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ALLELOPATHIC POTENTIAL OF *SERIPHIDIUM LEUCOTRICHUM*: A WEED OF THE FAMILY ASTERACEAE FROM SKARDU REGION, PAKISTAN

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ABSTRACT

The study of alleopathic potential of *Seriphidium leucotrichum* (Krasch. ex Ladyg.) K. Bremer & Humphries ex Y.R. Ling, on seed germination and early seedling growth of four test plant species including *Triticum aestivum* (wheat), *Zea mays* (maize), *Brassica compestris* (mustard) and *Pennisetum americanum* (millet), was conducted under laboratory conditions. Aqueous extract of the air-dried plants of *S. leucotrichum* at different concentrations i.e. (25, 50, 75 and 100% of stock solution) inhibited germination of the test species in the order: *Brassica compestris*> *Zea mays*> *Triticum aestivum*> *Pennisetum americanum.* Root and shoot growth of the test species was also reduced by the extracts in the order: *Brassica compestris*> *Zea mays*> *Triticum aestivum* > *Pennisetum americanum.* It was observed that the soil application of the aqueous extract had considerable delaying effect on *Triticum aestivum* growth while shoot spray or root dip treatment had no such effects. Decaying shoot of *S. leucotrichum* in sandy–loam at 5, 10 and 20g/400g soil caused considerable inhibition of germination and seedling growth of *Pennisetum americanum* at high concentration (20 g/400g soil). Bioassay of the ether extract of *S. leucotrichum* exhibited three zones of inhibition at Rf values 0.7-0.8, 0.8-0.9 and 0.9-1.0 while a promoter was detected between Rf value 0.4-0.5. Paper Chromatography was employed for the identification of phenolic constituents and as a result, caffeic acid, p-coumaric acid and gallic acid were identified while one constituent remained unknown. In this connection, the possible role of the phenolic compounds through their allelopathic effect in driving the structure of natural communities is discussed.

Key-words: Allelopathy, *Seriphidium leucotrichum*, mustard, wheat, maize, millet, seedling growth.

INTRODUCTION

Seriphidium leucotrichum (Krasch. ex Ladyg.) K. Bremer & Humphries ex Y.R. Ling is a perennial, basally woody herb that belongs to the subgenus *Seriphidium* of the genus *Artemisia* and the family Asteraceae, commonly seen growing at dry hillside slopes in fine gravely soil away from glaciers in the subalpine zone from 3000 to 4500 m of N. E. Afghanistan, Soviet Union (Pamir) and N. W. Pakistan (Breckle *et al*., 2010). *Seriphidium leucotrichum* is often seen, forming more or less pure populations on the dry slopes of the mountains. Formation of pure populations indicates the allelopathic potential of the species. Allelopathy plays a significant role in the intra-specific and inter-specific competition and also involves determining the type of inter-specific associations. Plants release allelochemicals into the environment through root diffusates, leaching by rains and decomposition of plant parts in soil and by volatilization (Rice, 1984; Inderjit and Dakshini, 1995; Inderjit, 1998; Inderjit and Duke, 2003; Einhellig, 2008). Such chemicals are present in almost all plant's tissues including leaves, stems, roots, flowers, seeds bark and buds (Weston and Duke, 2003). The plants may exhibit inhibitory or rarely stimulatory effects on the germination and growth of other plant species growing in the immediate neighborhood (Einhellig and Rasmussen, 1975; Shaukat *et al*., 1985; Ahmed and Wardle, 1994; Burhan and Shaukat, 1999; Rebaz *et al*., 2001; Shaukat and Siddiqui, 2001; Shaukat *et al*., 2002; Tajuddin *et al*., 2002). A wide variety of secondary compounds have also been implicated in allelopathic action, including phenolic compounds, alkaloids, terpenoids, flavonoids, steroids, glycosides and tannins. Secondary metabolites usually have inhibitory effects on crops (Whittakar and Feeny, 1971; Mandava, 1985; Stupnicka-Rodzynkiewicz, 2004, Li *et al*., 2010; Chotsaeng *et al*., 2017). Phenolic compounds often constitute the major allelopathic agents in weeds and other allelopathic plant species (Inderjit, 1998; Ferreira *et al*., 1998; Burhan and Shaukat, 2000; Inderjit and Duke, 2003; Li *et al*., 2010). A number of weed species have been reported for possessing allelopathic potential. According to Holm (1978) around 200 species of twelve families are most important weeds throughout the world. In this account, the family Asteraceae is known in many parts of the world for comprising weed species. These species contain ecologically important bioactive compounds of survival value in ecosystems (Meepagala *et al.*, 2003). Among them, several species belong to the genus *Artemisia* which have been reported to have allelopathic potential (Yun, 1999; Alexa *et al*., 2004; Chaves and Escudero, 2006; Khanh *et al*., 2007) while *S. leucotrichum* an allied member of the genus *Artemisia* is consequently suspected (based on field observations) to have allelopathic potential and could be involved in the suppression of the other species

growing in its vicinity. The objectives of this study were multifold as follows: 1) to examine the effect of aqueous extract of *S. leucotrichom* on the germination and early seedling growth of four crop species, namely *Brassica compestris* L. (mustard), *Pennisetum americanum,* (bullrush millet), *Triticum aestivum* L. (wheat) and *Zea mays* L. (maize), 2) to assess the effect of decaying *S. leucotrichum* on the germination and seedling growth of four test species (as above), 3) to perform coleoptile straight growth bioassay to test the presence of inhibitors and promoters in *S. leucotrichum* , and finally 4) to identify the phenolic compounds present in *S. leucotrichum.*

MATERIALS AND METHODS

Seriphedium leucotrichum (Krasch. ex Ladyg.) K. Bremer & Humphries ex Y.R. Ling was collected from Skardu and air-dried in the laboratory. Because of its more or less pure populations, it was suspected to have the allelopathic effects on the germination and seedling growth. Therefore, a laboratory experiment was performed to test its phytotoxicity on four test species including *Brassica compestris* L. (mustard), *Pennisetum americanum* (L.) Schumann (bullrush millet), *Triticum aestivum* L. (wheat) and *Zea mays* L. (maize). Aqueous extract of *S. leucotrichum* was used to check its effect on germination and early seedling growth of the four test species.

Effect of Aqueous Extract on Germination and Early Seedling Growth

Air dried material of *S. leucotrichum* was chopped into small pieces to prepare an aqueous extract. 10 g chopped material of *S. leucotrichum* was soaked in 100 mL of distilled water for 24 h to obtain stock solution (S) which was subjected to decant. Stock solution was used further for making solutions of various concentrations i.e. 25, 50, 75 and 100%.

The seeds of four test species: *Triticum aestivum* L., *Zea mayz* L., *Brassica compestris* L., and *Pennisetum americanum* (L.) Schumann were sterilized with 0.3% calcium hypochlorite ($Ca(ClO)_2$. Whatman #.1, filter paper was placed in 9-cm diam., sterile Petri plates. 20 seeds of each test species were placed on the Petri plates. Petri plates were kept at laboratory temperature (25± 1º C) and the humidity approximately 50 to 55%. Five mL stock solution (S) of different concentration i.e 75, 50 and 25%, were poured in the plates. Small amounts of respective solutions were added when it was obvious that Petri plates were drying out. Germination was recorded daily while seedling growth was recorded at 120 h. Speed of germination (S) for each species in the treatments and controls was determined using the following formula developed by Khandakar and Bradbeer (1983).

$$S = \{ {}^{N}/_{1} + {}^{N}/_{2} + {}^{N}/_{3} + \ldots\ldots + {}^{N}/_{N} \} \times 100$$

Where N_1, N_2, N_3….N_n are proportion of seeds in a treatment which germinated on day 1,2,3…..N following the commencement of experiment; the index S ranges from 0 (when no seed ever germinated) to 100 (If all seeds germinated on the first day). The program GVSSS developed by one of us (S.S.S) in C++ was used for this computation of germination speed and is available on request.

Coleoptiles Bioassay

10 g air dried shoot part of *S. leucotrichum* was blended in 200 ml distilled water. The centrifuged homogenate was adjusted to pH 3 with 0.5 N H_2SO_4 and extracted three times with peroxidase free ether and evaporated to dryness using argon gas. Two ml (80 %) ethanol was added to the dry material and was streaked on Whatman #1., filter paper. Duplicate 10 cm wide chromatograms were developed in the solvent system; isopropanol- ammonia - water (10: 1: 1, v/v/v) by ascending chromatography. After the solvent had moved 30 cm from the origin, they were taken out and dried in a chromatogram dryer. The dried chromatograms were divided into 10 equal size strips. The strips were assayed for growth regulators using wheat coleoptiles straight growth test by Nitsch and Nitsch (1956). Five mm segments of 3 days old dark grown wheat coleoptiles (after removal of tip 1.5mm) were excised and put into the distilled water for 1 h. Subsequently, 10 coleoptiles segments were placed in between two strips of the chromatograms of the same Rf value and kept in 11.5 cm diameter Petri plates over two layers of tissue papers moistened with 4 mL (0.02 M) citrate phosphate buffer (pH 4.8) solution. Controls were also kept similarly. After 48 h of growth in dark, length of coleoptiles segments were measured.

1. Chromatography

Ether extract of *S. leucotrichum* was evaporated to dryness and was dissolved in 2 mL of 80% ethanol, loaded on TLC plate. Chromatograms were developed in a system of n-butanol: acetic acid: water (50:2:48) by ascending chromatography on silica gel TLC (F254) plates using various phenolics as reference compounds. Phenolic principles were detected using Rf-values, ferric chloride-ferric cyanide reagent and UV light (+NH_3 vapours) (Harborne, 1973).

2. Statistical Analysis

Data sets obtained through experimentation were subjected to analysis of variance (ANOVA) using completely randomized design CRD (lab experiments) and randomized complete block design RCBD (greenhouse experiment). Percentage data were arcsine transformed prior to analysis. As a follow up of ANOVA, Fisher's, Least significant difference (LSD) test and Duncan's multiple range test were performed at p=0.05. In data sets where significant heteroscedasticity occurred, log(x) transformed values were used in the ANOVA. Computer programs for the analyses were developed in Microsoft FORTRAN-77 by the second author (S.S.S.) of the current paper. The statistical procedures of Zar (2008) was followed.

RESULTS

Effects of Aqueous Extract on Germination and Seedling Growth of the Test Species

Germination of three species was inhibited by various concentrations of the aqueous extract (P at the most 0.05) over the control (Table 1) Inhibitory effect increased with the increase in concentration. Different species were affected with different extent; the degree of inhibition varied in the order: *Brassica compestris> Zea mays> Tritecum aestivum> Pennisetum americanum.* Seedling growth of two test species affected adversely and growth reduction was found greater at higher concentration (Table 1). Root and shoot growth of *B. compestris* and *Zea mays* were inhibited to a greater extent compared to *Triticum aestivum and Pennisetum americanum*. Generally, root growth was reduced to a greater degree than the shoot growth.

Phytotoxicity of decaying *S. leucotrichum*

Germination of *Pennisetum americanum* was significantly (P<0.001) reduced at high concentration (10 and 20 g/400 g soil) of decaying *S. leucotrichum* (Table 2). Germination percentage declined sharply with the increase in concentration. Similarly, both root and shoot growth were significantly (P<0.001) suppressed at all the concentrations of the decaying shoot material compared to controls. These inhibitory effects increased with the increase in concentration.

Table 1. Phytotoxic effects of *S. leucotrichum* extract on final germination % and speed of germination. Concentrations are % of Stock solution; 0%= control. Values are Means ± SE

Species	Concentration of extract			
	0 Germ.% Speed	25 Germ.% Speed	75 Germ.% Speed	100 Germ.% Speed
P. americanum	97.2 ± 5.5 69.4	86.9 ± 5.3 62.5	80.0 ± 4 56.8	62.0 ± 3.8 46.9
T. aestivum	98.0 ± 5.0 59.0	79.5 ± 4.5 54.4	70.0 ± 3.5 49.0	56.2 ± 3.0 43.1
Z. mays	84.5 ± 4.3 53.7	72.0 ± 4.2 48.4	78.3 ± 4.3 50.4	49.4 ± 3.2 36.7
B. compestris	78.2 ± 5.2 50.4	62.5 ± 3.8 49.4	40.0 ± 2.5 31.8	25.5 ± 3.0 22.4

Wheat coleoptiles bioassay

Wheat coleoptiles bioassay disclosed three significant inhibitory bands at Rf- values of, 0.7-0.8, 0.8-0.9 and 0.9-1,0 while one significant promoter was detected at Rf-value of 0.4-0.5 (Fig. 1).

Chromatography

Five significant bands were identified during chromatography of aqueous extract of *S. leucotrichum*, under UV light on TLC plates. Maximum wave lengths of the bands after treatment with ferric chloride-ferricyanide were appeared with minor differences to the standards of phenolic compounds *i.e* p-coumaric acid, caffeic acids and gallic acid, while one band was found unknown (Table 3).

Table 2. Effect of aqueous extract of *S. leucotrichum* on shoots and roots length (cm.) of test species.

Species	Concentration (% stock solution)				
	0	25	50	75	100
P.americanum Root Shoot	 10.2 ± 1.25 11.4 ± 1.41	 10.0 ± 1.34 11.1 ± 1.28	 8.2.0 ± 0.67 8.9 ± 0.86	 8.2 ± 0.85 8.5 ± 0.73	 6.7 ± 0.74 7.3 ± 0.83
Z. mayz R S	6.5 ± 0.52 7.0 ± 0.44	4.0 ± 0.25 4.5 ± 0.31	3.9 ± 0.92 3.6 ± 078	1.8 ± 0.15 1.9 ± 0.21	0.5 ± 0.18 0.4 ± 0.15
T. aestivum R S	7.4 ± 0.61 7.8 ± 0.65	7.0 ± 0.38 7.2 ± 0.42	6.5 ± 0.63 6.8 ± 0.66	6.4 ± 0.49 6.5 ± 0.48	5.5 ± 0.46 5.8 ± 0.52
B. compestris R S	6.0 ± 0.66 6.5 ± 0.43	4.0 ± 0.34 4.2 ± 0.47	4.0 ± 0.34 4.2 ± 0.47	1.0 ± 0.25 1.2 ± 0..14	0.7 ± 0.24 0.8 ± 0.33

R= Root; S= Shoot

Table 3. Rf-values (x100) of phenolic constituents in ether fraction of aqueous extract of *S. leucotrichum*.

Reagent: Ferric chloride-ferric cyanide			
Compound	Rf-values	Reagent	UV-light
Unknown 1	96.24	Bluish	Blue
Caffeic acid	68.75	Dark blue	Bright blue
p-Coumaric acid	72.35	Blue	Bright violet
Gallic acid	5.33	Bluish brown	Bluish brown

DISCUSSION

S.leucotrichum is a weed species belongs to the family *Asteraceae* (Breckle *et al*., 2010) that has not been reported previously for allelopathic interference. Present study clearly indicates the allelopathic potential of the species as indicated by various experiments. For instance, the aqueous extract of *S. leuacotrichum* produced differential inhibitory effect on germination and early seedling growth of the four test crop species. The inhibitory effects on test species are probably due to the varied secondary metabolites such as phenolic constituents (Whittakar and Feeny1971; Mandava, 1985; Stupnicka-Rodzynkiewicz, 2004, Chotsaeng *et al*., 2017). Phenolic constituents have strong potential of inhibition (Burhan and Shaukat, 1999; Dambolena *et al*., 2012). They are responsible of inhibition of germination and early seedling growth (Kuiter, 1989; Ishikura *et al*., 2001) but due to morphological and physiological differences among the species they might respond differentially.

The application of an aqueous extract of *S. leuacotrichum* was found to have significant impact on the growth of *Triticum aestivum* in the soil. The prominent growth reduction in the soil can be attributed to direct absorption and upward translocation of inhibitory compounds through roots thereby causing detrimental impact on growth. Furthermore, the decaying plant material changes microbial community structure and composition (Shaukat and Siddiqui, 2001; Siddiqui *et al*., 2002) following soil application of plant material which could suppress certain pathogenic organisms that in turn might result in better plant growth.

The late germination and maximum growth reduction in *Pennisetum americanum* were observed in the soil which may presumably be due to the release phytotoxic substances from the decaying *S.leuacotrichum*. Phytotoxins are active substances and are known to maintain their stability for considerable duration in the soil (Han *et al*., 2009). When present in enough concentration they can have adverse effects on the growth of other plant species. In this connection, Shaukat *et al.* (1985) reported adverse effects on the growth of millet plants by decaying *Citrullus colocynthis* while Burhan and Shaukat (1999) studied inhibitory effects of decomposing *Argemone mexicana*. Previous works carried out on the inhibitory effects of weeds by allelopathic plants strongly support the present findings and therefore it can safely be assumed that the phytotoxins released from the *S. leuacotrichum* plant under natural conditions can accumulate over the years in the soil in sufficiently high amounts and therefore they can play

a vital role as an edaphic variate exerting a detrimental impact on the growth and development of other neighboring plant species.

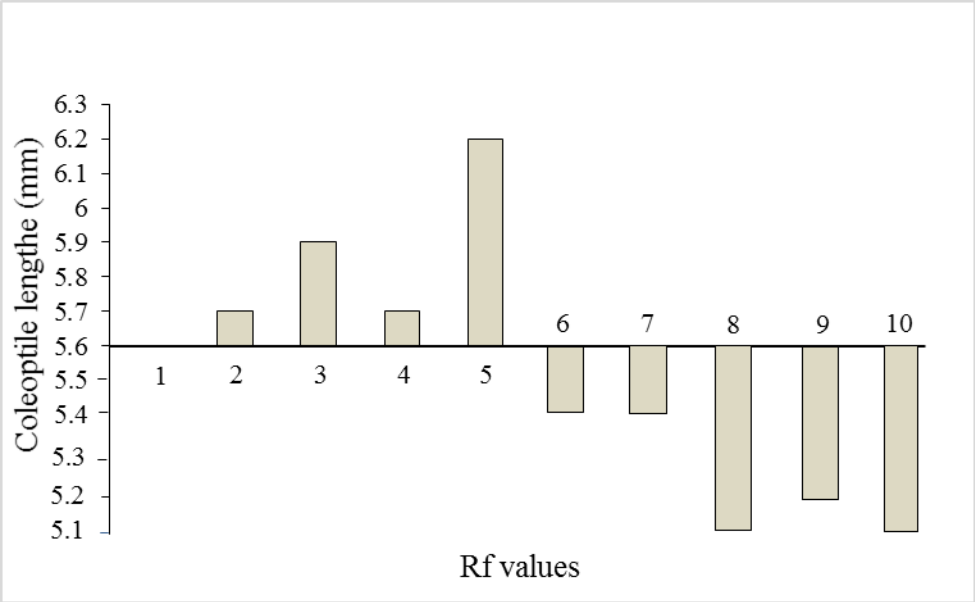

Fig.1. Wheat coleoptiles test showing inhibitors and stimulators at various Rf-values. The x-axis represents the coleoptiles length of control. The numbers on the X-axis represent Rf-values: 1, 0-0.1; 2, 0.1-0.2; 3, 0.2-0.3; 4, 0.3-0.4, 5, 0.4-0.5; 6, 0.5-0.6; 7, 0.6-0.7; 8, 0.7-0.8; 9 , 0.8-0.9; 10, 0.9-1.0.

The four inhibitory zones were observed during the wheat coleoptiles bioassays of *S. leuacotrichum* which are most likely described as phenolic substances while one significant promoter at Rf value of 0.4-0.5 could be a growth hormone such as indol acetic acid (IAA). Finally, the chromatographic study of an aqueous extract of *S. leuacotrichum* was performed to examine the presence of phenolic constituents and their identification in the species, resulting the Rf values of the observed band under UV light and subsequent spray of ferric-chloride ferric-cyanide reagent were found with minor error equal to the standards of three compounds including caffeic acid, p-coumaric acid and gallic acid while one remained unknown. It is also disclosed that *S. leuacotrichum* is an allied species of the genus *Artemisia* whose several species are known for phenolic components (Swiatek *et al*., 1998; Sengul *et al*., 2011; Baiceanu *et al*., 2015) therefore previous findings also support the results of the current study. The present investigation discloses the presence of considerably phytotoxic substances in the plant. It is also assumed that secondary metabolites other than phenolic constituents might also be important allelopathic agents occur in *S. leuacotrichum*. However, species grows abundantly in Gilgit-Baltistan region and could be responsible for the accumulation of phytotoxins in the soil when regularly added over the years and finally suppress other species in the vicinity in natural communities and also when occurring as a weed in the crops whose germination and growth may be retarded. Often, in disturbed habitats *S. leuacotrichum* eventually forms more or less pure population. Perhaps it expresses inter-specific competition (interference) due to its accumulated toxic allelochemicals in the associated soils. Thus, this species not only interferes with crop growth through its allelopathic action but could also be a significant regulating factor in determining the biodiversity of mountainous plant communities.

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