

Original Research Article

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## Evaluation of Histopathological Changes and Enzyme Bioassay during Interaction between *Verticillium fungicola* and *Agaricus bisporus*

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### ABSTRACT

*Verticillium fungicola* var. *fungicola* (Preuss) is the most important dry bubble disease causing pathogen of the *Agaricus bisporus* (Lange) Imbach. Therefore, present investigation carried out on *A. bisporus* host and *V. fungicola* pathogen; by covering an aspect of interaction, variability and management of *Verticillium* dry bubble disease in India. Host and pathogen easily distinguishable at interaction regions on the basis of hyphal width *i.e.* ranged from 4.0 to 6.5  $\mu\text{m}$  and 1.5 to 4.5  $\mu\text{m}$  respectively. Pathogen grow inter and intercellularly on host hyphae and resulting in damaged, coiling and lysis of host mycelia. During enzymatic bioassay in dual culture on inducing substrate and mushroom extracts, mycopathogen showed the production of different hydrolytic enzymes *i.e.* amylase, cellulose and chitinase but not lipase and pectinase by formation of clear zonation on substrate.

#### Keywords

*Verticillium fungicola*,  
*Agaricus bisporus*,  
Enzyme, Interaction,  
Histopathology.

#### Article Info

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### Introduction

The commercial production of edible mushroom converts different types of agricultural and household wastes into nutrition rich food which helps in addressing the problems of quality food, health and environmental sustainability. In view of increasing demand of high quality food with an increasing world population, mushrooms will be an important source of proteins that can replace meat and vegetables and milk products for a major part (Wani *et al.*, 2010). About 1.5 million species of fungus are known (Hawksworth, 1991) 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). In India, mostly four species of edible mushrooms *viz.*, *Agaricus bisporus* (white button mushroom), *Volvariella* spp. (paddy straw mushroom), *Pleurotus* spp. (oyster mushroom) and *Calocybe indica* (milky mushroom) are commercially cultivated.

Mushroom cultivation is affected by a large number of biotic and abiotic 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 perniciosa*, *Trichoderma* spp. and *Papulaspora byssina* are the predominant mycopathogens. Amongst these, *Verticillium fungicola* var. *fungicola* (Preuss) is the important pathogen of the *Agaricus bisporus* (Lange) Imbach 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).

## Materials and Methods

By dual culture method hyphal interactions between *A. bisporus* and *V. fungicola* were studied by growing five mm mycelia disk cut from actively growing colonies of both fungi and were placed six cm apart on the surface of 2% PDA in Petri plates and 0.2 % PDA on a clean and sterile glass microscope slide and incubated both at 25±1°C for nine days. The antagonist grew radially and overgrowth of *A.*

*bisporus* by *V. fungicola* began to occur by 5 to 7 days after inoculation. Mycelial samples from the interaction region in Petri plates were excised along with agar media and in case of glass microscope slide the mycelia at point of interaction directly examined and each one stained with methylene blue during examination. These samples were examined under high power objective of optical research compound microscope (40X). Records of the interactions between opposing colonies including hyphal width, contact or coiling were made after incubation. After one week, the slides were examined microscopically for lysis of *A. bisporus* mycelium by *V. fungicola*. Extracellular enzymes (*viz.* amylase, cellulase and chitinase) bioassays of pathogen during *V. fungicola* and *A. bisporus* interaction as per methods of Cattelan *et al.*, (1999) and Lima *et al.*, (1993). Fungal enzymes play an important role in host infection since they can localize on host structures to develop infection and eventually leading to disease. Hence, the qualitative bioassay was performed to estimate the presence of extracellular enzymes *i.e.* amylase, cellulose, chitinase, lipase and pectinase during interaction of *V. fungicola* and *A. bisporus* in dual culture.

## Results and Discussion

### Mode of parasitism under microscope

Hyphal interactions (Plate-1): the mycelia samples taken from the interaction zone of dual cultures in Potato dextrose agar. The hyphae of *V. fungicola* and *A. bisporus* in individual as well as in paired cultures could be readily distinguished on the basis of hyphal width. The hyphal width of host ranged from 4.0-6.5µm while mycopathogen ranged from 1.5-4.5 µm. Therefore, different hyphal width of both easily distinguish under interaction study and at interaction point host mycelium damaged and pathogen grow in and on

hyphae of host and coiling and lysis of host hyphae were also reported after two weeks of incubation.

During interactions studies the host and pathogen easily distinguish and at interaction point host mycelium damaged and pathogen grow in and on hyphae of host and coiling and lysis of host hyphae were also reported after two weeks of incubation. These findings are

in complete agreement with Pieterse (2005) and Sabharwal *et al.*, (2014) that depicted the clear difference in hyphal diameter of host and pathogens and easily distinguishing the two under interaction studies. Similarly, Dragt *et al.*, (1996) showed that hyphae of the mycopathogen coil around the host hyphae with firm adhesions but intra-hyphal growth can also be observed in dry bubble pathogen *Verticillium fungicola* var. *fungicola*.

**Table.1** Enzymatic bioassay during interaction of *Agaricus bisporus* and *Verticillium fungicola*

Sr. No.	Enzymatic activity *bioassay during host and pathogen interaction				
	Name of enzymes	Activity inducer medium	<i>Agaricus bisporus</i>	<i>Verticillium fungicola</i>	Both
1	Amylase	Substrate	+	+	+
		Mushroom extract	-	+	+
2	Cellulase	Substrate	+	+	+
		Mushroom extract	-	+	+
3	Chitinase	Substrate	-	+	+
		Mushroom extract	-	+	+
4	Lipase	Substrate	-	-	-
		Mushroom extract	-	-	-
5	Pectinase	Substrate	-	-	-
		Mushroom extract	-	-	-

\*Average of three replications, Where + : zone (enzyme activity) present and - : zone (enzyme activity) absent

## MICROSCOPICALLY EXAMINATION OF INTERACTION

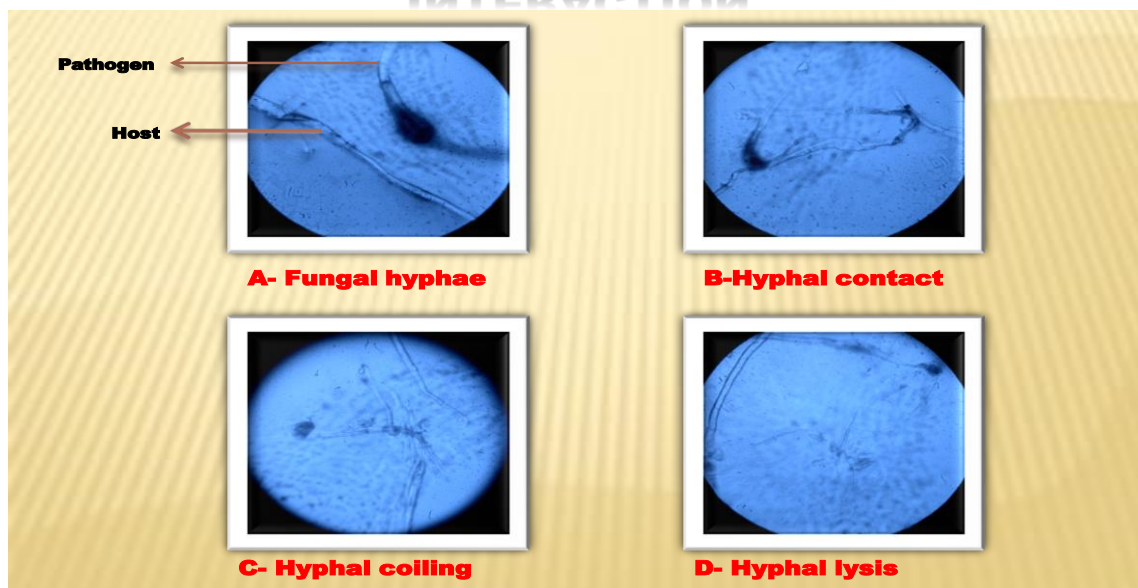
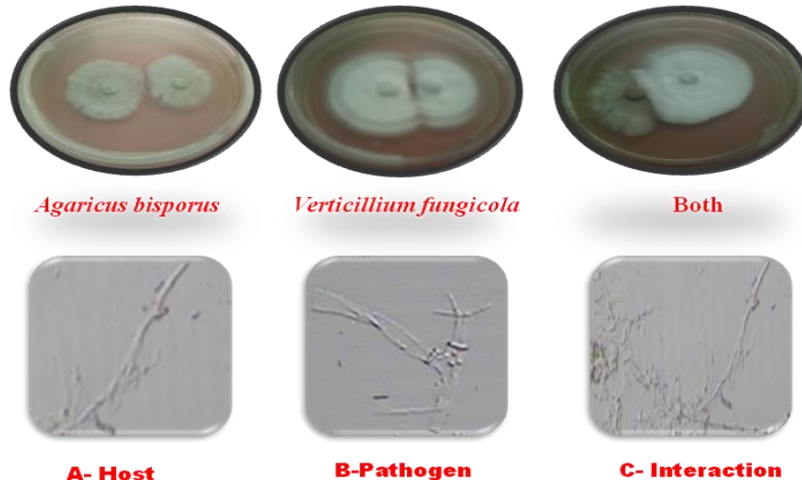


Plate.1

**HYPHAL INTERACTION IN DUAL CULTURE**



**Extracellular enzymes bioassays**

*V. fungicola* screened for its extracellular enzymes bioassay (Table-1) like amylase, cellulose, chitinase, lipase and pectinase activity by plate assay method during host–pathogen interaction. The qualitative assessment of mycopathogen singly as well as in dual culture on enzymatic activity inducer medium substrate and mushroom extracts showed the production of different hydrolytic enzymes *i.e.* amylase, cellulose and chitinase but not lipase and pectinase. *V. fungicola* singly as well as in dual culture showed the clear zonation on substrate and mushroom extract which are due to enzymatic activity of amylase, cellulose and chitinase while, rest one (lipase and pectinase) no any zonation are reported. The qualitative assessment of mycopathogen singly as well as in dual culture on enzymatic activity inducer medium substrate and mushroom extracts showed the production of different hydrolytic enzymes *i.e.* amylase, cellulose and chitinase but not lipase and pectinase. Therefore, we considered that the disintegration of host hyphae due to enzymatic activity of

mycopathogen. Similarly, Mills *et al.*, (2008) confirmed that *V. fungicola* produced a wide range of hydrolytic enzymes, which play a critical role in the infection process. On the other hand, Trigiano and Fergus, (1979) reported that *V. fungicola* is able to produce extracellular enzymes such as an amylase, lipase and cellulose.

During interactions studies the host and pathogen easily distinguish and at interaction point host mycelium damaged and pathogen grow in and on hyphae of host and coiling and lysis of host hyphae were also reported after two weeks of incubation. *V. fungicola* in dual culture showed the clear zonation on substrate and mushroom extract which are due to enzymatic activity of amylase, cellulose and chitinase.

Therefore present studies concluded in single pharos, it revealed that dry bubble disease of button mushroom was caused by *V. fungicola* due to producing various enzymes during interaction process and caused coiling and disintegration of host hyphae which leads to symptoms development.

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