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IN VITRO STUDIES ON DUAL CULTURE OF PROTOMYCES MACROSPORUS ON CORIANDRUM SATIVUM

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ABSTRACT

The dual culture of Coriandrum sativum infected by Protomyces macrosporus, raised on MS medium (Murashige and Skoog 1962) supplemented with NAA (8.0mg/l), kinetin(0.5mg/l) and 2,4-D(2.0mg/l) with ascorbic acid (2.0mg/l) and biotin (2.0mg/l) as additives yielded cottony growth of the fungus on the callus. Microscopic examination of 5 day old dual culture revealed profuse coenocytic, branched and intercalary mycelium.In 15-20 day old septate mycelium ,multinucleate cells were present,even turning in to thick walled resting chlamydospores ,which germinated to form synascus containing ascospores.

Key Words: *Coriandrum sativum , Protomyces macrosporus ,dual culture*

Introduction

Coriander (*Coriandrum sativum* L.) is one of the earliest spices known to mankind. It is cultivated in the field for green leaves and dry fruits used as a condiment. The plant belongs to the family Umbelliferae.The crop is raised in relatively lower atmospheric temperature conditions (22°C).

Coriander plants suffer from severe stem gall disease caused by the fungus *Protomyces macrosporus*. The disease is reported from several parts of India .A preliminary survey has shown that more than 50% loss in yield occurs due to this disease in Kota, Bharatpur, Dholpur, Sawai Madhopur and adjoining areas in Rajasthan. The disease appears in the form of tumour like swellings on stem ,leaf ,peduncle ,flowers and fruits(Plate:1,2,3,4). In instances of infection in inflorescence seed grain production is drastically reduced causing colossal loss to the crop. Severely infected plants loss their vigour and are deformed in their vegetative and reproductive growth forms. Infection occurs during January to March, when winter rains occur and foggy conditions prevail in the atmosphere. The disease appears in cold and humid conditions in the field.

Several aspects like epidemiology, histopathology, biochemistry and control of coriander gall caused by *P.macrosporus* have been studied (Mukhopadhyay and Pavgi 1971,Pavgi and Mukhopadhyay 1972,Gupta 1973, Tayal *etal.*1981,Goel *etal.*1983).Singh *etal.*1984 conducted survey of coriander fields in seven districts of Uttar Pradesh and reported disease intensity ranged between 7.5 to 24.4%.Paul 1992made a survey of coriander field in three districts of Himachal Pradesh, he also tested eight fungicides in different combinations as seed dresser against stem gall disease.Jain *etal.* 1994 recorded an increase in total soluble sugars and alpha amylase activity in galled tissues both *in* vivo and *in vitro.* Tripathi 1998 worked on stem gall management through soil treatment. Lakra 1999 and 2000 found best management of stem gall disease when seeds were treated with thiram@4gm/kgseeds. Masood *etal.*2004, Sharma and Sharma 2004 and Singhania *etal.*2006 also worked on occurence of the disease and its effect on seed production. Mishra *etal.*2017 worked on biochemical changes in coriander due to stem gall disease .However very little is known regarding the mode of infection and nature of infection and nature of its recurrence.

Present investigation deals with the successful establishment of dual culture of the fungus in association with the host callus and time related morphological studies of the fungus.

Dual cultures have been used frequently in plant pathology for growth of biotrophic fungi and *in vitro* expression of disease resistance. Arya and Shekhawat 1981 established dual culture of *Sclerospora graminicola* on pearl millet tissues *in vitro.* Kant and Goyal 1990 established

biotrophic culture of *Albugo candida* on Brassica *in vitro.* Diop *etal* .1994 also worked on dual and axenic culture of mycorrhizal fungi. Dquen Zhou 2001and Dodds 2010 worked on host specificity of biotrophic fungi *in vitro.* Morkus *etal.* 2011 conducted experiments on role of effectors of biotrophic fungi in infection.Katarzyna 2013 worked on dual cultures *in vitro* to evaluate pathogeicity of fungi. Deepak *etal* .2014 successfully established dual culture of *Alternaria burnsii* on cummin callus *in vitro.* Kabori *etal.* 2015 worked on growth of fungi in liquid culture.Abbas *etal.*2017 also conducted experiments on growth of fungi *in vitro.* However no studies have been published on dual culture of *Protomyces* on Coriander.

Materials and Methods

Establisment of dual culture: The stem explant of hypertrophied (galled) plant of *C. Sativum* were cut in to small (2-3mm) discs. These discs were surface sterilised with 0.1% HgCl2 for 2 min.,followed by repeated washings with sterile ditilled water. These were then placed aseptically on MS medium supplemented with NAA (8.0 mg/l), kinetin (0.5 mg/l), and 2,4-D (2.0 mg/l). The cultures were regularly subcultured on the same medium with ascobic acid (2.0 mg/l) and biotin (2.0mg/l) as additives for good fungal growth. 80-100 mg fresh weight of the callus containing small amount of the fungus was used as the inoculum on modified MS medium in every subculture. All the cultures were maintained in dark at 22*c temperature,70 % humidity and 6.8 Ph. The fungus from the dual culture was examined microscopically at different intervals during its growth period.

Results and Discussion

The callus appeared with in 15-20 days . The colour and texture of the fungus was light yellow and fragile (Plate 5). Cottony growth of the fungus was observed on its surface.With in 15 days the callus grew from 100 mg to 250 mg by fresh weight and the fungus covered most of its surface. The callus grew further and the fungus covered entire surface of the callus and descended on the surface of the culture medium (Plate 6).

Characteristics of the fungus:

Fungus grown on the surface of the callus was cottony white and compact . The mycelium was

intercellular , coenocytic , septate , and branched . After 15 – 20 days of the culture ,visible thickenings appeared on the cell walls along the septation . This resulted in the development of resting spores , the chlamydospores (Plate 7) . Chlamydospores were dark brown , spherical smooth walled and measured 40 – 60 microns in diameter .They germinated with the endospores passing through the exospore and formed an elongated more or less cylindrical vesicle leading to development of a dome shaped sac (Plate 8) . The nuclei inside the the sac undergo meiosis followed by second mitosis and developed in to a synascus which is naked without wall . Each synascus contained large number of haploid asci . Two ascospores fuse prior to germination and germinate afterwards to form diploid mycelium (Plate 8). These observations were also made by earlier workers *in situ* (Fitzapatrick 1930,Gauman 1952 , and Martin 1961).Sanchez *etal.* 2007 and Pankaj Trivedi 2008 evaluate the antagonistic properties of fungi *in vitro* . Engh I 2010 Studied *in vitro* cellular development of *Sordaria macrospora* . Gerphagon *etal.*2013 did some experiments on fungal parasitism *in vitro.* Timothy *etal.* 2014 studied effect of cow urine on the growth of *Fusarium lateritium in vitro.* Newcombe G *etal.*2016 studied life cycle of some dung fungi *in vitro.*

Information gained from the present studies will help in selecting disease resistant cell lines from dual cultures The whole life cycle of the fungus can be studied from dual culture .Such findings may help to suggest control of the disease.

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PLATE 1

Coriander plants showing infection of *P. macrosporus*

(various stages of gall development)

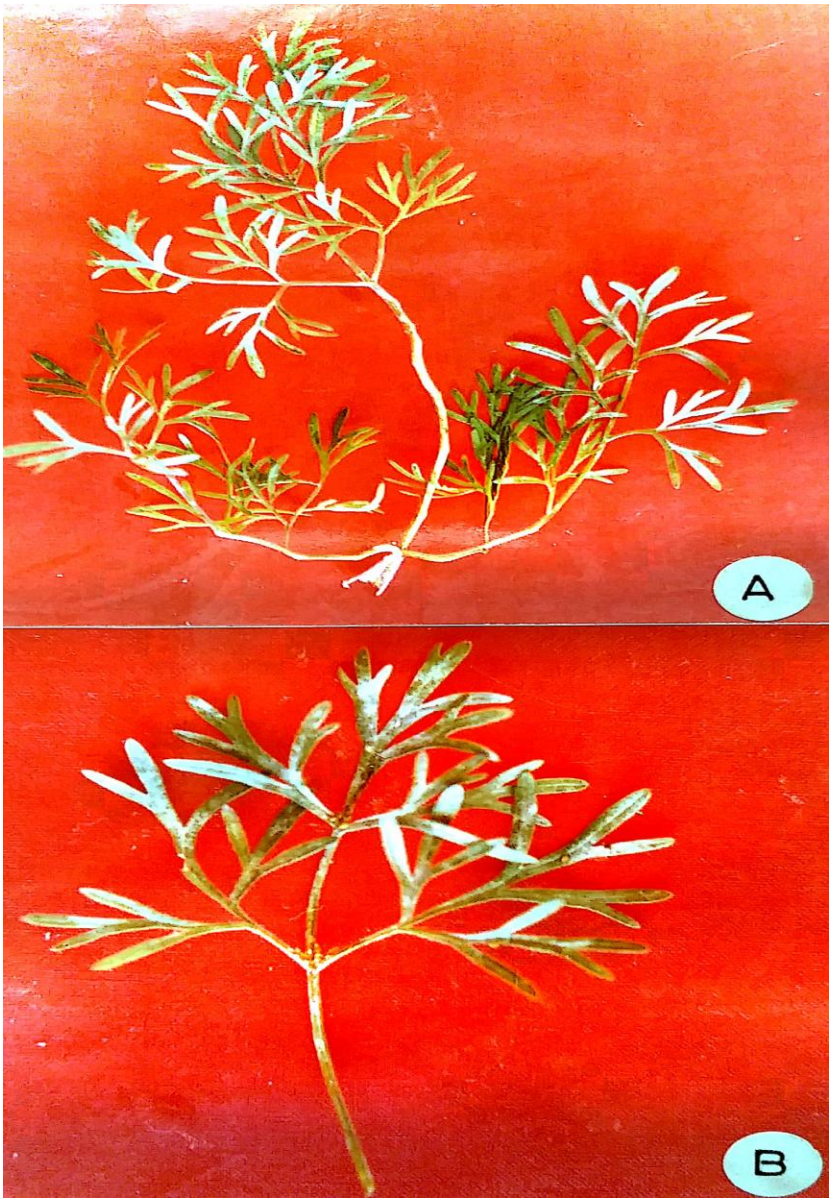

PLATE 2

Galled leaf of *Coriandrum sativum*

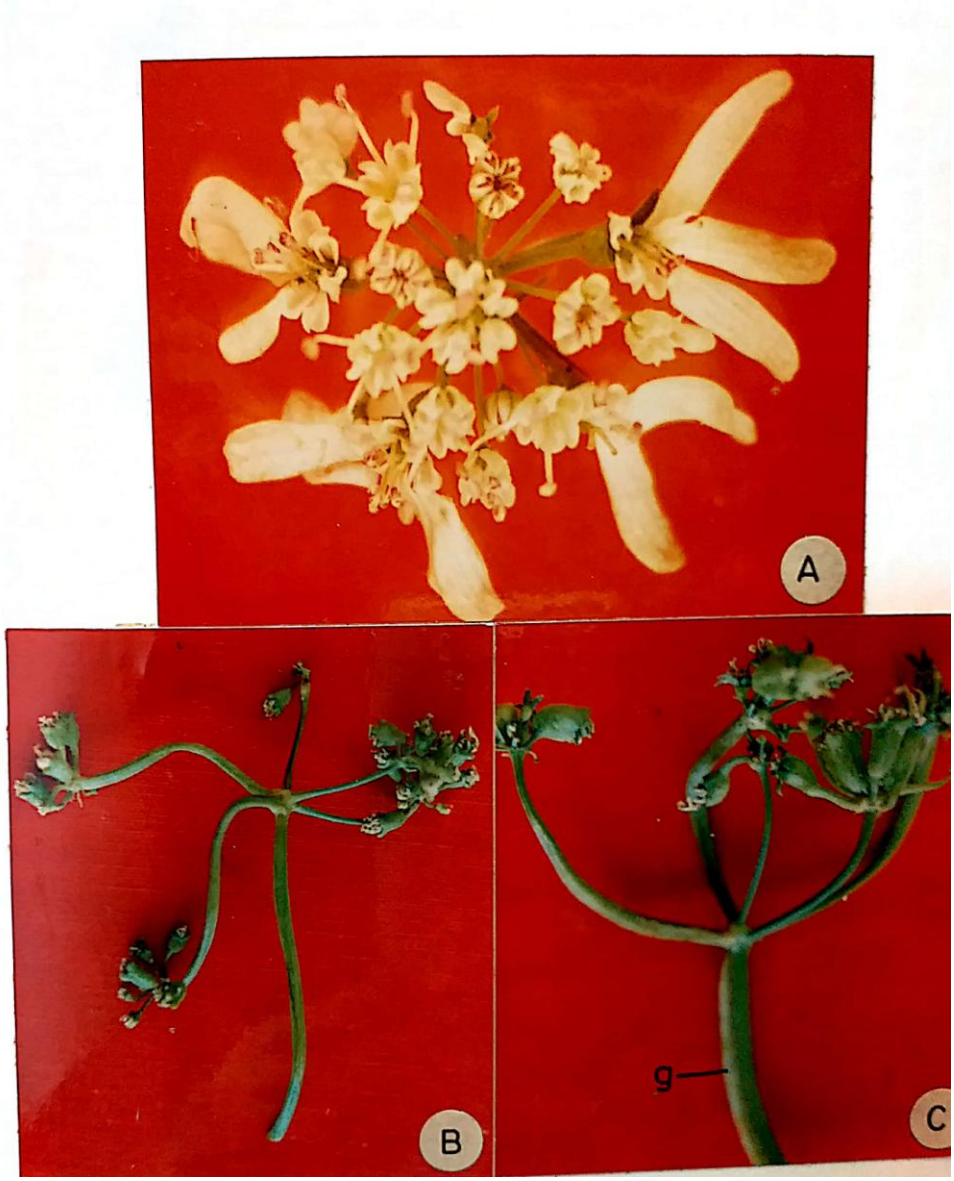

PLATE 3

(A) Normal (B, C) Galled flowers of *Coriandrum sativum*

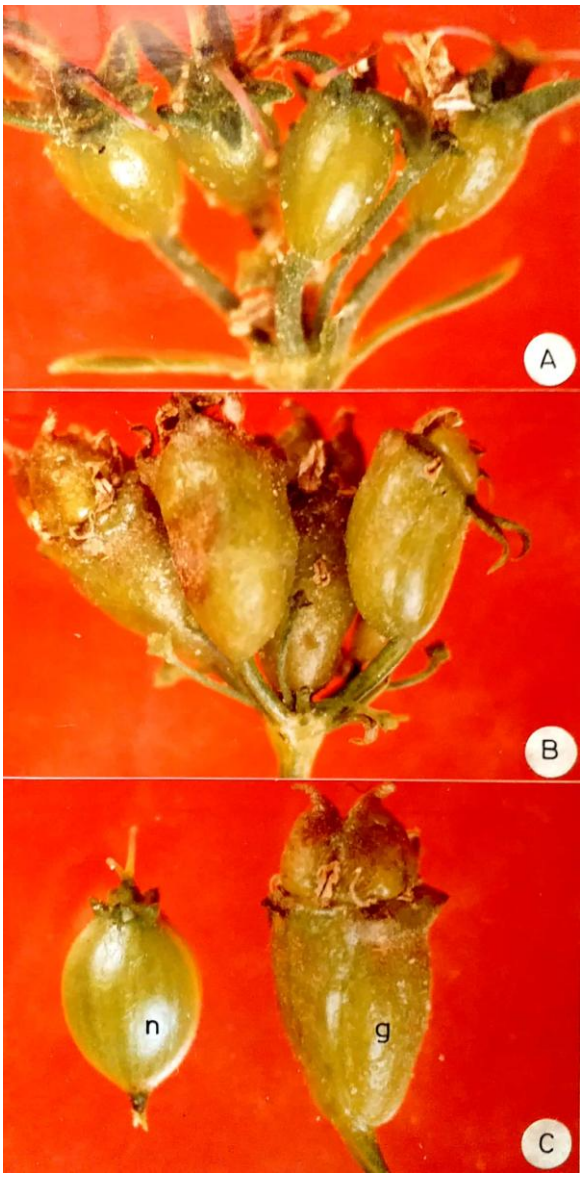

PLATE 4

(A)Normal (B,C)Galled fruits of *Coriandrum sativum*

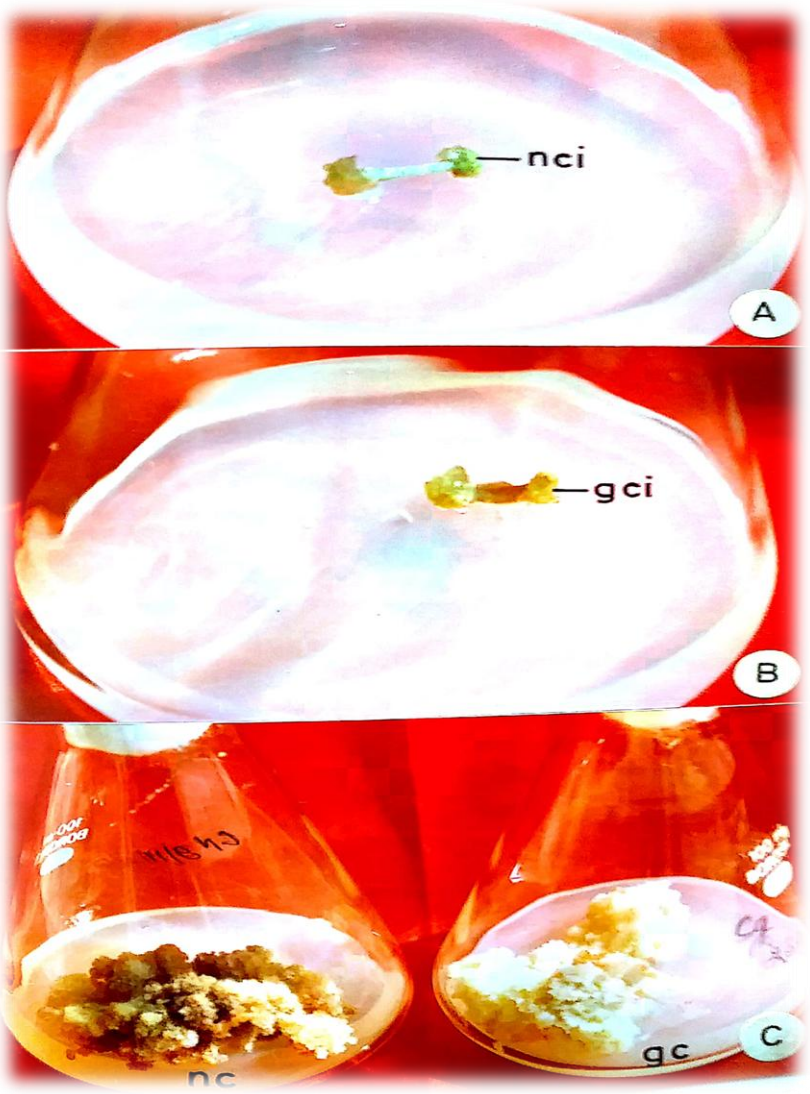

PLATE 5

(A-C)Normal and gall callus cultures of *Coriandrum sativum*

nc = normal callus gc = gall callus

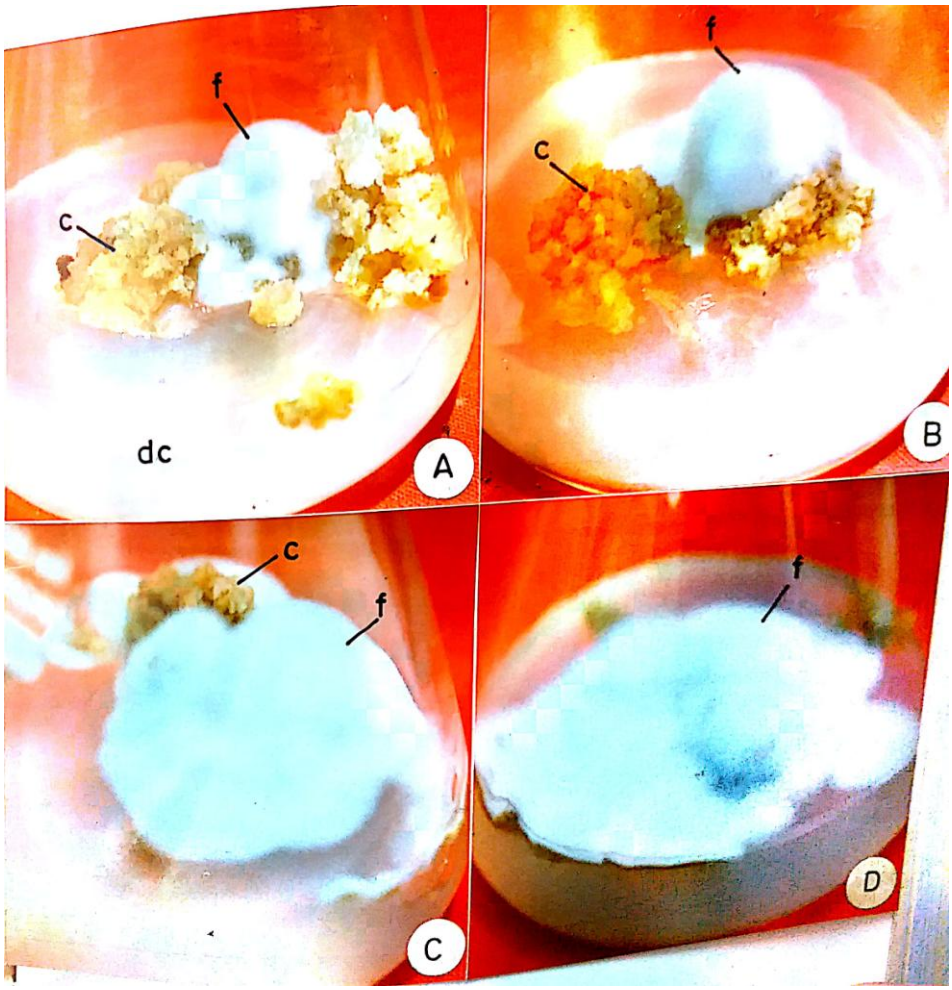

PLATE 6

(A-D)Dual culture of *Protomyces macrosporus* and *Coriandrum sativum*

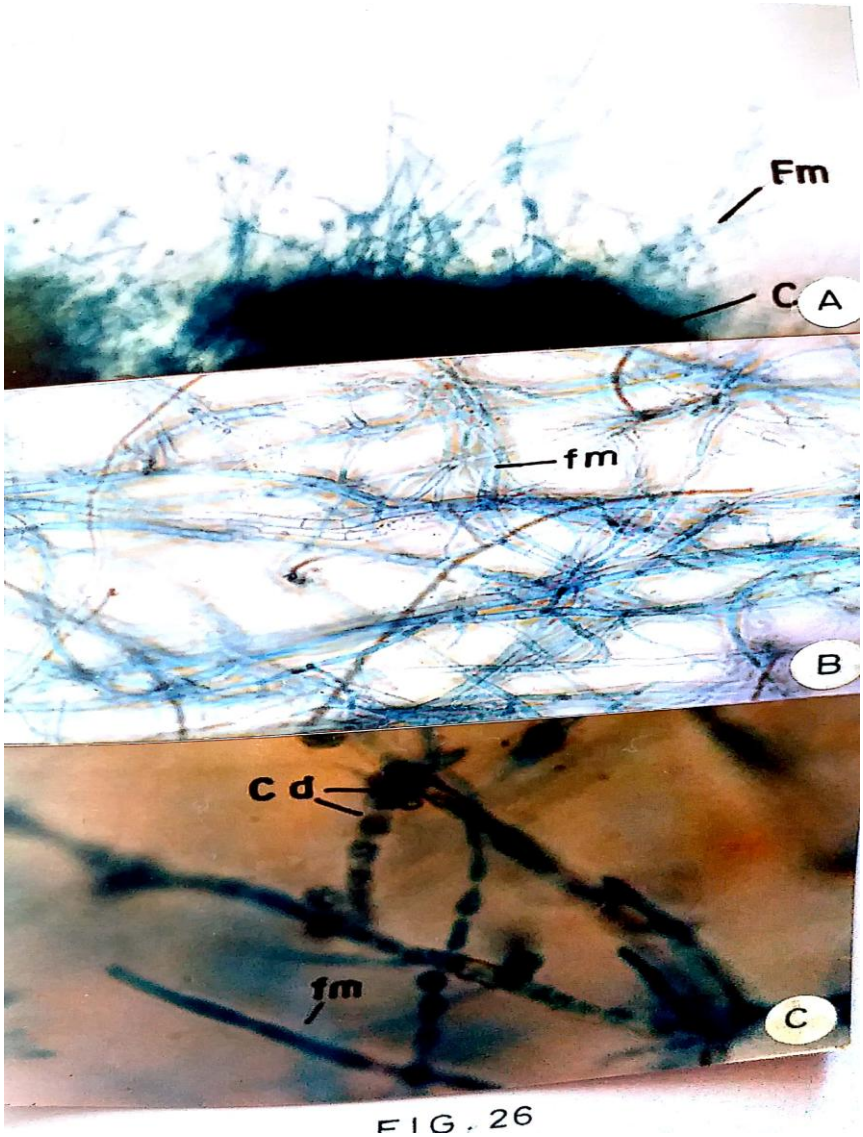

FIG. 26

PLATE 7

Fungal mycelium of *Protomyces macrosporus* from dual culture.

Fm-fungal mycelium Cd-chlamydospores

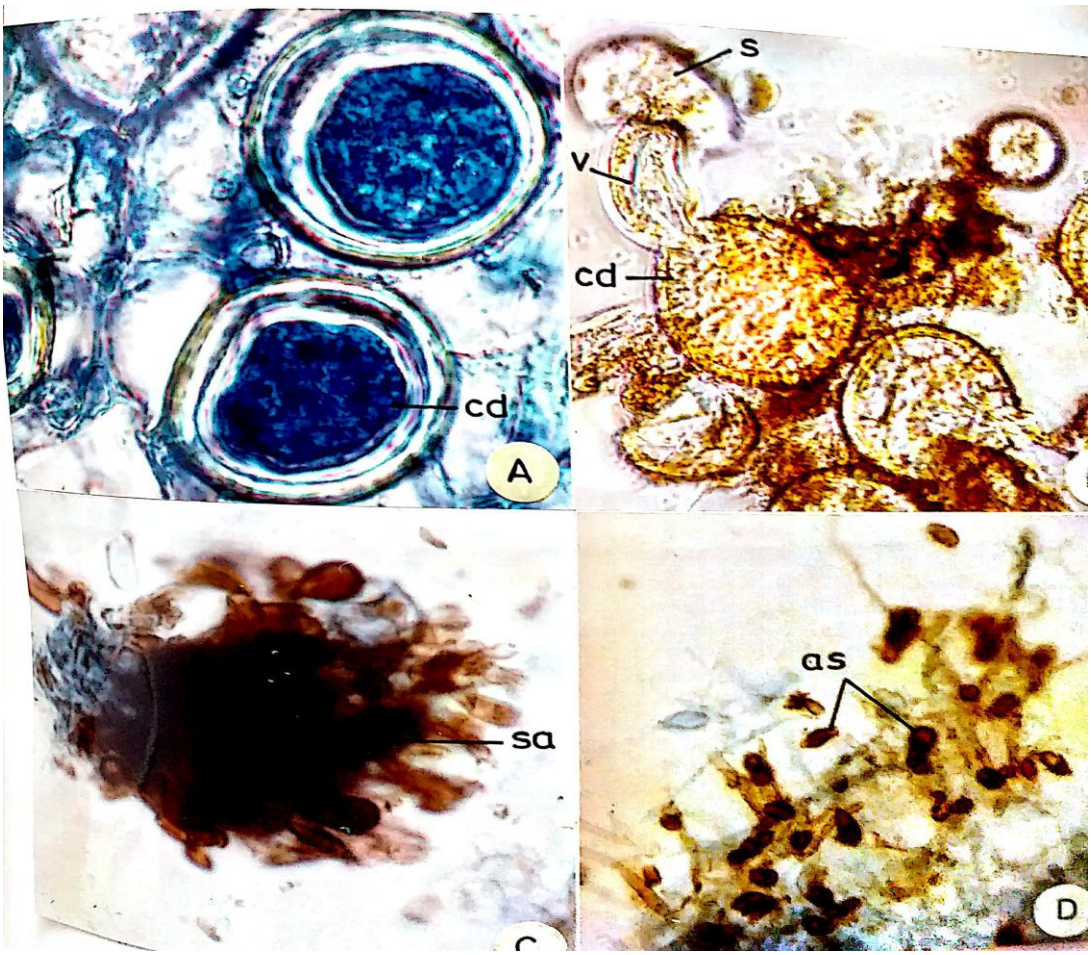

PLATE 8

A : Chlamydospores of *P.macrosporus1500**

B : Germinating Chlamydospores with vesicle and dome

Shaped sac*1500

C : Synascus with asci*150

D : Germinating ascopores*150