ABSTRACT

The efficiency of 2 methods for extraction of *Radopholus similis* from banana roots was compared with 10 g root samples. A greater number of nematodes was recovered using a method of maceration and sieving (Taylor and Loegering modified by Quimí and Villacís) than by a technique of maceration and incubation (Gowen and Edmunds). Also, this first method required less time for processing.

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NEMATICIDAL ACTIVITY OF THE FUNGICIDE 2,3,4,5-TETRACHLORONITROBENZENE [ACTIVIDAD NEMATICIDA DEL FUNGICIDA 2,3,4,5-TETRACLORONITROBENCENO]

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ABSTRACT

The fungicide 2,3,4,5-tetrachloronitrobenzene (TCNB) applied to Norfolk sandy loam at rates ranging from 0-0.1 g a.i./kg soil resulted in sharp declines in numbers of stunt *(Tylenchorhynchus claytoni)*, lance *(Hoplolaimus galeatus)*, root-knot *(Meloidogyne incognita)*, dorylaimoid and saprophagous nematodes in soil planted to M-8 cotton; higher rates produced little additional effect. A similar pattern of reduction was observed for numbers of lance nematodes and the larvae of *M. incognita* in the root, number of galls/g root and the galling index. Numbers of lesion *(Pratylenchus brachyurus)* nematodes in the roots increased above those in the control in soils with 0.0125 g TCNB/kg soil; however, higher concentration reduced the numbers so that roots from soils with 0.1-0.5 g TCNB/kg contained few of these nematodes. Application of TCNB to soil resulted in a marked improvement in the appearance of the root systems.

INTRODUCTION

Chloronitrobenzenes are among the oldest soil fungicides. While the fungicidal activity of these compounds is well known (4,6), their nematicidal efficacy has received little attention. There are indications, however, that these compounds affect

nematodes, Thus, pentachloronitrobenzene (PCNB) has been reported to inhibit or stimulate plant parasitic nematodes in soil depending on species and rate of application (1,3,7). We have shown that chloronitrobenzene fungicide, tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) possesses considerable nematicidal activity against a number of cotton parasites (2). This paper reports results of a study on the nematicidal activity of TCNB and a discussion of the relationship of its activity to that of other related fungicides.

MATERIALS AND METHODS

Soil for the study was a Norfolk sandy loam taken from the top 20 cm of a field under continuous cotton culture. The soil was naturally infested with stunt *(Tylenchorhynchus claytoni)*, root-knot *(Meloidogyne incognita)*, lance *(Hoplolaimus galeatus)* and lesion *(Pratylenchus brachyurus)* nematodes. The moist (60% field capacity) soil was sieved (2mm) and apportioned in 500 g quantities into polyethylene bags. Each bag of soil was thoroughly mixed with an amount of a granular formulation of TCNB calculated to have a concentration of: 0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.25, or 0.5 g of the fungicide / kg soil. The granular formulation was prepared by dissolving chemically pure 2,3,4,5-tetrachloronitrobenzene in xylene and adding the solution to attapulgite clay granules so that after complete evaporation of the solvent at room temperature (24-25 C) the formulation contained 10% (w/ w) of the chemical.

The contents of the polyethylene bags were transferred to 11-cm diam plastic pots and two M-8 cotton seeds were planted in each pot. Eight replications per concentration of fungicide were arranged in randomized complete blocks in the greenhouse.

Four wks after planting, the number of nematodes in 50 cm^3 of soil was determined by the molasses flotation-sieving technique (9). Cotton plants were removed from pots by washing soil from roots, and height of each plant and fresh weights of root and shoot systems were determined. Number of nematodes in the roots was assessed by incubating root systems from each pot for 72 hrs on a fiberglass screen in contact with 500 ml of water in 1 liter beakers. After incubation, the water was passed through a 38 um (400 mesh) screen and nematodes collected were washed into a counting dish.

Number of galls caused by *M. incognita* in each root system was recorded, and degree of galling was assessed using a scale in which 0 represented no galling and 10 severe galling (11). In addition, the general condition of roots was assessed subjectively by using a scale where 0 represented poor growth and appearance and 6 excellent development and appearance.

All data were analyzed following standard procedures for analyses of variance, and differences between means were evaluated for statistical significance with the modified Duncan's multiple range test (10). Except where otherwise stated, all differences referred to in the text were significant at the 5% level of probability or better. Values for the least significant difference (LSD) were also calculated and included on the graphs to facilitate interpretation. LD50 values were calculated following standard procedures (5) and are expressed as the mgs of TCNB per kg of soil required to achieve 50% kill of nematodes.

RESULTS

Number of stunt and saprophagous nematodes in soil (Fig. 1A) declined sharply with increasing concentrations of TCNB between 0 and 0.1 g/ kg; little or no additional

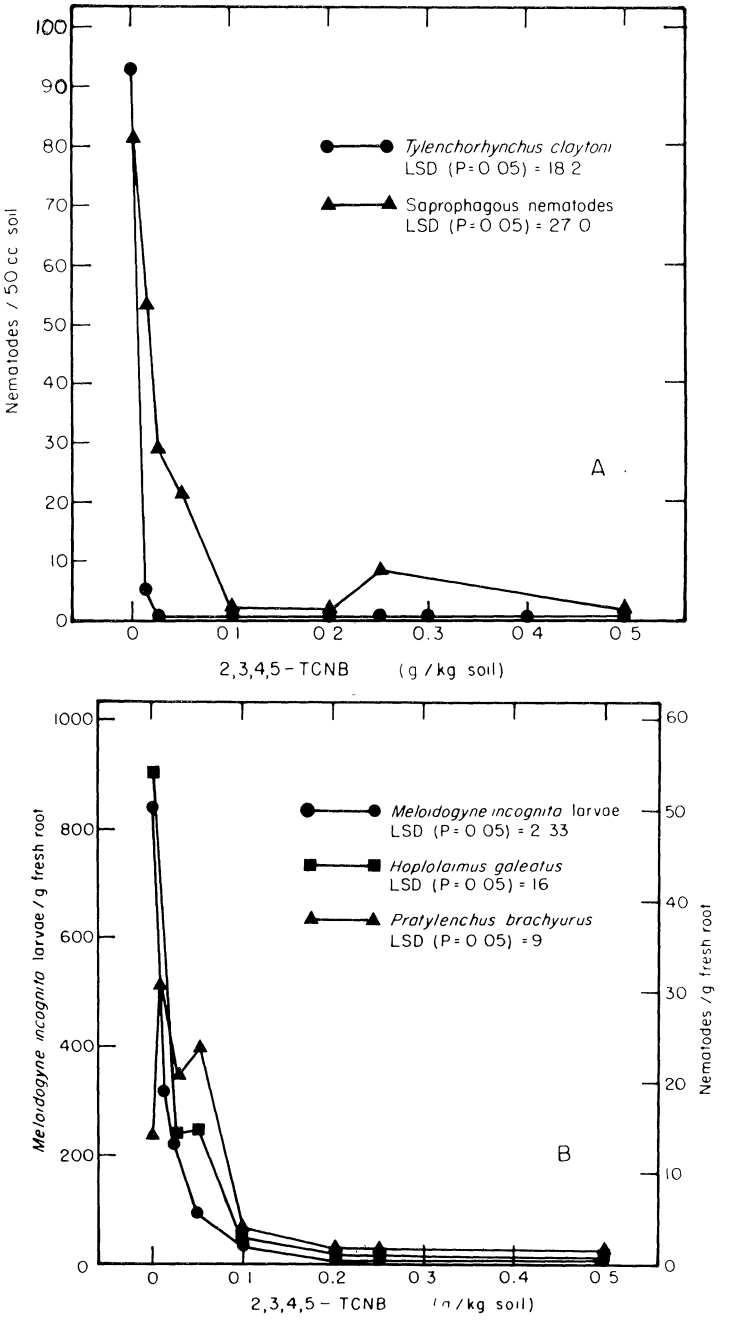
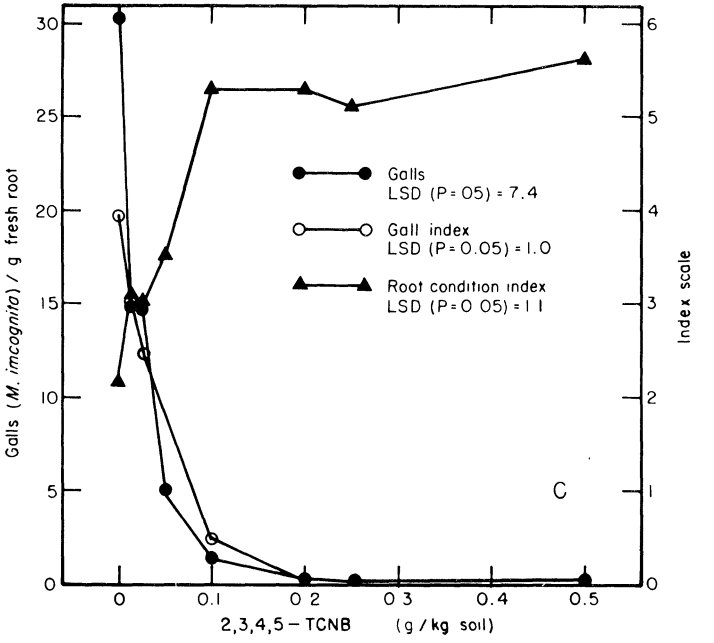
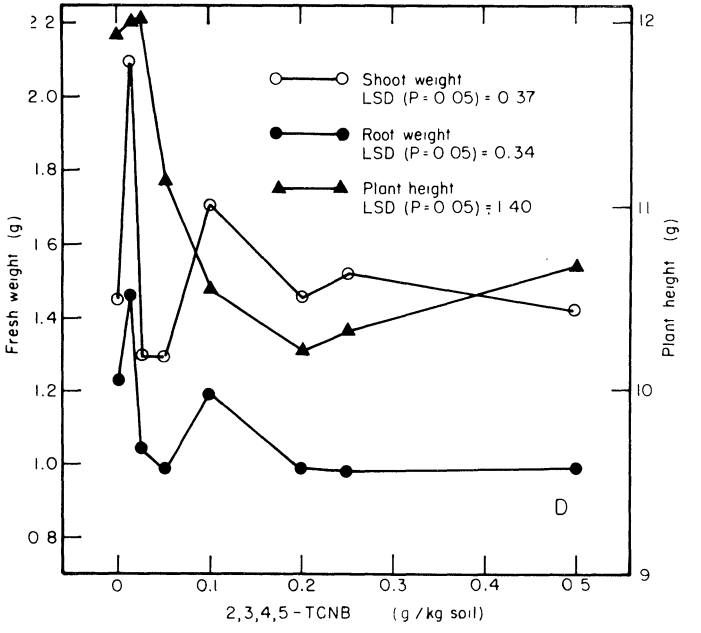


Fig. 1. Effect of 2,3,4,5-tetrachloronitrobenzene on nematode numbers (A,B), cotton root condition and galling reaction (C), and on growth of cotton plants (D).


Galls (M. imcognita) / g fresh root
Index scale
Galls
LSD (P = 05) = 7.4
Gall index
LSD (P = 0.05) = 1.0
Root condition index
LSD (P = 0 05) = 1 1
C
2,3,4,5 – TCNB (g / kg soil)


Fresh weight (g)
Plant height (g)
Shoot weight
LSD (P = 0 05) = 0 37
Root weight
LSD (P = 0 05) = 0.34
Plant height
LSD (P = 0 05) = 1 40
D
2,3,4,5 - TCNB (g / kg soil)

decrease in numbers was observed at higher concentrations. Numbers of lance, lesion, and dorylaimoid nematodes and larvae of *M. incognita* in soil were small ($< 10/50$ cm^3 soil); however, *(data not shown)* these nematodes also declined in a manner identical to that described for stunt and saprophagous nematodes.

Larvae of *M. incognita* and lance nematodes extracted from roots (Fig. 1B) declined sharply with increasing TCNB concentration over the range of 0-0.1 g/kg soil; very few of these nematodes were found in roots from soil with TCNB concentration < 0.20 g/kg. Numbers of lesion nematodes in the roots (Fig. 1B) increased sharply in response to the lowest TCNB concentration and declined in response to higher concentration so that they were almost eradicated with concentrations ≥ 0.2 g/kg soil.

The number of galls and the root-knot nematode index (Fig. 1C) declined sharply, paralleling the pattern observed for larvae of this nematode in the roots. Values for the root condition index (Fig. 1C) were inverse to those obtained for the root-knot nematode index. Roots from soil with 0.1 g TCNB/kg or higher were vigorous and exhibited no necrosis.

Plant height of cotton (Fig. 1D) was not significantly affected by TCNB concentrations ≤ 0.05 g/kg soil; however, higher concentrations resulted in shorter shoots compared with the control. Total fresh weight of the shoot system (Fig. 1D) increased in response to the lowest concentration of TCNB, but differences between all other concentrations and the control were not significant. Differences in fresh weight of root system (Fig. 1D) paralleled those for shoot weight; however, all differences between treatment and control for weights were not statistically valid.

DISCUSSION

Our results indicate that cotton has considerable tolerance for 2,3,4,5-tetrachloronitrobenzene. If the effective zone of treatment and activity of an in-furrow application is considered to be a cylinder 5 cm in radius in a row-ha, the concentrations used in the study were equivalent to rates between 1.5 and 65 kg a.i./ha (1.4-58 lbs a.i./acre). The nematicidal activity of this tetrachloronitrobenzene isomer compares well with commercially available nematicides which are applied at rates of 1-3 kgs/ha in in-furrow or narrow band applications.

In comparison to the nematicidal activity of other chloronitrobenzenes, 2,3,4,5-TCNB is considerably more active than 1,2,4,5-tetrachloro-3-nitrobenzene (2); the latter isomer in similar studies evidenced LD50 values of 200 mg/kg soil with most of the species of nematodes described in this paper. Results from 2,3,4,5-TCNB indicates that its corresponding values are less than 100 mg/kg soil for all nematodes except lesion. The 2,3,4,5-TCNB isomer is also considerably more nematicidal than pentachloronitrobenzene (PCNB) (1). A point of similarity between PCNB and 2,3,4,5-TCNB is the stimulatory effect of these compounds on lesion nematodes at low rates of application (1,3,7). The reason for this is not known. The greater tolerance by *Pratylenchus* spp. to these compounds could, however, lead to the preponderance of these nematodes over more susceptible species. While the greater activity of the tetrachloronitrobenzene isomers compared to PCNB may be attributed to their higher vapor pressures and, hence, greater fumigant activities (8), differences between the tetrachloro isomers must be based on other reasons, as yet unknown. The recorded differences are particularly intriguing in view of the absence of differences in fungicidal activity between the tetrachloronitrobenzene isomers (8).

The occurrance of fungicidal and nematicidal properties in 2,3,4,5-tetrachloronitrobenzene may be of particular value for treatment of soils in which dis-

ease complexes involving nematodes and fungi occur. This may be particularly relevant in view of the known activity of tetrachloronitrobenzenes against species of *Fusarium* involved in such complexes (6).

RESUMEN

El fungicida 2,3,4,5-tetrachloronitrobenceno (TCNB) se mezcló con un limo arenoso tipo Norfolk en dosis de 0-0.1 g i.a./kg de suelo lo que resultó en una disminución aguda en el suelo del número de *Tylenchorhynchus claytoni, Hoplolaimus galeatus, Meloidogyne incognita,* y en el de los nematodos saprófagos y dorilaimoideos; el uso de dosis más altas no resultó en disminuciones adicionales. Una reducción similar se observó también en el número de *H. galeatus* y en el de larvas de *M. incognita* en las raíces de algodón M-8 plantado en el suelo, así como en el número de nódulos/gm de raíz y en el índice de nodulación. El número de *Pratylenchus brachyurus* en las raíces aumentó sobre el del testigo en suelos con 0.0125 g TCNB/kg de suelo; sin embargo, concentraciones más altas del fungicida redujeron el número de este nematodo de tal manera que suelos con 0.1-0.5 g TCNB/kg tenían muy pocos. La adición de TCNB al suelo resultó en un mejoramiento señalado en la apariencia general del sístema radicular del algodón.

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