Allelopathy and Sowing Depth Effects on Agropyron Desertorum and Onobrychis Sativa

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Abstract

The allelopathic impact of Eucalyptus spp is a pivotal factor in plant-plant interactions, particularly in arid and semi-arid ecosystems. The consideration of sowing depth in plant species becomes increasingly significant when examining allelopathic effects. This study aimed to assess the allelopathic effects of Eucalyptus camaldulensis leaf powder in conjunction with sowing depth on the germination and growth behavior of *Agropyron desertorum* and *Onobrychis sativa*. Seeds of these species were collected from Yazd province, Iran. An experimental design was implemented employing a completely randomized design (CRD) with four replications and two factors. The first factor (allelopathy) comprised five levels: 0 (control), 5%, 1%, 2%, and 3% leaf powder of *E. camaldulensis* in 1000 g of soil. The second factor (sowing depth) included four levels: 0.5 (control), 1.5, 3, and 4.5 cm. Data analysis utilized one-way ANOVA and Duncan's multiple range tests. The results indicated that both allelopathy and sowing depth significantly impeded the growth of *A. desertorum* and *O. sativa*. Additionally, findings revealed that 3% leaf powder of eucalyptus exerted the most pronounced impact on the number of germinated seeds, stem length, and shoot weight in both species. Furthermore, a sowing depth of 4.5 cm was observed to be more inhibitory on the germination, shoot length, and shoot weight of *A. desertorum* and *O. sativa* compared to other depths. In conclusion, the study established that the interactions between allelopathy and sowing depth significantly constrained the growth and performance of *A. desertorum* and *O. sativa*.

Introduction

Eucalyptus spp., indigenous to Australia and belonging to the Myrtaceae family, have been widely imported to numerous countries owing to their high productivity, adaptability, and swift economic returns (Cossalter & Pye-Smith, 2003). Consequently, Eucalyptus spp. has become a successful invader (Forsyth et al., 2004), thriving even in arid and saline regions such as the Mazandaran province in Iran (Zhao-Jiang et al., 2011). Eucalyptus camaldulensis Dehnh., among the species, serves various purposes, including energy production, pulp fiber, solid timber, and environmental remediation. Despite its rapid growth potential over multiple rotations when planted in suitable environments (Eldridge et al., 1993), the cultivation of *E. camaldulensis* leads to adverse environmental consequences. Plantation activities not only disrupt soil water balance but also diminish soil productivity, resulting in reduced undergrowth vegetation biomass and simplified community structures, consequently diminishing biodiversity and promoting soil erosion. The phenomenon of allelopathy, particularly in relation to sowing depth constraints in plantations, is suggested to contribute to the threat posed by Eucalyptus to biodiversity and habitat quality (Florence, 1986). This study seeks to assess the allelopathic effects of *E. camaldulensis* leaf powder, the impact of sowing depth, and their interplay on the germination and growth of *Agropyron desertorum* and *Onobrychis sativa*.

Allelopathy assumes a vital role in shaping interactions between plant species and the development of specific plant communities (Huang et al., 2020). Plant species engage in complex, multi-directional relationships with neighboring species, involving competition for nutrients and water, as well as inhibition, stimulation, and interdependence (Ercisli et al., 2005; Jafariehyazdi & Javidfar, 2011). Chemical compounds released by allelopathy, such as phenolics, terpenoids, alkaloids, coumarins, tannins, steroids, and quinines, can influence plants through root exudation, leaf leaching, volatile emissions, and plant material decomposition. Additionally, allelopathy can indirectly impact plants by inhibiting microorganisms, including nitrogen-fixing and nitrifying bacteria and ectomycorrhiza (Rice, 1964). Recognizing the allelopathic effects of plants on one another is crucial for agricultural producers and natural ecosystem managers in planning vegetative projects for environmental restoration and rehabilitation (Jafari et al., 2004). Numerous studies have investigated the allelopathic effects of various plants, such as germination inhibition, plumule and radicle length, seedling growth retardation, and poor seedling survival (Djurdjevic et al., 2004; Turk & Tawaha, 2003). The establishment of eucalyptus plantations has been associated with bare ground surfaces and limited ground vegetation, particularly in tropical and sub-tropical regions where high soil nutrient demand poses challenges to soil quality and fertility (Lemenih & Kassa, 2014; Chanie et al., 2013).

Sowing depth significantly influences crop management practices, affecting crop stand establishment and yield. Inadequate sowing depth can lead to weak germination due to insufficient soil moisture at the topsoil layer (Desbiolles, 2002; Sturm et al., 2017). Seed burial depth influences germination and emergence by impacting moisture, temperature, and light exposure, potentially decreasing crop emergence and yield (Suresh & Vinaya Rai, 1987). Conversely, excessive sowing depth can negatively affect seedling emergence and grain yield unless cultivars adapted to deep sowing are employed. Soil contact is crucial for healthy crop emergence, with shallow sowing resulting in extensive temperature and moisture fluctuations, affecting germination and emergence. Deeper sowing, on the other hand, provides better access to moisture and minimizes temperature fluctuations (Turk & Tawaha, 2003).

Considering the pivotal roles of sowing depth and allelopathy in crop management practices, particularly in natural and agricultural ecosystems, this study aims to fill existing gaps by examining their effects on *A. desertorum* and *O. sativa*. The research investigates the allelopathic characteristics of *E. camaldulensis* and its impact on the growth of these two species, addressing aspects such as germination, allelochemical release processes, optimal sowing depth, and the interactive effects of allelopathy and sowing depth on seedling growth. The study contributes valuable insights to agricultural and environmental practices for the sustainable management of ecosystems.

Materials and methods

Experiment description

Site description

Species samples were gathered in Yazd, Yazd province, Iran, during October 2013 (see Fig. 1). Plant materials were subsequently dried at 70°C until a constant weight was achieved. The climate in this region is characterized as cold steppic to semi-desert, with a mean annual precipitation of 60 mm and a rainy season spanning from April to June. The average annual temperature is 40°C, with the minimum temperature recorded in December (-8°C) and the maximum temperature reaching + 45°C in June. The region's topography ranges from the highest point at 3970m in Shirkouh Mountain to the lowest point at 1450m on the margin of Kavir-e-Abarkouh (Jafari et al., 2004).

Plant species

Onobrychis sativa (Sainfoin)

Sainfoin, scientifically known as *Onobrychis sativa*, is native to Turkey, Iran, and Europe. Initially cultivated in the northern regions of France and the United Kingdom, it has since proliferated across warm-temperate Europe, extending as far north as Sweden, as well as in Asia, Mediterranean countries, and western North America. Sainfoin exhibits a broader optimal temperature range for germination and early seedling growth compared to most other forage legumes. It thrives particularly well in deep, well-drained calcareous soils with a pH level of 6.0, and it performs optimally in warm temperate climates, displaying vigorous seedling growth.

Agropyron desertorum

Agropyron desertorum, belonging to the Graminaceae family, is a pivotal species in various rangelands, predominantly characterized by high soil salinity. This plant is well-adapted to the distinctive feature of high salt content prevalent in most soils where it naturally grows (Koivisto & Lane, 2001).

Experimental design

The allelopathy and sowing depth experiments were conducted within a controlled greenhouse environment. Each experiment was meticulously designed to ensure precision and reliability, as outlined below:

Full allelopathy experiment

To investigate the impact of *E. camaldulensis* on the germination and seedling growth of *A. desertorum* and *O. sativa*, an exhaustive allelopathy experiment was conducted. Leaves of *E. camaldulensis* were procured from both agricultural lands and rangelands (natural habitats), shade-dried, and subsequently ground into a powdered form. Seeds of *A. desertorum* and *O. sativa* were sourced from Pakkanbazr Company of Esfahan.

The allelopathy experiment was designed with the first factor (allelopathic) having five distinct levels: 0% (control), 1%, 2%, 3%, and 5% of *E. camaldulensis* leaf powder by weight in 1000 grams of soil. Each experimental unit comprised plastic pots with dimensions of 20 cm in height, 10 cm in span, and a soil capacity of 1 kg. To eliminate the influence of additional factors such as salinity, sand was chosen as the soil texture, and thorough leaching of the soil was undertaken.

This meticulous experimental setup allowed for the systematic examination of the allelopathic effects of varying concentrations of *E. camaldulensis* leaf powder on the germination and seedling growth of *A. desertorum* and *O. sativa*, ensuring precise control over potential confounding factors.

Sowing depth experiment

In order to ascertain the optimal sowing depth for the cultivation of *Agropyron desertorum* and *O. sativa*, a dedicated sowing depth experiment was conducted. Fifteen seeds possessing specified characteristics were individually placed in each pot containing 1 kg of soil. The seeds were planted at four distinct depths: 0.5 cm (control), 1.5 cm, 3 cm, and 4.5 cm.

The pots, situated in the greenhouse, received regular irrigation at predetermined intervals. Daily counts of germinated seeds were conducted at specified times, enabling the measurement of both germination speed and germination percentage. To investigate the combined impact of allelopathy and sowing depth, the soil was infused with *E. camaldulensis* leaf powder. Subsequently, seeds were planted at the designated depths within the experimental plots and irrigated once daily to supplement natural moisture until seed germination.

Following a 10-day period, various parameters, including radicle length, shoot growth, germination percentage, germination speed, root length, shoot length, plant length, and vigor index of the seedlings, were meticulously measured. This comprehensive assessment aimed to elucidate the influence of sowing depth on the growth and development of *A. desertorum* and *O. sativa* under the additional consideration of allelopathic effects induced by *E. camaldulensis* leaf powder.

Measurement of growth parameters

The assessment of germination percentage and germination speed was conducted utilizing the equations outlined by Camberato and McCarty (1999):

$$GP = \frac{\sum G}{N} \times 100$$

(1) Measurement of Germination Percentage

GP represents germination percentage.

G stands for the number of germinated seeds.

N denotes the total number of seeds.

$$GR = \sum_{i=1}^{n} \frac{S_i}{D_i}$$

(2) Measurement of Germination Speed

Si represents the number of germinated seeds at each counting.

Di denotes the number of days until the nth counting.

n signifies the total number of counting events.

(3) Computation of Plant Length: Sum of Root Length and Shoot Length

(4) The vigor index serves as an indicator of plant health, encompassing multiple growth variables such as germination, root length, and shoot length. It is assessed to identify optimal environmental conditions conducive to the production of high-quality seeds

Vi= %Gr ×MSH/100

Vi stands for the vigor index.

MSH represents the mean of plant length (sum of root length and shoot length) per millimeter.

Gr denotes the germination percentage.

Statistical analysis

The data derived from the experiments underwent descriptive statistical analysis, including the calculation of mean values, standard deviation, germination index, and percentage, utilizing Microsoft Excel spreadsheet version 2013. To ascertain statistical significance in variables such as germination percent, germination speed, root length, shoot length, plant length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, and vigor index, a one-way analysis of variance (ANOVA) was employed through SPSS version 20. Post hoc comparisons were conducted at a 5% significance level using Duncan's multiple range tests.

Results

Allelopathy effects

The findings revealed a substantial impact of *E. camaldulensis* leaf powder on the growth indices of both assessed species (Fig. 2A to 2D and Table 1). In both *A. desertorum* and *O. sativa*, the root, shoot, and plant lengths, as well as the vigor index, exhibited significant decreases in treatments compared to the control. The leaf powder of *E. camaldulensis* induced a noteworthy reduction in growth indices at all concentrations, with the highest concentration (5%) demonstrating adverse effects on all measured parameters in both species. This implies a proportional decrease in growth indices with an increasing amount of *E. camaldulensis* leaf powder. Statistical analysis indicated a significant difference ($P < 0.01$) in root, shoot, and plant length among various treatments in both species. Seedlings treated with controls exhibited the highest root, shoot, and plant lengths, while those treated with a 5% concentration of *E. camaldulensis* leaf powder displayed the lowest values in both species.

Furthermore, the leaf powder of *E. camaldulensis* significantly influenced both root and shoot dry and fresh weights in both species. Figure 3A to 3D and Table 1 illustrate that root and shoot dry and fresh weights in both species were reduced compared to the control. At all concentrations, the root and shoot dry and fresh weights were significantly lower than the control. *E. camaldulensis* leaf powder exhibited adverse effects on all measured parameters in both species, with the maximum root and shoot dry and fresh weights recorded in the control group and the minimum observed with a 5% concentration of leaf powder.

The germination percentage and speed exhibited a significant reduction across all concentrations of *E. camaldulensis* leaf powder. In the case of *A. desertorum*, the maximum and minimum percentages and speeds of germination were observed in the control and with 5% leaf powder, respectively. Similarly, in *O. sativa*, the highest germination percentage and speed were recorded in the control, while the lowest were observed with 5% leaf powder (Fig. 4A to 4B and Table 1). The differences in germination percentage and speed were statistically significant among the treatments. Specifically, the control treatment showed the highest germination percentage, whereas the 5% leaf powder of *E. camaldulensis* resulted in the lowest values.

Table 1
Analysis of variance parameters germination and seedling growth under the influence of allelopathic treatments and sowing depth

S.O.V	species	df	Germination Percent %	Germination speed	Root Length (cm)	Shoot Length (cm)	Plant Length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root Dry weight (g)	Shoot Dry Weight (g)
Allelopathy (A)	*A. desertorum*	4	4523.4**	1.21**	228.7**	133.4**	698.6**	0.877**	0.55**	0.25**	0.044**
	O. sativa	4	1043.9^{**}	2.55^{**}	1.25^{ns}	66.32^{**}	260.4^{**}	0.601^{**}	1.99^{**}	0.03^{**}	0.04^{**}
Sowing depth (S)	*A. desertorum*	3	5189.5*	1.3**	215.2**	101.2**	602.8**	1.94**	0.58**	0.33**	0.047**
	O. sativa	3	3538.7^{**}	3.84^{**}	1.25^{ns}	32.34^{**}	328.6^{**}	0.111^{*}	0.80^{**}	0.01^{**}	0.05^{**}
A * S	*A. desertorum*	12	84.1*	0.06**	14.2**	4.7*	29.4*	0.061**	0.02*	0.007**	0.002*
	O. sativa	12	75.9^{*}	0.29^{*}	1.25^{ns}	2.23^{**}	12.81^{**}	o.80*	0.11^{*}	0.001^{ns}	0.01^{*}
Error	*A. desertorum*	60	42.4	0.025	2.6	1.9	5.2	0.021	0.01	0.002	0.001
	O. sativa	60	43.9	0.12	1.2	0.79	1.7	0.36	0.08	0.001	0.01

Note: "ns" indicates not significant. (*) and (**) denote a significant difference compared to the control at P < 0.05 and P < 0.01, respectively.

Sowing depth effects

The findings indicate a noteworthy impact of sowing depth on the growth indices of *A. desertorum* and *O. sativa* (Fig. 5A to 5D and Table 1). The root, shoot, and plant lengths, as well as the vigor index of both species, demonstrated a significant reduction with increasing sowing depth. The highest sowing depth (5 cm) adversely affected all measured parameters in both species. Statistical analysis revealed significant differences (P < 0.01) in root, shoot, and plant lengths among various treatments in both species. Seedlings subjected to a 0.5 cm sowing depth (control) exhibited the highest root, shoot, and plant lengths, whereas those sown at a 5 cm depth displayed the lowest values in both species.

Additionally, the results unveiled a significant effect of sowing depth on root and shoot dry and fresh weights in both *A. desertorum* and *O. sativa*. Figure 6A to 6D and Table 1 illustrate that root and shoot dry and fresh weights at all treatments (sowing depths of 1, 3, and 5 cm) were significantly lower than the control (sowing depth of 0.5 cm). Hence, sowing depth exerted a notable influence on root and shoot dry and fresh weights in both species, with the maximum values recorded in the control and the minimum observed at a sowing depth of 5 cm.

Moreover, the percent and speed of germination were significantly diminished with increasing sowing depth (Table 1). In *A. desertorum*, the maximum and minimum percentages and speeds of germination were recorded in the control and at a sowing depth of 5 cm, respectively. Similarly, in O. sativa, the maximum and minimum percentages and speeds of germination were observed in the control and at sowing depths of 3 cm (minimum germination percent) and 5 cm (minimum germination speed), respectively (Fig. 7A to 7B).

Allelopathy and sowing depth interactions

The findings regarding the interactions between allelopathy and sowing depth on the germination and seedling establishment of *A. desertorum* and *O. sativa* revealed a significant decrease in the germination and growth indices of both species (Table 1). The impact of *E. camaldulensis* leaf powder and sowing depth was notably observed in the root dry and fresh weight of both species across all treatments. In *A. desertorum*, the maximum root dry and fresh weight occurred under 5% leaf powder and a sowing depth of 1 cm, significantly differing from the control values for the same species. Conversely, in O. sativa, the minimum root dry and fresh weight was recorded under 3% leaf powder and a sowing depth of 5 cm. For shoot dry and fresh weight, the maximum values were observed in O. sativa under 5% leaf powder and a sowing depth of 1 cm, while the minimum values were found in *A. desertorum* under 3% leaf powder and a sowing depth of 5 cm.

Furthermore, the results indicated no significant interaction between allelopathy and sowing depth on root length for *O. sativa*, whereas such an interaction was observed for *A. desertorum* ($P > 0.01$). Table 1 illustrates that shoot and plant length exhibited a significant decline ($P > 0.01$) with an increase in the amount of *E. camaldulensis* leaf powder and sowing depth.

Discussion

Previous studies conducted in natural ecosystems, laboratories, and diverse farming systems have consistently identified allelopathy as a pivotal factor influencing plant community structure. Preliminary findings from our study affirm the suppressive impact of *E. camaldulensis* on germination, shoot and root elongation, nodulation, root diameter, and the development of lateral roots in bioassay species. Our observations underscore the inhibitory influence of *E. camaldulensis* on the germination and growth of *A. desertorum* and *O. sativa*, with the inhibitory effect intensifying with increasing concentrations of leaf powder. These results align with prior research, such as Ahmed et al., (2008), highlighting the inhibitory effects of allelopathic *E. camaldulensis* on various plant growth parameters.

The inhibitory impact on seedling growth can be attributed to the release of various phytotoxic compounds, including phenolics, sesquiterpenes, p-cymene, ascaridole, aritazone, and lactones, from the vegetative parts of living plants (Belz et al., 2007; Evans, 1997). Changes in DNA synthesis, mitochondrial metabolism, and cell mitotic indices contribute to the inhibition of root growth and development, consistent with findings by Lawan et al., (2011). The higher sensitivity of roots to allelochemicals compared to aerial parts of seedlings has been reported in various studies (Bughio et al., 2013; Das & Bandyopadhyay, 2011; Ercoli et al., 2007; Florence, 1986; Jafari et al., 2004).

Furthermore, our results highlight the allelopathic influence of *E. camaldulensis* leaf powder on germination and seedling growth in both *A. desertorum* and *O. sativa*. The inhibitory effects on seed germination are associated with alterations in physiological and biochemical processes essential for seed germination, including disruption of mitochondrial respiration. The disruption of mitochondrial respiration impedes seed germination by affecting glycolytic activity, crucial for utilizing stored carbohydrates to provide energy and carbon products for seedling development. Understanding allelopathic interactions between plants aids in proper planning for ecological restoration and agricultural projects (Mohamadi & Rajaie, 2009).

The reduction in weights, both fresh and dry, observed in our study can be attributed to allelochemicals diminishing plant water potential, inhibiting mineral uptake, and compromising ion uptake. The interference with protein and carbohydrate synthesis further contributes to the declined plant growth and weight. Our findings align with previous studies demonstrating the allelopathic characteristics of E. globulus leaf extract on germination and seedling growth of various plants (El-khawas & Shehata, 2005).

In the second experiment, we observed a reduction in plant establishment and germination percentage with increasing sowing depth. The depth of 0.5 cm was associated with the highest germination speed. This reduction in germination percentage at greater depths could be attributed to secondary dormancy in seeds, potentially linked to low gas exchange, leading to insufficient oxygen and elevated carbon dioxide levels during seed metabolism. The behavior of seed germination at greater depths may depend on the storage reservoir within the seed.

In conclusion, our study sheds light on the intricate interactions between allelopathy, sowing depth, and the growth parameters of *A. desertorum* and *O. sativa*. The inhibitory effects of *E. camaldulensis* on germination and seedling growth highlight the ecological significance of allelopathy in shaping plant communities. Understanding these interactions is essential for ecosystem management and planning agricultural practices in areas influenced by allelopathic species like *E. camaldulensis*.

Conclusions

Our study unequivocally establishes that allelopathy and sowing depth exert inhibitory effects on the growth indices of *Agropyron desertorum* and *Onobrychis sativa*. The investigation affirms the unfavorable interactions between sowing depth and allelopathy. Consequently, the evaluation of sowing depth and allelopathic conditions emerges as a crucial consideration for land managers. This assessment aids in obtaining the necessary germination percentage and promoting optimal growth when introducing such species into both natural and agricultural ecosystems. The recognition of these inhibitory factors contributes valuable insights for effective ecosystem management and informed decision-making in agricultural practices.

Declarations

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Author contribution

Setareh Bagheri: Contributed to fieldwork, participated in methodology development, and played a role in the analysis and writing of the paper. (25%)

Valiollah Raufirad: Engaged in the analysis and writing phases of the paper, contributing expertise in statistical analysis and discussion. (25%)

Ali Tavili: Contributed to the planning, methodology, and proposed topics, and actively participated in the reviewing and editing stages of the paper. (25%)

Mohammad Jafari: Involved in the planning, methodology, execution, and actively participated in the reviewing and editing stages of the paper. (25%)

All authors played integral roles in the manuscript's development, making substantial contributions to various stages, including data collection, methodology, statistical analysis, discussion, and the review and editing process. Each author provided crucial input to the drafts and granted final approval for publication.

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Data availability The data were personally collected and analyzed, originating exclusively from this study without external sources. The supporting data for the findings presented in this study can be obtained from the corresponding author upon reasonable request.

Competing Interest The authors assert that they have no known competing financial interests or personal relationships that could have influenced the work conducted in this research. In summary, the authors declare no competing interests.

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Figures

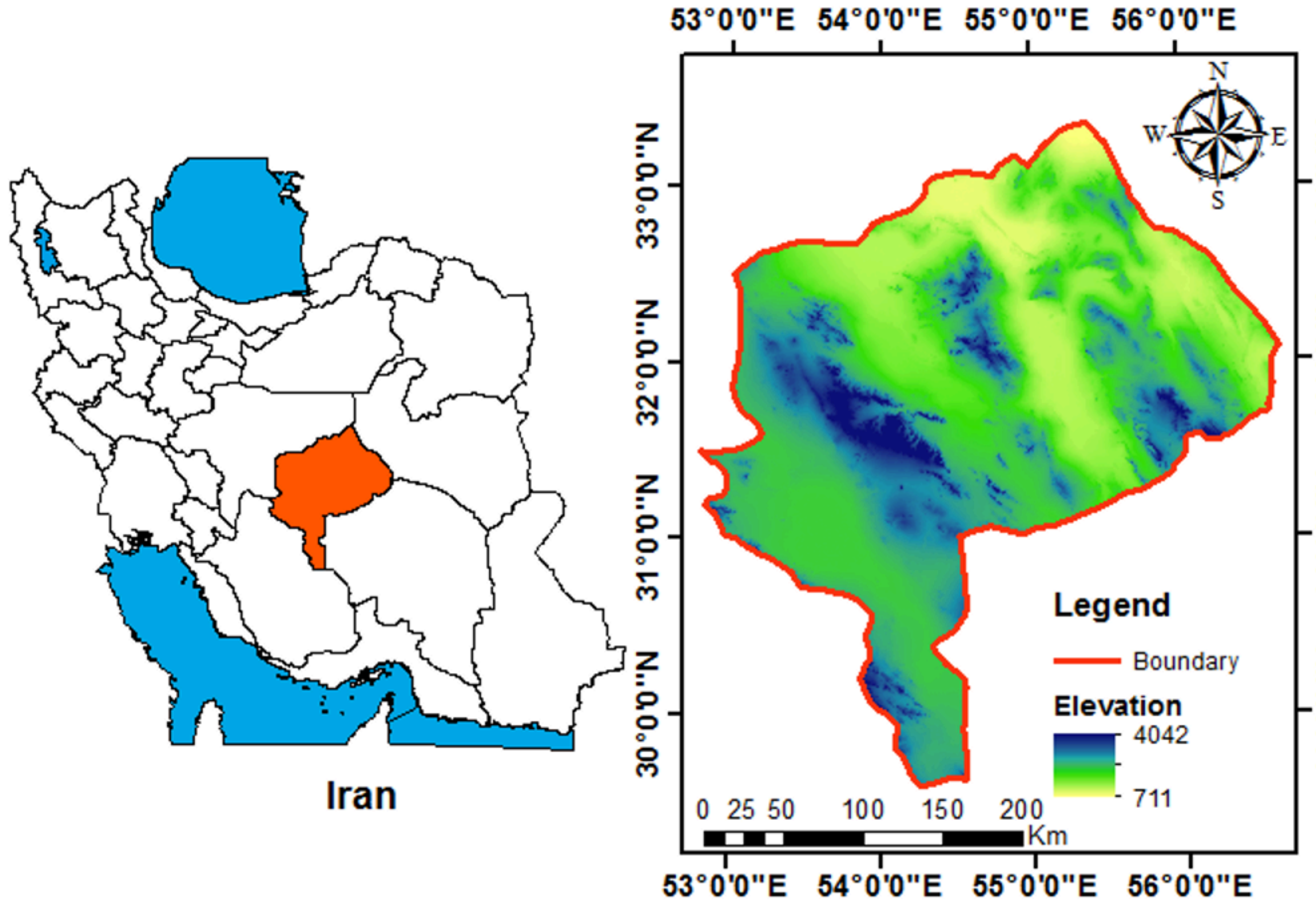

Figure 1

Collection Site of Species Samples in Yazd Province, Iran

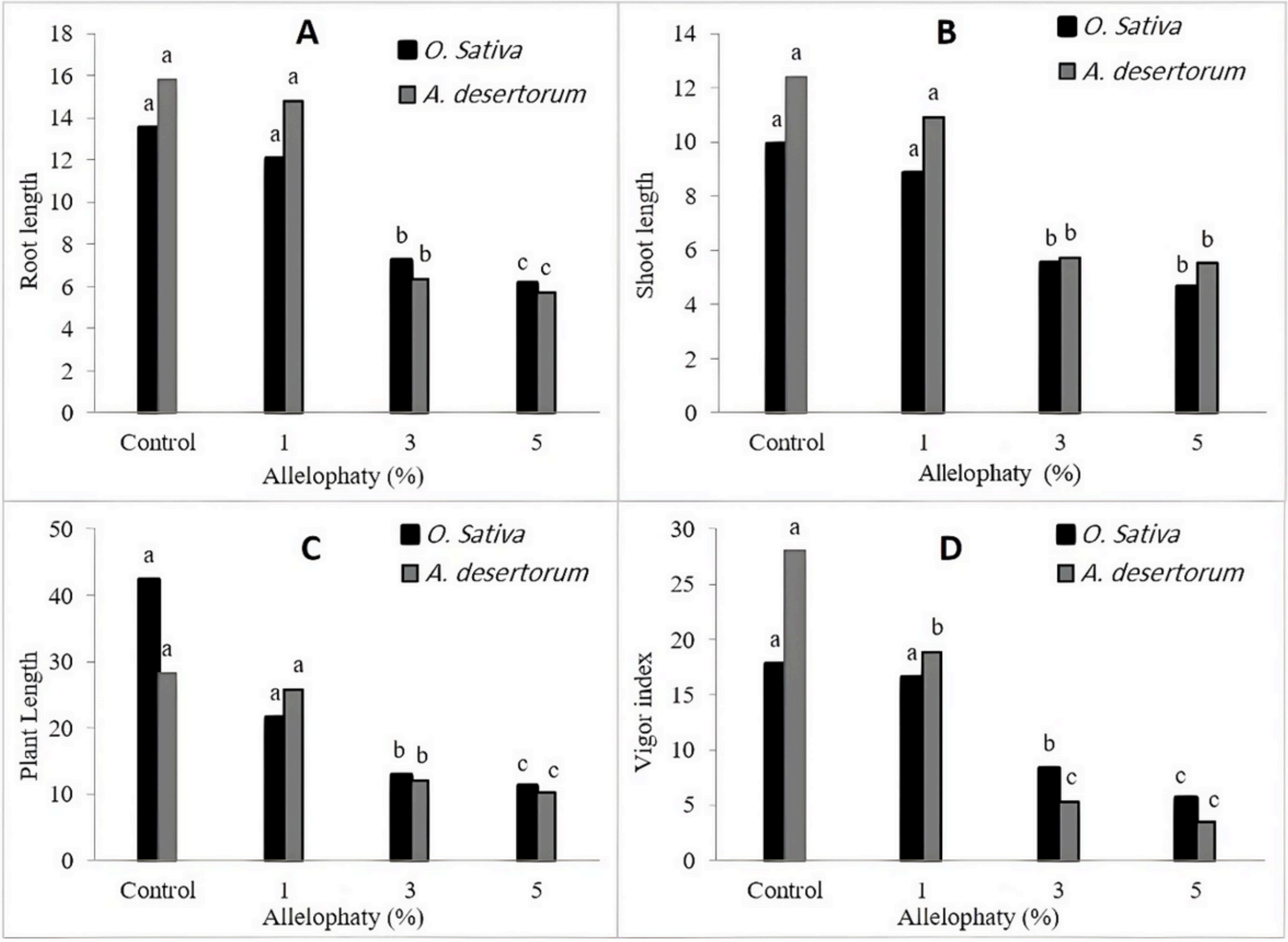

Figure 2

Allelopathic Effects of Eucalyptus camaldulensis Leaf Powder on Root, Shoot, and Plant Length, and Vigor Index in *A. desertorum* and*O. sativa*

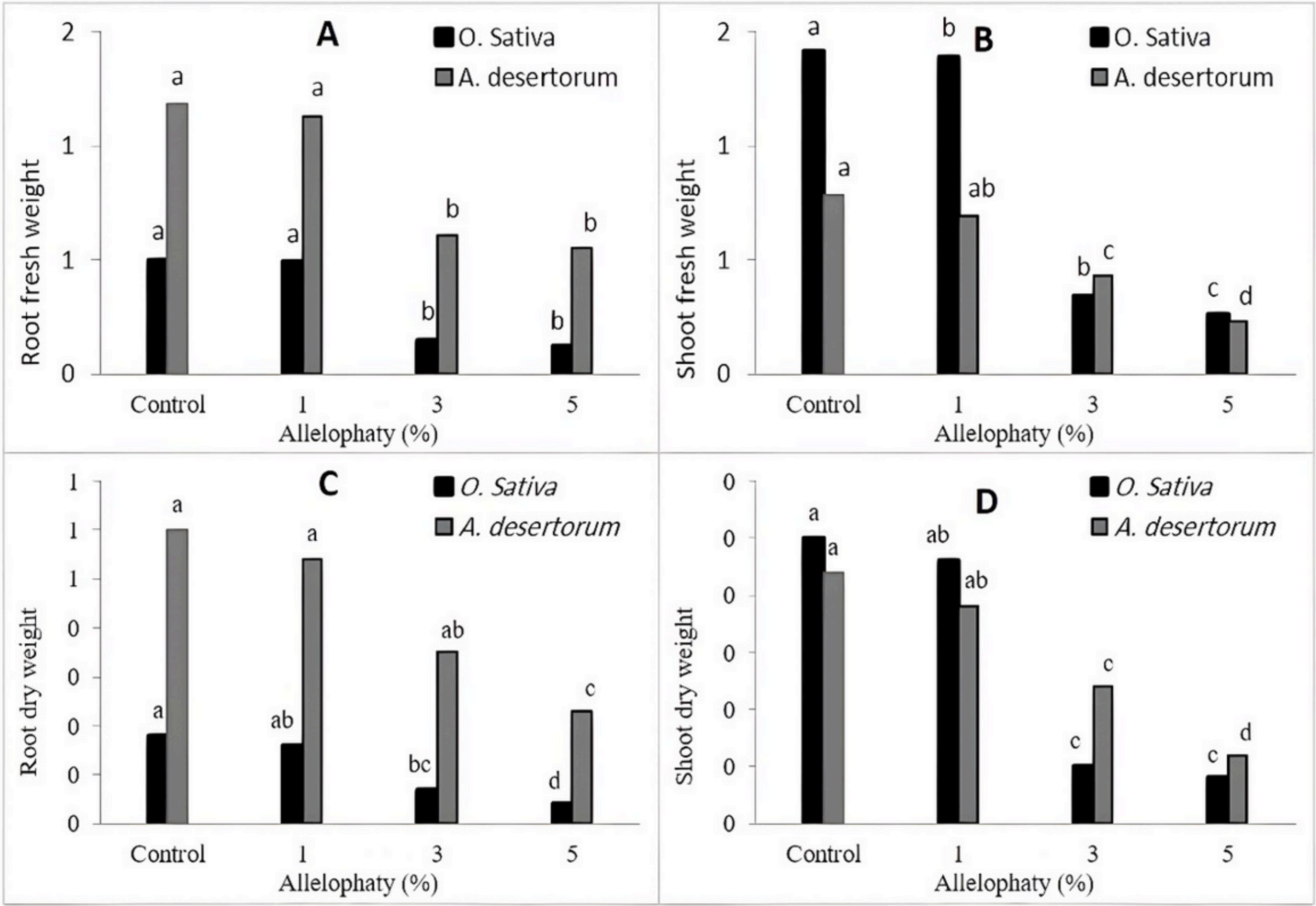

Figure 3

Allelopathic Effects of Eucalyptus camaldulensis Leaf Powder on Root and Shoot Dry and Fresh Weight in A. *desertorum* and *O. sativa*

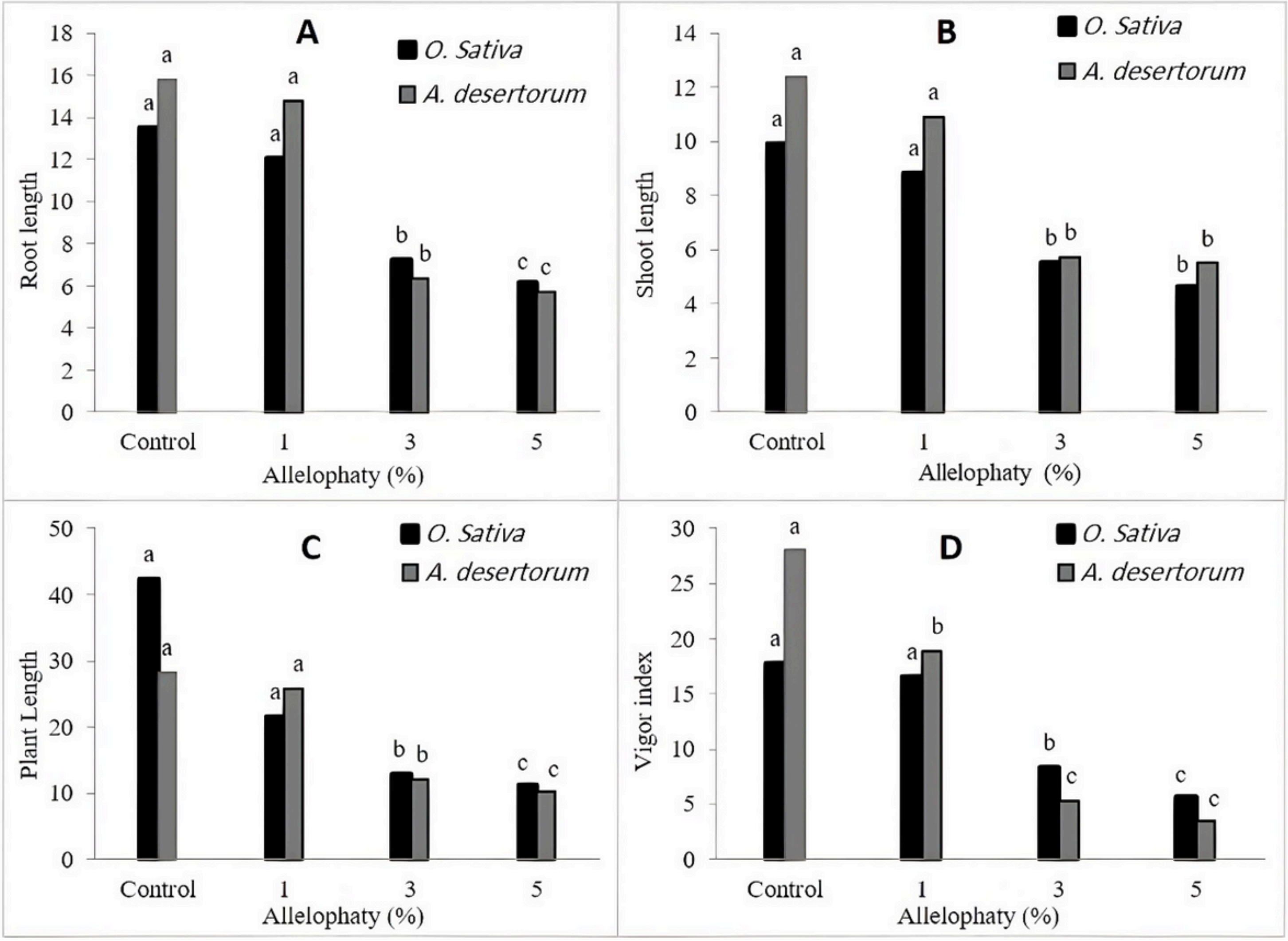

Figure 4

Allelopathic Effects of Eucalyptus camaldulensis Leaf Powder on Root, Shoot, and Plant Length, and Vigor Index in *A. desertorum* and *O. sativa*

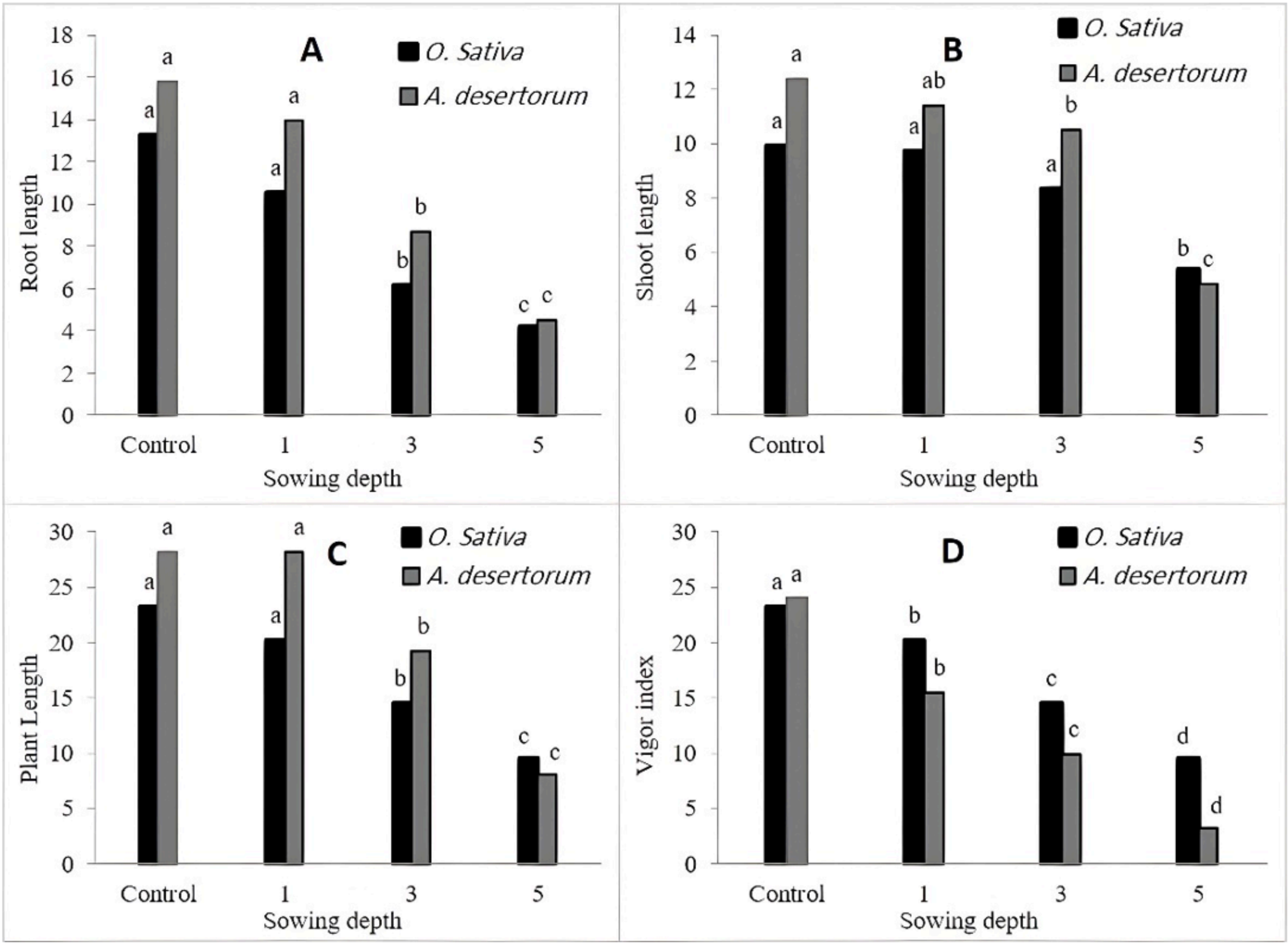

Figure 5

Influence of Sowing Depth on Root, Shoot, and Plant Length, and Vigor Index in *A. desertorum* and *O. sativa*

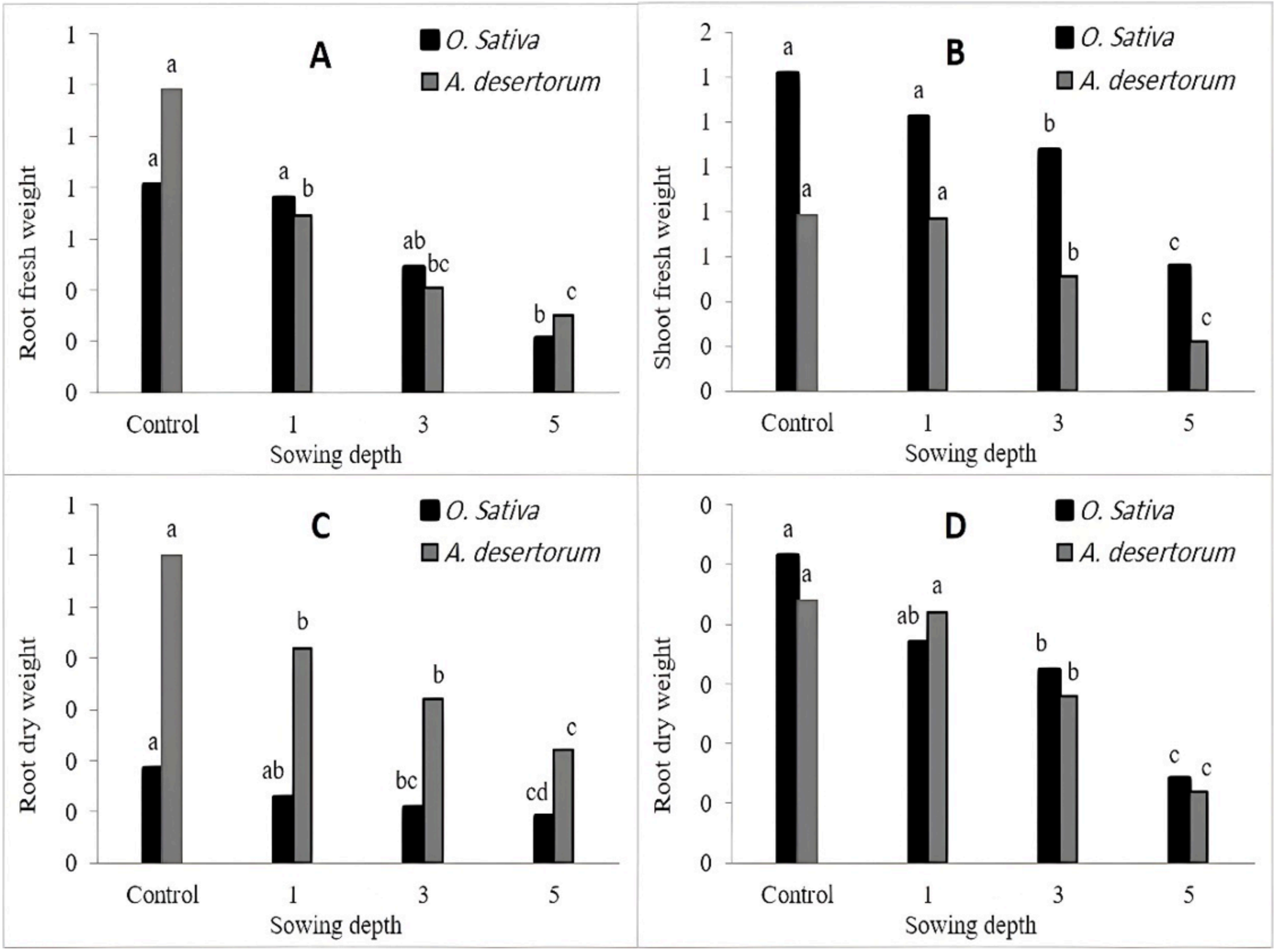

Figure 6

Impact of Sowing Depth on Root and Shoot Dry and Fresh Weight in *A. desertorum* and *O. sativa*

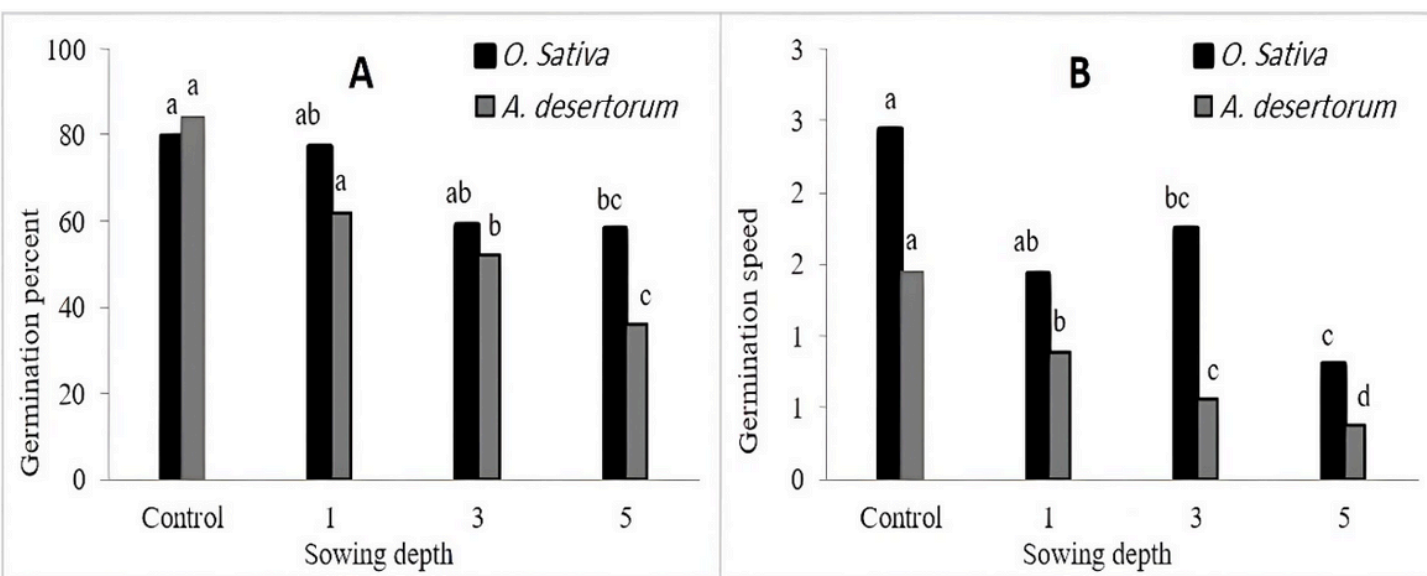

Figure 7

Impact of Sowing Depth on Germination Speed and Percentage in *A. desertorum* and *O. sativa*