In-vitro study of pathogenicity and colony characteristics of *Verticillium fungicola* isolates collected from different regions of Northern India

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ABSTRACT

A trial was conducted to study about pathogenicity and colony characteristics of different isolates of *Verticillium fungicola* under *in-vitro* condition. Growth of all isolates of *V. fungicola* was checked using potato dextrose broth medium at temperature range from 20-23^{o}C, in which 22^{o}C temperature was found most suitable for growth of all isolates of *V. fungicola.* Virulence assay was performed using sporophores of *A. bisporus* and diameter of the necrotic lesions was ranged from 19-28.5 mm.

INTRODUCTION

White button mushroom, *Agaricus bisporus,* derives its name from young shape. However, button is a initial development stage of mushroom which becomes like umbrella at full growth. It has pleasant taste and aroma. Morphologically, mushroom has fruiting body that can differentiate by sporocarps. Mushrooms have a pileus or cap which is fleshy, thick as well as membranous. It is found in different shapes (Jonathan and Adeoyo, 2011a).

Verticillium fungicola causes dry bubble disease in white button mushroom. This disease was first reported by Malthouse in 1901 and after further study he found that *Verticillium* sp. is associated with same which later named as *Verticillium malthousei.* Previously this disease was a minor only but now a days it's becoming a major problem in mushroom industry which causes many losses.

Two type of symptom were observed. Initial symptom was observed on casing layer and later on it spread on all the surface area and converted in to grey and yellow in colour. Later, some spots are formed on the surface of caps which later on become large patches. Contaminated casing soil, human beings, compost and water splash are main reasons for transmission of disease. (Kumar *et al*., 2014). In this study, morphological characteristics and pathogenicity of different isolates of *Verticillium fungicola* have been studied under *in-vitro* condition, to check most virulent isolates against *Agaricus bisporus.*

MATERIALS AND METHODS

Collection and isolation of *Verticillium fungicola* isolates:

A survey was carried out in February, 2016 to collect different samples of white button mushroom for isolation of different strains of *Verticillium fungicola* from selected locations of Northern India. A total number of 10 cities from 6 states of Northern India were surveyed. The samples collected from different locations were brought to laboratory for isolation and further study (Soosairaj *et al*., 2012). Standard method of isolation was followed to isolate *V. fungicola.*

One cultures of *V. fungicola* (ITCC No. 4909) was procured from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi, which was taken as control. Present work was carried out at Department of Plant Pathology, SHUATS, Prayagraj (UP), India, during April-May, 2016.

Colony characteristics of *V. fungicola* isolates:

All the samples have been isolated on potato dextrose agar (PDA) and colony characteristics *viz.*, growth pattern, colour, shape and texture, have been recorded after full growth on Petri plates.

Pathogenicity of different *V. fungicola* isolates against *Agaricus bisporus:*

a. By using potato dextrose broth medium:

Pathogenicity of *Verticillium fungicola* was checked with this method. For each isolate, ten ml of PDB (potato dextrose broth) and ten grams of mushroom compost were poured in conical flasks and inoculated with culture of *V. fungicola* of five mm diameter. After inoculation, all flasks were kept in incubator at 20-23^{O}C. Temperature was increased day by day to check the growth of *V. fungicola* on the medium and suitable temperature for growth was recorded.

b. By using sporophores of *A. bisporus:*

By using method of Bonnen and Hopkins (1997), virulence assay was performed with some modification. A fresh button mushroom fruiting body was used for *V. fungicola* isolate and placed into Petri plates which are used as a moist chamber. Before inoculation of *V fungicola,* stipes of all mushroom fruiting bodies have been removed to stop opening of cap and release of basidiospores from gills. Five mm discs of pathogen culture were placed on cap surface of button mushroom. All the Petri plates were kept in dark condition at 22°C and data were recorded at 5 DAI.

RESULTS AND DISCUSSION

Collection and isolation of *Verticillium fungicola* isolates:

Total 10 isolates of *V. fungicola* have been isolated by using standard method of isolation. Details are given below:

Table 1: Details of year, isolate code and geographic region different isolates of *V. fungicola*

Isolate code	Year	Geographic region
S_0	2016	New Delhi **(Control)**
S_1	2016	Ayodhya (Faizabad, UP)
S_2	2016	Kanpur (UP)
S_3	2016	Meerut (UP)
S_4	2016	Sonepat (Haryana)
S_5	2016	Hisar (Haryana)
S_6	2016	Udham Singh Nagar (Uttarakhand)
S_7	2016	Ludhiana (Punjab)
S_8	2016	Jammu (J&K)
S_9	2016	Solan (HP)
S_{10}	2016	Prayagraj (Allahabad, UP)

Colony characteristics of *V. fungicola* isolates:

Data depicted in Table 2 revealed that growth pattern of all isolates were disperse at early stage but later it became dense too in some of the isolates like S_0, S_4, S_7, S_8 and S_{10}. In case of colour of the mycelia, mostly isolates grown in white colour except two (S_0 and S_5) which growth was creamy. Shape of the colony of 6 isolates (S_1, S_2, S_3, S_5, S_6 and S_9) were round and for rest, it was round and wide too and texture of colonies of all isolates were smooth.

Table 2: Colony characteristics of different isolates of *Verticillium fungicola*

Isolate code of *V. fungicola* isolates	Colony characteristics			
	Growth pattern	Colour	Shape	Texture
S_0	Disperse & dense	White	Round & wide	Smooth
S_1	Disperse	Creamy	Round	Smooth
S_2	Disperse	White	Round	Smooth
S_3	Disperse	White	Round	Smooth
S_4	Disperse & dense	White	Round & wide	Smooth
S_5	Disperse	Creamy	Round	Smooth

S_6	Disperse	White	Round	Smooth
S_7	Disperse & dense	White	Round & wide	Smooth
S_8	Disperse & dense	White	Round & wide	Smooth
S_9	Disperse	White	Round	Smooth
S_{10}	Disperse & dense	White	Round & wide	Smooth

Pathogenicity of different *V. fungicola* isolates against *Agaricus bisporus:*

a. By using potato dextrose broth medium:

Table 3: Growth of *V. fungicola* isolates at different temperature by using broth compost medium

Sr. No.	*V. fungicola* isolates	Growth of *V. fungicola* @different temperature						
		20°C	20.5°C	21°C	21.5°C	22°C	22.5°C	23°C
1.	S_0	-	-	+	+ +	+ + +	+ + +	+ + +
2.	S_1	-	-	-	-	+	+ +	+ + +
3.	S_2	-	-	-	+	+ +	+ + +	+ + +
4.	S_3	-	-	-	-	+	+ +	+ + +
5.	S_4	-	+	+ +	+ + +	+ + +	+ + +	+ + +
6.	S_5	-	-	+	+ +	+ + +	+ + +	+ + +
7.	S_6	-	-	+	+ +	+ + +	+ + +	+ + +
8.	S_7	-	+	+ +	+ + +	+ + +	+ + +	+ + +
9.	S_8	-	+	+ +	+ + +	+ + +	+ + +	+ + +
10.	S_9	-	-	-	+	+ +	+ + +	+ + +
11.	S_{10}	-	-	+	+ +	+ + +	+ + +	+ + +

(- = no growth, + = growth start, + + = normal growth and + + + = full growth)

b. By using sporophores of *A. bisporus:*

At 5 DAI, data related to virulence assay was recorded (Table 4), which are given below:

Table 4: Pathogenicity of different isolates of *Verticillium fungicola* by using sporophores of *Agaricus bisporus*

Sr. No.	Isolate code of *V. fungicola* isolates/strains	Diameter of necrotic lesion (mm)

1.	S_0	20
2.	S_1	24.5
3.	S_2	26
4.	S_3	27
5.	S_4	28.5
6.	S_5	24.5
7.	S_6	25.5
8.	S_7	28
9.	S_8	28
10.	S_9	19
11.	S_{10}	20.5

In virulence assays, virulence potential of all *V. fungicola* isolates was very high for necrotic diameter. Same result was reported by Bonnen and Hopkins (1997). The larger growth rate of *V. fungicola* var. *aleophilum* ex-type strain at 30^{o}C compared with 20-24^{o}C for *V. fungicola* var. *fungicola* ex-type strain; initially this difference was wont to distinguish the two varieties (Gams and Van-Zaayen, 1982). In current study, none of V. fungicola isolates was able to grow @30^{o}C and mycelial growth rates were higher at 22^{o}C than at 30^{o}C.

However, the pathogenicity of *V. fungicola* var. *fungicola* as well as *V. fungicola* var. *aleophilum* to *Agaricus* sp. has been recorded by many authors (Largeteau *et al.*, 2006).

In current study, isolates were genetically homogeneous. This genetically clonal population possibly indicates fungicidal effect on fungal population shift toward more genetic homogeneity as well as resistance on fungicide. These findings suggested that fungicide has gradual selective pressure on pathogen population and within some years, the sensitivity rate will shift from low to moderate. Thus, the use of fungicides acts as special selection pressure such that after some years there will not be sensitive pathogen populations and the pathogen will form a more genetically clonal population (Largeteau *et al.*, 2006 and Largeteau *et al.*, 2008).

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