



Original article

Evaluation of the herbicidal potential of some fungal species against *Bidens pilosa*, the coffee farming weeds

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ABSTRACT

Weeds are the most productive limiting factor, especially in organic farming systems where the uses of synthetic herbicides are not allowed due to their negative impacts. Hence, synthetic herbicides need to be replaced with biological herbicides for weed management. Thus, the present study was designed to evaluate the herbicidal activity of conidia suspensions from *Aspergillus niger*, *Trichoderma asperlium*, *Trichoderma atroviride*, *Trichoderma hamatum*, *Trichoderma harzanium*, *Trichoderma longibrachatum* and *Trichoderma viride* against *Bidens pilosa* weed via a series of laboratory and lath-house conditions that laid out in a CRD and RCBD, respectively, with three replications for each bioassay. The results revealed that all fungi, except *T. longibrachatum*, had significantly reduced seed germination as well as early growth of the target weed compared to the untreated control. The inhibitory effects were measured to be varied among the types of conidia suspensions of fungal species and their level of concentration. The highest rate of inhibition was observed for conidia suspension from *A. niger* which suppressed with the maximum seed germination inhibitory level (65%) over control. Likewise, the plumule and radicle growth length of the target weed also significantly inhibited by the tested fungi (ranging from 10 to 85% and 34 to 97%) compared to the control, respectively. Based on their efficacy in the laboratory bioassay, the herbicidal potential of selected fungi was further evaluated in pot experiments. In contrast to laboratory observations, the effect of different fungal conidia suspensions on various growth parameters of the targeted weed was insignificant in the lath-house experiments. In conclusion, the application of *A. niger* displayed some potential green light to be investigated as a biocontrol agent with promising retarding in the germination and early growth of *B. pilosa*. Hence, we recommend further investigation of those fungi under field conditions on different coffee weed species.

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1. Introduction

Weeds are one of the most serious causes of economic losses in agricultural production (Kuang et al., 2016). They compete with crops for space, nutrients, water and light (Wu et al., 2017). They also directly influence on the affairs of humans more than any other pest in developing countries, like Ethiopia. The weed flora of Ethiopia is highly diverse and it is composed of a wide range of perennial

and annual grasses and broad-leaved weeds, sedges, parasitic and invasive weed species (Fasil, 2006). The high rainfall and hot humid climate in the coffee-producing country encourage the rapid and continuous growth of the noxious weeds, such as *Amaranthus* spp, *Cyperus rotundus* L., *Cynodon dactylon* L., *Digitaria* spp, *Bidens pilosa* L., *Galinsoga parviflora* Cav. and *Commelina* spp that seriously compete with the available resource. The yield loss assessment studies conducted in Jimma showed a yield loss of 60 to 80% for coffee (Eshetu et al., 2007). *Bidens pilosa* (Asteraceae) is a common annual broadleaf species that is widely distributed in agricultural and disturbed areas in tropical and subtropical regions of the world (Grombone-Guaratini et al., 2004). It is considered a “cosmopolitan” weed with a worldwide distribution that is mainly attributed to human activity and troublesome weed to at least 30 crops including coffee in over 40 countries and it is known to significantly reduce crop yields (Holm et al., 1991). As reported by (Ronchi et al.,

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2007), *B. pilosa* is a challenging weed in a coffee plantation, due to the fast growths (three times faster than similar plant species) and a single plant may produce 3000 to 6000 seeds per year which are reported to have no dormancy, remain viable for five to six years. In a weed survey conducted in Ethiopia (Sintayehu, 2019), *B. pilosa* was found in 31.58% of the fields surveyed, with high uniformity of distribution compared with other species.

Weed management is one of the major year-round operations that contribute to the high cost of production, especially difficult to employ organic products without the use of chemical herbicides (Labouisse et al., 2008). Conventional farming has mainly been dependent on intensive inputs of synthetic herbicides. The systems are often associated with problems such as disturbances of the environment, groundwater pollution and lethal effect on non-target organisms in the agroecosystems in addition to direct toxicity to users (Poudel et al., 2002; Prakash et al., 2008). Because of their environmental and toxicological effects, besides increasing synthetic herbicidal resistance among weeds, more alternative strategies against weeds must be developed which are less impact on the environment, and pose a smaller risk to human health (El-Rokiek and Eid, 2009). Due to these and other sustainability reasons, many countries are attempting to reduce the reliance on synthetic herbicides for weed management and led to the development and promotion of an organic farming system that accounts for the environment and public health as main concerns (Araújo et al., 2008).

Microorganisms and their metabolites have herbicidal activity and recently attracted more attention in the field of biocontrol research. Hence, they alleviate the effects of resistance and environmental problems associated with chemical herbicides (Guo et al., 2020). Biocontrol methods should be considered as components of an overall IWM to achieve efficiency and to reduce herbicide application to the minimum possible extent (Patel and Patel, 2015). According to Khattak et al. (2014), fungi are easy to isolate and culture comparative to plants, the attention of researchers has hence focused on the isolation of bioactive compounds from fungi. The herbicidal activity of fungal culture filtrates against various weeds was reported by Sica et al. (2016). Evidente et al. (2006) reported that *Drechslera gigantea*, a fungal pathogen isolated from *Digitaria sanguinalis* produced phytotoxins in liquid and solid cultures which were potential myco-herbicide of grassy weeds. Javaid et al. (2013) suggested that culture filtrates of different *Trichoderma* species contain compounds having herbicidal activity and can be used for parthenium weed management. As reported by (Harding and Raizada, 2015), the two species within *Sclerotinia* genus and three species within the genus *Phoma* have been received attention as potential agents for biological weed control. *Trichoderma* is a genus of soil-borne filamentous fungi widely used as a source of biocontrol agents in agriculture owing to effective antagonistic mechanisms such as myco-parasitism, antibiosis or competition against plant pathogens and nematodes. Despite having the enormous scientific evidence for the development of biocontrol activities of fungal species and proposed for weed management, their application in weed management especially in coffee production in Ethiopia received very little attention to date. Therefore, this study was designed to investigate the potential use of pathogenic fungal species against the germination and seedlings growth of *Bidens pilosa* under laboratory and lath-house conditions.

2. Materials and methods

2.1. Description of the study area

The laboratory and lath-house experiments were carried out in the laboratory of Jimma University, College of Agriculture and

Veterinary Medicine (JUCAVM), Jimma, Ethiopia, during the summer season of 2018/19. The testing site was located at an altitude of 1710 m above sea level and 7°, 33' N, and 36°, 57' E. The temperature and relative humidity were 18.4 ± 2.24 °C and $60.26 \pm 12.42\%$ for laboratory and 20.8 ± 2.22 °C and $48 \pm 22.26\%$ for lath-house during the experimental periods. The soil used for the pot experiment was clay-loam in texture with an organic matter content of 3.5–4% and a pH of 5.8–6.5.

2.2. Culturing and maintenance of fungal isolates

Six *Trichoderma* species namely *Trichoderma asperlium*, *Trichoderma atroviride*, *Trichoderma hamatum*, *Trichoderma harzanium*, *Trichoderma longibrachatum* and *Trichoderma viride*, which were used in this study, were acquired from culture collections of Ambo Plant Protection Research Center, Ambo, Ethiopia. *Aspergillus niger* was obtained from Jimma University, Pathology laboratory and included in the treatments. Each of the fungal species was sub-cultured on sterilized Potato Dextrose Agar (PDA) medium and incubated at 25 ± 2 °C for five days and then further transferred into a test tube contained PDA slants and maintained as the stock culture for further studies.

2.3. Growth media preparation and extraction of fungal conidia suspension

The growth medium (PDA) was used for culturing both *Aspergillus* and *Trichoderma* species which consisted of 15 g agar, 20 g dextrose, potato infusion from 200 g and 1000 mL sterile distilled water and autoclaved for 20 min at 121 °C and 15 psi for 15 min. Furthermore, the molten (autoclaved) medium was amended with 200 mg L⁻¹ of streptomycin to prevent bacterial proliferation (Davet and Rouxel, 1997). After thorough shaking, approximately 15 mL of the autoclaved media was poured into sterilized Petri plates (9-cm-diameter) aseptically. A full of inoculation loop of the spores/mycelium plague was placed in the center of each Petri plate containing the media and sealed with parafilm. Spores cultures in sealed Petri plates were incubated at a temperature (25 ± 2 °C) in darkness for 21 days. Afterward, conidia were harvested from the surface of solid media (Fig. 1) by pouring 5 mL of sterile distilled water containing a drop of Tween-80 (0.01%) into the fungal plate.

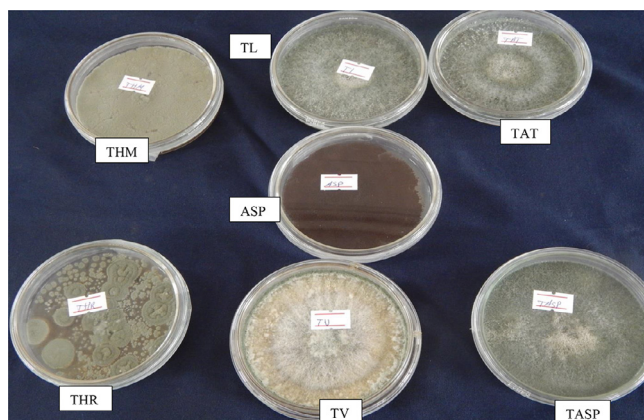


Fig. 1. Fungal species cultures used in screening for potential herbicidal activity after 21 days in artificial growth medium; ASP: *Aspergillus niger*; TASP: *Trichoderma asperlium*; TAT: *Trichoderma atroviride*; THM: *Trichoderma hamatum*; THR: *Trichoderma harzanium*, TL: *Trichoderma longibrachatum* TV: *Trichoderma viride*.

2.4. Preparation of fungal conidia suspension for bioassays

Conidia from each plate were scrapped by using a sterile spatula and the spore suspension was filtered through double sterilized muslin cloth and the filtrates were used as stock for conidia solution. Sterilized distilled water was added to the original filtrates to prepare a dilution. Conidia concentration was quantified using a haemocytometer (Superior Marienfeld, Germany) under the binocular light microscope (Labomed cxi mono) (400x) and adjusted to the concentration of 1×10^8 and 1×10^9 conidia mL^{-1} .

2.5. In-vitro bioherbicidal assays with fungal conidia suspension against *Bidens pilosa*

A bioassay experiment was conducted to study any inhibitory effects of fungi species against *B. pilosa* seed germination and early growth parameters. Seeds of *B. pilosa* were collected from the surroundings of Jimma University and surface sterilized by soaking them for five minutes in a 1% sodium hypochlorite (NaOCl) solution. Seeds were washed with sterile water for three minutes immediately before use to remove all chemical residues. The seeds of the tested weed were placed in 9-cm-diameter Petri dishes (20 seeds each) lined with filter paper (Whatman No. 1). Two spore concentrations (1×10^8 and 1×10^9 conidia mL^{-1}) were prepared from the stock suspension, based on the results of a preliminary experiment. Afterward, each Petri dish was moistened with a 5 mL suspension of respective fungal species having prescribed concentration levels. Sterile distilled water contains the culture medium and a drop of Tween-20 (0.01%) was used as untreated control. The whole experiment was laid out in a complete randomized design with three replications. The experiment was conducted twice and the averaged data were used for analysis.

2.6. In-vivo bioherbicidal assays of fungal conidia suspensions on *Bidens pilosa*

Potential herbicidal activities of tested fungal species were determined using *B. pilosa* plants as target weed. Conidia suspensions of fungal species exhibited with better herbicidal activity in the Petri dish experiment were further selected for pot experiments in lath-house. Mature seeds of *B. pilosa* were collected from surrounding JUCAVM. Agricultural soil from the Horticultural Demonstration Farm of JUCAVM was collected at 0–5 cm depth, which was sieved then autoclaved for one hour at 121 °C and 15 psi. Then 850 g of the autoclaved soils were used to fill in disinfected plastic pots (16 cm wide \times 20 cm height) and 15 pre-sterilized black-jack seeds were sown per pot. A week after emergence, pots were thinned to 10 plants per pot. The plants in pots were irrigated with tap water when it was necessary. When the seedlings were attained 3–4 true leaves stage (4-weeks-old weeds), the surfaces of the leaves were rubbed by sterile sand to enhance the entrance of fungal conidia suspensions. The formulation of each test material owing with a volume of 20 mL with a 1×10^9 conidia mL^{-1} concentration of the conidia suspension was applied at the same time in each pot uniformly as foliar sprays using hand sprayers. A drop of Tween-20 (0.01%) was mixed with the solutions to homogeneous dispersion and facilitates the absorption of the fungal conidia suspensions. Untreated control pots were treated with distilled water containing 0.01% of Tween-20. Four days after applying the suspension of fungal species, the second application frequency was done and in such a manner that the third application was performed 8 days after the first application (Fig. 2). The amount of conidia suspension solution required per pot was pre-adjusted by applying tap water until the whole plant parts were covered with water. The experiment was

laid out in a factorial arranged in RCB design with three replications.

2.7. Data collection

Seed germination count was taken in 24 h intervals for 7 days and when the seed germination became stable, the counting was terminated. The percent germination was carried out according to Abdul-Baki and Anderson (1973).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

The seed germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by the following formula:

$$\text{GI} = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

The plumule and radicle length was measured at the end of the germination period of 7 days, by taking five seedlings at random from each Petri dish. The average (cm) of five plumule and radicle lengths were calculated separately and employed for statistical analysis.

The seedling vigor indices (SVI) were determined according to the following formula (Abdul-Baki and Anderson, 1973):

$$\text{SVI} = (\text{Plumule length} + \text{Radicle length}) \times \text{Germination percentage}$$

In the lath-house experiment, 14 days after applying the conidia suspension, symptoms of damaged plants were visually estimated as the percent growth reduction and assessed according to EWRS (European Weed Research Society) scale, which ranges from 1 to 9, where 1 is for 100% weed control and 9 for no effects, other values were given between the two extremes, as indicated in (Pacanoski and Dimov, 2017). Afterward, seedlings per pot were carefully uprooted and the roots were washed with tap water to remove the soil load. After separating the seedlings into the shoot and root parts, shoot and root length were measured by using a ruler and the average was calculated and expressed in centimeter. Similarly, root, as well as shoot dry biomass of *B. pilosa* plants per

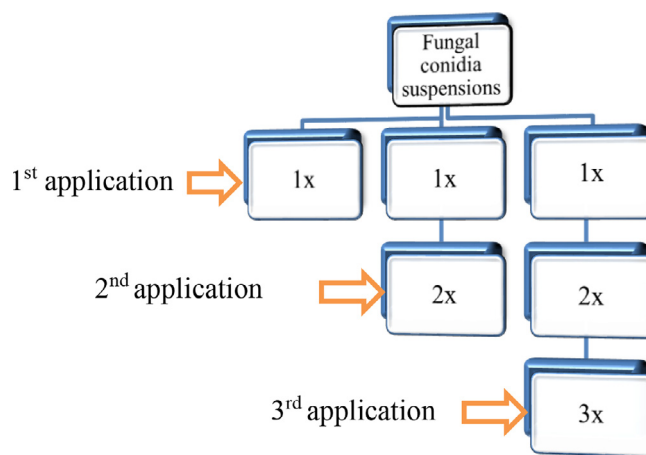


Fig. 2. Application frequencies of conidia suspensions from selected fungal species on *Bidens pilosa* weed per pots. All pots were treated the same day for the first application, then after 4 days of treatment, the application was repeated for the 2nd pots by excluding the first pot. Finally, the application was again repeated for the 3rd pot after 8 days of the first application. The same pattern was repeated three times for each block.

pot, were determined and quantified in gram. The efficacy of test preparation against the test plants was computed by applying the methods of Abbott (1925).

2.8. Data analysis

Before variance analysis, all data were tested for normality by the Kolmogorov-Smirnov test and homogeneity of variances by Levene's test. The observation from both experiments showed consistency and similarity; the averaged data were used for statistical analysis. The seven fungal species filtrate tested and the three levels of concentrations (0 , 1×10^8 and 1×10^9 conidia mL^{-1}) for laboratory and five fungi species and the four application frequencies (0 , $1x$, $2x$ and $3x$) for pot experiment, were considered as fixed factors of interest in the model. When the interaction effect of these fixed factors was significant ($p < 0.05$), data were subjected to a two-way analysis of variance (ANOVA) using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). For significant parameters, multiple comparisons among the treatment means were compared based on Tukey's honest significant difference (HSD) test at α 0.05.

3. Results

3.1. In-vitro bioassay

3.1.1. Inhibitory effects of conidia suspensions on seed germination of *Bidens pilosa*

Conidia suspensions of *Aspergillus* and *Trichoderma* species tested on *B. pilosa* for phytotoxicity effects showed variable results. According to the analysis of the variance table, the percentage of germination, germination index, plumule length, radicle length and seedling vigor index of the tested weed were affected by conidia suspension of the two fungal species and their concentration levels, which depicted significant differences ($p < 0.001$) among treatments (Table 1). All the of tested fungi, except *T. longibrachatum*, highly suppressed *B. pilosa* seed germination percentage with varying levels of inhibition (Table 2) in which the range of germination was 33.33% to 92%. That means the amounts of non-germinated seeds were ranged from 66.67% to 8%, respectively. The percentage of seed germination data showed that maximum germination (96.90%) was recorded from Petri dishes treated with sterilized water (control) which was followed by Petri dishes treated with conidia suspension of *T. longibrachatum* in which the mean germination was 92.50% (7.5% inhibition), while with conidia suspension of *A. niger* exhibited the mean germination of 33.33% (66.67% inhibition) at 1×10^9 conidia mL^{-1} . On the other hand, Petri dishes treated with the conidia suspension of *T. hamatum* at (1×10^9 conidia mL^{-1}) concentration levels had slightly affected the germination percentage of seeds of the targeted weed.

The result presented in (Table 2) also revealed that the number of days taken for starting seed germination was significantly delayed. An application of some fungal conidia suspensions, a long

period was taken to attain the maximum germination percentage of the target weed. The inhibitory potential of those fungi, however, was found to be varied very highly and significantly ($p < 0.001$) depending on the fungal species and their concentrations. Application of some fungal conidia suspensions at a high concentration level (1×10^9 conidia mL^{-1}), significantly delayed germination as compared to their lower concentrations (1×10^8 conidia mL^{-1}) levels. Such results were well suggested that when the concentration of materials increased the germination index were decreased. Seeds treated with higher concentrations of tested materials needed more time to germinate (induced lower germination rate). Accordingly, conidia suspensions of *A. niger*, *T. hamatum* and *T. viride* at 1×10^9 conidia mL^{-1} concentration level delayed the germination with the values of GI = 7.56, 14.56 and 16.53, respectively. Contrarily, an average base of the control treatment has the highest GI with 24.65, that indicating there were high germination rates. Some of the observations indicated that the *B. pilosa* seeds took significantly more time to attain complete germination when treated with conidia suspensions from most of the tested fungal species than those treated with distilled water.

3.1.2. Inhibitory effects of fungal conidia suspensions on early plant growth of *Bidens pilosa*

Similarly, the plumule and radicle length measured from randomly selected five seedlings after seven days of sowing were highly influenced by types of fungal conidia suspensions and their concentration levels. Highly significant differences ($p < 0.001$) were detected among the treatments (Table 2). In the early growth evaluation, it was evident that fungal conidia suspensions caused significant dose-dependent inhibitory levels. The highest inhibition percentage was recorded at the highest dose (1×10^9 conidia mL^{-1}) for some fungal species, except for *T. longibrachatum*, which showed statistical indifference regardless of concentration levels. All the rest treatments affected highly the plumule height of the target weed, which ranges from 10 to 85% inhibition as compared to sterilized water (control) treatments (Fig. 3). The mean data showed that the highest plumule height (4.38 cm) of the target weed was recorded for control which was followed by *T. harzanium*, whereas; the shortest plumule height (0.65 cm) was obtained as a result of treatment treated with *A. niger*.

In a similar pattern, fungal species and their concentrations also considerably influenced the radicle length of the target weed as presented in (Table 2) and statistically, there was a very highly significant ($p < 0.001$) difference among the treatments. All the conidia suspensions of fungal species showed a strong inhibitory effect (ranging from 38 to 97%) on the radicle length of the tested weed as compared to control. The mean data showed that the tallest radicle length (4.77 cm) was recorded for the control Petri dishes, while the shortest radicle length (0.11 cm) was calculated for Petri dishes treated with conidia suspension of *A. niger* at 1×10^9 conidia mL^{-1} concentration. Approximately 74% and 97% reduction in the radicle length was seen for this fungal extraction at 1×10^8 and 1×10^9 conidia mL^{-1} concentration as compared to control, respectively (Table 2). Radicle length was relatively

Table 1

Analysis of variance for the effect of different fungal conidia suspension against germination and seedling growth of *Bidens pilosa*.

Source of variation	df	Mean squares				
		GP	GI	PL	RL	SVI
Fungal species (S)	12	522.09***	33.23***	0.64***	0.59***	23796.16***
Concentration (C)	3	3417.06***	356.39***	41.06***	108.46***	2830515.60***
S × C	36	295.30***	19.83***	0.42***	0.62***	16136.85***
Error	40	17.30	1.03	0.03	0.07	1230.95
Total	62	–	–	–	–	–

Note: ***significant at $p \leq 0.001$; GP: Germination percentage; GI: Germination index; PL: Plumule length; RL: Radicle length; SVI: Seedling vigor index.

Table 2
Effect of fungal conidia suspensions on germination and early growth of *Bidens pilosa*.

Source of conidia suspensions	Concentration (conidia mL ⁻¹)	GP (%)	GI	PL (cm)	RL (cm)
<i>A. niger</i>	0	98.33 ^a	24.91 ^a	4.39 ^a	4.75 ^a
	1 × 10 ⁸	80.00 ^{cde}	20.82 ^{d-g}	3.73 ^b	1.23 ^{de}
	1 × 10 ⁹	33.33 ^f	7.56 ^j	0.65 ^f	0.11 ^f
<i>T. asperlium</i>	0	96.67 ^{ab}	24.41 ^a	4.41 ^a	4.72 ^a
	1 × 10 ⁸	91.67 ^{abc}	23.06 ^{a-d}	3.94 ^{ab}	2.33 ^{bc}
	1 × 10 ⁹	80.00 ^{cde}	19.51 ^{e-h}	1.91 ^{de}	0.34 ^f
<i>T. atroviride</i>	0	95.00 ^{ab}	24.59 ^a	4.34 ^a	4.77 ^a
	1 × 10 ⁸	85.00 ^{bcd}	20.97 ^{c-g}	3.67 ^b	1.86 ^{cd}
	1 × 10 ⁹	76.67 ^{de}	17.876 ^{gh}	1.69 ^e	0.28 ^f
<i>T. hamatum</i>	0	98.33 ^a	24.49 ^a	4.35 ^a	4.78 ^a
	1 × 10 ⁸	81.67 ^{cde}	21.25 ^{b-f}	2.89 ^c	1.20 ^{de}
	1 × 10 ⁹	70.00 ^e	14.56 ⁱ	1.04 ^f	0.25 ^f
<i>T. harzanium</i>	0	95.00 ^{ab}	24.64 ^a	4.38 ^a	4.74 ^a
	1 × 10 ⁸	91.67 ^{abc}	22.89 ^{a-d}	3.68 ^b	0.75 ^{ef}
	1 × 10 ⁹	76.67 ^{de}	18.35 ^{gh}	2.20 ^{de}	0.52 ^{ef}
<i>T. longibrachatum</i>	0	98.33 ^a	24.84 ^a	4.37 ^a	4.84 ^a
	1 × 10 ⁸	95.00 ^{ab}	24.29 ^{ab}	3.47 ^b	1.30 ^{de}
	1 × 10 ⁹	90.00 ^{abc}	22.40 ^{a-e}	2.29 ^d	0.56 ^{ef}
<i>T. viride</i>	0	96.67 ^{ab}	24.68 ^a	4.39 ^a	4.80 ^a
	1 × 10 ⁸	96.67 ^{ab}	24.12 ^{abc}	3.90 ^{ab}	2.95 ^b
	1 × 10 ⁹	76.67 ^{de}	16.53 ^{hi}	1.86 ^{de}	0.36 ^f
MSD, α 0.05		12.967	3.1586	0.5669	0.8261
CV (%)		4.843	4.763	5.654	11.731

Note: Means followed by the same letters in each column are not significantly different (*p* < 0.05) according to Tukey's test; *A*: *Aspergillus*; *T*: *Trichoderma*, GP: Germination percentage; GI: Germination index; PL: Plumule length; RL: Radicle length; SVI: Seedling vigor index.

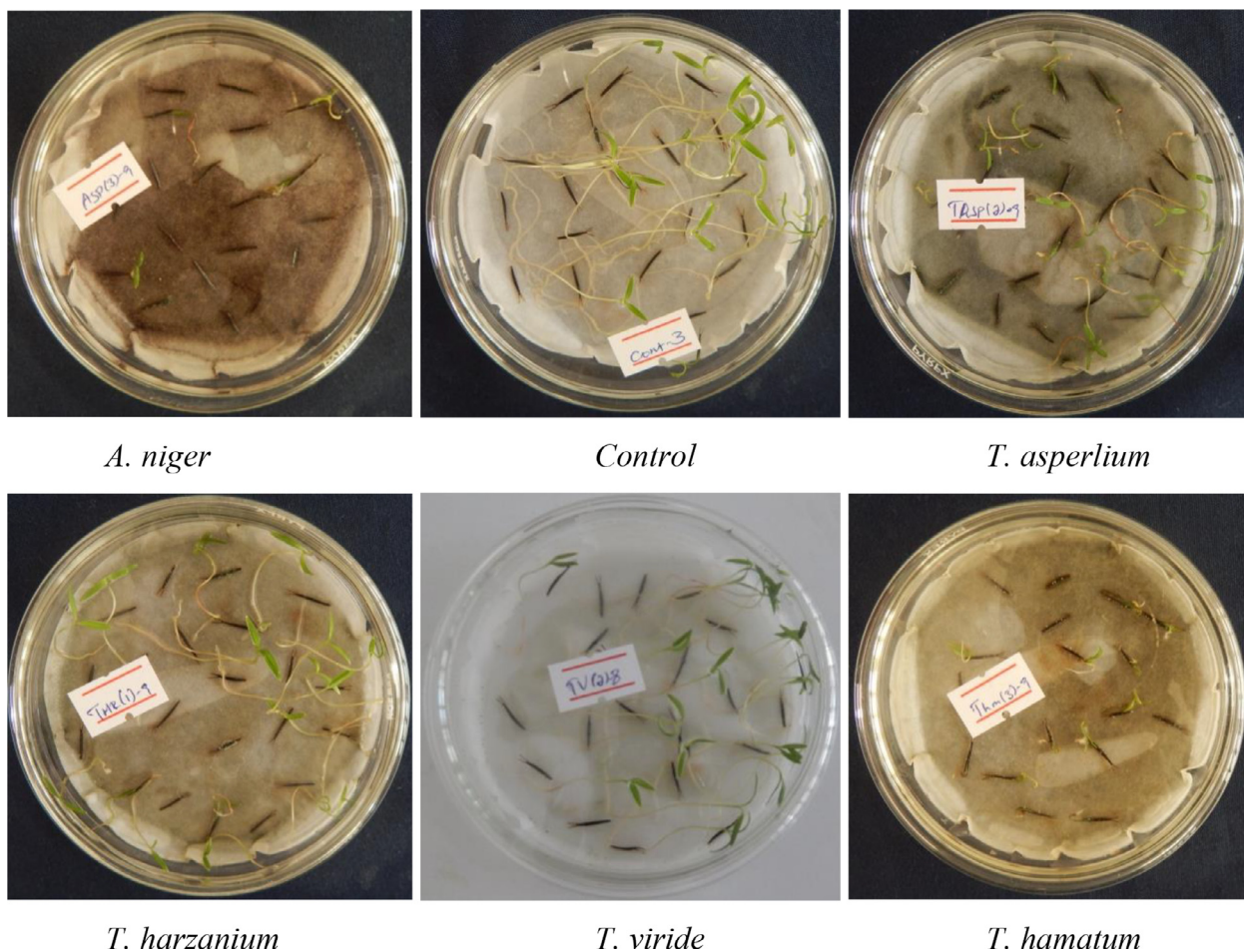


Fig. 3. Herbicidal activity of fungal conidia suspensions after seven days of treatment on germination and early growth of *Bidens pilosa*; *A*: *Aspergillus*; *T*: *Trichoderma*.

more sensitive to fungal conidia suspensions than the plumule length. So, about 97% of the fungal species were significantly

reducing radicle length (ranging from 51 to 97%) as compared to plumule height, for which about 57% of the tested fungal species

reduced the plumule height within a range of 34 to 85% as compared to control.

The analysis of variance showed that there was a clear significant effect of fungal species and their concentration levels on seedling vigor index (SVI) (Fig. 4) and statistically, there was a highly significant difference ($p < 0.001$) among the treatments. Almost all the conidia suspensions of the tested fungal species were highly inhibiting (ranging from 25 to 97%) the SVI of *B. pilosa*. The inhibition was concentration-dependent; so that as concentration increased, the seedling vigor index decreased for all fungal conidia suspensions. The least SVI value (25.77) was obtained from Petri dishes treated with *A. niger* at 1×10^9 conidia mL^{-1} concentration. Similarly, the inhibitory effects of *T. hamatum* on SVI were reaching a high reduction percentage at the concentration levels of 1×10^9 conidia mL^{-1} in comparison to control.

3.2. In-vivo bioassay

3.2.1. Inhibitory effects of fungal conidia suspensions on shoot and root length of *Bidens pilosa*

Based on their assessed efficacy in the laboratory bioassay, foliar application of conidia suspensions from five selected fungal species namely *A. niger*, *T. asperillum*, *T. atroviride*, *T. hamatum* and *T. viride* were further evaluated in the pot experiment on 4-weeks-old plants and applied 3 times applications at 4 days intervals under the lath-house conditions against seedling growth as well as dry biomass yields of *B. pilosa* plant. As post-emergence phytotoxicity potential, the effect of tested fungal species and their application frequency showed non-significant variations against seedling shoot height, 14 days after treatment (Table 3). Although the conidia suspensions of fungal species statistically did not induce a significant variation on shoot height of the target weed, conidia suspension of *A. niger* slightly inhibited shoot height up to 8% when compared with the control treatments. The tallest shoot (20.83 cm) was calculated from the pots treated with *T. viride* which was followed by the control (20.33 cm) on average shoot measurement.

Unlike the shoot height, the main effect of fungal species on root length was significant ($P = 0.0065$). However, the effect of application frequency, as well as the interaction of fungal species and application frequency on shoot dry was insignificant ($p = 0.5796$). Even though there was no significant difference among the treatments on root length regardless of application frequency, the conidia suspension of *A. niger* had herbicidal activity

causing up to 11.78% reduction in root length as compared to control. On the other hand, conidia suspension of *T. viride* slightly enhanced the root length of the target weed over control.

3.2.2. Effects of fungal conidia suspensions on the shoot and root dry biomass of *Bidens pilosa*

The result showed that only the main effect of fungal suspensions was significant on shoot dry weight, but the effect of application frequency, as well as the interaction of fungal species and application frequency on dry biomass, was insignificant (Table 3 and Fig. 5). Though the influence of fungal species was statistically insignificant, however, *A. niger* and *T. hamatum* exhibited slight herbicidal activities where the three times application reduced the shoot dry biomass by 17.78% and 13.65% as compared to the untreated control, respectively (Fig. 5). Similarly, these two fungi reduced the root dry biomass by 25% and 15% as compared to control, respectively.

4. Discussion

The present study was designed to try managing *Bidens pilosa* weed using alternative natural resources. For this purpose, conidia suspensions of seven pathogenic fungal species were evaluated against germination and early seedling growth of the target weed in a laboratory bioassay. The potential herbicidal properties of selected fungal conidia suspensions based on their efficacy in the laboratory bioassay were further evaluated under lath-house conditions on seedling length as well as dry biomass of the same weed. The results of the laboratory bioassays indicate that the herbicidal constituents present in these fungi are highly effective against the target weed and significantly reduced seed germination as well as early growth of the tested weed in a concentration-dependent manner. This finding is in agreement with those found by (Toderò et al., 2019), where the metabolites from *Phoma* spp showed an inhibitory effect on the germination of *B. pilosa* seeds. Similarly, the bioherbicidal activity of *Trichoderma* and *Aspergillus* species against parthenium has been well documented (Javaid et al., 2013, 2014). The herbicidal activity of *Alternaria japonica* prepared in different growth media against parthenium was reported by Javaid et al. (2017). Bashir et al. (2018) also found that secondary metabolites of *A. niger* reduced germination of parthenium seeds by more than 90% when used original metabolites. Recently, Ahmad et al. (2020) reported that *Aspergillus* spp reduced the rate

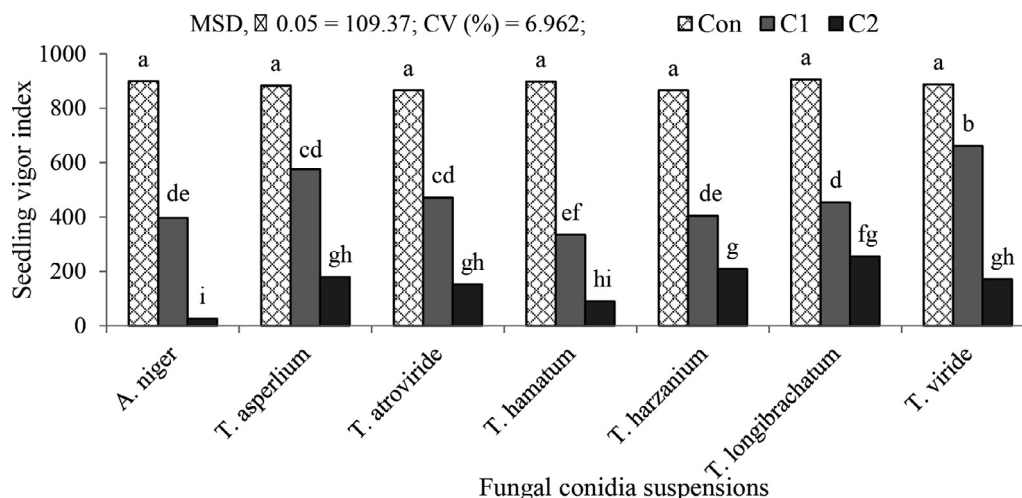


Fig. 4. Herbicidal efficacy of fungal conidia suspensions on *Bidens pilosa* weed seedling growth under laboratory conditions; means followed by the same letters are not significantly different ($p < 0.05$) according to Tukey's test; A: *Aspergillus*; T: *Trichoderma*; Con: Control; C2: 1×10^9 conidia mL^{-1} .

Table 3
Analysis of variance for the effect of different fungal conidia suspensions on seedlings growth of *Bidens pilosa* in the pot experiments.

Source of variation	df	Mean squares			
		Shoot length	Root length	Shoot dry weight	Root dry weight
Fungal species (S)	4	2.060 ^{NS}	13.728 ^{**}	0.482 [*]	0.085 ^{NS}
Application frequency (Fr)	3	0.711 ^{NS}	2.979 ^{NS}	0.197 ^{NS}	0.060 ^{NS}
S X Fr	12	0.973 ^{NS}	2.857 ^{NS}	0.111 ^{NS}	0.041 ^{NS}
Error	38	1.238	3.272	0.128	0.113
Total	59	–	–	–	–

Note: *,**significant at $p \leq 0.01$ and 0.001 , respectively; NS: Non significant.

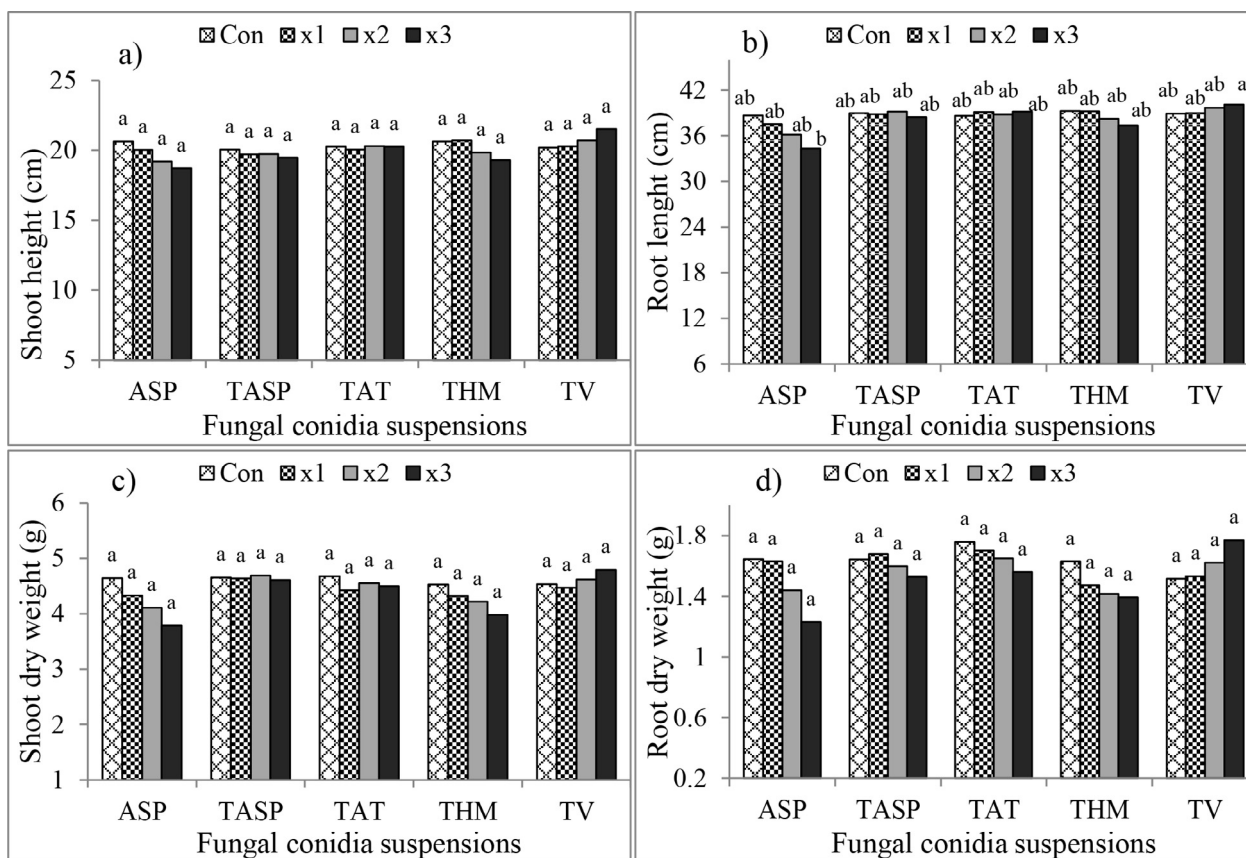


Fig. 5. Effect of foliar spray of conidia suspensions of five fungal species on shoot height (a), root length (b), shoot dry weight (c) and root dry weight (d) of *Bidens pilosa*; means followed by the same letters for a particular parameter are not significantly different ($p < 0.05$) according to Tukey's test; ASP: *Aspergillus niger*; TASP: *Trichoderma asperlium*; TAT: *Trichoderma atroviride*; THM: *Trichoderma hamatum*; TV: *Trichoderma viride*; Con: Control; 1x: sprayed once; 2x: sprayed twice; 3x: sprayed thrice.

of germination of *Chenopodium album*, *Avena fatua* and *Convolvulus arvensis*.

The results of the present study indicated that conidia suspensions of the tested fungal species exhibited variable herbicidal activity against the germination and early growth of the target weed. The conidia suspensions of *A. niger* showed better herbicidal activity, resulting in up to a 65% reduction in seed germination of the target weed. It implies that either applied conidia from the conidia suspensions or exudates released in the suspensions while the fungal growth was active to induce herbicidal activities against the tested weed (Bashir et al., 2018). As stated by Ahmad et al. (2020), the herbicidal effect of fungal filtrates is mainly due to the phytotoxins produced by fungal species.

On the other hand, the conidia suspension of *T. longibrachatum* failed to exhibit any pronounced adverse effect against the germination of *B. pilosa*. Plumule and radicle length of *B. pilosa* seedlings were, however, highly inhibited by all the tested fungi to variable extents. In this regard, *A. niger* was found to be most effective fol-

lowed by *T. hamatum*. This fact seems in line with earlier findings of Javaid and Ali (2011) reported that culture filtrates of *T. harzianum* and *T. pseudokoningii* significantly inhibited the shoot and root growth of *Avena fatua* seedlings. Similarly, Mohammed and Badawy (2020) currently reported the herbicidal activity of crude filtrates of fungal species that significantly reduced radicle length and plumule height of weeds over control. According to Ahmad et al. (2020), the herbicidal effect of fungal filtrates is mainly due to the phytotoxins produced by these fungi. In general, the conidia suspensions of *A. niger* showed remarkable herbicidal effects on the germination, plumule and radicle length of the target weed in a laboratory bioassay. This fungus showed approximately a 17% to 65% reduction in germination, a 15% to 85% reduction in plumule length and a 74% to 97% reduction in radicle length of the target weed. The present finding also revealed that radicle length was relatively more sensitive to conidia suspensions than the plumule length. This is in line with earlier findings by Akbar and Javaid (2010), who stated that the root growth was more susceptible to

the application of culture filtrates of *Drechslera* species than shoot length.

In contrast to the results obtained from Petri dishes under laboratory conditions, the effect of foliar spray with conidia suspensions of fungal species on seedlings length as well as dry biomass of *B. pilosa* was non-significant in pot experiments. The non-significant effect of the fungal species on the growth of Black-jack weed could be due to the age of the weed plants or exposure of the natural products to other environmental conditions. The results of the laboratory bioassays in the present study clearly indicate that the herbicidal potential of these fungi was highly effective against very young (less than a week) seedlings. The results of the present study are in line with the previous studies by Akbar and Javaid (2013), who stated that adverse effects of foliar spray on shoot biomass of *Rumex dentatus* were more pronounced in 1-week old than in 2-week old plants. Javaid et al. (2017) stated that herbicidal activity of fungal metabolites decreased with the age of the weed plants possibly because of increased resistance in old plants. The non-phytotoxicity of fungal culture filtrates or metabolites against *C. sativus* and *S. bicolor* in post-emergence has been reported by Daniel et al. (2018).

Although the effect of conidia suspensions of fungal species was the insignificant difference from the controls on the growth parameters of the target weed, the suspension of *A. niger* has slightly inhibited the shoot and root length approximately up to 8% and 12% over control, respectively. Likewise, suspensions of *A. niger* and *T. hamatum* slightly exhibited herbicidal activities and reduced the shoot dry biomass by 17.78% and 13.65% and root dry biomass by 25% and 15% over control, respectively. Similar results have been found in previous studies, where the metabolites of *Diaporthe schinii* showed herbicidal potential on *B. pilosa* (Brun et al., 2020). Ahmad et al. (2020) mentioned that foliar spray of culture filtrate of *Alternaria* spp. inhibited shoot growth of *Convolvulus arvensis*. On the other hand, *T. viride* showed an increase in some growth parameters rather than inhibition as compared to the control. Our investigation is supported by the work of (Machado et al., 2012) who reported that *Trichoderma* species promoted plant growth in agriculture by enhancing hormone production and increase of some nutrient absorption by the plant. Currently, Kakabouki et al. (2021) reported that *Trichoderma* species can enhance plant development, nutrient uptake and resistance to biotic and abiotic stresses.

5. Conclusion

The result of the present investigation revealed that conidia suspensions from different tested fungal species displayed great potential inhibitory effects on germination and early growth of *B. pilosa* weed. The conidia suspensions may contain compounds with some inhibitory potential, which upon release into the aqueous medium, inhibited germination and reduce early growth of the target weed. Therefore, it is pertinent to conclude that conidia suspensions of biocontrol fungal species, especially *A. niger* contains herbicidal constituents and can be used as pre-emergence herbicides for the management of *B. pilosa* in the organic coffee farming system, but further in-vitro and in vivo inclusively of field experiments needs to be performed to isolate and identify these herbicidal constituents for future practical application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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