ALLELOPATIC EFFECT OF AQUEOUS EXTRACTS FROM LEAVES OF *CEDRUS ATLANTICA* L. ON GERMINATION AND GROWTH OF WEED *BROMUS RUBENS* L.

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Abstract. This study was designed in order to investigate the allelopathic effect of *Cedrus atlantica* L. needles on weed *Bromus rubens* L. during 2020-2021. In order to find natural products of plant origin that can have a herbicidal action, aqueous extracts of the cedar leaves of the atlas *Cedrus atlantica* L. of different concentrations (1%, 2% and 3%) were compared to a control, and tested at different concentrations on the weed species *Bromus rubens* L. and a durum wheat variety GTA to investigate the selectivity effect under laboratory conditions. The results showed that the inhibitory effect of these extracts have significant effect on germination, the growth of coleoptiles and radicles and decrease the production of dry matter. Generally speaking, the inhibition efficiency increases with increasing extract concentration.
Keywords: *bio-herbicide, Bromus rubens L., Cedrus atlantica L., inhibition, allelopathy, durum wheat*

Introduction

The projections by FAO indicate that the global population is expected to rise to 9.7 billion by 2050, with a projected need of a 60% increase in agricultural production to meet the rising population (FAO, 2017). At the same time, crop losses due to weeds result in reduced yields in global production (McErlich and Boydston, 2014). Cereals have a great importance in agricultural research programs worldwide. They are the staple food of many developing countries, especially the Maghreb countries. Durum wheat variety is a staple food for millions of people especially in the Mediterranean Basin. Flour and semolina are used to make many traditional products, and different types of bread. Therefore, reducing chemical inputs without negatively affecting yield performance, represents a crucial goal to increase the environmental and economic sustainability of durum wheat production systems and also food safety for millions of people (Sall et al., 2019). The cereal sector is one of the main sectors of agricultural production in Algeria. Improving cereal production is crucial in developing the standard of living (Djermoun, 2018).

Due to the many abiotic factors, including annual climate change, and insufficient control of appropriate technical methods, durum wheat production is still insufficient and productivity is extremely low year after year, and Biotic factors such as pests, diseases and weeds also contribute to this (Rastoin and Benabderrazik, 2014). Weeds affect crop productivity for agricultural producers especially due to competition for inorganic nutrients leads to significant losses in crop yields and quality (Popp et al., 2013). Cover crops can suppress weeds within agricultural fields due to competitive and allelopathic effects. Glasshouse experiments were conducted to evaluate the relative proportions of allelopathic effects to the total weed inhibition (Sturm et al., 2018).

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The vast weed infestations interfere with plowing and harvesting implements and makes these operations troublesome. Mixing weed seeds with cereal seeds reduces the commercial quality of the harvested product. Therefore, it is necessary to effectively control the weeds of cereals. In order to successfully solve cultural and environmental problems (reduce crop losses and increase cereal production), development of an integrated weed management system requires detailed information on crop-weed interactions, including the impact of the relative competitive ability of the crop during various phases of development on weed growth. Most of the annual weeds produce a majority of seeds after completing their vegetative growth. Many seeds are retained in the soil seed bank, which creates problems after emergence in standing crops. Weed populations can be decreased by removing their seeds at maturity (Mahajan et al., 2020).

Weeds constitute major losses to crops which necessitates the use of control practices. Approaches to the biological control of weeds in arable crops and integration of biological weed control with other methods of weed management are broadly discussed (Ghosheh., 2005; Lamichhane et al.,2016; Abbas et al., 2017). Organic agriculture is now emerging as a sustainable alternative to traditional agriculture using environmentally friendly strategies such as the application of herbicides based on plant extracts (Nay and Zhang, 2019). The structural diversity and evolved biological activity of natural phytotoxins offer opportunities for the development of both directly used natural compounds and synthetic herbicides with new target sites based on the structures of natural phytotoxins. Natural phytotoxins are also a source for the discovery of new traditional herbicide discovery (Dayan and Duke, 2014).

In addition, several studies have shown that the ability to control weeds by a crop is very different (or variable) from one variety to another. This difference is partially explained by the ability of these crops to secrete chemicals that affect weed growth, and allelopathy (Morsli et al., 2017). Weeds are one of the most important biological constraints in agricultural production systems. These species are harmful at all stages of crop development. Weeds compete with crops for input resources like, nutrients, water, light and space. Weeds are also important reservoirs of pests and exert allelopathic effects towards cultivated plants. All these traits can affect the yield and quality of crop production (Kouakou et al., 2016).

The influence of weeds on the crop is increasing because of the adoption of broad spectrum herbicides reduce reliance on herbicides causing for environmental, health and regulatory reasons (Colbach et al., 2017). Non-chemical methods are often adopted as means of compensating for reduced herbicide efficacy, due to increasing resistance, rather than as alternatives to herbicides. Justifiably, herbicides are often seen as the easier option. Better recognition of the reasons why farmers are reluctant to use non-chemical alternatives is required to encourage farmers to adopt a longer-term approach to weed control (Moss, 2019). The objective of the present study is to examine the role of needles extract of Atlas cedar (*Cedrus atlantica* L.) on germination and growth of weeds considered to be dominant species in cereal fields.

Materials and methods

Collection and preparation of plant materials

To test the allelopathic effect of Atlas cedar, needles (*Cedrus atlantica* L.) were collected from the Belezma National Park (Batna, Algeria) (6°5’1” E, 35°33’23”N) and were washed with distilled water. The samples were air dried in the open air in the dark

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at room temperature for one month, then ground into fine powder and stored in plastic bags in a refrigerator until use.

Preparation of the extract

For the extraction by maceration, we opted for the protocol described by Rsaissi et al. (2013), The solution was prepared from Atlas cedar powder in sterilized flasks using different concentrations (1%, 2% and 3%) such as 5 g, 10 g, and 15 g in 500 ml of distilled water separately and were filtered using filter paper (Whattman n°1) after 24 h and left at room temperature in the dark. Aqueous extracts of cedar needles were placed in flasks and stored in a refrigerator at a temperature of 4 °C for further use.

Laboratory experiments

The seeds were first treated with ethanol (70%) for 15 min, then with sodium hypochlorite (NaOCl, 6%) for 5 min. Each treatment was rinsed with distilled water.

To test the inhibition of germination 10 seeds of *Bromus rubens* L. were used as test organisms and transplanted into petri dishes containing filter paper. In a laboratory bioassay, the effect of different concentrations (1%,2% et 3%) of the aqueous extracts Atlas cedar needles was studied on germination and early seedling growth of *Bromus rubens* L. Three petri dishes were set up, compared to the control dish (0% of seeds soaked in distilled water). Each treatment was repeated 4 times for a total of 16 petri dishes.

Three milliliters of extracts of different concentrations of *Cedrus atlantica* L. were added to each Petri plate. Treatment in a similar manner with distilled water, served as control. Ten seeds of *Bromus rubens* L. were placed in each petri dish (*Fig. 1*).

Figure 1. *Final germination test of Bromus rubens L.*

Incubation was carried out at 24 °C for 10 days in an oven. Germinated seeds were monitored daily, to understand germination kinetics and evolution. The experimental set-up chosen for the Petri dish were set up in randomized block design in four replications. In total, 16 petri dishes were monitored.

These parameters were also calculated from the formulae proposed by Getu (2009).

Germination and inhibition rate

Percent germination was computed using the following formula Getu (2009):

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$$Viability\ index\ (\%) = \frac{NG}{TG} \times 100 \qquad \text{(Eq.1)}$$

where NG = number of grains germinated and TG = total number of grains tested in each Petri dish.

The inhibition rate is calculated according to the following formula, the results are expressed as a percentage:

$$Inhibition\ rate\ IR\ (\%) = \frac{TG-NG}{TG} \times 100 \qquad \text{(Eq.2)}$$

During this time measurements have been made on the germination rate, the growth of the seedlings (average length of coleoptiles and radicles as well as the production of the dry matter).

Kinetics of germination

For better apprehending the physiological significance of germination behavior, the number of germinated seeds was counted over 10 days.

Greenhouse experiments

These experiments were conducted at the greenhouse to study the allelopathic effects of Atlas cedar needles on seedlings growth of *Bromus rubens* L., 10 seeds were placed in each pot. 3 ml of each extract solution was added. Sterile distilled water was used as a control, and each treatment has been replicated 4 times.

Treatments were arranged in completely randomized design with four replicates, in a follow-up 16 pots (*Fig. 2*).

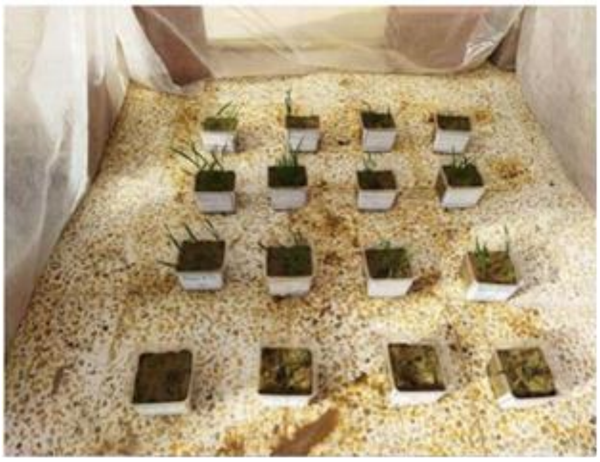

Figure 2. *General aspect of the experimental set-up*

After sowing, experiments were conducted to evaluate plant height, number of leaves and root length of seedlings were measured as well as seedling dry weight and Growth inhibition rate.

Statistical analysis was performed by using analysis of variance (ANOVA) followed by Newman–Keuls test to determine the differences among the means of all experimental groups and to establish significance at the 5% threshold averages among all groups.

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Results

The results of laboratory experiments after 10 days showed that the aqueous extract of Atlas cedar needles significantly reduced seed germination of weed compared to the control (*Table* 1). Effect of aqueous extracts of cedar needles on the germination rate increased with concentration. The highest seed germination was observed in the corresponding control (92.5%). However, the highest concentration (1% 2% 3%) displayed the lowest seed germination which was 75%, 70%, 50% respectively. Bromus seeds were highly affected by the aqueous extract of Cedar needles.

Table 1. *Analysis of variance on the effect of aqueous extracts of Cedrus atlantica L. on germination and morphological traits of Bromus rubens L.*

Source of variation	df	Sum of squares	Mean square	F	Pr > F	S
Germination (%)						
Treatment	3	36.6875	12.2292	2.5411	0.1055	NS
Error	12	57.7500	4.8125			
Total	15	94.4375				
Growth of weed radicle						
Treatment	3	15011.6250	5003.8750	110.3644	< 0.0001	THS
Error	12	544.0750	45.3396			
Total	15	15555.7000				
Growth of coleoptiles						
Treatment	3	7834.8075	2611.6025	24.3130	< 0.0001	THS
Error	12	1288.9900	107.4158			
Total	15	9123.7975				
Dry matter production per plant						
Treatment	3	0.0003	0.0001	20.0687	< 0.0001	THS
Error	12	0.0001	0.0000			
Total	15	0.0003				

The calculation of the germination inhibition rate shows that the highest percentage is obtained in the lot treated with aqueous extract of *Cedrus atlantica* L. leaves at a concentration of 3% with an IR of 45.9% Compared to the control (lots irrigated with distilled water) which has a loss rate of 7.5%, the aqueous extract had a higher inhibitory effect on weed seed germination with 3% concentration (*Fig. 3*).

Statistical analysis showed that at the 5% threshold the effect of the aqueous extract on the germination of weed seeds factor ($p = 0.000$), is not significant ($p < 0.1$) (*Table 1*).

Kinetics of germination

The results of the germination kinetics of the weed in the batches treated with the aqueous extract of cedar show that the reddish bromine exhibits a certain resistance (*Fig. 4*). This clearly shows the effectiveness of the aqueous extract of cedar on the weed. In fact we noticed that when the concentration of aqueous extract increases the germination kinetics slows down.

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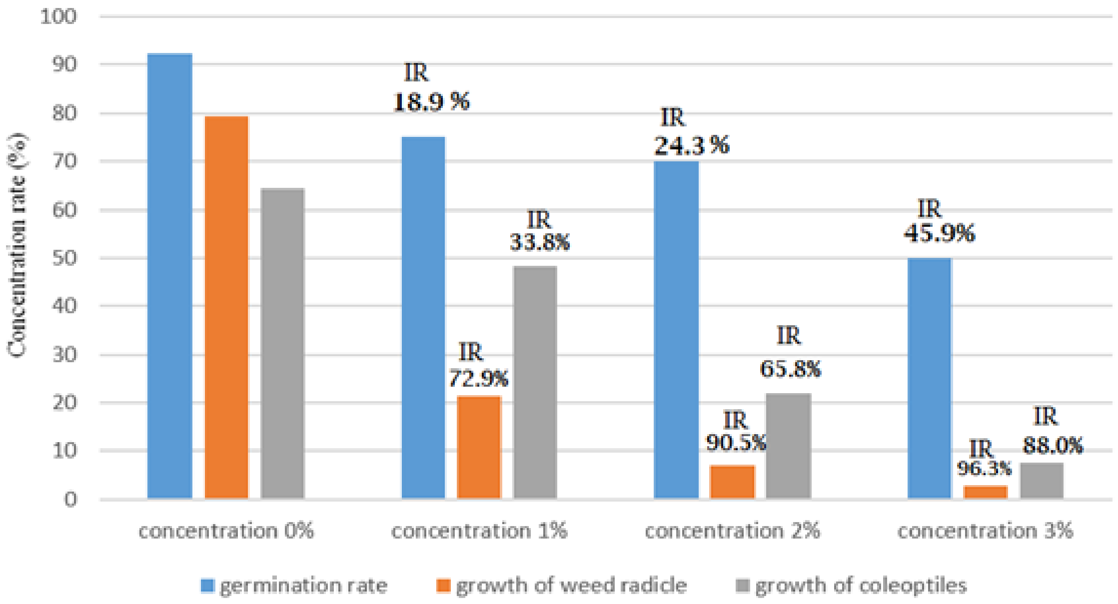

Figure 3. *Effect of aqueous extract of Cedar on different traits of Bromus compared to the control*

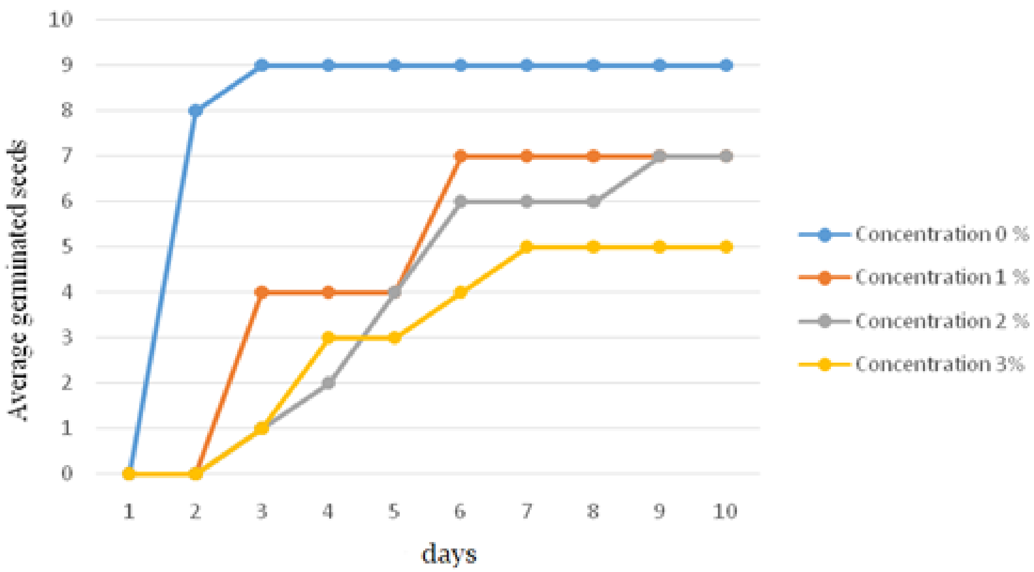

Figure 4. *Weed germination kinetics in the different treatments*

Effect of aqueous extracts of Cedrus atlantica L. on the growth of weed radicle

The results obtained on the allelopathic effect of the aqueous extract of *Cedrus atlantica* L. on the weed radicle (*Fig. 3; Table 2*), revealed that the control has an average length of the radicle of 79.45 mm for the 0% concentration, in batches treated with the aqueous extract at a concentration of 1% the length of the radicle is equal to 21.47 mm, it is 7.15 mm at concentration 2% and 2.92 mm at concentration 3%.

Variance analysis that weed radicle length shows a very highly significant difference ($p < 0.0001$) at the different concentration levels (*Table 1*).

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Table 2. *Comparison and classification of the means of the different parameters of the weed according to the different treatments*

Parameter	**Length of radicle**				**Length of coleoptile**				**Production of dry matter per plant**			
Modality	**Average**	**Homogeneous groups**			**Average**	**Homogeneous groups**			**Average**	**Homogeneous groups**		
Control	79.4500	A			64.5000	A			0.0134	A		
C1 (1%)	21.4750		B		48.2000		B		0.0088		B	
C2 (2%)	7.1500			C	21.9500			C	0.0056			C
C3 (3%)	2.9250			C	7.7000			C	0.0024			C

Effect of Cedrus atlantica L. extracts on the growth of Bromus coleoptiles

The average length of the brome coleoptiles affected by the aqueous cedrus extracts, (*Table 2*) shows three homogeneous groups, the first group (A) represents the control which has a coleoptile length of 64.5 mm, the second group (B) represents the 1% concentration with a length of 48.2 mm for the third group (C) represents concentrations 2% and 3% with a length of 21.95 mm and 7.7 mm respectively (*Fig. 3*).

The statistical analysis indicates that coleoptile length shows a very highly significant difference ($p < 0.0001$) at the different concentration levels (*Table 1*).

Effect of aqueous extracts of cedar on the Bromus dry matter

Figure 5 revealed that the decrease of the dry matter reddish bromus is a function of the concentrations of the extracts. The inhibition rate for the 1% concentration is 34.0%, 58.7% for the 2% concentration and for the 3% concentration is 82.0%.

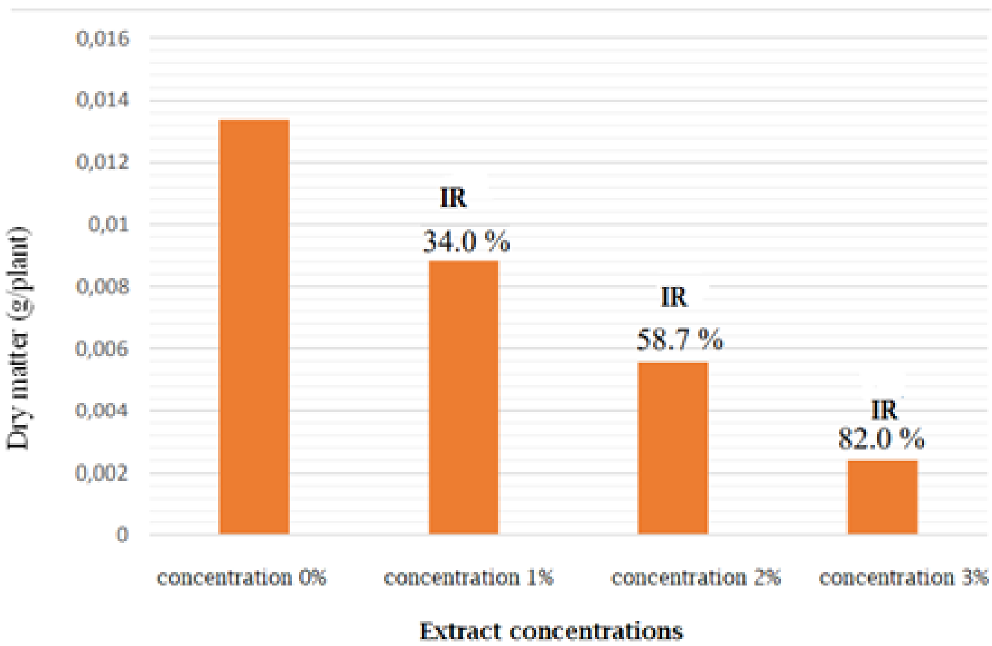

Figure 5. *Effect of aqueous extract of cedar on the Bromus dry matter compared to the control*

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Table 2 shows three homogeneous groups, the first group (A) represents the control with 0.0134 g/plant, the second group (B) represents the 1% concentration with 0.0088 g/plant, the third group (C) represents the 2% and 3% concentrations with averages of 0.0056 g/plant and 0.0024 g/plant, respectively.

The results of the variance analysis reveal that dry matter production shows a very highly significant difference ($p < 0.0001$) at the different concentration levels (*Table 1*).

Discussion

In the present study allelopathic effects of *Cedrus atlantica* L. were observed on germination, length of coleoptiles, radicles and the production of the dry matter of *Bromus rubens* L. Research of (Kruse et al., 2000) have affirmed that exposure of sensitive plants to allelochemicals can affect their germination. According to our results, the germination stops in the seed swelling stage with respect to the controls. For others, germination stops when the radicle begins to appear. Weeds enter into significant competition from the emergence stage and continue until maturity. The measurement of the above-ground biomass of reddish bromine is usually considered to be the best indicator of the effects of competition.

According to Gomes et al. (2017) allelopathy is an important ecological phenomenon in determining the structure and composition of plant communities influencing ecosystem dynamics. Currently, it has gained attention due to the potential applications of allelochemicals in agriculture. Weeds constantly compete with crop plants to cause a considerable loss in their productivity. Therefore, weeds have been documented as serious plant pests for a long time, interfering with the functions of plants and suppressing their growth and development (Jabran, 2015). The persistence of weeds is directly influenced by their capacity to interfere with other plants, either through competition for resources or through chemical interference mediated by plant-produced secondary metabolites, a phenomenon known as allelopathy. Recent research has focused on critically assessing the role of allelochemicals in plant succession and invasion, in both native and invaded ecosystems and their associations with other plants and the soil microbiome (Latif et al., 2022).

Wheat is the most grown crop in the world and has an economical significance for the humankind. Weeds have been shown to possess negative impacts on wheat growth and yield. This result is similar to previous findings on wheat by Abu-Romman et al. (2010).

The results compared to those of Sondhia and Saxena (2003) showed that the saponin isolated from *Xanthium strumarium* L. significantly inhibits the germination, root and stem length of *Vicia sativa* L. The results of this study were also in agreement with observations made by Batish et al. (2002), Turk and Tawaha (2003), Arslan et al. (2005), Nandal and Dhillon (2005), and Uremis et al. (2005) showing that the inhibition increases with increasing concentration of extracts. In this work we tested, at different concentrations, the effect of the aqueous extract of the needles of Atlas cedar (*Cedrus atlantica* L.) on the germination and seed growth of reddish bromine.

Allelopathy is a biological phenomenon in which the plant synthesizes one or more allelochemical compounds that influence germination, growth, metabolism, development, survival and the reproduction of other organisms (Hossain et al., 2012). According to Mali and Kanade (2014) and Edrisi and Farahbakhsh (2011), allelochemicals have beneficial or harmful effects on the target organisms and the

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community. Weeds can also affect crop growth by releasing allelochemicals in the culture environment.

Many works have been reported the beneficial as well as the harmful allelopathic effects of weeds and on plants seed germination (Khan et al., 2008; Ghodake et al., 2012), growth of root shoots (Dessalegne et al., 2013).

The results obtained in this study are promising showing clearly that the aqueous extract of the leaves of *Cedrus atlantica* L. has inhibitory effects on the germination and growth of the weed plant from the point of view of efficacy at low concentration.

The same observation is made for the kinetic parameter of germination which it has inhibited. The inhibitory effects increased as the extract concentration increased and allelopathy was manifested. The increase in the concentration of aqueous extract caused significant inhibitory effects on the germination and growth parameters. These same findings have been obtained by other researchers by Ahmed (2007) and Talhi et al. (2020). The results obtained for rootlet length, coleoptile length and dry matter production are similar to the results for germination. The diversity of allelopathic relationships discovered in plants opens up important prospects for human use. These natural substances could ultimately solve the weed problems that must always be managed through the use of synthetic herbicides (Hallett, 2005). While the majority of weed problems are brought under control in the short term by chemicals, long-term adverse effects should not be overlooked.

Controlling the use of plants and allelopathic substances in agriculture would provide natural herbicides, fungicides and insecticides that are supposed to be able to preserve the environment. In recent decades there has been an increase in demand for natural allelopathic compounds having selective toxicity to weeds and can be degraded by plants or soil microorganisms (Sodaeizadeh and Hosseini, 2012).

In addition to the inhibitory effect, allelochemicals can have a stimulating effect by promoting the growth of other plants. These molecules may also in other cases have no negative or positive effect, but these studies are small and receive less attention from researchers. Among other things, cereals remain too sensitive to competition from weeds that can significantly affect yield and cause important crop losses. Plants in a plot interfere with each other in different ways. In addition to the classic competition for water, nutrients, space and light, in recent years an influence induced by chemical molecules, called allelopathy have been shown (Kunz et al., 2016).

Conclusion

Aqueous extracts of the leaves of Atlas cedar (*Cedrus atlantica* L.) in increasing concentrations seem to have an inhibitory effect on physiological processes. Depending on the concentration of extracts from the leaves of Atlas cedar, they have an inhibitory effect, which negatively influences germination and growth of seeds.

The results presented in this paper allow a reasonable hope of use of plant extracts as herbicides in the biological control because the plant tested during this work showed real germination inhibitory properties. The application of plant extracts to control weed crop is an ecological strategy that could potentially be useful in agriculture and environmental safety.

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