Distribution of Plant Parasitic Nematode Community in Contaminated Egyptian Fields, a Case Study and Preliminary Heat Management.

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Abstract: Water sources is an important source for spreading the plant-parasitic nematodes (PPN) particularly irrigation water. The objective of current study is to assess the contamination of irrigation water with PPN by conducted a survey on occurrence of nematodes, their survival and infection potential under laboratory conditions. Two survival tests of collected PPN were carried out by pipetting collected nematodes around the root system of tomato plants (*Solanum lycopersicum* L.) cv. Super strain B. under expected (24±3°C) or unexpected greenhouse conditions (32±3°C) for two months. Statistical analysis of variance was used and least significant differences at 5% were detected. *M. incognita* successfully infected and reproduction on tomato plants under expected greenhouse conditions whereas, under unexpected greenhouse conditions (32±3°C) unable to infect and reproduce on tomato plants. **Conclusion:** water resource from three governorates is contaminated by plant nematodes and able to infect and reproduction. It is necessary to find methods used for the management contamination of water resource.
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1. Introduction

Nematodes are one of the most abundant groups of soil invertebrates. Nematodes often reaching several millions per square meter and more than four out of five metazoan individuals are nematodes[1]. Also, soil nematodes have profound effects on soil processes through their influence on the composition and activity of soil microflora[2]. Besides their role in decomposition and nutrient release in natural ecosystems and interaction with microflora[3].

Plant-parasitic nematodes are major pests in many countries, particularly in the tropics and subtropics, where they are recognized as the cause of serious yield losses on a wide range of crops[4]. Of the 50% of potential crop losses caused by pests, 12.3% is estimated to be caused by nematodes and more of this damage is in the developing than developed countries [5]. Among the plant parasitic nematodes, root-knot nematodes *Meloidogyne* spp., are economically the most important, limiting agricultural productivity and quality [6]. This nematode can cause 24–38% loss on a tomato crop where sequential cropping of susceptible crops is practised with up to four crops per year. In the absence of effective control root-knot nematodes can cause total crop failure [7].

In the previous century, free-living nematodes in drinking water were detected [8,9] and plant-parasitic nematodes being dispersed by irrigation water[10]. Contamination sources of irrigation water with plant-parasitic nematodes were investigated, including wells, boreholes, collected rainwater, ponds, lakes, dams, rivers, municipal water, runoff water, irrigation canals and drainage water in soilless culture[11]. The reuse of agricultural drainage water (ADW) is one of the main reasons to spread plant-parasitic nematodes from infected fields to clean or new reclaimed field. During the 1970s and 1980s, work was done by the then South African Department of Agriculture on the distribution and possible control of nematodes in irrigation water. Since 1992, a few or rarely researches have been done in this field, though [11]. In Egypt, information on the occurrence of plant parasitic nematodes in irrigation water is particularly rare.

The objective of this study was to evaluate ability of available collected plant parasitic nematodes from agricultural drainage water (ADW) to infect sensitive tomato plants under greenhouse conditions and to discuss possible strategies to prevent or control plant-parasitic nematode contamination of irrigation water in Egypt.

2. Materials and Methods

A survey **study:**

A survey was conducted in three Egyptian governorates (Sharkia, Kafr Elshekh and Menia) during 2018. Samples of water were sampled from irrigation canals, and municipal water.

1- Water sampling methodology:

To get large numbers of nematodes in irrigation canals, about 25- 40 Liters of water were passed through the two sieves, 60-mesh sieve and 500-mesh

sieve within a period of 2 hours, whereas lower water volume substantially gets lower numbers of nematodes according to [12] who determined the optimum size of sampling from an irrigation canal or plastic containers by dipping the containers in the water [13,14]. The number and volume of samples depend on the detection threshold that can be determined by means of trial and error[15]. Vegetable and citrus Irrigation Water Sources (IWS) or agricultural drainage water (ADW) samples were collected every 2 weeks from January 2018 to December 2018.

Sampling time:

Since fields in shallow irrigation system in Delta of Egypt, during exploded irrigation water waste periods, it is the optimum time to pass water through the sieves.

Sampling irrigation water for nematodes can be done very simply, such as by merely dipping a container in the water. The water concerned is then poured through sieves with different pore sizes to concentrate the nematodes.

Nematodes were extracted using a combination of serving and Baermann trays technique [16] and collected in a small volume of water[17]. For nematode identification, 1 ml of nematode suspension was pipetted into Hawksely counting slide and nematodes were examined by the aid of the of research microscope under 100X magnification. Based on morphology of adult and juvenile forms nematodes were identified according to[18,19].

Experimental design:

A greenhouse test was conducted to determine root penetration by plant parasitic nematodes in tomato plant (*Solanum lycopersicum* L.). Tomato plant was chosen because it is severely attacked by the plant parasitic nematodes as well as it's regional economic importance. Seeds of the susceptible tomato cv. Super Strain B were soaked in sterile distilled water in Petri dishes and kept in an incubator at 26 ±1 °C. After 48 hours seeds were germinated in clay pots of 20-cm diameter containing steam sterilized sandy soil. At the two leaf stage, seedlings were singly transplanted to formalin sterilized 20-cm diameter plastic pots filled with steam sterilized soil. This experiment was carried out in the soil texture i. e., sandy soil (95.7% sand), (1.2% silt) and (3.1% clay). One week after transplanting, when seedlings were approximately 10 cm in height, they were inoculated with Irrigation Water Sources (IWS) or agricultural drainage water (ADW) were obtained from the mentioned survey.

Control treatments (healthy plants) without nematode inocula. Each treatment was replicated three times. All treatments were arranged in a complete randomized block design in the greenhouse at 24±3°C., and all received similar horticultural treatments. On the other hand, same treatments kept at 32 ±3°C to estimate effect of temperature on root penetration by plant parasitic nematodes.

Plant parameters:

Two months after inoculation, plants were removed carefully from pots and data on plant growth (fresh and dry weight of shoot) were recorded. Roots and surrounding soil in the pots were soaked in tap water for two hours to facility removing adhering soil and keep egg masses on root surface. Roots were wrapped in tissue paper to prevent drying out during the steps of evaluation. Moreover, numbers of galls and egg masses were counted per root system under a dissecting microscope.

Nematodes were extracted from soil using a combination of sieving and Baermann trays technique[16]. Root-knot index was assessed using scale of 0 = No galling; 1 = 1-2 galls; 2 = 3 - 10 galls; 3 = 11 - 30 galls; 4 = 31-100 galls and 5 = more than 100 galls[20].

Moreover, fresh and dry weight of shoots were measured.

Statistical Analysis:

Means were compared by Duncan's multiple range test at $P \leq 0.05$ [21].

Results and Discussion

1- Sources of irrigation water contaminated with plant-parasitic nematodes and associated crops.

Plant parasitic nematode community associated with IWS and ADW:

Data in Table 1 on nematode survey and generic abundance demonstrated that, the highest number and nematode generic during the cultivated period for each crop. Total nematode population was mainly collected from irrigation water sources (IWS), agricultural drainage water (ADW) and small canal.

In Kassasein and Old Salyhia districts, with surface irrigation system, survey assessed the occurrence of *Meloidogyne* (*incognita*) spp. Goeldi with fields cultivated with tomato, eggplant and potato plants cultivated many times around the year. The citrus nematodes, *T. semipenetrans* Cobb was found in agriculture drainage water (ADW) and small irrigation canals in Old Salyhia district. While, lesion nematode, *Pratylenchus* spp. Flipjev, the stunt nematodes, *Tylenchorhynchus* spp. Cobb and *Tylenchus* sp. were found in each collected samples. In Wadi elmolaak district, the citrus nematodes, *T. semipenetrans* and lesion nematode, *Pratylenchus* spp. were found in ADW and small irrigation canals. Lesion nematode, *Pratylenchus* spp., the stunt nematodes, *Tylenchorhynchus* spp. and the spiral nematodes, *Helicotylenchus* spp. Steiner found in banana fields with surface irrigation system. Whereas, *Hoplolaimus* spp. was found in samples collected

from small canals. The same trend was observed with Kafr Saqr district, the lowest nematode species and numbers recovered from each contamination sources of irrigation water. In Kafr Elshekh and Menia governorates, the main nematode population collected from small canal used for filed irrigation while the lowest nematode population recovered from samples of ADW. as well as ADW.

Table 1. **Plant-parasitic nematode species recovered from collected samples of irrigation water sources and their associated cultivated plants in three Egyptian governorates, Egypt.**

Crops	Localities	Water source	Nematode species
Banana+*, Citrus ++, Cotton+ Eggplant+, Potato*, Tomato*, Egyptian clover - Rice -, Maize-, Citrus++, Potato+*, Sugar beat-, Watermelon*+Sugar cane-+	(Kassasein, Old Salyhia, Wadi Elmolaak and Kafr Saqr districts) Sharkia governorate, El -Hamoul district, Kafr Elshekh and Menia city, Menia governorates	Agricultural Drainage Water (ADW), Irrigation Water Sources (IWS) and Small canal	*Meloidogyne* spp.*, *Pratylenchus* spp.,*+ *T.semipenetrans, Tylenchorhynchus* spp.+*, *Helicotylenchus* spp.+, *Hoplolaimus* spp.+, *Tylenchus* sp.+*

During 2018, 473 samples were collected from crop fields of three Egyptian Governorates.
*Crops mainly associated with *Meloidogyne* spp., ++associated with *T. semipenetrans*, +* crops associated with nematode species collected from each other.

2- Tests for survival of plant-parasitic nematodes in water and their infectivity after survival.

Damage of root knot nematode (RKN), *M. incognita* and their infectivity to tomato plants (*Solanum lycopersicum* L.) cv. Super strain B under greenhouse conditions (24±3°C) were assessed in (Table 3). Results revealed that all treatments significantly ($P \leq 0.05$) infected by recovered from irrigation water sources as compared to check (control) treatment. Pots treated with recovered J2 of *M. incognita* successfully infected tomato plants and formation root galls. For plant growth, it could be concluded that *M. incognita* caused remarkable reduction in tomato growth response in terms of root fresh weight (30.55 %) and fresh shoot weight (14.06 %) as compared to healthy plants. On the other hand, pots infected with J2 showed fewer (16.67) and smaller galls (<2 mm). On the other hand, number of IJs of *M. incognita* increased to reach 52.34/100 g in each treated pot.

Table 2. **Greenhouse tests for survival of root –knot nematode, *M. incognita* (in water sources and drainage water) and their infectivity to tomato plants after survival.**

Treatments under 24±3°C	Fresh root weight (g)	Fresh shoot weight (g)	Root galls/Root	Egg masses /Root	Number of IJs/100g	Number of galls		
						Gall diameter (Root-knot nematode)		
	(Reduction %)	(Reduction %)				≥ 4 mm	< 4-2 mm	< 2 mm
Healthy tomato plants (Control)	12.83 a	17.42 a	0.00 a	0.00 a	0.00 a	0.00	0.00	0.00
Tomato plants infected with root – knot nematode, *M. incognita*	8.91 b	14.98 b	16.67	23.00 b	52.34	0.00	2.00	14.67
	(30.55)	(14.06)						

Same letter (s) in each column indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test.

$$\text{Reduction} \quad (\%) = \frac{\text{Control} \quad - \text{Treated}}{\text{Control}} \times 100$$

Root-knot index (RGI) or eggmasses index (EI): 0 = No galling; 1 = 1-2 galls; 2 = 3 - 10 galls; 3 = 11 - 30 galls; 4 = 31-100 galls and 5 = more than 100 galls (Taylor and Sasser, 1978).

Under unexpected greenhouse conditions (32±3°C), the RKN, *M. incognita*; stunt nematode *Tylenchorhynchus* spp.; the lesion nematodes, *Pratylenchus* spp. and *Tylenchus* sp. unable to infect and reproduce on tomato plants (Table 3). Only two uncompleted root galls formed on roots of tomato plants. While, other nematode species not detected in soil of treated pots.

$$\text{Reduction} \quad (\%) = \frac{\text{Control} \quad - \text{Treated}}{\text{Control}} \times 100$$

$$\text{Increase} \quad (\%) = \frac{\text{Treated} \quad - \text{Control}}{\text{Control}} \times 100$$

Table 3. **Greenhouse tests for survival of root –knot nematode, *M. incognita* (in water sources and drainage water) and their infectivity to tomato plants.**

Treatments under 32±3°C	Fresh root weight (g)	Fresh shoot weight (g)	Root galls/Root	Egg masses /Root	Number of J2/100g	Number of galls		
						Gall diameter (Root-knot nematode)		
	(Reduction %)	(Reduction %)				≥ 4 mm	< 4-2 mm	< 2 mm
Healthy tomato plants (Control)	11.94 a	16.12 a	0.00 a	0.00 a	0.00 a	0.00	0.00	0.00
Tomato plants infected with root –knot nematode, *M. incognita*	11.80 a	15.98 a	2.00 a	0.00 a	0.34 a	0.00	0.00	2.00
	(1.17)	(0.08)						

Same letter (s) in each column indicate no significant difference (P ≤ 0.05) between treatments according to Duncan's multiple range test.

$$\text{Reduction} \quad (\%) = \frac{\text{Control} \quad - \quad \text{Treated}}{\text{Control}} \times 100$$

Root-knot index (RGI) or eggmasses index (EI): 0 = No galling; 1 = 1-2 galls; 2 = 3 - 10 galls; 3 = 11 - 30 galls; 4 = 31-100 galls and 5 = more than 100 galls (Taylor and Sasser, 1978).

Collected nematode species from contaminated irrigation water were survival and infect and reproduce on tomato plants. On the other hand, unable to infect and reproduce on tomato plants under unexpected greenhouse conditions. Water contaminated incidentally when used for irrigation in commercial plant nurseries. As well as, contamination of wells, boreholes, collected rainwater, ponds, lakes, dams, rivers, municipal water, runoff water, irrigation canals and drainage water in soilless culture[11]. In a survey on detected plant pathogens from water resources, 17 species of *Phytophthora*, 26 of *Pythium*, 27 genera of fungi, 8 species of bacteria, 10 viruses, and 13 species of plant parasitic nematodes were found[15]. Large nematode populations in irrigation canals of south central Washington and in 1970 were found[12]. In addition, agriculturally-polluted irrigation water play part as a source of plant-parasitic nematode infestation[22] and nematode virus vectors may be spread by means of irrigation water [23].

Direct heat treatment of water is also very effective for the killing of nematodes in irrigation water [24]. However, the beneficial organisms present were also killed [25].

When rarely clean water sources is found, or cannot prevent contamination by plant parasitic nematodes, should physical, chemical, or combinations of treatments be considered[26, 27]. Over a century, sand filtration method used to purification drinking water[28] and used to elimination *Radopholus similis* and retained second-stage juveniles of *Globodera rostochiensis* [15]. Solar radiation used effectively to disinfest soil nematodes in hot climates[29].

Conclusion

From the previous results, it can be concluded that, used solar heat management reduced increase population density of economic nematodes from contaminated water sources. In the future, further water sources should be inspected and new techniques used for the management of nematodes.

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