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Allelopathic activity of extracts from leaves of *Ziziphus spina-christi* collected in five Tunisian ecotypes

M. Elaloui[1], I. Soltani[2], A. Laamouri[1], A. Ennajah[1], and S. Houcine[2]

[1]Institut National des Recherches en Génie Rural, Eau, Forêts (INRGREF), Ariana, Tunisia

[2]Ecole Supérieure d'Agriculture de Mograne (ESAM), Tunisia

ABSTRACT: This work was performed to investigate the potential allelopathic effects of *Ziziphus spina-christi* aqueous extracts (AE) on the seed germination and seedling growth of *Triticum durum* L. and *Raphanus sativus* L. Plant materiel (leaves) were collected from five ecotypes (INRGREF, Dgéuche, Kébeli, Nafta and Tozeur). Total phenolic and flavonoid contents have been done by Folin-Ciocalteu reagent and colorimetric assay respectively. The germination efficiency, plumule and radicule length of *Triticum durum* and *Raphanus sativus* were completely inhibited at the highest concentration (100 mg/L) of aqueous extracts. *Raphanus sativus* was more sensitive (89.92% - 85.29%), while *Triticum durum* was more adopted (90.699% - 88.17%) to aqueous extracts.
Extracts were more effective in reducing the early seedling growth than in suppressing the seed germination. The inhibitory effect of these extracts occurred much more on the root length. Considerable differences in total phenolic contents (860.20 µgEAG/gDW) and flavonoids (1989.52 µgEQ/gMS) were found. The *Ziziphus spina-christi* leaf extracts could justify the use of this specie as an herbicide and can give a great success in agriculture.

KEYWORDS: *Triticum durum*; *Raphanus sativus*; seedling development; germination inhibition; phenolic content; herbicide.

1 INTRODUCTION

Ziziphus spina christi (L.), known as sedra, was introduced a long time ago in Tunisia and are now well acclimatized in the country [1]. It is an evergreen tree or shrub with high of 20 m and 60 cm of diameter [2]. This specie has been used in folk medicine for the treatment of various diseases such as the chest pains, dandruff, fractures, headache and as mouth wash [3]. *Z. spina christi* leaves were wide (1.5 x 3.5 cm), glabrous or pubescent [4], [5]. They had been credited with many medicinal fields of applications, including immunity and nutrition [6]. Flavonoids extracted from Z. *spina christi* [7] were found to be 11.5µg/g Dw (quercetin) and 58.8µg/g DW (rutin).

Allelopathy, process involving secondary metabolites (allelochemicals) has been employed widely to protect crops and replace herbicides, fungicides and insecticides ([8], [9]). This phenomenon has received much attention as shown by the numerous reports on the subject such as *Salvia syriaca* and *Cardia draba* [10], *Dodonaea viscosa* [11], *Lactuca sativa* [12], *Azaddirachta indica* [13], *Cassia angustifolia* [14] and *Helanthus annuus* [15]. The extract obtained from *Z. jujuba* roots had no significant effect on apple seeds [16], but the *Z. joazeiro* fruit pulps had reduced lettuce germination from 98.75% to 82.5% [17]. *Z. spina-christi* has received little attention and the allopathic effects of extracts from leaves were unknown and lacking.

Allelopathy activity was studied on germination and early seedling growth of aqueous leaf extracts of five ecotypes (Tozeur, Degueche, Nefta, Kebili and INRGREF) on *Triticum durum* L. and *Raphanus sativus* L. seed germination, seedling (shoot and root) growths. The experiments were done under laboratory conditions. The polyphenols and flavonoids levels in *Z. spina-christi* was also carried out.

Corresponding Author: M. Elaloui

2 MATERIEL AND METHODS

2.1 LEAVES COLLECTION

The *Z. spina -christi* leaves (figure 1) were collected on Avril 2014, from different ecotypes (Touzeur, Degueche, Nefta, Kebili and INRGREF). The identification of plant materiel was done by one of the authors [5] and a voucher sample was deposited at the Herbario of National Institute for Research in Rural Engineering, Water and Forests (INRGREF) in Tunisia. Dried leaves were grounded by a mill equipped with a grid whose holes are 1.00 mm in diameter and stocked in plastic bags.

Fig 1. Leaves and powder of five Ziziphus spina christi ecotypes (Tozeur, Degueche, Nefta, Kebili and INRGREF).

2.2 METHODS

2.2.1 MACERATION

Ziziphus leaf extracts were obtained by macerating 5g, 20g, 40g, 60g and 100g powdered plant in 1L of UHQ water for 24 h. The macerate was centrifuged. The supernatant was filtrated using a Whatman filter paper and kept at 5°C in the obscurity until utilization [18].

2.2.2 BIOESSAIS

Triticum durum L. and *Raphanus sativus* L. seeds were sterilized with 2% sodium hypochlorite for 2 min before sowing, then rinsed four times with distilled water. Seeds (25) were arranged in Petri dishes (9 cm diameter) lined with two discs of Whatman No.1 filter paper. Two ml of aqueous extracts were added to each Petri dish. The control groups were each given 1 ml of deionized water. The Petri dishes were sealed with plastic wrap to prevent the loss of moisture and avoid contamination. The treatments were kept at a laboratory bench with 12 h supply of fluorescent light during the night. The germination percentage (GP), plumule (PL) and radicle length (RL) were recorded after seven days. Relative reduction or stimulation of seed germination and radicle length as affected by the allelopathic substance were calculated.

2.2.3 POLYPHENOL AMOUNTS

The contents of total phenolic of extracts from *Z. spina christi* leaves were performed using gallic acid as a standard. So, dilutions from 0 mg / L to 100 mg / L. was prepared from gallic acid (2g / L) solution. An aliquot (0.125mL) of a suitable diluted methanolic leave extract was added to 0.5mL of deionized water and 0.125mL of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6min, before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 760 nm. The absorption was performed in triplicate using a SHIMADZU UV-1800 spectrophotometer. Results were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW),

2.2.4 TOTAL FLAVONOID CONTENTS

Total flavonoids were measured by a colorimetric assay according to Popova et al. [19]

1 mL of 2% aluminium trichloride ($AlCl_3$) was mixed with 1 mL of plant extract and the volume was made up to 25 mL with methanol. The absorbance was measured at 420 nm in a Shimadzu 160-UV (Tokyo, Japan) spectrophotometer after incubation of 40 min.

2.2.5 STATISTIC ANALYSIS

Logiciel SAS (Statistics Analysis System) version 9.1 (2003). Results are means ± SD of three experiments. The one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed.

3 RESULTS

3.1 ALLECLOPAHIC LEAF EFFECTS

THE EFFECT OF DIFFERENT LEAF EXTRACTS ON SEEDS GERMINATION

The germination percent of *T. durum* and *R. sativus* seeds were significantly affected by different concentrations of leaf extracts from all *Z. spina-christi* ecotypes. A gradual decrease in seeds germination of *T. durum* and *R. sativus* was observed with gradual increase in aqueous leaf extracts concentrations (table 1). The highest level (77.33%) was recorded especially at the highest concentrations (100 mg/L).

Table 1 The effect of different concentrations of aqueous extract (0, 5, 20, 40, 60 and 20mg/L) of Z. spina christi leaves on germination of T. durum and R. sativus 10 days after plating the seeds under laboratory conditions. Only one untreated control bar (0 %) is used for all the different concentration levels LSD (P < 0.05)

C (g/L)		INRGREF	Kebeli	Deguech	Nafta	Tozeur
0	*T. durum*	98.67±2,31^{a}	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}
	R. sativus	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}
5	*T. durum*	97.33±2.31^{a}	98.67±2.31^{a}	97.33±2.31^{ba}	98.00±2.83^{a}	98.67±2.31^{a}
	R. sativus	98.67±2.31^{a}	98.67±2.31^{a}	98.67±2.31^{a}	97.33±2.31^{ba}	98.67±2.31^{a}
20	*T. durum*	94.67±2.31^{a}	94.67±2.31^{a}	93.33±2.31^{cb}	90.67±2.31^{b}	97.33±2.31b^{a}
	R. sativus	97.33±2.31^{ba}	94.67±2.31^{ba}	97.33±2.31^{a}	93.33±2.31^{b}	97.33±2.31^{ba}
40	*T. durum*	93.33±4.62^{a}	89.33±2.31^{b}	90.67±2.31^{c}	89.33±2.31^{b}	93.33±2.31^{bc}
	R. sativus	94.67±2.31^{cba}	89.33±6.11^{bc}	96.00±0.0^{a}	86.67±2.31^{c}	93.33±2.31^{b}
60	*T. durum*	92.00±4.00^{ba}	86.67±2.31^{cb}	89.33±2.31^{dc}	85.33±2.31^{b}	90.67±2.31^{dc}
	R. sativus	93.33±2.31^{bc}	86.67±2.31^{bc}	90.67±2.31^{b}	82.67±2.31^{c}	86.67±2.31^{b}
100	*T. durum*	86.67±4.6^{2b}	82.67±4.62^{c}	85.33±2.31^{d}	77.33±4.62^{c}	86.67±2.3^{1d}
	R. sativus	92.00±4.00^{c}	81.33±2.31^{d}	86.00±3.46^{c}	77.33±2.31^{d}	86.67±2.31^{b}

THE EFFECT OF DIFFERENT LEAF EXTRACTS ON MEAN GERMINATION TIME (MGT)

The MGT oscilled between 2.74 and 3.28 for *T. durum* and from 2.61 to 3.69 for *R. sativus*. The high values were observed on Nafta ecotype (Table 2).

Table 2. The effect of different concentrations of aqueous extract (0, 5, 20, 40, 60 and 20 mg/L) of Z. spina christi leaves on mean germination time of T. durum and R. sativus 10 days after plating the seeds under laboratory conditions. Only one untreated control bar (0 %) is used for all the different concentration levels. LSD (P < 0.05)

C (g/L)		INRGREF	Kebeli	Deguech	Nafta	Tozeur
	T. durum	1.01±0.02^{e}	1.01±0.02^{e}	1.01±0.02^{f}	1.01±0.02^{f}	1.01±0.02^{e}
0	*R. sativus*	1.22±0.13^{e}	1.22±0.13^{e}	1.22±0.13^{d}	1.22±0.13^{f}	1.22±0.13^{e}
	T. durum	1.15±0.12^{ed}	1.19±0.10^{ed}	1.29±0.08^{e}	1.31±0.08^{e}	1.10±0.05^{ed}
5	*R. sativus*	1.53±0.04^{d}	1.61±0.11^{d}	1.57±0.10^{c}	2.12±0.07^{e}	1.66±0.14^{d}
	T. durum	1.22±0.06^{d}	1.34±0.07^{d}	1.72±0.08^{d}	1.60±0.09^{d}	1.29±0.25^{d}
20	*R. sativus*	1.85±0.13^{c}	1.81±0.25d^{c}	1.92±0.08^{b}	2.38±0.15^{d}	2.00±0.04^{c}
	T. durum	1.97±0.03^{c}	2.15±0.10^{c}	2.34±0.15^{c}	2.26±0.17^{c}	1.80±0.06^{c}
40	*R. sativus*	2.29±0.18^{b}	2.06±0.12^{c}	2.06±0.10^{b}	2.80±0.07^{c}	2.16±0.02^{c}
	T. durum	2.58±0.11^{b}	2.74±0.30^{b}	2.63±0.06^{b}	2.80±0.22^{b}	2.43±0.15^{b}
60	*R. sativus*	2.69±0.14^{a}	2.49±0.27^{b}	2.56±0.12^{a}	3.16±0.08^{b}	2.39±0.15^{b}
	T. durum	2.97±0.14^{a}	3.02±0.03^{a}	2.97±0.07^{a}	3.28±0.08^{a}	2.74±0.08^{a}
100	*R. sativus*	2.87±0.26^{a}	3.05±0.05^{a}	2.61±0.34^{a}	3.69±0.11^{a}	2.97±0.19^{a}

THE EFFECT OF DIFFERENT LEAF EXTRACTS ON SHOOT LENGTH

As compared to control, except aqueous leaf extracts from Kebeli and Nafta ecotypes, which had a stimulatory effect on shoot lengths in low concentration (5mg/L), aqueous extracts from Z. *spina christi* leaves have a inhibiting effect on shoot lengths (Table 3).

Table 3. The effect of different concentrations of aqueous extract (0, 5, 20, 40, 60 and 20mg/L) of Z. spina -christi leaves on root length (cm) of T. durum and R. sativus 10 days after planting the seeds under laboratory conditions. Only one untreated control bar (0 %) is used for all the different concentration levels LSD (P < 0.05).

C (g/L)		INRGREF	Kebeli	Deguech	Nafta	Tozeur
	T. durum	9.35±1.18^{a}	9.35±1.18^{a}	9.35±1.18^{a}	9.35±1.18^{a}	9.35±1.18^{a}
0	*R. sativus*	9.21±0.42^{a}	9.21±0.42^{a}	9.21±0.42^{a}	9.21±0.42^{a}	9.21±0.42^{a}
	T. durum	9.31±0.99^{a}	9.86±0.63^{a}	6.75±1.5^{ba}	10.32±1.53^{a}	9.07±2.29^{a}
5	*R. sativus*	7.69±1.49^{a}	9.43±0.51^{a}	7.76±0.22^{a}	8.23±0.50^{a}	7.69±1.49^{a}
	T. durum	4.48±0.13^{b}	6.29±1.05^{b}	4.43±0.72^{bc}	4.27±0.88^{b}	4.48±0.23^{b}
20	*R. sativus*	4.08±1.02^{b}	7.08±1.33^{b}	5.02±2.07^{b}	4.16±4.39^{b}	4.08±1.02^{b}
	T. durum	3.86±0.45^{b}	3.63±0.25^{c}	2.53±0.40^{bc}	3.47±1.64^{b}	3.86±0.79^{b}
40	*R. sativus*	3.31±2.29^{cb}	5.40±1.81^{b}	3.95±0.57^{cb}	2.80±0.22^{b}	3.31±2.29^{cb}
	T. durum	2.55±0.28^{cb}	2.79±0.43^{c}	3.07±1.31^{bc}	2.63±0.48^{cb}	2.55±0.48^{b}
60	*R. sativus*	1.46±0.33^{c}	2.61±0.71^{c}	2.67±0.80^{c}	1.81±0.88^{b}	1.46±0.33^{c}
	T. durum	1.61±0.13^{c}	2.17±0.46^{c}	1.76±0.10^{c}	0.82±0.04^{c}	1.61±0.23^{b}
100	*R. sativus*	1.09±0.06^{c}	1.30±0.23^{c}	2.23±0.38^{c}	0.93±0.14^{b}	1.09±0.06^{c}

High concentrations (60g/l and 100g/l) greatly inhibit shoot length. In fact, the shoot length does not exceed 1.61 cm and 1.09 cm for *T. durum* and *R. sativus* respectively.

THE EFFECT OF DIFFERENT LEAF EXTRACTS ON ROOT LENGTH

As shown in table 4, the extract used at 40 g/L; 60 g/L and 100 g/L were the most inhibited of *T. durum* and *R. sativus*.

Table 4. The effect of different concentrations of aqueous extract (0, 5, 20, 40, 60 and 20mg/L) of Z. spina christi leaves on shoot length of T. durum and R. sativus 10 days after plating the seeds under laboratory conditions. Only one untreated control bar (0 %) is used for all the different concentration levels LSD (P < 0.05).

C (g/L)		INRGREF	Kebeli	Deguech	Nafta	Tozeur
0	*T. durum*	4.4±0.31^{a}	4.4±0.31^{a}	4.4±0.31^{a}	4.4±0.31^{a}	4.4±0.31^{a}
	R. sativus	6.22±0.20^{a}	6.22±0.20^{ba}	6.22±0.20^{a}	6.22±0.20^{a}	6.22±0.20^{a}
5	*T. durum*	4.71±0.31^{a}	4.49±0.14^{a}	6.98±1.90^{a}	4.07±0.21^{a}	4.17±0.38^{ba}
	R. sativus	5.92±0.71^{ba}	7.59±0.40^{a}	7.92±0.83^{a}	9.55±0.75^{a}	5.13±1.39^{a}
20	*T. durum*	3.27±0.30^{dc}	4.66±0.43^{a}	3.76±0.52^{b}	3.97±0.47^{a}	2.94±0.05^{bc}
	R. sativus	4.58±0.31^{cb}	6.34±0.43^{ba}	5.32±1.01^{cb}	1.20±0.16^{e}	5.46±0.33^{ba}
40	*T. durum*	3.96±0.48^{bac}	3.58±0.14^{b}	3.32±0.82^{b}	1.97±0.39^{b}	3.63±0.82^{bac}
	R. sativus	3.32±1.51^{c}	5.15±1.59^{b}	4.27±1.21^{c}	3.11±1.28^{c}	4.17±0.74^{b}
60	*T. durum*	3.38±0.07^{bdc}	3.05±0.23^{b}	3.05±0.69^{cb}	1.62±0.61^{b}	3.38±0.12^{bac}
	R. sativus	3.12±0.92^{c}	2.59±0.72^{c}	3.99±0.26^{c}	2.13±0.36^{cd}	2.16±0.15^{c}
100	*T. durum*	2.53±0.27^{d}	1.43±0.75^{c}	1.33±0.45^{c}	0.51±0.07^{c}	2.56±00.51^{c}
	R. sativus	0.71±0.24d	1.50±0.39^{c}	1.53±0.49^{d}	0.92±0.16^{e}	1.12±0.13^{c}

These results showed also that shoots appeared more sensitive to allelopathic effect than roots. The comparison between the five ecotypes showed that the degree of inhibition/stimulation was largely dependent on the concentration of the extracts being tested. For INRGREF ecotype, the highest inhibition (12.17%) was obtained at the concentration of 100g/l for *T. durum*, this value being more important for *R. sativus* seeds (Figure 2).

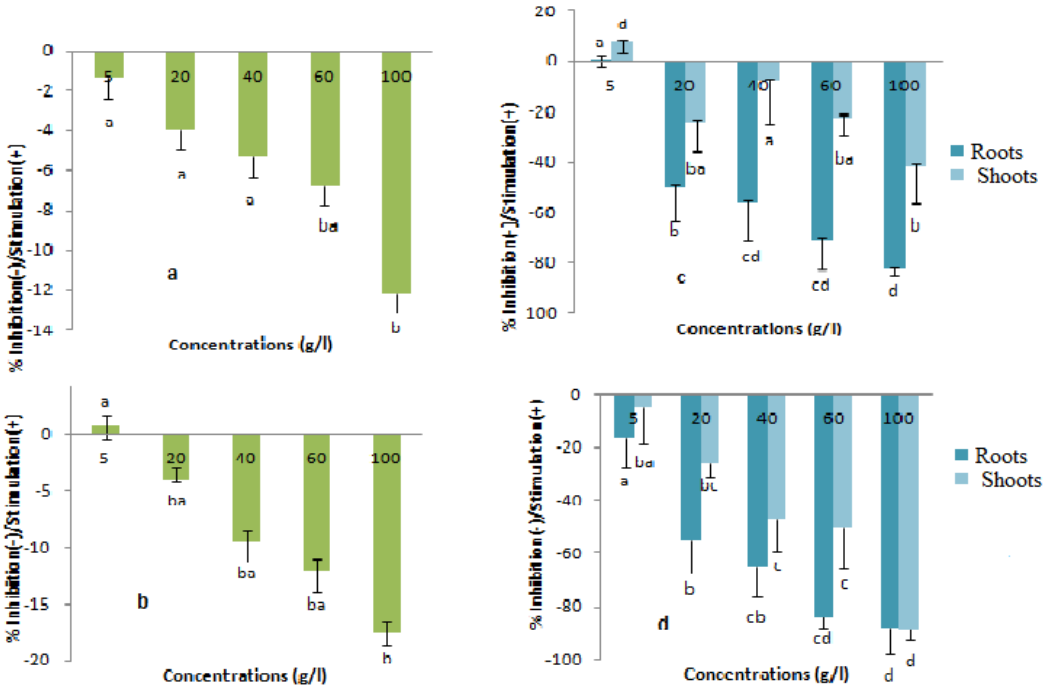


Fig. 2. Inhibitor leaf extract effects of Z. spina christi collected from INRGREFF a: effect on T. durum seeds germination; b: effect on R. sativus seeds germination; c: effect on root and shoot lengths, of T. durum; d: effect on root and shoot lengths, of R. sativus. The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

These effects became less important (12.11 %) on the seeds germination where treated by leaf extracts from Tozeur ecotype (Figure 3). The seedling growths were more sensitive and had the highest inhibition ratios (82.53 % and 85.74%) for root and (81.90% and 71%) for shoot lengths.

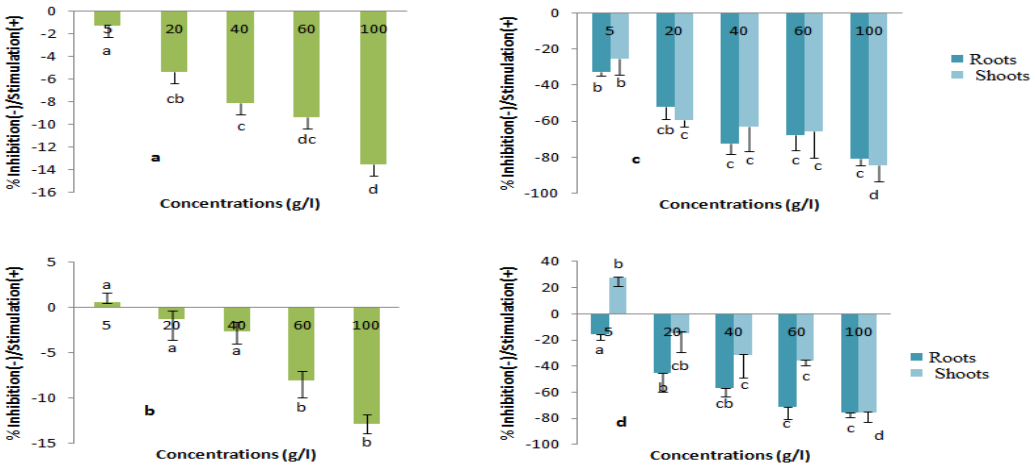


Fig. 3. Inhibitor leaf extract effects of Z. spina christi collected from Tozeur

a: effect on T. durum seeds germination; b: effect on R. sativus seeds germination; c: effect on root and shoot lengths, of T. durum; d: effect on root and shoot lengths, of R. sativus. The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

The reduction was more prominent for Deguech ecotype, the rate of inhibition riched levels between 12.83% and 13.5% for *T. durum* and *R. sativus* respectively (Figure 4). These extracts reduced significantly the root (80.61%) and the shoot (84.61%) growths of *T. durum* germinant. Those ratios decreased to 75.84% and 75.55% for *R. sativus* seeds.

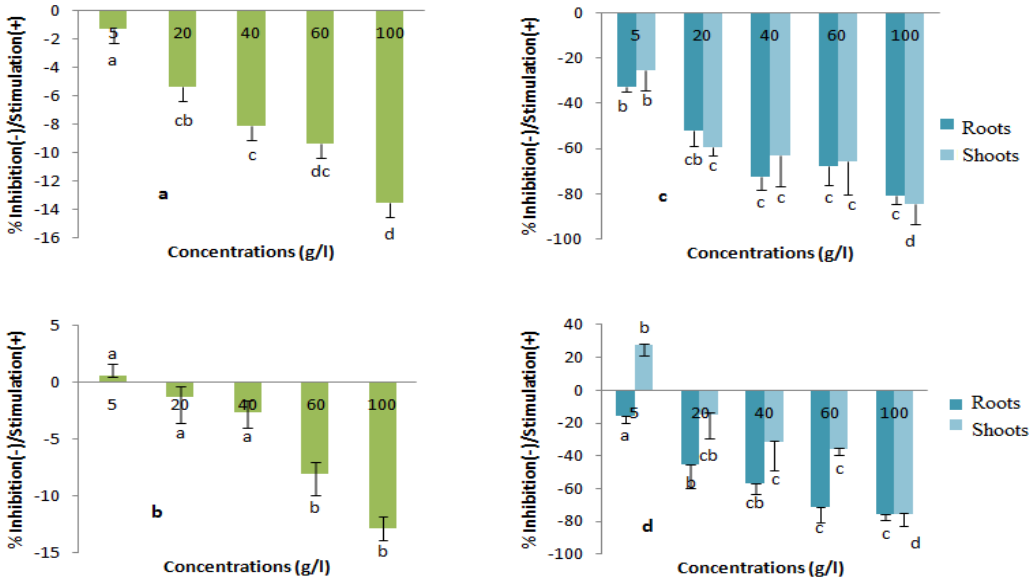


Fig. 4. Inhibitor leaf extract effects of Z. spina christi collected from Degueche

a: effect on T. durum seeds germination; b: effect on R. sativus seeds germination; c: effect on root and shoot lengths, of T. durum; d: effect on root and shoot lengths, of R. sativus. The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

High concentrations of Z*. spina-christi* leaves from Nafta ecotype negatively influenced seed germination rate, plumule and seedling length of *T. durum* and *R. sativus*. However, their effects were not considerable at low concentrations (Figure 5).

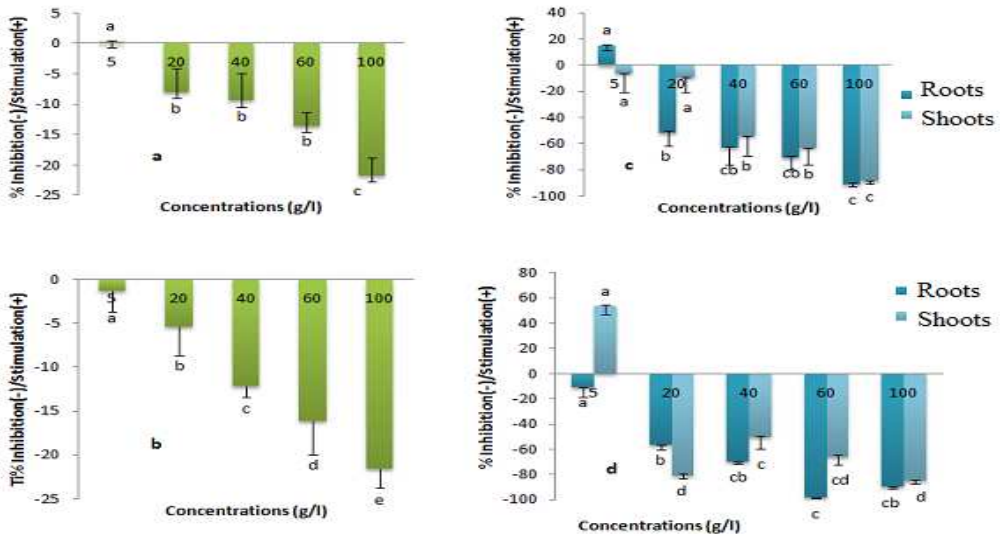


Fig. 5. Inhibitor leaf extract effects of Z. spina christi collected from Nafta

a: effect on T. durum seeds germination; b: effect on R. sativus seeds germination; c: effect on root and shoot lengths, of T. durum; d: effect on root and shoot lengths, of R. sativus. The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

These percentages were more important (16.22% and 17.56%) for *T. durum* and *R. sativus* seeds respectively using leaf extracts from Kebeli ecotype (Figure 6).

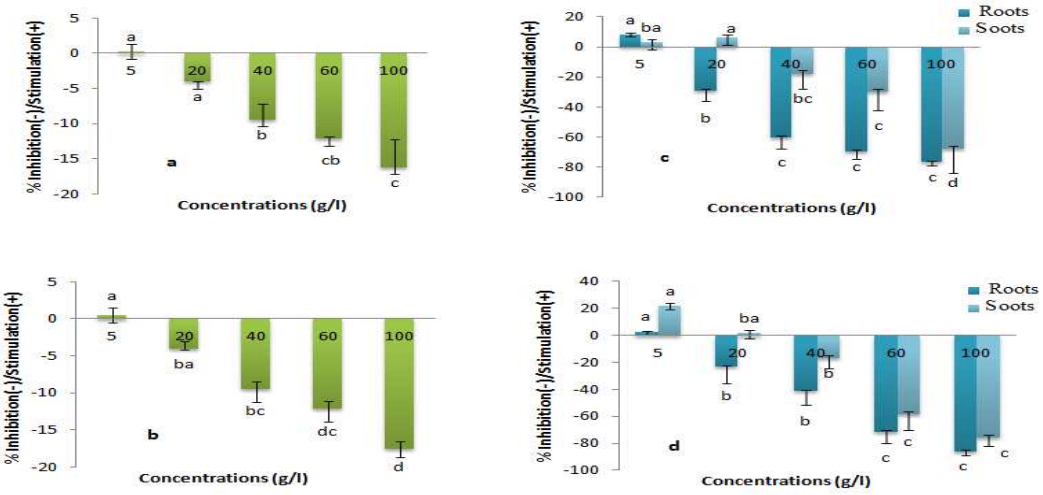


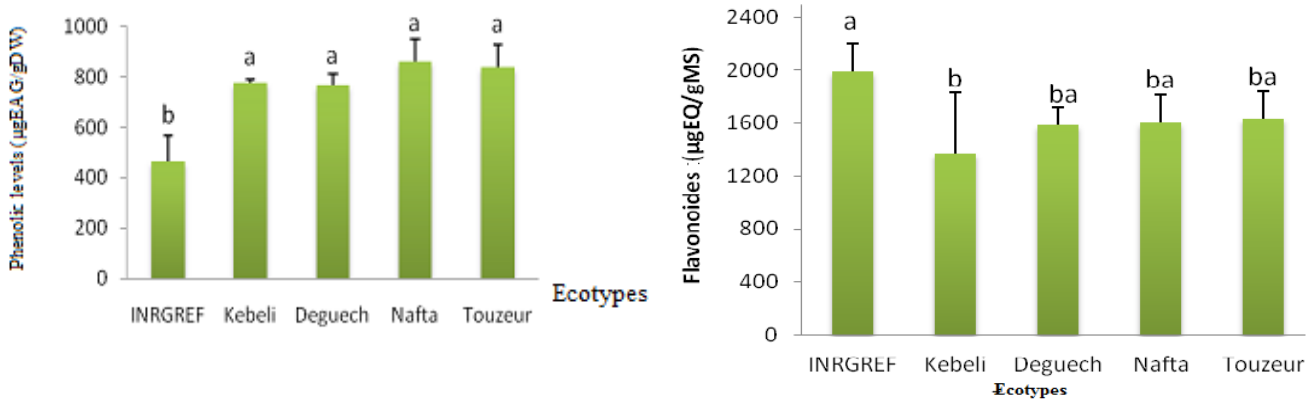
Fig. 6. Inhibitor leaf extract effects of Z. spina christi collected from Kebili

a: effect on T. durum seeds germination; b: effect on R. sativus seeds germination; c: effect on root and shoot lengths, of T. durum; d: effect on root and shoot lengths, of R. sativus. The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

This study proved the superiority of Nafta ecotype that had the higher allelopathic effects. Many authors such Blum et al. (2004) [20] had repotted that the allelopathic compounds were mainly phenolic compounds. For this raison a comparison of phenolic compounds of the five Z. *spina christi* ecotypes had been done.

3.2 TOTAL PHENOLIC AND FLAVONOID CONTENTS

The analysis of the total phenolic composition of *Z. spina christi* leaves varied from 764.22 µgEAG/gDW to 860.20 µgEAG/gDw. The highest rate was shown in the Nafta ecotype (Figure 7).



(a) (b)

Fig. 7. Polyphenol (a) and flavonoid (b) yield (%) of five Tunisian Ziziphus spina-christi ecotypes (INRGREF, Dgeuche, Kebeli, Nafta and Tozeur). The data are means values of three measurements. The confidence intervals were calculated at the threshold of 5%.

Flavonoid levels ranged from 1989.52 µgEQ/g DW (INRGREF) to 1371.61 µgEQ/g DW (Kébeli). Many studies, had demonstrated that numerous environmental factors especially abiotic stresses have a great influence on plants to synthesize secondary metabolites [21]. Basuny et *al.* (2013) [22] had mentioned that the fruits and leave were rich on 13 phenolic compounds.

4 DISCUSSION

Allelopathic effect of 60 and 100 mg/L aqueous extract besides the control from leaves of *Z. spina-christi was* clearly demonstrated on germination percentage, plumule and radicle length of *R. sativus* and *T. durum*. The degree of inhibition was largely dependent on the concentration of the aqueous extracts [23]. Roots appeared more sensitive to allelopathic effect than shoots. This inhibition could be caused by the presence of certain allelochemicals [24] that contribute to inhibition of gibberellin and indoleacetic acid function in meristimatic cell [25] t. These results were consistent with those reported elsewhere for other species as *Calotropis procera* and *Morettia philaeana* [8].

As we can see in these results, *Z. spina- christi* leaves of Nafta ecotype were a big source of phenols and flavonoids, compounds affected specially photosynthesis [26], membrane permeability [27] and germination [28].

5 CONCLUSION

The magnitude of reduction of seed germination, shoot and root length after treatment with *Z. spina -christi* extracts followed the order of *T. durum* > *R. sativus* > especially on seeding length. High levels of phenolic compounds on Z. *spina - christi* leaves especially Nafta ecotype could justify its inhibitory effect on germination rate and seedling length of the tested species. This ecotype could be used as an herbicide and can give a great success in agriculture.

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