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Allelopathic effect of Sidr (Ziziphus spina- christi (L.) Wild.) Leaf extracts on seed germination and seedling parameters

Abstract

Plants have several direct or indirect effects on other surrounding organisms in which it may be harmful or beneficial, in this study the potential allopathic effects of Ziziphus spina-christi (Sidr) leaf extracts were investigated alongside wheat (Triticum aestivum L), barley (Hordeum vulgare L.), cress (Lepidium sativum L.) and fenugreek (Trigonella foenum-graecum L.) Seeds for the observation of their germination, in which different concentrations (0%, 2.5%, 5.0%, 7.5%, and 10%) of sidr aqueous and methanolic extracts (70% methanol) were made and used against these seeds in vitro and their effect on germination was determined. This experiment showed a significant inhibitory effect of both extracts on seed germination at all concentrations and it became more effective as the concentration rises, as a result, sidr leaf extracts may demonstrate the use of this species as an herbicide and provide significant agricultural success

Keywords

sidr; barley; wheat; seed germination; water extract, methanolic extract

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RESEARCH ARTICLE

Allelopathic effects of *Ziziphus spina- christi* L. (Wild) Leaf extracts on germination and seedling growth of some plant species

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ABSTRACT

Background and objectives: plants have several direct or indirect effects on other surrounding plants in which it may be harmful or beneficial.

Methods: in this study the potential allopathic effects of *Ziziphus spina-christi* (Sidr) leaf extracts were investigated alongside wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), cress (*Lepidium sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.).Different concentrations (0%, 2.5%, 5.0%, 7.5%, and 10%) of sidr aqueous and methanolic extracts (70% methanol) were used *in vitro* and their effect on germination and seedling growth.

Results: This experiment showed a significant inhibitory effect of both extracts on seed germination at all concentrations and it became more effective as the concentration rises, as a result, sidr leaf extracts may demonstrate the use of this species as an herbicide and provide significant agricultural success.

Keywords; sidr; seed germination; water extract, methanolic extract.

INTRODUCTION

Allelopathy refers to the action as a promotion or inhibition effects of a plant on surrounding organisms, including crops and weeds, caused by the release of compounds from plant parts via root exudation, residue decomposition, volatilization, and leaching of compounds, as well as other mechanisms in both natural and agricultural systems(Goran, 2020). Allelochemicals are secondary metabolites that are produced by plants not required for the metabolic activities of the organism (growth and development) and allelochemicals are of plant defensive component line against herbivore (El-Amier *et al*., 2015).The word allelopathy is a combination of the Greek words allelo and pathos (which mean "mutual harm") in which allelopathy has the potential to affect many factors of plant physiology such as occurrence, development, plant transition, plant community structure, dominance, variety, and biomass production (Chou, 1999).

Allelopathy's characteristics is commonly associated with decreased seed germination and seedling growth, moreover, allelopathic suppression is a complicated process involving the interaction of different classes of compounds such as, flavonoids, phenolic compounds, alkaloids, terpenoids, carbohydrates, amino acids and steroids, with compounds with different substances having a greater allelopathic effect than individual compounds on its own (Yasir *et al*., 2018). Allelopathic weed suppression can be influenced by environmental and physiological stresses, herbicides, solar radiation and less-than-optimal nutrient, temperature levels and moisture, one of the plants known to have allelopathic effect is sidr (*Ziziphus spina-christi* L.) that is found in tropical and subtropical areas especially in places with high altitude

due to its high tolerance to elevated temperatures and drought (Zandiehvakili and Khadivi, 2021) it belongs to the family: Rhamnaceae and holds around 100 species of annual plant shrubs and trees worldwide (Johnston, 1963). *Ziziphus spina-christi* is a tree that can grow to a height of (5-12) meters and a width of (45) centimeters or more (El Amin, 1990). Sidr's seed coat is thick and woody, which measures about 1.38-2.0 mm. This may play a major role in the species' lack of or postponed seed germination , as a result its ability to occupy sites and compete for resources with other species may be hampered (AlshahraniThobayet, 2004)

The leaves of sidr are a basis of medicinal compounds used by the native residents to treat a variety of conditions (Al-Qarawi and Alshahrani, 2010). Leaves may have been used as a cleaning product as well as a supply of nutrition for animals. Compounds such as flavonoids, alkaloids, saponins, and other allelopathic compounds found in the species leaves may play a significant role in understanding the interaction is critical for making well-versed choices about the management of presented species (Nawwar *et al*., 1984). As a result, it is critical to investigate the species' allelopathic properties. The perennial plant tree *Ziziphyus spina-christi* (sidr) has been linked to a variety of medicinal applications, containing nutrition and immunity (Jiang *et al*., 2007). Moreover, allelopathy interaction has been commonly used to protect crops and substitute environmentally damaging fungicides, herbicides and insecticides. Other plants and microorganisms were inhibited in their development and growth by sidr leaf extracts, which is known as a medicinal plant that can be used to treat a variety of ailments such as ulcers, diabetes, and wounds due to a compound known as Ziziphyus.

MATERIALS AND METHODS

1. Plant preparation

a. Sidr Leaves Preparation

Sidr plant leaves were collected from Baghdad in summer 2021, air-dried for almost 3 days at 45^{o}C, in an air dryer cabinet in advanced plant physiology lab at collage of science-Salaheddin university then ground to a fine powder through 1 mm mesh, for crude extract extraction, methanol (70%) and water have been used as a diffusion medium.

b. Tested Seeds

The testing seeds of wheat (*Triticum aestivum* L), barley (*Hordeum vulgare* L.) were obtained from the Agriculture Research Center in Erbil city, while cress (*Lepidium sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.) were obtained from local market (qaysari bazar).

2. Preparation of Extracts

a. Aqueous Extraction

Aqueous extracts of sidr leaves, were obtained by measuring 50g of plant leaves powder and placed it in a conical flask, adding 500ml of distilled water and left on a shaker for 72 hours. Subsequently the mixture was nominated in three layers of muslin cloth to remove the large particles, then filtration of the mixture was carried out by filter paper and the extract was obtained. Placed into a 500 ml volumetric flask, then distilled water was added up to the 500 ml mark, getting a concentrated stock solution of 10%. Owing that, extracts at various concentrations (2.5%, 5.0%, and 7.5%) were prepared from 10% stock solution, placed in dark glass bottles, and keep in refrigerator (Goran, 2020).

b. Methanolic Extraction

Plant leaf powder (50g) was placed in a 1000 ml conical flask; 500 ml of 70% methanol was added and then the solution was left for 72 hours on shaker. When filtered three layers of muslin cloth was used to remove the large particles then after the filtrate was filtered by filter paper. The filtrate was concentrated using a rotary evaporator; the final extract was completed up by distilled water to reach 500 ml to get a 10% stock solution in a volumetric flask, furthermore different concentrations were made (2.5%, 5.0%, and 7.5%) and it was left in refrigerator for further use (Ndip et al., 2007).

3. Preparation of seeds for Germination

Testing seeds were placed in a sterile Petri dish lined with filter paper, water and methanolic extract were applied to these seeds in different concentration (2.5%, 5.0%, 7.5% and 10%) 5 ml each and the control get distilled water only. They were incubated for one week at 23^{O} C in which finally the measurement of plumule and radicle length, and germination percent of seeds for each species was calculated.

4. Statistical analysis

Complete Randomized Design (CRD) was used for compering the means. Petri dishes were distributed randomly in the cabinet. Seed percent germination and seedling growth parameters, radicle and plumule length in millimeters, were measured. The results were statistically analyzed using the graph pad software to test the signification of results. The germination percentage was calculated using the following equation:

$$\text{Germination \%} = \frac{\textbf{Number of seeds germinated}}{\textbf{total number of seeds sown}} \times 100$$

RESULTS

The obtained results in this study indicates that both Sidr leaf extracts assessed on the seed germination percent and seedling parameters, all seeds were grown in petri dishes and treated with different concentrations of the aqueous and methanolic extracts.

The germination percentage was significantly affected with the various concentrations of both extracts used, all plant seed growth shown a gradual decrease in germination percent with the increase of the extract's concentration as shown in (Table1 and 2) the highest germination percentage was observed in the control group and lowest was with 10% concentration.

Table 1: Percent germination of seeds in water extract of sidr leaves

plant seeds	Control	2.5%	5.0 %	7.5 %	10 %
Wheat	91%	82%	79%	74%	59%
Barley	100%	95%	80%	42%	7%
Fenugreek	84%	82%	56%	40%	33%
Cress	97%	85%	72%	55%	37%

Table 2: Percent germination of seeds in methanolic extract of sidr leaves

Moreover, the result obtained using methanolic extract showed a higher inhibition rate of germination percentage than aqueous extract.

plant seeds	Control	2.5%	5.0%	7.5%	10%
Wheat	77%	62%	38%	18%	15%
Barley	70%	23%	11%	5.0%	0%
Fenugreek	84%	55%	52%	40%	31%
Cress	90%	83%	70%	51%	11%

On the other hand, the length of radicle and plumule of treated seeds with both extracts were measured, longest radicle length was observed in the control group alongside the increasing concentration, in which 10% marked the shortest radicle length (Table 3&4) furthermore similar results were obtained with plumule in which the plumule length decreased with increasing the concentrations as shown in (Table 5&6) which indicates that the means of the data in different concentration is significant with the control group mean.

Table 3: Effect of Sidr leaf water extract on radicle length (mm)

Plant tested	N	Control	2.5%	5.0%	7.5%	10%
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Wheat	100	176.9* ± 100.1	48.08*± 4.7	24.6* ± 2.0	17.5* ± 1.6	13.7* ± 1.6
Barley	100	78.4* ± 1.7	70.9* ± 2.0	26.9* ± 1.8	8.6* ± 1.2	1.1* ± 0.5
Fenugreek	100	46.8*±1.48	38.48*±2.22	21.27*±1.63	14.4*±1.66	14.31*±2.1
Cress	100	32.8* ± 1.5	21.6* ± 1.6	12.8*± 1.1	9.8* ± 1.06	1.6* ± 0.5

(*) =P.Value <0.0001

Table 4: Effect of Sidr leaf methanolic extract on radicle length (mm)

Plant tested	N	Control	2.5%	5.0%	7.5%	10%
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Wheat	100	50.8* ± 4.4	22.7* ± 2.3	1.6* ± 0.4	0.04* ± 0.02	0.03* ± 0.02
Barley	100	47.0* ± 4.7	12.9* ± 1.3	2.02* ± 0.7	1.7* ± 0.8	0* ± 0
Fenugreek	100	45.88*±2.38	31.24*±172	8.5*±0.80	3.4*±0.4	0.63*±0.21
Cress	100	20.9* ± 1.3	16.4* ± 1.1	5.8* ± 0.6	2.2* ± 0.4	0.2* ± 0.1

(*) =P.Value <0.0001

Table 5: Effect of Sidr leaf water extract on plumule length (mm)

Plant tested	N	Control	2.5%	5.0%	7.5%	10%
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Wheat	100	50.77* ± 4.4	22.7* ± 2 .3	1.6* ± 0.4	0.04* ± 0.02	0.030* ± 0.08
Barley	100	103.7*± 1.2	86.54* ± 2.52	45.4* ± 3.0	19.0* ± 2.3	2.1* ± 1.0
Cress	100	46.80* ± 1.5	38.48* ± 2.22	21.2* ± 1.6	14.44* ± 1.6	14.31* ± 2.1

(*) =P.Value <0.0001

Table 6: Effect of Sidr leaf methanolic extract on plumule length (mm)

Plant tested	N	Control	2.5%	5.0%	7.5%	10%
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Wheat	100	52.2* ± 3.9	27.6* ± 2.9	5.4* ± 0.9	0.4* ± 0.1	0.6* ± 0.2
Barley	100	39.7* ± 4.7	9.0* ± 2.0	2.1* ± 0.7	1.7* ± 0.8	0* ± 0
Cress	100	45.9* ± 2.4	31.2* ± 1.7	8.5* ± 0.8	3.43*± 0.4	0.6* ± 0.2

(*) =P.Value <0.0001

As noted, both extracts have more inhibitory effect on radicle length than plumule and the methanolic extract has more inhibitory effect than the aqueous extract on the radicle and plumule length.

DISCUSSION

This study indicates that both (water and methanolic) extracts of sidr leaves in elevated concentration can suppress germination as well as seedling development, the result for all seeds showed the 10% extract concentration had the lowest germination percent comparing with concentration groups and control, which had the highest percent germination, and as noted the methanolic extract had more negative impact on tested seeds in comparing with water extract, this is explained by that the allelochemicals are dissolved in methanol much more that is in water and this is due to polarity, in which only polar compounds dissolve in water but for methanol polar and non-polar compound are dissolved and this explains the effectiveness of the methanolic extract more than the water as it is tested (Lapornik *et al*., 2005)

On other hand the study showed that radicle and plumule length of all seeds were affected by methanolic and water extract used, in which the results were similar and significant in all seeds. When using Sidr leaves water extract the radicle of all seeds in control group were the longest length but when applying the 2.5%-10% concentrations there was a significant decrease in length alongside the increasing concentration of extract, and the same result was obtained in the methanolic extract in which with increasing the extract concentration the radicle length decreases (Table 3&4).

Furthermore, plumule length in all seeds showed a significant decreasing as the concentration increases (Table 5&6), results showed that the radicle is more sensitive to the sidr extracts than plumule , based on previous studies radicle is more sensitive to allelopathic effect than plumule and this could be due to certain allelochemicals that effect plant hormones such as gibberellin and indoleacetic acid function in meristematic cell (Elaloui *et al*., 2017).

Results of the present study revealed that the methanolic

extract had more inhibitory effect on radicle and plumule development in all seeds than the aqueous extract, based on previous studies that conducted the amount of total phenolic content differ significantly among the various extracts of the Sidr leaves.

The above-mentioned results are in accordance with a previous investigation, that methanolic extract showed a highest phenolic contents, followed by aqueous extracts (Abdullah, 2013).The maximum amount of phenolic in Sidr fruit was investigated in methanol extract, followed by acetone and water extracts (Atik and Mohammedi, 2011). The difference in phenolic content between extracts can be determined by the nature of the extracting solvent as well as the chemical nature and availability of the compounds extracted (Bae *et al*., 2012).

According to some research findings, methanol and ethanol were superior extraction solvents for phenolics from plant materials than less polar solvents such as acetone and hexane (Mohsen and Ammar, 2009). Plant materials may contain phenolics in varying amounts ranging from simple (e.g., phenolic acids, anthocyanins) to highly polymerized (e.g., tannins). Furthermore, phenolics may be linked to other plant components such as carbohydrates and proteins(Dai and Mumper, 2010). And this explains why the methanolic extract had more effect than the water extract on the tested seed germination and development.

CONCLUSION

This study showed a clear divergence in the Sidr leaves extract efforts allelopathic effects being tested and this disparity has shown a clear effect on the germination and growth of tested seeds. The effect of both methanolic and aqueous extract of Sidr plant tested on the germination percent of tested plant seeds showed a great inhibitory effect in the percentage of germination of plant seeds. Both showed a difference in the impact on the growth of the seed's germination tests.

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