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Phenolic contentand allelopathic potential of leavesand rhizosphere soilaqueous extracts of white horehound (*Maribum vulgare*L.)

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Abstract - The present study was conducted to determine phenolic content and evaluate allelopathic effects of leaf and rhizosphere soil extractsof *Maribum vulgare*L.under laboratory conditions. Samples were collected from National Park of Djebel Zaghouan (Tunisia) in October 2016.Total phenolic and flavonoid content of methanolic and water extracts was determined by using Folin-Ciocalteau reagent and aluminum chloride method, respectively. The highest amounts of polyphenols (44.89±0.25 mg EAG/g DW) and flavonoids (24.6±1.07 mg QE/g DW) were shown by the leaf methanolic extracts. The total phenolic contents of the soil extracts, varied from 0.03±0.00 to 0.05±0.00 mg of gallic acid equivalent/g soil extract, and the highest concentration was found in methanol/water extract. Aqueous extracts concentrations of leaf (1, 2.5, 5.0 and 10.5 g mL^{-1}) and soil (5, 10, 30 and 50 g L^{-1}) were studied for their effects on seed germination and seedling growth of *Sinapis arvensis* L. (weed) and *Lactuca sativa*L. (cultivated).The results showed that the leaf extract and rhizosphere soil of *M. vulgare*significantly ($p<0.05$) affects the germination (%), mean germination time (MGT),root and shoot length of *S. arvensis* and *L. sativa*, and the effects were proportional to the concentration. The inhibition caused by the leaf and soil extracts was found to be more than shoot length.The results demonstrated that both leaf aqueous extracts and soil of tested species could be used as natural herbicides.

Keywords: Allelopathy, aqueous extracts, weed, germination, seedling growth.

1. Introduction

Synthetic chemicals have been a major contributor to the increase of agricultural productivity and food supply (Delcour et al. 2015). However, environmental pollution and potential damage to human health provoked by synthetic pesticides and weeds resistance to herbicides were regarded today as a real problem (Araniti et al. 2014). In this context, allelopathy offers an important tool for selective biological weed management (Kakati and Baruah 2013).Allelopathy is a phenomenon whereby secondary metabolites synthesized by fungi, viruses, microorganisms and plants influence biological and agricultural systems, which may be either stimulatory or inhibitory(Ghafarbi et al. 2012; Areco et al. 2014). Initially allelopathic studies were mainly done using leachates or extract in bioassays without soil (Lottina-Hennsen et al. 2006). However, from 1990's, allelopathic research shifted from merely laboratory work to field studies (Dakshini et al. 1999). Phenomenon of allelopathy is considered an attractive method for weed management due to its environmental friendliness (Samedani et al., 2013). The plant produces and releases several types of secondary metabolites including carbohydrates, flavonoids, phytosterols, tannins, coumestans, saponins, alkaloids, terpenoids, cyanogenic glycosides, etc. (Dalal et al. 2010; Khalaj et al. 2013). These secondary metabolites have multiple biological activities(Szczepanik et al. 2012).Thus, the use of secondary metabolites implicated in allelopathic interactions as sources for news agrochemical models could satisfy the requirements for crop protection and weeds management (Singh et al. 2003; Dayan et al. 2009).Allelochemicals are present in almost all plants and their tissues (leaves, stems, roots, flowers, seeds, bark, and buds), and are known to modify growth and development of plants, including germination and seedling growth (Scrivanti 2010; Khalaj et al. 2013). In all allelopathic phenomena, allelochemicals were translocated from one plant to another through various systems. The major translocation pathways of allelochemicals are volatilization of low–molecular weight compounds through atmosphere, root exudation and the decomposition of plant

materials into soil environments, and leaching from the leaves and other aerial parts and plant residues (Hiradate et al. 2010; Amri et al. 2012; Samedani et al. 2013). The interaction of allelochemicals with soil components upon release from the plant is important in determining whether inhibition of the target plant is likely to occur in the field (Blum 2011; Samedani et al. 2013). Soil plays an important role, as it is the matrix through which potential allelochemicals are adsorbed and pass (El-Darieret al., 2014). Furthermore, the activities of allelochemicals in the soil are strongly linked with physical, chemical, biological, and physicochemical properties of the soil, which in turn affect their adsorption and degradation (Gulzar and Siddiqui 2015). Indeed, soil pH, soil moisture and nutrient content significantly affect the concentrations of allelochemicals (Kruse and Strandberg 2000; Salhi et al. 2013).Phytotoxic potential of some plants and soils extracts on germination and growth of weed and cultivated species has been studied (Hosni et al. 2013; El-Darieret al. 2014).

The family of Lamiaceae consists of about 230 genera and 7100 species worldwide, and some species of this family are considered of high importance because of their uses in medicine, culinary, and cosmetics (Khaled-Khodja et al. 2014).The genus *Marrubium* L. belongs to the family Lamiaceae, and consists of about 97 species (Zaabat et al. 2011).In Tunisianflora, five*Marrubium* species had been recognized (*M. vulgare*, *M. supinum*, *M. aschersonii*, *M. deserti* and *M. alysson*) (Pottier-Alapetite 1981). A literature review on the chemical and biological aspects of *Marrubium sp* indicates antimicrobial, analgesic, anti-hypertensive, antidiabetic, antioxidant properties, among others, particularly related to the presence of diterpenoids, monoterpenes, flavonoids, sterols and phenylethanoid glycosides (Ahmed et al. 2010; Boulila et al. 2015). Many species of genus *Marrubium* are widely used in traditional and modern medicine (Stanković et al. 2011). Among them, only *M. vulgare* has been extensively investigated.*Marrubium vulgare* L. (Lamiaceae), commonly known as white horehound" in Europe, or "Marrubia" in Tunisia, is herbaceous perennial and medicinal plant(Chedia et al. 2014). It is indigenous to Mediterranean regions, central and Western Asia, North Africa and Southern Europe (Khaled-Khodja et al. 2014).Furthermore, *M. vulgare*has been reported to possess a wide range of several therapeuticeffects in many diseases, including analgesic, hypoglycemic, anti-inflammatory, expectorant, asorelaxant, antihypertensiveand anti-oedematogenic proprieties (Orhan et al. 2010; Bouterfas et al. 2014). The leaves and flowering stems of *M. vulgare* are traditionally used as diuretic, antispasmodic, antiseptic, anti-diabetic (Boudjelal et al. 2012). Indeed, various biological activities have been showed *M. vulgare*, such as antioxidant (Boulila et al. 2015), antifungal(Zarai et al. 2011), antibacterial (Dehbashi et al. 2015) and insecticidal activities (Pavela 2004). Phytochemical studies of *M. vulgare* have been reported to contain several active compounds namely phenolic acids, alkaloids, flavonoids, phenylpropanoid esters, coumarins, tannins, saponins and essential oils (Sahpaz et al. 2012; Kurbatova et al. 2013; Hamdaoui et al. 2013).

The aims of this study were: (i) to assess the phenolic contents of extracts ofleaves and rhizosphere soilsfrom*M. vulgare* and (ii) to investigate the potential allelopathic activities of the plant and soil extracts.

2. Materials and Methods

2.1. Plant materials and soil collection

The fresh leaves of *Marubium vulgare* L. were randomly collectedinOctober2016, at the vegetative stage, in the "National Park of Djebel Zaghouan"located in the Northeast of Tunisia (latitude36°22'19,757" (N), longitude 10°06'34,788" (E), altitude 820 m).The leaves were air-dried at room temperature (20±2°C) for one week,ground in Retsch blender mill (Normandie-Labo, Normandy, France), sieved through 0.5 mm mesh screen to obtain a uniform particle size. The powderwas stored at room temperature until used (Dallali et al. 2014).Soil samples were collected randomly from 0 to 15 cm depth, immediately put in polyethylene bags, brought to the laboratory, air-dried and sieved (2 mm mesh) to eliminate debris and root tissues (Priyadharsini and Dhanasekaran 2015). Plant and soil bioassay studies were conducted in Laboratory of Agronomic Sciences and the Environment, Department of Agricultural Production, Agricultural High School of Mograne, Tunisia.

2.2. Preparation of plant extracts

Plant extracts were prepared according the method as described by Neffati et al. (2011).Triplicate sub-samples (1g)were extracted with 20 mL of solvents with different polarities (water and methanol) respectivelyfor 24 h. Extraction was carried out using maceration at room temperature. The macerated

extracts were filtered through Wattman No.1 filter paper (Bärenstein, Germany)andwith a microfilter paper (Wattman, 0.45µm). The resulting solutions were evaporated under vacuum at 40°C usinga rotavapor (Buchi R-210) and the yield (%) of extraction was determined.Samples were stored at 4°C until use.

2.3. Total phenolic contentsin the plant extracts

Total phenolic content in the plant extracts was determined using the Folin–Ciocalteu reagent, following Singleton's method slightly modified (Dewantoet al. 2002).To 0.5 mL of extract at appropriate dilutions was added to 2.5 mL freshly diluted 10-fold Folin Ciocalteu reagent and 7.5 mL of Na_2CO_3 solution (7%), and the volume was made up to 5 mL with distilled water. After incubation for 90 min in dark, the absorbance was measured at 760 nm using a UV/Vis Jenway-6300spectrophotometer (Jenway Ltd.,United Kingdom)against a blank.The total phenolic content was expressed as mg Gallic acid equivalents per gram of dry weight (mg GAE g^{-1}DW) through the calibration curve with Gallic acid.

2.4. Total flavonoid contentin the plant extracts

The total flavonoids contentwere estimated according to the aluminum chloride colorimetric method (Djeridane et al. 2006). A diluted solution (1 mL) of each extract was mixed with a 2% solution of $AlCl_3$ (1 mL) in methanol. After incubation for 30 minat room temperature, the absorbance was measured at 430 nm using a UV/Vis Jenway-6300spectrophotometer (Jenway Ltd.,United Kingdom)against a blank sample. Total flavonoid content was expressed as mg quercetin equivalents per g dry weight (mg QE g^{-1}DW) using Quercetin calibration curve.

2.5. Soil physical-chemical analysis

The collected soil was analyzed for various physical-chemical parameters. The pH of saturated soil paste and electrical conductivity of the saturation extract were determined using a digital pHand conductivity meter. Organic carbon determined by rapid titration method (Walkey and Black 1934). Totalnitrogen was analyzed following Kjeldahl's method, soil particle size (sands, slits and clays) was determinedas per Aubert (1978).The total and active carbonate content was determined by the volumetric method (Afnor 1987).

2.6. Total phenolic contentsin the soil extracts

Total phenolic content was quantified as described by Gulzar and Siddiqui(2015) with some modifications. Dried soil (5 g) from each sample was extracted with 50 mL of methanol/water (80:20v:v) and water(agitation, 24h at 25°C; centrifugation, 5000 × g for 10 min). The extraction was repeated three times.The combined extracts were concentrated under vacuum rotary evaporator (Buchi R-210) at 40°C, and the residues were dissolved in methanol (5 mL) and filtered through microfilter paper (Wattman, 0.22 µm). The modified Folin-Ciocalteau method was used to determine the concentration of phenolics (mg GAE g^{-1} sol extract) in solution using Gallic acid as a calibration standard (Box 1983; Dewantoet al. 2002).

2.7. Allelopathic activity

2.7.1. Preparation of aqueous extracts

The allelopathic aqueous extracts concentrations were prepared by soaking the powder plant materials (1, 2.5, 5.0and 10.5g) in distilled water (100 mL) andthe dried soil (5, 10, 30 and 50g) was steeped in 1 L of distilled waterat ambient room temperature. After 24h, the mixtureswere filtrated through Wattman No.1 filter paper (Bärenstein, Germany) and microfilter paper (Wattman, 0.22 µm), and the resulting filtrates were centrifuged at 10,000 rpm for 15 min at 10°C (Eppendorf 5810R, Le Pecq, France). The supernatants were then collected and stored at 4°C in a refrigerator until use (Marichali et al. 2014).

2.7.2. Biological assays

The phytotoxic effects of aqueous extracts of leaf and rhizosphere sol of *M. vulgare* were evaluated using *Sinapis arvensis* L. (weed) and *Lactuca sativa*L. (cultivated) according to the method described by Sarkar et al. (2012) and Hosni et al. (2013).The seeds were surface-sterilizedwith 2% sodium hypochlorite solution for 2 min and washed with abundant distilled water. Sets of 20 seeds each with

triplicate per treatment were germinated in sterile glass Petri dishes (Ø = 90 mm) lined with two discs filter paper (Whatman No. 1, Bärenstein, Germany) wetted with 2 mL of each solution (plant aqueous extracts/sol aqueous extracts). Petri dishes were closed with Parafilm (Neenah, Wisconsin, USA) to prevent the loss of moisture and contaminations. Distilled water was applied to the control treatment. The germination conditions were as follow: for field mustardseeds, 25±1ºC, and for lettuce seeds, 22±1ºC, with natural photoperiod.Emergence of 1 mm of the radicle was used as the criterion for germination.Treatments were laid out in completely randomized design with three replications. After 7-days, the number of germinated seeds was recorded and the radicle length (cm) and shootlength (cm) were measured.Plant growth was evaluated at the end of the incubation period.The parameters used to evaluate germination and seedling growth were: germination percent (GP), mean germination time (MGT) and inhibition/stimulationpercent of radicle elongation and shoot length.The germination percentage(GP) was determined using the following equation from Moussavi-Nik et al. (2011):

$$\mathbf{GP}(\%) = \frac{\mathbf{Nt} \times \mathbf{100}}{\mathbf{N}}$$

WhereNt: number of germinated seeds in respective treatments. N: total number of seeds used in bioassay.Mean germination time (MGT) was calculated by the following equation the formula described by Demir et al. (2008):

$$\mathbf{MGT(days)} = \frac{\mathbf{\Sigma n.\, D}}{\mathbf{\Sigma n}}$$

Where n: Number of seeds which were germinated on day D, D: Number of days counted from the beginning of germination.The relative inhibition or stimulation of seed germination, and radicle and length as affected by the allelopathic substance were calculated according to Ladhari et al. (2013) as following:

$$\mathbf{Inhibition}(-)/\mathbf{Stimulation}\ (+)\% = \left(\frac{\mathbf{E} - \mathbf{C}}{\mathbf{C}}\right) \times \mathbf{100}$$

Where E: Extract (growth parameter measured in presence of *M. vulgare*or sol extract), C: Control (growth parameter measured in presence of distilled water).

2.8. Statistical analysis

All extractions and analyses were performed in triplicate for each sample, and the data expressed as mean ± standard deviation (SD). The means were compared by using the one-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $P < 0.05$. All analyses were performed using "SAS v 9.1" software package.

3. Results

3.1. Extraction yield, total phenolics and flavonoid contentsof plant extracts

3.1.1. Extraction yield

As shown in Table 1, the extraction yields (w/w, on dry weight basis) presented significant difference between the solvents, and ranging from 12.77 % to 23.60 %.The highest extraction yield was observed in the methanolic solution while the lowestthe water.

3.1.2. Total phenolic content

The results of total phenolics content of the four species investigated are given in Table 1. Total phenolics content of the plant extracts was expressed in terms of gallic acid equivalent,varied significantly ($p<0.05$) in the different extracts, and ranged from 25.04±0.02 to 44.89±0.25 mg GAE/g dry weight.The highest total phenolic content was observed in the methanolicextract.

3.1.3. Total flavonoids content

The total flavonoid content in methanol and water leafextract of *M. vulgare* was shown in Table 1. The total flavonoid content, expressed in quercetin equivalent, varied significantly ($p<0.05$) between the solvents, and ranged from17.18±0.20 to 24.6±1.07mg QE/g dry weight. As for the total phenolic content,the highest flavonoidscontent was registered in methanolextract, whereas the lowest amount of flavonoid concentration was found in waterextract.

Table1: Extraction yield, Total phenolic and flavonoids contents of methanol and waterextract of *M. vulgare*

Extracts	Extraction yield (%)	Total Phenolic content (mg GAE/g DW)	Total Flavonoids content (mg QE/g DW)
Methanol	23.60±1.15[a]	44.89±0.25[a]	24.6±1.07[a]
Water	12.77±0.67[b]	25.04±0.02[b]	17.18±0.20[b]

Means with the same letter in a column are not significantly different at $p < 0.05$.Data are mean ±S.D of three replicates

3.2. Total phenolic contents and soil characteristics

The analysis of the physical-chemical parameters has shown the soil isalkaline claytype,richer in organic matter (1.24%), total carbonate (32.63%), and characterized by a lowest nitrogen content (0.25 %),a slightly neutral pH (pH=7.97) and low electrical conductivity (0.57 mS cm^{-1}).

Table 2. Total phenolic contents and soil parameters measured (0–15 cm)

Soil characteristics	**Rhizosphere soil**
Phisical	
Clay (%)	57.46±0.21
Sand (%)	27.64±0.18
Silt (%)	14.90±0.16
Chemical	
Organic carbon (%)	1.24±0.12
Nitrogen (%)	0.25±0.03
Electrical conductivity (mS cm^{-1})	0.57±0.02
pH (H_2O)	7.97±0.13
Active $CaCO_3$(%)	13.22±0.10
Total $CaCO_3$(%)	32.63±0.15
Total Phenolic Content	
Methanol/water (mg GAE/g sol extract)	0.05±0.00
Water (mg GAE/g sol extract)	0.03±0.00

Total phenolic content of the sol extracts was expressed in terms of gallic acid equivalent, displayed a significant ($p<0.05$) variation between solvents, and ranged from 0.03±0.00 to 0.05±00 mg GAE/g sol extract (Table 2).The highest total phenolic content was observed in the methanol/water extract.

3.3. Allelopathic activity

The allelopathic effects of leaf and soil aqueous extract of white horehound (*M. vulgare*)were investigated against *Sinapis arvensis* (weed) and *Lactuca sativa*cultivated). The germination, growth of roots and shoots of the target seeds was influenced by these extracts.

3.3.1. Allelopathic activity of the plant aqueous extracts

3.3.1.1. Germination percentage

The aqueous extracts of *M. vulgare*on the germination of are shown in Table 3.The results of the experiment showed that aqueous extracts significantly ($P < 0.05$) reduced the germination of the both test target species when compared to control(distilled water). However, the highest germination percentage was observed in control treatment and the lowest was observed at 10.5 g mL^{-1}, respectively for *S. arvensis* and *L. sativa*.There was a drastic reduction in germination of seeds with increasing concentration of aqueous extracts.

3.3.1.2. Mean germination time

As shown in Table 3,mean germination time (MGT) of *S. arvensis* and *L. sativa*was influenced significantly ($P<0.05$) by the aqueous leafextracts. The MGT increased with the concentration of treatments. However, the lowest MGT was observed at 10.5g mL^{-1} (5.02±0.03 and 4.68±0.12days respectively)and the lowest was observed in control treatment (1.11±0.01 days).It shows that when concentration of extract increased the germination percentage decreased, while MGT increased.

Table3: Inhibitory effects of *M. vulgare* aqueous extracts on germination and Mean germination time of *S. arvensis* and *L. sativa*.

Concentrations (g mL^{-1})	Germination (%)		MGT (days)	
	S. arvensis	***L. sativa***	***S. arvensis***	***L. sativa***
Control	81.67±0.58^{a}	81.67±0.58^{a}	1.11±0.01^{e}	1.11±0.01^{d}
1	58.33±0.58^{b}	44.67±0.58^{b}	1.22±0.05^{d}	1.30±0.08^{c}
2.5	26.33±0.58^{c}	23.00±0.00^{c}	1.34±0.07^{c}	1.32±0.06^{c}
5.0	9.33±0.58^{d}	10.33±0.58^{d}	3.18±0.05^{b}	2.07±0.03^{b}
10.5	4.00±0.00^{e}	5.00±0.00^{e}	5.02±0.03^{a}	4.68±0.12^{a}

Means with the same letter in a column are not significantly different at $p < 0.05$.Data are mean ±S.D of three replicates.

Increasing aqueous extracts concentrations of *M. vulgare*caused a significant ($p<0.01$) and remarkable inhibitory effect on germination of *S. arvensis* and *L. sativa* (Fig. 1). The highest decrease was observed at 10.5 g mL^{-1}by about 95.10% and 93.88%, respectively in comparison to the control. The results indicated that seeds germination of *S. arvensis* was relatively more sensitive to *M. vulgare* leaf extract than the one of *L. sativa* (Fig.1).

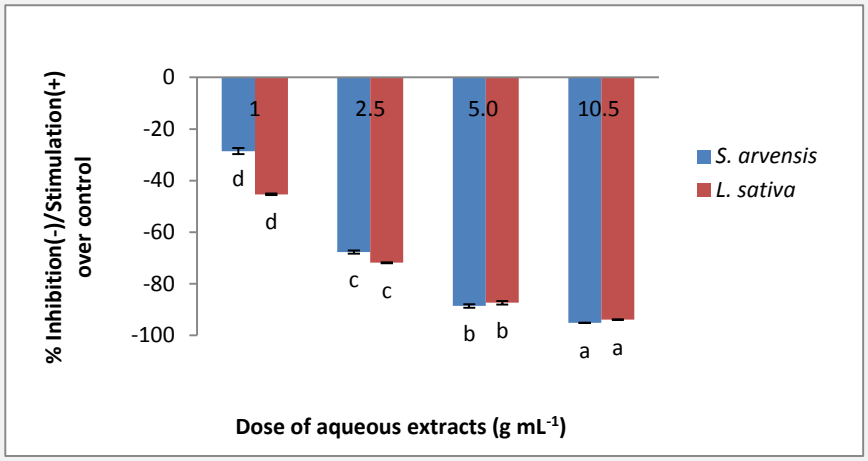

Fig.1. Inhibition (-)/stimulation (+) of germination seedsof *S. arvensis* and *L. sativa*, in presence of aqueous extracts of *M. vulgare*.

3.3.1.3. Root and Shoot length

Application of different concentrations of aqueous extracts of *M. vulgare*posed variable effects on root and shoots length of*S. arvensis* and *L. sativa* (Table 4). The leaf extracts proved most toxic to roots and shoots growth of both the test crops. The lowest concentrations (1 g mL^{-1}) and control treatment (distilled water) presented the highest root and shoot length respectively, while the lowest root and shoot length was registered in 10 g mL^{-1} for all extracts. The decrease in root and shoot length seemed to increase with increase in concentrations of the extract, and varied with target species.However, the results showed a stimulatory effect of the lowest concentration (1 g mL^{-1}) on the root and shoot growth of both test crops (Table 4).

Table 4. Effects of *M. vulgare*leaves aqueous extracts on Root and Shoot length of *S. arvensis* and *L. sativa.*

Concentrations (g mL^{-1})	Root length (cm)		Shoot length (cm)	
	S. arvensis	***L. sativa***	***S. arvensis***	***L. sativa***
Control	2.97±0.38^{a}	2.97±0.38^{a}	3.56±0.09^{b}	3.56±0.09^{a}
1.0	3.24±0.44^{a}	1.81±0.09^{b}	4.02±0.09^{a}	2.67±0.06^{b}
2.5	1.82±0.23^{b}	1.31±0.18^{c}	1.86±0.06^{c}	2.04±0.08^{c}
5.0	0.99±0.11^{c}	0.27±0.04^{d}	1.39±0.05^{d}	1.63±0.04^{d}
10.5	0.22±0.02^{d}	0.12±0.02^{d}	0.39±0.02^{e}	0.59±0.03^{e}

Means with the same letter in a column are not significantly different at $p < 0.05$.Data are mean ±S.D of three replicates

The degree of inhibition/stimulation was largely dependent on both the concentration of the extracts and the target species (Fig. 2 and Fig. 3).The highest concentration extract (10.5 g mL^{-1}) of*M. vulgare* was respectively inhibited root and shootgrowth of *S. arvensis*(92.64%and 89.14%,respectively) and *L. sativa* (95.90%and 83.54%,respectively). In contrast, root and shootlength of the target species was significantly stimulated by the lowest concentration (1g mL^{-1}).

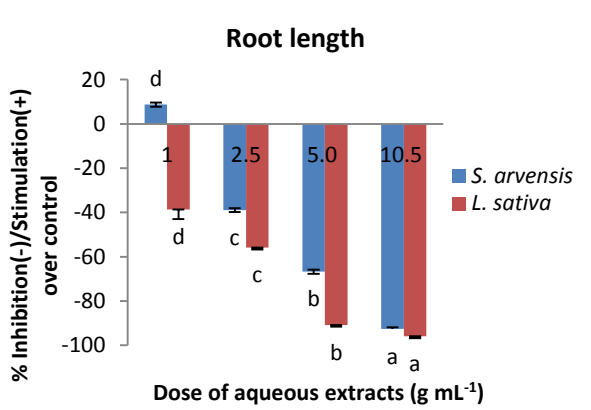

Fig.2. Inhibition (-)/stimulation (+) ofaqueous extracts of *M. vulgare* on root length of *S. arvensis* and *L. sativa.*

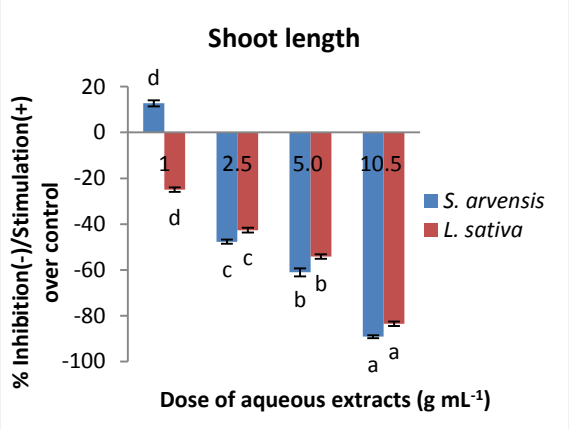

Fig.3. Inhibition (-)/stimulation (+) of aqueous extracts of *M. vulgare* on shoot length of *S. arvensis* and *L. sativa.*

3.3.2. Allelopathic activity of thesoil aqueous extracts

3.3.2.1. Germination percentage

As shown in Table 5,mean comparison indicated that germination percentage in both species decreased by increasing the sol aqueous concentration. There was significant differences among germination percentage at all species ($P<0.01$). The highest germination percentage was belonged to control treatment (distilled water) and the lowest one belonged to the highestconcentration(50 g L^{-1}).

3.3.2.2. Mean germination time

The mean germination time (MGT) ofcanary grass was influenced significantly ($P<0.05$) by sol aqueous extracts (Table 5).The MGT increased with the sol aqueous concentration. However, the highest MGT of *S. arvensis* and *L. sativa*was observed at 50 g L^{-1} treatment (3.86±0.20 and 4.11±0.10 days respectively) and the lowest was observed in control treatment (1.91±0.01 days). Results show that when concentration of extract increased the germination percentage decreased, while MGT increased.

Table5: Inhibitory effects of solaqueous extract on germination and Mean germination time of*S. arvensis* and *L. sativa*

Concentrations (g L^{-1})	Germination (%)		MGT (days)	
	S. arvensis	***L. sativa***	***S. arvensis***	***L. sativa***
Control	99.33±0.58^{a}	99.33±0.58^{a}	1.91±0.01^{e}	1.91±0.01^{e}
5	93.67±0.58^{b}	95.00±0.00^{b}	2.22±0.06^{d}	2.43±0.05^{d}
10	55.67±0.58^{c}	66.00±1.00^{c}	2.48±0.08^{c}	2.94±0.05^{c}
30	27.00±1.00^{d}	32.00±0.00^{d}	3.13±0.08^{b}	3.53±0.06^{b}
50	19.00±1.00^{e}	21.00±1.00^{e}	3.86±0.20^{a}	4.11±0.10^{a}

Means with the same letter in a column are not significantly different at $p < 0.05$. are mean ±S.D of three replicates

The inhibitory activity of different aqueous extracts fromrhizosphere soil of *M. vulgare* on seed germination of *S. arvensis* and *L. sativa* is shown in Fig.4. The sol aqueous extracts significantly reduced ($P<0.05$)germination of both the test crops.However, the inhibition increased with extract concentrations and varied with target species. The inhibition potentials of sol extracts at their highest concentration (50 g L^{-1}) were 80.87 and 78.86% for *S. arvensis* and *L. sativa* seed germination,and the lowest inhibition in seed germination (7.50 and 4.36%, respectively) over control were found for the highest concentration. Therefore, the sol extract had strong inhibitory effect on seed germination of *S. arvensis*.

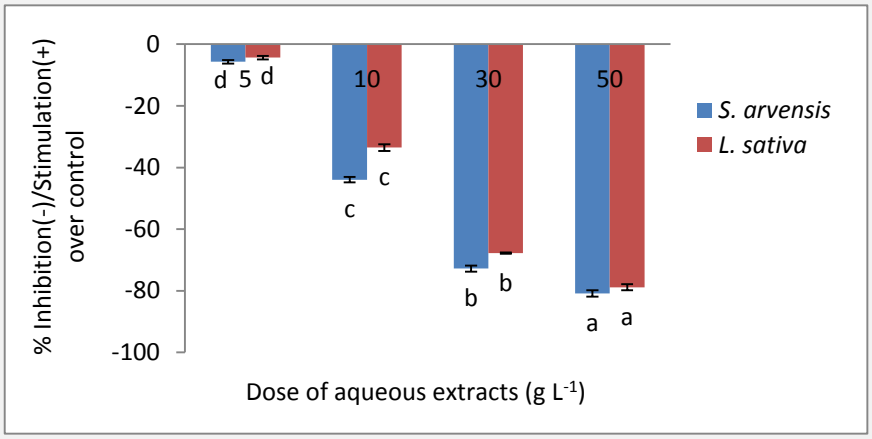

Fig.4. Inhibition (-)/stimulation (+)of germination seedsof *S. arvensis* and *L. sativa*, in presence of sol aqueous extracts.

3.3.2.3. Root and Shoot length

The inhibitory activity of sol aqueous extracts on seed germination of *S. arvensis* and *L. sativa*is shown in Table 6. Root and shoot length was significantly influenced by the treatments. Concentrations of 5 g L^{-1}soilaqueous extract and control treatment (distilled water) exhibited the highest root and shoot length of *S. arvensis* and *L. sativa*respectively, while the lowest root and shoot length was exhibited by 50g L^{-1}soil extract.

Table 6. Effects of sol aqueous extracts on Root and Shoot length of *S. arvensis* and *L. sativa*

Concentrations (g L^{-1})	Root length (cm)		Shoot length (cm)	
	S. arvensis	***L. sativa***	***S. arvensis***	***L. sativa***
Control	3.27±0.38^{b}	3.27±0.38^{a}	3.86±0.09^{b}	3.86±0.09^{a}
5	3.82±0.44^{a}	2.91±0.35^{a}	4.45±0.06^{a}	3.55±0.09^{b}
10	1.46±0.11^{c}	1.00±0.13^{b}	2.06±0.05^{c}	1.64±0.09^{c}
30	0.92±0.09^{d}	0.69±0.08^{b}	1.44±0.07^{d}	1.10±0.07^{d}
50	0.63±0.02^{d}	0.22±0.02^{c}	0.86±0.05^{e}	0.52±0.04^{e}

Means with the same letter in a column are not significantly different at $p < 0.05$.Data are mean ±S.D of three replicates

The inhibition potential of different aqueous extracts from sol on root and shoot growth shown Fig. 5 and Fig. 6. Root and shoot growth of *S. arvensis* and *L. sativa* was severely affected (P<0.01) by sol aqueous extracts. However, the inhibition increased with extract concentrations and varied with target species. Indeed, the reduction of root and shoot length was less in *S. arvensis* (weed) rather than *L. sativa* with respective control at increasing concentration of sol aqueous extracts. However, the results showed a stimulatory effect of the lowest concentration (5 g L^{-1}) on the root and shoot growth of both test crops.

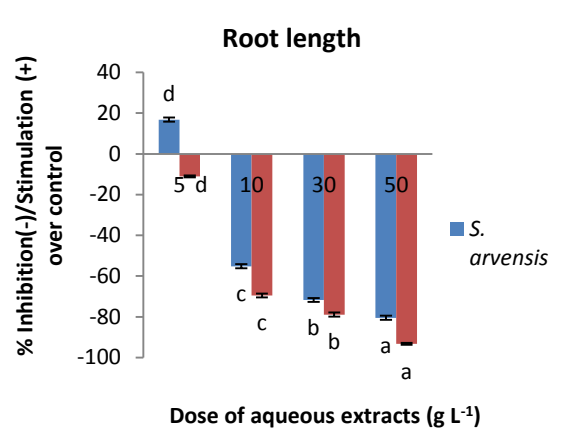

Fig.5. Inhibition (-)/stimulation (+) of soil aqueous extracts on root length of *S. arvensis* and *L. sativa.*

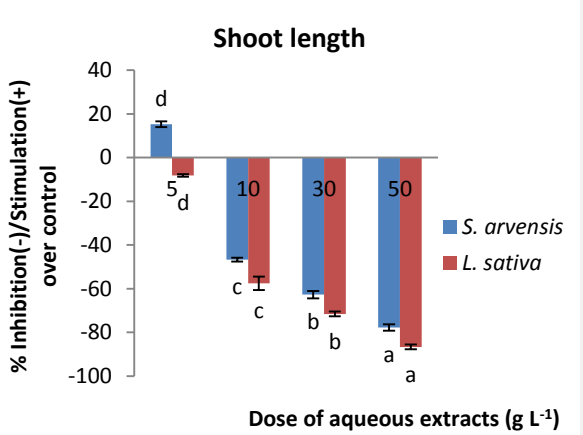

Fig.6. Inhibition (-)/stimulation (+) ofsoil aqueous extracts on shoot length of *S. arvensis* and *L. sativa.*

4. Discussion

Extraction is the main step for recovering and isolating phytochemicals from plant materials.In the present study, two solvents with different polarities (water and methanol) were used. the extraction yields presented significant difference (p<0.05) between the solvents. The highest extraction yield was observed in the methanolic solution. Then, the higher yield the methanolic solution might be contributed by other polar compounds besides phenolics, namely polysaccharides and plant debris (Dai and Mumper 2010;Lee 2007). In addition, extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method and solvent used,extraction time,pH, temperature, sample particle size, as well as the presence of interfering substances (Doet al. 2013; Ghedadba et al. 2014).

Total phenolics content of the plant and sol extracts was expressed in terms of gallic acid equivalent, varied significantly (p<0.05) in the different extracts.Several investigators have found phenolic and flavonoid compounds in the leaves of *M. vulgare* (Bouterfas et al. 2014; Boulila et al. 2015). The total phenolic and flavonoids content of certain *M. vulgare* samples has also been previously determined (Amessis-Ouchemoukh et al. 2014). Kohli and Batish (1994) reported the presence of a significantly high amount of phenolics in the rhizosphere sol of *Parthenium* (*P. hysterophorus* L.).Rashid et al. (2010) reported 69% soluble phenolics in the soil after the first week of incubation of kudzu litter and a 62% phenolic content after the sixth week. The variations in the distribution of the total phenolics can be partially due to genotypic factors that control accumulation of these compounds in the plant and edaphic factors (Schmidt et al. 2010). Moreover, other investigations suggested that the biotic and abiotic conditions can play an important role in the production and accumulation of phenolic compounds (Hosni et al. 2011).The method of extraction and quantification also influences the estimation of total phenols content (Lee et al. 2003).

The total flavonoid content, expressed in quercetin equivalent, varied significantly (p<0.05) between the solvents. Flavonoids are polyphenolic compounds with low molecular mass, found in leguminous, fruits, flowers, and leaves (Harbone and Williams 2000; Karioti et al. 2010), having several biological activities.Amjad and Shafighi (2013)reported that geographical climate differences, soil conditions and pesticide or herbicide use may contribute to variations flavonoid content of plants. Genotypic and

botanical origins of plant can influence the estimation of total flavonoids content (Schmidt et al. 2010;Tan et al. 2016). The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-zhao 2005). The season and post-harvest conditions have been recently reported as additional source of variance in the total flavonoids content (Dziri et al. 2012).

Allelopathy in agricultural practices has become more important in biological control of weeds (Al-Taisan 2014).Results showed that aqueous extracts of leaf and rhizosphere sol of *M. vulgare* significantly ($p<0.05$) inhibited germination and seedlings growth of *Sinapis arvensis* (weed) and *Lactuca sativa*(cultivated) and the effects were concentration specific; as the concentration of extracts increased the allelopathic effects were more pronounced. The present findings corroborate the earlier report by Marichali et al. (2014) who found that, the inhibitory effect extracts of *Carum carvi* on germination of some agricultural crops was proportional to the concentration of extract.The inhibition of the plant depends on the concentrations of the allelochemicals (Ashrafi et al. 2009).The inhibitory effect of the tested species on seed germination of *S. arvensis* and *L. sativa*may be related to the presence of allelochemicals compoundsincluding tannins, phenolic acidsand flavonoids, which prevented the growth of embryo, or caused its death due to chromosomal aberrations in dividing cells(Ladhari et al. 2013; Marichali et al. 2014).Also, many investigations have found that inhibitory substances involved in allelopathy are terpenoids and phenolic substances (Khanh et al. 2007). Phenolic compounds are rapidly adsorbed and/or oxidized by soil (Dalton et al. 1989; Makino et al. 1996), and correlated most strongly with growth inhibition of several plants (Inderjit 1996; Ohno et al. 2000). Furthermore, Siddiqui et al. (2009) reported that the toxicity might be due to synergistic effects rather than single.In fact,allelochemicals compounds which determine the success of seed germination may inhibit the germination by altering the membrane permeability, respiration, plant water balance, enzymes activities, DNA transcription and RNA translation, or alternatively, the combination of these factors (Amini and Yarnia 2014; Jelassi et al. 2016).The phytotoxic activity of allelochemicals is responsible for growth suppression of weeds (Farooq et al. 2013). The differences in the germination percentage between the tested species could be attributed to differences in the selective permeability of the seeds coat of *S. arvensis* and *L. sativa*to inhibitory substances (Hassan et al. 2012).

The phytotoxicity from leaf and rhizosphere soil of *M. vulgare* was evaluated using an aqueous extract bioassay on the mean germination time of *S. arvensis* and *L. sativa*. Results show that when concentration of extract increased the germination percentage decreased, while MGT increased (Table 3 and 5). The present findings corroborate the earlier report by Moosavi et al. (2011) and Hassan et al. (2012) who respectively found that, the mean germination time of *S. bicolor* and same botanical extracts was increased withincreased the concentration of extract. Root and shoot growth of the target species were significantly affected by the sol aqueous extracts. significantly ($P<0.05$) decreased by increasing the aqueous extract concentrations.Hosni et al. (2013)andMarichali et al. (2014)found inhibition in the length of root and shoot in various weedswith water extracts of flowerheads (*C. coronarium*) and caraway (*C. carvi*).Nasrine et al. (2011) stated that the plumule and radicle length of Bromus was completely inhibited at the highest concentration of aqueous extracts of the donor species level (10%).Decrease in root and shoot length can cause by inhibiting in cell division and elongation and or decreasing in hormones such as acetic acid and gibberellinn (Al-Taisan 2014).The highest inhibiting effect on radicle length of *S. arvensis* and *L. sativa*could be due to the high accumulation of allelochemicals in the top meristems of the plants (Gella et al. 2013).The two test species differed in their root and shoot growth sensitivity to allelopathic interference. The *M. vulgare* leaf and rizosphere sol extracts were more effective at inhibiting root and shoot length in *S. arvensis* (weed) than in *L. sativa* (cultivated). This suggests that attributes of seed germination and seedling growth of the weeds were differentially susceptible to the aqueous extracts of *M. vulgare* and sol (El-Darier and Youssef 2000). The different responses of bioassay species to *M. vulgare* leaf and rizosphere sol aqueous extracts might be due to evolutionary differences in resistance to allelopathic compounds among the target species (Hunter and Menges2002; Samedani et al. 2013).The study demonstrated that aqueous extract could be attributed to the inhibitory effects of rhizosphere soil allelopathic substances present in the extract.Gulzar and Siddiqui(2015)reported that rhizosphere soil is an active root zone of the soil, where allelochemicals from higher plants accumulate and where most of the interaction among microorganisms, roots of higher plants and even with microbes occur. Thus, Xuan et al. (2005) reported that during decomposition in the soil allelopathic plants release phytotoxic phenolics into the immediate

soil environment. The magnitude of suppressive activity in soil would be strongly associated with chemical type, concentration and the mode of decomposition of allelopathic plants added to the soil (Xuan et al. 2005). Therefore, the degree of phytotoxic effect of alleochemicals in soil is greatly influenced by soil properties and conditions.Once an allelochemical or a mixture of allelochemicals enters a soil system, processes such as adsorption-desorption, microbial decomposition, and leaching can modify its behavior (Kobayashi 2004). The phytotoxic activity of allelochemicals in soil is, therefore, a function of complex interactions among soil and plant factors (Samedani et al. 2013).Soil properties are the dominant factors determining the activity of allelochemicals in soil (Inderjit 2002). Soil factors might increase or decrease allelopathic activity. Thus,the phytotoxic activity of dehydromatricaria ester released from Canada goldenrod (*Solidago altissima* L.) depends on its concentration in soil water, which is affected by soil characteristics through its adsorption and degradation activity (Kobayashi et al. 2004). The phytotoxicity of allelochemicals is affected by soil factors (Kobayashi 2004). It has been reported that the activities of allelochemicals in the soil are strongly linked with physical, chemical, biological, and physicochemical properties of the soil, which in turn affect their adsorption and degradation (Hiradate et al. 2010; Gulzar and Siddiqui 2015). Indeed, the effectiveness of allelochemicals is greatly affected by soil organic substances, soil inorganic components and nutrients, microbes, soil chemical properties such as soil pH, and soil physical properties such as water retention (Inderjit and Mallik 1996; Schmidt et al. 2000; Hiradate et al. 2010). Application of allelopathic water extracts at high concentrations substantially suppresses the weed seed germination, root growth and shoot growth (Table 3 and 5). The results confirm the findings of Iqbal et al. (2009) and Shang and Xu (2012), showing that allelochemicals have an inhibitory and/or lethal effects on seed germination, growth and development of crops.However, the lowest concentrations extracts from rhizosphere soil of *M. vulgare* (1 g mL^{-1} and 5 gL^{-1}, respectively) found stimulated growth of *S. arvensis* and *L. sativa* root and shoot. This could probably be due to the induction of growth promoting hormones. This is in agreement with the result obtained by Scrivanti (2010) and Gella et al. (2013). Allelopathic compounds release in special circumstances and affect on seed germination, root and shoot growth, and soil micro-organisms (Hesammi 2013). Water extracts of plant derived allelochemicals have been used as potential herbicides in mixture with reduced dose of chemical herbicides (Cheema et al. 2012).

5. Conclusion

The present study revealed that methanolic and aqueous extracts of leaf and rizosphere soil of white horehound (*Maribum vulgare* L.) contains phenolics and flavonoids.The studyshowed that aqueous extracts of leaf and rizosphere soil of *M. vulgare*significantly influence seed germination and seedling growth of *Sinapis arvensis* and *Lactuca sativa*in laboratory condition.However, the allelopathic effects depend on target species, leafand soilextract concentrations. Hence, we can conclude that exist of inhibitory substances in aqueous extract leaves and rizosphere soil of white horehound would be the responsible for the observed effects. These extracts can be used as important source of natural herbicides to control weeds in crop fields.However, further studies under field conditions are necessary to evaluate the possible use of these sol and leaf extracts for their allelopathic potential, and determine the effect on other species.

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