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Aqeous Swine Cress (*Coronopus didymus*) Extracts Inhibit Wheat Germination and Early Seedling Growth

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Abstract

Suspected allelopathic activity of swine-cress (*Coronopus didymus* L.) was evaluated based on germination and early seedling growth of wheat (*Triticum aestivum* L.) in a laboratory bioassay. Aqueous root, leaf and whole plant extracts of swine-cress were applied at 0, 2.5, 5.0, 7.5 and 10% (w/v). Results revealed significant inhibition of germination and seedling growth of wheat for all extract sources and concentrations. Variable phytotoxicity was exhibited by different extract sources and leaf extract caused the greatest inhibition. Nonetheless, the degree of inhibition increased in a concentration dependent manner for all extract sources. Compared to the control all treatments increased the time to 50% germination, and mean germination time, with corresponding decreases in germination index and final germination percentage of wheat. Higher (10%) concentration of root, leaf and whole plant extracts reduced final germination by 36, 59 and 39%, respectively. Leaf extract caused more inhibition of wheat shoot length, root length and dry biomass than other extracts. Wheat seedling dry biomass was inhibited 73% by leaf extract, 65% by whole plant extract and 12% by root extract at 10% concentration. All extracts reduced chlorophyll contents and increased phenolics in wheat seedlings. The results suggest that the inhibitory allelopathic activity of swine-cress against germination and seedling growth of wheat should be explored under field conditions.

Key words: Allelopathy; Seed germination; Growth inhibition; Chlorophylls; Wheat

Introduction

Weeds compete with wheat crop for resources such as water, nutrients and sunlight. Moreover, weeds may cause inhibition of the growth of crop plants (Qasem and Foy, 2001). Allelopathic activity of many weeds on wheat has been reported by several authors (Mishra *et al.,* 2001; Dongre and Singh, 2007; Aziz *et al.,* 2008; Xingxinag *et al.,* 2009; Farooq *et al.,* 2011).

Several putative allelochemicals have been isolated from *Brassica* species and their allelopathic potential has been demonstrated in bioassays (Turk and Tawaha, 2003; Turk *et al.,* 2005; Norsworthy *et al.,* 2005; Ercoli *et al.,* 2007; Matloob *et al.,* 2010). Members of the Brassicaceae contain allelochemicals as glucosinolates that can be released into the environment, to affect seed germination and plant growth of neighboring species (Bones and Rossiter, 1996). Glucosinolates are degraded by the enzyme myrosinase, which results in the release of various hydrolysis products such as isothiocyanates, nitriles and others. These allelochemicals have been reported to interfere with basic physiological and biochemical processes of receiver plants (Weston and Duke, 2003). Release of these compounds into crop environment can have important implications for crop production (Qasem and Foy, 2001; Weston and Duke, 2003). This specific plant defense mechanism determines interactions with other organisms (Halkier and Gershenzon, 2006) and might be one of the key factors underlying the invasion success of *Brassica* species.

Identification of weed species with allelopathic potential and characterization of their adverse effects on associated crops are essential to understand weed-crop interactions in agro-ecosystems. It is reasonable to suspect that allelopathy could be involved in the suppression of other plants in the vicinity of glucosinolate-producing species. Resource competition is difficult to separate from allelopathy under field conditions. To overcome this problem, various laboratory screening techniques have been developed to measure allelopathy without the interference of resource competition (Inderjit, 2001). Many authors have successfully demonstrated the allelopathic potential of invasive plants using tissue extracts (Turk *et al.,* 2005; Ercoli *et al.,* 2007; Dongre and Singh, 2007; Xingxinag *et al.,* 2009). Considerable variation also occurs among weeds in their ability to produce and accumulate allelochemicals in different parts (Qasem and Foy, 2001). Moreover, allelopathic interactions are also dependent on the biological response capacity of donor and receiver species and the concentration of allelochemicals in the soil (Rice, 1984).

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Swine-cress (*Coronopus didymus* L.) is an important component of the weed flora of family Brassicaceae infesting crop fields in Pakistan (Ahmad and Sheikh, 2003). It grows profusely in cultivated as well abandoned fields, lawns, waste ground and vacant plots as a winter annual weed. Little information is available on the possible allelopathic interference of swine-cress on wheat. The objective of this preliminary investigation was to explore the ill effects of root, leaf and whole plant aqueous extracts of swine-cress on germination dynamics, early seedling growth and some biochemical attributes of wheat seedlings.

Materials and Methods

Extract Preparation

Mature swine-cress plants were collected from Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan and separated into different fractions (root, leaf and whole plant) and each fraction was separately ground with a sample mill (Cyclotec, Sweden). Ground samples were soaked in distilled water in 10 g/100 mL (w/v) ratio for 24 h at room temperature. The filtrate was passed through four layers of cheese cloth and was further filtered through a Whatman # 42 filter paper. The leachate was designated as stock solution of swine-cress having 10% concentration. It was used as such or diluted to make respective concentrations (2.5, 5.0 and 7.5%) as per treatment. The pH and electrical conductivity of these extracts were determined with the help of digital pH and conductivity meters (HI-9811, Hannah, USA). The osmotic potential of different extract concentrations was determined using formula:

$$\text{Osmotic potential (-MPa)} = \text{EC (dS m}^{-1}) \times -0.036$$

Total water-soluble phenolics in each concentration and source were determined as per Swain and Hillis (1959) using Folin-cicalteu reagent and are expressed as gallic acid equivalents. The chemical characteristics of aqueous swine-cress extracts of different plant parts are given in Table 1.

Bioassay

Seeds of wheat (cultivar "AARI-2011") were obtained from the Wheat Research Institute, Faisalabad. Seeds were surface sterilized with fungicide prior to sowing. Fifteen seeds were uniformly placed between two layers of a Whatman # 42 filter paper in sterilized Petri dishes (9 cm diameter). Five mL of extract solution (of root, leaf, and whole plant each at 2.5, 5.0, 7.5 and 10%) was added to the Petri dishes, while the control treatment received equal volume of distilled water. Half of the aqueous extract was applied to bottom filter paper receiving the seeds while remaining was applied to the covering filter paper. Thereafter, Petri dishes were covered with lid and placed on a steel rack in a well illuminated room at temperature and humidity of 20±5ºC and 55±5%, respectively. Equal volume of distilled water was applied to all the Petri dishes when the moisture content of the filter papers declined.

Inhibitory activity of aqueous swine-cress extracts was assessed in terms of germination dynamics, seedling growth, and biochemical attributes such as chlorophyll contents and total soluble phenolics. Germination counts were made on a daily basis according to AOSA (1990) until a constant count was achieved. Time to start germination (TSG) was recorded when the radicle and hypocotyl length of first seed was slightly above 2 mm. Mean germination time (MGT) was calculated according to Ellis and Robert (1981) as:

$$\text{MGT} = \frac{\sum \text{Dn}}{\sum \text{n}}$$

Where, n is the number of seeds emerging on day D, and D represents the number of days counted from the beginning of emergence. Time to 50% germination (T_{50}) was calculated according to the modified formula of Farooq *et al.* (2005):

$$T_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i} \right] \times (t_j - t_i)$$

Where, N is the final number of germinated seeds; and n_i and n_j are the cumulative number of seeds emerged by counts at the times t_i and t_j where ni<N/2<nj. Germination index (GI) was calculated as described by AOSA (1983):

$$\text{GI} = \frac{\text{No.of emerged seeds}}{\text{Days of first count}} + \text{-----} + \frac{\text{No.of emerged seeds}}{\text{Days of final count}}$$

Final germination percentage was taken as the ratio of number of seeds germinated to the total number of seeds sown and is expressed as percentage. Shoot and root length of five randomly selected seedlings from each replication was measured using a measuring tape at 18 days after sowing. Seedling fresh and dry biomass was recorded using a digital balance. For dry biomass, seedlings were oven dried at 70ºC for 48 h. Seedling vigor index was calculated according to the formula of Abdul-Baki and Anderson (1973):

$$\text{SVI} = \text{radicle length (cm)} \times \text{germination percentage}$$

Total soluble phenolics in wheat seedlings were determined as per Randhir and Shetty (2005) and expressed as gallic acid equivalents. Chlorophyll contents were extracted in 80% ice cold acetone and read out at 663 and 645 nm wavelength in a UV spectrophotometer (UV-4000, ORI, Germany). These are expressed as mg g^{-1} fresh leaf weight (Lichtenthaler, 1987). Percentage change over control was computed as under:

$$\text{Percent change over control} = \frac{\text{Treatment - Control}}{\text{Control}} \times 100$$

The overall effect of swine-cress on the growth of wheat crop was determined on the basis of average percent inhibition (API) as:

$$API = \frac{\text{Sum of percent reduction in all parameters}}{\text{Number of parameters}}$$

The value of API was used as the indicator of allelopathic potentials of swine-cress. Higher value of API indicates higher allelopathic potential of respective extract source and concentration and *vice versa*.

Statistical Analysis

The experiment was replicated four times in a completely randomized design under factorial arrangements and repeated in time. Analysis of variance was carried out for the data and the mean differences were separated using HSD Tuckey's test at 5% probability level (Steel *et al*., 1997). Graphical representation of the data was made and standard errors were computed using MS Excel.

Results

Germination

Aqueous extracts of swine-cress significantly inhibited germination of wheat over control (Fig. 1). Substantial delay in TSG, T_{50} and MGT was noticed for all extract sources (root, leaf, whole plant) of swine-cress. Increasing concentration of extracts markedly delayed TSG, T_{50} and MGT. At higher extract concentration (10%), delay in TSG was one day for root extract that was twofold for leaf and whole plant extracts. Likewise, T_{50} was prolonged to the tune of 12–70%, 15–88% and 12–85% by root, leaf and whole plant extracts, respectively (Fig. 1). Inhibition in final germination of wheat was 36, 59, and 39% for aforementioned extract sources at 10% concentration. MGT was also higher for 10% concentration in all the extract sources. Significantly lower germination indices over control were recorded under the influence of all extract sources. Nonetheless, leaf extract with 78% inhibition stood at top than 52 and 58% inhibition realized for root and whole plant extract at 10% concentration.

Seedling Growth

Seedling growth of wheat was adversely affected by aqueous swine-cress extracts (Fig. 2) and increasing extract concentrations suppressed seedling growth so that a strong correlation was observed between level of suppression and concentration of the extracts (Fig. 4b, c). Increasing extract concentration from 2.5 to 10% inhibited the shoot length of wheat in the range of 7–86%. Leaf extracts of swine-cress were more phytotoxic at 10% concentration, with 86% inhibition followed by whole plant (78%) and root (56%) extracts. The root length of wheat was inhibited to greater extent than shoot length (Fig. 2). Both leaf and whole plant extracts exhibited over 90% inhibition of root length of wheat seedlings over the control at the highest (10%) concentration. The respective inhibition for root extract was only 56%. Swine-cress extracts caused a marked reduction

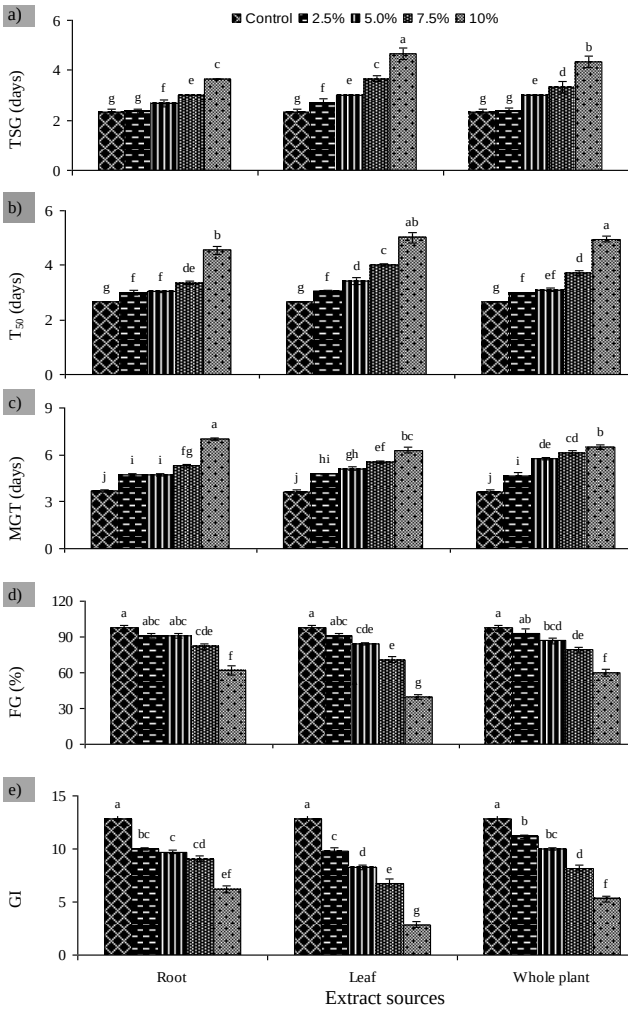

Fig. 1: Effect of different extract sources of swine-cress applied at different concentrations on (a) time to start germination, (b) time taken to 50% germination, (c) mean germination time, (d) final germination percentage and (e) germination index of wheat. Vertical bars above mean denote standard error of four replicates. Means with different letters differ significantly at 0.05 probability level by HSD Tuckey's Test

in fresh and dry biomass of wheat seedlings at all concentrations compared to control (Fig. 2). Seedling dry mass was decreased by 5–73% with leaf extract. Seedling vigor index (SVI) also declined in response to increasing concentration of different extract sources (Fig. 3). All swine-cress aqueous extracts caused significant ($p \leq 0.05$) average percentage inhibition (API; 10–78%) of wheat over control (Fig. 3). Leaf extract recorded highest API of 78% at 10% concentration.

Biochemical Attributes

Significant ($p \leq 0.05$) differences in biochemical attributes of wheat seedlings were recorded by aqueous extracts of

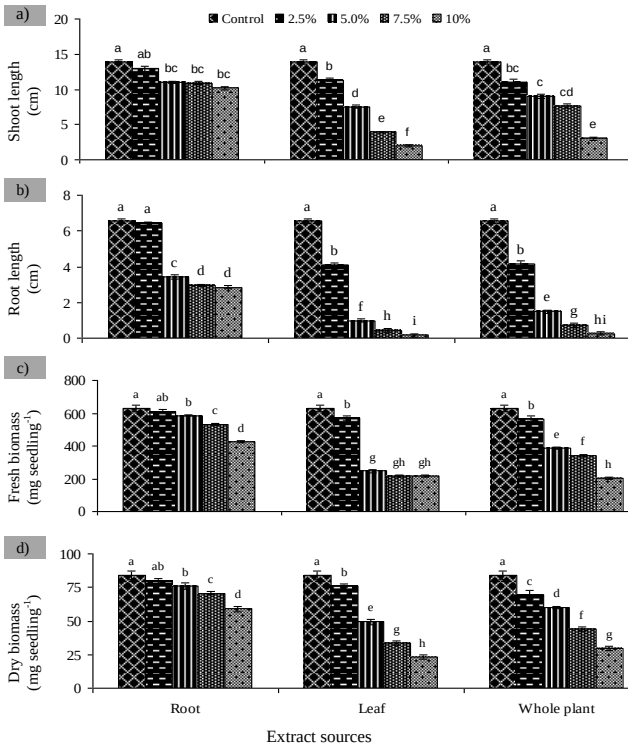

Fig. 2: Effect of different extract sources of swine-cress applied at different concentrations on (a) shoot length (b) root length (c) fresh biomass and (d) dry weight of wheat. Vertical bars above mean denote standard error of four replicates. Means with different letters differ significantly at 0.05 probability level by HSD Tuckey's Test

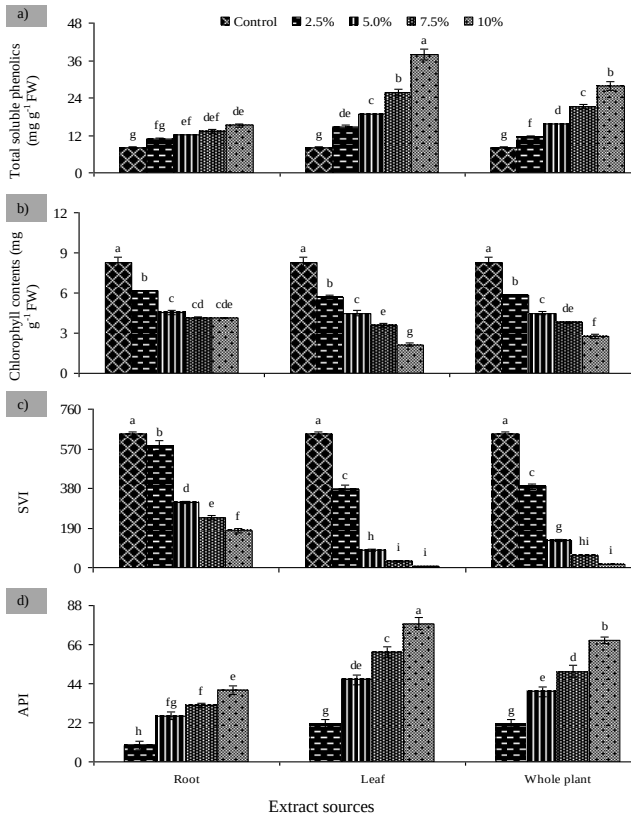

Fig. 3: Effect of different extract sources of swine-cress applied at different concentrations on (a) total soluble phenolics (b) chlorophyll contents (c) seedling vigor index and (d) average percent inhibition of wheat. Vertical bars above mean denote standard error of four replicates. Means with different letters differ significantly at 0.05 probability level by HSD Tuckey's Test

swine-cress (Fig. 3). Total chlorophyll content in wheat seedlings declined by 25–49, 50–74 and 29–66% over control for root, leaf and whole plant extracts, respectively (Fig. 3 and 4e). Wheat seedlings developing under increasing stress of swine-cress aqueous extracts showed more water soluble phenolics (Fig. 3). Root, leaf and whole plants extracts at 10% concentration recorded an increase of 85, 363 and 241% total soluble phenolics in wheat seedlings.

Discussion

Reduced seed germination is the most obvious allelopathic effect observed in bioassays (Khan *et al.*, 2005; Turk *et al.*, 2005; Samreen *et al.*, 2009; Alagesaboopathi, 2011). Retarded germination might be an outcome of interference of allelochemicals with the processes of cell division and elongation (Al-Wakeel *et al*., 2007). In this study, the concentration effect was highly pronounced and inhibition in germination attributes was intensified with increasing extract concentration (Fig. 1). Final germination inhibition was enhanced with increase in extract concentration and regression analysis revealed 82, 89 and 94% variability in germination percentage of wheat due to root, leaf and whole plant extracts of swine-cress, respectively (Fig. 4a). More pronounced inhibition at higher extract concentration is due to greater fraction of allelochemicals in concentrated extracts (Turk and Tawaha, 2003; Javaid *et al*., 2005). Chon and Kim (2004) concluded that higher extract concentration contains more quantity of allelochemicals, which enhance inhibitory activity of an extract. Our data depicted that that leaf extract had the strongest allelopathic effect on seed germination. Tefera (2002) found that the inhibitory allelopathic potential of parthenium leaf extract was more pronounced than other plant parts. Qasem and Foy (2001) stated that qualitative and quantitative differences in allelochemicals exist among different species and their parts.

Decrease in shoot and root length might be due to the inhibitory action of allelochemicals, either by creating physiological drought (Samreen *et al.*, 2009), with prevention of cell division and elongation (Al-Wakeel *et al*., 2007). Barkosky *et al*. (2000) reported alterations in the cell membranes as one of the foremost effect caused by allelochemicals, which provoke several other cross-stresses

Table 1: Chemical properties of different concentrations of aqueous swine-cress extracts

Extract source	Concentration (%)	pH	EC (dS m^{-1})	Osmotic potential (-MPa)	Total soluble phenol (µg mL^{-1} extract)
Root	2.5	5.8	2.8	0.10	116
	5.0	5.6	3.9	0.14	295
	7.5	5.4	4.6	0.17	432
	10	4.9	6.0	0.22	439
Leaf	2.5	5.1	4.6	0.17	353
	5.0	4.9	5.4	0.19	426
	7.5	4.7	6.8	0.24	486
	10	4.6	9.2	0.33	930
Whole plant	2.5	5.7	3.2	0.12	249
	5.0	5.2	4.8	0.17	420
	7.5	5.1	6.2	0.22	598
	10	5.0	7.8	0.28	679

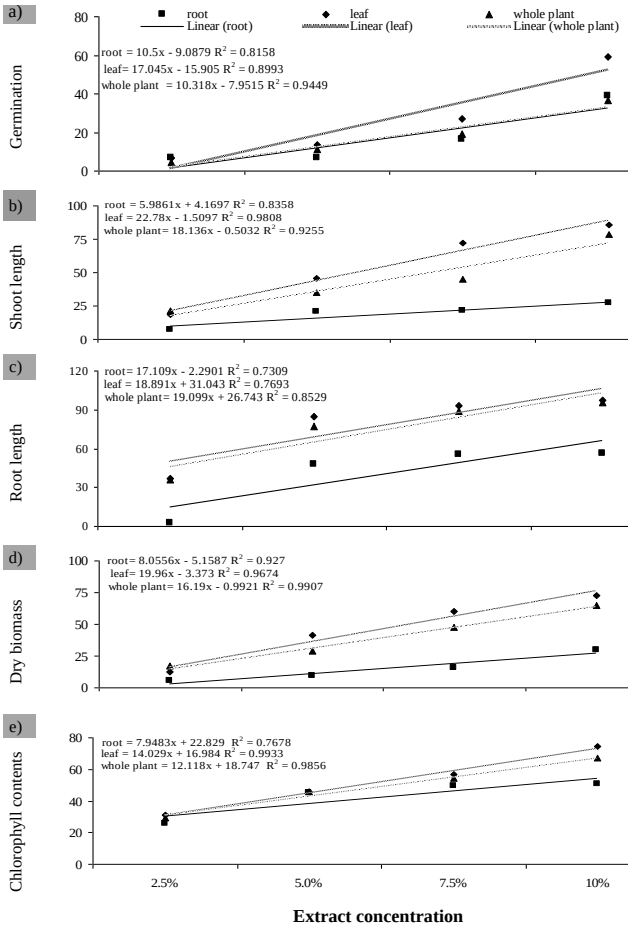

Fig. 4: Relationship of different aqueous extract concentrations of different parts of swine-cress to various germination and seedling growth attributes expressed as percentage inhibition over control

due to secondary effects like ROS damage to cell ultra-structures (Khaliq *et al.*, 2011, 2012) and lipid peroxiadation (Zeng *et al.*, 2001). Allelochemical activity of plants is measured by the sensitivity of roots in the bioassay (Alagesaboopathi, 2011). Root length of wheat appeared more sensitive to swine-cress allelochemicals than shoot length. Several other studies demonstrated that roots were more sensitive to allelochemicals than aerial parts of seedlings (Rahman, 2006; Punjani *et al*., 2006; Ercoli *et al*., 2007). This may be explained by the fact that after the seed, roots are the first to come in direct contact with allelochemicals, especially in bioassays. Stunted vegetative growth of seedlings due to phytotoxic activity of phytochemicals present in aqueous extracts of swine-cress resulted in lower fresh and dry weight of seedlings.

Decrease in chlorophyll contents is a common phenomenon of plants subject to allelochemicals, and this might be a subsequent response to these chemicals beside cellular damage. Huang (2010) stated that such a biochemical response occurs either by interference of allelochemicals with biosynthesis of photosynthetic pigments or their enhanced degradation by inducing oxidative stress via ROS, or through the integration of both. Reduction in chlorophyll was associated with higher total phenolics in wheat seedlings (Fig. 3) and is in line with Khaliq *et al.* (2011, 2012).

In conclusion, aqueous extracts of different parts of swine-cress were inhibitory to wheat germination and seedling growth, suggesting a possible allelopathic interference under field conditions. This provides a justification for its removal of this weed to ensure satisfactory wheat germination and seedling growth for a good stand establishment. Field studies under varying moisture regimes, nutrient levels and soil types need to be conducted to measure such effects in realistic conditions in wheat and possibly other crops.

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