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Allelopathic effects of native plant species *Dicranopteris dichotoma* on invasive species *Bidens pilosa* and *Eupatorium catarium*

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ABSTRACT

We studied the inhibitory effects of native plant *Dicranopteris dichotoma* (Thunb.) Bernh on the growth and development of two invasive weed species i.e. *Bidens pilosa* L. and *Eupatorium catarium* Veldkamp in Hainan Province, China. The effects of aqueous extracts of leaf, stem and root of *D. dichotoma* were examined on the seeds germination and seedlings growth of two invasive test species. Increasing extracts concentration of leave, stem and root of *D. dichotoma* decreased the root length, shoot length and dry weight of *E. catarium* as well as *B. pilosa*. The 1.5 % leaf extract of *D. dichotoma* proved most inhibitory to seeds germination, survival rate and dry weight of *E. catarium*, while inhibitory to seeds germination, survival rate, root and shoot length, and dry weight of *B. pilosa*. GC-MS analysis revealed the highest percentage of 2,3-Butanediol (34.65 %) in N-trimethane extract while in carbinol leave extract phenol,2,4-bis(1,1-dimethylethyl)- (28.36 %) was present in highest percentage. In N-trimethane extract of stem, the highest percentage was of 2,3-Butanediol (78.14 %). While in carbinol stem extract, there was highest percentage of Dodecanoic acid, 1,2,3-propanetriyl ester (41.52 %). In the root extract of N-trimethane the highest percentage was of 2,3-Butanediol, (R-(R*,R*)- (91.68 %). In carbinol root extract, the highest percentage was of Dodecanoic acid, 1,2,3-propanetriyl ester (69.25 %). Allelopathic effects of extracts from this native plant *D. dichotoma* on two invasive species may be caused by the high concentration of compounds identified by GC-MS analysis.

Key words: Allelopathy, *Bidens pilosa*, carbinol, chemical compounds, *Dicranopteris dichotoma*, GCMS, *Eupatorium catarium*, interaction, invasive species, leaf, N-trimethane, native species, root, seeds germination, seedling growth, stem.

INTRODUCTION

Biological invasions has caused tremendous damages to environment, agriculture and biodiversity of natural ecosystems worldwide (11,32,39,40,42,56). Some native plants should be utilized to inhibit the invasive species as effective biological prevention method to control the invasion (25,46). Asteraceae has many most troublesome, invasive weeds, because (i). produce small but numerous viable seeds every year, (ii). wind-assisted seed dispersal and (iii). rapid growth (3,28,30). *E. catarium* (Family 'Asteraceae') is perennial weed native to South America. and spreads through seeds. It causes heavy losses in pastures, croplands and gardens (30,43). *B. pilosa* (Family 'Asteraceae') is native to Americas and now widely distributed in other regions of the world. The seeds are short with stiff hairs, which get stuck in feathers, fur, or socks, etc. and spread throughout the

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warmer regions of the world (4,16). Many invasive species are found in China; among them, *B. pilosa* and *E. catarium* are widely found in Hainan Province due to tropical Island (Figure 1) (31,50). Both species are inhibitory to Chinese flora (5) and fauna. The growth of both these species is very fast that covers the empty areas of the fields (22,48).

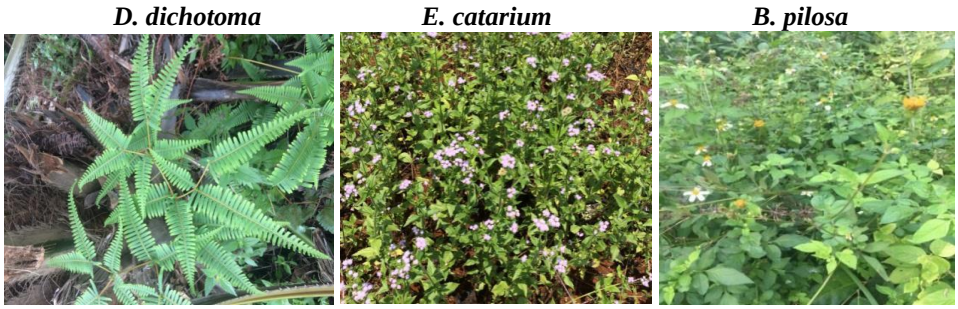

Figure 1. Photographs of Donor native plant (*D. dicholoma*) and Recipient weeds (*E. catarium* and *B. pilosa*).

The plants have detrimental effects on other plants through the production and action of toxic chemical compounds is called allelopathy. These compounds act as fighting agents against competing plants, are environmentally friendly for weed control thus leads to discovery of new herbicides (20). Nowadays, allelopathy has significant role in research involving sustainable agriculture, like biological weed control (13). Recently much progress has been made in identification of potentially important allelochemicals produced by crops. Many genes have been identified, which are involved in various chemical pathways to produce allelochemicals thus providing the potential to enhance the allelopathy by molecular breeding or by transgene technology, e.g. genes encoding enzymes of the highly potent phytotoxin sorgoleone in *Sorghum* spp. might be transgenically manipulated to enhance the allelopathic properties of sorghum crop (12,45). Understanding the interactions between the invasive plants and their novel natural enemies is the central aspect of mechanism that underlies the success of plant invasions and control of invasive species (14,24). To control two invasive species (*E. catarium, B. pilosa*), a native specie *D. dichotoma* in Hainan Island, China was selected to inhibit their establishment. *D. dichotoma* (Family 'Gleicheniaceae') is perennial fern native to Southern China, also distributed in tropical to temperate regions of the world (41). The plant specie can grow, resist and tolerate very poor to acidic soils. It dominates the plant communities, grows in layers and form pure patches in forest floor that plays an important role in ecosystem dynamics (18,47,52). *D. dichotoma* grows fast under high light intensity, which is important in the areas, where forests are removed due to anthropogenic pressure of agriculture production and increasing industrialization (39). *D. dichotoma* have allelopathic effects, preventing the growth of other plants (23,31).

This study aimed to test the allelopathic effects of different concentrations (0, 0.5, 1.0 and 1.5 %) of extracts from the native species *D. dichotoma* on two invasive species *E. catarium* and *B. pilosa* and to explore the possibility of plant *D. dichotoma* to control the growth of these two invasive species (31).

MATERIALS AND METHODS

Plant Materials

In July 2017 to June 2018, 5.0 kg fresh leaf, stem and root samples of *D. dichotoma* were collected from Yunlong Forestry Breeding Base, Hainan Province Forestry Institute (110°28'E 19°52'N) and altitude of 37.60 ± 1.5 m above sea level. The materials were washed with distilled water, air-dried in shade and cut into pieces (< 2cm) and then grinded into fine powder in Grinding machine. The samples were soaked in 4 L of distilled water for 2 days with stirring every15 min in at 25°C (53). The resulting water extract was filtered with three layered cheese cloth, then filtered by 11.0 mm double loop quality filter paper in conical flask. The extracts were stored at 4°C in clean plastic bottles until further use (2,15,34,51).

Extraction of Chemicals for GC-MS Analysis

For extraction, *D. dichotoma* samples were dried at 60 ℃ in oven till constant dry weight (7). Extracts for GC-MS analysis were prepared by separately suspending 50.0 g of each leaf, root and stem of *D. dichotoma* dry samples in 1.0 L distilled water for 48 h at 25 °C in sterile environment. The resulting water extract was first filtered with three layered cheese cloth, then filtered by 11.0 mm double loop quality filter paper and finally passed through microfiltration membrane micro pore (0.45 µm). The filtered supernatant was condensed to 1.0 ml at 70°C by rotary evaporation, then freeze-dried and finally extracted with N-trimethane and carbonyl and stored for GC-MS analysis.

Laboratory Bioassay

This experimental treatments consisted of 3-Factors: (i). 3-samples of *D. dicothoma* leaf, stems and roots, (ii). 2-invasive species *E. catarium* and *B. pilosa* and (iii). Extracts concentrations 4: control distilled water, 0.5 %, 1 % and 1.5 % g/ml. The treatments were replicated thrice in completely randomized design (24). Seeds of *E. catarium* and *B. pilosa* were sterilized in 2 % sodium hypochlorite for 15 min and rinsed thrice in distilled water (10). Fifteen uniform seeds of *E. catarium* and *B. pilosa* were kept equidistant for germination in sterilized 9 cm dia Petri dishes on filter paper (Whatman No. 1) (10). The Petri dishes were kept under laboratory conditions (temperature 24 ± 1°C and light during day) for 15-days (Figure 2,3). Samples were moistened with 10 ml of different concentrations of extracts as per treatments. Ten ml distilled water was added in dishes when moisture content of the filter paper decreased (1,8,9).

$$\text{Germination Index (GI)} = \Sigma\ (Gt/Dt),$$

Where, Gt: Number of seeds that germinated t days after planting, and Dt : Days after germination.

The magnitude of inhibition and stimulation was denoted by the response index (RI) and calculated as under:

$$RI = T/C-1,$$

Where, T: Treatment data and C: Control data. RI > 0 indicates stimulation and RI < 0 indicates inhibition (54).

GC-MS analysis

The extracts of the *D. dichotoma* leaf, stems and roots were freeze-dried and extracted with N-trimethane (52) and carbonyl. Then, the extracts contents were analyzed using a Thermo Quest TRACE GC/MS system (Trace 2000, Thermo Finnigan) equipped

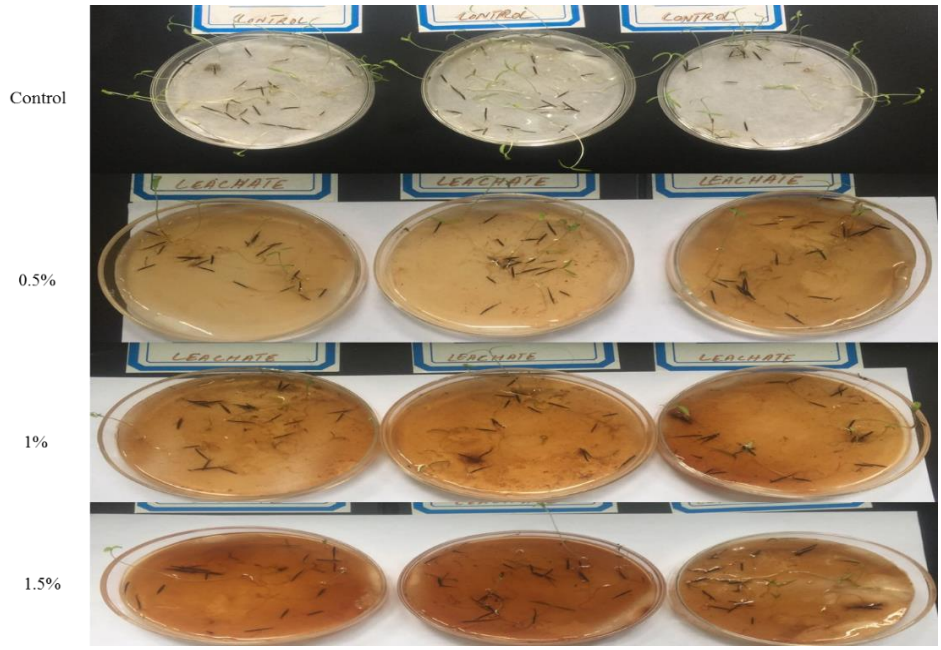

Figure 2. Effects of *D. dichotoma* leaf, stem and root leachates 0 %, 0.5 %, 1 % and 1.5 % on *B. pilosa*.

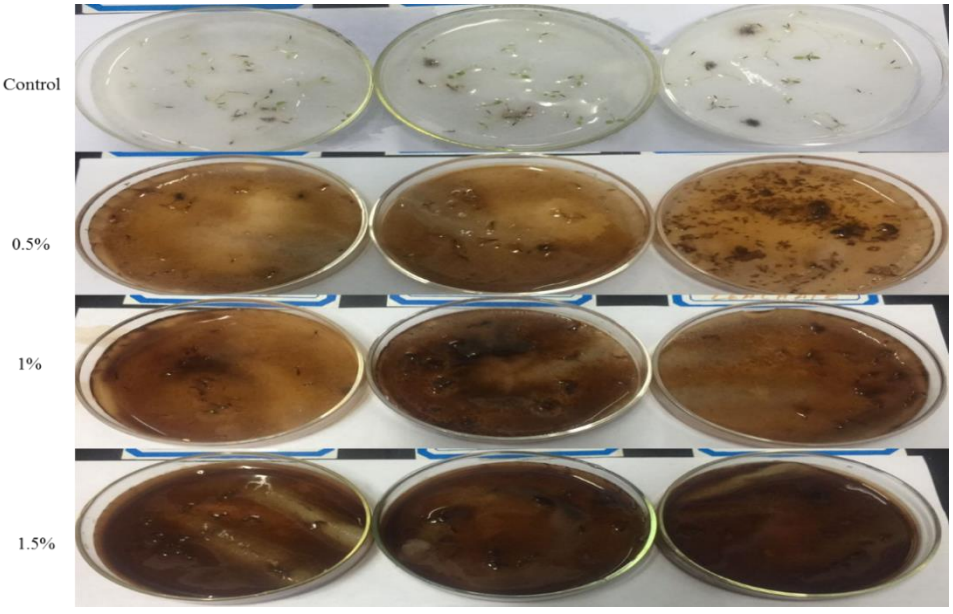

Figure 3. Effects of *D. dichotoma* leaf, stem and root leachates 0 %, 0.5 %, 1 % and 1.5 % on *E. catarium.*

with a programmable split injector (port temperature of 250°C throughout the run) and X calibur analysis software. A PLB-5 (30 M×0.025MM×0.25µM) GC column was used with following temperature program: 70 to 270 °C at rate 10°C min^{-1}. Helium was the carrier gas at a flow rate of 1 ml min^{-1}. The sample injection volume was 1 µL. The Thermo Quest TRACE mass spectrometer was equipped with an ion source (EI+, 70 eV) and operated in full scan mode (59.60 - 480.40 atomic mass units). MS condition were as follows: inlet line temperature, 350 mA; and solvent delay, 3min. Relative quantity of the chemical compounds present in each of the extracts of *D. dichotoma* leaf, stems and roots was expressed as percentage based on peak area produced in the chromatogram (36,54).

Statistical Analysis

The data was statistically analyzed using the one way analysis of variance (ANOVA) by statistics 8.1 software.

RESULTS AND DISCUSSION

The allelopathic inhibitory effects of one plant on another through release of chemicals into the environment has been reported (17,33,45,51). For bioassays, plant extracts (allelochemicals exudates) of different concentrations are used in allelopathy studies. The allelopathic potential of plants is due to allelochemicals, whose, synthesis continues throughout the year (21,44).

Seed germination and seedling growth

B. pilosa : The stem and leaf extracts of *D. dichotoma* decreased the seed germination of *B. pilosa* and decrement increased with the increase in extract concentration. The seed germination inhibition was high (84.85 %) in leaves extract at 1.5 % concentration (Figure 5), while, low in stem extraction (32.65 %) at 0.5 % concentration. It is clear from the result that 1.5 % leaf extract increased the magnitude of inhibition (95.83 %). The results for root length showed the maximum inhibition (95.83 %) with highest (1.5 %) leaf extract concentration (Figure 5). The leaf extract at 1.5 % also caused highest inhibition (95.83 %) in shoot length. The result revealed that dry weight significantly decreased to 92.36 % by 1.5 % leaf extract. Thus leaf extracts of *D. dichotoma* significantly inhibited the seed germination and other growth parameters of *B. pilosa*.

E. catarium**:** Results showed that 1.5 % leaves extract of *D. dichotoma* was most inhibitory (84.98 %) to seed germination followed by 1.5 % root extract (82.41 %) (Figure 4). The survival was completely inhibited (100 %) at 1.5 % leave extract, while, minimum inhibition (91.88 %) was with 1.5 % stem extract. The 1.5 % root extract caused maximum inhibition (90.90 %) in root length, while, minimum inhibition (54.29 %) was with 0.5 % stem extract. The shoot length was most inhibited (89.47 %) by 1.5 % root extract. In dry weight, highest inhibition (100 %) occurred with 1.5 % leaves extract (Figure 4). The increasing concentrations of extracts increased the level of inhibition for all parameters i.e. concentration dependent. These results are correlated with findings of Xuan, (52) who reported significant inhibition in germination and dry weight of radish. There was little inhibition from the root and stem extract. The leaf extract of *D. dichotoma* significantly decreased the seed germination, survival and dry weight of *E. catarium* (Figure 4).

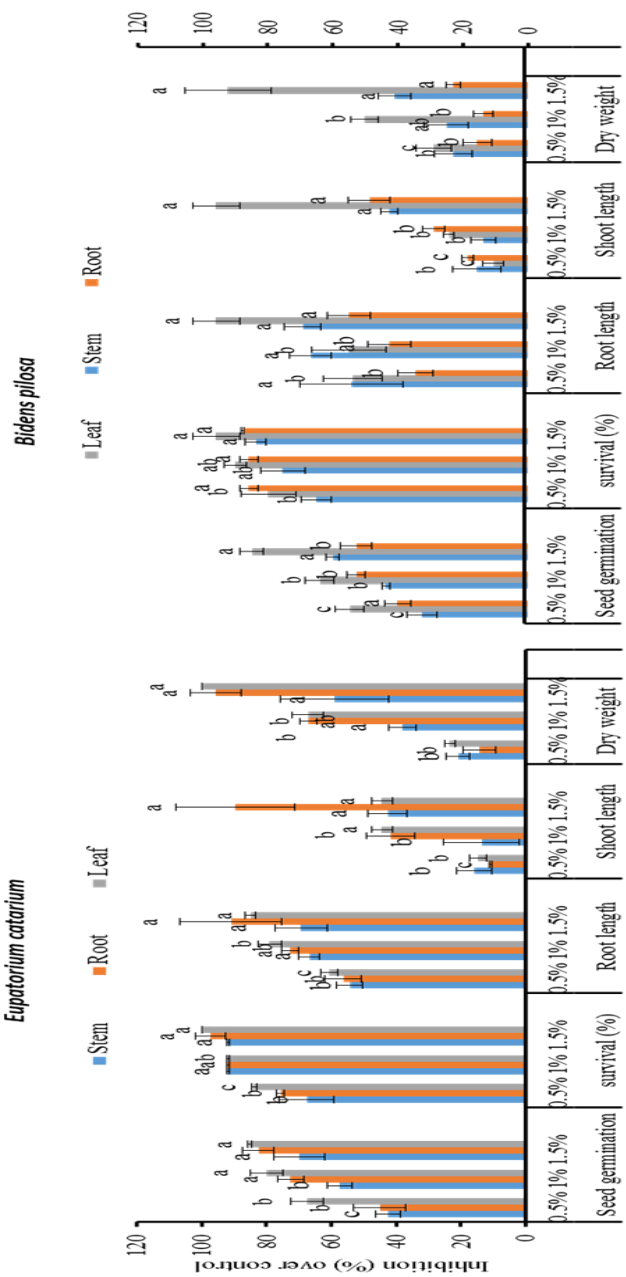

Figure 4. Effects of *D. dicothoma* leaf, stem and root extract on seed germination, survival rate, root and shoot length and dry weight of *E. catarium*. Where a, b and c indicate significant difference with in the treatments

Figure 5. Effects of *D. dichotoma* leaf, stem and root extract on seeds germination, survival (%), root and shoot length and dry weight of *B. pilosa*.Where a, b and c indicate significant difference within the treatments.

The root extract of *D. dichotoma* slightly inhibited the root and shoot length of *E. catarium*. These results corroborate the findings of Kil and Lovett (27), who reported inhibition of seed germination and seedling growth of some herbaceous plants (chick pea, maize, pea and teff) by aqueous leaf extracts of *Eucalyptus camandulensis* Dehnh. These results are also in accordance with findings of Rafiqul *et al.* (38), which confirmed the strong inhibitory effects with increasing concentrations of extract.

GC-MS Analysis of leaf, stem and root extract from *D. dichotoma*

To investigate the chemical composition of leaf, stem and root extracts from *D. dichotoma*, Gas chromatography mass spectroscopy analysis was done by N-trimethane and carbinol extracts separately. The total ion chromatogram (TIC) of N-trimethane extract and carbinol extract of *D. dichotoma* leaf, stem and root showing the detailed tabulations of GC-MS analysis of the extracts are given in Table 1-3. In N-trimethane extract of leaf, the highest percentage was of 2,3-Butanediol (34.65 %), while, in carbinol leaves extract, 2,4-bis (1,1-dimethylethyl) was in highest (28.36 %) quantity (Table 1).

Table 1. GC- MS analysis of *D. dichotoma* leaves aqueous extract

S. No.	Compound name	Relative content (%)
	N-trimethane extract	
1	2(R),3(S)-1,2,3,4-Butanetetrol	0.33
2	Glycidol	0.09
3	Propylene Glycol	0.80
4	2,3-Butanediol	34.65
5	1,2-Propanediol, 3-methoxy-	0.34
6	2-Cyclopenten-1-one	0.25
7	Silane, ethyldimethyl-	0.26
8	2-Butanol, 1-methoxy-	0.32
9	Cyclopropanecarboxylic acid, 2-pentyl ester	0.16
10	1-Methoxy-2-methyl-3-butene	0.52
11	2-Ethylacrolein	0.30
12	1,2-Cyclohexanedione	7.09
13	1H-Imidazole-1-ethanol	1.85
14	Glycerin	2.21
15	Sorbic Acid	0.59
16	2(1H)-Pyridinone, 6-hydroxy-	4.13
17	Homopiperazine	0.27
18	Phenol, 2-methoxy-	0.34
19	Maltol	0.69
20	1,2-Dimethyl-3-ethyldiaziridine	0.43
21	1,2-Benzenediol	10.51
22	Catecholborane	2.06
24	2-Methoxy-4-vinylphenol	0.27
25	1H-Imidazo(4,5-d)pyridazin-7-ol	0.09
26	1,2-Benzenediol, 4-methyl-	0.13
27	Propane, 2-(ethenyloxy)-	4.49
28	Ethyl 2,3-epoxybutyrate	0.65
29	4,5-Dimethyl-2-pyrimidone	0.97
30	2-Cyclohexen-1-ol	0.97

31	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	0.17
32	cis-3-Hexenoic acid	13.25
33	4,5-Dihydro-3-furoic acid	3.33
34	Hydrazine, 1-ethyl-1-(1-methylpropyl)-	0.41
35	.beta.-d-Mannofuranoside, methyl	0.36
36	2-Trimethylsilyl-1,3-dithiane	0.82
37	Benzeneacetic acid, 4-hydroxy-3-methoxy-	0.49
38	Phenol, 2-ethyl-4-methyl-	0.65
39	Cyclohexanone, 4-hydroxy-	0.60
40	Methoxyacetic acid, 1-cyclopentylethyl ester	0.48
41	1-Naphthalenol, decahydro-4a-methyl-	0.37
42	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	0.10
43	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.29
44	Silane, (bromomethyl)-	0.17
45	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	0.16
46	2-Amino-5-methyl-4-oxo-3,4-dihydropyrimidine	0.22
47	3-Cyclohexene-1-methanol, .alpha.,4-dimethyl-.alpha.-(4-methyl-3-pentenyl)-, (R-(R*,R*))-	0.09
48	Androst-5-en-3-ol, 4,4-dimethyl-, (3.beta.)-	0.13
49	(1R,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	0.45
50	Bicyclo(3.1.0)hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1S-(1.alpha., 3.beta. ,5.alpha.))-	0.64
51	Androst-8-en-3-ol, 4,4,14.alpha.-trimethyl-17-(2-bromo-1-methylethyl)-	0.07
52	Cyclopropanecarboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	0.13
53	1H-Cycloprop(e)azulene,decahydro-1,1,7-trimethyl-4-methylene , (1aR(1a.alpha.,4a.alpha.,7.alpha.,7a.beta.,7b.alpha.))-	0.66
54	Carbamic acid, ((4-amino-2-methyl-5-pyrimidinyl)methyl)-, ethyl ester	0.20
	Carbinol Extract	
55	Phenol, 2,4-bis(1,1-dimethylethyl)-	28.36
56	Octadecane, 1-iodo-	27.34
57	Heptadecane, 2-methyl-	24.04
58	Octacosane	20.26

The highest percentage of compounds in N-trimethane extract of stem was of 2,3-Butanediol (78.14 %) (Table 2). The highest percentage of Dodecanoic acid, 1,2,3-propanetriyl ester (41.52 %) was in carbinol extract of stem (Table 2). In N-trimethane extract of root, the 2,3-Butanediol, (R-(R*,R*) was found in highest (91.68 %) amount. In carbinol extract of root, the highest percentage (69.25 %) of Dodecanoic acid, 1,2,3-propanetriyl ester was found (Table 3). These results agree with Qin *et al*. (36) who analyzed the chemicals in leaf extracts of *D. dichotoma*.

The 2,3-Butanediol was found present in three extracts (leave, root and stem) of *D. dichotoma,* while, 4,5-Dimethyl-2-pyrimidone, Bicyclo (3.1.0) hexan-3-ol,4-methylene-1-(1-methylethyl), [1S-(1.alpha.,3.beta.,5.alpha.)] and Octacosane were found in leaf and stem extracts. Dodecanoic acid, 1,2,3-propanetriyl ester, 3-Dibenzofuranamine, 4-Dibenzofuranamine, Benzo (cd) indol-2(1H)-one, 6-(4-methyl-2-nitrophenylsulfonylamino), Benzamide, N-(4-bromophenyl) -3-bromo were found in stem and root extracts of *D. dichotoma*. This suggested that these substances are synthesized by plants and released

Table 2. The GC- MS analysis of *D. dichotoma* stem aqueous extracts.

S. No.	Compound name	Relative content (%)
	N-trimethane extract	
1	2,3-Butanediol, (R-(R*,R*))-	8.21
2	2,3-Butanediol	78.14
3	2-Butenediamide, (Z)-	1.56
4	4,5-Dimethyl-2-pyrimidone	6.36
5	Cyclopentane	1.94
6	Illudol	1.42
7	Bicyclo(3.1.0)hexan-3-ol,4-methylene-1-(1-methylethyl)-,(1S-(1.alpha.,3.beta.,5.alpha.)	1.43
8	Geranylgeraniol, tert-butyldimethylsilyl ether	0.94
	Carbinol Extract	
9	Octacosane	0.06
10	Pentadecane, 2-methyl-	0.07
11	Dodecanoic acid, 1,2,3-propanetriyl ester	41.52
12	3-Dibenzofuranamine	2.10
13	Benzamide, 2-bromo-N-(2-(3-fluorophenyl)-5-benzoxazolyl)-	20.05
14	4-Dibenzofuranamine	0.90
15	Benzo(cd)indol-2(1H)-one, 6-(4-methyl-2-nitrophenylsulfonylamino)-	9.81
16	Pivalamide, N-(4-chlorophenyl)-	1.79
17	Propanamide, N-(3-chlorophenyl)-2,2-dimethyl-	3.89
18	7,7-Dibutoxyheptanoic acid, butyl ester	1.77
19	5-Acetyl-2-(3-bromo-benzoylamino)-4-methyl-thiophene-3-carboxylic acid ethyl ester	7.22
20	Carbamic acid, N-(3-chloro-4-methoxyphenyl)-, glycidyl ester	3.44
21	(R)-Prophos	7.23
22	Fumaric acid, 2-heptyl isohexyl ester	0.04
23	Benzamide, N-(4-bromophenyl)-3-bromo-	0.10
24	Pentadecane, 2,6,10,14-tetramethyl-	0.01
25	1-(5-Ethyl-tetrahydrofuran-2-yl)-3,3-dimethyl-butan-2-one	0.01

in to the surrounding environment through leaf, stem and root. In present study, germination and growth inhibition of two invasive test species may be due to the presence of 2,4-bis (1,1-dimethylethyl) phenol in applied leaf extract. These results are in accordance to previous reports. Earlier studies have reported that 2, 4-bis (1, 1-dimethylethyl) phenol was most inhibitory to rice, lettuce and barnyard grass (37) and it was also the main allelochemical of barnyard grass. This allelochemical is also present in *Rehmannal glutinosa* that affects the *Sesamum indicum* (29,49). It is also present in *C. equisetifolia* litter, root and soil that affect *V. mangachapoi*, *T. Lampas* and *C. inophyllum* (52). The 2,4-bis (1,1-dimethylethyl) phenol allelochemical was also present in *D. dichotoma* leaf extract, that adversely affected the *E. catarium* and *B. pilosa* growth. The leaf extract of *D. dichotoma* was more inhibitory to *E. catarium* and *B. pilosa* than stem extract. The octacosane compounds were present in leaves extract in highest percentage than stem extract of *D. dichotoma*. This study also showed that the amount and

variety of potentially allelopathic compounds present in the leaf, stem and roots of *D. dichotoma* differed and the potential allelopathic compounds in leaf were more diverse than in the stem and root.

Table 3. The GC- MS analysis of *D. dichotoma* root aqueous extracts.

S. No.	Compound name	Relative content (%)
	N-trimethane extract	
1	2,3-Butanediol	4.40
2	2,3-Butanediol, (R-(R*,R*))-	91.68
3	Spiro(2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran)-7,2'-(oxirane)	3.93
	Carbinol Extract	
4	3-Dibenzofuranamine	0.15
5	Dodecanoic acid, 1,2,3-propanetriyl ester	69.25
6	Benzo(cd)indol-2(1H)-one, 6-(4-methyl-2-nitrophenylsulfonylamino)-	2.83
7	3-Triisobutyloxyhex-4-yne	0.70
8	Dodecanoic acid, ethenyl ester	0.38
9	2,5-Dimethyl-4-phenylpyridine	1.20
10	2,6-Dimethyl-4-phenylpyridine	1.03
11	Lauric anhydride	2.21
12	Coumarin, 4-difluoroboroxy-3-(3-(4-dimethylamino)phenyl-1-oxo-2-propenyl)-	2.50
13	4-Nitrophenyl laurate	2.29
14	Benzenamine, 4-methoxy-N-(triphenylphosphoranylidene)-	8.60
15	4-Dibenzofuranamine	1.04
16	Fumaric acid, 3,5-dichlorophenyl isohexyl ester	1.54
17	Fumaric acid, isohexyl 2,4,5-trichlorophenyl ester	1.51
18	Benzamide, N-(4-bromophenyl)-3-bromo-	0.34
19	Fumaric acid, 2-formylphenyl isohexyl ester	1.34
20	Fumaric acid, cyclobutyl hexyl ester	1.50

The chemical analysis of leaves extract of *D. dichotoma* showed that it contained high amount of 2,3-Butanediol, Phenol, 2,4-bis(1,1-dimethylethyl)-, Octadecane, 1-iodo-, Heptadecane, 2-methyl- and Octacosane. It is assumed that these compounds are responsible for significant effects on *B. pilosa*. Likewise, the root extract contains 2,3-Butanediol and Dodecanoic acid, 1,2,3-propanetriyl ester in high concentration, which significantly controlled the *E. catarium*. Further, the effects of individual chemical compounds on the growth parameters of *B. pilosa* and *E. catarium* may be studied to find the most potent compound, which may lead to development of bio-herbicide, preventing the invasive species (36).

According to current results, the 1.5 % concentration of leaf extracts of *D. dichotoma* was very inhibitory to different parameters (seed germination, survival rate, root and shoot length and dry weight) of *B. pilosa*. The highest concentration of leaf extract (1.5 %) of *D. dichotoma* was very inhibitory to seed germination, survival rate and

dry weight of *E. catarium.* While 1.5 % root extract was inhibitory to *E. catarium* root and shoot length. The trend in overall data shows that increasing the concentration of extracts, increased the level of inhibition for all parameters (7).

It is clear from the results that the leaf extract of *D. dichotoma* have significantly inhibited the seed germination and other growth parameters of *B. pilosa*. While no significant inhibition was recorded for root and stem extract. The leaf extract of *D. dichotoma* significantly inhibited the seed germination, survival rate and dry weight of *E. catarium* (7). The root extract of *D. dichotoma* also slightly inhibited the root and shoot length of *E. catarium*. In present study, germination and growth inhibition of two test invasive weed species may be due to presence of 2,4-bis (1,1-dimethylethyl) phenol in applied leaf extract. These results are in accordance with previous reports. Earlier studies have reported that 2, 4-bis (1, 1-dimethylethyl) phenol was most inhibitory to rice, lettuce and barnyard grass (36) and it was also the main allelochemical in barnyard grass. This allelochemical is also present in *Rehmannia lglutinosa* that adversely affected the *Sesamum indicum* (29,49). It is also present in *C. equisetifolia* litter, root and soil that affects the *V. mangachapoi, T. Lampas* and *C. inophyllum* (52,55). The 2,4-bis (1,1-dimethylethyl) phenol allelochemical is also present in *D. dichotoma* leaf extract that affected the *E. catarium* and *B. pilosa*. Another allelochemical octacosane, was also isolated from the leaf and stem extracts *D. dichotoma* (Tables 1, 3).

The atrazine nano-encapsulation improves the pre-emergence herbicidal activity against *Bidens pilosa*, without long-term residual effects on *Glycine max* (L.) Merr.) (35), residual effects of herbicide are present, comparative to safe biological control with allelopathic inhibitory effects.

CONCLUSIONS

The 1.5 % leaf extract of D. dichotoma was the most inhibitory to the seeds germination, survival (%) and dry weight of E. catarium, but was also inhibitory to all studied attributes [Seeds germination, shoot and root length, dry weight and plant survival (%)] of *B. pilosa*. These results showed that *D. dichotoma* has the potential to prevent the invasion of *E. catarium* and *B. pilosa*.

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