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EFFECT OF DIFFERENT LEVELS OF LIME AND PH ON MYCELIAL GROWTH AND PRODUCTION EFFICIENCY OF OYSTER MUSHROOM (*PLEUROTUS* SPP.)

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Abstract

pH is an impotant factor for good production of Oyster mushroom. Most of the mushrooms grow and perform well at pH near to neutral or light basic. Lime ($CaCO_3$) is an important constituent in mushroom cultivation, commercial cultivation of mushroom depends upon proper adjustment of pH of substrate. Most of the substrates used for the cultivation of mushroom have pH approximately near to neutral i.e., 7 in Pakistan. In this study, Oyster mushroom (*Pleurotus* sp.) was grown on cotton waste with different levels of lime in substrate like 0%, 2%, 4% and 6% of substrate weight at pH 7.2, 7.8, 8.2 and 8.7 respectively, to find out the most suitable pH/level of lime for mycelial growth and fructification of mushroom. Data regarding number of days for mycelial growth, number of days taken for initiation of primordia, number of pinheads, number of days taken to reach at harvesting stage after primordia initiation, number of days taken to reach at harvesting stage, total number of fruiting bodies, yield of mushroom in 1st flush (g), yield of mushroom in 2nd flush (g) and total yield of mushroom (g) was recorded. It was observed that treatment containing 2% lime completed mycelial growth after 18 days of inoculation of spawn, number of days taken for initiation of primordia (3 days), number of days taken to reach at harvesting stage after primordia initiation (3 days), number of pinheads (30.8), time taken to reach picking stage after spawning (25 days), yield of mushroom in 1st flush (41.02g), yield of mushroom in 2nd flush (15.3g) and total yield of mushroom was recorded 56.32g and 9.57g on fresh and dry weight basis, respectively. In conclusion use of 2% lime is good for the production of Oyster mushroom (*Pleurotus* spp.) using cotton waste as a substrate. The Oyster mushroom grows well and give best yield at pH slightly basic in nature.

Introduction

Mushroom is an attractive crop to cultivate in developing countries for many reasons. One of the most charming points would be that they are grown on agricultural wastes. It enables us to acquire substrate materials at low prices or even for free and to conserve our environment by recycling wastes Khan *et al*., 2012). Oyster mushroom (*Pleurotus* spp.) can grow and utilize various kinds of substrate materials than any other mushrooms (Cohen *et al.*, 2002).Oyster mushroom (*Pleurotus* spp.) is commonly known as Dhingri in Pakistan and India because of its oyster like shape.The genus *Pleurotus* belongs to family Tricholomataceae and has about 40 well-recognized species, out of which 12 species are cultivated in different areas of Pakistan and India. *Pleurotus* is an efficient lignin- degrading mushroom and can grow and yield well on different types of lignocellulolosic materials. Cultivation of oyster mushroom is very simple and low cost production technology, which give a continuous growth with high biological efficiency. Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various tropical regions of the country (Ahmed *et al*., 2009). It can be cultivated in containers like jars, basins, trays, plastic bags and other similar substances by providing artificial controlled conditions (Quimio, 1998). Mycelium of fungi (mushroom) obtain nutrients from substrate at specific level of pH (Sarker *et al*., 2007), Lime is used in cultivation of mushroom to enhance the pH of substrate. Rapid mycelial growth of mushroom (*Pleurotus sajor-caju*) takes place at pH 6.4-7.8 (Iqbal & Shah, 1989). Mycelial growth is the growth of hyphae of fungi in substrate, these are binucleate thread like structures composed of large no. of septa (cells). When mycelium mature primordia formation take place which are small out growth of mycelium and on muturation becoms fruit body of mushroom. Mushrooms growers in Pakistan usually cultivate oyster mushroom on cotton waste. They usually add lime to adjust pH level. Quantity of lime used is ranged between 2-6%, which results in variations in yield. Therefore, present study was conducted to determine the most suitable lime concentration to adjust appropriate pH level for better oyster mushroom production.

Materials and Methods

The study was carried out in Mushroom laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad during (2010-2011). The emphasis of study was to investigate the effect of different pH on growth and production of oyster mushroom by using cotton waste as a substrate material. The different levels of pH 7.2, 7.8, 8.2 and 8.7 were used by using, 0% lime (T_0), 2% lime (T_1) 4% lime (T_2) and 6% lime (T_3).The spawn of Oyster mushroom (*Pleurotus* spp.) was prepared on sorghum grains using standard procedure (Jiskani *et al*., 2007). The substrate was prepared by soaking cotton wastes throughly in water, containing 2% 4% and 6% lime ($CaCO_3$) and was allowed to ferment for 3 days under anaerobic conditions. Cotton waste was filled in polypropylene bags (4x6") after the adjustment of its moisture contents. Substrate weight of each bag was 200g.The substrate was pasteurized for one an hour by using drum system (on small scale cultivation of

mushroom commonly used in Pakistan) and spawning of substrate was done @ 1% of substrate on wet weight basis. The temperature (20 to 30ºC) and relative humidity (70-80%) was maintained in growth room till the harvesting of mushroom (Khan *et al*., 2009; Bano *et al*., 1979). Number of days for mycelial growth, number of days taken for initiation of primordia, number of pinheads, number of days taken to reach at harvesting stage after primordia initiation, number of days taken to reach at harvesting stage, total number of fruiting bodies, yield of mushroom in 1st flush (g), yield of mushroom in 2^{nd} flush (g) and total yield of mushroom (g) was recorded and the experiment was laid out according to the completely randomized design with five replications. The data collection was analyzed statistically by using the analysis of variance technique (LSD test at 5% probability) was applied to compare the difference among the treatment (Steel & Torrie, 1984).

Results and Discussion

Influence of different quantities of lime on the growth of oyster mushroom (number of days for mycelial growth): The white thread like structure in substrate that provides nutrition to the mushroom is known as mycelium. Statistical data regarding number of days taken to complete mycelial growth of mushroom is shown in Table 1. The comparison of treatments means showed that number of days taken for complete mycelial growth differs significantly. Treatments T_0, T_1, T_2 and T_3 took maximum number of days 18.40, 19.00, 21.60 and 28.60 to complete mycelial growth at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 took maximum number of days (28.60 d) to complete mycelial growth followed by T_2 (21.60 d), T_1 (19 d) and T_0 (18.40 d) with pH 8.2, 7.8 and 7.2, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with minimum number of days (19.00 d and 18.40 d) to complete mycelial growth but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. similar results are computed by Shah *et al.,* (2004) investigated the growth of *Pleurotus* species on wheat straw, rice husk and saw dust and find out that spawn running (mycelial growth) took 2-3 weeks after spawning (inoculation) of substrate. Shahid *et al.,* (2006) studied the effect of different methods of compost preparation and lime concentration on the yield of *Pleurotus sajor-caju* and concluded that mycelial growth of mushroom was completed in 23 days with yield of 295g/1.5kg substrate through wetting wheat straw + 2% lime concentration. Hernandez *et al*., (2003) investigated the cultivation of oyster mushroom (*Pleurotus* species) using wooden crates, for composting a mixture of 70% grass, *Digitaria decumbens*), and 30% coffee pulp, combined with 2% $Ca(OH)_2$. They concluded that *P. ostreatus* can grow on lignocellulosic, non-composted, non-pasteurized substrate with an initial pH of 8.7, and that composting for 2 to 3 days improves the biological efficiency. Ibekwe *et al*., (2008) carried out a study to investigate cultivation of oyster mushroom (*Pleurotuso streatus*) on various substrates viz., rice, corn, milletand rye good sources of carbohydrates. Results indicated that mycelia growth of mushroom was highest in millet while on rye it was lowest. Optimum growth of mycelium was achieved at pH 6.5. Further results of study indicated that proper selection of substrate and optimal environmental conditions help in the best production of oyster mushroom. Mycelium of fungi (mushroom) obtain nutrients from substrate at specific level of pH (Sarker *et al.,* 2007), pH of substrate can be increased by adding lime in it. The maximum quantity of lime for oyster (*Pleurotus* spp.) mushroom production is 2%. Too much quantity of lime reduces nutrients uptake ability of fungi and mycelial growth of mushroom. Without using lime in substrate mycelium of oyster mushroom has ability to grow and nourish successfully.

Number of days taken for initiation of primordia: Primordia (pinheads) are the tiny fruit bodies of mushroom with a size of greater than (0.01mm). They appeared after the completion of mycelial growth of mushroom. pH and environmental conditions of the substrate affects directly to the growth of primordia. Data regarding no. of days taken for initiation of primordia is shown in Table 2. The comparison of treatments means showed that number of days taken for primordia initiation differ significantly. Treatments T_0, T_1, T_2 and T_3 took maximum number of days 3, 3, 5 and 6.8 d for initiation of primordia after complete mycelial growth at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 took maximum number of days (6.8 d) for the initiation of primordia followed by T_2 (5 d), T_1 (3 d) and T_0 (3 d) with pH 8.2, 7.8 and 7.2, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with minimum number of days (3 d) for initiation of primordia but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Ahmad (1986) studied the cultivation of oyster mushroom on waste material of corn industry and concluded that *Pleurotus ostreatus* took time of 17-20 days for complete spawn running while pinheads formation started after 23-27 days of spawning. Khan *et al*., (2001) described the cultivation of oyster mushroom using different ligno cellulosic substrates and found that pinhead formations take place after 7-8 days while sporocarps formation take place after 10-12 days of spawn running. Shah *et al*., (2004) investigated oyster mushroom cultivation and observed that pin heads like structure are formed after 6-7d of spawn running. Khan *et al.,* (2004) studied the cultivation of *Pleurotuso streatus* on different lingo cellulosic substrates and the results showed that the highest mean yield of 680.9g was observed on substrate containing rice husk 50% + cotton waste 50%. It was also observed that pinhead formed after 17-29 days of spawning.

Number of pinheads: The Table 3 described the statistical data about number of primordia (pinheads) formed. Analyses of variance for number of pinheads showed that number of primordia differ significantly. The comparison of treatments means showed that treatments

T_0, T_1, T_2 and T_3 showed maximum number of primordia (pinheads) 28.2, 30.8, 20.4 and 11.8 at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment means of T_1 (2% lime) with pH 7.8 showed maximum number of primordia (pinheads) 30.8 followed by T_0 (28.2), T_2 (20.4) and T_3 (11.8) at pH 7.2, 8.2 and 8.7, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 maximum number of primordia 30.8 and 28.2, but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Khan *et al.*, (2001) examined the cultivation of oyster mushroom using different lingo cellulosic substrates and found that pinhead formations take place after 7-8 days while sporocarps formation take place after (10-12) days of spawn running. Further studies reviled that highest yield of mushroom was observed in cotton waste and the formation of pinhead and fruiting bodies per bag were also more in cotton waste. Kimenju *et al*., (2009) described the suitability of different substrates for the good production of oyster mushroom. Ten different substrates viz. hyacinth, maize cobs, coconut fiber, finger millet straw, banana fiber, saw dust, rice straw, bean straw and wheat straw were studied for better production of oyster mushroom. 250g of substrate was filled in plastic bags. Result of experiment showed that the control took (28 days) while maize cobs, sawdust and coconut fiber took time for pinheads formation (19, 22 and 23 days) respectively. Bean, finger millet rice and wheat straws showed maximum biological efficiency of mushroom in decreasing order 106, 92, 85 and 77%, respectively. Stipe length of mushroom was observed longest in bean straw followed by finger millet straw, banana fiber and maize cobs while it was observed shortest in mushroom grown on sawdust. As compared to the control, yield of mushroom was, 80, 78, 76, 73 and 68%, was higher in bean straw, rice straw, millet straw, wheat straw and banana fiber, while the yield of mushroom on sawdust was observed 60% lowered than control. At the end of experiment suitability of substrate in descending order was observed as follows bean < rice < finger millet < wheat straws.

Table 1. Comparison of treatments means taken to complete mycelial growth (number of days).

Treatments	Means
T_0 : 0% lime	18.40c
T_1: 2% lime	19.00c
T_2: 4% lime	21.60b
T_3: 6% lime	28.60a

Treatments means with different letters differ significantly at p<5%

Table 2. Comparison of treatments means for initiation of primordia (number of days).

Treatments	Means
T_0 : 0% lime	3.00c
T_1: 2% lime	3.00c
T_2: 4% lime	5.00b
T_3: 6% lime	6.80a

Treatments means with different letters differ significantly at p<5%

Table 3. Comparison of treatments means for number of pinheads.

Treatments	Means
T_0 : 0% lime	28.20b
T_1: 2% lime	30.80a
T_2: 4% lime	20.40c
T_3: 6% lime	11.80d

Treatments means with different letters differ significantly at p<5%

Table 4. Comparison of treatments means at harvesting stage after primordia initiation.

Treatments	Means
T_0 : 0% lime	3.00c
T_1: 2% lime	3.00c
T_2: 4% lime	4.80b
T_3: 6% lime	5.60a

Treatments means with different letters differ significantly at p<5%

Number of days taken to reach at harvesting stage after primordia initiation: The data regarding no. of days to reach at harvesting stage after the initiation of primordia is shown in Table 4, analysis of variance for treatments means showed that no. of days taken to reach at harvesting stage after primordia initiation differ significantly. The comparison of treatments means showed that treatments T_0, T_1, T_2 and T_3 took maximum no. of days 3, 3, 4.8 and 5.6d to reach at harvesting stage after the formation of primordia at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 took maximum no. of days (5.6 d) to reach at harvesting stage followed by T_2 (4.8 d), T_1 (3 d) and T_0 (3 d) with pH 8.2, 7.8 and 7.2, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with minimum number of days (3d) to reached at harvesting stage after initiation of primordia but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively.These results are in lined with the results of Khan *et al.*, (2001) examined the cultivation of oyster mushroom using different lingo cellulosic substrates and found that pinhead formations take place after 7-8 days while sporocarps formation take place after 10-12 days of spawn running. Shah *et al*., (2004) studied the cultivation of oyster mushroom and observed that pinheads like structure are formed after 6-7d of spawn running. Ragunathan & Swaminathan (2003) designed an experiment to compare the yield of different species of oyster mushroom viz. *Pleurotus sajor-caju, Pleurotus platypus* and *Pleurotus citrinopileatus.* The different species of oyster mushroom were grown on various agricultural wastes like coir fiber, cotton stalk, sorghum stover and mixtures of these substrates. The results indicated that primordial initiation started after 21-30 days of spawning and the highest yield of mushroom *P. sajor-caju* and *P. citrinopileatus* was observed in cotton stalks, while *P. platypus* showed their maximum

production on sorghum stover. Khan *et al*., (2004) carried out an experiment for the cultivation of *P. ostreatus* on different ligno cellulosic substrates and the results showed that rice husk 50% + cotton waste 50% gave the highest mean yield of 680.9g. It was also concluded that after 17-29 days of spawning pinhead formation took place in *P. ostreatus* (oyster mushroom).

Number of days taken to reach at harvesting stage (number of days): The number of days taken to reach at harvesting was recorded to determine the lifecycle of mushroom. Statistical data regarding total number of days taken to reach at harvesting stage is represented in Table 5. Analysis of variance for treatments means showed that total no. of days taken to reach at harvesting stage differs significantly. The comparison of treatments means showed that treatments T_0, T_1, T_2 and T_3 took maximum no. of days 24, 25, 31.4 and 41 days to reach at harvesting stage at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 took maximum number of days to reached at harvesting stage 41d followed by T_2 (31.4 d), T_1 (25 d) and T_0 (24 d) at pH 8.2, 7.8 and 7.2, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with total number of days to reached at harvesting stage 25d and 24d respectively, but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Heltay (1987) observed that fruit bodies appeared after 49 days of spawning of *P. florida*using different lingo cellulosic substrates like rye, barley, and wheat bran. Khan *et al*., (2001) examined the cultivation of oyster mushroom on different cellulosic substrates. It was observed that after spawn running pinhead formations take 7-8 days while sporocarps formed after 10-12 days. Dundar & Yildiz (2009) carried out a comparative study on *Pleurotu sostreatus* (jacq.) *P. kumm*.cultivated on different agricultural lignocellulosic wastes and observed that shortest total harvest time (THT), maximum yield and the highest biologic efficiency degree (BED) 166.18% was observed on soyabeen stalks + lentals stalks (100:20; w:w). Kim *et al*., (2008) studied mycelial growth rate of *Pleurotus* species. The results indicated that addition of bacterial culture strain P-7014 and its supernatant to the mushroom growing media resulted in fast mycelial growth of mushroom. Mycelial growth rate of *Pleurotuseryngii* was increased up to 1.6 fold and primordial formation take place one day earlier than the normal. Moreover, it was supposed that addition of bacteria had beneficial applications for commercial mushroom production, which appreciably reduced total number of days for cultivation of about 5 ± 2 days compared with uninoculated which took 55 ± 2 days.

Table 5. Comparison of treatments means to reach at harvesting stage (number days).

Treatments	Means
T_0: 0% lime	24.40c
T_1: 2% lime	25.00c
T_2: 4% lime	31.40b
T_3: 6% lime	41.00a

Treatments means with different letters differ significantly at p<5%

Table 6. Comparison of treatments means of formation of Total number of fruiting bodies.

Treatments	Means
T_0 : 0% lime	24.20b
T_1: 2% lime	26.80a
T_2: 4% lime	16.40c
T_3: 6% lime	7.80d

Treatments means with different letters differ significantly at p<5%

Total number of fruiting bodies: The Fruiting bodies of mushroom is also known as sporophore. The production of healthy fruit body of mushroom depends upon good environmental conditions especially humidity and temperature of mushroom growth room. Most the species of oyster mushroom grow and give maximum production on more than 85% of humidity and 15-25°C temperature (Jandaik & Kapoor, 1976; Hasan *et al*., 2010). Data regarding total number of fruiting bodies formed after primordia initiation is presented in Table 6. Analysis of variance for treatments means showed that total number of fruiting bodies differs significantly. The comparison of treatments means showed that maximum number of fruits bodies observed in treatments T_0, T_1, T_2 and T_3 were 24.2, 26.8, 16.4 and 7.4 at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 showed minimum number of fruit bodies 7.4 d followed by T_2 (16.4), T_0 (24.2) and T_1 (26.8) at pH 8.2, 7.2 and 7.8, respectively. Treatment T_1 showed maximum number of fruit bodies (26.8) and differs significantly from T_0, T_2 and T_3 respectively. These results agreed with the results Shah *et al*., (2004) who investigated the cultivation of oyster mushroom on 50% sawdust plus 50% wheat straw, 75% sawdust plus 25% leaves, 50% wheat straw plus 50% leaves, 100% sawdust plus 100% wheat straw and 100% leaves. The temperature was maintained at 25°C for spawn running and 17-22°C for fruit body formation. The results indicated that spawn running take 2-3 weeks after inoculation, while small pinhead formed after 6-7 weeks of spawn running. The fruit bodies appeared after 3-6 days after pinhead formation and take 27-34 days after spawn inoculation. 100% Sawdust gave highest yield 646.9g, biological efficiency of 64.69% with 22.11 (number of fruiting bodies).

Yield of mushroom in 1st flush (g): The Table 7 represents the data about total yield of 1st flush (break) of mushroom on fresh weight basis.Analysis of variance for treatments means showed that mushroom yield of 1st break differs significantly. The comparison of treatments means showed that treatments T_0, T_1, T_2 and T_3 showed fresh weight of mushroom 38g, 41.02g, 22.18g and 14.53g at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 showed minimum yield of mushroom (14.53g) in 1st break followed by T_2 (22.18g), T_0 (38g) and T_1 (41.02g) at pH 8.2, 7.2 and 7.8,

respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with fresh weight 41.02g and 38g respectively in 1st break of mushroom, but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Shahid *et al.*, (2006) studied the effect of different methods of compost preparation and lime concentration on the yield of *Pleurotus sajor-caju* and concluded that the most rapid mycelial growth (23 days), least duration of pin setting (48 days), maximum number of flushes (6) and the highest yield (295g/1.5kg substrate) was obtained from wetting wheat straw + 2% lime concentration. Muhammad & Khan (1993) studied cultivation of oyster mushroom using cotton waste as substrate material. They used two species of oyster mushroom i.e., *Pleurotus sajor caju* and *Pleurotus ostreatus.* Their results indicated that mycelial growth of *Pleurotus ostreatus* was faster than *Pleurotus sajor caju*. Khan *et al.*, (2012) reported that oyster mushroom showed relatively more yield on cotton waste (296.25g) as compared to other substrates. Further studies indicated that *Pleurotus ostreatus* give maximum yield of mushroom in first and third flush while the highest yield of *Pleurotus sajor-caju* was observed in second flush of mushroom. In our experiment maximum yield and quality of mushroom was obtained in first flush of mushroom it was might be due to the availability of maximum nutrients in substratum.

Yield of mushroom in 2nd flush (g): Flush of mushroom is also known as break of mushroom. The duration and yield of breaks depends upon type of strain, substrate and environmental conditions of mushroom growth room. Data regarding yield of mushroom in 2nd flush on fresh weight basis is shown in Table 8. Analysis of variance showed that mushroom yield of 2nd break differs significantly. The comparison of treatments means showed that treatments T_0, T_1, T_2 and T_3 showed fresh weight of mushroom 15.08g, 15.3g, 9.99g and 8.84g at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 showed minimum yield of mushroom (14.53g) in 2nd break followed by T_2 (9.99g), T_0 (15.08g) and T_1 (15.3g) at pH 8.2, 7.2 and 7.8, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with fresh weight 15.3g and 15.08g respectively in 2nd break of mushroom, but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Manan (2000) studied the cultivation of oyster mushroom on different cellulosic materials like cotton waste, paper waste, wheat straw and paper waste + wheat straw. He fined maximum yield of mushroom 198.67g and 29.253g on cotton waste and wheat straw respectively. However, papers waste gave no yield where amended with wheat straw gave mushroom yield of 58.953g. Muhammad & Khan (1993) studied. However. cultivation of oyster mushroom using cotton waste as substrate material. They used 2 species of oyster mushroom *Pleurotus sajor-caju* and *Pleurotus ostreatus.* Their results indicated that mycelial growth of *Pleurotus ostreatus* was faster than *Pleurotus sajor-caju*. Further studies indicated that *Pleurotus ostreatus* give maximum yield of mushroom in first and third flush while the highest yield of *Pleurotus sajor-caju* was observed in second flush (break) of mushroom.

Total yield of mushroom (g): The Table 9 represents data about total yield of mushroom on fresh weight basis. Analysis of variance showed that mushroom yield of treatments means differ significantly. The comparison of treatments means showed that treatments T_0, T_1, T_2 and T_3 gave fresh weight of mushroom 53.08g, 56.32g, 32.17g and 23.37g at pH 7.2, 7.8, 8.2 and 8.7 respectively. Treatment T_3 (6% lime) with pH 8.7 showed minimum yield of mushroom (23.37g) followed by T_2 (32.17g), T_0 (53.08g) and T_1 (56.32g) at pH 8.2, 7.2 and 7.8, respectively. Treatments with 2% (T_1) and without lime addition (T_0) showed same level of significance at pH 7.8 and 7.2 with fresh weight of mushroom 56.32g and 53.08g respectively, but differ significantly from T_2 (4% lime, pH 8.2) and T_3 (6% lime, pH 8.7) respectively. These results are similar with the results of different scientists. Shahid *et al.*, (2006) studied the effect of different methods of compost preparation and lime concentration on the yield of *Pleurotus sajor-caju* and concluded that the most rapid mycelial growth (23 days), least duration of pin setting (48 days), maximum number of flushes (6) and the highest yield (295g/1.5kg substrate) was obtained from wetting wheat straw + 2% lime concentration. Khan *et al*., (2004) carried out an experiment for the cultivation of *P. ostreatus* on different ligno cellulosic substrates and the results showed that rice husk 50% + cotton waste 50% gave the highest mean yield of 680.9g. Hernandez *et al*., (2003) studied oyster mushroom (*Pleurotus* species) cultivation by using wooden crates for composting a mixture of 70% grass, (*Digitaria decumbens*), and 30% coffee pulp, combined with 2% $Ca(OH)_2$. Crate composting considerably modified the temperature pattern of the substrate in process, as compared to pile composting. In this method of composting lower temperatures and less homogeneous distributions was observed and biological efficiencies varied from 59.79% to 93% in 2 harvests. Statistical analysis showed significant differences between the treatments, composting times and in the interactions between these 2 factors. It was concluded that it is possible to produce *P.ostreatus* on a ligno cellulosic, non-composted, non-pasteurized substrate with an initial pH of 8.7, and that composting for 2 to 3 days improves the biological efficiency. Total yield of mushroom was observed maximum in treatment T_0 and T_1 as mushroom find maximum metabolic activities at pH 7.2 and 7.8 respectively.

Table 7. Comparison of treatments means of yield of mushroom in 1st flush (g).

Treatments	Means
T_0 : 0% lime	38.00b
T_1: 2% lime	41.02a
T_2: 4% lime	22.18c
T_3: 6% lime	14.53d

Treatments means with different letters differ significantly at p<5%

Table 8. Comparison of treatments means of Yield of mushroom in 2nd flush (g).

Treatments	Means
T_0 : 0% lime	15.08a
T_1: 2% lime	15.30a
T_2: 4% lime	9.99b
T_3: 6% lime	8.84b

Treatments means with different letters differ significantly at p<5%

Table 9. Comparison of treatments means for Total yield of mushroom (g).

Treatments	Means
T_0 : 0% lime	53.08a
T_1: 2% lime	56.32a
T_2: 4% lime	32.17b
T_3: 6% lime	23.37c

Treatments means with different letters differ significantly at p<5%

Conclusion

Different levels of lime were studied in substrate preparation for effective cultivation of Oyster mushroom (*Pleurotus* spp.). The treatment with 2% lime showed best results regarding number of days to complete mycelial growth and yield of mushroom followed by treatments containing no lime, 4% and 6% of lime in substrate preparation. The results showed that cotton waste containing 2% lime was proved one of the best for cultivation of oyster mushroom compared to other variables of lime with respect to number of days for mycelial growth, number of days taken for initiation of primordia, number of pinheads, number of days taken to reach at harvesting stage after primordia initiation, number of days taken to reach at harvesting stage, total number of fruiting bodies and overall production of was observed better in treatment containing 2% lime than all the other variables. It is concluded that Oyster mushroom give well enough yield on cotton waste containing 2% lime as compared to other levels of lime.

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