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Allelopathic effect of pepper mint on growth and seed production of redroot pigweed (*Amaranthus retroflexus*)

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Key words: Allelopathy, extract, concentration, foliar application, pigweed.

Abstract

Weeds are one of the most important factors in reducing plant growth, however current methods for controlling them are not effective or have environmental problems. Allelopathic is emphasized recently as an effective way to control weeds. Therefore, a RCBD based factorial experiment was conducted in three replicates in greenhouse condition to evaluate allelopathic effect of Pepper mint on growth and seed production of redroot pigweed in 2013. Evaluated factors were: (1) Pepper mint extracts from different organs: (leaves, stems, mixture of leaves and stems), (2) Different concentrations of extracts: (0 (control), 10%, and 20%, (3) Different methods of extract using: soil and foliar application. The results showed that all extracts of Pepper mint caused significant decreasing on the growth and performance of pigweed. Pepper mint leaf extract 20% caused the highest reduction in pigweed plant height and shoot dry weight and showed 68.3, 86.6% less than control, respectively. Foliar application of leaf 20% extract concentration showed the highest reduction in plant height in comparison with control (68 %). Both 10% and 20% concentrations decreased leaf area, seed number per plant, seed weight per plant and 1000 kernel weight as 58.2, 57.8, 63.8 and 14.7%, compared to control, respectively and reduction in attributes was higher with increasing concentration of the extracts. Results indicated the strong controlling effect of Pepper mint extract on pigweed growth and seed production.

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Introduction

Allelopathy is any direct or indirect, useful or useless effects of plants on other ambient plants germinating and growing via created chemical materials and its transmittal. These compounds are usually secondary products of plant or waste products derived from plant primary metabolic pathways (Rice, 1984).

Weeds are important components of agricultural ecosystems which directly and indirectly compete with crops in order to more utilize resources such as soil, water, nutrients and light and in most cases reduce the quantity and quality of product. Because of similar food needs and root systems of weeds, often monocotledon weeds compete with cereals and broadleaf weeds with broadleaf crop plants (Gutillo *et al.,* 2004). Nowadays, the use of herbicides is restricted due to increasing weed resistance to herbicides, environmental impacts and contamination of water resources (Vyvyan, 2002).

Some medicinal plants are considered a good source of allelochemicals which will be useful in developing natural herbicides and pesticides. Allelopathic compounds found in medicinal plants such as alkaloids, flavonoids, phenols, tannins and glycosides can act as a growth inhibitory substances (Oliva *et al.,* 2001). Allelopathy understanding can be encompassing the new key strategy of weed management. In other words, allelopathic attribute of medicinal plants in weed control is a new hope to avoid using of hazardous chemical substances and it's a convenient alternative to reduce the risks of agro ecosystems (Irshad and Cheema, 2006).

Azizi *et al.* (2006) showed that germination of *Centura ovina* and *Bromus tectorum* were completely inhibited by *Bunium persicum* essential oils at 700 and 1000 ppm respectively, where, *Descurainia sophia* seeds were more sensitive to *Bunium persicum* essential oils and its germination completely inhibited at 500 ppm and more. Furthermore, germination of *Bromus tectorum*, *Centura ovina* and *Descurainia sophia* completely inhibited by 2000, 1000 ppm 500 ppm of *Bunium persicum extracts*, respectively.

Khan *et al.* (2008) showed the inhibitory effect of aqueous extract of *Eucalyptus camadulensis* on six weed species and proposed that the aqueous extract of this species can be used as an herbicide for weeds control. Azizi *et al.* (2008) showed that ethanol extract of thyme and peppermint can limit or stop seed germination of pigweed, wild lettuce and purslane weed. stem aqueous extract of invasive tobacco increased seedlings growth of Juniper, over 88%, while aqueous extracts had a negative effect on root dry weight (Alshahrani, 2008).

Ramezani *et al.* (2008) showed that essential oils of rosemary have significant effect on germination of pigweed and purslane weeds. Saharkhiz *et al.* (2009) found the significant decreasing allelopathic effect of essential oils of *Carum copticum* L., *Cuminum cyminum* L., *Rosmarinus officinalis* L., and *Zataria multiflora* Boiss., on germination and growth of *Cynodon dactylon* L., *Festuca arundinacea* Schreb., and *Lolium perenne* L., but *Zataria multiflora* essential oil was the most effective one that completely inhibited germination and growth. Qasemi (2009) in the evaluating of the allelopathic effect of some medicinal plants on lambsquarters and pigweed in the greenhouse conditions showed that lavender and rosemary have high toxicity on germination and growth of two weeds, and an increase of more than 16 g per kg pot soil of Capparis and lavender extracts , reduces their growth. In the examining of the allelopathic effect of *Parthenium hysterophorus* on germination and growth of onion, it's discovered that plumule is more sensitive than radicle. Root extract decreased germination of onion as 37%, and length of radicle and plumule as 13% and 17%, respectively (Wakjira, 2009). Hamedanian *et al.* (2010) in the evaluating of the *Atriplex canescens* allelopathic effect on germination of *Salsola rigida* found that extract of leaves and fruits of *A. canescens* decreased germination of *Salsola rigida*. Further more, results also indicated that increasing in

concentration of fruit and leaves extract can reduce germination of *Salsola rigida*, where as the highest percentage of germination is obtained from control while the lowest from the 100% treatment. In other research allelopathic effects of *Andrographis panykulath* on the germination of sesame are assessed and its found that plant extract have a significant effect on the germination, especially leaf extract had more efficiency than stem and root extracts (Alagesaboopathi, 2011).

Pukclai and Noguchi (2011) reported that *Piper sarmentosum* Roxb has significant effect on germination of weed seeds, whereas extract of this plant in 0.03 ml concentration was efficient to inhibit weed shoot and root growth and increasing the extract concentration increased inhibition and among the studied plants, alfalfa seedling was the sensitive ones. Jalali *et al.* (2012) showed that increasing in concentrations of aqueous extract from different parts of redroot pigweed and glycyrrhizin plants, significantly decreased rate of germination, length of radical and hypocotyls, and increased time from 10% to 90% of germination in maize and chickpea. Also, between two plants, glycyrrhizin indicated more allelopathic effects on germination traits. So, the aim of this experiment is evaluating allelopathic effects of extracts obtained from different organs of pepper mint on growth and seed production of red root pigweed.

Materials and method

The study was conducted in Faculty of Agriculture, Islamic Azad University, Tabriz branch, Iran, located at 5 km far from Tabriz in lohgtitude38^{o} 3' N , and latitude 46^{o} 27', and 1360 meter altitude, at 2013. This study was done using factorial experiment based on randomized complete block design with 3 replications. Evaluated factors include (1) Pepper mint extracts from different organs at three levels: extracts from the leaves, stems, mixture of leaves and stems, (2) Different concentrations of extracts, prepared from Pepper mint organs at three levels: at concentrations of 10%, 20%, and distilled water (control), (3) Different methods of extract using at two level: Soil and foliar application. Experiments were performed on redroot pigweed seeds.

Germination Experiment

In order to guarantee of seeds viability, ISTA (International Seed Testing Association) rules used in control conditions with distilled water and uniformity of germination has been evaluated. , Scarification methods were used to break seed dormancy of Pigweed seeds. Extraction procedure: after cleaning Pepper mint, related components, divided into two parts as stems and leaves, and after drying in the open air, they grinded to powder . In order to prepare the extract, 20 g. of plant material soaked in 80 ml distilled water for 24 hours, then smoothed and centrifuged, so the extract concentration became 20% stock and then it is used as main concentration to make 10% extract. Greenhouse experiments were implemented in controlled environment, equipped with a ventilation system (temperature 19-35^{o}C, Humidity 40-70%).

Greenhouse Experiment

Cultivation environment: to perform the test, identical pots were used with a volume of 10 L. and a diameter of 25 and height of 30 cm. The pots were filled by 1/3 of sand and 2/3 of field soil and FYM mixture. 50 Redroot pigweed seeds were planted at the depth of 0.5 cm. Every 3 days, the pots were irrigated until plant establishment. Seedling thinned after germination and growth and then 5 plants remained in each pot and irrigation began with peppermint extract which captured from different organs at the test concentrations, was added to each pot, foliar and soil application, every 15 days for four times, each time at a rate of 80 ml. Each plant attributes like plant height, shoot dry weight, leaf area, seed number per plant, seed weight per plant and 1000 kernel weight measured.

Statistical analysis

ANOVA was done by MSTAT-C. The comparison of means was made using Duncan's Multi Range test at 5%, and the graphs were drawn using Excel software.

Results and discussion

Organ extracts effect were significant on all the characteristics except the leaf area and grain weight. Effect of extract concentration was significant on all reviewed traits. Interaction of concentrations * organs effect were significant on pigweed plant height and shoot dry weight. Interaction of concentration * method of use, and tripartite treatment combination of concentration* method of use * organs were significant only for plant height (Table 1).

Table 1. Variance analysis of Pepper mint extract effect on growth and seed production of pigweed in greenhouse.

SOV	df	plant height	leaf area	shoot dry weight	seed number per plant	Seed weight per plant	1000 kernel weight
Organ Extract (A)	2	70.974**	58.079	1.671*	2320898*	0.135**	0.110
Concentration (B)	2	10298.684**	11762.810**	300.654**	38926167**	5.584**	1.212**
B*A	4	38.310*	44.471	1.463*	624087	0.041	0.118
method of use (C)	1	2.756	272.925	0.240	71235	0.000	0.036
A*C	2	9.709	14.437	0.441	495862	0.036	0.047
B*C	2	48.058*	408.836	0.465	274562	0.025	0.303
A*B*C	4	43.747*	49.537	0.706	13584	0.045	0.052
Error	36	12.580	214.941	0.489	22284	0.023	0.155
CV (%)		8.19	27.53	15.46	20.25	15.83	12.37

* and**: significant at 5% and 1% levels, respectively

Plant height: All treatments related to use of extracts caused a significant reduction in plant height. Average comparison of plant height affected by foliar and soil application of Pepper mint organs extracts showed that in foliar treatment, 20% concentration of the leaf extract had the highest effect of decreasing on the plant height. It seems that 20% concentration of the leaf extract had not significant difference with treatment of a 20% concentration mixture of stem and leaf extracts, and these two treatments reduced plant height 68.3 and 66.3%, respectively. Between two used methods observed no significant difference at concentration of 20%, leaves and mixture of leaves and stems decreased 60.2 and 63%, respectively. The soil application of 10% concentration of leaf extract, had the most decrease of 67% in plant height, and then had not significant difference with 20% concentration of mixed leaves and stems extract. In the leaf extract, there was no significant difference between extract concentrations of 10 and 20% soil application, where as in the foliar extracts, with increasing of concentrations, decrease effect of the extract were greater on plant height. In the stem extract, there was no significant difference between soil and foliar application of extracts 10 and 20 %. In mixed extract of leaves and stems and both application methods, increasing of extract concentration, decreased significantly plant height (Fig. 1). Bajalan *et al.* (2013) reported that Lamiaceae family has strong allelopathic properties and increasing of extract concentration, increased the effectiveness of plant extract. Monoterpenes are one of the most important compounds found in Pepper mint (Turner and Croteau, 2004). Monoterpenes like 1.8-cineole, reduced both the mitotic index and elongation of the cells, and the amount of reduction depends on the concentration. Elongation of cell is more sensitive than division of cell to allelopathic compounds (Yoshimura, 2011), therefore the effect of terpenes can reduce the plant height.

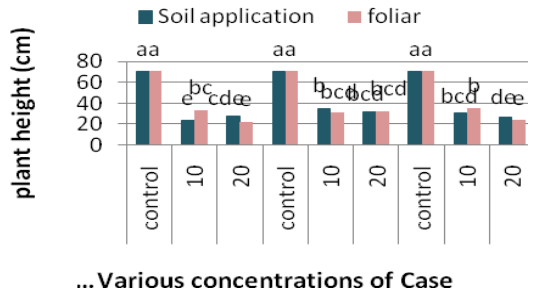

Fig. 1. Comparison of plant height affected by foliar * soil application * different concentrations of Peppermint organs extracts.

Leaf area: Average comparison of leaf area affected by different concentrations of Pepper mint extracts showed that concentrations of 10 and 20% had significant effect on redroot pigweed leaf area. Highest leaf area in control condition was equivalent to 82.27 cm². Concentrations of 10 and 20% of extractions reduced pigweed leaf area 47.2 and 58.5% compared to control, respectively. In treatment with 10 and 20% concentrations, pigweed had the highest and minimum leaf area, respectively (Fig. 2). Allelopathic compounds can interfere with the polymerization and de-polymerization of microtubules, and resulting in reduced cell division. Numerical and structural changes of chromosomes is under effect of allelopathic compounds related to the disorganization of spindle fiber, which leads to lack of double, c-mitosis, nuclear fragmentation, de-formation due to multipolar, to cluster and thickening of chromosomes (Adhikary, 2013). Reducing cell division and growth of cells, causing to be reduced plants leaf area which are exposed to allelopathic compounds (El-Khatib *et al.*, 2004).

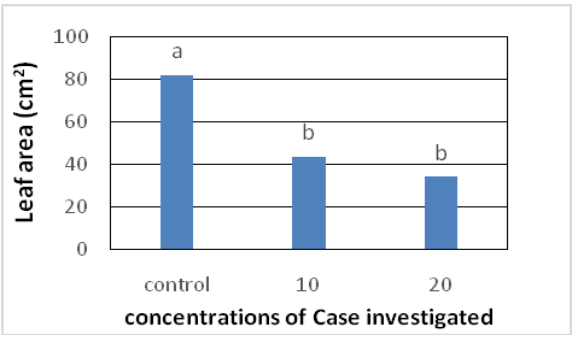

Fig. 2. Comparison of leaf area affected by different concentrations of Pepper mint extracts.

Shoot dry weight: Average comparison of shoot dry weight affected by different concentrations of Pepper mint leaves, stems and mixture of leaves and stems, extracts, showed that all treatments of extracts usage caused a significant reduction in shoot dry weight of pigweed. In extracts of Pepper mint leaves and stems, there was not significant difference between concentrations of 10 and 20% in terms of shoot dry weight. Where as in the mixture of stem and leaf extracts, 20% concentration had more redaction effect on shoot dry weight. In the mixture of stem and leaf extracts, 20% concentration reduced the shoot dry weight of pigweed by 79.2%, where as this redaction in 10% concentration was 67.1%. Concentrations of 10 and 20% Pepper mint leaf extract reduced 77.9 and 86.6%, and 10 and 20% concentrations of Pepper mint stem extract reduced 77.7 and 71.6% of shoot dry weight, respectively. In this study, no significant difference observed between leaves, stems and mixed of leaves and stems extract at concentration of 20%, and at concentration of 10% of Pepper mint leaves and stems extract showed more allelopathic properties than 10% concentration of mixture of stems and leaves extract. In general, 20% concentrations of Pepper mint leaf extract had greatest reduction in shoot dry weight, with 86.6% reduction (Fig. 3).

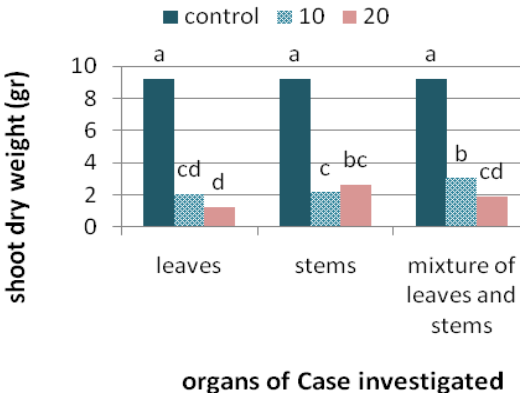

Fig. 3. Comparison of shoot dry weight affected by different concentrations * different organs.

Allelopathic compounds in mature plants are leading to a reduction in chlorophyll content and protein content of leaf. Allelopathic compounds would lead to

a reduction in chlorophyll content, either by preventing the synthesis of chlorophyll or its degradation. Therefore, allelopathic compounds can stop the process of photosynthesis, which eventually will lead to a reduction in plant growth (Abdul Raoof and Siddiqui, 2013).

Seed number per plant: Average comparison of seed number per plant affected by different organs of Pepper mint showed that treatments of leaves had significant difference with stems and mixture of leaves and stems which reduced the number of pigweed seeds per plant 19.2 and 19.7%, respectively. No significant difference observed between stem and mixture of leaf and stem extracts (Fig. 4).

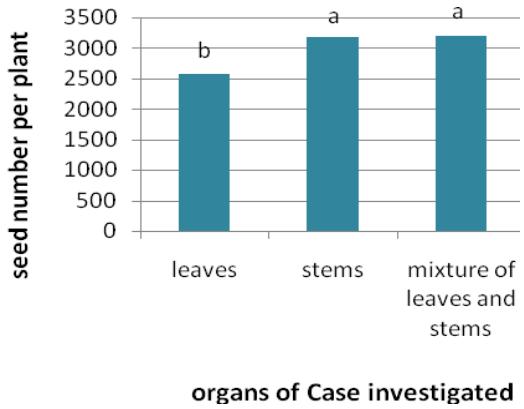

Fig. 4. Comparison of seed number per plant affected by different organs.

Average comparison of seed number per plant affected by different concentrations of Pepper mint extract showed that, highest seeds per plant of pigweed were equivalent to 4670 in control and both 10 and 20% concentrations had a significant effect on reduction of the trait. Furthermore extract concentration increasing, increased allelopathic properties, so that 20% extract concentration reduced the number of seeds per plant, 57.8% compared with control, and 10% extract concentration reduce the number of seeds per plant of pigweed, 50.3% compared to controls (Fig. 5).

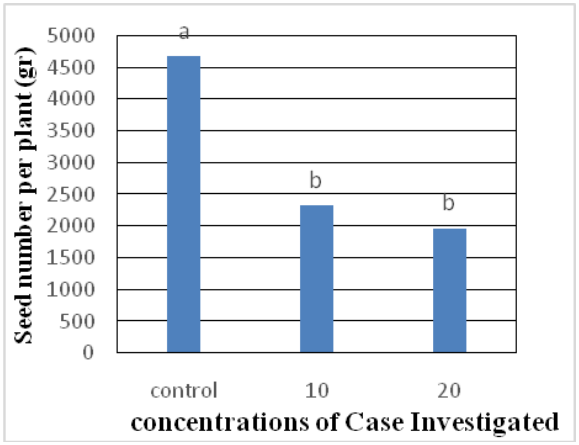

Fig. 5. Comparison of seed number per plant affected by different concentrations.

Allelopathic compounds limit rate of photosynthesis rate and subsequently respiration and limitation in protein synthesis, photosynthetic pigments and change of biosynthesis pathways (Yang *et al*., 2002), changes in chloroplast and mitochondrial membranes, nutrient uptake, stopping of cells mitotic activity (De Neergard and Porter, 2000), disruption at the hormonal system and the closure of xylem and disruption of transport (Colpas *et al*., 2003), disruption of enzymatic activities, increasing of abscisic acid level (De Neergard and Porter, 2000). In general, these compounds leads to a decrease in overall growth of the plant, production of initial flowers, reproduction parts of the flower and inoculation and finally endosperm cell dividing and assimilate transportation into these cells. Therefore, allelopathic compounds not only decrease vegetative growth of plants parts but also lead to reduce the number and weight of seeds.

Seed weight per plant : According to the results of average comparison of seed weight per pigweed plant affected by examined organs, stems and mixed of leaves and stems treatments of Pepper mint, reduced seed weight per plant of pigweed, 99.4% compared to treatments of leaves (Fig. 6). Average comparison of seed weight per plant affected by different concentrations showed that all treatments related to use of this medicinal plant extract, has resulted in to significant reduction of seeds per plant weight. There

was a significant difference in seed weight per plant of pigweed at both 10 and 20% concentrations of extracts. Highest seed weight per plant was equivalent to 1.6 g in control. Treatment with 10 and 20% concentrations reduce the seeds weight per pigweed plant, 55.9 and 63.8%, respectively. 20% concentration, caused the greatest reduction in seed weight per plant (Fig. 7).

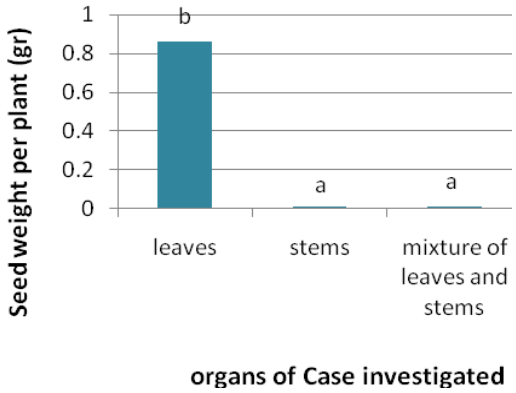

Fig. 6. Comparison of seed weight per plant affected by different organs.

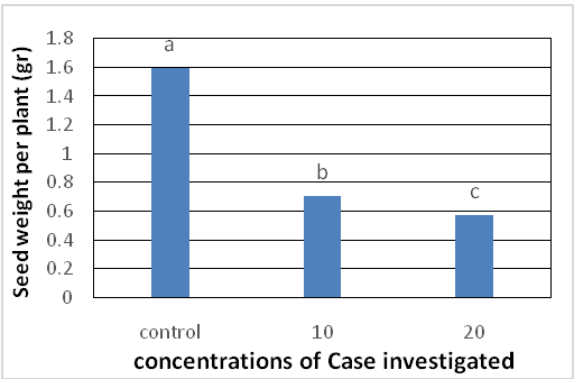

Fig. 7. Comparison of seed weight per plant affected by different concentration.

1000 kernel weight: Average comparison of 1000-kernel weight affected by different concentrations showed that, both extracts with concentrations of 10 and 20% had a significant effect on this attribute and maximum 1000-kernel weight was equivalent to 3.468 g in control. In extracts with 10 and 20% concentrations, reduction effect compared to the control was 9.6 and 14.7 %, respectively. Increasing of extract concentration increased their allelopathic properties (Fig. 8). Allelopathic interference in shoot growth and crop production of plant is a complex process, which can affect all aspects of the development from cell division to photosynthesis and material transfer and reduce the crop yield. For example, reduction in elongation of plant is because of allelopathic compounds interfering in cell division. Possibly this leads to reduce the absorption of minerals and nutrient transport from the roots to other parts of the plant. Furthermore this redaction in growth can be because of allelochemical interference in the synthesis of proteins and hormones (El-Khatib *et al.*, 2004). Interaction activity of allelochemical on the growth of plants is also related to reduction of photosynthesis operation. The decrease in photosynthesis leads to reduction the amount of carbohydrates, which ultimately, leads to the reduction in dry matter accumulation in plant (De Neergard and Porter, 2000).

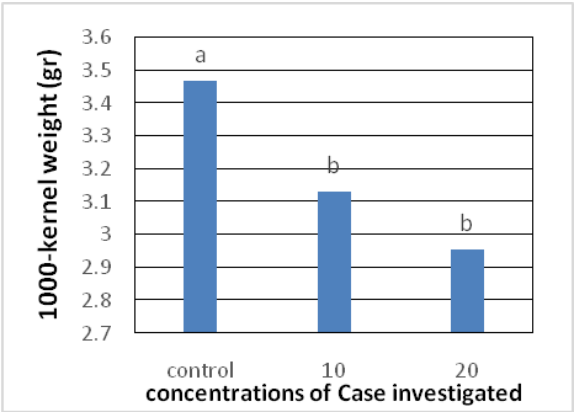

Fig. 8. Comparison of 1000-kernel weight affected by different organs concentration.

Conclusion

The results of this study demonstrated that the material produced from different organs of Pepper mint plant can affect growth and seed production of pigweed and this is an emphasis on different allelochemical existence in Pepper mint organs and influence of these materials on pigweed different characteristics. This study proves that increasing of Pepper mint aqueous extract concentrations caused significant decrease in surveyed traits, which this reduction was at least 58.2% in leaf area, 57.8% in the number of seeds per plant, 63.8% in the seed weight

per plant, and 14.7 in the weight of seed. Leaf extract caused the highest reduction effect, and reduced 86.6% of shoot dry weight, and 68% of plant height. Foliar application of leaf extract with 20% concentrations reduced 68.3 % in plant height. Eventually, with more investigation on different concentrations of Pepper mint plant organs and its effect on pigweed growth stages and other similar weeds, this valuable medicinal plant can play significant role in establishment of this plant applications in control of weeds and this can be leads to promising results, in order to produce organic farming of agricultural products and natural origin herbicides.

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