HISTOPATHOLOGY OF ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD INFECTING GUAVA SEEDLINGS ROOT

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Abstract

Guava roots naturally infected with root-knot nematodes (*Meloidogyne javanica*) were fixed, sectioned and examined with a microscope. It was observed that the nematode juveniles developed and moved normally within root tissue. The cortex was completely destroyed. Cells showing hypertrophy and hyperplasia were common. Females deposited eggs into a gelatinous egg mass, while the males were present in the rhizosphere soil.

Introduction

With the rapid increase of guava area cultivation the incidence of pest including nematodes also increases (Lim *et al.*, 1986; Razak and Lim, 1987; Jindapunnapat *et al.*, 2013). The most prominent symptoms of nematode infestation in guava are stunted growth, yellowing of leaves, and defoliation. The quality and quantity of fruit produced by plant parasitic nematodes infected trees were also affected. The fruits looked glossy, smooth green in appearance.

In order to control nematode infection from spreading to farms it is important to control nematodes in nurseries before transplanting as in a matter of few months their population can reach dangerous levels (Gaur and Meher, 1994; Khan *et al.*, 2014a,b).

Histological studies on fruit trees roots infested with plant nematodes have been conducted from time to time (Khan *et al.*, 2004; 2007; 2009; 2010, Sayed *et al.*, 2008; 2010). Samad *et al.* (2012) reported histological changes in guava seedling roots infested by *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949.

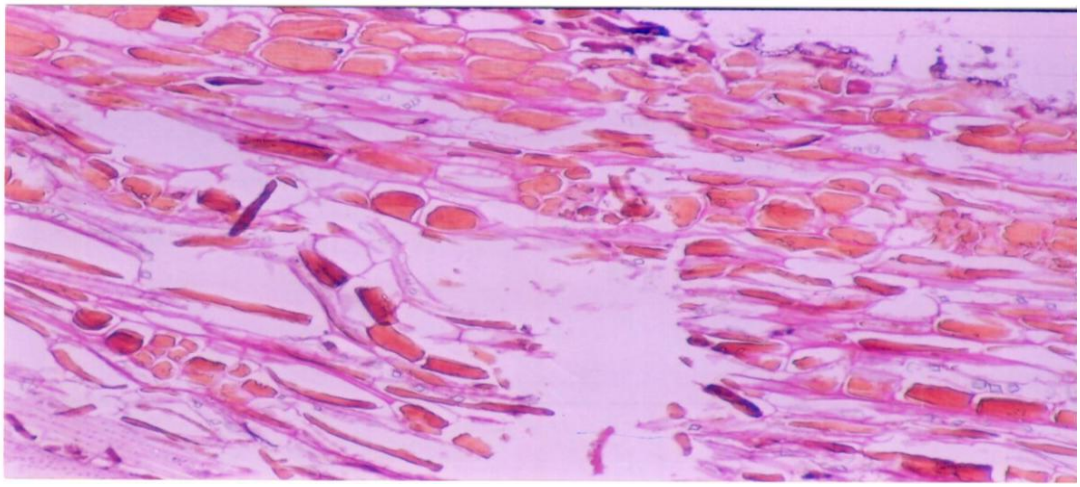
The objective of this investigation was to study the histological changes caused by *M. javanica* to guava seedlings roots in Sindh, Pakistan.

Materials and methods

Four nurseries of Matiari district (Sindh), 1. Deh Dari, 2. Matiari town, 3. Oderolal, 4. Khaibar were visited to assess the incidence of nematode infected guava seedlings. Forty polybag seedlings from Matiari town were taken to observe the severity of galling on the roots as the seedlings at Matiari town nursery appeared to be stunted with yellowing leaves. Infected roots were washed thoroughly to clean the debris under running water and observed for the presence of root-knots. Females from root-knot were identified using perineal pattern and male from the soil were identified using head shape and stylet morphology (Eisenback and Hirschmann, 1981). Pieces of naturally infected gall roots of guava var. safeda were fixed in F.A.A. for 24 h and processed with histological technique according to the standard method of Sass (1964). Dehydration was carried out in a serial concentration of known volume of ethanol. Dehydrated root tissue were then infiltrated and embedded in paraffin wax at 52°C for 12 days. During the wax infiltration process, air bubbles were removed from root tissue under vacuum. Using a rotary microtome 6-8 µm thick sections of root were gently cut, stained by haematoxylin and eosin, later mounted in Canada balsam. Photographs were taken employing camera mounted on a research microscope Nikon (Optiphot-2) in the Department of Zoology, University of Karachi, Pakistan.

Results and Discussion

During the survey of nurseries the slow decline of seedling was rampant. All the seedlings showed poor growth and were infested by root-knot nematodes. The presence of root-knot nematodes was indicated by heavily galled exposed roots around the base of the plant. Those seedlings with heavy infestation could be easily pulled out from pots or polythene bags revealing roots with knots, disfigured and blackened. This is most indicative of root-knot infection. In most samples there was reduced root system. Perineal patterns of saccate



female and measurement of males collected from the rhizosphere soil, the species identified was *Meloidogyne javanica*. Histological root sections of guava var. safeda showed that the nematode *M. javanica* juveniles

Fig. 1. Root cells with sections of female (⟶) (x50)

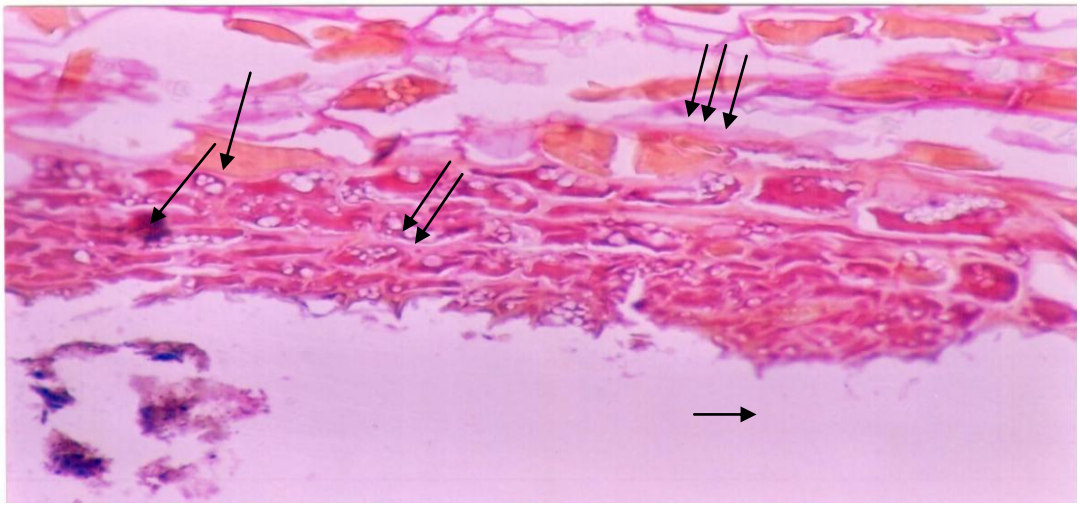


Fig. 2. Cortex cells of root showing hypertrophy (⟶) and hyperplasia (⇉) and cells with gelatinous egg masses (⇉) (x50)

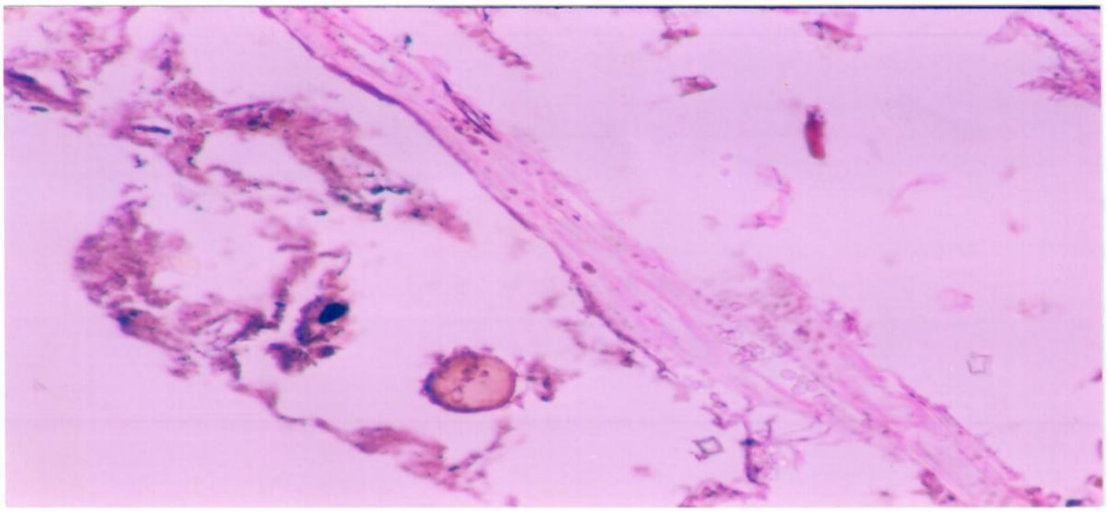


Fig. 3. Cortex of guava root completely destroyed (x100) by the nematodes.

developed and reproduced normally within. Cells with female sections were prominent (Fig. 1). Cells showing hypertrophy and hyperplasia were common with different size of gelatinous egg masses (Fig. 2). The cortex was completely destroyed (Fig. 3) which is a major component of ground tissue of root and is mainly meant for storage of water.

Nematodes have been reported as important pests of guava (Iwahori *et al.*, 2009; Musarrat *et al.*, 2007; Khan *et al.*, 2012). Similarly (Martinez), 1973 and Martin (1959) reported root-knot nematodes to be major pests of guava in Hawaii and Rhodesia.

Meloidogyne (Taylor and Sasser, 1978) is a major pest of different crops in Pakistan (Maqbool, 1992; Aatif *et al.*, 2016) and root galling is generally associated with it.

The trees of guava generally have a lifespan of more than 20 years. Orchards when once infested reduce production of fruit and quality. Pre-plant control can be most effective because in most cases the nematodes are protected by the host root tissue, the trees which are damaged during the first year after planting generally causes stunted growth and at the same time not all chemical nematicides are recommended for post-plant application for guava trees.

The spread of plant nematodes in Sindh nurseries can be accelerated by the farmers and nurseries staff who are not aware of the existence of nematodes aspects. It appears necessary to use sterilized soil for the seedlings soil bags and pots.

Therefore, as suggested earlier (Bertland and Nyczepir, 1989; Nyczepir, 1991) it is essential to give a healthy start to the seedlings to prevent nematodes causing major root damage.

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