

ALTERNATIVE MANAGEMENT OF A PROBLEMATIC WEED OF WHEAT *Avena fatua* L. BY METABOLITES OF *Trichoderma*

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ABSTRACT

Wild oat (*Avena fatua* L.) is a problematic weed of wheat (*Triticum aestivum* L.) in Pakistan. The present study was designed to evaluate the herbicidal activity of culture filtrates of four *Trichoderma* spp., namely *T. harzianum*, *T. pseudokoningii*, *T. reesei* and *T. viride*, against this weed species. In a laboratory bioassay, original (100%) and diluted (50%) culture filtrates of *T. harzianum* and *T. pseudokoningii* significantly reduced shoot and root growth of *A. fatua* seedlings. Only original culture filtrates of *T. reesei* exhibited significant effects, while the effect of filtrates of *T. viride* was insignificant against shoot and root growth of the target weed species. Generally, original concentrations of culture filtrates of all *Trichoderma* spp., except *T. harzianum*, significantly reduced various parameters of root and shoot growth of wheat seedlings. In foliar spray bioassay, pot-grown 1-wk and 2-wk old *A. fatua* and wheat seedlings were sprayed with 100% culture filtrates of the four *Trichoderma* spp. thrice with 5 d interval each. Culture filtrates of all except *T. viride* significantly diminished root and shoot biomass of *A. fatua* plants in 1-wk old plants. The effect of foliar spray on root and shoot growth of wheat was insignificant. Present study concludes that metabolites of *T. harzianum*, *T. reesei* and *T. pseudokoningii* contain herbicidal constituents for the management of *A. fatua*.

Key words: Alternative herbicides, *Avena fatua*, fungal metabolites, *Trichoderma* spp.

In the literature, there are many examples of fungal products having herbicidal activities. AAI-toxin is a phytotoxin produced by *Alternaria alternata* (Fr.) Keissl. pathovars that infect tomato. Its mode of infection was reported to be inhibition of RNA synthesis. It is active on many weed species (Abbas *et al.*, 1995). Two phytotoxic metabolites prehelminthosporal and dihydropore, were isolated from the cultural filtrates of the fungus *Bipolaris* sp. showing herbicidal activity against *Sorghum halepense* (L.) Pers. (Parmar and Devkumar, 1993). Three herbicidal metabolites, 3-hydroxybenzylalcohol, 2-methyl hydroquinone and epiepoformin were isolated from a soil-borne fungus *Scopulariopsis brumptii*. Similarly, a phytotoxic metabolite trans-4 aminoproline isolated from culture filtrates of *Ascochyta caulina* was found to be very effective in controlling *Chenopodium album* (L.) (Evidente *et al.*, 2000). Evidente *et al.* (2005) isolated a new phytotoxic trisubstituted naphthofuroazepinone from the culture filtrates of *Drechslera siccans*, named drazepinone, and characterized as a 3,5,12 a-trimethyl-2,5,5a,12a-tetrahydro-1H-naphtho[2',3':4,5]furo[2,3-b]

azepin-2-one. Assayed at 2 µg µL⁻¹ solution, the novel metabolite proved to have broad-spectrum herbicidal properties. *Phyllosticta cirsii*, a fungal pathogen isolated from diseased *Cirsium arvense* leaves and evaluated as a biocontrol agent of this noxious perennial weed, produces different phytotoxic metabolites with potential herbicidal activity when grown in liquid cultures. Phyllostictines A–D, four novel oxazatricycloalkenones, were isolated from this pathogen (Evidente *et al.*, 2008). Recently Javaid and Adrees (2009) reported that metabolites of *Alternaria alternata*, *Fusarium oxysporum*, *F. solani*, *Drechslera hawaiiensis*, *D. australiensis* and *D. rostrata* were highly effective in controlling the growth of the noxious weed *Parthenium hysterophorus*.

Trichoderma spp. are among the most frequently isolated soil fungi and present in plant root ecosystems (Harman *et al.*, 2004). To date, most of the studies on *Trichoderma* spp. have been conducted with respect to their activity as biological control agents. Their efficacy has been demonstrated for the management of nematode and fungal diseases under field conditions (Sahebani and Hadavi, 2008; Hanada *et al.*, 2009). The modes of action as biological control agents include mycoparasitism, antibiosis, competition, enzyme activity and induced plant defense (Howell, 2003). In addition to their role as biocontrol agents, *Trichoderma* spp. have also been

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used in a wide range of commercial enzyme productions, namely, cellulases, hemicellulases, proteases, and β -1,3-glucanase (Sadhasivam *et al.*, 2008; Kovacs *et al.*, 2009). However, weed control by using *Trichoderma* spp. is still a relatively unexplored field and the few known studies in this area are restricted to *T. virens* only (Hutchinson, 1999; Heraux *et al.*, 2005). The present study was, therefore, designed to evaluate the herbicidal activity of culture filtrates of four *Trichoderma* spp.: *T. harzianum*, *T. pseudokoningii*, *T. reesei* and *T. viride*, against *Avena fatua*, a problematic weed of wheat.

MATERIALS AND METHODS

Procurement of *Trichoderma* species

Pure cultures of four species of *Trichoderma*: *T. harzianum*, *T. pseudokoningii*, *T. reesei* and *T. viride* were procured from the Fungal Culture Bank of the Institute of Plant Pathology, University of the Punjab, Lahore, Pakistan. These cultures were maintained on Malt Extract Agar (MEA) medium and stored at 4 °C in a refrigerator.

Preparation of cultural filtrates of the test fungi

Malt extract broth medium was autoclaved at 121 °C in 250 mL conical flasks at 100 mL medium in each flask. Flasks were inoculated with 5 mm agar discs of each of the four test fungi from margins of actively growing fungal colonies. Inoculated flasks were incubated under static conditions at 25 °C for 4 wk. After 4 wk, the cultures were filtered through sterilized muslin cloth followed by Whatman filter paper N° 1. These filtrates were preserved at 4 °C in a refrigerator. The cultural filtrates were used within 1 wk of filtration to avoid any contamination or chemical alteration (Javaid and Adrees, 2009).

Laboratory bioassay

Mature seeds of *A. fatua* were collected from wheat fields of the University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan. Seeds were dried in the sun, packed in paper bags and stored at room temperature. Ten seeds of *A. fatua* and wheat var. Sehr were placed in 9 cm diameter sterilized Petri plates with sterilized filter papers moistened with 3 mL of original (100%) and diluted (50%) crude fungal extract of different *Trichoderma* species. The negative control treatment received 3 mL of sterilized distilled water. Since fungal metabolites were obtained using 2% malt extract broth, and filtrates were also used as 50% dilutions. Therefore, two positive control treatments with 3 mL of 2% and 3 mL of 1% malt extract broth were also included. Each treatment was replicated four times. The Petri plates were arranged in a completely randomized design in a growth room maintained at 18 \pm 2 °C and 10:14 h photoperiod. Plants were harvested after

12 d. Data regarding germination, and root/shoot growth in terms of length and fresh biomass were recorded. Plants were dried at 60 °C to constant weight and dry weight was recorded (Javaid and Adrees, 2009).

Foliar spray bioassay

Experiment was conducted in February-March 2009 at the University of the Punjab, Lahore (31°57'N;74°31' E) Pakistan. Plastic pots of 8 cm diameter and 12 cm deep were filled with 350 g of sandy loam soil with 0.69% organic matter, pH 7.8, N 0.035%, available P 6.3 mg kg⁻¹ and available K 100 mg kg⁻¹. The micronutrients B, Mn, Fe, Cu and Zn were 1.06, 22.8, 10.8, 1.9 and 1.3 mg kg⁻¹ of soil, respectively. Ten seeds of *A. fatua* and wheat var. Sehr were sown in each pot. After germination, pots were divided into two sets to perform the foliar spray on 1-wk and 2-wk old seedlings. All the pots were arranged in a completely randomized design under natural environmental conditions.

Original culture filtrates of the four selected *Trichoderma* species were sprayed on 1-wk and 2-wk old *A. fatua* and wheat seedlings. Both of the sets were sprayed 3-times with an interval of 5 d. Treatment in a similar manner with distilled water spray served as control. All the plants were harvested after 40 d growth. Plants were carefully uprooted and washed under tap water. Roots were separated from shoots. Data regarding length and dry biomass of shoot and root were recorded.

Statistical analysis

All the data were subjected to ANOVA followed by Duncan's Multiple Range Test ($P \leq 0.05$) to delineate mean differences (Steel and Torrie, 1980).

RESULTS

Laboratory bioassay

The effect of culture filtrates of all the *Trichoderma* species was insignificant on germination of *A. fatua* seeds (Table 1). Maximum shoot length of 115 mm was recorded in negative control (water). Decreases in shoot length in 1% and 2% malt extract treatments were insignificant. Culture filtrates of all the *Trichoderma* species, except *T. viride*, significantly reduced shoot length. Highest toxicity was recorded due to the culture filtrate of *T. pseudokoningii*, followed by *T. harzianum* and *T. reesei*, respectively. The original culture filtrates (100%) of *T. pseudokoningii*, *T. harzianum*, *T. reesei* and *T. viride* reduced the shoot length of *A. fatua* by 80, 73, 62 and 11%, respectively; as compared to the negative control (Table 1). Culture filtrates of 50%, as well as 100%, of *T. pseudokoningii* and *T. harzianum* significantly suppressed shoot fresh and dry biomass of *A. fatua* as compared to negative

Table 1. Effect of culture filtrates of four *Trichoderma* species on germination and growth of *Avena fatua* in laboratory bioassays.

Fungal species	Concentration	Germination	Shoot	Shoot	Shoot	Root	Root	Root
			length	fresh weight	dry weight	length	fresh weight	dry weight
		%	mm	mg		mm	mg	
Control (H ₂ O)	0	100a	115a	48a	5.5ab	196a	51a	5.1bc
Control (1% ME)	0	100a	108ab	43a	5.9ab	161c	47a	4.9c
Control (2% ME)	0	100a	101ab	46a	6.2a	178b	50a	5.4abc
<i>T. harzianum</i>	50	91a	47c	20b	2.6de	34f	15bc	1.9d
	100	88a	31cd	14b	2.0e	14g	8d	1.0d
<i>T. pseudokoningii</i>	50	100a	26d	15b	2.4de	19g	12cd	1.4d
	100	94a	23d	11b	1.7e	10g	8d	1.2d
<i>T. reesei</i>	50	100a	91b	41a	5.3ab	112e	48a	6.1ab
	100	100a	44c	22b	3.1d	18g	17b	1.9d
<i>T. viride</i>	50	100a	103ab	40a	4.7bc	146d	49a	6.3a
	100	100a	102ab	48a	4.9bc	119e	48a	5.1bc

In a column, values with different letters show significant differences as determined by Duncan's Multiple Range Test ($P \leq 0.05$). ME: malt extract.

control. Similarly, the negative effect of 100% filtrates of *T. reesei* was significant on shoot fresh and dry biomass. Culture filtrates of *T. viride*, however, failed to exhibit any pronounced effect on shoot biomass of *A. fatua* (Table 1).

Root length was reduced due to 1% and 2% malt extract by 18 and 9%, respectively. Culture filtrates of all four tested *Trichoderma* species significantly reduced root length. The adverse effects of *T. pseudokoningii* and *T. harzianum* filtrates on root length were more pronounced than the effects of *T. reesei* and *T. viride*. There was 95, 93, 91 and 39% reduction in root length due to 100% culture filtrates of *T. pseudokoningii*, *T. harzianum*, *T. reesei* and *T. viride*, respectively, as compared to negative control. Malt extract medium exhibited insignificant effects on

root fresh and dry biomass. The effect of culture filtrates of the four *Trichoderma* species on fresh and dry biomass of roots of *A. fatua* was generally similar to that observed on root length (Table 1).

None of the treatments exhibited significant effects on germination of wheat seeds. Except for 50% of culture filtrates of *T. harzianum*, all treatments significantly reduced shoots length as compared to negative control. Similarly, original culture filtrates (100%) of all the *Trichoderma* spp. except *T. harzianum* significantly reduced shoot biomass of wheat seedlings. The effects of culture filtrates of the different *Trichoderma* species evaluated were in general similar for both root and shoot growth parameters (Table 2).

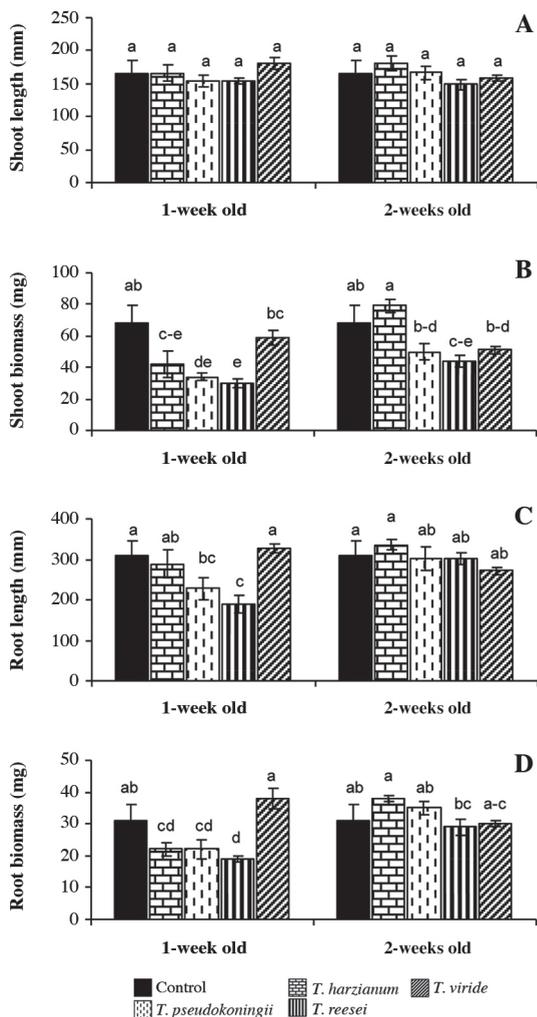
Table 2. Effect of culture filtrates of four *Trichoderma* sp. on germination and growth of wheat in laboratory bioassays.

Fungal species	Concentration	Germination	Shoot	Shoot	Shoot	Root	Root	Root
			length	fresh weight	dry weight	length	fresh weight	dry weight
		%	mm	mg		mm	mg	
Control (water)	0	100a	129a	86b	25ab	60a	63abc	18ab
Control (1% ME)	0	100a	97de	65cde	20cde	43cd	54bcd	16abc
Control (2% ME)	0	100a	112b	79bc	22bcd	47c	55bcd	14cd
<i>T. harzianum</i>	50	85a	121a	104a	26a	55ab	76a	19a
	100	90a	104bcd	76bcd	22bcd	49bc	60bc	16abc
<i>T. pseudokoningii</i>	50	95a	106bcd	78bc	23abc	40de	50cd	14cd
	100	95a	87f	64de	17e	39de	52bcd	14cd
<i>T. reesei</i>	50	90a	93ef	63de	19de	43cd	63abc	20a
	100	90a	75g	58e	18e	35e	43d	12d
<i>T. viride</i>	50	100a	108bc	74bcde	22bcd	55ab	66ab	16abc
	100	100a	98de	68cde	20cde	43cd	56bcd	16abc

In a column, values with different letters show significant differences as determined by Duncan's Multiple Range Test ($P \leq 0.05$). ME: malt extract.

Foliar spray bioassay

Even though a minimum effect of the different *Trichoderma* extracts was observed on the shoot length of *A. fatua*, there was a clear suppressing effect on shoot biomass of the extracts when they were sprayed 1 wk after germination. The effect of all except *T. viride* culture filtrates was significant on shoot biomass. There was a 9-56% reduction in shoot biomass due to foliar spray of culture filtrates on 1-wk old plants, while 2-wk old plants were comparatively tolerant to foliar spray treatments. In 2-wk old plants, foliar spray of only *T. reesei* culture filtrates significantly reduced the shoot biomass by 35% (Figure 1B).

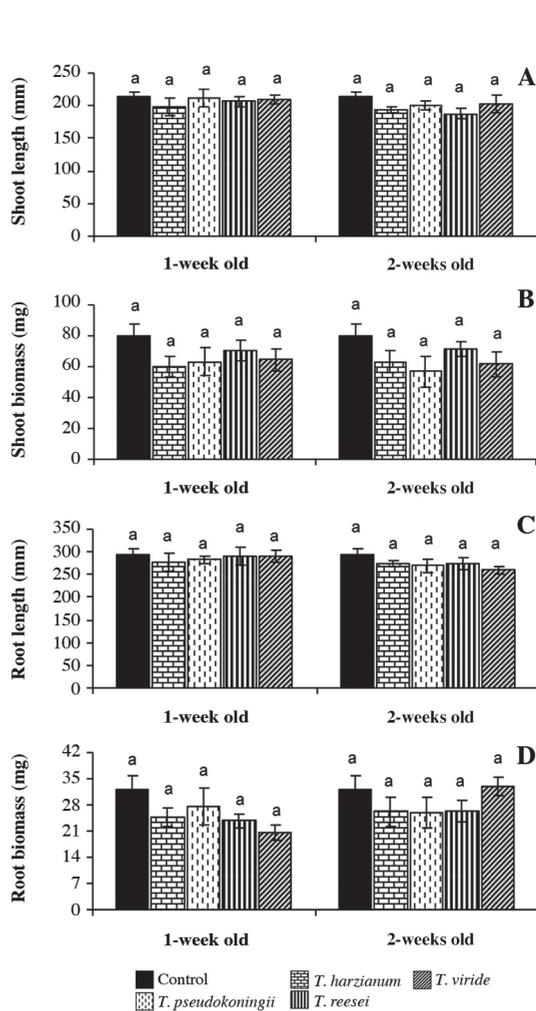


Values with the same letters show insignificant differences as determined by Duncan's Multiple Range Test at $P \leq 0.05$.

Figure 1. Effect of foliar spray of cultural filtrates of four species of *Trichoderma* on growth of 1-wk and 2-wk-old *Avena fatua* plants. Vertical bars show standard errors of means of three replicates.

Root length was significantly reduced by 26% and 39% due to foliar spray of *T. pseudokoningii* and *T. reesei*, respectively, in 1-wk old *A. fatua* plants. Root biomass of *A. fatua* plants was also susceptible to foliar spray of culture filtrates at 1-wk growth stage. At this growth stage, foliar spray with culture filtrates of *T. pseudokoningii*, *T. reesei* and *T. viride* significantly reduced root biomass. None of the foliar spray treatments showed a significant effect on root length and biomass in 2-wk old plants (Figures 1C and D).

The effect of foliar spray of culture filtrates of all the four *Trichoderma* spp. on various parameters of root and shoot growth of wheat was insignificant (Figure 2).



Values with the same letters show insignificant differences as determined by Duncan's Multiple Range Test at $P \leq 0.05$.

Figure 2. Effect of foliar spray of cultural filtrates of four species of *Trichoderma* on growth of 1-wk and 2-wk-old wheat plants. Vertical bars show standard errors of means of three replicates.

DISCUSSION

Culture filtrates of the four *Trichoderma* spp. tested: *T. harzianum*, *T. pseudokoningii*, *T. reesei* and *T. viride*, exhibited herbicidal activity against *A. fatua*. These findings are in agreement with those of the few studies carried out earlier in this field (Hutchinson, 1999; Heraux *et al.*, 2005). The earlier studies regarding the herbicidal activity of *Trichoderma* spp. were restricted to the use of *T. virens* in combination with chicken manure (Hutchinson, 1999; Heraux *et al.*, 2005). However, the present study reveals that the metabolites of other *Trichoderma* species also exhibit herbicidal activity. *Trichoderma* spp. produces a range of secondary toxic metabolites, including gliovirin, gliotoxin, viridian, and viridiol, of which viridiol is strongly phytotoxic (Jones and Hancock, 1987; Jones *et al.*, 1988).

Metabolites of *T. harzianum* exhibited herbicidal activity against *A. fatua* in both laboratory and foliar spray bioassays. Production of toxins belonging to the trichothecene class has been reported from *T. harzianum* (Sivasithamparam and Ghisalberti, 1998). Trichothecenes have been proven to be phytotoxic (Harris *et al.*, 1999). These are sesquiterpenoid epoxides and represent a large family of secondary toxic metabolites produced by a variety of filamentous fungi (Ueno, 1980). Many of the toxic properties of the trichothecenes are attributed to their ability to inhibit protein and DNA synthesis (McLaughlin *et al.*, 1977) and to induce apoptosis in eukaryotic cells (Okumwai *et al.*, 1999). Recently, Gallo *et al.* (2004) isolated and characterized a putative trichodiene synthase (*tri5*) gene in *T. harzianum* required for trichothecene biosynthesis. Another compound trichosetin, a tetramic acid-containing metabolite produced in the culture of *T. harzianum*, is known to exhibit phytotoxic activity (Marfori *et al.*, 2003). Vital staining of trichosetin-treated *Nicotiana tabacum* BY-2 cells, with rhodamine 123, showed a weaker green fluorescence compared to controls, indicating damaging effects on mitochondria. FDA-PI staining, to determine cell viability, indicated that cells of the trichosetin-treated roots were mostly dead (Marfori *et al.*, 2003). *T. harzianum* also synthesizes 6-pentyl- α -pyrone (6PP), a lactone with coconut-like aroma characteristics, is used in food and pharmaceutical products (Rocha-Valadez *et al.*, 2006). This compound is also known to exhibit phytotoxic activity (Rocha-Valadez *et al.*, 2006), and may derive from 6PP's ability to bind to or permeate cell membranes (Poole and Whitaker, 1997).

Culture filtrates of *T. pseudokoningii* proved highly toxic, resulting in significant growth suppression of *A. fatua* plants in both the laboratory bioassay and the foliar spray trial. *T. pseudokoningii* culture can produce

complete cellulase enzymes, namely endoglucanase, cellobiohydrolase, and β -glucosidase (Ma *et al.*, 1990), which may be responsible for damage to the cell wall, resulting in reduced plant growth. In addition, a low molecular weight peptide, termed short fiber generating factor (SFGF), derived from the culture supernatant of *T. pseudokoningii*, decomposes cellulose by oxidative reaction, which differs from the hydrolysis of cellulose by cellulases. The alcoholic hydroxyl groups in cellulose can be oxidized by SFGF, which leads to destruction of the hydrogen bond network in cellulose and cleavage of glycosidic linkages. Both effects led to the depolymerization of cellulose and the formation of short fibers, further reducing groups in residual cellulose, making the cellulose substrates more susceptible to hydrolysis by cellulases (Wang *et al.*, 2003). Certain other bioactive chemical substances, such as acetophenone derivatives 2',4'-dihydroxy-3'-methoxymethyl-5'-methylacetophenone and 2',4'-dihydroxy-3',5'-dimethylacetophenone (Astudillo *et al.*, 2000), Pseudokonins KL III and KL VI (Rebuffat *et al.*, 2000), and Harzianin HK VI (Rebuffat *et al.*, 1996) have also been isolated from culture filtrates of *T. pseudokoningii*, and may be responsible for the herbicidal activity of the fungus.

Culture filtrates of *T. reesei* were found to be comparatively less toxic than filtrates of *T. harzianum* and *T. pseudokoningii* in laboratory bioassays. Generally, only 100% culture filtrates of *T. reesei* exhibited significant effect against shoot and root growth of the target weed species and the effect of 50% culture filtrates were insignificant. However, in the foliar spray bioassay, the adverse effect of culture filtrates of *T. reesei* on 1-wk old *A. fatua* plants was similar to that of culture filtrates of *T. harzianum* and *T. pseudokoningii*. Trichodermin, a member of the trichothecene toxin family – a group of sesquiterpenes, is produced by *T. reesei* in culture medium. This secondary metabolite may be responsible for herbicidal activity against *A. fatua*.

CONCLUSIONS

The present study concludes that *Trichoderma* spp. namely *T. harzianum*, *T. pseudokoningii* and *T. reesei* produce certain secondary metabolites in the culture medium that exhibit herbicidal activity against *A. fatua*, a problematic weed of wheat. Further studies are in progress regarding the isolation and identification of potential herbicidal constituents in the culture filtrates of various tested *Trichoderma* species.

RESUMEN

Alternativas de manejo de una maleza problemática de trigo *Avena fatua* L. por metabolitos de *Trichoderma*. La avena silvestre (*Avena fatua* L.) es una maleza problemática del trigo (*Triticum aestivum* L.) en Paquistán. El presente estudio se diseñó para evaluar la actividad de filtrados de cultivos de cuatro *Trichoderma* spp.: *T. harzianum*, *T. pseudokoningii*, *T. reesei* y *T. viride*, contra esta especie. En un bioensayo de laboratorio, filtrados de cultivos originales (100%) y diluidos (50%) de *T. harzianum* and *T. pseudokoningii* redujeron significativamente el crecimiento de brotes y raíces de plántulas de *A. fatua*. Sólo los filtrados de cultivos originales de *T. reesei* exhibieron efecto significativo mientras el efecto de filtrados de *T. viride* fue insignificante contra el crecimiento de brotes y raíces de la maleza. Las concentraciones originales de filtrados de todos los cultivos de *Trichoderma* spp. excepto *T. harzianum* redujeron significativamente varios parámetros de crecimiento de brotes y raíces de plántulas de trigo. En el bioensayo de aspersión foliar, plántulas de *A. fatua* y trigo de 1 y 2 semanas de edad cultivadas en macetas se asperjaron con filtrados de cultivo 100% de las cuatro especies de *Trichoderma* tres veces con 5 d de intervalo cada uno. Los filtrados de cultivo de todos excepto *T. viride* redujeron significativamente la biomasa de raíces y brotes de plantas de *A. fatua* en plantas de 1 semana de edad. El efecto de la aspersión foliar sobre el crecimiento de raíces y brotes de trigo fue insignificante. El presente estudio concluye que los metabolitos de *T. harzianum*, *T. reesei* y *T. pseudokoningii* contienen constituyentes herbicidas para el manejo de *A. fatua*.

Palabras clave: herbicidas alternativos, *Avena fatua*, metabolitos fúngicos, *Trichoderma* spp.

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