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Influence of various substrate treatment methods on yield and biological efficiency of *Pleurotus florida* mushroom

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Abstract

Due to their excellent flavour and taste, *Pleurotus* species, which flourish in temperate and subtropical environments, are well-liked among edible mushrooms. Oyster mushrooms could be grown on a variety of substrates due to their powerful enzymatic properties. *Pleurotus* sp. grows more easily, more profitably, and more nutrient-densely on a variety of organic waste raw materials and environmental factors. Sterilization of the substrate is one of the most crucial steps in oyster mushroom cultivation. There have been numerous reports of contaminated mushroom substrate. Suitable sterilization techniques must be used on the substrates. There are several treatments for mushroom substrate that can be used to manage the frequent moulds that infest edible mushrooms. Sterilization of the substrate is necessary to get rid of pathogenic and rival microorganisms and to encourage the mycelial growth of the desired mushroom species. The goal of the current study was to identify the best treatment strategy for the chosen substrates. For substrate treatment for *Pleurotus florida* cultivation, hot water, boiling water, steam sterilization in an autoclave, and chemical treatment methods were used. It was observed that the steam sterilization method was superior to other methods as it supported relatively short spawn run duration, amazing yield, and high biological efficiency.

Keywords: Agricultural residues; Functional foods; Fungicides; *Pleurotus florida*; Sterilization

1. Introduction

Mushrooms have probably been used as food since the dawn of civilization. Around the globe, over 200 mushroom species have long been consumed as functional foods. With the passage of time, a greater understanding of the nutritional value of mushrooms and its medicinal value has increased [1, 2]. *Pleurotus* species, which thrive in temperate and subtropical environments, are popular among edible mushrooms due to their excellent flavour and taste. Because of their strong enzymatic properties, oyster mushrooms could be cultivated on a variety of substrates. *Pleurotus* sp. is simpler to cultivate, more nutritious to eat, and more cost-effective to grow on various organic waste raw resources and weather conditions [3,4]. Oyster mushrooms have significant economic, medicinal, and nutritional value [5,6]. These are widely and regionally grown on non-composted lignocellulosic substrates. Numerous studies on different substrates have reported the successful cultivation of *Pleurous* sp., by using paddy straw, maize stalks and cobs, vegetable plant residues, bagasse, sawdust, chopped office papers, cardboard, plant fibres, and so on [7,8].

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One of the most important steps in oyster mushroom cultivation is substrate sterilization. There have been several reports of mushroom substrate contamination. Substrates must be sterilized using an appropriate method. Several mushroom substrate treatments are available to control the common moulds found in edible mushrooms [9,10]. Substrate sterilization is required to eliminate pathogenic and competitive microorganisms and to promote mycelial growth of desired mushroom species. Sterilization of substrates is primarily conducted to avoid the presence of pathogens that appear to compete for nutrient uptake [11]. The composition of substrates used to grow various mushrooms varies greatly, as does the preparation of substrates used for each cultivated species [12,13]. Most substrates are boiled in water for a set amount of time. Ideally, all seeds, nematodes, insects, and other organisms that thrive at the temperatures used to grow the mushrooms must be killed by the efficient procedure [14]. Steam sterilization, immersion in hot water, and chemical treatment methods are described in the oyster mushroom cultivation protocol [15]. However, very few studies have been published in the literature to evaluate the efficacy of these sterilization methods that influence mushroom's quality and quantity [16]. In this context, the current study was conducted to determine the most appropriate treatment method for the selected substrates.

2. Material and methods

2.1. Obtaining a spawn sample and processing selected agro-industrial residues

Pleurotus florida spawns were obtained for the first time from Peeper Agro Industry in Vimanapura, Bengaluru, India, and it's pure culture was grown on potato dextrose agar plates (pH 7.2). Bread grass (*Brachiaria brizantha*), soybean straw, saw dust, rice straw, wheat straw, cotton straw, and sugarcane bagasse were collected in dried form. All substrates, with the exception of sawdust, were cut into fine pieces and pulverised with a grinder [2].

2.2. Design of consortiums for selected substrates

The chosen substrates were combined to form a specific consortium or formulation design, yielding 15 formulations in total. The first formulation was entirely composed of dried bread grass (*Brachiaria brizantha*). Second formulation was entirely composed of soybean straw. Third formulation was entirely composed of saw dust. Fourth formulation was entirely made of rice straw. Fifth formulation was entirely composed of wheat straw. Sixth formulation was made entirely of cotton straw. Sugarcane bagasse was used entirely in the seventh formulation. Consortium no. 8 consisted of all of the chosen substrates, each of which contributed 14.29%. Consortium no. 9 was made up of 8% bread grass, 15% soybean straw, 25% saw dust, 20% rice straw, 15% wheat straw, 10% cotton straw, and 7% sugarcane bagasse. 20% bread grass, 25% soybean straw, 15% saw dust, 8% rice straw, 7% wheat straw, 10% cotton straw, and 15% sugarcane bagasse made up consortium no. 10. 25% bread grass, 15% soybean straw, 8% saw dust, 20% rice straw, 15% wheat straw, 7% cotton straw, and 10% sugarcane bagasse made up consortium no. 11. Consortium number 12 was made up of 3% bread grass, 12% soybean straw, 17% saw dust, 32% rice straw, 16% wheat straw, 9% cotton straw, and 11% sugarcane bagasse. Consortium no. 13 was made up of 13% bread grass, 11% soybean straw, 15% saw dust, 12% rice straw, 7% wheat straw, 3% cotton straw, and 39% sugarcane bagasse. Consortium no. 14 was made up of 10% bread grass, 10% soybean straw, 10% saw dust, 10% rice straw, 10% wheat straw, 40% cotton straw, and 10% sugarcane bagasse. Finally, consortium no. 15 contained 7% bread grass, 10% soybean straw, 15% saw dust, 20% rice straw, 25% wheat straw, 15% cotton straw, and 8% sugarcane bagasse [2, 17,18,19,20].

2.3. Different treatment methods for prepared substrates

Five different treatment methods were used for the substrates prepared in aforementioned combinations.

- Hot water treatment

Each substrate consortium was placed in a net bag, tied with thread, and dipped to pasteurize in hot water at 70 ^{o}C in a large container for 60 minutes. The excess water was then drained by hanging the bag for 20 hours. Following that, the contents of each bag were spread on different plastic sheets to evaporate excess moisture, resulting in a moisture content of 55 to 60% [16].

- Boiling water treatment

In this method, each consortium of substrates was immersed in boiling water for 60 minutes before repeating the method described above [15].

- Steam sterilization in an autoclave

The prepared substrate consortiums were placed in net bags, tied with thread, and soaked in fresh water for 8 hours. The extra water was removed. Each formulation was individually wrapped in cloth before being sterilized in an autoclave at 121 °C for 20 minutes. The sterilized contents of the cloths were placed in a laminar air flow chamber for 4-5 hours [2].

- Chemical treatment method

Fungicide solutions A and B were prepared and labeled separately. The solution A contained Bavistin (50 ppm) and Formalin (500 ppm). SAAF™ (UPL Limited, Gujarat) was used in solution B (500 ppm). The substrate bags were allowed to soak in these chemical solutions for 18 hours. The solution was then drained from each net bag, and a moisture content of 55-60% was maintained in the wet substrates before spawning [16].

- Ordinary water/control: By this method the substrates were treated in simple water [13].

2.4. Spawning, incubation and harvesting

An equal quantity of spawns was added while filling the sterilized substrates into each polypropylene bag of 100-gauge thickness. In this way, all the bags were packed and tied with pieces of thread. Then, with the tip of a dull pen, uniform holes were made in all the bags. The holes were approximately 2-3 mm in size. Bags were incubated at 28 ℃ and a relative humidity of 80-85% was maintained in the cropping room under dark conditions until a complete spawn run was achieved. The fruiting bodies of *Pleurotus florida* were harvested by twisting to uproot them from the base [2, 21, 25].

2.5. Observation and calculations

The time required for complete substrate colonization is called spawn run duration. The observation was recorded for time taken for spawn run. The biological efficiency (BE) for each substrate consortium was calculated as follows [2, 10-15].

Biological efficiency (%) = (Fresh weight of mushroom / Dry weight of substrate) × 100

2.6. Data analysis

All experiments were performed in three replications. Data was analyzed in MS-Excel 2013 software [2].

3. Results and discussion

3.1. Effect of different treatment methods

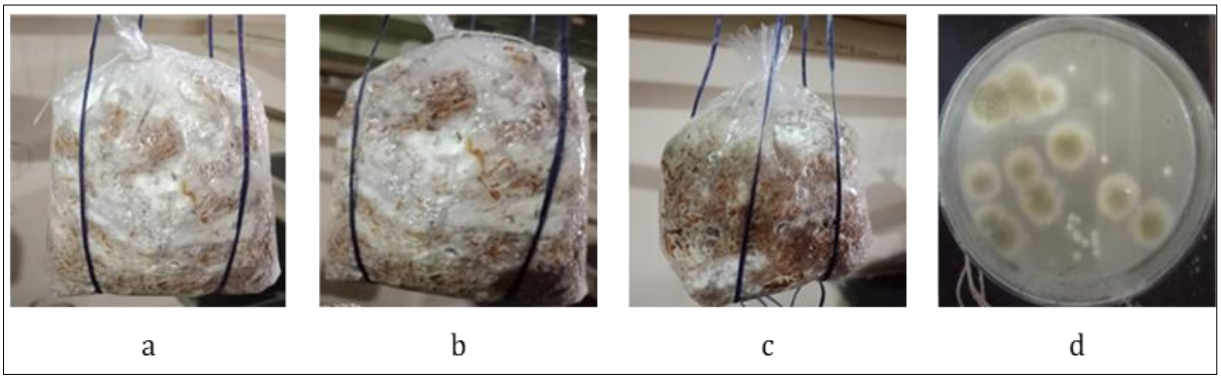

Figure 1a Substrates treated by steam sterilization method, b: Substrates treated by fungicide solution A, c: Substrates treated by hot water, d: Contaminants isolated from the substrates treated with hot water

During the incubation period, no contamination was observed in the steam sterilized bags. The highest contamination of *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Mucor* sp. and *Rhizopus* sp. was observed in substrates treated in hot water, while substrates treated in boiling water had the lowest contamination of these fungi. However, minor

contamination of these fungi was observed in chemically treated substrates. The substrates treated with ordinary water (control) without any heat or chemical treatment completely failed to support the growth of *Pleurotus florida* mycelium, resulting in the formation of a large number of maggots and infesting molds in the bags. Figure 1 (a, b and c) depicts the bags processed by different treatments. Figure 1 (d) depicts the isolated contaminants. According to Sharma et al., *Aspergillus* sp., *Aspergillus niger*, *Fusarium* sp., *Mucor* sp., and *Trichoderma* sp. were direct competitor species and might spread disease in cultivated oyster mushrooms [22]. Lopez-Arevalo et al. also found the presence of fungal contaminants like *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Cunninghamella* sp. [23].

3.2. Measurement of observed parameters

Spawn run duration was compared across substrate treatment methods (Figure 2). *Pleurotus florida* mycelium was completely spread after 10 days in the ninth consortium, and the bag was fully transformed into white. The growth of the fruiting body was initiated after 13 days, and the complete fruiting body appeared after 16 days. Figure 3 depicts the appearance of fruiting bodies with their different growth stages on steam sterilized consortium of substrates. According to Khan et al., complete spawn running on substrates like sawdust, wheat straw, rice husk, etc. takes 15 to 20 days [11].

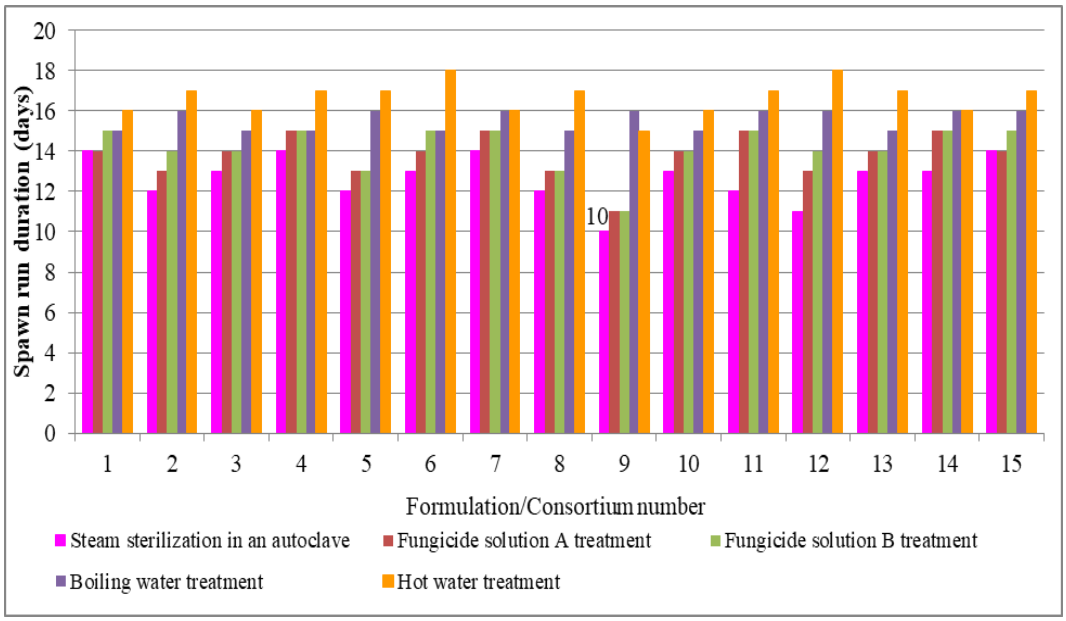

Figure 2 The time required for complete substrate colonization compared with all substrate treatment methods

Figure 3 Appearance of fruiting bodies with different stages of development on steam sterilized consortiums

3.3. Effect of different substrate treatment methods on yield and biological efficiency

According to the findings, steam sterilization in an autoclave was one of the best substrate treatment methods for all designed consortiums and it supported the production of maximum yield in each consortium. Table 1 shows the yield of *Pleurotus florida* that was obtained using various substrate treatment techniques on all consortiums. The consortium no. 9 composed of dried bread grass (8%), soybean straw (15%), saw dust (25%), rice straw (20%), wheat straw (15%), cotton straw (10%), and sugarcane bagasse (7%), treated by steam sterilization method, had the highest biological efficiency (75.55%) than any other method. Figures 4 and 5 show the biological efficiencies obtained by various treatment methods for consortiums 1–7 and 8–15, respectively. Based on yield, steam sterilization was found to be the most effective method for treating substrates, followed by treatment with fungicide solutions A and B, boiling water, and hot water treatment.

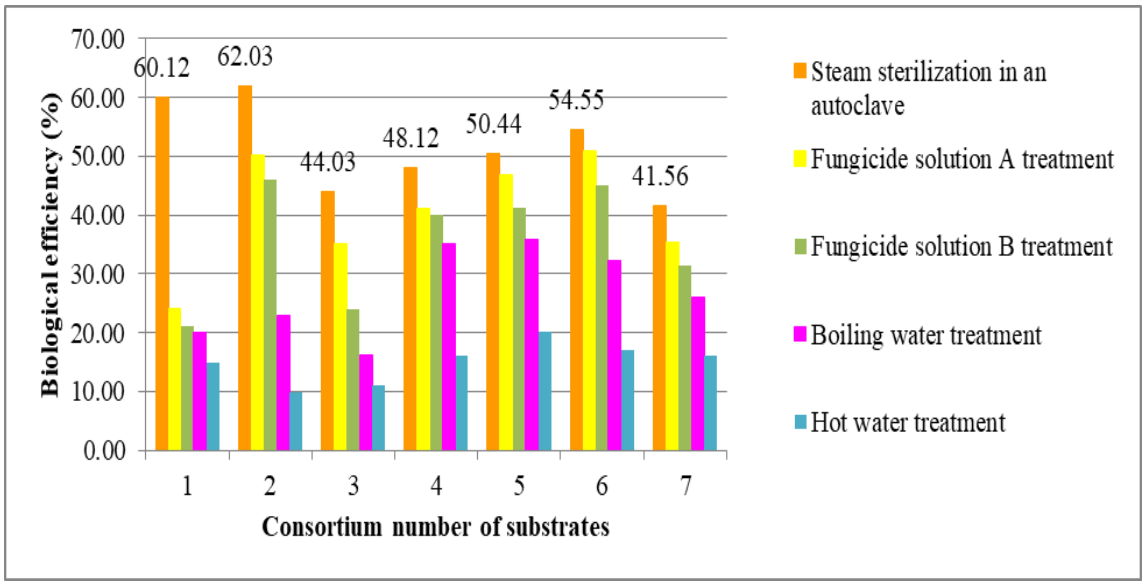

Figure 4 Biological efficiencies obtained by various treatment methods for formulations 1–7

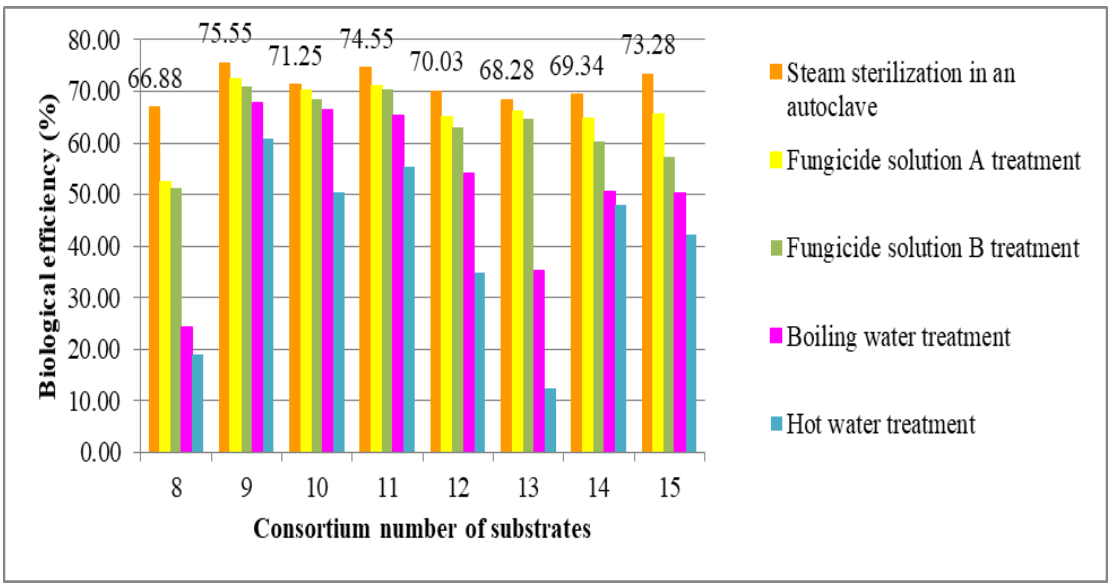

Figure 5 Biological efficiencies obtained by various treatment methods for consortiums 8–15

Table 1 Yield and biological efficiency (B.E.) of *Pleurotus florida* obtained using various substrate treatment techniques on all consortiums

Formulation/ Consortium number	Steam sterilization in autoclave		Fungicide solution A treatment		Fungicide solution B treatment		Boiling water treatment		Hot water treatment	
	Yield (gm)	B.E. (%)	Yield (gm)	B.E. (%)	Yield (gm)	B.E. (%)	Yield (gm)	B.E. (%)	Yield (gm)	B.E. (%)
1	180.36	60.12	72.30	24.10	63.33	21.11	60.36	20.12	45.03	15.01
2	186.09	62.03	150.33	50.11	138.24	46.08	69.09	23.03	30.00	10.00
3	132.09	44.03	105.33	35.11	72.12	24.04	48.33	16.11	33.36	11.12
4	144.36	48.12	123.09	41.03	120.36	40.12	105.33	35.11	48.66	16.22
5	151.32	50.44	140.67	46.89	123.96	41.32	107.34	35.78	60.69	20.23
6	163.65	54.55	153.18	51.06	135.09	45.03	97.02	32.34	51.27	17.09
7	124.68	41.56	106.02	35.34	94.02	31.34	77.94	25.98	48.66	16.22
8	200.64	66.88	157.68	52.56	153.66	51.22	73.05	24.35	56.94	18.98
9	226.64	75.55	217.26	72.42	213.02	71.01	203.07	67.69	182.12	60.71
10	213.75	71.25	210.45	70.15	205.68	68.56	199.44	66.48	151.08	50.36
11	223.65	74.55	213.24	71.08	211.29	70.43	196.35	65.45	166.32	55.44
12	210.09	70.03	195.33	62.11	189.00	63.00	162.69	54.23	104.34	34.78
13	204.84	68.28	198.33	66.11	194.01	64.67	105.51	35.17	36.99	12.33
14	208.02	69.34	194.58	64.86	180.84	60.28	151.44	50.28	143.61	47.87
15	219.84	73.28	196.92	62.64	171.54	57.18	151.14	50.38	126.81	42.27

Yield is average of three replications (n=3).

There are numerous different kinds of mushrooms available around the world, and only 280 species of the 2,500 known edible varieties are grown in India [1, 15]. The actual disinfection of the mushroom substrate is the most crucial unit process. It takes a lot of time and effort to prepare the mushroom substrate by disinfecting the agricultural residues because the process must be done in a clean environment [15, 24, 25]. The disinfection procedure is a crucial step in mushroom growth for a number of reasons. Lignin is said to act as a barrier to the availability of carbohydrates because it is encrusted with both cellulose and hemicellulose in the cell wall [26]. In order to overcome this barrier and obtain a high level of fermentable sugars for mushroom growth, hydrothermal treatment is crucial. The substrate is pasteurized at 65 °C for 6–8 hours to soften the texture and eradicate mesophilic microorganisms [15, 16]. While mould spores can be killed at temperatures above 80 °C, they are stable at 65 °C. Most importantly, substrate disinfection is carried out to eradicate rival mould species like *Trichoderma*, *Coprinus*, *Penicillium*, and *Aspergillus* that emerge during colonization and fruiting. One of the most prevalent and destructive diseases in the mushroom farming industry is green mould, which is brought on by these species. It is possible to create low-cost, energy-efficient substrate treatment techniques by utilizing solar energy to generate hot water and steam, which would otherwise go to waste in the fight against the energy crisis. There aren't many reports on microwave pasteurization of substrate. Microwave energy can be used to quickly and uniformly disinfect a large amount of substrate after soaking because it can quickly and ubiquitously heat any quantity of substance. The creation of low-cost, suitable pasteurization equipment is what mushroom growers urgently need to do in order to address their most pressing issues [15].

4. Conclusion

For the efficient and smooth cultivation of *Pleurotus florida* mushrooms, substrates must be treated properly. The steam sterilization method was found to be more effective with fairly short spawn run duration, truly amazing yield, and the greatest biological efficiency than other methods. In our study, the different substrate treatment methods had an impact

on the spawn run duration, mushroom yield, and biological efficiency of *Pleurotus florida* mushrooms. Steam sterilization was therefore determined to be the best method for treating substrates for *Pleurotus florida* cultivation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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Author's short Biography

Dr. Mukundraj Govindrao Rathod (Ph.D. & MH-SET): At Yeshwant College of Information Technology in Parbhani, Maharashtra, India, Dr. Mukundraj G. Rathod is the in-charge Principal. In addition, he heads this college's Biotechnology and Bioinformatics department. More than 77 research papers, including review articles in various peer-reviewed international and national journals and proceedings of conferences in various research fields, haveindeed been published by him. He has 301 citations in Google Scholar, an i10 index of 10, and a H index of 10, which is impressive. He has an amazing H index of 4, with 31 Scopus citations. He had given oral talks and posters at numerous conferences and seminars. He has also reviewed publications for a number of reputable scientific publishers. He had donated seven crucial industrial cultures to the National Center for Cell Science's Microbial Culture Collection in Pune, Maharashtra, India, for use by the general public. He is currently the lead researcher on a study supported by Swami Ramanand Teerth Marathwada University in Nanded through the Rajiv Gandhi Science and Technology Commission's application scheme for science and technology (Government of Maharashtra). According to the AD Scientific Index 2023, he recently held the 17th rank among the top 20 scientists at Swami Ramanand Teerth Marathwada University, Nanded, and its jurisdiction.

	**Prof. Dr. (Mrs.) Anupama Prabhakarrao Pathak** (Ph.D. & MH-SET): Dr. Anupama P. Pathak was previously serving as the School of Life Sciences' director and currently serving as the microbiology department's head at Swami Ramanand Teerth Marathwada University, Nanded. More than 200 research papers, including review articles, in various peer-reviewed international, national journals and conference proceedings were published by her. She has 774 citations in Google Scholar, an i10 index of 28, and a H index of 15, which is really impressive. She has an amazing H index of 8, with 239 Scopus citations. More than 110 16S rRNA gene sequences and numerous industrially significant bacterial cultures are among her contributions. She had finished two research projects in microbiology on extremophiles that were funded by the University Grants Commission of New Delhi. She is a member of the university's Microbiology Board of Studies. She is a renowned scientist who, according to the AD Scientific Index 2023, is ranking currently in ninth place of the top 20 scientists at Swami Ramanand Teerth Marathwada University, Nanded, and its jurisdiction.