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Lentinula edodes : AN EFFECTIVE ANTIMICROBIAL CONTROL AGAINST PLANT PATHOGENS - A REVIEW

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ABSTRACT
In considering the enormous rise in the occurrence of diseases resistant to antibiotics, it is crucial to consider alternative therapies. The main causes of significant losses each year in plant are diseases are fungi, bacteria, viruses, and nematodes. Bio control agents can therefore be employed to treat these plant pathogens and enhancing the positive responses of plants. These biocontrol agents are an alternate approach to manage plant diseases that will help to minimise the overuse of agricultural pesticides. *Lentinula edodes* an edible mushroom has antimicrobial property against different plant pathogen and can play a role of effective biocontrol agent. They produce volatile chemicals that have a direct harmful impact on pathogens. They reduce the use of chemicals, less phyto toxic and improve soil health.

INTRODUCTION

A huge diversity of disease and pest attacks almost all type of crops in the environment. Beyond any doubt it is right, that use of chemical based insecticides to control plant disease has remarkably increased the crop production to the point that our nation has not only achieved self-sufficiency but also an exporter of various agricultural products to the world. These chemical pesticides have been used broadly in agriculture for diseases and pest management from past few decades (Hickey, 1986). The chemical based pesticides are very quick in action but relentless use of these chemicals in crop production is detrimental to the ecosystem and environment (Muto *et al*., 2005), and is also responsible for emergence of new isolates which are resistant to the fungicides (Parra and Ristaino, 2001). Hence there is a prime need to look for the natural antimicrobial agents for a sustainable agriculture crop production.

Organic way to control plant diseases has gained a lot of attention these days. For minimizing damage to the environment use of natural products for pest management is favorable for sustainable crop production (Stanley *et al*., 2002). Different sources are investigated for antimicrobial compounds including prokaryotes, marines, fungi, animals etc. (Fallarero *et al*., 2014). Different types of plant extracts can be used to control plant diseases *i.e.*, neem extract, garlic extract, onion extract *etc.* against *Alternaris solani* (Yadav *et al*., 2017). In past few years, mushrooms are being an important and interesting part of search for being an antimicrobial agent (Singh *et al*., 2014). 14,000 mushroom species have been outlined but only 2,000 species of them are edible (Hawksworth, 2001). The basidomycetes show various biological activities and low toxicity, which makes them a promising source of bioactive molecules (Arnone *et al*., 1997). Globally, *Lentinula edodes* (Shiitake) is one of the most important commercially cultivated edible mushroom in the world, it rank second after *Agaricus bisporus*. Cultivation of shitake mushroom is gaining limelight as it is known not only for having high nutritional values and medical properties but also show potent antimicrobial activity against the plant pathogenic microorganism.

Fungi have two different culture types

Solid-state fermentation (SSF) and submerged fermentation (SmF), which are essentially differentiated by the substrates they employ. The optimum method for fungi to ferment in order to produce a high output of certain bioactive chemicals is SSF, which uses solid substrates like bran, bagasse, and paper pulp (Vikineswary *et al*., 1997, Subramaniyam and Vimala, 2012). Additionally, recent research has indicated that SSF produces more stable and substantial amounts of antibiotics than SmF (Vikineswary *et al*., 1997). The postharvest substrate known as spent mushroom substrate (SMS) continues to have a number of bioactive elements created during the growth of mycelium and the development of fruiting bodies, including extracellular enzymes, antibiotics, secondary metabolites, and carbohydrates. SMS is quite similar to a sort of solid-state fermentation SSF that is utilized to make a natural

antibiotics based on its growth features. SMS of *Lentinula edodes* also contain a range of bioactive substances with antimicrobial activity *i.e.* lentinamicin (octa-2, 3-diene-5,7 diyne-1-ol), β-ethyl phenyl alcohol and lentin, an antifungal protein (Ngai and Ng, 2003) and Unlike most other recycling methods, SMS do not have to be lined in cropping area for long time to be reused as a carrier for bio fertilizer and bio control agent (Shitole *et al*., 2014).

The aim of present article is to summarize the literature of *Lentinula edodes* in vivo as well as in vitro experiments in reference to their History, classification, characteristic features and antimicrobial activity, which has the potential to be used as a bio pesticide and can be used in future as an effective bio agent to protect plant from disease, improve yield, conserve ecosystem, natural resources and a step towards sustainable agriculture system.

History, Botanicals classification and General characteristics of *Lentinula edodes*

Mushrooms have been consumed for centuries for their sensory characteristics and culinary attributes. In world there are thousands of species of mushroom cultivated for human consumption however, only about 25 varieties are cultivated for their edible characteristics and medicinal properties (Ng and Tan, 2017) few of them are *Agricus bisporus, Pleurotus ostreatus, Lentinula edodes, Volvariella volvacea, Calocybe indica etc*. These mushrooms are grown throughout the world, and require different methods and environment for cultivation. Substrate ultization is also an important aspect for example *Pleurotus ostreatus* can be grown on a variety of straw but wheat is best substrate for its cultivation (Pant *et al*., 2020).

Out of these edible mushroom Shiitake mushroom (*Lentinula edodes*) is one among the six popular edible mushrooms in the world contributing 17 per cent production in terms of tonnes (Chang and Miles, 2004; Miles and Chang, 1997) and according to Diallo *et al*. In 2020 it is most widely cultivated mushroom which accounts for 25% of global mushroom output. *Lentinula edodes* also known as Shiitake, is the second most cultivated mushroom species globally owing its unique palatability. It was first described in 1877 by mycologist Miles Joseph Berkeley and since then has been given several scientific names. In addition, it is known as saw tooth oak mushroom, oak wood mushroom, golden oak mushroom, black forest mushroom and black mushroom. It is thought that this mushroom may have first been cultivated as long ago as 1000 AD, in China and is still a highly sought-after mushroom in traditional medicine. Shiitake mushroom species are the edible mushroom which have originated from East Asia and cultivated and consumed in numerous Asian nations (Chittaragi *et al*., 2018).It approximately represents 25% of worldwide mushroom production. Shiitake is a complete nutritional food type because it has low lipid content, high protein content, vitamins, minerals and fibers (Akesowan 2016).

The taxonomic classification according to Ainsworth 1973, this mushroom belongs to the Kingdom:Fungi;Division: Eumycota;SubDivision:Basidiomycota;Class:Hymenomycetes; Order:Agaricales; Family:Tricholomataceae; Genus: Lentinula. The species which are commercially cultivated in different parts of the world are *Lentinula aciculospora, L. boryana, L. edodes, L. guarapiensis, L. lateritia, L. raphanica etc*. Out of these species *L. edodes* is widely cultivated in India.

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Lentinula edodes is well known for its medicinal properties, which includes polysaccharides, terpenoids, sterols and lipids making it effective against infection, tumors. It contain β-glucan which helps the body against various bacteria, fungi, viruses and most importantly use as natural immunostimulant for cancer treatment in Japan since 1980. β- Glucans are also effective against allogeneic, syngeneic, and even autochthonous tumors (Petravic *et al*., 2010). Due to its high water content and soft texture it was mostly consumed in dried form *Lentinula edodes* is a white rot fungus that secretes a class of ligno-cellulolytic enzymes, which permit it to grow on ligno-cellulosic substrates rich in lignin (Leatham, 1986).

In recent decades a great increase in the International cultivation of *Lentinula edodes* has been noted due to its significant tolerance to varied agro climatic conditions. It is now cultivated commercially all around the world as its cultivation is a nonpolluting and ozone friendly activity where agricultural/industrial wastes are used and reprocessed. Previously it grows on decaying wood of deciduous trees particularly on shii, oak, poplar, ironwood, mulberry *etc*. but after knowing all the precious properties of this mushroom is grown at commercial level also. It can be grown on various substrates including sawdust, wood chips, wheat straw, paddy straw, pine needle, finger millet straw, *etc*. *Lentinula edodes* is a magical crop to be cultivated in developing countries, the first point would be that they can be grown on variety of agricultural residues and it converts a high percentage of the substrate to fruiting bodies, increasing profitability.

Under laboratory condition to obtain pure culture sterilized PDA is use at temperature of $25 \pm 2°C$ while Malt extract media give a good result at different temperature ranges but best grows at 30°C (Furlan *et al*., 1997). The growth of Shitake requires high humidity (80-90%) and high temperature (25-30%) for vegetative growth called spawn running and lower temperature (18-25°C) for fruit body formation. The substrate on which *Lentinula edodes* is grown, also affects the mushroom production.

Antifungal properties of *Lentinula edodes*

According to the research by Bianco in 1981 the action of L. *edodes* on fungi is only mentioned in relation to the yeast Candida albicans in the literature, and a substance extracted from *L. edodes* known as Cortinellin shown antibiotic efficacy against this yeast. This is the first time that the growth of phytopathogenic fungus has been shown to be inhibited by *L. edodes* mycelial extract.

The compound lentin extracted from Leninula edodes inhibits the growth of various fungus species which are Physalospora piricola, Botrytis cinerea, Mycosphaerella arachidicola Ngai and Ng. (2003).

Edible mushrooms culture filtrates were also tested for antifungal efficacy against various plant diseases. Chen and

Huang (2011) observed that *Colletotrichum higginsianum*'s spore germination was entirely inhibited by the culture filtrates of *Lentinula edodes* and Clitocybe nuda and also it was found that *Ganoderma lucidum, L. edodes*, and *C. nuda* were compounds that had the ability to entirely suppress *Alternaria brassicicola* spore germination. Later on the culture filtrate Coprinus comatus, *L. edodes*, Tremella aurantialba, and C. nuda were also found to inhibit the germination of *Phytophthora capsici* spores completely. Istifadah and Herawati in 2019 also found that by adding bacteria from spent mushroom substrate of shiitake and oyster mushrooms to the planting media may prevent tomato leaves from developing the early blight disease.

In study conducted in 2015 by Zanardo *et al*., it was concluded that the fruiting body of *Lentinula edodes* significantly reduced the spore germination of *Colletotrichum sublineolum*, the causal agent of anthracnose in sorghum.

Kang *et al*. (2017) reported that *Lentinula edodes* wasted mushroom substrate (SMS), produced by sawdust bag culture, when utilised as a material to control the pepper *Phytophthora* blight disease. WESMS of *L. edodes* suppresses *Phytophthora* blight disease in pepper seedlings by 65%, accelerated plant development by over 30%, and inhibited *Phytophthora capsici* mycelia growth. High performance liquid chromatography (HPLC) analysis of this substrate revealed that oxalic acid was the primary acid component in this.

The impact of treatment with a hot water extracts from the SMS of *Lentinula edodes* on plants' ability against pathogenic infection. Spraying rice with *L. edodes* SMS extract was followed by inoculating those leaves with rice blast fungus conidia. Treatment with the SMS extract inhibited the growth of lesions. Extract notably prevented the conidia of *Pyricularia oryzae* from germinating (Ishihara *et al*., 2018).

In a study conducted by Istifadah in 2018, the potential and application method of SMS of *Lentinula edodes, Volvariella volvaceae*, and *Pleurotus ostreatus* mushrooms was examined in order to control the basal rot disease caused by *Fusarium oxysporum cepae*. She noted that the SMS of these mushrooms lessened the severity of the basal rot disease.

Priya *et al*. (2019) studied eight mushroom fungi, including *Auricularia polytricha, Coprinus comatus, Ganoderma lucidum, Volvariella volvaceae, Lentinus edodes, Pycnoporus sanguineus, Schizophyllum commune*, and *Trametes versicolor*, against spore germination and mycelial growth of *Colletotrichum capsici*, the mycelial growth of *C. capsici* was inhibited by *Ganoderma lucidum, Auricularia polytricha*, and *Lentinus edodes* to the highest antifungal levels (54.81%, 53.70%, and 45.55%, respectively), with maximum inhibition zones of 4.86 mm, 2.86 mm, and 4.86 mm, respectively, according to results from the dual culture technique .

Herawati and Istifadah investigated the efficacy of *Lentinula edodes* and *Pleurotus ostreatus* SMS against plant diseases in 2019. They observed that the SMS of both mushrooms may suppress *Rhizoctonia solani* development in vitro and reduce tomato seedling damping off disease as well.

By using gas chromatography-mass spectrometry, Muto *et al*., 2022 analyse a volatile chemical emitted by Shiitake SMS and discovered octan-3-one as a significant volatile ingredient. Octan-3-one suppressed Alternaria sooty spot of cabbage and grey mould of tomato in addition to having antimicrobial effects on a variety of bacterial and fungal plant diseases. Because *A. brassicicola* spores began to germinate after octan-3-one was removed, it was determined that the substance has fungistatic properties. The similar study was done by Fujita *et al*. (2021) and reported that the volatile compound of *Lentinula edodes* shows inhibition in growth of *A. brassicicola*.

Antibacterial properties of *Lentinula edodes*

Pacumbaba *et al*. (1999) determined the inhibitory activity of the mycelial leachate of *L. edodes* on the growth of several important species of plant pathogenic bacteria in the laboratory. The mycelial leachate applied as soil drench prevented symptom expression of soil born bacterial pathogens, suggesting that the shiitake mycelial leachate contains an antibiotic component.

In 1999, a study was conducted by Hirasawa *et al*., in which three different antibacterial compounds were extracted from dried Shiitake mushrooms (*Lentinus edodes*) using chloroform, ethyl acetate, or water. They found that these compounds have powerful antibacterial properties that are effective against oral strains of *Streptococcus spp*., *Actinomyces spp*., *Lactobacillus spp., Prevotella spp*., and *Porphyromonas spp*.

According to the study of Hatvani (2001) a sulfur-containing molecule in culture filtrate of *Lentinula edodes* called lenthionine has antibacterial and antifungal properties, and its derivative bis [(methylsulfonyl) (methyl disulphide)] has potent inhibitory effects against Bacillus subtilis. Later on Ishikawa *et al*. (2001) and Hassegawa *et al*. (2005) provided data demonstrating *Lentinula edodes* antibacterial activity against *B. cereus, S. aureus, E. coli* and *Bacillus subtilis* respectively.

Between *Pleurotus ostreatus, Lentinula edodes*, and *Hypsizigus tessulatus, Lentinula edodes* had the highest degree of antibacterial activity. The extracts minimum inhibitory concentration values demonstrated that they are still active at concentrations as low as 1 mg/ml to as high as 9 mg/ml. The most effective antibacterial activity has been demonstrated by *Lentinula edodes* (Chowdhury *et al*., 2015).

In a 2015 study, Zanardo et al. found that the aqueous extract from the fruiting body of *Lentinula edodes* significantly decreased the in vitro growth of *Xanthomonas axonopodis* pv. *passiflorae*, the causative agent of bacterial spot in passion fruit.

Kwak *et al*., 2016 reported that the plant pathogenic bacterium *Ralstonia solanacearum* is effectively combated by the culture filtrate of *Lentinula edodes*. The results of further HPLC fractionation revealed that the active substances were organic acids. Oxalic acid, which made up the majority of the nine organic acids found in the culture filtrate of *L. edodes*, had antibacterial action against nine distinct phytopathogenic bacteria. This shows that an eco-friendly plant disease management agent is the water extract of used *L. edodes* substrate.

The extracts made with methanol of *Lentinula edodes*, *Pleurotus ostreatus*,and *Agaricus bisporus* shows antibacterial activities against E*nterococcus faecalis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* were

also examined by Taofiq *et al.*, 2016.

Lentinula edodes mycelia culture filtrate foliar spray considerably reduced the occurrence of bacterial spots on tomato leaves in a research by Kaur *et al*. (2016) but it was ineffective in vivo.

According to Rodrigus *et al*. (2021), strawberry plants that have been infected with the leaf spot caused by Xanthomonas fragariae when sprayed with *Lentinula edodes* polys-accharides, propolis extracts, acibenzolar-s-methyl, or distilled water (control) they may be able to eradicate strawberry leaf spot successfully.

Antiviral properties of *Lentinula edodes*

Indirect *Lentinula edodes* antiviral activity was described by Tsunoda and Ishida (1969), in watery extract from the fruit body and spores. According to Sasaki *et al*. (2001) study the VSA virus (vesicular stomatitis virus) was effectively inhibited by the extract from the shiitake fruit body, which includes strong plant viral inhibitors.

The *Lentinula edodes* fruit body contains a chemical that Kobayashi *et al*. (1987) reported as having an inhibitory effect on plant virus infection. It was said that this material was a protein. There have been several reports of compounds from the fruit bodies of *L. edodes* and mycelia having antiviral effects on plants. These antiviral compounds have been subjected to the test for preventing viral infection in plants.

Lentinula edodes extracts were reported to be effective against cowpea aphid-borne mosaic virus (Di Piero *et al.*, 2010). In another research by Wang *et al.*, 2013 Tobacco seedlings were exposed to the antiviral properties of *Lentinula edodes*, sulfated lentinan and lentinan, and it was discovered that the fungus may be used to suppress Tobacco Mosaic Virus [TMV].

In vitro Groundnut Bud Necrosis Virus testing was done on the culture filtrate made from the mycelia extracts of *Coprinopsis cinerea, Ganoderma lucidum*, and *Lentinula edodes*. According to the findings of Sangeetha *et al.*, 2020 spraying the mixed culture filtrate could decrease the severity of the pathogenic variations and the virus frequency.

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