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Pathogenicity of *Colletotrichum capsci* on chilli by seed inoculation technique

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ABSTRACT

Anthracnose of chili caused by *Colletotrichum capsci*, is one of the important fungal pathogens of chilli. It is a wide spread disease in India as well as Odisha made in the laboratory of the Department of Plant Pathology, Institute of Agriculture Sciences, SOADU and Bhubaneswar during 2019-20. On Isolation of the causal organism and proving the pathogenicity, epidemiology and management of disease in vitro condition.

Key words: *Colletotrichum*, isolation, *in vitro,* pathogenic

Detection of pathogen from seeds of chilli

Ten species of *Colletotrichum* have been reported from chilli seeds which include, *Colletotrichum capsici, C.coccodes, C. dematium, C. gloeosporioidies, C. piperatum. C. multisetorum, C. graminicola, C. lindemuthionum, C. multiacutatum* and *C. lini* (Yu et al., 1987; Suryanarayan and Bhombe, 1961; Grover and Bansal, 1970; Rout and Rath, 1972; Chaurasia, 1976; Sangchote and Juangoanich, 1984; Kumar and Mahmood, 1986; Dhyani et al., 1990; Khodke and Gahukar, 1995 and Kulshrestha *et al*., 1976, respectively).

Anjili *et al.* (2005) observed that young mature and ripe fruits of C. papaya collected from farmer's fields in Abdulapur, Gokulpur and markets in Meerut and Mawana, Uttar Pradesh, in India were analysed for associated mycoflora. Isolation on Potato Dextrose Agar and Czapek's Dox Agar media showed that ripe fruits harboured more fungi. Sterilized or unsterilized seeds (100 to 400 seeds per sample), collected from different localities and cultivars were examined by blotter and agar plate methods. The percentage of occurrence and incidence varied between methods, places and cultivars. The fungi isolated from fruits and seeds consisted of 61 species belonging to 32 genera. Of these Aspergillus, Alternaria (15 spp.), Colletotrichum and Fusarium (3 spp.) each were dominant.

Basak *et al.* (1991) Reported that seed mycoflora of composite samples of diseased fruits of different types of fruit rot viz., black rot, Botryodiplodia rot, basal rot, anthracnose 1, anthracnose 2, Curvularia rot, Fusarium rot, Phomopsis rot, Periconia rot caused by Alternaria capsici-annui Savulescu and Sandu-Ville and A. tenuis Auct.: Botryodiplodia the obromae Pat.; *Cercospora capsici* Heald and Holf; *Colletotrichum capsici* (Syd.) Butler and Bisby 1: *C. gloeosporioides Penz*. 2; *Curvularia geniculata* (Tracy and Earle) Boedijn, C. lunata (Wakker) Boed., *Curvularia* spp.; Fusarium spp. [F. equiseti (Corda) Sacc., F. moniliforme Sheldom, F. oxysporum Schecht, F. solani (Mart) Appel and Wr.]; Phomopsis capsici (Magnaghi) Sace; Periconia byssoides Person respectively were directly collected from the farmers' fields of three selected locations were detected by blotter method.

Singh *et al.* (2009) reported that fungus is seed borne in nature and cause significant losses. Standard blotter method is found to be the best for detection of pathogen from seed.

Hemannavar *et al.* (2009) evaluated that seed samples were collected from different chilli growing districts of northern Karnataka revealed that the fungi viz., *Colletotrichum capsici*, species of *Cercospora*, Alternaria, Penicillium and Aspergillus. *Colletotrichum capsici* were the most predominant fungi encountered (71.24%). Among different seed health testing methods, standard blotter method was found to be most efficient forth quick and accurate diagnosis of *Colletotrichum capsici.*

The method suggested by Agarwal and Sinclair (1987) was followed

Examination,20g seeds per sample were drawn and divided into four fractions of 5g each. Each fraction was spread on bottom of the petri dish and examined with the help of a hand lens or if needed under stereo binocular microscope.

The inspected seed materials were categorized as follows:

a) Deformed seeds (shriveled or inflate)

b) Discolored seeds (black or brown)

c)damaged seeds (insect or mechanically)

d)impurities

i)inert material

ii)plant debris

e) apparently healthy seeds

seeds and impurities of each category were pooled separately and weighted on electronic balance and percent content by weight was calculated.

Seed washing test:

The Seed Washing test suggested by Agarwal and Sinclair (1987) was followed. from each sample 200 seeds taken at random were divided two groups of 100 seeds and were immersed in 20ml distilled water in 100ml.conical flask and shaken for 15 minutes on a mechanical shaker. The seeds were discarded and sediment was responded in 2ml of Lacto phenol. A drop of suspension was placed at the center of the hemocytometer and spores of different fungi were counted in 10 squares, chosen at random under stereo binocular microscope. Spores load per seed was calculated by using following formula:

Spore load per seed = $(N \times V)/(X \times N)$

Where, N =Total number of spores counted/number of squares

X=Volume of mounting solution between the cover glass and above the square covered

V=Volume of the mounting fluid added to the sediment

N=Number of seeds washed

Isolation of pathogen collected seed samples:

The pathogen was isolated from the leaf sample collected from the field in the following procedures. First the samples were washed with distilled water to remove the foreign materials. A small diseases portion (4-5mm) of leaf along with healthy portion from the periphery were cut into pieces and

surface sterilized with sodium hypochlorite (o.1%) solution for 2mnts followed by subsequent washing in sterile distilled water for 3times to remove residues of sodium hypochlorite from cut pieces. Then the diseased cut pieces were aseptically transferred in the center of PDA plates as well as PDA slant.

Purification and identification of fungi:

The fungus growth found in PDA plate and PDA slant was further sub cultured by taking the hyphal tip from the periphery of young colonies and transferred to PDA slants under aseptical condition. This process was repeated for 2-3 times till the pure culture of the test fungus was found. The pure culture of the fungus was periodically sub –cultured to maintain the viability and vigor of the fungus.

Isolation of pathogen collected seed samples

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Conclusion:

In order to determine the parasitic nature of fungus *Colletotrichum capsci*, Pathogenicity test was carried out. Chili plants were transplant in pots containing sterilized soil and watering was done at regular period of time. The pathogenicity experiment was carried by seeds artificially inoculated with pure culture of the fungus and sown in pot.

Control (plants without fungus inoculation only spraying sterile water to injured plant parts) Such inoculated plants were covered with polythene bags with a moistened cotton swab inside each bag to maintain humidity and kept in dark for 48 hours at temperature of 25.c and 80-100%RH. After the end of 48hours at temperature, the pots were kept in green house under natural condition. Regular observations were made for the appearance and development of disease symptoms. Five replications were taken for each treatment. The disease symptoms developed in each treatment was compared with control and the degree of pathogenicity was calculated. After development of typical disease symptoms in inoculated plants, the fungus was re-isolated. Its morphological characters were studied again and compared with that of the isolate obtained originally from the naturally infected plants. Chilli seed infection reduces both quality and quantity. As the chilli fruit are sold in market either green chilli or dry chilli. Due to infection both quality as well as quantity of produce reduced. The *Colletotrichum capsici* may initiate the anthracnose disease as primary organism but other fungi may be associated with the infected cite as secondary micro-organism to make the disease a more complex one and aggravate the infection leading to heavy loss in produce.

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