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Effect of solid and liquid media inoculations of spawn on yield and quality parameters of oyster mushrooms (*Pleurotus ostreatus*) grown on different substrate in Guyana

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ABSTRACT

Oyster mushrooms (*Pleurotus ostreatus*) convert lignocellulosic materials present in agricultural waste into high quality protein rich food. This study investigated the yield performance of *P. ostreatus* on different substrate such as wheat straw, paddy straw, and sugarcane bagasse. A total of three trials were conducted with solid and liquid media inoculum: trials 1 and 2 were inoculated with liquid oyster mushroom culture, while trial 3 was inoculated with oysters' mycelium grown on a petri dish. In trial 1, wheat straw substrate produced the most fruiting bodies with the highest average pin yield, but sugarcane bagasse produced mushrooms with the excellent fruit body quality. Paddy straw had the greatest pin yield in trial 2, but sugarcane bagasse produced the most fruiting bodies, and wheat straw substrate produced mushrooms with the best fruit body quality. However, in trial 1 and 2, results were at par statistically. In trial 3, Only paddy straw substrate produced fruiting bodies. Elemental analysis of the mushrooms harvested produced variable results. For trial 1, sugarcane bagasse produced mushrooms with the highest calcium content, whereas mushrooms grown in wheat straw substrate had the highest iron and copper content. The opposite results are seen in trial 2 where mushrooms cultivated in sugarcane bagasse had the highest iron and copper content, and wheat straw substrate produced mushrooms with very high calcium content. Elemental analysis of the substrates before cultivation showed that wheat straw had the highest average calcium concentration, whereas sugarcane bagasse initially had the highest iron content and copper content. After harvesting, sugarcane bagasse substrate had the highest average calcium content, while paddy straw substrate had the highest average iron and copper content. Our data suggest that growth parameters are affected by the type of substrate. We suggest exploring the substrate combinations for mushroom cultivation to increase the yield of mushroom fruiting bodies and their growth parameters.

Keywords: Oyster mushrooms, agriculture waste, growth parameters, elemental analysis

Mushrooms were traditionally wild-harvested, but commercial cultivation has taken over during the past century due to their nutritional and medicinal value, and their potential for generating income through trade. Approximate per capita annual consumption of mushroom is 5 kg by consumers and this is expected to increase as consumers become more aware of their health benefits (Royse *et al*., 2017).

Oyster mushrooms (*Pleurotus ostreatus*) are the second most cultivated edible mushroom globally, after shiitake mushrooms (*Lentinula edodes*) (Royse *et al*., 2017; Singh *et al*., 2020). Oyster mushrooms have an eccentric short stem or stipe, and the fruiting body lacks chlorophyll, which means that it is unable to produce food. Instead they secrete enzymes into the environment to break down complex organic materials

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into simple compounds that they then absorb as nutrients (Kamthan & Tiwari, 2017). The appropriate substrate for edible mushrooms comprises hemicellulose, cellulose, and lignin. Agricultural waste products are, therefore, an excellent substrate for mushroom cultivation providing energy, nutrition, and structure (Josephine, 2014; Adebayo and Martinez-Carrera, 2015). They are abundant in carbon, which is mycelium's primary food supply. The most frequently used agriculture waste products (substrates) include paddy, wheat and coffee straw, sawdust, banana leaves and many others (Kamthan & Tiwari, 2017). Substrates and supplements are important in mushroom cultivation because they enhance the physical and enzymatic accessibility of the ingredients for fungus growth and development (Jeznabadi *et al*., 2016).

The quality of substrate directly affects the nutritional composition of mushroom fruit body. Kamthan & Tiwari (2017) reported that the lipid content of oyster mushrooms gown on wheat straw was higher, compared to paddy straw substrate, while the oyster mushrooms grown on paddy straw had a higher protein content than on wheat straw.

Oyster mushrooms grow at moderate temperatures ranging from 20^{o}C to 30^{o}C and 55-70% relative humidity (Nongthombam *et al*., 2021). The fruiting bodies comprise as much as 90% water sourced from the substrate, while humidity in the growing environment prevents the fruiting mushrooms from drying (Morais *et al*., 2000; Sánchez, 2010). Oyster mushrooms are simple to cultivate and are rich in several nutrients on dry weight basis including 18%-37% proteins, 1%-8% fats, high content of linoleic acids, minerals, and vitamins such as thiamine, riboflavin, ascorbic acid, and folic acid (Kamthan & Tiwari, 2017).

This research investigated the most suitable substrate for edible oyster mushroom cultivation. The study intended to study the effect of different lingo-cellulosic agriculture waste in Guyana, and inoculation of substrates with solid or liquid culture on yield potential and composition of nutritional qualities such as calcium, iron and copper, etc. in oyster mushrooms (*P. ostreatus*) grown in three different organic substrates.

MATERIALS AND METHODS

Rice straw, wheat straw, and sugarcane bagasse were used to cultivate *P. ostreatus* as per the method described by Jaikaran *et al*. (2017) for the preparation of the mushroom mother culture and Seecharran *et al*. (2018) for the cultivation of mushroom, which included spawn preparation, substrate preparation, and cultivation using the three substrates. Ansari *et al*. (2016) method was used for the preparation of vermicompost. The preparation and growth of mushrooms were done at the mushroom house located at the University of Guyana, Turkeyen Campus, Guyana during four months from November, 2022 to February, 2023.

The parameters of mushrooms measured included the number of pins and fruit bodies, size of fruiting bodies, fresh and dry weight of fruiting bodies, and elemental composition of fruiting bodies and substrates (Seecharran *et al*., 2018). Analysis of mushroom samples for Ca, Fe and Cu was done at the Government Analyst Food and Drug Department, and the Department of Chemistry, University of Guyana.

Preparation of the mushroom mother culture

Precisely 39.0g of powdered potato dextrose agar (PDA) to make 1 litre medium and heated until completely dissolved. The media was autoclaved at

121°C for 15 minutes and 5g of antibiotics (Amoxicillin) was added after cooling to 50-55°C to inhibit bacterial growth. The media was poured into Petri dishes and 0.8-1cm mycelial discs of pure cultures of *P. ostreatus* was used to inoculate the plates (Jaikaran *et al*., 2017) and incubated at room temperature.

Preparation of the mushroom spawn

The preparation of mushroom spawn was done using white millet bird seeds. 500g of white millet bird seeds were soaked for 12 hours and then boiled in water for 10-15 minutes. The seeds were drained and allowed to air dry for 12 hours, and poured into transparent glass bottles. The mouth of the bottles was sealed with cotton plugs, autoclaved at 121°C for 2 hours and left to cool to room temperature. After cooling, the bottles were inoculated with the culture in a sterilized environment. For trials 1 and 2, bird seeds were inoculated with liquid oyster culture. For trail 3, 0.8-1 cm mycelial discs were used to inoculate. The bottles were shaken to ensure even distribution of the discs and stored in a clean, dark area until mycelia had fully colonized the bottles (Seecharran *et al*., 2018).

Preparation of vermicompost

Vermicompost was obtained from a vermicomposting unit of the university, where stones were added at the first layer (bottom layer) in the composting unit (1/5 of the container), and a similar height of sand was added to the top of the stone layer. Garden soil of the same height was added on top of the stone layer. 100g of the dried crushed leaves were added as the fourth layer (Ansari *et al*., 2016). 100g of cattle dung was weighed and added as the fifth layer into the composting unit. The cattle dung layer was covered with the same quantity of dried leaves. Earthworms were added to commence the decomposition process. The mixture was agitated regularly for aeration and watered to maintain moisture. 100g of cattle dung and 100g of dried leaves were added two days per week to the container for one month. Small amounts of water were continuously added until the surface of the compost became black and granular in appearance. This indicated that vermicomposting was almost complete. At this stage, watering was ceased for seven days before vermicompost was harvested. Harvesting was done from the top layer, and the bottom layer was left undisturbed (Ansari *et al*., 2016).

Preparation of substrate for mushroom cultivation

Substrate for cultivation of mushroom was prepared following the method of Seecharran *et al*., (2018). 200g of straw and 100g of vermicompost were weighed and placed into a container. 2g of lime was taken into a beaker with 60 ml of distilled water. The solution was added to the substrate and mixed thoroughly. A small amount of the substrate mixture was removed and wrapped in an aluminum foil for further analysis. The remaining substrate mixture was divided into four equal portions, each placed into autoclave polyethylene bags and labeled accordingly. The bags were autoclaved for 1 hour, and left to cool. 10 g of bird seed spawn was added to each bag. The bags were closed using cotton plugs, and stored in a sterile, dark area to allow for mycelium growth. Relative humidity of the room was maintained by gentle misting with water. After mycelium had colonized the bags, slits were made on the sides of the bags. The bags were watered daily and emerging fruiting bodies were observed. Same steps were followed for the bagasse and wheat straw substrates also.

Harvesting

Mature fruiting bodies were harvested by gently twisting the stipe. The substrate was continuously watered and aerated following the initial harvest allowing the progression of additional harvest (Second and third flushes). The measurements for each of the

parameters included the size of fruiting bodies, texture, number of pins and clustered fruiting bodies, color, and fresh & dry weights of fruit bodies, were recorded. The substrates used and fruiting bodies harvested were prepared for elemental analysis by drying at 60-70^{o}C until a constant weight was achieved. The dried fruiting bodies were grinded into powder and digested using the wet-chemical microwave sample preparation technique outlined in the CEM MARS6 method note compendium for plant tissue. The elemental (Ca, Fe and Cu) composition was then determined by Agilent 240 Flame Atomic Absorption Spectrometer.

RESULTS AND DISCUSSION

In this study, oyster mushrooms were cultivated in three substrates, paddy straw, sugarcane bagasse and wheat straw. Fruiting bodies were successfully produced on all substrates for trials 1 and 2, where the spawn was prepared using liquid culture. However, for trial 3, solid culture was used to inoculate the spawn. Physical growth parameters for the fruiting bodies were observed two weeks following inoculation (Singh *et al*., 2021).

In trial 1 and 2, wheat straw substrate produced the most fruiting bodies with the highest average pin yield. However, sugarcane bagasse produced mushrooms with the best growth parameters, that is the largest average cap diameter and stipe length. In trial 2, paddy straw had the greatest pin yield, while wheat straw substrate produced mushrooms with the largest cap diameter and stipe length (Table 1). Hoa *et al.* (2015) reported that sugarcane bagasse was among the most suitable substrates for oyster mushroom cultivation, producing the best fruit body parameters. However, Seecharran *et al*. (2018) found rice straw supplemented with vermicompost and agriculture wastes as a suitable substrate for the cultivation of *P. ostreatus*. These studies along with the current findings indicate that the substrate type has an direct impact on yield. One-way ANOVA showed that there were no significant differences in the pin yield produced by the different substrates for trials 1 and 2 (p=0.347). There was a significant difference in the cap diameter produced by the different substrates (p-value = 0.0395) and between cap diameter and cap thickness (p-value = 1.26e-05) (Table 1).

Table 1. Number of fruiting bodies and pin yield at harvest

Substrate	*No. Fruit bodies	*No. Pin yield	*Cap Diameter (mm)	*Cap thickness (mm)	*Stipe length (mm)	*Stipe thickness (mm)
			Trial 1 (vermicompost + liquid inoculation)			
Paddy straw	2.67±0.67	28.33±6.96	18.16±1.58	1.12±0.12	14.33±0.72	2.42±0.20
Sugarcane bagasse	1.00±0.00	8.33±3.38	45.84±5.60	2.20±1.18	28.52±2.53	5.52±0.61
Wheat straw	3.50±1.50	32.67±8.41	23.79±2.19	0.94±0.07	17.07±0.77	2.77±0.23
			Trial 2 (vermicompost + liquid inoculation)			
Paddy straw	3.00±0.00	32.00±9.00	26.14±2.69	1.03±0.07	19.24±1.03	3.74±0.66
Sugarcane bagasse	4.33±1.33	28.67±7.22	16.03±2.19	1.01±0.09	16.69±1.00	2.19±0.31
Wheat straw	2.33±0.88	7.33±2.19	52.43±7.55	1.48±0.11	21.19±2.81	5.62±0.64
			Trial 3 (vermicompost + solid inoculation)			
Paddy straw	0.67±0.67	10.33±1.33	17.79±2.87	1.09±0.10	10.09±1.30	2.42±0.23
Sugarcane bagasse	0	0	0	0	0	0
Wheat straw	0	0	0	0	0	0

* (Mean ± SE)

Edible mushrooms can be cultivated on lingo-cellulosic waste materials. Kamthan & Tiwari (2017) found that wheat straw had the highest hemicellulose concentration of 39%, sugarcane bagasse had the highest lignin concentration of 18-24%, and paddy straw had the highest cellulose concentration of 41%. *Pleurotus* produces hydrolyzing and oxidizing enzymes, such as cellulases, xylanases, and ligninases, that degrade substrates containing lignin, cellulose and hemicellulose into soluble compounds of low molecular weight (Lim *et al.*, 2013; Kamthan & Tiwari, 2017). The fungal hyphae absorb these soluble compounds, which is then transformed into fresh new mushroom tissues. *P. ostreatus* has the ability to grow on a wide variety of substrates, has few environmental requirements and produces high yields (Josephine, 2014). Mushroom cultivation on agricultural by-products, various forms of vegetation or dried plant debris is often performed after pasteurizing or sterilizing the medium before spawn is added. This ensures that the dried substrate becomes hydrated and softened, making it more digestible for the mushroom mycelium, and the chemicals or heat used during sterilization and pasteurization can reduce or eradicate populations of potential bacteria and other contaminants the spawn may encounter as it colonizes the medium (Cotter 2014; Bellettini *et al*., 2019).

Colonization of substrates by saprophytic mushrooms is affected by the availability of essential nutrients. Nitrogen is needed for polysaccharide, protein and nucleic acid synthesis, and mushroom productivity and biomass can increase by supplementing the substrate with nitrogen (Nunes *et al*., 2012). Glucose is used by fungi for energy and as a carbon source, providing the carbon atoms that make up the skeleton of the organic molecules that comprise the cells (Chang and Miles, 2004). Minerals present in the substrates are consumed by the growing mycelium, and then moved to the sporophores. Potassium, phosphorus and magnesium are essential for fungal growth and development (Campos *et al*., 2009). Minerals, such as calcium and iron, stimulate mycelial growth and fruiting body formation (Adams *et al*., 2022). Copper is a micro-element, vital for normal growth of mushrooms at concentrations ranging from 10-6 to 10-7 M; higher concentrations cause toxicity (Chang and Miles, 2004).

In this study, the calcium, iron and copper content of the mushroom and substrate samples were analyzed. For trial 1 and 2, mushrooms grown in the sugarcane bagasse had the highest calcium content, whereas mushrooms produced by the wheat straw had the highest iron and copper content (Table 2). Interestingly, the mushrooms produced by paddy straw substrate in trial 3 ranked second overall for calcium content, and third for iron and copper content. Lignocellulosic materials generally have low mineral content and supplements are required to be added to provide them with essential minerals to enhance mushroom production (Bellettini *et al.*, 2019). Previous studies have supported that substrate combinations and added supplements can be most suitable for high yield production of oyster mushrooms. Vetayasuporn *et al*. (2006) found that the substrate combination of bagasse and sawdust as a mixed substrate showed great potential for use as raw material and provided an economically acceptable substrate for *P. ostreatus* cultivation. The combination of supplements and substrate type is valuable for mushroom production because it enhances the physical and enzymatic accessibility of the nutrients for fungal growth and development (Jeznabadi *et al*., 2016). Similarly, Maheswari *et al*. (2021) found that the combination of sugarcane bagasse, saw dust, and wheat bran was the best appropriate substrate for oyster mushroom cultivation.

The substrates, before and after mushroom cultivation, were analyzed for calcium, iron and copper content. Before the inoculation and growth of edible

Table 2. Concentration of Ca, Fe, and Cu in the oyster mushroom fruiting bodies

Substrate	Calcium (Mg/L ± SE)	Iron (Mg/L ± SE)	Copper (Mg/L ± SE)
	Trial 1 (vermicompost + liquid inoculation)		
Paddy straw	10.57 ± 0.25	15.37 ± 0.87	3.03 ± 0.03
Sugarcane bagasse	14.33 ± 0.23	15.20 ± 1.02	3.97 ± 0.17
Wheat straw	12.76 ± 1.22	17.40 ± 0.64	3.98 ± 0.05
	Trial 2 (vermicompost + liquid inoculation)		
Paddy straw	17.48 ± 0.60	21.35 ± 0.78	4.09 ± 0.11
Sugarcane bagasse	14.29 ± 6.90	35.54 ± 2.87	6.68 ± 0.85
Wheat straw	306.17 ± 23.87	32.09 ± 2.39	5.47 ± 0.03
	Trial 3 (vermicompost + solid inoculation)		
Paddy straw	63.61 ± 3.12	32.09 ± 12.66	5.27 ± 0.13

Table 3. Concentration of Ca, Fe, and Cu in the initial and final substrates

Substrate	Calcium (Mg/L ± SE)	Iron (Mg/L ± SE)	Copper (Mg/L ± SE)
Paddy straw initial	278 ± 20.57	233.35 ± 42.76	2.93 ± 0.42
Paddy straw final	370.57 ± 6.41	352.37 ± 16.61	3.50 ± 0.10
Sugarcane bagasse initial	84.40 ± 2.52	482.99 ± 20.25	18.54 ± 1.29
Sugarcane bagasse final	2025.57 ± 63.19	287.24 ± 36.03	2.13 ± 0.31
Wheat straw initial	2471.67 ± 75.42	272.15 ± 23.40	11.21 ± 0.70
Wheat straw final	1969.65 ± 8.48	243.38 ± 14.12	2.36 ± 0.03

oyster mushrooms on the various substrates, wheat straw had the highest average calcium content, followed by paddy straw substrate and sugarcane bagasse (Table 3). Sugarcane bagasse initially had the highest iron content and copper content. After the inoculation and growth of oyster mushrooms, sugarcane bagasse substrate had the highest average calcium content, while paddy straw substrate had the highest average iron and copper content. Increase in mineral content in the post-harvest substrate can be attributed to biodegradation of the substrate by enzymes produced by the mushroom mycelia (Seecharran *et al*., 2018).

CONCLUSION

During this study, we tried to compare yield and quality parameters of mushroom fruit bodies when spawn was prepared with solid and liquid cultures. In Trial 1 and 2, results on wheat straw and sugarcane bagasse did not differed significantly, although the number of pin heads and quality parameters numerically differed but were statistically at par. The substrates inoculated with solid culture media only had minimal growth of *P. ostreatus* fruiting bodies on the paddy straw substrate. There was a significant difference in the growth parameters of oyster mushrooms cultivated on the three different substrates. Moreover, mushrooms fruit bodies with the highest calcium, iron and copper content were produced on sugarcane bagasse and wheat straw. When the substrates were inoculated spawn made with solid culture media, mushrooms produced by paddy straw substrate in trial 3 showed second highest calcium content, and the lowest iron and copper content. The study showed that the spawn preparation method and substrate combination both has significant effect on

yield and quality parameters of oyster mushroom produced. It is recommended that substrate combinations for mushroom cultivation be explored further for increased yield of mushroom fruiting bodies and better quality parameters.

REFERENCES

1. Adams, A., D. Seecharran, and A. Ansari. 2022. Growth of oyster mushroom using sawdust and agriculture waste as substrates. *Mushroom Research* **31(1):** 73–80.

2. Adebayo, E.A. and D. Martinez-Carrera. 2015. Oyster mushrooms (*Pleurotus*) are useful for utilizing lignocellulosic biomass. *African Journal of Biotechnology* **14(1):** 52-67.

3. Ansari, A.A., S. Jaikishun, M.A. Islam, S.K. Kuri, K. Fiedlerand and D. Nandwani. 2016. Principles of vermitechnology in sustainable organic farming with special reference to Bangladesh. *Sustainable Development and Biodiversity* **9:** 213-229.

4. Bellettini, M.B., F.A. Fiorda, H.A. Maieves, G.L. Teixeira, S. Ávila, P.S. Hornung, A.M. Júnior, and R.H. Ribani. 2019. Factors affecting mushroom *Pleurotus spp. Saudi Journal of Biological Sciences* **26(4):** 633-646.

5. Campos, J.A., N.A. Tejera and C.J. Sánchez. 2009. Substrate role in the accumulation of heavy metals in sporocarps of wild fungi. *Biometals* **22(5):** 835-41.

6. Chang, S.T., and P.G. Miles. 2004. *Mushrooms: Cultivation, nutritional value, medicinal effect and environmental impact*. CRC Press LLC.

7. Cotter, T. 2014. Organic mushroom farming and mycoremediation: Simple to advanced and experimental techniques for indoor and outdoor cultivation. Pp 251-365. *Chelsea Green Publishing*.

8. Hoa, H.T., C.L. Wang and C.H. Wang. 2015. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **43(4):** 423-34.

9. Jaikaran, S., D. Seecharran, and A.A. Ansari. 2017. Growth and bioremediation properties of oyster mushroom (*Pleurotus ostreatus*) using gold and bauxite mining soils in Guyana. *Mushroom Research* **26(1):** 57-67.

10. Jeznabadi, E.K., M. Jafarpour, and S. Eghbalsaied. 2016. King oyster mushroom production using various sources of agricultural wastes in Iran. *International Journal of Recycling of Organic Waste in Agriculture* **5:** 17-24.

11. Josephine, R.M. 2014. A review on oyster mushroom (*Pleurotus spp*). *International Journal of Advanced Biotechnology and Research* **1:** 7-9.

12. Kamthan, R. and I. Tiwari. 2017. Agricultural wastes-potential substrates for mushroom cultivation. *European Journal of Experimental Biology* **7(5):** 31.

13. Lim, S. H., Y.H. Lee, and H.W. Kang. 2013. Efficient recovery of lignocellulolytic enzymes of spent mushroom compost from oyster mushrooms, *Pleurotus spp*., and potential use in dye decolorization. *Mycobiology* **41(4):** 214-220.

14. Maheswari, S., P. Rajarajan, P.M. Pandian and B.B. Krishnan. 2021. Yield performance of mushroom (*Pleurotus Ostreatus*) on different treatment of sugarcane bagasse and saw dust and its nutrient analysis. *Plant Cell Biotechnology and Molecular Biology* **22(8):** 7-13.

15. Morais, M.H., A.C. Ramos, N. Matos and E.J.S. Oliveira. 2000. Note. Production of

shiitake mushroom (*Lentinus edodes*) on lignocellulosic residue. *Food Science and Technology International* **6(2):** 123-128.

16. Nongthombam, J., A. Kumar, B.G. Ladli, M. Madhushekhar and S. Patidar. 2021. A review on study of growth and cultivation of oyster mushroom. *Plant Cell Biotechnology and Molecular Biology* **22(5&6):** 55-65.

17. Nunes, M.D., J.M. da Luz, S.A. Paes, J.J. Ribeiro, M.D. da Silva and M.C. Kasuya. 2012. Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. *Journal of Food Research* **1(2):** 113.

18. Royse, D. J., J. Baars and Q. Tan. 2017. Current overview of mushroom production in the world. *Edible and medicinal mushrooms: technology and applications*, Zied P.C. and Gimenez A.P. (eds), pp 5-13, John Wiley & Sons Ltd, Chichester, UK.

19. Sánchez, C. 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied microbiology and biotechnology* **85:** 1321-1337.

20. Seecharran, D., A. Ansari, G. Subramanian, A. Nankishore, H. Sambhu and B. Punu. 2018. An Investigation into culture and propagation of edible mushrooms on different organic substrates. *Mushroom Research* **27(1):** 45-53.

21. Singh Manjit, Shwet Kamal, Ved Parkash Sharma. 2020. Status and trends in world mushroom production-III-World Production of Different Mushroom Species in 21st Century. *Mushroom Research* **29 (2):** 75-111

22. Singh, S., D. Seecharran and A.A. Ansari. 2021. Growth and nutritional content of *Pleurotus ostreatus* using different substrates (rice straw, vermicompost and vermiwash). *Mushroom Research* **30(2):** 175-196.

23. Vetayasuporn, S., P. Chutichudet and K. Cho-Ruk. 2006. Bagasse as a possible substrate for *Pleurotus ostreatus* (Fr.) kummer cultivation for the local mushroom farms in the Northeast of Thailand. *Pakistan Journal of Biological Sciences* **9(13):** 2512-2515.