# Lignocellulosic Wastes Used for the Cultivation of *Pleurotus ostreatus* Mushrooms: Effects on Productivity

Gonca Düzkale Sözbir,[a,]* İbrahim Bektaş,[a] and Ayhan Zülkadir [b]

The potential for using cotton seed hulls (CSHs) and walnut shells (WSs) as new, essential substances for substrate preparation in the cultivation of *Pleurotus ostreatus* was studied. Substrates prepared with oak sawdust alone (OS) and with mixtures of OS and CSHs and WSs in different ratios were compared, and their effects on the earliness, total time, yield, and biological efficiency (BE) were determined. The nitrogen (N) content of the substrates prepared using CSHs and WSs alone was high, so the C:N ratio of the substrates diminished as the proportions of CSHs and WSs in the mixtures were increased. The highest yields were obtained from substrates containing the maximum amount of N. The highest yield and highest biological efficiency were obtained for a mixture of 25OS:75CSHs, indicating that the yield in the substrates increased as the amount of CSHs in the mixtures increased. The morphological characteristics were influenced by the various substrates and their various ratios. The properties of mushroom cultivation in bags were related to the nitrogen content, as indicated by the C:N ratios. The results indicated that CSHs and WSs could be used as new, essential substances in the preparation of substrates for the cultivation of *Pleurotus ostreatus*.

*Keywords: Mushroom; Cotton seed hulls; Walnut shells; Pleurotus ostreatus*

*Contact information: a: Department of Forest Industry Engineering, Faculty of Forestry, KSU, 46060, Kahramanmaras, Turkey; b: Agricultural Research Institute, 46060, Kahramanmaras, Turkey;*
** Corresponding author: goncaduzkale@gmail.com*

## INTRODUCTION

*Pleurotus* spp. mushroom production has increased extensively worldwide during the last few decades (Royse 2002). Generally, the mushrooms are grown on pasteurized wheat or rice straw, and they can be cultivated on a large variety of substrates that contain lignin and cellulose (Das and Mukherjee 2007). The mushrooms are a potential source of valuable protein, and their mycelium has the ability to bioconvert various lignocellulosic materials effectively (Zadrail and Dube 1992). The mushrooms are an easily colonizable genus that cultures on various residues as substrates. This capability is a very important property because the rapid industrialization occurring worldwide is resulting in increasing amounts of waste generation. *Pleurotus* spp. are able to help in managing lignocellulosic wastes whose disposal has become a problem (Das and Mukherjee 2007). The several million tons of agricultural wastes are being disposed of by means of incineration, land applications, and land filling. The eliminating by incineration of wastes was adversely affect the environment because of the dirty gas emission. These wastes have a biorenewable energy resource and can be turned into high-value organic biomaterials such as nutritious food (Iscia and Demirer 2007).

Cotton waste is one of the lignocellulosic wastes that results from large scale planting in countries including China, Brazil, India, Pakistan, Turkey, and Australia, where it is often considered as an "energy source". The cotton production in the 2004 to 2005 harvesting season was 116.7 and 23 million bales in the world and in the United States (Iscia and Demirer 2007), respectively. Additionally, there is over 2.25 million tons of cotton waste generated every year in United States (Holt *et al*. 2000). Walnut shell is another lignocellulosic waste to be considered here. Walnut production in 2009 was approximately 2.2 million tons in the world (Pirayesh *et al.* 2012). Walnut shell composes 67% of the total weight of the fruit body and this waste is about 1.5 million tons annually (Martinez *et al*. 2003).

*Pleurotus ostreatus* mushrooms can produce fruit bodies on the straws of rice, wheat, bazar, ragi, sorghum, maize, weed plants, wood (*e.g.*, poplar and oak), cotton stalks, poplar sawdust, beer grain, waste paper, and hazelnut (Wang *et al*. 2001; Yildiz *et al*. 2003; Das and Mukherjee 2007). Media made from a mixture of different portions of lignocellulosic wastes provides advantages that has affected several properties such as cultivation time, biological efficiency, firmness, yield, total number of fruiting bodies, and fruiting body average weight per bag (Ruiz-Rodriguez *et al*. 2010; Alananbeh *et al.* 2014).

The aim of the current work was to determine the effects of oak wood sawdust (OS), cottonseed hulls (CSHs), walnut shell (WSs), and their mixtures on the yield and quality of *P. ostreatus* mushrooms. In this paper, the results of an assessment of the influence of waste substrates on the earliness and morphological characteristics of *P. ostreatus* mushrooms are presented.

## EXPERIMENTAL

### Materials

The substrates used in this study were industrial wastes. To determine which substrates and substrate ratios were suitable for the cultivation of *Pleurotus ostreatus*, various waste materials and combinations were tested. Walnut shells and oak sawdust were supplied by walnut shell growers in the vicinity of the Kahramanmaras Province in Turkey. Air-dried walnut shells were milled with a grinder. Cotton linter was obtained from an olive oil factory in the vicinity of Kahramanmaras (Dok Company). The mushroom strain used in this study was the commercial strain of *P. ostreatus*. The mycelium of *P. ostreatus* was supplied by the mushroom spawn company Agromycel (Denizli, Turkey).

### Methods

Cultures were grown and maintained on antibiotic malt agar and stored in an incubator at 25 °C for three weeks. After the culture run was completed, the mycelium was added to sawdust in bottles for spawning (five months). Homogeneous substrate mixtures were obtained by mixing in the component materials based on their dry weights (Table 1).

The substrate mixtures were wetted for 3 d to increase their moisture content to suitable levels, as determined by the palm test method (Kwon and Kim 2004). The mixed substrate was put in heat-resistant polypropylene bags (40 x 60 cm) and sterilized in an autoclave at 121 °C for 90 min. After the substrate was sterilized, its moisture content, pH, and carbon (C) content were determined. The total nitrogen (N) content of the substrates was determined *via* the Kjeldahl method, and the C:N ratios were calculated. The correction factor 4.38 was used to determine the total protein content from the total nitrogen content (Breene 1990). Later, the substrate samples were left to cool to ambient temperature and were immediately inoculated with 5% oak sawdust spawn for cultivation. The inoculated substrate was placed in polyethylene bags (1 kg of substrate per bag) and incubated at room temperature until colonization was completed. Afterwards, the samples were subjected to conditions of 25 °C and 95 to 90% relative humidity for spawn running.

**Table 1.** Substrates and Mixing Ratios

| Substrates and Mixing Ratio | Symbol |
|---|---|
| Oak sawdust | OS |
| Cotton seed hull | CSH |
| 75% oak sawdust:25% cotton seed hull | 75OS:25CSH |
| 50% oak sawdust:50% cotton seed hull | 50OS:50CSH |
| 25% oak sawdust:75% cotton seed hull | 25OS:75CSH |
| Walnut shell | WS |
| 75% oak sawdust:25%walnut shell | 75OS:25WS |
| 50% oak sawdust:50% walnut shell | 50OS:50WS |
| 25% oak sawdust:75% walnut shell | 25OS:75WS |

After the mycelium colonization was completed, the bags were exposed to conditions of 18 ± 2 °C and 80% to 90% relative humidity in a controlled room. After one flush of mushroom was harvested, the spawn run time, earliness, yield, biological efficiency, fresh weight, and physical properties (diameter of stalk, mm; length of stalk, mm; diameter of pileus, mm; thickness of pileus, mm) of the mushrooms were also determined. Mushroom yield (g) was calculated by adding the weights of the mushrooms in each of the bags and dividing by the number of bags to obtain the average weight. Biological efficiencies were defined as the percentage of the fresh weight of harvested mushrooms over the dry weight of substrate, as explained by Zervakis and Balis (1992).

In all experiments, a completely randomized design with 10 replicates per treatment was applied. The results were evaluated by analysis of variance (ANOVA) and Duncan tests to populate homogeneity groups that showed significant differences at the 95% confidence level.

## RESULTS AND DISCUSSION

The humidity and some chemical properties of the substrates, such as pH, protein, C, and N contents, and C:N ratios, are given in Table 2. Average of humidity and some chemical properties of 5 replications were calculated for each of the substrates. The highest humidity was found in CSHs, followed by 25OS:75CSH, 50OS:50CSH, and 75OS:25CSH, in that order. In addition, the protein content of the substrates prepared using mixtures of OS and WSs was lower than those of the others. The C contents of the CSHs and the mixtures of CSHs with OS were lower than those of the other substrates. The substrates prepared from OS alone contained 1.14% N, and the N content of the substrates decreased as the OS content in the mixtures increased. The mixture of OS had the highest C:N ratio, followed by 75OS:25WS and 50OS:50WS (Table 2).

**Table 2.** Average Humidity and some Chemical Properties of the Substrates Prepared from OS, CSHs, and WSs Alone and their Mixtures with OS at Various Ratios

| Substrates | Humidity (%) | pH | Protein (%) | C (%) | N (%) | C/N ratio |
|---|---|---|---|---|---|---|
| OS | 64.86 | 7.75 | 4.99 | 41.86 | 1.14 | 36.78 |
| CSH | 65.82 | 7.1 | 9.11 | 40.22 | 2.08 | 19.37 |
| 75OS:25CSH | 65.10 | 6.42 | 6.00 | 41.45 | 1.37 | 30.21 |
| 50OS:50CSH | 65.34 | 6.33 | 7.05 | 41.04 | 1.61 | 25.54 |
| 25OS:75CSH | 65.58 | 7.41 | 8.06 | 40.63 | 1.84 | 22.06 |
| WS | 50.10 | 6.21 | 6.44 | 43.17 | 1.47 | 29.37 |
| 75OS:25WS | 61.17 | 7.24 | 5.34 | 42.19 | 1.22 | 34.55 |
| 50OS:50WS | 57.48 | 6.73 | 5.69 | 42.52 | 1.30 | 32.60 |
| 25OS:75WS | 53.79 | 6.69 | 6.09 | 42.84 | 1.39 | 30.89 |

The effects of the morphological and chemical properties of the substrates on the characteristics of *Pleurotus ostreatus* mushroom production, particularly mycelium running, yield, and BE, have been examined in recent studies (Yildiz *et al*. 2003; Das and Mukherjee 2007). In the present study, the humidity in the mixtures of OS with CSHs was higher than that in the mixtures of OS with WSs. This is because CSHs have greater water holding capacity than WSs (Mohamed 1993; Ayrilmiş *et al.* 2013). Moisture content is a very important factor affecting the cultivation of *P. ostreatus* mushrooms because it influences yield and mushroom production (Wang *et al.* 2001). In earlier studies, the moisture content of the substrates was adjusted to 43% to 75% (Wang *et al.* 2001; Hernandez *et al*. 2003). In this study, the moisture content of the substrates was, essentially, within the same range. *Pleurotus ostreatus* mushrooms were produced in a pH range between 6.21 and 7.75, similar to pH ranges used by Yildiz *et al*. (2003). The C:N ratios increased when the amount of OS increased in the mixtures of OS with CSHs and WSs (Table 2). The C:N ratio depended on the sources of both the C and the N. The high N contents positively affected yield and productivity. According to Rizki and Tamai (2011), the N content affected spawn running time, primordial initiation, and biological efficiency. The low C:N ratios of the substrates increased yield and BE such as CSH and 25OS:75CSH in substrates. In this study, the best results were obtained at C:N ratios of 19.00 and 22.00. Other studies also have reported a positive correlation between growth and low substrate C:N ratios (Philippoussis *et al*. 2001; Stamets 2005; Narain *et al*. 2008).

Mushrooms were harvested from the substrate obtained with one flush. Earliness and total time were affected by the substrate mixtures ($p < 0.01$). Earliness was shorter in CSH and WS substrates, and the earliness (d) in the substrates increased as the amount of OS in the mixtures was increased. The maximum total time was obtained from the OS mixtures, and the total time in the substrates increased as the amount of OS in the mixtures was increased. Yield was affected by the substrate mixtures ($p < 0.01$), varying between 13.41 and 113.16 g/kg substrate.

The highest yield was achieved with the mixture of 25OS:75CSH. The yields from 75OS:25WS, 50OS:50WS, and 25OS:75WS were lower. BE varied significantly, as indicated by its values of 2.52% in 25OS:75WS and 36.87% in 25OS:75CSH (Table 3).

**Table 3.** Earliness, Total Time, Yield, and Biological Efficiency (be) of the Substrates Prepared by OS, CSHs, and WSs Alone and their Mixtures with OS in Different Ratios

| Symbol | Earliness (days) | Total time (spawn run time and earliness) | Yield (g/kg substrate) | BE (%) |
|---|---|---|---|---|
| OS | 111.23d** | 211.23f** | 41.45a** | 11.80ab** |
| CSH | 58.26b | 121.26c | 87.03b | 25.46bc |
| 75OS:25CSH | 111.5d | 187.50e | 32.10a | 11.65ab |
| 50OS:50CSH | 101.89d | 177.89e | 34.14a | 11.91ab |
| 25OS:75CSH | 72.63bc | 148.63d | 113.16b | 36.87c |
| WS | 27.68a | 53.68a | 40.77a | 7.73a |
| 75OS:25WS | 97.00d | 173.00e | 28..67a | 6.14a |
| 50OS:50WS | 79.00c | 151.00d | 16.92a | 4.40a |
| 25OS:75WS | 28.00a | 98.00b | 13.41a | 2.52a |

**Significant at 0.01 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Duncan's mean separation test.

The earliness days of WSs and the mixtures of WSs with OS were less than those of the CSHs and the mixtures of CSHs with OS substrates. In addition, the earliness and total time in the substrates increased as the amount of OS in the mixtures was increased. Other studies also have indicated that high extractives content in substrates limits the growth of microorganisms (Mahesh and Satish 2008; Mutai *et al.* 2009), and the oak sawdust (OS) that was used contained a high amount of extractives (Szczepkowski *et al*. 2007).

The highest yield was observed for the mixture of 25OS:75CSH. The yield in the substrates increased as the amount of CSHs in the mixtures increased because of the larger water content of the CSHs (Mohamed 1993). Also, Ayrilmiş *et al.* (2013) observed that the yield in the substrates decreased as the amount of WSs in the mixtures increased because the WSs had less water absorption capacity. The biological efficiency directly affected the yield. Several substrates were used to cultivate *Pleurotus* spp., and the BE values varied from 17% to 79% (Dhanda *et al*. 1996; Wang *et al*. 2001, Rizki and Tamai 2011).

Table 4 shows the fresh weight, diameters of stalk and pileus, lengths of stalk, and thickness of pileus. The fresh weight, length of stalk, and diameter of pileus were affected by the substrate mixtures ($p < 0.01$), but the diameters of stalk and thickness of pileus were not affected by the substrate mixtures. The highest fresh weight was obtained from the mixture of 23.87 g in 75OS:25CSH. The maximum diameter of stalk and length of stalk were observed for the CSHs. The largest diameter of pileus (102.98 mm) was observed from the mixture of 75OS:25CSH, and the best thickness of pileus (18.48 mm) was obtained from the mixture of 25OS:75CSH.

**Table 4.** Fresh Weight and Morphological Characteristics of *Pleurotus ostreatus* on Selected Substrates

| Symbol | Fresh weight (g) | Diameter of stalk (mm) | Length of stalk (mm) | Diameter of pileus (mm) | Thickness of pileus (mm) |
|---|---|---|---|---|---|
| OS | 18.86bc** | 14.18a$^{ns}$ | 39.82ab** | 87.52cd** | 9.85a$^{ns}$ |
| CSH | 12.94abc | 72.35a | 54.13c | 78.49bc | 6.19a |
| 75OS:25CSH | 23.87c | 15.78a | 44.26abc | 102.98d | 9.93a |
| 50OS:50CSH | 18.84bc | 13.07a | 32.37a | 83.90c | 9.60a |
| 25OS:75CSH | 17.07bc | 10.93a | 52.42bc | 84.98cd | 18.48a |
| WS | 11.41ab | 8.76a | 41.92abc | 60.09a | 7.20a |
| 75OS:25WS | 5.61a | 8.17a | 36.96a | 62.07ab | 5.44a |
| 50OS:50WS | 16.06abc | 12.03a | 41.55abc | 84.66cd | 8.54a |
| 25OS:75WS | 13.41abc | 10.58a | 51.45bc | 81.53c | 7.79a |

**Significant at 0.01 level and $^{ns}$ Non-significant level in ANOVA, and mean values with the same lower-case letters are not significantly different according to Duncan's mean separation test.

The mixtures of OS with CSHs and OS alone in the substrates had greater fresh weight than the WSs in the substrates and their mixtures. In other words, yield depended on the water-holding capacity of the substrates. Most of the mixtures of OS with CSHs and CSH alone in the substrates were found to have better morphological properties than the mixtures of OS with WSs and WSs alone. In this study, some results (such as stalk length, pileus diameter) were in agreement with those reported in another study (Oseni *et al*. 2012). Onyango *et al*. (2011) indicated that large mushrooms were expected to be of good quality and have high production rates, but another study considered this only a slight advantage because such mushrooms tend to break during wrapping (Shen and Royse 2001).

The present investigation indicates that the substrates and mixing ratios affected earliness, yield, biological efficiency, fresh weight, and morphological properties of mushroom. Some mixings provide advantages, for example, a mixture of 25OS:75CSH increased yield and biological efficiency and mixtures of 75OS:25CSH improved fresh weight of *Pleurotus ostreatus* mushrooms.

## CONCLUSIONS

1. The results of the present study support the efficient production of *Pleurotus ostreatus* mushrooms on substrates that are mixtures of CSHs and WSs. In addition, these substrates can be used as alternatives to wood.
2. Utilizing these waste products as substrates for the production of mushrooms would reduce the adverse environmental effects of these waste products.
3. An economical strategy for converting waste products into a nutritious food source is represented in this study.

## REFERENCES CITED

Alananbeh, M. K., Bouqellah, A. N., and Al Kaff, S. N. (2014). "Cultivation of oyster mushroom Pleurotus ostreatus on date-palm leaves mixed with other agro-wastes in

Saudi Arabia," *Saudi Journal of Biological Sciences* 21(6), 616–625. DOI: 10.1016/j.sjbs.2014.08.001

Ayrilmis, N., Kaymakci, A., and Ozdemir, F. (2013). "Physical, mechanical, and thermal properties of polypropylene composites filled with walnut shell flour," *Journal of Industrial and Engineering Chemistry* 19(3), 908-914. DOI: 10.1016/j.jiec.2012.11.006

Breene, W. M. (1990). "Nutritional and medicinal value of specialty mushrooms," *Journal of Food Protection* 53(10), 883-894.

Das, N., and Mukherjee, M. (2007). "Cultivation of *Pleurotus ostreatus* on weed plants," *Bioresource Technology* 98(14), 2723-2726. DOI: 10.1016/j.biortech.2006.09.061

Dhanda, S., Sodhl, H. S., and Phutela, R. P. (1996). "Nutrition and yield evaluation of oyster mushroom *Pleurotus* sp.," *The Indian Journal of Nutrition and Dietetics* 33(1), 275-278.

Hernandez, D., Sanchez, E. J., and Yamasaki, K. (2003). "A simple procedure for preparing substrate for *Pleurotus ostreatus* cultivation," *Bioresource Technology* 90(2), 145-150. DOI: 10.1016/S0960-8524(03)00118-4

Holt, G. A., Barker, G. L., Baker, R. V., and Brashears, A. (2000). "Various parameters of cotton gin by-products produced from the gin processing machinery," *Proceedings of the Beltwide Cotton Conference* 1595-1602.

Iscia, A., and Demirer, G. N. (2007). "Biogas production potential from cotton wastes," *Renewable Energy* 32(5), 750-757. DOI:10.1016/j.renene.2006.03.018

Kwon, H., and Kim, S. B. (2004). "Mushroom grower's Handbook. 1: Bag cultivation," in: *Oyster mushroom Cultivation*, Mushworld, Republic of Korea, 160-173.

Mahesh, B., and Satish, S. (2008). "Antimicrobial activity of some important medicinal plant against plant and human pathogens," *World Journal of Agricultural Sciences* 4(S), 839-843.

Martinez, M. L., Moiraghi, L., Agnese, M., and Guzman, C. (2003). "Making and some properties of activated carbon produced from agricultural industrial residues from Argentina," *Journal Argentina Chemistry Society* 91(4-6), 103-108.

Mohamed, S. S. (1993). "Effect of some degosypolisation treatments of cottonseed meal on its functional properties," *Consejo Superior de Investigaciones Científicas* 44(4-5), 279-283.

Mutai, C., Bii, C., Vagias, C., Abatis, D., and Roussis, V. (2009). "Antimicrobial activity of *Acacia mellifera* extracts and lupine triterpenes," *Journal of Ethnopharmacology* 123(1), 143-148. DOI: 10.1016/j.jep.2009.02.007

Narain, R., Sahu, R. K., Kumar, S., Garg, C. S., Singh, and Kanaujia, R. S. (2008). "Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate," *Environmentalist* 29(1), 1-7. DOI: 10.1007/s10669-008-9174-4

Onyango, B. O., Palapala, V. A., Arama, P. F., Wagai, S. O., and Gichimu, B. M. (2011). "Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*)," *American Journal Food Technology* 6(5), 395-403. DOI: 10.3923/ajft2011.395.403

Oseni, O., Dlamini, S. O., Earnshaw, D. M., and Masarirambi, M. T. (2012). "Effect of substrate pre-treatment methods on oyster mushroom (*Pleurotus ostreatus*) production," *International Journal of Agriculture & Biology* 14(2), 251-255.

Philippoussis, A., Zervakis, G., and Diamantopoulou, P. (2001). "Bioconversion of lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp.," *World Journal of Microbiology and Biotechnology* 17(2), 191-200. DOI: 10.1023/A:1016685530312

Pirayesh, H., Khazaeian, A., and Tabarsa, T. (2012). "The potential for using walnut (*Juglans regia* L.) shell as a raw material for wood-based particleboard manufacturing," *Composites: Part B* 43 (1) 3276–3280.

Rizki, M., and Tamai, Y. (2011). "Effects of different nitrogen rich substrates and their combination to the yield performance of oyster mushroom (*Pleurotus ostreatus*)," *World Journal of Microbiology and Biotechnology* 27(7), 1695-1702. DOI: 10.1007/s11274-010-0624-z

Royse, D. J. (2002). "Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size and time to production," *Applied Microbiology and Biotechnology* 58(4), 527-531. DOI: 10.1007/s00253-001-0915-2

Ruiz-Rodriguez, A., Soler-Rivas, C., Polonia, I., and Wichers, J. H. (2010). "Effect of olive mill waste (OMW) supplementation to Oyster mushrooms substrates on the cultivation parameters and fruiting bodies quality," *International Biodeterioration and Biodegradation* 64(1), 638-645. DOI: 10.14720/aas.2014.103.1.05

Shen, Q., and Royse, D. (2001). "Effects of nutrient supplement on biological efficiency, quality and crop cycle time on maitake (*Griofola frondosa*)," *Applied Microbiology and Biotechnology* 57(1-2), 74-78. DOI: 10.1007/s002530100748

Stamets, P. (2005). "Mycelium running: How mushroom can help save the world," Ten Speed Press, Berkeley and Toronto p. 574.

Szczepkowski, A., Nicewicz, D., and Koczon, P. (2007). "The relationship between tree health and chemical composition of beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) wood of Polish provenances," *Acta Scientiarum Polonorum* 6(3), 77-88.

Yildiz, S., Yildiz, U. C., Gezer, E. D., and Temiz, A. (2003). "Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom," *Process Biochemistry* 38(3), 301-306. DOI: 10.1016/S0032-9592(02)00040-7

Wang, D., Sakoda, A., and Suzuki, M. (2001). "Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain," *Bioresource Technology* 78(3), 293-300. DOI: 10.1016/S0960-8524(01)00002-5

Zadrail, F., and Dube, H. C. (1992). "The oyster mushroom. Importance and prospects," *Mushroom Resource* 1(1), 25-32.

Zervakis, G., and Balis, C. (1992) "Comparative study on the cultural characters of *Pleurotus* species under the influence of different substrates and fruiting temperatures," *Micologia Neotropical Aplicade* 5(1), 39-47.

Article submitted: February 25, 2015; Peer review completed: May 15, 2015; Revised version received and accepted: May 28, 2015; Published: June 11, 2015.
DOI: 10.15376/biores.10.3.4686-4693