NOTAS CIENTÍFICAS

Nematicidal effect of volatile organic compounds (VOCs) on the plant-parasitic nematode *Meloidogyne javanica*

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ABSTRACT

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Previous studies have demonstrated that volatile organic compounds (VOCs), produced by the yeast *Saccharomyces cerevisiae*, were able to inhibit the development of phytopathogenic fungi. In this context, the nematicidal potential of the synthetic mixture of VOCs, constituted of alcohols and esters, was evaluated for the control of the root-knot nematode *Meloidogyne javanica*, which causes losses to crops of high economic value. The fumigation of substrate containing second-stage juveniles with VOCs exhibited nematicidal effect higher than 30% for the lowest concentration tested (33.3 µL g^{-1} substrate), whereas at 66.6 and 133.3 µL g^{-1} substrate, the nematode mortality was 100%. The present results stimulate other studies on VOCs for nematode management.

Aditional Keywords: phytonematode, nematicide, fumigation, alternative control

RESUMO

Fialho, M.B.; Bessi, R.; Inomoto, M.M.; Pascholati, S.F. Efeito nematicida de compostos orgânicos voláteis (COVs) sobre o nematóide parasita de planta *Meloidogyne javanica.* ***Summa Phytopathologica,*** v.38, n.2, p.152-154, 2012.

Estudos anteriores demonstraram que compostos orgânicos voláteis (COVs), produzidos pela levedura *Saccharomyces cerevisiae*, são capazes de inibir o desenvolvimento de fungos fitopatogênicos. Neste contexto, foi avaliado o potencial nematicida da mistura sintética de COVs, constituída por alcoóis e ésteres, para o controle do nematóide das galhas *Meloidogyne javanica*, que causa perdas em culturas de alto valor econômico. A fumigação do substrato, contendo juvenis de segundo estádio, com COVs apresentou efeito nematicida superior a 30% na menor dosagem testada (33,3 µL g^{-1} de substrato), enquanto que nas dosagens de 66,6 e 133,3 µL g^{-1}, a mortalidade atingiu 100%. Os resultados encorajam maiores estudos envolvendo COVs no manejo de nematóides.

Palavras-chave adicionais: fitonematóide, nematicida, fumigação, controle alternativo

The javanese root-knot nematode *Meloidogyne javanica* is a major plant pathogen widespread in the tropics. As the management of *M. javanica* by traditional methods is laborious and infested areas frequently become not viable for agricultural use, the development of new control methods is desirable.

In previous studies, VOCs produced by the yeast *Saccharomyces cerevisiae*, or their artificial mixtures, inhibited several phytopathogenic fungi (1, 2). The effect of VOCs on plant parasitic nematodes is scarcely studied. Thus, an *in vitro* assay was carried out in order to evaluate the effect of an artificial mixture of VOCs, identified from the yeast, on the survival of *M. javanica* juveniles.

The artificial mixture of VOCs was prepared by using standard chemicals (Sigma-Aldrich), based on the results of the analysis of gaseous atmosphere produced by *S. cerevisiae* (1). The analysis was carried out by Fialho et al. (1) by using gas chromatography with mass spectrometric detection (GC-MS). The concentration of each compound present in the mixture was based on its relative proportion (%), estimated by the area in relation to all other components of the mixture (Table 1). The population of *M. javanica* was maintained in soybean plants in a greenhouse. *M. javanica* egg masses were stained by immersing the roots in phloxine B solution (0.0015%) for 20 min. Then, the egg masses were hand-picked, transferred to hatching chambers and maintained at 30 °C (4). A suspension of second stage juveniles (J2) of the nematode was obtained after 24h and used as inoculum. Commercially available polystyrene plates (60 mL volume) divided in half by a wall were used for the assay. On one side of the plate, a total of 2.3 mL of the suspension containing 1,000 juveniles (J2) was added to 1.5 g of the substrate vermiculite. On the opposite

Table 1. Composition of VOCs produced by *S. cerevisiae*, grown on potato dextrose agar, identified by using GC-MS (1).

Peak	Compound	Retention time (min)	Area (units)	Relative (%)
1	Ethanol	2.10	21,478	85.3
2	Unidentified	2.91	376	1.5
3	Ethyl acetate	3.05	450	1.8
4	3-methyl-1-butanol	5.15	1,736	6.9
5	2-methyl-1-butanol	5.23	611	2.4
6	Phenylethyl alcohol	27.15	181	0.7
7	Ethyl octanoate	28.30	362	1.4

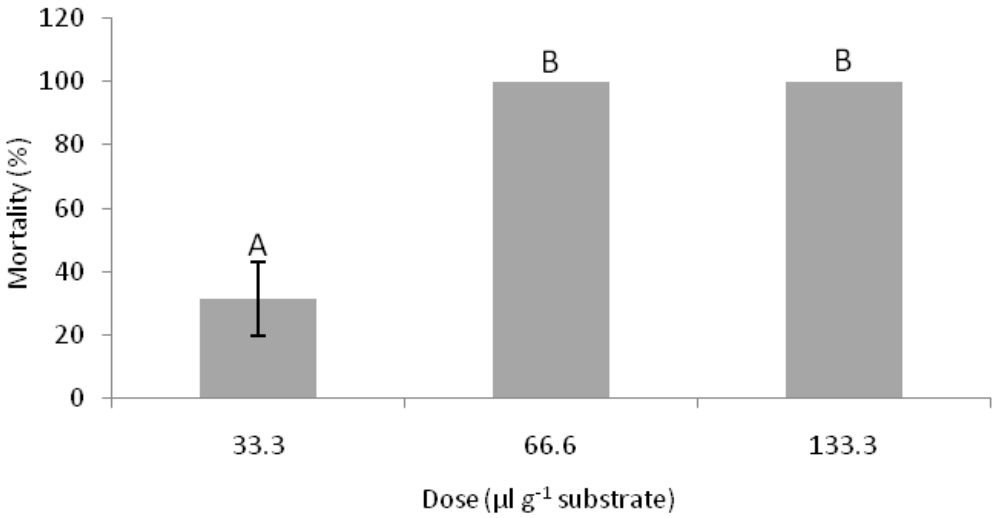

Figure 1. Nematicidal effect of the artificial mixture of VOCs, identified from *S. cerevisiae*, on *Meloidogyne javanica*. The average values of 10 replicates (SD) were calculated as percentage of death compared to control plates in the absence of volatiles. Means followed by the same letter do not differ according to Tukey's test (p £ 0.01)

side, different volumes of the artificial mixture of VOCs (50, 100 and 200 µL) were added to a cotton wool to obtain the concentrations of 33.3, 66.6 and 133.3 µL g^{-1} substrate. Immediately after the addition of compounds, the plates were sealed and maintained at 27 °C in the dark. The control treatment consisted of plates containing the nematodes in the absence of VOCs. A total of 10 replicates were conducted for each treatment. After five days, the fumigated vermiculite was collected and transferred to a modified Baermann system (4), where it was maintained for five days at 30 °C. This procedure recovered only live nematodes after the treatment, which were counted using a stereoscope.

RESULTS AND DISCUSSION

An average of 40 and 27 nematodes were recovered, respectively, from the control and at the concentration of 33.3 µL g^{-1}. In contrast, at 66.6 and 133.3 µL g^{-1} no nematodes were recovered. At the lowest concentration tested, the VOCs treatment resulted in the mortality of nearly one third of the nematodes when compared to control after 5 days of fumigation, while at higher concentrations all nematodes died (Figure 1).Previous work showed that the artificial mixture of VOCs or some of the components used alone were able to reduce the development of phytopathogenic fungi (2). The present assay proved that VOCs of *S. cerevisiae* also cause high mortality of plant parasitic nematodes.

There is limited research on the effect of microbial VOCs on plant-parasitic nematodes. The biofumigation with VOCs produced by the fungus *Muscodor albus* showed nematostatic and nematicidal effect on the nematodes *Meloidogyne chitwood*, *M. hapla*, *Paratrichodorus allius* and *Pratylenchus penetrans* in the roots and rhizosphere of beans, potatoes and pepper (5).

Grimme et al. (3) found that fumigation with volatiles produced by *M. albus* caused 74 and 100% mortality of *M. incognita* in the soil after 72 hours and 7 days of treatment, respectively. In *in vitro* assays, 5 ml mL^{-1} of the artificial mixture of VOCs caused 100% mortality after 24h, while in *in vivo* assays, the concentration of 1.67 ml per cm^3 of sand resulted in fewer galls on tomato roots. The formulation described in those experiments contained compounds such as 3-methyl-1-butanol, 3-methyl-1-butanol acetate and some esters of propanoic acid, whereas in the present work the main compounds were ethanol, 3-methyl-1-butanol and 2-methyl-1-butanol. The last two compounds were responsible for most antimicrobial activity against phytopathogenic fungi (1, 2). However, the specific compounds responsible for the mortality of the plant-parasitic nematodes have not been identified yet.

The present information stimulates further studies to evaluate the potential of these compounds in the control of nematodes and open

perspectives for a new alternative control method, as the soil fumigation with methyl bromide is no longer available due to high toxicity and damage to the ozone layer.

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