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*Full Length Article*

# Detection of a Virus Disease on White Button Mushroom (*Agaricus bisporus*) in Ankara, Turkey

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## ABSTRACT

Numerous virus-like symptoms including reduced yield, deformed and brownish sporophores were observed on white button mushrooms (*Agaricus bisporus*) at a mushroom house in Ankara. The disease agent detection was accomplished using growth tests of mycelium on agar, direct electron microscopy and double-stranded RNA (dsRNA) analysis. Diseased sporophore tissues were grown on potato dextrose agar (PDA) and brown colonies developed irregularly and slowly on PDA. Agarose gel electrophoresis of total nucleic acid fractions revealed the presence of three dsRNAs in diseased sporophores. Rod-shaped particles (34 nm) in diseased mushrooms were observed. These findings proved a virus infection in diseased mushrooms in Ankara. Our results showed that disease was likely to be La France disease caused by La France virus. This is the first record of presence of a virus infection in Central Anatolia Region in Turkey. 

**Key Words:** White button mushroom; *Agaricus bisporus*; Virus; Disease

## INTRODUCTION

Roughly 35 mushroom species have been cultivated commercially worldwide. Of these, 20 are cultivated on an industrial scale. A majority of cultivated species are both edible and possess medicinal properties. Among them, white button mushroom [(*Agaricus bisporus*) (J. Lge) Imbach)] is the most widely cultivated mushrooms in the world, which account for 35-45% of the total mushroom production. Large scale white button mushroom production is centered in Europe (mainly Holland & France), North America (USA, Canada) and S.E. Asia (China, Korea, Indonesia, Taiwan & India) (Chang, 1999). Annual white button mushroom production is 27000 tons in Turkey and main regions include Marmara, Aegean, Mediterranean and Central Anatolia (Anonymous, 2009). Nonetheless mushroom production is low in Turkey. One of the most important reasons for this is low demand in addition to inadequate standards in freshness, color, structure, size, yield and marketing.

There are various biotic and abiotic agents causing damages to mushrooms. Among these disease factors, virus diseases are one of the major limiting factors. Virus as a cause of disease of mushrooms was first described in 1948 and since then various diseases have been described. In the 1960s, many outbreaks were reported and considerable losses in yield were attributed to virus disease. Mushroom virus diseases have been recognized in many mushroom-growing countries all over the world and have probably been present as long as mushrooms were grown (Dieleman-van Zaayen, 1979). Sinden and Houser (1950) for the first time noticed the symptoms of a very infectious disease of mushrooms (*A. bisporus*) leading to large scale losses in yield, which was named "La France Disease". Gandy and Hollings (1962) demonstrated that it was a virus disease.

At present, five species of virus are associated with mushrooms worldwide. La France disease, caused by La France isometric virus (LIV), also known as X-disease, dieback, watery stipe and brown disease, is among the most serious infectious pathogens of *A. bisporus*. LIV is an unclassified virus (Goodin *et al*., 1997). Associated with La France disease is a mushroom bacilliform virus (MBV), a sole member belonging to the genus *Barnaviridae* and commonly found as a double infection with LIV affected mushrooms (Revill *et al*., 1999; Romaine, 2000). Mushroom virus X (MVX) is a disease of the *A. bisporus*. It seriously affected the UK mushroom industry in 2000/01 and has been reported in Ireland, Holland and a number of other mushroom growing countries. The dsRNAs of MVX are likely to represent a complex of more than one virus (Adie *et al*., 2004). Vesicle virus (VV) also appears to be a benign virus that is widely distributed in commercial mushrooms (Romaine, 2002). *A. bisporus* endornavirus 1 (AbEV1) is a fourth virus that represents the first endornavirus known to infect edible mushrooms (Maffettone, 2007; Woodhall *et al*., 2009).

There are very few studies concerning virus detection in button mushrooms in Turkey (Fidan *et al*., 1998 & 2000). In this study, virus detection was done using agar test, electron microscopy and double-stranded RNA (dsRNA)

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analysis from the virus infected *A. bisporus* with high incidence in a mushroom house in Ankara.

## MATERIALS AND METHODS

**Growth rates of mycelium on agar:** Approximately 0.5 $cm^3$ of diseased and healthy sporophore tissues were grown on potato-dextrose agar (PDA) and incubated at 25-26°C for 20 days (Koons *et al*., 1989). The colony growth was assessed with regard to colony color, morphology and growth rate.

**Isolation of dsRNA:** Nucleic acids were extracted as described preciously by Sonnenberg *et al*. (1995). Sporophore tissues were homogenized in mortar and pestle with 1.5x extraction buffer (250 mM NaCl, 200 mM Tris-HCL, pH 7.5, 1% SDS & 1 mM EDTA, 1/2 w/v) and filtered with a cheesecloth. The liquid was extracted with an equal volume of phenol/chloroform. Extraction was centrifuged at 12000x*g* for 1 h at 4°C and the supernatant liquid was adjusted to 15% ethanol in 1xSTE buffer, 30 mg CF-11 Cellulose was added. The suspension was occasionally vortexed on ice for 1 h and then centrifuged 10000x*g* for 30 sec. Supernatant was discarded and the pellet was washed with 1.5 mL STE buffer (100 mM NaCl, 50 mM Tris-HCl pH 8.0, 1 mL EDTA) containing 15% ethanol. The dsRNA was eluted with 200 µL 1xSTE at three times, precipitated with an aqual volume of isopropanol and incubated -20°C for 2 h. The suspension was centrifuged 10000 x*g* for 1 h and washed with 1 mL of 70% cold ethanol and dried. The pellet suspended in 100 µL sterilized distillated water and electrophoresed in 1% agarose gel with STE buffer. Molecular weights of the dsRNAs were estimated from their electrophoretic mobilities relative to the *BstE* II restriction endonuclease fragments of lambda DNA (Koons *et al*., 1989).

**Electron microscopy:** A 50 g sample of mushrooms was homogenized in 0.015 M phosphate buffer (pH 7.0) containing 5% polyvinylpyrrolidone (PVP) and 0.02 M 2-mercaptoethanol (2-ME). The homogenate was centrifuged at 15000×*g* for 10 min and supernatant adjusted top pH 4.8 with 0.5 M citric acid. After 30 min the precipitate was collected by centrifugation and resuspended in 0.05 M citrate buffer (pH 7.0) for 24 h. The resuspended material was again precipitated with citric acid and resuspended as before. This extract was then centrifuged at 180000×*g* for 2 h. The pellet was finally resuspended in 1 mL of citrate buffer. The concentrated preparations were then negatively stained with 2% potassium phosphotungustate (PTA) and examined in electron microscope (Pasmore & Frost, 1974).

## RESULTS AND DISCUSSION

Various symptoms were observed in the mushroom house, including distortions of the sporophores, elongation of the stalks, brown caps and thickened barrel-shaped stipes (Fig. 1). Affected crops showed a patchy appearance associated with death of the mycelium. A high crop loss was also observed in the mushroom house. Our observed symptoms were determined as diagnostic symptoms of La France virus disease by several researchers (Schistler *et al*., 1967; Dieleman-Van Zaayen, 1972; Fletcher *et. al*., 1989; Koons *et al*., 1989).

**Fig. 1: Brown, thickened, barrel-shaped stipes with small pleus (left), elongated stipe with small cap (center), malformed and misshapen fruit body (right).**

**Fig. 2: An irregular and brownish colony of diseased *A. bisporus*.**

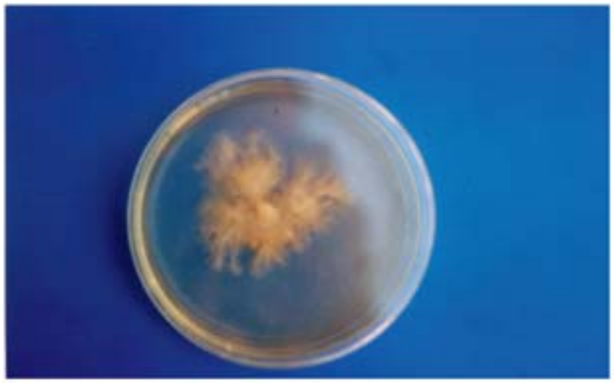


**Fig. 3: Regular and white colonies of healthy *A. bisporus*.**

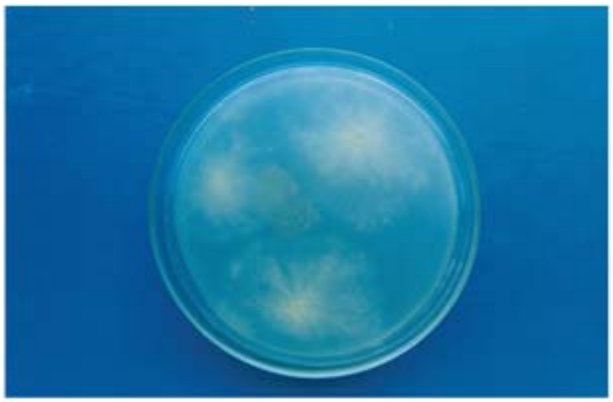


**Fig. 4: DsRNA patterns in diseased mushrooms (B) and molecular weight markers (A).**

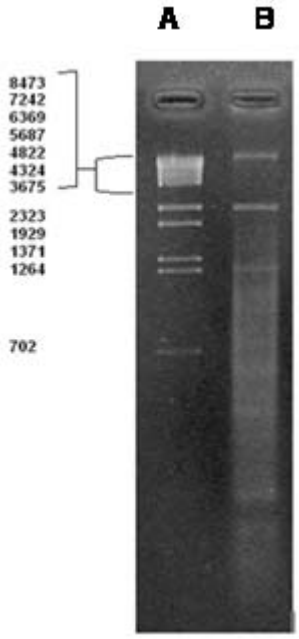
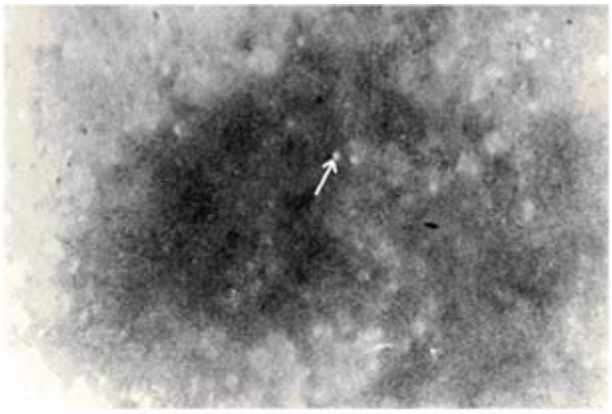



**Fig. 5: Virus particles extracted from diseased mushrooms: Arrow indicate the stained particle (X 48000).**



Diseased sporophore tissues were grown on PDA and brown colonies developed irregularly and slowly on PDA (Fig. 2). Average diameter of colonies was measured as 19.4 mm. In healthy mushrooms; however, colony formation was regular, colonies were white in color (Fig. 3) and average colony diameter was measured as 27.6 mm. Several researchers also reported that virus-infected mycelia of *A. bisporus* developed sparsely, colony colors were brown and colony formation was irregular (Schistler *et al*., 1967; Romaine *et al*., 1993; Fidan *et al*., 1998).

Agar growth assay is one of the tests to diagnose viral infections of mushrooms and that even a minimum of 15% retardation in mycelia development in virus-infected mushrooms in comparison to mycelia development in healthy mushrooms would indicate the presence of a viral infection (Fletcher *et al*., 1989). In addition, virus-infected mycelia typically develop slowly and have brown color (Koons *et al*., 1989; Romaine *et al*., 1993; Chang & Miles, 2000). Slow and abnormal mycelia development is characteristics of viral disease (Romaine & Schlagnhaufer, 1995). The reports mentioned above supported that abnormal cultural characteristics of our samples were due to viral infection.

Agarose gel electrophoresis and ethidium bromide staining of total nucleic acid fractions revealed the presence of three dsRNAs in diseased sporophores that were absent in healthy sporophores. Three dsRNAs (over 8473, 2450 & 1350 kb) were only found in symptomatic mushroom sporophores (Fig. 4). Some researchers detected apparently healthy mushrooms (2400, 2350, 15 & 6.5 kb) dsRNAs (Harmsen *et al*., 1989; Romaine & Schlagnhaufer, 1989; Grogan 2003), although some others did not detect it in healthy mushrooms (Deahl *et al*., 1987; Wach *et al*., 1987; Koons *et al*., 1989; Romaine *et al*., 1993). Our findings agreed with those who reported dsRNAs in healthy mushrooms. Detected bands (2350 & 1350 kb) were also typical for LIV-infected mushrooms, but not characteristic for MVX-infected ones (Harmsen *et al*., 1989; Grogan *et al*., 2003). Reports show up to 12 and 26 dsRNAs (0.27-4.5 kb) in LIV-infected mushrooms (Wach *et al*., 1987; Harmsen *et al*., 1989; Goodin *et al*., 1992; Romaine & Schlagnhaufer, 1995; Sonnenberg *et al*., 1995; Revill & Wright, 1997) and MVX-infected mushrooms (Grogan *et al*. 2003; Rao *et al*. 2007), respectively. The dsRNA genome of virus has been reported in fungi (Ihrmark *et al*., 2002). Their infestation may vary from crop to crop and also large dsRNA concentration differences can be observed between different fruit bodies within the same crop (Sonnenberg *et al*., 1995). Growth temperature has a pronounced effect on the accumulation of dsRNAs in *A. bisporus* (Koons *et al*., 1989). Romaine and Goodin (2002) indicated that the detection of duplex RNA molecules in symptomatic mushroom tissues constitutes the most convincing evidence for the LIV disease of viruses. Hence presence of dsRNAs in diseased mushrooms indicated that they were virus infected.

The researchers isolated three types of virus particles from fruit bodies exhibiting La France disease symptoms; isometric particles with a diameter of 25 nm and 34-36 nm and bacilliform particles, size 19 x 50 nm (Hollings, 1962; Dieleman-van Zaayen, 1972; Goodin *et al*., 1992; Van der Lende *et al*., 1994). The 19 x 50 nm bacilliform virus [Mushroom bacilliform virus (MBV)] was not detected in healthy mushrooms and was not detected in all cases of La France disease (Dieleman-van Zaayen, 1979; Romaine & Schlagnhaufer, 1995). There is a wealth of circumstantial evidence implicating a dsRNA genome, 36-nm-diameter isometric virus (LIV) in the etiology of La France disease (Goodin *et al*., 1992). In this study, we also observed spherical particles about 34 nm in diameter (Fig. 5) and not

observed rod-shaped particles in both diseased and healthy mushrooms.

Consequently, abnormal growth of mycelium on agar, absence of dsRNAs in healthy mushrooms, presence of virus-typical two dsRNAs and observation of spherical particles in diseased mushrooms indicated that the symptoms in mushrooms were virus originated. All these data indicated that the symptoms were viral in nature. These findings indicated that the disease symptoms likely related to La France disease caused by La France virus. However, further studies are needed to support the results.

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