Screening of Fungal Extracts for Weed Germination and Growth Inhibitory Activity

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Abstract

The screening for weed germination and growth inhibitory activity of crude extracts at 35 mg/ml from twenty fungal species was examined in this study. The activity was tested against *Mimosa pigra* Linn. and *Echinochloa crus-galli* [L.] Beauv.. Results indicated that crude extracts from *Aspergillus fischeri* TISTR 3272 and *Asp. usamii* TISTR 3258 showed remarkable germination and growth inhibition on both weeds. *Fusarium poae* TISTR 3321 and *Asp. candidus* TISTR 3268 extracts exhibited excellent weed germination inhibition on *M. pigra* whereas crude extracts from *F. solani* TISTR 3436 and *Asp. niger* TISTR 3274 showed strong germination inhibition only against *E. crus-galli*. All fungal extracts exhibited different degrees of growth inhibitory activity on root of both weeds as compared with the control. The growth inhibitory effects on root were also found to be higher than that on shoot. The results from this study revealed potential fungal species that could be used as a source of natural herbicides in the future.

Keywords : Fungal extracts; Germination inhibitory activity; Growth inhibitory activity

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Introduction

Weeds are one of the most serious causes of economic losses in agricultural production. The exploitation of synthetic herbicides for weed control has been increasing. However, their heavy application in crop fields has resulted in environmental and medical problems. Natural herbicides which are more eco-friendly, biodegradable, and less toxic from plants and microorganisms are needed.

Fungi are well recognized for their ability to produce diverse biologically active metabolites including herbicides (Saxena and Pandey, 2001). Therefore, screening for fungal products with herbicidal activity has been one of the most interested areas in weed management research. Some fungal metabolites are toxic to certain weeds, e.g. maculosin produced by *Alternaria alternata* which is host-specific to spotted knapweed (Steirle et al., 1988). Some are toxic against both monocotyledonous and dicotyledonous weeds, e.g. cornexistin from *Paecilomyces variotii* (Pearce, 1997) and prehelminthosporal from *Helminthosporium sp.* (Pena-Rodriguez et al., 1988). These compounds have been used as candidates in developing eco-friendly herbicides.

In this study, the extracts from twenty fungal species collected at the Thailand Institute of Scientific and Technological Research (TISTR) were tested for their germination and growth inhibitory activity on monocotyledonous and dicotyledonous weeds, *Echinochloa crus-galli* [L.] Beauv. (barnyard grass) *and Mimosa pigra* Linn., respectively. These weeds are commonly found in many agricultural fields in Thailand. The activities of the extracts on both weeds were measured under controlled laboratory conditions. The main objective of this project is to reveal potential fungal species which could have a promising future for their use in weed control.

Materials and methods

Fungi

Twenty fungal species, *Aspergillus candidus* TISTR 3268, *Asp. clavatus* TISTR 3384, *Asp. fischeri* TISTR 3272, *Asp. flavus* TISTR 3366, *Asp. japonicus* TISTR 3261, *Asp. kawachii* TISTR 3194, *Asp. niger* TISTR 3274, *Asp. niveus* TISTR 3262, *Asp. terreus* TISTR 3109, *Asp. usamii* TISTR 3258, *Alternaria alternata* TISTR 3435, *Fusarium moniliforme* TISTR 3175, *F. poae* TISTR 3321, *F. solani* TISTR 3436, *Penicillium citrinum* TISTR 3437, *P. pinophilum* TISTR 3386, *P. striatisporum* TISTR 3358, *Trichoderma aureoviride* TISTR 3330, *T. koningi* TISTR 3331 and *T. viride* TISTR 3161, were obtained from the Thailand Institute of Scientific and Technological Research (TISTR) in live cultures. The cultures were maintained by subculturing on Potato Dextrose Agar (PDA) slants at room temperature in tightly capped culture tubes.

Culture Fermentation and Preparation of Crude Extracts

Two-stage broth fermentation of each culture was used as follows: A spore suspension of each 7- to 10-day-old culture was prepared by pipetting 5 ml of sterile normal saline into a slant culture, and gently agitating the slant with a pipet tip. One milliliter of the suspension was transferred into 100 ml of sterile stage I medium in a 500 ml erlenmeyer flask. The stage I cultures were incubated at ambient temperature (25°C) on a rotary shaker at 250 rpm for 2 days. Approximately, 10 ml of each actively growing stage I culture were transferred to 100 ml of fresh medium in a 500 ml erlenmeyer flask. The stage II cultures were further incubated on a shaker for 5 days. All fermentation experiments were carried out in Sabouraud Dextrose Broth (SDB) for both stages. Aseptic techniques were performed throughout the processes.

600 Ml of each 5-day-old stage II culture broth were chromatographed on a column packed with 50 g of Amberlite XAD-16 resin, using 600 ml of methanol, followed by 300 ml of

acetone as eluents. All methanol and acetone extracts were combined and concentrated to dryness. The extracts at 35 mg/ml in methanol were prepared and further evaluated for their germination and growth inhibitory activity against *E. crus-galli* and *M. pigra.*

Bioassay

Seeds of *E. crus-galli* and *M. pigra* were obtained from National Weed Science Research Institute, Department of Agriculture, Thailand.

For weed germination inhibition assay, 1.5 ml of each extract were pipetted onto 5 cm Petri dishes lined with Whatman # 1 filter paper. After allowing methanol to evaporate, 2.3 ml of distilled water were added to the Petri dishes. In the control, the same amount of methanol was used instead. To each treated dish, 25 seeds were added and the dishes were covered with the lids. All of the Petri dishes were placed in a growth chamber at 30˚C for 5 days. Three replicates were conducted for each extract. The percent germination inhibitory activity was calculated as follows:

Weed germination inhibitory activity (%) = $100 - [(N_{treatment}/N_{control}) \times 100]$

Where $N_{treatment}$ is the number of germinating seeds treated with each fungal extract, and $N_{control}$ is the number of germinating seeds in the control.

For weed growth inhibition assay, 2 ml of each extract were pipetted into glass tubes (23 x 147 mm) containing 1 g of cellulose powder from spruce. After allowing methanol to evaporate in a vacuum chamber for overnight, 3 ml of distilled water were added. Methanol was used as a control. To each tube, 5 seeds were added and the tubes were covered with Para film. All of the tubes were placed in a lit growth chamber at 30˚C. After 5 days, the lengths of root and shoot were measured. Three replicates were conducted for each extract. The percent growth inhibitory activity was calculated as follows:

Weed growth inhibitory activity (%) = $100 - [(L_{treatment}/L_{control}) \times 100]$

Where $L_{treatment}$ is the average of the root (shoot) length of five

seedlings in each tube treated with fungal extracts, and $L_{control}$ is the average of the root (shoot) length of five seedlings in the control.

Results and discussions

The germination inhibitory activity of fungal extracts

Results in Table 1 showed that among the extracts from twenty fungal species, *Asp. fischeri* TISTR 3272 extract showed the strongest germination inhibition (100%) on both weeds and *Asp. usamii* TISTR 3258 extract was the second most effective (92% and 100% inhibition on *E. crus-galli* and *M. pigra*, respectively).

The other three fungal extracts having different degrees of germination inhibition on both weeds as compared with the control were *Asp. candidus* TISTR 3268, *Asp. japonicus* TISTR 3261, and *F. solani* TISTR 3436 extracts. The extract of *Asp. candidus* TISTR 3268 completely inhibited *M. pigra* germination but showed weak inhibition (15%) on *E. crus-galli*. However, the extracts of *F. solani* TISTR 3436 and *Asp. japonicus* TISTR 3261 exhibited stronger germination inhibition on *E. crus-galli* (91% and 63%, respectively) than that on *M. pigra* (61% and 37%, respectively).

F. poae TISTR 3321 and *Asp. flavus* TISTR 3366 extracts were found to have strong germination inhibition only on *M. pigra* (80% and 74%, respectively). On the other hand, the extract of *Asp. niger* TISTR 3274 showed strong germination inhibition (91%) on *E. crus-galli* with no promising inhibition on *M. pigra.*

Other eleven fungal extracts including *Asp. clavatus* TISTR 3384, *Asp. kawachii* TISTR 3194, *Asp. niveus* TISTR 3262, *Asp. terreus* TISTR 3109, *F. moniliforme* TISTR 3175, all three *Penicillium spp.*, and three *Trichoderma spp.* showed very weak or no germination inhibition on both weeds.

The growth inhibitory activity of fungal extracts

The growth inhibitory activity of the fungal extracts on root and shoot elongation was studied. Results in Table 2 showed that all fungal extracts inhibited root growth of both weeds with different

degrees as compared with the control. Seven fungal extracts suppressing root elongation of both weeds over 80% were *Asp. candidus* TISTR 3268, *Asp. fischeri* TISTR 3272, *Asp. japonicus* TISTR 3261, *Asp. niger* TISTR 3274, *Asp. terreus* TISTR 3109, *Asp. usamii* TISTR 3258, and *F. solani* TISTR 3436.

It was also observed that the growth inhibitory activity of all fungal extracts on root was stronger than that on shoot. *Asp. fischeri* TISTR 3272 and *Asp. usamii* TISTR 3258 extracts were the two most effective on growth inhibition of both weeds whereas the extract of *Asp. kawachii* TISTR 3194 was the weakest.

Based on the results of this study, the extracts with remarkable weed growth and germination inhibitory activity were revealed and should be further examined for their efficacy and safety in weed control. Among twenty screened fungal extracts, *Asp. fischeri* TISTR 3272 and *Asp. usamii* TISTR 3258 extracts displayed the strongest inhibitory activity on weed germination and growth of *E. crus-galli* and *M. pigra*. Analysis of active metabolites in these fungi should be further investigated as well.

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Table 1 The germination inhibitory activity (%) of fungal extracts

Fungal extracts at 35 mg/ml	**Germination inhibitory activity (%)*on**	
	***M. pigra* Linn.**	***E.crus-galli* [L.] Beauv.**
Asp. candidus TISTR 3268	100	15 ± 2
Asp. clavatus TISTR 3384	0	0
Asp. fischeri TISTR 3272	100	100
Asp. flavus TISTR 3366	74 ± 11	1 ± 2
Asp. japonicus TISTR 3261	37 ± 7	63 ± 19
Asp. kawachii TISTR 3194	0	0
Asp. niger TISTR 3274	3 ± 6	91 ± 8
Asp. niveus TISTR 3262	0	0
Asp. terreus TISTR 3109	3 ± 5	0
Asp. usamii TISTR 3258	100	92 ± 4
Alt. alternata TISTR 3435	19 ± 3	0
F. moniliforme TISTR 3175	3 ± 6	0
F. poae TISTR 3321	80 ± 4	0
F. solani TISTR 3436	61 ± 6	91 ± 8
P. citrinum TISTR 3437	0	0
P. pinophilum TISTR 3386	0	0
P. striatisporum TISTR 3358	3 ± 5	0
T. aureoviride TISTR 3330	4 ± 4	0
T. koningi TISTR 3331	0	0
T. viride TISTR 3161	3 ± 5	0
Control	0	0

*Data are presented as mean ± SD from three replicates.

Table 2 The growth inhibitory activity (%) of fungal extracts

Fungal extracts at 35 mg/ml	Growth inhibitory activity* (%) on			
	M. pigra Linn.		*E.crus-galli* [L.] Beauv.	
	root	shoot	root	shoot
Asp. candidus TISTR 3268	86 ± 1	45 ± 32	82 ± 11	66 ± 19
Asp. clavatus TISTR 3384	83 ± 1	44 ± 14	56 ± 15	42 ± 28
Asp. fischeri TISTR 3272	85 ± 4	54 ± 30	95 ± 2	73 ± 9
Asp. flavus TISTR 3366	64 ± 14	12 ± 29	75 ± 8	75 ± 3
Asp. japonicus TISTR 3261	87 ± 1	12 ± 12	84 ± 13	77 ± 9
Asp. kawachii TISTR 3194	28 ± 26	-6 ± 16	52 ± 10	10 ± 31
Asp. niger TISTR 3274	82 ± 5	9 ± 21	92 ± 1	73 ± 2
Asp. niveus TISTR 3262	65 ± 20	24 ± 31	85 ± 9	47 ± 6
Asp. terreus TISTR 3109	83 ± 8	26 ± 16	81 ± 2	47 ± 13
Asp. usamii TISTR 3258	89 ± 6	68 ± 27	94 ± 3	89 ± 12
Alt. alternata TISTR 3435	59 ± 11	29 ± 19	83 ± 6	56 ± 19
F. moniliforme TISTR 3175	57 ± 5	8 ± 22	78 ± 1	50 ± 8
F. poae TISTR 3321	57 ± 9	24 ± 18	74 ± 9	45 ± 3
F. solani TISTR 3436	86 ± 4	27 ± 24	90 ± 11	73 ± 22
P. citrinum TISTR 3437	80 ± 4	0 ± 11	75 ± 6	18 ± 6
P. pinophilum TISTR 3386	57 ± 11	-3 ± 14	71 ± 13	55 ± 7
P. striatisporum TISTR 3358	52 ± 14	26 ± 25	76 ± 7	57 ± 15
T. aureoviride TISTR 3330	74 ± 16	49 ± 14	77 ± 8	65 ± 25
T. koningi TISTR 3331	60 ± 9	-2 ± 3	73 ± 4	35 ± 15
T. viride TISTR 3161	70 ± 9	21 ± 16	85 ± 5	74 ± 13
Control	0	0	0	0

*Data are presented as mean ± SD from three replicates.

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