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Phytotoxic Impact of Mesquite (*Prosopis juliflora*) Pericarps on Seed Germination and Seedling Growth of Lettuce (*Lactuca sativa*)

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Abstract: Phytotoxic effects of mesquite (*Prosopis juliflora)* pericarps on seed germination and seedling growth of lettuce (*Lactuca sativa*) were investigated. Aqueous extracts of 10, 20, 30, 40, 50 and 60 g of dry mesquite pericarps in a litre of distilled water were used. Final seed germination percentage and germination rate, evaluated with corrected germination rate index, and days to 50% of the final seed germination, were significantly reduced. Likewise were the radicle and the hypocotyl lengths, five days after germination. The magnitude of the reduction increased with the increase of extract concentration. Mannitol solutions with the same osmotic potential and pH as the aqueous extracts resulted in significantly higher values of these traits than their corresponding extracts. This indicates that mesquite pericarp contains allelochemicals which could detrimentally affect seed germination and seedling growth of lettuce.

Key words: Mesquite; lettuce; allelopathy; pericarp

INTRODUTION

Mesquite *(Prosopis juliflora* (Sw.) DC is an evergreen , fast growing and nitrogen-fixing tree, tolerant to arid conditions and saline soils (Pasiecznik *et al.* 2001). It produces a variety of valuable products and services that include construction materials, charcoal, soil conservation and rehabilitation of degraded and saline soils. It ameliorates some soil characters, through significant pH reduction and increase in potassium, nitrogen, phosphorus and organic matter (El-Keblawy and Al-Rawai 2007). However, it has invaded millions of hectares of rangeland and

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was rated in 2004 as one of the world's top 100 least wanted species (Mwangi and Swallow 2005). The species allelopathic effect on natural plant communities and soil chemical characters were described by El-Keblawy and Al-Rawai (2007). Larger individual trees and greater densities of mesquite have significant negative impacts on the associated plants, where annuals are inhibited more than perennials.

Mesquite pods make an excellent cattle feed (Douglas and Hart 1984). Hence, it could be incorporated in the soil as part of animal manure. However, it was reported that its pericarp has allelopathic effects on seed germination and seedling growth of Bermuda grass (*Cynodon dactylon*) (Al-Humaid and Warrag 1999), and autoallelopathic effects on the same parameters (Warrag 1994). As yet, it is not known whether it has such effects on vegetable crops.

Lettuce *(Lactuca sativa* L.) is an important dietary leafy vegetable crop (Doğan and Salman 2007). It is frequently used in bioassay studies (Nakano *et al*. 2001; Nakano 2007; Valerio *et al*. (2007).

Thus, this study was conducted to investigate the allelopathic potential of mesquite pericarp on seed germination and seedling growth of lettuce. Such information may add to a better understanding of the allelopathic mechanisms to improve crop productivity and environmental protection through synthesis of novel agrochemicals with remarkable herbicidal activity.

MATERIALS and METHODS

Ripe dry pods were collected from four mesquite trees. Each tree was used as a replicate. The pods were washed with distilled water and oven dried at 45 ºC for 72 hours. They were then broken into small portions and the seeds were discarded. Samples of these pericarps were soaked in distilled water at 10, 20, 30, 40, 50 and 60g L^{-1} at 27 ± 2 ºC for 24 hours. The mixtures were filtered through a l.0 mm wire mesh and then through Whatman No.1 fitter paper. The pH and the osmotic potential_of the extracts and a distilled water (control) were determined with an Orion

Digital Lonalyzer (Model 501) and a freezing point depression osmometer (Osmette, Model 5004, Precision System Inc.), respectively (Table 1). The extracts and the distilled water (control) were put in plastic bottles and kept refrigerated at 4 ± 1ºC. Hereafter extract concentration refers to the weight of dry pericarp per litre of distilled water.

Table 1. Osmotic potential and pH of mesquite (*Prosopis juliflora*) pericarp aqueous extracts

Aqueous extract (g L^{-1})	Osmotic potential (- m Pa)	pH
0 (distilled water)	0.002	6.74
10	0.030 ± 0.001*	6.46 ± 0.02*
20	0.049 ± 0.003	5.69 ± 0.01
30	0.082 ± 0.001	5.23 ± 0.02
40	0.115 ± 0.002	4.97 ± 0.03
50	0.140 ± 0.002	4.86 ± 0.03
60	0.171 ± 0.001	4.65 ± 0.04

*Average of four samples ± standard error of the mean

The first two experiments were carried out concurrently to determine the effect of pericarp aqueous extracts on seed germination and early seedling growth components of lettuce. The two subsequent experiments were also carried out concurrently to determine whether the effects observed in the first two experiments were due to the pH, the osmotic potential and /or the phytotoxicity of the extracts.

In the first experiment, 6 ml of each extract of the pericarp from each tree and the distilled water (control) were dispensed in a 95 × 12 mm plastic Petri dish lined with Whatman No.1 filter paper. The dishes were then placed in polyethylene bags, to prevent evaporative water loss, and arranged in a completely randomized design inside an oven maintained at 23 ± 2 ºC. After an hour, as the temperature of the solutions equilibrated with the oven, 100 seeds of lettuce ‘Dark Green’ were uniformly distributed in each Petri dish. The seeds were examined daily under a dissecting microscope fitted with a micrometer. Germinated seeds were counted and discarded daily until no further germination took place for five successive days. A seed was considered germinated when the radicle protruded by ≥ 3 mm.

In the second experiment, some seeds were put in a Petri dish lined with moistened Whatman No.1 filter paper and kept in an oven maintained at 23 ± 2 ºC. A day later, l0 seeds with the radicles just starting to appear were transferred to each of a set of Petri dishes prepared and treated with the mesquite pericarp extracts and distilled water (control) as specified previously. With a dissecting microscope, the radicle and hypocotyls lengths were measured daily for five days. The average length was then calculated for each dish.

For the third and fourth experiments, mannitol solutions with the same osmotic potentials as the extracts (10, 20, 30, 40, 50 and 60g L^{-1}) (Table 1) were prepared using the following formula (Ibanze and Passera 1997):

$$W = PVM/ RT$$

where W is the weight of mannitol (g), P is the desired osmotic potential (bar), V is the volume (L), M is the molecular weight of mannitol (182.17g), R is the gas constant (0.08205) and T is the absolute temperature (ºC + 273).

Then, the pH of the mannitol solutions were adjusted to those of respective extracts as shown in Table 1 by the addition of few drops of dilute hydrochloric acid. Using these solutions and the extracts, seed germination count (experiment 3) and radicle and hypocotyl lengths (experiment 4) were determined as described previously.

The seed germination rate was evaluated by the corrected germination rate index (CGRI) and time (in days) to 50% of the final germination percentage (GT50). The CGRI was calculated as follows (Hsu *et al.* 1985):

$$CGRI = 100\ DGP/ND*FGP$$

where DGP is the summation of daily germination percentages, ND is the total number of days of germination and FGP is the final germination percentage.

The final seed germination percentages and the GT50 were arcsine and square root $(\times + 0.5)^{1/2}$ transformed, respectively. Analysis of variance and mean separation were performed at the 5% level using Duncan's multiple range test (Gomez and Gomez 1984) for the data of experiments 1 and 2. On the other hand, student t-test was performed for the data of experiments 3 and 4 between each of the mesquite pericarp aqueous extract and the corresponding mannitol solution.

RESULTS and DISCUSSION

Both acidity and osmotic pressure (the negative of osmotic potential), increased with the increase of the mesquite pericarp aqueous extract concentration, expressed as weight of pericarp soaked in a litre of distilled water (Table1). This indicates that the extracts contained acidic compounds that lowered the solutions pH and osmotic potentials.

The results of experiment 1 showed that the aqueous extracts of mesquite pericarp had significant ($P<0.05$) effect on lettuce seed germination components. The final seed germination percentage and the CGRI decreased significantly with the increase of the extract concentration, and the control had the highest value (Table 2). The CGRI is a widely used index that evaluates germination rate for relative comparison (Nurdin and Fulbright 1990). The GT50, an index used for evaluation in meaningful biological units (Angus *et al*. 1981), increased significantly with the increase of the extract concentration. This result indicates lower germination rate with the increase of extract concentration (more days to reach GT50) (Table 2).

Table 2. Effect of distilled water and mesquite (*Prosopis juliflora*) pericarp aqueous extracts on final seed germination percentage (FSGP), corrected germination rate index (CGRI), days to 50% of the final germination (GT50) of lettuce (*Lactuca sativa*)

Aqueous extract (g L^{-1})	FSGP	CGRI	GT50 (days)
0	94.7A	82.6A	2.24C
10	88.5A	76.0AB	2.27C
20	62.3B	69.8B	2.51C
30	41.9C	44.5C	3.41B
40	28.2D	30.7D	3.52B
50	14.8E	22.6DE	3.80A
60	3.0F	13.1E	3.87A

The values of FSGP were arcsine transformed and those of GT50 were square root transformed $(X+0.5)^{1/2}$ for analysis

Means in the same column followed by the same letter(s) are not significantly different at the 5% level, using Duncan's multiple range test.

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Similarly, the results of experiment 2 showed that the aqueous extracts of mesquite pericarp had significant ($P<0.05$) effect on lettuce early seedling growth components. The radicles were significantly longer in the control than in the extracts (Table 3). Likewise were the hypocotyls, with the exception of the least concentrated extract. Both radicles and hypocotyls decreased in length with the increase of extract concentration (Table 3). However, the radicles were more affected, as indicated by the progressive decline in the radicle: hypocotyl length ratio. The radicle tips were darker in colour in the extract than in the control, with the colour becoming more conspicuous with the increase of extract concentration.

Similar effects of the aqueous extracts of mesquite pericarp on seed germination and seedling growth were reported for mesquite (Warrag 1994) and Bermuda grass (Al - Humaid and Warrag 1999). To find the factor(s) implicated, solutions of mannitol with the same osmotic potential and pH as the pericarp aqueous extracts were used for comparison in experiments 3 and 4.

Table 3. Effect of distilled water and mesquite (*Prosopis juliflora*) pericarp aqueous extracts on radicle and hypocotyl lengths 5 days after seed germination of lettuce (*Lactuca sativa*)

Aqueous extract (g L^{-1})	Radicle length (mm)	Hypocotyl length (mm)
0	91.4A	54.8A
10	72.1B	52.6AB
20	65.4BC	45.7BC
30	56.0C	40.8C
40	31.9D	38.2CD
50	19.2E	29.7DE
60	2.4F	23.5E

Means in the same column followed by the same letter(s) are not significantly different at the 5% level, using Duncan's multiple range test.

Both mannitol solutions and extracts resulted in a progressive decrease of seed germination and seedling growth of lettuce with the increase of concentration (Tables 4 and 5). However, with the exception of the least concentrated solutions, CGRIs were significantly higher in the mannitol solutions than in their corresponding extracts (Table 3). Regarding GT50, the differences were significant in the three most concentrated solutions only. This was probably due to the wide intervals of data collection. Although both radicles and hypocotyls decreased in length with the increase of concentration, the decrease in radicles was steeper (Table 5). While the mannitol solutions resulted in significantly longer radicles than in all corresponding extracts, this was exhibited by hypocotyls in the three most concentrated solutions only (Table 5). The radicle tips were not as dark in the mannitol as in the pericarp aqueous extracts. These effects could have been induced by unsuitable pH, low osmotic potential and/or the allelopathic action of these extracts (Rice 1984; Putnam 1986; Nakano *et al*. 2001).

The significantly higher seed germination percentage and seed germination rate and the longer radicles and hypocotyls in the mannitol solution than in most of their corresponding pericarp extracts could have eliminated the implication of the pH and the osmotic potential. Hence, apparently it seems that mesquite pericarp contains water soluble allelochemicals that could cause inhibition of seed germination, reduction of seed germination, and radicle and hypocotyl growth rate of lettuce. Also, these allelochemicals seem to be responsible for the dark colour of radicle tips, a phenomenon reported with other plant species exposed to phytotoxins (Yang 1982; Weston and Putnam 1985; Warrag 1994 and 1995; Al-Humaid and Warrag 1998). The isolation and identification of these allelochemicals and subsequently understanding their mechanisms would help enrich the limited research in this area (Putnam 1986).

CONCLUSION

Mesquite (*Prosopis juliflora* (Sw.) DC.) pericarp aqueons extracts detrimentally affect lettuce (*Lactuca sativa* L.) 'Dark Green' seed germination and seedling growth. Apparently, the mesquite pericarp contains water soluble allelochemicals that brought about these effects. Thus, it is advisable not to grow lettuce in the vicinity of mesquite trees or incorporate manure containing mesquite pods in soil intended to be sown with lettuce. The isolation and identification of these mesquite allelochemicals may lead to improvement of crop productivity and protection of the environment through synthesis of agrochemicals with remarkable herbicidal activity.

Table 4. Final seed germination percentage (FSGP), corrected germination rate index (CGRI) and days to 50% of the final germination (GT50) of lettuce (*Lactuca sativa)* in the aqueous extracts of mesquite (*Prosopis juliflora*) pericarp and mannitol solutions

Osmotic potential (-mPa) pH	0.036 6.46	0.049 5.69	0.082 5.23	0.115 4.97	0.140 4.80	0.171 4.65
			FSGP *			
Aqueous extracts	85.6 A	60.3 B	44.7B	30.9B	12.8B	2.6 B
Mannitol solutions	82.4A	79.5A	71.3A	65.0A	61.2A	52.8A
			CGRI (day $^{-1}$)			
Aqueous extracts	71.3 A	62.4 B	46.1 B	34.8 B	25.7 B	11.2B
Mannitol solutions	75.6 A	70.9 A	64.3 A	56.0 A	52.5A	44.5A
			GT 50 (days)			
Aqueous extracts	2.16A	2.70A	2.95A	3.48A	3.61A	3.79A
Mannitol solutions	2.20A	2.56A	2.77A	2.82B	2.96B	3.14B

* The values of FSGP were arcsin transformed and those of GT50 were square root transformed $(X+0.5)^{½}$ for analysis. Means in the same column for the same variable followed by the same letter are not significantly different at 5% level, according to Duncan's multiple range test

Table 5. Radicale and hypocotyl length of lettuce (*Lactuca sativa)* seedling in pericarp aqueous extracts of mesquite (*Prosopis juliflora*) and mannitol solutions, 5 days after germination

Osmotic potential (-mPa)	0.036	0.049	0.082	0.115	0.140	0.171
pH	6.46	5.69	5.23	4.97	4.80	4.65
			Radicle length (mm)			
Aqueous extracts	68.4B	54.8B	45.1B	30.2B	21.4B	5.3B
Mannitol solutions	85.3A	81.3A	73.0A	67.8A	62.6A	57.9A
			Hypocotyl length (mm)			
Aqueous extracts	49.3A	42.2A	39.2A	34.6B	22.5B	2.3B
Mannitol solutions	51.7A	44.7A	42.0A	40.3A	36.5A	24.8A

Means for each variable in the same column followed by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

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التأثير المثبط لغلاف ثمار المسكيت على إنبات بذور ونمــــو بادرات الخس

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المستخلص :- تمت دراسة التأثيرات المثبطة لغلاف ثمار المسكيت على إنبات بذور ونمو بادرات الخس. إستخدمت المستخلصات المائية لـ 10 و20 و30 و40 و50 و60 جرام من غلاف ثمار المسكيت الجافة فى 1 لتر ماء مقطر. حدث إنخفاض معنوى فى النسبة المئوية للانبات النهائى ومعدل الانبات الذى تم تقييمه بموشر معدل الانبات المصحح وعدد الايام الى 50% من إنبات البذور النهائى. كما حدث تأثير مماثل على طول الجذير والسويقة الجنينية السفلى خمسة أيام بعد الانبات. ازدادت قيم الانخفاض بإزدياد تركيز المستخلص. نتج عن إستخدام محاليل المانيتول، التى لها نفس الجهد الأسموزى والأس الهايدروجينى مثل المستخلصات المائية، قيم أعلى معنوياً لهذه الصفات من المستخلصات المقابلة. يدل هذا على أن غلاف ثمار المسكيت تحتوى على مواد كيميائية يمكن أن تؤثر تأثيرا" ضاراً على إنبات بذور ونمو بادرات الخس.

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