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RESEARCH ARTICLE

Studies on basal bulb rot of onion caused by *Fusarium oxysporum* f.sp. *cepae*

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ABSTRACT

Basal bulb rot of onion causes considerable damage. In recent years Fusarium basal rot of onion has assumed a serious problem and hence the studies were undertaken on this disease with the objectives *viz.*, isolation of the causal organism, its identification, pathogenicity, morphological and cultural characters. The causal organism was isolated from infected bulbs of onion. The pathogenicity of onion basal bulb rot pathogen was proved by soil inoculation method. The fungus culture was identical as *Fusarium oxysporum* f.sp. *cepae*. on the basis of morphological characters of the pathogen. Among the various cultural media used an, excellent mycelial growth and sporulation were observed on PDA, Richards agar and were followed by Czapeck's dox agar, host leaf extract, coon's agar and A. and H. medium.

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INTRODUCTION

Onion (*Allium cepa* L.) a bulbous, biennial herb, is one of the most important vegetable crops grown in India. In India onion is one of the most important among various Allium crops grown and comprises 772.8 ha area and 12,970.1 MT of production in 2010-11. In Maharashtra it occupied an area of about 170 ha and production 2800 M. tones (Anonymous, 2011).

Onion crop is attacked by many diseases, which cause loss in quality and quantity. Among these diseases, the basal bulb rot caused by *Fusarium oxysporum* f.sp. *cepae* is the most widespread and economically important disease of onion. In India, the occurrence of this disease was first reported from Rajasthan.

The disease is characterized by symptoms as wilting and rapid dying back of leaves from the tips of the plants near maturity. Infected plants can be pulled out easily because they have a retarded root system. Affected roots are dark brown, flattened, hallow and transparent the present studies were undertaken to isolate and identify the pathogen causing basal bulb rot of onion, to prove the pathogenicity of the isolated pathogen and to study the morphological and cultural characters of the pathogen.

MATERIAL AND METHODS

Isolation of the pathogen :

Isolation of the pathogen associated with basal rot of onion was done by tissue isolation method on PDA. For isolation of the pathogen, the affected portion of the bulb samples were cut in to suitable pieces, washed thoroughly in tap water so as to remove soil and other adherent particles. The pieces were then disinfected by 0.1 per cent sodium hypochloride solution for one minute followed by rinsing in three changes of sterilized water to remove the traces of mercuric chloride solution and were dried on sterilized blotting paper. Three to four such pieces were then plated aseptically on sterilized potato dextrose agar medium in each Petriplate. The Petriplates were incubated at room temperature ($28 + 1^0$C). Soon as growth of fungus was noticed, well isolated fungal growth free from contamination was transferred to agar slants by hyphal

tip method. By single spore isolation technique, the fungus *Fusarium oxysporum* was purified and agar slants bearing pure culture of fungus growth were maintained for further studies.

Pathogenicity test :

Pure culture of the fungus was multiplied separately on sand-maize medium (sieved fine river Sand 80 g, maize meal 20 g and 50 ml water) in conical flasks. After ten days of incubation the mass cultures of the fungus were uniformly mixed with sterilized soil which was already mixed with FYM in the proportion of 3 : 1. Six earthen pots were treated with 5 per cent copper sulphate ($CuSO_4$) solution. Out of six pots, four pots were filled with *F. oxysporum* inoculated soil and remaining two pots were kept as control which were filled in with sterilized uninoculated soil. The inoculated soil in the pots was incubated for 15 days at room temperature, frequently stirred and watered so that, the fungus could colonise in the soil. Then four to five seedlings of onion were transplanted in each pot and observations were recorded daily for disease development and continued till complete rotting of the plant.

Morphological characters :

Morphology of the fungus was studied of 5-10 days old culture grown on potato dextrose agar medium by adoption of slide culture technique. Morphological characters *viz.*, septation, shape and colour of different morphostructures like mycelium, micro and macro-conidia, sporodochia, chlamydospores etc. were recorded under compound microscope and measurements were done wherever necessary with the help of occular micrometer calibrated with stage micrometer.

Cultural characters :

The fungal culture isolated was grown on different media in order to study its growth characters and ability to sporulate. The different fifteenth media used for these studies were peptone glucose agar, host leaf extract, leonian agar, potato dextrose agar, czapeck's dox agar, asthana and hawker's medium, coon's agar, waksmans special medium, ashby's medium, yeast extract glucose agar, richards agar, tap water agar, sach's medium, malt extract and oat meal agar.

Different media were prepared freshly in the laboratory under aseptic conditions and sterilized plates in triplicate for each medium were poured with equal quantity (20 ml) of media. The plates were inoculated with uniform discs of mycelium of seven days old culture of *Fusarium oxysporum* which were removed by cork borer and were placed at the centre of plates. The plates were incubated at 28 + 1^0C temperature in an incubator and observations on mean colony diameter, sporulation, colour and growth characters were recorded seven days after inoculation.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Table 1 : Cultural characters of *Fusarium oxysporum* f.sp. *cepae* on different media

Sr. No.	Name of the medium	Mean colony diameter (mm)*	Sporulation	Characters
1.	PDA	87	++++	Circular margin compact mycelium, aerial hyphe, cottony white growth
2.	Coon's Agar	63	+++	White, cotton fuzz like growth, mycelium grow well
3.	Leonian Agar	70	+	Flat growth ,margin smooth poor aerial mycelium.
4.	Sachs medium	00	–	–
5.	Richards Agar	80	++++	Colony circular, very thin mycelium, raised growth at centre
6.	Peptone glucose agar	76	+++	Slightly white,very thin mycelial growth.
7.	Asthana and Hawker's medium	78	+++	White compact growth with entire margin.
8.	Waksmans medium	75	+	White to skin colour, very compact growth at centre.
9.	Ashby's agar	60	+	Fuzzy fur like mycelia growth.
10.	Tap water agar	00	–	–
11.	Czapeck's dox agar	62	++++	Sligtly pinkish and raised mycelium growth at centre
12.	Oat meal agar	65	++	White brown coloured ,flat and loose mycelial growth.
13.	Host leaf extract	85	++	Colony circular with entire margin, thin and skin colour mycelial growth.
14.	Yeast extract glucose agar	52	++	Creamish, brown coloured mycelial growth, raised mycelium at centre
15.	Malt extract agar	75	+	Slightly red, oval to circular growth, thin mycelium.
	S.E.m. ±	0.73	–	–
	CD at 5%	2.10	–	–

++++ = Excellent, +++ = Good, ++ = Moderate + = Scanty - = No sporulation

Sporulation :

1. 40-50 = Micro and macroconidia/microscopic field – Excellent
2. 30-40 =Micro and macroconidia/microscopic field – Good
3. 20-30 = Micro and macroconidia/microscopic field – Moderate
4. Below 20 =Micro and macroconidia/microscopic field – Scanty

Isolation of the pathogen :

Isolation was made from infected bulbs of onion which yielded a growth of *Fusarium oxysporum* f.sp. *cepae*. The growth of fungus from infected tissue was distinctly visible after three to four days in Petriplates containing the potato dextrose agar medium. The pure culture of fungus was obtained by single spore isolation method on potato dextrose agar (PDA) in plates and was maintained on PDA slants.

Pathogenicity :

Pathogenicity test was carried out in earthen pots by soil inoculation with *Fusarium oxysporum* f.sp. *cepae*. Typical symptoms observed were yellowing and eventual dieback of the leaves, drying of leaves and in advance stage, basal portion of plants completely rotted and entire plant got collapsed on ground, which was noticed 15 days after transplanting in the pots. Uninoculated plants remained healthy.

Earlier some workers have studied the pathogenicity of *Fusarium oxysporum* on onion. Mudiyanselage *et al.* (2009) studied the pathogenicity of 32 isolates of *Fusarium* spp. on five commercial cultivars of welsh onion and proved that five *Fusarium oxysporum* isolates had higher virulence to *Allium* spp. Davis (2008) reported the pathogen, *F. oxysporum* f.sp. *cepae* causing onion and garlic basal rot. Kehr *et al.* (1962) isolated *Fusarium oxysporum* f.sp. *cepae* from root and proved its pathogenicity. It is pathogenic over a wide range of temperature (20 to 38°C).

Morphology of the pathogen and its identification :

Aerial mycelium is white cottony to slightly pink in colour, circular colony with entire margin. Microconidia were observed, their size was 5-12 µm in length without septation and their shape was oval to kidney shaped Macroconidia were also observed. Their size ranged from 4 to 7 µm wide and 20-35 µm long. Number of septa were usually four to five, gradually attenuate toward apex, falcate shaped. Chlamydospores usually abundant, composed of one or two round cells and have thick cell wall and were formed in or on older mycelium. All these observations were found similar with those reported by Cramer (2000), Hanzawa (1914) and Delahaunt and Stevenson (2004). Earlier, Rodriguez and Regina (2008) studied the morphology of *Fusarium culmorum* and observed that fungal colonies grew rapidly on PDA and its colour became brown to dark brown with age.

Cultural study of *Fusarium oxysporum* f.sp. *cepae* :

The growth characters of the fungus on various media are presented in Table 1. The cultural media evaluated for growth characters exhibited varying degrees of growth rates ranging from 52 to 87 mm. An excellent mycelial growth was observed on potato dextrose agar (PDA) followed by host leaf extract and richard agar, while good growth was observed on asthana and hawkar's medium, peptone glucose agar, waksman's medium, malt extract agar, while moderate growth was observed on leonian agar, oat meal agar, coons agar, czapeck's dox agar, ashby's agar. mycelium growth was not observed on Tap water agar and Sachs medium. These results are mostly similar to the observations made by Yogehswari (1948), Patel and Prasad (1964), Jhamaria (1972), Raghuwanshi (1995) and Mandhare (1997).

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