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Assessment of Auto-allelopatic Potential of Broomcorn (*Sorghum vulgare* var. *technicum*)

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Abstract. Broomcorn as an ancient crop is a variety of *Sorghum vulgare* planting in Iran to produce traditional cleaning broom. Miyaneh is one of the major broomcorn-growing regions in where farmers crop it intensively since old ages. The crop is so profitable that they prefer not to include any other crops in rotation. One of the problems in continuous cropping might be related to crop auto-toxicity. To evaluate of broomcorn auto-allelopathic potential on its seed germination and initial growth, a sequence of laboratory and greenhouse experiments were conducted by aqueous extract of broomcorn roots and shoots, instant and burnt residues and root exudates using by Equal Compartment Agar Method as well. Shoot extracts were more auto-toxic than roots'. Root exudates were negatively more effective on coleoptile growth than radicle. Burning of residue could be considerable depression of auto-toxic potential of broomcorn. Auto-toxicity was also reduced by residue aging up to 30 days.

Keywords: auto-toxicity, allelopathy, sorghum, residue management, ECAM

1. Introduction

Auto-alleopathy is beneficial or harmful effect of a plant species on itself (Mighani, 2004). Likewise, auto-toxicity is defined as intra-specific negative allelopathy which takes place when a plant releases chemical substances that inhibit or delay germination and seedling growth of the other plant from the same species (Pantum, 1985). This phenomenon has been confirmed to be in various plants such as lucerne, cotton, soybean, wheat, barley, rye, rice, oats, and so on, by several investigators. On the other hand, allelopathy of *Sorghum* species was well-known against other crops and weeds (Solymosi, 2004). Sorghum residues have been used for weeds' biological management. Also, it is evidence that sorghum germinating seeds release allelopathic chemicals from roots and foliages which negatively affects wide range of plants (Mighani, 2004). Zahid *et al*. (2001) used successfully Sorghaab (sorghum stem water extract) as the natural weed inhibitor for weed management in spring mung bean field. In addition, sorghum stems, leaves, and roots are inhibitive components on wheat radicle growth and allelopathic potential may occur within and among various sorghum hybrids (Ben-Hammouda *et al.*, 1995).

Broomcorn (*Sorghum vulgare* (L.) Moench var. *technicum*), is a type of sorghum that is used for making brooms and whiskbrooms (Carter *et al*., 1990). The first report of broomcorn allelopathic potential goes back to its interaction with weeds such as *Amaranthus retroflexus* and *Chenopodium album* which was proven to be inhibitive on the mentioned weeds germination and initial growth (Mardan, 2009). Aqueous broomcorn roots, stems and leaves extracts sampled 20, 50, and 80 days after planting, affected negatively wheat, fat hen, and redroot pigweed seed germination. Stem extracts had the least allelopathic effects on both weeds (Latifi *et al.*, 2010). Broomcorn root extracts was inhibitive on lentil seed germination even in 2.5% concentration (Valizadeh *et al.*, 2010). Likewise, Stems were less allelopathic on chickpea than leaves and roots. Broomcorn different organs' extracts had partially equal effect on corn seed germination, but root was

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less effective than stems and leaves (Valizadeh *et al.*, 2010). Also, instant and burnt residues of broomcorn inhibited considerably seed germination and negatively affected initial growth of wheat, barley, and tomato seedlings (Hashemizadeh *et al.*, 2010). Broomcorn's 3-month old root exudate was very inhibitive on wheat seeds germination and initial growth. Also, shoot extracts with the same age totally prevented wheat seed germination (Hashemizadeh *et al.*, 2011).

As broomcorn is a very strategic plant in Miyaneh region, Iran. It is planting frequently due to the high economic benefits, mostly with no crop shifting (Jamshidi, 2003). The auto-toxic potential of the crop might be easily ignored in the process and the previous crop might negatively be allelopathic on the next crop by affecting on seed germination and seedling establishment. The current study was carried out to evaluate possible auto-allelopathic potential of broomcorn various organs in different stages.

2. Material and Methods

2.1. Sampling, plant extract preparation and bioassay tests

Shoots and roots of broomcorn (cv. Nikabad) were sampled 1, 2, 3 and 4 months after planting, separated, washed with distilled water and dried in oven (50 °C for 72 h), divided into small pieces, blended and sifted by 20-mesh sieve. Twenty grams of powdered plant material were separately suspended in 200 ml distilled water and shaken for 24 hours by a horizontal rotary shaker in room temperature. Centrifugation was performed using a Mikro 22 R centrifuge (Hettich, Germany) at 6000 rpm for 30 minutes at 10˚C) and considered as 10% concentration. The 2.5, 5 and 7.5% concentrations were obtained by adding distilled water. Broomcorn seeds were disinfected superficially by 70% ethanol for 1 minute and 2.5% sodium hypochlorite solution for 3 minutes followed on four times washing by sterile distilled water. Twenty seeds of broomcorn were placed in Petri dishes with sterile filter paper inside and 5 ml extract were added on and incubated in 25 ± 1 ˚C in dark condition. Seed germination, radical and coleoptile length, seedling fresh and dry weight were recorded after 15 days incubation.

2.2. Root exudates assessment

The ECAM (Equal Compartment Agar Method) was employed for assessment of root exudates of broomcorn seedlings (Wu *et al.,* 2000). Briefly, surface-sterilized seeds germinated in light at 25˚C for 24 hours. Twelve pre-germinated seeds were uniformly selected and aseptically sown on the agar surface with the embryo up, in three rows on one half of a glass beaker (500 ml) pre-filled with 30 ml of 0.4% water agar as donor. The beaker was wrapped with a piece of parafilm and incubated in 25 ˚C/13 ˚C alternatively for day and night. After seven days, the donors were removed and 12 broomcorn pre-germinated seeds were aseptically sown on the agar surface in three rows on the other side of the beaker as receivers. After sowing receiver broomcorn seeds, the beaker was again wrapped with parafilm and placed back in the growth cabinet for 10 days continuous growth. The radicle and shoot lengths of both donor and receiver seedlings were measured after 10 days of receiver growth.

2.3. Residue's allelopathic potential evaluation

Broomcorn instant and burnt residues were sampled immediately after harvest and one month after that and 5 and 10% aqueous residues extracts were prepared using above-mentioned method. Bioassay method, incubation circumstances and also measured traits was the same with part 2.1.

2.4. Greenhouse studies

Twenty broomcorn surface sterilized seeds were planted in pots (20 cm diameter) filled with autoclaved (121 ˚C, 15 minutes), sand, soil, peat (1:1:1) soil mixture incubated in a greenhouse at 25 ± 3 ˚C temperature and 70 ± 5% relative humidity. After four days, emerged plants were abated to 10 by selecting equally well-developed seedlings. Every three days, the seedlings were irrigated with 2.5, 5, 7.5 and 10 % aqueous broomcorn extracts from the 5th day. Fifteen days after, root and stem length and fresh and dry weight of seedlings were measured. Distilled water was used for check plants irrigation.

2.5. Experimental design and data analysis

The experiment was conducted based on completely randomized design with three replications. The first experiment was in factorial. Inhibition percentage of germination and growth of receiver plants were calculated as (control – data with donor)/control*100. The data were analysed using GLM procedure by SAS software and Duncan's Multiple Range Test was used for mean comparisons.

3. Results and Discussion

In the first experiment, triple interaction between sampling stage, plant organ, and extract concentration was significant in all studied traits. On the whole, shoots were more auto-toxic than roots, especially in third sampling stage with 100% preventing on seed germination, even in the most diluted concentration. Also, the 4-month old shoots extract completely prevented seed germination but 2.5%. In addition, 7.5 and 10% shoot extract concentrations in the first and second sampling stages were totally inhibitive on seeds germination. Seemingly, the auto-toxic chemicals are increasing up to 3 months but depressing afterwards, both in shoots and roots. Also, in the second to fourth sampling stages, root extracts was 100% preventive on seed germination, but one-month old roots extracts was less auto-toxic. The least inhibitive effect of root extracts was observed in the first stage sampling. Even germinated influenced seeds produced weak seedlings in size and weight influencing by root and shoot extracts. This phenomenon was more obvious in shoots than roots (Table 1).

Table 1 - Reduction in germination, radicle and coleoptile length of receiver broomcorn seeds exposed to the aqueous extracts of roots, shoots and roots (inhibition %)

Traits	Sampling stage	Root extracts concentrations (%)				Shoot extracts concentrations (%)			
		2.5	5	7.5	10	2.5	5	7.5	10
germination	1	17.2 ± 5.3 ^{gh*}	27.6 ± 5.3 ^{fg}	37.9 ± 8.3 ^{ef}	41.3 ± 5.9 ^{ef}	16 ± 1.2 ^{gh}	36 ± 6.9 ^{ef}	100 ^{a}	100 ^{a}
	2	19.2 ± 7.5 ^{gh}	38.4 ± 7.6 ^{ef}	80.7 ± 9.3 ^{bc}	100 ^{a}	28.5 ± 6.2 ^{fg}	78.5 ± 10.7 ^{bc}	100 ^{a}	100 ^{a}
	3	26.9 ± 6.6 ^{fg}	73.0 ± 8.6 ^{c}	88.4 ± 0.8 ^{ab}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
	4	10.7 ± 2.3 ^{h}	46.4 ± 10.7 ^{de}	57.1 ± 10.7 ^{d}	100 ^{a}	28.5 ± 6.3 ^{fg}	100 ^{a}	100 ^{a}	100 ^{a}
radicle length	1	9.0 ± 5.2 ^{i}	9.4 ± 5.2 ^{i}	27.08 ± 1.9 $^{e-h}$	40.3 ± 4.7 $^{c-e}$	12.5 ± 8.2 ^{hi}	15.3 ± 6.2 $^{g-i}$	-	-
	2	15.3 ± 5.1 $^{g-i}$	30.2 ± 5.9 $^{d-f}$	58.8 ± 9.1 ^{b}	-	5.8 ± 4.7 ^{i}	19.3 ± 8.2 $^{f-i}$	-	-
	3	28.5 ± 9.7 $^{d-g}$	58.1 ± 6.9 ^{b}	63.2 ± 4.3 ^{b}	-	-	-	-	-
	4	5.1 ± 4.6 ^{i}	54.8 ± 9.7 ^{bc}	61.6 ± 5.1 ^{b}	-	41.7 ± 8.1 ^{cd}	-	-	-
coleoptile length	1	13.8 ± 4.6 ^{hi}	26.2 ± 3.3 $^{e-h}$	34.4 ± 5.3 $^{d-g}$	49.7 ± 5.3 ^{c}	18.6 ± 7.7 ^{hi}	39.4 ± 4.2 $^{c-f}$	-	-
	2	8.6 ± 1.6 ^{i}	27 ± 5.3 $^{e-h}$	48.1 ± 4.6 ^{cd}	-	18 ± 7.1 ^{hi}	47.3 ± 10.3^{cd}	-	-
	3	24.8 ± 8.6 ^{gh}	52 ± 4.3 ^{c}	74.4 ± 7.2 ^{b}	-	-	-	-	-
	4	26.6 ± 5.1 $^{e-h}$	40.3 ± 6.3 $^{c-e}$	50.2 ± 9.5 ^{c}	-	25.6 ± 2.7 $^{f-h}$	-	-	-
fresh weight	1	6.4 ± 3.7 ^{i}	11.4 ± 3.1 ^{i}	20.9 ± 4.5 ^{hi}	40.4 ± 4.8 $^{e-g}$	20.7 ± 3.5 ^{hi}	32.6 ± 9.8 ^{gh}	-	-
	2	28.9 ± 8.3 ^{gh}	35.5 ± 5.6 ^{fg}	72.1 ± 9.2 ^{b}	-	13.9 ± 7.3 ^{i}	53.1 ± 8.6 $^{c-e}$	-	-
	3	31.7 ± 7.7 ^{gh}	59.8 ± 14.3 $^{b-d}$	66.9 ± 7.1 ^{bc}	-	-	-	-	-
	4	31.1 ± 7.4 ^{gh}	48.8 ± 7.6 $^{d-f}$	68 ± 4.2 ^{b}	-	52.8 ± 8.2 $^{c-e}$	-	-	-
dry weight	1	10.8 ± 4.3 $^{e-h}$	19.9 ± 3.1 $^{c-g}$	21.8 ± 3.1 $^{b-f}$	30.9 ± 3.1 $^{b-d}$	28.5 ± 4.7 $^{b-d}$	34.9 ± 2.7 ^{b}	-	-
	2	12.3 ± 3.7 $^{e-h}$	16.6 ± 6.4 $^{d-h}$	31.6 ± 7.4 ^{bc}	-	4.7 ± 4.3 h	24.8 ± 4.6 $^{b-e}$	-	-
	3	9.6 ± 1.4 $^{f-h}$	29.7 ± 9.2 $^{b-d}$	35.7 ± 6.9 ^{b}	-	-	-	-	-
	4	6.7 ± 1.3 ^{gh}	18.4 ± 4.0 $^{c-h}$	27.7 ± 4.5 $^{b-d}$	-	11.9 ± 4.1 $^{e-h}$	-	-	-

* All measurements are in inhibition percentage (mean ± standard deviation)
Values in the same column followed by the same letter are not significantly different at 0.01 probability level (Duncan test)

Totally, root exudates were less auto-toxic than extracts. They were more effective on coleoptile growth than radicle, lessening 30.56 and 6.69% of coleoptile and radicle length, respectively comparing check plants. The seedlings received root exudates had 14.1 and 7.7% less dry and fresh weight, respectively.

Instant immediate residues in 5 and 10% concentrations could markedly prevent broomcorn seed germination. The auto-toxic effect was reduced to 46.1 and 27.9% in 5 and 10% of burnt aqueous residue extracts, respectively. Thirty-day old instant residues in 10% concentration were still toxic with 20%

inhibitive effect on germination, but in the low concentrations they were far less toxic (3.4% germination inhibitive). 30-days old residues in 5% concentration were almost ineffective on coleoptile growth, but negatively positively affected radicle growth and fresh weight, whereas the dry weight of seedlings were decreased up to 5.2.

Burnt residues in higher concentration caused only 7.6 per cent percent seed germination, even at the highest studied concentration. Instant 30-day residue in 5% extract concentration had positive effect on radicle production, but almost no effect was observed on coleoptile growth. Totally, burnt residues were less toxic on seed germination, most probably duo to affecting the auto-toxic chemicals by burning process. 30-day old instant residues had little effect on dry weight (averagely 6%) in both studied concentrations, but immediate instant residue in 5% concentration could reduce seedlings dry weight up to 29.8%. However, burning residues were still effective on seedling growth by producing weak seedlings with small radicle and coleoptile comparing check seedlings. Coleoptile was more impressible than radicle (Table 2).

Table 2 - Reduction in germination, radicle and coleoptile length of receiver broomcorns exposed to the aqueous extracts of different ages of instant and burnt residues (inhibition %)

Residue types	Residue ages	Concentration (%)	Germination	Radicle length	Coleoptile length	Fresh weight	Dry weight
Instant	1-day	5	84.6 ± 6.6 ^{a}	67.7 ± 2.1 ^{a}	34.7 ± 12.3 ^{d}	46.3 ± 8.5 ^{ab}	29.8 ± 5.6 ^{b}
		10	100 ^{a}	-	-	-	-
	30 -day	5	3.4 ± 1.9 ^{d}	49.1 ± 9.5 ^{b}	0.75 ± 9.2 ^{e}	54.4 ± 9.2 ^{a}	5.2 ± 2.2 ^{c}
		10	20.6 ± 5.9 ^{cd}	21.4 ± 6.06 ^{d}	25.6 ± 6.1 ^{d}	45.5 ± 9.5 ^{ab}	6.3 ± 1.9 ^{c}
Burnt	1-day	5	26.9 ± 7.6 ^{bc}	43.3 ± 6.8 ^{b}	73.8 ± 2.5 ^{b}	33.7 ± 8.6 ^{bc}	40.3 ± 8.4 ^{a}
		10	46.1 ± 3.3 ^{b}	74.4 ± 2.1 ^{a}	89.4 ± 2.9 ^{a}	48.6 ± 7.7 ^{ab}	47.8 ± 8.6 ^{a}
	30-day	5	3.8 ± 7.6 ^{d}	24.9 ± 9.1 ^{d}	57.9 ± 3.3 ^{c}	19.5 ± 8.6 ^{c}	27.8 ± 7.5 ^{b}
		10	7.6 ± 1.5 ^{d}	63.5 ± 6.3 ^{a}	82.2 ± 3.1 ^{a}	41 ± 6.3 ^{ab}	37.8 ± 4.3 ^{ab}

* All measurements are in inhibition percentage (mean ± standard deviation)
Values in the same column followed by the same letter are not significantly different at $p < 0.01$ (Duncan test)

In the greenhouse studies, we encountered with the same effect of the extracts on coleoptile and radicle. Coleoptiles were more sensitive than radicle responsing to the extracts (Table 2). The produced seedlings were weaker than the ones did not received extracts.

Table 3 - Reduction in germination, radicle and coleoptile length of broomcorn seedlings exposed to the aqueous extracts of different concentrations of shoot extracts (inhibition %)

Concentration (%)	Radicle length	Coleoptile length	Fresh weight	Dry weight
2.5	8.2 ± 3.9 ^{c}	29.2 ± 3.2 ^{c}	12.6 ± 7.6 ^{c}	22.2 ± 6.3 ^{d}
5	11.3 ± 1.6 ^{c}	34.4 ± 2.2 ^{b}	34.7 ± 3 ^{b}	34.3 ± 3.5 ^{c}
7.5	23.1 ± 4.2 ^{b}	39.3 ± 3.2 ^{ab}	37.7 ± 1 ^{b}	44.4 ± 1.7 ^{b}
10	33.5 ± 4.1 ^{a}	46 ± 3.9 ^{a}	51 ± 1.5 ^{a}	54.6 ± 3 ^{a}

* All measurements are in inhibition percentage (mean ± standard deviation)
Values in the same column followed by the same letter are not significantly different at $p < 0.01$ probability level (Duncan Test)

Findings showed the obvious high auto-toxicity in broomcorn. Although, allelopathic effects of broomcorn were previously recorded on some weeds and crops (Mardan, 2009; Latifi *et al.*, 2010; Hashemizadeh *et al.*, 2010, 2011 and Valizadeh *et al.*, 2010), this is the first report of its auto-toxicity. Considering the cropping system of broomcorn in Miyaneh and farmers interest to continuously plant this crop in the same farm, there should be enough accuracy of shifting programs. The harmful auto-toxic potential of broomcorn should be seriously considered. Also, special crop residue management like burning can reduce the negative effect of residues on broomcorn germination and growth, but its auto-toxicity remains to some extent and the seedlings growing in burned residues farms could be considerably weak.

More investigation on in-toxicity of crop residues during the time up to 8 months that the next crop should be cropped is suggestible. The chemicals dynamics in the soil should be studied to clarify the pure and real effectiveness of chemical fates released from the instant and burnt residues, too.

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