



PHYTOTOXICITY OF *PLANTAGO MAJOR* EXTRACTS ON GERMINATION AND SEEDLING GROWTH OF PURSLANE (*PORTULECA OLERCEUS*)

A. F. Al-obaidi

Horticulture science Department, Collage of Basic Education/Haditha, Anbar University, Iraq

Abstract

Plantago major L. (Plantaginaceae family) have been used as herbal remedies for centuries in almost all over the world and in the treatment of a number diseases. This study aims to assess the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane. Total phenols, tannins, saponins, flavonoids and alkaloids were determined in *P. major*. Furthermore, concentrations of 2.5, 5, 10, 20, and 40 mg.ml⁻¹ of both alcoholic and aqueous extracts were prepared to study their phytotoxic effect on the germination and seedling growth of *Portuleca olerceus* weed. In our study, showing the germination of *P. olerceus* was completely inhibited (96.30 mg.ml⁻¹) under treatment of *P. major* methanolic extracts at 40 mg ml⁻¹. Moreover, both radicle and plumule were strongly inhibited (87.20 and 74.29 mg.ml⁻¹, respectively) under the same treatment. This could be attributed to the high content of bioactive constituents. Therefore, this species can be used in the method of biological control of weeds. In addition, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

Key words: Allelopathy, *Plantago major*, *Portuleca olerceus*, Phytochemical

Introduction

At present there is a lot of emphasis on finding new methods to fight weeds and concept of competition between plant species has been improved with that of plant allelopathy (Razzaq *et al.*, 2010; Wang *et al.*, 2015). Allelopathy involves the effects of one plant on another because of the chemicals it releases, or the breakdown products of their metabolites (Willis, 1994). There are some examples of plant toxins among the plant secondary compound classes of alkaloids, terpenes, and especially phenolics (Aasifa, 2014). Phytotoxicity assays has been reported to be an important approach for identifying plants that are likely to be a source of vital herbivorous compounds (Scognamiglio *et al.*, 2013; Trezzi *et al.*, 2016).

The allelopathic effects of crop plants or crop residues on weeds benefit farmers, which can cause significant economic losses (Reinhardt *et al.*, 1994). There is competition for weed crops for moisture, nutrients, space and light, which negatively affects crop yield (Kadioglu *et al.*, 2005). It has been reported that

the predominant species of weed allelochemicals stop crop production but sometimes also stimulate seed growth, germination and crop production (Narwal, 1994, Goncalves and Romano, 2016).

Management methods that reduce the requirement for herbicides are needed to reduce adverse environmental impacts. Herbicides can cause crop injury (Bilalis *et al.* 2001). Moreover, there is a keen interest in developing alternative methods of natural weed control in organically grown crops (Bilalis *et al.* 2010), as weed control remain one of the most significant agronomic challenges in the production of organic crops. Weed management is often the most troublesome technical problem to be solved in organic farming, especially in poorly competitive crops like vegetables (Peruzzi *et al.*, 2007; Trezzi *et al.*, 2016). Cultivation and hand hoeing are common practices used in organically grown leek crops.

Portulaca oleracea L. (purslane) is a common troublesome weed worldwide. Despite being considering a poor competitor, it can quickly establish and easily regenerates by vegetative reproduction method

(Mohamed and Hussein, 1994). *Plantago* is the largest genus within the Plantaginaceae family comprising approximately 275 annual and perennial species distributed all over the world (Goncalves and Romano, 2016). *Plantago major* L. (*Plantago major* ssp. *Major* L.) is a perennial plant that belongs to the Plantaginaceae family and is found in fields, lawns, and on the roadsides. It can become about 10-60 cm high, but the size varies a lot depending on the growth habitats. The leaves grow in rosettes, and they are ovate to elliptical with parallel venation (5–9) (Boulos, 2002). In Asia and Europe, the aerial parts of *P. major* is often used as herbal remedies in the treatment of a number of diseases related to the skin, respiratory and digestive organs, reproduction, and against infections (Samuelsen, 2000).

Phytochemical investigation of the genus revealed the presence of polysaccharides, phenylpropanoid glycosides, alkaloids, triterpenes, flavonoids and phenolic acids as the main bioactive compounds present in the aerial parts (Ronsted *et al.*, 2000; Taskova *et al.*, 2002; Haddadian *et al.*, 2014; Tarvainen *et al.*, 2010). The aim of the present study was to evaluate the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane.

Materials and Methods

Plant Material

Plantago major L. was collected from canal banks in Al Anbar city (Iraq) during their vegetative stage (February 2018). The identification of species was done according to Boulos (2002). The plant material was handily cleaned, washed several times with distilled water to remove dust and other residues, dried in room temperature in shaded place for several day till complete dryness and ground into powder, then preserved in well stopped bottles (AOAC, 1990).

Phytochemical Analysis

Plantago major was collected and prepared as previously mentioned. Total phenolics, flavonoids and alkaloids were estimated using spectrophotometric techniques adapted by Harborne (1973), Sadasivam and Manickam (2008) and Boham and Kocipai-Abyazan (1994), respectively. Tannins were determined according to Van-Buren and Robinson (1969), while Saponin content was estimated by the method adopted by Obadoni and Ochuko (2001).

Allelopathy bioassay

Weed seed source

The seeds of *Portulaca oleracea* were collected from cultivated land from Al Anbar, Iraq. Seeds were sterilized by 0.3% sodium hypochlorite for 3 minutes,

washed several times by distilled water, dried at room temperature for 7 days and reserved in paper bag until further use (Sampietro *et al.*, 2009; Uremis *et al.*, 2005).

Preparation of extracts

For bioassay tests, aqueous and methanol extracts were prepared to obtain various concentrations of 2.5, 5, 10, 20, and 40 mg.ml⁻¹ (w/v). The solutions were filtered through double layers of muslin cloth followed by Whatman No. 1 filter paper. The pH of the mixtures was adjusted to 7 with 1 M HCl, and then mixtures were stored in a refrigerator at 4°C until further use (Rice, 1972).

Germination bioassay

For germination experiment, 25 seeds were placed in each filter paper in addition to 10 ml of tested extract for each Petri dish (90 mm diameter). The control treatment was designed with distilled water. Germinated seeds were counted daily starting from the first day of treatment. The design of the experiment was randomized complete block with three replicate. The experiment repeated three times and the inhibition percentage was calculated.

Seedling growth bioassay

The seeds of *Portulaca oleracea* were germinated in the dark at room temperature for 2 days. 25 germinated seeds were placed in Petri dishes lined with two layers of filter paper (Whatman No. 1) and 10 ml of different extracts (2.5, 5, 10, 20, and 40 mg.ml⁻¹) were added. Moreover, a control treatment was designed with distilled water. The design of the experiment was randomized complete block with three replicate. The experiment repeated twice, the radicle and plumule lengths of seedlings were measured on a tenth day and growth inhibition for radicle and plumule lengths were calculated.

Results and Discussion

Phytochemical Constituents

Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues (Turk and Tawaha, 2003; Soyler *et al.*, 2012). The phytochemical constituents of *Plantago major* is presented in table (1). *Plantago major* contained high contents of phenolics (132.2 mg/g dry weight) and tannins (28.7 mg/g dry weight), While contained relatively contents of alkaloids (10.6 mg/g dry weight), saponins (15.8 mg/g dry weight) and flavonoids (14.8 mg/g dry weight).

This results is supported with the study of Kolak *et al.* (2011) and Miser-Salihoglu *et al.* (2013). In addition,

Table 1: Concentrations of the active organic compounds estimated in *Plantago major*.

Plant species	Active organic compounds (mg.g ⁻¹ dry weight)				
	Phenolics	Tannins	Alkaloids	Flavonoids	Saponins
<i>Plantago major</i>	132.2±2.35	28.7±0.89	10.6±0.05	14.8±0.21	15.8±0.06

this results relatively comparable to those reported in *Senecio glaucus* as described by El-Amier *et al.* (2014) with the exception of phenols less, but higher than those reported by Kobeasy *et al.* (2011) on same species and El-Amier *et al.* (2016) on *Euphorbia terracina* as well as El-Amier and Abdullah (2014) on some wild plants (*Calligonum polygonoides*, *Cakile maritima* and *Senecio glaucus*).

Allelopathic effect of *P. major* extracts on *P. oleracea* germination

Allelopathy is some plant's affecting the others, either positively or negatively, by exuding chemicals (Chon *et al.*, 2003). In the present study, the allelopathic effect of shoot extracts (aqueous and methanol) on the germination percentage of *Portuleca olerceus* at 4 DAT was shown in fig. 1. It is observed from the Figure that the methanolic extract of *Plantago major* exhibited higher germination inhibition of *Portuleca olerceus* than the aqueous extract. This could be attributed to the methanol polarity that has ability to extract a wide variety of active components compared to water (Oskoueian *et al.* 2011). The degree of inhibition was significantly increased in a concentration-dependent manner. The aqueous extract of *P. major* at 40 mg ml⁻¹ inhibited the germination of *P. oleracea* by about 30.24%, while the lowest concentration (2.5 mg ml⁻¹) inhibited the germination by 4.60%. On the other hand, *P. major* methanolic extract showed a highest inhibition of germination at 40 mg ml⁻¹, while at 2.5 mg ml⁻¹ exhibited lowest inhibition percentage (20.37%).

Many plant species showed inhibitory effects on *P.*

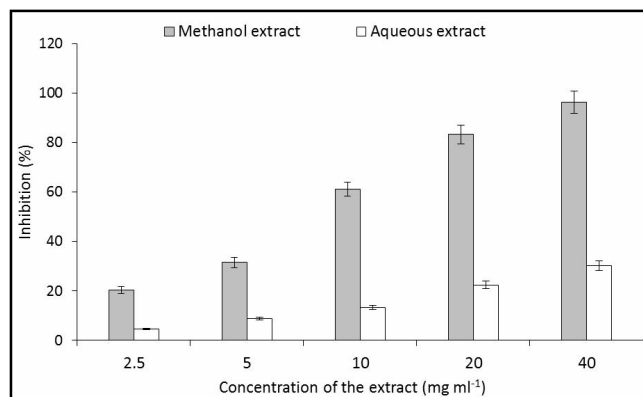


Fig. 1: The allelopathic effect of both aqueous and methanolic *Plantago major* extracts on the germination inhibition percentage (mean value) with the error bars of *Portuleca olerceus* ten days after treatment.

oleracea germination such as *Medicago sativa* and *Vicia cracca* (Koloren, 2007), *Salvia macrochlamys* (Erez and Fidan, 2015), wheat and rye straw (Boz, 2003). Aqueous extract of some plant species may contain some toxic substances (Habib and Abdul Rehman, 1988). These substances probably inhibit the germination and seedling growth of other plants species (Al-Charchafchi *et al.*, 1987), which was due to their interference with indol acetic acid metabolism, or synthesis of protein and ions uptake by the plants (Hussain and khan, 1988).

Allelopathic effect of *P. major* extracts on *P. oleracea* seedling growth

Allelopathy offers potential for biorational weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials. Under appropriate conditions, allelochemicals often exhibit selectivity, similar to synthetic herbicides (Weston, 1996).

The allelopathic effect of both aqueous and methanolic extracts on *Portuleca olerceus* radicle growth after ten days of treatment revealed that there was significant variation between different extracts. However, the degree of inhibition significantly increased in a dose-dependent manner (fig. 2). The aqueous extract of *P. major* showed 52.34% at 40 mg ml⁻¹, while showed the lowest inhibition percentage of radicle growth (3.5%) at 2.5 mg ml⁻¹ (Figure 2). On the other side, the methanolic extracts from *P. major* at 40 mg ml⁻¹ inhibited the radicle growth of *Portuleca olerceus* by 87.20%, while at the lowest concentration (2.5 mg ml⁻¹), *P. major* extract showed the lowest inhibition percentage (19.21%) of radicle growth (fig. 2).

The phytotoxic effect of both methanolic and aqueous extracts from the studied *Plantago* species on *Portuleca olerceus* plumule growth revealed slight significant variation between two extracts. However, there was a very large difference between different concentrations (fig. 3). The aqueous extract from *P. major* showed the highest inhibition percentage of *Portuleca olerceus* plumule growth (48.69%) at 40 mg ml⁻¹. While, at 2.5 mg ml⁻¹ *P. major* extract inhibited the plumule growth by 4.11%. On the other hand, the methanolic extract of *P. major* exhibited high inhibition (74.29%) *P. olerceus* plumule growth at 40 mg ml⁻¹. While the lowest concentration (2.5 mg ml⁻¹) of *P. major* extract inhibited

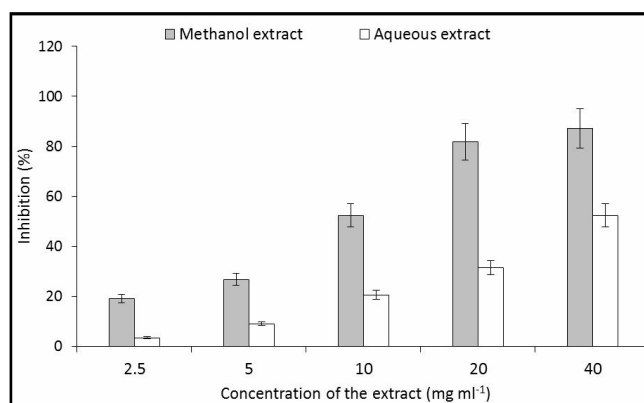


Fig. 2: The allelopathic effect of both aqueous and methanolic *Plantago major* extracts on the radicle growth inhibition percentage (mean value) with the error bars of *Portuleca olerceus* ten days after treatment.

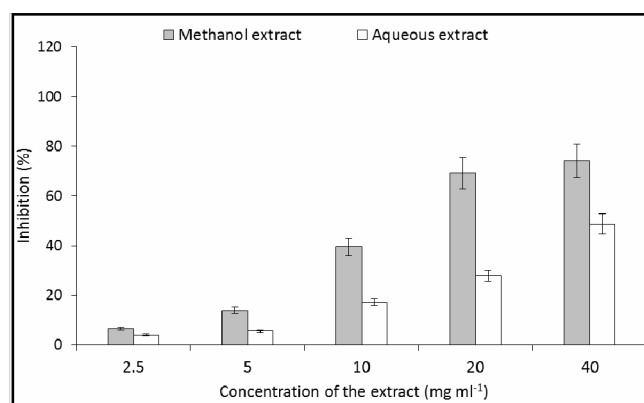


Fig. 3: The allelopathic effect of both aqueous and methanolic *Plantago major* extracts on the plumule growth inhibition percentage (mean value) with the error bars of *Portuleca olerceus* ten days after treatment.

the plumule growth by 60.95% (fig. 3). Phytochemical investigation of the genus revealed the presence of phenylpropanoid glycosides, alkaloids, triterpenes, flavonoids and phenolic acids as the main bioactive compounds present in the aerial parts (Taskova *et al.*, 2002; Haddadian *et al.*, 2014; Tarvainen *et al.*, 2010).

The allelopathic effect of *P. major* could be attributed to several bioactive compounds that act in a synergistic manner or to compounds which regulate one another such as flavonoid, phenolic acids, saponin, alkaloids and tannins. *Plantago* species was reported to contain several bioactive secondary metabolites such as vanillic acid, iridoid glycoside (aucubin), caffeic acid derivatives, chlorogenic acid, ferulic acid, *p*-coumaric acid and triterpenes (oleanolic acid, ursolic acid) (Long *et al.*, 1995; Samuelson, 2000; Chiang *et al.*, 2002). Many of these compounds were reported as allelochemicals (Cheema *et al.*, 2013). Generally, the reduction in the seedling growth of *P. olerceus* in this study may be attributed to

reduction in cell division of the seedlings, altering the ultrastructure of the cells as well as led to alteration of the ion uptake, water balance, phytohormone balance, photosynthesis, respiration and inactivate several enzymes (Li *et al.*, 2010; Fahmy *et al.*, 2012).

Conclusion

In conclusion, the aim of this study was to assess the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane. In our study, showing the germination of *Portuleca olerceus* was completely inhibited under treatment of *P. major* methanolic extracts at 40 mg ml⁻¹. Moreover, both radicle and plumule were strongly inhibited under the same treatment. This could be attributed to the high content of bioactive constituents. Therefore, this species can be used in the method of biological control of weeds. In addition, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

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