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ALLELOPATHIC POTENTIAL OF *ARGEMONE OCHROLEUCA* FROM DIFFERENT HABITATS ON SEED GERMINATION OF NATIVE SPECIES AND CULTIVATED CROPS

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Abstract

Allelopathy has been regarded as a mechanism for the successful exotic plant invasion, but this mechanism has not been evaluated for *Argemone ochroleuca* Sweet; an invasive weed in rangelands and farmlands of the Arabian Peninsula. We investigated whether wild native range plant species (*Farsetia aegyptia* Turra and *Salvia aegyptiaca* L.) and forage crops (*Hordeum vulgare* L. and *Medicago sativa* L.) respond differently to potential allelopathic effects of aqueous extracts from roots and shoots of *A. ochroleuca* growing in two habitats; rangelands and farmlands. Almost all the germination indices were sensitive enough to establish the allelopathic potential of aqueous extracts. Inhibition of seed germination of the test species showed species-specific; concentration, organ and habitat dependent response with highest inhibition occurring at 100% concentration of shoot extract from rangeland habitat. Seed germination of *F. aegyptia* was the most sensitive to different aqueous concentrations extracted from the two habitats, whereas *H. vulgare* seed germination was the least sensitive. The results suggest different organs of *A. ochroleuca* exhibit sufficient allelopathic potential in different habitats.

Key words: Alien species, Invasive weed, Phytotoxicity, Aqueous extract, Germination indices.

Introduction

During the last two centuries, human disturbance has degraded the ecosystems (Gurevitch & Padilla, 2004) and have been accidently and deliberately dispersing and introducing plants to ecosystems beyond their native geographical region, causing biological invasion (Mack *et al*., 2000). Biological invasions have caused more species extinctions than did human-induced climate change (Gurevitch & Padilla, 2004). It is a form of biological pollution which can occupy almost all the habitats of the ecosystem like natural, semi-natural or agricultural habitats (Podda *et al*., 2011) and is responsible for native biodiversity loss and ecosystem depletion (Wilcove *et al*., 1998). This biological pollution is responsible for homogenizing the world's flora and fauna (Mooney & Hobbs, 2000) and is known to decrease native plant species diversity and productivity (Powell *et al*., 2011).

Allelopathy is one of the mechanisms which provide means for widespread and mass expansion for the exotic invasive species (Callaway & Aschehoug, 2000; Hierro & Callaway, 2003). Several hundred allelochemicals released from invasive species are known to affect the emergence or survival of both crops and native species in the invaded habitats (Einhellig, 2002; Hierro & Callaway 2003). The effects of chemicals involved in allelopathic interference are dependent on the type of plant tissues and habitats of invasive species (Bais *et al*., 2003; Jefferson & Pennacchio, 2003). Allelochemicals are present in all plant tissues including leaves, roots, stems and seeds (Batish *et al*., 2006). They are often water soluble and are released into the soil environment through foliar leaching, via root exudation, and after decomposition or volatilization of plant residues (Tawaha & Turk, 2003; Dastagir & Hussain, 2015) and can thus break the dynamic beneficial plant, soil and microorganism interaction in the rhizosphere of the associated species (Napoli *et al*., 2008**)**. Consequently, different plant organs of the same invasive species vary in their allelopathic effect on wild plants and cultivated crops (Cipollini & Greenawalt, 2016).

Pale Mexican pricklypoppy (*Argemone ochroleuca* Sweet, Papaveraceae) is a noxious weed of various agricultural crops and pastures in semi-arid and arid regions of Africa, Asia and Australia (Wilson *et al*., 1995; Parsons & Cuthbertson, 2001; Karlsson *et al*., 2003; Kalwij *et al*., 2008; Carbutt, 2012). It has the ability to invade disturbed areas and roadside verges, where it rapidly regenerates after rain and spread from viable seed bank (Wagner *et al*., 1999; Karlsson *et al*., 2003; Kalwij *et al*., 2008). In the Arabian Peninsula, it is currently spreading in many terrestrial and aquatic habitats such as mountains, roadsides, farmlands, wastelands, wetlands and ephemeral streams (Chaudhary & Al-Jowaid, 1999; Howladar *et al*., 2015). This widespread in different habitats could be related to its allelopathic potential or chemical interference. Like many other exotic invasive species, it accounts for loss of native species diversity in invaded habitats (Milton & Dean, 1998; Alemayehu, 2012). It is unpalatable and not eaten by either domestic or wild animals, and even if the plant material is mixed with hay or seeds mixed with grains may poison feeding animals (Cullen *et al*., 2012).

In view of the recent advances in allelochemistry as biologically and ecologically a sound explanation for plant invasion, it is assumed that the negative response of seeds or seedlings is related to phytotoxicity of the extract applied. To our knowledge, there is no existing information about the allelopathic potential of *Argemone ochroleuca* tissues or extracts. Therefore, we aim to explore the allelopathic potential of *A. ochroleuca* with an emphasis on the inhibition of seed germination of two forage crops (*Hordeum vulgare* cv. Gustoe) and alfalfa (*Medicago sativa* cv. CUF 101), and two wild native range species (*Farsetia aegyptia* Turra and *Salvia aegyptiaca* L.) by different tissue extracts (roots and shoots) collected from different two habitats (rangelands and farmlands) in the semi-arid region of Saudi Arabia. We tested the following hypothesis: (i) The root and shoot fresh aqueous extracts of *A. orchroleuca* would reduce seed germination of each of the four target species, and that this reduction will increase with increasing concentration. (ii) The shoot extracts have more inhibitive potential than their counterparts of root ones. (iii) The fresh aqueous extracts of *A. ochroleuca* from rangeland habitat have more phytotoxic effect than the farmland habitat due to more stress conditions in rangeland.

Materials and Methods

Collection of plant material: The fresh roots and shoots of *A. ochroleuca* Sweet (Papaveraceae) were collected from two different habitats (rangelands and farmlands) in spring 2014 at the flowering stage from mature individuals in Taif, Saudi Arabia. Plant parts (shoots and roots) were separated and chopped into small pieces up to three cm of length and then air dried for three days. The chopped material was grinded into powder form and kept at 2^0C until extraction. Four species i.e., two wild natives viz., *F. aegyptia* Turra and *S. aegyptiaca* L. and two forage crops namely Barley (*H. vulgare* L. cv. Gustoe) and Alfalfa (*M. sativa* L. cv. CUF 101) were selected as test plant species. The two wild native species were chosen based on their absence in the infested spots and their predominant presence in non-infested spots of the same area. Selection of the two forage crops was based on their common cultivation in Taif farms where *A. ochroleuca* was the commonly an associated weed.

Water extraction of *Argemone ochroleuca*: Aqueous extracts of shoots and roots were prepared separately by soaking 12.5gm/100ml (W/v) of the dried powdered plant materials in distilled water for 24 hours which acted as 100% concentration. The mixture was then filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter paper, covered tightly and stored in a refrigerator. Five dilution extracts, ranging from 20% to 100% in 20% incrimination were prepared using distilled water. Thus, there were six concentrations of each extract to be tested for germination bio-assay i.e., 0% as control, 20%, 40%, 60%, 80% and 100%.

Germination bioassay: Thirty seeds of *F. aegyptia, S. aegyptiaca*, barley and alfalfa were placed in 11cm diameter petri dishes lined with Whatman no. 4 filter paper. The petri dishes were moistened with 7ml of different extract concentrations of *A. ochroleuca*. Control Petri dishes were also maintained in each experiment using only distilled water, i.e. without plant extracts. Petri dishes were placed in a germinator at 25± 1^oC for 12/12 hours of light/dark photoperiod upto16 days (the time when no further seeds germinated). Seeds were considered germinating when the radical emerged by rupturing the seed coat. During this period, the petri plates were observed daily and distilled water or extract concentrations were added to the respective dishes to avoid drying out of the germinated seeds if any. After 14 days of experiment, total germination, final germination percentage, speed of germination, and speed of accumulated germination were calculated.

Statistical analysis: The bioassay experiments were conducted as completely randomized design (CRD) with five replications. The experiments were repeated twice to avoid any experimental error and data were averaged before performing statistical analysis. The data generated in each experiment were analyzed using statistical package IBM SPSS software version 16.0 for windows. The data were analyzed as three-factor factorial experiments (habitat, plant organs and concentration of aqueous extracts) in a CRD design. When the analysis of variance (ANOVA) revealed significant treatment differences, a Tukey test ($p<0.05$) was used for mean separations at 5% level of probability (Steel & Torrie, 1980).

Results

In most cases, ANOVA indicated significant ($p<0.001$) effect of habitat, plant organ and extract concentration and their interactions on seed germination of test species (Tables 1 and 2). Results showed that *A. ochroleuca* shoot and root aqueous extracts of both habitats have inhibitive effect on seed germination of all the target species (Fig. 1). The germination inhibition was dependent upon plant organ, extract concentration, and habitat. Germination at all concentrations of shoot extract was lower than in the root ones, and decreased with increasing concentration. For example, when seeds of *F. aegyptia* were exposed to the lowest concentration of *A. ochroleuca* shoot aqueous their germination was 15% when compared to 84% in their corresponding root extract (Fig. 1a). However, the effect of shoot extract from rangeland habitat has more inhibitive effect and increased with the increase in concentration (Fig. 1).

The seed germination of both native species *F. aegyptia* and *S. aegyptiaca* were completely inhibited above 40% concentration of all the extracts from the two habitats leading to zero germination. The only exception is 10% germination of *S. aegyptiaca* at 60% root extract in farmland habitat (Fig. 1b). Next to control, *F. aegyptia* showed a maximum germination of 84% when exposed to 20% concentration of *A. ochroleuca* root extract collected from rangeland habitat (Fig. 1a) followed by 65% of its germination at 20% of shoot and root extract from farmland habitat (Fig. 1a).

Barley was the only target species whose seeds germinated in all the extract concentrations from both habitats but decreased with the increase in concentration (Fig. 1c). Germination of alfalfa was significantly inhibited above 40% concentration for both habitats but inhibition was more pronounced in shoot extracts compared to its respective root extracts (Fig. 1d). With respect to barley and alfalfa, all the extracts at 20% concentration of both habitats did not show any prominent inhibition in germination.

Table 1. Analysis of variance of the effect of habitat, plant organ type and concentration of the aqueous extracts of *Argemone ochroleuca* on total germination of two native range species *Farsetia aegyptia* (a) and *Salvia aegyptiaca* (b)

Source of variation	DF	*F* Value	*P* Value
(a) *Farsetia aegyptia*			
Habitat (A)	1	22.330	<0.0001
Organ (B)	1	136.639	<0.0001
Concentration (C)	5	2095.941	<0.0001
A × B	1	84.680	<0.0001
A × C	5	14.577	<0.0001
B × C	5	72.078	<0.0001
A × B × C	5	71.089	<0.0001
(b) *Salvia aegyptiaca*			
Habitat (A)	1	.522	0.4720
Organ (B)	1	45.339	<0.0001
Concentration (C)	5	354.767	<0.0001
A × B	1	.766	0.3841
A × C	5	1.554	0.1813
B × C	5	16.459	<0.0001
A × B × C	5	1.992	0.0876

Table 2. Analysis of variance of the effect of habitat, plant organ type and concentration of the aqueous extracts of *Argemone ochroleuca* on total germination of two forage crops *Hordeum vulgare* (a) and *Medicago sativa* (b).

Source of variation	DF	*F* Value	*P* Value
(a) *Hordeum vulgare*			
Habitat (A)	1	8.576	0.0041
Organ (B)	1	81.931	<0.0001
Concentration (C)	5	206.663	<0.0001
A × B	1	14.901	<0.0001
A × C	5	6.968	<0.0001
B × C	5	8.089	<0.0001
A × B × C	5	7.234	<0.0001
(b) *Medicago sativa*			
Habitat (A)	1	31.920	<0.0001
Organ (B)	1	88.666	<0.0001
Concentration (C)	5	1046.850	<0.0001
A × B	1	12.334	<0.0011
A × C	5	16.202	<0.0001
B × C	5	40.355	<0.0001
A × B × C	5	4.276	0.0015

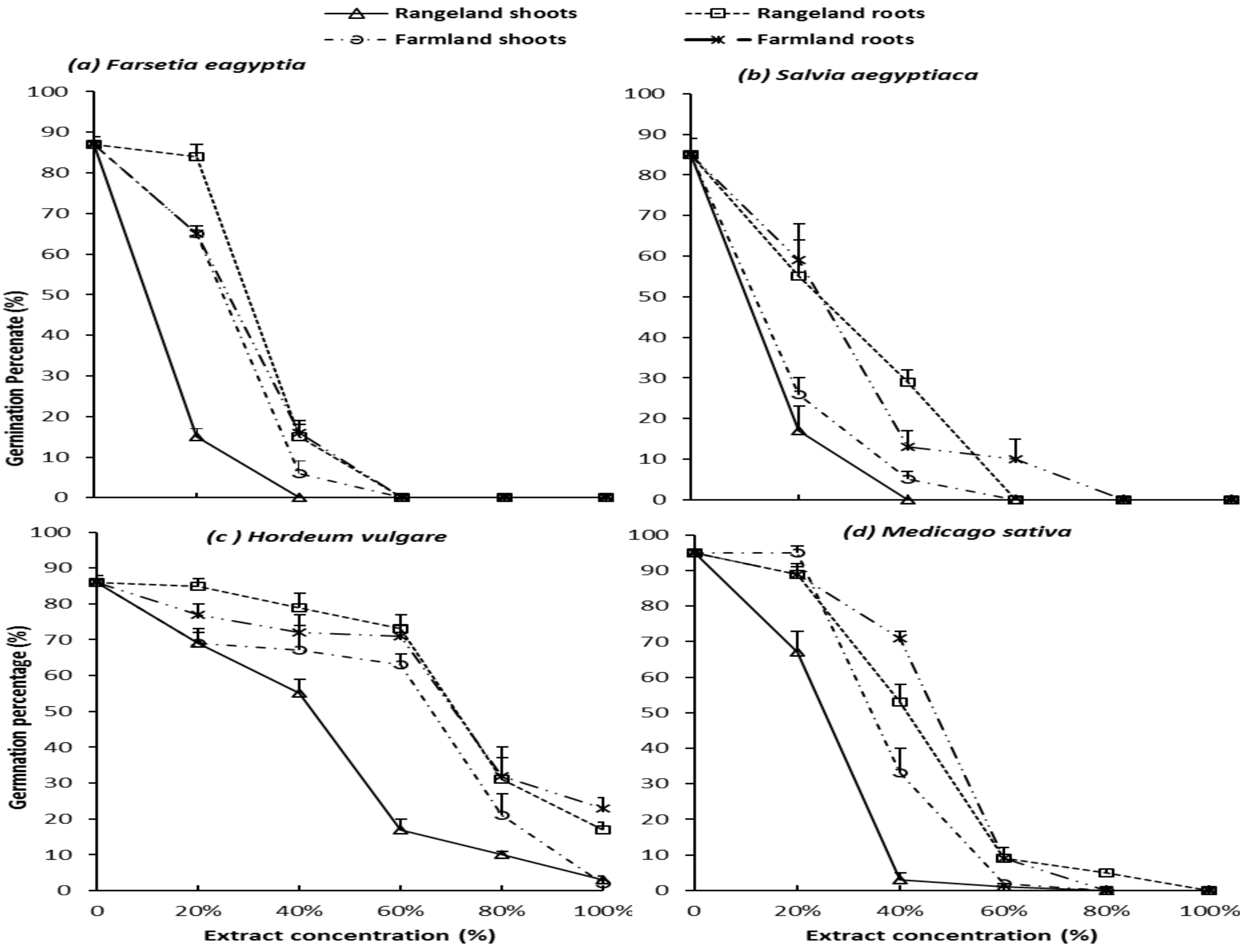

Fig. 1. Germination percentage for *Farsetia aegyptia* (a), *Salvia aegyptiaca* (b), *Hordeum vulgare* (c), and *Medicago sativa* (d) after sixteen days of treatment to five extract concentrations of *Argemone ochroleuca* shoot and root system collected rangeland and farmland habitats. Each bar represents the means (±SE) of five replicates.

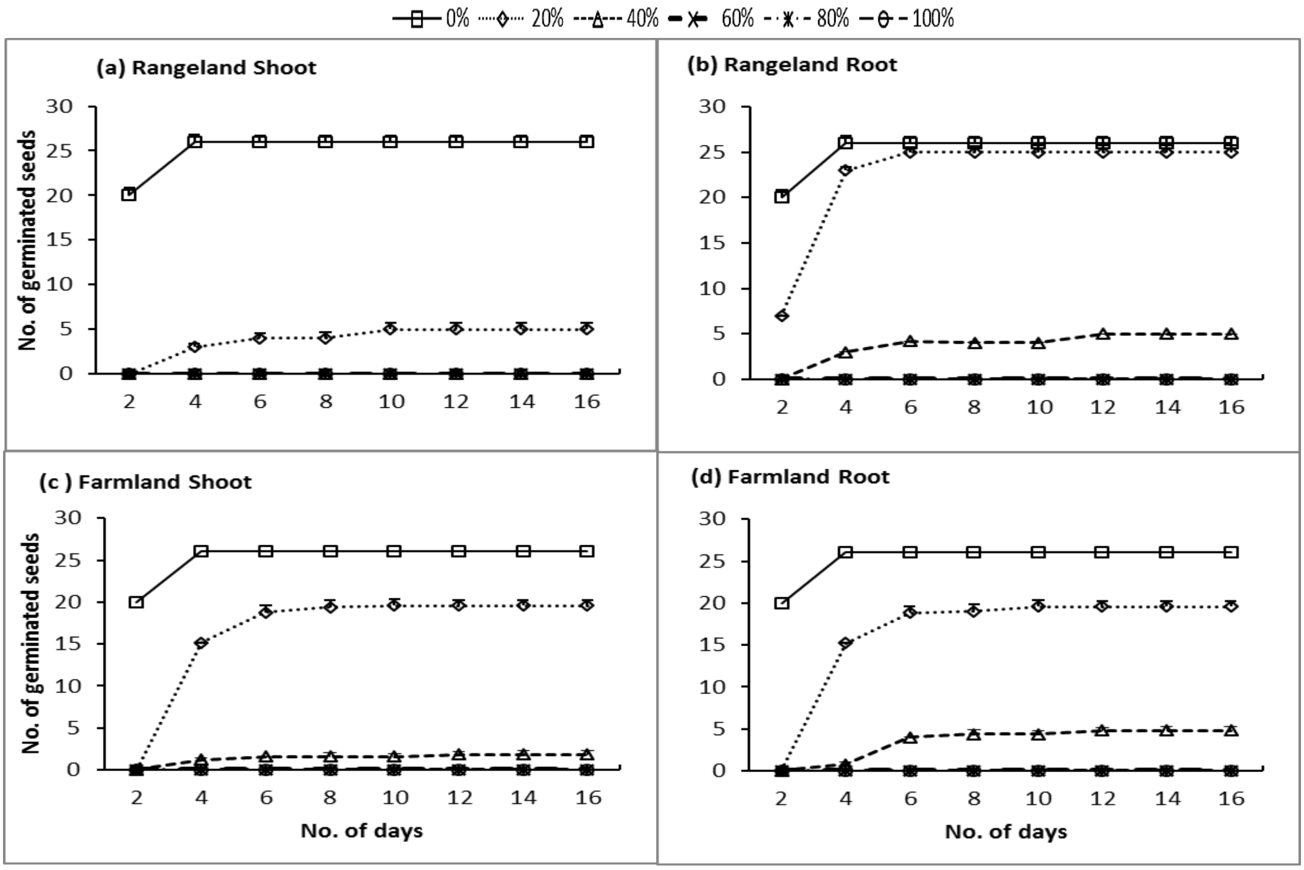

Fig. 2. Mean number of germinated seeds (out of thirty) for *Farsetia aegyptia* through time when treated with different concentration of *Argemone ochroleuca* extracts of rangeland shoots (a), rangeland roots (b), farmland shoots (c) and farmland roots (d). Each bar represents the mean (±SE) of five replicates.

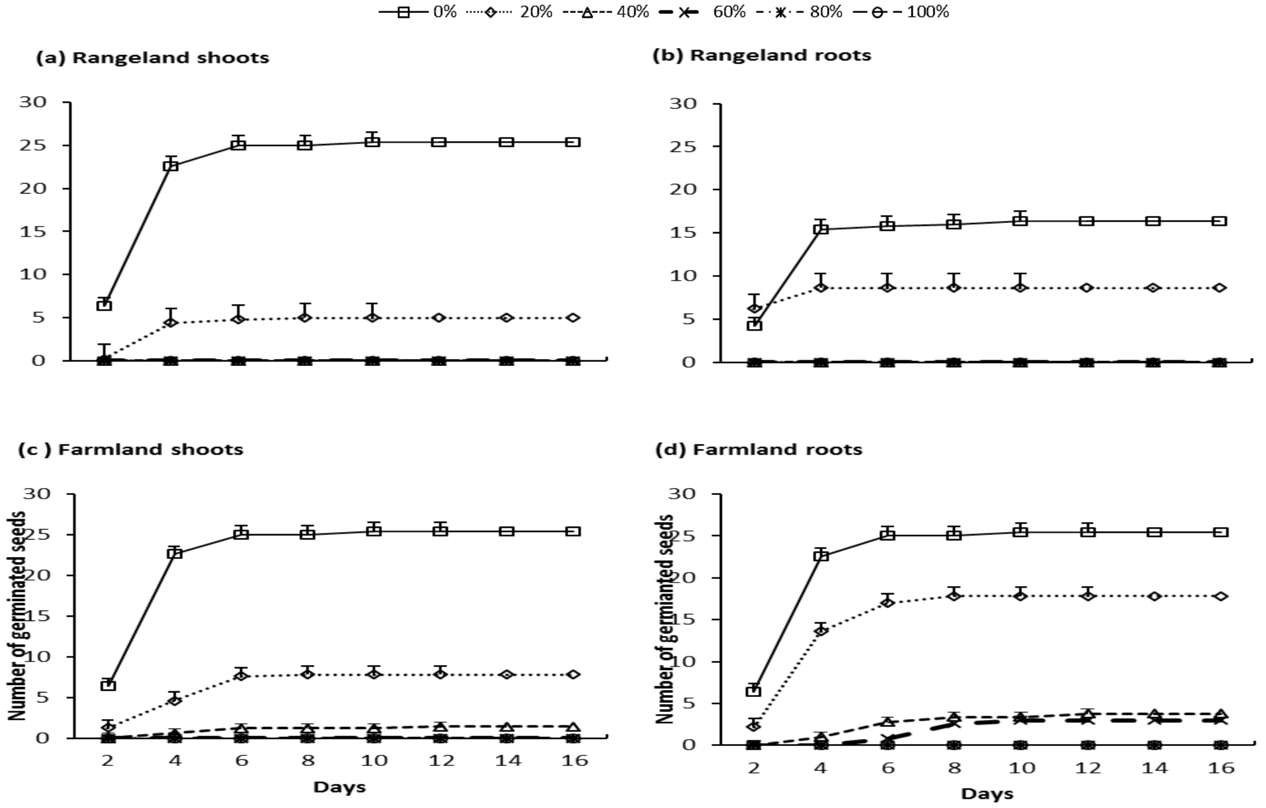

Fig. 3. Mean number of germinated seeds (out of thirty) for *Salvia aegyptiaca* through time when treated with different concentration of *Argemone ochroleuca* extracts of rangeland shoots (a), rangeland roots (b), farmland shoots (c) and farmland roots (d). Each bar represents the mean (±SE) of five replicates.

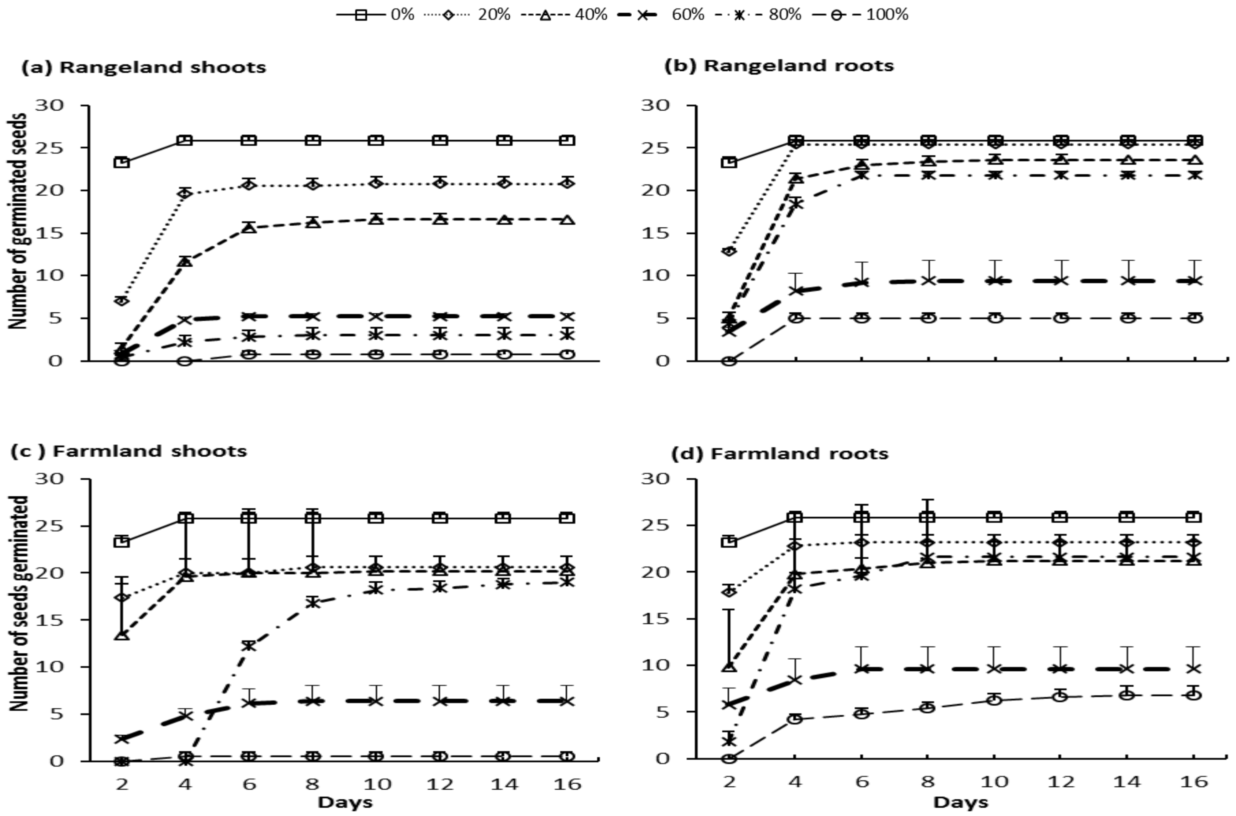

Fig. 4. Mean number of germinated seeds (out of thirty) for *Hordeum vulgare* through time when treated with different concentration of *Argemone ochroleuca* extracts of rangeland shoots (a), rangeland roots (b), farmland shoots (c) and farmland roots (d). Each bar represents the mean (±SE) of five replicates.

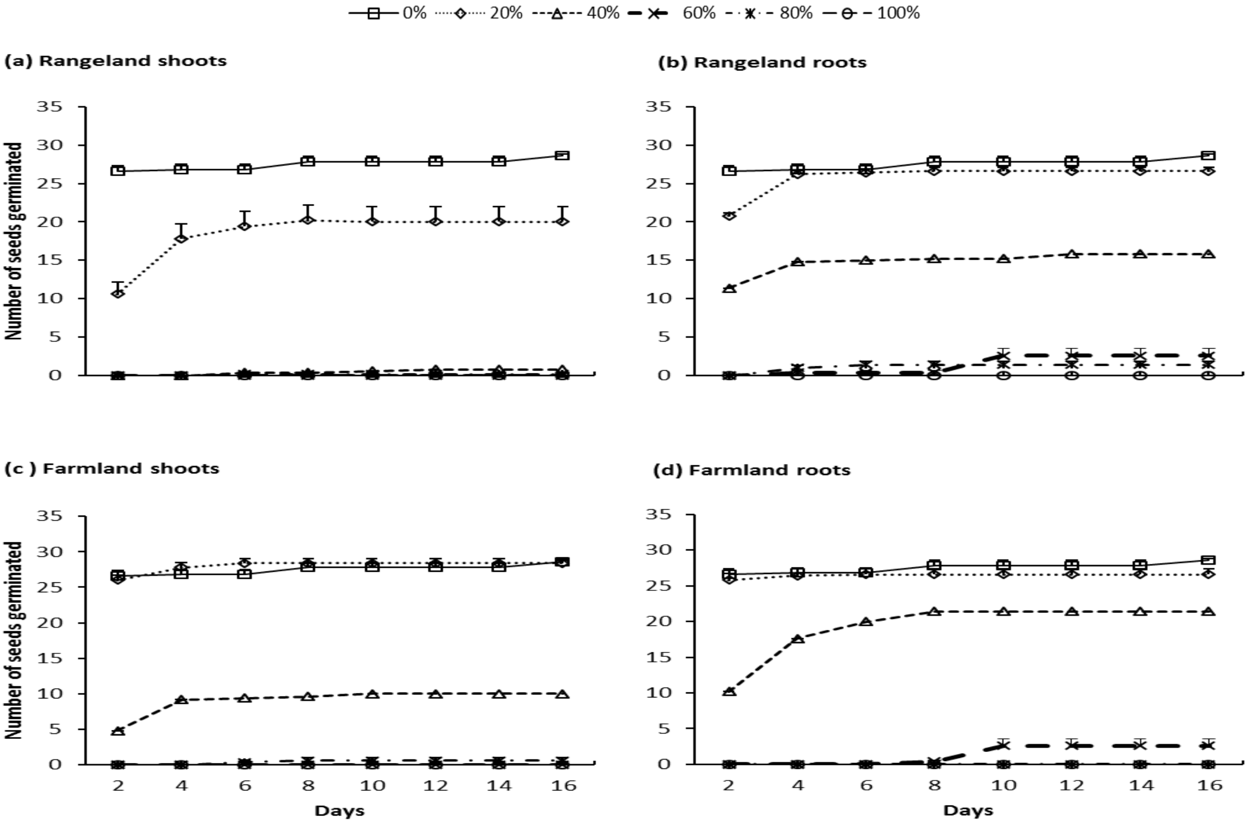

Fig. 5. Mean number of germinated seeds (out of thirty) for *Medicago sativa* through time when treated with different concentration of *Argemone ochroleuca* extracts of rangeland shoots (a), rangeland roots (b), farmland shoots (c) and farmland roots (d). Each bar represents the mean (±SE) of five replicates.

The delay in mean germination with respect to time was concentration dependent (Figs. 2-5). Most of germination in *F. aegyptia* and *S. aegyptiaca* were delayed upto10th day of experiment for all shoot and root extract concentrations of both rangeland and farmland habitat compared to their controls wherein most of their germinations occurred in day 4 and day 6 respectively (Figs. 2 and 3). Barley was the only target species wherein no delay was occurred in germination compared to its control (Fig. 4). The only exception was at 60% farmland shoot extract where in it was delayed up to 12th day of the experiment (Fig. 4c). Among all the target species, alfalfa was the only species wherein the germination was delayed up to 14th day of the experiment (Fig. 5).

Discussions

The results of the current study indicate that allelopathic properties could be one of the potential reason of serious infestations of *A. ochroleuca* in the rangelands and farmlands of Saudi Arabia. The aqueous extracts of roots and shoots of *A. ochroleuca* growing in rangelands and farmlands greatly inhibited the seed germination of native range species *F. aegyptia* and *S. aegyptiaca* and cultivated crops; barley (*H. vulgare*) and alfalfa (*M. sativa*). This inhibition was dependent on growing habitat and plant organ types of *A. ochroleuca*. Additionally, the response to different plant extract concentrations was not consistent within all tested species. Among all the tested species, barley was the least affected. This may be due to potential phytotoxicity of barley (Ben-Hammouda *et al.*, 2002) that could counter the allelopathic effect of *A. ocrhroleuca*. Wu *et al.* (1998) reported that seed germination of wheat (*Triticum aestivum*) was less affected by ryegrass (*Lolium rigidum* Gaud.) extracts.

One of the key factor responsible for the dominance of any invasive plant species is its phytotoxicity which inhibits seed germination and seedling development of native species and economic crops (Bais *et al*., 2003; Hierro & Callaway, 2003; Powell *et al*., 2011; Sayed *et al*., 2016). *A. ochroleuca* extract from rangeland habitat showed higher inhibitory effect than the farmland habitat. It may be due to abiotic stress of the rangeland habitat. Stress caused due to abiotic factors like high temperatures or low water content or by nutrient deficiencies also magnifies allelopathic process in plants. If allelopathic potential of invasive species is considered as competitive plant strategy in stressful habitats (Grime, 2001; Ridenour & Callaway, 2001), we would expect plant organs of invasive species in natural habitats to be more toxic than their counterparts in agricultural lands. Einhellig (1987) noticed the decline and death of a seemingly healthy white birch tree adjacent to *Ailanthus altissima* tree in 4-6 weeks during an extended drought with high temperature. Both root and shoot extracts of *A. ochroleuca* growing in rangelands showed higher inhibitory effects than those of farmlands. However, shoot extracts of both habitats had more inhibition on seed germination of all tested species than that of root extracts. This may be related to the variation in chemical composition between the different tissues of *A. ochroleuca* in Saudi Arabia (Al-Hayyan, 2006), and more concentrations of dissolved compounds in shoots than in roots as shown for *A. mexicana* (Brahmachari *et al*., 2013). *Argemone mexicana*, another related species contains phenolic compounds including p-hyxdroxybenzoic acid, vanillic acid and salicylic acid (Burhan & Shaukat, 1999) which have been frequently implicated in allelopathy (Barkosky & Einhellig, 2003; Chandra *et al*., 2007; Ghareib *et al*., 2010). Alagesaboopathi (2013) reported that aqueous leaf extracts of *A. mexicana* showed inhibitory effect on seed germination of *Sorghum bicolor*. Paul & Begum (2010) reported that the aqueous extracts of *A. mexicana* root and leaf could reduce the germination of Lentil (*Lens culinaris*). In addition, these studies were carried out on aqueous extracts of either leaves or roots of *A. mexicana* growing in the same habitats in tropical regions.

Different quantities of same compounds can be found in *A. ochroleuca* shoot and root parts from rangeland and farmland habitats and may be responsible for germination inhibition of the test plants. Al-Hayyan (2006) also isolated three major alkaloid compounds from shoots of *A. ochroleuca* which might have contributed to the strong inhibition of shoot extracts compared to root ones. Similarly, several studies have documented greater inhibition effects of leaves and/or stem extracts than that of roots of weedy species on seed germination and growth of native and crop species (Turk *et al*., 2003; Xuan *et al*., 2004; Hussain *et al*., 2007; Li & Jin, 2010; Suwal *et al*., 2010; Hussain *et al*., 2011).

In conclusion, results demonstrated that *A. ochroleuca* from rangeland habitat had more phytotoxic potential than from farmland habitat. Both shoot and root part of the plant material released the water-soluble phyotoxins that decreased the germination of most of the test species. But the effect of shoot part was more than its corresponding root part. Barley (*H. vulgare*) being the least affected species, this could be used as a candidate species for cultivation in *A. ochroleuca* infested farmland. Phytotoxic activity of *A. ochroleuca* increases with the increase in concentration.

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