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Screening and evaluation of herbicidal metabolites produced by *Trichoderma* spp.

Wenfeng Kuang*, Chengfan Wang and Weili Mao

Shanghai W.L.H. Bio-tech Corp., 351 Guoshoujing Road, Building 1, Room620, Zhangjiang Hi-tech Park, Pudong District, Shanghai, China.

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Laboratory bioassays were conducted to screen and evaluate the potential herbicidal metabolites produced by *Trichoderma.longibrachiatum* (Tr673), *T. harzianum* (Tr319), *T. viride* (Tr347) *T. koningii* (Tr324) and *T. asperellum* (Tr85) on inhibiting seed germination (SGe) and shoot/root growth (SGr/RGr) of seedlings of *Porslane (Portulaca oleracea), Barnyardgrass (Echinochloa crus-galli), and Amaranthus retroflexus L*. The culture filtrates generated from fermented liquids with each of the *Trichoderma* strains were diluted into 10 and 20% by adding sterilized water, which was then applied onto the weed seeds individually. The IR (%) of SGr/RGr of seedlings were significantly higher ($P \leq 0.05$) in the treatments with Tr673 culture filtrate generated from potato dextrose broth (PDB) for all treated weed seeds compared to those in the treatments generated from other culture media with Tr673 or Tr347. The IR (%) on SGe and SGr/RGr of seedlings in the treatment with Tr673 culture filtrate generated from PDB + 0.4% sodium glutamate were significantly higher ($P \leq 0.05$) than those generated from PDB alone. The result of the bioassay with the crude extract (62.5 $mg \cdot L^{-1}$) generated from Tr673 culture filtrate showed a similar effect on decreasing the SGr/RGr rates (%) of the weeds and cucumber compared to those with the chemical herbicide-glyphosate.

Key words: *Trichoderma,* herbicidal activity, metabolites, inhibiting, crude extract.

INTRODUCTION

About 842 million people or around 14% of the world's population suffered from chronic hunger due to not getting enough food to conduct an active life from 2011 to 2013 (FAO, 2013). Weed is one of the most serious causes of economic losses in agricultural production. Losses of agriculture caused by weeds are about 5 to 10% in developed countries, while losses can be up to 20 to 30% in developing or emerging countries (FAO, 2006). *Portulaca oleracea, Amaranthus retroflexus* and *Echinochloa crus-galli* are the world's worst weeds (Holm et al., 1991).

Due to lack of labor for weeding and other economic reasons, the use of chemical herbicides has been increased and herbicides have made great contribution for farming on weed control and increasing yields of food production. However, it has become a serious

*Corresponding author. E-mail: kuangzou@hotmail.com.

problem that repeated use of the same chemicals, or chemicals with the same mode of action would lead to the selection and buildup of resistant pest populations (Carey et al., 1995; Daou and Talbert, 1999). We are facing the challenges on weed control with synthetic agro-chemicals such as emergence of weeds resistant to herbicides (Yuan et al., 2007; Llewellyn et al., 2009), and concerns about the side effects caused by herbicides in food, soil, ground water and atmosphere (Rial-Otero et al., 2005).

The use of microbes for pest management is one of the most effective biocontrol strategies in agriculture (Ahmad et al., 2011). Natural herbicides are eco-friendly, biodegradable, and less toxic to plants and beneficial microorganisms. They are biosynthesized through specialized pathways and exhibit a wide range of biocontrol activities (Hanson, 2003). Fungi are known to produce diverse active metabolites including herbicidal metabolites (Saxena and Pandey, 2001). It has been one of the most important research areas in weed management to screen and evaluate herbicidal metabolites from fungal products. Fungi such as *Alternaria, Fusarium, Coletotrichum*, etc. can produce phytotoxin (Andolfi et al., 2005; Pedras et al., 2009; Junko and Kenji, 1995). Culture medium is an important influence factor of herbicidal activities, and different carbon and nitrogen sources have different effects on the herbicidal activity of these fungi (Eduardo et al., 2013).

Trichoderma may act as symbionts of plants and have been studied as biopesticides and biofertilizers due to their abilities to protect crops from weeds and promote vegetative growth (Hill et al., 1995; Harman, 2000, 2006). *Trichoderma* spp. is a well-known producers of secondary metabolites with different biological activities, not only potential antibiotics, but also mycotoxins and more than 100 metabolites with antibiotic activities such as pyrones, terpenes and metabolites derived from amino acids and polypeptides (Leclerc et al., 1998; Mukherjee et al., 2012). However, *Trichoderma* products for weed control are still commercially limited and few known studies in this area are restricted to *T. virens* only (Hutchinson, 1999; Heraux et al., 2005 a, b). In this study, we designed to screen and evaluate the herbicidal activities of the culture filtrates produced by *Trichoderma longibrachiatum* (Tr673), *T. harzianum* (Tr319), *T. viride* (Tr347), *T. koningii* (Tr324) and *T. asperellum* (Tr85).

MATERIALS AND METHODS

Trichoderma strains and seeds of weeds and cucumber

The strains of Tr673 (*T. longibrachiatum*), Tr319 (*T. harzianum*), Tr347 (*T. viride*), Tr324 (*T. koningii*) and Tr85 (*T. asperellum*) used in this study were provided by Shanghai WanLiHua BioTech Company. Unless otherwise noted, all the strains were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit) at 4°C. Weed seeds of *P. oleracea, A. retroflexus* L and *B. campestris* L. and cucumber (BiYu 2) were purchased from agricultural markets.

Medium and stock solution

1. Potato Dextrose Broth (PDB, $g \cdot L^{-1}$): 20 potato-powder, 20 dextrose, 3 K_2HPO_4, 1.5 $MgSO_4$ and 0.005 to 0.01 VB_1).
2. Molasses yeast-extract medium (MYM, $g \cdot L^{-1}$): 30 molasses, 10 yeast extract.
3. Synthetic medium (SM, $g \cdot L^{-1}$): $MgSO_4$ 0.2; KCL 0.15, KH_2PO_4 0.9; NH_4NO_3 3.0; dextrose
4. Sabouraud's dextrose broth (SDB, $g \cdot L^{-1}$): Dextrose 40, mycological peptone 10.
5. Stock solution of glyphosate (62.5 $mg \cdot L^{-1}$): 30% glyphosate aqueous solution (Wynca Group) glyphosate 62.5 µl and SDW 300 ml.

Preparation of culture filtrates

Plugs (5-mm) of each *Trichoderma* strain collected from actively growing margins of PDA cultures were transferred into 1.0 L flasks containing 200 ml of PDB, MYM, SM and SDB. Inoculated flasks were put onto a shaking incubator at 28°C and 200 r/min for 6 days. The culture filtrate was individually filtrated and the pH of the culture filtrates were adjusted to 7.0 by adding 0.5 $mol \cdot L^{-1}$ NaOH or 0.5 $mol \cdot L^{-1}$ HCl. To obtain the cell free supernatants, the culture filtrates were set on a centrifuge at 10,000×g for 15 min at 4ºC and then passed through a 0.2 µm filter (Sartorius) separately. The 10 and 20% diluted culture filtrates were made by adding SDW.

Laboratory bioassays

Screening of herbicidal metabolites generated from Trichoderma culture filtrates

To screen the herbicidal activities of the culture filtrates generated from each of the *Trichoderma* strains, *in vitro* bioassays were conducted according to the filter paper method described by Deba et al. (2007) and Xu et al. (2009). Seeds of *P. oleracea, A. retroflexus* L, and *B. campestris* L were immersed in 1% sodium hypochlorite for 10 min followed by 3 times of washings with SDW for surface sterilization. A total of 20 seeds of each of the weeds were placed into Petri plates (9-cm) with two layers of filter papers (Whatman No. 1) on the bottom. For the test of inhibiting seed germination (SGe), the filter papers were wetted with 5 ml of 10 and 20% of each of the culture filtrates, respectively. For the control treatment, the filter papers were wetted with 5 ml of sterilized PDB.

Each of the treatments has 3 replicates. The Petri dishes with treated seeds were put into a growth chamber at 25°C with a 12-h photoperiod for 7 days. Data of inhibiting rate (IR, %) of SGe was recorded for the total viable seeds.

The IR (%) of SGe was calculated as follows:

$$\text{The IR (\%) of SGe} = 100 - [(N^*\text{-treatment}/N\text{-control}) \times 100] \quad (1)$$

Where N*-treatment is the number of germinated seeds of different treatments; N-control is the number of germinated seeds in the control.

For the test of inhibition shoot/root growth (SGr/RGr) of seedlings, the filter papers of all Petri dishes were wetted with 5 ml of SDW first, after all the seeds geminated, the filter papers were again wetted with 5 ml of 10 and 20% of each of the culture filtrates, respectively. For the control treatments of chemical and

blank, 5 ml of glyphosate stock solution and SDW were added, respectively. Data of IR (%) of SGr/RGr of seedlings was collected 5 days after the culture filtrates were applied.

The IR (%) of SGr/RGr of seedlings was calculated as follows:

The IR (%) of SGr/RGr of seedlings = 128100 - [(L*-treatment/l-control)×100] (2)

L*-treatment is the average of the root/shoot length of 15 seedlings picked randomly from each of the Petri dishes of different treatments. L-control is the average of the root/shoot length of 15 seedlings picked randomly from each of the Petri dishes of control treatment.

Screening of a culture medium for optimal production of herbicidal metabolites

In order to screen a medium for optimal production of herbicidal metabolites, PDB, MYM, SM and SDB were prepared and inoculated with Tr673 and Tr347. As described previously under the preparation of culture filtrates, 10% diluted culture filtrates generated from each of the growth media were separately prepared and applied onto the seeds of *A. retroflexus.* Data of IR (%) of SGr/RGr of seedlings was recorded 5 days after the seeds were treated.

For optimal production of herbicidal metabolites, a single-variable optimization strategy utilized to determine the C-source and N-source in the culture media with Tr673. To screen the C- source, 2% of soluble starch, sucrose, lactose, glycerol, xylose or maltose were separately used to replace the glucose in original PDB. After finalizing the C-source, 0.4% of amino acids of tryptone, arginine, glycine, sodium glutamate, urea or aspartic acid were separately added into PDB with a selected C-source. As described previously under the preparation of culture filtrates, 10% diluted culture filtrate was separately prepared and applied onto the seeds of *A. retroflexus* L. Data of IR (%) of SGr/RGr of seedlings were recorded 5 days after the seeds were treated.

Test of the herbicidal activity with Tr673 crude extract

The crude extract of Tr673 was obtained by utilizing macroporous adsorption method. The conditions for adsorption of macroporous adsorption resin HZ806 (Shanghai Huazhen Sci. & Tech. Co., Ltd) were a processing of volume as 32 bed volumes (BV), pH value of 4, and flow rate of 2 BV/h; and those for desorption of HZ806 were a 50:50 (*v*/*v*) ratio of ethanol to water. The crude extracts were finally obtained by adding 50% of ethanol elution and a process of vacuum distillation.

The dried crude extract (5 g) was dissolved with 10 ml of acetone/water (50:50, *v*/*v*) and the solution was then diluted into the concentrations of 7.8, 15.6, 31.3, 62.5, 125 and 250 $mg.mL^{-1}$ with appropriate amount of SDW. Sterilization of the extracts was made by passing the solutions separately through a 0.2 µm filter (Sartorius). As described previously under screening of herbicidal metabolites generated from Trichoderma culture filtrates, an *in vitro* bioassay was conducted with the weeds of *P. oleracea, E. crus-galli, A. retroflexus* L, *B. campestris* L. and cucumber, 5 ml of each of diluted crude extract solutions and stock solution of glyphosate were added into each of the correct Petri dishes. Five days after seed treating, the data were collected.

Statistical analysis

A completely randomized design was used for all of the bioassays and the data was analyzed by ANOVA (analysis of variance). The common difference of the treatments was considered to be significant at the 5% level ($P \leq 0.05$).

RESULTS

Screening of herbicidal metabolites with *Trichoderma* culture filtrates

The results of the bioassay for screening and evaluation of the herbicidal metabolites (Table 1) showed that there were no significant differences on the inhibitory rates (IR%) of seed germination (SGe) among the treatments with 10 and 20%, respectively of diluted culture filtrates generated from the strains ofTr324, Tr85, Tr319, Tr347 and the SDW control, but the parameters of the IR (%) of SGe in each of these treatments were significantly lower ($P \leq 0.05$) than those in the treatment with the strain of Tr673. The IR (%) of shoot/root growth (SGr/RGr) of seedlings in the treatment with 10 and 20%, respectively of diluted culture filtrates generated from the strains of Tr347 were significantly higher ($P \leq 0.05$) than those in the treatments with the strains of Tr324, Tr85 and Tr319, but the parameters of the IR (%) of SGr/RGr of seedlings in each of these treatments were significantly lower ($P \leq 0.05$) than those in the treatment with the strain of Tr673.

Screening of a culture medium for optimal production of herbicidal metabolites

To screen a culture medium for optimal of herbicidal metabolites, 4 media of PDB, PYM, SM and SDB were tested. The results indicated (Figure 1) that the IR (%) of SGr/RGr of seedlings were significantly higher ($P \leq 0.05$) in the treatments with the culture mediainoculated with Tr673 for all treated weed seeds compared to those inoculated with Tr347, except in the treatments with the culture media of SM on *A. retroflexus*; SM, MYM, SDB on *E. crusgalli*; SM and MYM on *P. oleracea* for SGr, and MYM on *A. retroflexus*; SM, MYM on *E. crusgalli*; SM on *P. oleracea* and SM on *B. campestris* for RGr. There were no significant differences on the IR (%) of SGr/RGr of seedlings between each of the culture media inoculated with Tr673 and Tr347. The IR (%) of SGr/RGr of seedlings were significantly higher ($P \leq 0.05$) in the treatments with PDA inoculated with Tr673 for all treated weed seeds compared to those in the treatments with other culture media inoculated with Tr673 or Tr347. The results of determining the C-source with different culture media showed (Figure 2) that there were no significant differences between the treatments with the C-sources of glucose (original PDB) and sucrose on the IR (%) of SGr of seedlings, but the parameters of the IR (%) of SGr in each of the two treatments were significantly higher ($P \leq 0.05$) than those with each of other C-sources.

Table 1. Effect of herbicidal metabolites generated from *Trichoderma* culture filtrates on inhabiting SGe and RGr/SGr of seedlings of *A. retroflexus*, *P. oleracea* and *B. campestris* L.

Treatment		Inhibitory rate (%)								
		A. retroflexus			*P.oleracea*			*B. campestris* L.		
Strains	**C*(%)**	**SGe**	**RGr**	**SGr**	**SGe**	**RGr**	**SGr**	**SGe**	**RGr**	**SGr**
Blank PDB (Ctrl.)	20	3^{c}	-1.88^{ef}	-1.65^{de}	2.0^{c}	-4.03^{de}	$-.56^{d}$	13^{b}	-1.79^{e}	-4.30^{e}
Tr347-*T. viride*	10	12^{c}	4.38^{d}	2.16^{d}	2.0^{c}	4.40^{c}	4.27^{cd}	13^{b}	32.51^{c}	41.47^{c}
	20	25^{b}	12.69^{c}	10.55^{c}	2.0^{c}	9.19^{c}	8.84^{c}	16^{b}	14.27^{d}	8.39^{d}
Tr324-*T. koningii*	10	10^{c}	-0.12^{de}	1.52^{d}	2.0^{c}	3.27^{cd}	4.38^{cd}	12^{b}	0.56^{e}	-2.57^{e}
	20	9^{c}	0.81^{de}	-3.75^{de}	2.0^{c}	5.50^{c}	5.14^{cd}	14^{b}	$.69^{e}$	1.02^{de}
Tr85-*T. asperellum*	10	4^{c}	-6.11^{f}	-28.15^{f}	2.0^{c}	-5.16^{e}	-7.36^{e}	16^{b}	-2.83^{e}	-4.95^{e}
	20	7^{c}	2.5^{ed}	-5.01^{de}	2.0^{c}	3.51^{cd}	-0.60^{d}	16^{b}	-0.37^{e}	-4.56^{e}
Tr319-*T. harzianum*	10	9^{c}	0.02^{de}	-2.03^{de}	2.0^{c}	4.45^{c}	1.20^{d}	17^{b}	-0.95^{e}	-3.95^{e}
	20	4^{c}	-0.17^{de}	$.25^{de}$	2.0^{c}	5.45^{c}	3.62^{cd}	16^{b}	-0.49^{e}	-7.36^{e}
Tr673-*T .longibrachiatum*	10	26^{b}	34.8^{b}	22.93^{b}	12^{b}	30.10^{b}	30.95^{b}	18^{b}	62.74^{b}	60.83^{b}
	20	46^{a}	80.5^{a}	71.91^{a}	48^{a}	71.31^{a}	68.63^{a}	46^{a}	77.68^{a}	76.75^{a}

According to Duncan's Multiple Range Test ($P \leq 0.05$, n= 5), means with the same letter among the treatments are not significantly different from each other. C*: Concentration.

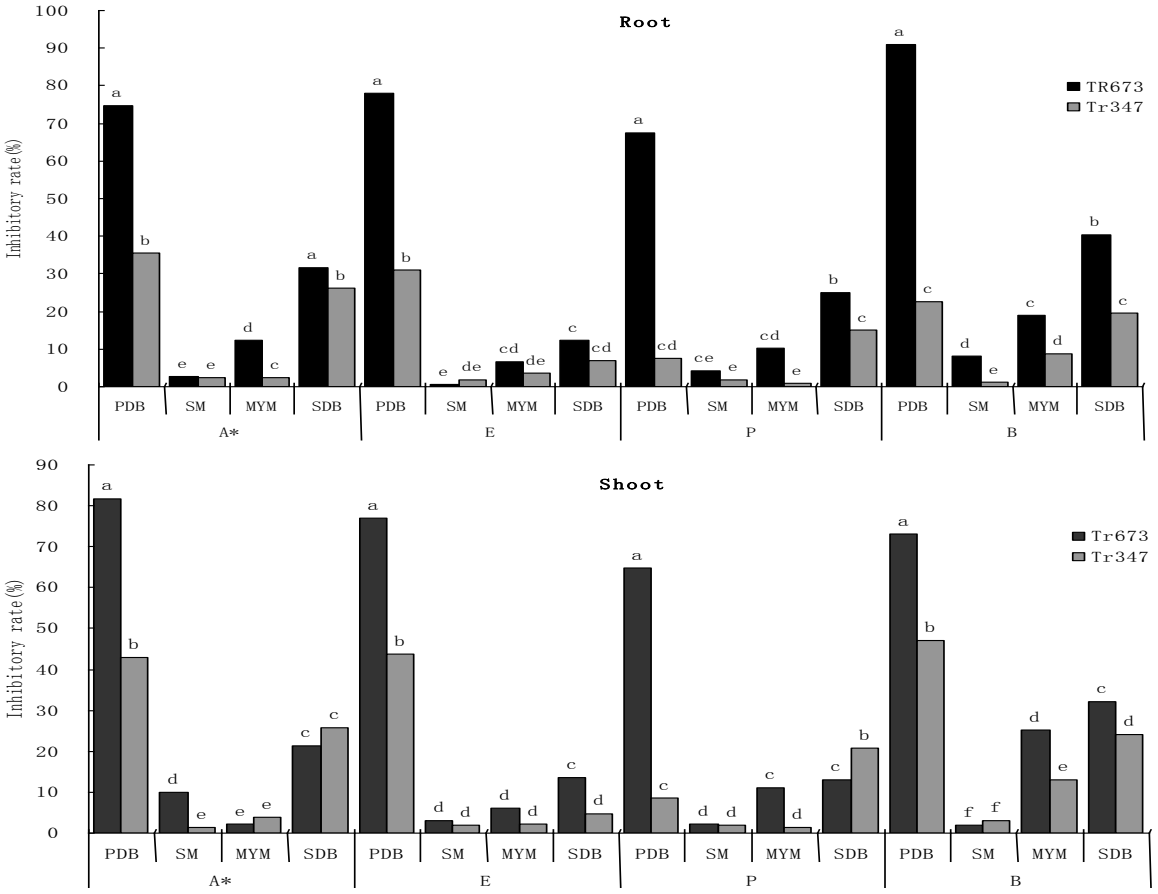

Figure 1. Effect of herbicidal metabolites generated from Tr673 and Tr347 in different culture media on inhabiting root/shoot growth of seedlings. According to Duncan's Multiple Range Test ($P \leq 0.05$, n=5), means with the same letter among the treatments are not significantly different from each other. *: A, *A. retroflexus,* E, *E. crusgalli, P, P. oleracea,* B, *B. campestris.*

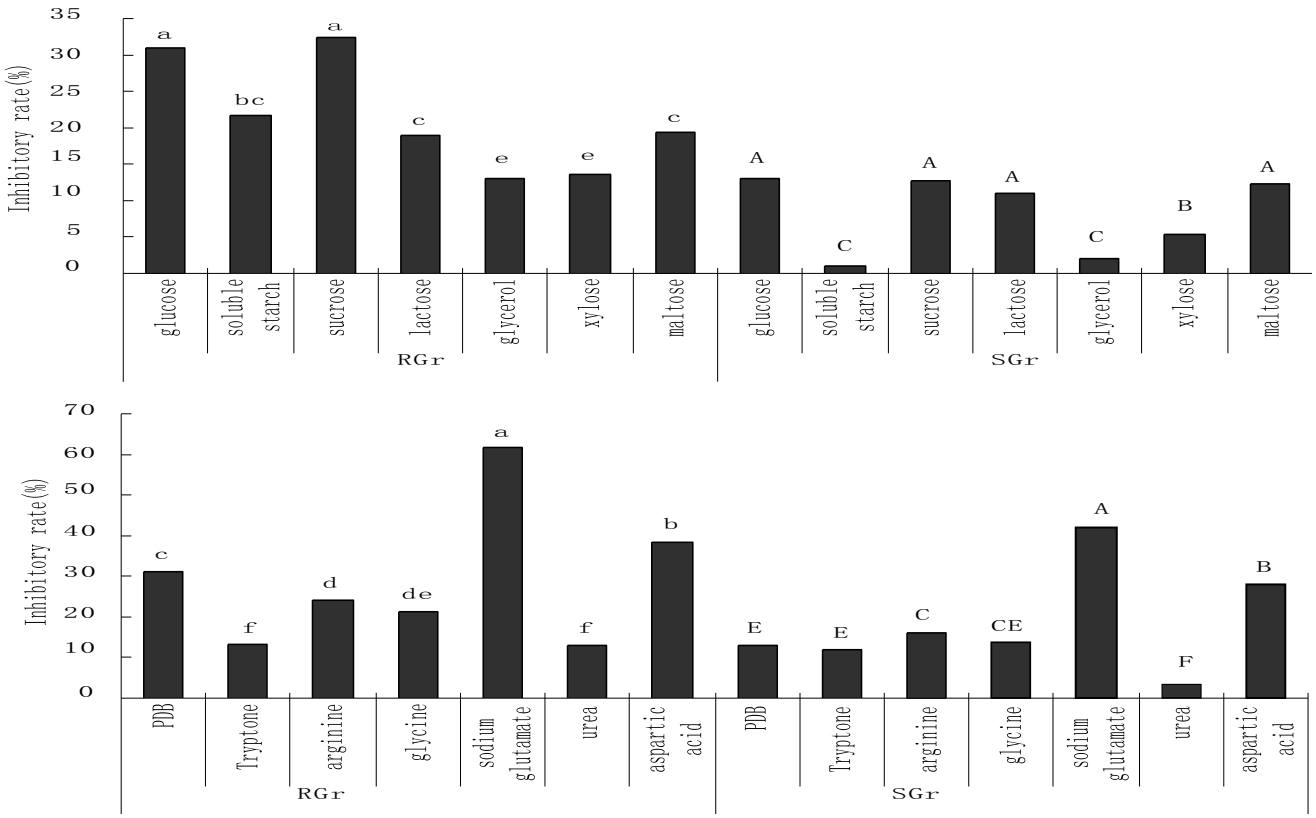

Figure 2. Effect of herbicidal metabolites generated from Tr673 culture filtrates of PDB with different C-sources and N-sources on inhabiting RGr/SGr of seedlings. According to Duncan's Multiple Range Test (P≤ 0.05, n= 5), means with the same letter among the treatments are not significantly different from each other different from each other.

Similarly, there were no significant differences among the treatments with the C-sources of glucose, sucrose, lactose and maltose on the IR (%) of RGr of seedlings, but the parameters of the IR (%) of RGr in these four treatments were significantly higher (*P ≤ 0.05*) than those with soluble starch, glycerol or maltose. The results indicated that glucose is one of the best C-sources used in the culture medium of PDB for optimal production of herbicidal metabolites.

For the test of determining the N-sources added into PDB, the results showed (Figure 2) that 4% of sodium glutamate is the best N-source added into PDB to increase the production of herbicidal metabolites, it had a significantly higher (*P≤ 0.05*) IR (%) on both SGr and RGr of seedlings than any of other individual N-source added into PDB or PDB alone.

Test of the herbicidal activity with Tr673 crude extract

The results of the test with Tr673 crude extract showed that the IR (%) of SGr/RGr of seedlings was significantly higher (*P* ≤ 0.05) in the treatment with diluted solution at the concentration of 125 $mg \cdot L^{-1}$ than those in the treatments with diluted solutions at lower concentrations of 7.8, 15.6, 31.3 and 62.5 $mg \cdot L^{-1}$ and the stock solution of glyphosate, but the parameters of the IR (%) of SGr/RGr of seedlings in this treatment were significantly lower (*P ≤ 0.05*) than those in the treatments with diluted solutions at higher concentrations of 250 and 500 $mg \cdot l^{-1}$ (Table 2).

DISCUSSION

As a biocontrol agent, *Trichoderma* has been extensively used in agriculture worldwide for crop protection due to its antimicrobial activities on suppressing a broad range of phytopathogens (Harman, 2000, 2006). The diverse type of secondary metabolites including gliovirin, gliotoxin, viridian, and viridiol produced by *Trichoderma* spp. has also been demonstrated on pest control (Jones and Hancock, 1987). Harziphilone and fleephilone were used as HIV, regulation of virion expression/Rev response element (RRE) binding inhibitors produced by T. harzianum (Qian-Cutrone et al., 1996). The volatile compound of 6-pentyl-2H-pyran-2-one produced by *T. harzianum* is active to

Table 2. Effect of herbicidal activity of Tr673 crude extract on inhibiting RGr/SGr of seedlings.

Treatment*	Inhibitory rate (%)									
	P. oleracea		*E. crus-galli*		*A. retroflexus* L		*B. campestris* L		*Cucumber*	
	SGr	RGr	SGr	RGr	SGr	RGr	SGr	RGr	SGr	RGr
500	100.0^{a}	100.0^{a}	98.7^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
250	94.3^{b}	100.0^{a}	74.3^{b}	100.0^{a}	97.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	87.7^{b}	100.0^{a}
125	70.7^{c}	87.7^{b}	56.0^{d}	88.0^{b}	82.3^{c}	83.7^{b}	93.0^{b}	92.3^{b}	66.0^{c}	86.3^{b}
62.5	52.0^{d}	65.7^{c}	38.7^{e}	66.0^{d}	44.3^{e}	50.7^{c}	55.0^{c}	78.3^{c}	40.0^{d}	59.7^{c}
31.3	34.3^{f}	40.3^{e}	9.7^{f}	36.0^{e}	25.0^{f}	28.7^{d}	28.7^{d}	47.7^{e}	9.3^{e}	32.7^{d}
15.7	0.0g	10.7^{f}	-0.7g	-0.7^{f}	1.0g	1.0^{e}	1.3^{e}	14.3^{f}	1.7^{f}	10.3^{e}
7.8	-2g	0.7g	-1.3g	-0.3^{f}	0.0g	0.0^{e}	2.0^{e}	-0.7g	-2.7 ^{f}	1.3^{f}
Glyphosate**	44^{e}	49.7^{d}	63.39^{c}	74.7^{c}	53.0 ^{d}	49.7^{c}	54.3^{c}	70.3^{d}	39.3^{d}	61.0^{c}

According to *Duncan's* Multiple Range Test ($P \leq 0.05$, n= 5), means with the same letter among the treatments are not significantly different from each other. *, sample concentration at mg·L^{-1}; **, in a concentration at 62.5 mg·L^{-1}.

suppress *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici* (Scarselletti and Faull, 1994) and viridiol is strongly phytotoxic (Jones and Hancock, 1987). Chicken manure composted with *T. virens* exhibited its herbicidal activity on inhibiting the growth of *Setaria viridis* and *A. retroflexus* in the fields of rye cover crop (Heraux et al., 2005a). In this study, the culture filtrates generated from the strains of TR673 and Tr347exhibited strong herbicidal activities on inhibiting SG and RGr/SGr of seedlings of tested weeds. These findings are in certain agreement with the results of the studies conducted by Hutchinson (1999) and Héraux et al. (2005a, b).

However, it was the first time to report the observation that the culture filtrate produced by Tr673, a strain of *T. longibrachiatum*, exhibited a stronger herbicidal effect on suppressing the growth of tested weeds compared to other *Trichoderma* strains. It also indicates that *T. longibrachiatum* might produce herbicidal compounds, which will be detected in the subsequent studies.

Nusrat et al. (2013) observed that the maximum biomass of *T. harzianum* was produced in PDB, but the minimum was produced in water broth. Harman et al. (1991) reported that maximum fresh and dry weight of biomass was obtained during fermentation process with PDB followed by TSM, Czapek-Dox and MEB. In present study, it was observed that Tr673 culture filtrate generated with PDB showed a significant herbicidal effect on inhibiting RGr/SGr tested weed seedlings compared to those with MYM, SM and SDB. It indicated that PDB is one of the most suitable media for *Trichoderma* spp. to produce secondary metabolites with anti-pest activities. The role of carbon sources added in culture media is to provide energy for the growth of microorganisms and the basic carbon skeleton for microorganisms to synthesize secondary metabolites (Demain, 1986). Glucose and sucrose are monosaccharides and disaccharides respectively, which can be utilized by microorganisms for cell growth and in the processes of metabolisms (Demain, 1986). Leclerc et al. (1998) found that glucose is superior to sucrose as a growth-stimulating carbon source to be utilized by *Trichoderma* strains. They also found that when adding glutamic acid to the culture medium with *T. longibrachiatum*, the production of acidic peptides can be increased. In this study, we found that when 0.4% sodium glutamate was added to the culture medium, the herbicidal activity of Tr673 culture filtrate was markedly increased, which revealed that sodium glutamate might be one of important precursors of the herbicidal metabolites.

It is important to know the characteristics of chemical targets during the process of macroporous adsorption, which include polarity and molecular weight of the targets and the resin properties of polarity, surface area and diameter of pore. Non-polar resins can easily absorb non-polar substances in polar solvents, while polar resins can absorb polar targets from non-polar solvents (Zhang et al., 2011). We found in this study that actively herbicidal metabolites can be collected and enriched since HZ 806 have a large surface area and is matched to the target herbicidal metabolites in polarity. The result of the bioassay indicated that the crude extract had the same herbicidal effect on inhibiting SG and RGr/SGr of the weeds compared with Tr673 culture filtrate and the chemical herbicide of glyphosate.

Trichoderma metabolites, such as IAA and 6 - PP, can significantly promote the growth of the plant in moderate concentration (Vinale et al., 2008). Harman (2006) reported that *Trichoderma* spp. can decrease wilt incidence in chickpea plants and increase root development in numerous of other plants.

Narasimha et al. (2013) reported that seed treatment with *T. asperellum* enhanced the vigour index of tomato seedlings. In this study, we observed that the treatment

with Tr85 culture filtrate did not exhibit herbicidal effect on inhibiting SG and RGr/SGr of the weeds, however it showed a significant effect on increasing SGr of *A. retroflexus.* This indicated that Tr85 might be considered as a strain for plant growth promotion. Nowadays, the genus of *Trichoderma* has a great number of fungal strains that act as biocontrol agents, the antagonistic properties of which are based on the activation of multiple mechanisms (Benitez et al., 2004). The findings described in this study could be considered as a basic knowledge and references for the further studies in this area.

Conflict of Interests

The authors have not declared any conflict of interests.

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