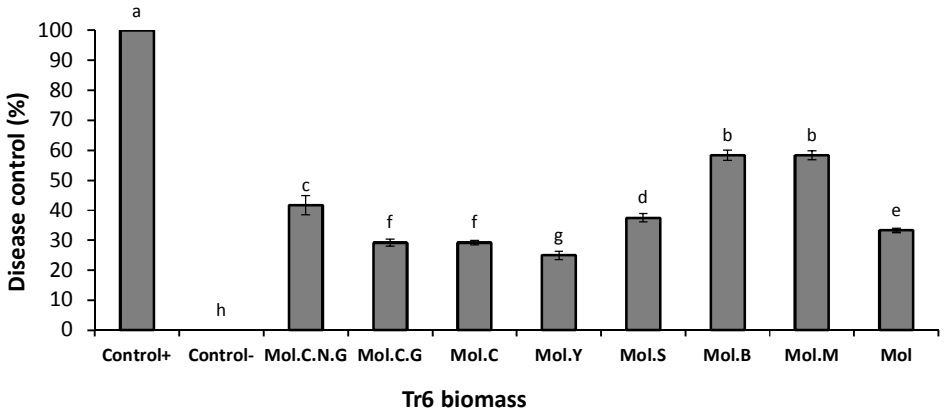

Figure 4 Control of phytophthora damping-off of cucumber using *Trichoderma* isolate grown in the various growth media. Bars with the same letters are not significantly different (Duncan test, $P \leq 0.05$).

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Table 2 Effect of *Trichoderma harzianum* Tr6 biomass on the growth parameters of the cucumber plant.

Tr6 biomass derived from media	Lenght of shoot (cm)	Lenght of root (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)
Control	14.90 ± 0.10 g	1.77 ± 0.25 d	1.16 ± 0.22 e	0.41 ± 0.10 e
Mol. C. N. G.	14.83 ± 0.27 g	2.13 ± 0.32 cd	1.42 ± 0.07 e	0.54 ± 0.07 de
Mol. C. G.	16.17 ± 0.61f g	2.47 ± 0.25 c	1.38 ± 0.01 e	0.65 ± 0.05 cd
Mol. C.	32.60 ± 0.53 b	3.06 ± 0.05 b	5.15 ± 0.05 b	0.85 ± 0.05 a
Mol. Y.	34.67 ± 0.04 a	4.60 ± 0.36 a	6.83 ± 1.30 a	0.93 ± 0.11 a
Mol. S.	25.10 ± 0.50 e	2.57 ± 0.40 c	3.00 ± 0.21 d	0.70 ± 0.02 bc
Mol. B.	29.70 ± 0.75 c	2.30 ± 0.26 c	4.03 ± 0.05 c	0.83 ± 0.08 ab
Mol. M.	27.70 ± 0.93 d	2.30 ± 0.20 c	4.08 ± 0.29 c	0.55 ± 0.07 de
Mol.	17.23 ± 0.45 f	2.50 ± 0.20 c	1.84 ± 0.31 e	0.58 ± 0.09 cd

Mol = Sugar beet molasses; C = Corn steep liquor; N = NaCl; G = Glucose; Y = Yeast extract; S = Soybean extract; B = Baker's yeast and M = Malt extract.
** Values followed by the same letters are not significantly different (Duncan test, $P \leq 0.05$).

Furthermore, the maximum fresh weight of shoot and root was related to Mol. Y. medium's final product. In total, the results obtained from this study indicated that Mol. C. medium for spore production and Mol. M. and Mol. B. media for disease control were superior to the others. However, the results of laboratory assays were different, as Mol. M. medium had the most influence on antagonistic activity of Tr6 in dual culture test. Although Mol. M. medium had no superlative effect on plant growth factors, it had a better effect than other treatments except Mol. Y. medium.

The second stage of trials for optimum medium selection

Generally, the essential aspects of mass production of biocontrol agent are maximum biomass production of Tr6, while maintaining biocontrol activity. Therefore, this stage was carried out to select optimum culture medium in high spore production and effective control of disease as a supplementary stage done according to the previous experiments. For this purpose, Mol. M., Mol. B., Mol. C., and Mol. C. B. M. media were used. According to the results, Mol. C. medium followed by Mol. C. B. M. (with 1.83×10^{10} spore/gdw) and Mol. M. (with 1.65×10^{10} spore/gdw) had the highest spore production though there was no significant difference between the Mol. C. B. M. and Mol. M. media (Fig. 5). Furthermore, Mol. C. B. M. medium reduced disease by 62.5%, but this reduction was not significantly different from Mol. M. and Mol. B. media (Fig. 6). Therefore, Mol. M. medium had the most influence on the growth and biocontrol performance of Tr6.

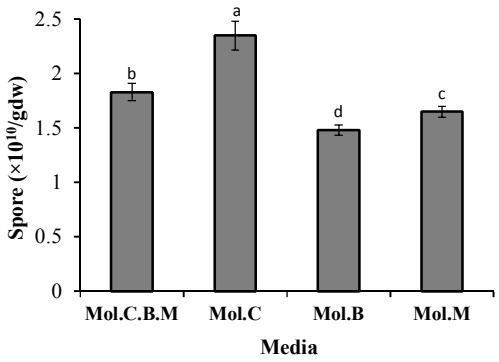

Figure 5 Spore production by *Trichoderma harzianum* Tr6 in the selective liquid media. Bars with the same letters are not significantly different (Duncan test, $P \leq 0.05$)

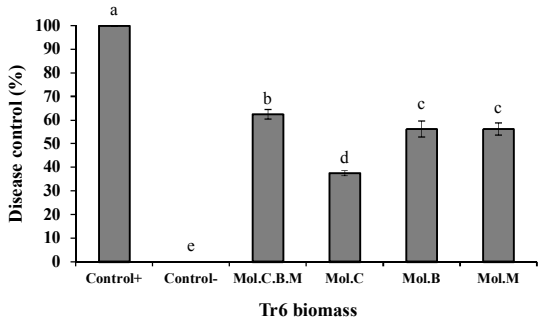

Figure 6 Control of phytophthora damping-off of cucumber by *Trichoderma* isolate grown in the selective media. Values followed by the same letters are not significantly different (Duncan test, $P \leq 0.05$).

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Discussion

Trichoderma spp. develop under different environmental and nutrient conditions. This potency facilitates their mass production *in vitro* conditions on various low-cost substrates. The present study suggests that different carbon and nitrogen sources of the medium affect growth and sporulation and the efficacy of Tr6 inoculum as a biocontrol agent against *P. drechsleri*. Similar findings were also observed in other studies (Monga, 2001; Said, 2009; Kobori *et al.,* 2015; Rai and Tewari, 2016; Reaz Mahamud, 2019). The increment of spore production in different media was in agreement with the findings obtained by some researchers (Chaudhari *et al.,* 2011; Khandelwal *et al.,* 2012; Onilude *et al.,* 2013; Emerson and Mikunthan, 2015; Kobori *et al.,* 2015; Reaz Mahamud, 2019). Different liquid media were used for *Trichoderma* spp., and maximum biomass was produced in a short time inappropriate liquid media. The substrate is important, and usually, agricultural or industrial waste is considered an economic resource. Mass scale production of *Trichoderma* would have great potential for commercial application. There is abundant literature on using conventional synthetic media like glucose, cellulose, soluble starch, and molasses to produce *Trichoderma.* However, the cost of these raw materials for biocontrol agents' commercial production is a major limitation. Generally, biocontrol agents are mass-produced by solid fermentation technology. Some of the disadvantages of this method are the high volume of the substrate required, contamination during fermentation, and the long time required for fermentation. On the contrary, liquid fermentation will help in the mass production of microorganisms under axenic conditions within a short time. *T. harzianum*, a proven biocontrol agent against soil-borne plant pathogens, was mass multiplied using molasses as substrate. Use of inexpensive substrates such as molasses helps reduce the cost of production of biomass. In this work, low-cost and availability of materials were the mainsprings for the selection of media. Our results indicate that the highest spore yield of Tr6 was obtained in Mol. C. medium. Previously, this medium supplemented with NaCl has been used for abundant production of conidia in *Aspergillus* and *Fusarium* spp (Vezina *et al.,* 1965). Carbon is one of the most important factors for the development of mycelial growth of *Trichoderma* spp. Agosin *et al.* (1997) demonstrated that limitation of carbon appeared to trigger cell differentiation in *T. harzianum* strain P1 (Agosin *et al.,* 1997), and the most productive of spores also occurs in nitrogen shortage condition (Thomas *et al.,* 2013; Mascarin and Jaronski, 2016; Mishra et al., 2016). The composition of sugar beet molasses include water, sucrose (mainly), fructose, raffinose and, acids. Corn steep liquor is rich in carbohydrates (lactose, starch, and glucose) and contains nitrogen, vitamins, and minerals. Therefore, less nitrogen and more carbon in this medium result in greater spores by antagonist isolate. Indeed, Tr6 produced poor spores when grown on the medium Mol. C. G. (Table 1) and increasing glucose concentration did not affect sporulation than other media. This result was contrary to several authors' findings (Agosin *et al.,* 1997; Monga, 2001; Said, 2009). The main reason for this different observation is the difference between our cultural conditions and other studies. Also, in the second stage, malt extract and baker's yeast did not positively affect sporulation in the medium Mol. C. B. M. This result indicates that suitable conditions for vegetative growth reduce spore generation. However, it is better in terms of sporulation in comparison with other treatments. This outcome indicates that different cultures and growth media showed variability in biomass production of the biocontrol agents. This ability has been suggested as the main reason for the ubiquitous nature of *Trichoderma. In vitro* surveys indicated that all media affect antagonistic activity, consistent with previous findings (Mustafa *et al.,* 2009). The results of our experiments about the effect of growth media on the efficacy of Tr6 to control phytophthora damping-off of cucumber indicate

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that the nutrition sources and its composition can affect the performance of Tr6 for controlling some plant pathogens. *T. harzianum* protected the seedlings from damping-off disease. Subash *et al.* (2014) showed that *T. harzianum* has grown in sugarcane bagasse, and talcum powder protected the seedlings from the damping-off disease in chili plants. As demonstrated in this study, the positive effect of media on antagonists' ability to increase plant growth factors follows Subash *et al.* (2014). The success of using *Trichoderma* as biocontrol agents is dependent on complex tripartite interactions between plants, pathogens, and *Trichoderma* (Hanhong, 2011). *Trichoderma* spp. are being used in reasonably large quantities in agriculture, both for plant disease control and yield increases (Harman, 2006). *Trichoderma* can protect plants against various plant pathogens using various mechanisms like mycoparasitism of plant pathogens, induction of resistance mechanisms in plants (Vinale *et al.,* 2008; Shoresh *et al.,* 2010), siderophores production (Qi and Zhao, 2013). Therefore, these fungi have been applied to a wide range of plant species for growth enhancement, positively affecting plant weight, crop yields, and disease control. For example, Alizadeh *et al.* (2013) studied the effectiveness of combining these biocontrol agents on induced resistance in cucumber and the model plant *Arabidopsis thaliana*. The enhanced protection in cucumber by the combination of *T. harzianum* Tr6 and *Pseudomonas* sp. Ps14 is most likely due to the two biocontrol agents' activation of different signaling pathways (Alizadeh *et al.,* 2013). Also, *Trichoderma* species can improve plant growth through mechanisms such as auxins production (Björkman, 2004; Contreras-Cornejo *et al.,* 2009), solubilization of several plant nutrients (Li *et al.,* 2015), and reduced ethylene production through decreased 1-aminocyclopropane-carboxylic acid (A. C. C.) (Gravel *et al.,* 2007). These features of *Trichoderma* also depend on environmental factors, especially nutrition source (growth medium). Also, the effect of nutrients in increasing the efficiency of antagonistic metabolites production can be investigated in the biological control surveys. Due to these results and reported positive features of the isolate such as resistance induction, antibiosis, and control of various plant pathogens such as *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Alizadeh *et al.,* 2013), *B. cinerea* (Delkhah and Behboudi, 2015), and *P. drechsleri* (Delkhah and Behboudi, 2015) applying low-cost agro-wastes to mass production of Tr6 are suggested. This property might be useful in biocontrol programs of some soil-borne plant pathogens and promote plant growth. Several species of *Trichoderma* positively affect plants by stimulating plant growth and protecting them from pathogens.

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بهبود کارآیی بیوکنترلی *Trichoderma harzianum* Tr6 در برابر *Phytophthora drechsleri*، عامل بیماری مرگ گیاهچه در گیاه خیار

ژیلا دلخواه و کیوان بهبودی*

گروه گیاه‌پزشکی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: behbodi@ut.ac.ir
دریافت: ۲۳ تیر ۱۳۹۹؛ پذیرش: ۲۹ فروردین ۱۴۰۰

چکیده: عوامل بیوکنترل پاسخ رشدی و بیوکنترلی مختلفی تحت تأثیر عوامل فیزیکوشیمیایی دارند. محیط کشت یکی از این عوامل است. در این مطالعه تأثیر هشت محیط کشت بر رشد و کارآیی آنتاگونیستی Tr6 *Trichoderma harzianum* در برابر *Phytophthora drechsleri* بررسی شد. یک میلی‌لیتر از سوسپانسیون اسپور Tr6 حاوی 10^8 اسپور در این محیط‌های کشت رشد داده شد. pH محیط‌ها در ۵/۵ تنظیم شد. تیمارها تحت شرایط شدت نوری ۲۰۶ لوکس و تکان ۱۳۰ دور در دقیقه در دمای ۲۸ °C به‌مدت ۱۰ روز نگه‌داری شدند. اسپورزایی بیشینه ($2/5 \times 10^{10}$ اسپور در گرم وزن خشک) در محیط کشت Mol. C. (ملاس چغندرقند و شربت ذرت) مشاهده شد. بیش‌ترین بازدارندگی از بیمارگر مربوط به محیط کشت Mol. M. (ملاس چغندرقند و عصاره مالت) بود. Mol. M و Mol. B. (ملاس چغندرقند و مخمر نان) بیش‌ترین تأثیر را در کاهش بیماری (۵۸/۳۳٪) داشتند. Mol. M تأثیر فوق‌العاده‌ای روی عوامل رشدی گیاه نداشت اما تأثیر بهتری در مقایسه با تیمارهای دیگر به‌جز Mol. Y. (ملاس چغندرقند و عصاره مخمر) داشت. مرحله دوم برای انتخاب مطلوب‌ترین محیط کشت از نظر اسپورزایی بالا، کنترل مؤثر بیماری و توسعه رشد گیاه به‌طور هم‌زمان انجام شد. برای این‌منظور، محیط‌های کشت Mol. M.، Mol. B.، Mol. C. و Mol. C. B. M. استفاده شدند و Mol. M به‌عنوان محیط کشت مؤثر بر رشد و کارآیی بیوکنترلی Tr6 نشان داده شد. Mol. C، Mol. C. B. M و Mol. M با $2/35 \times 10^{10}$، $1/83 \times 10^{10}$ و $1/65 \times 10^{10}$ اسپور در گرم وزن خشک به‌ترتیب بیش‌ترین تولید اسپور را داشتند. هیچ تفاوت معنی‌داری بین Mol. C. B. M. و Mol. M. وجود نداشت. علاوه بر این، Mol. C. B. M بیماری را به‌میزان ۶۲/۵٪ کاهش داد اما این کاهش به‌طور معنی‌داری متفاوت از Mol. M و Mol. B. نبود. بنابراین، Mol. M، محیط کشت مؤثری بر رشد و کارآیی بیوکنترلی Tr6 است.

واژگان کلیدی: آنتاگونیست، بیوکنترل، *Phytophthora drechsleri*، تولید انبوه، *Trichoderma*

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