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Original Article

Phytotoxicity of Some Medicinal Plants Residues on Emergence and Establishment Criteria of Canola

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Abstract

The effect of the phytotoxicity of black cumin, dragonhead, dill and soybeans was investigated on the emergence and early growth criteria of canola. Experiment was conducted as factorial based on a completely randomized design with three replications under greenhouse conditions, Shahrekord University, 2016. The experimental factors consisted of four species of plants (soybean, black cumin, dragonhead and dill), two fertilizer types (organic and chemical fertilizers) and two levels of plant residue (without and with residue). The results showed that the black cumin residue in soil treated with both organic and chemical fertilizers reduced the dry weight of canola seedling. In soil fertilized with organic manure, the effects of all four plants residues were the same. The chlorophyll b and carotenoid contents of canola seedling significantly decreased in soil with chemical fertilizer and black cumin or dragonhead residues. In soil fertilized with organic manure, there were no significant differences in the effects of the soybean, black cumin and dill residues. In general, the cultivation of autumn canola should be delayed until the plant residues from black cumin, dragonhead, dill and soybean have been removed or until their effects have dissipated after harvest.

Keywords: Allelopathy, Medicinal plant, Photosynthetic pigment, Phytotoxicity

Introduction

Canola (*Brassica napus* L.) is the third largest source of oil after soybeans and palms [1]. According to the FAO [2], the area harvested in the world is 34740403 hectares and the average yield is 2194 kg/ha. Canola has traits that benefit from winter rainfall and can be rotated with cereals if needed [3].

In moderate climates, canola seeds should be sown six weeks before the first frost. Late planting can cause the plant to fail to store sufficient food and increases the risk of winter frostbite [4]. The timing of canola planting is important and should occur immediately after the harvest of summer plants.

The type of tillage and determination of the amount of residue left on the soil surface are factors that contribute to agricultural success and influence the quantity and quality of crop yield [5]. Additionally, the conservation of plant residue on the soil surface limits water evaporation and clogging of the soil, reduces soil hardening and erosion and increases water and air permeability [6]. The optimum conservation of plant residue on the soil surface can improve soil moisture and influence yield [7]. High amounts of plant residue can have an inhibitory effect on yield and yield components. In this regard, excessive amounts of plant residue from a previous crop can reduce the number of seeds per spike and the number of spikes per plant [8].

Different crops have different abilities in terms of nutrient uptake and release of nutrients through their plant residue. Putting them into a crop rotation system can provide a good balance of nutrients in the soil. Beneficial effects of crop rotation include an increase in soil organic carbon, prevention of erosion and soil compaction, improvement of soil structure, reduction of pests, diseases and weeds, and an increase the water retention capacity of the soil [9].

Allelopathy is a process by which a plant releases toxins to the surrounding environment to make it competitive with other plants [10]. It is the result of the production of

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active compounds during plant growth and in plant residue which has a direct or indirect effect on the growth and development of individuals of the same species or other species after their transformation and entry into the environment [11].

A plant can release bioactive chemicals from the leaves, stems and roots that decompose into its surroundings. These biologically active chemicals are often referred to as being allelochemical because they affect the environment in a positive or negative manner [12]. Studies have shown that products with allelopathic properties can reduce the growth, development and yield of other crops in the field [13].

Allelopathy increases the thickness and reduces the length and weight of roots, altering the structure of the cell wall, permeability of the cell membrane and ultimately leading to a decrease in cell division [14]. This allelopathic property can be used to control weeds [15]. Allelopathic compounds are classified as secondary plant substances or by-products of the metabolic pathways of plants and include terpenes, tannins, alkaloids, flavonoids, quinones and phenols [16].

Azirak and Karaman [17] have reported that thymol, carvacrol and carvone showed a high inhibition effect on weed seedling, even at low concentrations. Also, *Salvia leucophylla* Greene produces several volatile monoterpenoids (camphor, 1, 8-cineole, beta-pinene, alpha-pinene, and camphene) that potentially act as allelochemicals. These monoterpenoids produced by *S. leucophylla* could interfere with the growth of other plants in its vicinity through inhibition of cell proliferation in the root apical meristem [18]. Rostaei *et al*. [19,20] reported that the seed of dill and black cumin contains thymol, carvacrol and carvone compounds.

Canola is a major plant in crop rotations that is cultivated for its valuable oil in semi-arid areas. During the selection of plants for crop rotation, the effects of allelopathy should be considered. The present study was conducted to investigate the effects of soybeans [*Glycine max* (L.) Merr.], black cumin [*Nigella sativa* (L.) Merr.], dragonhead (*Dracocephalum moldavica* L.) and dill (*Anethum graveolens* L.) residues on the emergence and early growth traits of canola.

Material and Methods

The experiment was carried out at the agricultural research farm of Shahrekord University. Each medicinal plant was cultivated in separate plots on 24 May 2016. The experimental factors consisted of four species of plants (soybean, black cumin, dragonhead and dill), two fertilizer types (organic and chemical fertilizers) and two levels of plant residue (without and with residue). The experiment was conducted as a factorial layout based on a completely randomized design with three replications. In some plots, organic manure (broiler litter) was used and, in other plots, chemical fertilizers (urea and triple superphosphate) were mixed with the soil before sowing. The amount of N, P, and K of broiler litter was 19.1, 6.9, and 12.9 g kg^{-1}, respectively.

Irrigation and weeding were done uniformly for all experimental units. The plants were harvested on 6 October 2016. After harvest, the residue of each plants were collected from the soil surface. Thereafter a composite sample was prepared from the rhizosphere soil of each plant.

The canola was sown in the soil prepared using a 2 mm sieve. The plants residues were crushed into small pieces (1 to 2 cm). About 500 g of soil was poured into each pot (14 cm in diameter and 9.5 cm in height). In treatments containing residue, 25 g of plant residue was added to the pot and thoroughly mixed with the soil. On 31 October, 10 canola seeds were sown at 3-cm depths in each pot. For treatments without plant residue, only 500 g of soil treated with organic or chemical fertilizer was poured into a pot and the canola was planted. For the control, plowed soil was used.

Canola irrigation was done based on water requirements and environmental conditions in the greenhouse. Seedling emergence was counted from the onset of emergence to ten days after planting. After three weeks, the canola seedlings were removed from the pots and the root length, leaf length, root dry weight, leaf dry weight and leaf area were measured. Image software was used to calculate the leaf area. In order to determine the dry weight, samples were placed in an oven for 48 h at 72 °C and the dry weight then was measured.

The chlorophylls and carotenoids were assessed based on the method developed by Souto *et al*. [21]. One gram of fresh leaf tissue was shredded into small pieces in a Chinese mold containing 80% acetone. The mixture was completely dissolved and the volume was adjusted to 20 µL using 80% acetone. The solution then was passed through a filter paper and a sample volume was poured into the cuvette of the spectrophotometer. The adsorption was read at 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for the carotenoids. The values were expressed as mg/g of the fresh weight using the Lichten-Thalor method (Eqs. (1), (2) and (3)).

$$\text{Chlorophyll a} = (12.25\times A_{663}) - (2.79\times A_{647}) \qquad (1)$$

$$\text{Chlorophyll b} = (21.50\times A_{647}) - (5.10\times A_{663}) \qquad (2)$$

$$\text{Total carotenoids} = [(1000\times A_{470}) - (1.82\times Cl_a) - (85.02\times Cl_b)]/198 \qquad (3)$$

The emergence percentage (EP) is calculated [22] as:

$$EP = \left(\frac{n}{N}\right)\times 100 \qquad (4)$$

where n is the final number of germinating seeds and N is the total number of seeds.

The emergence rate (ER) can be calculated using the Maguire formula [23] as:

$$ER = \Sigma(\frac{Ni}{Ti}) \quad (5)$$

where Ni is the number of germinated seeds in the n count and Ti is the time from the beginning of planting to the n count.

The average of each parameter was divided into the mean of that parameter from the control treatment. The percentage of change of each parameter was statistically compared to the control using SAS 9.1 software. The comparison of means was done using the least significant difference (LSD) test at the 5% probability level.

Results

The effects of previous crop, plant residue, fertilizer type and the interaction of previous crop × plant residue and previous crop × fertilizer type were significant for the emergence of canola (Table 1). The EP for canola in soil without plant residue was the same as that for previous crop type. With residue, the amount of germination sharply decreased, with the greatest decrease being for black cumin residue at an average of 18.33% (Fig. 1A). In soil with organic manure, the effect of soybean and dill residues on canola EP was similar to those for the chemical condition, but black cumin and dragonhead residues were less effective with organic fertilizer (Fig. 1B).

The ANOVA results showed that the effect of plant residue and the interaction of previous crop × plant residue on the germination rate were statistically significant at *P*= 0.001 and *P* = 0.05, respectively (Table 1).

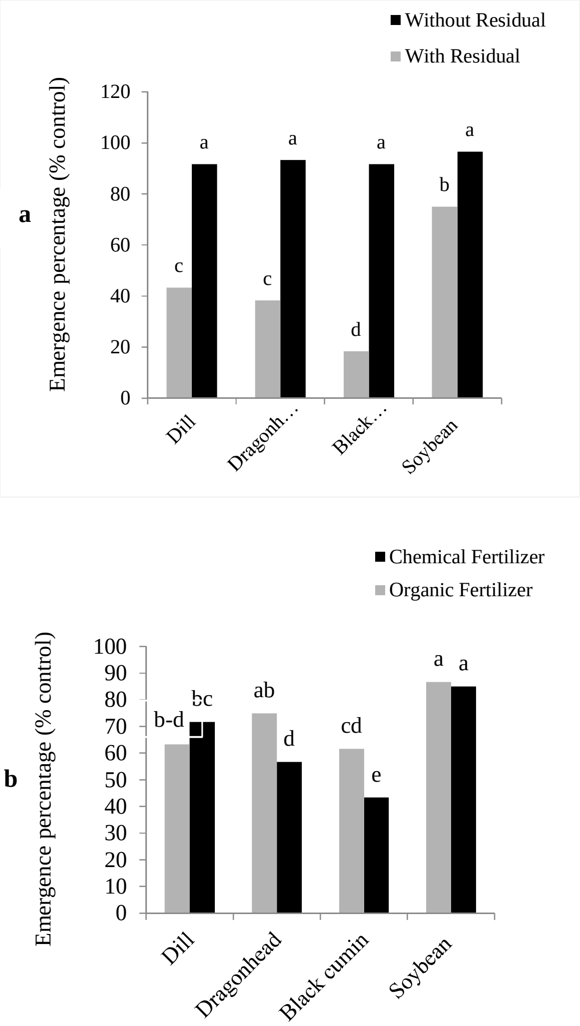

Fig. 1 Mean comparisons for the interaction effects of plant type × plant residue (a) and plant type × fertilizer type (b) on canola emergence percentage. Different letters indicate significant differences at *P* <0.05 by LSD test.

Table 1 Analysis of variance (MS) for toxicity effect of soybean, black cumin, dragonhead and dill residual on growth properties of canola.

Source of variation	Emergence	Emergence rate	Root length	Leaf length	Leaf area
Plant type (P)	***	ns	**	***	***
Plant residue (R)	***	***	***	***	***
Fertilizer type(F)	*	ns	*	***	***
P×R	***	*	***	***	***
P×F	*	ns	***	***	*
R×F	ns	ns	ns	ns	*
P×R×F	ns	ns	***	***	***
	Root weight	Leaf weigh	Chlorophyll a	Chlorophyll b	Carotenoid
Plant type (P)	*	***	**	***	***
Plant residue (R)	***	***	***	***	***
Fertilizer type(F)	**	***	***	***	***
P×R	ns	***	**	***	***
P×F	**	**	**	***	**
R×F	*	***	***	***	***
P×R×F	ns	*	**	***	**

*, ** and ***Significant effect at *P*< 0.05, *P* < 0.01 and *P* < 0.001, respectively. ^{ns}: not significant.

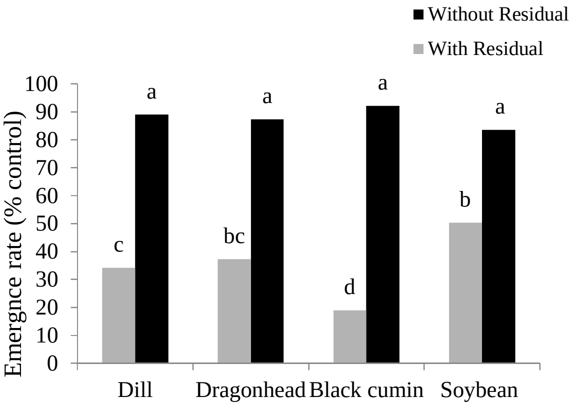

Fig. 2 Mean comparisons for the interaction effects of plant type × plant residue on canola emergence rate. Different letters indicate significant differences at $P < 0.05$ by LSD test.

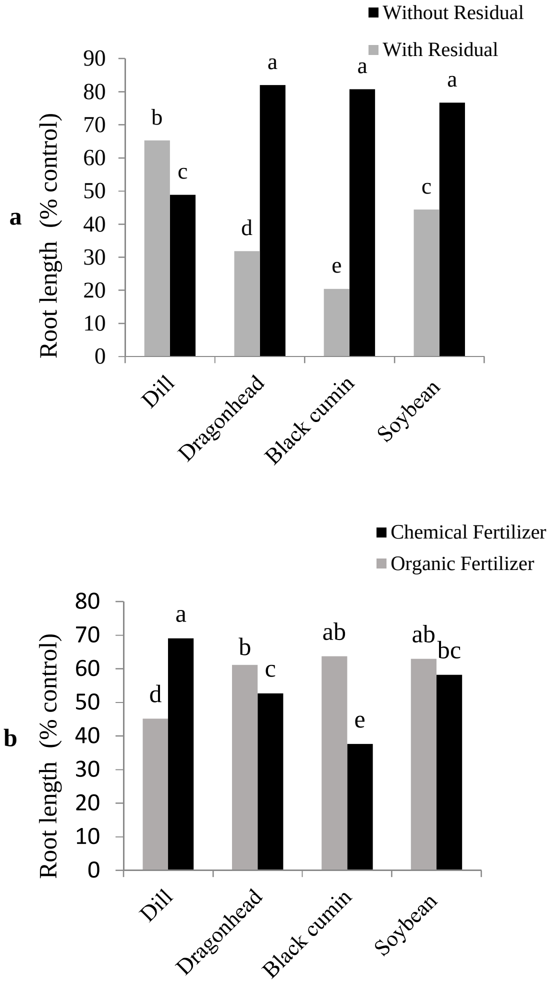

Fig. 3 Mean comparisons for the interaction effects of plant type × plant residue (a) and plant type × fertilizer type (b) on canola root length. Different letters indicate significant differences at $P < 0.05$ by LSD test.

In the absence of residue, the canola ER showed no significant difference in response to plant type. The presence of plant residue significantly reduced the canola ER (50% to 81%). The greatest inhibitory effect on ER was observed for black cumin residue, followed by dill and dragonhead (Fig. 2).

The effects of previous crop, plant residue and fertilizer type on canola root length were statistically significant at $P = 0.01$, $P = 0.001$ and $P = 0.05$, respectively (Table 1). The interaction of previous crop × plant residue, previous crop × fertilizer type and previous crop × plant residue × fertilizer type were significant ($P < 0.001$; Table 1). Black cumin, dragonhead and soybean residues significantly reduced the root length of canola. For dill residue, although the root length decreased in both nutritional conditions, this decrease was greater without residue (Fig. 3a). Organic fertilizer decreased the inhibitory effects of black cumin, dragonhead and soybean residues on the length of the canola root, but this was not observed for dill. The residue of dill with organic fertilizer showed a greater decrease in root length than without organic fertilizer (Fig. 3b).

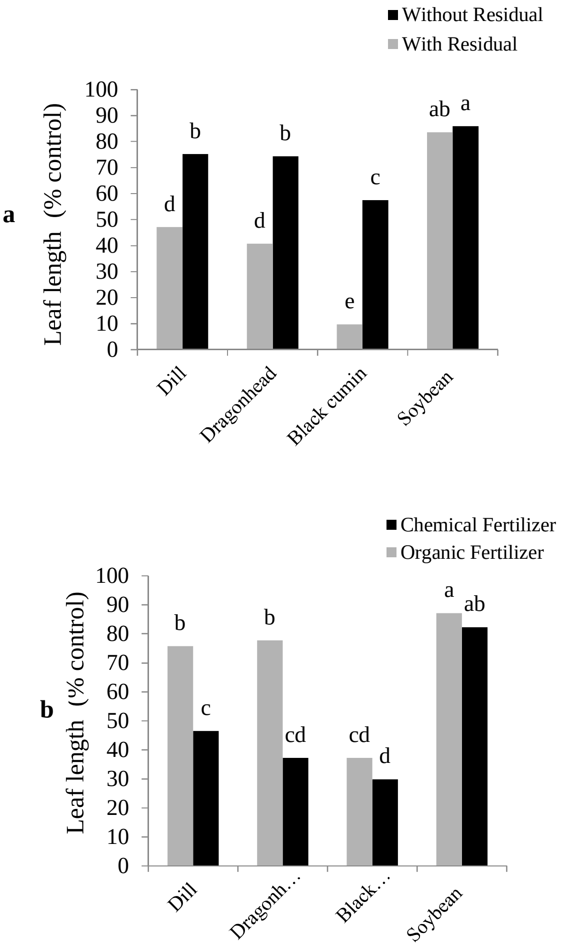

Fig. 4 Mean comparisons for the interaction effects of plant type × plant residue (a) and plant type × fertilizer type (b) on canola leaf length. Different letters indicate significant differences at $P < 0.05$ by LSD test.

The greatest decrease in root length was recorded for black cumin residue without organic fertilizer (Table 2). In each column, means with different letters indicate significant differences at $P< 0.05$ by LSD test.

The length of the canola leaf was significantly affected by previous crop, plant residue and the interactions of previous crop × plant residue, previous crop × fertilizer type and previous crop × plant residue × fertilizer type ($P< 0.001$; Table 1).

The longest leaf length was observed in canola grown after a soybean crop. The three other plants significantly decreased the length of canola leaf (Fig. 4a). The plant residues for dragonhead and dill had similar effects on the leaf length of canola, but the black cumin residue significantly decreased it.

A decrease of 90% was observed in canola leaf length with black cumin residue compared to the control (Fig. 4a). The leaf length of canola grown after harvesting of soybean and black cumin showed no significant response to fertilizer type. However, after harvesting of dragonhead and dill, the inhibitory effects on the leaf length of canola with organic fertilizer was less than without organic fertilizer (Fig. 4b). The interactions shown in Table 2 indicate that the maximum decrease in canola leaf length was observed for soil with black cumin residue and chemical fertilizer followed by black cumin with organic fertilizer and dragonhead and dill residues with chemical fertilizer.

The leaf area of canola was influenced by the previous crop, plant residue, fertilizer type, the interaction effects of previous crop × plant residue and previous crop × plant residue × fertilizer type ($P < 0.001$). The interaction of previous crop × fertilizer type and plant residues × fertilizer type were significant at the 0.05 probability level (Table 1). The highest leaf area observed in comparison with the control was after soybeans and dill, respectively. However, in the presence of four plant residues, the leaf area of canola decreased compared to the control and the smallest leaf area was observed in soil with black cumin residue (Fig. 5a). Black cumin residue with organic fertilizer showed less inhibitory effect on canola leaf area than with chemical fertilizer. With the other three plant residues with chemical fertilizer the decrease was less (Fig. 5b). Organic and chemical plant residues showed similar effects on the leaf area of canola (Fig. 5c).

The effect of fertilizer type and the interaction of previous crop × fertilizer type were significant on the dry weight of canola roots at $P = 0.01$. The effect of previous crop and the interaction of fertilizer type × previous crop on the root dry weight was statistically significant at the 0.05 probability level, but the effect of plant residue on the dry weight of canola root were significant at $P< 0.001$ (Table 1). Organic fertilizer decreased the inhibitory effect of dragonhead, black cumin and dill residue on root dry weight (Fig. 6a).

Table 2 Mean comparisons for the effects of plant type, plant residue and fertilization type on root length, leaf length, leaf area, leaf weight, and photosynthesis pigments of canola (Percentage compared to control).

Residue density	Plant type	Root length	Leaf length	Leaf area	Leaf weight	Chlorophyll a	Chlorophyll b	Carotenoids
			Chemical Fertilizer					
Without residue	*Soybean*	66.33 b	70 b	165.42 a	85.60 bc	52.79 d	49.3 d	63.4 d
	Black cumin	72.25 b	57.2 c	74.09 d	47.44 ef	52.26 d	44.3 d	56.9 d
	Dragonhead	72.47 b	57.5 c	78.32 cd	56.29 e	28.86 f	28.7 e	36.6 e
	dill	72.17 b	74.6 b	108.93 b	43.97 f	42.51 e	45.4 d	58.2 d
With residue	*Soybean*	50.03 c	93.8 a	29.43	13.78 gh	6.97 gh	5.8 fg	7.5 fg
	Black cumin	2.90 f	2.6 e	10.41 h	1.87 i	0.17 h	0.3 g	0.4 g
	Dragonhead	32.94 de	17 d	34.49 fg	5.86 hi	2.04 gh	0.4 g	0.5 g
	Dill	65.92 b	18.4 d	67.46 d	16.86 g	8.58 g	8.9 f	11.5 f
			Organic Fertilizer					
Without residue	*Soybean*	87.07 a	100.9 a	94.9 bc	97.28 a	86.97 ab	107.8 a	138.8 a
	Black cumin	89.27 a	57.6 c	74.36 d	88.70 ab	76.16 c	71.6 c	91.9 c
	Dragonhead	91.53 a	91.1 a	61.15 de	78.17 cd	80.22 bc	74.9 bc	95.9 bc
	Dill	25.58 e	75.7 b	104.36 b	70.87 d	92.94 a	80.5 b	102.9 b
With residue	*Soybean*	38.79 d	73.3 b	43.76 ef	6.58 g-i	1.64 gh	1.9 g	2.4 fg
	Black cumin	38.06 d	17 d	18.86 gh	1.77 i	0.54 gh	0.5 g	0.7 g
	Dragonhead	30.85 de	64.5 cb	18.85 gh	14.48 gh	4.29 gh	4.4 fg	5.7 fg
	Dill	64.68 b	75.9 b	28.89	3.99 hi	1.34 gh	1.6 g	2.04 fg

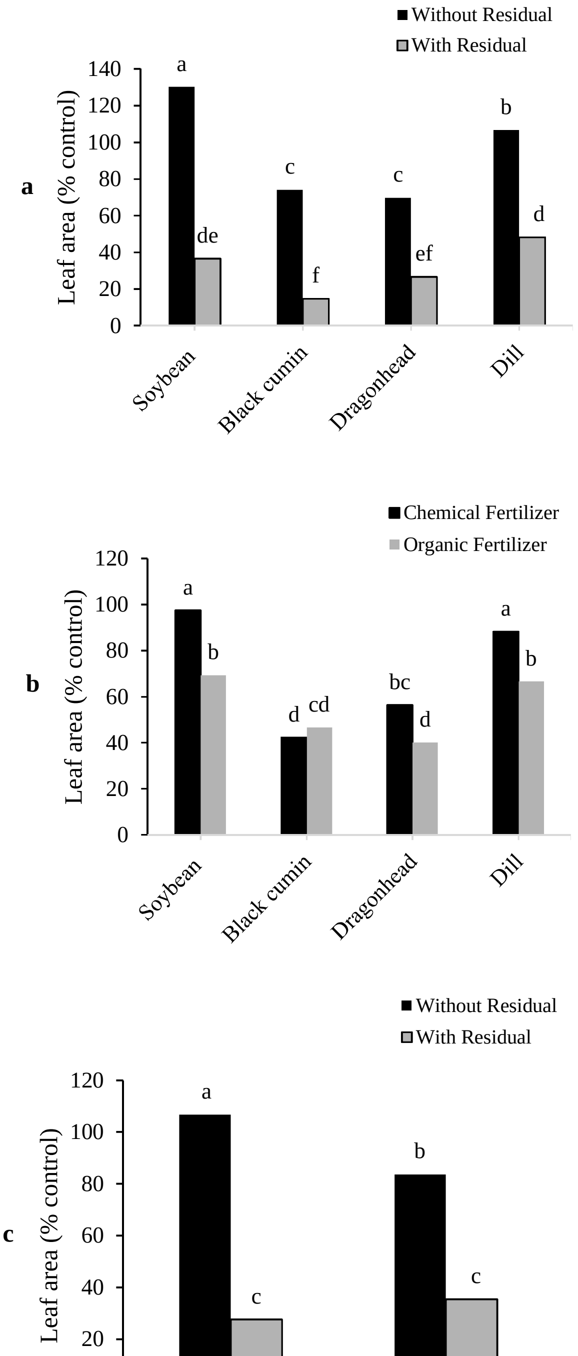

Fig. 5 Mean comparisons for the interaction effects of plant type × plant residue (a), plant type × fertilizer type (b), and fertilizer type × plant residue (c) on canola leaf area. Different letters indicate significant differences at $P<0.05$ by LSD test.

It should be noted that the canola cultivated in soil treated with chemical fertilizer and black cumin residue had the lowest germination rate; thus, its average would be low (Fig. 6a). The dry weight of canola root in without residue was similar for the organic and chemical treatments (Fig. 6b).Leaf dry weight was affected by previous crop, plant residue, fertilizer type and the interactions of previous crop × plant residue and plant residue × fertilizer type ($P < 0.001$). The interactions of previous crop × fertilizer type and previous crop × plant residue × fertilizer type were statistically significant at the 0.01 and 0.05 probability levels, respectively (Table 1). The lowest leaf dry weight was observed in black cumin residue, but the residue of the three other plants had the same effect on canola leaf dry weight (Fig. 7a).

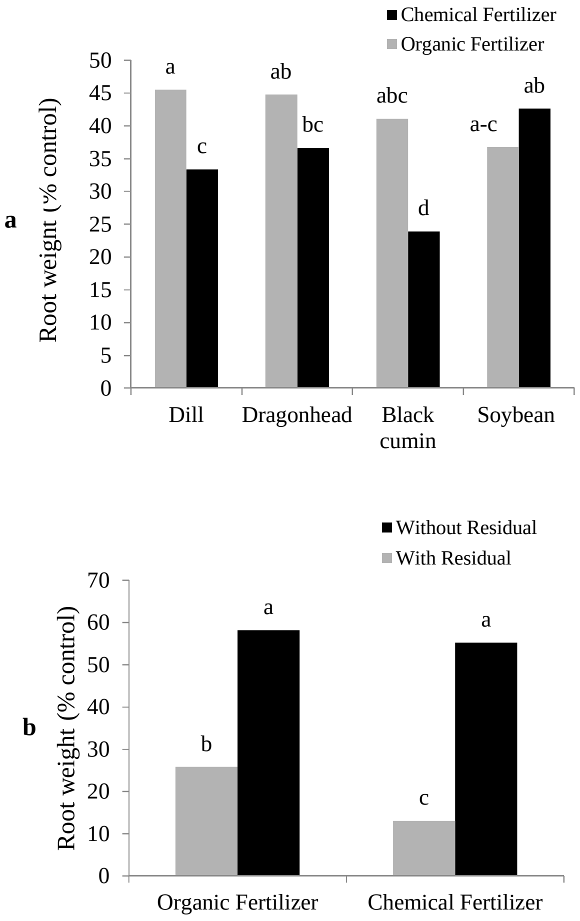

Fig. 6 Mean comparisons for the interaction effects of plant type × fertilizer type (a) and plant residue × fertilizer type (b) on canola root weight. Different letters indicate significant differences at $P<0.05$ by LSD test.

The effect of organic soybean residue on canola leaf dry weight was similar to the chemical condition, but black cumin, dragonhead and dill residues with organic fertilizer were less effective in inhibiting the dry weight of canola (Fig. 7b). The canola in soil with organic and chemical fertilizers had the lowest and highest decreases in leaf dry weight, respectively (Fig. 7c).

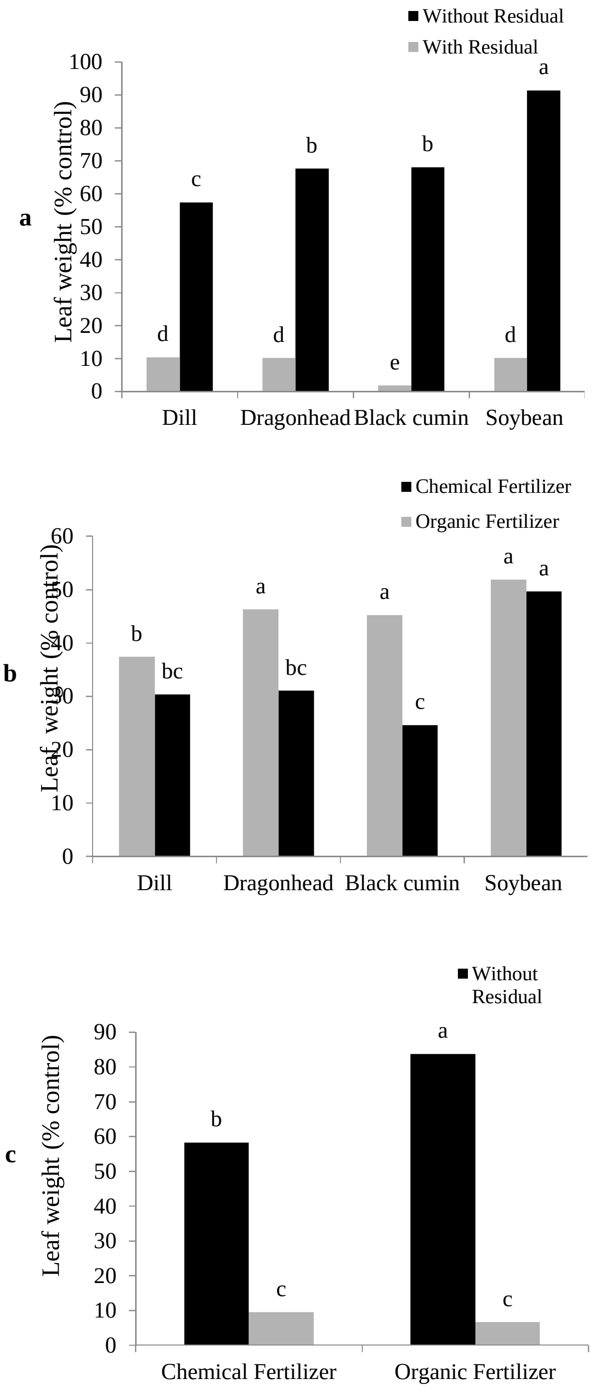

Fig. 7 Mean comparisons for the interaction effects of plant type × plant residue (a), plant type × fertilizer type (b), and fertilizer type × plant residue (c) on canola leaf weight. Different letters indicate significant differences at $P < 0.05$ by LSD test.

The chlorophyll a content was influenced by previous crop and the interactions of previous crop × fertilizer type, previous crop × plant residue and previous crop × plant residue × fertilizer type ($P < 0.01$; Table 1). Canola in soil with chemical fertilizer and black cumin residue had a higher phytotoxic effect on chlorophyll degradation (Table 2). The highest rate of degradation of canola chlorophyll was observed in black cumin residue, while the other three plants had the same effects (Fig. 8A). The decrease in the chlorophyll a content was considerably less under organic than chemical conditions (Fig. 8b). Organic manure showed a much lower inhibitory effect than chemical fertilizer (Fig. 8c).

The effects of previous crop, plant residue, fertilizer type and the interactions of previous crop × fertilizer type, previous crop × plant residue, plant residue × fertilizer type and previous crop × plant residue × fertilizer type on chlorophyll b of canola were significant ($P < 0.001$). Canola grown in soil with chemical fertilizer and black cumin residue had the lowest chlorophyll b content (Table 2).

The decrease in chlorophyll b was the same for dragonhead and soybean residue and was greater for black cumin residue (Fig. 9a). The maximum inhibitory effects on canola chlorophyll b content was recorded in rotation with dragonhead and black cumin treated with chemical fertilizer, but the soybeans and dill treated with chemical fertilizer had similar effects on the chlorophyll b content (Fig. 9B). Plant residue with organic and chemical conditions had similar effects on the chlorophyll b content of canola (Fig. 9c).

The carotenoid content was influenced by the effect of previous crop, plant residue, fertilizer type and the interactions of previous crop × plant residue and plant residue × fertilizer type (Table 2). The interaction of previous crop × fertilizer type and previous crop × plant residue × fertilizer type were significant as well. The lowest and highest carotenoid contents were observed for soil with black cumin residue treated with chemical fertilizer (Table 2). The carotenoids of canola grown in the residue of all four plants were similar (Fig. 10a).

The lowest carotenoid content was observed in canola rotated with dragonhead treated with chemical fertilizer. The three other plants showed similar effects on carotenoid content (Fig. 10b). The carotenoid content in soil without plant residue and with organic manure were almost 100 times higher than the organic soil with the plant residues (Fig. 10c).

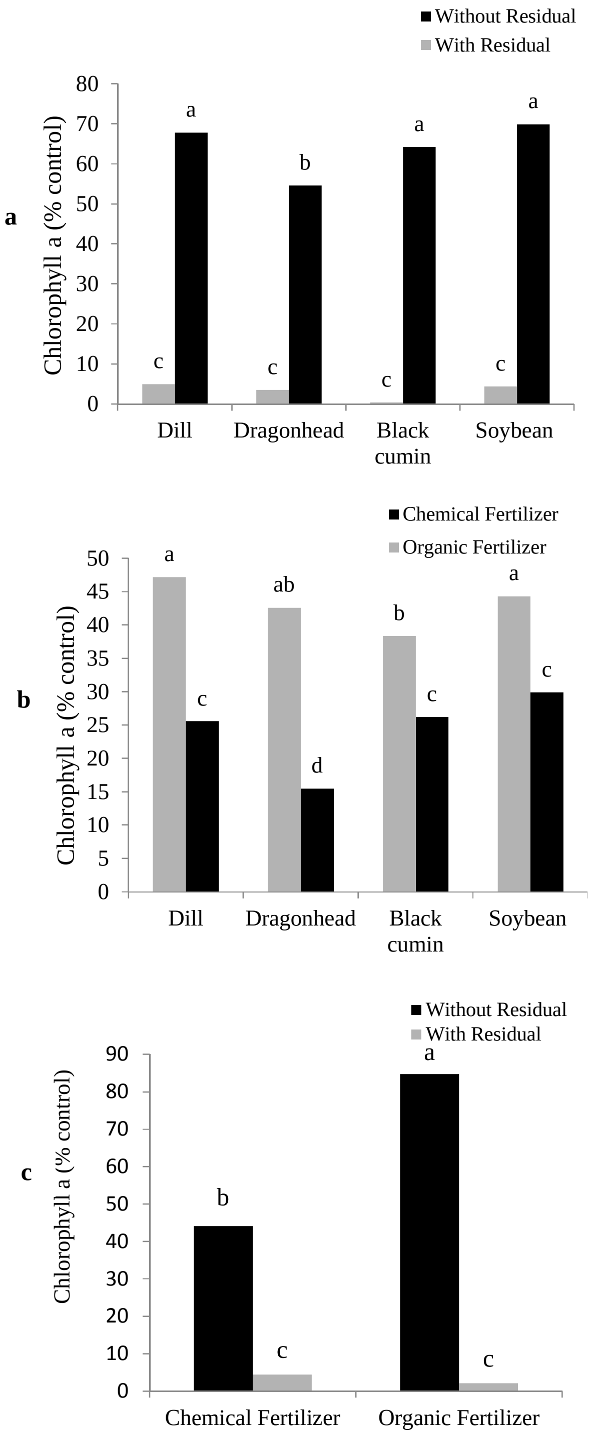

Fig. 8 Mean comparisons for the interaction effects of plant type × plant residue (a), plant type × fertilizer type (b), and fertilizer type × plant residue (c) on canola chlorophyll a. Different letters indicate significant differences at *P* <0.05 by LSD test.

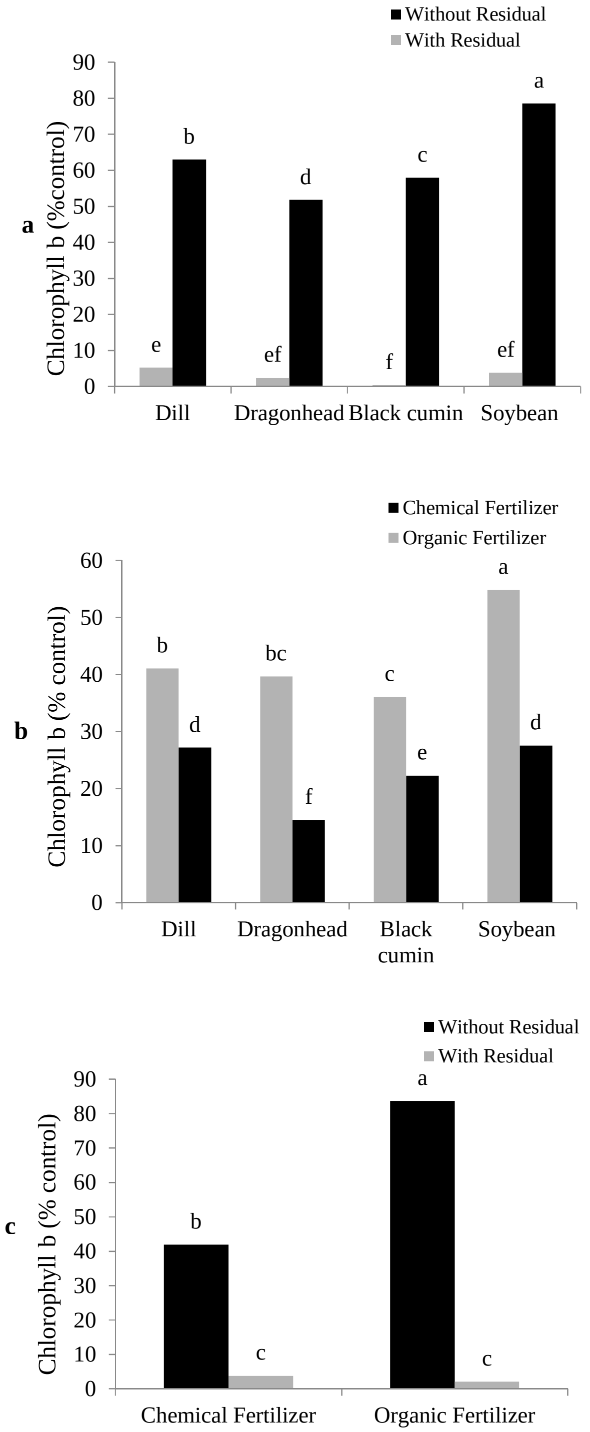

Fig. 9 Mean comparisons for the interaction effects of plant type × plant residue (a), plant type × fertilizer type (b), and fertilizer type × plant residue (c) on canola chlorophyll b. Different letters indicate significant differences at *P* <0.05 by LSD test.

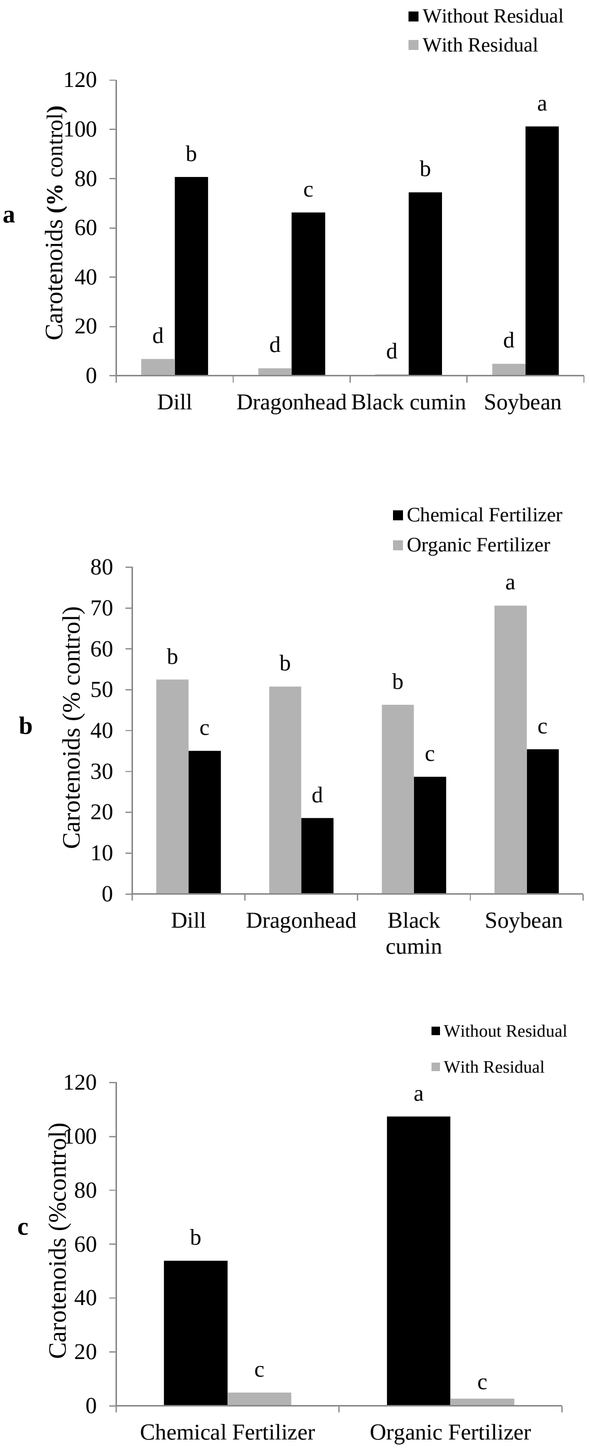

Fig. 10 Mean comparisons for the interaction effects of plant type × plant residue (a), plant type × fertilizer type (b), and fertilizer type × plant residue (c) on canola carotenoids. Different letters indicate significant differences at $P<0.05$ by LSD test.

Discussion

The results of the present study indicated that the residues of soybeans, black cumin, dragonhead and dill had the potential to produce allelopathic effects on canola. The greatest sensitivity was related to black cumin residue and fertilizer from the previous crop in rotation. It was found that organic manure reduced the negative allelopathic effects (Tables 3 and 4). The EP of canola in soil with plant residue, especially black cumin residue, significantly decreased (Fig. 1).

The reason for the decrease in EP is that chemical compounds with allelopathic properties decrease stimulation of gibberellin hormones and indole-3-acetic acid [24]. Saberi *et al.* [25] showed that the inhibitory effects of allelochemicals on gibberellin are the main cause of a decrease in germination. In this regard, enzyme activity decreases the alpha-amylase, which plays a role in seed germination [26]. Fenandez *et al.* [27] indicated that different bioassays for allelopathy primarily related to changes in the rate and percentage of germination and seedling growth due to the allelopathic effect of plants. Mohammaddoost-Chamanabad *et al.* [28] indicated that charlock (*Sinapis arvensis* L,) and acanthus (*Gundelia tournefortii* L.) extracts had significant effects on seed germination in canola.

The root and leaf length of canola were significantly decreased in soil with plant residue. The lowest root and leaf lengths were observed in canola grown in soil with black cumin residue and chemical fertilizer (Table 2). Apparently, black cumin residue produced the greatest decrease in cell division in canola. This effect was partially moderated by organic manure from the previous crop (Fig. 3). A decrease in gibberellin and alpha-amylase during germination decreases the reserves transferred to seedlings and the efficiency of conversion of transferred reserves to seedling tissue [29]. Allelopathic compounds were shown to effect root growth, reducing water absorption in plants, decreasing the length of the seedlings [30].

Treatments that deceased root length also decreased dry weight, so it can be deduced that root and leaf length and weight traits are directly related. In this study, the correlation between length and the root and stem weight was 0.39 and 0.51 ($P < 0.0058$ and $P < 0.0002$, respectively). The leaf area of canola significantly decreased in soil with black cumin residue (Fig. 5). Azirak and Karamen [17] demonstrated that inhibition of division Spindle prevents cell division. The presence of allelopathic compounds in the rhizosphere is associated with inhibition of mitochondrial respiration of DNA [31].

The combination of these events produced seedlings with shorter radicles and plumules and lower dry weights with allelopathic compounds. Soil treated with organic fertilizer without residue had less effect on the degradation of canola pigments, but soil fertilized chemically and black cumin residue severely decreased the canola pigments. Pirasteh-Anosheh *et al.* [32]

concluded that increasing the concentration of an aqueous extract of rosemary (*Rosmarinus officinalis* L.), licorice (*Glycyrrhiza glabra* L.), chamomile (*Matricaria chamomilla* L.) and eucalyptus (*Eucalyptus* sp.) decreased the germination percentage and chlorophyll content of wheat seedlings.

Conclusions

The results of this study indicated that soybean, black cumin, dragonhead and dill residues hada phytotoxic effect on the growth and establishment of canola seedlings. The greatest inhibitory effect was achievedfor black cumin. In semi-arid areas, the early sowing of winter crops is important for the efficent use of rainfall and the timing of the growing season. Although the low organic matter content of the soil in such areas is a good reason for incorporation of plant residue, concerns about the decreasing temperature and possible frost damage to canola limits the opportunity for removal of previous crop residue. The added value of medicinal plants and their growing demand has led to the expansion of their cutivation area, but when they are used in rotation with canola, the residue of medicinal plants can have phytotoxic effects on canola seedlings. When planting these crops before canola cultivation, the application of organic manure can ameliorate the inhibiting effects of plant residue on canola as the next crop. However, the removal of their residue from the field before canola cultivation is the best way to reduce the allelopathic effects of medicinal plant residue.

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