

Nematicidal Activity of the Fungicide Ethazole

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Abstract: The nematicidal activity of the fungicide ethazole was studied under greenhouse conditions in which a nematode-infested, Norfolk sandy loam was planted to cotton (var. = 'Rowden'). At planting time, the fungicide was incorporated in the soil at rates of 0-100 mg/kg soil. When assayed 4 weeks after planting, plant-parasitic nematodes in soil and roots occurred in decreasing numbers with increasing concentrations of the fungicide; the degree of control obtained was from 60-100% with the four highest concentrations. Results indicate that ethazole possesses nematicidal activity at rates recommended for field application. *Key Words:* nontarget effects, terrazole, chemical control.

The effects of fungicides on nematodes have received little attention, despite occasional reports that some of these compounds possess stimulatory or repressive properties towards nematodes. Chlorinated nitrobenzene fungicides, depending on dosage and compound, have been shown either to stimulate (1, 7), or kill plant-parasitic and other nematodes. Carbamate-type fungicides have been found to stimulate the hatching of cyst-nematode eggs and to reduce the number of larvae of these nematodes in soil (2, 6). Systemic benzimidazole fungicides have antihelminthic properties (3, 4, 5).

The fungicide ethazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) is marketed in the United States under the name Terrazole® for the control of soilborne

fungal pathogens which cause seedling diseases in cotton, soybean, peanut, and other row crops. In the past, we have observed beneficial responses following applications of this fungicide to soybean fields with no serious seedling-disease problems. Since these responses could be due to effects of the fungicide on plant-parasitic nematodes, we investigated the effects of ethazole on these parasites under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Laboratory study: The direct toxicity of ethazole was determined *in vitro* by using a culture of the microbivorous nematode *Pelodera chitwoodi* (Bassen) Dougherty. An emulsion of the fungicide was prepared by dissolving 1 gm of technical ethazole (Olin Corporation) in 1 ml of Tween 20 which was then increased to 1 liter with water. A series of dilutions of the stock emulsion

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were prepared to provide concentrations of 10, 20, 40, 50, 75, and 100 $\mu\text{g}/\text{ml}$.

Five, vigorous, adult nematodes were placed in each of a series of 2-cm diam Syracuse dishes. Each dish contained 1 ml of one of the fungicide concentrations. Each dilution was represented by a total of three, 3-dish replications. The Syracuse dishes were maintained at 27 C on 10 ml of water within a covered standard petri plate. A treatment with water alone and one with 0.1 ml Tween 20/liter were included to serve as controls. Nematodes were observed 12 and 24 h after initiation of the test, and the percentage of dead nematodes was recorded.

Greenhouse experiments: A stock emulsion of ethazole for evaluation in soil was prepared by dissolving 10 gm of technical grade fungicide in 5 ml of Tween 20 and adding water to make 1 liter. This emulsion contained 10 mg/ml ethazole. A series of aqueous dilutions were prepared from the stock emulsion so that when 10 ml were added to 500 gm of soil, the resultant concentrations of ethazole were: 0, 5, 10, 20, 40, 60, 80, and 100 mg/kg soil. A solution containing 5 ml/liter of Tween 20 was also prepared and tested at the rate of 10 ml/kg of soil to determine the effect of the emulsifier on nematodes.

Soil for the study was a Norfolk sandy loam from a field under cotton monoculture. The soil was naturally infested with *Hoplolaimus galeatus* (Cobb) Sher, *Tylenchorhynchus claytoni* (Steiner), *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven, and *Meloidogyne incognita* (Kofoid and White) Chitwood. The soil also contained a variety of tylenchoid (*Ditylenchus* + *Tylenchus*), dorylaimoid, and saprophagous nematodes. The soil was sieved (2-mm sieve), moistened to 0.5 bar, and placed in 500-gm portions in plastic bags. Each bag received 10 ml of the appropriate ethazole emulsion. The bags were shaken vigorously and the soil transferred to 11-cm diam plastic pots. Two cotton (*Gossypium hirsutum* L.) seeds of cultivar 'Rowden' were planted in each pot and allowed to grow under greenhouse conditions. There were 8 replicates/treatment. Pots were arranged in a randomized complete block design.

After 4 weeks of plant growth, 50 cm^3

of soil were collected from each pot for determination of nematode populations by the molasses-flotation-sieving technique (8). Cotton roots were carefully washed and weighed, the number of *M. incognita* galls counted, and the galling index (9) value estimated. The general appearance and condition of the roots were also evaluated by using a scale of 0-5 on which 0 represented poor development with considerable necrosis, and 5 represented excellent growth with no necrosis. The heights of plants and the fresh weights of shoot systems were also determined. The numbers of nematodes in the roots were determined by incubating 10 gm of fresh roots on a 2-mm screen barely covered with water in a beaker; after 72 h, the water was passed through a 38- μm (400-mesh) sieve and the nematodes collected were washed into a counting dish.

All data were analyzed according to standard procedures for analysis of variance. Least significant differences were calculated and included in the graphs for ease of interpretation.

RESULTS AND DISCUSSION

Tween 20 did not injure *Pelodera chitwoodi* in the *in vitro* test. The percent of *P. chitwoodi* found dead after 12 h (Fig.

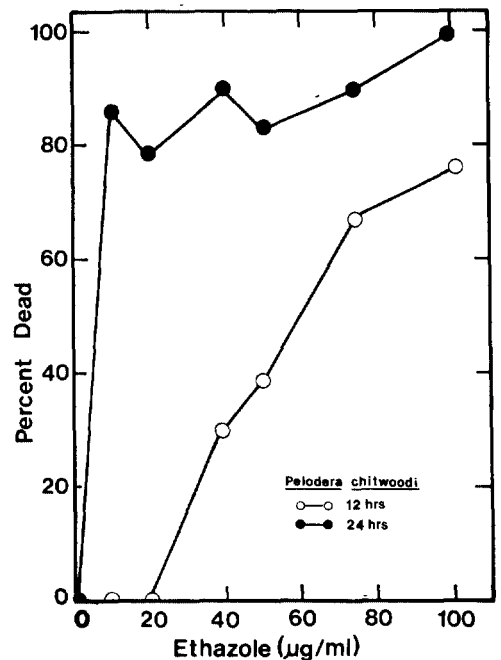


FIG. 1. Effect of ethazole on *Pelodera chitwoodi* in *in vitro* test.

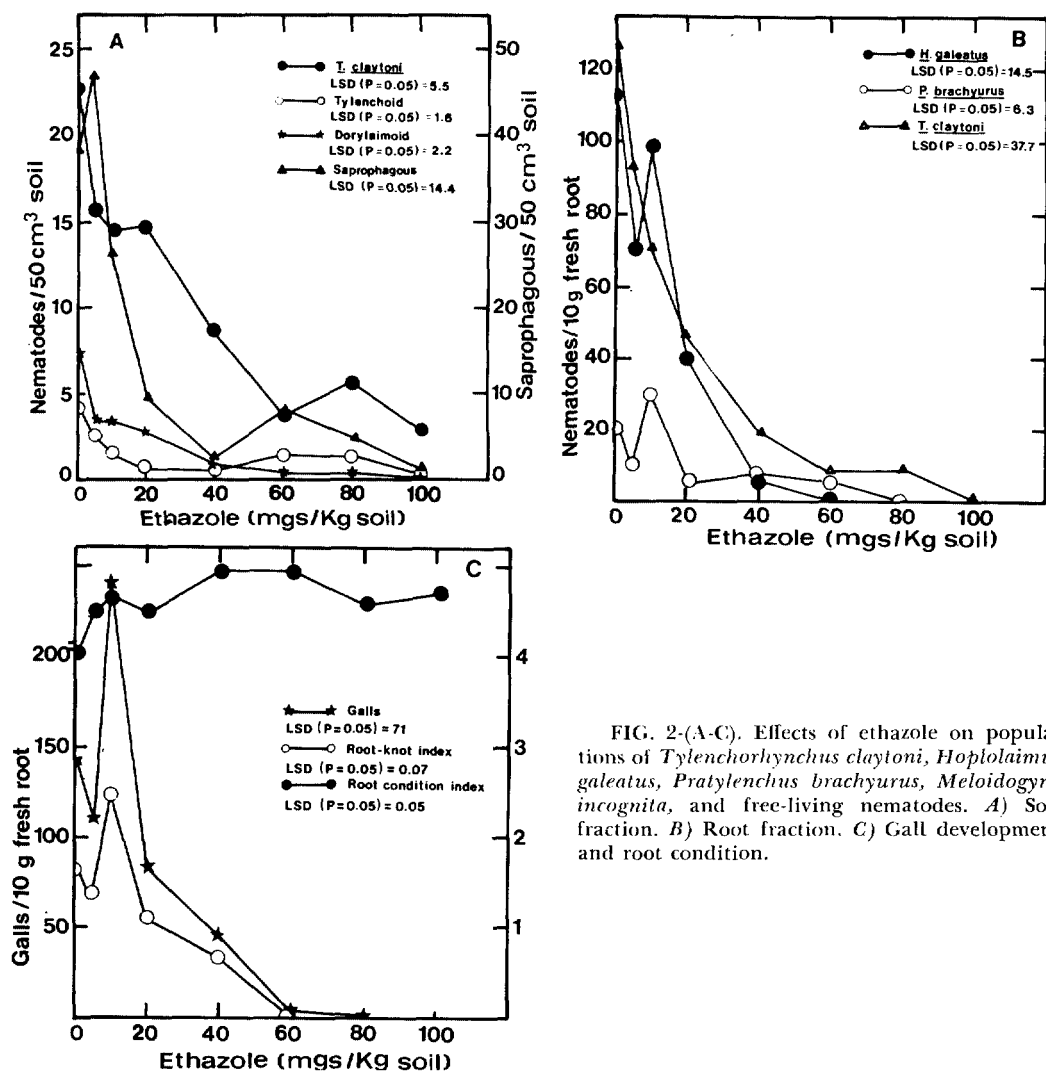


FIG. 2-(A-C). Effects of ethazole on populations of *Tylenchorhynchus claytoni*, *Hoplolaimus galeatus*, *Pratylenchus brachyurus*, *Meloidogyne incognita*, and free-living nematodes. A) Soil fraction. B) Root fraction. C) Gall development and root condition.

1) increased almost linearly in response to ethazole concentrations above 20 $\mu\text{g/ml}$; all nematodes in dishes with less than 20 $\mu\text{g/ml}$ of the fungicide were alive. The mean percent of nematodes killed after 24 h varied from 79-100%, in dishes containing ethazole, with no constant pattern between treatments.

Tween 20 applied alone to the soil had no effect on the numbers of nematodes in the roots or in soil. The only nematodes found in significant numbers in the soil were *T. claytoni*, tylenchoids (*Tylenchus* + *Ditylenchus*), and free-living forms. Numbers of nematodes in soil declined (Fig. 2-A) sharply and proportionately in response to ethazole concentrations. However, the

sharpest reductions in numbers occurred in the range of concentrations of 0-40 mg/kg; differences between means for the three highest concentrations of the fungicide were not significant.

Significant numbers in the roots were only found for *H. galeatus*, *P. brachyurus*, and *T. claytoni* (Fig. 2-B). The highest numbers were observed for *H. galeatus* and *T. claytoni*. The response to ethazole concentration followed the pattern found for soil populations. Few or no nematodes of any species were collected from roots grown in soil with more than 40 mg/kg ethazole.

The number of galls/10 gm fresh root (Fig. 2-C) increased in response to 10 mg/kg ethazole only to decline again at higher

concentrations so that there were no galls on roots from the pots with the three highest rates of the fungicide. Values for the root-knot index for *M. incognita* paralleled the pattern described for gall number .

The general condition of the roots (Fig. 2-C) was better in all treatments with ethazole, and no discernible pattern of differences was observed with different fungicide rates. Differences in root and total plant weights between treatments were not significant; however, suppression of elongation (plant height) was observed at the three highest concentrations of the fungicide.

Our results show that the fungicide ethazole possesses nematocidal activity. The fungicide is most commonly used as an in-furrow application at rates of 0.6-2.25 kg/ha and is usually delivered in mixtures with pentachloronitrobenzene (PCNB), another fungicide. Since the volume of soil treated with in-furrow applications is equivalent to a cylinder 3-4 cm in radius and the length of a single-row hectare, the rates for ethazole are in the range of 20-70 kg/ha, on a broadcast basis. The rates used in these experiments represent those for the volume of soil in an in-furrow application that would be in intimate contact with the fungicide. The rates are, consequently, those of equivalent broadcast applications. Our results strongly suggest that good nematocidal activity is probably attained in the seed furrow when the fungicide is applied at recommended rates. The observed nematode control may explain the beneficial effects of the fungicide noted in fields with no significant damping-off problem (un-

published observations by the authors). Our results also indicate that the nematocidal activity of ethazole could be used to reduce rates of standard nematocides when applied in-furrow, or to complement insecticides with moderate nematocidal activity to attain good nematode control during the early phases of seedling development in crops such as cotton.

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