PLANT PARASITIC NEMATODES: HIDDEN ENEMY OF BLACK PEPPER (*Piper nigrum* L.)

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Introduction

Plant parasitic nematodes are serious threats to crop production, causing an estimated loss of US $125 billion per year worldwide (Chitwood, 2003). Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging nematodes of crop production worldwide. The impact of this nematode genus is enhanced by its wide host range of more than 5,000 plant species and it causes severe economic losses in many agricultural and horticultural crops (Ntalli *et al.*, 2010). It has been estimated that global losses amount to US $78 billion due to RKN (Chen *et al.*, 2004).

Many plant parasitic nematodes are associated with black pepper in major growing countries including India. Among them, burrowing nematode, *Radopholus similis* (Cobb, 1893, Thorne, 1949) and root-knot nematode, *Meloidogyne* sp. (Goeldi, 1892), which causing slow decline and root-knot disease to black pepper. Significant reduction in the growth and yield was observed in black pepper plants infested with varying populations of nematodes summarized by Devasahayam *et al.*, 2012. Though exact crop loss estimates are not available, the yield losses due to nematode attack may range from 39 to 65% under field conditions.

Slow decline disease

Causing agent: Slow decline disease of black pepper due to feeder root damage caused by *R. similis* and *M. incognita* or *Phytophthora capsici* either alone and in combination. There is a gradual reduction in the vigour and productivity of the vine which leads to death over a period of few years and hence called slow decline (Fig. 1).

Fig. 1 : Slow decline disease in black pepper

Symptoms: The nematodes produce small, elongate lesions on the young tender roots, and later these lesions coalesce and cause extensive root rotting (Fig. 2). The primary symptoms are pale yellow, whitish, discolouration leaves, typical orange to purple colured lesion on young roots, root system exhibits extensive rotting, main roots are devoid of fine feeder roots that rot quickly, extensive necrosis of longer lateral roots develops, yellow patches that later turn as barren standard that have lost their vines or standard supporting dead vines without any leaves. These symptoms are well pronounced when soil moisture is depleted. In general foliar yellowing and defoliation were low during July and high during April-May.

Fig. 2: Rotting in black pepper root due to *R. similis*

Feeding mechanisms: *R. similis* penetration occurred preferentially through the root tips, after 10 days nematodes took up a feeding position intercellularly and intracellularly and cortex cells immediately around the nematodes were necrotic. Nuclei and nucleoli of cells upon which nematodes fed showed increased size. Large necrotic lesions were detected throughout the cortex after 20 days nematode penetration. Eggs were found in the necrotic cortical tissues. By 30 days nematodes had reached vascular tissues and some xylem vessels were seen plugged with a "gum-like substance" (Freire and Bridge, 1985).

Root-knot disease

Causing agents: Root-knot nematode feeding on plant roots, resulting large galls or "knots" can form throughout the root system of the plants is known as root-knot disease (Fig. 3). Root-knot disease due to plant infested with root-knot nematodes (RKN), *Meloidogyne* spp. In Kerala, mainly two species of RKN, *M. incognita* and *M. javanica* infesting black pepper in the fields. The second stage juvenile of the root-knot nematode are most commonly encountered in soils. They are vermiform and infective stage.

Fig. 3: Root-knot disease in black pepper

Symptoms: RKN infested plant leaves exhibits dense yellowish disclouration, root system became heavily galled, egg masses with females enclosed deep within the roots, galls are smooth and bigger size in few cultivars but small galls in many cultivars. While, the most diagnostic RKN damage occurs below ground, numerous symptoms can also be observed above ground. Severely affected plants leaves showed

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yellowing, chlorosis and stunting in the fields, resulted crop yields are reduced.

RKN population in roots of pepper reaches maximum during April - May and minimum during December and January. A low soil temperature coupled with adequate soil moisture and availability of fresh tender roots help in the buildup of its population during September-October. The nematode was detectable throughout the year. Various factors like, rainfall and temperature influenced nematode populations.

Feeding mechanisms: Unlike most other plant-parasitic nematodes RKN females are globose and sedentary at maturity. Once they establish a feeding site, they permanently remain at that location within the plant root. The RKN feeding site is actually a group of cells known as "giant-cells". When a nematode initially penetrates a plant cell with its stylet, it injects secretory proteins that stimulate changes within the parasitized cells. Parasitized cells rapidly become multinucleate (contain many nuclei) as nuclear division occurs in the absence of cell wall formation. This process is considered to be "uncoupled" from cell division. Cells never actually divide into new cells; they just get bigger and contain more nuclear material. This allows the giant-cell to produce large amounts of proteins which the nematode will then ingest. Giant-cells also act as nutrient sinks, funneling plant nutrients to the feeding nematode. RKN does not feed from the cells directly. It forms a feeding tube (from the oesophageal gland cell secretions), secreted from the stylet into the plant cell cytoplasm, which acts as a sieve to filter the cytosol that the nematode ingests. As the name implies, giant-cells can grow very large in size. Triggered by nematode oesophageal gland cell secretions, an increase in the production of plant growth regulators has been demonstrated to play a role in this increase in cell size and division. Root cells neighbouring the giant-cells also enlarge and divide rapidly, presumably as a result of plant growth regulator diffusion, resulting in gall formation.

As the female nematode enlarges, its posterior region may break the epidermis of the root, and the eggs are deposited into a gelatinous egg mass. Mature RKN females (pearly white in color) can be observed without magnification. Second-stage juveniles (J_2) and males can only be observed with the aid of a microscope. Generally, females of RKN have a globose body, with a short "neck," containing their stylet, metacorpus and oesophageal gland cells.

The motile J_2 stage is attack growing root tips and enter roots intercellularly, behind the root cap. They move to the area of cell elongation where they initiate a feeding site by injecting oesophageal gland secretions into root cells. These nematode secretions cause dramatic physiological changes in the parasitized cells, transforming them into giant-cells. Root-knot nematode males also are vermiform but they do not harm to plants.

Management

The control measures should be taken up during early stages of the disease. If nematodes become established in deep-rooted, control is difficult and options are limited. Nematode management in black pepper warrants a sustainable approach integrating several strategies to bring down nematode populations below economic injury levels.

Cultural methods: Use of nematode-free, certified planting material and phytosanitation can reduce nematode spread to a great extent. Uprooting and destruction of diseased vines along with root mass and replanting after 9-12 months have to be undertaken in nematode infested plantations. This will reduce the source of inoculum and spread of the pathogens. Denematization of nursery mixture either through solarization or fumigation with chemicals is highly effective in reducing the nematode load for production of healthy rooted cuttings. The potting mixture should also be fortified with biological control agents after sterilization.

Exclusion of nematode susceptible intercrops and supports or standards can minimise nematode infestations in the field. Support trees such as *Ailanthus malabarica* (Dennst.) Alston., *Artocarpus heterophyllus* Lam., *Garuga pinnata* Roxb., *Macaranga indica* Wight and *Erythrina variegata* L. are not susceptible to RKN and can be grown as support trees. Several inter crops like banana, ginger and turmeric and a large number of weeds are susceptible to these nematodes and may be avoided in black pepper plantations. Application of neem cake @ 2 kg/ vine is highly effective against *M. incognita*. Mulching plant basins with Guatemala grass (*Imperata cylindrica* (L.) Beauv.) or *Gliricidia* leaves also reduces populations of *M. incognita* and *R. similis*.

Host resistance: IISR developed black pepper variety, Pournami which is resistant to *M. incognita* can be cultivated in RKN infested areas. *Piper colubrinum*, which is resistant to *R. similis* can used as a root-stock to graft cultivated pepper plants.

Biological control: Promising isolates of VAM such as *Glomus mossae*, *G. fasciculatum*, *G. etunicatum*, *Acaulospora laevis* and *Gigaspora margarita*, which are suppressive to nematodes can be incorporated with nursery soil mixture for better establishment and production of nematode free planting materials. Antagonistic fungi such as *Paecilomyces lilacinus* and *Pochonia chlamydosporia* (=*Verticillium chlamydosporium*) could be utilized for the management of nematodes in the nursery and field. Several bacteria such as *Pseudomonas* sp., *Bacillus* spp. and *Pasteuria penetrans* are also promising for the suppression of nematode populations and growth of plants. Endophytic bacteria such as *Bacillus megaterium* and *Curtobacterium luteum* suppress *R. similis* populations and enhance growth of black pepper in the nursery.

Chemical control: As a preventive measure, black pepper planting materials can be treated with granular nematicides like phorate or carbofuran @ 0.1g a.i./plant once in 2 months. In plantations, application of carbofuran or phorate @ 3g a.i./vine in May/June and again in September/October is effective for remission of foliar yellowing and reduction in nematode populations (except Kerala). Drenching of Carbosulfan 0.1 % @ 50 ml /poly bag containing potting mixture for the management of plant parasitic nematodes in black pepper rooted cuttings in the nursery.

However, the chances of rehabilitating severely affected vines by application of nematicides are slim because of the heavy damage already caused to the root system and the inability of such plants to put out fresh roots for quick rejuvenation. Hence, the preventive measures are to be undertaken during the initial stages of infestation.

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