http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (11): 1374-1376, 2001

Standardization of Medium for the Production of Maximum Phytotoxic Activity by *Fusarium oxysporum* f. sp. *ciceris*

Iftikhar A. Khan, S.S. Alam and [1]A. Jabbar
Phytopathological Chemistry Lab. NIAB, Jhang road, Faisalabad, Pakistan
[1]Department of Chemistry, Islamia University, Bahawalpur, Pakistan

Abstract: Five liquid media were studied for the production of dry weight\ spores of *Fusarium oxysporum* f. sp. *ciceris* and pH/ phytotoxicity of the culture filtrates after each 7 days incubation up to 28 days. Maximum increase in pH was observed in minimal medium (MM) at 21 days. The media potato dextrose broth (PDB) and minimal medium containing chickpea root extract (MM+) were found suitable for sporulation of the fungus, while maximum dry weight of the fungus was obtained in PDB medium. Phytotoxicity assay of these culture filtrates revealed that the maximum activity was produced in PDB medium, while ethyl acetate phases of the culture filtrates from Czapek dox (CZ) medium and minimal medium (MM) showed maximum phytotoxic activity.

Key words: Phytotoxicity, culture filtrates, *Fusarium oxysporum* f. sp. *ciceris*, minimal medium, Czapek dox, PDB.

Introduction

Fusarium wilt of chickpea caused by *Fusarium oxysporum* Schlect. Fr. f. sp. *ciceris* (Padwick) Matuo & Sato (FOC) has been reported at 10-50% incidence in dry areas of Pakistan during the last several years, while in irrigated belts of Punjab, farmers have shifted to other crops only due to this single disease (Ikramul Haq and Farhat, 1992). The fungus is also reported from all areas of chickpea cultivation, including Bangladesh, Burma, California, Ethiopia, India, Malavi, Mexico, Morocco, Pakistan, Peru, Syria, Tunisia, Turkey, USSR and Spain (Nene *et al.*, 1984).

Pathogenic fungi may often damage their host plants by producing toxins, which cause various symptoms including necrosis, chlorosis, wilting, water soaking and eventually the death of plants (Scheffer, 1983). One criterion of the importance of a toxin in a disease syndrome caused by a pathogen is that toxigenicity should be related to pathogenicity or virulence. Toxins can be used for screening of resistance, selection for resistance in tissue culture for which resistant plants may be regenerated and genetical engineering plants to destroy the toxic compound e.g. genotypes of oats that were resistant to the victorin toxin produced by *Helminthosporium victoria* were also resistant to fungus (Wheeler and Luke, 1955) and partially purified toxins of *H. oryzae*, the pathogen of brown spot of rice, were used to select resistant calli. They were able to regenerate resistant plants that were heritable and stable (Vidhyasekaran, 1990). Toxin production by a fungus can be studied by growing it on a suitable medium and testing culture filtrates at various times for toxicity. Alam *et al.* (1989) also demonstrated that *A. rabiei*, a pathogen of chickpea, produced toxic culture filtrates when grown for 12 days on Czapek-dox liquid medium supplemented with an aqueous extract of chickpea seeds.

The objective of the present studies was to standardize the suitable medium for the production of phytotoxins of *F. oxysporum* f. sp. *ciceris*.

Materials and Methods

Isolation, Identification and Maintenance of FOC: FOC isolate was obtained from wilted seedlings of chickpea. Collar regions from diseased seedlings were cut, surface sterilized with 2% sodium hypochlorite for 2 min., rinsed in distilled water and placed on petri plates containing Komada's medium (KM), specific for *Fusarium oxysporum* (Komada, 1975). Plates were incubated at 25 ± 2 °C in dark for 5-7 days. FOC colonies appeared on KM medium were subcultured and single spored on PDA. Identification of the isolates was confirmed on carnation leaf agar medium (CLA) and later was maintained on CLA (Fisher *et al.*, 1982) and chickpea agar meal (2% chickpea meal and 2% agar) and were stored in liquid nitrogen at −15 °C in deep freezer for further use. Pathogenicity of the isolate was done by pot method (Nene *et al.*, 1981) on susceptible cultivar Aug-424.

Standardization of medium for the production of maximum phytotoxicity: Following liquid media were prepared: 1) Minimal medium (MM) as described by Hanif & Ikram (1998). 2) Minimal medium supplemented with chickpea root extract (MM+). 3) Czapek dox medium (CZ). 4) Czapek dox medium containing chickpea root extract (CZ+). 5) Potato dextrose broth (PDB). The media were distributed in 50 ml aliquots in 250 ml conical flasks and, after autoclaving at 121°C for 15 min and cooling, inoculated with 2mm disc of 7 days old culture of FOC. The flasks were incubated at 25°C in dark without shaking and culture filtrates were harvested in triplicate at 7, 14, 21 and 28 days by filtering through muslin cloth and were studied for their pH, phytotoxicity and spore concentration. Phytotoxicity of the culture filtrates was determined by the cut seedling method (Huang and Hartman, 1998). The mycelial mats were taken in 50ml conical flasks and placed in oven at 90 °C for 72 hrs and then weighed on an analytical balance.

Extraction with ethyl acetate: Culture filtrates obtained from each medium after 21 days (100 mL) were adjusted to a pH of 3.0 using 2M sulphuric acid (H_2SO_4) and partitioned three times into half the volume of ethyl acetate. The ethyl acetate phases (upper part) were combined and dried over anhydrous sodium sulphate (Na_2SO_4). They were filtered through Whatman filter paper No.1 to eliminate the sodium sulphate powder and the filtrate was evaporated to dryness at 30 °C using a vacuum evaporator (Büchi Rotavapor, model R110). The residues were dissolved in 3 ml ethanol. From these stock solutions 50 µl, 100 µl, and 150 µl were pipetted into small vials, diluted with 5 ml distilled water in three replicates and phytotoxicity were determined by cut seedling method against two weeks old chickpea cuttings of Aug-424 cultivar.

Results and Discussion

The fungus was pathogenic to chickpea and caused complete wilting after twenty days of sowing. It produced typical symptoms i.e., vascular discoloration at collar region, drooping of leaves and finally the wilting.

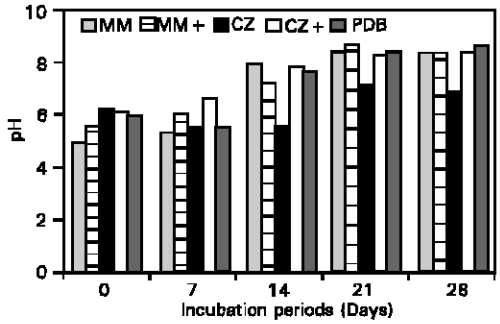

Fig. 1: pH of culture filtrates of FOC from different media at varying incubation periods.

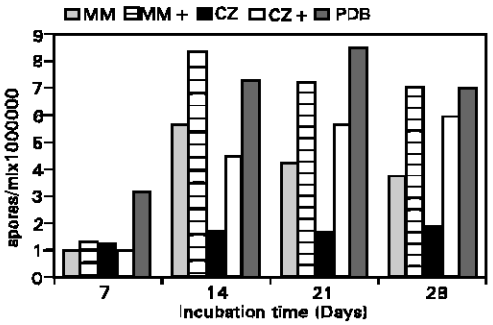

Fig. 2: Spores\ml of FOC on different media at various incubation periods.

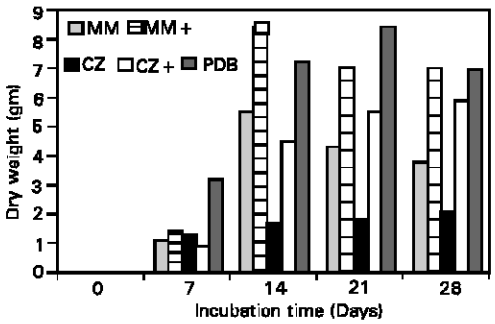

Fig. 3: Dry weight of FOC on different media at varying incubation time

pH: Culture filtrates of different media showed variable pH (Fig. 1) with increase in incubation period. Culture filtrates in CZ and PDB media were found to decrease first at 7 days incubation time and then increased up to 21\28 days respectively. The pH continuously increased for 28 days in CZ+ (8.56) and PDB (8.73) media. The maximum pH was found in MM+ (8.82) at 21 days and PDB media. In CZ medium least change in PH was observed. The increase in pH of culture filtrates indicated that most of the metabolites produced by the fungus might be basic in nature.

Spore production: The spore production (Fig. 2) in MM and MM+ media increased up to 14 days then decreased with further incubation, while in CZ and CZ+ a continuous increase was observed up to 28 days incubation. In PDB medium spore

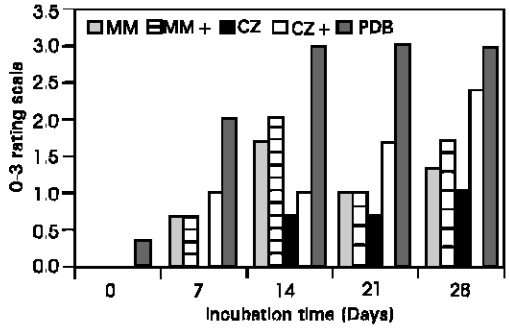

Fig. 4: Activity of culture filtrates of FOC from different media at various incubation time (O=healthy, 1=yellowing\burning, 2=drooping, 3=wilting)

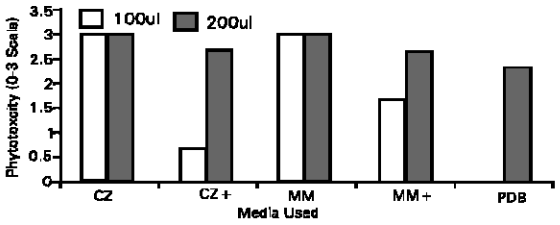

Fig. 5: Activity of ethyl acetate phases extracted from culture filtrates of different media.

concentration increased up to 21 days and then decreased at 28 days. Maximum spores were produced in MM+ (8.5x 10^6 at 14 days) and PDB (8.5x 10^6 at 21 days), followed by CZ+ (6.05 x 10^6 at 28 days), MM (4.23 x 10^6 at 21 days) and in CZ (1.92 x 10^6 at 28 days). The results showed that the media containing chickpea root extract (MM+ and CZ+) or any organic supplement like PDB produced more spores as compared to the simple media (CZ and MM). This showed that organic supplements were most favorable for the growth of the fungus, as Woltz and Engelhard (1973) reported that *F. oxysporum* cultured on organic nitrogen source was more virulent than the nitrate nitrogen.

Dry weight: The dry weight (Fig. 3) of the fungus increased in MM, MM+, CZ+ and PDB media up to 14 days and for 21 days in CZ medium then decreased with further incubation. The decrease in the dry weight of fungus has also been reported in *Ascochyta rabiei* (Alam *et al.*, 1989). Maximum dry weight was produced in PDB medium (576 mg at 21 days), followed by MM (403 mg at 21 days), MM+\ CZ+ (343 mg at 21 days) and in CZ medium (306 mg at 21 days). Less dry weight of the fungus on MM+/CZ+ as compared to MM might be due to the presence of antifungal phenolic compounds in host root extract (Alam & Strange, 1995), which might inhibit the growth of fungus.

Phytotoxicity: Phytotoxicity assay (Fig. 4) on chickpea seedlings revealed that PDB medium produced maximum phytotoxicity at 14, 21 and 28 days. The phytotoxic activity of the culture filtrate of MM and MM+ media increased up to 14 days then decreased with further incubation, while the phytotoxicity of the culture filtrates from CZ and CZ+ media constantly increased up to 28 days. The order of phytotoxic

activity produced by FOC in these media was as follows: PDB > CZ+ > MM+ > MM > CZ. When the culture filtrates were extracted in ethyl acetate at pH 3.00, the order of ethyl acetate phase phytotoxicity was reversed and was as follows CZ\MM > CZ+\MM+ > PDB (Fig. 5). This showed that the fungus growing on the media containing organic supplements like chickpea root extract e.g., in MM+, CZ+ and PDB, produced different phytotoxic metabolites. Being different, the polarity of these compounds was also different from the metabolites produced on CZ\MM media, so ethyl acetate did not recover these compounds, furthermore the toxic metabolites produced on CZ/MM are easier to identify and purify than other media containing organic supplements. The present study showed that organic supplements like chickpea root extract, potato starch\ nutrient broth could be useful for the production of spore and mycelial growth and Czapek dox/ minimal could be used for the production of phytotoxic activity of FOC.

References

Alam, S.S., J.H. Bilton, A.M.Z. Slawin, D.J. Williams, R.N. Sheppard and R.N. Strange, 1989, Chickpea blight: Production of he phytotoxins solanapyrones A and C by *Ascochyta rabiei*. Photochem., 28: 2627-30.

Alam, S.S. and R. N. Strange, 1995. Identification of medicarpan, maackiaan and formononetin from germinating seed of chickpea. Pak. J. Phytopathol., 7: 38-40.

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson, 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathol., 72: 151 -153.

Hanif, M. and Ikramul Haq, 1998. Nitrate non-utilizing mutants (nit mutants) of *Fusarium oxysporum* f.sp. *ciceris*. Pak. J. Phytopathol., Vol., 10: 57-77.

Ikramul Haq and Farhat F. Jamil, 1992. Screening of chickpea lines in the wilt sick plot and effect of environmental temperature on wilt incidence. Proceedings of COMSTECH-NIAB, International workshop on agroclimatology, pests and diseases and their control. November, 21-26, 1992, Faisalabad, Pakistan.

Huang, Y. H. and G.L. Hartman, 1998. Reaction of selected soybean genotypes to isolates of *Fusarium solani* f.sp. *glycines* and their culture filtrates. Phytopathol., 82: 999-1002.

Komada, H., 1975. Development of selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Pl. Prot. Res., 8: 114 - 124.

Nene, Y.L., M.P. Haware and M.V. Reddy, 1981. Chickpea diseases: Resistant screening techniques. ICRISAT Information Bullion, 10.

Nene, Y.L., V.K. Sheila and S.B. Sharma, 1984. A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.) pathogens. ICRISAT, Pulse progress report, 32: 19.

Scheffer, R.P., 1983. Toxins as chemical determinant of plant diseases. p. 1-40. In Toxins in plant pathogenesis. J.M. Daly and B.J. Darval, Eds. Academic Press, Sydney, 181.

Vidhyasekaran, P., D.H. Ling, E.S. Borromeo, F.J. Zapata and T.W. Mew, 1990. Selection of brown spot-resistant rice plants from *Helminthosporium oryzae* toxin-resistant calluses. Ann. Appl. Biol., 117: 515-523.

Wheeler, H.E. and H.H. Luke, 1955. Mass screening for disease-resistant mutants in oats. Sci., 122, 1229.

Woltz, S.S. and A.W. Engelhard, 1973. Fusarium wilt of Chrysanthemum: Effect of nitrogen source and lime on disease development. Phytopathol., 63: 155-157.