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A Comparative Study of Different Inoculation Techniques for *Verticillium* Wilt of Brinjal

BY

K. SIVAPRAKASAM[1] and C. K. SOUMINI RAJAGOPALAN[2]

ABSTRACT

A comparative study of five inoculation techniques has heen made under controlled conditions of infection. Among the inoculatien methods tried, hypodermic injection in the hypocotyl region of plants appears to be the ideal method for artificial inoculation.

INTRODUCTION

In India, *Verticillium* wilt was reported on brinjal from Maharashtra (Patel *et al.*, 1949) and Tamil Nadu (Natarajan *et al.*, 1968). Different inoculation techniques have been reported for inducing wilt disease under artificial conditions of infection. A comparative study of five techniques has been made and the results are reported here.

MATERIALS AND METHODS

Monosporic isolates of *Verticillium dahliae* were made from infected cotton and brinjal plants. Because of the high pathogenic ability of cotton isolate over brinjal isolate (Sivaprakasam, 1972) the cotton isolate was utilized for inoculation. The inocula were multiplied in the laboratory using Czapek-Dox broth at 20-24°C for 7 days in 250 ml Erlenmeyer flasks. The spores were separated from mycelial mat by keeping the flasks in a shaker for one hour and filtering through double layers of muslin cloth. This culture filtrate containing spores was diluted to the required concentration. This inoculum will be referred as spore inoculum.

The inocula were multiplied as in previous case using Czapek-Dox broth for 7 days. But no effort was made to separate the mycelial mat. The entire contents were poured out and macerated in a waring blender for 10 minutes. A slurry liquid containing spores, mycelium and microsclerotia was obtained, which was diluted to the required concentration. This inoculum will be referred to as macerated fungus inoculum.

The inocula were multiplied in sand maize medium (95:5) in 250 ml Erlenmeyer flasks for three weeks. This inoculum will be referred as sand-maize inoculum.

1. Assistant Plant Pathologist, Regional Research Station, Kovilpatti, 2. Associate Professor of Plant Pathology, Agricultural College and Research Institute, Madurai,

Hypodermic injection:

Spore inoculum was delivered from the syringe to form a bead of suspended conidia at the tip of the needle. The needle was inserted into the lower stem just above the soil level at approximately 45° to the stem until the tip of the needle is just visible. Plants injected with sterile water or Czapek's solution served as checks

Root dip inoculation:

The roots of brinjal seedlings after washing free of adhering soil particles were placed for 15 minutes in a macerated fungus inoculum. Control plants were placed in sterile water or Czapek's solution for a corresponding time. The treated plants were planted on sterilized soil.

Soil inoculation:

a) **Soil drench inoculation:** The macerated fungus inoculum was poured on the soil around the base of the plants at the rate of 100 ml per pot. Sterile water or Czapek's solution was poured in the soil around the base of control plants.

b) **Sand - maize inoculation:**

The sand-maize inoculum was transferred to pots containing brinjal plants at 5 per cent inoculum level. Control plants were treated with the same quantity of sand-maize medium without the fungus.

Foliage inoculation:

The macerated fungus inoculum was atomized on to the unabraded foliage of brinjal seedlings, care being taken to prevent the inoculum contaminating the soil and other parts of the plant. Sterile water of Czapek's solution was atomized on the foliage of control plants Pots were covered with polythene bags for 2 days to provide adequate humidity.

In all inoculation methods except sand-maize inoculation an uniform concentration of 10^7 spores per ml was used. For every treatment five replications were maintained, at the rate of 2 plants in a pot. All the pots were incubated in soil temperature tank maintained at 24 ± 1°C and periodical observations were recorded

RESULTS AND DISCUSSION

Among the inoculation techniques tried the root dipping technique was found to be the most effective in inducing wilt as was also the experience of Wiles (1952) and Erwin *et al.*, (1965). But most of the plants inoculated by

TABLE. 1. Different methods of inoculation and the incubation period.

Method of inoculation	Incubation period (days)
Root dip inoculation	6
Hypodermic injection	8
Soil drench inoculation	15
Sand-maize inoculation	25
Foliage inoculation	12

this technique died before becoming established However, hypodermic injection of pathogen into the hypocotyl region was found to give consistent results and induce symptom in 8 days. (Table 1) The value of this method of inoculation over others lies in its rapidity (Brinkerhoff, 1949). It was estimated that one person could inoculate at least 700 plants in 8 hr in the field and certainly more in the green house (Bugbee and Presley, 1967). But this method becomes ineffective if the seedlings to be inoculated are too young. In such cases introduction of sand-maize inoculum into the soil or pouring macerated fungus inoculum around the plant seemed to have practical utility. Spray inoculation of the foliage brought about onset of symptoms in the inoculated leaves in 12 days after inoculation. The possibility of the leaves being primary infection sites has received relatively little attention. [Foliage infection was obtained under artifical conditions of injection following inoculation of red clover (Sackston, 1959) and tomato and brinjal (Provvidenti and Schroeder, 1959).

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