Estimation of Teliospores of *Tilletia indica* from Field Soil

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ABSTRACT

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Teliospores of *Tilletia indica* were estimated in the soil by pouring an infested soil suspension through a series of test sieves with pore sizes of 600, 250, 105, 63, and 25 μm. The residue on the 25-μm sieve was resuspended in water, and teliospores were enumerated under a stereobinocular microscope. Recovery of teliospores ranged from 79.9 to 93.9%. Teliospores were present in all of 50 field soil samples collected in Punjab.

Additional keywords: *Neovossia indica*

Teliospores of *Tilletia indica* Mitra (syn. *Neovossia indica* (Mitra) Mundkur), the causal agent of Karnal bunt of wheat, survive in the soil for more than 2 yr and serve as the source of infection in subsequent years (4). The estimation of teliospores in the soil can help to predict potential crop losses. Datnoff et al (2) employed a modified flotation bubbling system using glycerol for the estimation of teliospores from infested soil but did not report estimations from naturally infested soil. We report a simple, less time-consuming technique—a slight modification of Cobb's (1) method commonly used to extract nematodes from soil—that has been used successfully to estimate teliospores.

MATERIALS AND METHODS

To determine the efficiency of teliospore recovery, we infested soil with a suspension containing 2.2×10^4 teliospores per milliliter; 1, 5, 10, 15, and 60 ml of the stock suspension was added per 100 cm^3 of sandy loam soil. Each treatment was repeated three times. The soil was thoroughly mixed and 500 ml of tap water was added; after 2 min, the soil was homogenized by hand and large pebbles were removed. Water (1 L) was added, and the soil was suspended and passed through a sieve with a 600-μm pore size. This was repeated three times with 500 ml of water to the soil settled at the base of the first trough and retained on the sieve. The suspension collected in the second trough was passed through a set of three sieves with pore sizes of 250, 105, and 63 μm. The last washing was passed through a sieve with a 25-μm (500-mesh) pore size, with the sieve at an inclined angle. The residue on the sieve containing the teliospores was resuspended in 250 ml of water; 50 ml of this suspension was diluted to 250 ml, and teliospores were enumerated from this diluted suspension. Each time, 5 ml of this suspension was added to a counting dish with 64 squares, and teliospores were counted by means of stereobinocular microscope (×50). The count was made three times for each sample.

The method was used to estimate teliospores in soil samples from 50 fields in different agroclimatic regions of Punjab. Soil was taken from 10 locations in each field (down to 15 cm) and combined, and 1 kg of the combined soil was used as the sample for that field. The samples were air-dried and stored at room temperature, and 250 cm^3 of each sample was used for further extraction and enumeration as described above.

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Table 1. Teliospores of *Tilletia indica* recovered from infested sandy loam soil using test sieves

Teliospores per 100 cm^3 of soil before sieving	Teliospores recovered after sieving[a]	Percent recovery
2.2×10^4	17,575.8	79.9
1.1×10^5	88,165.0	80.2
2.2×10^5	196,202.0	89.2
3.3×10^5	309,705.0	93.9
1.32×10^6	1,188,000.0	90.0
		av. 86.6

[a] Average of three replications.

Table 2. Teliospores of *Tilletia indica* recovered from naturally infested field soils

District of Punjab	No. of samples	Teliospores per 250 cm^3	
		Range	Average
Gurdaspur	10	6,000–31,000	16,700
Ludhiana	10	3,000–50,500	18,200
Patiala	17	2,000–40,000	14,400
Sangrur	13	3,000–16,000	5,400

RESULTS AND DISCUSSION

Teliospores were recovered from 79.9–93.9% (average 86.6%) of the infested sandy loam soil (Table 1); the percent recovery was increased by resuspending the residue after each sieving. Datnoff et al (3), using a glycerol extraction technique to quantify the teliospores of *T. indica* in Mexican soil, recovered 52% of inoculum added to the soil. The technique we used is simpler and more efficient, and larger amounts of soil can be handled.

Teliospores were found in all 50 soil samples collected from different agroclimatic regions of Punjab, but the quantity varied considerably—from 2,000 to 50,500 per 250 cm^3 of soil (Table 2), possibly reflecting variations in disease incidence the previous year. Karnal bunt inoculum thus is widely distributed in the soils of Punjab and may serve as a source of infection for subsequent wheat crops (6).

The method can be used to estimate teliospores in soil from fields and to determine the relationship between teliospore density and disease incidence. Teliospores of *T. indica* average 35.5 μm in size (5) and thus are retained on a 500-mesh (25-μm) sieve. The morphology, color, and size of the teliospores easily distinguish them from clay and silt particles. This method could be useful in many ecological and epidemiological studies.

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