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A study on controlling *Setaria viridis* and *Corchorus olitorius* associated with *Phaseolus vulgaris* growth using natural extracts of *Chenopodium album*

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Abstract: The effects of water extracts of *Chenopodium album* leaves and roots on the growth of grass weed (*Setaria viridis*) and broad leaf weed (*Corchorus olitorius*) grown with beans (*Phaseolus vulgaris*) in greenhouse pots were studied in the National Research Centre, Giza, Egypt. In this experiment fresh leaf and root extracts and their corresponding dry leaf and root extracts at different concentrations were used. There were significant inhibitions in the dry weights of *S. viridis* and *C. olitorius* by all extracts at the flowering stage of beans and at harvest. The inhibition effect of all *C. album* extracts on both weeds (dry weight/pot) depended on the extracted plant organ (leaf or root), its fresh or dry form as well as its concentrations. The inhibition caused by the leaf extract was much higher on weed growth than that of root extract. A higher concentration of fresh leaf extract (25%) had the highest significant inhibition effect. The results also indicated that *C. olitorius* was more sensitive to the extracts than *S. viridis*. On the other hand, the inhibition effect of the extracts on the growth of both weeds was accompanied by increased bean growth and yield/plant. The analysis of both leaf and root extracts of *C. album* revealed that the total content of polyphenols and flavonoids in the leaf extract was more than triple that of the root extract. The results suggested that the fresh leaf extract of *C. album* may be a possible tool for the development of weed control using natural herbicides.

Key words: beans, *Chenopodium album*, *Corchorus olitorius*, flavonoids, leaf extract, polyphenols, root extract

Introduction

The bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for direct human consumption. The consumption of the common bean in the world made up 50% of all other legumes consumed (Graham *et al*. 2003). It contains large amounts of protein, phosphorus, iron, vitamin B, fiber, and is free of cholesterol. Weeds compete with crop plants for light, water and minerals causing damage to crop yield (Lehoczky and Reisinger 2003). El-Rokiek *et al*. (2013) recorded more than 47% reduction in bean yield due to weed competition. Therefore, weeds are considered to be an important factor in reducing crop yield.

Allelopathic plants produced allelochemicals that may be released into the surrounding environment in high amounts, affecting neighboring species (Weston 1996; Singh *et al*. 2003 and 2005). *Chenopodium album* L. is a widespread weed in field crops as well as orchards in Egypt. This weed is one of many noxious weeds that possess allelopathic potential to affect plant growth and crop production (Hamayun *et al*. 2005; Singh *et al*. 2003 and 2009; El-Rokiek *et al*. 2010). *Chenopodium album* has been reported to possess high allelopathic activity against corn, bean and wheat (Szaryas 2000; Alam *et al*. 2002; Bagheri *et al*. 2013). Aqueous leachates of *C. album* plant parts (roots, whole plants and leaves) affect the germination and initial growth of *Cassia occidentalis* L. (a weed) and *Phaseolus aureus* (a crop) by significantly decreasing plant height, biomass, chlorophyll and protein content (Batish *et al*. 2006). Rezaie and Yarnia (2009) reported that *C. album* extract severely reduced root and shoot dry weight, root length and biomass as well as crop establishment of safflower. The aim of the present experiment was to study the allelopathic effect of leaf and root extracts of *C. album* on the growth of two infested weeds, *Corchorus olitorius* L. (broad leaf weed) and *Setaria viridis* (L.) P. Beauv. (grass weed) grown with beans.

Materials and Methods

Collection of *Chenopodium album*

Preparation of dry leaf and root materials

Chenopodium album was collected from Egyptian fields and gardens at the flowering stage. The leaves and roots

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were separated, washed with tap water several times, then with distilled water to remove dust. Fresh weight of leaves at 25 g corresponded to 5.33 g dry leaf and fresh weight of root at 25 g corresponded to 9.90 g dry root. All the quantities needed for the experiment were calculated and weighed. The separated parts were left in the shade till dry. Then they were powdered and kept till use.

Preparation of fresh leaves, roots and the water extract

Chenopodium album was collected as previously mentioned. The leaves and roots of *C. album* were separated (750 g for each), washed with tap water several times, then with distilled water to remove dust. Next, they were cut into fine particles and transferred to labeled beakers. Three liters of distilled water were added, and allowed to soak for 24 h. Then the produced leaf and root extracts were collected and filtered through a very fine mesh and pressed carefully for complete extraction. The produced extracts (leaf and root) were at 25% concentration and then diluted with distilled water, to a concentration of 12.5% for each extract. This step was repeated with the corresponding dry, finely powdered leaves and roots that had previously been prepared. The process of extraction was repeated according to need so; the extracts were fresh.

Growing beans and weeds

The study was carried out in the greenhouse of the National Research Centre, Dokki, Cairo, during two successive seasons, March, 2013 and March, 2014. Bean seeds cv. Giza 3 were used in the experiment. The pots, 30 cm in diameter and 30 cm in height, contained equal amounts of sieved soil (2 : 1 v/v clay and sand). Bean seeds were selected for uniformity by choosing those of equal size and with the same colour. Seeds of beans were sown 2 cm deep, and germinated at average maximum and minimum temperatures of 27.5±1 and 15.5±1°C. All pots were infested with the same weight of seeds (0.03 g) of *C. olitorius* and *S. viridis* and mixed thoroughly at a depth of 2 cm in the soil. Beans and weeds were sown at the same time. The cultivated beans were thinned two weeks after sowing so that three homogeneous seedlings were left per pot. Ammonium nitrate and super phosphate (2 : 1 w/w) were added to each pot during plant growth. The prepared fresh leaf and root extracts were used at concentrations of 12.5 and 25% and the corresponding dry weights of leaf were 2.66 and 5.33 g and of the root 4.95 and 9.90 g extracts.

The pots were sprayed three times during the three weeks to obtain maximum effect, starting with 15-day-old plants. The experiment consisted of 13 treatments including: five untreated controls, *S. viridis* only, *C. olitorius* only, *S. viridis* and *C. olitorius*, only beans and beans with two weed species (unweeded treatment). The other eight treatments were: fresh leaf extract at two concentrations, and corresponding dry leaf extract at two concentrations, fresh root extract at two concentrations and corresponding dry root extract at two concentrations. The pots contained beans and the two weed species were sprayed with the fresh leaf and root extracts of *C. album* at 12.5 and 25% and their correspondence in dry weight (2.66 and 5.33 g for dry leaf extract and 4.95 and 9.90 g for dry root extract). Each pot was sprayed with 150 ml of the extract. Each treatment was represented by nine pots. The pots were distributed in a completely randomized design.

Weeds and beans data

Weeds

Weed samples were taken from each of the three pots at the flowering stage and at harvest (all weed samples in each pot were pulled up). They were then oven dried at 60°C for determination of dry weight (g/pot). The dry weights of grown weeds were recorded.

Beans data

For the three plants in each pot that were pulled up (three pots in each stage), plant height, number of leaves, and number of flowers as well as dry weight (g/plant) were recorded at the flowering stage for each individual crop plant. At harvest, the number and weight of green pods per plant were taken from three pots of each treatment and the other three pots were left for dry yield. The number of dry pods/plant, the number of seeds/pod, weight of seeds/plant and weight of 100 seeds were recorded in dry yield.

Chemical analysis of *Chenopodium album* extracts

Preparation of plant extracts

Plant extracts were prepared according to a standard protocol. Prepared plant material (10 g) was transferred to dark-coloured flasks and mixed with 200 ml methanol, and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and the residue was re-extracted with an equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40°C using a Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4°C (Stanković 2011).

Determination of total phenolic contents in the plant extracts

The concentration of phenolics in the plant extracts was determined spectrophotometrically (Singleton *et al*. 1999). A methanolic solution of the extract, at a concentration of 1 mg · ml^{-1}, was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of the methanolic solution of the extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using a spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate

for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg · ml^{-1}) from the calibration line, then the content of phenolics in the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of sample).

Determination of flavonoid concentrations in the plant extracts

The content of flavonoids in the examined plant extracts was determined spectrophotometricaly (Quettier *et al*. 2000). The sample contained 1 ml of the methanol solution of the extract at a concentration of 1 mg · ml^{-1} and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using a spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg · ml^{-1}) on the calibration line, then, the content of flavonoids in the extracts was expressed in terms of rutin equivalent (mg of RU · g^{-1} of sample).

Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA), the mean values were compared at 5% level of significance (Snedecor and Cochran 1980).

Results

Effect of *Chenopodium album* extracts on weeds

The results in Table 1 show that different extract treatments of *Chenopodium* reduced the dry weight of both *S. viridis* and *C. olitorius* in comparison to two untreated weed species associated with beans (mixed control). The reduction was recorded at the flowering stage and at harvest. The reduction in the dry weight of the two weeds was consistent during the experimental period with all treatments. The reduction in *C. olitorius* was higher than *S. viridis*. At harvest, *S. viridis* was reduced by about 90.3% with the fresh leaf extract (25%) compared to untreated weeds associated with beans. The corresponding result in *C. olitorius* was about 95.1% (Table 1).

Effect of *Chenopodium album* extracts on bean growth and yield

Growth parameters

The results in Table 2 reveal significant increases in plant height of beans due to spraying with fresh and dry leaf extract as well as fresh and dry root extract of *C. album*. The increase in plant height at the flowering stage as well as at harvest reached maximum value by using 25% leaf extract. The number of leaves/plant as well as the number of flowers/plant increased significantly compared to the untreated, unweeded control (weeds associated with beans) by all extracts at both the flowering stage and harvest. The fresh leaf extract was more effective. The most significant increase in dry weight at both flowering and harvest was recorded with fresh leaf extract (25%) as compared to the untreated, unweeded control.

Table 1. Effect of *Chenopodium album* extracts on dry weight of *Setaria viridis* and *Corchorus olitorius* in beans (average of the two seasons)

Treatment	Extract concentration [%]	Dry weight [g/pot]			
		at flowering		at harvest	
		S. viridis	*C. olitorius*	*S. viridis*	*C. olitorius*
Setaria viridis	–	1.385 a	N/A	3.605 a	N/A
Corchorus olitorius	–	N/A	0.838 a	N/A	5.756 a
S. viridis + *C. olitorius*	–	1.277 b	0.733 b	3.492 a	4.952 b
Beans only	–	N/A	N/A	N/A	N/A
Beans + *S. viridis* + *C. olitorius*	–	0.815 c	0.682 bc	3.267 b	4.352 c
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves fresh	12.50	0.392 i	0.174 f	0.904 g	0.260 f
	25.00	0.323 j	0.113 f	0.318 h	0.212 f
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves dry	2.66	0.546 g	0.435 e	1.948 e	0.623 e
	5.33	0.433 h	0.190 f	0.966 g	0.486 ef
Beans + *S. viridis* + *C. olitorius* + *C. album* root fresh	12.50	0.723 e	0.453 de	2.112 e	1.940 d
	25.00	0.650 f	0.527 d	1.614 f	1.557 d
Beans + *S. viridis* + *C. olitorius* + *C. album* root dry	4.95	0.781 d	0.642 c	2.863 c	1.833 d
	9.90	0.733 e	0.611 c	2.399 d	1.693 d
LSD at 5%		0.029	0.082	0.216	0.386

N/A = not applicable; mean values in the same column for each trait followed by the same lower-case letter are not significantly according to Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$

Table 2. Effect of *Chenopodium album* extracts on different growth parameters of beans

Treatment	Extract concentration [%]	At flowering				At harvest		
		plant height [cm]	number of leaves/ plant	number of flowers/ plant	dry weight/ plant [g]	plant height [cm]	number of leaves /plant	dry weight/ plant [g]
Setaria viridis	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Corchorus olitorius	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A
S. viridis + *C. olitorius*	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beans only	–	42.16 b	7.00 a	6.00 b	2.331 a	55.66 b	15.00 a	6.322 a
Beans + *S. viridis* + *C. olitorius*	–	34.00 f	3.83 f	3.00 e	1.238 d	41.26 f	5.66 f	2.915 g
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves fresh	12.50	40.00 c	4.72 d	6.50 b	1.753 c	53.50 bc	9.66 cd	5.393 d
	25.00	43.66 a	6.16 b	8.16 a	2.190 ab	60.00 a	15.50 a	6.516 a
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves dry	2.66	35.41 e	4.80 d	5.50 c	1.575 c	51.33 cd	9.16 d	5.061 e
	5.33	38.83 cd	5.22 c	6.50 b	2.044 b	53.00 c	11.25 b	6.019 b
Beans + *S. viridis* + *C. olitorius* + *C. album* root fresh	12.50	38.08 d	4.72 d	5.00 d	1.713 c	50.00 d	9.26 d	4.926 e
	25.00	39.16 cd	6.00 b	5.75 c	1.796 bc	51.00 cd	10.33 c	5.680 c
Beans + *S. viridis* + *C. olitorius* + *C. album* root dry	4.95	36.48 e	4.33 e	4.53 d	1.653 c	47.10 e	7.00 e	4.569 f
	9.90	38.10 d	5.13 c	5.46 cd	1.733 c	49.26 de	7.66 e	4.829 e
LSD at 5%		1.36	0.33	0.71	0.269	2.29	0.88	0.281

N/A = not applicable; mean values in the same column for each trait followed by the same lower-case letter are not significantly according to Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$

Green and dry yield of beans plant

Chenopodium album leaf and root extracts induced significant increases in the number of green pods/plant as well as the weight of green pods/plant of beans in comparison to the untreated, unweeded control (Table 3). There was an observable significant increase with a higher concentration of fresh leaf extract and corresponding dry leaf extract. In general, the increase in number and weight of green pod/plant caused by fresh leaf and root extract was remarkable, especially when 25% leaf extract was used.

The effect of leaf and root extracts of *C. album* on the number of dry pods/plant as well as the number of seeds/pod of beans showed good results when compared to the untreated, unweeded control (Table 3). The ability of *C. album* extract to increase yield/plant was variable depending on the type of extract, whether it was fresh or dry, leaf or root as well as its concentration. In general, fresh leaf and root extracts were significantly more effective. The increase in seed weight/plant (seed yield/plant) was concentration dependent. Increasing the concentration of the extract from 12.5 to 25% caused a more significant increase in bean yield/plant. Spraying fresh leaf extract at 25% was the most effective. A similar trend was obtained in the weight of 100 seeds.

Table 3. Effect of *Chenopodium album* extracts on yield and yield components of beans

Treatment	Extract concentration [%]	Number of green pods /plant	Weight of green pods/ plant [g]	Number of dry pods	Number of seeds/pod	Seed yield/ plant [g]	Weight of 100 seeds [g]
Setaria viridis	–	N/A	N/A	N/A	N/A	N/A	N/A
Corchorus olitorius	–	N/A	N/A	N/A	N/A	N/A	N/A
S. viridis + *C. olitorius*	–	N/A	N/A	N/A	N/A	N/A	N/A
Beans only	–	6.53 c	15.20 bc	7.16 a	5.00 a	12.29 b	33.82 a
Beans + *S. viridis* + *C. olitorius*	–	4.56 f	7.30 f	4.83 e	2.55 e	6.13 f	21.78 f
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves fresh	12.50	5.00 e	14.43 c	5.10 de	3.16 c	8.70 de	30.28 b
	25.00	7.50 a	18.56 a	7.30 a	4.55 b	14.23 a	35.40 a
Beans + *S. viridis* + *C. olitorius* + *C. album* leaves dry	2.66	5.00 e	10.76 e	5.16 de	3.00 d	7.80 e	28.07 cd
	5.33	7.00 b	15.86 b	6.73 b	4.33 b	10.80 c	30.16 c
Beans + *S. viridis* + *C. olitorius* + *C. album* root fresh	12.50	5.10 e	10.88 e	5.39 d	2.85 de	8.48 de	25.42 e
	25.00	5.63 d	12.65 d	5.80 c	3.41 c	10.78 c	29.30 cd
Beans + *S. viridis* + *C. olitorius* + *C. album* root dry	4.95	4.99 e	10.50 e	4.88 e	2.75 de	7.20 e	24.90 e
	9.90	5.50 d	11.53 e	5.26 d	3.11 c	9.36 d	27.84 d
LSD at 5%		0.37	1.11	0.36	0.34	0.90	2.14

N/A = not applicable; mean values in the same column for each trait followed by the same lower-case letter are not significantly according to Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$

Table 4. Total polyphenois and total flavenoids in leaf and root extracts of *Chenopodium album*

Part of the plant	Total polyphenol [mg gallic acid/100 g dry weight]	Total flavonoids [mg rutin/100 g dry weight]
Leaves	550.00	1,880
Roots	171.22	590

Table 4 shows that the total polyphenol and flavonoid content in the leaves of *C. album* was greater than that in the root. The quantity of polyphenols and flavonoids in the leaves was triple or even higher than that found in the root.

Discussion

Many plant products are known to inhibit germination and growth of other plants. Therefore, these products can be a possible tool for controlling weeds and may be used as natural herbicides (Mahmood and Cheema 2004; Singh *et al*. 2005).

The results of the current study reveal that spraying different extracts of *C. album*, significantly inhibited the growth of *S. viridis* and *C. olitorius* associated with beans in comparison to the untreated, unweeded control (Table 1).

The phytotoxic effects of *Chenopodium* spp. have been well documented by Szaryas (2000); Alam *et al*. (2002); Batish *et al*. (2006); Rezaie and Yarnia (2009); Shahrokhi *et al*. (2011); Abdul Majeed and Muhammad (2012) and Bagheri *et al*. (2013). The phytotoxic inhibition of the extracts on weed growth may be attributed to the presence of some allelochemicals in the extracts. It has been found that *C. album* extract contains some allelochemicals such as cinnamic acid amide alkaloid as a racemic mixture, named chenoalbicin 1 (Cutillo *et al*. 2004), some phenolic compounds and lignan (Cutillo *et al*. 2006).

In this current work we used fresh leaves and roots and their corresponding dry materials, so the differences between fresh and dry materials are not due to the quantity of materials but to the type of the extract.

The results also show that the inhibition of weed growth depended on the extracted plant organ, whether it was leaf or root, fresh or dry, as well as its concentration. The leaf extract showed the highest inhibition effect at 25%. The data also indicate that *C. olitorius* (broad leaf weed) was more susceptible to allelopathic leaf and root extract of *C. album* than *S. viridis* (grass weed). The inhibition in both weed growth was consistant during the experimental period in comparison to two untreated weed species associated with beans (unweeded control). Previous studies carried out by our group using fresh and dry leaf extracts have shown specific inhibitory activity against weeds especially spraying with fresh leaf extract (El-Rokiek and Eid 2009; El-Rokiek and El-Nagdi 2011). Mallik *et al*. (1994); Mojab *et al*. (2003); Hegazy and Farrag (2007) found that the aerial part of *Chenopodium* spp. contain flavonoids, saponins and tannins. Our results were confirmed by Chatterjee *et al*. (2012). Vaidya *et al*. (2014) attributed the differences between fresh and dry tissues to the thermostability of flavonoid groups during leaf drying and extract preparation.

In the current work the chemical analysis of both leaf and root extract was found to contain polyphenols as well as flavonoids (Table 4). The results in Table 4 point out that the total polyphenol and flavonoid content in the leaves of *C. album* highly exceeded that in the root. The quantity of polyphenols and flavonoids in the leaves was triple, or even higher than that determined in the root. Hence, a correlation between higher amounts of total polyphenols, flavonoid content and weed growth inhibition may be associated with a potential allelopathic property of *C. album*. This may explain the more inhibitory effect of leaf extract on weed growth than that of root extract. El-Khatib *et al*. (2004) obtained similar results. Several workers attributed the inhibition in weed growth by different plant extracts to the presence of some allelochemicals such as phenols, flavonoids and/or alkaloids (Chon and Kim 2004; El-Rokiek and Eid 2009; Ghareib *et al*. 2010; El-Rokiek and El-Nagdi 2011; El-Rokiek *et al*. 2014).

It was observed that there are great differences between the percentage of reduction in dry weight of *S. viridis* in pots of the untreated, unweeded control (pots that contain beans and the two weed species) and that treated with *Chenopodium* extracts as compared to untreated pots that contain *S. viridis* alone (Table 1). The percentage of reduction in dry weight of *S. viridis* reached 91.17% at harvest with 25% leaf extract and 9.37% in the untreated control. The corresponding results in *C. olitorius* were 96.31 and 24.39. This indicates that the reduction in weed growth may be primarily attributed to the allelopathic effect of *C. album* extracts and not to competition between weeds.

The reduction in the growth of both weeds in treated pots decreased their competition against beans and accordingly this reduction was accompanied by increases in bean growth. The increase in the growth of beans was reflected in the increase of both green and dry yield which was represented by the number of green pods, weight of green pods, the number of dry pods, the number of seeds/pod, seed yield/plant and weight of 100 seeds (Tables 2 and 3). It has often been reported that controlling weeds decreased the competition of weeds against crops and consequently increased growth and yield of the crop plants (Ngouajio *et al*. 1997; Blackshaw *et al*. 2000; El-Metwally *et al*. 2010; El-Rokiek *et al*. 2013).

Conclusions

This work indicates that fresh leaf and root extracts of *C. album* are potential bioherbicides against *S. viridis* and *C. olitorius*. The leaf extract of *C. album*, which contains many more polyphenols and flavonoids than the root extract, was more effective than root extract against the two weeds and increased bean yield. Future studies will be required to better understand the difference between the effects of leaf and root fractions.

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