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**ORIGINAL PAPER**

# Evaluation of phytotoxic activity of *Melia azedarach* L. extracts on *Lactuca sativa* L.

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## Abstract

*Phytotoxic effects of aqueous and alcoholic extracts obtained from different parts (leaves, leaves & wood mix, fruits) of Melia azedarach L. on germination and growth of Lactuca sativa L. seeds were investigated under laboratory conditions. Ultrasonic and classic reflux methods were used for obtaining extracts from the vegetable material. The inhibitory effect on the germination of lettuce seeds as well as on seedling growth varied with concentration and extract type. Thus, fruit extract showed the most pronounced inhibitory effect followed by leaves extract and leaves and wood mix extract. The 5% w/v ethanolic extracts obtained by using reflux method showed a significant inhibitory effect on lettuce seeds germination and on seedlings.*

**Keywords:** phytotoxicity, *Melia azedarach* L., germination, reflux extraction, ultrasounds extraction

## Introduction

The presence of weeds in crops has always represented a big problem for cultivators. Synthetic herbicides extensively used for weed management, proved in time to be harmful for the environment - soil, water and food - consequently affecting public health (S. Wahab, [1], Mancini & al. [2]).

These facts increased the interest of researchers towards finding alternative weed management strategies, which could be offered by the allelopathy (R.J. Petroski, D.W. Stanley, [3]). Allelopathy is a mechanism used by plants species to achieve an edge between them and the others growing in close proximity (D. R. de Souza & al. [4]). The tools used for this are chemical compounds called allelochemicals that can be located in any part of the so-called donor species (leaves, flowers, roots, fruits, or stems).Target species exposed to toxic allelochemicals (phytotoxins) exhibit various symptoms from wilting, chlorosis (foliar yellowing) to potential death depending on the way the toxins act, as they can either inhibit nutrient uptake or attack a naturally symbiotic relationship and destroy the plant source of a nutrient (S. Abu-Romman & al.[5])

Applying the allelopathy principles in agriculture by using plant extracts or natural compounds as herbicides, environmental friendly and cost effective alternatives to synthetic herbicides as well as healthy food products can be obtained.

*Melia azedarach L.* (Meliaceae)**,** also known as Chinaberry tree or syringa tree (common name in South Africa), is a tree growing in North and South America, Asia, North

Australia and Africa. Extracts of *M. azedarach* L. have been tested for various activities such as: antiviral (A.A. Barquero & al. [6], S.M. Villamil & al. [7], antihelmintic (J.M. Watt & al. [8]), antibacterial (Saleem R. & al. [9]), insecticidal (Akhtar Y. & al. [10]), antifungal (Ashraf H., Javaid A., [11], Carpinella M.C. & al. [12]), nematicidal (Ntalli N. G. & al. [13]).

Often planted as an ornamental shade tree, *M. azedarach* L. is considered in South Africa an invasive plant and it can be found on the list of the species that are meant to be destroyed (J. Morris [14]). Therefore we initiated this study to check the phytotoxic activity of *M. azedarach* L. extracts.

In this work we tested three concentrations (5%, 2.5% and 1.25% w/v) of extracts from leaves, leaves and wood mix, and fruits of *M. azedarach* L. were tested. The influence of the solvent (water and ethanol) and of the extraction method (reflux and ultrasounds) on phytotoxic activity of the extracts were also studied, in order to assess the best way of extracting phytotoxic compounds from plant.

## Materials and methods

***Extracts preparation***

The vegetable material (supplied by Syringa Bioscience Pty. Company, South Africa), dried and grounded into powder (5 g), was extracted with 100 ml solvent (distilled water or ethanol 70% ) for 1 hour by means of ultrasounds or reflux to obtain a 5% w/v extracts.

For ultrasounds extracts, vegetable material and solvent were added in an Erlenmeyer flask, sealed and sonicated in an ultrasonic cleaning bath (Langford Sonomatic, operating at 33 kHz and 50 W power).

The aqueous extracts obtained through both methods were filtered through paper filter and then filtered through mixed cellulose ester membrane filter for sterilization (0.45 μm, ADVANTEC, Toyo Roshi Kaisha, Ltd., Japan) before applying on seeds.

After extraction, ethanolic (70%) extracts were paper filtered and the alcohol was evaporated at low pressure using a rotary evaporator. The obtained residue was dissolved in water (up to 100 ml) to get a stock solution and then filtered through filter paper.

The other extract concentrations (2.5 and 1.25% respectively) were obtained by diluting the 5% extracts. All extracts were filtered through mixed cellulose ester membrane filter for sterilization right before applying on seeds.

To calculate the yields of dry extracts per 5 grams of plant, the residue obtained after solvent (water or ethanol 70% v/v) removal was dried in an oven at $100^0$C for 1 hour and weighed.

***Phytotoxicity assay***

Lettuce (*Lactuca sativa* L., *Asteraceae*, seeds, Mefim AGRO SRL, Romania) was used as target species in this experiment because it has a short germination time and is sensitive to phytochemicals (J. Morris [14], F.A. Macias [15]). Before tests, seeds were sterilized in 3% $H_2O_2$ for 30 min and then washed many times with distilled water.

5 ml extract was added in 9 cm diameter Petri dishes, lined with filter paper and 20 salad seeds were disposed on it (D.R. Richardson [16]). Controls received distilled water. Two replicates were prepared for each sample.

Experimental dishes were incubated at room temperature (20-$22^0$C), in the dark.

Results for germination test were recorded after 5 days, when all seeds from control plate germinated. Germination speed (S) (J.D. Maguire [17]) and response index (RI) (F. Pellissier [18], G.B. Williamson & al. [19]) were used for evaluation of phytotoxic activity of extracts on lettuce seed germination.

$$S = 2(5d_1+4d_2+3d_3+2d_4+d_5) \quad (1)$$

where $d_{1-5}$ number of seeds geminated in day 1, day2… etc.

$$RI = (test/control)-1 \quad (2)$$

where test = number of seeds from test germinated after 5 days,
control = number of seeds from control plate germinated after 5 days.
If $-1 < RI > 0$, then the effect is inhibition;
If $0 < RI > 1$, effects is stimulation.

For growth tests, 10 lettuce seeds germinated under control conditions were transferred to fresh Petri dishes and treated with *M. azedarach* L. extracts (5 ml extract for each Petri dish). A seed with 0.2 cm radicle was considered germinated. After 5 days, when the controls showed good growth, the shoot and root elongations were measured (H. Kato-Noguchi [20]).

The inhibition percent (R. Tsao & al. [21]) was calculated using the formula below.

$$\text{Inhibition (\%)} = (L_{control}-L_{treatment})/L_{control} \times 100 \quad (3)$$

where $L_{control}$ = radicle length of salad seedlings in the control,
$L_{treatment}$ = radicle length of salad seedlings treated with syringa extract.

***Statistical analysis***

Data were subjected to one way analysis of variance and the mean values were separated at $P<0.05$ by applying 2-sample t-test. The statistical analysis was done using ORIGIN 6.0 version program.

## Results and discussion

### Phytotoxic effects of *Melia azedarach* L. extracts on germination of lettuce seeds

The obtained results show that the 5% aqueous extracts totally suppressed germination of salad seeds in case of reflux extraction (Table 1); 2.5% extracts also inhibited significantly germination compared to control.

**Table 1**. Response index for aqueous and alcoholic extracts

| Extract conc. % | Aqueous extracts | | | | | |
|---|---|---|---|---|---|---|
| | ultrasound extraction | | | reflux extraction | | |
| | leaves | leaves+wood mix | fruits | leaves | leaves+wood mix | fruits |
| 5 | $-0.50^{b}$ | $-0.42^{c}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ |
| 2.5 | $-0.26^{b}$ | $-0.10^{ns}$ | $-0.65^{c}$ | $-1^{c}$ | $-0.92^{a}$ | $-0.85^{a}$ |
| 1.25 | $-0.02^{ns}$ | $-0.05^{ns}$ | $-0.76^{b}$ | $-0.58^{ns}$ | $0^{ns}$ | $-0.71^{c}$ |
| | Alcoholic extracts | | | | | |
| 5 | $-1^{a}$ | $-0.38^{c}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ |
| 2.5 | $-1^{a}$ | $-0.26^{c}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ | $-1^{a}$ |
| 1.25 | $-0.76^{a}$ | $-0.20^{ns}$ | $-0.82^{a}$ | $-0.96^{a}$ | $-1^{a}$ | $-1^{a}$ |

a) $P<0.001$, b) $P<0.01$, c) $P<0.05$, ns - not significant (t-test)

The 5% and 2.5 % alcoholic extracts (leaves and fruit) resulted in total inhibition of lettuce seeds germination, while 1.25% extracts had lower but also significant effect.

Lower response index values for reflux extracts reflect a more potent inhibitory activity of these when compared to ultrasounds obtained extracts.

The ethanolic extracts of syringa had a more pronounced herbicidal effect on salad seeds germination compared to the aqueous extracts.

Data obtained using germination speed for phytotoxic activity evaluations of extracts (Fig. 1, 2) matches with those obtained from the response index.

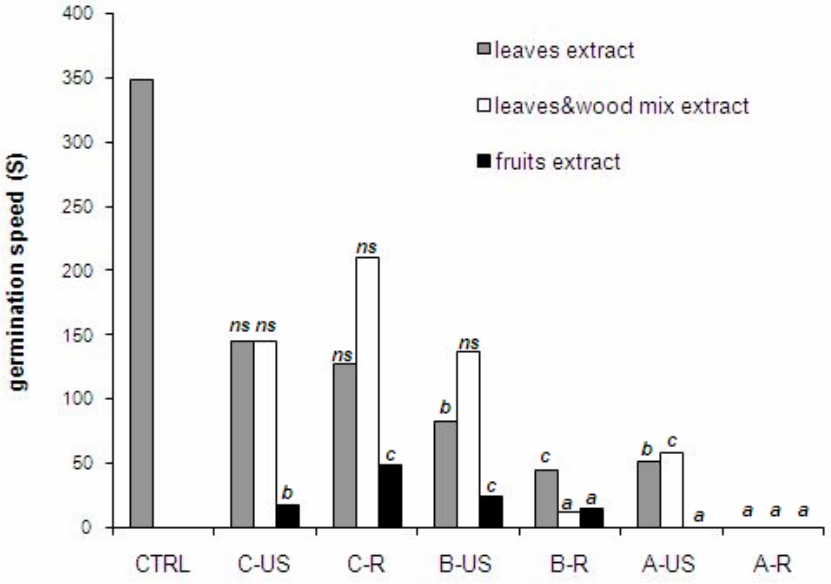


**Figure 1**. Effects of aqueous *M. azedarach* L. extracts on lettuce seed germination. A: 5% concentration, B: 2.5% concentration, C: 1.25% concentration, US: ultrasounds extraction, R: reflux extraction, CTRL: control; a) P<0.001, b) P<0.01, c) P<0.05, ns - not significant (t-test)

In the case of treatment with 5% aqueous extracts obtained by reflux (Fig. 1), no lettuce seed germinated; fruit extracts appeared to be the most efficient inhibitor, followed by leaf extracts.

Speed germination was inhibited by all leaf and fruit ethanolic extracts tested (Fig. 2). By contrast, the mixed leaf and wood extracts had only modest inhibitory activity.

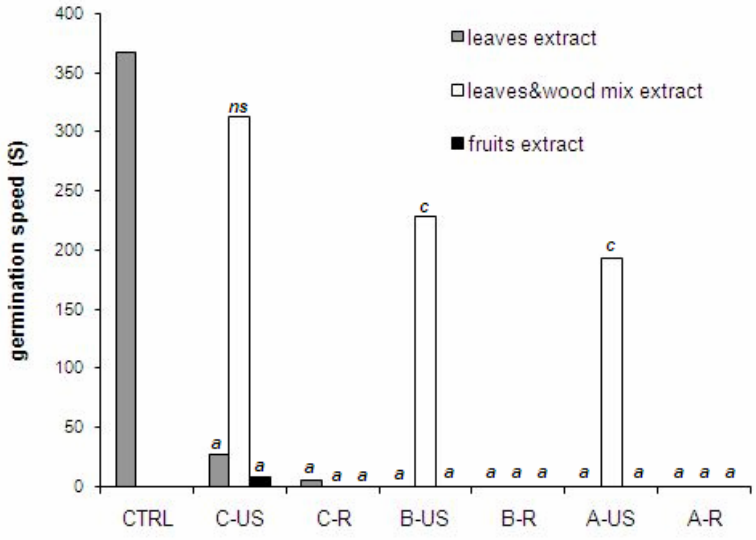


**Figure 2**. Effects of alcoholic *M. azedarach* L. extracts on lettuce seed germination. A: 5% concentration, B: 2.5% concentration, C: 1.25% concentration, US: ultrasound extraction, R: reflux extraction; CTRL: control; a) P<0.001, b) P<0.01, c) P<0.05, ns - not significant (t-test)

## Phytotoxic effect of *M. azedarach* L. extracts on the growth of lettuce seedlings

Significant phytotoxic effect on seedling roots was noted for the leaves and fruits extracts (table 2).

**Table 2**. Inhibition (%) of *Melia azedarach* L. extracts on root and shoot lettuce seedlings

| Extract/ method | Inhibition percent (%) for root seedlings compared to control | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | leaves extract (conc.%) | | | leaves and wood mix (conc.%) | | | fruits (conc.%) | | |
| | 5 | 2.5 | 1.25 | 5 | 2.5 | 1.25 | 5 | 2.5 | 1.25 |
| Aqueous/US 50 | 77.46[a] | 68.70[a] | 57.28[a] | 76.05[a] | 56.80[a] | 21.60[a] | 83.50[a] | 60.00[a] | 55.40[a] |
| Aqueous /reflux | 88.13[a] | 89.10[a] | 70.43[a] | 90.27[a] | 95.71[a] | 93.00[a] | 100.0[a] | 94.81[a] | 94.68[a] |
| Alcoholic/US 50 | 100.0[a] | 99.80[a] | 32.14[a] | 100.0[a] | 99.71[a] | 40.00[a] | 100.0[a] | 49.64[a] | 31.07[a] |
| Alcoholic /reflux | 100.0[a] | 69.35[a] | 80.46[a] | 100.0[a] | 62.45[a] | 56.32[a] | 100.0[a] | 90.09[a] | 54.78[a] |
| | Inhibition (%) for shoot seedlings | | | | | | | | |
| Aqueous/US 50 | 41.56[c] | 0[ns] | 0[c] | 21.25[b] | 0[ns] | 0[ns] | 73.40 [a] | 21.00[b] | 10.00[ns] |
| Aqueous /reflux | 77.75[a] | 64.08 [a] | 0[b] | 71.06[a] | 80.31[a] | 45.80[b] | 100.0 [a] | 89.76[a] | 86.87[a] |
| Alcoholic/US 50 | 100.0 [a] | 99.70 [a] | 42.93[a] | 100.0[a] | 99.73[a] | 59.78[a] | 100.0 [a] | 58.69[a] | 37.50[a] |
| Alcoholic /reflux | 100.0 [a] | 83.21 [a] | 82.50[a] | 48.47[a] | 14.50[ns] | 12.20[ns] | 43.13[a] | 2.29[ns] | 11.07[ns] |

a) P<0.001, b) P<0.01, c) P<0.05, ns - not significant (t-test)

Root growth inhibition was higher than 92.9% for 5% extracts, 78% for 2.5% extracts, and 58.4% for 1.25% extract concentration. All seedlings died when ethanolic 5% extracts (leaves, leaves and wood mix and fruits) were applied. Water extracts also inhibited root growth with up to 50%. Reduction of root length and necrosis of the root was observed in all the other cases.

5% alcoholic extracts seemed to be a very effective inhibitor on shoot growth too; all seedlings treated died (table 2). The 2.5% extracts inhibited shoot growth by more than 85%, when compared to control.

Aqueous extracts obtained by reflux reduced the shoot growth by more than 80% compared to the aqueous extracts obtained using the ultrasounds-assisted extraction method. In the case of aqueous 2.5% and 1.25% leaves and leaves and wood mix extracts, values calculated for inhibition percent were negative. The explanation is that sample shoots were even longer than control, suggesting a stimulation effect. There is literature references indicating that shoot growth is less affected than root growth in allelopathic studies, confirmed by our results (S.R. Devi & al. [22]).

Inhibitory effect on the germination of lettuce seeds as well as on seedling growth varied with extract type as follows: fruit extract > leaves extract > leaves and wood mix extract.

The yields of the dry extract obtained per 5 grams vegetable material (leaves, leaves and wood mix, fruits) with water and alcohol 70% v/v, by reflux and ultrasounds extraction methods are shown in table 3.

**Table 3**. Yields of the extracts obtained

| Vegetable material | Aqueous extracts | | Alcoholic extracts | |
|---|---|---|---|---|
| | (g dry extract/ 5 grams of vegetable material) | | | |
| | ultrasounds extraction | reflux extraction | ultrasounds extraction | reflux extraction |
| leaves | 1.503 | 0.501 | 1.094 | 1.147 |
| leaves+wood mix | 0.884 | 0.969 | 0.824 | 1.768 |
| fruits | 2.871 | 1.534 | 1.594 | 1.628 |

Fruits extracts yielded the highest values (table 3); the yields for ultrasounds aqueous extracts was higher than yield of reflux aqueous extracts; in case of alcoholic extracts yields obtained were lower for ultrasounds obtained extracts compared to reflux obtained alcoholic extracts. Some explanations will be possibly found after a chemical characterization of the extracts.

## Conclusions

The results presented in this paper showed that *M. azedarach* L. contains phytotoxic compounds that inhibit germination and growth of *Lactuca sativa* L., thus showing a good allelopathic potential.

Alcoholic extracts were more effective than the aqueous extracts, so alcohol is a better extraction solvent for phytotoxic compounds from *Melia azedarach*. As extraction method, classic reflux proved to be better than ultrasounds extraction.

We found that 5% concentration of extracts totally inhibited the germination of lettuce seeds and also the growth of seedling root and shoot.

Further studies are needed to determine the phytotoxic effects of *M. azedarach* L. extracts in natural environment too, with the final purpose of application of our results in agriculture.

The results may also serve South African authorities to develop a strategy for using *M. azedarach* L. plants as sources of potential herbicides instead of destroying them.

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## References

1. S. WAHAB – Biotechnological approaches for crop pest and disease management. *J. Biopest*., **2(2)**, p. 115-134, (2009).
2. F. MANCINI, A.J. TERMORSHUIZEN, J.L.S. JIGGINS, A.H.C. van BRUGGEN, Increasing the environmental and social sustainability of cotton farming trough farmer education in Andhra Pradesh. *Indian Agric* Systems, **96**, p. 16-25, (2008).
3. R.J. PETROSKI, D.W. STANLEY, Natural compounds for pest and weed control. *J. Agric.. Food Chem*., **57(18)**:8171-8179, (2009).
4. D. R. de SOUZA, M.L. MARTINS, F.M.S. CARMO, A multiscale model for plant invasion through allelopathic suppression. *Biological Invasions*, **12(6)**, p. 1543-1555, (2010)
5. S. ABU-ROMMAN, M. SHATNAWI, R SHIBLI, Allelopathic effect of spurge Euphorbia hierosolymitana on wheat Triticum durum. *American-Eurasian Journal of Agricultural and Environmental Science*. **7(3)**, p. 298-302, (2010)
6. A.A. BARQUERO, L.E. ALCHE and C.E. COTO, Antiviral activity of meliacine on the replication of a thymidine kinase-deficient mutant of *Herpes simplex virus type* 1 alone and in combination with acyclovir. *Int. J. Antimicrob. Ag.*, **9**(**1**), 49-55 (1997).
7. S.M. VILLAMIL, L.E. ALCHE and C.E. COTO, Inhibition of *herpes simplex virus type*-1 multiplication by meliacine, a peptide of plant origin. *Antivir. Chem. Chemoth.*, **6**(**4**), 239-244 (1995).
8. J.M. WATT and M.G BREYER-BRANDWICK**,** *The medicinal and poisonous plants of southern and eastern Africa,* 2-nd Ed., E. & S Livingstone, London, , p. 745, (1962).
9. R. SALEEM, S.I. AHMED, S M.S. HAMIM, S. FAIZI, B.S. SIDDIQUI, Antibacterial effect of Melia azedarach flowers on rabbits. *Phytother. Res*., **16(8)**, p. 762-764, (2002).
10. Y. AKHTAR, Y.-R. YEOUNG, M.B. ISMAN, Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. *Phyochem. Rev.*, **7**, p. 77-88, (2008).
11. H. ASHRAF, A. JAVAID, Evaluation of antifungal activity of *Meliaceae* family against *Macrophomina phaseolina*. *Mycopath* **5(2)**, p.81-84, (2007).
12. M.C. CARPINELLA, L.M. GIORDA, C.G. FERRAYOLI, C.G. FERRAYOLI, Antifungal effects of different organic extracts from *Melia azedarach L*. on phytopahogenic fungi and their isolated active compounds. *J. Agric Food Chem.*, **51**, p. 2506-2511, (2003).
13. N.G. NTALLI, U. MENKISSOGLU-SPIROUDI, I. GIANNAKOU, Nematicidal activity of powder and extracts of *Melia azedarach* fruits against *Meloidogyne incognita. Ann. App.l Biol*., **156**, p. 309-317,. (2010).

14. J. MORRIS, *From Ravage to Resource: Adding Value in the Control of Invasive Plants*. BookSurge Publishing, USA, (2009).
15. F.A. MACIAS, Isolation and phytotoxicity of terpenes from *Tectona grandis*. *J. Chem Ecol*., **36**, 396-404, (2010).
16. D.R. RICHARDSON, *Allelopathic effects of species in the sand pine scrub of Florida.* PhD dissertation, Univ. of South Florida, Tampa, (1985).
17. J.D. MAGUIRE, Speed of germination aid in selection and evaluation for seedling emergence and vigor. *Crop Sci*., **2**(**2**), 176-177 (1962).
18. F. PELLISSIER, Allelopathic inhibition of spruce germination. *Acta Oecol.*, **14** (**2**), 211-218 (1993).
19. G.B. WILLIAMSON and D. RICHARDSON, Bioassays for allelopathy: Measuring treatment responses with independent controls. *J. Chem. Ecol.*, **14**(**1**), 181-187 (1988).
20. H. KATO-NOGUCHI, Assessment of allelopathic potential of shoot powder of lemon balm. *Sci Hortic*.-Amsterdam, **97**(**3-4**), 419-423 (2003).
21. R. TSAO, F.E. ROMANCHUK, C.J. PETERSON and J.R. COATS, Plant growth regulatory effect and insecticidal activity of the extracts of tree of heaven *(Ailanthus altissima* L.). *BMC Ecol.*, **2**(**1**), 1-6 (2002).
22. S.R. DEVI, F. PELLISSIER and M.N.V PRASAD*, Allelochemicals.* In *Plant Ecophysiology*, M.N.V. Prasad, ed., John Wiley & Sons, Inc., New York, pp. 253-302 (1997).