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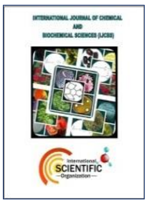

Biocidal allelopathic effects in vitro of aqueous and organic fractions extracts of *Visnaga daucoides* and *Ricinus communis* on a noxious weed (*Phalaris canariensis*) and a cultivated plant (*Lactuca sativa*) during seed germination and initial seedling growth

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Abstract

As crop pests become resistant to current pesticides, certain biologically active compounds in the plant with a biopesticidal effect have been of increasing interest to scientists. Therefore the biocidal allelopathic potential of aqueous and organic phases of aqueous extract of two wild plants (*Visnaga daucoides* and *Ricinus communis*) was tested on the germination and growth of the weed phalaris (*Phalaris canariensis*) and the lettuce crop (*Lactuca sativa*). The results showed that the aqueous fraction and the organic phase of ethyl acetate of R. communis and the organic phases of ethyl acetate and hexane of V. daucoides, had a very significant inhibitory power against the germination of phalaris weed seeds, without negatively affecting the germination of lettuce seeds. Furthermore, the growth of lettuce was not too much affected by the aqueous fraction of R. communis and the ethyl acetate phase of V. daucoides. However, both fractions very strongly inhibited the growth of the radicle and shoot of the phalaris weed. These extracts of these two wild plants could have a selective bioherbicidal effect on dicotyledonous weeds.

Keywords: allelochemicals, biocontrol, plant-plant interaction, bioherbicide, weed management.

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1. Introduction

Agriculture faces critical challenges to ensure high crop production and quality, while preserving the environment and human health. However, since pest control is mainly based on chemical control, the use of agrochemicals, including the misuse of synthetic plant protection products, is one of the pressures that agriculture exerts on the environment. The harmfulness of chemical pesticides requires the search for alternative methods of control, including biological control. For this purpose, the valorization of the allelopathic potentialities in plant protection can be a promising way. Allelopathy has been defined by [1] as "any direct or indirect, positive or negative, interaction between plants (including microorganisms), through chemicals released into the environment".

Allelochemicals are secondary metabolites including phenolic acids, flavonoids, terpenoids and alkaloids, which are responsible for the allelopathic potential of plants [2]. Allelopathic activity is believed to arise from different chemicals acting additively or synergistically [3]. Allelochemicals are highly inhibitory to the neighboring plants [4]. Indeed, many plant species synthesize molecules capable of inhibiting the germination and growth of neighboring plants [5]. This allelopathic interaction gives an extra benefit to the donor plants to dominate in their vicinity. Allelochemicals are produced from roots, stems, leaves, flowers, or seeds [6]. The release of allelopathic compounds occurs through four different pathways: volatilization, leaching, root exudation, or decomposition of plant residues, including roots [7]. Most of these allelochemicals released from donor plants are compounds that are already active on target plants [6]. Allelochemicals released into the environment directly affect the target plant by blocking the metabolism necessary for plant life [8]. These allelochemicals influence the regular metabolic processes of other plants, including breathing, cellular division, growth, development, productivity, and enzymatic activity [9], [10], [11].

The biosynthesis of allochemicals is often dependent on environmental stimuli [12]. Indeed, it has been reported that the environmental and geographical conditions, such as hydric stress and season of collection, influence the production of these specialized compounds [13]. However, the environmental stresses arising from the interaction among plants are also important for the production of other allelochemicals [14]. Some derivatives of chemical compounds from plants and microorganisms that provide good crop protection against weeds, pests and diseases have been used to formulate biopesticides [15]. Crop pest control appears to be a privileged area for the exploitation of allelopathins in agriculture. In plants, seed germination and seedling performance are the main life stages usually affected by allelochemicals: frequent negative allelopathic effects are seed germination inhibition [16], seed germination delay [17] and seedling growth inhibition [18].

Several examples of plant extracts have shown strong allelopathic potential against weeds. In certain works, it has been noted that water extracts of some plants allow the control of weeds in cotton [19] and wheat culture [20]. More recently, it has been shown that the aqueous extract of *Carica papaya* can be used for weed control of seeds and pre-germinated seeds of Avena fatua [21]. In other works, non-volatile organic extracts have been tested for their biocidal effects. Thus, for example, [22] investigated the phytotoxic activity on germination and growth of soft wheat seedlings, testing the effect of four organic solvent fractions (n-hexane, chloroform, ethyl acetate, 1-butanol) and crude (1:10) aqueous fractions of whole *Euphorbia dracunculoides* plants, using distilled water and 0.05% (v/v) dimethyl sulfoxide as a control. The final germination percentage of wheat was not affected. Nevertheless, root and shoot elongation and biomass accumulation in different organs of wheat were significantly delayed. Furthermore, [23] showed that the herbicidal activity of the crude methanolic extract of the aerial parts of *Cynara cardunculus* inhibited seed germination of several weeds (*Trifolium incarnatum*, *Silybum marianum*, and *Phalaris minor*), as well as the growth of their seedlings, and induced necrosis or chlorosis in their plants.

Furthermore, although the pharmacological and/or pharmaceutical properties of the Apiaceae *Visnaga daucoides* Gaertn and the Euphorbiaceae *Ricinus communis* L. are found in scientific literature, research articles relating to their allelopathy are less reported. *V. daucoides* is a short annual or biennial herb [24]. It is distributed abundantly throughout the world as an introduced species [25], and it is found mainly in North Africa, Europe, Atlantic Islands, southwestern Asia, North America and Eastern Mediterranean region [26]. It is a valuable herbal plant that is frequently collected for medicinal purposes [27]. This plant is also popular in the treatment of vitiligo and psoriasis, and used as a lithotriptic agent. It is generally used to dilate bronchial, urinary, and blood vessels without affecting blood pressure. It is also internally used as an emmenagogue to regulate menstruation, as a diuretic, and in the treatment of vertigo, diabetes, and kidney stones. An infusion of the aerial parts has also been used to treat headaches [28].

R. communis is originally native to north-eastern Africa and the Middle East. It has escaped cultivation and become naturalized as a weed almost everywhere in the world under tropical or subtropical climate [29]. However, the roots, leaves and seed extracts of this plant are famous for their medicinal properties. Indeed, Ricinus communis is traditionally used as antidiabetic, anti-inflammatory, antiasthmatic, anticancer and antimicrobial [30]. The plant is reported to possess antioxidant, anti-inflammatory, antidiabetic, central analgesic, antitumour, larvicidal, and antiasthmatic activity [31], [32]. Furthermore, R. communis is an oilseed crop; it is primarily of economic interest as a source of castor oil, used for the production of high-quality lubricants because of its high proportion of the unusual fatty acid ricinoleic acid [33]. The major phyto-constituent reported in this plant are rutin, gentisic acid, quercetin, gallic acid, kaempferol-3-O-beta-d-rutinoside, kaempferol-3-O-beta-d-xylopyranoid, tannins, Ricin A, B and C, ricinus agglutinin, Indole-3-acetic acid and an alkaloid ricin [34], [35].

The objective of this study is to test the allelopathic potential of different aqueous extract fractions of two wild medicinal plants (*Visnaga daucoides* and *Ricinus communis*) on the germination and growth of a noxious monocotyledonous weed (*Phalaris canariensis*) and of a dicotyledonous crop (*Lactuca sativa*). The ultimate goal of this work is to test the possibility of their use as bioherbicides from plant extracts that can be used as a potential method for weed control.

2. Materials and methods

2.1. Plant material

Samples of *Ricinus communis* and *Visnaga daucoides* wild plants were collected randomly at the Northwestern Morocco. Vegetative parts were cut at the flowering stage. Water-soluble extracts (mainly foliage) were tested on germination and growth of canary grass (*Phalaris canariensis* Retz) and lettuce (*Lactuca sativa* L). Canary grass is a common agronomic weed that competes with crops, exudes plant inhibitors, and serves as an alternate host for plant diseases.

2.2. Preparation of the plant extracts

2.2.1. Crude aqueous extract: The plants were dried at a temperature of 45°C for 4-5 days in a ventilated oven then, milled, and sieved through a 20–40 mesh. The powder was held in paper bags and stored in the dark under laboratory conditions before being used. 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% w/v) of the sample used.

2.2.2. Partition of the total extract: 100 ml of the crude extract (4%) was transferred to a separating flask and washed three times with 50 ml of hexane. In a similar manner, the partition of the remaining pellet with ethyl acetate was carried out exhaustively. The remaining fraction constituted the aqueous phase. All extracts had been concentrated with a rotary evaporator under a vacuum at low temperature (40 °C). The fractions obtained were stored in the refrigerator at 4 °C.

2.3. Phalaris and Lettuce germination and growth test: For the fractions in the two organic solvents (hexane, ethyl acetate), a 10 ml volume is placed in a Petri dish which is then left open overnight (12 hours) to evaporate the solvent. Then, a 10 ml volume of distilled water is placed in the Petri dish, the bottom of which is then scraped with a spatula to solubilise the organic deposit. In parallel, other Petri dishes are prepared in which 10 ml aliquots of the aqueous fraction are placed. Finally, other Petri dishes containing 10 ml of distilled water each are used as controls. 10 sterilized canary or lettuce seeds were placed in each Petri dish, in the presence of a plant extract or the control. Petri dishes were sealed with parafilm, to prevent water evaporation and eventually to prevent seed contamination, and they were maintained under laboratory conditions (room temperature 18 °C at midday with diffused light during day) for 10 days. The experiments were conducted under completely randomized design with three replicates. Each procedure was repeated three times.with five replicates.

2.4. Observations and measurements :

Seed germination percentages and growth of seedlings (radical and hypocotyl length) were calculated according to the following formulas :

% inhibition = 100 x (N-n)/N

N: number of seeds germinated in the control at 10 days

n: number of seeds germinated in the treatment at 10 days

Average root length (cm):

$$ARL = \frac{\sum_i^n RL_i}{n}$$

Average shoot length (cm):

$$ASL = \frac{\sum_i^n SL_i}{n}$$

n: number of seeds germinated in the treatment at 10 days;

SL(i): shoot length I

RL(i): root length i

3. Results and Discussions

3.1. Effect of Ricinus communis (R.c.) extract fractions on seed germination and seedling growth of phalaris and lettuce:

Only the aqueous phase ($AP_{[R.c.]}$) and the ethyl acetate organic phase ($EAOP_{[R.c.]}$) of *Ricinus communis* extract showed a very high inhibitory power on canary seed germination, with an inhibition of 100% and 96% respectively (Fig.1). However, no significant inhibition of lettuce germination was recorded with all tested fractions of the same extract.

The results illustrated in figures (Fig.2 and Fig.3) show that only the aqueous phase "$AP_{[R.c.]}$" fraction did not inhibit the growth of lettuce, whereas it exerted a very significant inhibition on that of phalaris. Moreover, with this fraction, the growth of lettuce was higher than that of phalaris. In contrast, the other tested fractions of the R.c. extract, notably the "$EAOP_{[R.c.]}$" fraction, significantly reduced the growth of both lettuce and phalaris.

According to our results, it turned out that the aqueous extract of *Ricinus communis*, showed an excellent inhibiting power on phalaris, without affecting too much the lettuce. In parallel, the ethyl acetate organic phase of R. communis was more cytotoxic than the hexane phase, on the germination and growth of both phalaris and lettuce. In other works, it was noted that, compared to several organic fractions, water was a better solvent for extraction of germination-inhibiting compounds [22].

Reductions in seedling growth might be due to the involvement of the phenols, which can suppress protein and nucleic acids synthesis and inactivate several enzymes in growing plants [36]. R. communis is rich in phenol [37]. The major phenolic compounds isolated from its leaves are the monoterpenoids (1,8-cinole, camphor and α-pinene) and sesquiterpenoids (β- caryophyllene), gallic acid, quercetin, gentisicacid, rutin, epicatechin and ellagic acid [38]. Phenolics are a major category of compounds responsible for allopathic activity. Phenolic allochemicals can lead to an increase in cell membrane permeability, thus disrupting the regulatory mechanisms of nutrient uptake and therefore affecting normal plant growth [39]. Also, the phenolic allelochemicals could inhibit cell division and alter the cell ultrastructure [40]. Similarly, the presence of phenolic compounds could be the cause of reduced germination and growth of seedlings [41]. In addition, some flavonoids, including rutin and quercetin, have been found to exert an inhibitory effect on radish seedling germination and growth [42].

3.2. Effect of Visnaga daucoides (V.d.) extract fractions on seed germination and seedling growth of phalaris and lettuce:

The organic phases of hexane ($HOP_{[V.d.]}$) and ethyl acetate ($EAOP_{[V.d.]}$) as well as the aqueous phase ($AP_{[V.d.]}$) of *Visnaga daucoides* showed a significant inhibitory effect on the germination of phalaris, with percentages of inhibition of 93, 100 and 78% respectively (Fig.4). However, the germination of lettuce seeds was not influenced by the different fractions.

The growth of phalaris and lettuce seedlings was significantly inhibited by the organic fraction in hexane and especially by the organic fraction in ethyl acetate of *Visnaga daucoides*. However, the aqueous phase was more selective and induced a very significant inhibition of phalaris weed growth, while lettuce growth was not affected (Fig.5 and Fig.6).

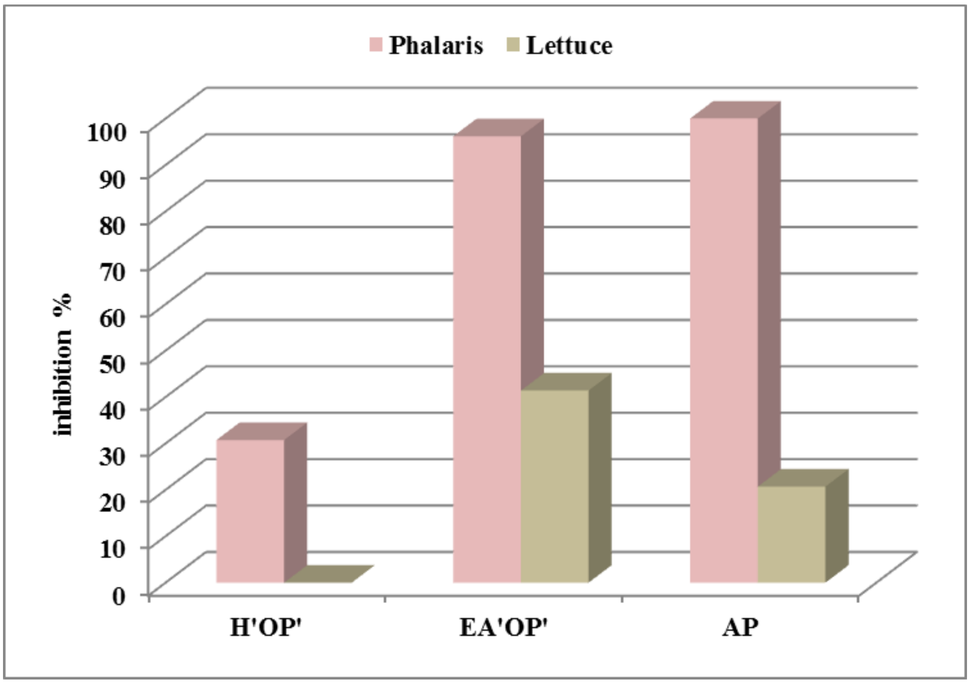
Compared to the water phase, the ethyl acetate and hexane organic phases of *Visnaga daucoides* were inhibitorier to phalaris germination. However, the germination of lettuce seeds was not influenced by the three different fractions of V. daucoides. In addition, the ethyl acetate organic phase was found to be inhibitorier to phalaris root growth, while it did not affect lettuce root growth. Other researchers have shown that the ethyl acetate fraction of *Croton argenteus* showed high activity on germination and growth, and these effects take place by means of mitochondrial metabolism alterations and increase the oxidative stress, leading the seedling death [43]. It is known that commercial herbicides cause cellular death by an oxidative stress increase. This is a consequence of interference in the Photosystem II (PSII) flow or in inhibition of antioxidant substances, including carotenoids [44].

Furthermore, it seems that the inhibitory power can change depending on the polarity index of the solvent used. The solubility of the phenolic compounds depends on the polarity and the properties of the extraction solvent [45], [46]. Organic solvents with high polarity are more effective in the extraction of phenolic compounds [47]. The organic fractions also exhibited different levels of phytotoxicity against the species tested, which might arise due to the variable chemical nature of the compounds used for extraction. Water is a polar compound while n-hexane is non-polar in nature. [48] noted that the reduction in germination percentage was exacerbated with increasing polarity of the extract, suggesting an increase in active ingredients with increasing polarity. [49] showed that the quality and quantity of phytochemicals can vary with the change in extraction solvent. These researchers showed that allelochemicals can be extracted with different solvents (water, methanol, ethyl acetate, chloroform, and petroleum ether) and noted that all soluble fractions of *Ampelocissus latifolia* possess cytotoxic allelopathic substances for wheat root growth. [22] reported that the variable phytotoxicity of the different aqueous and organic fractions of Euphorbia dracunculoides could be due to the different extraction efficiencies of the solvents used, explaining the qualitative and quantitative differences of the phytotoxins extracted in the different fractions.

Our results also showed that the V. daucoides organic phase of hexane has a significant inhibitory effect on the germination and growth of phalaris, while it only has an inhibitory effect on the elongation of lettuce seedlings. As the organic phase of hexane contains mainly lipids and V. daucoides is rich in essential oils, it is possible that these caused the inhibition of germination and growth of the tested seeds. [50] showed that eucalyptus essential oil has strong inhibitory potential against weeds, and found that with increasing its concentration, germination percentage, radicle length, plumule length, seedling height, primary root length, and primary pedicle length, decreased significantly. Also [51] showed that the essential oils of *Cinnamomum zeylanicum*, *Lippia sidoides* and *Cymbopogum nardus* inhibited seed germination and root growth of seedlings of *Bidens pilosa*.

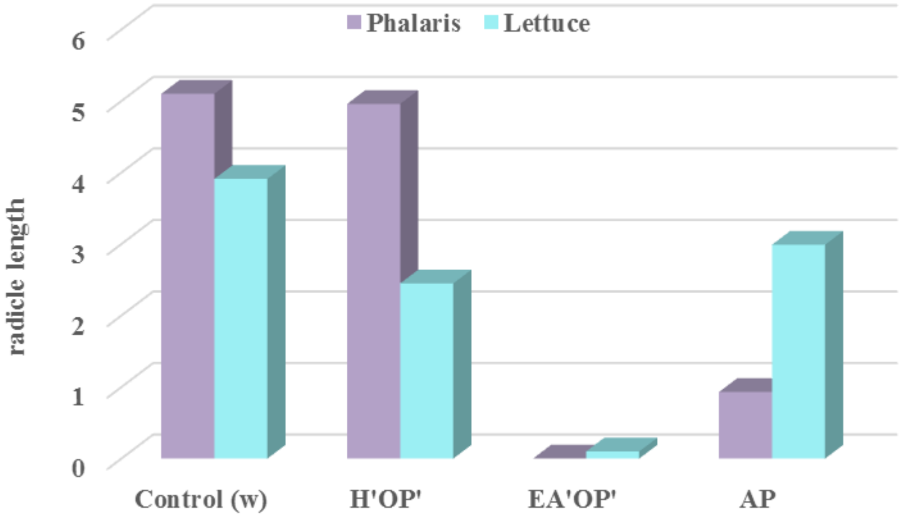
The presence of high levels of secondary metabolites in a given species can enhance its allelopathic potential because many of these compounds interfere with various mechanisms of action and could influence a number of target sites [52]. Thus, some secondary plant metabolites influence germination or plant growth by multiple mechanisms. [1] reported that allochemicals suppress the mitotic activity of young cells, resulting in inhibition of seed germination which has been attributed to disruption of mitochondrial respiration [53] and disruption of the activity of metabolic enzymes involved in glycolysis and the oxidative pentose phosphate pathway [54]. [55] showed also that some allelopathic compounds interact with the mitochondrial membrane and directly alter mitochondrial respiration. Plant development is regulated by six main types of hormones: auxins, gibberellins, cytokinins, ethylene, abscisic acid, and brassinosteroids, which can be negatively affected by the presence of allelochemicals. Changes in these growth regulators may influence seed germination and seedling growth [56]. According to several studies, some oxygenated monoterpenes show a strong inhibitory activity on germination and root elongation by causing anatomical and physiological changes in the seedlings: reduction of certain organelles such as mitochondria, accumulation of lipid globules in the cytoplasm, which may be due to inhibition of DNA synthesis or rupture of the membranes. In addition, alkaloids, flavonoids have the ability to inhibit the action of certain plant enzymes such as ATPase, or block the progress of certain phenomena such as oxidative metabolism, membrane transport, reduction of the synthesis of certain proteins and lipids [57], [58], [59]. Others explain the action of some secondary plant metabolites such as benzoxazolinones as inhibiting substances of oat coleoptile auxin [60]. The suppression of indole acetic acid (IAA) degradation by various phenols was reported by [61]. [62] indicated that the highest level of allelopathic suppression may occur when maximum levels of phytotoxins coincided with early stages of plant growth. The reduction in shoot and root lenghts may be due to reduced cell division and elongation due to the presence of allelochemicals [63]. Indeed, many substances with allelopathic effect act as inhibitors of auxins and gibberellins, which may induce a reduction in root elongation and development [64], because of the fact that the root is in direct contact with the soil and absorbs many allelochemicals [65], [66]. Furthermore, these allelopathic compounds prevent roots from absorbing water and mineral salts from the soil [67].

Inhibition of shoot and root growth of the plant species tested in response to allelopathic products is a good indicator of phytotoxicity. Indeed, it has been shown that a number of abnormalities, mainly in the root system, have been observed, with primary roots being shriveled, defective and, in some cases, practically or totally absent. This type of growth inhibition by allelopathic plant extracts has been reported in many works for example by [68], [69], [70].



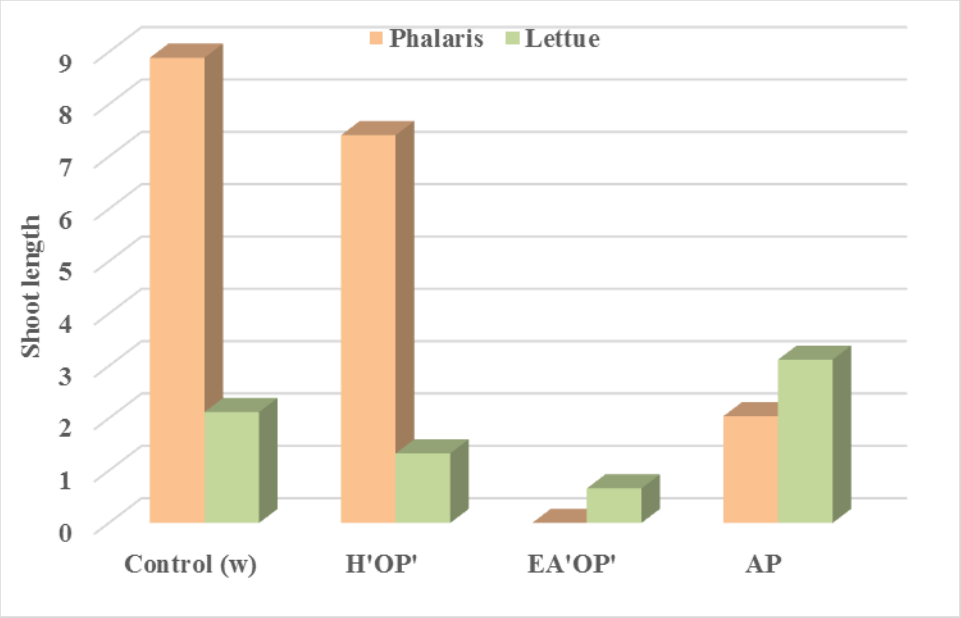
H'OP': Hexane organic phase ; EA'OP': Ethyl acetate organic phase ; AP: Aqueous phase.

Figure1: *Ricinus communis* aqueous and organic fractions effect on the germination inhibition percentage of Phalaris and Lettuce



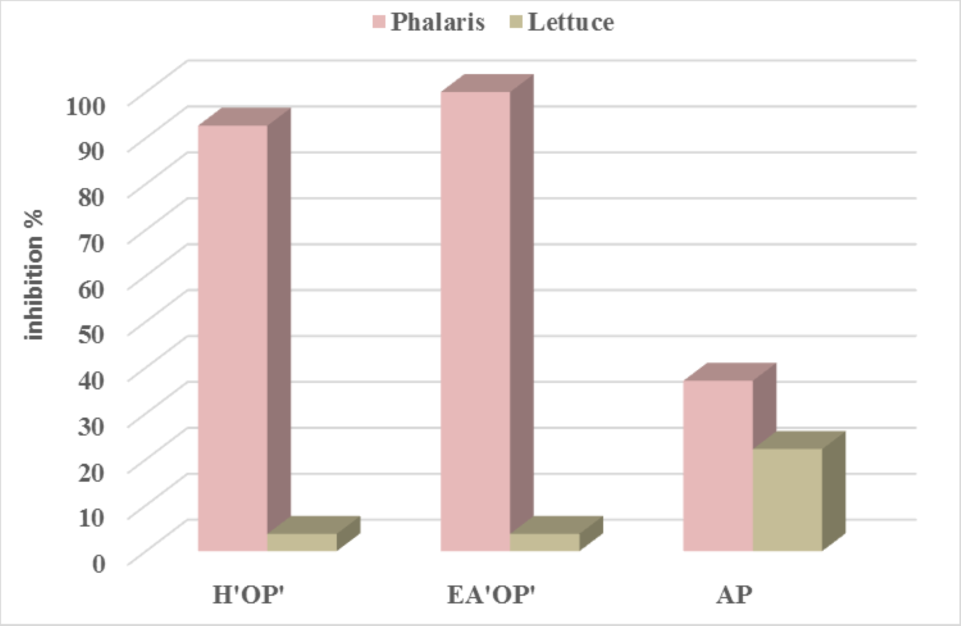
H'OP': Hexane organic phase; EA'OP': Ethyl acetate organic phase; AP: Aqueous phase.

Figure 2: Ricinus *communis* aqueous and organic fractions effect on radicle growth of Phalaris and Lettuce



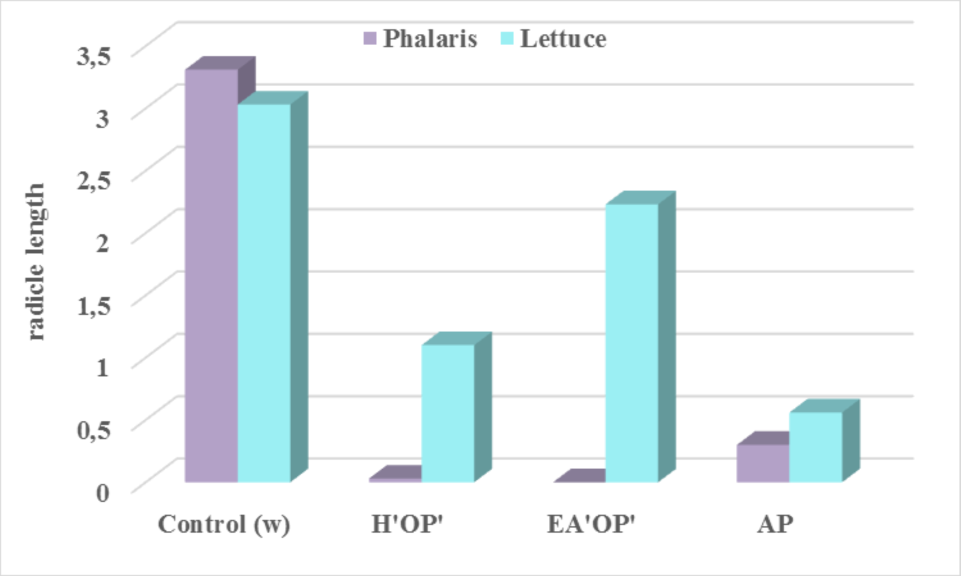
H'OP': Hexane organic phase; EA'OP': Ethyl acetate organic phase; AP: Aqueous phase.

Figure 3: *Ricinus communis* aqueous and organic fractions effect on shoot growth of Phalaris and Lettuce



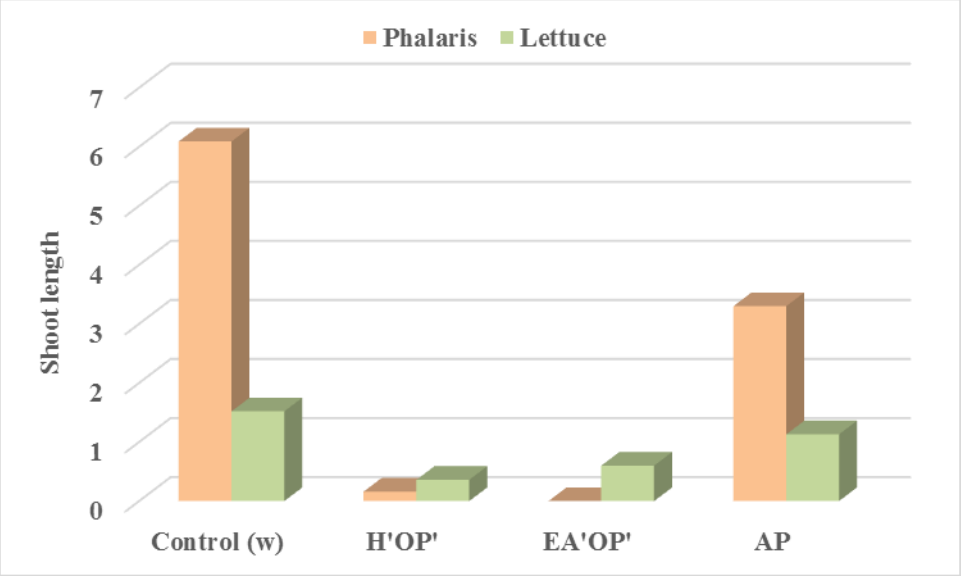
H'OP': Hexane organic phase; EA'OP': Ethyl acetate organic phase; AP: Aqueous phase.

Figure 4: *Visnaga daucoides* aqueous and organic fractions effect on the germination inhibition percentage of Phalaris and Lettuce



H'OP': Hexane organic phase; EA'OP': Ethyl acetate organic phase; AP: Aqueous phase.

Figure 5: ***Visnaga daucoides*** **aqueous and organic fractions effect on radicle growth of Phalaris and Lettuce**



H'OP': Hexane organic phase; EA'OP': Ethyl acetate organic phase; AP: Aqueous phase.

Figure 6: ***Visnaga daucoides*** **aqueous and organic fractions effect on shoot growth of Phalaris and Lettuce**

4. Conclusions

The aqueous phase of *Ricinus communis* and the ethyl acetate organic phase of *Visnaga daucoides* have good inhibitory powers on the germination and growth of the phalaris weed, without affecting the lettuce crop. Therefore, they have allelopathic properties and possess allelochemicals. The variable phytotoxicity of the different aqueous and organic fractions of R. communis and V. daucoides might be due to the different extraction efficiencies of the solvents used, explaining for qualitative and quantitative differences in extracted phytotoxins in the different fractions. These plants could be used for isolation and identification of allelochemicals to be used as natural herbicides for weed management for sustainable agriculture. The identification of these allelochemicals may contribute to the discovery of natural substances with herbicide potential.

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