



Article

# Allelopathic Interactions between Seeds of *Portulaca oleracea* L. and Crop Species

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**Abstract:** Allelopathy is described as the interference to plant growth resulting from chemical interactions among plants and other organisms mediated through the release of bioactive secondary metabolites. Since only a few studies have been reported about the role of seed allelopathy, an experiment was designed to evaluate the interactions among seeds of *Portulaca oleracea* L. and the crop species common bean (*Phaseolus vulgaris* L.), onion (*Allium cepa* L.), sugar beet (*Beta vulgaris* L.), broad bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) on seed and seedling growth parameters. The results indicated that *P. oleracea* seeds had a negative effect on the germination of *P. vulgaris* and *A. cepa*. Conversely, germination of *P. oleracea* in the presence of *P. vulgaris*, *A. cepa*, and *B. vulgaris* seeds was strongly reduced with a higher inhibitory effect found for the seeds of *A. cepa*. The highest negative effect on root and shoot length was observed in *P. vulgaris*. Seedling vigor of all crop species decreased in the presence of *P. oleracea*. Our results suggest that seeds of *P. vulgaris*, *A. cepa*, and *B. vulgaris* exhibited high allelopathic effects against seeds of *P. oleracea* and can be used as potential bio-herbicides in future screening programs.

Keywords: allelopathy; bio-herbicide; germination inhibition; weeds



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# 1. Introduction

Weeds are a threat in all cropping systems. These undesirable plants decrease input efficiency, interfere with agricultural practices, impair the quality of plant products, deplete resources such as soil nutrients, moisture, and space allocated to crop plants, and ultimately cause heavy losses in plant production [1]. It has been estimated that the economic damage of weeds is more than 100 billion dollars worldwide [2]. Therefore, eradicating or decreasing the harmful effects of weeds on crop plants is the main target of weed management.

Allelopathy is a natural process that can be considered as a tool for biological weed control in agriculture [3,4]. According to the International Allelopathy Society, allelopathy is defined as any process in which the secondary metabolites produced by plants affect the growth and development of biological systems [5]. Approximately 100,000 secondary

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metabolites have been identified to date in plants [6]. A smaller number of these are described as bioactive allelochemicals and are generally classified as members of specific chemical families that include phenolics, terpenoids, glycosteroids, and alkaloids [7]. These compounds are present in various concentrations in many plant parts, including leaves, stems, roots, flowers, seeds, rhizomes, pollen, bark, and buds [8], and are released through root exudates, leaching, volatilization, and decomposition of plant residues [9,10].

Several researchers have reported that some plant growth inhibitors from allelopathic plants can inhibit weed growth [11]. Consequently, allelopathic plants and allelochemicals can also be applied in the biological and non-synthetic chemical control of weeds; thus, introducing a new generation of environmentally friendly weed inhibitors and reducing the costs of crop productivity [12,13].

Purslane (*Portulaca oleracea* L.) is a summer annual C<sub>4</sub> weed from the Portulacaceae family and is a very troublesome weed worldwide. This weed has been ranked as the 9th worst weed in the world, recorded in 45 crops in 81 countries [14]. It can severely decrease the yield of plants such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), and other vegetables [14]. This weed species has been identified as an allelopathic plant containing terpenes [15], tannins [16], saponins [17], alkaloids [18], phenolic acids, and flavonoids [19,20]. Silva et al. [21] indicated that leaves and roots of *P. oleracea* had detrimental effects on the germination and growth of *Allium cepa* L., *Brassica oleracea* L., *Raphanus oleracea* L., and *S. lycopersicum*. In addition, leaf and root aqueous extracts of *P. oleracea* adversely affected the activities of antioxidant enzymes and photosynthetic pigments of *Cucurbita pepo* L. So far, no study has reported the allelopathic effect of *P. oleracea* seeds on seed germination and seedling growth of other species. For this reason, the aim of the present study was to investigate the allelopathic effect of *P. oleracea* on seed germination and growth of *Phaseous vulgaris* L., *A. cepa, Beta vulgaris* L., *Vicia faba* L., and *Pisum sativum* L.

# 2. Materials and Methods

### 2.1. Experimental Design

In addition to *P. oleracea* seeds, common bean (*P. vulgaris* L. cv. Nassau), onion (*A. cepa* L. cv. Blanca de Pompei), beet (*B. vulgaris* cv. conditiva), broad bean (*V. faba* cv. Muchamiel.), and pea (*P. sativum* cv. Dulce de Provenza) seeds (Semillas Battle, Molins de Rei, Barcelona, Spain) were surface sterilized with hypochlorite (10%) and then soaked eight times with distilled water.

We collected matured seeds of *P. oleracea* from natural communities in Zanjan, Iran (36°41′ N and 48°23′ E; altitude 1634 m), which were stored at room temperatures (15–19 °C, 20–35% humidity) until the start of the experiment. Each replicate consisted of a Petri dish (11 cm diameter) with 25 seeds (only weed or only crop) or 50 seeds (weed + crop) on two layers of filter paper moistened with 3 mL of distilled water. Four replicates were established for each treatment combination and the Petri dishes were placed randomly within a climate-controlled room at 25 °C for 14 days with 12 h light per day. The crop seeds were placed at regular intervals between the weed seeds. Petri dishes with only weed or crop seeds were used as controls. The number of germinated seeds was recorded daily up to 14 days, and each seed was considered germinated when the protrusion of the radicle was visible [22].

Germination percentage was estimated by using the following equation:

$$GP = 100*(NG/NT),$$

where NG is germinated seeds and NT is total seeds [23].

The root and shoot length of all germinated seeds were measured using a metric ruler. Seedling vigor index was evaluated with equation:

$$SVI = (s + r)G$$

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where s and r are the shoot and root length (in cm), respectively; G is the percentage of germination [24].

Coefficient of allometry (CA) = radicle length/plumule length [25].

Inhibition (–) or stimulation (+) = GST - GSC/GSC\*100,

where GST is germination of seeds in treatments (seed close to seed); GSC is germination of seeds in control.

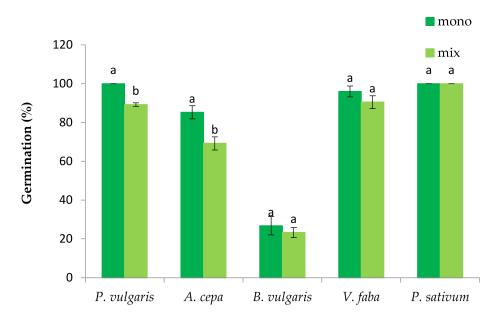
## 2.2. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and means were compared using Duncan's multiple-range tests ( $p \le 0.05$ ). The Software SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) was used to conduct all the statistical analysis. Excel software was used to obtain figures.

## 3. Results

## 3.1. The Effect of P. oleracea on the Germination and Seedling Growth of Crop Species

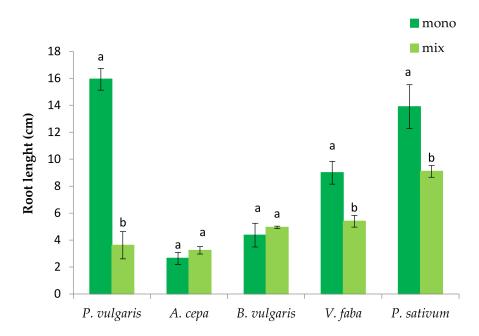
The presence of *P. oleracea* caused differential responses in the germination percentage of crop species. The results revealed that the germination percentage of *P. vulgaris* and *A. cepa* seeds was reduced by the presence of *P. oleracea* seeds. In contrast, *B. vulgaris*, *V. faba*, and *P. sativum* were not affected (Figure 1). The germination rates of *P. vulgaris* and *A. cepa* decreased marginally with the presence of *P. oleracea*, while *B. vulgaris*, *V. faba*, and *P. sativum* were not influenced (Figure S1).



**Figure 1.** Germination percent of crop species grown together with *P. oleracea* seeds (mono = only crop plant; mix = crop grown together with *P. oleracea*). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

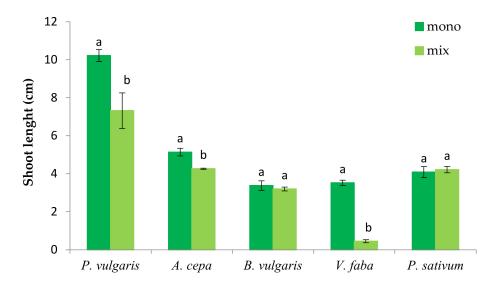
The proximity of *P. oleracea* strongly affected the root length of *P. vulgaris*, *V. faba*, and *P. sativum*. The root length of these plants was reduced by 77%, 39%, and 34% in comparison with their respective controls. This weed species had the highest inhibitory effect on the root length of *P. vulgaris*. In contrast, variation of the root length of *A. cepa* and *B. vulgaris* was not influenced by the presence of *P. oleracea* (Figure 2).

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**Figure 2.** Root length of crop species grown together with *P. oleracea* seeds (mono = only crop plant; mix = crop grown together with *P. oleracea*). Bars represent means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

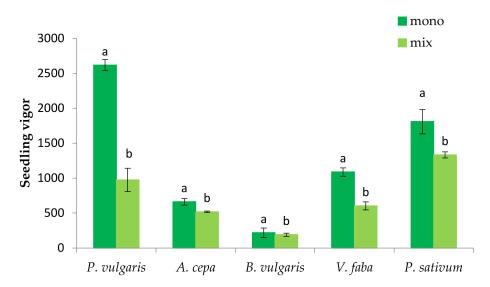
Shoot length of *P. vulgaris*, *A. cepa*, and *V. faba* was decreased by *P. oleracea* seeds. Among these crop species, *P. oleracea* had less effect on reducing the shoot length of *A. cepa* (Figure 3). There were no significant differences (p > 0.05) in the shoot length of *B. vulgaris* and *P. sativum* grown with *P. oleracea*.



**Figure 3.** Shoot length of crop species grown together with *P. oleracea* seeds (mono = only crop plant; mix = crop grown together with *P. oleracea*). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant differences between treatments at the 5% level using Duncan's multiple-range test.

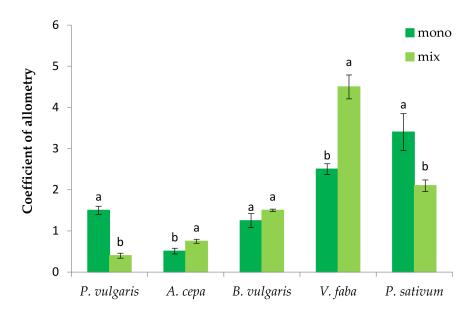
Seedling vigor of all crop species decreased in the presence of *P. oleracea*. This weed showed the highest inhibitory effect on seedling vigor of *P. vulgaris* (<60%), but the seedling vigor of *A. cepa* and *B. vulgaris* were also reduced in the presence of *P. oleracea* (Figure 4).

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**Figure 4.** Seedling vigor of crop species grown together with *P. oleracea* seeds (mono = only crop plant; mix = crop grown together with *P. oleracea*). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

The coefficient of allometry of crop species was also affected by the presence of *P. oleracea*. The coefficient of allometry of *P. vulgaris* and *P. sativum* was negatively affected by the proximity of *P. oleracea*, but *A. cepa* and *V. faba* showed a comparably higher coefficient of allometry in the presence of *P. oleracea*, and the strongest increase was observed in *V. faba* (Figure 5).



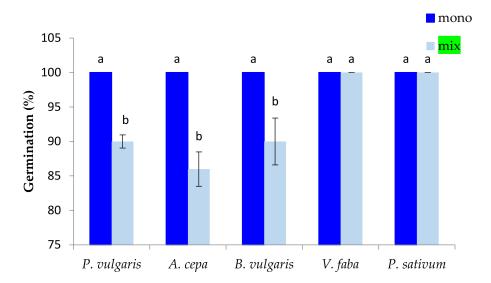
**Figure 5.** Coefficient of allometry of crop species grown together with P. oleracea seeds (mono = only crop plant; mix = crop grown together with P. oleracea). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

# 3.2. The Effect of Crop Species on the Germination and Seedling Growth of P. oleracea

The seeds of *A. cepa*, *B. vulgaris*, and *P. vulgaris* exerted inhibitory influences on the germination percent of *P. oleracea*. In particular, *A. cepa* had the highest effect on *P. oleracea* germination of -13.5%. In contrast, the presence of *P. sativum* and *V. faba* had no effect on the germination of this weed (Figure 6). Additionally, the germination rate of *P. oleracea* was

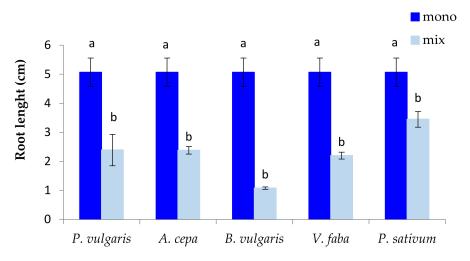
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reduced by *P. vulgaris*, *A. cepa*, and *B. vulgaris* (Figure S2). As a consequence, the proximity of *P. vulgaris* and *A. cepa* with *P. oleracea* exhibited a mutual inhibition (Figure S3).



**Figure 6.** Percent germination of *P. oleracea* grown with crop seeds (mono = weed alone; mix = weed grown together with crop). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

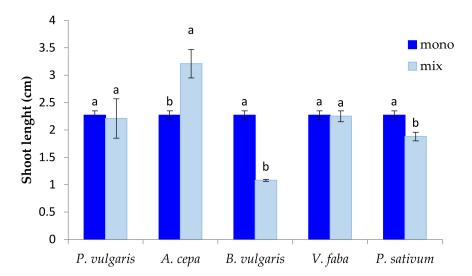
The root length of *P. oleracea* decreased when grown in close proximity to all the crop species. The highest reduction was recorded in the presence of *B. vulgaris* (Figure 7).



**Figure 7.** Root length of *P. oleracea* grown with crop seeds (mono = weed alone; mix = weed grown together with crop). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

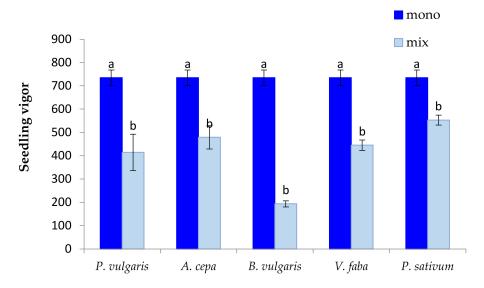
The shoot length of *P. oleracea*, which was not affected by the presence of *V. faba* and *P. vulgaris*, was lower when grown near *B. vulgaris* and *P. sativum*. Additionally, the shoot length of *P. oleracea* was stimulated by the association with *A. cepa* (>29% compared to mono (Figure 8).

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**Figure 8.** Shoot length of *P. oleracea* grown with crop seeds (mono = weed alone; mix = weed grown together with crop). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

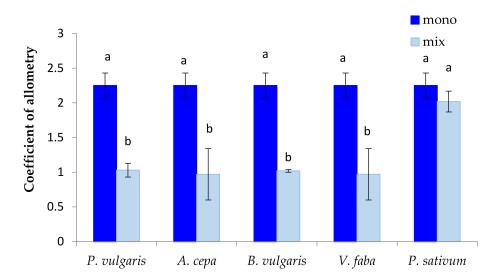
Similar to the root length results, the proximity of all crop seeds had a severe impact on the seedling vigor of *P. oleracea*. Among crop plants, the highest effect on seedling vigor of *P. oleracea* was observed by *B. vulgaris* (Figure 9) and the seedling vigor of *P. oleracea* had a small, but significant decrease grown in close proximity to *P. sativum*.



**Figure 9.** Seedling vigor of *P. oleracea* grown with crop seeds (mono = weed alone; mix = weed grown together with crop). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

The presence of *P. vulgaris*, *A. cepa*, *B. vulgaris*, and *V. faba* strongly decreased the coefficient of allometry of *P. oleracea*. In contrast, the coefficient of allometry for *P. oleracea* did not differ from the control when this weed was grown with *P. sativum* (Figure 10).

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**Figure 10.** Coefficient of allelometry of *P. oleracea* grown with crop seeds (mono = weed alone; mix = weed grown toghether with crop). Bars represent the means of 4 replicates  $\pm$  SE. Bars topped by the same letter indicate no significant difference between treatments at the 5% level using Duncan's multiple-range test.

#### 4. Discussion

Reduced germination in A. cepa and P. vulgaris as a result of the allelopathic potential of P. oleracea indicated that this weed species probably possess allelochemicals which exhibited phytoinhibitory effects on these crops. Alkaloids from seeds of *P. oleracea* such as dopa, dopamine and noradrenaline [26], and monoterpenes are widely known to modify seed germination and seedling growth [27]. Furthermore, inhibition of seed germination may be attributed to the presence of inhibitory allelochemicals. The latter can exert inhibitory effects by affecting cell division and cell elongation [28], and mobilization of stored compounds [29]. Therefore, the cultivation of A. cepa and P. vulgaris is not recommended on the farms with P. oleracea. Among crop species, the germination of P. sativum, V. faba, and B. vulgaris in the presence of P. oleracea was not affected, because these plants were not influenced by P. oleracea. In contrast, the germination of P. oleracea decreased in the presence of P. vulgaris, A. cepa, and B. vulgaris. The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds. As a result, the allelopathic potential of these plants can be used to suppress P. oleracea. There are some phytochemical constituents in the seeds of *P. vulgaris*, such as alkaloids, flavonoids, tannins, terpenoids, and saponins, which alter mitochondrial structure and function, leading to the inability of the cells to use the storage materials [30]. Dadkhah [31] reported that foliar aqueous extracts of B. vulgaris had significant herbicidal effects on seedling and plant growth of P. oleracea, and similar results were observed by El-Shora et al. [32]. According to our results, the presence of P. oleracea also strongly decreased the root length of crop plants. Since roots are sensitive to any chemical changes in their surroundings, they may respond more quickly. Reduced length in roots and shoots might be due to reduced cell division and abnormalities in growth hormones [18]. Alkaloids are among major allelopathic compounds in *P. oleracea*. Alkaloids have been observed to inhibit plant growth by several mechanisms, including interference with DNA, enzyme activity, protein biosynthesis, and membrane integrity in developing plants [33]. Flavonoids also affect the breakdown of auxin by IAA oxidases and peroxidases [34-36] and impact polar auxin transport [35,37,38], thereby affecting the root growth of target species. For example, flavonols identified from lettuce function as allelopathic inhibitors of seedling growth [39].

Seedling vigor was evaluated as a component of vegetative performance or fitness of a plant species. Seedling vigor of all crop species and *P. oleracea* significantly decreased in

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the presence of each other. These results are in agreement with those of Kiran et al. [40], who found that the aqueous extract of *Psoralea corylifolia* L. seeds decreased the seedling vigor of maize (*Zea mays* L.). Dhungana et al. [41] evaluated the allelopathic potential of soybean (*Glycine max* L.) root extract and maize on beggarticks (*Bidens* spp.) and goosegrass (*Eleusine* spp.), reporting that seedling vigor and weed germination were decreased.

The coefficient of allometry of *P. oleracea* decreased in the presence of crop species. Seeds of *V. faba* and *P. vulgaris* contain a high amount of phenolic compounds [42,43], potent inhibitors of cell division, able to decrease radicle and seedling growth [44], thus reducing the coefficient of allometry. Our results confirmed that the studied crop species can be categorized into two groups according to their sensitivity to the inhibitory potential of *P. oleracea*. The first group include *P. vulgaris*, *B. vulgaris*, and *A. cepa*, with a higher inhibitory activity on *P. oleracea* seed germination, and the second group represented by *P. sativum* and *V. faba* with no inhibitory effect on seed germination of the weed.

#### 5. Conclusions

The allelopathic potential of *P. oleracea* was demonstrated against *P. vulgaris* and *A. cepa* plants. Since seed germination is a pivotal stage in the lifecycle of higher plants, the release of inhibitory substances from seeds of *P. oleracea* may impact the competitive ability of the neighboring plant or crop species during the establishment stage. On the other hand, seeds of *P. vulgaris*, *A. cepa*, and *B. vulgaris* exerted a higher reduction in the germination of *P. oleracea*. Therefore, aqueous extracts or selected allelochemicals of *P. oleracea* can be developed as bio-herbicides for controlling weeds, as well as some crop species with allelopathic potential can be used to suppress weeds, thereby decreasing synthetic herbicide dependency in conventional weed management [45].

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10 .3390/app11083539/s1.

**Author Contributions:** Conceptualization, A.R.Y., N.G., P.M., and M.I.; methodology, S.R., M.P., and S.V.; formal analysis, S.R., M.P., and S.V.; investigation, S.R., M.P., and S.V.; writing—original draft preparation, S.R. and M.P.; writing—review and editing, A.R.Y., N.G., P.M., S.V., and M.I.; supervision, A.R.Y., N.G., P.M., and M.I.; funding acquisition, A.R.Y., N.G., P.M., and M.I. All authors have read and agreed to the published version of the manuscript.

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