

PLANT PARASITIC NEMATODES ASSOCIATED WITH SOME ORNAMENTAL PLANTS

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ABSTRACT

An assessment of parasitic nematodes on ornamental plants was conducted in the University of Karachi, Karachi, Pakistan, from August to December 2018. Ten different ornamental plants viz., Christ Plant (*Euphorbia milii*), Copper Leaf (*Acalypha wilkesiana*), Jungle Flame (*Ixora coccinea*), Rose (*Rosa indica*), Champa (*Plumeria obtusa*), China rose (*Hibiscus rosa-sinensis*), Good luck tree (*Cordyline terminalis*), Jasmine (*Jasminum officinale*), Marigold (*Tagetes patula*) and Ulta Ashok (*Polyalthia longifolia*) were assessed for association with plant-parasitic nematodes. Seven of the eleven genera encountered in this study were identified up to species level, viz., *Pratylenchus coffeae* ((Zimmermann, 1898), Filipjev & Schuurmans Stekhoven, 1941), *Hoplolaimus indicus* (Sher, 1963), *Longidorus siddiqii* (Aboul Eid, 1970), *Rotylenchulus reniformis* (Linford & Oliveira, 1940), *Tylenchorhynchus vulgaris* (Siddiqi, 1961), *Aphelenchus avanae* (Bastian, 1865), *Hemicriconemoides communis* (Edward and Misra, 1963). Ornamental plants in which new host associations were recorded included *Euphorbia milii*, *Acalypha wilkesiana*, *Ixora coccinea*, *Polyalthia longifolia*, *Plumeria obtusa*, *Hibiscus rosa-sinensis*, *Cordyline terminalis* and in *Tagetes patula*.

Keywords: Plant-parasitic nematodes, Ornamental plants, Karachi, Pakistan, new host association.

INTRODUCTION

Nematodes being complex and diverse occur in every environment as free-living as well as parasitic species. Many of them live as plant parasites and animal parasites, while others are beneficial for the environment. Nematodes can be herbivorous, carnivorous, or parasitic, and include both generalists and specialists. Nematodes play a particularly critical role in decomposition and nutrient recycling (Neher, 2010).

Free-living species inhabit all types of biomes. Based on their feeding habits free-living species are commonly grouped into the following types: plant-eaters, fungi eaters, bacteria eaters, animal predators, unicellular eukaryote feeders, and omnivores (Yeates *et al.*, 1993). While parasitic nematodes live off of their host and can cause diseases in various types of plants and animals they infect. Plant-Parasitic Nematodes are abundantly found associated with different types of plants. The parasitic phases of their life-cycle occur inside the host body (plant). The pre-parasitic phase or the free-living phase happens in the outer environment (soil) or an intermediate host, a second host (Moens and Perry, 2011).

The plant-parasitic feeding process damages the root system, reducing water and nutrient absorption ability of the plant. It causes diseases such as root rot, cysts, and lesions and provide invading opportunities to other pathogens as well (Back *et al.*, 2002). A few diagnostic symptoms appear except the ones beneath the ground. Thus, to diagnose precisely sampling of soil and plant material from suspected sites is required. Above-ground symptoms are rarely sufficient to diagnose root problems. However, the problems are always noticed from abnormal plant appearances. Below-ground symptoms are more useful in diagnosing galls, abbreviated roots, necrotic lesions or root rotting in the roots (Bridge and Starr, 2007).

The objective of this study was to isolate and identify Plant-parasitic nematodes associated with some ornamental plants of Pakistan.

MATERIALS AND METHODS

Sampling, Extraction and Isolation

The ornamental plants chosen as hosts have been planted within the premises of University of Karachi, Karachi. Ten plants were chosen randomly, that made up twenty samples; two from each host plant. Host plants chosen included Christ Plant (*Euphorbia milii*), Copperleaf (*Acalypha wilkesiana*), Champa (*Plumeria obtusa*), China rose (*Hibiscus rosa-sinensis*), Good luck tree (*Cordyline terminalis*), Jasmine (*Jasminum officinale*), Marigold (*Tagetes patula*), Jungle Flame (*Ixora coccinea*), Rose (*Rosa indica*), and Ulta Ashok (*Polyalthia longifolia*).

During August to December 2018, damp soil samples with some fresh little roots were collected and stored in labelled plastic bags. To extract nematodes from the soil samples, Cobb's sieving and decanting method (Cobb,

1918) was used. Three different samples were collected depending on the mesh size (36 μ , 100 μ , and 350 μ). Contents from 36 μ sample were observed for root-knot nematodes and 100 μ contents were observed for cyst nematodes and larger nematodes. The 350 μ sample was subjected to Baermann's funnel method (Baermann, 1917) to isolate nematodes. Samples of 100mL were collected from funnel after 48 hours for quantitative and qualitative analyses.

Quantitative and Qualitative Analysis

For quantitative analysis, three 5mL aliquots from 100mL samples of 350 μ contents were observed one by one under a stereomicroscope. The nematodes were counted in a zig-zag manner from one block to another in a counting chamber. The average number of nematodes per 100mL from three different readings was recorded (Table 1). Nematodes were heat-killed (Hooper, 1986) and then preserved by adding Tri-ethanolamine formaldehyde (TAF) to analyze them qualitatively (Courtney *et al.*, 1955). Nematodes (genera) were observed through temporary mounting. Each sample cavity was made TAF free and left in the incubator for slow dehydration, after adding 1.25% Glycerin, at 55-60°C for 3-5 days (Seinhorst, 1959).

Then these were subjected to measurements to identify them using their different morphological and anatomical features (De Man, 1884). Thus seven species were identified through permanent mounting technique (Siddiqi, 2000). The results were illustrated using a Nikon Eclipse E400 compound microscope, to which a drawing tube was attached. They were photographed with Nikon DS, film camera, attached to the microscope.

RESULTS AND DISCUSSION

Soil samples collected to analyze the plant-parasitic nematodes associated with these host plants showed the given quantitative results (Table 1) and qualitative results (Table 2). Some new nematodes and hosts associations are recorded for the first time in Pakistan (Table 3).

Table 1. Locations of Host Plants along with their Quantitative results.

| HOST PLANTS | LOCATION (University of Karachi) | QUANTITATIVE RESULTS (Average number of nematodes/100mL) |
|-------------------------|---|---|
| <i>P. obtusa</i> | Department of Genetics | 196 |
| <i>P. obtusa</i> | Department of Genetics | 814 |
| <i>H. rosa-sinensis</i> | Botanical Garden | 149 |
| <i>H. rosa-sinensis</i> | Botanical Garden | 273 |
| <i>E. milii</i> | Department of Food Science and Technology | 1300 |
| <i>E. milii</i> | Department of Food Science and Technology | 1093 |
| <i>A. wilkesiana</i> | Botanical garden | 712 |
| <i>A. wilkesiana</i> | Botanical garden | 989 |
| <i>C. terminalis</i> | Department of Food Science and Technology | 83 |
| <i>C. terminalis</i> | Department of Food Science and Technology | 80 |
| <i>J. officinale</i> | Department of Genetics | 165 |
| <i>J. officinale</i> | Department of Genetics | 141 |
| <i>I. coccinea</i> | Botanical garden | 235 |
| <i>I. coccinea</i> | Botanical garden | 299 |
| <i>T. patula</i> | Botanical Garden | 128 |
| <i>T. patula</i> | Botanical garden | 350 |
| <i>R. indica</i> | Department of Genetics | 880 |
| <i>R. indica</i> | Department of Genetics | 675 |
| <i>P. longifolia</i> | Department of Food Science and Technology | 622 |
| <i>P. longifolia</i> | Department of Food Science and Technology | 260 |

The analysis of soil samples collected from ten different ornamental plants revealed various nematode types - spiral, stunt, needle, dagger, lesion, sheath, lance, reniform and free-living.

Based on some morphological and anatomical characters eleven different genera were identified. The genera identified in this study include *Aphelenchus* (Bastian, 1865), *Aphelenchoides* (Fischer, 1894), *Helicotylenchus* (Steiner, 1945), *Hemicriconemoides* (Chitwood & Birchfield, 1957), *Hoplolaimus* (von Daday, 1905), *Longidorus*

(Thorne, 1961), *Pratylenchus* (Filipjev, 1936), *Rotylenchulus* (Linford & Oliveira, 1940), *Tylenchorhynchus* (Cobb, 1913), *Tylenchus* (Bastian, 1865) and *Xiphinema* (Cobb, 1913).

Table 2. Qualitative Results (Genera identified).

| HOST PLANTS | QUALITATIVE RESULTS (Nematode genera identified from the samples) |
|-------------------------|---|
| <i>P. obtusa</i> | <i>Aphelenchus</i> , <i>Hemicriconemoides</i> , <i>Tylenchorhynchus</i> , Free-living. |
| <i>P. obtusa</i> | <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Pratylenchus</i> , <i>Hemicriconemoides</i> , Root-Knot larvae, Free-living |
| <i>H. rosa-sinensis</i> | <i>Rotylenchulus</i> , <i>Tylenchid</i> , <i>Hemicriconemoides</i> , <i>Pratylenchus</i> , <i>Longidorus</i> , <i>Xiphinema</i> , Root-Knot larvae, Free-living |
| <i>H. rosa-sinensis</i> | <i>Aphelenchoides</i> , <i>Aphelenchus</i> , <i>Hemicriconemoides</i> , Free-living |
| <i>E. milii</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Pratylenchus</i> , <i>Hoplolaimus</i> , Free-Living. |
| <i>E. milii</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , Free-Living. |
| <i>A. wilkesiana</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchoides</i> , <i>Xiphinema</i> , <i>Longidorus</i> , <i>Helicotylenchus</i> , Free-Living. |
| <i>A. wilkesiana</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Longidorus</i> , <i>Xiphinema</i> , <i>Helicotylenchus</i> , Free-Living. |
| <i>C. terminalis</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , Root-Knot larvae, Free-living |
| <i>C. terminalis</i> | <i>Rotylenchulus</i> , <i>Aphelenchus</i> |
| <i>J. officinale</i> | <i>Rotylenchulus</i> , <i>Aphelenchus</i> , Free-living |
| <i>J. officinale</i> | <i>Rotylenchulus</i> , Free-living |
| <i>I. coccinea</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Xiphinema</i> , <i>Helicotylenchus</i> , <i>Longidorus</i> , <i>Aphelenchoides</i> , <i>Hemicriconemoides</i> , Free-Living. |
| <i>I. coccinea</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Tylenchus</i> , <i>Helicotylenchus</i> , <i>Hemicriconemoides</i> , <i>Xiphinema</i> , Free-Living. |
| <i>T. patula</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Helicotylenchus</i> , <i>Longidorus</i> , <i>Xiphinema</i> , Root-Knot larvae, Free-living |
| <i>T. patula</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchoides</i> , <i>Helicotylenchus</i> , <i>Tylenchid</i> , <i>Pratylenchus</i> , Root-knot larvae, Free-living |
| <i>R. indica</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , Free-Living. |
| <i>R. indica</i> | <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , Free-Living. |
| <i>P. longifolia</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , Free-Living. |
| <i>P. longifolia</i> | <i>Rotylenchulus</i> , <i>Tylenchorhynchus</i> , <i>Aphelenchus</i> , <i>Pratylenchus</i> , Free-Living. |

Seven of these genera were identified up to species level, viz., *Pratylenchus coffeae* (Zimmermann, 1898), Filipjev & Schuurmans Stekhoven, 1941), *Hoplolaimus indicus* (Sher, 1963), *Longidorus siddiqii* (Aboul Eid, 1970), *R. Rotylenchulus reniformis* (Linford & Oliveira, 1940), *Tylenchorhynchus vulgaris* (Siddiqi, 1961), *Aphelenchus avanae* (Bastian, 1865), *Hemicriconemoides communis* (Edward and Misra, 1963).

Many of the associations recorded in this study were reported for the first time in Pakistan. The specimens of *P. coffeae* (Zimmermann, 1898), Filipjev & Schuurmans Stekhoven, 1941) in *E. milii* and *P. longifolia* were recorded for the first time. *H. indicus* (Sher, 1963) in *E. milii*, *H. communis* (Edward and Misra, 1963) from *I. coccinea*, *P. obtusa*, and *H. rosa-sinensis* were also reported in this study. The associations of *T. vulgaris* (Siddiqi, 1961) with *E. milii*, *P. obtusa*, *I. coccinea*, *T. patula*, *C. terminalis*, and *P. longifolia* were also reported for the first time.

Similarly, *A. avenae* (Bastian, 1865) was also found to be associated with *E. milii*, *A. wilkesiana*, *P. obtusa*, *H. rosa-sinensis*, *C. terminalis*, *P. longifolia*, and *I. coccinea*. *L. siddiqii* (Aboul Eid, 1970) species were reported in *H. rosa-sinensis* and *T. patula*. And species of *R. reniformis* (Linford & Oliveira, 1940) in *C. terminalis* were also reported for the first time in Pakistan.

Most of the nematodes found in abundance in the soil samples of these ornamental plants had not been reported before. *Ixora coccinea* appeared to be associated with the largest number (nine) of nematodes and *T. patula* with only two pathogenic nematodes (Table 3). *P. longifolia*, *P. obtusa* and *C. terminalis* associated with three pathogenic nematodes. *A. wilkesiana* and *E. milii* associate with five nematodes each. *Tylenchorhynchus* and *Aphelenchus* were

found to be more widely spread with six and five plants, respectively. Whereas, *Hemcrinemoides* associated with two plants (*Plumeria obtusa* and *Ixora coccinea*) and *Xiphinema* with only one, *A. wilkesiana*.

Table 3. Host Records.

| ORNAMENTAL HOST PLANTS | NEMATODES ENCOUNTERED |
|-------------------------------|---|
| <i>Plumeria obtusa</i> | <i>Aphelenchus</i> Bastian, 1865 <i>Hemicriconemoides</i> Chitwood & Birchfield, 1957 <i>Tylenchorhynchus</i> Cobb, 1913 |
| <i>Hibiscus rosa-sinensis</i> | <i>Longidorus</i> Thorne, 1961 |
| <i>Euphorbia milii</i> | <i>Aphelenchus</i> Bastian, 1865 <i>Hoplolaimus</i> Von Daday, 1905 <i>Pratylenchus</i> , Filipjev, 1936 <i>Rotylenchulus</i> Linford & Oliveira, 1940 <i>Tylenchorhynchus</i> Cobb, 1913 |
| <i>Acalypha wilkesiana</i> | <i>Aphelenchus</i> Bastian, 1865 <i>Aphelenchoides</i> Fischer, 1894 <i>Helicotylenchus</i> Steiner, 1945 <i>Longidorus</i> Thorne, 1961 <i>Xiphinema</i> Cobb, 1913 |
| <i>Cordyline terminalis</i> | <i>Aphelenchus</i> Bastian, 1865 <i>Rotylenchulus</i> Linford & Oliveira, 1940 <i>Tylenchorhynchus</i> Cobb, 1913 |
| <i>Ixora coccinea</i> | <i>Aphelenchus</i> Bastian, 1865 <i>Aphelenchoides</i> Fischer, 1894 <i>Helicotylenchus</i> Steiner, 1945 <i>Hemicriconemoides</i> Chitwood & Birchfield, 1957 <i>Longidorus</i> Thorne, 1961 <i>Rotylenchulus</i> Linford & Oliveira, 1940 <i>Tylenchorhynchus</i> Cobb, 1913 <i>Tylenchus</i> Bastian, 1865 <i>Xiphinema</i> Cobb, 1913 |
| <i>Tagetes patula</i> | <i>Longidorus</i> Thorne, 1961 <i>Tylenchorhynchus</i> Cobb, 1913 |
| <i>Polyalthia longifolia</i> | <i>Rotylenchulus</i> Linford & Oliveira, 1940 <i>Tylenchorhynchus</i> Cobb, 1913 <i>Aphelenchus</i> Bastian, 1865 |

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