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RESEARCH ARTICLE

Herbicidal efficacy of culture filtrates of *Alternaria brassicicola* and *Alternaria gaisen* against parthenium weed

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Abstract: Background: *Parthenium hysterophorus* L. is one of the top ten worst weeds globally and is recorded in the global database of invasive species. **Objective:** The current study was aimed to evaluate the herbicidal potential of *Alternaria brassicicola* (Schwein.) Wiltshire and *Alternaria gaisen* Nagano. culture filtrates against a problematic weed *P. hysterophorus*. **Methods:** *A. brassicicola* and *A. gaisen* culture filtrates were tested *in vitro* against the test weed. *A. gaisen* culture filtrates were found most effective against the test weed, and this test fungus was partitioned with various fractions viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol were isolated. *In vitro* bioactivity of these fractions were tested against *P. hysterophorus*. The most productive *n*-hexane fraction was subjected to GC-MS analysis, and thirteen compounds were identified. **Results:** *A. gaisen* original (100%) and diluted (50%) culture filtrates showed significant herbicidal activity against *P. hysterophorus*. However, culture filtrates of *A. gaisen* suppressed the germination, root and shoot growth of the test weed to a greater extent compared with culture filtrates of *A. brassicicola*. Original culture filtrates of *A. gaisen* significantly reduced germination of *P. hysterophorus* by 88% as compared to diluted concentration by 56% in comparison with control. On the other hand, original and diluted culture filtrates of *A. brassicicola* reduced the germination of *P. hysterophorus* by 69% and 50%, respectively, over control treatment. The *n*-hexane fraction was found more effective in suppressing the *P. hysterophorus* growth as compared to other fractions. Both 0.10% and 0.05% concentrations of *n*-hexane fraction significantly inhibited *P. hysterophorus* seedlings germination by 88% and 81%, respectively. The *n*-hexane fraction was subjected to GC-MS analysis, and thirteen compounds were identified. Among these, ocimene (27.63%); benzene 1-ethyl-3-methyl- (20.30%) and *n*-hexadecanoic acid (10.27%) were major compounds. **Conclusion:** The present study concludes that *A. gaisen* culture filtrate has substantial herbicidal potential against *P. hysterophorous*.

Keywords: Alternative herbicides; Fungal metabolites; GC-MS analysis; *in vitro*; Organic fractions

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1. Introduction

Parthenium (*Parthenium hysterophorus* L.), family Asteraceae, is a devastating and dangerous weed of many economically important crops (Ray, Gour, 2012). It is among the top ten worst weeds worldwide and is included in the world catalogue of aggressive species (Callaway, Ridenour, 2004; Kapoor, 2012; Khan et al., 2020). In 1955, it was accidentally introduced through imported food grains in the subcontinent (Hassanein et al., 2008). It is also responsible for significant losses in the agricultural and forestry sector (Knox et al. 2006). *P.hysterophorus* also caused asthma, diarrhea and skin allergy in humans (Natukunda et al., 2020).

Use of commercial synthetic herbicides is considered as the most consistent and common technique for managing weeds. Another approach to control the weeds is to isolate natural herbicidal components from fungi culture filtrates (Zhang et al., 2010). Fungal metabolites can be used as a convenient biological pesticides tool (Cipriani et al., 2009; Sands, Pilgeram, 2009; Javaid et al., 2017; 2022).

The members of Deuteromycetes (fungi imperfecti), specifically *Alternaria* spp. have an extensive distribution in nature and act as saprophytes, endophytes, weak facultative parasites, and plant pathogens (Thomma, 2003). Some metabolites from *Alternaria* fungus are labelled as phytotoxins and mycotoxins for plants and animals, respectively (Duke, Dayan, 2011). *Alternaria* spp. metabolites have drawn the attention of many pharmacologists, chemists and plant pathologists in research programs due to the exhibition of various biological activities including herbicidal potential (Bräse et al., 2009; Tsuge et al., 2013; Bashir et al., 2018). However, studied regarding herbicidal activities of *A. brassicicola* and *A. gaisen* against parthenium weed are lacking. Therefore, this study was planned to explore the herbicidal efficacy of culture filtrates of *A. brassicicola* and *A. gaisen* for Parthenium weed biocontrol.

2. Materials and methods

2.1 Preparation of fungal culture filterate

For the preparation of fungal culture filtrates, malt extract broth (2%) 200 mL was autoclaved at 121 °C for 15 minutes in 500 mL flasks and cool at room temperature. Five-millimetre discs of *A. brassicicola* and *A. gaisen* were added to these flasks and incubated for 15 days at 25 °C. The fungal cultures were filtered through a sterilised muslin cloth and sterilised Whattman No. 1 filter paper (Javaid et al., 2017). The filtered test fungal metabolites were centrifuged at 600 rpm for 5 minutes and then re-filtered with millipore filter paper.

2.2 *In vitro* bioassay of fungal culture filterate

In vitro assessment of fungal metabolites herbicidal activity against test, weed species was carried out following the protocol of Javaid and Ali (2011a; 2011b). Nine-centimetre pre-sterilized Petri plates were taken and lined with sterilised filter papers. Twenty surface-sterilised seeds of *P. hysterophorus* were set in all experimental Petri plates. Two millilitres of 100% and 50% concentrations of culture filtrates of *A. brassicicola* and *A. gaisen* were transferred to every Petri plate. The diluted concentration was prepared using sterilised distilled water in a suitable amount (Akbar, Javaid, 2013). In Petri plates of control treatment, two millilitres of distilled water were added. Each treatment was replicated three times. All the plates were incubated at 25 °C in an incubator with ten h daily light period for seven days. After that, data about germination, shoot and root length and seedling's fresh weight were noted.

2.3 Fractionation of culture filtrates of *A. gaisen*

A. gaisen culture filtrates were found very effective in reducing the *in vitro* growth of *P. hysterophorus*. Fifty millilitres of this fungal culture filtrate was taken for partitioning (Jabeen et al., 2014). Bioactive compounds in this filtrate were separated with several organic solvents like *n*-hexane, chloroform, ethyl acetate and *n*-butanol using a separating funnel. All the separated fractions were evaporated on a rotary evaporator which resulted in 0.02 g *n*-hexane, 0.01 g chloroform, 0.12 g ethyl acetate and 0.14 g *n*-butanol fraction.

2.4 *In vitro* bioassay with isolated fractions of culture filtrates of *A. gaisen*

The *in vitro* bioactivity of these four isolated fractions was studied. Two concentrations (0.10% & 0.05%) of each fraction were tested against *P. hysterophorus*. The experiment was conducted by the addition of 0.07 mg and 0.03 mg of all crude organic fractions and raised the final volume to 15 mL. Control medium was without any extract. All the treatments were replicated thrice.

2.5 Gas chromatography-mass spectrometry (GC-MS) analysis

A. gaisen culture filtrates *n*-hexane fraction was analysed on GC-MS chromatograph (GC-MS-QP 2010) to separate bioactive compounds against *P. hysterophorous*. This *n*-hexane fraction was filtered through nylon membrane filters of 0.22 µm pore size and 47 mm diameter by using filtration assembly. Chromatograph separated with the DB-5MS capillary column (0.25 µm, 0.25 mm, 30 m) was used to analyse the sample. Helium gas was used as a carrier gas and following program temperatures 40 °C for 5 min, 40-70 °C at 2 °C/min, 70 °C for 2 min, 70-120 °C at 3 °C/min, 120-150 °C at 5 °C/min, 150-220 °C at 10 °C/min and then 220 °C for 2 min were applied. The temperatures of the detector and injector were 250 °C and 200 °C, respectively. The mass detector conditions were: 70 ev ionisation voltage, m/z 29-540 mass scanning range and 230 °C base temperature. GC peak areas were used to compare the percentage configuration of volatile constituents. Qualitative analysis was done on software NIST Library 2010 (Sureshkumar et al., 2012) based on mass spectra, comparison of indices and retention times with the analogous data in the previous literature.

2.6 Statistical analysis

All the obtained data were statistically analysed at 5% level of significance by ANOVA (analysis of variance) and Duncan's Multiple Range Test (Steel, Torrie, 1980).

3. Results and discussion

In this study, the effect of culture filtrates of *Alternaria* spp. viz. *A. gaisen* and *A. brassicicola* were observed on germination and seedling growth of *P. hysterophorus*. Herbicidal effect of original (100%) and diluted (50%) culture filtrates of *A. gaisen* was more pronounced than culture filtrates of *A. brassicicola*. The original and diluted concentrations of culture filtrates of *A. gaisen* showed remarkable effect by inhibiting the germination of parthenium by 88% and 56%, respectively, compared with control. Whereas original culture filtrate of *A. brassicicola* reduced *P. hysterophorus* germination by 69% and its diluted culture filtrate reduced germination by 50% of the tested weed specie as compared to the control treatment (Figure 1).

In case of other growth parameters also both concentrations of *A. gaisen* showed a significant reduction in shoot length, shoot fresh weight, root length, root fresh weight and the decrease was 84-99%, 65-99%, 81-96% and 59-98% respectively (Figure 2A & B).

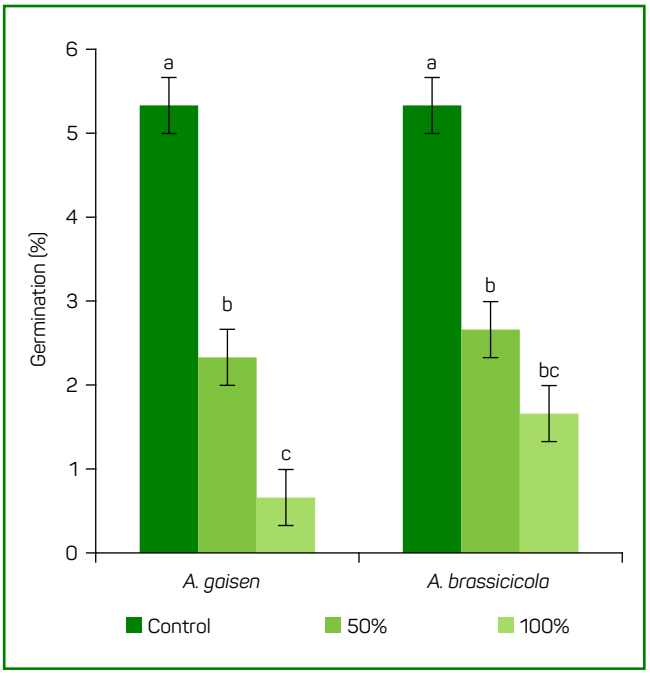

Figure 1 - Effect of 100% and 50% concentrations of *A. gaisen* and *A. brassicicola* metabolites on germination of Parthenium seeds

On the other hand, culture filtrates of *A. brassicicola* reduced these growth parameters by 63-80%, 25-40%, 62-73%, 52-59%, respectively (Figure 3A & B). The metabolites of *A. alternata, Fusarium solani*, *Drechslera rostrata, Trichoderma viride, T. pseudokoningii* and *T. harzianum* significantly retarded the parthenium and other weed species seed germination (Javaid, Ali 2011a; 2011b). Culture filtrates of *Cladosporium oxysporum*, *Macrophomina phaseolina* and *Fusarium equisetti* also significantly inhibited parthenium's *in vitro* germination (Idrees, Javaid, 2008).

Results regarding the herbicidal activity of *n*-hexane, chloroform, ethyl acetate and *n*-butanol fractions of *A. gaisen* culture filtrates against the test weed species *P. hysterophorous* are illustrated in (Figure 4). Data obtained after a seven-day incubation period revealed that the *n*-hexane fraction displayed the best herbicidal activity compared to other tested organic fractions. The applied concentrations of *n*-hexane fraction viz. 0.10% and 0.05% significantly inhibited the germination of test weed by 88% and 81%, respectively. This variable herbicidal expression of the applied fractions of fungal metabolites might cause the solubility of various compounds in different organic solvents (Zonno et al., 2008). The herbicidal efficacy of *Alternaria* species could be owed to the production of some secondary metabolites, some of which are powerful mycotoxins (Thomma, 2003). Like *A. alternata* produced a phytotoxin AAL-toxin which has the potential of suppressing the growth of numerous weeds (Abbas et al., 1995).

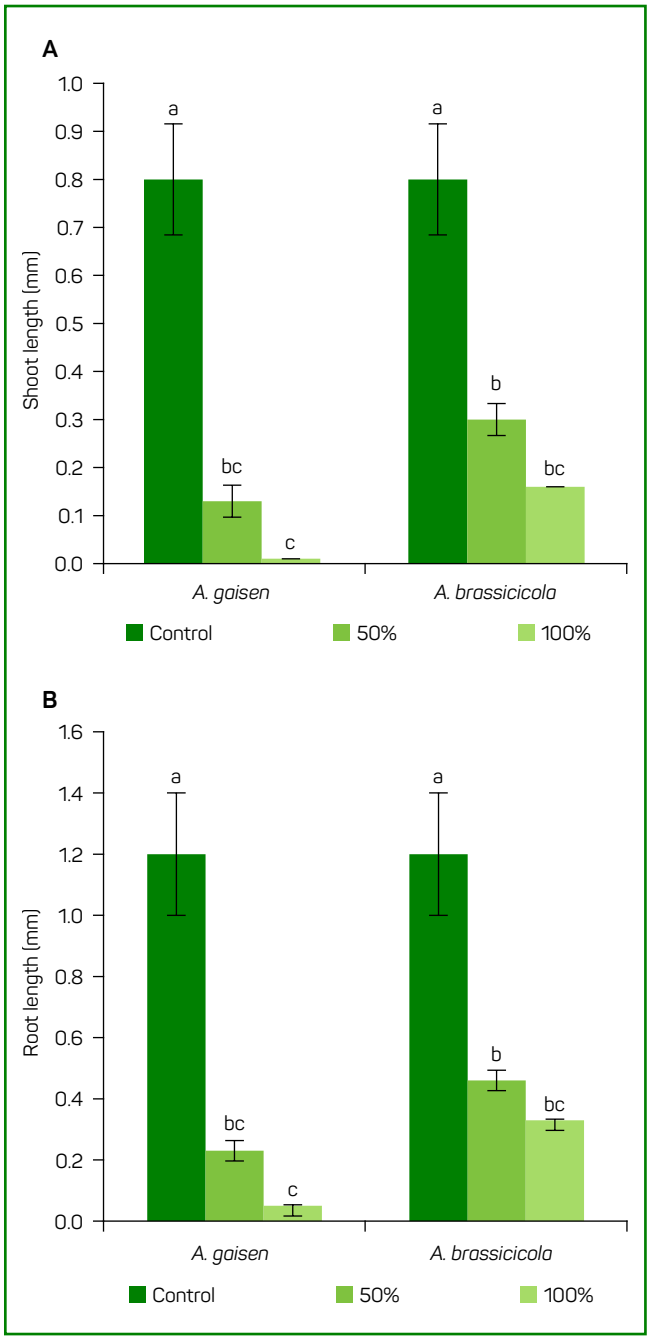

Figure 2 A, B - Effect of 100% and 50% concentrations of *A. gaisen* and *A. brassicicola* metabolites on root & shoot length of Parthenium seeds

Thirteen compounds were identified in the GC-MS analysis of *n*-hexane fraction of *A. gaisen* culture filtrates (Table 1). The compounds ocimene (27.6%), benzene 1-ethyl-3-methyl- (20.3%) and *n*-hexadecanoic acid (10.22%) were identified as major constituents. Other identified compounds were 3-trifluoroacetoxypentadecane (6.4%), trichloroacetic acid 2-tetradecyl ester (6.40%), Z,Z,Z-,4,6,9-nonadecatriene (4.4%), 2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol (4.1%), 2-nitrohept-

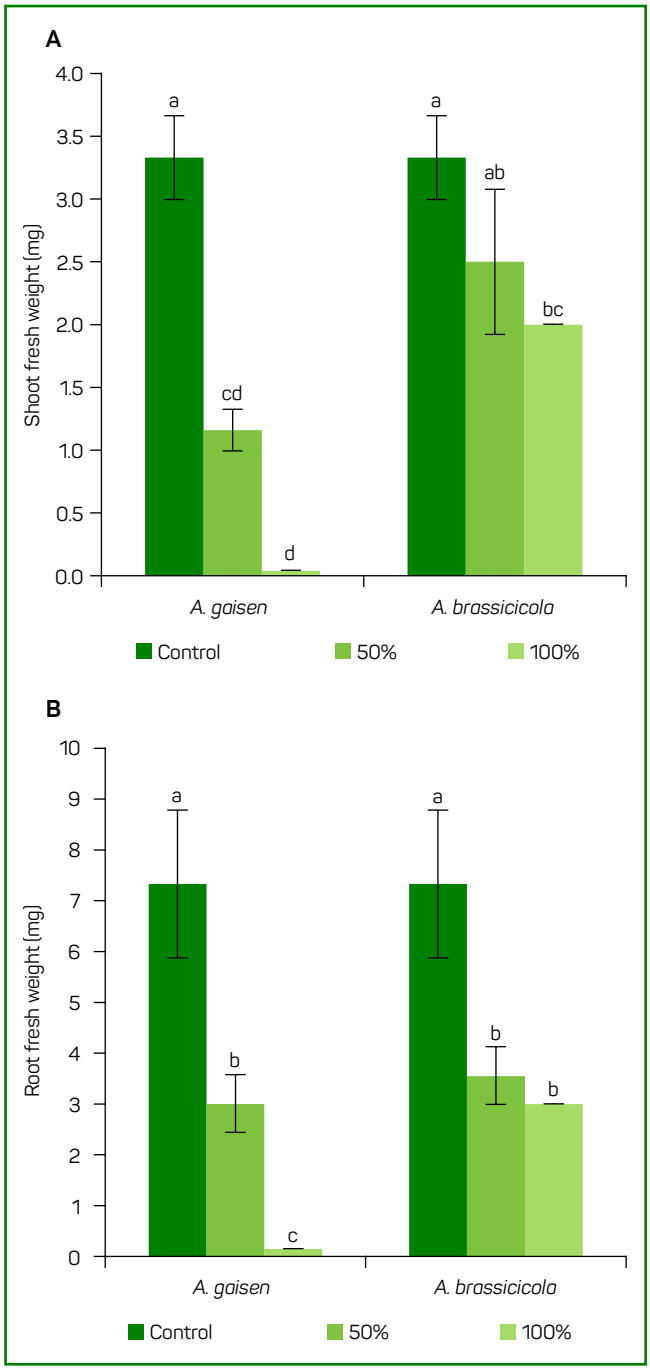

Figure 3 A, B - Effect of 100% and 50% concentrations of *A. gaisen* and *A. brassicicola* metabolites on root & shoot fresh weight of Parthenium seeds

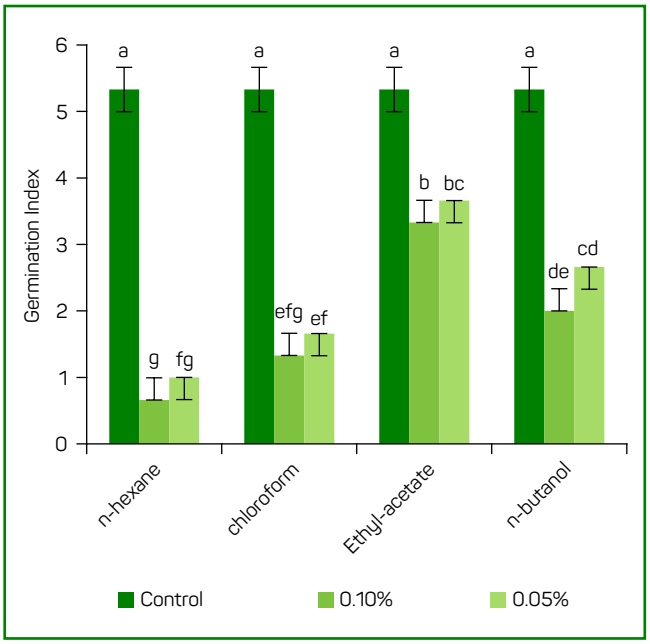

Figure 4 - Result of various organic fractions concentrations of *A. gaisen* metabolite's on *in vitro* growth of *P. hysterophorus*

2-en-1-on (4.1%), Z,Z,Z-1,4,6,9-nonadecatetraene (4.0%), oleic acid (3.2%), 3-trifluoroacetoxytetradecane (3.2%), nonadecane (3.1%) and 3,7,11,15-tetramethyl-2-hexadecene-1-ol (2.7%). Javaid et al. (2019) also identified ten bioactive compounds from *n*-hexane fraction of *A. japonica*. Ocimene a monoterpene compound is well known for its antimicrobial activity. The antimicrobial effects of ocimene were also tested on economically important microbes, and promising results were found (Pirbalouti et al., 2016; Mahdian et al., 2017). The second major isolated compound was benzene 1-ethyl-3-methyl- which is an aromatic hydrocarbon). This compound possesses substantial antifungal and antibacterial property (Vukovic et al., 2007). *n*-hexadecanoic acid a saturated fatty acid was also found in the GC-MS analysis in the present study. *n*-hexadecanoic acid was isolated from many plants and hold herbicidal and fungi toxic effects (Kordali et al., 2009; Shirani et al., 2017). 3-trifluoroacetoxypentadecane a bioactive compound produced fungi-static effects against *Aspergillus terreus*. Oleic acid, an unsaturated fatty acid identified in the present study, was found to establish resistance in plants and microorganism and be used as a pesticide (Pohl et al., 2011). The substantial herbicidal effects of 3,7,11,15-tetramethyl-2-hexadecen-1-ol isolated from *Mikania micrantha* were also deliberated by Ni et al. (2007).

4. Conclusion

This syudy conclude that the herbicidal potential of *A. gaisen* against *P. hysterophorus* could be attributed to the presence of many significant compounds present in this tested fungus. Furthermore, *A. gaisen* can effectively be used as an alternative to chemical herbicides to manage this problematic weed *P. hysterophorus*.

Authors' contributions

All authors have read and agreed to the published version of the manuscript. KJ, and AJ: conceptualization.

Table 1 - GC-MS analysis of *n*-hexane fraction of culture filtrates of *A. gaisen*

Sr. #	R. Time	Compound Name	Molecular Formula	Molecular Weight	Peak Area (%)
1	**5.223**	Benzene 1-ethyl-3-methyl-	C_9H_{12}	120	20.304
2	5.81	Ocimene	$C_{10}H_{16}$	136	27.632
3	7.651	Trichloroacetic acid, 2-tetradecyl ester	$C_{16}H_{29}Cl_3O_2$	360	6.404
4	8.909	2-Nitrohept-2-en-1-ol	$C_7H_{13}NO_3$	159	4.092
5	10.345	3-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	310	3.208
6	12.132	2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	$C_{11}H_{200}$	168	4.148
7	12.831	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	6.427
8	13.32	Z,Z,Z-,4,6,9-Nonadecatriene	$C_{19}H_{34}$	262	4.397
9	14.295	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260	4.004
10	15.626	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{400}$	297	2.742
11	17.813	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.277
12	20.084	Oleic acid	$C_{18}H_{34}O_2$	282	3.246
13	21.422	Nonadecane	$C_{19}H_{40}$	269	3.119

TK: methodology, investigation, data curation, writing, original draft preparation. KJ, TK, AJ, and SI: software, formal analysis. KJ, AJ and SI: validation. KJ, and TK: writing—review and editing.

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