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A MEDIUM FOR *IN VITRO* PRODUCTION OF TOXINS BY *PHYTOPHTHORA CAPSICI* FROM BLACK PEPPER

Several plant pathogenic organisms are known to produce toxic substances which produce all or part of disease syndromes on the host and some related plants. The toxic metabolites produced by the pathogens play a significant role in pathogenesis. *Phytophthora* spp. are no exception to it (Savel'eva and Rubin, 1963). Siedel (1961) studied the nutrient requirement for the toxin production of *Phytophthora infestans* and found that varying amounts of toxins were produced in different media. Lee (1973) utilized the toxic metabolite of *P. palmivora* for screening black pepper varieties. In the present study, an attempt is made to find out a suitable medium for toxin production by *P. capsici* from pepper.

For *in vitro* production of toxin by *P. capsici*, seven day old, 5 mm culture disc of the fungus was inoculated in 250 ml conical flasks containing 100 ml of media. Five different liquid media viz., Richards+yeast extract (2.5%) broth, potato dextrose broth, oat meal broth, corn meal broth and a synthetic liquid medium for *Phytophthora* (Singh, 1975) were used for the study. After inoculation the flasks were incubated at 22.0 ± 1°C for 15 days. Then the cultures were filtered using Whatman No. 1 filter paper and the filtrate was centrifuged at 1000 g for 15 minutes and the pellets were discarded. The supernatant liquid was dialysed against glass distilled sterile water for two hours. The efficacy of different propagule free dialysed culture filtrate was tested by inoculating the leaves of one year old pepper plant of the variety Panniyur-1. The propagule free dialysed culture filtrates collected from different liquid media were placed evenly in the surface of five leaves of uniform age at the rate of 0.05 ml per leaf, after gently pricking the area with sterile needle. Sterile dialysed uninoculated liquid media served as control. For comparing the symptom, five leaves of one pepper plant were inoculated with culture disc of 5 mm diameter on the lower surface and swabbed with sterile moist cotton. After inoculation, plants were covered with polythene bags to provide high humidity.

Table 1. Comparison of different media on toxin production by *P. capsici* (Toxin assayed as average diameter of necrotic spots formed on the leaves of *Piper nigrum* variety Panniyur-1)

Name of media	Mean symptom devtlopment, mm				
	1st day	2nd day	3rd day	4th day	5th day
1 Richards + Yeast extract broth	4.20	10.28	13.19	14.81	14.81
2 Potato dextrose broth	2.18	6.75	9.25	10.25	10.25
3 Com meal broth	1.71	5.28	7.75	8.56	8.56
4 Oat meal broth	1.52	4.50	7.13	7.63	7.63
5 Synthetic medium for *Phytophthora*	2.14	6.44	8.69	9.56	9.56
CD (0.05)					1.654

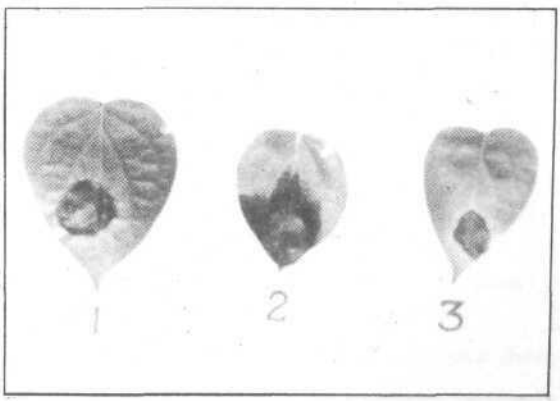

Fig 1. Symptom expression on the leaves of *Piper nigrum* (Panniyur-1). 1. Natural infection; 2. Inoculation with *P. capsici* culture disc; 3. Assay of propagule-free dialysed culture filtrate of *P. capsici*

The propagule free toxic metabolite obtained from all the five liquid media produced necrotic spots which are quite typical as that of artificial and natural infection of *P. capsici* (Fig 1), while the control plants were free from infection. Lesions developed 24 hours after treatment with culture filtrate. At this stage, the maximum necrotic area of 4.2 mm was noticed on leaves treated with pathogen free dialysed culture filtrate from Richards + yeast extract broth, the minimum necrosis was observed in oat meal broth (1.52 mm) (Table 1). The lesions expanded quickly up to 4th day of inoculation and ceased to develop further. Statistical analysis of the 5th day data showed that the maximum necrotic area on the leaves was obtained in the propagule free dialysed culture filtrate of Richards + yeast extract broth (14.81 mm) followed by potato dextrose broth (10.25 mm) (Table 1).

In this study, the symptom expression observed in leaves treated with culture filtrate was almost same as was noticed in natural infection by the pathogen (Fig 1), thus fulfilling the criteria prescribed for a phytotoxin by Graniti (1972). From the study, it can be reasonably presumed that the propagule free dialysed culture filtrates of *P. capsici* in different media have toxic metabolites which are responsible for symptom expression by *P. capsici* in black pepper. This finding is in conformity with the results obtained by Lee (1973).

Gilchrist and Grogan (1977) reported the use of yeast extract for obtaining high quantity of toxic metabolite in synthetic media. Since, the symptoms produced by the toxic metabolite produced by *P. capsici* in the culture media are same as that of the pathogen, this can be used for varietal screening of pepper against *P. capsici* throughout the year unlike the artificial inoculation with the pathogen which is usually seen mainly during rainy season. Further, unlike the pathogen in this case there is no chance of spread of the disease by artificial inoculation.

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BACTERIAL WILT OF PUMPKIN (*CUCURBITAMOSCHATA* POIR) AND SNAKEGOURD (*TRICHOSANTHESANGUINA* L.) INCITED BY *PSEUDOMONAS SOLANACEARUM*(SMITH) SMITH FROM INDIA

Recently a serious wilt symptom was noticed in some of the pumpkin and snakegourd plants grown in the vegetable garden of the College of Horticulture, Thrissur, Kerala. The characteristic symptom of the disease was the drooping of the leaves followed by rapid wilting of the entire plant. Brown vascular discolouration of stem and root tissues was also observed.

The diseased plants were subjected to routine laboratory studies. On ooze test, profuse bacterial ooze was noticed from the fresh cut surface of infected portion. The bacterium was isolated on potato dextrose agar medium and then on triphenyl-tetrazolium chloride (TTC) medium. Greyish white with light pink centered slimy colonies were obtained on TTC medium. Artificial inoculation on healthy plants showed typical wilt symptoms. Cross inoculation of the bacterium on solanaceous plants also gave the same wilt symptoms. The cultural, biochemical and physiological characters of the bacterial isolate were studied according to Breed *et al.* (1957), Buchanan and Gibbons (1974). When the results of the study were combined with pathogenicity test and cross inoculation study, the organism causing the wilt of pumpkin and snakegourd could be identified as *Pseudomonas solanacearum* (Smith) Smith. A perusal of literature revealed that bacterial wilt of pumpkin caused by *Pseudomonas solanacearum* was reported by Date *et al*, (1992) from Japan. There is no other record of this bacterium causing diseases of pumpkin and snakegourd from India. So occurrence of bacterial wilt of pumpkin (*Cucurbita moschata* Poir) and snakegourd (*Trichosanthes anguina* L.) incited by *Pseudomonas solanacearum* (Smith) Smith is recorded for the first time from India.

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