SUPPLEMENTARY MATERIAL

Direct nematicidal effects of methyl jasmonate and acibenzolar-S-methyl against *Meloidogyne incognita*

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Abstract: The aim of this study was to examine the nematicidal properties of two defence inducers against the root-knot nematode *Meloidogyne incognita*. A direct-contact bioassay was applied to evaluate the nematicidal effects of acibenzolar-S-methyl (ASM) and methyl jasmonate (MEJA) on second-stage juveniles (J2). Nematodes were incubated in different concentrations of these compounds, and the number of immobile nematodes were counted after 24 h and 48 h post incubation. Tap water was then added to verify whether the nematodes recovered or remained dead at 72 h. The percentage of dead nematodes was used as indicator for the toxicity of the different solutions. Our results show that ASM, in the formulation of Bion® and MEJA both have nematicidal properties.

Keywords: Acibenzolar-S-methyl; Bion®; *Meloidogyne incognita*; Methyl jasmonate; Nematicidal

1. Experimental

The *Meloidogyne incognita* population used in our study was originally isolated from a banana plant in Malaysia and maintained in the greenhouse at KU Leuven on tomato (*Solanum lycopersicum*) cv. Marmande plants (accession 10618 – Aveve) grown in soil in pots. The culture was initiated from a single egg mass and inoculum of *M. incognita* was collected from infected roots by the maceration-sieving method followed by a modified Baermann method (Speijer & De Waele 1997). To obtain a more homogeneous inoculum, second-stage juveniles (J2) collected at the first day were discarded as these contained juveniles that did not hatch freshly. The J2 used for the experiments were collected after 5 days in tap water, rinsed on a 25 µm mesh sieve with fresh tap water, and then suspended in tap water to meet the required number of J2 per µl.

A direct-contact bio-assay was used to evaluate the nematicidal effects of acibenzolar-S-methyl (ASM) and methyl jasmonate (MEJA). A solution of MEJA (Sigma-Aldrich) was prepared with demineralised water to obtain a final concentration of 2 mM. Bion® (Syngenta, active compound 50% ASM). This was dissolved in demineralised water to obtain a final concentration of 2.5 mM of ASM. Since all solutions were prepared in demineralised water, the effect of demineralised water on the mortality rate of the nematodes was also tested.

Acetic acid was also included in the bio-assay and served as a positive control, as acetic acid is known to kill nematodes (Wuyts et al. 2006). As negative control, both demineralised and tap water were used.

In each well of a 96-well plate, 50 µl of the different solutions were pipetted and diluted with tap water in such a way that a single row contained a solution of one compound, and every well was sequentially a 1:1 dilution of the previous well. Then, an average of about 40 J2 in 5 µl of tap water was added to each well. At 24 h and 48 h after incubation, the number of immobile (typically rod-shaped) nematodes was counted (Mukhtar et al. 2013). Immediately after counting at 48 h, 200 µl of tap water was added to each well and 24 h later verified if the nematodes had recovered or remained dead. At all times, the 96-well plate was kept at 26°C in the dark. The percentage of dead nematodes was calculated and used as an indicator of the nematicidal effect of the compounds.

The concentration in which 50% of the nematodes were killed was calculated (LC50; Wuyts et al. 2006). For each compound at least three independent experiments with three replicates were carried out.

References

Mukhtar T, Kayani MZ, Hussain MA. 2013. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Ind. Crops Prod. 42: 447-453.

Speijer PR, De Waele D. 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines. INIBAP Montpellier.

Wuyts N, Swennen R, De Waele D. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. Nematology 8: 89–101.