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TERAPÉUTICA

Impact of natural extracts on target and non target soil organisms

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Aqueous extracts from the chinaberry tree (*Melia azedarach*, Meliaceae), eucalyptus, (*Eucalyptus globulus*, Mirtaceae), castor oil (*Ricinus communis*, Euphorbiaceae) and trichilia (*Trichilia glauca*, Meliaceae) were tested against a soil target species (*Nacobbus aberrans*, Nematoda:Nacobbidae) and two non target soil organisms (*Eisenia foetida* and *Dendrobaena octaedra*, Annelida:Oligochaeta). Nematodes were assayed *in vitro* and *in vivo*, while worms were tested *in vivo*. Significant mortality of *N. aberrans* ($p<0.05$) was observed *in vitro* with the four extracts. The best results were obtained with both Meliaceae. When galls number was registered *in vivo*, it was shown that pepper plants treated with *M. azedarach* extract were the less affected ($p<0.05$). The effect of the other treatments was significantly lower. No degree of damage against the non target species was shown with any of the extracts. These results suggest that application of *M. azedarach* extracts would be a good alternative to manage *N. aberrans* in soils with worms populations.

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INTRODUCTION

Plants synthetize different secondary metabolites, terpenoids, alkaloids, flavonoids, etc. Most of them, the allelochemicals, play different roles in the communication among plants and invertebrates. Some of these compounds, the allomones, act as a chemical defence against herbivores. This natural advantage of plants is useful to look for pesticides, effective and environmentally safe (SCHOONHOVEN *et al.*, 1998).

Horticultural crops are frequently attacked by nematodes, pests which live in the soil, together with other pests and with beneficial worms (Annelida, Oligochaeta). *Nacobbus aberrans* (Nematoda, Nacobbidae), the false root knot nematode, causes galls in the roots of different orchard crops, with great economic losses. It was reported for the first time in Buenos Aires almost 25 years ago and at present it is distributed in different regions of Argentina, favoured by its polyphagous habits (DOUCET, 1999).

Diverse biological, genetic, chemical and cultural alternatives had been used as strategies to reduce its damage (VEREMIS *et al.*, 1997). At present, the importance of horticultural organic production (ASAPROVE, 2004), which avoid synthetic pesticides applications, increased the research on botanical pesticides with potential use for nematode management (SOLER SERRATOSA *et al.*, 1996; LOVANG and WILDT PERSSON, 1998; DIAS *et al.*, 2000). Then, in this paper, we show the impact of four plant extracts at two trophic soil fauna

Figure 1. Extraction of *Nacobbus aberrans* infective larvae from soil.

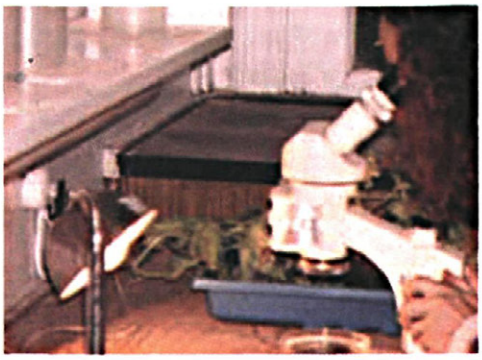

Figure 2. *In vitro N. aberrans* bioassay: evaluation of *N. aberrans* mortality.

levels, the target organism *N. aberrans* and the worms *Eisenia foetida* and *Dendrobaena octaedra* (Annelida, Oligochaeta).

MATERIALS AND METHODS

1. Plant material:

Aerial parts, free of agrochemicals, from the chinaberry tree (*Melia azedarach*, Meliaceae), eucalyptus, (*Eucalyptus globulus*, Mirtaceae), castor oil (*Ricinus communis*, Euphorbiaceae) and trichilia (*Trichilia glauca*, Meliaceae) were tested.

2. Obtention of the extracts:

Ten hundred grams of each powdered plant material were extracted with 100 ml of water to obtain aqueous extracts (10% P/V).

3. Organisms assayed:

3.1.Target organism: *Nacobbus aberrans* (Nematoda: Nacobbidae) second stage larvae, from a colony reared in our laboratory, were extracted from soil (Figure 1). An aqueous suspension with 100 infective larvae per ml was obtained with HUSSEY and BARKER technique (1973).

3.2. Non target organism: Adults of the worms *Eisenia foetida* and *Dendrobaena octaedra* (Annelida, Oligochaeta), reared in our laboratory, were tested.

Figure 3. *In vivo N. aberrans* bioassay: pepper seedlings at the beginning of the test.

Figure 4. *In vivo N. aberrans* bioassay: pepper plants at the end of the test.

4. Bioassays with target organisms:

4.1) *In vitro* bioassay: It was carried out in a growth chamber at 22+2°C, 65+5%RH and photoperiod 12:12 h. A complete randomized design with four repetitions was used. Each repetition consisted of a plastic dish 2.5 cm diameter and 0.8 cm height. One mililiter of each extract 10% (P/V) was added to each dish, depending upon the treatment. Controls received water. One mililiter of the *N.aberrans* suspension (100 larvae/ml) was also added to every dish. Forty eight hours later, mortality was evaluated (Figure 2).

4.2) *In vivo* bioassay: A complete randomized design with four repetitions was used. Each repetition consisted of a pot with 500cc of sterilized soil and a pepper seedling (*Capsicum annuum* CV California Wonder) (Figure 3). Nematode inoculation was achieved watering each pot with 10 ml of the nematode suspension (100 larvae/ml). Two days after the inoculation, plants began to be watered twice a week with 30 ml of each extract, while the controls received only water. The remaining days, enough water was added to each pot just to maintain the adequate humidity. Two months later (Figure 4), the aerial part was cut off and the galls number in the roots was determined. The *in vivo* bioassay was carried out in a growth chamber at 22 + 2 °C, 65 + 5 % RH and photoperiod 12:12 h.

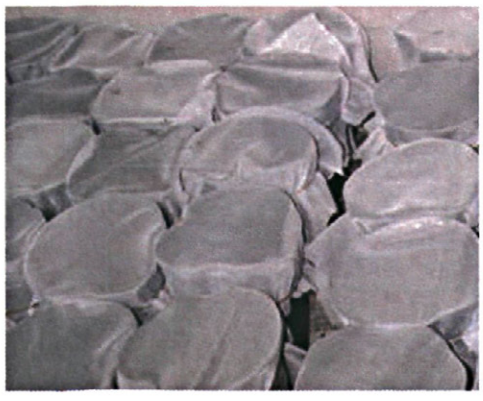

Figure 5. Bioassay with *Eisenia foetida* and *Dendrobaena octaedra*.

5. Bioassays with non target organisms:

Two identical bioassays were carried out, one for *Eisenia foetida* and another for *Dendrobaena octaedra*. A complete randomized design with four repetitions was used in both cases. Each repetition consisted of a 500 cc pot with sterilized soil enriched with compost. Twice a week, 30 ml of each extract was added to each treated replica, while the controls received only water. The pots were covered with gauze to avoid the worms to escape (Figure 5). Sixty days later, worms mortality was determined.

5. Statistical analysis:

ANOVA and LSD Test were used to analyze data ($p<0.05$).

RESULTS AND DISCUSSION

The impact of the products assayed on the target organism *N.aberrans* is shown in Figure 6 and 7 while Figure 8 shows their bioactivity on the non target organisms *E.foetida* and *D.octaedra*.

The mortality (%) *in vitro* 48 h after the application of the plant extracts indicates that the four extracts had nematicide properties (Figure 6). All of them had significative differences with the control ($p<0.05$). However the higher nematicide activity is attributed to both Meliaceae (*M.azedarach* and *T.glauca*). These results agree with those from other authors who investigated the bioactivity of aqueous extracts against other pests. LOVANG and WILDT-PERSSON (1998) showed the effect of aqueous extracts of *M.azedarach* and *Trichilia emetica* against pathogens of tomato, bean and maize while SOUZA *et al.* (2001) showed that Meliaceae plants had insecticidal activity the silverleaf whitefly. RAO *et al.* (1998) managed the nematode *Meloidogyne incognita* with *Ricinus communis* extracts.

When extracts were assayed *in vivo*, significative differences were observed in pepper plants treated with *E. globulus* and *M.azedarach* (Figure 7). It is possible that the penetration of infective larvae into the roots could

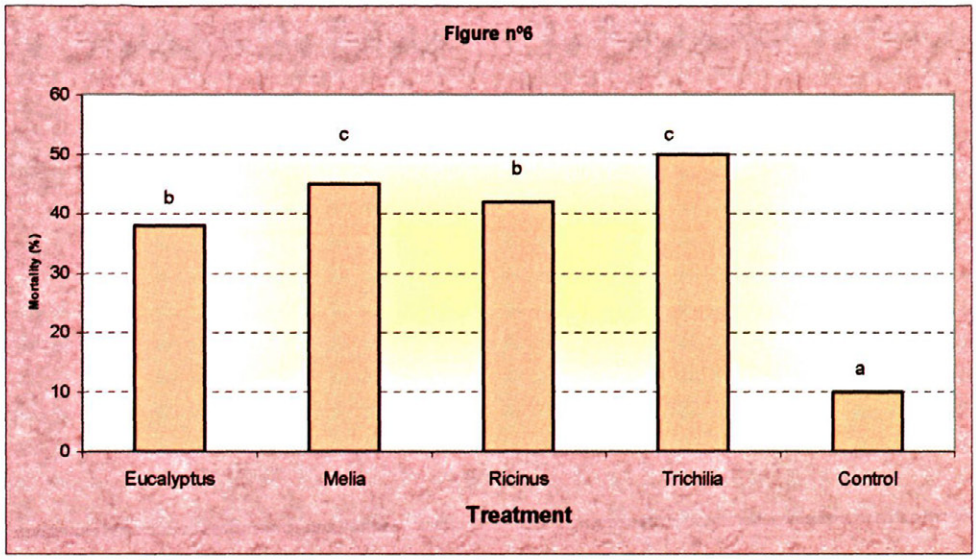

Figure 6. *In vitro N. aberrans* bioassay: Mortality of *N. aberrans* after 48 hs of treatment with plant extracts.

have been affected by the extracts. However, the mode of action of these compounds is not yet completely understood (BREUER *et al.*, 2003). *E. globulus* contains monoterpenoids, compounds which had shown nematicide activity (SOLER SERRATOSA, 1996), while *M.azedarach* contains a limonoid, another terpenoid (CARPINELLA *et al.*, 2003). The absence of bio-

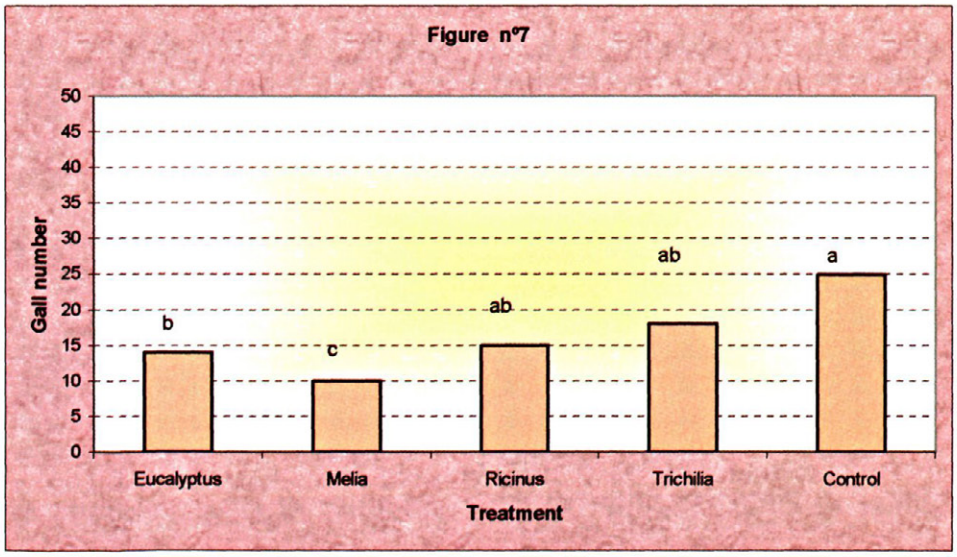

Figure 7. *In vivo N. aberrans* bioassay: Galls number produced by *N. aberrans* after 60 days of treatment with plant extracts.

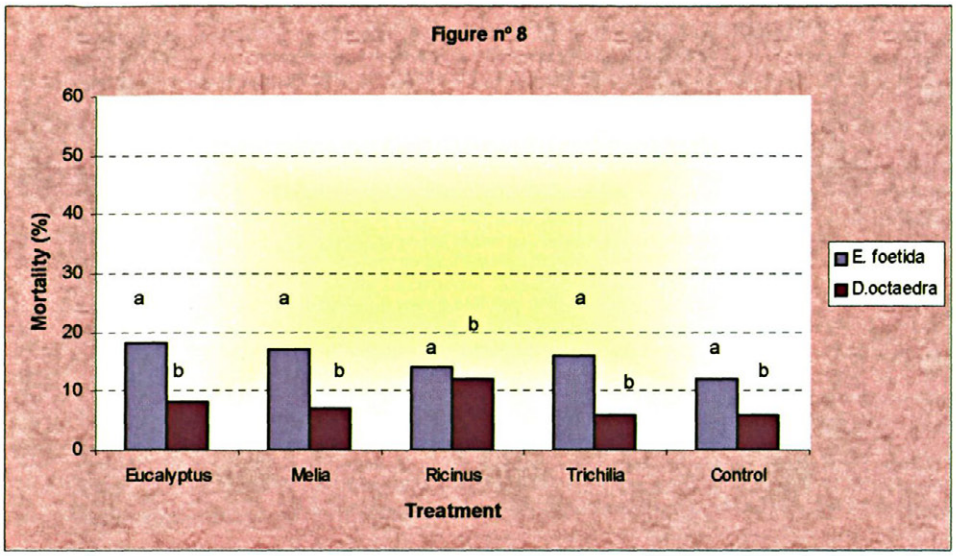

Figure 8. Bioassays with non target organisms: Mortality of *Eisenia foetida* and *Dendrobaena octaedra* after 60 days of treatment with plant extracts.

activity in the case of *T. glauca* , activity which was shown *in vitro*, could be due to a lower bioavailability of the extract when it was applied to the soil.

Figure 8 shows that none of the extracts had negative side effects on the non target organisms *E. foetida* and *D. octaedra*. Similar results had been obtained by ROSSNER and ZEBITZ (1986) who treated the soil with neem, a Meliaceae which contains the terpenoid azadirachtina. It is possible that the earthworms could have detoxified the extracts, avoiding their negative effects.

The fact that no damage against the earthworms was shown, while the nematode was controlled by *M. azedarach* extract, both *in vitro* and *in vivo*, suggest that the application of *M. azedarach* extracts would be a good alternative to manage *N. aberrans* in soils with worms populations.

CONCLUSIONS

None of the extracts tested had negative effects on the non target soil species. Taking into account the excellent performance of *M. azedarach* against the soil target species, *N.aberrans*, this extract could be considered an appropriate alternative for its control in organic productions.

RESUMEN

MAREGGIANI G., N. ZAMUNER, M. MICHETTI, D.FRANZETTI, M. COLLAVINO. 2005. Impacto de extractos naturales sobre organismos objetivos y no objetivos de suelo. *Bol. San. Veg. Plagas*, **31**: 443-448.

Se evaluó la actividad de extractos acuosos de paraíso (*Melia azedarach*, Meliaceae), eucaliptus, (*Eucalyptus globulus*, Mirtaceae), ricino (*Ricinus communis*, Euphorbiaceae) y trichilia (*Trichilia glauca*, Meliaceae) sobre una especie objetivo de suelo (*Nacobbus aberrans*, Nematoda:Nacobbidae) y dos organismos no objetivo de suelo (*Eisenia foetida* y *Dendrobaena octaedra*, Annelida: Oligochaeta). Los bioensayos con nematodos se efectuaron *in vitro* e *in vivo*, mientras que los referidos a lombrices se realizaron *in vivo*. Se observaron diferencias significativas en la mortalidad de *N. aberrans* con los cuatro extractos estudiados *in vitro* ($p<0.05$). Los mejores resultados se obtuvieron con Meliaceae. Al registrar el número de agallas *in vivo*, se vió que las plantas de pimiento tratadas con el extracto de *M. azedarach* fueron las menos afectadas ($p<0.05$). El efecto de los restantes tratamientos fue significativamente menor. No se observaron efectos perjudiciales sobre las especies no objetivo con ninguno de los extractos. Estos resultados sugieren que la aplicación de extractos de *M. azedarach* podría resultar una buena alternativa para el manejo de *N. aberrans* en suelos con poblaciones de lombrices.

Palabras clave: *Melia azedarach*, *Eucalyptus globulus*, *Ricinus communis*, *Trichilia glauca*, *Nacobbus aberrans*, *Eisenia foetida*, *Dendrobaena octaedra*, plaguicidas botánicos.

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