Occurrence of *Verticillium chlamydosporium* Goddard in a black pepper (*Piper nigrum* L.) garden in Kerala, India[1]

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ABSTRACT

Verticillium chlamydosporium was isolated and identified for the first time from cases of a semi-endoparasitic nematode, *Trophotylenchulus piperis*, from an infested black pepper (*Piper nigrum*) garden in Calicut District of Kerala, India. The fungus suppressed hatching of root knot nematode (*Meloidogyne incognita*) eggs by 41.4 per cent within 5 days in an *in vitro* bioassay and appears promising for the control of root knot nematodes of spice crops.

Key words : biological control, black pepper, *Meloidogyne incognita*, *Piper nigrum*, root knot nematode, *Trophotylenchulus piperis*, *Verticillium chlamydosporium*.

The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949 and root knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 cause severe root damage to black pepper (*Piper nigrum* L.), an important spice crop of India leading to 'slow decline' disease (Mohandas & Ramana 1991). *Trophotylenchulus piperis* Mohandas, Ramana & Raski, 1985, a semi-endoparasitic nematode, is also widely prevalent in black pepper gardens of Kerala and Karnataka in India (Ramana & Mohandas 1987; 1989). However, the present recommendation of nematicide application is not well accepted and practiced by growers. Biological control of plant parasitic nematodes has gained much attention in recent years and is most essential in spice crops as there is a global demand for spices with 'zero pesticide residues'. The present study was a part of the ongoing work to identify and develop native isolates of efficient biocontrol agents against plant parasitic nematodes of spices.

Soil and root samples from a black pepper garden in the District Agricultural Farm, Koothali (Calicut District, Kerala) were collected regularly for monitoring the population fluctuations of *T. piperis*. During routine microscopic observations, most of the intact cases of this nematode were found

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infected with fungal mycelium. Such cases were collected from infected roots, rinsed in sterile distilled water and plated in 1% water agar in petridishes. Three to four days after incubation at 20°C, when the fungal growth was evident, the mycelium was transferred to Potato Dextrose Agar medium (PDA) and incubated at 20°C for 3-5 days. Fungal mycelium from these cultures were stained with 1% cotton blue for further microscopic observations. Slide culture studies were conducted on Corn Meal Agar (CMA) medium to study the characteristics of fungal sporulation.

An experiment was also conducted to test the efficacy of the isolated fungus in suppressing egg hatching in *M. incognita* population. Egg masses were collected from root knot nematode infected black pepper plants grown in greenhouse and axenised using 1% streptomycin sulphate. Ten egg masses each were placed on specially designed sieves as described by Kanwar, Kumar & Bajaj (1994). These were kept in small petridishes (5 cm dia) containing sufficient sterile water. Fungal macerate of 10 days old culture was prepared in 250 ml of sterile distilled water. About 3 ml of the fungal suspension was added onto each sieve and three replications were maintained. An equal quantity of distilled water alone was added in control. The petridishes were maintained at room temperature (29.2 - 32.2°C) and nematode hatching was assessed at regular intervals by counting the number of juveniles that migrated into the petridishes. The estimated control was worked out based on the equation (1-T/C) x 100, where T and C are the means of juveniles hatching in treatment and control, respectively (Tigano-Milani *et al.* 1995). The egg masses were taken and examined under a microscope for fungal colonisation after 5 days.

Microscopic observations of the fungus in slide culture showed evidently the verticillate phialides and phialospores, characteristic of the opportunistic fungus, *Verticillium chlamydosporium* (Deuteromycetes : Moniliales) (Fig. 1a). The fungus produced distinct multicellular, thick walled chlamydospores abundantly in PDA (Fig. 1b). This fungus was originally isolated from clay loam garden soil at Ann Arbon, Michigan by Goddard (1913) and was subsequently reported from several countries (Morgan-Jones, Godoy & Rodriguez-Kabana 1981). In India, *V. chlamydosporium* was recorded in the excreta of some animals by Singh & Singh (1972). However, this is probably the first report of *V. chlamydosporium* from Kerala and also from rhizosphere of black pepper in association with *T. piperis*.

Preliminary studies on its pathogenicity on egg masses of *M. incognita* were also encouraging. The fungus suppressed hatching of root knot nematode eggs by 41.4 percent over a period of five days (Table 1). The rate of suppression showed a declining trend with time probably because of decrease in fungal load when nematode suspensions containing mycelial bits were removed for assessing the daily hatch. However the hatching was on par for different intervals, except for the 4th day, as the value for that internal represented the cumulative hatch for two days. The fungus treated egg masses showed heavy colonization by *V. chlamydosporium* and most of the eggs did not hatch (Fig. 1 c & d). Willcox & Tribe (1974) first reported this fungus as a parasite of plant parasitic nematodes. Subsequently, it

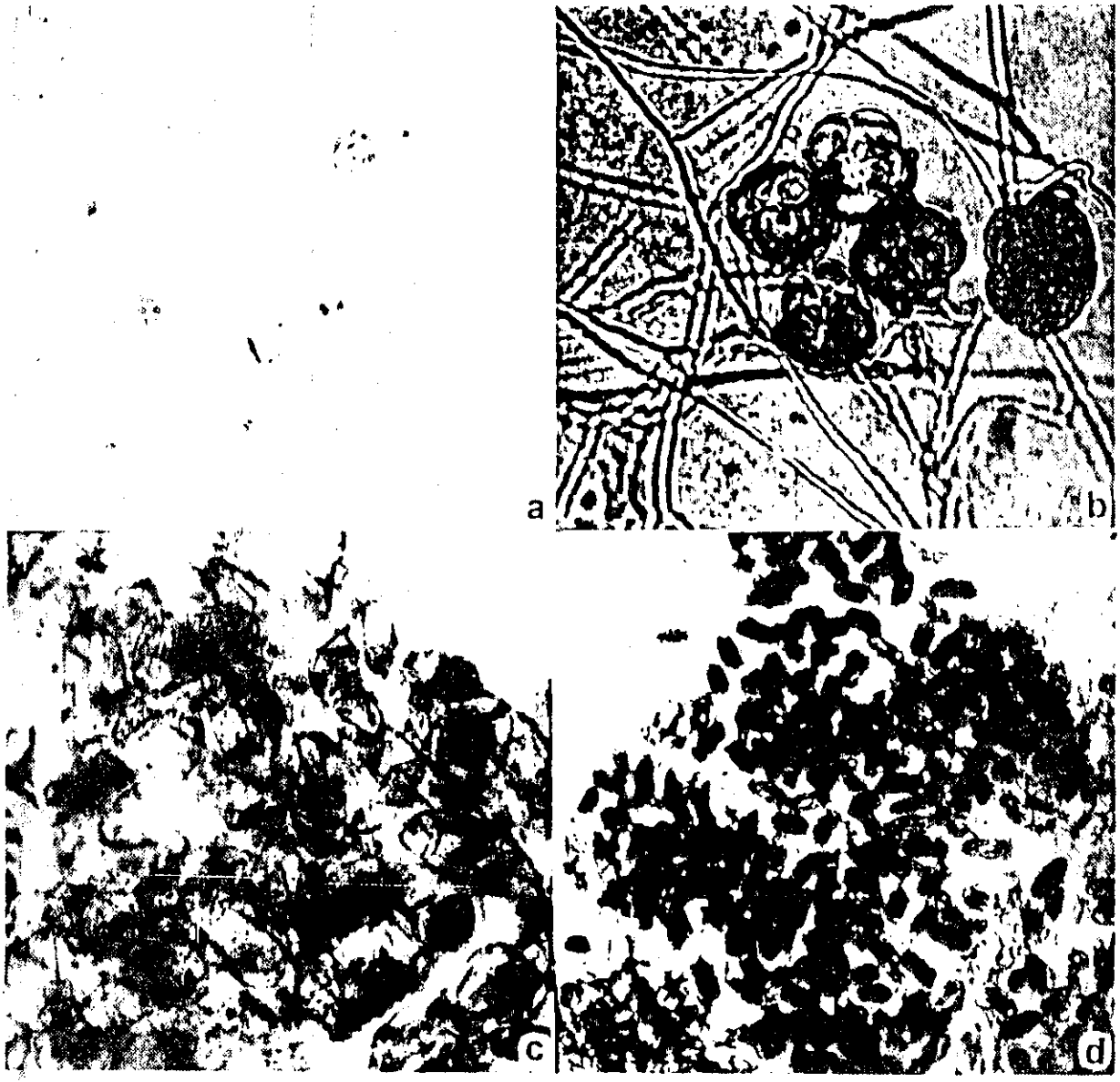

Fig. 1. a. Verticillate phialospores of *Verticillium chlamydosporium* b. Chlamydospores of *V. chlamydosporium* c. Root knot nematode egg mass with empty eggs (control) d. Root knot nematode egg mass colonized by *V. chlamydosporium* showing unhatched eggs.

was reported to be a potential parasite of cyst nematodes (Kerry & Crump 1977; Clyde 1992) and root knot nematodes (Morgan-Jones, Godoy & Rodriguez-Kabana 1981; Leij & Kerry 1991; Leij, Kerry & Dennehy 1993; Mertens & Stirling 1993). *V. chlamydosporium* readily colonizes rhizosphere and rhizoplane, infects adult females and egg masses and reduces nematode multiplication by inhibiting egg hatching. However, the level of parasitism may vary depending on the stage of embryonic development in the nematode eggs. *V. chlamydosporium* needs to be further evaluated in soil as a biocontrol agent to control root knot nematodes of spice crops. Since *V.*

Table 1. Pathogenicity of *Verticillium chlamydosporium* on egg masses of *Meloidogyne incognita*

Interval[a]	No. of juveniles hatched			Estimated control (%)
	Treated[b]	Untreated[b]	Mean[bc]	
1	228.55	504.66	339.62 b	56.3
2	258.82	391.74	318.41 b	33.8
4	451.85	746.44	580.76 a	39.6
5	206.06	278.61	239.33 b	28.5
Total	1161.45*	1963.36	-	41.4

[a] Number of days after fungal treatment

[b] Mean of juveniles transformed to logarithmic scale for analysis and retranslated

[c] Means followed by the same letter are not significantly different by Duncan's Multiple Range Test

*Significantly different (P=0.05) from that of untreated

chlamydosporium is a potential parasite of cyst and root knot nematodes, which are of economic importance in several agricultural and horticultural crops of India, the occurrence of a native isolate of this fungus is of much significance.

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