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Factors affecting vegetative growth and asexual reproduction of *Phytophthora nicotianae* var. *nicotianae*

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Effects of solidified synthetic, semisynthetic and natural media on vegetative growth and sporangia production of *Phytophthora nicotianae* var. *nicotianae* were investigated. Oat-meal agar and Bean extract agar supported good vegetative growth. On brinjal fruit extract agar and synthetic CDA growth was comparable. On semisynthetic PDA growth was poorest. Sporangia production was significantly higher on BEA and BFEA than the other media tested. Sporangia production on mycelial mats grown on different nutrient media and infected host tissue blocks flooded with distilled water, tap water or 0.01M KNO_3 solutions was examined. Mycelial mats grown on OMA and flooded with 0.01M KNO_3 solution caused production of large number of sporangia. Tap water and distilled water supported moderate and poor sporangia production respectively. The results suggests that the brinjal isolate of the pathogen produced sporangia in presence of liquid medium containing salts.

Key Words : Brinjal fruit rot, *Phytophthora nicotianae*, post-harvest disease, asexual reproduction

INTRODUCTION

Fruit rot caused by *Phytophthora nicotianae* var. *nicotianae* is the most prevalent post-harvest disease of brinjal fruits in West Bengal (Guha Roy *et al.*, 1986). Besides post-harvest fruit rot the pathogen is known to cause rot of fruits in the field (Jain *et al.*, 1982) and damping off of seedlings (Guha Roy *et al.*, 1988). Information regarding the environmental and nutritional factors affecting vegetative growth and asexual reproduction of the pathogen is insufficient. The

objective of this work was to examine the factors affecting mycelial growth and sporangium production of *Phytophthora nicotianae* var *nicotianae*.

MATERIALS AND METHODS

A highly virulent strain of the pathogen was obtained from Kalyani University stock culture collection. The organism was maintained on Oat-meal agar slopes (OMA) at 20°C with monthly subculturing.

Linear growth of the organism was measured by transfering inoculum discs of PDA grown 7-day old culture of the pathogen to different agar media in 9 cm Petridishes. The plates were incubated at 28°C in the dark and the radial increment of the mat was measured at regular intervals from triplicate Petridishes.

For determination of growth as measured by dry weight increments, the organism was grown in different broth media. The media were seeded with inoculum discs of mycelial mat and incubated at 28°C in the dark. The mycelium was harvested at intervals and dried in an oven at 90°C till constant weight.

Induction of sporangia was investigated following the method of Gooding and Lucas (1959) with certain modifications. Mycelial discs from infected fruit tissues were cut out with sterilized cork borer, the agar base was scraped off and the mats were placed on glass slides kept in 9 cm Petridishes and flooded with different treatment solutions and incubated at different environmental conditions. The average number of sporangia produced per microscopic field was determined based on observation of 10 fields.

RESULTS AND DISCUSSION

Effect of nutrient media on vegetative growth

Effects of different nutrient media on the mycelial growth of the pathogen were examined. The organism was grown on Bean extract agar (BEA), Brinjal fruit extract agar (BFEA), Czapecks Dos agar (CDA), OMA, and potato dextrose agar (PDA) and the linear growth was measured on indicated days. The production of sporangia on these nutrient media was determined after 6 days of incubation The results are shown in Fig. 1,a. It is evident that both OMA and BEA were very good for vegetative growth and within 4 days the surface of the Petridish (90 mm.) was nearly or fully covered. On CDA and BFEA the linear growth was moderate and covered 50 percent of the surface of the Petridish after 4 days. Poor linear growth was observed on PDA. It is apparent from the data (Fig. 1,b.) that the synthetic CDA supported no sporulation. Sporangia production on CDA and PDA was moderate. However, BFEA and BEA supported very good sporulation.

Induction of sporangia in liquid media

The mycelial discs of the sporangium grown on different agar media or from infected host tissues were aseptically flooded with water or different treatment solutions and incubated at 28°C in the dark. At intervals the mycelial discs were examined under light microscope and the number of sporangia per microscopic field was counted. Average number per ten fields were determined, expressed as number per mm^2 and presented in Fig. 2. It is evident that flooding of the mycelial discs induced production of significant number of sporangia within two days irrespective of the source of the discs. Abundant easily harvestable sporangia were produced after 8 to 10 days of incubation in the submerged condition. It was of interest that mycelial discs obtained from OMA produced maximum number of sporangia irrespective of the treatment solutions. The mycelial discs from host tissue also produced abundant sporangia in different liquid solutions, but the number were less than OMA discs and greater than the number produced by the other mycelial discs. Moreover, of the three liquid media KNO_3 (Fig. 2,C) solution induced highest number of sporangia in OMA discs and deionised distilled water (Fig. 2,A) induced less number of sporangia than the tap water (Fig. 2,B).

It is evident from the study that the brinjal isolate of *Phytophthora nicotianae* var. *nicotianae* grew well on CDA containing sucrose but poorly on PDA. There are several reports that vegetative growth of *Phytophthora* species including *Phytophthora nicotianae* var. *nicotianae* is stimulated by sucrose, but somewhat inhibited by glucose (Singh, 1975, Shepherd and Pratt, 1973 ; Hohl, 1983). In several other species of *Phytophthora*, however, glucose has been found to be most stimulatory for growth (Vujicic and Colhoun, 1966).

There are reports that some species of *Phytophthora* forms sporangia abundantly on agar media and sparsely or not at all in liquid media, whereas many species forms sporangia abundantly in both liquid and solid media (Brasier, 1965). The brinjal isolate used in this study sporulated on solidified natural media. The total number of sporangia, however, were too low and since they were embedded in the medium, harvesting of intact sporangia from agar media was found to be quite difficult. When the mycelial discs of the test isolate were flooded with water or different treatment solutions abundant sporangia were produced within a short time which could be harvested easily by decanting the ambient liquid. The results are in conformity with previous works by many authors using different species and isolates of *Phytophthora* (Gooding and Lucas, 1959 ; Zentmyer and Erwin, 1970 ; Ribeiro, 1983). Of the test flooding solutions maximum sporulation was obtained in 0.01M KNO_3 solution using OMA agar grown mycelial mats (Fig. 2,C). Similar results have been obtained by other investigators using various species of *Phytophthora*.

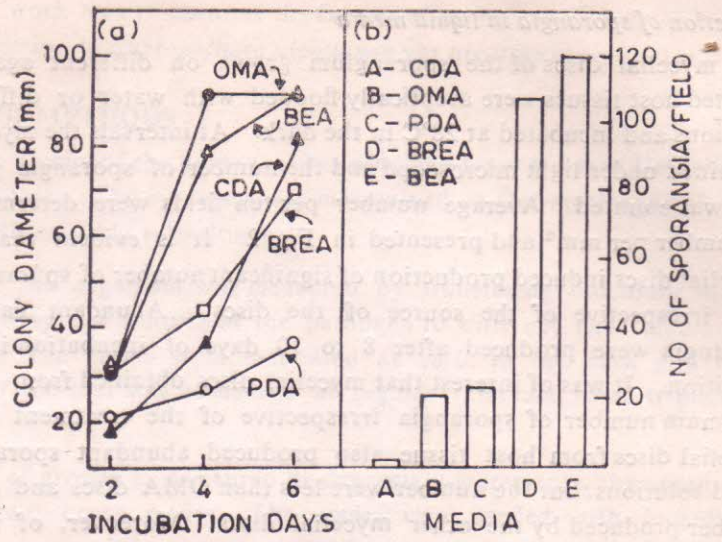

Fig. 1- Effects of different nutrient media on the linear growth (a) and sporangia production (b) by *P. nicotianae* var. *nicotianae* in vitro.

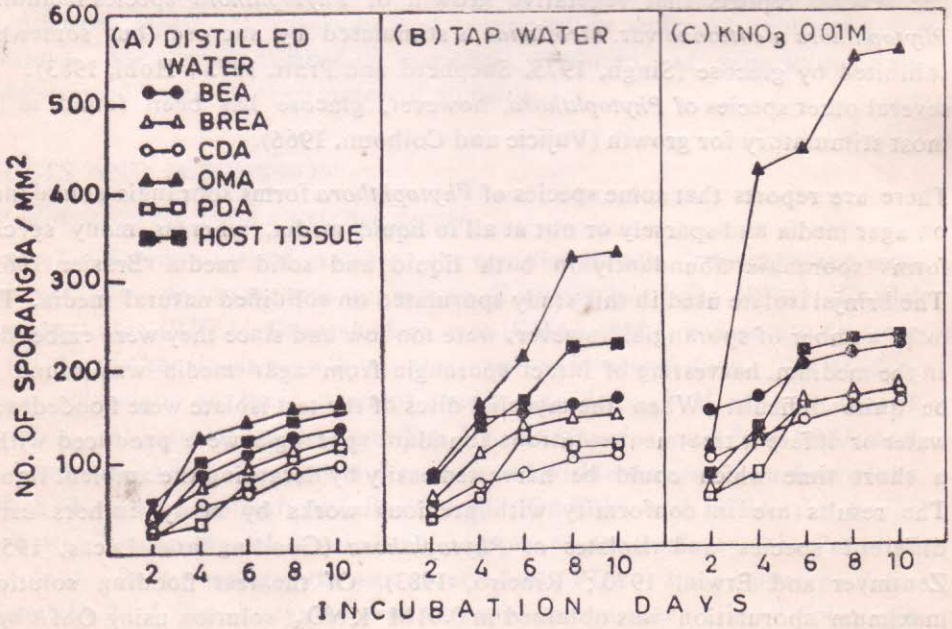

Fig. 2. Effects of nutrient source of mycelial discs and flooding solutions on the induction of sporangia in *Phytophthora nicotianae* var *nicotianae*.

Wills (1954) working with tobacco isolate of *Phytophthora nicotianae* var. *nicotianae* reported that no sporangia were formed in distilled water. Gooding and Lucas (1959) reported good sporulation of the same pathogen in distilled water. It was of interest that eggplant isolate of the pathogen used in this study sporulated well both in distilled and tap water.

Good sporulation in tap water but not in distilled water by many *Phytophthora* species is thought to be due to the presence of dissolved salts in tap water (Ribeiro, 1983). The brinjal isolate produced abundant sporangia in tap water and nitrate solutions which suggest that dissolved salts and ions are necessary for induction of sporangia in this isolate.

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