

The Potential Allelopathic Effects of *Varthemia iphionoides* and the Identification of Phenolic Allelochemicals

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Received: July 6, 2015 Revised: August 23, 2015 Accepted: August 27, 2015

Abstract

The allelopathic effects of aqueous extracts from *Varthemia iphionoides* (Compositae) leaves were tested on germination and early seedling growth of wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), tomato (*Solanum lycopersicum* L.), and pepper (*Capsicum annuum* L.). The experiments were conducted in petri dishes and treatments were arranged in a completely randomized design with eight replications. *V. iphionoides* aqueous extract was applied in 1.25, 2.5, 5.0 and 10% concentrations and water was used as a control. Germination was observed daily and, after 7 days, radicals length and seedlings dry weight were recorded. Total germination percentage, germination rate, seedling vigor index, and seedling dry weights were significantly inhibited by *V. iphionoides* aqueous extracts. The germination percentage of chickpea seeds was almost unaffected by the treatment. The extract enhanced germination and seedling dry weights in chickpea at the lowest extract concentration. Tomato and wheat were the most sensitive to *V. iphionoides* extract followed by pepper and barley. On the other hand, chickpea and lentil were less affected. Using LC-ESI-MS/MS analysis, ten phenolic compounds were identified in *V. iphionoides* leaves. The results of the current study suggested that *V. iphionoides* aqueous extract possesses allelopathic properties and extract activities are species-specific and concentration-dependent.

Keywords: Allelopathy, germination, phenolic compounds, seedling vigor index, *Varthemia iphionoides*, aqueous extracts.

1. Introduction

Allelopathy is defined as any direct or indirect harmful or beneficial effects of one plant species on another through the release of phytochemicals (allelochemicals) under natural conditions (Rice, 1984). Allelochemicals are present in almost all plant organs and are released into the environment via a variety of processes, including root exudation, volatilization, leaching, and decomposition of plant residues (Inderjit and Duke, 2003; Weston and Duke, 2003).

The phenylpropanoid pathway is the main source of allelochemicals in plants. These allelochemicals are diverse in structures and mode of actions and are known to inhibit growth, development, and several physiological processes in receptor plants (Gniazdowska and Bogatek, 2005; Lorenzo *et al.*, 2008); in consequences, affecting competition and dominance within plant communities and

contribute to the success of invasive species (Abu-Romman and Ammari, 2015; Callaway and Ridenour, 2004; Ens *et al.*, 2009). Some plant species are able to inhibit their own kind through the release of phytotoxic compounds (Singh *et al.*, 2009). Allelopathy is currently practiced in organic agriculture as a biological and eco-friendly practice of weed control instead of using herbicides (Hoagland *et al.*, 2008; Khaliq *et al.*, 2010; Vyvyan, 2002).

Varthemia iphionoides Boiss is a member of the Compositae family and is widely distributed in Jordan mainly in semidry lands (Al-Eisawi, 1982). *V. iphionoides* is a 30-80 cm long bushy-perennial herb with many aromatic and sticky stems (Feinbrun-Dothan, 1977). Aqueous extracts of *V. iphionoides* are used in folk medicine of the eastern Mediterranean region as infusion against diabetes mellitus and gastrointestinal disorder (Afifi *et al.*, 1997). Furthermore, flavonoids isolated from this medicinal plant exhibited antimicrobial activity and

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* **Abbreviations:** GR: germination rate; LC-ESI-MS/Ms.: liquid chromatography-electrospray ionization-tandem mass spectrometry; LSD: least significant difference; SVI: seedling vigor index.

antiplatelet activity on human blood (Afifi *et al.*, 1991; Afifi and Aburjai, 2004; Al-Dabbas *et al.*, 2006).

V. iphionoides has become a noxious weed in field crops in Jordan and inhibition zones around this plant in the fields were noticed (Abu-Romman, personal observations). Therefore, it would be reasonable to propose that *V. iphionoides* plants exhibit allelopathic effects. Hence, this study was designed to determine whether aqueous extracts of *V. iphionoides* leaves exhibit allelopathic potential on germination characteristic and early seedling growth of wheat, barley, chickpea, lentil, tomato, and pepper. In addition, we identified phenolic compounds in *V. iphionoides* leaves using LC-ESI-MS/MS analysis.

2. Materials and Methods

2.1. Plant material and extract preparation

Leaves of *Varthemia iphionoides* were collected in August, 2013 from wild population in Al-Salt, Jordan (32°12'N latitude; 35°74'E longitude; 800 m above sea level). Leaves were well cleaned with tap water. Leaf tissues were oven-dried at 50 °C for three days and ground to a fine powder.

A hundred grams of tissue powder were used to make aqueous extract by soaking in 1 L of distilled water for 24 h at room temperature. The resultant extract was first filtered through three layers of cheesecloth to remove tissue debris, followed by a second filtration through Whatmann No. 1 filter paper. The extracts obtained were considered to be the stock extract (10 % w/v). The stock extract was diluted to give final concentrations of 1.25, 2.50, 5.00 and 10 % (w/v). Distilled water was used as a control.

2.2. Bioassay

The agricultural crops selected were wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), tomato (*Solanum lycopersicum* L.), and pepper (*Capsicum annum* L.). All seeds were surface-sterilized in 70% ethanol for 10 min and then by soaking in 2% sodium hypochlorite for 5 min followed by rinsing several times in sterile water.

Fifteen seeds of each species were evenly placed on three-layer filter papers (Whatmann No. 1) in a sterilized 9-cm Petri dishes. All Petri dishes were placed in a dark incubator at 25 ± 1 °C.

Initially, filter papers were moistened with 10.0 ml of respective extract concentration as treatment and distilled water as control. Two ml of the extract solution for the treatment and 2.0 ml of distilled water for the control were added daily. Germination was observed daily over a 7-day period. Germination was considered to occur only after the radical had protruded beyond the seed coat by at least 1.0 mm. Seven days after sowing, the germination percentage was calculated using the formula: [(germinated seeds/total seeds) × 100]. The rate of germination (GR) was calculated according to the formula of Noor *et al.* (1995): $[\Sigma G/t]$, where G is the number of

seeds germinated at every day and t is the total germination period in days.

Seedling Vigor Index (SVI) was calculated using the formula: [germination % × radical length (cm)] (Abdulbaki and Anderson, 1973). Dry weight of seedlings were measured 7 days after germination by drying seedlings in an oven at 50 °C until the seedlings are completely dry and their weights are constant.

2.3. Identification of phenolic allelochemicals

Identification and quantification of phenolic allelochemicals were performed using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

The analysis of phenolic compounds in *V. iphionoides* extract was carried out using Agilent 1100 chromatography system (Agilent 1100, Agilent Technologies, Wilmington, DE, USA) equipped with a diode array UV detector. The samples were injected into a Thermo C₁₈ reversed phase column (pore size 5 µm, 250 × 4.6 mm i.d. Thermo Fisher Scientific, San Jose, CA, USA).

The analysis employed a gradient solvent system using aqueous formic acid (1%) as solvent (A), and methanol/acetonitrile/formic acid mixture (89.5/9.5/1 v/v/v) as solvent (B). A seven-step linear gradient elution for a total run time of 65 min was carried out using the solvent gradient as follows: 0-10 min 90-70 solvent A and 10-30% solvent B, 10-15 min isocratic, 15-25 min 60 solvent A and 40% solvent B, 25-40 min 50% solvent A and 50% solvent B, 40-50 min to 100% solvent B, 50-55 min 90% solvent A and 10% solvent B and 55-65 min isocratic. An injection volume of 15 µL at constant flow of 0.75 mL/min was employed. The entire flow from the HPLC was directed into the triple-quadrupole mass spectrometer (API 3200; MDS Sciex, Concord, ON, Canada). The mass spectral data were acquired on a negative ion mode. The ESI conditions were as follows: capillary voltage of 4000 V, the ion source was Atmospheric Pressure Chemical Ionization, a cone voltage of 70 V. The dry temperature was 350°C and the drying gas, N₂, had a flow rate of 4.0 L/min. Product ion scans for mass were performed by low-energy collision (10 eV) and helium was used as the nebulizer gas with a flow rate of 40 psi. Diode array UV detector was used to scan mode between 200 and 400 nm to evaluate contents of individual phenolic compounds. A maximum absorbance of 280 nm was selected to determine the contents of the individual phenolic compounds. The mixture of external standards was used to quantify the contents of individual phenolic compounds. All LC-ESI-MS/MS data were processed by Bruker Daltonics data analysis software (version 4.0).

2.4. Statistical analysis

The experiment was designed to have eight replications using a completely randomized design. All values were expressed as percentages compared to the control. Means are separated using the LSD test, and statistical significance was evaluated at $P < 0.05$.

3. Results

The effects of aqueous extract at different concentrations from *V. iphionoides* on germination percentage of six test plant species are shown in Figure 1. The results illustrated that germination percentage of all test species except for chickpea was significantly altered. The response toward the inhibitory potential of the aqueous extracts varied among the examined species. At all concentrations of aqueous extract; the final germination percentages of wheat, barley, and tomato were significantly reduced with each increase in the concentration of *V. iphionoides* aqueous extracts. Pepper germination percentage was only significantly reduced at 5% and 10% extract concentrations. The final germination percentage of chickpea was not significantly altered in response to *V. iphionoides* aqueous extracts. The lentil germination percentage was significantly reduced at only 5% and 10% extract concentration (Figure 1).

The application of *V. iphionoides* aqueous extracts had significant effects on the germination rate (GR) of all the examined species (Figure 2). GRs of wheat, barley, tomato, and pepper were significantly suppressed by all applied concentrations of the extract. At 10% extract concentration, the GRs relative to the control were 55.7%, 46.4% and 54.8% for wheat, tomato and pepper, respectively.

An enhancement in GR compared to the control was recorded for chickpea when treated with 1.25% extract concentration. The GR of chickpea was only significantly reduced at the highest extract concentration (10%) that was used in the experiment. GR of lentil had the lowest values at 10% extract concentrations. However, lentil and chickpea GRs were least inhibited regardless of the aqueous extract concentrations compared to wheat, barley, tomato, and pepper.

The Seedling Vigor Index (SVI) of all the examined species was significantly inhibited in response to *V. iphionoides* aqueous extract treatment (Figure 3). SVI recorded the lowest values in tomato and wheat followed by pepper and barley. On the other hand, SVI of lentil and chickpea was only significantly lowered by the application of the highest extract concentration (10%). It was noted that the lowest concentration (1.25%) of aqueous extract stimulated chickpea SVI compared to the control. Tissue browning in the radical of tomato seedlings was noticed at 5 and 10% concentrations of *V. iphionoides* aqueous extract (data not shown).

The seedling dry weights were also significantly affected in response to the aqueous extracts of *V. iphionoides* (Figure 4). Among all examined crops, the dry weights of tomato and wheat seedlings were the most negatively affected, followed by pepper and barley. The dry weight of lentil seedlings was less affected compared to the previously mentioned crops. A slight increase in the dry weight of chickpea seedlings was observed at 1.25% extract concentration. However, the dry weight of chickpea seedlings was only reduced significantly when treated with the highest aqueous extract concentration.

The determination of phenolic compounds in *V. iphionoides* leaf extract was performed using LC-ESI-MS/MS analysis. The results presented in Table 1

revealed the presence of ten phenolic compounds in *V. iphionoides* leaves, i. e. *p*-hydroxybenzoic acid, ferulic acid, vanillic acid, tyrosol, chlorogenic acid, *p*-coumaric acid, naringin, quercetin, sinapic acid and rosmarinic acids. Among these phenolic compounds, vanillic acid was the most prominent in leaves of *V. iphionoides*, followed by *p*-coumaric acid, tyrosol and ferulic acid.

Table 1. The nature and concentration of phenolic compounds identified by LC-ESI-MS/MS analysis from leaves of *V. iphionoides*.

Phenolic compound	Concentration ($\mu\text{g g}^{-1}$ dry eaves)
<i>p</i> -Hydroxybenzoic acid	2.4
Ferulic acid	28.8
Vanillic acid	139.6
Tyrosol	36.4
Chlorogenic acid	7.94
<i>p</i> -Coumaric acid	47.2
Naringin	0.104
Quercetin	1.42
Sinapic acid	2.88
Rosmarinic acids	4.36

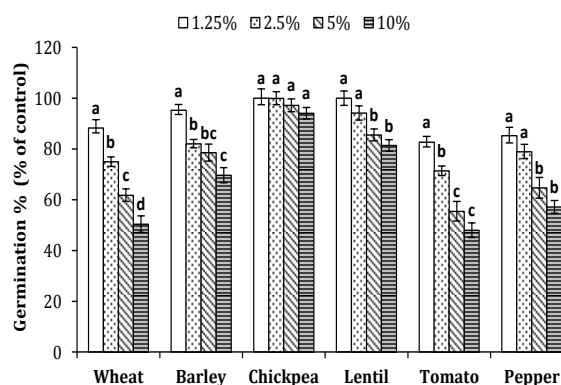


Figure 1. Effect of aqueous extract at different concentrations from *V. iphionoides* leaves on final germination percentages of six agricultural crops. Results are presented as the percentage of control plants. Different letters within the same crop species indicate significant differences using LSD test at $P < 0.05$.

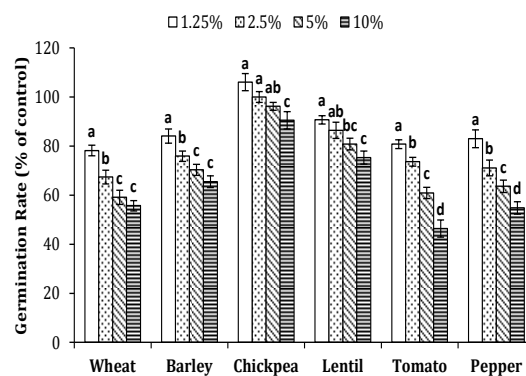


Figure 2. Effect of aqueous extract at different concentrations from *V. iphionoides* leaves on germination rates of six agricultural crops. Results are presented as the percentage of control plants. Different letters within the same crop species indicate significant differences using LSD test at $P < 0.05$.

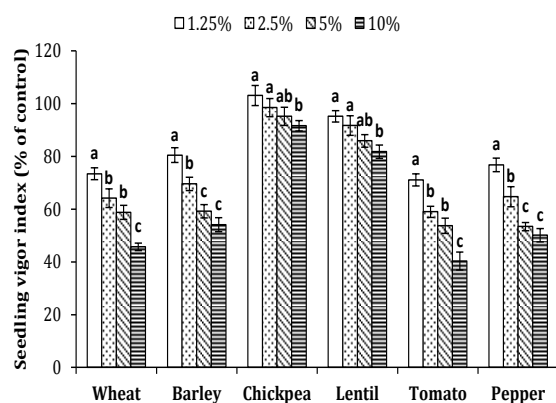


Figure 3. Effect of aqueous extract at different concentrations from *V. iphionoides* leaves on seedling vigor indexes of six agricultural crops. Results are presented as the percentage of control plants. Different letters within the same crop species indicate significant differences using LSD test at $P < 0.05$.

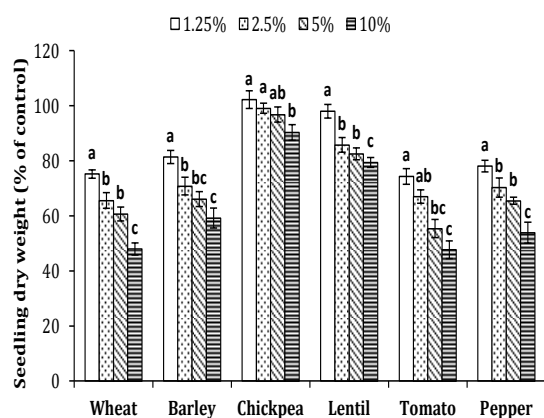


Figure 4. Effect of aqueous extract at different concentrations from *V. iphionoides* leaves on seedling dry weights of six agricultural crops. Results are presented as the percentage of control plants. Different letters within the same crop species indicate significant differences using LSD test at $P < 0.05$.

4. Discussion

The present results indicate that aqueous extracts of *V. iphionoides* plants contain indeed growth inhibitors that are capable of reducing germination and growth of the studied species. The responses of the studied species to the aqueous extracts of *V. iphionoides* were markedly different. This indicates that the degree of allelopathic interference of *V. iphionoides* aqueous extracts were quite species-specific. Similar results were previously reported with different target species and allelochemical sources (Bhowmik and Inderjit, 2003; El Ayeb *et al.*, 2013; Mutlu and Atici, 2009; Vrchatová *et al.*, 2011). The different responses among species and even within species to allelochemicals result mainly from the target plant genetic makeup, which is responsible for the physiological and the biochemical characteristics (Kobayashi, 2004; Prati and Bosendorf, 2004). Moreover, the differential variation in the target species responses to allelochemicals could result in part to the biometric parameters of the plant seeds (Pellissier, 2013).

With exception of chickpea, final germination percentages of the test species were reduced and were proportional to the extract concentrations applied. Moreover, the germination process was delayed in response to the treatment with *V. iphionoides* aqueous extract. However, treating chickpea seeds with the lowest extract concentration showed slight enhancement of GR. The effect of aqueous extracts on SVI were almost similar to those observed in GR.

Allelochemicals are known to influence the metabolic and physiological processes during seed germination (Abu-Romman, 2011; Chon *et al.*, 2005; Rashid *et al.*, 2010). Phenolic allelochemicals were shown to delay seed imbibition and inhibit germination enzymes, which, therefore, resulted in slowing down starch degradation and sucrose hydrolysis during germination (Lara-Núñez *et al.*, 2009; Politycka and Gmerek, 2008; Singh *et al.*, 2009; Tawaha and Turk, 2003).

Stimulation of GR and SVI by lower concentration of the extract was previously reported (Duke *et al.*, 2006; Liu and Chen, 2011; Sampietro and Vattuone, 2006; Singh *et al.*, 2008). Lower concentrations of allelochemicals were shown to stimulate amylase activity and therefore increased solubilization of starch during the germination process of some species (Rizvi *et al.*, 1989; Singh *et al.*, 2009).

Seedling dry weights were negatively affected by treatments with aqueous extracts of *V. iphionoides*. Allelochemicals were shown to impose oxidative stress on the target species. This oxidative stress interferes with cell division, cell elongation, and phytohormone induced growth (Golisz *et al.*, 2008; Jacob and Sarada, 2012; Nishida *et al.*, 2005).

The legumes examined in the present study were found relatively resistant to the allelopathic *V. iphionoides* plant. Some plant species possessed detoxification mechanisms to cope with allelochemicals (Weir *et al.*, 2004). These detoxification mechanisms include the oxidation, hydroxylation or glucosylation of the phytotoxic compounds (Inderjit and Duke, 2003; Sicker *et al.*, 2001; von Rad *et al.*, 2001).

Phenolics are the most important and common allelochemicals known to play a significant role in the ecosystem (Batish *et al.*, 2007; Li *et al.*, 2010). The phytotoxicity of phenolic allelochemicals was reported as a result of their ability to disrupt normal metabolic processes in the plant (Weir *et al.*, 2004). Via LC-ESI-MS/MS analysis, ten phenolic compounds with varied concentrations were detected in *V. iphionoides* leaves. Among them, vanillic acid, *p*-coumaric acid, tyrosol and ferulic acid were dominant (Table 1). *V. iphionoides* plants were reported to contain remarkably high levels of total phenolic contents compared to other medicinal plants within the same Compositae family (Alali *et al.*, 2007). The allelopathic impacts of some phenolic compounds identified in the present study were previously documented. Blum and Gerig (2005) reported that vanillic, *p*-coumaric and ferulic acid inhibited transpiration, water utilization, leaf area expansion and dry weight of cucumber seedlings. These phenolic acids were also reported to reduce chlorophyll content of soybean plants (Patterson, 1981). Moreover, ferulic and *p*-

coumaric acids were shown to enhance lipid peroxidation and modulate antioxidant system in maize and soybean seedlings (Devi and Prasad, 1996; Doblinski *et al.*, 2003).

5. Conclusion

In conclusion, *V. iphionoides* showed an allelopathic potential on the crop plants under investigation. The effects of allelochemicals produced in this plant are both species-specific and concentration-dependent. The magnitude of reduction in seed germination and seedling dry weights after treatment with *V. iphionoides* aqueous extracts followed the following order: tomato > wheat > pepper > barley > lentil > chickpea. These findings are valuable for avoiding the cultivation of some crop species (e.g., tomato and wheat) in fields infested with *V. iphionoides* plants. LC-ESI-MS/MS analysis revealed the presence of several phenolic compounds in *V. iphionoides* leaves that possibly contribute to the observed phytotoxicity of this medicinal plant. The present results are obtained under laboratory conditions. Laboratory bioassays were commonly used as a first step toward exploring the potential allelopathic effects of a certain plant species (Inderjit and Callaway, 2003). However, field studies are still needed to test the allelopathic properties of *V. iphionoides* under more natural conditions. Further studies on isolation, purification and evaluation of the candidate allelochemicals present in *V. iphionoides* are required to gain a better understanding of its physiological and biochemical mechanisms of action.

Acknowledgement

The authors are grateful to Aya Awad, Noor Ateyat and Bayan Al-Momany for their skilled technical assistance and to Jarah Al-Zoubi for sample collection from the field. We would also like to thank Ashraf Mutlaq (Pharmaceutical Research Center - Jordan University of Science and Technology) for performing the chemical analysis.

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