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Improvement of Strains of Two Oyster Mushroom Cultivars Using Duel Culture Technique

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Abstract: In Sri Lanka, only a few mushroom species are popular among growers and among these, Oyster mushrooms (*Pleurotus* sp.) are cultivated widely at present. There is a growing demand for new and improved mushroom strains with better characters including yield. The present study was conducted with the objective of improving mushroom strains using duel culture hybridization technique. Hybridization was achieved by duel culture technique of monospore cultures of two strains (Lanka Oyster and American Oyster) of Oyster mushrooms (*Pleurotus ostriatus*). Among twenty five combinations of mono spore cultures, twenty three hybridized successfully. Maximum growth of hybridized mycelia was obtained at 28°C. A_3L_3, A_4L_1, A_4L_4, A_3L_4 and A_2L_4 monospore combinations were selected as the best hybrids showing higher growth rate. Although these hybrids did not vary significantly in colonizing spawn medium and inoculated bags, the noticeable differences were observed for the formation of pinhead and number of fruiting bodies. Morphology of fruiting bodies of these hybrids was also did not differ considerably. Low biological efficiency was noticed in all five hybrids indicating their unsuitability to grow on saw dust medium.

Key words: Duel culture · Hybridization · Pleurotus · Oyster mushrooms · Strain improvement

INTRODUCTION

Since commercial cultivation of mushrooms was started, the growth and rapid development of the mushroom industry was stimulated in the 1960's [1]. It became popular among people in Sri Lanka as an agribusiness only in the late 1980's [2]. In Sri Lanka, only a few mushroom species are popular among growers and among those, oyster mushrooms (*Pleurotus* sp.) are the mostly cultivated mushrooms at present. Oyster mushrooms are one of the most delicious foods due to their high nutritional value, very good taste and medicinal value [3,4]. Two oyster mushroom varieties mostly preferred by growers as well as consumers in Sri Lanka are American oyster (*Pleurotus ostriatus*) and Lanka oyster (*Pleurotus* sp).

Both strains have the ability to grow on different compost media easily. There are some good and bad characters found in these mushroom verities as par as cultivators and consumers are concerned. The fruiting bodies of American oyster are creamy white, while Lanka oyster appeared somewhat grayish dark. American oyster has higher growth rate and good texture than Lanka Oyster. In contrast, Lanka oyster comparatively has high yielding ability, larger fruiting bodies and longer harvesting period and shelf life.

The experiment on new and improved varieties is needed because of the consumer preference for new mushroom strains and cultivator's problems with existing mushroom types. The limitation of successful mushroom breeding programs in Sri Lanka for new and improved mushroom species became one of the major setback in popularizing mushroom cultivation at present. Productivity and quality of widely cultivated mushrooms are mainly dependent on strain make up, therefore, many different strain types are constantly produced, aiming at higher yield and improved quality attributes [5, 6]. Simple techniques such as selection (Monospore and Multispore) and cross breeding (hybridization) can be easily adopted to improve the characters of the cultivated lines. The major aim of hybridization is to combine desirable characteristics from different strains and create variability in the existing germplasm. Breeding high yielding strains has traditionally been accomplished by

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trial and error and large numbers of hybrids can be obtained by pairing monosporic cultures, need to be cultivated to evaluate the production characteristics [7]. In this research study, we tried to develop new mushroom strains crossing two widely grown Oyster mushrooms (American oyster and Lanka oyster) by hybridization technique.

MATERIALS AND METHODS

The experiment was conducted in the research laboratory, Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna during February to August 2008. Two types of oyster mushroom strains selected for this study were American Oyster (*Pleurotus ostriatus*) and Lanka Oyster (*Pleurotus* sp.).

Spore Print: The newly opened fleshy and healthy mushroom was selected to prepare a spore print. The cap of the mushroom was laid flat with the gills down on a sterilized black paper lining a sterilized petri dish. The petri dish was sealed properly and the entire setup was placed in an undisturbed area about 6 hours. Then the resulted spore print was washed to a test tube using sterilized distilled water and spore suspension was prepared. By using spreading method, the serially diluted spores were transferred to PDA media. Then the single spore cultures were prepared and maintained in PDA slants and were stored for future use.

Hybridization/Cross Breeding: Five single spore cultures of American Oyster and five Lanka Oyster cultures were randomly selected and twenty five combinations of the above cultures were established to test the compatibility as presented in the Table 1. The crosses between homokaryotic cultures were performed in duel culture technique by placing actively growing mycelia/agar plugs of single spore cultures of above two strains approximately one cm apart in the center of a 100 mm petri dish of PDA. Three replicates were used for each combination and arranged in a completely randomized design.

After these homokaryons were fused and developed heterokaryotic mycelium, a sample of mycelia was transferred to fresh agar medium. The crosses were considered positive if dikaryotic hyphae clamp connections could be observed under 100x magnification at the growing margin of either side of the interacting strains. The length of hybridized area was measured.

Table 1: Combinations of two monospore cultures of American oyster (A) and Lanka oyster (L)

	Lanka Oyster				
American Oyster	L_1	L_2	L_3	L_4	L_5
A_1	A_1L_1	A_1L_2	A_1L_3	A_1L_4	A_1L_5
A_2	A_2L_1	A_2L_2	A_2L_3	A_2L_4	A_2L_5
A_3	A_3L_1	A_3L_2	A_3L_3	A_3L_4	A_3L_5
A_4	A_4L_1	A_4L_2	A_4L_3	A_4L_4	A_4L_5
A_5	A_5L_1	A_5L_2	A_5L_3	A_5L_4	A_5L_5

Performances of Hybridized Mycelia under Different Temperature Levels: The effect of temperature on the growth of hybridized strains was tested under different temperature levels of 28°C, 30°C, 32°C and room temperature 28±2°C. The temperature levels were maintained using incubators with four replicates. Radial mycelial growth was recorded every other day. Based on the growth performance, A_2L_4, A_3L_3, A_3L_4, A_4L_1 and A_4L_4, hybridized strains were selected and used for further experiments.

Production of Spawns: The colonization rate of the five selected strains was studied on finger millet (*Eleucine coracana*) spawn substrate mixed with $CaCO_3$ @ 1% and glucose @ 0.05%. The mixture was adjusted at the moisture of 60% [8] and then filled in polypropylene bags. After autoclaving, bags were inoculated with strains and incubated in dark at room temperature (28°C). Treatments were replicated four times and were arranged in a Randomized Complete Block Design. The mycelia development was observed visually and the percentage of medium colonization by a particular strain was recorded calculating number of days required for the 100% colonization of the spawn substrate.

Fruiting and Harvesting: The compost mixture was prepared by mixing saw dust 100 kg + Rice bran 10 kg+ Mung bean powder 1 kg + $CaCO_3$ 2 Kg + $MgSO_4$ 200g with sufficient amount of water. Each substrate was filled in polypropylene bags and their mouths were plugged by inserting water absorbing cotton with the help of plastic rings. The bags were autoclaved at 121°C at 15-20 *psi* pressure and allowed to cool. After sterilization, next day, the bags were inoculated with the spawns of improved strains at the rate of 5% per bag according to the dry weight of substrates. The bags were then incubated for spawn run under complete darkness at the room temperature (28°C). The experiment was laid out in RCBD with three replicates and five treatments. In about 28 days,

when the mycelium reached the bottom the bag was cut open and kept in the cropping room for initiating fruiting. The bags were watered two to three times in a day during cropping. When pin heads grew into mature fruit bodies, harvesting was done.

Total weight of fruiting body was taken during each harvesting and weighed using a weighing scale. The biological efficiency was worked out as yield of mushroom strain against the dry weight of each bag as follows.

$$Biological\ efficiency \frac{Total\ weight\ of\ fruit\ bodies}{Total\ weight\ of\ substrate(compost)of\ spawning}\times 100$$

Time was recorded in days for the completion of growth of mycelium on substrates bags and appearance of pinheads in different strains. Average number of fruiting bodies formed in bags inoculated with each strain was also recorded.

All laboratory experimental data were analyzed using SAS software (SAS Institute, Cary, NC) and means were separated by Duncan's multiple range test (P=0.05).

RESULTS AND DISCUSSION

The two mushroom monospore cultures were successfully hybridized and produced heterokaryotic mycelium in 23 combinations out of 25 and the success rate was 92%. The high rate of mating observed in these crosses could be due to morphological structures come from the same genus [9]. However, more detailed studies are needed for a stronger evidence to support this assumption on mating compatibility. The length of the hybridized area may indicate the strength of compatibility between two strains. According to the Figure 1, hybridization length along the margin of interacting cultures was highest in A_2L_4 combination however not significantly different (P=0.05) from other combinations. There was no hybridized area seen in A_2L_2 and $A_5\ L_1$ combinations and therefore did not form any kind of heterokaryotic mycelium (Fig. 1).

The growth of the heterokaryotic mycelia differed significantly among different temperature levels (P=0.05). The maximum growth was recorded in 28°C and significantly differed from other temperature levels (Table 2). At higher temperatures (32°C)' heterokaryotic mycelia showed poor growth and significantly different (P=0.05) from other temperature levels tested.

Table 2: Growth of heterokaryotic mycelia at different temperature levels

	Mean mycelial growth (mm)				
Combination	RT[1]	28°C	30°C	32°C	Mean[2]
A_2L_4	65.67	79.00	66.00	44.67	63.84^{abc}
A^3L_4	63.50	77.67	72.33	54.00	66.88^{a}
A^2L_3	70.00	62.00	58.67	44.67	58.84^{cd}
A_5L_5	64.50	65.00	61.00	47.50	59.50^{bcd}
A_4L_1	68.00	79.67	63.67	46.67	64.50^{ab}
A_1L_2	66.67	60.00	52.67	47.00	56.59^{cdef}
A_4L_5	60.00	64.00	58.33	43.00	56.33^{cdef}
A_2L^5	57.00	71.33	67.00	48.33	60.92^{bcd}
A_3L_1	64.00	64.00	60.00	40.00	57.00^{cde}
A_4L_3	63.67	68.33	67.00	50.33	62.33^{abcd}
A_3L_5	60.00	36.67	66.67	51.00	53.59^{fg}
A_5L_4	59.50	54.00	54.33	41.00	52.21^{g}
A_1L_4	62.00	70.00	58.67	47.33	59.50^{bcd}
A_3L_3	67.67	77.00	59.33	47.00	62.75^{abc}
A_1L_5	55.67	66.00	62.50	48.00	58.04^{cd}
A_1L_3	51.50	65.00	58.33	42.50	54.33^{efg}
A_5L_3	53.00	64.50	60.00	51.00	57.13^{cde}
A_4L_4	71.67	77.50	61.67	45.00	63.96^{ab}
A_1L_1	43.67	65.00	59.00	52.33	55.00^{def}
A_3L_2	62.00	62.00	59.33	49.33	58.17^{cd}
Mean[2]	61.48^{b}	66.43^{a}	61.32^{b}	47.03^{c}	-

[1]RT = Room temperature (28±2°C)

[2]Values within a column or row followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

The mycelial growth was also different among different heterokaryotic combinations (P=0.05). Highest mycelial growth was observed in A_3L_4 followed by A_4L_1, A_4L_4, A_2L_4 and A_3L_3 (Table 2). The interaction between temperature levels and heterokaryotic mycelia was significant (P=0.05).

It is evident from the results that heterokaryotic mycelia arise from different combinations of single spore cultures of American Oyster and Lanka Oyster responded differently to different temperature levels. Mycelial growth is one of the characteristics need to study in order to select improved strains for the commercial cultivation [10]. Therefore, based on the growth of the resulted dikaryons, combinations of A_2L_4, A_3L_3, A_3L_4, A_4L_1 and A_4L_4 were selected for further studies.

There was no correlation observed between the length of hybridized area and the growth of hybridized strains and therefore, hybridized area has no validity when selecting improved strains for commercial cultivation.

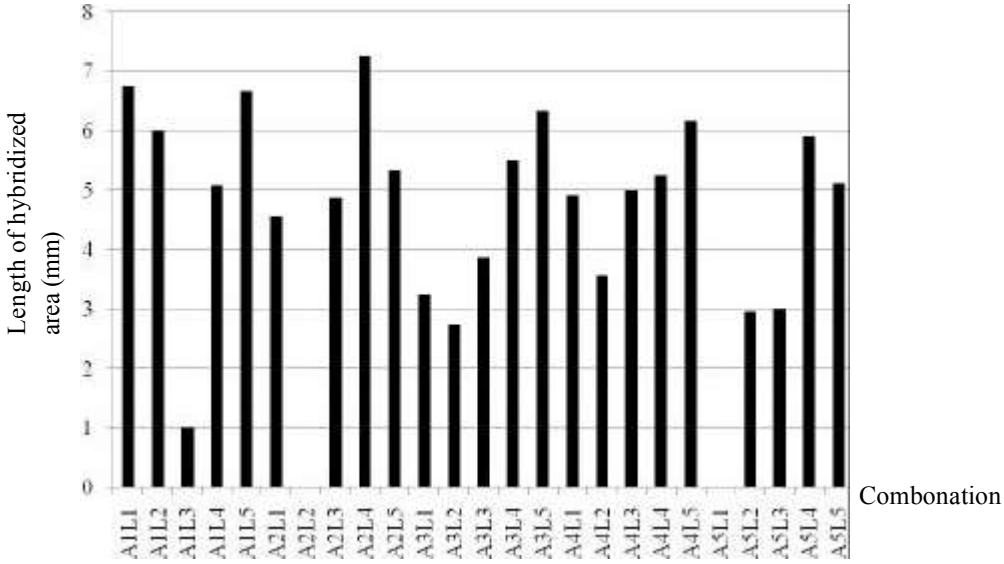

Fig. 1: Length of hybridized area of different strains four days after the inoculation

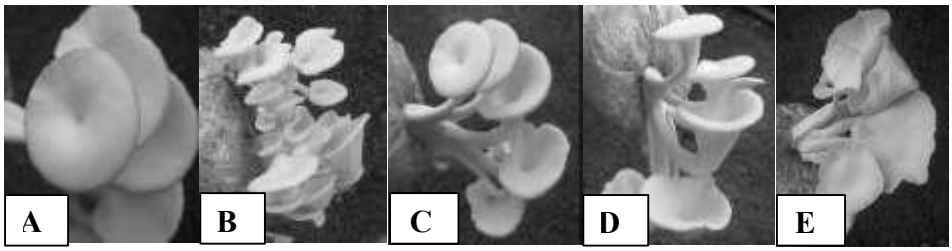

Plate 1: Fully grown fruiting bodies formed in five selected hybrid strains A_2L_4 (A), A_3L_3 (B), A_3L_4 (C), A_4L_1 (D) and A_4L_4 (E).

Table 3: Days for complete colonization of spawn medium, completion of spawn running, pinheads formation and average number of fruiting bodies of selected hybridized strains

Combination	Days for complete colonization of spawn medium	Days for completion of spawn running	Days for pinheads formation after opening bags	Average number of fruiting bodies
A_2L_4	31.3^{ns}	26^{ns}	18^{a1}	18^{b}
A_3L_3	32.0	25	17^{a}	26^{a}
A_3L_4	30.7	24	10^{b}	07^{d}
A_4L_1	31.7	24	18^{a}	14^{c}
A_4L_4	31.0	26	09b	12^{c}

[1]Values within a column followed by a same letter are not significantly different at P=0.05 according to Duncan's multiple range test.
ns = not significant at P = 0.05

No considerable morphological differences were observed in fruiting bodies of five hybrid strains. The pileus and stalk of all five strains were white which is the colour of American Oyster mushrooms. The pileus margin of A_4L_4 was somewhat wavy appearance while other strains had smooth margin around the cap. In addition, compared to other strains, the size of fruiting bodies were very small in A_3L_3 which in turn formed large number of fruiting bodies (Plate 1). As the consumption of fresh mushrooms is steadily increasing, the demand for premium quality products at the point of sale mainly focuses on whiter, firmer mushrooms [11, 12 & 13] which was observed in the resulted hybrid strains quite promising.

The spawn running, pinheads formation and average number of fruiting bodies of selected strains are presented in the Table 3. Those selected strains when tested for their rate of mycelial colonization in spawn medium, complete colonization was observed within 31 to 32 days. There was no significant difference observed among

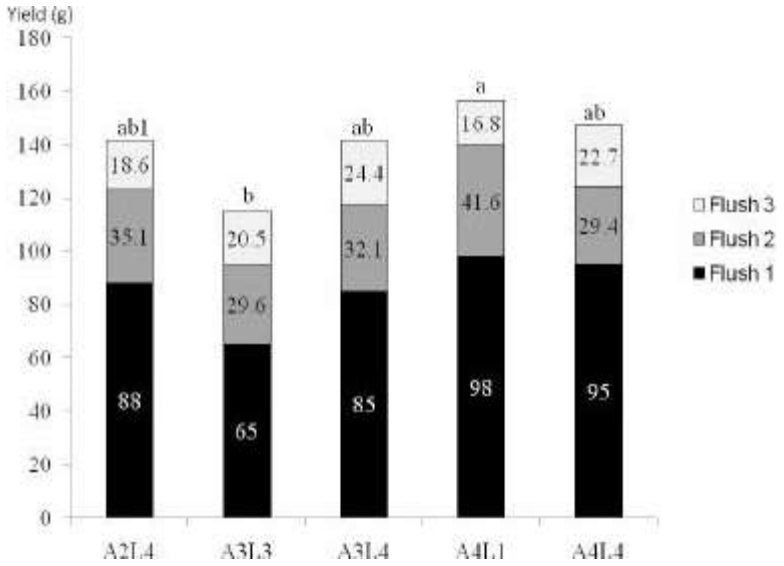

Fig. 2: Accumulated and individual flushes yield of five selected hybrid strains
[1]Columns (mean overall yield) not sharing common letters are significantly different at P=0.05

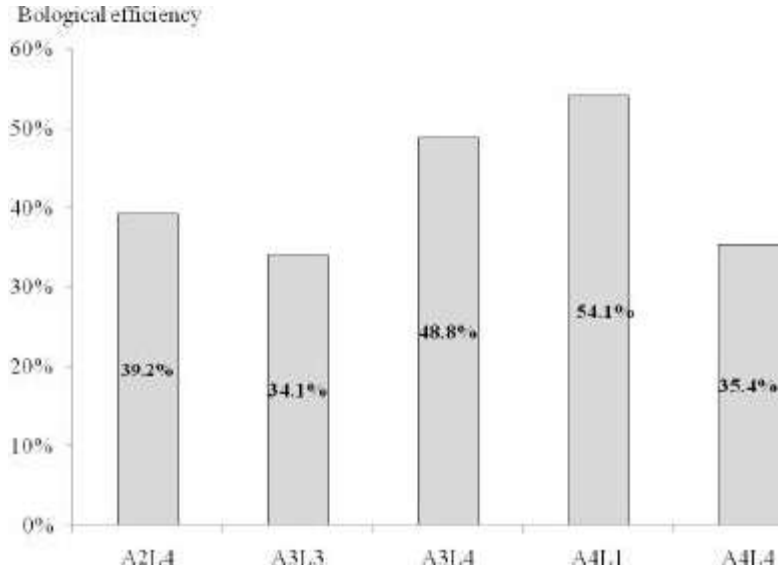

Fig. 3: Biological efficiency (%) of selected hybrid strains on Finger millet medium
*Columns not sharing common letters are significantly different at P=0.05

strains for the rate of colonization of spawn medium. *Pleurotus* species usually colonize spawn medium completely in 21 days [2] however, improved strains had taken more number of days in this study to colonize the medium 100%. The mycelial colonization rate can be varied with many factors including inherent genetic differences within strains. However, any improved strain can be preferred to have better colonization in order to produce spawns fast.

All strains had colonized the sterile compost bags 24 to 26 days of spawn run depending on the strain. Although no statistical difference was noted in the number of days taken for the spawn run among the five hybrids tested, fast colonization was achieved in the A_2L_4 and A_4L_4 strains. Similar results were also recorded by Tan [14] who reported that the spawn running took three weeks in their study with *Pleurotus* species.

The pinheads were formed in different time periods in tested strains. In A_3L_4 and A_4L_4, it took significantly

less number of days (10 and 9 days. respectively) after opening of bags, compared to other three strains (Table 3). However, total number of days taken from inoculation to pinheads formation was much higher (34 and 35 days) in these strains compared to observation made by Ahmad [15] who stated that *P. ostreatus* completed spawn run in 17-20 days on different substrates and time for pinheads formation was noted as 23-27 days. Similarly, Quimio [16] also reported that fruiting bodies of *P. ostreatus* appeared 3-4 weeks after inoculating of spawns.

The average number of fruiting bodies formed differed significantly (P=0.05) among strains (Table 3). Maximum number of fruiting bodies formed in A_3L_3 was 26 and which was significantly on par with other strains. The lowest value was recorded in A_3L_4 (07). Interestingly, even though, A_3L_3 had more number of fruiting bodies in the first flush, the accumulated yield was the lowest in this strain (Table 3 and Fig. 2).

The spawn running, pinheads and fruiting bodies formation are three important phases in the cultivation of mushroom, require proper humidity and temperature. The number of days taken for each of the above can be varied greatly with the type of strain and cultivation condition [17].

The harvest of improved strains has been realized during three flushes. The maximum yield was obtained in first flush than the second and third flushes (Fig. 2) for all five strains. The lowest quantity of mushrooms has been harvested in the third flush. As it is evident from the data presented in fig. 2, significant differences (P=0.05) among strains were observed in accumulated yields. Maximum average yield (156.4 g) was estimated from A_4L_1on which about 63% recorded in the first flush. However, this value did not differ significantly with three other strains A_2L_4, A_3L_4 and A_4L_4.

The biological efficiency was worked out against the dry weight of each substrate. It is clear from the Figure 3 that A_4L_1 showed highest biological efficiency (54.09 %) followed by A_3L_4 (48.8%).

The lowest biological efficiency value was observed with A_3L_3 and A_4L_4 (34.09% and 35.4%, respectively) and did not differ significantly (P = 0.05) from each other.

Biological efficiency indicates strain's ability to utilize the medium to a more usable form. Among selected strains, only one strain showed more than 50% biological efficiency on saw dust medium which is not good indication of a good character of an improved strain. According to Shah *et al*. [3] biological efficiency of *P. ostreatus* observed in saw dust based medium was maximum compared to other media tested. The maximum utilization of substrate depends on the type of strain grown on a particular medium and therefore, some strains may perform well in one medium while other may not. The strain do not show high biological efficiency, may perform well in another medium. Therefore, if the improved strain shows acceptable characteristics, their yield and biological efficiency need to be studied on different compost media.

CONCLUSION

Twenty three combinations out of 25 successfully formed heterokaryotic mycelia indicating 92% success rate. There was no correlation between the length of hybridized area and mycelial growth. Better growth of heterokaryotic mycelia resulted from duel culture of American Oyster and Lanka Oyster was noted at 28 °C. Colonization of spawn medium and inoculated bags were somewhat similar in five hybrid strains however, noticeable differences observed with days for pinhead formation and average number of fruiting bodies formed. All five strains had low biological efficiency on saw dust medium and was found unsuitable to grow.

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REFERENCES

1. Pathak, V.N., N. Yadav and M. Gaur, (Eds). 1998. Mushroom production and processing technology, H S Offset Printers, New Delhi.
2. Amarasinghe, D.M., 1995. *Bimmal Wagawa* (Sinhala), Sara printers, Sri Lanka.
3. Shah, Z.A., M. Ashraf and Ch, M. Ishtiaq 2004. Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates (Wheat Straw, Leaves, Saw Dust), Pakistan Journal of Nutrition, 3(3): 158-160.
4. Diana, F., D. Indrea, A.S. Apahidean, M. Apahidean, R. Pop, Z. Moldovan, D. M'niuiu, R. Ganea and I. Paven, 2006, Importance of substrate disinfection on oyster mushroom (*Pleurotus* sp.) culture, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 34: 48-53.

5. Tschierpe, H.J., 1983. Environmental factors and mushroom strains. Mushroom J., 132: 417-429.
6. Burton, K.S., 1990 The quest for quality. Mushroom Journal, 212: 228-291.
7. Eger, G., 1978. Biology and Breeding of *Pleurotus*, pp: 479-519, In S.T. Chang and W.A. Hayes (Eds). The Biology and cultivation of edible mushrooms. Academic Press, New York.
8. Yang, X.M., 1986. Cultivation of edible mushroom in China, Agriculture printing house, Beijing, China.
9. Rosanina, A, A. Noorllidah and S. Vikineshwary, 2007. Strain improvement of edible mushrooms by mating. In the Abstracts of the Proc. of the Asian Mycologial Congress (AMC 2007) and Xth International Marine and freshwater mycology symposium (IMFMS), 266 P, (Eds.) S. Kamaruzaman, Z.A.M. Ahmad, S. Vikineshwary and J.Y. Tin, 02-06 December 2007, Park Royal, Penang, Malaysia.
10. Ko, H.G., H.G. Park, S.H. Park, W.C. Chang, S.H. Kim and W.M. Park, 2005. Comparative study of mycelial growth and basidiomata formation in seven different species of the edible mushroom genus, Hericium, Bioresource Technology, 96: 1439-1444.
11. Mac Canna, C. and T.R. Gormley, 1968. Quality assessment of mushrooms: relationship between moisture loss, color and toughness of harvested cultivated mushrooms, Mushroom Science, 7: 485-492.
12. Rama, T., K. Barton and J. Vincent, 1995. Changes to the surface texture of mushroom during post harvest storage, Mushroom Science., 14: 729-752.
13. Philippoussis, A. and G. Zervakis, 2000, Cultivation of edible mushrooms in Greece: presentation of the present status and analysis of future trends, Mushroom Science, 15:43-848.
14. Tan, K.K., 1981. Cotton waste is a fungus (*Pleurotus)* good substrate for cultivation of *Pleurotus ostreatus:* the oyster mushroom. Mushroom Science, 11: 705-710.
15. Ahmad, I., 1986. Some studies on oyster mushroom (*Pleurotus* sp.) on waste material of corn industry. M.Sc thesis. Department of plant Pathology, University of Agriculture, Faisalabad.
16. Quimio, T.H., 1978, Indoor cultivation of *Pleurotus ostriatus*. Philippines Agriculturist, 61: 253-262.
17. Sohi, H.S. and R.C. Upadhyay, 1989. Effect of temperature on mycelial growth of *Pleurotus* species and their yield performance on selected substrates. Mushroom Science, 12: 49-56.