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Bacterial Rot of Paddy Straw Mushroom and its Management

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ABSTRACT

Background: Paddy straw mushroom is widely cultivated in Odisha for their unique aroma, texture and growth habit. Bacterial diseases contributed to low production and storability of *Volvariella* sp. So the present study was carried out to identify the pathogen and undertake suitable measures.

Methods: A study was undertaken on the bacterial disease of *Volvariella* sp. incited by bacterium. Different types of symptoms associated with marketable mushroom buds of *Volvariella* sp. leading to rotting of the buds within 24-48 hours of harvest were recorded. Fourteen plant extracts and twelve antibiotics were tested against the bacterium infecting *Volvariella* sp.

Result: *Serratia marcescens* found to be first report causing bacterial rot. Among the plant extracts evaluated, *Trigonella foenum* and *Aloe barbadensis* were found to be most effective in inhibiting the growth of bacterium with a 15 mm zone of inhibition in each case. Among the 12 antibiotics tested, Ciprofloxacin and cefaclor inhibited the growth of the bacterium with 50.3 mm and 65.6 mm zone of inhibition, respectively. Rest of the antibiotics differed in their action against the bacterial pathogen.

Key words: Antibiotics, *Klebsiella pneumonia,* Paddy straw mushroom diseases and Plant extract, Yellowish browning rot.

INTRODUCTION

Mushrooms are basically fungi, which have a fleshy and spore-bearing fruiting body Verma *et al.* (2017). The key elements of the global mushroom industry are edible and medicinal mushrooms. Vegetable flesh is another name for edible mushrooms Jaiswal *et al*. (2024). There are about 2000 mushroom species in nature, but only about 25 are commonly used as food (Chatterjee and Patel, 2016). The countrys old tradition of cultivated mushrooms originated in China (Boa, 2004). Mushroom production worldwide was increased to 10,378,163 metric tons in 2016. Currently, countries with the highest mushroom production are China, USA, Poland, Netherlands, India, France, Spain, Canada, Mexico *etc*.

Medicinal or edible mushrooms are often recognized as nutritious foods. Nutraceuticals confirm to attract the growers and researchers attention. The global mushroom production has increased more than 27-fold in the last 35 years from approximately 1 billion kg in 1978 to approximately 27 billion kg in 2012 which is remarkable especially in comparison to the global human population which has increased 1.7-fold in the same duration from approximately 4.2 billion in 1978 to approximately 7 billion in 2012 Royse, (2014). According to Royse *et al.* (2017), people currently consume 5 kg of mushrooms per year on average. In particular, proteins, minerals and vitamins as well as bioactive substances like steroids, polysaccharides and phenolic compounds are abundant in mushrooms Okhuoya *et al.,* (2010). It is the best food choice for patients with heart disease and high blood pressure because of its high potassium-to-salt ratio. According to Nongthombam *et al.* (2021), a variety of metabolites found in mushroom species have been shown to exhibit anticancer, antioxidant, antigen toxic, antiplatelet aggregating, antihyperglycemic, antimicrobial and antiviral effects. In India Punjab, Haryana, Uttar Pradesh, Rajasthan, Jammu and Kashmir and Odisha are the major mushroom-producing states. From 2010-17, the mushroom industry in India have reported an average growth of 4.3% per annum. Out of total mushroom produced, the major contributors are white button (73%) followed by oyster (60%), paddy straw (7%) and milky mushroom (3%). Mushrooms as compared to vegetables, per capita consumption of mushroom is meager and data indicates that it is less than 100g/year in 2016-17. India's mushroom industry increased by exporting 1054 quintals of white button mushrooms as reported by Sharma *et al.,* (2017).

Mushrooms with their medicinal, nutritional and savory qualities are seen as the future of vegetables in today's health-conscious society. They offer high digestibility and abundant protein making them a potential meat substitute Pavel (2009). In addition, they are a fantastic source of vitamin D which is not found in other food supplements Pehrsson *et al.* (2003). Vitamin D and protein deficiencies are amongst the most prevalent malnutrition problems of

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India and several other developing nations Srivastava *et al.* (2023). Mushrooms like all other crops are impacted by a variety of biotic and abiotic factors. The production of mushrooms is vulnerable to a variety of competing organisms and weed fungus Brown (1937); Davis (1938). Nematodes, bacteria and other pollutants have been documented to harm mushrooms both directly and indirectly. During the period of incubation of the spawn and cultivation of the oyster mushroom *Pleurotus ostreatus*, *Trichoderma harzianum*, *Coprinus* spp., *Aspergillus niger* and *Penicillium* spp. were collected Akhter *et al.,* (2017) which caused specific diseases in mushroom sporophores. A large no of other fungi grows within the mushroom bed as competitors significantly affecting the yield of mushrooms. Many fungal diseases can affect commercial mushroom crops Fletcher and Gaze, (2008). Similarly, few viral diseases have been reported to be associated with edible mushrooms Sharma, (2017); Sharma *et al.,* (2007). However, bacteria are considered as major threat to mushroom production. Many bacterial diseases have been reported from all over the world in the fruit bodies of *A. bisporus, A.bitorquis, Pleurotus* spp*., Volvariella sp., Lentiluna edodes, Flamulina velutipes* and *Auricularia* spp. According to Fermor, (1986), losses from mushroom spoilage caused by the bacterial blotch (*Pseudomonas tolaasii*) are estimated to be 5–10% of the total mushrooms cultivated. On the other hand, the traditional cultivation methodology of *Volvariella* sp. provides enough scope for development and colonization of mushroom mycelium and fruiting bodies by wide range of bacterial pathogen as prevalence of high moisture and warm temperature provides ideal ecological condition for colonization of bacterial pathogen within the mushroom substrate. The research on bacterial pathogens is limited to bacterial rot of *Volvariella* sp. caused by *Pseudomonas* sp. Kannaiyan, (1978).

Lack of research on occurrence of bacterial diseases of *Volvariella* sp. led to deterioration in marketable mushrooms quality and storability. The present study was carried out to explore the possibilities of bacterial pathogens responsible for causing rotting of marketable mushrooms of *Volvariella* sp.

MATERIALS AND METHODS

The experiment was carried out in the Department of Plant Pathology, Institute of Agricultural Sciences, SOADU, Bhubaneswar in the year 2021. The infected samples were collected from different sources and mushrooms showing water-soaked lesion developed at the tip of the vulva which enlarged and extended towards the base of the fruiting body were selected. The infected area was sunken as it became thin. The internal tissues resembled yellow to light brown in color. As the disease advanced, the vulva became slimy and sunken circular to irregular spot developed on the cap or pileus of the mushroom. Below the cap the gills looked water-soaked and in advance case of infection the gills were disintegrated and these symptoms were recorded (Fig 1). Bacteria associated with symptom category were isolated. Apparently, infected mushrooms were selected for isolation of the bacteria for the purpose. Each mushroom was surface disinfected by cleaning the mushroom surface with a cotton swab soaked in 70% ethyl alcohol. The surface disinfected mushrooms were longitudinally cut by a surgical knife sterilized by alcohol. A five-millimeter size tissue from the diseased area was cutoff and placed in a drop of sterile water for about 15-20 minutes or till the water became turbid. A loop full of bacterial suspension was taken and cross-streaked on the dry surface of a nutrient agar plate for the isolation of bacteria. The plates were kept in an upside-down position in a BOD incubator maintained at 27±1º c for 24-48 hours for the development of bacterial colonies. One or two frequently growing colonies having distinct colony characters were selected. A selected colony was picked up with the help of an inoculating needle and cross-streaked on another NA plate for purification. The procedure was repeated until all the colonies developed on the media surface resembled to that of the first selected colony. A single well-separated colony was transferred to NA slant and assigned with an isolated number. To assess the

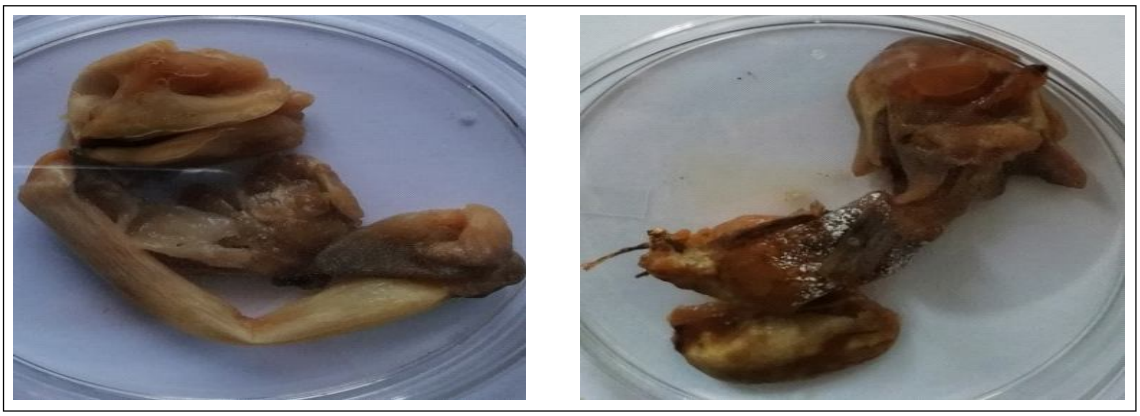

Fig 1: Symptoms of complete rotting of fruiting body of *Volvariella sp.*

pathogenicity of bacterial isolate, fresh, apparently healthy mushroom buds at the egg stage was collected from mushroom growers and placed them in new polythene bags and brought to the laboratory Pathogenicity test was conducted as described by Kumar. *et al.* (2018). The pure bacteria colonies obtained from the colonies growing on the nutrient agar were later identified based on morphological and biochemical tests as reported by CMI (2010). DNA extraction and BLAST analysis of 16S rRNA sequence homology was conducted as described by Joko *et al.* (2014). This sequence was submitted to the GeneBank database under accession number OQ410475. Water-based crude extracts were collected from fourteen plant species (Table 1) chosen for their common use in Ayurvedic medicine. Plant extracts on the growth of test bacteria were conducted according to Gonelimali *et al.,* (2018).

Twelve different antibiotic discs which were commercially available (Hi –media) were tested against the test bacteria. Bacterial suspension was transferred and spread on NA plates following the same procedure as described for testing of plant extracts. A set of three numbers of antibiotic discs were placed on the previously inoculated plates. The inoculated plates with antibiotic disc were incubated at 27±1°C for 24-48 h. Zone of inhibitions from the center of the antibiotic disc were measured in millimeter. (mm).

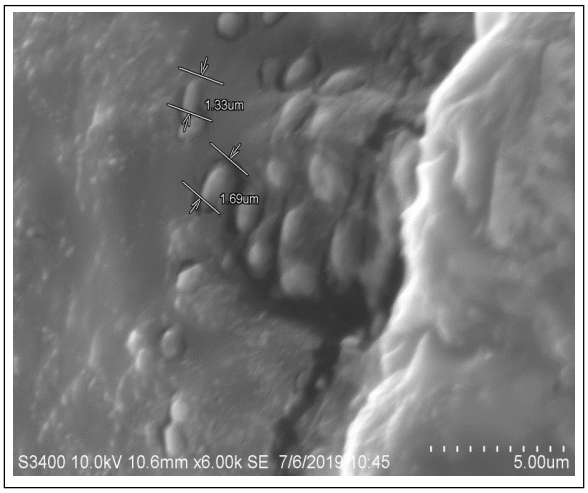

Fig 2: Scanning electron micrograph of test bacteria.

RESULTS AND DISCUSSION

Bacteria associated with *Volvariella* mushroom

One bacterial isolate was detected exibiting browning of the bulbous base of the *Volvariella* sp. was the major diagnostic symptom.The symptoms were exhibited in diseasesd mushrooms and not present in healthy mushrooms which looks white from base to stipe. The SEM of the bacteria was taken as described earlier which revealed that the associated bacteria was rod-shaped and capsulated measuring 1.33-1.69 µm (Fig 2). Browning of the bulbous base linked to the vulva and fungal mycelium suggests that multiple pathogens association in paddy straw mushroom diseases including a novel symptom, complete rotting was found associated with one isolate. Systematic studies are required for better conclusive results. Similar type of symptom were not earlier reported and hence it was the first report of this paddy straw mushroom disease.

Identification of bacterial isolates based on morphology, utilization of carbohydrates, biochemical characterization and molecular test

Morphological characterization revealed bacterial isolate was rod-shaped, capsulated, measuring 1.33-1.69 µm. No flagella could be detected during gram staining technique. The colonies on nutrient agar appeared as circular, convex, glistering and pink to red in color. Production of acid from carbohydrate was also revealed that the bacterium was

Table 1: Plant extracts used for test bacteria.

Scientific name of the plant	Common name	Parts of plant used
Elettaria cardamomum	Cardamom	Seed
Curcuma longa	Turmeric	Rhizomes
Zingiber officinale	Ginger	Rhizomes
Trigonella foenum	Fenugreek	Seed
Amomum sabulatum	Black Cardamom	Seed
Ocimum sanctum	Tulsi	Leaf
Syzygium aromaticum	Clove	Flower
Psidium guajava	Guava	Leaf
Clitoria teunalis	Clitoria	Flower
Aloe barbadensis	Alovera	Leaf
Cinnamomum verum	Cinnamon	Bark
Laurus nobilis	Bay laurel	Leaf
Tridax procumbens	Tridax daisy	Leaf
Piper nigrum	Black pepper	Seed

Table 2: Biochemical test.

Biochemical test	IAS 1001
ONPG	-ve
Esculin hydrolysis	+ve
Citrate utilization	+ve
Melonate utilization	+ve
Lysine utilisation	+ve
Ornithine utilisation	+ve
Urease	+ve
Phenylalanine deamination	-ve
Nitrate reduction	+ve
H2S production	+ve
Vogesproskauer's	-ve
Methyl red	-ve
Indole	-ve
Gelatin liquification	-ve
Casein hydrolysis	+ve
Oxidase test	-ve
Growth at 27°C	+ve
Growth at 47°C	-ve

Table 3: Utilization of carbohydrates by test bacteria.

Carbohydrates	IAS 1001
Lactose	+ ve
Xylose	+ ve
Maltose	+ ve
Fructose	+ ve
Dextrose	+ ve
Galactose	+ ve
Raffinose	+ ve
Trehalose	+ ve
Melobiose	+ ve
Sucrose	+ ve
L-arbinose	+ ve
Mannose	+ ve
Rhamnose	+ ve
Cellobiose	- ve
Melezitose	- ve
α methyl-D-mannoside	- ve
Xylitol	- ve
D-Arabinose	+ ve
Sorbose	+ve

found positive in reaction against xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melobiose, sucrose, L-arabinose, manose, rhamnose, cellobiose and sorbose. Results of the biochemical test revealed that the isolate showed positive reaction with regard to esculin hydrolysis, citrate, malonate, lysine and orthinine utilization; urease production, nitrate reduction, H2S production and casein hydrolysis. The growth of bacteria at 27°C was positive and at 47°C red coloration disappeared and producing light yellow colored colony (Table 2 and 3). The amplification of the 16S rRNA of this bacterial strain (accession number OQ410475) was generated. A similarity search of the 16S rRNA gene sequence against the NCBI GeneBank database showed a 99 to 100% homology to known sequences of *Serratia marcescens*. A phylogenetic tree (Fig 3) was constructed using sequences in databases corresponding to strains of *Serratia* group which one you have followed in which *Serratia marcescens* was found and the isolate was identified as *Serratia marcescens.* In the present study, *Serratia marcescens* caused browning of bulbus base followed by rotting of sporophore of market samples of *Volvariella sp*. In India Kannaiyan, (1978) reported *Pseudomonas sp*. bacterial rot disease of *Volvariella* sp. Because *pseudomonas tolasii* due to its widespread distribution, was mainly studied pathogen. It affects a variety of mushroom hosts including *A. bisporus, A. bitorquis, Pleurotus ostreatus* and *Pleurotus eryngii* causing significant losses to mushroom cultivation Bradbury, (1987); Soler-Rivas *et al.* (1999). Munsch *et al.* (2000) was also reported the association of *Pseudomonas costantinii* with formation of brown blotch disease of *Agaricus bisporus*. The present revealed the association of *Serratia marcescens* which was responsible for causing the browning and rotting of commercially cultivated paddy straw mushrooms (*Volvariella* sp.) was found to be the first report in india.

Pathogenic ability of *Serratia marcescens*

Serratia marcescens induced 100% rot in 72 hours, while the healthy (Mushrooms) control remained unaffected. Upon reisolate matched with the original bacterial culture.

Sensitivity of *Serratia marcescens* to different plant extracts

Crude plant extracts with water base were collected from fourteen plant species and tested against the *Serratia marcescens*. Zone of inhibition of bacterial growth was recorded and presented (Table 4) and (Fig 4). *Trigonella foenum and Aloe barbadensis* plant extracts were found most effective in inhibiting the growth of the bacterium. Whereas, *Psidium guajava*, *Elettaria cardamomum*, *Curcuma longa* and *Tridax procumbens* extract could not inhibit the growth of the test bacterial species. The rest of the plant extracts differed in their effectiveness against *Serratia marcescens*. Since plant extracts recorded potent antibacterial activity against harmful bacteria are considered to be acceptable as eco-friendly alternatives. As paddy straw mushroom is produced in 15 days, the use of chemicals were banned for use in mushroom beds. Paddy straw mushroom grown by using paddy straw as substrate. Therefore, mixing plant parts or their extracts along with the substrate having antibacterial properties was found better proposition for the management of bacterial diseases as they inhibit the bacterial growth to establish itself within the mushroom beds.

In the present study, sensitivity of *Serratia marcescens* to fourteen plant extracts were studied out of which the bacterium showed sensitivity to ten plant extracts. Todoroviæ

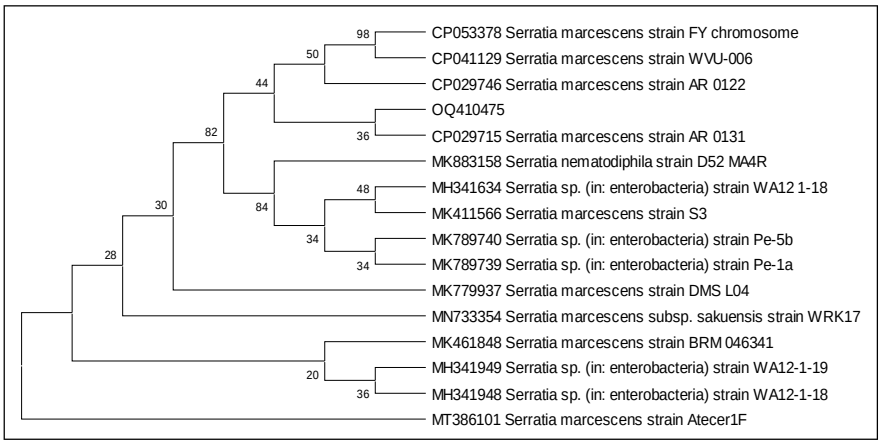

Fig 3: Phylogenetic tree using sequences in databases corresponding to strains of *Serratia* group.

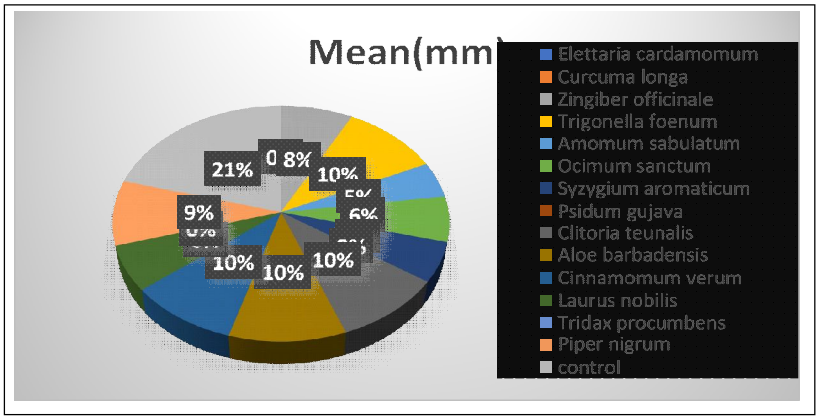

Fig 4: Chart showing the effect of plant extract inhibition the growth of *Serratia marcescens.*

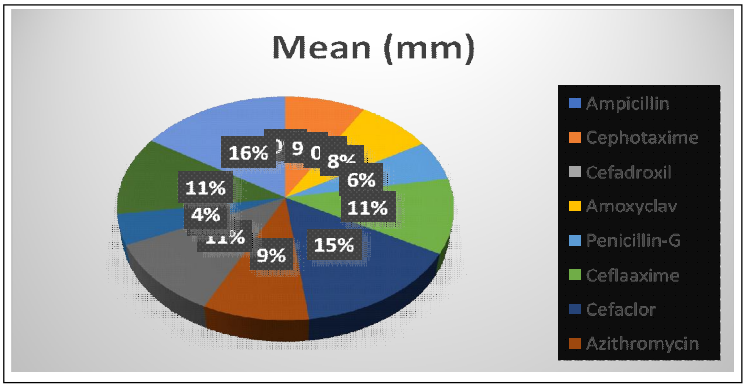

Fig 5: Chart showing the sensitivity of *Serratia marcescens* to different antibiotics.

et al., (2016) tested essential oil of twenty plants extracts *Pseudomonas tolaasii,* the causal agent of a bacterial brown blotch disease of cultivated mushrooms and observed that *P. tolaasii* was most sensitive to peppermint oil. *Trigonella foenum* plant extract inhibited the growth of the test bacterial pathogens of paddy straw mushrooms because Trigonellin is the essential oil present in *Trigonella foenum.* Confirmative studies are required with regard to the temperature sensitiveness of the active ingredients and toxicity against the mycelial growth of mushroom fungus before giving final recommendation for use.

Sensitivity of the *Serratia marcescens* to different antibiotics

Commercially available antibiotic discs were tested against the bacterial species by recording growth inhibition zone

Table 4: Effect of plant extracts against *Serratia marcescens.*

Scientific name of the plant	Mean radial growth of bacterium (mm)	Mean growth inhibition (%)
Elettaria cardamomum	0	0
Curcuma longa	0	0
Zingiber officinale	11.033	36.733
Trigonella foenum	15.067	50.2
Amomum sabulatum	7.1	23.633
Ocimum sanctum	9.067	30.2
Syzygium aromaticum	8	26.633
Psidium guajava	0	0
Clitoria teunalis	14.2	47.3
Aloe barbadensis	14.967	49.867
Cinnamomum verum	14.067	46.833
Laurus nobilis	9.1	30
Tridax procumbens	0	0
Piper nigrum	12.967	43.767
Control	30	100
C.D. at P≤0.05	0.318	1.14
SE(m) ±	0.11	0.393

Table 5: Evaluation of antibiotics on the growth of *Serratia marcescens.*

Antibiotics	Mean radial growth of bacterium (mm)	Mean growth inhibition (%)
Ampicillin	0	0
Cefuroxime	37.167	53.033
Cefadroxil	0	0.0
Amoxyclav	35.167	50.233
Penicillin-G	25.267	36.08
Cefotaxime	47.6	67.967
Cefaclor	65.567	93.637
Azithromycin	40.953	58.5
Erythromycin	48.133	68.567
Cefoperazone	0	0.0
Clarithromycin	19.267	27.467
Ciprofloxacin	50.3	71.833
Control	70	100
C.D. at P≤0.05	0.358	0.542
SE(m) ±	0.123	0.186

formed on inoculated media plates. The results presented in (Table 5 and Fig 5) revealed that among different antibiotics Cefaclor recorded maximum zone of inhibition followed by Ciprofloxacin. Two test antibiotics could not inhibit the growth of bacterial species. Among the twelve antibiotics tested, the bacterium was found sensitive to nine antibiotics. The highest inhibition zone of 65.6 mm of bacterial growth of *Serratia marcescens* was recorded in case of Cefaclor whaere as Clarithromycin recorded the lowest zone of inhibition.

Worldwide survey has shown that representative of Bacterial illnesses infects every major genus of edible mushrooms including Agaricus, Pleurotus, Lentinus, Flammulina, *Volvariella* and Auricularia. As more intense farming techniques are adopted, pseudomonadales are the primary cause of most mushroom crop losses. Manipulation of the environment in the growing room and application of regular drenching of chlorinated water to casing layer has been suggested by Fermor, (1986). Application of terramycin 9 mg per square feet, streptomycin (200 ppm), oxytetracycline (300 ppm), kasugamycin and kanamycin were found effective in managing the bacterial diseases of mushrooms as reported by Sharma *et al.* (2007). All the above recommendations have been tried in management of bacterial diseases of commercially grown mushrooms other than *Volvariella* sp. The cultivation methods for *Volvariella* sp. seems to create an environment suitable for the growth and establishment of various bacterial pathogens. In the present study Cefaclor and Ciprofloxacin antibiotics were found effective against the bacterial species. Detection of bacterial pathogens present in the mushroom bed and application of selected antibiotics is thought to be a difficult proposition in managing bacterial diseases of *Volvariella* sp. Therefore, more investigations are required for selection of a broad-spectrum antibiotic.

CONCLUSION

The bacterial pathogen was isolated from Volvariella Mushroom exhibited bacterial rot symptom. The bacterial isolates were studied for their pathogenic ability in causing rot of apparently healthy mushroom buds. Several morphological and biochemical characteristics of bacterial isolate were studied and identified as *Serratia marcescens* and the bacterium was studied for its sensitivity to different plant extracts and antibiotics. Study of the sensitivity of bacterium against Fourteen different plant extracts revealed that it was more sensitive to plant extract of *Trigonella foenum* and *Aloe barbadensis* whereas the plant extracts of *Psidium guajava, Tridax procumbens, Curcuma longa* with *Elettaria cardamomum* were found to be ineffective in inhibiting the growth of bacterium. Among 12 antibiotics, cefaclor was found to be most effective in inhibiting the growth of the bacterium whereas ampicillin and Cefoperazone were found to be ineffective. The rest of the antibiotics differed in their sensitivity to the bacterium. More studies required to determine the suitable measures combining sanitation, integrated management of bacterial diseases in addition to use of plant extracts and antibiotics.

Conflict of Interest

Authors do not have any conflict of interest to declare.

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