

ASSOCIATION OF PATHOGENS WITH RHIZOME ROT OF GINGER IN KERALA

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Abstract : During the survey for disease incidence, a total 288 diseased samples were collected from 195 ginger fields spread all over Kerala during 1984 and 1985. Individual samples were plated on special media like P₁₀ VP and Peptone PCNB medium besides nutrient agar and potato dextrose agar for isolation and identification. Of them, 19.79% samples yielded *Pythium aphanidermatum* and *P. myriotylum*, 6.6% gave *Fusarium Oxysporum* f.sp. *zingiberi* and *F. solani*, and 26.71% had *Pseudomonas solanacearum*. None of the samples yielded more than one kind of pathogen. Pathogenicity of the isolates was evaluated. Symptoms became apparant after 6 days in case of *Pseudomonas solanacearum* 10 days in *Pythium* spp. and 16 days in *Fusarium* spp.

Keywords : Association, Rhizome rot, Ginger

Rhizome rot of ginger (*Zingiber officinale* Rosc.) caused by *Pythium* spp. was known to exist from 1918 in the Malabar and South Kanara districts of South India (Thomas, 1938). Besides *Pythium aphanidermatum* (Edson) Fitzp; Subramanian 1919, *Pythium myriotylum* Drech. and *Pythium vexans* de Bary (Ramakrishnan, 1949), other pathogens reported to cause rhizome rot are *Fusarium oxysporum* Schlecht f. *zingiberi* Trujillo from Madhya Pradesh (Haware *et al.*, 1967); *Fusarium solani* (Mart.) Sacc. from Karnataka (Kumar, 1977); and *Pseudomonas solanacearum* E.F. Smith from Kerala (Sarma *et al.*, 1978; Mathew *et al.*, 1979). The ginger crop suffers from rhizome rot in field involving fungi and bacteria which ultimately lead to rhizome rot.

Hence, the present study was undertaken to identify the organisms associated with rhizome rot and to compare their pathogenicity, symptomatology under artificial inoculation in pots.

MATERIALS AND METHODS

Collection of samples

During the survey for disease incidence, a total of 796 samples (288 diseased and 508 healthy), mostly cultivars like Maran, Himachal Pradesh and Nadan were collected at random from 195 ginger fields spread all over Kerala and from Peruvannamuzhi Experimental Farm of National Research Centre for Spices, Calicut during 1984 and 1985, and were brought to the laboratory for isolation.

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Isolation

Individual infected specimens of ginger, viz. pseudostem and rhizomes were washed and cut into small pieces of 1-1.5 cm². These pieces were surface disinfected in 0.1 per cent mercuric chloride (Hg Cl₂) for 1 min. Four pieces of pseudostems and rhizomes from each sample were placed in petriplates of CMA containing P₁₀ VP (Tsao and Ocana, 1969) for *Pythium*, Nash and Snyder peptone PCNB medium (1962) for *Fusarium* spp. and PDA for general fungi; the plates were incubated at 26°C. The CMA amended with P₁₀VP petriplates were incubated in the dark for 96 hours. The hyphal tips of fungi growing from these pieces were transferred to PDA slants for maintenance.

For pieces of infected pseudostem were placed in a small quantity of sterile distilled water for 20 minutes to allow bacteria to ooze out. A loopful of 'bacterial suspension' was streaked on to nutrient agar and the plates were incubated at 26°C for 48 hours. Discrete colonies were transferred on yeast extract glucose carbonate agar (YDCA) slants and stored in sterile distilled water.

Pathogenicity

Pathogenicity of the isolates of *Pythium* spp. *Fusarium* spp. and *Pseudomonas solanacearum* was evaluated on hypocotyl tissue of 30 days' old sprouts grown in 12 × 15 cm size polythene bags on a standard pot mixture. Four hours before inoculation, bags were watered to field capacity in order to distribute the inoculum uniformly throughout the soil.

Isolates of *Pythium* spp. *Fusarium* spp. were grown separately on potato dextrose agar and *Pseudomonas solanacearum* on YDCA petriplates. Seven days old cultures of *Pythium* spp. and *Fusarium* spp. were harvested by washing mycelial growth with oospores/conidia and then suspended in 100 ml of tap water and maintained by a magnetic stirrer. The mycelial/spore suspension was added to the bags around the hypocotyl @ 50 ml per bag. A 24 h old bacterial growth was harvested with a cotton swab and applied at the collar region of pseudostem with prick injury.

Two plants were independently inoculated with the isolates of *Pythium*, *Fusarium* and *Pseudomonas* and suitable controls maintained either by pouring water or rubbing with a cotton swab. When the symptoms became apparent, the same were recorded from the infected plants and re-isolations made from them. Fungi in the genus *Pythium* were identified with the use of Waterhouse's Monograph (1968) and keys of Hendrix and Papa (1974), and *Fusarium* spp. with Booth's key (Booth, 1971 and 1977).

RESULTS AND DISCUSSION

Isolation

Out of 288 diseased samples, 19.79 per cent samples yielded *Pythium aphanidermatum*, *Pythium myriotylum*, 6.6 per cent *Fusarium oxysporum* f.sp. *zingiberi*, *Fusarium solani* and 26.71% *Pseudomonas solanacearum*. Of them, 93% isolates of *Pythium* spp., 79% of *Fusarium* spp. and 88.31% of *Pseudomonas solanacearum* were pathogenic. The association of *Pythium aphanidermatum* was more compared to *P.*

myriotylum, whereas there was no difference in *F. oxysporum* f.sp. *zingiberi* and *F. solani* (Table 1).

The individual samples plated for isolation yielded only one organism, i.e., either *Pythium* spp. or *Fusarium* spp. or *Pseudomonas solanacearum*. However, the samples collected from the same field yielded all the three pathogens.

Pathogenicity

The symptoms became apparent after 6 days at 28°C in case of plants inoculated with *Pseudomonas solanacearum* 10 days with *Pythium aphanidermatum*/*myriotylum* and 16 days with *Fusarium oxysporum* f.sp. *zingiberi*/*F. solani*. The symptoms exhibited for which those fungi/bacteria were isolated were typical of those occurring on plants. However, the time taken for expression of symptoms varied from isolate to isolate because the inoculations were carried out during 1984 and 1985 from September to December under pot culture conditions.

Symptomatology

The curling of leaf margins with drooping was noticed five days after inoculation in plants inoculated with *Pseudomonas solanacearum*. Yellowing started from the lowermost leaf and progressed upward until all leaves gave a golden yellow appearance. The milky exudate was present in infected pseudostem and rhizome, whereas in plants inoculated with *Pythium* spp., they became pale yellow followed by yellowing of the tips of the leaves and the infection spread along with the leaf margin. The pseudostem became soft, pale brown at collar portion. The conspicuous symptoms exhibited by

TABLE 1 : Association of microflora with rhizome rot of ginger in Kerala

| Districts | No. of samples collected | No. of diseased samples | No. of healthy samples | Associated pathogens | | |
|----------------|--------------------------|-------------------------|------------------------|---|---|---|
| | | | | <i>Pseudo-</i> <i>monas</i> <i>solana-</i> <i>cearum</i> | <i>Pythium</i> <i>aphani-</i> <i>dermatum/</i> <i>myriotylum</i> | <i>Fusarium</i> <i>o.f.sp.</i> <i>zingiberi/</i> <i>solani</i> |
| Wynad | 162 | 135 | 27 | 19 | 16 | 4 |
| Cannanore | 35 | 7 | 28 | 3 | 6 | 1 |
| Pathanamthitta | 55 | 34 | 21 | 16 | 3 | 2 |
| Kottayam | 10 | 7 | 3 | 0 | 0 | 0 |
| Ernakulam | 15 | 13 | 2 | 0 | 3 | 4 |
| Idukki | 43 | 0 | 43 | 0 | 0 | 0 |
| Quilon | 34 | 10 | 24 | 4 | 0 | 0 |
| Trivandrum | 28 | 6 | 22 | 0 | 3 | 1 |
| Malappuram | 39 | 16 | 23 | 5 | 8 | 0 |
| Palghat | 43 | 12 | 31 | 1 | 8 | 0 |
| Trichur | 105 | 32 | 73 | 8 | 8 | 1 |
| Calicut | 200 | 3 | 197 | 1 | 0 | 0 |
| NRCS Farm* | 28 | 28 | 14 | 20 | 2 | 6 |
| Total | 796 | 288 | 508 | 77 | 57 | 19 |

*Samples collected from experimental field of ginger at NRCS Peruvannamuzhi farm, Calicut.

plants inoculated with *Fusarium* spp. were yellowing of leaves followed by drooping and drying of plant. The discolouration was also noticed at the hypocotyl portion of pseudostem.

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