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Evaluation of natural chemical compounds against root-lesion and root-knot nematodes and side-effects on the infectivity of arbuscular mycorrhizal fungi

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Abstract

The survival of two species of plant parasitic nematodes: the root-lesion nematode *Pratylenchus brachyurus*, and the root-knot nematode *Meloidogyne javanica*, was evaluated in saturated atmospheres of 12 natural chemical compounds. The infectivity of two isolates of arbuscular mycorrhizal fungi: *Glomus mosseae* and *Glomus intraradices*, under identical experimental conditions, was also determined. All the compounds tested exerted a highly significant control against *M. javanica* and among them, benzaldehyde, salicilaldehyde, borneol, p-anisaldehyde and cinnamaldehyde caused a mortality rate above 50% over *P. brachyurus*. The infectivity of *G. intraradices* was inhibited by cinnamaldehyde, salicilaldehyde, thymol, carvacrol, p-anisaldehyde, and benzaldehyde, while only cinnamaldehyde and thymol significantly inhibited mycorrhizal colonization by *G. mosseae*.

Introduction

Plant parasitic nematodes attack a wide range of crop species. The chemical control of these pests has traditionally been done through the direct application of nematicides or biocides to agricultural soils. The deleterious effects of soil fumigation treatments compounded by groundwater contamination to the environment have revealed the need for alternative chemical control methods. In the rhizosphere, soil desinfestation can generate additional negative effects on the populations of soil beneficial microbiota, causing stunting and growth inhibition on many crops (MacDonald and Reichmuth, 1996). Thus the importance of evaluating soil treatments on these populations. In the pool of identified beneficial microorganisms, there are arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, actinomycetes and antagonistic saprophytic fungi (Linderman, 1992).

The use of natural low-molecular weight volatile compounds for the control of soil-borne plant pathogens is considered a potential alternative to the use of commercial soil biocides, but information concerning their effects on beneficial soil microbiota is scarce (Bauske et al., 1993; 1994; Canullo et al., 1992). In this work, 12 chemical compounds of different structure and origin, including alcohols (thymol, geraniol, borneol, carvacrol, 2-octanol), aldehydes (benzaldehyde, citral, cinnamaldehyde, p-anisaldehyde, salicilaldehyde), a non-aromatic carboxilic acid (octanoic acid), and a non-aromatic disulfide mercaptan (allyldisulfide) were tested on the survival of the root-lesion nematode *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Steckhoven and of the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, as well as on the infectivity of two arbuscular mycorrhizal fungi: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe and

Glomus intraradices Schenck and Smith, both registered in the European Bank of Glomales as BEG 116 and BEG 72 respectively.

The microorganisms evaluated were separately included in sodium alginate films for the experimental study (Rodríguez-Kábana et al., 1994). Among the compounds tested, 11 are natural products from botanical sources and only 2-octanol, a non-aromatic alcohol, is not of direct natural origin, but is easily obtained from castor oil produced by the castor bean plant (*Ricinus communis* L.) after an alcaline hydrolysis followed by a destillation process. Thymol (5-methyl-2-isopropylphenol) is obtained from the essential oil of thyme (*Thymus vulgaris* L.) as well as carvacrol (5-isopropyl-2-methylphenol). The latter is also found in the oil of origanum (*Origanum vulgare* L.). Borneol (1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol) is found in the oil of rosemary (*Rosmarinus officinalis* L.) and of many other Labiatae, geraniol (trans-3,7-dimethyl-2,6-octadien-1-ol) is produced by rose (*Rosa* spp.), citral (3,7-dimethyl-2,6-octadienal) is found in the oil of lemon grass (*Cymbopogon* spp.) and in balm (*Melissa officinalis* L.). p-Anisaldehyde (4-methoxybenzaldehyde), benzaldehyde (benzoic aldehyde) and cinnamaldehyde (3-phenyl-2-propenal) are found in fennel (*Foeniculum vulgare* L.), in kernels of bitter almond (*Prunus dulcis* L.) and in cinnamon (*Cinnamomum cassia* L.) respectively. Salicilaldehyde (2-hydroxybenzaldehyde) can be obtained from the bark of willow (*Salix alba* L.). The non-aromatic carboxilic acid caprylic acid (n-octanoic acid) is a natural component of coconut oil and of palm nut oil, and the non-aromatic mercaptan allyldisulfide is produced by garlic (*Allium sativum* L.).

Materials and methods

Alginate films were prepared by applying different 2% sodium alginate solutions which contained suspensions of the microorganisms to be evaluated to polyvinylchloride (PVC)-coated fiberglass screens (1.5 mm^2 grid square) measuring 2.5 $\times$ 2 cm, following the method described by Rodríguez-Kábana et al. (1994). One solution contained adults and larvae (50 $\pm$ 14 per film) of the root-lesion nematode *P. brachyurus* cultured monoxenically on carrot (*Daucus carota* L.) disks (Moody et al., 1973). A second solution contained *M. javanica* larvae (63 $\pm$ 15 per film) freshly extracted from a tomato (*Lycopersicon esculentum* L.) galled root system. The other two solutions were prepared by including spores of arbuscular mycorrhizal fungi as described by Calvet et al. (1996). One contained *G. mosseae* and the other one *G. intraradices* resting spores (58 $\pm$ 14 per film and 448 $\pm$ 56 per film, respectively) extracted in a blender from mycorrhizal root fragments of one-year-old leek (*Allium porrum* L.) pot cultures. Solutions of the chemical compounds (Aldrich Chemical Company, Inc.) were prepared using 250 µl per 100 ml distilled water (2500 ppm) for liquids. For the two solid compounds tested, thymol and borneol, 250 mg and 17.6 mg were respectively used per 100 ml distilled water. Films were suspended in sealed 100 ml plastic pots, and submitted to saturated atmospheres of natural chemical compounds applied in a water solution (1 ml concentrated solution of the chemical compound + 4 ml distilled water). Five millilitre distilled water were added to no-compound control pots. There were seven replications for each combination of compound and microorganism, except for *M. javanica*, with only six films per each chemical compound tested. The sealed atmospheres were maintained for four days at ambient temperature, fluctuating between 19 and 22 °C, in the dark. Films were then removed. Those containing specimens of the root-lesion nematode *P. brachyurus* were immediately observed under the microscope and survival of the nematode was assessed. The criteria established for the assessment of *P. brachyurus* mortality under the microscope was to consider dead specimens those that were immobile with their body vacuolated and granuluous with loss of transparency after the first reading, and no recovery of mobility after transfer to sterile water for 24 h (second reading). In order to determine the survival of both arbuscular mycorrhizal fungi and of *M. javanica*, two bioassays were performed. Films containing mycorrhizal spores or larvae of the root-knot nematode were used as individualized inocula in leek plantlets for mycorrhizal films and in tomato plantlets for *M. javanica* films, respectively. Plants were grown in 400 ml plastic containers filled with a pasteurized sandy soil under greenhouse conditions. Leeks were cut after six weeks growth and mycorrhizal root colonization determined using the grid line intersect method (Giovannetti and Mosse, 1980) after clearing and staining the roots following the method described by Phillips and Hayman (1970) modified by Koske and Gemma (1989). Tomato plants were cut after two months growth and the percentage of galled root system was estimated (Barker, 1985).

Results and discussion

All the compounds evaluated decreased the survival of the root-lesion nematode (Figure 1) and significantly reduced root galling by *M. javanica* following a bioassay test with tomato (Figure 2). Five of them: benzaldehyde, salicilaldehyde, borneol, p-anisaldehyde, and cinnamaldehyde caused a mortality rate above 50% on *P. brachyurus*, the latter being the most effective, followed by borneol and p-anisaldehyde. The quantification of root galls in the root systems of tomato plants inoculated with *M. javanica* alginate films showed a different nematicidal effect of the saturated atmospheres on the root-knot nematode. Caprylic acid, thymol, benzaldehyde, salicilaldehyde, p-anisaldehyde, and cinnamaldehyde exerted the most effective control as no galls appeared in the root systems (Figure 2). Allyldisulfide reduced root galling to 20% and citral to 30%, compared with the 100% determined in plants inoculated with no-compound control films. Geraniol and 2-octanol caused a reduction of 50% approximately on root galling and borneol and carvacrol were the least effective against *M. javanica*. It is noteworthy that cinnamaldehyde caused the highest mortality to both nematode species. From the nematode standpoint this compound would be the most interesting, but unfortunately, it was inhibitory for arbuscular mycorrhizal fungi. When comparing the survival of the root-knot and the root-lesion nematode, the latter showed the highest survival rate for most compounds, with the exception of borneol, suggesting that root-knot nematodes are more vulnerable and easier to control with these compounds. However this observation needs further testing. The mode of action of these compounds over nematode inactivity and mortality is unknown. It is likely that several mechanisms are involved. Citral and benzaldehyde had already been used in greenhouse and microplot experiments by Bauske et al. (1994) for the effective control of *M. incognita*.

The effects recorded on the infectivity of arbuscular mycorrhizal fungi inocula varied depending on the fungus (Figures 3 and 4). *Glomus mosseae* spores were more tolerant to the treatments (Figure 4). Only cinnamaldehyde and thymol significantly inhibited mycorrhizal colonization by *G. mosseae,* but among them, cinnamaldehyde completely prevented mycorrhizal colonization. The alginate films treated with thymol still caused a root colonization percentage of 36 in leek plants. The infectivity of *G. intraradices* mycorrhizal films was inhibited by carvacrol, thymol, salicilaldehyde and cinnamaldehyde (Figure 3). Benzaldehyde and p-anisaldehyde caused a significant reduction of the root colonization percentage by *G. intraradices*. The rest of the compounds tested did

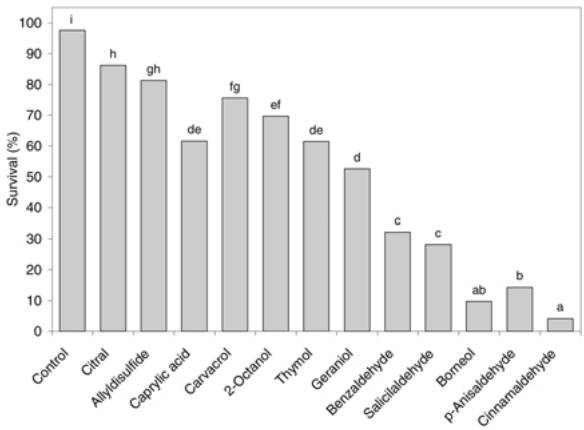

Figure 1. Survival of *P. brachyurus* after four days exposure to saturated atmospheres of 12 natural chemical compounds. The concentration per 100 ml distilled water was 250 µl for all the compounds except for the two solids, 250 mg for thymol and 17.6 mg for borneol. Data are means of seven replications and were transformed to Arcsin$\sqrt{x}$ for ANOVA. Data in the same column followed by the same letter do not differ according to Duncan's multiple range test.

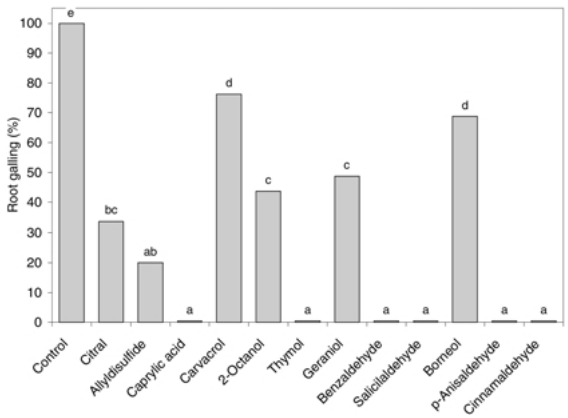

Figure 2. Percentage of root galling of 2-month-old tomato (*Lycopersicon esculentum* L.) plants inoculated with *Meloidogyne javanica* alginate films subjected to four days exposure to saturated atmospheres of 12 chemical compounds. The concentration per 100 ml distilled water was 250 µl for all the compounds except for the two solids, 250 mg for thymol and 17.6 mg for borneol. Data are means of six replications and were transformed to Arcsin$\sqrt{x}$ for ANOVA. Data in the same column followed by the same letter do not differ according to Duncan's multiple range test.

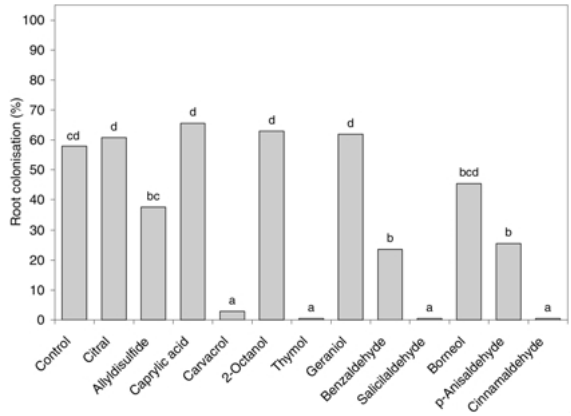

Figure 3. Mycorrhizal root colonization percentage of 2-month-old leek (*Allium porrum* L.) inoculated with *Glomus intraradices* alginate films subjected to four days exposure to saturated atmospheres of 12 chemical compounds. The concentration per 100 ml distilled water was 250 µl for all the compounds except for the two solids, 250 mg for thymol and 17.6 mg for borneol. Data are means of seven replications and were transformed to Arcsin$\sqrt{x}$ for ANOVA. Data in the same column according to the same letter do not differ according to Duncan's multiple range test.

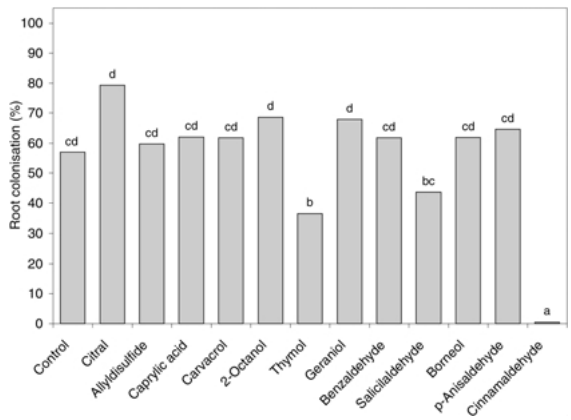

Figure 4. Mycorrhizal root colonization percentage of 2-month-old leek (*Allium porrum* L.) inoculated with *Glomus mosseae* alginate films subjected to four days exposure to saturated atmospheres of 12 chemical compounds. The concentration per 100 ml distilled water was 250 µl for all the compounds except for the two solids, 250 mg for thymol and 17.6 mg for borneol. Data are means of seven replications and were transformed to Arcsin$\sqrt{x}$ for ANOVA. Data in the same column followed by the same letter do not differ according to Duncan's multiple range test.

not affect arbuscular mycorrhizal fungus infectivity. The direct application of benzaldehyde and citral significantly reduced natural mycorrhizal root infection of cotton plants in the greenhouse experiment conducted by Bauske et al. (1994), but the indigenous AM fungi colonizing the root, were not identified to species nor quantified in the soil used. The morphology of AM fungi spores is most probably the cause of their different tolerance to the toxic effects of the compounds, *G. mosseae* chlamydospores are irregularly shaped bigger spores (110–310 µm diameter) with a unique thick wall while *G. intraradices* chlamydospores are smaller globose spores (40.5–190.5 µm diameter) and have one or more (up to four) thin laminated walls (Schenck and Pérez, 1988). Among the natural compounds tested, the best candidates for a practical control application would be those exerting effective control on the target plant parasitic nematode, and not affecting the infectivity of mycorrhizal fungi. This could be the case of caprylic acid, a compound that exerts the maximum control on the root-knot nematode and does not affect mycorrhizal infectivity of both arbuscular mycorrhizal fungi tested, although it proves to be ineffective for the control of the root-lesion nematode. Aldehydes like benzaldehyde and p-anisaldehyde controlled *M. javanica* completely, and also reduced the survival of *P. brachyurus* to low rates. Root colonization by *G. mosseae* was unaltered after these treatments and the internal infection caused by *G. intraradices*, decreased but was not inhibited. These compounds can still be considered in future studies for their nematicidal potential on root-knot nematodes. On the other hand, borneol exerted a good control of *P. brachyurus* and did not affect the infectivity of the arbuscular mycorrhizal fungi, and can thus be considered a good control chemical for the root-lesion nematode, but inefficient against *M. javanica* because the percentage of galled root system was nearly 70 when tomato plants were inoculated with borneol-treated films.

The identification of natural compounds useful in pest control and inoquous to beneficial soil microbiota can support new strategies for the management of soil-borne plant pathogens in agriculture. The experimental bioassays performed in this work must be considered a rapid first step evaluation of potential nematicides against soil microbiota under controlled conditions, and the information obtained from the screening of several substances must be confirmed by long-term application rates and field trials.

Acknowledgements

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