EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2025, 26(10), 15–25
https://doi.org/10.12912/27197050/209607
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Received: 2025.07.29
Accepted: 2025.09.15
Published: 2025.10.01

Allelopathic potential of aqueous extracts of *Marrubium vulgare*, *Dittrichia viscosa* and *Eucalyptus camaldulensis* on some durum wheat weeds

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ABSTRACT

The presence of weeds in a cereal field is detrimental to the crop on several levels. The discovery of natural herbicides represents a potential solution for reducing the harmful effects on the environment. In this context, three plant species have been selected to assess their allelopathic potential: *Marrubium vulgare, Dittrichia viscosa* and *Eucalyptus camaldulensis*. The aim is to test their effectiveness as natural herbicides, by studying their influence on the germination and development of seedlings of certain weeds present in wheat crops. Aqueous leaf extracts at concentrations of 5% and 10% were prepared for each species. These extracts were applied to the seeds of four weed species (*Avena sterilis, Phalaris brachystachys, Biscutella auriculata, Centauria melitensis*) and to a variety of durum wheat, *Triticum turgidum* (Karim variety). The results show that both concentrations have a significant effect on seed germination and seedling growth compared with the control. Inhibition increased with extract concentration, although this response varied according to the plant species used. At 10%, *Dittrichia viscosa* extract inhibited *Avena sterilis* seed germination and growth by over 81%, a greater effect than that observed with *Eucalyptus camaldulensis* and *Marrubium vulgare*, which showed inhibition of over 75%. In contrast, *Marrubium vulgare* extract proved particularly effective against *Phalaris brachystachys*, with germination inhibition reaching 100%. Phytochemical analysis of the aqueous extracts revealed the presence of secondary metabolites such as flavonoids, glycosides, terpenes and saponins. These bioactive compounds, notably those contained in *Dittrichia viscosa* and *Marrubium vulgare*, could be promising new bioherbicides against certain wheat weeds.

Keywords: Allelopathic, inhibition, bioherbicide, bioactive compounds, weeds.

INTRODUCTION

Wheat is a staple food in many developing countries, particularly in the Maghreb. In Morocco, the cereals industry is one of the pillars of agricultural production. It plays a central role in the national food system and the national economy. However, the presence of weeds in wheat fields causes a number of problems: they compete directly with the crop for water, nutrients and light, which hampers wheat growth. In addition, heavy infestation complicates ploughing and harvesting, and the mixing of weed seeds with those of the wheat alters the commercial quality of the produce (Tanji, 2005).

Competition between weeds and crops is also responsible for significant yield losses, estimated at between 20 and 30%, with major economic

repercussions, particularly in cereal (Hussain et al., 2007). Since the 1950s, agriculture has relied heavily on the use of herbicides and pesticides to control weeds and maintain high yields, as these products offer a quick and effective solution. However, their intensive use has led to adverse effects on human health and the environment (Weih et al., 2008), prompting researchers to explore sustainable alternatives, particularly of biological origin. Biological control is a promising alternative approach to agricultural pests, diseases and weeds (Mason, Spanner, 2006). Among these approaches, allelopathy -the ability of certain plants to release chemical compounds that inhibit the growth of other species - has shown great potential for weed management under real-life conditions (Olofsdotter, 2001). Numerous studies have revealed that the ability of a crop to suppress weeds varies considerably between varieties, this variation being partly attributed to the production of allelopathic compounds (Olofsdotter, 2001). In this context, the present study aims to evaluate the allelopathic power of two concentrations of aqueous extracts from three plants: *Eucalyptus camaldulensis, Marrubium vulgare and Dittrichia viscosa*, on the germination and growth of some common durum wheat weeds (*Avena sterilis, Phalaris brachystachys, Biscutella auriculata, Centauria melitensis*). The aim is also to identify, by phytochemical screening, the compounds potentially responsible for this allelopathic effect, in order to valorize them as natural bioherbicides, economical, easy to use by farmers, and capable of improving wheat production while preserving the environment.

MATERIALS AND METHODS

Plant material

To test allelopathic effects, three plants (*Marrubium vulgare, Dittrichia viscosa and Eucalyptus camaldulensis*) were harvested in the green state in the Tghat region of Fez during the month of April - May 2023. Samples of the four weeds and of Karim durum wheat were collected after seed maturation from 3 stations located in the outskirts of the city of Fez (Table 1 and Table 2).

Preparation of aqueous plant extracts

The leaves of *Marrubium vulgare*, *Dittrichia viscosa*, and *Eucalyptus camaldulensis* were dried in an oven for 72 hours at 50 °C to preserve the plants allelochemical composition. The dried leaves were then ground using an electric grinder. The crushed plant material was stored in paper bags labeled with the species name, date, and place of collection, and kept at room temperature in the dark until use. A 5 g and 10 g sample of each plant was macerated in 100 mL of distilled water and stirred for 1 hour using a shaker. After 48 hours, the solutions are filtered and stored in hermetically sealed, labeled bottles in a refrigerator at 4 °C. Each bottle is labeled with the species name, concentration, and date of preparation.

Germination test

To assess the effect of each plant extract on germination, the seeds are disinfected with 10% bleach for 2 minutes, then rinsed three times with distilled water. After sterilizing the petri dishes, 25 seeds from each weed species studied were placed in a petri dish on two sheets of filter paper soaked with 5 mL of prepared plant extract (5% or 10%) and 5 mL of distilled water for the control, with three replicates. The petri dishes were immediately sealed with parafilm and incubated at 18 °C with a 12 hour photoperiod for 20 days.

Table 2. Botanical families of weeds

Species name	Botanical family
Avena sterlis (L.)	Poaceae
Phalaris brachystachys (L.)	Poaceae
Biscutella auriculata (L.)	Brassicaceae
Centaurea melitensis (L.)	Asteraceae

Table 1. Wheat weed survey stations

Stations	Latitude	Longitude	Altitude
Station1 (Laajajra)	34.078159 N	-5.022944 W	440 m
Station 2 (Douiete)	34.0372941 N	-5.135006 W	385 m
Station 3 (Ain Beida)	33.988358 N	-4.995331 W	462 m

Measurements and observations

After 20 days of incubation, the experiment is stopped, and the germination percentage for each species in each Petri dish is determined. A seed is considered germinated when it develops a coleorhiza in monocotyledonous species or a radicle in dicotyledonous species. After determining the number of seeds that have germinated in each dish, the lengths of the root (LR) and aerial (LAP) parts are measured using graph paper.

The percentage of seed germination, as well as the root and aerial lengths for each Petri dish, are calculated using the following formulas :

$$PG\ (\%) = \frac{n}{N} \times 100 \quad (1)$$

$$LAP = \frac{\sum_{i=1}^{i=n} Li}{n} \quad (2)$$

$$RL = \frac{\sum_{i=1}^{i=n} Ri}{n} \quad (3)$$

where: PG – percentage of germination; n – number of seeds germinated in a petri dish for 20 days; N – total number of seeds per petri dish; LAP – mean length of aerial part; Li – length of aerial part; RL – average root length; Ri – length of roots.

The percentages of inhibition of germination and growth of roots and aerial parts are calculated according to the following formula: (Dhima et al; 2006).

$$I\ (\%) = \frac{C-E}{C} \times 100 \quad (4)$$

where: I (%) – percentage of inhibition; C – average of controls; E – average of extract-treated boxes.

Phytochemical screening

Phytochemical screening involves detecting the different families of metabolites specialized in plants through qualitative characterization reactions. These reactions are based on precipitation or coloration by specific reagents. The results are classified according to appearance as follows: frankly positive reaction +++; positive reaction ++; moderately positive reaction +; negative reaction.

- Tannin revelation test – in a test tube, 1 mL of extract is added to 1 mL of 1% $FeCl_3$. In the presence of tannins, a greenish or blackish-blue coloration develops (Judith., 2005). Differentiation between gallic and catechic tannins is performed using the following methods.
- Catechic tannins – 1 mL of concentrated hydrochloric alcohol is added to 5 mL of extract and boiled for 15 minutes. In the presence of catechic tannins, a red precipitate soluble in amyl alcohol is formed (Judith ., 2005).
- Gallic tannins – 15 mL of Stiany reagent is added to 30 mL of extract, and the mixture is heated in a water bath for 15 minutes, then filtered. The filtrate is saturated with 5 g of powdered sodium acetate, followed by the dropwise addition of 1 mL of a 1% $FeCl_3$ solution. The precipitate obtained indicates the presence of gallic tannins (Judith., 2005).
- Flavonoid revelation test – 1 mL of hydrochloric alcohol is added to 1 mL of extract, along with a few magnesium chips and 1 mL of isoamyl alcohol. The appearance of a pink-orange coloration in the isoamyl alcohol supernatant layer indicates the presence of flavones, a purplish-pink color indicates flavonones, and a red color indicates the presence of flavanols and flavanonols (Judith, 2005).
- Alkaloid revelation test – this test is performed using precipitation reactions with general alkaloid reagents (Dohou et al., 2003).
- Test: 1 mL of Mayer's reagent is added to 1 mL of extract. The formation of a soft yellow color indicates the presence of alkaloids.
- Saponoside revelation test – 5 mL of distilled water is added to 0.5 mL of extract, and the mixture is shaken vigorously for 5 minutes. Persistent foaming is an indicator of saponosides (Dohou et al., 2003).
- Steroidal heterosides – 10 mL of anhydrous chloroform is added to 10 mL of evaporated extract, mixed with 5 mL of acetic anhydride. Then, a few drops of sulfuric acid (H_2SO_4) are added, and the mixture is stirred. The development of a purplish-green coloration indicates the presence of steroidal heterosides (Judith, 2005).
- Tri-terpene heterosides – 2 mL of chloroform and 3 mL of sulfuric acid are added to 5 mL of extract. The appearance of a reddish-brown coloration in the interphase layer indicates the presence of tri-terpene heterosides (Judith., 2005).
- Sterol revelation test (Shalkowski Test) – 1 mL of H_2SO_4 is added to the extract. If a brownish-red or purple ring forms at the contact zone of

the two liquids, this indicates the presence of sterols (Zekri, 2017).

- Test for revealing cardiac glycosides – 1 mL of chloroform is added to 1 mL of extract. The appearance of a reddish-brown coloration after the addition of H_2SO_4 indicates the presence of cardiac glycosides (Ammor, 2020).
- Test for revealing oses and holosides – 2 to 3 drops of concentrated sulfuric acid are added to 1 mL of extract. After 5 minutes, 3 to 4 drops of alcohol saturated with thymol are added. The appearance of a red coloration reveals the presence of oses and holosides (Diallo, 2005).
- Mucilage revelation test – 5 mL of absolute alcohol is added to 1 mL of extract. A positive reaction is indicated by the formation of a flaky precipitate (Diallo, 2005).

RESULTS

Effect on *Avena sterilis*

Germination rate and growth of roots and aerial part

The analysis of variance reveals that germination percentage, root length, and aerial part length are significantly influenced by the aqueous plant extracts of *D. viscosa*, *M. vulgare*, and *E. camaldulensis*. A comparison of the averages for germination, root length, and aerial part length shows that the control group exhibits the highest values compared to the other extracts and concentrations. The 10% *D. viscosa* extract exhibited the lowest averages for seed germination, root length, and aerial part length when compared to the control (Table 3).

Inhibition rate

The highest germination inhibition was observed at the 10% concentration for the aqueous extract of *D. viscosa*, exceeding 81%, while the lowest effect at the same concentration was seen for the aqueous extract of *E. camaldulensis*, which inhibited only 25.66% at 5% and 37.68% at 10%. Therefore, the reductions in germination and growth caused by the extracts were greater at the 10% concentration. Regardless of the concentration, the lowest inhibition of root length and aerial part length was exerted by the *E. camaldulensis* aqueous extract, followed by the *M. vulgare* extract. The highest inhibition was achieved by the *D. viscosa* extract, which reached 69.22% for root length and over 55% inhibition for aerial part length (Figure 1).

Effect on *Phalaris brachystachys*

Germination rate and growth of roots and aerial parts

The germination percentage (PG), root length (RL), and aerial part length (LAP) were significantly affected by the aqueous extracts of the plants (*D. viscosa, M. vulgare*, and *E. camaldulensis*). However, the difference in concentration of the *D. viscosa* and *M. vulgare* extracts was not significant for germination and root growth. In contrast, for the *E. camaldulensis* extract, the variation in concentration had a significant effect on both germination and growth.The results show that the germination rate of *Phalaris brachystachys* seeds (97.33%) and the growth of roots and aerial parts were very high in the control compared with the other extracts. In the case of the *M. vulgare* extract, no germination or growth occurred at a 5% concentration (Table 4).

Table 3. Effect of *E. camaldulensis, D. viscosa* and *M. vulgare* extracts on germination (PG), root length (RL) and length of aerial part (LAP) of *Avena sterilis*

Extracts	Species name	Concentration	PG (%)	RL (cm)	LAP (cm)
Aqueous extracts	*E. camaldulensis*	5 %	74,67±4.62^{a}	3,23±0.07^{a}	7.71±0.05^{a}
		10 %	61.33±2.31^{b}	2.72±0.12^{b}	6.99±0.10^{ab}
	D. viscosa	5 %	37.33±2.31^{c}	2.77±0.15^{b}	5.83±0.15^{c}
		10 %	18.67±2.31^{d}	1.72±0.11^{c}	3.44±0.05^{d}
	M. vulgare	5 %	48.00±4.00^{ab}	2.77±0.15^{b}	6.74±0.05^{b}
		10 %	24.00±4.00^{ac}	2.06±0.12^{ab}	5.95±0.15^{ab}
Control		0%	98,66±2.31^{e}	5.60±0.20^{d}	8.25±0.15^{e}

Note: Means followed by the same letter in the same column are not significantly different at $P < 0.05$. *E. camaldulensis: Eucalyptus camaldulensis; D. viscosa: Dittrichia viscosa; M. vulgare: Marrubium vulgare*

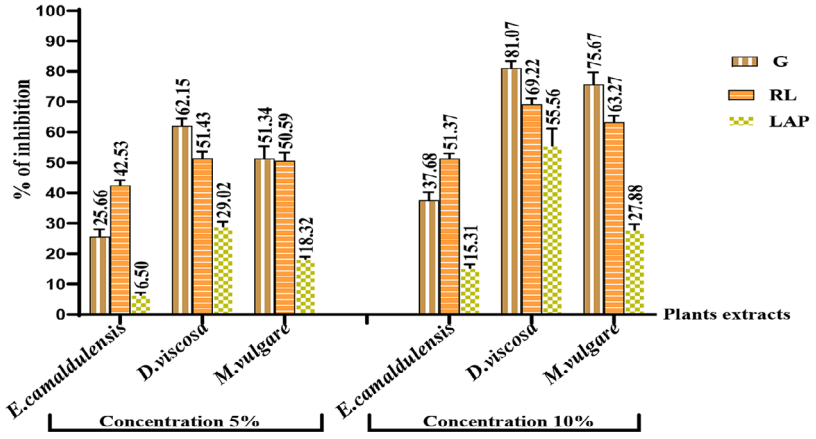

Figure 1. Inhibitory effect of *E. camaldulensis, D. viscosa* and *M. vulgare* extracts at 5% and 10% concentrations on germination (G), root length (RL) and above-ground length (LAP) of *Avena sterilis*

Table 4. Effect of *E. camaldulensis*, *D. viscosa* and M. *vulgare* extracts on germination (PG), root length (RL) and length of aerial part (LAP) of *Phalaris brachystachys*

Extracts	Concentration	PG (%)	RL (cm)	LAP (cm)
E. camaldulensis	5 %	48.00±4.00^{b}	2.43±0.15^{a}	5.06±0.05^{a}
	10 %	9.33±2.31^{a}	1.57±0.20^{b}	3.24±0.11^{b}
D. viscosa	5 %	9.33±2.31^{a}	0.77±0.06^{c}	1.81±0.10^{c}
	10 %	6.67±2.31^{a}	0.53±1.15^{c}	1.27±0.11^{cd}
M. vulgare	5 %	0.00+0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}
	10 %	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}
Control	0%	97.33±2.31^{ab}	5.06±0.08^{e}	8.05±0.07^{f}

Note: Means followed by the same letter in the same column are not significantly different at $P < 0.05$; *E. camaldulensis: Eucalyptus camaldulensis; D. viscosa: Dittrichia viscosa; M. vulgare: Marrubium vulgare.*

Inhibition rate

The comparison of inhibition rates of the aqueous extracts shows that the *E. camaldulensis* extract inhibits germination by more than 50% at a concentration of 5% and 90.41% at 10%. In contrast, the *D. viscosa* extract inhibits germination by 90.41% at 5% and over 93% at 10%, while the *M. vulgare* extract causes total inhibition of germination. Inhibition of root and aerial growth is greater at 10% than at 5%, with the inhibitory effect of the *D. viscosa* extract being stronger than that of the *E. camaldulensis* extract at both concentrations (Figure 2).

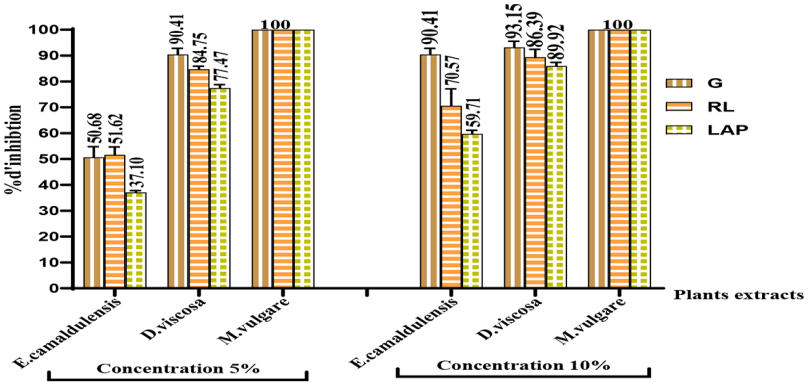

Figure 2. Inhibitory effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts at 5% and 10% concentrations on germination (G), root length (RL) and length of aerial part (LAP) of *Phalaris brachystachys*

EFFECT ON *BISCUTELLA AURICULATA*

Germination rate and growth of roots and aerial part

Percentage germination (PG), root length (RL), and length of the aerial part (LAP) of *Biscutella auriculata* seeds were significantly affected by aqueous plant extracts of *D. viscosa*, *M. vulgare*, and *E. camaldulensis* compared to the control. The highest averages for the three variables (PG, RL, LAP) were observed in the control, while the lowest were found in *M. vulgare* at concentrations of 5% and 10% (Table 5).

Inhibition rate

Aqueous extracts of all three plants inhibit seed germination, as well as root and aerial growth, but the degree of germination inhibition varies by species. For all extracts, inhibition increases at a concentration of 10% compared to 5%. The aqueous extract of *M. vulgare* was the most inhibitory, reducing germination of *Biscutella auriculata* seeds by 90.09% at the 5% concentration and 95.04% at the 10% concentration. *E. camaldulensis* had the weakest inhibitory effect, with inhibition reaching 69% at the 5% concentration and 76.19% at the 10% concentration. Inhibition of root length was greater with *M. vulgare* extract, exceeding 77% at the 5% concentration and over 80% at the 10% concentration. In contrast, *E. camaldulensis* inhibited only 45.10% of root length at 5% and 53.73% at 10%. Inhibition of above-ground growth by *M. vulgare* extract was higher at both concentrations compared to *D. viscosa* extract, which inhibited over 73% at 5% and 75.93% at 10%. The effect of *E. camaldulensis* was the lowest, with inhibition reaching only 51.22% at the 10% concentration (Figure 3).

EFFECT ON *CENTAURIA MELITENSIS*

Germination rate and growth of roots and aerial part

The percentage of germination (PG), root length (RL), and length of the aerial part (LAP)

Table 5. Effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts on germination (PG), root length (RL) and length of aerial part (LAP) of *Biscutella auriculata*

Extracts	Species name	Concentration	PG (%)	RL (cm)	LAP (cm)
Aqueous extracts	*E. camaldulensis*	5 %	16.67±1.15^{a}	0.93±0.06^{b}	1.10±0.10^{a}
		10 %	13.33±1.15^{a}	0.79±0.06^{b}	1.00±0.10^{a}
	D.viscosa	5 %	6.67±2.31^{b}	0,49±0.07^{c}	0.55±0,05^{b}
		10 %	5.33±2.31^{b}	0.41±0.04^{c}	0.49±0,05^{b}
	M. vulgare	5 %	5.33±2.31^{b}	0.39±0.03^{c}	0.50±0.10^{b}
		10 %	2.67±2.31^{b}	0.33±0.06^{c}	0.41±0.04^{b}
Control		0%	56.00±4.00^{e}	1.70±0.10^{a}	2.05±0.08^{c}

Note: Means followed by the same letter in the same column are not significantly different at $P < 0.05$. *E.camaldulensis: Eucalyptus camaldulensis; D. viscosa :Dittrichia viscosa; M. vulgare: Marribium vulgare.*

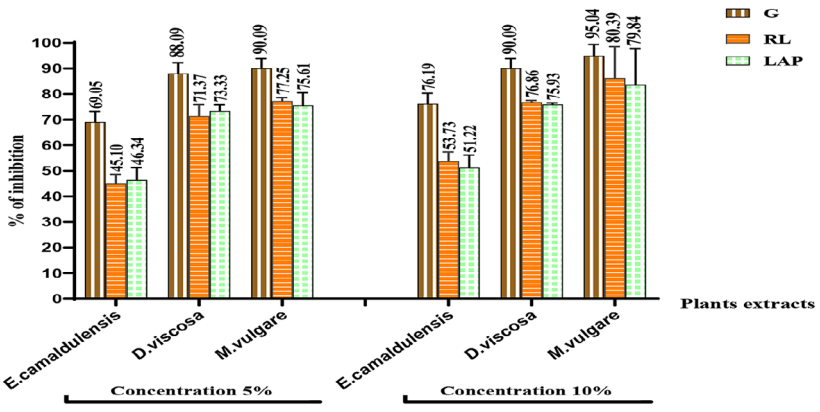

Figure 3. Inhibitory effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts at 5% and 10% concentrations on germination (G), root length (RL) and length of aerial part (LAP) of *Biscutella auriculata*

Table 6. Effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts on germination (PG), root length (R L) and length of aerial part (LAP) of *Centaurea melitensis*

Extracts	Species name	Concentration	PG (%)	RL (cm)	LAP (cm)
Aqueous extracts	*E.camaldulensis*	5 %	18.67±2.31^{a}	1.70±0.08^{bd}	2.68±0.07^{e}
		10 %	9.33±2.31^{b}	1.45±0.05^{d}	2.48±0.07^{e}
	D. viscosa	5 %	14.67±4.62^{a}	1.35±0.05^{cd}	2.49±0.03^{e}
		10 %	8.00±0.00^{b}	1.10±0.01^{ab}	1.59±0.08^{d}
	M. vulgare	5 %	9.33±2.31^{b}	1.25±0.05^{bc}	1.49±0.08^{d}
		10 %	8.00±0.00^{b}	0.97±0.03^{a}	1.29±0.03^{d}
Control		0%	53,33±6.11^{c}	2.93±0.15^{f}	3.63±0.09^{ac}

Note: Means followed by the same letter in the same column are not significantly different at $P < 0.05$; *E.camaldulensis: Eucalyptus camaldulensis; D. viscosa: Dittrichia viscosa; M. vulgare: Marribium vulgare*.

of *Centaurea melitensis* seeds were significantly affected by aqueous plant extracts (*D. viscosa*, *M. vulgare*, and *E. camaldulensis*) compared to the control. The variables studied showed higher values in the control group than in the other extracts, with the highest germination and growth benefits observed with the *Eucalyptus camaldulensis* extract (Table 6).

Inhibition rate

All aqueous extracts inhibit germination and growth of *Centaurea melitensis* seedlings, but comparison of the results shows that the 5% concentration of *M. vulgare* extract inhibits 82.49% of seed germination, higher than that of *D. viscosa* aqueous extract which reaches 72.49%, and the effect of *E. camaldulensis* extract decreases to 65.42%. At 10%, *D. viscosa* and *M. vulgare* extracts are the most inhibitory, reaching 84.99% inhibition. At 10% concentration, extracts of all three species show a greater inhibitory effect on LR and LPA than at 5% concentration. At 10%, the inhibition exerted by *M. vulgare* extract on root growth exceeds 66% and reaches 62.34% in the case of *D. viscosa* extract and only 50.39% for *E. camaldulensis*. At 10%, this extract inhibits 31.68% of the aerial part; this effect increases to 56.10% with *D. viscosa* extract and 64.37% in the case of *M. vulgare* (Figure 4).

EFFECT ON THE KARIM VARIETY OF DURUM WHEAT (*TRITICUM TURGIDUM*)

Germination rate and growth of roots and aerial part

The extracts did not significantly affect wheat germination, but they did affect the length of its aerial and root parts at $P < 0.05$. The mean root and above-ground length of the control was higher than that of the other extracts, and the lowest mean root length was recorded for *M. vulgare* at 10% concentration, but for above-ground length, the 10% *D. viscosa* extract produced the lowest value (Table 7).

Table 7. Effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts on germination (PG), root length (RL) land length of aerial part (LAP) of durum wheat (Karim variety)

Extracts	Species name	Concentration	PG (%)	R L (cm)	LAP (cm)
Aqueous extracts	*E. camaldulensis*	5 %	96.00±0.00^{a}	4.20±1.14^{a}	6.60±0.14^{c}
		10 %	94.00±2.83^{a}	3.95±0.21^{a}	6.54±0.17^{c}
	D. viscosa	5 %	96.00±0.00^{a}	4.40±0.06^{ab}	5.70±0.14^{bc}
		10 %	90.00±2.83^{b}	3.80±0.14^{b}	4.76±0.20^{b}
	M. vulgare	5 %	96.00±0.00^{a}	3.81±0.15^{b}	6.00±0.14^{bc}
		10 %	94.00±2.83^{a}	3.67±0.15^{b}	5.67±0.80^{bc}
Control		0%	98.00±2.83^{a}	5.45±0.35^{c}	7.10±0.14^{ac}

Note: Means followed by the same letter in the same column are not significantly different at $P < 0.05$. *E. camaldulensis: Eucalyptus camaldulensis; D. viscosa: Dittrichia viscosa; M. vulgare: Marribium vulgare*.

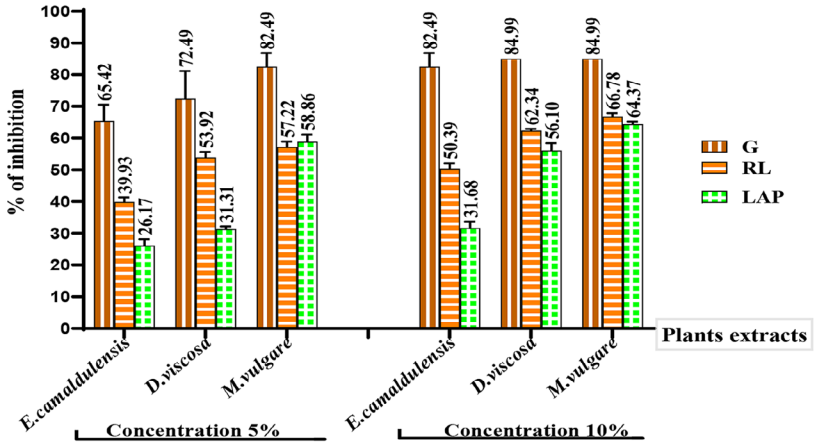

Figure 4. Inhibitory effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts at 5% and 10% concentrations on germination (G), root length (RL) and length of aerial part (LAP) of *Centauria melitensis*

Inhibition rate

For both concentrations, the plant extracts showed low inhibition of germination and growth of roots and aerial parts of durum wheat seeds. The highest inhibition of germination was 8.16% recorded for *D. viscosa* at the 10% concentration. At this concentration, the aqueous extract of *M. vulgare* had the highest inhibitory effect on root length, at 31.28%, compared with the other extracts, but for the length of the aerial part, the *D. viscosa* extract exerted a higher inhibition of 32.95%. The aqueous extract of *E. camaldulensis* had the lowest inhibitory effect in both concentrations (Figure 5).

Phytochemical screening results

The results of the phytochemical screening tests on the various aqueous extracts from the leaves of the plants used (*M. vulgare*, *D. viscosa,* and *E. camaldulensis*), as shown in the Table 8, highlight the presence of several chemical compounds. Tannins and flavonoids are present in all the aqueous extracts tested, with higher concentrations in *D. viscosa* and *E. camaldulensis* extracts. Sterols and terpenes are detected in higher concentrations in *D. viscosa* and *M. vulgare* extracts, while alkaloids are only present in *E. camaldulensis* leaves at low concentrations. Moss indices show that saponin content is medium in *D. viscosa* leaves and low in *M. vulgare* extract, but absent in *E. camaldulensis*. Cardiac glycosides are present in all extracts, with a high concentration in *M. vulgare* leaves, while oses and holosides are absent in *D. viscosa* extract and present at medium and low concentrations in *E. camaldulensis* and *M. vulgare* extracts, respectively. Mucilages are not detected in the *E. camaldulensis* extract and are present at medium levels in the other two extracts. Steroidal heterosides were absent in all the aqueous extracts studied,

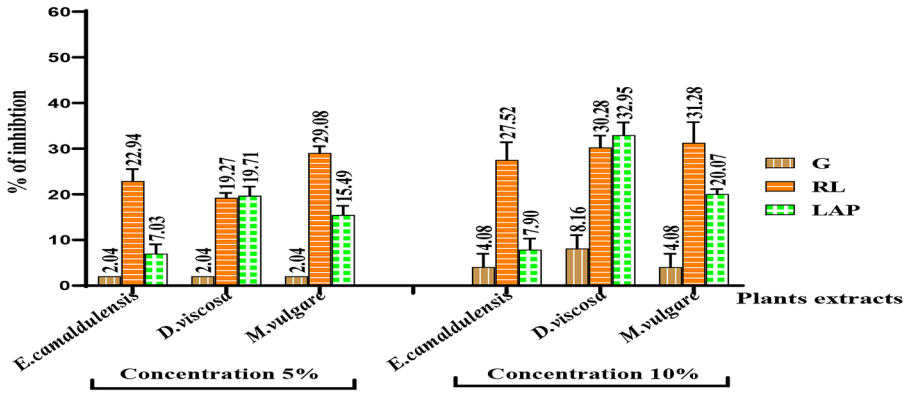

Figure 5. Inhibitory effect of *E. camaldulensis*, *D. viscosa* and *M. vulgare* extracts at 5% and 10% concentrations on germination (G), root length (RL) and above-ground length (LAP) of durum wheat (Karim variety)

Table 8. Phytochemical screening of aqueous extracts of *D.viscosa, M. vulgare* and *E.camaldulensis*

Specification	*D. viscosa*	*E. camaldulensis*	*M. vulgare*
Tannins: - Catechic tannins - Gallic tannins	+++ ++ -	+++ ++ -	+ +++ -
Flavonoids	++	++	++
Alkaloids (Mayer test)	-	+	-
Saponosides	++	-	+
Cardiac glycosides	++	++	+++
Oses and holosides	-	++	+
Mucilages	++	-	++
Sterols and terpenes	+++	+	+++
Steroid heterosides	-	-	-
Triterpene heterosides	++	+	+++

while triterpene heterosides were present in high concentrations in the *M. vulgare* extract and at medium levels in the *D. viscosa* extract. Phytochemical characterization of the aqueous extracts studied is essential for identifying the bioactive molecules responsible for the allelopathic effect.

DISCUSSION

The three species (*Eucalyptus camaldulensis*, *Marribium vulgare* and *Dittrichia viscosa*) affected weeds and the tested durum wheat variety to varying degrees. The inhibitory effects of these plant extracts were observed on seed germination and seedling growth. (Kruse et al., 2000) demonstrated that when sensitive plants are exposed to allelochemicals, seed germination is inhibited in some seeds, while in others, germination is interrupted at the start of radicle emergence. Kruse et al. (2000) also showed that the effects of allelochemicals are manifested through morphological changes, most often observed in the early stages of development, and include inhibitory effects on tigelle and radicle elongation.

For each allelopathic species, inhibition increases as the concentration rises from 5% to 10%. This increase is greater in all tests when *M. vulgare* and *D.viscosa* extracts are used. The varying effects of the extracts on seed germination and seedling growth can be attributed to differences in the concentrations and physicochemical properties of the allelopathic species, likely due to the presence of specific allelochemicals. However, according Arslan et al. (2005) have shown that inhibition increases with increasing extract concentration. At 10%, the inhibition rate of weed seeds (*Avena sterilis, Phalaris brachystachys, and Biscutella auriculata*) obtained from *M. vulgare* and *D. viscosa* extracts exceeds 75% in all tests, which is higher than that of *E. camaldulensis* and the control. These results are consistent with those of the inhibitory effect of parthenin, a substance isolated from the leaves of *Parthenium hysterophorus*, on *Avena sterilis* seed germination and seedling development. Batish et al. (2002) and

Machado (2007) also found that *Avena sterilis* germination was completely inhibited by extracts (5%) of leaves of the allelopathic species *Limnanthes alba Hartw*. Ex *Benth, Vigna sesquipedalis* (L.). It is recognized that Under natural conditions, seed germination is both a biochemical and physiological process. On first contact with an exogenous stimulus such as water, the seed initiates the synthesis and secretion of an enzyme, amylase, which is involved in the degradation of starch (or albumins), providing the embryo with the energy it needs to begin the germination process (Regnault-Roger et al., 2008). The ability to inhibit seed germination is a complex process, and several hypotheses can be put forward, including the ability of certain molecules found in extracts to inhibit or reduce the action of the amylase enzyme (Hu et al., 2017) or by other mechanisms such as inhibition of the electron transport system and seed swelling or inhibition of translation and transcription (Pal et al., 2020)

The various extracts had a low inhibition of durum wheat seed germination, reaching only 2% for *E. camaldulensis* and a maximum inhibition of 8% for *D. viscosa* extract. These results concur with those of Dogan (2004). The latter demonstrated that Raphanus sativus extracts do not affect wheat germination. Contrary to these results, Machado (2007) obtained 77% germination inhibition for the

extract (5%) of Limnanthes alba Hartw. ex Benth. The plant extracts used exert a moderate effect on root growth and the aerial part of durum wheat seeds, and these results can be compared with those of Hegab et al. (2008), who also found that chard extract (*Beta vulgaris* L.) inhibited wheat seedling growth at concentrations of 8% and 12%.

Phytochemical screening of the extracts revealed a number of secondary metabolites (flavonoids, tannins...). The inhibitory effect on germination could be due to these substances. Several studies have demonstrated the toxic action of these metabolites, for example a number of acids such as o-coumaric acid, p-coumaric acid and chlorogenic acid have been identified by high-performance liquid chromatography (HPLC) as being responsible for the observed allelopathic effect (Chon et al., 2005). phenolic acids and flavonoids in leaf extracts of *Chrysanthemum morifolium* have an inhibitory allelopathic activity (Beninger et al., 2003). these flavonoids exert an inhibitory effect on root growth by inhibiting ATPase activity in the plasma membranes of Avena sterilis roots (Mao et al., 2006). This is consistent with our results, which show that aqueous extracts of all three plants containing flavonoids inhibit root and aerial growth. Abdelgaleil & Hashinaga (2007) reported that sesquiterpenes in *Magnolia grandiflora* leaf extract inhibited germination of wheat (*Triticum aestivum*), lettuce (*Lactuca sativa*), radish (*Rhaphanus sativus*) and onion (*Allium cepa*). Lettuce seeds are the most sensitive, compared with wheat, radish and onion seeds, which are affected by a high concentration of sesquiterpenes. These results concur with the analyses obtained, which show that terpene-rich extracts of *D. viscosa* and *M. vulgare*, compared with *E. camaldulensis* extract, also have a high rate of inhibition of weed germination and a low effect on the durum wheat variety.

Inhibition of the parameters studied also varied with concentration, being higher at 10%. These allelopathic effects of different doses of *E. camaldulensis* leaf extract on three plants (*Vigna unguiculata, Cicer arietinum, Cajanus cajan*), chosen as experimental models, were also studied and confirmed (Ahmed et al., 2008).

CONCLUSIONS

The results obtained are encouraging and suggest that the bioproducts formulated, in particular from the aqueous extracts of *Marrubium vulgare* and *Dittrichia viscosa*, are particularly effective in inhibiting weed germination and growth. All the extracts tested exerted a significant inhibitory effect on seed germination, as well as on the development of weed roots and aerial parts. However, their impact on durum wheat germination was more moderate. The inhibition observed increases with extract concentration, reaching its maximum at 10%. In addition, extracts of *Dittrichia viscosa* and *Marrubium vulgare* showed a higher inhibitory power than *Eucalyptus camaldulensis*, probably due to their richness in secondary metabolites. These extracts are therefore promising candidates for effective biological weed management. In order to optimize their use and better understand their mechanism of action, it would be relevant to identify the bioactive compounds responsible for the bioherbicidal effect using HPLC, and to study their mode of action on weed germination and growth. It would also be interesting to extend the allelopathic tests to other sites and weed species, and to reproduce these experiments under in situ conditions in order to assess their effectiveness in a real environment

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