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Research Article

ALLELOPATHIC EFFECTS OF CARDARIA (*LEPIDIUM LATIFOLIUM*) AND FLIXWELD (*DESCURAINIA SOPHIA*) ON GERMINATION AND GROWTH OF BLANKET FLOWER (*GAILLARDIA)*

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ABSTRACT
Allelopathy is one of the most important ecosystem processes, which affect many aspects of plant growth. Despite, widespread allelopathic effects on plants growth; the impact of this process on ornamental plants has been investigated rarely and there are negligibility reports about the allelopathic impact on ornamental plants. Thus, this study was performed to evaluate the allelopathic effects of Flixweld and Cardaria with different concentrations (In vitro condition with concentrations of 0.31% and 0.62%, in greenhouse condition with concentrations of 5% and 10% and with control treatment) on germination, growth and flower characteristics in blanket flower. According to the results of this investigation, majority of studied traits in blanket flower showed significantly decrease influenced by weeds extractions. Germination percentage, seedling length and dry weight of seedling in blanket flower were zero due to no seed germination affected by extraction with 0.62% concentration. Also, the weeds extraction with concentration of 0.31% reduced seed germination and seedling length of blanket flower, significantly. In this study, cardaria extraction had no effect on number of healthy leaves of blanket flower, but 10% extraction of flixweld reduced 26.7% of healthy leaves numbers in Blanket flower. On the other hand, flowers diameter of blanket flower were not affected by Flixweld extraction while, 10% extractions of cardaria reduced this trait to 26% in comparison with control treatment. Leaf area and number of flowers per plant in blanket flower influenced by both extractions of cardaria and flixweld showed significant reduction. In cardaria extraction, only 10% concentration caused significant reduction about 49.7% in the leaves, while both 5% and 10% concentrations of flixweld caused 19.4% and 39.8% significant reductions in leaves area of blanket flower. Also, number of flowers in blanket flower affected by 10% extractions of cardaria and flixweld, reduced about 14.7% and 51.8%, respectively.

***Keywords*:** *Allelopathy, Cardaria, Flixweld, Blanket Flower*

INTRODUCTION
Weeds compete with crop plants for different environmental factors. Also, weeds reduce growth of crop plants by releasing allelopathic compounds (Ullah *et al*., 2013). Weeds can affect plant growth through release of allelopathic compounds, thus evaluation of chemical effects in precedent plants, remnants, companion plants and even self toxicity in plants will be necessary for agricultural ecosystems (Shinwari *et al*., 2013). Even weeds remnants in soil could have negative effect on plant growth in following years (Katoch *et al*., 2012). Allelopathy caused reduction in plant growth through competition for light, water and food (Saberi *et al*., 2013). Farajollahi *et al*., (2012) reported that allelopathy has an important role in the interaction between species and within species and can determine the type of relationship between species. Allelopathy is an important ecological mechanism which, influences on plants dominance, constitution of plant communities, plant climax and production force of agricultural ecosystems (Sanderson *et al*., 2013). Allelopathic compounds release in special circumstances and affect on seed germination, root and shoot growth and soil Micro-organisms (Hesammi, 2013). Allelopathic compounds which determine the success of seed germination can be responsible for alteration in plant water ratio, membranes penetrance, enzymatic activity of proteins and carbohydrate degradation during seed germination. Also, during seed germination; efficiency of carbohydrates transfer into meristematic places is reduced. Alteration in proteins metabolism have an important role on seed germination and seedlings

growth influenced by treatments of leachate, extractions and remnants of allelopathic plants (Ghayal *et al.*, 2013). Allelopathic compounds affect on many physiological processes; including chlorophyll production, respiration, hormonal balance, protein synthesis, membrane permeability, and plant water balance (Prabhakaran and Maharaj, 2013). Negligibility researches have been done about allelopathic effects of plants ornamental (Nodehi *et al.*, 2003). Thus, the aim of this study was to study allelopathic effects of both weeds of cardaria and flixweld on germination and growth of blanket flower.

MATERIALS AND METHODS

This experiment was performed in 2014 at greenhouse and laboratories of Agricultural College of Tabriz Branch, Islamic Azad University, Tabriz, Iran. This factorial experiment based on a completely randomized design were carried out with three replications in two separate experiments of laboratory and greenhouse to study seed germination, growth and production of flower in blanket flower under effects of shoots allelopathic extractions of both flixweld and cardaria. The first factor was weeds of cardaria and flixweld and the second factor was different concentrations of shoots extraction of these two weeds.

In greenhouse condition, the extractions were used with concentrations of 0, 5 and 10%. These concentrations were used in laboratory condition to assess seed germination in flowers of ornamental plant. But seeds did not germinate due to influenced by extractions. Thus, first attempt was reduction in dose of the minimum concentration to half (1.25 and 2.5%) which these concentrations were also led to lack of seed germination. Thereby, germination occurred by reducing the concentration to half of the lowest concentration (0.62 and 0.31%). So these two levels of concentrations with control treatment (distilled water) were tested for germination. Shoots of cardaria and flixweld were collected from the fields on September 2013. These shoot were comminuted, after rinsing and drying in shade. Then, for preparation of 1/10 aqueous extractions from plant samples, 10g. of dried shoots of flixweld and cardaria immersed in 90 mL. of distilled water and after 24 hours, the resulting mixture was used for extraction. The obtained solution was centrifuged at speed of 10,000 rpm for 15 minutes and was regarded as the mother extraction. Concentration of 5% was prepared by adding distilled water to the mother extraction. The extractions were poured into a glass and were covered by foil paper and also were kept in refrigerator during the experiment.

In laboratory condition, petri dishes after disinfection with ethanol were placed in an electric oven at 110°C for 24 hours in order to eliminate potential pathogens, then filter papers and petri dishes were placed in electrical hood under ultraviolet radiation (UV) for 12 hours. Germination test was conducted according to ISTA rules in winter 2013. For this purpose, 25 seeds of blanket flower were placed separately within each petri dish with two layers of disinfected Whatman filter paper which, one layer was on top of the seeds and the other one was under the seeds. Bulk of water and used extraction in a sample were determined by adding water until filter papers become wet in petri dishes, but there is no free water, which was equivalent to 5cc. Thereby, 5cc of each extractions were added to each petri dishes based experimental treatments and to prevent degradation of water and extraction, petri dishes were completely blocked with Para film. Germinator temperature during the seed germination test was about 25-20°C. During the experiment, water and extraction of petri dishes were controlled once every two days. The number of germinated seeds was counted at the end of the tenth day. In greenhouse, extraction effects of flixweld and cardaria shoots with different concentrations on growth and flowering in blanket flower were evaluated. In greenhouse experiment, after preparing nursery using soil with manure and pulp of tea and straws to improve soil, sowing was done for blanket flower. Seedlings were transferred into pots, when height of seedlings reaches to 4-5 cm. After establishment of blanket flower, extraction treatments were applied in pots based on the planting map. The amount of extraction was used about 30 cc which; was added once every 10 days and continued until 50 days. After flowering in two ornamental plants on April, measurements of studied traits were attempted at each plant. Studied traits were included number of healthy leaf per plant, number of yellow leaf, leaf area, and leaf dry weight, number of flower per plant, length of flowering stem, flower dry weight, root dry weight and average of flower diameter.

Analysis of variance for studied traits and mean comparisons of the data by using the Duncan test at 5% probability level were performed using MSTAT-C software, and drawing shapes were done using Excel software.

RESULTS AND DISCUSSION

Variance analysis of studied traits in blanket flower under laboratory conditions showed that, concentrations of extraction had significant effect at 1% probability level on percentage of seed germination; seedling length and seedling dry weight. Interaction of weed type× weed density was significant at 5% probability level for seedling dry weight (Table 1). Variance analysis of studied traits in blanket flower under greenhouse conditions showed that, weed type had significant effect at 1% probability level on number of healthy leaf per plant, number of flower per plant, flower dry weight, shoot dry weight and plant biomass. The effect of extraction concentration was only significant at 1% probability level for number of flower per plant. The interaction of weed type×weed density was significant at 1% probability level for leaf area and number of flower per plant, also this interaction was significant at 5% probability level for number of leaf and flower diameter (Table 2).

Table 1: Analysis of variance of blanket flower traits in laboratory experiment

S.O.V	df	seed germination percentage	seedling length	seedling dry weight
Weeds type	1	22.222ns	0.007 ns	0.001 ns
Extract concentration	2	2299.556**	0.158**	0.012**
Weed ×Extract concentration	2	6.222 ns	0.006 ns	0.002*
Error	12	6.222	0.006	0.001
CV (%)		18.4	7.24	2.27

*ns, * and **: non significant, significant at 5 and 1% probability levels, respectively.*

Table 2: Analysis of variance of blanket flower traits in greenhouse experiment

S.O.V	df	Number of healthy leaf	Leaf area	Number of flower	Flower diameter	Flower dry weight	Shoot dry weight	biomass
Weeds type	1	2628.850**	1670.1 ns	5.556**	0.01 ns	0.991**	3.505**	9.599**
Extract concentration	2	124.081 ns	2348.851 ns	19.580**	0.075 ns	0.212 ns	0.527 ns	0.238 ns
Weed ×Extract concentration	2	945.902*	8110.067**	6.741**	2.136*	0.018 ns	0.055 ns	0.099 ns
Error	12	164.166	616.61	0.457	0.331	0.066	0.31	0.174
CV (%)		11.45	15.76	8.37	13.24	23.35	18.99	10

*ns, * and **: non significant, significant at 5 and 1% probability levels, respectively.*

Germination Experiment

Germination Percentage of Blanket Flower: In this study, percentage of seed germination in blanket flower showed significant decrease due to affected by both concentrations of 0.31% and 0.62%. Concentration of 0.31% extraction declined 87.2% of seed germination. Concentration of 0.62% extraction was prevented from seed germination (Figure 1). There are several investigations which demonstrate, allelopathic compounds reduce seed germination of plants significantly or prevent from germination, and stages of germination and seedlings growth have been stated as sensitive stages to allelopathic compounds (Alagesaboopathi, 2010). Seed germination after water absorption, depend on initiation of RNA transcription, production of enzymes and proteins, decomposition in storage contexture of seed and transfer it to apex of rootlet and shoot which is active site for division and elongation of cells. All of these processes are affected by allelopathic compounds (Hegab *et al*., 2008). Edrisi *et al*., (2011) in assessment of allelopathic effect of Flixweld observed that extractions of this plant reduced wheat germination. Samadi and Lotfi (2011) also found that flixweld extraction declined germination of alfalfa.

Seedling Length of Blanket Flower: According to the results of mean comparisons, seedling length of Blanket flower influenced by different concentrations of weeds extraction, and applying weed extraction even concentration of 0.31% extraction caused significant decrease in seedlings length of Blanket flower. In treatment of 0.31% weed extraction, seedling length was 0.65cm which in comparison with control treatment (1.9cm) represented 65.7% of decrement. Also, in concentration of 0.62% extraction, seedling length was zero because of no seed germination (Figure 2). Researchers have reported ions absorption and growth are processes which need high energy in plant cells. Therefore, inhibition of seedling growth under allelopathic stress can be due to reduction in ions absorption (Gniazdowska and Bogatek, 2005). During seed germination, prompt augmentation in glycolytic activity with enhancement of respiration rate is observed. This glycolytic activity is necessary and essential for transmission of stored carbohydrates to supply energy and carbon compounds for produce new tissues during germination. One of the mechanisms of allelopathic compounds in respiration inhibition is cluttering in activity of metabolic enzymes which is interfered in glycolysis and oxidative pentose phosphate pathway. Glycolytic enzymes of aldolase and Glycophosphate isomerase with glucose-6-phosphate dehydrogenase which are catalyzed the first steps of oxidative pentose phosphate pathway, have decrement in their activity; in presence of soil phenolic compounds (Weir *et al*., 2004). Therefore, reduction of requirement energy for seedling growth, through halting of respiratory processes can also be a factor in decrement of seedling growth.

Seedling Dry Weight of Blanket Flower: In this study, 5% extraction concentration of cardaria had no significant effect on seedling dry weight of blanket flower. Also, in 10% concentration of cardaria extraction, seedling dry weight was zero due to no seed germination of blanket flower. Dry weight of blanket flower illustrated significant decrease due to affected by both concentrations of 5% and 10% of flixweld extraction. 5% concentration of flixweld extraction declined 78% of dry weight in blanket flower in comparison with control treatment. In 10% concentration of flixweld extraction, seedlings dry weight were zero due to no seed germination of Blanket flower (Figure 3). Transmission of seed storage compounds is also dependent on decomposition of these compounds by enzymes. Researches have indicated that allelopathic compounds are stopped activity of starch-degrading enzymes in seed (Han *et al*., 2008). Under this condition, seedling will be faced with carbohydrate deficiency for growth. On the other hand, developmental processes of seedling are also disrupted due to affected by allelopathic compounds. Seedling growth is dependent on division and elongation of cells in embryo which allelopathic compounds disrupt these processes in different ways (Li *et al*., 2010).

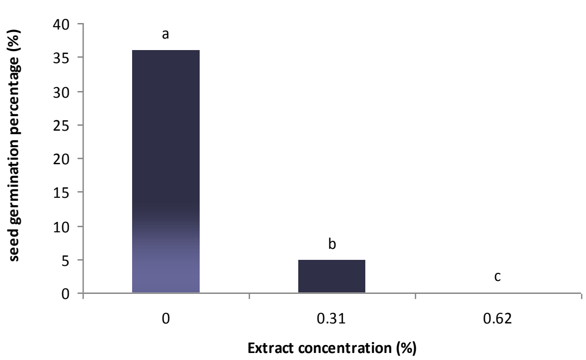

Figure 1: Effects of extract concentration on blanket flower seed germination percentage

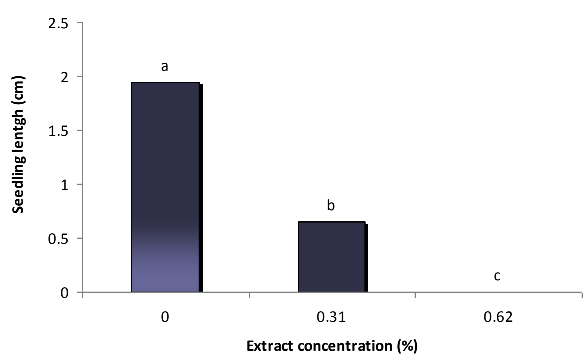

Figure 2: Effects of extract concentration on blanket flower seedling length

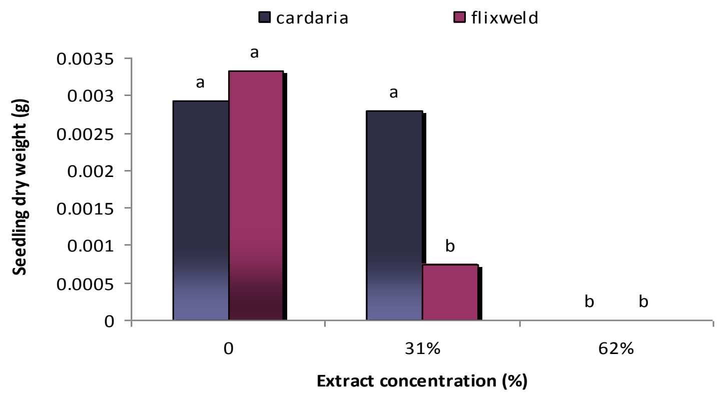

Figure 3: Effects of weeds extract concentration on blanket flower seedling dry weight

Greenhouse Experiment

Number of Healthy Leaf in Blanket Flower: Mean comparisons of number of healthy leaf in blanket flower demonstrated that cardaria extraction had no significant effect on number of healthy leaf in blanket flower but flixweld extraction caused significant reduction in number of healthy leaf in blanket flower. In control treatment, number of healthy leaf in blanket flower was 112.9, while in 10% extraction of flixweld, number of healthy leaf with 26.7% of decrease reach to 84 leaves. 5% extraction of flixweld had no significant effect on number of healthy leaf in blanket flower (Figure 4). Production of leaves per plant is dependent on production of primordial leaf, which has direct relationship with plant growth. Each factor that reduces plant growth will decline leaf production by decrement of primordial leaves (Coder *et al.*, 2011). Allelopathy is some factors which can decrease production of primordial leaves by affect on various processes of plant growth, but on the other hand; allelopathic compounds accelerates leaves senescence (Chen *et al.*, 2013). With acceleration of leaves senescence, number of healthy leaves per plant is reduced.

Leaf Area of Blanket Flower: According to the results of mean comparisons; leaf area of blanket flower represented significant decrement due to affected by extraction of cardaria and flixweld. In evaluation of cardaria extraction effect was observed that 5% concentration had no significant effect on leaf area of blanket flower. But, concentration of 10% extraction caused significant decrease in leaf area of blanket flower. 10% concentration of cardaria extraction declined 49.7% of leaf area in blanket flower. In flixweld extraction, both concentrations of 5 and 10% caused significant reduction in leaf area of blanket flower. Concentrations of 5% and 10% flixweld extraction reduced leaf area of blanket flower with rate of 19.4% and 39.8%, respectively; in comparison with control treatment (Figure 5). It's observed that threshold concentration of flixweld extraction effect is lower than cardaria extraction; various investigations have been illustrated that, sensitivity of plant traits to plants allelopathic extraction is different (Mahmood *et al.*, 2013). Thus, different extractions have various effects on traits. However, according to the results of this study; both weeds extractions had decreasing effect on leaf area of Blanket flower. Mechanisms of decrement in plants leaf area can be varied by different plant extraction. Leaf area per plant is dependent on both number of leaf and area per leaf. In this study was found that flixweld extraction declined number of leaves in blanket flower, while cardaria extraction had no effect on number of leaves in Blanket flower. cardaria extraction had greater impact on cell development. On the other hand, allelopathic extraction of plants can reduce leaves growth by decrement in division and growth leaf cells which cause to reduction of leaf area. Li *et al.*, (2010) reported that allelopathic extraction decrease plants leaf area by reduction of cells growth. With acceleration of leaves senescence can also be a factor in reducing the leaf area affected by allelopathic extraction (Chen *et al.*, 2013). However, the results of this study displayed decrease effect of cardaria and flixweld extractions on leaf area of blanket flower.

Leaf is assimilate supplier organ of plant which its reduction has significant influence on plants growth such as ornamental plants.

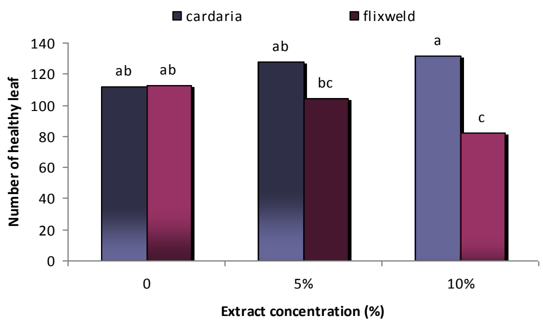

Figure 4: Effects of weeds extract concentration on blanket flower number of healthy leaf

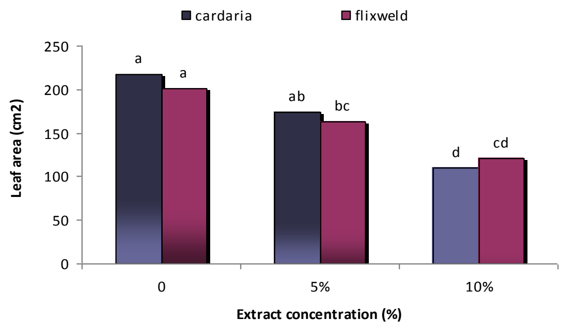

Figure 5: Effects of weeds extract concentration on blanket flower leaf area

Number of Flower per Plant in Blanket Flower: In this study, number of flowers per plant in blanket flower declined due to affected by extractions. Mean comparisons of flowers number in blanket flower affected by different concentrations of weeds extraction, demonstrated that both 5% and 10% concentrations of cardaria and flixweld extractions caused significant reduction in number of flowers per plant. But, decrement rate in number of flower influenced by flixweld extraction was more than cardaria extraction. Number of flowers in blanket flower affected by 5% and 10% concentrations decreased with rate of 13.6% and 14.7%, respectively. There were no significant differences between 5% and 10% of cardaria extractions from the view point of flowers number. 5% and 10% of flixweld extractions increased number of flowers per plant with rate of 36.7% and 51.8%, respectively. According to these results, with augmentation in concentrations of flixweld extraction, further reduction was observed in flowers number of blanket flower (Figure 6). In general, it was observed that flixweld extraction had further reduction effect in number of flowers per blanket flower. Flowers production in ornamental plants is dependent on assimilate presence (Tilly-Mándy *et al*., 2009). As it was observed, allelopathic extractions of both flixweld and cardaria caused significant reduction in leaf area of blanket flower which can decline amount of requirement assimilate for its production and growth. On the other hand, there are researches that indicated; allelopathic compounds reduce flowering with direct affect on terminal buds (Khalaj *et al*., 2013).

Flower Diameter of Blanket Flower: Flower size is one of the factors which affect on quality of ornamental flowers. In this study, flixweld extraction had no significant effect on flower diameter of blanket flower, but cardaria extraction caused significant decrease in this trait. According to the results, in terms of no applying of cardaria extraction; flower diameter of blanket flower was 5cm. In treatment of 5% cardaria extraction, flower diameter of blanket flower was 4.1cm. But, this treatment did not have significant difference with treatment of no applying of cardaria extraction. In 10% cardaria extraction, flower diameter of blanket flower was 3.7 cm, which mean comparisons of this trait indicated significant reduction in flower diameter due to influenced by 10% cardaria extraction. 10% cardaria extraction declined flower diameter of blanket flower with rate of 26% in comparison with non-applied of extraction (Figure 7). Flowers growth per plant is dependent on amount of entered assimilate into flowers. Transmission rate of assimilate from leaves to flowers can decrease due to reduction in photosynthesis rate and assimilate transmission. Researches have been shown that allelopathic compounds reduce rate of assimilate production by effecting on processes which related to photosynthesis (Blanco, 2007). On the other hand, it was observed that some allelopathic compounds decrease chlorophyll synthesis which is main photosynthetic pigment in plant (Benyas *et al*., 2010). Therefore, assimilate production which is essential for flowers growth reduces due to affected by reduction in photosynthesis rate. But on the other, allelopathic compounds decline transmission rate of assimilate by effecting on transport processes such as assimilate loading to phloem and its transport into phloem (Mazid *et al*., 2011). Under these conditions, amount of received assimilate by flowers will reduce. In some research also found that allelopathic compounds decrease sink growth by reduction in sink capacity for receiving assimilate (Asaduzzaman *et al*., 2010; Ul-Subtain *et al*., 2014; Hale, 2007).

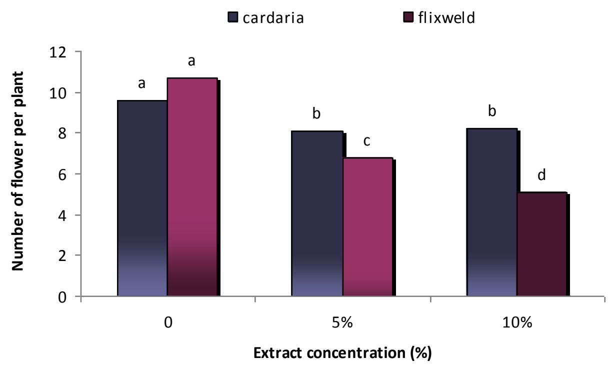

Figure 6: Effects of weeds extract concentration on blanket number of flower

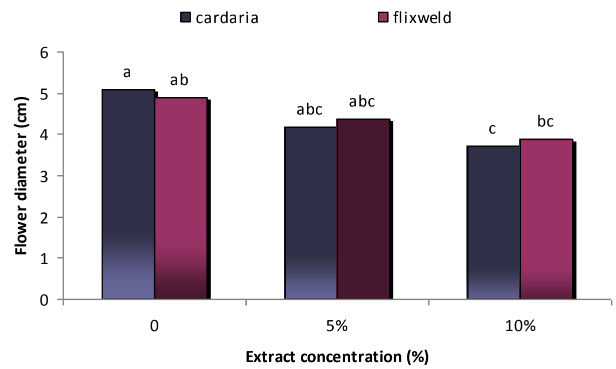

Figure 7: Effects of weeds extract concentration on blanket flower diameter

Flower Dry Weight of Blanket Flower: In this study, flower dry weight in treatment of flixweld extraction was less than treatment of cardaria extraction. In cardaria extraction, dry weight of flowers was 1.33g, while in flixweld extraction dry weight of flowers was 0.86g, which was lower than cardaria extraction with rate of 35.3% (Table 3). Thus, in this study it was found that despite non-significant effect of flixweld extraction on flower size, reduction in flower dry weight is due to decrease in number of flowers. Differences in allelopathic properties of various plants depend on different factors. Also, composition and its amount will vary in different plants (Labbafi *et al*., 2010).

Therefore, alteration in amount and quality of composition will be caused to different plant toxicity. But, sensitivity of different traits to different plants extraction can be varied. So that, extraction of a plant can have more impact on a trait in comparison with other extraction on other trait of the same plant (Mahmood *et al*., 2013).

Shoot Dry Weight of Blanket Flower: In this study, shoot dry weight of blanket flower in cardaria treatment was higher than flixweld treatment. In flixweld treatment, shoot dry weight was 2.4g per plant, while in cardaria treatment was 3.3g per plant. Shoot dry weight in cardaria treatment was 37.5% higher than flixweld treatment (Table 3).

Table 3: Mean comparison of the effect of weeds extract on some traits of blanket flower

	Blanket flower		
	Flower dry weight (g)	**Shoot dry weight (g)**	**Biomass (g)**
Cardaria	1.33 a	3.3 a	4.9 a
Flixweld	0.86 b	2.4 b	3.4 b

Different letters in each column indicate significant differences using Duncans test at the 5% probability level.

Blanket Flower Biomass: According to the results, biomass of Blanket flower in cardaria treatment was higher than flixweld treatment. In flixweld treatment, biomass of blanket flower was 3.4g per plant, while in cardaria treatment was 4.9 g per plant. Shoot dry weight in cardaria treatment was 44.1% higher than flixweld treatment (Table 3).

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