



ALLELOPATHIC EFFECTS OF SOME PHYTO-EXTRACTS ON GERMINATION AND EARLY DEVELOPMENT OF *PHALARIS CANARIENSIS* AND *LACTUCA SATIVA*

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Abstract

The use of allelopathic effects, especially bioherbicides, in biological control can reduce the negative impacts of synthetic pesticides on the environment. In this sense, aqueous extracts of the leaves of thirteen medicinal plants from northwestern Morocco were tested at 4% for their biocidal power on the germination of canary grass (*Phalaris canariensis*) and lettuce (*Lactuca sativa*). The results showed that fig (*Ficus carica*) and oregano (*Origanum compactum*) extracts alone met the criteria of an effective biological herbicide. Indeed, the rate of reduction of the germination of the canary grass by the extracts of fig tree and oregano were respectively 90 and 95%; whereas the germination of the lettuce was not affected. After dilution of the extracts of these two plants, the allelopathic effect was maintained on the germination and growth of canary grass. In general, the effects were significant from the 1% concentration and the rate of reduction increased with the concentration of the extracts. Thus, the aqueous extracts of *Ficus* and *Origanum* showed good herbicidal efficacy on germination and growth of monocotyledonous weeds (represented by canary grass) and selectivity towards lettuce (representing the dicotyledons).

Keywords: bioherbicide, germination, growth, plant aqueous extract, weed control.

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1. Introduction

Plant communities are partly governed by interactions between species. One of the interactions between plants is negative interference, which can be indirect (attraction or maintenance of organisms affecting neighbouring plants) or direct (competition, allelopathy) [1]. Allelopathy is one of the survival strategies that involves the release of allelochemicals into the surrounding environment, in order to influence (usually inhibit; although stimulation is also included in the broad definition of allelopathy) the germination/growth/development of neighbouring species that share the same habitat. Indeed, it is reported [2, 3] that allelopathy is a sub-discipline of chemical ecology that refers to the effects of chemicals produced by plants and micro-organisms on plant growth and development in agricultural systems or in natural communities. This phenomenon can be viewed as a part of biological control in which plants are utilized to diminish the power and advancement of different plants [4, 5]. Natural products

identified as allelochemicals are reported to be present in almost all plant parts [6, 7]. Therefore, it is suggested that allelopathic plants could be used as bioherbicides for weed control in agriculture [8]. Indeed, these compounds offer great potential for weed control, either directly or their chemistry could be used as a model for developing new herbicides.

Weeds are unwanted plants that have no economic use and are difficult for farmers to manage. They affect the growth and development of crops and thus limit their productivity and yield [9, 10]. Furthermore, they have an indirect effect by harboring pests and diseases of plants [11]. They limit the availability of light, moisture, space to crops and deteriorate their quality [12]. In this case, the use of synthetic chemical herbicides plays an effective role in managing the growth and multiplication of weeds [13]. However, recent researches showed that the frequent and massive use of these chemical products presents risks to

human health [14]. In addition, some weeds develop resistance to chemical herbicides [15, 16]. Indeed, 253 weed species worldwide have shown resistance to herbicide active molecules [17], such as glyphosate, the most common weed killer [18]. For these reasons, the development and use of natural herbicides has attracted the interest of researchers who have demonstrated the phytotoxic effect of natural substances, mainly secondary metabolites extracted from plants which could be exploited by humans for their herbicidal effect [19]. Turk & Tawaha [20] noted that water extracts from the fresh parts of *Brassica nigra* have the ability to inhibit germination and radicle length of *Avena fatua*. Elkenany & Eldarier [21] showed that the germination of *Phalaris minor* is sensitive to the aqueous extract of the leaf part of *Lantana camara*. BenGhnaya *et al.* [22] noted that the effect of the aqueous extract of the leaf part of *Eucalyptus erythrocorys* was more severe on weeds (*Sinapsis arvensis* L. and *Phalaris canariensis* L.) than on the crop (*Triticum durum* Desf.). These results indicate also that seedling growth, particularly root elongation, was the most sensitive indicator for evaluating the effects of the extracts with respect to seed germination. Benzarti *et al.* [23] showed that aqueous extracts of the leaves of *Verbena officinalis* and *Aloysia citrodora* inhibited the germination and elongation of the radicle of *Phalaris canariensis*.

Our objective is to test the allelopathic power of leaves aqueous extracts from thirteen Moroccan plant species on canary grass (*Phalaris canariensis*) as a representative plant of the monocotyledonous and on lettuce (*Lactuca sativa*) as a representative of the dicotyledonous.

2. Materials and methods

2.1. Plant Material

To test the allelopathic effect, the leaves of 13 medicinal plants (table 1) were collected in the northwestern regions of Morocco during the period May-August. Leaves foliar aqueous extracts of these species have been tested on germination and growth of Canary grass (*Phalaris canariensis* L.) and lettuce (*Lactuca sativa* L.).

2.2. Preparation of Plant Extracts From Leaves

Healthy leaves of plants were dried at a temperature of 45°C for 4-5 days in a ventilated oven and then directly ground with an electric grinder. The powder (leaf crush) was sieved through a 20–40 mesh, put into paper bags and stored in the dark at room temperature (25°C) in the laboratory. Four grams of each grind was dissolved in 100 ml of distilled water and magnetically stirred for one hour. The mixture was filtered through a piece of muslin cloth. The precipitate was removed and the resulting solution was then centrifuged for 10 minutes at 3000 rpm. The supernatant was collected and filtered through a Wathman N1 filter paper. The filtrate obtained is the crude extract (4%) of the sample used.

2.3. Phalaris and Lettuce Germination and Growth Test

Germination of canary grass (*Phalaris canariensis*) and lettuce (*Lactuca sativa*) seeds was carried out in Petri dishes containing 5 ml of one of the extracts (tests) or distilled water (control). Each treatment was repeated three times. Ten canary grass or lettuce seeds were placed in each petri dish,

which were immediately sealed with Para-film and incubated for 10 days at 20°C and under a 12-hour photoperiod. Subsequently, the extracts of the plants having shown an herbicidal effect (at 4%) were then retested at different dilutions (2%, 1% and 0.5%). The median effective dose (ED 50) is the dose or concentration that produces a effect in 50% of cases. ED50 values were calculated by plotting concentration on a log scale (X) and the reduction response on the Y axis. The data appear linear in a semi-log graph paper. ED50 is extensively applied for investigating the phytotoxic effect or toxic efficacy of several allelochemicals [24] and herbicides [25].

2.4. Observation and measures

The number of germinated seeds was counted in each Petri dish after ten days of incubation and the percentage of germination inhibition was determined according to the formula [26].

$$\% \text{ germination inhibition} = 100 * (N-n) / N$$

With N: number of germinated seeds in the control at 10 days and n: number of germinated seeds in a treatment.

The evaluation of the herbicidal efficacy of the plant extracts was judged according to the scale established by the commission of biological tests of the Scientific Society of Phytiatrics and Phytopharmacy:

- 95 to 100% : Very good efficiency
- 80 to 95% : Good efficiency
- 60 to 80% : Average efficiency
- 40 to 60% : Low efficiency
- <40% : Effectiveness without practical interest.

The percentage of growth inhibition (radicle and aerial part) was also calculated:

$$\% \text{ growth inhibition} = 100 * (\text{length of control} - \text{length in test}) / \text{length of control}$$

2.5. Statistical Analyzes

Statistical analyses were performed with the SAS program. An arcsine transformation of the square root of the germination inhibition percentage and a conversion of negative means to zero values (0) were adopted before submitting the data to statistical analysis. The Student-Newman and Keuls test, with a probability of 5%, was applied for the comparison of means.

3. Results and Discussions

A highly significant difference was noted between the effects of the different extracts. Thus, a total inhibition of seed germination of *Phalaris canariensis* was recorded with the aqueous extracts of *Lavandula sp.* Moreover, an inhibitory effect higher than 80% was noted for canary grass with the aqueous extracts of *Ficus carica*, *Myrtus communis*, *Origanum compactum* and *Ocimum basilicum*, which corresponds to a good to very good herbicidal efficiency. In contrast, the aqueous extracts of *Mentha pulegium*, *Nicotiana*

glauca, *Peganum harmala* and *Pinus pinaster* showed only poor herbicidal efficacy or no practical value (inhibitory effect <40%) on weed germination (Table 2). Concerning the effect on *Lactuca sativa*, its germination was not significantly affected by the tested plants, except for the aqueous extract of *Lavandula* sp, which exerted a very significant inhibitory action on the lettuce. Indeed, some extracts had no (0% for *Ficus carica*, *Juniperus communis*, *Mentha pulegium*) or very weak (0 to 7% for *Nicotiana glauca*, *Origanum compactum* and *Peganum harmala*) to weak inhibition (11 to 23% for *Myrtus communis*, *Mentha rotundifolia*, *Nerium oleander* and *Medicago sativa*) effects on the lettuce. In addition, although aqueous extracts of *Ocimum basilicum* and *Pinus pinaster* caused reduction rates of over 40% on lettuce, they were not very effective in terms of herbicidal effect on the canary grass weed (Table 2). According to the results obtained, it was found that only the aqueous extracts of *Ficus carica* and *Origanum compactum* among the 13 tested plants meet the criteria of a bio-herbicide. Indeed, these two species showed a good herbicidal efficacy against canary grass seeds while the germination of lettuce was not affected (reduction rate of 0 and 7% respectively).

Subsequently, these two aqueous extracts were retested at different dilutions (0-4%) on germination and growth of *Phalaris canariensis* and *Lactuca sativa*.

The results showed that there was no significant effect on the germination of lettuce. On the other hand, it was found that from the concentration of 1%, both extracts exerted a significant inhibitory effect on the germination of canary grass. This effect was reflected in reduction rates ranging from 81.5 to 96.3% by *Ficus* and from 31.0 to 93.1% by *Origanum* (Table 3). Concerning the growth of the canary grass (Table 4), a significant inhibiting effect was recorded for the root part from the concentration of 0.5% of the *Ficus* and *Origanum* extracts. This altering effect increases with the increase of the concentration of the extracts, to reach at the dose of 4%, reductions of 79 and 97% respectively. On the other hand, a significant inhibitory effect on the growth of the canary grass aerial part was recorded from 0.5% of the two extracts tested. At the highest concentration (4%), the reduction rate reached its maximum of 66 and 80% for *Ficus* and *Origanum* respectively (Table 4).

In the case of *Lactuca sativa* (Table 4), it was found that at 4% of *Ficus* and from 1% of *Origanum* extracts, a significant inhibitory effect on radicle growth was recorded. The maximum reduction was recorded at the concentration of 4% (76 and 90% for *Ficus* and *Oregano*, respectively). On the other hand, a significant inhibitory effect was also recorded on the aerial part growth, starting at 4% for *Ficus* and 1% for *Oregano*. The reduction rate reached its maximum of 64 and 73% for these plants aqueous extracts respectively (Table 4). The elongation of the root and that of the aerial part of the canary grass was significantly affected from 0.5% of the aqueous extracts of *F. carica* and *O. compactum*. On the other hand, the growth of lettuce was also affected by these species. In fact, a significant inhibitory effect on the growth of the root and aerial part of *Lactuca sativa* was recorded at 4% and from 1% of *F. carica* and *O. compactum* extracts respectively.

The cytotoxic effect of *Ficus carica* was also demonstrated by Zaman et al [27] who reported germination reduction rates About et al., 2022

of 57, 56, 46 and 38% for *Trifolium repens*, *Triticum aestivum*, *Lactuca sativa* and *Zea mays* respectively. However, the extract of another *Ficus* species (*F. benghalensis*), inhibited germination of *Zea mays* and *Helianthus annuus*, without causing any inhibitory effect on *Vigna radiata* [28].

Some secondary plant metabolites influence germination or plant growth by multiple mechanisms. They can act as growth regulators, interfering in metabolic pathways, or in the production of chlorophyll, having effects on the uptake of luminous energy, and changing the pattern of growth, multiplication and maintenance of cells [29, 30]. It has been reported that allochemicals suppress the mitotic activity of young cells, resulting in inhibition of seed germination [6] which has been attributed to disruption of mitochondrial respiration [31] and disruption of the activity of metabolic enzymes involved in glycolysis and the oxidative pentose phosphate pathway [32]. In this sense, Abraham et al. [33] showed that some allelopathic compounds interact with the mitochondrial membrane and directly alter mitochondrial respiration. According to Almeida et al. [34], some oxygenated monoterpenes showed strong inhibitory activity on germination and root elongation of radish. It is well known that these compounds have phytotoxic effects that can cause anatomical and physiological changes in seedlings: reducing some organelles such as mitochondria, accumulation of lipid globules in the cytoplasm, may be due to inhibition of DNA synthesis or rupture of membranes [34].

In addition, the radicle elongation in lettuce and some weeds could be inhibited by phenolic compounds at higher concentration, which is caused by the blocking of the amylase activity [35], these compounds block also the progress of certain phenomena such as oxidative metabolism, membrane transport, reduction of the synthesis of certain proteins and lipids. Other works explain the action of some secondary plant metabolites such as benzoxazolinones as inhibiting substances of oat coleoptile auxin [36, 37]. The suppression of indole acetic acid (IAA) degradation by various phenols was reported by Lee et al. [38].

Calculated ED50

We calculate ED50 by a simple extraction of the inconu x from the numerical equations noted in each of the graphs below (Figures 1; 2; 3), thus giving y the value 50.

Table 1: Species studied and their botanical family

Scientific name	Vernacular Name	Botanical family
<i>Ficus carica</i> L.	Fig	Moraceae
<i>Juniperus communis</i> L.	Juniper	Cupressaceae
<i>Lavandula</i> sp	Lavender	Lamiaceae
<i>Medicago sativa</i> L.	Alfalfa	Fabaceae
<i>Mentha pulegium</i> L.	Penny royal	Lamiaceae
<i>Mentha rotundifolia</i> (L.)	Fragrant mint	Lamiaceae
<i>Myrtus communis</i> L.	Myrtle	Myrtaceae
<i>Nerium oleander</i> L.	Pinklaurel	Apocynaceae
<i>Nicotiana glauca</i> Graham.	Glaucous tobacco	Solanaceae
<i>Ocimum basilicum</i> L.	Basil	Lamiaceae
<i>Origanum compactum</i>	Oregano	Lamiaceae
<i>Peganum harmala</i> L.	Pegane	Zygophyllaceae
<i>Pinus pinaster</i> Aiton	Pine	Pinaceae

Table 2: Allelopathic biocidal effect of aqueous extracts of some natural plants on seed germination of *Phalaris canariensis* and *Lactuca sativa*

plants tests	Reduction of germination (%)	
	<i>Phalaris canariensis</i>	<i>Lactuca sativa</i>
<i>Ficus carica</i> L.	90 a	4 d
<i>Juniperus communis</i> L.	54 bc	0 d
<i>Lavandula</i> sp	100 a	96 a
<i>Medicago sativa</i> L.	44bc	23 c
<i>Mentha pulegium</i> L.	8 d	0d
<i>Mentha rotundifolia</i> (L.) Huds	57 bc	12 cd
<i>Myrtus communis</i> L.	96 a	11 cd
<i>Nerium oleander</i> L.	67 bc	17 cd
<i>Nicotiana glauca</i> Graham.	9 d	4 d
<i>Ocimum basilicum</i> L.	96 a	56 b
<i>Origanum compactum</i> Benth.	95 a	7 d
<i>Peganum harmala</i> L.	32 cd	7 d
<i>Pinus pinaster</i> Aiton.	27 cd	40bc

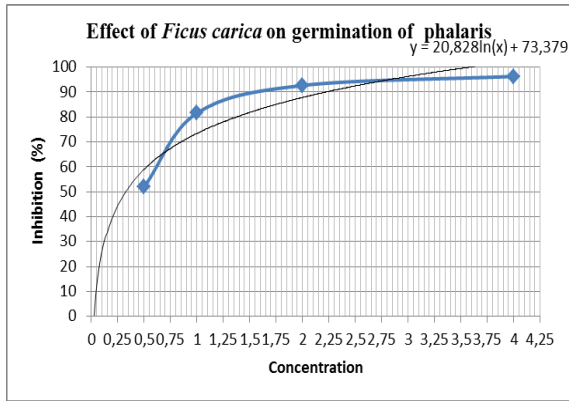
Table 3: Effect of aqueous extracts of two medicinal plants at different concentrations on germination of *Phalaris canariensis* and *Lactuca sativa*.

Plants	Concentrations (%)	(% Réduction)	
		<i>Phalaris canariensis</i>	<i>Lactuca sativa</i>
<i>Ficus carica</i>	0	0 c	0 a
	0,5	51,88 cb	0 a
	1	81,55 b	3,53 a
	2	92,66 a	7,18 a
	4	96,3 a	7,18 a
<i>Origanum compactum</i>	0	0 c	0 a
	0,5	6,83 c	-3,51 a
	1	31,05 cb	0 a
	2	65,52 b	3,41 a
	4	93,16 a	6,83 a

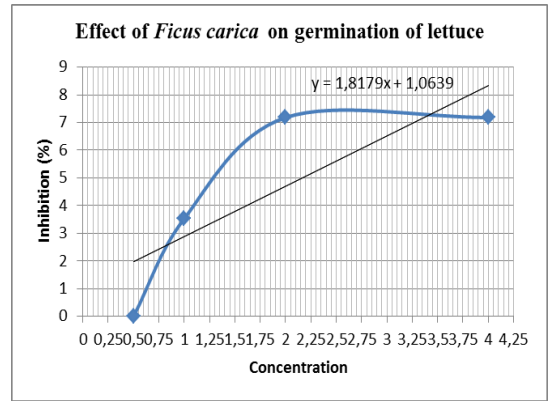
Table 4: Effect of aqueous extracts of two medicinal plants at different concentrations on radicle and aerial part length of *Phalaris canariensis* and *Lactuca sativa*

Plants aqueous extracts	Concentrations (%)	<i>Phalaris canariensis</i>		<i>Lactuca sativa</i>	
		LMR (cm)	LMA (cm)	LMR (cm)	LMA (cm)
<i>Ficus carica</i>	0	4,02 a	4,45 a	4,48 a	1,69 a
	0,50	1,25 b	2,40 b	4,19 a	1,96 a
	1	0,98 c	2,76 b	4,03 a	2,21 a
	2	0,66 c	1,50 c	3,53 a	1,56 a
	4	0,83 c	1,51 c	1,07 b	0,60 b
<i>Origanum compactum</i>	0	4,36 a	9,45 a	1,45 a	1,55 a
	0,50	0,67 b	4,19 b	1,36 a	1,16 a
	1	0,88 b	5,31 b	0,80 b	0,75 b
	2	0,25 b	2,46 c	0,24 b	0,65 b
	4	0,13 b	1,83 c	0,15 b	0,41 c

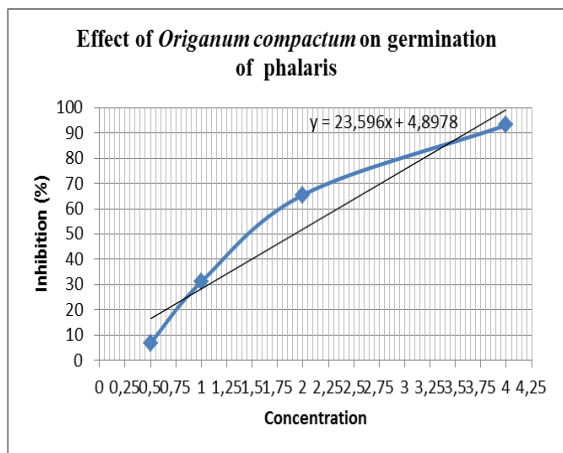
LMR: Average length of radicle; LMA: Average length of aerial part



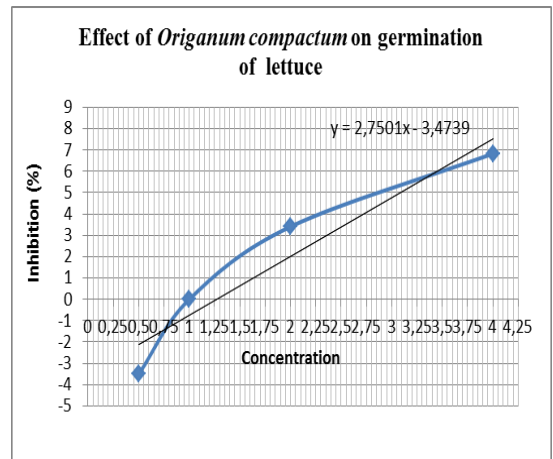
ED50= 0,32



ED50= 26,91

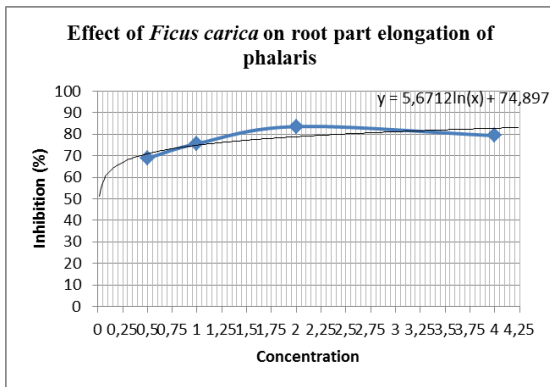


ED50= 1,91

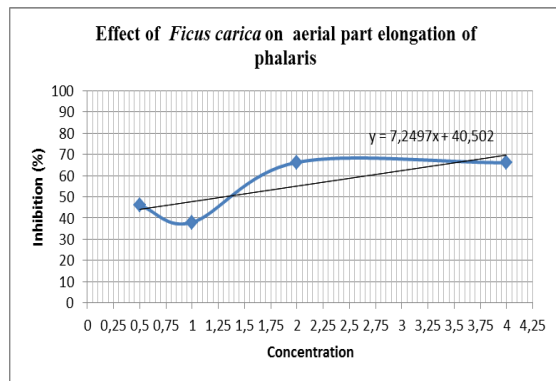


ED50= 19,44

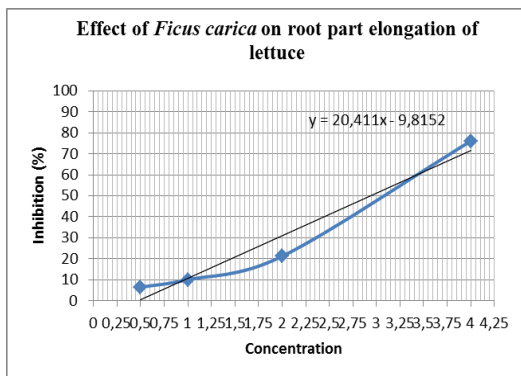
Figure 1: Effect of *Ficus carica* and *Origanum compactum* on seed germination of *Phalaris canariensis* and *Lactuca sativa*



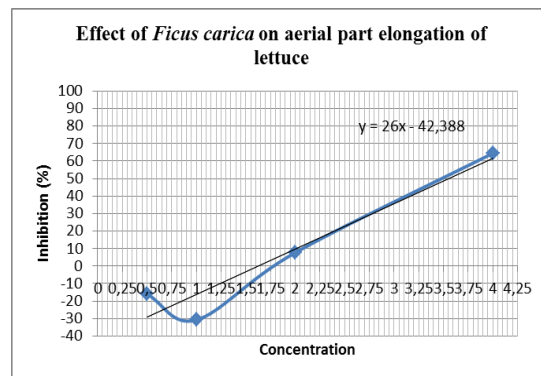
ED50= 0,012



ED50= 1,31



ED50= 2,93



ED50= 3,55

Figure 2: Effect of *Ficus carica* on root and aerial parts elongation of *Phalaris canariensis* and *Lactuca sativa*

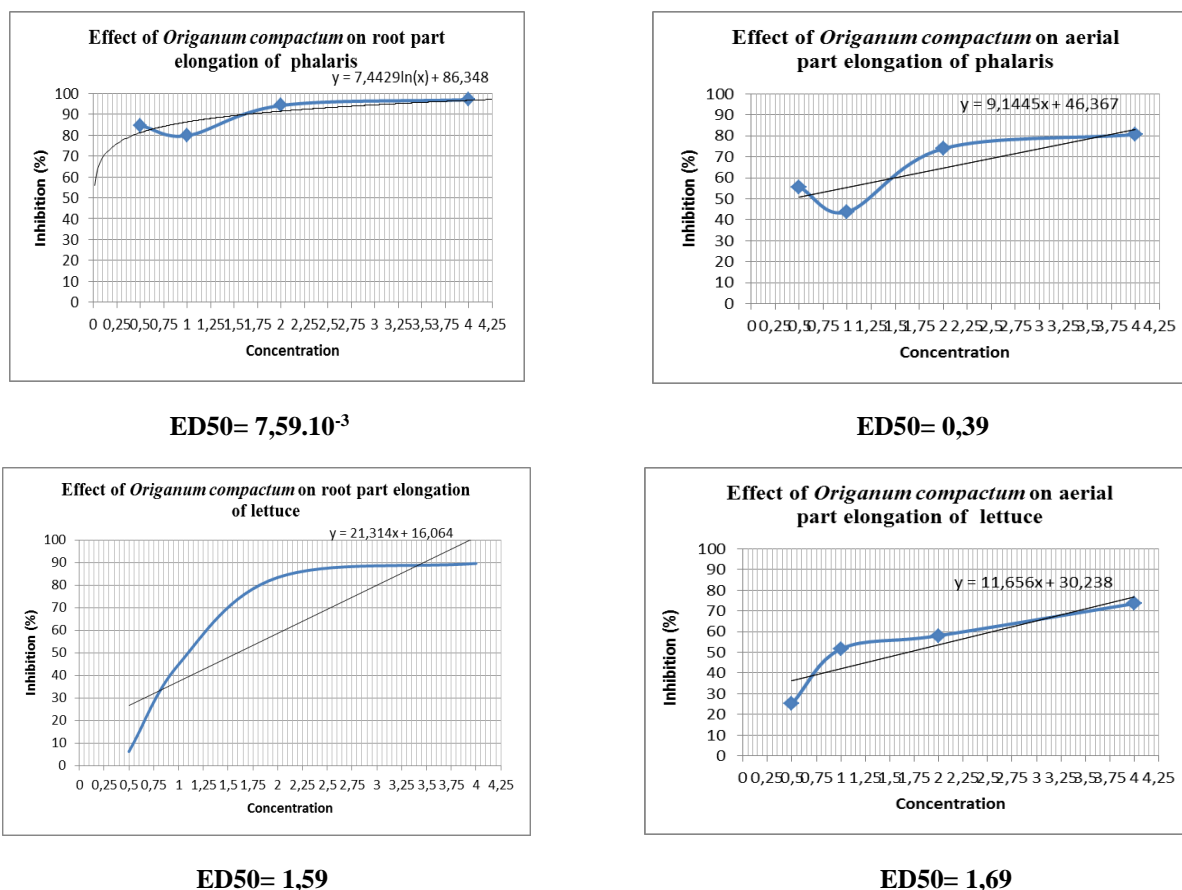


Figure 3: Effect of *Origanum compactum* on root and aerial parts elongation of *Phalaris canariensis* and *Lactuca sativa*

In seed germination of *Phalaris canariensis* and *Lactuca sativa*, aqueous extracts of the two species tested caused 50% inhibition (ED50) at the following concentrations: 0.32 and 26.91% (*Ficus carica*) and 1.91 and 19.44% (*Origanum compactum*). The comparison of ED50 clearly indicates that both aqueous extracts are more phytotoxic on phalaris seed germination than lettuce.

Root part elongation: ED50 values for root elongation were highly variable, ranging from 7.59 .10⁻³ % and 0,012 for *Phalaris canariensis* treated respectively with *Origanum compactum* and *Ficus carica* to 1,59 and 2.93 % for *Lactuca sativa* treated, respectively with *O. compactum* and *F. carica*, making it impossible to establish a hierarchy of phytotoxicity. In particular, *O. compactum* (7.59 .10⁻³) was more toxic than *F. carica* in inhibiting phalaris. Whereas, for lettuce, the strongest effect was caused by *O. compactum* (1,6%) followed by *F. carica* (2.93%).

Elongation of aerial part: ED50 values for elongation of aerial part varied from 0.39% and 1.31 for *Phalaris canariensis* treated respectively with *Origanum compactum* and *Ficus carica* to 1.69 and 3.55% for *Lactuca sativa* treated respectively with *O. compactum* and *F. carica*. It is clear that

the aqueous extracts of the two plants tested are more phytotoxic on the elongation of the aerial part of phalaris than that of lettuce. Furthermore, the strongest effect was caused by *O. compactum* with an ED50 of 0.39 and 1.69% for phalaris and lettuce respectively; followed by *F. carica* with an ED50 of 1.31 and 3.55% for phalaris and lettuce, respectively. When seed germination is not inhibited, we observed other effects on seedling growth (inhibition or stimulation). In this sense, it has been shown that the effect of allelochemicals is manifested by morphological variations that are most often observed in the early stages of development, effects on radicle and coleoptile elongation [4], so the different effects of extracts on seed germination and seedling development can be explained by the differences in quantities (concentrations) and physicochemical characteristics (allelopathic species) that possibly involve specific allelochemical substances [39].

In most cases, the reduction in seedling length can be attributed to the reduction in the rate of cell division and elongation due to the presence of allelochemicals in the aqueous extracts [40]. Dore et al. [41] reported that a lot of substances with allelopathic effect act as inhibitors of auxins

and gibberellins which are phytohormones responsible for cell elongation in plants, which can induce a reduction in root development, something that will prevent the roots from absorbing water and mineral salts from the soil, and consequently affect the growth, and yield [42]. Aqueous extracts of allelopathic plants have more pronounced effects on root growth than on shoot growth [43]. Other researchers [3, 44] also found that root length was the most sensitive trait to allelochemicals. However, this can be explained by the fact that roots are the first to absorb allelochemicals from the environment [20]. Blum [45] reported that allelopathic compounds are most often phenolic compounds and have an inhibitory effect on seed germination and sprout growth, these substances affect fundamental mechanisms of target plants such as respiration and protein synthesis [6], photosynthesis [46, 47], membrane permeability [48], cell division and germination [5]. They target hormones (auxin, gibberellins, abscisic acid) that control the major stages of the plant life cycle (germination, growth, and flowering) and membranes (disruption of their permeability) [49]. They can also influence the biological nitrogen fixation and reduce the number and size of nodules, which affect the growth of the host culture [50, 51]. They also can disturb the weeds growth by inhibiting the synthesis of proteins, the transport of amino acid and by decreasing the integrity of their RNA and DNA [52, 53]. The qualitative and quantitative distribution of phenolic compounds in plants may be influenced by different technical (method and solvent of extraction), environmental (climate, humidity, temperature, dehydration and light intensity), and biological (genotype, organ and ontogeny) factors [54].

4. Conclusions

Extracts of some plant species showed negative allelopathic effects on the germination of *Phalaris canariensis*, without having a significant negative effect on the germination of *Lactuca sativa*. Indeed, we tested the allelopathic effect on the germination and growth of canary grass and lettuce of aqueous extracts at different concentrations of two species (*Ficus carica* and *Origanum compactum*) that we retained in this work because they met the criteria of a bioherbicide. The plant extracts of *F. carica* and *O. compactum* did not show a significant effect on lettuce germination. However, they had a significant inhibitory effect on canary grass germination, and this effect was reflected in reduction rates that increased with increasing concentrations. The allelochemicals responsible for the allelopathic properties of plants need to be identified for use as natural herbicides in weed management.

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