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Allelopathic effects of perennial sow thistle (*Sonchus arvensis* L.) on germination and seedling growth of maize (*Zea mays* L.)

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ABSTRACT

In laboratory and green house conditions, the allelopathic effects of Perennial sow thistle (*Sonchus arvensis* L.) were analysed on germination and seedling growth of maize (*Zea mays* L.). Aqueous leachates of leaves, stem, roots and whole plant were prepared by soaking them in distilled water in a ratio of 1:20 (w/v). All leachate leachates (whole plant, leaves and stem) of *S. arvensis* significantly reduced the germination and seedling growth of maize. The stem leachates were most inhibitory; hence, these were further diluted to 1-4% concentrations. A linear increase in germination (%) of maize was observed with decrease in concentration of stem extract from 4% to 1% in laboratory conditions. Furthermore, plant residues were incorporated in soil @1-4%, these reduced the emergence and seedling growth of maize with maximum inhibition at 4% concentration. The laboratory analysis of stem and leaves leachates identified 4-phenolics (quercetin, hydrogenic acid, ferulic acid and coumaric acid) in stem and two phenolics (quercetin and vanillic acid) in leaves. It was concluded that allelopathic effects of plant parts of *S. arvensis* inhibited the seed emergence and seedling growth of maize, however, the stem leachates caused higher suppression in seedlings emergence and seedling growth of maize seeds.

Keywords: Allelopathy, bioassays, leachates, germination, phytotoxic, phenolics, pot culture, seedling growth, *Sonchus arvensis*, *Zea mays*.

INTRODUCTION

Perennial sow thistle (*Sonchus arvensis* L.**)**, *Asteraceae* family (25) is prevalent in moist, humid, sub humid and arid areas of the world (20). It is widely distributed in cultivated crops fields, disturbed areas, waste ground, woods, sloughs, meadows, lawns, roadsides, ditches, beaches, river and lake shores (35,37). The *S. arvensis* causes serious problems in cultivated crops (5). Weeds, competes with crops for light, moisture, space and nutrients and drastically reduces the crop growth and yield (6,33). Weed-crop competition affects the crop quality and yield and causes economic losses (4,29). Allelopathy plays significant part in weed-crop competition (28,40) and weed-crop interference (10). Plants may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in their immediate vicinity (19). Because the weeds usually exhibit allelopathic interactions through release of water soluble allelochemicals from their leaves, stems, roots, rhizomes, flowers, fruits and seeds (4,9). These chemicals mostly affect the plants in early growth stages (3,27). Previously, no studies have been done in Pakistan on the allelopathic effects of Perennial sowthistle on maize. Therefore,

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this study aimed to assess the allelopathic effects of Perennial sowthistle on seed germination and seedling growth of maize.

MATERIALS AND METHODS

Fully mature plants of Perennial sowthistle were collected from our Campus, Islamia University of Bahawalpur, Pakistan (29.39°N, 71.68°E and 116 m above the sea level) during March and April, 2015. Plants were dried under shade at room temperature for one week. The dried plants were separated into leaves, roots, stem and whole plant.

Preparation of leachates

Fresh plant parts were weighed and immersed in tap water in ratio of 1:20 (w/v) for 24 h at room temperature (18). Each aqueous extract was further sieved through 10, 25 and 50 mesh size sieves to filter the impurities. In preliminary trials, it was observed that Perennial sowthistle's stem exhibited strong allelopathic effects. Therefore, owing to their greater inhibitory activity, different concentrations (1-4%) were made from the stem extract with distilled water. After 24 h, the solutions were filtered and centrifuged at 12000 rpm and leachates were collected and were individually bottled and tagged. The inhibitory effects of these leachates were tested on maize seeds germination and seedling growth.

Bioassay

The roots, stems, leaves and whole plant leachates of Perennial sowthistle were tested on germination and seedling growth of maize and distilled water was used as control. The seeds were surface sterilized with 1.5% (v/v) sodium hypochlorite solution for 1 min and washed thrice with distilled water. Fifteen maize seeds were placed evenly on Whatman No. 1 filter paper lined petri dishes (9 cm dia). In each petri dish, 5 mL of leachate or distilled water was applied as per the treatment. To minimize moisture loss, the petri dishes were sealed with parafilm and kept at room temperature day (32.6 ± 3.7^0C) and night (23.8 ± 3.2^0C). Subsequently, the water or leachates were added to each petri dish as needed. The seeds sprouted in 2 days and the data for growth parameters were recorded after 10-days.

Pot culture

The soil was collected from an area, not infested with Perennial sowthistle plants. Soil was dried in shade for 1 week and sieved, while the dried plant material was crushed (not grinded) and mixed in soil at 1, 2, 3 and 4% (w/w). Residue mixed soil was placed in plastic pots (19 cm dia) and these were kept in green house for further decomposition for one month. Maize seeds were surface-sterilized with 1.5% (v/v) sodium hypochlorite solution for 1 minute and washed 3 times with distilled water to make them pathogen free. After complete decomposition, 10 seeds of maize per pot were sown at 205 cm depth and watered immediately after sowing. Fourteen days after emergence, the seedlings were uprooted and washed with water. The seedlings were dried with tissue paper and root length, shoot length, fresh root weight and fresh shoot weight were recorded. To measure dry weights, the roots and shoots were oven dried at 65 °C for 72 h to obtain a constant weight.

(i). Germination (%) was calculated as under;

Germination percentage = (Germinated seeds/Total seeds sown)/100

(ii). Mean germination time (MGT) was calculated as per equation of Ellis and Roberts (13)

$$MGT = \sum (D_n) / \sum n$$

Where, n: Number of germinated seeds or emerged seedlings on day D and Dn: Total number of days after the beginning of germination.

(iii). Time taken to 50% seeds germination (T_{50}): It was calculated following Coolbear (11) and later modified by Farooq *et al*. (14):

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i}$$

Where, N: Final number of germinated seeds and n_i, n_j: Cumulative number of seeds germinated by counts at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

(iv). Seedling vigour index: It was calculated as per the equation of Abdul–Baki and Anderson (1).

SVI = Germination percentage × Radical length (cm)

(v). Root and shoot length (cm) was measured with Measuring Scale.

Total soluble phenolics

Total soluble phenolics were determined as per the method of Randhir and Shetty (32). The total phenolics were measured as Gallic acid equivalent. One ml of each leachate (leaves and stem) and 1 ml of 95% ethanol was combined in a test tube. Then 5 ml of distilled water along with 0.5 ml of folin-ciocalteu phenol reagent was added. The solution was incubated for 8 min and then 1 ml of 5% Na_2CO_3 was added, thoroughly mixed and was kept in dark for 1.0 h. The samples were vortexed and absorbance was measured at 750 nm on ultra violet spectrophotometer.

Phytotoxins

In bioassays, the stem and leaves leachates of Perennial sow thistle were more suppressive in nature, hence, the leachates were chemically analysed on a Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan) to identify and quantify the suspected phytotoxins. The conditions of separation are listed in Table 1. The peaks were detected by a UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for identification and quantification. Standards of phenolics were prepared at different concentrations. Vanillic acid and 4-hydroxymethyl benzoic acid were identified by their retention time with authentic standards.

Table 1. HPLC conditions to determine the phytotoxins in stem and leaves aqueous leachates of *S. arvensis*.

Sr. No	Parameters	Characteristic
1	Column dimensions	25 cm length x 4.6 mm diameter, particle size of 5 µm
2	Diatomite	Supelco wax 10
3	Attenuation	0.01 ppm
4	Rate of Recorder	10 mm min^{-1}
5	Detector	SPD-10A vp-detector
6	Detection	UV, 280 nm
7	Flow Rate	0.25 mL min^{-1}
8	Volume injection sample	50 µL
9	Type of Column	Shim-pack CLC-Octadecyl Silicate (ODS) (C-18)
10	Mobile Phase	Isocratic; 100% methanol
11	Temperature	25 ˚C

Statistical Analysis

The experiment was laid out in completely randomized design (CRD) replicated thrice. The recorded data was analysed statistically using Fisher's analysis of variance method and means with significant F values were compared using least significant difference (LSD) test at 0.05 probability level (36).

RESULTS AND DISCUSSION

LAB BIOASSAY

Germination and seedling growth

All the aqueous leachates significantly reduced the seeds germination (%), germination index and seedling vigour index of maize than control (Figure 1). Maximum germination, germination index and seedling vigour index were recorded in control treatment as 77.5%, 6.29 and 321.25 respectively. While maximum inhibition over control in all these parameters was recorded in stem leachates treated seeds. Aqueous leachates of the leaves, stem, root and whole plant significantly affected time to 50% germination (T_{50}) and mean germination time (MGT) (Figure 2). Minimum time taken to 50% germination was observed in control treatment, while, the maximum increase over control, in time taken to 50% germination was in seeds treated with stem leachates. Maximum upsurge in mean germination time was recorded in seeds treated with whole plant aqueous leachates followed by stem leachates.

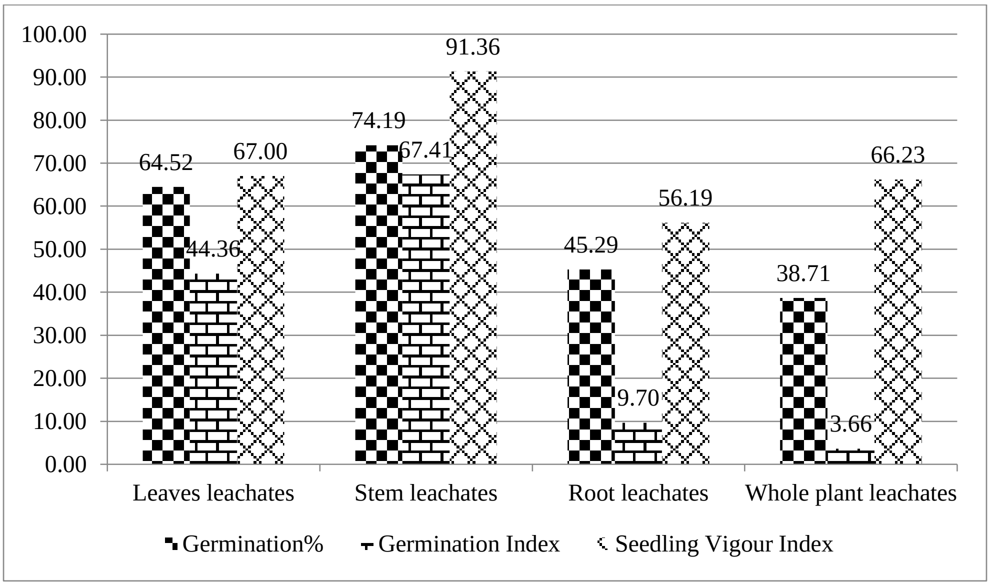

Figure 1. Inhibitory effects of *S. arvensis* leachates over the control on germination, germination index and seedling vigour index of *Z. mays*

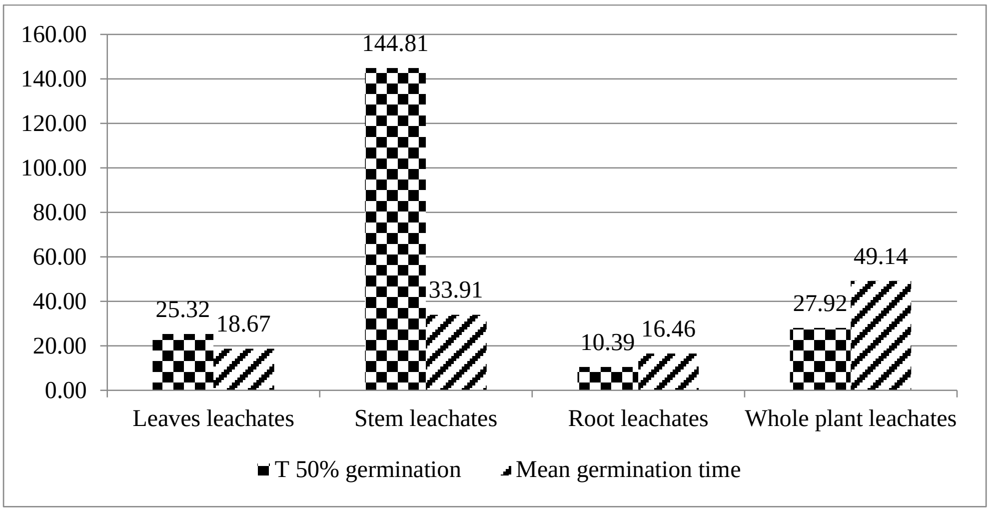

Figure 2. Inhibitory effects of *S. arvensis* leachates over the control on time taken to 50% germination and mean germination time of *Z. mays* seeds.

Inhibition percentage in root, shoot and seedling length in *Z. maize* due to various leachates of *S. arvensis* is displayed in Figure 3. All types of aqueous leachates significantly inhibited the root, shoot and seedling length of maize; however stem leachates drastically reduced the root, shoot and seedling length followed by whole plant leachates.

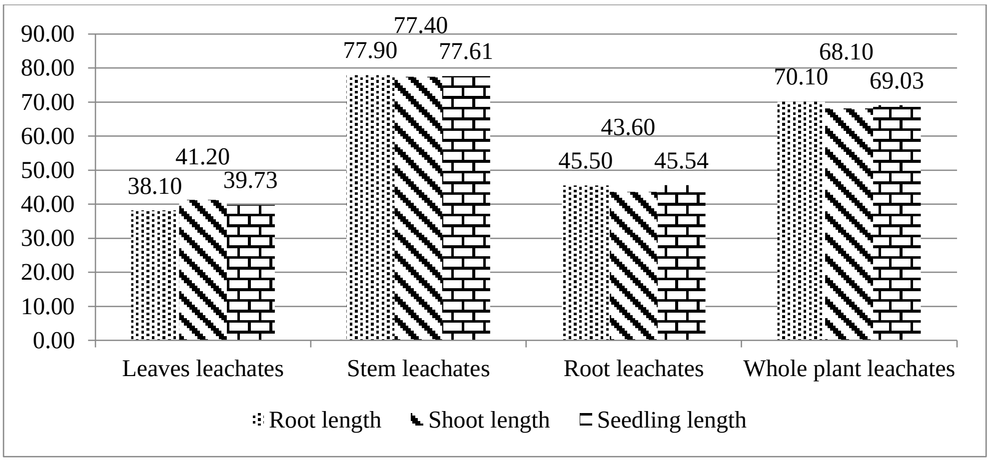

Figure 3. Inhibitory effects of *S. arvensis* leachates over the control on root length, shoot length and seedling length of *Z. mays*.

Owing to maximum reduction in germination and seedling indices by the stem and leaves leachates, both these leachates were analysed for phenolics and flavonoids through quantitative HPLC. The results showed that maximum total phenolics were detected in stem leachates than leaves leachates (Table 2, 3) which showed that inhibition in germination and seedling indices was most probably due to the presence of more phenolics in stem leachates.

Table 2. Quantitative high-performance liquid chromatography analysis of the concentrations and retention time of phenolic and flavonoid compounds in the aqueous leachates of *S. arvensis* stem.

Compound	Concentration (mg ml^{-1})	Retention time (min)
Quercetin	0.71	2.60
Hydrogenic acid	4.60	15.12
P-coumaric acid	2.56	19.86
Ferulic acid	8.92	23.39

Table 3. Quantitative high-performance liquid chromatography analysis of the concentrations and retention time of phenolic and flavonoid compounds in the aqueous leachates of *S. arvensis* leaves.

Compound	Concentration (mg ml^{-1})	Retention time (min)
Quercetin	1.35	3.03
Vanillic acid	23.25	13.23

We found that the aqueous leachates of Perennial sow-thistle were inhibitory to the germination and growth indices of maize. These results were supported by the findings of Garima *et al*. (15) who stated that aqueous leachates of different parts of Purple nutsedge (*Cyperus rotundus* L.) strongly suppressed the emergence and seedling growth of rice (*Oryza sativa* L.). Similarly, Babar (7) reported that chickpea (*Cicer arietinum*) seeds treated with aqueous leachates of different plant parts of onion weed (*Asphossdelus tenuifolius* cav.) took more time for germination. Our results are in accordance with findings of Shahrokhi *et al.* (34) who showed that Bindweed (*Convolvulus arvensis* L.) aqueous leachates from leaf and stem reduced the germination (%) of barley *(Hordeum vulgare* L.) seeds. Similarly our results are in agreement with those of Dhole *et al*. (12), who reported that *Alternanthera sessilis* L. strongly suppressed the seed germination of *S. vulgare*. Zhen *et al*. (42) stated that the water leachates of the Alligator-weed (*Alternanthera philoxeroides* Mart.) inhibited the germination of crop plants. Umer *et al.* (39) found that leave, stem and root aqueous leachates of sow thistle (*Sonchus aspera* L.) had inhibitory effects on growth of wheat (*Triticum aestivum* L.) and peas (*Pisum sativum* L.). Our findings were also in accordance with Mubeen *et al*. (26), who reported that stem aqueous extract of crowfoot grass (*Dactyloctenium aegyptium* L.) was more suppressive than other leachates on the rice seeds germination and seedling growth. Similarly, Ankita *et al.* (6) documented that leaves, roots and stems aqueous leachates of common sow thistle (*Sonchus oleracea* L.) suppressed the seed germination, seedling length and seedling dry weight of wheat (*Triticum aestivum* L.), which increased progressively with the increasing concentration of leaves, roots and stems aqueous leachates.

Effects of stem leachate

Different concentrations of stem leachates of Perennial sow thistle showed significant effects on the germination indices of the maize (Table 4). There was a gradual decrease in the germination percentage of maize seeds with the increasing concentration of stem leachates. Stem leachates at 4% concentration caused the maximum reduction (40.0%) in germination as compared to other concentrations. The data revealed that all the concentrations of stem extract increased the time taken to 50% germination than control, but the most significant increase was recorded with the 4% stem leachates. The maximum mean germination time (6.31 days) for maize seeds was noted in 4% stem extract as compared to other concentrations. The germination index was significantly higher (15.8) in control than various concentrations of stem leachates. The seedling vigour index of maize seed significantly decreased with the increasing concentration of leachates.

Table 4. Effects of *S. arvensis* stem leachates concentrations on germination indices of *Z. mays.*

Stem leachates concentrations (%)	Germination %	T 50% germination	Mean germination time	Germination Index	Seedling Vigour Index
Control	85.00a	0.77b	5.34d	15.8a	295.25a
1	60.00b	2.70a	5.78b	10.6b	123.25b
2	67.00b	2.48ab	5.70b	8.20c	81.50bc
3	67.50ab	2.42cb	5.47bc	6.50d	49.75c
4	60.00b	2.71dc	6.31a	4.80e	32.25c
LSD at 5%	25.069	1.182	0.347	1.937	57.63

Different concentrations of stem leachates also significantly reduced the root, shoot and seedling length as compared to control, but the highest reduction was noted with 4% concentration of stem leachate (Table 5). Allelopathic effects increased with the increasing concentration of allelochemicals i.e. was concentration dependent. More inhibitory effects were observed at higher concentrations as compared to lower concentrations.

Table 5. Effects of *S. arvensis* stem leachates concentrations on the seedlings growth of *Z. mays.*

Leachates concentration (%)	Root length	Shoot length	Seedling length
Control	24.97a	24.65a	49.65a
1	21.70b	19.40b	41.10b
2	11.75c	12.82c	24.5c
3	8.05d	11.15c	19.60ab
4	8.02d	8.02d	16.07d
LSD (5%)	2.129	2.13	3.695

These results showed that stem of Perennial sow thistle has greater allelopathic effects than other parts of the plant. The greater number of growth inhibitors detected in the stem explains the stronger inhibitory activity. These results were supported by the findings of Acciaresi *et al*. (2), who reported that the aqueous leachates of many weeds species had inhibitory effects on the seed germination, root, shoot and seedling length of many field crops. These results were also similar to findings of Kadioglue *et al*. (21), they stated that water leachates of different plant parts of weeds (*Solanum nigrum* L., *Chenopodium album* L. and *Matricaria chamomilla* L). inhibited the and final seed germination (upto 10%, 20% and 22.5%, respectively) of lentil (*Lens culinaris*), chickpea

(*Cicer arietinum*) and wheat (*Triticum aestivum* L.). Punjani (30) stated that leachates of Chilean mesquite (*Prosopis chilensis*) @ 0, 1, 3 and 5% concentrations significantly inhibited the seed germination and seedling growth of rice.

POT CULTURE

Effects of soil incorporated residues

The effects of different concentrations of Perennial sow thistle soil incorporated residues on the emergence (%) of maize are presented in Table 6. The results showed that emergence (%) of maize seedlings was significantly higher (100 %) in Perennial sow thistle free soil followed by the 2 % soil incorporated residues (92 %). Minimum emergence (60 %) of maize was recorded in 4% soil residues of Perennial sow thistle. Maize seedlings took minimum time (3.52 days) to complete 50 % emergence in control, whereas maximum (5.76 days) was recorded in 4 % soil residues of Perennial sow thistle. Germination index (GI) was maximum (10.73) in control treatment. Minimum mean germination time (7.02) was observed in the control, while the seeds took maximum mean germination time MGT (8.10 days) in 4 % soil residues of Perennial sow thistle. The infested soil significantly inhibited the root length, shoot length, root and shoot dry weight, seedling dry weight and seedling vigour index of maize.

In all cases, the largest seedlings in terms of root and shoot length were found in the control that had no Perennial sow thistle residues. Our results indicated that higher the amount of decomposed residues, the greater is the reduction in root and shoot length as well as in seedling vigour index of maize seedlings. Similarly, minimum dry weights of root, shoots and seedlings were also observed in the 4% soil residues as compared to other concentrations of Perennial sow thistle soil residues as well as the control treatment (Figure 4 & 5).

Table 6. Effect of doses (%) of soil incorporated stem residues of *S. arvensis* on the germination indices of *Z. maize*.

Concentration of Soil incorporated residues of *S. arvensis*	Germination (%)	T 50% germination	Mean germination time	Germination Index	Seedling Vigour Index
Control	100a	3.52b	7.02d	10.73a	11110a
1% (10 g residues/kg soil)	100a	3.53b	7.2cd	9.47a	8435ab
2% (20 g residues/kg soil)	92a	5.07a	7.52bc	6.89b	5437.5bc
3% (30 g residues/kg soil)	83a	5.06a	7.92ab	5.99b	5247.5bc
4% (40 g residues/kg soil)	60b	5.76a	8.1a	3.02c	2937.5c
LSD (5%)	29	1.01	0.45	2.06	3532.2

These findings are supported by the results of Katoch *et al.* (22) who reported impaired growth of rice (*Oryza Sativa* L.) and maize (*Zea mays* L.) seedlings germination and root, shoot length by allelopathic effects of plant residues of dominant weeds (*Eupatorium adenophorum*, *Ageratum conyzoides* and *Lantana camara*) in North western Himalayan region. Qayyum *et al*. (31) also stated that the leachates of purple nutsedge (*Cyperus rotundus* L.) weed inhibited the seed germination and seedling growth of rice. Our findings agree with Tanveer *et al.* (38) who also reported that minimum GI and germination (%) of rice seeds, when treated with leaf leachates of common cocklebur (*Xanthium strumarium* L.). Our results are also in agreement with earlier studies, where residues of *Ageratum conyzoides* adversely affected the crop growth (8,41) by releasing water-soluble phenolic acids into the soil environment. These results are also similar to Zuo *et al*. (43). Dhole *et al*. (12) reported that strong inhibitory effects of extract of Sessile Joyweed (*Alternanthera sessilis* L.) on seed germination and seedling growth of maize.

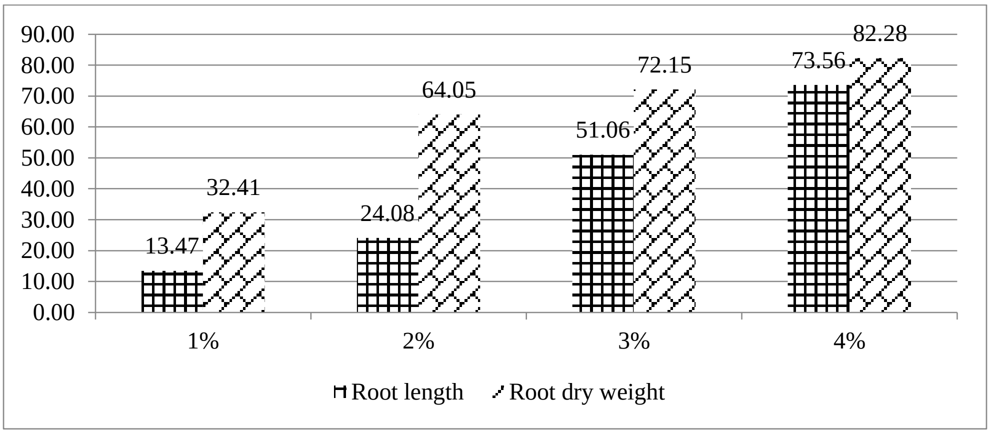

Figure 4. Inhibitory effects of concentrations of *S. arvensis* stem leachates over the control on root length and root dry weight of *Z. mays*

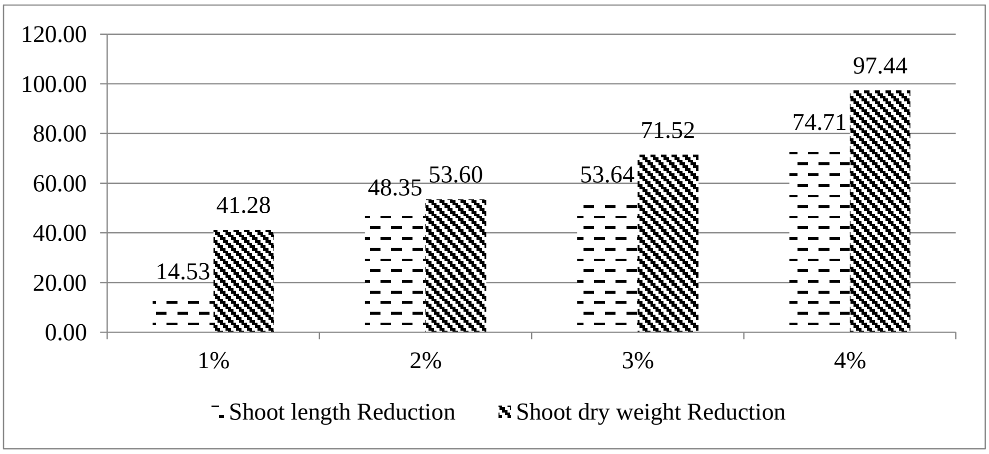

Figure 5. Inhibitory effects of concentrations of *S. arvensis* stem leachates over the control on shoot length and shoot dry weight of *Z. mays*.

Determination of phytochemicals

The quantitative determination of some phytochemical constituents of Perennial sow thistle stem is summarized in Table 2. Among the compounds detected were: Quercetin (0.71 mg ml^{-1}), Hydrogenic acid (4.6 mg ml^{-1}), Ferulic acid (2.56 mg ml^{-1}) and Coumaric acid (8.92 mg ml^{-1}). The HPLC analysis of the leaves of Perennial sow thistle confirmed the detection of two phytochemical compounds (Table 3): Quercetin (1.35 mg ml^{-1}) and Vanillic acid (23.25 mg mL^{-1}), respectively. Phenolic acids are found in large amount in plants and soils and are allelochemicals (16,23). The combination of phenolic acids have additive suppressive action and or synergistic suppressive action (16,24).

CONCLUSIONS

The aqueous leachates of Perennial sow thistle were allelopathic. The allelochemicals present in the donor plant drastically inhibited the seed germination and seedling growth in maize. Therefore, this weed should be removed from the fields in initial stages of growth to prevent the crop damage from this weed. Our current results were obtained under laboratory conditions, however, the assessment of the allelochemicals activities of Perennial sow thistle under field conditions is imperative for future recommendations.

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