SHORT COMMUNICATION

Phytotoxic effects of *Melia azedarach* L. (Meliaceae) fruit extract on weeds and crops

S.M. PALACIOS*, M.C. CARPINELLA, G. N. DIAZ NAPAL, C.E. VACCARINI[1] and C.G. FERRAYOLI[1]

Laboratorio de Química Fina y Productos Naturales, Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Camino a Alta Gracia Km 10, 5000, Córdoba, Argentina.

Phone (Office): 540351 4938000, FAX (Office): 540351 4938061. E-mail: sarapalacios@ucc.edu.ar

(Received in revised form: ----------)

ABSTRACT

In our continuous search for bioactive products obtained from plants with agrochemical prospects, the extract of *Melia azedarach* L. fruits was tested for the phytotoxicity against weeds and crops. In a paper disk assay, this extract inhibited the seed germination of *Avena sativa, Brassica napus*, *Chenopodium album, Lactuca sativa* and *Sorghum halepense* and the 50% germination inhibitory doses (GID_{50}) were 0.27, 16.5, 2.88, 7.85 and 1.31 mg/ml, respectively. *Melia* fruit extract, also inhibited the seedlings growth and 50% growth inhibitory doses ($GrID_{50}$) were 0.59, 1.86, 5.59, 3.98 and 1.03 mg/ml, respectively.

The effects of crushed *M. azedarach* fruit material mixed with the soil were examined on germination, radicle and shoot length of *A. sativa* and *S. halepense* in assay for 30 days. A GID_{50} of 0.56 and 3.51 % (w/w) was determined for *A. sativa* and *S. halepense*, respectively, while 10 % (w/w) concentration completely inhibited the root and shoot length in both species. These results indicate that phytotoxic compounds are present in *M. azedarach* fruits.

Key words: *Avena sativa, Brassica napus*, *Chenopodium album,* germination, *Lactuca sativa*, lettuce, *Melia azedarach,* mustard, oat, Phytotoxicity, *Sorghum halepense*, weeds.

INTRODUCTION

Plant secondary metabolites are chemical defences of plants to protect against herbivorous insects (21), pathogens (7) and to improve the plant competition through allelochemical effects (16). Recently the application of natural phytotoxic compounds to improve the crop productivity through an eco-friendly control of weeds has become increasingly important, to minimizes the harmful effects of synthetic herbicides in agricultural production (24).

Melia azedarach L. (Meliaceae) is a deciduous tree growing in temperate and tropical regions, widely used for ornamental and timber purposes. Its fruits extract has strong antifeedant effects on a variety of insects from different orders (3, 8,9,22,23). The most potent antifeedant compound present in this extract was isolated and identified as the limonoid, meliartenin (5). The fruit extract also have antifungal (4,7) and antiviral effects (1) due to lignanes and limonoids, respectively. Recently, *M. azedarach* has been recognized as the most promising plant, after *Medicago* and 7 other species (*Piper methysticum* L., *Azadirachta indica* A. Juss., *Leucaena glauca* Benth., *Ageratum conyzoides* L., *Galactia pendula* Pers., *Eupatorium canabium* L. and *Oryza sativa* L.) for use as cover mulch to control weeds in rice fields (24). Allelopathic studies have shown that aqueous leaf and root extracts from *M. azedarach* completely inhibited the germination of radish seeds (15), although the phytotoxic effects of fruit extracts have not yet been tested.

Since *M. azedarach* fruit extract is a promising source of natural pesticides for organic and conventional agriculture (9), its possible phytotoxic effect was investigated on three crops (*Avena sativa* L., *Brassica napus* L. and

* Correspondence author. [1]Centro de Excelencia en Productos y Procesos de la Provincia de Córdoba CEPROCOR, Alvarez de Arenales 230, B° Juniors, Cordoba, 5014, Argentina, S.M. Palacios and M.C. Carpinella are members of CONICET.

Lactuca sativa L.) and two weeds (*Chenopodium album* L. and *Sorghum halepense* (L.) Pers.). The weed species are widespread and represent a significant threat to agricultural production world wide and especially in the Pampa region of Argentina. Although compounds toxic to mammals have been reported from some chemotypes of *M. azedarach* (20), but its fruit extract has not exhibited either oral, dermal or ocular toxicity (6, 8).

MATERIALS AND METHODS

Crushed ripe fruits of *M. azedarach* collected in Córdoba, Argentina (a sample identified as CORD 229 has been deposited in the Botanic Museum, FCEFyN, Universidad Nacional de Córdoba) were Soxhlet-extracted with ethanol after fat removal with hexane. The ethanol was then evaporated and the resulting viscous extract (yield: 30.3 g/100g of fruit) was diluted with deionized water to make 0.02, 0.1, 0.5, 1, 10 and 20 mg/ml solutions (10). The pH of extract solutions ranged from 4.5 to 5 and osmolality ranged from 330-467 mOsm/l.

Seeds of *A. sativa, B. napus* and *L. sativa* were purchased from Semillería Florensa in Córdoba while seeds of *C. album* and *S. halepense* were supplied by Facultad de Agronomía, Universidad Nacional de Córdoba.

Paper disk bioassay

Twenty seeds of *A. sativa* and *L. sativa* var. Grand rapids Waldman green, 30 seeds of *B. napus* var. *napobrassica* and 15 seeds of *C. album* and *S. halepense* were placed in each Petri dish (9 mm dia) containing a moist filter paper with 10 ml aqueous *M. azedarach* fruit extract solutions. The control contained only deionized water. Three replicate assays for each preparation were performed. Both treatment and control dishes were kept at 25 ± 2°C under 16-8h light-dark cycle for 7 days. Thereafter, the number of germinated seeds was determined. Germination rates were averaged and the data were transformed into the inhibition percentage respect to the control. The dose resulting in 50% inhibition of germination (GID_{50}) was determined by Probit analysis.

The seedlings resulting from the germination assay were oven-dried at 75°C for 48 h and the dry weight was recorded. Data were normalized by the number of seedlings per replicate, averaged and the growth inhibition percentage respect to control was calculated. The dose resulting in 50% growth inhibition ($GrID_{50}$) was determined by Probit analysis.

Soil Assay

Each treatment was performed in pots (7.5 cm dia and 10 cm high) contained 200 g potting soil (2:2:1 sand-loam-peat, volume basis), to which 5, 10, 20 or 40 g of dried and crushed *M. azedarach* fruits were added (17). Controls contained only potting soil. Each treatment including the control, was watered and allowed to drain overnight before sowing, 1 cm deep and 2 cm apart, with 15 seeds of *A. sativa* or *S. halepense*. Treatments were replicated three times. Pots were kept in a greenhouse [25-30°C and 70-80% relative humidity]. The shoot emergence rate was monitored every 7 days. Seedlings were grown in the greenhouse for 30 days, thereafter root and shoot length was measured and averaged. Percent inhibition was calculated respect to control.

Statistical analysis

Analysis of variance by the Kruskal–Wallis test was performed for the soil assay data. Comparisons between treatments were made at 0.05 probability level of significance.

RESULTS AND DISCUSSION

The extract inhibited the germination of all tested species with varying effectiveness (GID_{50} = 0.27-16.5 mg/ml). The inhibition was stronger in monocotyledon species [*A. sativa* and *S. halepense* (GID_{50} = 0.27 and 1.31 mg/ml, respectively)], than in dicotyledons [*B. napus*, *C. album* and *L. sativa* where GID_{50} values were 16.5, 2.88, 7.85

mg/ml, respectively (Table 1)]. The germination of *B. napus* and *L. sativa* was less affected than *C. album* weed. Unfortunately, selectivity was not observed between the *A. sativa* and *S. halepense.* The effective dose of *M. azedarach* required to inhibit the germination of *L. sativa* was comparable to aqueous extract of the Japanese medicinal plant *Houttuynia cordata* Thumb (19).

The inhibitory effect of *M. azedarach* extract on the growth of emerged plants was noted for all species and is reflected in the $GrID_{50}$ values (Table 1). The highest effect was observed for *A. sativa* ($GrID_{50}$ = 0.59 mg/ml), while the least susceptible species was *C. album* ($GrID_{50}$ = 5.59 mg/ml). These results indicated that, if germination occurs, the development of seedling is highly affected by the *M. azedarach* extract, which may be due to toxic factors present in the fruit extract (9).

Comparing the GID_{50} with the $GrID_{50}$ for each species, it was seen that the germination *A. sativa* and *C. album* was more inhibited than their growth; while *L. sativa, B. napus* and *S. halepenses* germination was less affected than their seedling growth.

The phenolic compounds significantly inhibits the seed germination, plant growth and other plant physiological processes (11,12). Phenolics [*p*-hydroxybenzoic, vanillic and ferulic etc] present in *Chrysanthemum morifolium* (18) and in *Allium ursinum* (12) are phytotoxic allelopathic agents in natural and agro-ecosystems (2). Such compounds are also present in *M. azedarach* fruit (4,7,14) suggesting that they could be the chemicals responsible for the inhibitory effects in this study. The coumarin scopoletin isolated from the *M. azedarach* fruit extract is antifungal compound (4) inhibits the growth of tobacco (*Nicotania tabacum*) and sunflower (*Helianthus annuus*) (13). Thus this compound also contributes to the phytotoxic effects of *M. azedarach* fruit extract.

In soil assay, crushed *M. azedarach* fruit strongly inhibited the seed germination and seedling growth of *A. sativa* and *S. halepense*. The 10 and 20 % (w/w) concentrations, completely inhibited the germination of *A. sativa.* At 10 % (w/w) the seed germination of *S. halepense* was also completely inhibited, while at 20 % (w/w) inhibition of germination was 73%. The doses of 0.56 ($1.2\ 10^{-2}$, 25.5) and 3.51 (1.89, 6.49) g% (w/w) caused 50% inhibition in germination of *A. sativa* and *S. halepense* seeds, respectively. These crushed fruit doses potentially yield 0.13 and 0.88 mg of extract respectively, showing a logical ratio with the GID_{50} obtained in the paper disk assay (Table 1).

Strong root and shoot inhibition was also observed in soil assay. Crushed fruit inhibited the root growth of *A. sativa* seedlings and inhibition was 38% at both 2.5 and 5 % (w/w) (Fig. 1), these concentration also caused 15 and 27% shoot growth inhibition. In *S. halepense,* 2.5 and 5 % (w/w) drastically inhibited the root length (50 and 90%), respectively, while at 20 % (w/w) 93% root inhibition and 36% shoot inhibition were observed (Fig. 2). At 10 g% (w/w) complete inhibition of root and shoot length was observed in both tested specie, *A. sativa* and *S. halepense*.

Our results showed that *M. azedarach* fruit extract was phytotoxic to test crops and weed species and inhibited their germination and seedling growth. These findings open the opportunity for further studies on the possibility to exploit *M. azedarach* fruit extract or crushed fruit.

ACKNOWLEDGEMENTS

G. N. Diaz Napal gratefully acknowledges receipt of a fellowship from CONICET.

REFERENCES

1. Barquero, A.A., Michelini, F.M. and Alché, L.E. (2006). 1-Cinnamoyl-3,11-dihydroxymeliacarpin is a natural bioactive compound with antiviral and nuclear factor-κB modulating properties. *Biochemical and Biophysical Research Communications* **344**: 955-962.

2. Blum, U., Wentworth, T.R., Klein, K., Worsham, A.D., King, L.D., Gerig, T.M., Lyu, S.W. (1991). Phenolic acid content of soils from wheat-no till, wheat-conventional till, and fallow-conventional till soybean cropping systems. *Journal of Chemical Ecology* **17**: 1045-1068.

3. Carpinella, M.C., Defago, M.T., Valladares, G. and Palacios, S.M. (2003). Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. *Journal of Agricultural and Food Chemistry* **51**: 369-374.

4. Carpinella, M.C., Ferrayoli, C.G., and Palacios, S.M. (2005). Antifungal synergistic effect of scopoletin, a coumarin isolated from *Melia azedarach* L. *Journal of Agricultural and Food Chemistry* **53**: 2922-2927.

5. Carpinella, M.C., Ferrayoli, G.C., Valladares, G., Defago, M. and Palacios, S.M. (1999). Potent limonoid insect antifeedant from *Melia azedarach. Bioscience Biotechnology and Biochemistry* **66**: 1731-1736.

6. Carpinella, M.C., Fulginiti, S., Britos, S., Oviedo, M.M., Alonso, R.A. and Palacios, S.M. (1999). Acute toxicity of fruit extracts from *Melia azedarach* L. in rats. *Revista de Toxicología*. **16**: 22-24.

7. Carpinella, M.C., Giorda, L.M. and Palacios S.M. (2003). Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. *Journal of Agricultural and Food Chemistry* **51**: 2506-2511.

8. Carpinella, M.C., Miranda, M., Almirón, W.R., Ferrayoli, C.G., Ludueña Almeida, F. and Palacios, S.M. (2007). *In vitro* pediculicidal and ovicidal activity of an extract and oil from fruits of *Melia azedarach* L. *Journal of the American Academy of Dermatology* **56**: 250-256.

9. Carpinella, M.C., Defagó, M.T., Valladares, G. and Palacios, S.M. (2006). Role of *Melia azedarach* L. (Meliaceae) for the control of insects and acari: Present status and future prospects. In: *Naturally Occurring Bioactive Compounds*, (Eds. M.K. Rai and M.C. Carpinella) pp. 81-123. Elsevier, Amsterdam, The Netherlands.

10. Coria, C., Almirón, W., Valladares, G., Carpinella, C., Ludueña, F., Defago, M. and Palacios S. (2008). Larvicide and oviposition deterrent effects of fruit and leaf extracts obtained from *Melia azedarach* L. on *Aedes aegypti* (L.). *Bioresource Technology* **99**: 3066-3070.

11. Chon, S.-U., Jang, H.-G., Kim, D.-K., Kim, Y.-M., Boo, H.-O. and Kim Y.-J. Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae* **106**: 309-317.

12. Djurdjevic, L., Dinic, A., Pavlovic, P., Mitrovic, M., Karadzic, B., and Tesevic, V. (2004). Allelopathic potential of *Allium ursinum* L. *Biochemical Systematics and Ecology* **32**: 533-544.

13. Einhellig, F.A., Rice, E.L., Risser, P.G. and Wender, S.H. (1970). Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. *Bulletin of the Torrey Botanical Club* **97**: 22-33.

14. Harborne, J.B. and Baxter, H. (1983). *Phytochemical Dictionary. A Handbook of Bioactive Compounds From Plants*. Taylor & Francis, London. pp 791.

15. Hong, N.H., Xuan, T.D., Eiji, T., Hiroyuki, T., Mitsuhiro, M. and Khanh, T. D. (2003). Screening for allelopathic potential of higher plants from Southeast Asia. *Crop Protection* **22**: 829-836.

16. Inderjit and Duke, S.O. (2003). Ecophysiological aspects of allelopathy. *Planta* **217**: 529-539.

17. Inderjit (2006). Experimental complexities in evaluating the allelopathic activities in laboratory bioassays: A case study *Soil Biology & Biochemistry* **38**: 256-262.

18. Kil1, B.-S. and Youb, S.L. (1987). Allelopathic effects of *Chrysanthemum morifolium* on germination and growth of several herbaceous plants. *Journal of Chemical Ecology* **13**: 299-308

19. Lin, D., Sugitomo, Y., Dong, Y., Terao, H. and Matsuo, M. (2006). Natural herbicidal potential of saururaceae (*Houttuynia cordata* Thunb) dried powders on paddy weeds in transplanted rice. *Crop Protection* **25**: 1126-1129.

20. Oelrichs, P.B., Hill, M.W., Vallely, P.J., MacLeod, J.K. and Molinski, T.F. (1983).Toxic tetranortriterpenes of the fruit of *Melia azedarach*. *Phytochemistry* **22**: 531-534.

21. Schoonhoven, L.M., Jermy, T. and van Loon, J.J. (1998). *Insect-Plant Biology: From Physiology to Evolution.* Chapman and Hall, London, pp 409.

22. Valladares, G., Defago, M.T., Palacios, S.M. and Carpinella, M.C. (1997). Laboratory evaluation of *Melia azedarach* (Meliaceae) extracts against the Elm leaf beetle (Coleoptera, Chrysomelidae). *Journal of Ecomical Entomology* **90**: 747-750**.**

23. Valladares, G., Ferreyra, D., Defago, M.T., Carpinella, M.C. and Palacios, S.M. (1999). Effects of Argentinian *Melia azedarach* on *Triatoma infestans* (Hemiptera, Reduviidae). *Fitoterapia* **70**: 421-424.

24. Xuan, T.D., Shinkichi, T., Khanh, T.D. and Chung, I.M. (2005). Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. *Crop Protection* **24**: 197-206.

Table 1. Germination inhibitory effect of *Melia azedarach* fruit extract

	Germination inhibition (%)			
	Treatment[a]			
	0.02	0.1	0.5	1
Avena sativa	72	47	34	70
Brassica napus	-10	14	33	36
Chenopodium album	39	20	65	86
Lactuca sativa	14	10	34	66
Sorghum halepense	21	44	70	84

[a]: doses in g of *M. azedarach* fruit extract/100 ml

Table 2. Inhibitory effect of *Melia azedarach* fruit extract on seedlings growth

	Biomass (% growth inhibition)				
	Treatment[a]				
	0	0.02	0.1	0.5	1
Avena sativa	0.479 (0)	0.359 (25)	0.163 (66)	0.062 (87)	0.244 (49)
Brassica napus	0. 496(0)	0.479 (3)	0.197 (60)	0.153 (69)	0.143 (71)
Chenopodium album	0.040 (0)	0.031 (22)	0.024 (39)	0.022 (46)	0.018 (56)
Lactuca sativa	0.225 (0)	0.218 (3)	0.172 (23)	0.099 (56)	0.070 (69)
Sorghum halepense	0.218 (0)	0.155 (29)	0.103 (53)	0.148 (32)	0.139 (36)

[a]: doses in g of *M. azedarach* fruit extract/100 ml

Table 3. Inhibition of *Melia azedarach* fruit extract on germination and seedlings growth

Species	GID_{50}	$GrID_{50}$
	(mg/ml)	
Avena sativa	0.27	0.59
Brassica napus	16.5	1.86
Chenopodium album	2.88	5.59
Lactuca sativa	7.85	3.98
Sorghum halepense	1.31	1.03

GID_{50}: Effective dose for 50% germination inhibition. $GrID_{50}$: effective dose for 50% growth inhibition

Table 4.Effects of crushed *Melia azedarach* fruit mixed with soil on seedlings growth

Dose (%w/w)	Lenght (mm)			
	Avena sativa		*Sorghum halepense*	
	root	shoot	root	shoot
0	95	158	131	94
2.5	60	135	68	90
5	60	112	12	82
10	0	0	0	0
20	0	0	62	62

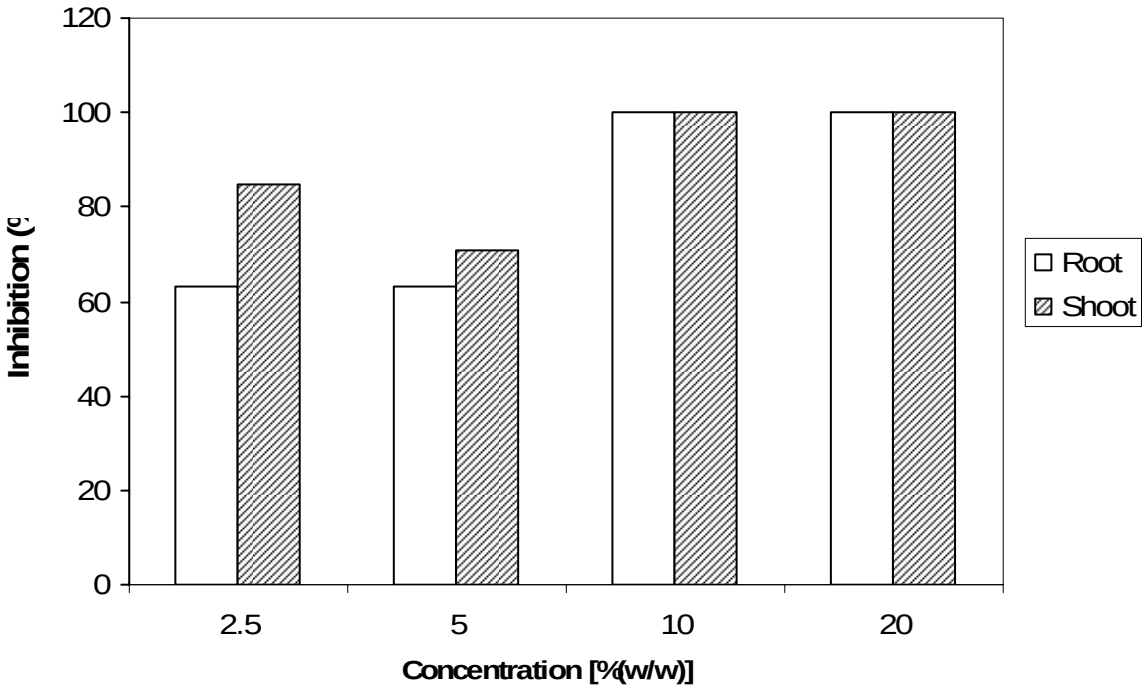

Figure 1. Effects of different concentrations of crushed *Melia azedarach* fruit mixed in soil on root and shoot length of *Avena sativa* at 30 days after sowing. Kruskal-Wallis test, data followed by the same letter are significantly different according to Dunn's test ($p < 0.01$).

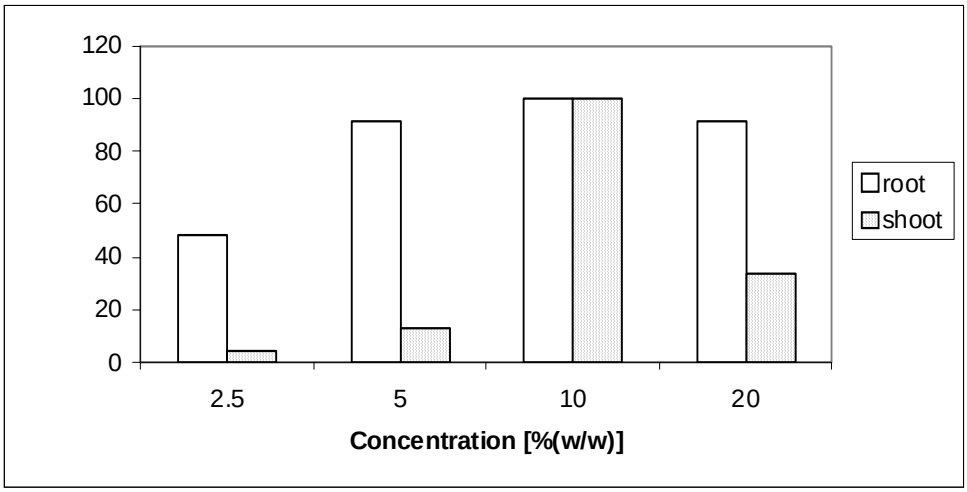

Figure 2. Effects of crushed *Melia azedarach* fruit concentrations mixed in soil on root and shoot length of *Sorghum halepense* at 30 days after sowing. Kruskal-Wallis test, data followed by the same letter are significantly different according to Dunn's test ($p < 0.01$).