

Allelopathic effects of nettle, chamomile and dandelion on germination and seedling growth of weeds (wild mustard, common mallow, canary grass)

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ABSTRACT

In Petri plate Lab. bioassays, we studied the effects of aqueous extracts (0, 5 and 10%) of nettle (*Urtica dioica* L.), chamomile (*Matricaria chamomilla* L.) and dandelion (*Taraxacum officinale* L.) as donor species on germination and seedling growth of wild mustard (*Sinapis arvensis* L.), common mallow (*Malva neglecta* Waller.) and canary grass (*Phalaris minor* Retz.) as recipient species. We found that weed species differed from each other in terms of germination and seedling growth. The aqueous extracts inhibited the seed germination and seedling growth of weeds. The 10% chamomile extract significantly reduced germination percentage, root length and seedling dry weight in all weeds. The effect inhibitory of nettle extract on seed germination and seedling growth was more pronounced than dandelion extract.

Key words: Allelopathy, aqueous extracts, canary grass, chamomile, common mallow, dandelion, *Malva neglecta*, *Matricaria chamomilla*, nettle, *Phalaris minor*, seed germination, seedling growth, *Sinapis arvensis*, *Taraxacum officinale* *Urtica dioica*, wild mustard.

INTRODUCTION

The increased use of chemical herbicides for weed control has caused so many problems, hence, researchers are trying to find new alternate methods. The biodynamic approaches may help us to resolve this issue. Briefly, the biodynamic agriculture is a holistic, spiritual ethical and ecological approach, in which farmers treat the soil, plant, and livestock in ecological manner (17). Similar to organic farming, the biodynamic agriculture also excludes the use of herbicides, pesticides or genetically modified organisms. For weed control, biodynamic agriculture encourages the use of new generation of natural herbicides, produced from allelochemicals, to create conditions for the development of sustainable agriculture (31). The concept of allelopathy was introduced by Hans Molisch to describe both the beneficial and the harmful chemical interactions of plants and microorganisms (22). Now allelopathy is defined as direct or indirect harmful interactions of chemicals and their breakdown metabolites released by donor plants, which influence the physiological processes and development of target plants (28, 29). Thus allelopathy may be used for weeds control to overcome the challenges of environmental pollution and herbicide resistance etc. (19). Allelochemicals are present all parts of plants, hence, found in plant extracts and in decomposing plant residues into the soil (2) and

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interfere with growth of other plants. They influence the photosynthesis, respiration, biochemical metabolism and even at molecular level of protein and nucleic acid synthesis (8) and thereby affect the seed germination and seedling development (1, 16, 23). Seeds germination is the most sensitive to allelochemicals, hence, widely studied in allelopathic research (3). The allelopathic potential of numerous plants and their efficacy for weeds control have been evaluated under laboratory and field conditions. Dikic (11, 12) reported that caraway (*Carum carvi* L.), coriander (*Coriandrum sativum* L.) and fennel (*Foeniculum vulgare* Mill.) inhibits the on growth of weed species (*Alinsoga parviflora*, *Echinochloa crus-galli* and *Galium molugo*). Similarly, Petrova et al., (25) indicated that lavender (*Lavandula angustifolia* Mill.) flower extract and horse mint (*Mentha longifolia* (L.) Huds.) leaf extract decreased the germination and seedling growth rate in Johnson grass (*Sorghum halepense* (L.) Pers.) and curly dock (*Rumex crispus* L.), respectively. In a study Chung et al., (9) used rice (*Oryza Sativa* L.) allelopathy to control barnyard grass (*Echinochloa crusgalli* P. Beauv.) in paddy. The rosemary (*Rosmarinus officinalis* L.) had considerable inhibitory effect on crap (*Acroptilon repens*) and pigweed (*Amaranthus retroflexus*) germination (26). Sorghum and berseem clover (*Trifolium alexandrinum* L.) aerial parts extracts reduced the seed germination dry weight and density of bindweed (*Convolvulus arvensis* L.) (7, 21). Wild mustard, common mallow and canary grass are the most noxious weeds in Iran and their infestation causes major yield losses in many crops. The nettle, chamomile and dandelion are allelopathic species, which can be used to develop natural herbicides. Chamomile is used in medicinal, culinary and cosmetic industries. The nettle gives best fibre for cloth manufacture. Therefore, this study was aimed to determine the allelopathic effects of chamomile, nettle and dandelion aqueous extracts, on germination and seedling growth of wild mustard, common mallow and canary grass weeds.

MATERIALS AND METHODS

This laboratory experiment was conducted in Department of Weed Science at the Islamic Azad University, Science and Research Branch, Tehran, Iran (Longitude: 51°, 31', Latitude: 35° 78', altitude: 1670 m above sea level). The treatments consisted of 3-factors: (i). Donor spp.: 3 (nettle (*Urtica dioica* L.), chamomile (*Matricaria chamomilla* L.) and dandelion (*Taraxacum officinale* L.), (ii). Aqueous extracts concentrations 3 (0, 5 and 10%) and (iii). Recipient weed spp. 3 [wild mustard (*Sinapis arvensis* L.), common mallow (*Malva neglecta* Waller.) and canary grass (*Phalaris minor* Retz.)]. The treatments were replicated thrice in complete randomised design.

The chamomile, nettle and dandelion plants were collected at flowering stage from our experimental fields in summer months of 2015, washed with distilled water and air dried at room temperature in shade for 2-weeks. These dried whole plant samples were ground into fine powder using electrical grinder and stored in air tight containers before making aqueous extracts.

Aqueous extracts: These were extracted by soaking 10 g powdered samples in 100 ml sterile distilled water (10% w/v) overnight at room temperature. Then, these solutions were filtered through Whitman No. 1 filter paper, poured into dark glass bottles, labelled and kept in refrigerator at 4° C.

The dried samples were also extracted by soxhlet extractor at 90° C using 70% ethanol for 18 h. The polar extract was evaporated to obtain crude ethanol extract, which was further fractionated into hexane, chloroform, ethyl acetate and methanol extracts. Afterwards, each extract was evaporated to dryness under fuming hood to obtain semi-solid samples. To detect the chemical compounds present in chamomile, nettle and dandelion weeds, gas chromatography-mass spectrometry (GC-MS) analysis was done using a PerkinElmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m×0.25 mm inner diameter, ×0.25 µm film thickness; maximum temperature, 350° C), coupled to a Perkin Elmer Clarus 600C MS. Ultra-high purity helium was used as carrier gas at a constant flow rate of 1.0 ml/ min. The injection, transfer line and ion source temperatures were all 290° C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed from 60° C (hold for 2 min) to 280° C at a rate of 3° C/min. The crude samples were diluted with appropriate solvent (1:100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The composition of crude extract constituents was expressed as percentage of peak area. The identification and characterization of chemical compounds in various crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

The weed seeds were supplied from Iran Research Institute of Plant Pathology, Tehran, Iran. The seeds were surface-sterilized using 5% sodium hypochlorite for 3 min and 70% ethanol for 30 s. Next, the seeds were rinsed several times with sterile distilled water. The seeds were dried under laminar flow for 10 min. Then 50-seeds of each specie were placed onto two layers of filter paper, in 9 cm petri dishes. The stock solutions (10% w/v) were diluted with sterile distilled water to give the final concentrations of 5 and 10%. The petri dishes were moistened with 10 ml aqueous extract and sterile distilled water was used as control. Then petri plates were wrapped with parafilm and placed in germinator [18/13° C day/night and 12 h photoperiod] conditions for 2-weeks.

At the end of experiment i.e. after 2-weeks, germination (%), shoot and root length and seedling dry weight were measured.

Germination (%) was calculated as under:

$$\text{Germination percentage} = (\text{Germinated seed}/\text{Total seed}) \times 100$$

Plants materials were dried in oven at 50° C for 48 h and weighed by digital scale.

Statistical Analysis: The significant differences in mean germination (%), shoot and root length and seedling fresh, dry weight in different treatments was tested using one-way analysis of variance (ANOVA), and Duncan's multiple range tests. All statistical analyses were performed using the Statistical Analysis System (SAS) software at $P \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical components in organic extract of chamomile (*Matricaria chamomilla* L.), nettle (*Urtica dioica* L.) and dandelion (*Taraxacum officinale* L.) were analysed by GC-MS. The GC-MS analysis confirmed the presence of compounds in in all plants extracts (Table 1). The major constituents in chamomile extract were trans-trans-

Table 1. The compounds detected in in chamomile, nettle and dandelion using GC-MS

Species	Compounds	%
Chamomile (<i>Matricaria chamomilla</i> L.)	trans-trans-matricaria ester	39.93
	cis-calamenene	22.99
	β -farnesene	12.54
	β -maaliene	7.98
	Spathulenol	0.84
	β -elemene	0.74
	Sativene	0.74
	δ -elemene	0.54
	β -caryophyllene	0.34
	α -ylangene	0.23
	β -sesquiphellandrene	0.22
	Caryophyllene oxide	0.17
	Bicylogermacrene	0.14
	Dendrolasin	0.14
	Phytol	25.01
	Rutin	21.33
	Nonanal	15.3
Nettle (<i>Urtica dioica</i> L.)	Phtaleic acid	7.44
	Isorhamnetin	4.78
	Ellagic	4.25
	Carvacrol	3.51
	Syringic	2.78
	Quercetin	1.56
	Myricetin	1.45
	Vanillic	1.25
	β -Iovov ϵ	0.9
	Kaempferol	0.78
	Fumaric	0.68
	5-O-Caffeoylquinic acid	2.56
	Caffeic acid pentoside	2.46
Dandelion (<i>Taraxacum officinale</i> L.)	Luteolin O-rutinoside	2.36
	Luteolin	2.33
	Caffeic acid hexoside	1.33
	Chicoric acid isomer	1.25
	4,5-di-O-caffeoylquinic acid	1.25
	Luteolin O-acetylhexoside	1.25
	Chicoric acid isomer	0.98
	Luteolin O-hexoside	0.84
	trans-Caffeic acid	0.78
	3,5-di-O-caffeoylquinic acid	0.36
Luteolin 7-O-glucoside	0.26	

matricaria ester, cis-calamenene and β -farnesene, along with other minor constituents. There were 17 compounds in nettle extract and phytol, rutin, nonanal and naringin were major constituents (Table 1). While in dandelion extract, 13 constituents were detected and 5-O-Caffeoylquinic acid, caffeic acid pentoside, Luteolin O-rutinoside and luteolin were the major constituents (Table 1).

The main effects of donor plants (nettle, chamomile and dandelion) extracts on germination and seedling growth of 3- recipients weed species [wild mustard (*Sinapis arvensis* L.), common mallow (*Malva neglecta* Waller.) and canary grass (*Phalaris minor* Retz.)] are shown in Table 2. The minimum germination (%) in wild mustard was found, when chamomile aqueous extract was used. The chamomile aqueous extract also drastically reduced the seedling length, shoot or root length and seedling dry weight (Table 2). In common mallow, the dandelion aqueous extract reduced the germination (%) and seedling length, however, the reduction was more in root length and seedling dry weight. Regarding canary grass, chamomile aqueous extract reduced the germination (%), seedling length, root length and dry weight, but shoot length was most affected. According to the mean values (Table 2), irrespective of weed species, chamomile aqueous extract reduced the germination, seedling length, root length and seedling dry weight followed by dandelion > nettle i.e the dandelion aqueous extract was more inhibitory to shoot growth compared with chamomile or nettle (Table 2).

Table 2. The mean effects of nettle, chamomile and dandelion aqueous extracts on germination and seedling growth of three recipients weed species.

Recipient weed species	Germination (%)	Seedling length (cm)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)
<i>Nettle (Urtica dioica L.)</i>					
Wild mustard	79.33	13.49	5.52	7.20	51.66
Common mallow	24.77	3.74	2.90	0.85	3.62
Canary grass	59.00	9.97	4.78	5.18	40.58
Mean	54.37	9.07	4.40	4.41	31.95
<i>Camomile (Matricaria chamomilla L.)</i>					
Wild mustard	59.00	9.97	4.78	5.18	40.58
Common mallow	26.33	4.51	3.20	1.42	4.68
Canary grass	26.66	5.59	3.59	2.19	3.04
Mean	37.33	6.69	3.85	2.93	16.10
<i>Dandelion (Taraxacum officinale L.)</i>					
Wild mustard	70.44	11.32	5.38	6.96	41.53
Common mallow	26.11	3.58	2.51	1.11	3.05
Canary grass	32.11	5.67	3.42	2.25	4.51
Mean	42.89	6.86	3.77	3.44	16.36

Wild mustard (*Sinapis arvensis* L.), Common mallow (*Malva neglecta* Waller.) and Canary grass (*Phalaris minor* Retz.)

The interaction between different aqueous extracts and their concentrations (Table 3). showed that the maximum germination (%) of wild mustard treated was in control (distilled water), followed by 5 or 10 % nettle extracts. In other words, 5 or 10 % nettle extracts caused the minimum inhibition in germination in wild mustard seeds. On the other hand, the maximum germination (%) inhibition was in common mallow petri plates treated with 10% nettle or chamomile aqueous extracts (Table 3). Likewise the wild mustard seedlings were longest in distilled water (control), 5 or 10% nettle aqueous extracts.

Table 3. The effect of nettle, chamomile and dandelion aqueous extracts on germination and seedling growth of three recipients weed species.

Recipient weed species	Donor aqueous extracts Conc. (%)	Germination (%)	Seedling length (cm)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)
<i>Nettle (Urtica dioica L.)</i>						
Wild mustard (<i>Sinapis arvensis</i> L.)	Control	88.66a	14.80a	6.30a	8.50a	53.80a
	5%	79.66b	12.98b	5.39c	7.29c	51.18b
	10%	69.66c	12.69bc	4.88e	5.81e	50.00c
Common mallow (<i>Malva neglecta</i> Waller.)	Control	26.66fgh	5.40hi	4.37f	1.03lm	7.66j
	5%	24.33gh	4.43kl	3.62j	0.81mn	2.30q
	10%	23.33h	1.40p	0.70r	0.70n	0.90t
Canary grass (<i>Phalaris minor</i> Retz.)	Control	67.00d	11.85d	5.30cd	6.51d	47.48d
	5%	64.00d	10.46e	5.24cd	5.22f	40.33g
	10%	46.00e	7.60f	3.80hi	3.80g	33.93i
<i>Chamomile (Matricaria chamomilla L.)</i>						
Wild mustard (<i>Sinapis arvensis</i> L.)	Control	67.00d	11.85d	5.30cd	6.51d	47.49d
	5%	64.00d	10.46e	5.24cd	5.22f	40.32g
	10%	46.00e	7.60f	3.80hi	3.80g	33.94i
Common mallow (<i>Malva neglecta</i> Waller.)	Control	29.33fgh	6.81g	5.21d	1.60k	5.3l
	5%	26.33fgh	3.71m	2.20o	1.50k	4.48m
	10%	23.33h	3.00no	2.18o	1.16l	4.26mn
Canary grass (<i>Phalaris minor</i> Retz.)	Control	28.00fgh	6.51g	3.89h	2.62h	3.91mno
	5%	26.33fgh	5.62h	3.28k	2.34i	3.17p
	10%	25.66gh	4.64jkl	3.02lm	1.62k	2.04qr
<i>Dandelion (Taraxacum officinale L.)</i>						
Wild mustard (<i>Sinapis arvensis</i> L.)	Control	67.00d	12.39c	5.74b	8.18b	43.90e
	5%	76.00bc	11.94d	5.61b	6.38d	42.06f
	10%	68.33d	10.69e	4.80e	6.33d	38.62h
Common mallow (<i>Malva neglecta</i> Waller.)	Control	26.66fgh	4.74jk	3.72ij	1.40k	6.35k
	5%	26.66fgh	3.41mn	2.01p	1.02lm	1.55rs
	10%	25.00gh	2.60o	1.79q	0.81mn	1.26st
Canary grass (<i>Phalaris minor</i> Retz.)	Control	34.33f	6.48g	4.20g	2.51hi	5.87kl
	5%	32.00fg	5.45hi	3.13kl	2.28i	3.93mno
	10%	30.00fgh	5.09ij	2.93m	1.96j	3.72nop

Means with the same letter are not significantly different.

Contrarily, the shortest seedlings were observed when common mallow seeds were treated with 10% nettle aqueous extract. In general, irrespective of donor or recipient species, increase in aqueous extract concentration significantly reduced the seedling growth. Similar results were found in shoot and root length. Wild mustard seedlings treated with distilled water produced the maximum shoot and root length. While, the minimum shoot and root length of common mallow was found with 10% nettle extract (Table 3). The maximum dry weights of wild mustard seedlings was observed with distilled water, 5 or 10% nettle aqueous extracts, respectively. By contrast, the minimum value was found in common mallow seedlings treated with 10% nettle extract (Table 3).

The inhibitory/stimulatory effects of nettle, chamomile and dandelion aqueous extracts on germination (%), seedling length, shoot length, root length and seedling dry weight of recipient weed species as percentage values over control treatment (water) were calculated and illustrated (Figures 1, 2, 3). In all cases, nettle, chamomile and dandelion aqueous extracts showed inhibitory effects on germination parameters, except the dandelion extracts, which stimulated the germination (%) in wild mustard (Figure 1). In other words, application of 5 and 10% dandelion aqueous extract increased the germination (%) of wild mustard by 13.43 and 1.99%, respectively (Figure 1). In all studied traits, application of 10% aqueous extract proved more inhibitory to germination and seedling growth parameters as compared to 5% extracts. There was no significant difference between the control treatment and 5% dandelion aqueous extract on common mallow germination (%). In other words, 5% dandelion aqueous extract neither increased nor decreased the germination (%) in common mallow (i.e. no inhibition/stimulation).

In general, there was variability between the recipient weed species in terms of germination parameters in response to different aqueous extracts and their concentrations. In addition, aqueous extracts differed from each other with regard to their potential to inhibit the seed germination. This might be mainly due to the different compositions of these extracts, which suggests presence of different allelochemicals or concentrations of allelochemicals. It has been reported that the extract of leaves, stems, roots, fruits and even seeds contain water soluble phytotoxins, which affects the seed germination and seedling growth of other plants (6). In several studies, inhibitory effects of water extract of plants have been documented (13,18,24). The aqueous extracts of sunflower (*Helianthus annuus*) residues inhibited the barley (*Hordeum spontaneum*) germination, root and shoot growth (4). It is known that chamomile plant contains different secondary metabolites (carotenoids, valeric acid, salicylic acid, coumarin derivatives, tannins, and flavonoids) which could act as allelochemicals (14). Moreover, the nettle plants also contains tannins, organic acids (formic acid and acetic acid, glycolic acid, glyceric acid), carotenoids, xanthophyll, flavonoids, phytosterols such as β -sytosterol, amine compounds (32). Similar compounds have been reported in dandelion extract (10).

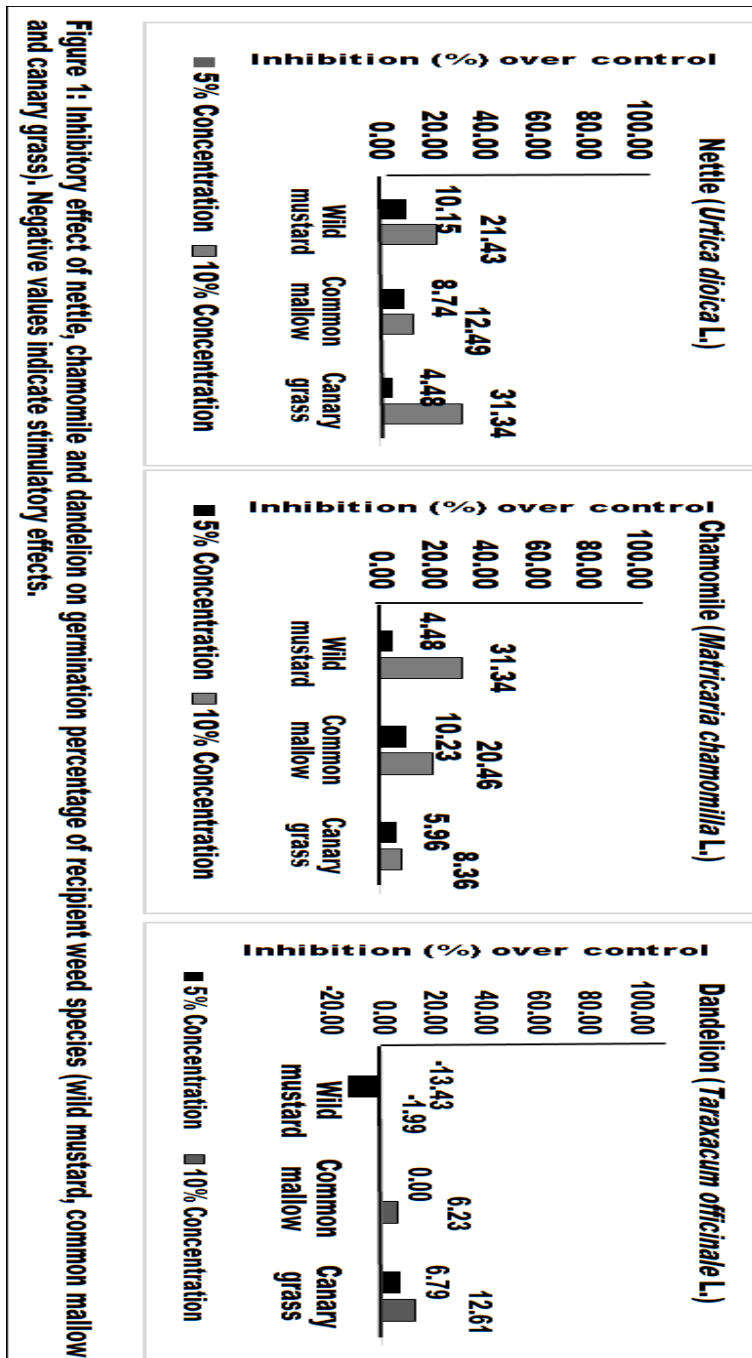


Figure 1: Inhibitory effect of nettle, chamomile and dandelion on germination percentage of recipient weed species (wild mustard, common mallow and canary grass). Negative values indicate stimulatory effects.

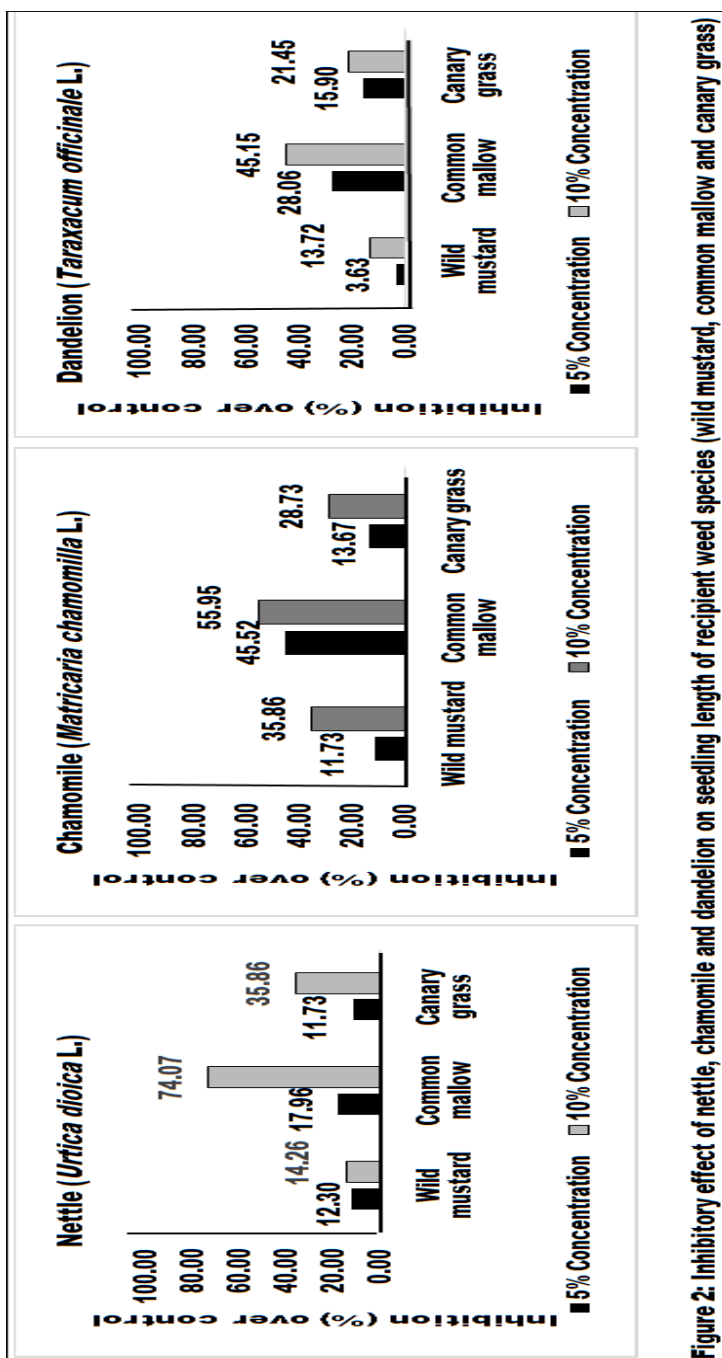


Figure 2: Inhibitory effect of nettle, chamomile and dandelion on seedling length of recipient weed species (wild mustard, common mallow and canary grass)

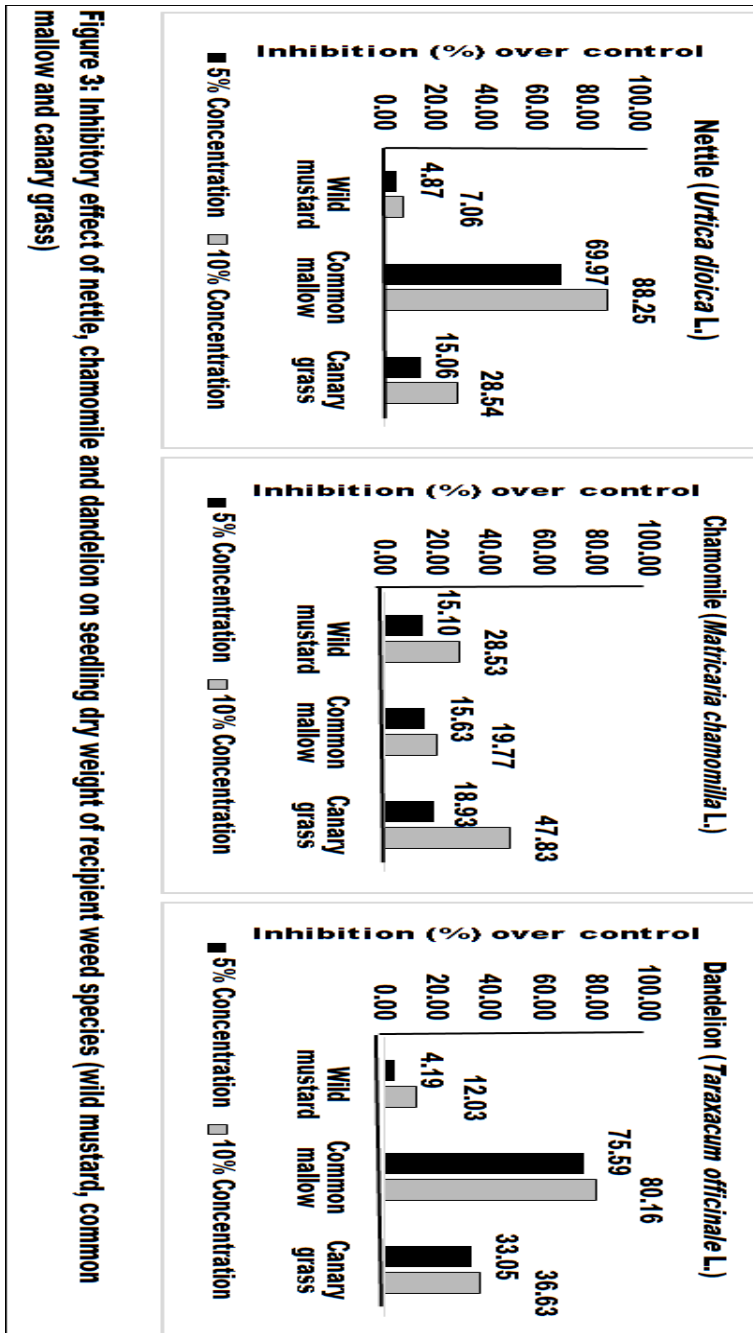


Figure 3: Inhibitory effect of nettle, chamomile and dandelion on seedling dry weight of recipient weed species (wild mustard, common mallow and canary grass)

These compounds probably affects the hormones involved in germination such as gibberellin (20) or activity of some enzymes such as amylases and proteinases, which are essential for seed germination (30). Therefore, reduction in germination (%) due to application of these allelochemicals is expected. Reduction in seedling growth found in this study is in agreement with Babar *et al.* (5) results, who stated that chickpea seeds soaked in root extract of *Asphodelus tenuifolius* took more time for germination. Similarly, Tawaha and Turk (32) in a study also observed inhibitory effects of allelochemicals on water imbibition in wild barley (*Hordeum leporinum*), focussing on allelopathic effects of black mustard (*Brassica nigra*). Reduction in root growth in *Trianthema portulacastrum* seedlings has also been reported by Randhawa *et al.* (27) who studied the effects of sorghum water extract on weed seed germination. Reduction in root and shoot length might be due to the allelochemicals present in aqueous extracts extracted from nettle, chamomile and dandelion plants. Our results confirm Hussain and Reigosa (15) findings, who found the inhibitory effects of allelochemicals on root and shoot length of *Dactylis glomerata*, *Lolium perenne* and *Rumex acetosa*. The reduction in root growth probably had effects on physiological and biological functions of the plants such as absorption of water and nutrients required by plant for its survival. This might have contributed to the decrease in dry weight.

CONCLUSIONS

The aqueous extracts from chamomile and nettle, inhibited the germination and seedling growth of test weeds. Plant residues of these two species could be spread in fields, so that their leachates containing allelochemicals could reduce the seed germination and consequently the weeds population. Besides the aqueous extracts may be prepared from these plants as source of natural weedicide for weeds control. However, further studies are required to identify and isolate the most effective allelochemicals from these plants and develop the natural-product based herbicides to control aggressive weeds.

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