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# Development of *Trophotylenchulus piperis* in Black Pepper Roots

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**Abstract.** Development of *Trophotylenchulus piperis* Mohandas *et al.*, 1985 was studied on black pepper roots under laboratory conditions. Second stage juveniles of *T. piperis* entered the roots within 3 days after inoculation. Further development of the juvenile involved the formation of a spherical bulb at midbody length which enlarged on the dorsal side giving a characteristic shape to the adult female. Between 30 and 40 days after inoculation, the protective covering of the nematode case started developing. The fully matured female was covered by a spherical case during 40-50 days after inoculation. The female laid eggs inside the case numbering from 25 to 35 per case. *T. piperis* completed its life cycle on black pepper roots within 55 days at a room temperature of 24-32°C.

**Keywords:** Black pepper, life cycle, *Piper nigrum*, *Trophotylenchulus piperis*.

## INTRODUCTION

Black pepper, *Piper nigrum* L., a spice of export value and cultivated in tropical countries, is subjected to infection by different species of nematode (Ramana and Mohandas, 1987). Mohandas and Ramana (1982) reported the occurrence of a semi-endoparasitic nematode, *Trophotylenchulus floridensis* on black pepper. Later, this nematode was described as a new species, *Trophotylenchulus piperis* by Mohandas *et al.* (1985). *T. piperis*, the pepper nematode as it is commonly called, is one of the predominant species infecting black pepper in all the major black pepper growing areas of Kerala and Karnataka States (Ramana and Mohandas, 1987, 1989). However, little information exists on its parasitism, biology and nature of damage to black pepper. Hence an experiment was conducted to study the mode of parasitism and development of this nematode on black pepper roots under artificial inoculation.

## MATERIALS AND METHODS

Black pepper (cv. Panniyur 1) seedlings were raised in sterile sand. Twenty days after germination, one seedling each was planted in small plastic vials (50 ml capacity) containing sterilized soil mixture. Each seedling was inoculated with 25 second stage juveniles ($J_2$) of *T. piperis*, collected from cases on black pepper roots. A total of 80 plants were inoculated and maintained at room temperature in the laboratory. The number of days required for the penetration of juveniles was observed by uprooting 5 plants at one day intervals for the first 5 days. A further 5 plants were uprooted for examination at an interval of 5 days and washed in running tap water to remove adhering soil particles. Roots were cut, stained in boiling acid fuchsin-lactophenol for 3 minutes, cooled to room temperature and cleared in pure lactophenol for one day to remove the excess stain. Stained roots were observed under the stereomicroscope to study the development of different stages of the nematode.

## RESULTS AND DISCUSSION

*Trophotylenchulus piperis* is a sedentary semi-endoparasite with vermiform second stage juveniles. The juveniles penetrated black pepper roots within 3 days of inoculation. They preferred fibrous roots to the main root. On penetration, they were seen perpendicular to the roots surface and on establishing feeding sites, acquired an orientation parallel to the root, one-tenth of the body being inside the root cortex and remaining portion protruding outside the root (Fig. 1A).

Five days after inoculation, the length of the juveniles reduced and body width started increasing (Fig. 1B). At this stage, the entry point can be seen clearly on the roots as a brownish discoloration. Darkening of the root surface has been observed in black pepper root due to the infection of *Radopholus similis* (Venkitesan and Setty, 1977) and of *Pratylenchus zeae* (Sundararaj and Mehta, 1992). Such changes in colour are attributed to the release of phenolic compounds from the roots and their further oxidation into quinon.

During the next 10-20 days, the juveniles further increased in size. The increase in the body width started from the middle region of the nematode posteriorwards (Fig. 1C). As a result, the nematode body curved ventrally, while the anterior end remained slender and straight. However, not many changes were seen in the posterior terminal part of the body which remained as a pointed tail.

Twenty to 25 days after inoculation the anterior part of the nematode body elongated and coiled (Fig. 1D). The curvature at the middle of the body became more pronounced by 25-30

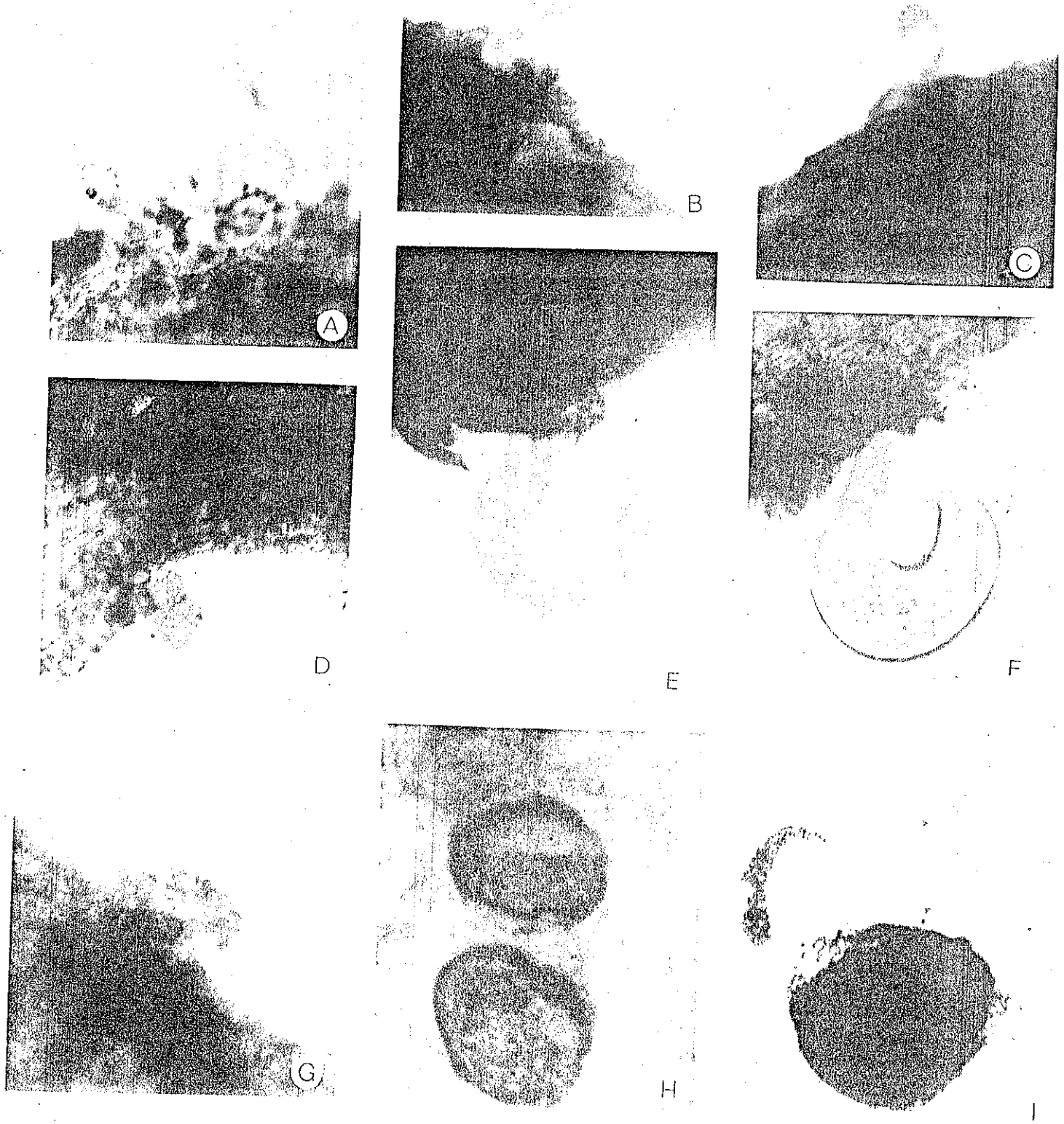


Fig. 1. A-I. Developmental stages of *Trophotylenchulus piperis* on black pepper roots.

days after inoculation (Fig. 1E). At this stage, the nematode body looked like a semi-circle parallel to the longitudinal axis of the root (Fig. 1F). The entire head region was inside the root and the tail was not prominent at this stage.

Between 30-40 days after inoculation, the protective covering of the nematode i.e. the case, started developing. Initially it was like a transparent jelly, probably secreted by the specific glands in the developing preadult female (Fig. 1G). Such glands have been reported in its close relative *Tylenchulus semipenetrans* (Jones, 1981) and in *Meloidogyne* spp. (Hussey, 1989). Cohn and Kaplan (1983) have suggested that the capsules or cases of *Trophotylenchulus floridensis* on *Pinus clausa* roots were of plant origin. We are not certain whether the cases of *T. piperis* are of plant or nematode origin.

The development of cases was completed and the females were covered with a brownish, spherical case during 40-50 days after inoculation (Fig. 1I). In some instances the female was seen attached to the root by its elongated anterior end, protruding out of the case (Fig. 1J). The anterior end was like a thin tube with its bulbular structure at its base. Usually the bulb is enclosed within the case and the region above the basal bulb increases in length.

Once the development of the case was completed the female laid eggs inside the case, numbers of eggs per case ranging from 25-35. Soil particles were seen adhered to the root near nematode cases due to some secretions by the female during its development. During the dry season, the slimy nature of the secretion may prevent desiccation of eggs and juveniles.

At 50-55 days after inoculation, most of the cases were empty indicating that eggs had hatched into $J_2$s which had escaped. Active $J_2$s were recovered from the soil during this period. Under field condition, $J_2$s were also found in soil adjacent to empty cases adhering to the roots. Remnants of the cases were observed as scars on the root with sometimes half of the female's anterior end still inside the root. Soil particles adhered at regions where cases were attached to roots. These regions showed some necrosis due to cellular hypertrophy. More studies are needed to understand the nature of interactions between *T. piperis* and black pepper. Most results in the present study support the earlier observations reported for *T. floridensis* on pine (Cohn and Kaplan, 1983) and *T. obscurus* on coffee (Vovlas, 1987). Our study showed that the $J_2$s of *T. piperis* entered the black pepper roots within 3 days after inoculation and completed their life cycles within 55 days at room temperatures of 24-32°C.

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## LITERATURE CITED

**Cohn, E. and D. T. Kaplan 1983.** Parasitic habits of *Trophotylenchulus floridensis* (Tylenchulidae) and its taxonomic relationship to *Tylenchulus semipenetrans*. *Journal of Nematology* **15,** 514-523.

**Hussey, R. H. 1989.** Disease inducing secretions of plant parasitic nematodes. *Annual Review of Phytopathology* **27,** 123-141.

**Jones, M. G. K. 1981.** Host cell responses to endoparasitic nematode attack: Structure and function of giant cells and syncytia. *Annals of Applied Biology* **97,** 353-372.

**Mohandas, C. and K. V. Ramana 1982.** *Trophotylenchulus floridensis* Raski, a new endoparasite of *Piper nigrum* L. from Kerala. *Journal of Plantation Crops* **10,** 53-54.

**Mohandas, C., K. V. Ramana and D. J. Raski 1985.** *Trophotylenchulus piperis* n. sp. parasitic on *Piper nigrum* L. in Kerala, India. *Revue de Nématologie* **8,** 97-102.

**Ramana, K. V. and C. Mohandas 1987.** Plant parasitic nematodes associated with black pepper (*Piper nigrum* L.) in Kerala. *Indian Journal of Nematology* **17,** 62-66.

**Ramana, K. V. and C. Mohandas 1989.** Endoparasitic nematodes infecting roots of black pepper (*Piper nigrum* L.) in two districts of Karnataka, India. *International Nematology Network Newsletter* **6,** 33-35.

**Sundararaj, P. and U. K. Mehta 1992.** Histopathology of sugarcane roots infested with *Pratylenchus zeae*. *Afro-Asian Journal of Nematology* **2,** 80-83.

**Venkitesan, T. S. and K. G. H. Setty 1977.** Pathogenicity of *Radopholus similis* to black pepper (*Piper nigrum*). *Indian Journal of Nematology* **7,** 17-26.

**Vovlas, N. 1987.** Parasitism of *Trophotylenchulus obscurus* on coffee roots. *Revue de Nématologie* **10,** 337-342.