



# 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<sup>-1</sup> 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<sup>-1</sup>) under treatment of *P. major* methanolic extracts at 40 mg ml<sup>-1</sup>. Moreover, both radicle and plumule were strongly inhibited (87.20 and 74.29 mg.ml<sup>-1</sup>, 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<sup>-1</sup> (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<sup>-1</sup>) 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*.

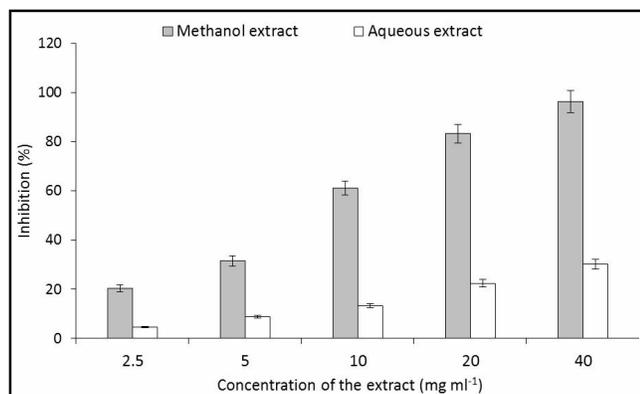
Plant species	Active organic compounds (mg.g <sup>-1</sup> dry weight)				
	Phenolics	Tannins	Alkaloids	Flavonoids	Saponins
<i>Plantago major</i>	132.2±2.35	28.7±0.89	10.6±0.05	14.8±0.21	15.8±0.06

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<sup>-1</sup> inhibited the germination of *P. oleracea* by about 30.24%, while the lowest concentration (2.5 mg ml<sup>-1</sup>) inhibited the germination by 4.60%. On the other hand, *P. major* methanolic extract showed a highest inhibition of germination at 40 mg ml<sup>-1</sup>, while at 2.5 mg ml<sup>-1</sup> 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.

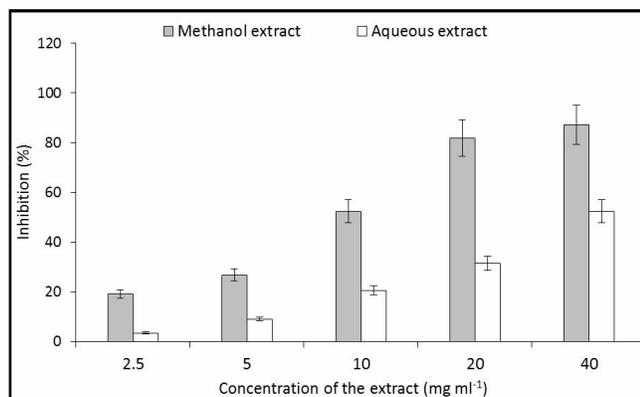
*oleracea* germination such as *Medicago sativa* and *Vicia cracca* (Koloren, 2007), *Salvia macrochlamys* (Erez and Fidan, 2015), wheat and rye straw (Boz, 2003). Aqueous extract of some plant species may contain some toxic substances (Habib and Abdul Rehman, 1988). These substances probably inhibit 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

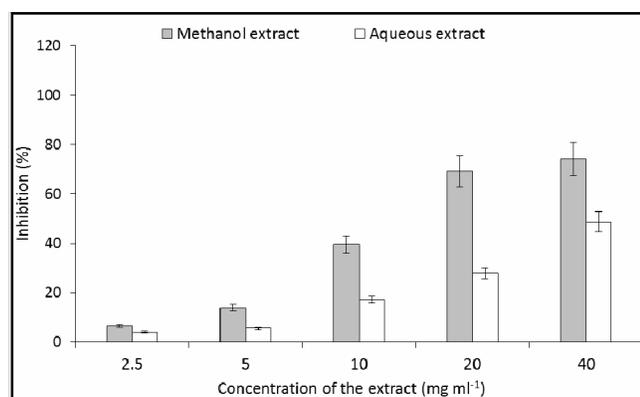
Allelopathy offers potential for biorational weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials. Under appropriate conditions, allelochemicals often exhibit selectivity, similar to synthetic herbicides (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<sup>-1</sup>, while showed the lowest inhibition percentage of radicle growth (3.5%) at 2.5 mg ml<sup>-1</sup> (Figure 2). On the other side, the methanolic extracts from *P. major* at 40 mg ml<sup>-1</sup> inhibited the radicle growth of *Portuleca olerceus* by 87.20%, while at the lowest concentration (2.5 mg ml<sup>-1</sup>), *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<sup>-1</sup>. While, at 2.5 mg ml<sup>-1</sup> *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<sup>-1</sup>. While the lowest concentration (2.5 mg ml<sup>-1</sup>) 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<sup>-1</sup>. 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.

## References

- Aasifa, G. (2014). Allelopathic effect of aqueous extracts of different part of *Eclipta alba* (L.) Hassk. on some crop and weed plants. *Journal of Agricultural Extension and Rural Development*, **6**: 55–60.
- Al-Charchafchi, F.M.R., F.M.J. Redha and W.M. Kamel (1987). Dormancy of *Artemisia herba alba* seeds in relation to endogenous chemical constituents. *Journal of Biological Sciences Research, Baghdad/Iraq*, **18**: 1-12.
- AOAC (1990). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, Virginia, USA.
- Bilalis, D., P. Efthimiadis and G. Katagiannis (2001). The phytotoxicity of various graminicides in durum wheat in Greece. *Journal of Agronomy and Crop Science*, **187**: 121–126.
- Bilalis, D., P. Papastylianou, A. Konstantas, S. Patsiali, A. Karkanis and A. Efthimiadou (2010). Weed-suppressive effects of maize–legume intercropping in organic farming. *International Journal of Pest Management*, **56**: 173–181.
- Boham, B.A. and R. Kocipai-Abyazan (1994). Flavonoids condensed tannin from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science*, **48**: 458-463.
- Boulos, L. (2002). Flora of Egypt. Vol. 3. (Verbenaceae-Compositae). AL-Hadara Publishing, Cairo, Egypt.
- Boz, Ö. (2003). Allelopathic effects of wheat and rye straw on some weeds and crops. *Asian journal of plant Sciences*, **2(10)**: 772-778.
- Cheema, Z.A., M. Farooq and A. Wahid (2013). Allelopathy: current trends and future applications. Springer-Verlag

- Berlin Heidelberg 2013, pp 113–143.
- Chiang, L.C., W. Chiang, M.Y. Chang, L.T. Ng and C.C. Lin (2002). Antiviral activity of *Plantago major* extracts and related compounds *in vitro*. *Antiviral Research*, **55**: 53–62.
- Chon, S.U., Y. Kin and J.C. Kee (2003). Herbicidal potential and quantification of causative allelochemicals from several compositae weeds. *Weed Research*, **43**: 444–450.
- El-Amier, Y.A. and T. J. Abdullah (2014). Allelopathic effect of four wild species on germination and seedling growth of *Echinochloa crus-galli* (L.) P. Beauv. *International Journal of Advanced Research*, **2(9)**: 287–294.
- El-Amier, Y.A., A.M. Abdelghan and A. Abed Zaid (2014). Green synthesis and antimicrobial activity of *Senecio glaucus* - Mediated silver nanoparticles, *Research Journal of Pharmaceutical, Biological and Chemical*, **5**: 631–642.
- El-Amier, Y.A., O.N. Al-Hadithy, H.L. Abdulhadi, E.M. Fayed (2016). Evaluation of antioxidant and antimicrobial activities of *Euphorbia terracina* L. from Deltaic Mediterranean coast, Egypt. *Journal of Natural Products and Resources*, **2(2)**: 83–85
- Erez, M.E. and M. Fidan (2015). Allelopathic effects of Sage (*Salvia macrochlamys*) extract on germination of *Portulaca oleracea* seeds. *Allelopathy Journal*, **35(2)**: 285–296.
- Fahmy, G.M., N.A. Al-Sawaf, H. Turki, H.I. Ali (2012). Allelopathic potential of *Pluchea dioscoridis* (L.) DC. *Journal of Applied Science Research*, **8**: 3129–42.
- Goncalves, S. and A. Romano (2016). The medicinal potential of plants from the genus *Plantago* (Plantaginaceae). *Industrial Crops and Products*, **83**: 213–226.
- Habib, S.A. and A.A. Abdul-Rehman (1988). Evaluation of some weed extracts against dodder on alfalfa (*Medicago sativa*). *Journal of Chemical Ecology*, **14**: 443–452
- Haddadian, K., K. Haddadian and M. Zahmatkash (2014). A review of *Plantago* plant. *Indian Journal of Traditional Knowledge*, **13(4)**: 681–5.
- Harborne, J.B. (1973). Phytochemical methods, Chapman and Hall, Ltd., London, pp. 49–188.
- Hussain, F. and T.W. Khan (1988). Allelopathic effects of Pakistani weed *Cynodon dactylon* L. *Journal of Weed Science Research*, **1**: 8–17.
- Kadioglu, I., Y. Yanar and U. Asav (2005). Allelopathic effects of weed extracts against seed germination of some plants. *Journal of Environmental Biology*, **26(2)**: 169–73.
- Kobeasy M.I., M. Abdel-Fatah, S.M. Abd El-Salam and Z.M. Mohamed (2011). Biochemical studies on *Plantago major* L. *International Journal of Biodiversity and Conservation*, **3**: 83–91.
- Kolak, U., M. Boga, E. Akalin Urusak and A. Ulubelen (2011). Constituents of *Plantago major* subsp. *intermedia* with antioxidant and anticholinesterase capacities. *Turkish Journal of Chemistry*, **35**: 637–645
- Koloren, O. (2007). Allelopathic effects of *Medicago sativa* L. and *Vicia cracca* L. Leaf and root extracts on weeds. *Pakistan Journal of Biological Sciences*, **10**: 1639–1642.
- Li, Z., Q. Wang, X. Ruan, C. Pan and D. Jiang (2010). Phenolics and plant allelopathy. *Molecules*, **15**: 8933–52.
- Long, C., C. Moulis, E. Stanislas and I. Fouraste (1995). L'aucuboside et le catalpol dans les feuilles de *Plantago lanceolata* L., *Plantago major* L. et *Plantago media* L. *Journal De Pharmacie De Belgique*, **50**: 484–8.
- Miser-Salihoglu, E., G. Akaydin, E. Caliskan-Can and S. Yardim-Akaydin (2013). Evaluation of antioxidant activity of various herbal folk medicines. *Journal of Nutrition and Food Sciences*, **3(5)**: 1–9.
- Mohamed A.I. and A.S. Hussein (1994). Chemical-composition of purslane (*Portulaca oleracea*). *Plant Food and Human Nutrition*, **45(1)**: 1–9.
- Narwal, S.S. (1994). Allelopathy in Crop Production. Scientific Publisher, Jodhpur, India, pp: 288.
- Obadoni, B.O. and P.O. Ochuko (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in edo and delta states of Nigeria. *Global Journal of Pure Applied Science*, **8**: 203–208.
- Oskoueian, E., N. Abdullah, S. Ahmad, W.Z. Saad, A.R. Omar and Y.W. Ho (2011). Bioactive Compounds and Biological Activities of *Jatropha curcas* L. Kernel Meal Extract. *International Journal of Molecular Sciences*, **12(9)**: 5955–5970.
- Peruzzi, A., M. Ginanni, M. Fontanelli, M. Raffaelli and P. Bàrberi (2007). Innovative strategies for on-farm weed management in organic carrot. *Renewable Agriculture and Food Systems*, **22**: 246–259
- Razaq, Z.A, K. Cheema, K. Jabran, M. Farooq, A. Khaliq, G. Haider and S.M.A. Basra (2010). Weed management in wheat through combination of allelopathic water extract with reduced doses of herbicides. *Pakistan Journal of Weed Science Research*, **16(3)**: 247–256.
- Reinhardt, C.F., R. Meissner and N. Labuschagne (1994). Allelopathic interaction of *Chenopodium album* L. and certain crop species. *South African Journal of Plant and Soil*, **11**: 45–49
- Rice, E.L. (1972). Allelopathic effect of *Andropogon virginicus* and its persistence in old field. *American Journal of Botany*, **59**: 752–5.
- Ronsted, N., E. Gobel, H. Franzyk, J. S. Rosendal and C.E. Olsen (2000). Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides. *Phytochemistry*, **55**: 337–348.
- Sadasivam, S. and A. Manickam (2008). Biochemical methods, 3rd Ed., New Age International Limited, New Delhi.
- Sampietro, D.A., C.A.N. Catalan and M.A. Vattuone (2009). Isolation, identification and characterization of allelochemicals natural products. Enfield, NH, USA:

- Science Publishers; 2009.
- Samuelson, A.B. (2000). The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. *Journal of Ethnopharmacology*, **71**: 1–21.
- Scognamiglio, M., B. D'Abrosca, A. Esposito, S. Pacifico, P. Monaco, A. Fiorentin (2013). Plant growth inhibitors: allelopathic role or phytotoxic effects. Focus on Mediterranean biomes. *Phytochemistry Reviews*, **12**(4): 803–830
- Soyler, D., E. Canýhoř, N. Temel and M. Hajyzadeh (2012). Determination of chemical fungicide against soil borne fungal diseases of capers (*Capparis ovata* Desf. var. *herbacea*) during early stages. *Pakistan Journal of Agriculture Science*, **49**: 345-348.
- Tarvainen, M., J.P. Suomela, H. Kallio and B. Yang (2010). Triterpene acids in *Plantago major*: identification, quantification and comparison of different extraction methods. *Chromatographia*, **71**: 279–284.
- Taskova, R., L.J. Evstatieva, N. Handjieva and S. Popov (2002). Iridoid patterns of genus *Plantago* L. and their systematic significance. *Zeitschrift für Naturforschung*, **57c**: 42–50.
- Trezzi, M.M., R.A. Vidal, A.A. Balbinot, H.V. Bittencourt and A. Souza (2016). Allelopathy: driving mechanisms governing its activity in agriculture. *Journal of Plant Interaction*, **11**: 53–60.
- Turk, M.A. and A.M. Tawaha (2003). Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop Protection*, **22**: 673-677.
- Uremis, I., M. Arslan and A. Uludag (2005). Allelopathic effects of some *Brassica* species on germination and growth of cut leaf ground cherry (*Physalis angulata* L.). *Journal of Biological Science*, **5**: 661–665.
- Van-Buren, J.P. and W.B. Robinson (1969). Formation of complexes between protein and tannic acid. *Journal of Agricultural and Food Chemistry*, **17**: 772-777.
- Wang, H., Y. Zhou, Y. Chen, Q. Wang, L. Jiang and Y. Luo (2015). Allelopathic Potential of Invasive *Plantago virginica* on Four Lawn Species. PLoS ONE, **10**(4): e0125433. <http://doi.org/10.1371/journal.pone.0125433>
- Weston, L.A. (1996). Utilization of allelopathy for weed management in agroecosystems. *Agronomy Journal*, **88**: 860-866.
- Willis, R.J. (1994). Terminology and trends in allelopathy. *Allelopathy Journal*. **1**(1): 6-28.