

ALLELOPATHIC EFFECTS OF *ACACIA TORTILIS* (FORSSK.) HAYNE SUBSP. *RADDIANA* (SAVI) BRENAN IN NORTH AFRICA

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Abstract

Allelopathic and autotoxicity effects of aqueous extracts from under the canopy soil and from different parts of *Acacia tortilis* subsp. *raddiana* were studied. These effects were measured in terms of germination rate and radicle length of barley (*Hordeum vulgare*), softwheat (*Triticum sativum* L.) and hardwheat (*Triticum aestivum* L.) with two varieties and *Acacia tortilis*. The experiments were conducted in the dark at an average temperature of 25°C for cereal species and 30°C for *Acacia* seeds which represents the optimum temperature of germination. Different extracts (from soil under canopy, leaf, fruit, shoot and root) significantly reduced germination and seedling growth of the tested species. However, this allelopathic effect varied with the extract source. The inhibitory effect was more pronounced in fruit and leaf extracts compared with root, shoot and soil extracts. These results strongly suggest that allelopathy may be a possible mechanism controlling the timing of cereal and *Acacia tortilis* germination and seedling establishment.

Introduction

Nowadays, *Acacia tortilis* subsp. *raddiana*, a species with a circum-saharan geographical distribution, is one of the most uncommon species and still present in North African regions. Indeed, the region of Bled Talah (whose name could be translated as "the country of *Acacia*") in the south of Tunisia constitutes today an ecosystem where the species continues to grow on a large scale. It grows on an area of 16488 ha. This area, where *Acacia tortilis* may be considered as a keystone species (Aronson *et al.*, 1993), represents a considerable interest by conditioning vegetation dynamics and structure in the Bled Talah region (Abdallah *et al.*, 2008). This characteristic may have originated firstly from its ability to fix atmospheric nitrogen and thus improve soil fertility and secondly from its improvement of soil water availability by the reduction of potential evapotranspiration, mainly under canopy. On the other hand, this species, contributes largely to the reduction of soil erosion with its strong root system.

Recently, there has been a great interest in diversified agricultural production systems to obtain improved crop protection and to increase productivity and profitability offered by many intercropping systems. Agroforestry has drawn considerable attention because of its potentialities to maintain or increase the biological productivity in areas characterized by high energy input and large-scale impractical agriculture (Kidd & Pimentel, 1992). It is often assumed that appropriate agroforestry systems can provide the essential ecological functions needed to ensure sustainability and maintain microclimatic and favorable influences (Wiley, 1979; Ofori & Stern, 1987). Such benefits may outweigh their greater use of water in areas of limited water availability.

The traditional *Acacia tortilis* subsp. *raddiana* agroforestry system practiced for many decades in the Bled Talah region can be seen as a complex and dynamic resilient system reacting to a wide variety of long term external changes and short-term disturbances related to climate, topography, soils texture and geomorphological variability.

In the Bled Talah region, agroforestry systems are based on cereal culture in association with the spontaneous presence of *Acacia* trees. The most important

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local cereals used are barley (*Hordeum vulgare* L.), softwheat (*Triticum sativum* L.) and hardwheat (*Triticum aestivum* L.).

The ecological significance of phytotoxins in old field succession and in other natural communities has attracted the attention of many workers (May & Ash, 1990; Choesin & Boerner, 1991). Among the allelochemicals which take part in such interactions are phenols, terpenes, glucosides, alkaloids, amino acids and sugars (Harborne, 1989).

Researches showed that *Acacia* trees are known as a versatile source of components with bioactive properties (Reigosa *et al.*, 1984; Rafiqul Hoque *et al.*, 2003), suggesting a large inhibitory potential in *Acacia* genus which dominates the dry south Saharan regions of Africa (Barnes *et al.*, 1996). The allelopathic mechanisms are very important in the Mediterranean region, where the climate favors allelochemical production (Rice, 1984). Most assessments of allelopathy or autotoxicity involve bioassays of plant or soil extracts based on seed germination and seedling growth, especially root growth. Bioassays regarding the effects of aqueous plant tissue and putative allelopathic compounds on seed germination and early seedling growth have typically been used to document the presence or absence of autotoxicity under laboratory conditions.

The objective of the present study were to: (i) elucidate the possible allelopathic potential of various parts of *Acacia tortilis* in barley, hardwheat and softwheat, and (ii) to study the possibility of the autotoxic effect of *Acacia tortilis* in an Agroforestry ecosystem of the Tunisian arid zones.

Material and Methods

Donor and receptor plants: To study the allelopathic potential of *Acacia tortilis*, three cereals were used: barley (*Hordeum vulgare* L.), softwheat (*Triticum sativum* L.) and hardwheat (*Triticum aestivum* L.) with two varieties 'Karim' and 'Om rabii'. In this study, the selection of the species was made in accordance to the characteristics of the agroforestry system in the Bled Talah region, located under the arid bioclimate of southern Tunisia.

Parallely, since research showed the low recruitment of *Acacia tortilis* under natural conditions, the possibility of the autotoxic effect of this species was also evaluated. Seeds of different species were collected during the 2008 summer from the National Park of Bou Hedma (34.39° N, 9.48° E), which follows the Saharian fringes of Tunisia (Fig. 1). This area is characterized by low arid bioclimate

(Emberger, 1955), and generally praised for edaphoclimatic characteristics (Chaieb, 1989). The region receives a total mean annual rainfall of 180 mm, occurring particularly between November and March. The mean temperature varies from 32 to 36°C in summer and from 4 to 7°C in winter.

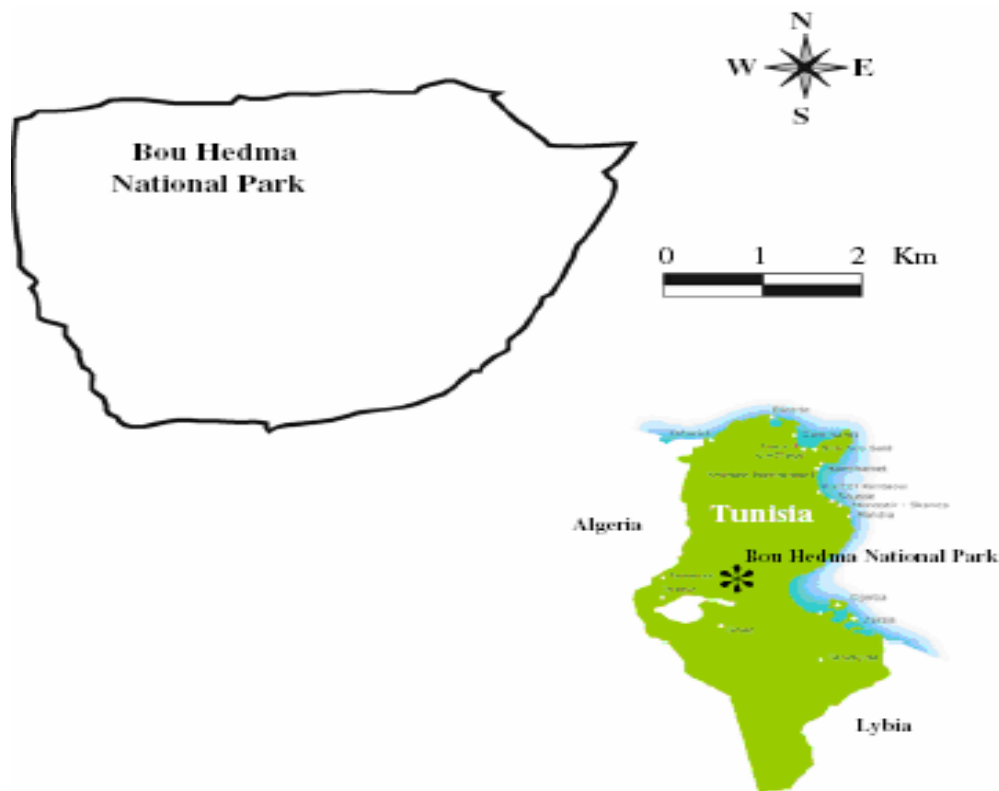


Fig. 1. Geographical context of study.

Extract preparation: Since research showed that compounds are concentrated in different parts of the *Acacia* tree (Table 1), roots, stems, leaves, fruits and seeds of *Acacia tortilis* were collected. A total of 500 g of the plant material were weighed, crushed and soaked in 500 ml of distilled water for 24 h, then homogenized and filtered using Whatman No. 1 filter paper.

A total of 500g of soil sample collected from under the canopy of *Acacia tortilis* were soaked in 500 ml distilled water for 24 h and, after shaking in an electric shaker for 30 min, were passed through Whatman No. 1 filter paper.

Extract bioassay: Extracts were tested for phytotoxicity on seed germination of receptor species. Germination tests were carried out on Whatman N°3 filter paper placed on 12cm sterile Petri dishes. Eight replicates of twenty-five seeds per species were placed in each Petri dish and 10 ml of distilled water (a control) or the various extracts (from roots, stems, leaves, fruits and soil) were added. Petri dishes were placed in the incubator (scientific bioblock model) in dark at 25°C for cereal species, and 30°C for *Acacia* seeds which represents the optimum temperature of germination. Seven days after seeding, germination

counts and central radicle length were measured and recorded. Seeds were considered germinated when the radicle extended through the seed coat (Warrag, 1994).

Statistical analysis: All collected data were subjected to variance analysis (One-way ANOVA) followed by Tukey's HSD-tests. ANOVAs were conducted with SPSS version 12. Probability values lower than 0.05 were considered as statistically significant.

Results and Discussion

Effects of the different extracts from different parts of *Acacia tortilis* on germination and radicle growth of cereal species: Extracts from plant parts and under canopy soil were phytotoxic to the radicle growth and germination of receptor plants. However, the inhibition magnitude varied between them (Tables 2 & 3). A maximum reduction occurred with fruit extracts, whereas, soil extracts showed a slightly significant inhibition. For all treatments, results showed that *Triticum aestivum* var. 'Om rabii' was more tolerant to different extracts compared to other receptor plants. Leaf and root extracts inhibited receptor plant more than stem and soil extracts.

Table 1. *Acacia tortilis* as conveyors of allelopathic impacts.

Part used	Identified constituents	Literature source
Leaf, Petiole	Tanins, phenols	Abdulrazak <i>et al.</i> , 2000
Fruit	Phenols	Bitende & Ledin, 1996
Leaf, Root, Stem	Phenolic compounds	Coder Kim, 1999
Leaf	Total phenolics	Dube <i>et al.</i> , 2001
Leaf	Phenolic compounds	Nakafeero <i>et al.</i> , 2007
Leaf	Phenolic and tanin	Rubanza <i>et al.</i> , 2005
Pods	Phenolic compounds	Shayo & Uden, 1999
Leaf	Phenolic compounds	Sundramoorthy & Kalra, 1991

Table 2. Effects of diluted extracts from soil and different parts of *Acacia tortilis* on the final germination percentage (%) of various test plants. Mean values which are not followed by the same letter are statistically significant ($p < 0.05$) as determined by Tukey's HSD-test.

Treatments	Species			
	<i>Hordeum vulgare</i>	<i>Triticum sativum</i>	<i>Triticum aestivum</i> var. 'Karim'	<i>Triticum aestivum</i> var. 'Om rabii'
Control	94 ± 1.63 ^a	93.5 ± 1.5 ^a	90 ± 2.23 ^a	91 ± 2.33 ^a
Soil	73 ± 1.69 ^b	76 ± 2.08 ^b	78 ± 2.44 ^{bc}	80 ± 2.56 ^b
Stem	60.5 ± 4.18 ^b	71 ± 4.64 ^{bc}	77 ± 2 ^{bc}	79.5 ± 1.89 ^b
Root	48.5 ± 2.47 ^{bc}	67.5 ± 5.97 ^{bc}	76 ± 1.94 ^{bc}	79.5 ± 1.89 ^b
Leaf	55 ± 2.98 ^{bc}	57.5 ± 3.09 ^{cd}	60 ± 2.33 ^c	62.5 ± 3.27 ^c
Fruit	44.5 ± 3.83 ^c	44 ± 3.39 ^d	36 ± 2.66 ^d	61 ± 2.76 ^c

Table 3. Effects of diluted extracts from soil and different parts of *Acacia tortilis* on the radicle growth (cm) of various test species. Mean values which are not followed by the same letter are statistically significant ($p < 0.05$) as determined by Tukey's HSD-test.

Treatments	Species			
	<i>Hordeum vulgare</i>	<i>Triticum sativum</i>	<i>Triticum aestivum</i> var. 'Karim'	<i>Triticum aestivum</i> var. 'Om rabii'
Control	14.35 ± 0.89 ^a	13.7 ± 0.84 ^a	11.7 ± 0.63 ^a	12.96 ± 0.38 ^a
Soil	11.3 ± 0.64 ^b	10.65 ± 1.25 ^b	9.07 ± 0.58 ^b	11.2 ± 0.31 ^b
Stem	12.97 ± 0.74 ^a	10.11 ± 0.59 ^{bc}	8.05 ± 0.87 ^b	10.14 ± 0.82 ^{bc}
Root	9.66 ± 0.28 ^b	8.06 ± 0.94 ^c	8.02 ± 0.66 ^b	8.23 ± 0.41 ^{cd}
Leaf	9.1 ± 0.86 ^b	8.75 ± 0.5 ^{bc}	7.22 ± 0.5 ^b	6.48 ± 0.51 ^d
Fruit	3.62 ± 0.12 ^c	2.89 ± 0.27 ^d	1.83 ± 0.41 ^c	3.93 ± 0.63 ^c

Our results are closely related with the findings of Nakafeero *et al.*, (2007) who stated that the presence of alkaloids and phenolic compounds in the leaves of *Acacia tortilis* species implies that it has the potential to inhibit seed germination. The results are also in conformity with those reported elsewhere for other species in a variety of plant families (Igboanugo, 1988; Sundramoorthy & Kalra, 1991; Kil & Yun, 1992; Macias *et al.*, 1992; Noor *et al.*, 1995; Al-Humaid & Warrag, 1998; Jayakumar & Manikandan, 2005; Javaid & Anjum, 2006; Shafique *et al.*, 2007).

The phenomenon of the presence of biochemical inhibitors associated with fruit structure is widespread in the plant kingdom (Khan, 1982; Hedge & Miller, 1990). Allelopathic metabolites leached out from woody plants often suppress the undergrowth species sharing the same habitat (Chou & Lee, 1991). Many woody species are reported to have phytotoxins (Akram *et al.*, 1990; May & Ash, 1990; Kil & Yun, 1992). Chou & Lee, (1991) showed that bamboo, *Phyllostachys edulis* (Poaceae) contains significant amounts of allelopathic compounds that can inhibit the growth of undergrowth weeds.

The presence of allelopathic substances in the soil is often determined by a number of important factors. These include the density at which the leaves fall, the rate at which this material decomposes, the distance from other plants, and, finally, the quantity and distribution of the annual rainfall (Mann, 1987; Escudero *et al.*, 2000; Nilsson *et al.*, 2000). The decomposition of plant material is then dependent on leaf tissue quality (C:N and C:P

ratios), temperature, rainfall and the presence of certain micro-organisms (Friedman *et al.*, 1977; Newman & Miller, 1977; Ito *et al.*, 1998). Soil type and its pH are also important (Saxena & Sharma, 1996) in determining whether or not allelopathic substances are present in the soil and if they are in sufficiently high enough concentrations to affect other plants.

Allelopathic effects generally produce an inhibition of germination and early growth of seedlings (Akram *et al.*, 1990; Kil & Yun, 1992). While we did not investigate the specific mode of action, many other studies demonstrated inhibition occurring through limiting cell division, respiration, photosynthesis or by disrupting membrane regulation (Macias *et al.*, 1992).

The present study showed that water extracts of different parts of *Acacia tortilis* inhibits the germination and the radicle length of receptor plant. By this time, farmers may have incurred significant opportunity costs by investing in agroforestry systems, since research showed the allelopathic potential of *Acacia tortilis* on crops.

Autotoxic effect: Our results suggest that seed germination and radicle growth of *Acacia tortilis* was inhibited, to varying degrees by extracts of root, stem, leaf, flower, fruit and the under canopy soil of *Acacia tortilis* subsp. *raddiana* (Table 4). A maximum reduction occurred with fruit and leaf extracts with 5.1 and 6.1 % respectively for germination rate and 2.79 and 2.85 cm for radicle growth.

Table 4. Effects of diluted extracts from soil and different parts of *Acacia tortilis* on germination percentage (%) and radicle growth (cm) of *Acacia tortilis*. Mean values which are not followed by the same letter are statistically significant ($p < 0.05$) as determined by Tukey's HSD-test).

Treatments	Percentage of germination (%)	Radicle growth (cm)
Control	28.4 ± 1.33 ^a	7.25 ± 0.54 ^a
Soil	20.7 ± 1.17 ^b	5.92 ± 0.28 ^b
Stem	13.9 ± 0.63 ^c	5.78 ± 0.4 ^b
Root	12.4 ± 1.16 ^c	4.86 ± 0.44 ^b
Leaf	6.1 ± 1.18 ^d	2.85 ± 0.28 ^c
Fruit	5.1 ± 0.67 ^d	2.79 ± 0.08 ^c
p-value	$p < 0.001$	$p < 0.001$

To the best of our knowledge, although there is evidence that *Acacia tortilis* inhibits the germination and seedling growth of a number of crop species (Nakafeero *et al.*, 2007), there is no previous report on the autotoxic effect of *Acacia tortilis* subsp. *raddiana* (Table 1). Our results agree with the findings of some authors (Chung & Miller, 1995; Hall & Henderlong, 1989; Hedge & Miller, 1990) who showed that Alfalfa (*Medicago sativa* L.) (*Leguminosae*) plants are known to contain water-soluble substances that are autotoxic to the same species. The autotoxicity of foliage on the seed germination and seedling growth of mesquite (*Prosopis juliflora*), has been reported (Warrag, 1994). Some perennials such as *Asparagus officinalis* (Yang, 1982), some *Citrus* spp. (Burger, 1981) and peach, *Prunus persica* (Patrick, 1955) were, at least partially, accounted for by autotoxicity. This is also responsible for the inhibition of seed germination and/or retardation of seedling growth exhibited by some annuals including corn, *Zea mays* (Martin *et al.*, 1990) and wheat, *Triticum aestivum* (Jessop & Stewart, 1983) under successive cropping using conservation tillage systems.

On the other hand, Chou & Yang (1982) showed that leachates of the bamboo, *Phyllostachys edulis* contain significant amounts of allelopathic compounds that can suppress the growth of undergrowth weeds. The leaf leachates of *Leucaena leucocephala* suppress the growth of weed species found underneath the canopy; however, these leachates do not affect the seedling of *L. leucocephala* itself.

Contrary to our results, Saxena *et al.*, (1996) reported that water-soluble extracts isolated from the roots and shoots of pearl millet also stimulated the rate of its own seed germination at low concentrations. This mechanism would undoubtedly improve species chances of establishing itself by permitting it to germinate and make use of limited resources, such as water, as soon as it is possible.

Finally, evidence is provided that *Acacia tortilis* subsp. *raddiana* is considerably autotoxic under the arid bioclimate of North Africa. This finding confirms the observation that *Acacia* seedlings grow poorly under *Acacia* canopy in natural ecosystem (data not shown).

Conclusion

The studies provide the evidence that *Acacia tortilis* subsp. *raddiana* has allelopathic potential. Again, its inhibitory effect on agricultural crops in the absence of fungi and bacteria is an added evidence for allelopathy. The allelochemicals present in *Acacia tortilis* subsp. *raddiana* can have an allelopathic inhibitory effect on different agricrops, including trees and weeds species

associated with *Acacia* plantations and also different agroforestry systems in field conditions. *Acacia tortilis* also exhibits autoallelopathy.

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