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Phytonematode Diversity of Spinach and its Control in Godumakunta Village of Telangana

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Abstract

Phytonematodes, are a serious pests on majority of crops, causing huge economic losses. Leafy vegetable crops, such as spinach, coriander, amaranthus etc have also been reported to be heavily infected with different species of phytonematodes. In this regard, not many studies have been conducted on spinach crop to isolate and identify phytonematodes. In the present investigation, a field study, using crop beds of spinach was conducted. From the spinach soil, two specific phytonematodes, *Radopholus* and *Criconemella* were identified. To control the phytonematode, infection, organic amendment such as cowdung was added to the test soil, and synthetic nematicide, such as phorate was added to the control soil and both were tested for their nematicidal activity. Spinach soil infected with phytonematodes showed a drastic improvement after the addition of cowdung and similarly phorate treated control also exhibited a notable improvement in the phytonematode population. Compared to both the treatments, cowdung treated spinach soil showed a considerable reduction in the phytonematode population, suggesting that this could be a better organic alternative to synthetic nematicides. This study successfully isolated and identified spinach specific phytonematodes and also recommends that cowdung can be a natural nematicide as it seems to contain certain nematicidal toxins.

Key words : Godumakunta village, Phytonematodes, Spinach soil, Cowdung, *Radopholus*, *Criconemella.*

The plant parasitic nematodes are generally called as eelworms, phytonematodes, phytohelminthes or simply plant nematodes. They parasitize both animals and plants. Nematodes are widespread in distribution and found in soil, fresh water and salt water, wherever organic matter exists, from oceans to mountains, from arctics to tropics and are said to be "Ubiquitous'[27] The majority of the plant species, which account for the major world's food supply, is susceptible to attack from phytonematodes which are capable of causing sustainable economic losses in the quantity and quality of the crops[5,13] . The crop losses caused by phytonematodes in economic terms estimated about $ 157 billion annually to the world agriculture[4]. The main objectives of phytonematodes management are usually a matter of reducing the nematode population by one or more methods or integration of one or more methods to a low level so that the damage is negligible or at an economically acceptable level[21]. (Based on these studies, it is quite evident that, phytonematodes, infect quite a majority of plants, leading to huge economic losses. However, not many studies have been reported on spinach crop. This study aims to isolate and identify phytonematodes specific to spinach and also to manage their infection using organic amendments such as cow dung.

For the present study, the field was divided into two parts, one part received normal ploughing (20 cm deep), whereas the other part received deep ploughing (40 cm deep). Both parts were further divided into 3×3 m 2 beds and a buffer zone of 0.25 meter left between the beds. Different beds, which were replicated five times and this experiment was carried out using the experimental design of Randomized Complete Block Design (RCBD) and made them as the plots for detailed study.

1) Control soil without plants (CSP)
2) Test soil samples with plants (TSSP)
3) Organic amendment treated soil (Cowdung) (OASC)

Immediately after the treatment, the beds were watered for ensuring proper decomposition of the organic additives. Five replicates for each treatment were arranged in a random manner according to the table of Khan and Khanum.[14] Three week old seedlings of spinach, were raised in pots filled with autoclaved soil and transplanted after two week of the treatment. Spinach were sown directly and necessary caring such as watering, weeding etc. were also done throughout the experiment which was terminated after three months of the treatment. Soil sampling was taken at the depth of 0-15 cm both prior and after termination of the experiment from each bed with the help of a soil sampler to count the initial and final population of phytonematodes. These samples were collected and brought to the laboratory in polythene bags and were mixed thoroughly. A representative soil sub-sample of 250 g was used for isolating the nematodes with the help of Cobb's sieving and decanting method along with modified Baermann funnel technique[23]. The nematodes were counted with the help of counting dish[7]. For each sample aliquots of 100 ml were used for counting and from this, 5 ml suspension was used after thorough stirring. For these experiments, different beds were maintained as such and were thoroughly prepared for the next growing season. Three week old seedlings of spinach, were raised in autoclaved soil and transplanted without giving any further treatment to the beds except the ploughing treatment (normal and deep ploughing). The seeds of spinach, were sown directly. The final data of nematode population were determined after three months at the termination of the crop.

Identification of Nematodes :

The classic method of extraction of

nematodes from soil is conducted following the method of Jenkins[11]. The soil sample is mixed thoroughly, but gently when tumbling, to homogenize the nematodes within the soil. A measured volume of soil (either 100 cm^3 or 250 cm^3) is rinsed through a 864 μm (20 mesh) sieve into a large pitcher. The filtrate is mixed with a pressurized water spray to fill the pitcher. After allowing the water and soil in the pitcher to settle for 20 seconds, the suspension is poured over a 38 µm (400 mesh) sieve held at a 45° angle. Material captured on the sieve is rinsed into a 100 mL centrifuge tube and centrifuged for 3minutes at 1,700 rpm. The supernatant is poured off and the pellet is re suspended in a 1.328 M sucrose solution (specific gravity = 1.10) before a repeated centrifugation at 1,700 rpm for 3 minutes. Following centrifugation, the supernatant is poured over a 25 μm (500 mesh) sieve and rinsed with water to remove any traces of sucrose. The resulting material captured on the sieve can be examined under a light microscope for identification and quantification. An alternative method to centrifugation of the soil sample is a modified Baermann tray or

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Secernentea
Order	:	Tylenchida
Family	:	Pratylenchidae
Genus	:	*Hirschmanniella*
Species	:	*Hirschmanniella*

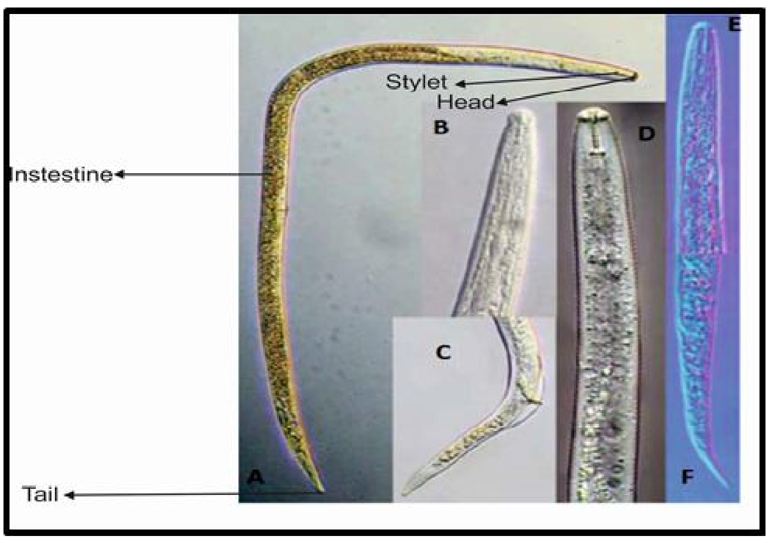

Fig. 1

Radopholus mature female whole body (A), male anterior head (B), male tail (C), female anterior end (D); *Hirshmanniella* anterior end (E) and tail region (F).

Magnification: 10x – 40x

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Secernentea
Order	:	Tylenchida
Family	:	Criconematidae
Genus	:	*Macroposthonia* de Man, 1880

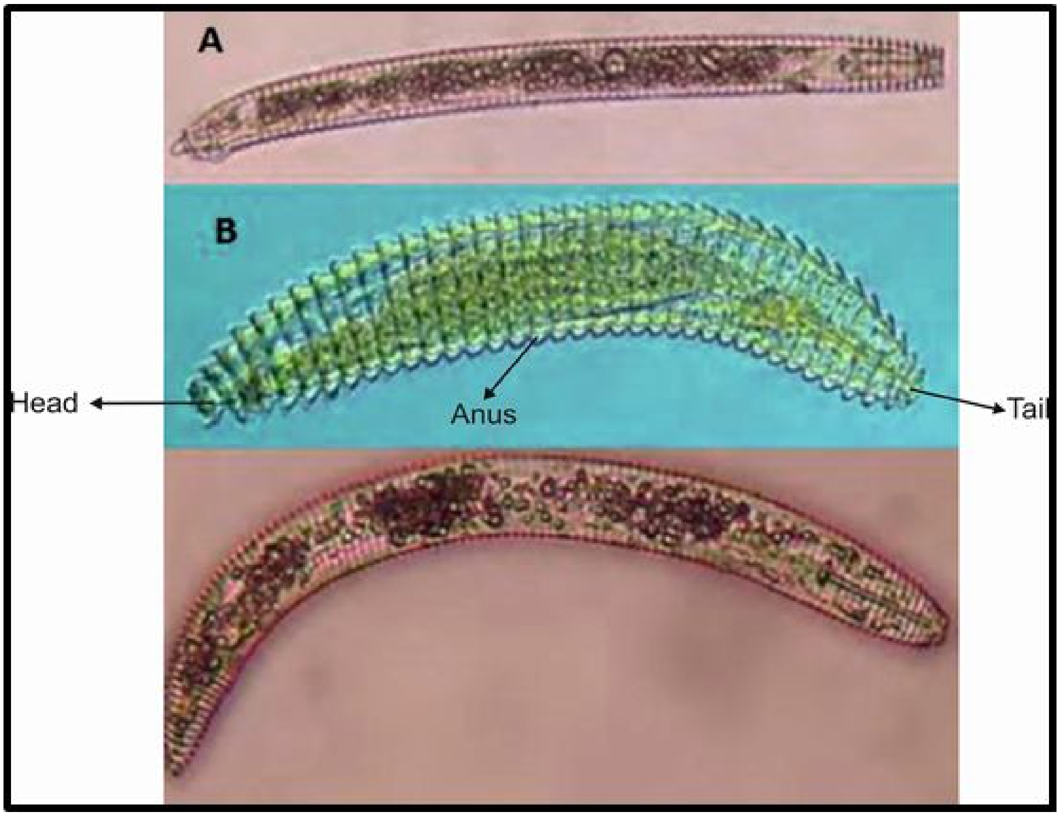

Figure 2.
Entire body of *Hemicriconemoides* (A), *Criconema* (B) and *Criconemella* (C). Magnification: 10x – 40x

Table-1. Treatment of Spinach soil with Cowdung and Phorate Control

S.No		Phytonematode population	
		Untreated	Treated
1	Experimental	20	15
2	Control	10	8

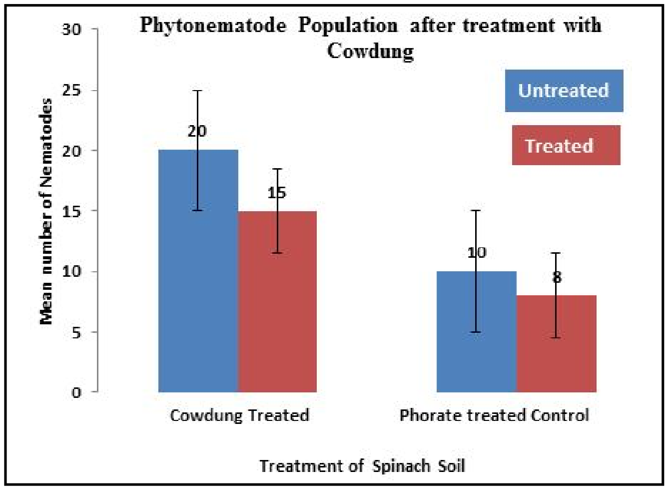

Fig. 3 Mean numbers of nematodes per 473 cc (1 pit) soil-20cm before and after the treatment of Cowdung

Table-2. Treatment of Spinach soil with Cowdung and Phorate Control

S.No		Phytonematode population	
		Untreated	Treated
1	Experimental	15	12
2	Control	8	6

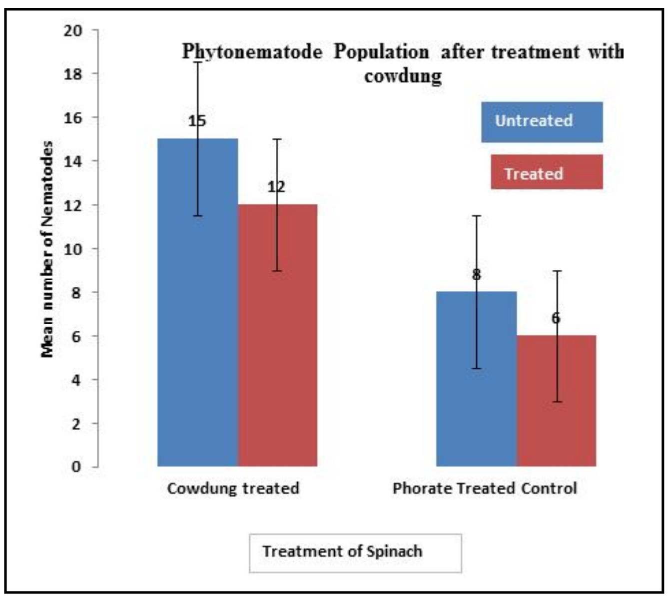

Fig. 4. Mean numbers of nematodes per 473 cc (2pit) soil-40cm

funnel. In this case, the required volume of soil is rinsed through an 864 μm sieve and over a 38 μm sieve, just as with the centrifugation method. The captured material is rinsed into a coffee filter placed within a plastic bowl or funnel and supported by a screen. The water level is brought up to at least 1.0 cm above the coffee filter and allowed to incubate for 24 hours. Following incubation, the filter and screen are removed from the bowl and the water left in the bowl or funnel base is poured over a 25 μm sieve. The material contains only live, mobile nematodes and can be observed under a light microscope.

In the present study, two phytonematode populations such as *Radopholus* (Fig. 1) *and Criconemella* (Fig. 2) which are specific to Spinach have been identified. The classification and morphological details have been presented below.

The spinach soil was treated with natural cowdung and the control soil was treated with chemical nematicide phortae. After the addition of cow dung there was a reduction in phytonematode population in the spinach soil. Similarly when the control bed was treated, with phorate there was a reduction in phytonematodes. In the experimental beds, cowdung was added to the plants as one bed which was at a distance of 20 cm (Table-1, Fig. 3) and a second one at a distance At a distance of 40cm from the root-knot the phytonematode population was decreased when compared to 20cm distance of root-knot (Fig. 4)of 40cm (Table-2, Fig. 4).

In the present investigation, field trial of the population of phytonematodes was conducted in the Godumkunta village of Telangana. In the untreated beds where spinach was seeded, phytonematodes multiplied freely but was significantly reduced in all cow dung treated beds. The test nematicide, phorate was found to be very effective than the organic amendments. Deep ploughing (40cm deep) also brought down the population of phytonematodes. After the application of cow dung into the soil, the growth of the phytonematodes were decreased when compared to the test crops grown soil. The results were in confirmation with those obtained by various other workers[1,12,17,22,24] who reported that the application of organic amendments and depth of ploughing, played an important role in reducing the population of plant parasitic nematodes as the nematodes are exposed to solar heat[18,23,24]. It was observed that deep summer ploughing in May-June resulted in reduction in population of cereal cyst nematodes and increased yield of wheat crops. Most probably the deep ploughing disturbs the ecological set up of nematodes and exposes the lower strata of soil to the external unfavorable environmental factors like solar heat and desiccation, thus adversely affecting the nematode populations[2,16]. The results obtained here with respect to the efficiency of organic amendment, cow dung are in conformity with those obtained by several other workers[3,9,10,15,19,20,26]. The longer persisting nematicidal effects of different organic amendments such as cow dung seems to be because of the fact that these are made up of complex organic substances which decompose rather slowly[6,8] and thus release nematotoxic-substances for longer durations. Therefore the present nematicidal property of cowdung may be attributed to certain complex compounds present in cowdung.

In the present study, nematodes *Radopholus and Criconemella* were specific in spinach soil were isolated and identified. The nematode population density was decreased from first day to 30th day in all the leafy vegetable crops at 40cm distance from root - knot. When compared to control (without leafy vegetables), the soil which contain with leafy vegetables have less nematode number. The phytonematode diversity changes were studied by mixing the cow dung into the all soil samples collected and checked for the population of nematodes. After the application of cow dung into the soil, the growth of the phytonematodes was decreased when compared to the test crops grown soil.

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