Short communication

Effect of nutrient media on radial growth of
Fusarium oxysporum f. sp. chrysanthemi

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Abstract

Chrysanthemum flower (Chrysanthemum morifolium Ramat.) is popularly designated as “Queen of the east”, or autumn queen (as its bloom in November-December). F. oxysporum f. sp. chrysanthemi causing vascular wilt of chrysanthemum is one of the most devastating pathogens infecting this flower crop. This particular disease gains more significance in terms of economic loss as it can attack the crop at all the growth stages ranging from nursery to flowering. Considering the seriousness of the disease and economic importance of the crop study of this pathogen becomes more essential in all aspects, i.e., cultural, biochemical and molecular aspects. In this experiment an attempt was made to record reaction of F. oxysporum f. sp. chrysanthemi in terms of cultural characteristics on seven different culture media. Data recorded after 10 days of inoculation, exhibited that Corn Extract Agar was the best media with 89.33 mm radial growth, while as, Czapek’s Dox Agar supported least radial growth of 58.33 mm. The test fungus sporulated on all the culture media under test, but, RSA and CEA supported maximum sporulation.

Keywords: Chrysanthemum, vascular wilt, radial growth, sporulation, culture media

Introduction

The word chrysanthemum, owes its origin to two Greek words, i.e., ‘chrysos’ (gold) and ‘anthemon’ or ‘anthos’ (flower). Chrysanthemum flower (Chrysanthemum morifolium Ramat.), belonging to the family Asteraceae (Compositae) is a partly woody erect perennial herb or sub shrub. This beautiful flower is popularly designated as “Queen of the east”, Mums, or autumn queen (as its bloom in November-December). In India, it is popular in the name of Guldaudi and Chandramallika etc. This magnificent flower holds a quite significant place in the global ornamental market, as it stands to the second position just after rose. The crop is vulnerable to several abiotic and biotic stresses including insect-pests, weeds, and pathogens (as, fungi, bacteria, viruses, etc.). Among all the known pathogens, F. oxysporum f. sp. chrysanthemi (FoC) is causing vascular wilt is one of the most devastating pathogens attacking all the growth stages (ranging from nursery to flowering). This devastating threat is of global importance, with its wide spread nature, i.e., occurrence is reported from all the chrysanthemum growing areas of word, including India.

The seriousness of the pathogen increases with its nature of parasitism and survival. As, this facultative saprophyte can survive in soil up to six years in the absence of susceptible host. Considering the nature of damage and survival ability of the fungus, use of resistant varieties is the only economical and available practical solution. But unfortunately, most of the resistant varieties become susceptible after some years because of breakdown in their resistance and evolution of variability in the pathogen. Therefore, to address the seriousness of this fungal pathogen an attempt was made to study the cultural characteristics on seven different culture mediums viz., Czapek’s Dox Agar (CDA), Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Richard’s Agar (RA), Rose Bengal Agar (RBA), Nutrient Agar (NA) and Corn Extract Agar medium (CEA). The results of this experiments can be utilized to identify the best culture media, which can be further exploited for isolation, routine culturing and sporulation studies.
Material method
Isolation of the fungal pathogen
Identification of the pathogen
a) Cultural characteristics.
b) Microscopic studies.
c) Pathogenicity testing.

Preparation and testing cultural characters on various culture media
The present studies were carried out under laboratory conditions at during 2017-18 in Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh (India). To carry out this experiment all the aforementioned media (Table no. 1) prepared in 1 liter of water and sterilized at 1.1 kg/cm² pressure (121.6 °C) for 15 min and poured in 90 mm sterilized Petri plates. Such Petri plates were inoculated with five mm disc cut from the periphery of actively growing culture and incubated at 25±2 °C. Each treatment was replicated thrice. The radial growth of the fungus was recorded at 3, 7 and 10 days after inoculation. The data for sporulation was recorded 10 days after inoculation with the help of hemocytometer.

Results
Results of the study showed suitability of all the tested media with reference to their radial growth, proving the ability of FoC to exploit and grow on diverse nutritional conditions. But interestingly, there was a remarkable variation spotted, in terms of radial growth and sporulation over all the tested media. However, a significant maximum radial growth (after 10 days of inoculation) was recorded on Corn Extract Agar (89.33 mm) followed by Richards’s Agar (86.66 mm) and Potato dextrose agar (82.66 mm), meanwhile, RSA and CMA were statistically at par in terms of radial growth. While, the least radial growth was recorded on Czapek’s Dox Agar (58.33 mm) followed by RBA (Rose Bangal Agar) and NA (Nutrient Agar) (Table 1).

The present study showed the variation of F. oxysporum when they were grown on same or different culture media. PDA and Richards Agar were found to be most similar in terms of radial growth. While, the least radial growth was observed best on Potato Agar medium followed by Richard’s agar medium. The test fungus sporulates in all the media under test, but interestingly none of all these can said to be excellent sporulation media as sporulation on all growth media is more or less same. But at the same time, RSA and CEA were the best among all to support maximum sporulation. Hence, according to the present finding CMA, RSA and PDA can be recommended for further cultural and sporulation studies.

Discussion
The present finding is corroborative with Gangadhara et al., 2004; Anjaneya 2002 [1], they studied the variation of Fusarium oxysporum when they were grown on same or different culture media. PDA and Richards Agar were found to be most suitable for their culture. Ingle (1995) [3] are observed similar result in F. udum. Khan et al., (2011) [4] conducted in-vitro studies to check the growth of the F. oxysporum f. sp. ciceri on different solid medium. The fungal growth was observed best on Potato Agar medium followed by Richard’s agar medium. Nath et al., (2016) [5] examined nine isolates of F. oxysporum in laboratory to study the effect of different culture media. The fungus was grown well on oat meal agar medium among seven culture media tested. Rao et al., (1989) [7] also showed good growth of F. oxysporum on Potato Dextrose Agar and Richards’ agar.

Table 1: Media composition media used during the studies, following media with given composition were used

| Media name               | Ingredients                           | Quantity |
|--------------------------|---------------------------------------|----------|
| Czapek’s Dox Agar (CDA)  | Cane sugar                            | 30.00 g  |
|                          | Monopotassium phosphate               | 1.0 g    |
|                          | Magnesium sulfate                     | 0.5 g    |
|                          | Potassium chloride                    | 0.5 g    |
|                          | Iron sulfate                          | 0.01 g   |
|                          | Agar                                  | 15.00 g  |
| Corn Meal Agar (readymade)| Corn meal                             | 50.00 g  |
|                          | Dextrose                              | 2.00 g   |
|                          | Agar                                  | 15.00 g  |
| Corn Meal Agar (by corn extract)| Maize seed                           | 200.00 g |
|                          | Dextrose                              | 20.00 g  |
|                          | Agar                                  | 15.00 g  |
| Potato Dextrose Agar (PDA)| Peeled potato                         | 200.00 g |
|                          | Dextrose                              | 20.00 g  |
|                          | Agar                                  | 20.00 g  |
| Richards’s Agar (RSA)    | Potassium nitrate                     | 10.00 g  |
|                          | Potassium dihydrogen phosphate        | 5.00 g   |
|                          | Magnesium sulphate                    | 2.50 g   |
|                          | Ferric chloride                       | 0.2 g    |
|                          | Sucrose                               | 50.00 g  |
|                          | Agar                                  | 15.00 g  |
| Rose Bengal Agar (RBA)   | MgSO₄.7H₂O                            | 0.2 g    |
|                          | K₂HPO₄                                | 0.9 g    |
|                          | NH₄NO₃                               | 1.0 g    |
|                          | KCl                                   | 0.15 g   |
|                          | Rose Bengal                           | 0.15 g   |
|                          | Glucose                               | 3.0 g    |
|                          | Agar                                  | 20.00 g  |
| Nutrient Agar (NA)       | Peptone                               | 5.00 g   |
|                          | Beef extract                          | 3.00 g   |
|                          | Sodium chloride                       | 8.00 g   |
|                          | Agar                                  | 15.00 g  |

*Water 1000 ml was used in all the media
### Table 2: Reaction of culture media on radial growth of *Fusarium oxysporum* f. sp. *chrysanthemi*

| Different culture media         | Sporulation | 3 days after | 7 days after | 10 days after | Colony characters                                                                 |
|--------------------------------|-------------|--------------|--------------|---------------|----------------------------------------------------------------------------------|
| Czapek’s Dox Agar (CDA)        | ++          | 19.33        | 33.33        | 58.33         | Circular white yellow colonies of aerial mycelium                                |
|                                |             | (26.06)      | (35.23)      | (49.86)       |                                                                                  |
| Corn Meal Agar (readymade)     | ++++        | 32.00        | 52.66        | 85.33         | Dull white regular mycelial colony                                               |
|                                |             | (34.42)      | (46.51)      | (67.60)       |                                                                                  |
| Corn Meal Agar (by corn extract)| ++++       | 42.00        | 65.33        | 89.33         | Deep white regular mycelial colony                                               |
|                                |             | (40.37)      | (53.94)      | (70.92)       |                                                                                  |
| Potato Dextrose Agar (PDA)     | +++         | 34.00        | 60.00        | 82.66         | White colour colonies of aerial mycelium with regular margin.                    |
|                                |             | (35.63)      | (50.76)      | (65.42)       |                                                                                  |
| Richards’s Agar (RSA)          | +++         | 30.00        | 63.33        | 86.66         | Bright white colony with profuse aerial mycelium, margins of the colonies appear irregular |
|                                |             | (33.19)      | (52.72)      | (68.64)       |                                                                                  |
| Rose Bengal Agar (RBA)         | ++          | 26.00        | 50.00        | 80.00         | Pink colour, uplifted cushion like mycelial colony with wavy margins            |
|                                |             | (30.63)      | (44.98)      | (63.42)       |                                                                                  |
| Nutrient Agar (NA)             | +           | 32.00        | 57.33        | 77.66         | Light yellow white colonies with regular colonies                                |
|                                |             | (34.42)      | (49.20)      | (61.79)       |                                                                                  |
| C.D (P<0.05)                   |             | 4.08         | 6.07         | 8.34          |                                                                                  |
|                                |             | (2.52)       | (3.59)       | (5.53)        |                                                                                  |
| C.V.                           |             | 7.50         | 6.29         | 5.90          |                                                                                  |
|                                |             | (4.24)       | (4.26)       | (4.89)        |                                                                                  |

### Conclusion

It is clear from the foregoing discussion that *F. oxysporum* f. sp. *chrysanthemi* preferred Corn Meal Agar (CMA), Richards’s Agar (RA) and Potato Dextrose Agar (PDA) for its maximum radial growth in comparison to others.

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