

Physiological variability in *Verticillium fungicola* isolates collected from different mushroom farms of Haryana state

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ABSTRACT

Verticillium fungicola is a serious pathogen causing dry bubble disease in button mushroom (*Agaricus bisporus*). Present investigations were carried out on different isolates of pathogen by covering an aspect of physiological variability in pathogen isolates. The isolates of *V. fungicola* collected from different mushroom farms of Haryana state, coded as MHS (Hisar), BFT (Fatehabad), NJN (Jind), RHT (Rohtak), TPN (Panipat), BSN (Sonipat), FDB (Fridabad) and SKK (Kurukshetra) and pathogenicity was proved on *A. bisporus*. All isolates showed physiological variations on PDA at pH regimes 6.0 - 7.5 and 15 - 25 °C temperature after 12 days of incubation. Among the evaluated isolates maximum growth was recorded in BSN (44.67 mm), followed by TPN (43.83 mm), FDB (43.33 mm), SKK (42.17 mm), RHT (41.50 mm) and NJN (38.00 mm), while the rest one such as MHS (35.83 mm) and BFT (34.83 mm) showed minimum radial growth at 25±1°C after 12 days of incubation. On the other hand, at 15±1°C the radial growth of all *V. fungicola* isolates was significantly reduced i.e. 10.00 (BFT), 10.83 (MHS), 11.33 (NJN), 12.50 (RHT), 17.50 (TPN), 18.00 (BSN), 16.17 (FDB) and 14.67 mm (SKK) after 12 days of incubation. *V. fungicola* isolates grow at pH regimes of 6.0 to 7.5. However, the maximum radial growth was achieved in all the isolates when pH was 6.5 followed by 6.0, 7.0 and 7.5. Among isolates the maximum growth was recorded at pH 6.5 in BSN isolate (44.83 mm), followed by TPN (44.00 mm), FDB (42.17 mm), SKK (41.50 mm), RHT (41.33 mm) and NJN (38.50 mm), while the minimum radial growth was recorded in isolates MHS (37.17 mm) and BFT (35.67 mm) at 25±1°C after 12 days of incubation.

Keywords: *Agaricus bisporus*, isolate, temperature, variability, *Verticillium fungicola*

Mushroom is a macro-fungus that may grow above or below the ground with a distinctive fruit body that can easily be seen by naked eyes and easily picked up by hands. Mushroom imparts diversification in any farming system and helps in addressing the problems of quality food, health and environmental sustainability. Mushroom farming is today being practiced in more than one hundred countries. The most fascinating concept of mushroom science is “The cultivation of highly nutritious fruit bodies of

excellent taste from waste”. Therefore the cultivation of edible mushrooms converts different types of agricultural and household wastes into nutrition rich food and their commercial production is a great source of income in almost every part of the world (Wani *et al.*, 2010). Generally, the mushrooms are edible fungi however someone is unpalatable and few of them are poisonous in nature. There is about 1.5 million species of fungus are known and out of these, it has been estimated that 14,000 species produce fruiting bodies

that are desirable to be considered as mushrooms (Hawksworth, 2001). About 7,000 species of edible mushrooms are known, out of which 200 are experimentally grown and 10 have been produced at the industrial scale (Chang and Miles, 2004).

The *A. bisporus* mushrooms cultivation is a highly specialised process and commercial growing is done under strictly controlled environmental conditions. In India, the major mushroom producing states are Himachal Pradesh, Punjab, Haryana, Maharashtra, Jammu and Kashmir, Andhra Pradesh, Tamil Nadu, Orissa, U.P. and Karnataka (Sharma *et al.*, 2017). However, in North India, its cultivation is seasonal with poor hygiene and sanitation that leads to the build up of pathogens. In Haryana, majority of the seasonal mushroom growers lack hygienic conditions that lead to the build up of serious diseases and pests causing considerable yield loss (Khanna *et al.*, 2003). Mushroom cultivation is affected by a large number of living and nonliving factors. Fungi, bacteria, viruses, nematodes, insects and mites are different biotic factors that damage the mushroom crop directly or indirectly (Sharma *et al.*, 2011). Among the various factors responsible for low production and productivity of mushroom in our country, fungal diseases play a major role. The fungal pathogens, *Verticillium fungicola*, *Mycogone perniciosus*, *Trichoderma* spp. and *Papulaspora byssina* are the predominant mycopathogens. Amongst these, *Verticillium fungicola* var. *fungicola* (Preuss) is most important disease causing agent of *Agaricus bisporus* (Lange) Imbach button mushroom and annual losses to the growers are estimated to be 2-4% of total revenue (Berendsen *et al.*, 2010). The pathogen induces various symptoms like bubbles (undifferentiated spherical masses), bent and/or split stipes (blowout) and spotty caps. Inoculation of *A. bisporus* crop with isolates of *V. fungicola* var. *fungicola* of various degrees of aggressiveness showed that the more aggressive isolates induced higher numbers of bubbles (Largeteau and Savoie, 2008). The *Verticillium* dry bubble is the most prevalent disease and if left

uncontrolled in the mushroom growing environment; the disease can wipe out an entire crop in 2–3 weeks (Sharma *et al.*, 2002). Moreover, the disease may be devastating for years following the initial infection because spores are capable of resting in debris and re-infecting crop year after year (Berendsen *et al.*, 2010).

Dry bubble was first detected in a commercial planting in North America in 1981. However, literature on dry bubble has been published in India as early as 1960, proving that it has been an economic problem for mushroom growers during 20th century. It mainly affects three different species of mushrooms viz. *Agaricus bisporus*, *A. bitorquis* and *Pleurotus ostreatus*. Though, the infection by *V. fungicola* does not decrease the weight of the mushrooms, but has the potential to decrease the total number of mushrooms produced (Berendsen *et al.*, 2010). *V. fungicola* when grown on potato-dextrose agar at room temperature, the colonies are white and the underside of the plates is colourless to yellow. Environmental conditions within the growing area can greatly affect the rate of growth and spread of dry bubble. However, it is known that any environmental alterations which affect dry bubble will similarly affect the mushroom crop which is its host. Siwulski *et al.* (2011) reported that mycelium growth of the *V. fungicola* isolates greatly affected by medium pH (5.5 to 7.5) and temperature (15 to 30°C) and mycelial growth fastest at 25°C and 5.5 pH. Dry bubble favours warm, humid environments for growth and coincides with environmental preferences of white button mushroom, its primary host, which favours a RH of 85% and warm temperatures of 25°C. Dry bubble disease management relies mainly on hygiene and prevention of introducing inoculums on mushroom farms. Berendsen *et al.* (2010) reported that dry bubble disease may be suppressed to some degree, if relative humidity is lowered by 10% and temperature lowered up to 5 to 10°C; the growth of pathogenic mycelia and germination of spores may be slowed with minimal impact to harvest.

Meagre information is available on *V. fungicola* in India, especially regarding physiological variability for effectively management of disease. Therefore, keeping this in view; the present studies were undertaken with the objectives of variability studies in terms of physiological parameters of *Verticillium fungicola* isolates.

MATERIALS AND METHODS

The present investigation entitled “physiological variability in *Verticillium fungicola* isolates collected from different mushroom farm of Haryana state” was carried out at Mushroom Technology Laboratory, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, during 2015-2017 seasons. Hisar is situated at a latitude of 29°10'N, longitude 75° 46'E and an altitude 215.2 m above mean sea level and fall in semi-tropical regions of Western Zone of India. Details of the materials used and methodology adopted during the course of this investigation are given below.

In the present study, 17 diseased samples in triplicate were collected from different parts of the Haryana, out of these *V. fungicola* was isolated from eight samples. Samples were washed thoroughly with tap water, dried and then kept in paper bags for further isolation of pathogen. The fungi was cultured on fresh potato dextrose agar medium for isolation, purification and pathogenicity test. The pathogen was purified and maintained by repeated sub-culturing of each isolates after every month and kept in a refrigerator at 4°C for the further studies. Isolation of the *V. fungicola* was made from the infected fruiting bodies, which showing typical symptoms of dry bubble disease. The diseased fruiting bodies were first examined for the associated pathogen by teasing the diseased portion with the help of a needle and observed under microscope. For isolation of the causal fungus, five mm small disc segments were cut from the infected sporophore with the help of sterilized cork borer, surface sterilized with 0.1% mercuric chloride for 30 seconds followed by rinsing thrice with sterilized

distilled water, blotter dried and inoculated under aseptic conditions on PDA medium in sterilized Petri dishes and incubated at 25±1°C (Sabharwal and Kapoor, 2014). The pathogen culture was purified by hyphal tip culture method (Pathak, 1972). The pure culture was obtained and maintained by repeated sub-culturing at monthly intervals. The stock culture in PDA slants was stored at 4±1°C in a refrigerator. The repeated sub-culturing was done for further studies to avoid the possible loss of pathogenic behavior of the test pathogen.

In vitro the effect of different temperature regimes on radial growth of *V. fungicola* isolates were studied on PDA. The sterile Petri plates (90 mm) containing 25 ml of medium having three replication were inoculated with five mm mycelial disc of each isolate taken from periphery of 12 days old actively growing colony on PDA plates. Inoculated Petri plates were kept in BOD incubator at 15, 20 and 25°C temperature. The radial growth of the isolates was recorded at four days interval upto 12 days of incubation and average radial growth of each isolate was measured.

The effect of different pH regimes on growth of *V. fungicola* isolates were studied on PDA medium under *in vitro* conditions. The pH range of medium were adjusted to 6.0, 6.5, 7.0 and 7.5 using 0.1 HCl or 0.1 NaOH and buffered with citrate phosphate buffer (Dhingra and Sinclair, 1986). Sterile Petri plates (90 mm) containing 25 ml of medium were inoculated with five mm mycelial disc of each isolate. The Petri plates were kept in BOD incubator at 25±1°C. The radial growth of the isolates was recorded at four days interval up to 12 days of incubation and the average radial growth of each isolate was measured.

RESULTS AND DISCUSSION

During physiological variability studies in *V. fungicola*, the 17 diseased samples of white button mushroom were collected from different parts of the Haryana and from these *V. fungicola* was isolated

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Fig. 1. *Verticillium fungicola* isolates collected from different locations

from eight samples. Isolation of *V. fungicola* was done from the diseased samples and pathogenicity was proved on white button mushroom. The cultures were purified and maintained on potato dextrose agar medium (Plate-1). The purified *V. fungicola* isolates were coded (Table-1) as MHS (Hisar), BFT (Fatehabad), NJN (Jind), RHT (Rohtak), TPN (Panipat), BSN (Sonipat), FDB (Fridabad) and SKK (Kurukshetra).

The observation regarding influence of different temperature regimes on mycelial growth (mm) were

recorded under *in vitro* conditions by culturing the fungus in Petri plates on potato dextrose agar medium. The radial growth was recorded for each treatment and data thus obtained after analyses were compared with the reference pathogen presented in table 2. The perusal of the results indicated that all the *V. fungicola* isolates could grow at all the temperature regimes (15 to 25 °C) evaluated in this study. However, the maximum growth was observed at 25±1°C followed by 20±1°C and 15±1°C in all the evaluated isolates after 12 days of incubation (Plate 2). Among the evaluated isolates maximum growth

Table 1. Collection of dry bubble disease samples from different parts of the Haryana

Isolates of <i>Verticillium fungicola</i>	White button mushroom diseased samples collection		
	Isolates Code	Village/ City	District
VF-1	MHS	Matarshyam	Hisar
VF-2	BFT	Bhattu	Fatehabad
VF-3	NJN	Narwana	Jind
VF-4	RHT	Rohtak	Rohtak
VF-5	TPN	Taharpur	Panipat
VF-6	BSN	Bainyapur	Sonipat
VF-7	FDB	Fridabad	Fridabad
VF-8	SKK	Sudha mushroom lab	Kurukshetra

Table 2. Effect of different temperature regimes on radial growth of *Verticillium fungicola* isolates

Sr. No.	Temp.(°C)	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		MHS				BFT				NJN			
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	15±1	1.17	2.97	10.83	4.99	1.27	2.17	10.00	4.99	1.23	4.17	11.33	5.58
2	20±1	2.13	6.83	23.00	10.66	1.33	6.67	22.50	10.66	2.00	6.00	24.33	10.78
3	25±1	2.97	11.50	35.83	16.77	3.67	12.67	34.83	16.77	3.50	11.67	38.00	17.72
4	Mean	2.09	7.10	23.22		2.09	7.17	22.44		2.24	7.28	24.56	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	1.36	1.36	2.36		0.78	0.78	1.36		1.89	1.89	3.28	
Sr. No.	Temp.(°C)	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		RHT				TPN				BSN			
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	15±1	1.33	5.33	12.50	6.39	1.50	5.17	17.50	8.06	1.17	5.00	18.00	8.06
2	20±1	3.67	14.50	33.17	17.11	4.17	13.67	36.00	17.94	4.17	12.00	36.33	17.50
3	25±1	4.67	17.00	41.50	21.06	5.67	22.17	43.83	23.89	6.33	22.33	44.67	24.44
4	Mean	3.22	12.28	29.06		3.78	13.67	32.44		3.89	13.11	33.00	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	0.91	0.91	1.57		0.76	0.76	1.32		0.91	0.91	1.58	
Sr. No.	Temp.(°C)	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		FDB				SKK				DMR			
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	15±1	1.17	4.33	16.17	7.22	1.50	5.17	14.67	7.11	1.93	5.40	18.50	8.61
2	20±1	3.50	12.83	35.17	17.17	3.67	13.17	34.00	16.94	4.57	14.17	37.33	18.69
3	25±1	6.50	23.00	43.33	24.28	5.50	20.00	42.17	22.56	7.67	26.17	45.00	26.28
4	Mean	3.72	13.39	31.56		3.56	12.78	30.28		4.72	15.24	33.61	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	1.28	1.28	2.21		1.29	1.29	2.23		1.18	1.18	2.05	

*Average of three replications, A- Temp. treatments, B- Observation of isolates

Table 3. Effect of different pH regimes on radial growth of *Verticillium fungicola* isolates

Sr. No.	pHScale	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		MHS			BFT			NJN					
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	6.0	2.50	10.00	31.33	14.61	3.00	10.50	30.33	14.61	3.17	9.50	32.83	15.17
2	6.5	3.00	11.50	37.17	17.22	3.67	12.67	35.67	17.33	3.50	11.67	38.50	17.89
3	7.0	1.50	6.83	22.83	10.39	1.33	6.67	22.50	10.17	2.00	6.00	24.33	10.78
4	7.5	1.17	1.33	10.83	4.44	1.17	2.17	10.00	4.44	1.17	4.17	11.33	5.56
5	Mean	2.04	7.42	25.54		2.29	8.00	24.63		2.46	7.83	26.75	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	1.21	1.40	2.43		0.69	0.80	1.38		1.78	2.05	3.56	
Sr.No.	pHScale	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		RHT			TPN			BSN					
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	6.0	4.00	14.33	35.33	17.89	4.67	20.00	39.33	4.67	4.50	14.83	40.33	19.89
2	6.5	4.67	17.00	41.33	21.00	6.50	23.00	44.00	6.50	5.50	20.00	44.83	23.44
3	7.0	3.67	13.83	31.33	16.28	3.50	12.83	36.00	3.50	3.67	13.17	36.00	17.61
4	7.5	1.33	5.33	12.50	6.39	1.17	5.83	17.50	1.17	1.67	5.17	18.33	8.39
5	Mean	3.42	12.63	30.13		3.96	15.42	34.21	3.96	3.83	13.29	34.88	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	0.71	0.82	1.43		0.84	0.97	1.67		0.84	1.18	2.04	
Sr. No.	pHScale	Radial growth* (mm) of <i>Verticillium fungicola</i> isolates											
		FDB			SKK			DMR					
		4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean	4DAI	8DAI	12DAI	Mean
1	6.0	5.00	15.33	39.17	19.83	5.00	18.33	37.83	20.39	5.83	16.17	41.17	21.06
2	6.5	6.33	22.33	42.17	23.61	5.67	22.17	41.50	23.11	6.83	23.17	45.00	25.00
3	7.0	4.17	12.00	35.17	17.11	4.17	13.67	34.00	17.28	4.33	12.50	36.33	17.72
4	7.5	1.33	5.00	16.17	7.50	1.83	5.67	14.67	7.39	2.50	7.50	18.50	9.50
5	Mean	4.21	13.67	33.17		4.17	14.96	32.00		4.88	14.83	35.25	
	Factors	A	B	A×B		A	B	A×B		A	B	A×B	
	CD at 5%	1.20	1.38	2.40		1.00	1.16	2.00		0.97	1.12	1.93	

*Average of three replications, A- pH treatment, B- Observation of isolates

was recorded in BSN (44.67 mm), followed by TPN (43.83 mm), FDB (43.33 mm), SKK (42.17 mm), RHT (41.50 mm) and NJN (38.00 mm), while the rest one such as MHS (35.83 mm) and BFT (34.83 mm) showed minimum radial growth at $25\pm 1^\circ\text{C}$ after 12 days of incubation. Thus a temperature of $25\pm 1^\circ\text{C}$ was found to be the most suitable for mycelial growth of *V. fungicola* isolates which showed maximum radial growth. On the other hand, at $15\pm 1^\circ\text{C}$ the radial growth of all *V. fungicola* isolates was significantly reduced *i.e.* 10.00 (BFT), 10.83 (MHS), 11.33 (NJN), 12.50 (RHT), 17.50 (TPN), 18.00 (BSN), 16.17 (FDB) and 14.67 mm (SKK) after 12 days of incubation.

To find out the suitable pH for the growth of *V. fungicola* isolates, the potato dextrose agar medium pH was adjusted to different pH levels *viz.*, 6.0, 6.5, 7.0 and 7.5. The data presented in table 3, indicate that *V. fungicola* isolates grow at pH regimes of 6.0 to 7.5. However, the maximum radial growth was achieved in all the isolates when pH was 6.5 followed by 6.0, 7.0 and 7.5. Among isolates the maximum growth was recorded at pH 6.5 in BSN isolate (44.83 mm), followed by TPN (44.00 mm), FDB (42.17 mm), SKK (41.50 mm), RHT (41.33 mm) and NJN (38.50 mm), while the minimum radial growth was recorded in isolates MHS (37.17 mm) and BFT (35.67 mm) at $25\pm 1^\circ\text{C}$ after 12 days of incubation (Plate 3). So, the pH of 6.5 was found to be the most suitable for mycelial growth of *V. fungicola* isolates after 12 days of incubation when compared with reference pathogen. On the other hand, at pH 7.5 the radial growth of all *V. fungicola* isolates was significantly reduced *i.e.* 10.00 (BFT), 10.83 (MHS), 11.33 (NJN), 12.50 (RHT), 17.50 (TPN), 18.33 (BSN), 16.17 (FDB) and 14.67 mm (SKK) after 12 days of incubation.

Isolates when grown on different temperature regimes (15 to 25°C) showed that these can grow in this range. However, the maximum radial growth was observed in *V. fungicola* isolates at $25\pm 1^\circ\text{C}$ followed by $20\pm 1^\circ\text{C}$ and $15\pm 1^\circ\text{C}$. Amongst isolates the

maximum growth was recorded in BSN (44.67 mm), followed by TPN (43.83 mm), FDB (43.33 mm), SKK (42.17 mm), RHT (41.50 mm) and NJN (38.00 mm), while the isolates MHS (35.83 mm) and BFT (34.83 mm) showed minimum growth at $25\pm 1^\circ\text{C}$ after 12 days of incubation. Our findings supported the work of Sabharwal and Kapoor, (2014) who observed that the optimum temperature for the mycelial growth of the mycopathogen was 25°C , while poor growth was recorded at 15°C and 20°C and the least growth was at 30°C . *V. fungicola* isolates can grow at pH regimes of 6.0 to 7.5. However, the maximum radial growth was achieved in all the isolates when pH was 6.5 followed by 6.0, 7.0 and 7.5. At pH 6.5 isolate BSN showed maximum growth (44.83 mm), followed by TPN (44.00 mm), FDB (42.17 mm), SKK (41.50 mm), RHT (41.33 mm) and NJN (38.50 mm), while minimum radial growth was recorded in the isolates MHS (37.17 mm) and BFT (35.67 mm) at $25\pm 1^\circ\text{C}$ after 12 days of incubation. Similarly, Siwulski *et al.* (2011) reported that mycelium growth of the *V. fungicola* isolates is greatly affected by medium pH (5.5 to 7.5) and temperature (15 to 30°C), being maximum at 25°C and 5.5 pH. However according to Fletcher *et al.* (1989) the pathogen grows best at 24°C . On the contrary Sharma and Kumar, (2000) reported that the radial mycelial growth of *V. fungicola* in the pH range from 4 to 8 varied from 54.4 to 78.18 mm and maximum being at pH 5.0. Similarly, Tan *et al.* (1994) observed that optimal pH for the development of mycopathogen ranged from 5.0 to 5.6.

CONCLUSION

V. fungicola isolates also responded differentially when subjected to different temperature regimes. Though *V. fungicola* isolates can grow at all the temperature regimes evaluated in the study, however the maximum growth was observed at $25\pm 1^\circ\text{C}$ followed by $20\pm 1^\circ\text{C}$ and $15\pm 1^\circ\text{C}$. The isolates BSN showed maximum radial growth (44.67 mm), followed by TPN (43.83 mm), FDB (43.33 mm), SKK (42.17 mm), RHT (41.50 mm) and NJN (38.00 mm), while

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isolates MHS (35.83 mm) and BFT (34.83 mm) showed minimum radial growth after 12 days of incubation at 25±1°C. The pathogen growth also varied with pH of the medium. Isolate BSN showed maximum radial growth (44.83 mm), followed by TPN (44.00 mm), FDB (42.17 mm), SKK (41.50 mm), RHT (41.33 mm) and NJN (38.50 mm), while the isolates MHS (37.17 mm) and BFT (35.67 mm) showed minimum radial growth at pH 6.5 when plates were incubated at 25±1°C for 12 days.

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