ARMOREX IN-VITRO ASSAY AGAINST NEMATODES

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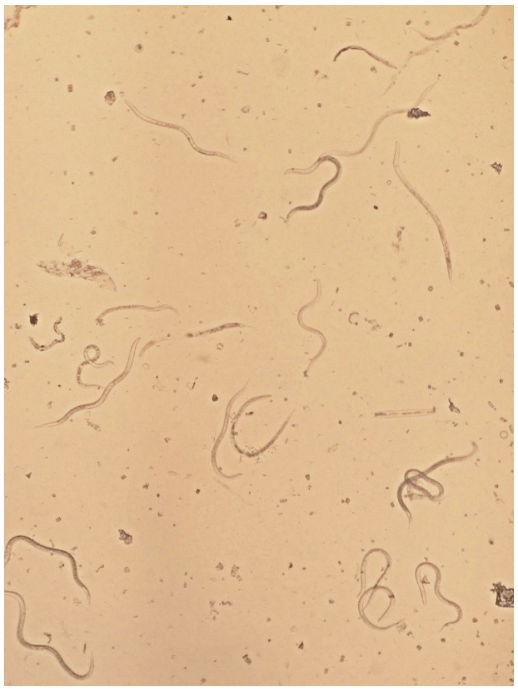
Armorex was evaluated for its biopesticidal potential against *Steinernema Feltiae* (a species of nematode commonly found in soil).

In-vitro growth chamber experiments were used to study the effect of Armorex on the mortality of third-stage juveniles (J3) of *S. feltiae*. The third-stage juveniles are the only stage of this species which exist freely in soil. They are considered to be more resistant to environmental conditions than the other three stages, meaning that a dose that can kill J3s would likely be lethal to the other stages. The mobility of the J3 stage of *S. feltiae* makes them good candidates for study. In the laboratory this tendency to move is useful because changes in motion or complete cessation of movement indicate impairment or death.

Method

Polystyrene 24-well microliter plates were used for the study. 1,000 µL of distilled water was placed into each well using a micropipette. 12 wells were used for the trials and 6 for the controls. One row of 6 wells was left empty between the test and control sections to reduce any potential spillage or cross-contamination. The nematodes were purchased in nutrient media that was shaped into small beads. Each bead contained approximately 7,000 nematodes. One bead was placed into each well of the microliter plate and the bead was broken up to allow the nematodes to move freely.



The nematodes were allowed to sit this way for 30 minutes, and then were checked to ensure that they were moving about and appeared healthy. A photo of untreated nematodes is shown here.

Note the curvature of the nematode bodies in this picture, which was taken while they were in motion prior to treatment.

To begin the test, 800 µL of Armorex was placed into a beaker containing 1000 mL of distilled water to give a dilution rate of 800 parts per million (800 ppm). Since Armorex already has surfactant in it, no additional surfactant was needed to dissolve it in water.

500 µL of this mixture was placed into each of the 12 test wells containing the nematodes. The 6 control wells were left alone. The microliter plates were then left on a level surface at 22º C for 6 hours.

After 6 hours had passed, each well was checked. 200 µl of the contents were placed onto a slide and then observed using a stereomicroscope to count how many nematodes had ceased movement. 200 µl was taken three times from the same well, and the results were considered to be representative of the results for the entire well (which had 1,500 µl total). Nematodes that had become rigid were considered to be dead, while those that were still moving, however slowly, were counted as alive. The controls were evaluated first, followed by the test wells. The percentage of dead nematodes was averaged to give an LD or lethal dose percentage. For example, if half of the nematodes were found to be dead, then the well was counted as having an LD 50. The LD results from all 12 wells were averaged.

Results

After 6 hours had passed, the 6 control wells had LD 0 – all nematodes observed were still motile and appeared healthy. After 6 hours with the 800 ppm Armorex added, the 12 test wells had an average LD of 97.5. That is, only 2.5% of the nematodes were still moving. The lowest LD among the 12 trials was 90, and the highest 100. Photos illustrating the nematodes after they had ceased movement is shown here.

This photo shows nematodes that have been treated with Armorex and have ceased moving completely. In most cases their bodies have straightened into large arcs.

Conclusions

Armorex has good potential as a biopesticide against third-stage juveniles of *S. feltiae*. At an application rate of 800 ppm, almost all nematodes were killed within 6 hours' time.