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Phytochemical and allelopathic effect of aqueous extracts of *Cenchrus ciliaris* L. on seed germination of *Lolium rigidum* G., *Daucus carota* L. and *Torilisnodosa* L.

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Abstract:
Background: The application of biological weed control is an alternative strategy to ensure agricultural sustainability since its improvement and expansion will contribute to limiting excessive use of herbicides.
Materials and Methods: In this study, the allelopathic effect of five ecotypes of Cenchrus ciliaris L. on seed germination of three weed species (Lolium rigidum G., Daucus carota L. and TorilisnodosaL.) was tested through aqueous extracts obtained from aerial part. Experiments were carried out under laboratory conditions using eleven different concentrations (0, 6.5, 7.5, 12.5, 14, 15.5, 17, 18.5, 20, 21.5 and 23%).
Results: The results emphasis that the extracts of the different accessions affect significantly ($p<0.05$) the germination of the target plants seeds depending on the interaction between species and concentration level.The highest germination was obtained at the control treatment and decreases as the concentration level increases. Moreover, T. nodosa was the most sensitive to the C. ciliaris aqueous extract with an inhibition percentage that reached 100% for 12.5 g/l. The application of concentrations more than 20% of different accessions of C. ciliaris totally inhibits the germination of L. rigidum seeds in a highly significant manner. For concentrations higher than 14%, a total and significant reduction ($p<0.05$) in seed germination of D. carota was recorded.
Conclusion: This study showed that extracts of the aerial part of C. ciliaris are very rich in phenolic compounds and could be used as a natural antioxidant. It has also shown that aqueous extracts of this species have allelopathic effects and could be applied in agro-ecosystems to reduce the use of highly redoubtable industrial herbicides.
Key Word: Cenchrus; Allelopathy; Seed germination; Aqueous extracts; Phenolic compounds.

I. INTRODUCTION

The phenomenon of allelopathy encompasses all types of direct and indirect chemical interactions by releasing some allelochemicals, mainly secondary metabolites among plants (Bachheti et al., 2020). Thus, efforts are being made to create new environmentally friendly strategy of weed management. Allelopathy has profound implications for weed management leading to the reducing of environmental contamination (Mushtaq et al., 2020). It is widely acknowledged that the presence of high levels of secondary metabolites in a given species can enhance its allelopathic potential since many of these compounds interfere with various mechanisms of action and could influence a number of target sites (Lotina-Hennsen et al., 2006). Preliminary phytochemical screening experiments are commonly performed to promote a guidance of substantial phytochemicals that may be involved in the allelopathic activity of plant extracts (Carvalho et al., 2019).

The genus *Cenchrus* L.(*Poaceae*) comprises about 20 species, predominantly fairly well distributed throughout warmer parts of the New World and with some additional species in Asia and Africa (De-Lisle 1963; Mabberley, 2008). *Cenchrus ciliaris* L. commonly known as buffelgrass, is a valuable perennial grass native to West Asia, India and the whole African continent (M'seddi et al., 2002) usually grown in arid and semi-arid regions (Laishram et al., 2020). Agronomically, *C. ciliaris* has been extensively evaluated in various studies for its reseeding potential (Kriwa et al., 2010), performance in mixtures (Mganga et al., 2010), yield and yield components (Ojo et al., 2020) and allelopathic effects (Kriwa et al., 2012). Leachates from the leaves and roots of *C. ciliaris* were shown to reduce germination rates of seeds and the radicle length of several plants (Hussain et al., 2010). There is some evidence that buffelgrass can release allelopathic chemicals (Kannan and Priyal,

2015), which alter soil properties to the extent that germination and growth of other plants is inhibited (Cheam, 1984; Nurdin and Fulbright, 1990).

The aims of this study are to (1) determinate the polyphenols and flavonoids contents of *C. ciliaris* aerial part extracts collected from different regions in Tunisia and to (2) investigate their allelopathic activities.

II. MATERIAL AND METHODS

Sample prospection and collection

Based on data published by Pottier-Alapetite (1981) on the range of Tunisian spontaneous *C. ciliaris* L. species, prospecting missions were carried out during 2010 and 2011 in different bioclimatic zone in central Tunisia (Dar Khera El Grin, Chrichira Haffouz, El-Houareb, Elkarma and Chott Meriem) (Table1). Five ecotypes of *C. ciliaris* L. were collected at vegetative growth stage from their natural habitats. The plant aerial part was air-dried at room temperature (20±2 °C). The dried parts were ground in a Mixer Mill (MM 200, Retsch GmbH, Germany), sieved through 0.5 mm mesh screen to obtain a uniform particle size. The powder was stored at room temperature until used (Dallali et al., 2014).

Table 1. Origin of plant material

Codes	Abbreviations	Longitude	Latitude	Altitude (m)	Locality	Bioclimatic zone*
E.S.A.M.01.731.01.01	CC1	9°53.274’	35°39.369’	126.2	Kairouan/Chebika/Dar Khera El Grin	LSAFW
E.S.A.M.01.731.01.02	CC2	9°39.429’	35°37.956’	256.4	Kairouan/Haffouz/Chrichira	ASTW
E.S.A.M.01.731.01.03	CC3	9°45.422’	35°32.623’	182	Kairouan/Chebika/El-Houareb	LSAFW
E.S.A.M.01.731.01.04	CC4	9°48	35°34.522’	170	Kairouan/Chebika/El-karma	LSAFW
E.S.A.M.01.731.01.05	CC5	10°33.453’	35°54.726’	40	Sousse/Chott Meriam	SAIMW

*Bioclimatic zone is defined according to Emberger’s (1976). LSAFW: Lower Semi-Arid with Fresh Winter, ASTW: Arid Superior with Temperate Winter, SAIMW: Semi-arid inferior with mild winter.

Total phenolic content

The total phenolic content of aerial parts was assessed using the Folin-Ciocalteu reagent, following Singleton and Rosi’s (1965) method. The standard curve was prepared by gallic acid solutions in methanol. The concentration of total phenolic compounds in the extracts was determined as µg of gallic acid equivalent using an equation obtained from the standard gallic acid graph, and expressed as mg gallic acid/g dry weight of the plant material (mg GAE/g DW). The data were expressed as the mean of triplicate analyses.

Total flavonoid content

The total flavonoid contents of plant samples were determined according to aluminum chloride colorimetric method (Djeridane et al., 2006). 1 mL of each extract was mixed with 1 mL of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 min, the absorbance was measured at 430 nm. Quercetin was chosen as a standard curve, the levels of total flavonoid contents in the sample extracts were determined in triplicate, respectively. Total flavonoids were expressed as mg quercetin equivalent/g DW (mg QE/g DW).

Allelopathic activity

The different extracts were prepared by soaking eleven different weighed amounts of air-dried aerial part of *Cenchrus ciliaris*: 6.5, 7.5, 12.5, 14, 15.5, 17, 18.5, 20, 21.5 and 23g per 100 mL of distilled water at room temperature. Distilled water was used as control. The extracts were filtered through filter paper to get the aqueous extracts. Before conducting the germination test, 5% sodium hypochlorite (NaOCl) solution was used to surface sterilize the seeds, which were soaked for 10 min to avoid fungal contamination and subsequently rinsed thoroughly with sterilized distilled water.

For each treatment, twenty-five (25) seeds were placed in petri dishes (Ø = 90 mm) upholstered with two layers of filter paper (Whatman No. 1) and moistened initially with 2 mL of distilled water or assigned test solution (Marichali et al., 2014) and incubated at 30 °C. The germination percentage (GP) was calculated according to Moussavi-Nik et al., (2011): $GP\ (\%) = Nt \times 100/N$; Where Nt is the number of germinated seeds in respective treatments and N is the total number of seeds used in bioassay.

Statistical analysis

All experiments were conducted in a randomized complete block design (RCBD) with three replicates per treatment. The differences between individual means were deemed to be significant at $p<0.01$. Data were analyzed by a Plabstat software Version 3A (Utz 2011), and followed by mean comparison by the LSD test.

III. RESULT AND DISCUSSION

Extract yield, total phenolic and flavonoid content

The extracted yield from areal part of Tunisian endemic *C. ciliaris* by methanol is reported in Table 2. The extraction yields (w/w, on dry weight basis) presented significant difference among accessions and ranged from 13.49% to 17.36%. The highest extraction yield was obtained by CC5 while the lowest was by CC4. To the authors' knowledge, there is no study in the literature which investigated the extracted yield from the areal parts of this spontaneous Tunisian endemic species collected from different regions.

Total phenolics content of the plant extracts varied significantly between accessions (p<0.01) and ranged from 2.243 to 3.228 mg GAE/g DW. The highest total phenolic content was observed for CC2 while the lowest was recorded for CC5. Similarly, total flavonoids content expressed in quercetin equivalent, varied significantly (p<0.01) between accessions and ranged from 1.120 to 2.761 mg QE/g DW. These variations are probably due to the presence of considerable genetic diversity and by the environmental conditions in which the plants are grown.

Table 2. Extract yield, total phenolic and flavonoids contents of methanol extract of *C. ciliaris*.

Accessions	Extraction yield (%)	Total phenolic content (mg GAE/g DW)	Flavonoids content (mg QE/g DW)
CC1	16.08^{b}	2.243^{c}	1.305^{c}
CC2	15.28^{b}	3.228^{a}	1.805^{b}
CC3	13.55^{c}	2.265^{c}	1.120^{d}
CC4	13.49^{c}	2.920^{b}	2.761^{a}
CC5	17.36^{a}	2.327^{c}	1.354^{c}
Moyenne	15.15	2.396	1.669
SEM	1.04	0.16	0.09

Means followed by the same letter do not differ statistically at p<0.01

Effect of interaction genotype × concentration on seed germination

Statistical analysis shows that the interaction genotype × concentration has a significant effect (p<0.05) on *Lolium rigidum* G., *Daucus carota* L. and *Torilisnodosa*L. seed germination (Table 3, 4 and 5).

Table 3. Effect of interaction genotype × concentration on seed germination of *Lolium rigidum* G.

Concentrations (%)	CC1 (%)	CC2 (%)	CC3 (%)	CC4 (%)	CC5 (%)	Mean
0	100.00	100.00	100.00	100.00	100.00	100. 00^{a}
6.5	74.67	74.67	61.33	76.00	92.00	75.73^{b}
7.5	52.00	72.00	62.67	49.33	77.33	62.67^{c}
12.5	53.33	48.00	33.33	30.67	49.33	42.93^{d}
14	52.00	41.33	28.67	26.67	38.67	37.47^{de}
15.5	44.00	36.00	26.67	25.33	30.67	32.53^{ef}
17	33.33	26.67	24.00	22.67	28.00	26.93^{fg}
18.5	30.67	17.33	24.00	14.67	25.33	22.40^{g}
20	26.67	0.00	26.67	0.00	20.00	14.67^{h}
21.5	0.00	0.00	24.00	0.00	5.33	5.87^{i}
23	0.00	0.00	0.00	0.00	0.00	0.00^{i}
Mean	42.42^{a}	37.82^{a}	37. 39^{a}	31.39^{b}	42.42^{a}	38.29

Standard error genotype = 1.35; Standard error concentration = 2.00; Standard error genotype × concentration = 4.46. Means followed by the same letter do not differ statistically at p<0.01.

Table 4. Effect of interaction genotype × concentration on seed germination of *Daucus carota* L.

Concentrations (%)	CC1 (%)	CC2 (%)	CC3 (%)	CC4 (%)	CC5 (%)	Mean
0	40.00	42.67	53.33	44.00	34.67	42.93^{a}
6.5	29.33	33.33	28.00	26.67	30.67	29.60^{b}
7.5	21.33	22.67	25.33	21.33	18.67	21.87^{c}
12.5	21.33	17.33	16.00	16.00	16.00	17.33^{d}
14	0.00	0.00	0.00	0.00	12.00	2.40^{e}
15.5	0.00	0.00	0.00	0.00	0.00	0.00^{e}
17	0.00	0.00	0.00	0.00	0.00	0.00^{e}

18.5	0.00	0.00	0.00	0.00	0.00	0.00[e]
20	0.00	0.00	0.00	0,00	0.00	0.00[e]
21.5	0.00	0.00	0.00	0,00	0.00	0.00[e]
23	0.00	0.00	0,00	0.00	0.00	0.00[e]
Mean	10.18[a]	10.55[a]	11.15[a]	9.82[a]	10.18[a]	10.38

Standard error genotype = 0.60; Standard error concentration = 0.89; Standard error genotype × concentration = 1.99. Means followed by the same letter do not differ statistically at $p<0.01$.

Table 5. Effect of interaction genotype × concentration on seed germination of *Torilisnodosa*L.

Concentrations (%)	CC1 (%)	CC2 (%)	CC3 (%)	CC4 (%)	CC5 (%)	Mean
0	100.00	100.00	100.00	100.00	100.00	100.00[a]
6.5	25.33	49.33	56.00	46.67	49.33	45.33[b]
7.5	18.67	41.33	52.00	29.33	44.00	37.07[c]
12.5	0.00	26.67	21.33	16.00	28.00	18.40[d]
14	0.00	20.00	0.00	16.00	14.67	10.13[e]
15.5	0.00	0.00	0.00	0.00	14.67	2.93[f]
17	0.00	0.00	0.00	0.00	14.67	2.93[f]
18.5	0.00	0.00	0.00	0.00	0.00	0.00[f]
20	0.00	0.00	0.00	0.00	0.00	0.00[f]
21.5	0.00	0.00	0.00	0.00	0.00	0.00[f]
23	0.00	0.00	0.00	0.00	0.00	0.00[f]
Mean	13.09[c]	21.58[ab]	20.85[ab]	18.91[b]	21.58[ab]	19.71[b]

Standard error genotype = 0.86; Standard error concentration = 1.27; Standard error genotype × concentration = 2.84. Means followed by the same letter do not differ statistically at $p<0.01$.

The analysis of variance revealed that germination percentage was significantly affected ($p<0.05$) by the different genotypes and the different concentrations of the extracts. The highest seed germination was recorded in the control treatment reaching 100%, for *L. rigidum* G., and *T.nodosa* and 42.93% for *D. carota* L. Increasing the aqueous extract concentrations resulted in delayed germination when compared with distilled water. In the control treatments, the seed germination reached 100% except for *D. carota*, which the highest germination percentage was observed for CC3 (53.33%). *Torilisnodosa*L. exhibited the highest inhibitory effect followed by *Daucus carota* L. and *Lolium rigidum* G. For *L. rigidum*, the highest seed germination, while being close to the control treatment, was shown by the lowest extract concentration (6.5%) and varied between 61.33% for CC3 and 92% for CC5. This indicates that there might be inhibitory compounds in water extracts, which delayed the germination process of target species.

The lowest seed germination of *L. rigidum* G. seeds was observed for CC5 decreasing from 100 to 5.33% as the concentration of the extract increased from 0 to 21.5%. Germination was completely prevented at 23% concentration (Table 2). However, for *Daucus carota* L., the seed germination was affected by the extract concentration and only CC5 was able to germinate at 14% with a percentage of 12%. Whereas nil germination occurred when concentration levels exceeded the 14% (Table 4). For *T.nodosa*, results show that the seed germination was affected at 12.5% of the extract concentration reaching 16% for CC4. By increasing concentration, inhibition degree increased. No germination occurs for concentrations above 17% for all *Cenchrus* accessions (Table 5).

Seed germination is considered as a critical stage especially under stress conditions. These differences on allelopathic activity on germination stage can be due to the difference in the quantitative and qualitative composition of extracts depending on the plant part, on the geographical growing area and the harvesting period. Many grasses exhibit allelopathy against the associated species by releasing of water-soluble phytotoxins through rain, dew or irrigation water.

The present study revealed that the aqueous extracts from aerial part of *Cenchrus ciliaris* L. obtained by soaking reduced germination of the tested species. The percentage of seed germination decreased corresponding to the increasing extract concentration. In addition, our bioassay indicated that the allelopathic effects of *C. ciliaris* extract on test species are concentration and genotypes dependent. The interaction between different concentrations and different genotypes were significant ($p<0.05$) for the germination of the three species.

Allelopathic potential is often verified by testing their influence on seed germinability and seed viability. Aqueous extracts of the aerial parts of *C. ciliaris* L. exhibited phytotoxic activity and effectively inhibit the seed germination of *L. rigidum* G., *D. carota* L. and *T. nodosa* L. depending on the applied

concentration. These findings are consistent with the findings of Mushtaq et al., (2020) who reported that allelopathy includes inhibitory activities and is a concentration-dependent phenomenon.

The obtained data strongly suggest that the decrease in the germination capacity of tested species was influenced by the increase in concentrations of aqueous extracts from *C. ciliaris* L., which were in accordance with previous studies reporting the allelopathic effects of *C. ciliaris* L. against the growth of *Pennisetum americanum*, *Setaria italica*, *Lactuca sativa* (Hussain et al., 2010), *Dichanthim annulatum* (Parwani and Mankad, 2013), *Cichorium pumilum* and *Trifolium alexandrinum* (Rouz et al., 2015).

Similar results have been reported in other species suggesting that water extract of barley (*Hordeum vulgare* L.) has been effective in suppressing rigid ryegrass (*Lolium rigidum* L.) germination (Kotzamani et al., 2021), and *Brassica oleracea* L. was reported to be effective on weeds *Amaranthus retroflexus* L., *Chenopodium album* L., *Solanum nigrum* L. as well as cultivated species such as *Zea mays* L. and *Beta vulgaris* L. germination (Kural and Özkan, 2020).

The strong relationship between phenolic content and allelopathic activity in plants is well acknowledged (Fatholahi et al., 2020). The high phytotoxicity exhibit by *C. ciliaris* accessions is conferred by various identified phytochemicals, including phenols, flavonoids, saponins, alkaloids (Kannan and Priyal, 2015). Arowosegbe et al., (2012) attributed the inhibitory effect of the aqueous extract of *Aloe ferox*Mill. to such chemical constituents. *Cenchrus ciliaris* L. is a particularly aggressive grass, by virtue of its extensive root system competing with associated species for water and nutrients. That is why it appears to be allopathic by secretion of phytotoxic chemicals that inhibit germination and growth of other plants (Vora, 2003).

IV. CONCLUSION

It can be concluded that aqueous extract of aerial part of *C. ciliaris* L. has an allelopathic effects on seed germination of the three tested species (*L. rigidum* G., *D. carota* L. and *T. nodosa* L.). Further studies should be conducted in the natural environment where these species grow in association. In addition, a separation and identification of allelochemicals present in *C. ciliaris* L. should be investigated in order to provide an alternative strategy for biological weed control.

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