

Allelopathic Effects of Sorghum on Milk Thistle (*Silybum marianum* L.) Seed Germination and Growth

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ABSTRACT

Crop residues are well known for their chemical (allelopathic) effects on crops. The allelopathic potential of the aqueous extracts and chopped residual of sorghum (*Sorghum bicolor* L.) on germination, seedling growth, relative water content, seedling dry weight, seedling length and antioxidant enzyme activity namely catalase (CAT), peroxidase (POD) and Superoxide Dismutase (SOD) of *Silybum marianum* was studied. Petri dish and pot experiments were performed in completely randomized design with three replications. Five concentrations of the aqueous extracts and foliar application of the sorghum [control (water distilled), 5, 10, 15 and 20 w/v] and sorghum chopped (0, 5, 10 and 20 g dry chopped / 100 g soil) were used in the experiments carried out in the laboratory and greenhouse, respectively. Petri dish trial showed that the different extract levels reduced total germination percentage (GP), germination rate (GR) and seedling growth. Moreover, relative water content (RWC), dry weight, and seedling length, antioxidant enzyme activity except CAT of milk thistle bioassay were inhibited when growth in soil incorporated with oven-chopped residual of sorghum. The inhibitory effects often depend on the concentration. Finally, sorghum residues had an enormous potential to suppress elements of germination and seedling growth of milk thistle. Hence, this soil incorporation of allelopathic crop residues could be employed as an important agent for crop rotation management.

Keywords: Allelopathy, *Silybum marianum*, Sorghum, Germination, Antioxidant enzyme activity, Relative water content

INTRODUCTION

Milk thistle (*Silybum marianum* Gaertn.) is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina (Khan *et al.*, 2009) and is a medicinal plant in Iran. Its current distribution includes most temperate areas of the world (Chambreau and MacLaren, 2007). Production of high-quality milk thistle achenes depends on conditions of cultivation that directly influence the quality of final product (Khan *et al.*, 2009).

The capability of some plant species to affect surrounding plants has been well documented since antiquity. This phenomenon is known as allelopathy (from the Greek *allelon*= of each other, *pathós*= to suffer) (de Albuquerque *et al.*, 2010). Allelopathy can affect all physiological factors, e.g. growth, plant canopy succession, survival, extension and crop production (Ferguson *et al.*, 2004). Crop residues is the name given to plant material left in the field for decomposition after the harvesting of a crop is over (Kumar and Goh, 1999). These residues can pose a chemical (allelopathic) as well as a physical effect on the growth and development of subsequent crops (Lovett and Jessop, 1982; Purvis *et al.*, 1985; Mason-Sedun *et al.*, 1986). Numerous plants possess allelopathic properties, including many crops: Wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L. Moench), and rye (*Secale cereale* L.) (Rahimi *et al.*, 2006).

Allelochemicals have mostly negative effects on crop plants such as: delayed or complete inhibition of germination, reduced plant population, stunted and deformed roots and shoots, deranged nutrient absorption, lack of seedling vigour, reduced tillering, chlorosis, wilting, and increase susceptibility to disease (Walker and Elliott, 1986; Waller, 1987). However, the main impacts of phytotoxins on crop plants are: inhibition of nitrification and biological nitrogen fixation, and inhibition or stimulation of germination, growth and yield (Bogatek *et al.*, 2006).

Sorghum (*Sorghum bicolor*) is also a great forage crop which has a high potential in forage production in Iran. Mature sorghum produces a large number of water-soluble allelochemicals (Moosavi *et al.*, 2011). Guenzi and McCalla (1962) tested various hot- and cold-water extracts of oats, corn, wheat, sorghum, sweet clover, and soybean and reported a wide range of inhibition of germination, shoot growth, and root growth of wheat. Subsequently, Guenzi and McCalla (1966a, b) isolated various allelochemicals from crop residues and their associated soils. Yang *et al.*, (2004) reported that the decrease in rice productivity of crops following the first rice was due mainly to the allelopathic effects of decaying rice residues.

The relationship between allelochemicals and environmental factors are a key for the growth of plants under rotation (Mamolos *et al.*, 2001). Rotation systems and allelopathic interactions between plants-plants would be important to exploit allelopathy in optimising the production of rotation systems. Examples from field crops, medicinal plant and forage crops provide evidences for the role allelopathy plays in crop rotation systems. In conclusion, the selection of certain plant sequences under standard environmental conditions may lead to suppression of crops and avoid yield decline (Mamolos *et al.*, 2001). The objective of this study was to determine the effect of sorghum residue from Speedfeed cultivar on germination, seedling growth and physiological parameter of milk thistle under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were conducted during 2012-2013 to evaluate the allelopathic effects of sorghum on the germination of milk thistle. The experiments were arranged in a completely randomized design with three replications. The experiments included three separate stages: Sorghum planting and preparation of extracts from vegetative growth stage of sorghum, laboratory bioassays and greenhouse bioassays.

Preparation of Water Extract Solutions

Sorghum shoots (leaf and stem) were sampled at the vegetative stage of plants that were grown in the greenhouse. The plant material was dried in an oven at 40°C for 72 h. Extracts were prepared by soaking appropriate amounts of chopped plant materials (5, 10, 15 and 20 g) in 100 ml distilled water at room temperature. After 24 hours, the solutions were filtrated and centrifuged at 9000 rpm, after that the clean and pure extracts were collected.

Laboratory Bioassays

Germination tests of milk thistle was performed in petri dishes in lab germinator based on ISTA rules (2003), for seven days at 25°C. Twenty five healthy milk thistle seeds were put in petri dishes and sorghum extracts were applied to each individual petri dish; distilled water was used as control treatment. After 7 days, germination percentage, germination rate, shoot, root and seedling length and dry weights were measured. Polyethylene glycol (PEG) was not used in this study because the extract solution concentrations did not exceed 50 milliosmoles (about - 0.11 Mpa) (Bell, 1974).

Greenhouse Bioassays

I) Bioassay in Greenhouse by Using Sorghum Residues

These studies were conducted to determine whether sorghum shoot residual would have any effect on the growth of test plants under field-like conditions. Pre-weighed 110 g of soil analyzed as 16% sand, 62% silt, 22% clay having 1.5% organic matter were mixed with 0, 5, 10, 15, and 20 g of milk thistle dried shoots tissue. The soil was flame-sterilized for 1 h before use and was placed on saucers to prevent the loss of water-soluble toxic substances. This treatment mixture was thoroughly mixed and placed in rectangular plastic pots with 100 cm² soil surface. Then, ten seeds of milk thistle were planted per pot. These pots were maintained at 25±5°C. Plants were harvested six weeks after planting. These producers were are consistent with those used for the allelopathic studies of other species (Bhowmik and Doll 1982, 1984; Touchette *et al.* 1988; Iqbal *et al.*, 2004). Finally, the relative water content (RWC) was calculated according to Barrs and Weatherley (1962).

II) Bioassay in Greenhouse by Using Sorghum Extracts Spraying

To determine the effect of foliar application of sorghum leaf crude extract on milk thistle growth, a post-emergence application test was performed. Sorghum shoot extracts at 5, 10, 15 and 20 mg/ml water (w/v) were sprayed in 110 L water using knap sack sprayer on foliage of test plants seedlings at 20 d after sowing. Three weeks after spraying, the shoots of each plants were collected to determine activity measurements of antioxidant enzymes. SOD activity was measured by the methods of guaiacol colorimetry, POD activity was assayed by the guaiacol method, and CAT activity was assayed by the permanganate titration. The detailed steps were as descried (Wheeler *et al.*, 1990).

Statistical Analysis

Data of laboratory and greenhouse bioassays were subjected to data transformation in order to uniform the variance of data. The SAS 9.2 software and Excel 2010 were used for analyzing the data of the experiment and drawing graphs.

RESULTS AND DISCUSSION

Results of analysis of variance for germination experiment showed that germination percentage, germination rate, shoot and root growth and dry biomass of milk thistle seedling was significantly affected by different concentrations of sorghum extracts (Table 1).

Table 1- Analysis of variance allelopathic effect of sorghum water extracts on growth parameters on Milk thistle

SOV	GP (%)	GR	SL	RL	SDW	RDW	RWC		CAT	POD	SOD
							Shoot	Root			
Extract	80.91**	3.12**	1.60**	13.13**	0.9**	0.03**	0.42**	1.36**	0.43**	0.82*	1.62*
Error	2.14	0.10	0.14	1.20	0.005	0.003	0.09	0.064	0.05	0.06	0.08

* and ** significant at 5 and 1%, respectively.

Germination percentage: GP, germination rate: (GR), Shoot length: (SL), Root length: (RL), Shoot dry weight (SDW), Root dry weight (RDW), relative water content (RWC), Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD).

Table 2. Allelopathic effect of sorghum water extracts on germination parameters on Milk thistle

Concentrations of Extract (w/v)	Measured traits					
	GP (%)	GR	SL (cm)	RL (cm)	SDW (gr)	RDW(gr)
Control	44.42 a	3.14 a	1.46 a	4.72 a	0.29 a	0.20 a
5 %	51.04 a	3.09 a	1.65 a	3.91 ab	0.33 a	0.11 b
10 %	34.96 b	2.28 b	1.36 a	2.43 b	0.00 b	0.00 c
15 %	13.46 c	0.95 c	0.41 b	0.37 c	0.00 b	0.00 c
20 %	17.50 c	1.24 c	0.00 b	0.00 c	0.00 b	0.00 c

In columns, means followed by same letter are not significantly different at p = 0.05.

Germination percentage (GP), germination rate (GR), Shoot length (SL), Root length (RL), Shoot dry weight (SDW), Root dry weigh (RDW).

Seed Germination

Germination percentage (GP) increased with the concentration of treatments; the highest GP was observed at 5% extract (Table 2). The additive effect on germination percentage was observed when 5% sorghum aqueous extracts were used. With increase in concentration, GP was reduced. Sorghum extracts in concentrations of 15 and 20% decreased germination of milk thistle seeds to 69.70 and 60.60%, respectively, compared to control treatment (Table 2).

The GR was observed in control treatment and the lowest was observed in 15% of sorghum extracts. Treatments with 5% to 20% extract decreased GR of milk thistle to 1.60, 27.38, 69.74 and 60.50%, respectively (Table 2). Generally, 15% extract was more inhibitory than 20% extract.

Sorghum extract treatments not only decreased germination but also decreased shoot, root, seedling length, dry weight and dry matter accumulation in milk thistle seedlings. The inhibitions were relatively enhanced with the increasing amount of each extract concentrations. Results indicated that root length was relatively more sensitive to allelochemicals compared to the shoot elongation (Table 2). Root length was significantly influenced by the treatments. Treatments with 5, 10, 15 and 20% extracts decreased dry weight of milk thistle 17.16, 48.51, 92.16 and 100%, respectively (Table 2). The most effective reduction among all treatments was observed at 20% extract (Table 2). In addition, inhibitory effect of rest of the concentrations was significant except effect of 5 and 10% extract on shoot length. The inhibitory potential of extracts generally enhanced with increase in concentration (Table 2). Concentrations of 15 and 20% sorghum extracts exhibited the lowest length, and 5% sorghum extracts exhibited the highest shoot length (Table 2). The decrease in shoot length of milk thistle seeds was 71.91 and 100% by 15% and 20% sorghum extracts in comparison with control (Table 2).

Seedling growth was decreased due to decrease in seedling dry weight (SDW). Sorghum aqueous extracts of all of the applied concentrations significantly suppressed the seedling dry weight of milk thistle. Early seedling dry weight of milk thistle was generally reduced significantly by extracts of 10% and of higher concentrations. The additive effect on SDW was observed when 5% sorghum aqueous extract was used. Sorghum extracts in concentrations of 5%

increased SDW of milk thistle seeds up to 13.79%, compared to control treatment (Table 2). The reduction pattern in RDW was highly similar to SDW (Table 2).

Results suggested that suppressive effects on germination traits and seedling growth characters of milk thistle were imposed by the release of phytotoxic allelochemicals in the sorghum extracts. Results were supported by the findings of Khaliq *et al.* (2009) who reported that evident suppressive effects of water extracts of sorghum on germination percentage, germination rate and root length of *Cichorium intybus* as compared with control. reduction in root and shoot length by application of sorghum water extracts might be due to inhibitory effects of allelopathic compounds present in the extracts of sorghum. Reported that several allelochemicals have been isolated from sorghum residues for example hydroxamic acids suppressing the germination and growth of many grass species (Vaughn and Boydston, 1997). The reductions in seedlings root and shoot length may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts (Buckolova, 1971). Results support the findings of Randhawa *et al.* (2002) who recorded significant reduction in root length and dry weights of *Trianthema portulacastrum* by the application of sorghum water extract.

Greenhouse Investigation

Analysis of variance showed that sorghum extracts concentration had significant effects on all studied traits (Table 1). Sorghum residual stress made significant changes on leaf and root RWCs (Table 3). The basic effect of the investigated extract was generally similar in shoot and root RWC. The highest mean increase of leaf RWC was observed in 15% extract with 74.85% and the lowest decreased (7.97%) was detected in 20 % extract treatment (Table 3). In addition, higher reduction in root RWC was observed in 20% extract treatment with maximum increase of 3.35% in 5% extract compared to control (Table 3).

Table 3. Allelopathic effect of Sorghum residue soil incorporation on Milk thistle

Sorghum residue	Measured traits			
	RWC (%)		H (cm)	DW (gr)
	Leaf	Root		
Control	37.38 c	62.76 a	19.18 a	10.11 a
5 %	39.66 c	64.86 a	11.67 b	9.97 a
10 %	48.66 b	63.50 a	10.12 b	6.89 b
15 %	65.36 a	31.90 b	7.73 c	5.87 b
20 %	34.40 c	22.50 b	6.41 c	4.92 c

In columns means followed by same letter are not significantly different at p = 0.05. Relative water content (RWC), height (H), dry weight (DW).

Table 4. Allelopathic effect of sorghum water extracts foliar application on activities of antioxidant enzymes of milk thistle

Water extracts as foliar application (w/v)	Measured traits		
	CAT mg protein/min	POD mg protein/min	SOD mg protein/min
Control	24.70 a	9.13 a	17.40 a
5 %	22.87 b	8.62 b	16.22 b
10 %	23.82 b	8.44 b	16.18 b
15 %	23.82 b	7.82 c	16.08 c
20 %	23.86 b	7.68 c	16.05 c

In columns means followed by same letter are not significantly different at $p = 0.05$. Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD).

Incorporation of sorghum residual affected the growth of milk thistle at all treatments. The plant height (H) of milk thistle decreased progressively with increased concentration and a 66.57% reduction in plant height with respect to control was observed at 20 g residual per soil (Table 3). The decrease in height and plant dry weight (DW) of milk thistle was dependent to concentration. Twenty gr sorghum residual inhibited DW as 51.33% of the control; roots were malformed with a corkscrew-like appearance (Table 3). Sorghum residual treatments not only decreased (H) but also decreased (DW) accumulation in milk thistle plants. Decreased H was resulted by decreased DW (Table 3). Generally, increase in the concentration increased the inhibitory potential of the extracts. At concentration of 20% extract, (H) was decreased by 80% compared to control (Table 3).

Our results suggested that sorghum residue contained a plant growth inhibitory substance, or substances, in amounts sufficient to suppress the growth of milk thistle. Phytotoxicity of dried sunflower residues and leaf powder has been reported (Narwal, 1999; Batish *et al.*, 2002). Incorporation of sunflower residues in the soil reduced the growth of sorghum, soybean and canary grass. All parts of sorghum plant such as roots, leave and stem as well as germinating seeds release phytotoxins that can suppress weed growth. Incorporation of (in situ) whole sorghum plant or its various parts alone or mixed with each other was found to suppress weed growth in wheat. Cheema *et al.*, (2004) stated that sorghum mulch (10-15 t/ha⁻¹) decreased the dry weight of purple nutsedge up to 38-41%, compared to control. In addition, macro and micronutrient absorption and IAA oxidase in plant root cells is inhibited by various allelochemicals (Yang *et al.*, 2004), which may lead to the observed reductions in DW and RWC of germinating mustard seedling. Allelopathy by sorghum frequently harms wheat when the crops are grown in rotation (Roth *et al.*, 2000). Tilled sorghum residues often delayed the development of the following wheat crop but did not affect grain yields, probably because allelopathic compounds degraded in the soil. No-till sorghum stubble had little effect on stand establishment, but frequently reduced the grain yield of wheat, probably because the allelopathic compounds leached slowly (Roth *et al.* 2000).

The effect of allelopathy stress on the activities of antioxidant enzymes is shown in (Table 4). The results revealed an increase in CAT activity and a decrease in SOD and POD activities in leaf of milk thistle under different sorghum extract concentrations. A gradual decrease was observed in SOD and POD activities under sorghum extract concentrations in comparison with the control (Table 4). In addition, CAT activity under 5 to 20% treatments were not significant between extract concentrations (Table 4). The maximum increase in the CAT activities was observed in the 20% extract (8% fold), while the minimum increase was observed in the 5% extract (4.15 fold) compared to the control (Table 4). On the other hand, a significant decrease was observed in SOD and POD activities under 20% extract treatment (7.78% and 15.88%, respectively) when compared to the control (Table 4).

The results shown in Table 3 were consistent with those of Cruz-Ortega *et al.* (2002), who reported that allelochemicals cause an increase in the activity of antioxidant enzymes and suggested that increasing induction of these enzymes was necessary to prevent lipid peroxidation. The results for enzyme CAT were consistent with those of Penner and Ashton (1967), who showed that the exposure of sunflower to 2-benzoxazolinone caused an increase in the activity of catalase. A decrease of POD and SOD activities in response to allelochemicals had also been reported by Yan *et al.* (2013) for wheat. This finding was consistent to that observed for other allelochemicals as reported by Qian *et al.* (2009), except that the trend was decreasing activity with increasing concentration of the extract. It could be speculated that at high concentrations, the allelochemical might directly inhibit oxidizing enzymes in some way, leaving the plant vulnerable to oxidative damage as reported by Qian *et al.* (2009).

CONCLUSIONS

Present study concludes that the sorghum extracts and residues have potential to suppress seed germination, seedling growth, antioxidant enzyme activity, and biochemical responses of milk thistle. The results of this study provided evidence that sorghum had allelopathic potential and could be used as a ground cover plant or as an agent for crop rotation management. A well-planned crop-rotation system can help producers avoid many of the problems associated with selective plants, such as decreased competition, weeds control, plant diseases, slow early season growth and decrease crop yield. So planting milk thistle in fields which were previously cultivated by sorghum, was not recommended, and farmers should highly notice how much of sorghum straws remain in the field before planting any crop, specially milk thistle.

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