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MORTALITY OF THE SOYBEAN CYST NEMATODE IN AQUEOUS EXTRACTS OF NEEM PLANT PARTS

by

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Summary. The effect of an aqueous extract (10 g/200 ml of distilled water) of vegetative and propagative parts of neem (*Azadiractina indica*) was tested on the mortality of juveniles of *Heterodera glycines*. Mortality induced by aqueous extract of the branches, leaves and seeds was 99, 97 and 99.9% respectively, not statistically different among them. In a second test, the number of females on the root system was determined thirty days after the incorporation of 15 g of whole leaves/kg of soil or 10 g of each of ground branches, whole seeds and ground seeds/kg soil. The number of females recovered after incorporation of whole leaves, ground branches, whole seeds and ground seeds was 1, 32, 9.1 and 0.8/root system respectively, the differences being statistically different (5%). The number of females in the control roots was 61, indicating the presence of toxic compounds in neem.

Heterodera glycines occurs extensively in Brazil and causes the economically important soybean disease known as "yellow dwarf" which results in severe economic losses (Lima *et al.*, 1992; Monteiro and Morais 1993). Because it is a cyst nematode it has a high survival capacity and is disseminated readily, thus making it difficult to control. Crop rotation and the use of resistant cultivars are the main methods of control, although the latter method cannot be used for extended periods due to the high genetic variability of the nematode (Dias *et al.*, 2000). Conventional chemical control is expensive and may be detrimental to the environment. The incorporation of organic plant material into the soil could be an alternative and acceptable method of control. The neem tree (*Azadiractina indica*) is a source of natural pesticides (Singh *et al.*, 1996; Johnson *et al.*, 1996) and has been reported to reduce populations of several species of nematodes attacking soybean (Vyas 1993). No studies have been reported on the effects of neem extracts on *H. glycines* reproduction. Hence, the studies reported were undertaken.

Materials and methods

Seeds of neem (*Azadiractina indica*, A. Juss) were obtained from five year old trees in Mirassol, São Paulo and branches and leaves from trees in Viçosa, Minas Gerais. Ten gram quantities of branches, leaves and seeds were ground by hand in a mortar and pestle, agitated for several hours in 200 ml of distilled water, filtered; then the filtrate was used immediately.

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The population of *H. glycines* Ichinone was obtained from Ponte Nova, Minas Gerais, Brazil and maintained on soybean, *Glycine max* (L) Merr. cv. FT-Cristalina, in pots in a glasshouse. For the experiment, soybean roots were carefully removed from the pots, immersed in water to remove excess soil and then placed on two sieves in series (the first 0.85 mm and the second 0.15 mm). The sieves were washed with a strong stream of running water to remove the female cysts from the root system. The females retained on the second sieve were homogenised in a tissue grinder and the eggs collected on a sieve (0.025 mm) and centrifuged in a sucrose solution (454 g/l) at 2200 rpm for 1 min. The egg suspension was sieved (0.025 mm) and the aqueous suspension placed in a hatch chamber (Lima and Ferraz, 1985). After hatching, the suspension was adjusted, using a stereoscopic microscope, to contain 600 juveniles/ml.

The inverted tube method of Kimura *et al.* (1981) was used to study the effect of neem on the viability of eggs. One ml of each neem suspension was transferred to a tube (5 x 1.5 cm) containing 1.5 ml of Tween-20 (0.1%) (Sigma) and 0.5 ml of nematode suspension. The tubes were incubated in the dark for 48 h at 26 °C, sealed with tissue paper, inverted over a Petri dish, containing 5 ml solution of streptomycin solution (1 mg/ml) to prevent infection from other microorganisms, and reincubated for 48 h in the dark at 26 °C. The number of nematodes in each Petri dish was counted using a stereoscopic microscope. All the nematodes which were found in the Petri dish were considered to be alive since they were sufficiently mobile to pass through the tissue paper. Control for each assay was distilled water. The percent mortality in the neem extracts was calculated.

Seeds and vegetative parts of neem were hand triturated and incorporated in a 1:1 mixture of sterile soil and sand (10 g/kg). The nematode suspension was prepared as described above except that 1000 eggs were used. The soil mixture was placed in 2 l plastic pots. One recently germinated soybean seedling cv. FT-Cristalina was placed in each pot and after four days it was inoculated with 4,000 eggs of *H. glycines*. Thirty days after inoculation the roots were removed to determine the number of females per root system. The control was soil mixture without neem.

The experiment was a randomized block design with six replicates; data were statistically analyzed by the Duncan test (5%).

Results and discussion

High mortality of juveniles occurred with all parts of neem (Table I). Also, the number of *H. glycines* females recovered 30 days after inoculation into the soil was significantly reduced. Significant difference ($p<0.05$) was observed in the mortality induced by the aqueous extract of each of ground branches, leaves and seeds (Table I). The highest nematicidal activity was, as expected, from the ground seeds as they contain the highest amounts of active compounds, compared to leaves and branches (Rodrigues and Jham, unpublished). Ground seeds produced a higher re-

TABLE I - *Mortality* of Heterodera glycines *induced by aqueous extracts of neem organs along with the number of females found in roots thirty days after inoculation*

Neem organs	% mortality of the nematode	Number of nematode females in the root system
Control (distilled water)	0.0 a	61.0 a
Ground branches	98.8 b	0.8 b
Ground seeds	99.3 b	9.1 bc
Whole seeds	–	32.0 b
Ground leaves	97.1 b	–
Whole leaves	–	1.0 c

Averages followed by the same letter in a row indicates no significant difference by Duncan's Multiple Range Test at P = 5%.

sponse as compared to the whole seeds due to higher contact with the active components. These results (at same dosage) are in agreement with those reported in the literature. Rossner and Zebitz (1986) utilizing ground seeds and leaves (1% v/v, neem leaves: soil) obtained a significant reduction of *Meloidogyne arenaria* and *Pratylenchus penetrans* in tomato. Vyas (1993) reported a significant reduction of soybean nematodes *Helicotylenchus* sp., *Pratylenchus* sp, *Xiphinema* sp. and *Aphelenchus* sp. at doses of 2 g of neem/kg of soil.

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