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Allelopathic effects of weeds extract on seed germination of *Trachyspermum copticum L.*, *Foeniculum vulgare* and *Cuminum cyminum*

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Abstract

Seed germination stage is one of the most basic growth stages in medicinal plants that may be adversely affected by the chemicals released from other plants in the soil environment. The present study was performed to investigate the allelopathic effects of weeds, including (*Xanthium strumarium, Amaranthus retroflexus* and *Chenopodium album)* as common weeds in medicinal plant's farms, on seed germination parameters, total dry weight and allometry coefficient of *Trachyspermum copticum L.*, *Foeniculum vulgare* and *Cuminum cyminum L.* in laboratory conditions. Treatments included extracts of all organs of three weed species, applied on the seeds of the medicinal plants at three concentrations of zero (distilled water), 50 and 100% by volume. The results showed that the extracts of mentioned weed species had a significant inhibitory effect on germination characteristics of the studied medicinal plants. The greatest negative impact on seed germination was recorded for the extract of *Amaranthus retroflexus*, but *Trachyspermum copticum L.* showed a better resistance to the extract of *Amaranthus retroflexus* as compared to *Foeniculum vulgare* and *Cuminum cyminum*.

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Introduction

The world's need for medicinal plants as medicines, cosmetics and as a food supplement for improving human welfare is rising day by day. Allelopathic is the interaction of produced plant chemicals on each other.

Allelopathy is one of the major problems in most agricultural lands so that many weed species affect the germination and growth of other plants by producing secondary metabolites (Anowar Razvy *et al.*, 2011; Mizutani, 1999) and the Production of secondary metabolites in plants depends on environmental conditions (Shams *et al.*, 2013).

The reduction of plant yield is not only due to weed interference in field but allelopathic agents can play an effective role in this regard (Iqbal and Wright, 1999).

In the farms, *Xanthium strumarium* (Rough Cocklebur), *Amaranthus retroflexus* (Redroot Amaranth) and *Chenopodium album* (Lamb's Quarters) as weed species cause a sharp decline in the yield of cultivated plants (Azizi and Fuji, 2006).

Allelopathic activity of Redroot Amaranth has been fully proven. Saponins, phenolics, chlorogenicacid, scopoline and benzoic acid are allelopathic compounds of Redroot Amaranth (Inderjit and Duke, 2003).

According to the new researches, seven sesquiterpens have been reported for Redroot Amaranth. The inhibitory effects of these compounds on the germination of Lettuce at minimum concentration have been demonstrated by biochemical studies (D'Abrosca *et al.*, 2006).

Aldehydes, alkaloids, apo-carotenoids, flavonoids, chlorogenic acid and saponins have been reported by the studies conducted on Lamb's Quarters (Della Greca *et al.*, 2004).

The aqueous extract of the roots and shoots of Lamb's Quarters reduced the growth of coleoptiles in Maize (El-Khatib *et al.*, 2004).

According to the results reported by (Mallik *et al.*, 1994), new compounds were identified in Lamb's Quarters, including chlorogenic acid and seven phenolic compounds, having a toxic effect on the germination of Radish.

Rough Cocklebur is also one of the weeds, threatening medicinal plants so that it severely competes in the fields through changing the physiological characteristics of medicinal plants (Wassom *et al.*, 2002).

(Cutler and Cole, 1983) reported that potassium carboxyactractyloside and hypoglyaemic, extracted from plant residues of Rough Cocklebur, strongly inhibited the growth of hypocotyls of wheat. The extracts of Rough Cocklebur also inhibited the germination of pepper, pumpkin and white clover (Kadioglu, 2004).

In general, *Xanthium strumarium* (Rough Cocklebur), *Amaranthus retroflexus* (Redroot Amaranth) and *Chenopodium album* (Lamb's Quarters) are common weeds having high effect on the growth and germination of crops (Azizi and Fuji, 2006). There is little information on the mechanism between their allelopatic substance and medicinal plants growth in farms. The fight against weeds requires spending high costs but it can be done easily through recognizing the allelopathic agents and its mechanism.

Therefore, the current study was aimed to investigate the harmful effects of secreted allelopathic substances and the extracts of *(Chenopodium album, amaranthus retroflexus* and *Xanthium strumarium*) on seed germination and growth of *Trachyspermum copticum L.*, *Foeniculum vulgare* and *Cuminum cyminum*.

Materials and methods

The present research was conducted at the laboratory of medicinal plants, Faculty of Agriculture of Arak University in 2008. The experiment was performed in a completely randomized design with four replications.

Treatments were included extracts of *Xanthium*, *Amaranthus retroflexus*and *Chenopodium album* at three concentration levels of 0, 50, and 100%. The medicinal species studied in this research were as follows: *Trachyspermum copticum L.*, *Foeniculum vulgare* and *Cuminum cyminum*.

The aqueous extracts of weeds were prepared from all plant parts (seeds, stems, leaves, and roots). The plants were collected from the field and then were dried in an electric oven at 60 °C and were powdered by grinding. 100 ml distilled water was added to 100 grams of powder of each plant and then it was placed on a shaker for 72 hours to prepare the main solution (100%).

The treatments included the solutions of 100% (100% main solution), 50% (50% main solution+50% distilled water), and distilled water was considered as control.

To prevent fungal infection, solution containers, the petri dishes, and seeds were disinfected by soaking in sodium hypochlorite 10% for 10 minutes and to remove the effect of sodium hypochlorite they were washed five times with distilled water. Then, 100 seeds of medicinal plants were placed on Whatman filter paper No. 2, inside the Petri dishes and placed into germinator with a temperature of 23+2 °C.

In this experiment, the traits presented in Table 2 were measured and germinated seeds were counted daily. A 2-mm radicle was considered as seed germination and evaluation ended when the number of germinated seeds did not differ in two consecutive counts. The length of radicle and plumule was measured on the eleventh day.

Data analysis was performed by SPSS15 software and mean comparisons were done by Duncan's Multiple Range Test at a significance level of 5%.

Results and discussion

Germination percentage

According to the results of ANOVA, the effect of the type of extract and different levels of extract was significant on germination percentage.

Table 1. Definition and formula related to the variables related to seed germination used in this study. N (the number of germinated seeds), D (the number of days after germination), SFW (seedling fresh weight), SDW (seedling dry weight), RL (radicle length) and PL (plumule length).

	Variable	Formula	References
UG	Ultimate Germination	The maximum number of seeding's that germinated during the experiment.	
GR	Germination rate	$GR = \sum_{i=1}^{d} \frac{Ni}{Di}$	(Saxena *et al.*, 1996)
MPUG	Mean Period of Ultimate Germination	$MPUG = \frac{\sum_{i=1}^{d} NiDi}{UG}$	(Saxena *et al.*, 1996)
IP	Inhibitory percent	$= 100 - \frac{\text{UG in aqueous extracts (\%)}}{\text{UG in distilled water (\%)}}$	(Saxena *et al.*, 1996)
SVI	Seed vigour index	SVI= UG × Mean (RL+PL)	(Biradar *et al.*, 2007)
TWC	Total water capacity	$TWC = \frac{SFW - SDW}{SFW} \times 100$	(Samad *et al.*, 2008)
CA	Coefficient of allometry	Radicle length/Plumule length	(Saxena *et al.*, 1996)

Mean comparison of the effects of Lamb's Quarters, Redroot Amaranth and Rough Cocklebur extracts showed that Lamb's Quarters at concentration level of 50 had the lowest effect on the reduction of seed germination percentage compared to Redroot Amaranth while at the same concentration Redroot Amaranth and Rough Cockleburhad the highest effect.

Seed germination was reduced to 100% by the extract of Redroot Amaranth and Rough Cockleburat concentration level of 100 but a reduction of 95% was recorded for Lamb's Quarters extract, indicating a lower inhibitory effect.

Therefore, according to the obtained results, the increased extract concentration caused decreased germination percentage of the studied medicinal plants. Our results are in agreement with the findings of (D'Abrosca *et al.*, 2006; Mallik *et al.*, 1994).

Previous researches indicate that weed extracts with a negative impact on the induction of germination hormones such as gibberellin and the activity of specific enzymes including amylase and proteinase which are essential for the germination process cause reduced germination percentage (Kruse *et al.*, 2000). Reduced germination percentage caused by Rough Cocklebur extract is due to the chlorogenic acid and phenolic in the extract (Mallik *et al.*, 1994). In addition, the inhibitory effects of Rough Cocklebur extract on germination percentage may be due to the toxic effects of folic acid in the extract (Einhelling, 2008). Therefore, the cultivation of medicinal plants is not recommended on the farms with the mentioned weeds.

Table 2. Effect of *Chenopodium album*, *Amaranthus retroflexus* and *Xanthium strumarium* extracts at concentration levels of 0, 50 and 100 on *Carum copticum*, *Foeniculum vulgare* and*Portulaca oleraceae* seed germination. Data followed by a different letter were significantly different (P≤ 0.05) according to the Duncan Multiple Range Test.

Plant	Weed	Substance level	UG	GR	MPUG	IP	SVI	CA	TWC
T. copticum	*Ch. album*	0	71 D	11.9 F	7.5 BCD	30 B	2.6 EF	4.6 B	0.70 B
		50	20 BC	1.7 C	13.2 EF	72 C	1.4 BC	0.8A	0.90 C
		100	16 B	1.1 BC	9.8 CDE	77 C	1.39 B	0.82A	0.93 C
	A. retroflexus	0	70 D	11.9 F	7.5 BCD	0.0 A	3.0 F	4.8 B	0.92 C
		50	20 BC	1.3 C	16.5 F	72 C	1.5 BC	1.3 A	0.95 C
		100	0.0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
	X. strumarium	0	70 D	11.9 F	7.5 BCD	0.0 A	3 F	4.8 B	0.92 C
		50	26 C	1.5 C	5.1 ABC	63 A	1.7 BCD	1.3 A	0.93 C
		100	0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
F. vulgare	*Ch. album*	0	70 D	8.5 E	10.1 CDE	0.0 A	3.0 F	4.8 B	0.92 C
		50	18 B	1.5 B	13.4 EF	75 C	2.2 DE	4.6 B	0.89 C
		100	0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
	A. retroflexus	0	70 D	8.5 E	10.1 CDE	0.0 A	3.0 F	4.8 B	0.92 C
		50	0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
		100	0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
	X. strumarium	0	70 D	8.5 E	10.1 CDE	0.0 A	3.0 F	4.8 B	0.92 C
		50	21 BC	2.2 B	3.4 AB	71 C	1.9 CD	7.4 C	0.88 A
		100	0 A	0.0A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
C.cyminum	*Ch. album*	0	70 D	5.5 D	12.6 DEF	0.0 A	3.0 F	4.8 B	0.92 C
		50	0 A	0.0 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
		100	0 A	0.00 A	0.0A	100 D	0.0 A	0.0 A	0.00 A
	A. retroflexus	0	70 D	5.5 D	12.6 DEF	0.0 A	3.0 F	4.8 B	0.92 C
		50	0 A	0.00 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
		100	0 A	0.00 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
	X. strumarium	0	70 D	5.56 D	12.6 DEF	0.0 A	3.0 F	4.8 B	0.92 C
		50	0 A	0.00 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A
		100	0 A	0.00 A	0.0 A	100 D	0.0 A	0.0 A	0.00 A

In this research, the highest and the lowest germination percentage caused by different concentrations of weed extracts were related to Ajwain and Cumin, respectively (table 3). This indicates that the seeds of Ajwain have a better resistance against the germination inhibiting factors in the weed extracts.

Germination rate

The results of mean comparisons with Duncan's Multiple Range Test showed that the extracts of Redroot Amaranth and Rough Cockleburat concentration level of 100 dramatically reduced the germination rate (GR).

Lamb's Quarters extract at low concentration had less impact on reducing the GR compared to the control group, indicating the lower allelopathic effects of Lamb's Quarters extract compared to Redroot Amaranth and Rough Cocklebur (table 2).

According to the results, the highest (maximum) GR in control group (distilled water) was related to Ajwain while the lowest GR was recorded for Cumin. The same was found for different concentrations of weed extract. Ajwain showed a better resistance against high concentrations of weed extracts in comparison with Fennel and Cumin.

(ZH and SG, 2012) showed that small seeds were more exposed to allelochemicals due to having a large contact surface and are more vulnerable to the allelopathic effects of weeds. Our results showed that seed size had no effective role in this regard since the seeds of Ajwain were smaller than that of Fennel and Cumin (Table 3).

However, the highest resistance against allelochemicals was related to Ajwain and it is probably due to its specific physiological properties.

As seen in Table 2, allelopathic effects not only reduce the germination but also cause a delay in germination, affecting the species competition (T. Nilsen and M. Orcutt, 1996).

Allelochemichals in the extract lead to the slowing of plant vital processes due to the reduction of seed respiration which ultimately reduce the GR.

Mean Period of Ultimate Germination

According to the results of Table 2, significant differences were found among the germination onset of the seeds affected by different concentrations of weed extracts.

Among the treatments, Rough Cocklebur and Redroot Amaranth extracts at concentration level of 100% increased the mean period of ultimate germination (MPUG) (table 2), Our results are in consistence with the findings of (Rezaei *et al.*, 2008), who studied the effect of Redroot Amaranth extract on the germination of Rapeseed.

In other words, Rough Cocklebur had the most impact on MPUG compared to Lamb's Quarters (table 4).

Therefore, MPUG increased by increase of weed extract concentration. This may be due to the reduced respiration caused by allelochemicals in the extract, ultimately leading to reduced energy for vital plant processes such as germination.

Among the studied species, the lowest MPUG was recorded for Ajwain (Table 3) because Ajwain showed higher resistance against allelochemicals and for this reason, its high germination rate reduced the MPUG.

Inhibitory percent

Triterpens are one of the allelopathic compounds in Redroot Amaranth. Evidence has shown that terpens inhibit cell elongation and cell division and also are considered as germination inhibiting factors. Sesquiterpenes, with nerolidol skeleton, are one of the germination inhibiting factors in lettuce (D'Abrosca *et al.*, 2006).

The Inhibitory percent (IP) of Redroot Amaranth extract increased by increasing the concentration of

the extract as compared to control group, germination was inhibited to 100% in the seeds of Ajwain, Fennel, and Cumin (Table 2). This is due to the presence of sesquiterpens in Redroot Amaranth extract and this compound is considered as the most important reason for allelopathic effects of Redroot Amarant (D'Abrosca *et al.*, 2006).

Seed germination of the studied medicinal species decreased by increasing the concentration of Rough Cocklebur extract as Rough Cocklebur extract at concentration level of 100 increased the IP up to 100% in Ajwain, Fennel, and Cumin compared to control group (Table 2).

Table 3. Mean values of seed germination characteristics of *T. copticum*, *Foeniculum vulgare and Cuminum cyminum* caused by weed extracts. Data followed by a different letter were significantly different (P≤ 0.05) according to the Duncan Multiple Range Test.

Plant	UG	GR	MPUG	IP	SVI	CA	TWC
T. copticum	32.56 A	4.59 A	7.46 A	57.11 A	1.62 A	2.05 A	0.69 A
Foeniculum vulgare	27.67 B	3.24 B	5.23 B	60.67 A	1.46 B	2.93 A	0.50 B
Cuminum cyminum	23.33 C	1.84 C	4.20 C	66.67 A	1.00 C	1.60 B	0.31 C

Also, Lamb's Quarters extract at concentration level of 100 reduced the seed germination of Fennel and Cumin up to 100% as compared to control group but Ajwain was less affected (70%). The allelopathic effect of Lamb's Quarters extract may be due to flavonoids in its extract, reducing seed germination by inhibition of energy transmission.

The studied medicinal species showed different resistance against IP of different weed extracts and the highest resistance was obtained for Ajwain compared to Fennel and Cumin.

Seed vigor index

Seedling length and germination percentage of the studied medicinal species decreased by increasing the concentration of weed extracts. As a result, seed vigor index (SVI) decreased, indicating that seedling resistance depends on the toxicity of weed extract.

According to the obtained results, different concentrations of weed extracts significantly affected SVI so that weed extracts at high concentration level had the most impact on the reduction of SVI compared to control group. The extract of Redroot Amaranth had the most impact on reducing the seed viability of the study medicinal species. This result is in agreement with the findings of (D'Abrosca *et al.*, 2006) who studied the effect of Redroot Amaranth extract on the seed viability of lettuce.

Among the weed extracts at low concentration level, the lowest impact on SVI of medicinal species was recorded for Lamb's Quarters extract (Table 4).

The highest SVI was obtained for the seeds of Ajwain, resulting from its genetic and physiological characteristics (Table 3).

Seedling fresh weight

According to the results, different concentrations of weed extract significantly affected seedling fresh weight. The lowest seedling fresh weight was obtained for Rough Cocklebur and Redroot Amaranth extracts, caused by the concentration level of 100 (Table 2). Reduced seedling fresh weight of Corn and Soya, caused by Lamb's Quarters extract, also has been reported (El-Khatib *et al.*, 2004).

Reduced absorption of storage nutrients required for growth is of the factors reducing seedling fresh weight.

Allelopathic compounds are effective on cell division and the synthesis of proteins and hormones, resulting in the decrease of cell growth and ultimately the

reduction of seedling fresh weight (Shahrokhi *et al.*, 2011).

Phenolic compounds in the weed extracts cause reduced ATP production by decreasing mitochondrial respiration. Also, phenolics have the ability to change the mitochondrial membrane and prevent the transmission of required energy for vital growth processes. This irregularity followed by a series of physiological effects leads to reduced growth and ultimately seedling fresh weight (Yang *et al.*, 2002).

Coefficient of allometry (Radicle length/Plumule length)

Previous results indicate the negative effect of Redroot Amaranth extract on Coefficient of allometry (CA). In the current experiment, CA decreased by increasing the concentration of Lamb's Quarters, Rough Cocklebur, and Redroot Amaranth extracts (table 2).

Table 4. Mean values effect of *Chenopodium album*, *Amaranthus retroflexus* and *Xanthium strumarium* extracts on seed germination. Data followed by a different letter were significantly different (P≤ 0.05) according to the Duncan Multiple Range Test.

Weed	UG	GR	MPUG	IP	SVI	CA	TWC
Chenopodium album	29.44 B	3.36 B	7.40 C	61.56 C	1.51 B	2.27 B	0.58 C
Amaranthus retroflexus	25.56 A	3.02 A	5.19 B	63.56 B	1.17 A	1.74 A	0.41 A
Xanthium strumarium	28.56 B	3.30 B	4.30 A	59.33 A	1.40 B	2.57 C	0.51 B

Our results are in agreement with the findings of (Shahrokhi *et al.*, 2011), who reported the negative effect of Redroot Amaranth essential oil on the growth of wheat seedlings.

Redroot Amaranth extract at concentration level of 100 reduced CA of Fennel, Anjowan, and Cumin as compared to control group (table 2) and Redroot Amaranth had the most negative impact on CA (table 4).

Also, Lamb's Quarters extract at concentration level of 100, reduced the CA to 100% in Fennel and Cumin as compared to control group. In the Lamb's Quarters extract, the main cause of allelopathic effects is Chlorogenic acid in the extract in which phytotoxin has been identified as the main compound (Mallik *et al.*, 1994).

Redroot Amaranth and Rough Cocklebur extracts also contain high levels of phenolic compounds. Phenolic compounds are the main inhibitor of cell division which ultimately reduces radicle and seedling growth (Mao *et al.*, 2006). A higher CA ratio was obtained in Ajwain as compared to Fennel and Cumin.

Total water capacity

Seedling water content was significantly affected by different concentrations of weed extracts so that Redroot Amaranth and Rough Cocklebur extracts at concentration level of 100 reduced total water capacity to 100%, as compared to control group (table 2). Redroot Amaranth had the most negative impact on CA (table 4). Our results were confirmed by (Smitht *et al.*, 2001) who investigated the allelopathic effects of Redroot Amaranth extract on seedling growth of *Carya illinoinensis*.

The extracts at concentration level of 50 had no significant effect on seedling water content (table 2).

Conclusion

The results of this experiment showed that under laboratory conditions, Lamb's Quarters, Redroot Amaranth and Rough Cocklebur extracts affected the seed germination of Ajwain, Fennel and Cumin but Redroot Amaranth extract had the most negative

impact on germination characteristics of the studied medicinal species.

According to the obtained results, the need for the weed control of the aforementioned weeds before cultivation and during seedling growth and establishment becomes more and more clear. In addition to the farm experiments in order to find the allelopathic potential of the mentioned weeds, the investigation of allelopathic effects of other weeds on germination characteristics (properties) of the studied medicinal species under both laboratory and farm conditions seems necessary.

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