Effect of Decon7 on viability of root-rot nematodes (*Meloidogyne* spp.) under *in vitro* test conditions.

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Nematodes, the most abundant multicellular animals on the planet, are unsegmented microscopic roundworms that inhabit soil, freshwater, and marine environments. Many feed on micro-organisms, while some are important parasites of human, animals or plants. Root-rot nematodes in specific of the genus *Meloidogyne* spp. have a worldwide distribution and can infect hundreds of plant species, including vegetables, fruits, and grasses, thus causing billions of dollars in damage every year to agricultural crops. Although nematodes are found in many soil types, damage from infections is frequently higher in warm and sandy soils. All also stages of plant growth are susceptible to attacks, but above ground symptoms may not be visible until plants are well developed. Such symptoms consist of stunting, yellowing, and wilting of plants, which may eventually die particularly in hot and dry weather. Belowground symptoms consist of characteristic knot or gall-like swellings, which are formed by nematodes penetrating roots with their stylet and inducing the infected and their neighboring plant cells to grow larger. The resulting 'giant cells' act as nutrient sinks from which nematodes can subsequently feed, but also block the movement of water and nutrients to the above parts, thus causing the plants to wilt and wither.

To date more than 100 species of *Meloidogyne* have been described, but the most frequently encountered species in agricultural crops are *M. incognita, M. javanica, M. arenaria,* and *M. hapla.* Although their infection biology may slightly differ depending on the species, root-rot nematodes typically survive in the soil as dormant eggs, which under favorable conditions hatch to second-stage juveniles (J2s) that move towards root tips and invade them in the zone of elongation. Once inside the roots, the juveniles puncture plants cells with the spear-like stylets and establish a permanent feeding site that eventually leads to the formation of the giant cells. J2s are sedentary (non-infectious) and have to progress to the adult stage in order to induce the formation of giant cells and become truly parasitic to the plants. During adulthood, they also undergo sexual dimorphism, giving rise to large round females that remain in the giant cells and lay eggs into an egg sac on the root surface, and vermiform (worm-shaped) motile males that leave the plant roots in search of new hosts. Notably, females are sedentary whereas the vermiform is the only infectious stage of root-rot nematodes.

Since adults are mostly buried within the root tissue, they are less susceptible to nematocides, and thus most of the control management practises target their eggs and the free-living juveniles. Therefore, in order to assess the nematicidal properties of Decon 7 (D7), we exposed eggs and nematode J2 hatchlings (250 individuals per ml) to 0.5%, 1% and 5% of D7 for 24 hour at 25°C

in dark, and under constant shaking at 150 rpm. The nematodes used in our assays were collected from infected tomato roots and represented a mixture of different *Meloidogyne* species. Under the assay conditions, all concentrations of D7 tested caused over 99% mortality in J2 hatchlings and over 99% inhibition of egg hatching (Figure 1). Moreover, treatments with 5% D7 further resulted in lysis of the hatchlings, indicating the strong nematicidal effects of D7 even at relatively low concentrations. In contrast, egg hatching and viability of the hatchlings in control treatments in which no D7 was added into the solution was nearly 100%. Dead J2s are easy to distinguish from alive ones, as the former appear like immobile straight rods floating in the solution (Figure 1). To further confirm that D7 has truly nematicidal and not just nematostatic effects, one day after exposing the eggs and J2 hatchlings to the various concentrations of D7, about 30 eggs and hatchlings were washed multiple times with sterile Milli-Q water and incubated for 12 hour at 25°C in the dark under constamnt shaking at 150 rpm. None of those eggs hatched and none of hatchlings were recovered, indicating that D7 causes mortality in nematodes. The present *in vitro* assays stimulate further studies to evaluate the potential of D7 in the control of nematodes in the fields, as an alternative to soil fumigation with methyl-bromide or other commercially available and higher priced nematicides.

Figure 1. Nematicidal effects of different concentrations of Decon 7 (D7) on eggs and second-stage juveniles (J2s) of root-rot nematodes (*Meloidogyne* spp.). Dead J2s appear as immobile straight rods floating in the solution, whereas alive ones as curved vermiforms due to their constant movement. Exposure of the nematodes for 24h to D7 results in over 99% mortality even at low concentrations of 0.5%, whereas at 5% J2s are completely lysed and thus are absent from the pictures. Juveniles inside eggs are also lysed at 5% D7, and thus many eggs appear to be emptied in their content.