INT. J. BIOL. BIOTECH., 21 (1): 65-68, 2024.

ROOT-KNOT NEMATODES (*MELOIDOGYNE INCOGNITA*) INFECTING YOUNG POMEGRANATE SHRUBS ROOTS IN KARACHI

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ABSTRACT

Root-knot nematodes are one of the major constraints in pomegranate cultivation, besides several other plant nematodes. Diseases incited by root-knot nematodes are of economic importance to the farmers and nurseries owners. In the present study roots of 6-10 months old pomegranate shrubs with obvious stunting and yellowing of leaves from Karachi were histopathologically examined in detail. Some cells of the epidermis and outer cortex were completely destroyed. Both hyperplasia and hypertrophy were present. Almost all cells were filled with sections of larvae, females and egg masses. Necrosis was present along with giant cells at feeding sites.

Key Words: Pomegranate, root-knot nematode, histopathology, Karachi nurseries.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit bearing deciduous shrubs belonging to the family Lythraceae, subfamily Punicoideae. It is widely cultivated in Middle East, North and tropical Africa, central Asia, Indian subcontinent Mediterranean Basi and caucasus region (Morton, 1987).

Several nematodes have been associated with pomegranate in Pakistan (Khan *et al*., 2005, 2008, 2011; Hussain *et al*., 2016; Nasira *et al*., 2011), however only a few cause severe loss to pomegranate plantation. Some nematode species can be lethal to the plant especially in early stage depending on the intensity of infestation and favorable environmental condition. In an early study Singh *et al*. (2019) reported 17.3% yield loss due to plant nematodes. The worms damage the root system thus causing difficulty in absorption of soil nutrients and water (Speijer and Ssango, 1999).

Amongst the nematode (*Meloidogyne incognita*) is the most important species threatening the pomegranate trees and moisture favors the rapid multiplication of root-knot nematode species (Pawar *et al*., 2013). Its fruit juice is popular in Middle East, Europe, Canada and United States. Besides it is used in baking, meal garnishes, alcohol beverages, wine smoothies and cooking (Nikki, 2007). The present communication deals with the histopathology of *Meloidogyne incognita* associated with young pomegranate shrubs from Karachi nurseries.

MATERIALS AND METHODS

To record nematode infection and histopathological study pomegranate shrub roots were collected from 6-10 months old shrubs from a nursery located on University road, Karachi. The shrubs were stunted with yellowing leaves. Roots were kept in polythene bags and directly send to the laboratory for examination. Root-knot *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 was identified according to Ferris (1999). Roots were gently washed and were cut into 2-4mm long sections, which were then fixed in FAA solution (2-4 parts formalin as 37% formaldehyde, 1.6 parts of acetic acid, 60 parts ethanol 95% and 80 parts of distilled water) (Southey, 1986).

The sections were dehydrated in tertiary butyl alcohol (TBA) (Johansen, 1940). Later dehydrated root tissues were embedded in paraffin oil and butyl alcohol (1:1) for almost 2-3hrs, and then replaced with pure melted paraffin wax kept in oven for 3 days at 52ºC. During the wax infiltration process, air bubbles were removed from the root tissue under vacuum. Using rotary microtome the blocks were cut 12µ.The paraffin strips were placed on slides and kept in incubator until the water evaporates. The slides were stained in Eosin and Haematoxylin. The best slides were selected and observed under microscope for histopathological study.Photomicrographs were prepared using an

automatic photographic camera mounted on a research microscope Nikon Optiphot-2.

RESULTS AND DISCUSSION

The second stage *Meloidogyne incognita* juveniles enter the pomegranate roots by puncturing action of the stylet. They move intercellularly and settle in the vascular region (Fig. 1).The hatching of larvae is effected by pH, moisture and temperature of soil (Velloso *et al*., 2022). Once the root cap cells are punctured other juveniles move in through this entrance point. Some cellsof the epidermis and outer cortex were seen to be completely destroyed (Fig. 2). In some cells two hypertrophised nucleoli were present. Both hyperplasia and hypertrophy were present simultaneously. The giant cells are formed on feeding sites, large vacuole with completely destroyed cytoplasm was observed (Fig. 3). Almost all cells were filled with sections of larvae, females sections and egg masses of various sizes, necrosis was distinct (Fig. 4).

Plant growth and the root development had adverse effect due to gall formation. The presence of giant cells was similar to that reported by Yousif (1979). Plants infected with root-knot nematode are easily vulnerable to secondary plant infection especially fungi (Gomes *et al*., 2014). Culture practices such as soil solarization, steaming and flooding can be used successfully in reducing the population (Schwarz *et al*., 2020). The nematode spreads rapidly through water, agricultural tools and plant matter thus using sterilized soil can prevent the nematode from spreading (Philbrick *et al*., 2020).

In order to control such a destructive nematode studies conducted go unnoticed due to unawareness of farmers and nurseries owners.

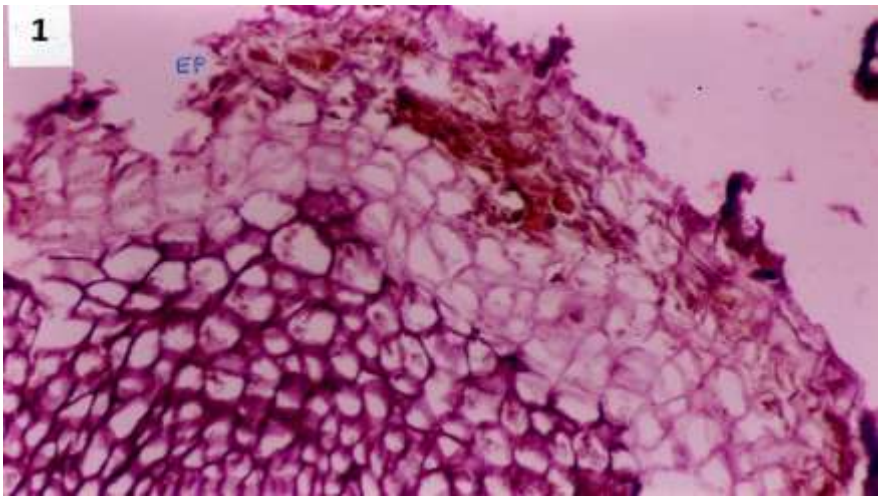

Fig. 1. Longitudinal section of pomegranate root showing entry point of juveniles in the root (x100) (EP: Entering point).

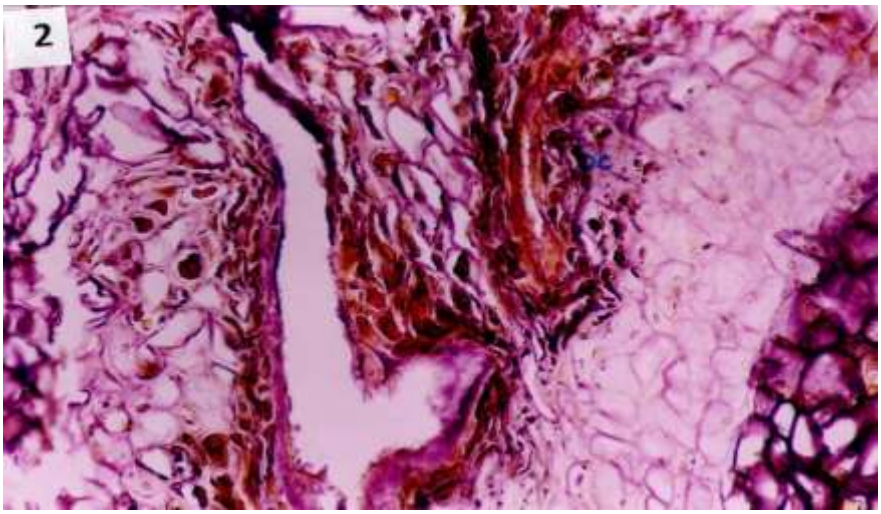

Fig. 2. Transverse section of pomegranate root showing some completely destroyed cells (x100) (DC: destroyed cells)

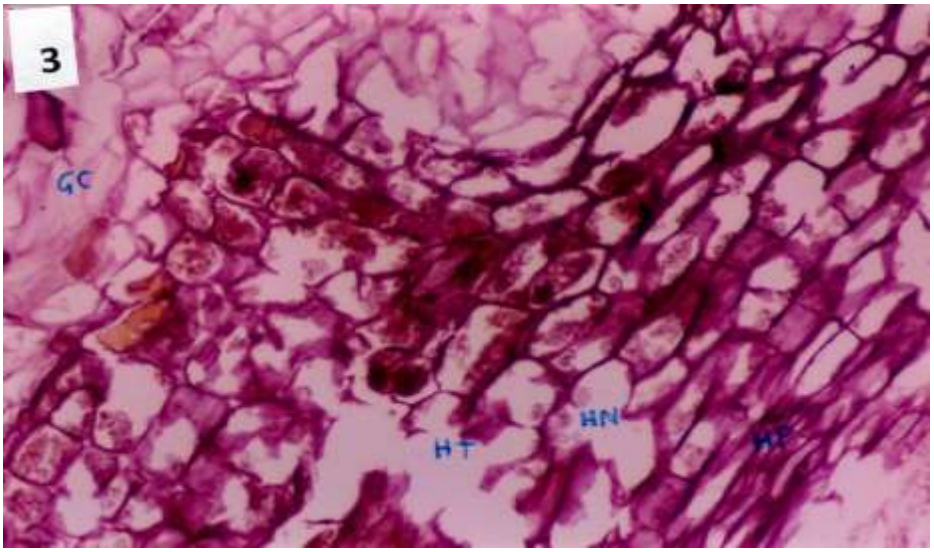

Fig. 3. Transverse section of pomegranate roots with hyperthrophised nucleoli, hypertrophy, hyperplasia and giant cells (x100) (HN: Hyperthrophised nucleoli, HT: Hypertrophy, HP: Hyperplasia, GC: Giant cells).

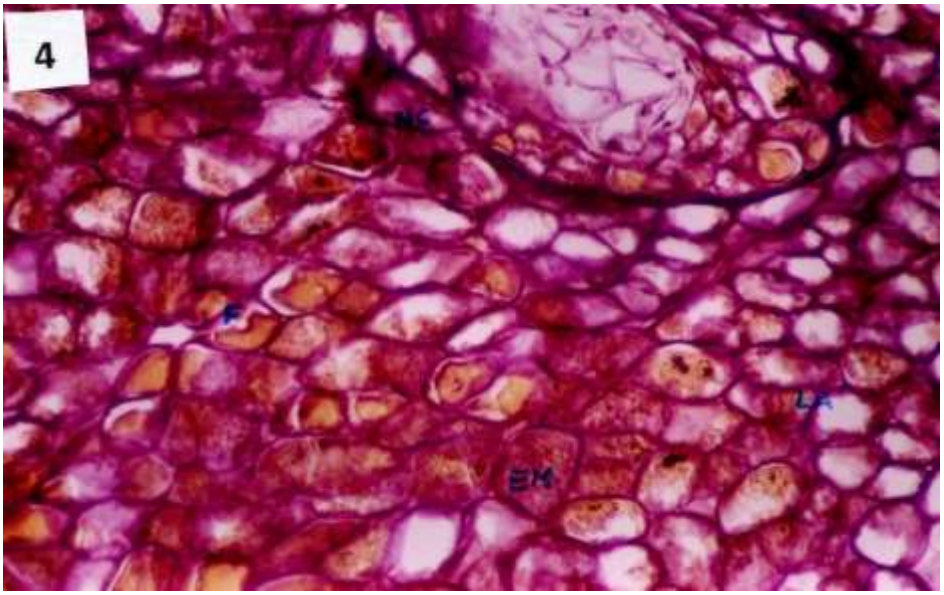

Fig. 4. Transverse section of pomegranate roots with cells filled with larvae, egg masses and female sections (x100) (LA: Larvae, NC: Necrosis, EM: Egg masses, FS: Female sections).

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(*Accepted for publication November 2023*)