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Full Length Research Paper

Cultivation of *Pleurotus ostreatus* on *Grevillea robusta* leaves at Dilla University, Ethiopia

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Mushrooms consumption has generated interest in man from early civilization. Mushrooms have a unique texture and flavour that are not found in other food crops. In addition, mushrooms cultivating is a promising new industry, with many new businesses developing every year. Cultivation of saprophytic edible mushrooms may be the currently economical biotechnology for lingo-cellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution. Therefore, the present study was undertaken to assess the growth of *Pleurotus ostreatus* on the *Grevillea* leaves of solid waste disposal in order to reduce environmental pollution by bioconversion of this waste into health food. *Grevillea* leaves were good substrate for oyster mushroom cultivation. The fruit bodies produced on this substrate were large in size and many in number. Therefore, cultivation of oyster mushrooms on this substrate can contribute to solving the food supply scarcity and quality problem beside removing solid waste pollutant from the environment.

Key words: Fruit body, *Grevillea robusta*, mushroom cultivation, *Pleurotus ostreatus,* spawn.

INTRODUCTION

The word mushroom is used in all part of world to describe the fruiting bodies of saprophytic, mycorrhizal and parasites fungi, belonging to the order of Basidiomycetes or Ascomycetes. Basidiomycetes or Ascomycetes can be found in soils rich in organic matter and humus, moist wood, animal waste after heavy rain or a sudden change of temperature and soon after a few hours or days they disappear, leaving no sign, but vegetative mycelium (Zied et al., 2011). Oyster mushrooms (*Pleurotus ostreatus*) are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Banik and Nandi, 2004). Oyster mushroom is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes.

Mushrooming or mushroom cultivation refers to the intentional and directed production of mushrooms, substituting wild collection in the fields and forests with a harvest in defined conditions of growing, resulting in strict quality control, food safety without risk of consumption of poisonous or toxic species, and with guarantees on the

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benefits generated by these fungi (Zied et al., 2011). The cultivation of edible mushrooms is actually an alternative biotech which is fast, environmentally friendly and feasible to recycle organic by-products from agribusiness into high nutritional and medicinal quality food both with respect to the amount of protein or minerals and selected substances with medicinal and pharmacological properties, for example the presence of β-glucans like lentinan, and thus it can contribute significantly to food for humans. The most cultivated mushrooms in the world are *Agaricus bisporus* (Champignon or button mushroom), *Lentinula edodes* (Shiitake) and *P. ostreatus* (oyster mushroom) because of its oyster like shape and other *Pleurotus* species. World-wide mushroom cultivation is dominated by the production of *A. bisporus* which is followed by *L. edodes* and *P. ostreatus* (Chang, 1999).

The mushroom production is a global and expanding industry, its world production is 6535542 ton in 2009 (FAO, 2011). Oyster mushroom is the second most popular mushrooms after button mushroom all over the world and its cultivation has increased rapidly during the last decade (Royse, 2002; Shelly et al., 2009; Adejoye et al., 2006). Oyster mushroom is rich in proteins, vitamin, and minerals and popularly called the vegetarian's meat because it has the same nutrients with meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) as they contain all the nine essential amino acids required for human body (Hayes and Haddad, 1976). Cultivation of *Pleurotus* spp. as edible mushrooms is becoming important throughout the world because of their ability to grow at temperatures of 10-35°C (Zadrazil, 1978; Yildiz et al., 1998) and on various lignocellulosic materials such as rotten wood, wood residues and most of agricultural wastes (Stamet, 2000; Straatsma et al., 2000).

Grevillea robusta (*Proteaceae*), commonly known as "silky oak", is native to Australia (Ritchie et al., 1965; Cannon et al., 1973). So far, alkyl resorcinols, macro cyclic phenols and cinnamic acid derivatives have been reported to be constituents of this plant. *G. robusta* is popular among farmers due to its fast growth, ability to tolerate heavy pollarding and pruning of branches and because it mixes well with other crops (Muchiri et al., 2002). Furthermore, the species has a proteoid root system (cluster of roots that develop in soils deficient of phosphorus) and hence is believed to compete less for mineral with crops making it ideal for planting on small farms sizes (Akycampong et al., 1999). The species has important uses including construction material, fuel wood, shade, fodder, soil erosion control and soil fertility improvement (FAO, 2001). It has been every well adopted and forms a near monoculture in central Kenya highlands, particularly in Kirinyaga district where it was reported to be grown on about 96% of the farms (Tyndall, 1996). Currently, it is a major timber species in small-scale farms where it significantly contributes to household income (Holding et al., 2006).

Pleurotus species are characterized by a white spore print attached to decurrent gills, often with an eccentric (off center) stipe, or no stipe at all. They always grow on wood in nature, usually on dead standing trees or on fallen logs. *Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities (Gunde-Cimerman, 1999).

Composting is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization (Mckinley and Vestal, 1984). Unlike undeveloped countries where mushrooms food consumption is increasing (Kurtzman, 2005; Gregori et al., 2007; Neyrinck et al., 2009), in Ethiopia, mushroom eating habit is very poor (Dawit, 1998). Information on nutritive value and sensory properties of edible oyster mushroom foods cultivated on agricultural residues in Ethiopia is limited. Such information is important to facilitate the popularization of mushroom cultivation, processing, marketing and consumptions. Mushroom cultivation is a useful method of environmental waste management and waste disposal.

Moreover, *P. ostreatus* are good fungi for cultivation in Ethiopia because of they are efficient degraders of lingo-cellulosic materials, easy to grow with simple technology, can complete a full cycle in three to four weeks and the raw materials are abundantly available. *Grevillea* leaves in Ethiopia particularly in Dilla town and surrounding areas are removed as solid waste. Cultivation of *P. ostreatus* mushroom on this substrate should have a good acceptance from the consumer and could be a good opportunity for small producers to embark in an enterprise. It is necessary to keep on promoting the benefits of this product, one of the main problems to start this project was the resistance to change the traditional ways of production so a new culture of sustainable agriculture needs to be developed. It is very important to do more research to develop a system that can be adapted completely to by the rural producers and urban of Ethiopians. Large amounts of freely available *Grevillea* leaves from trees as solid waste offer a potential alternative substrate source for mushroom cultivation in the Ethiopia. As a result, it is possible to convert through cultivation this waste into highly nutritious mushrooms with medicinal properties. Therefore, the present study was undertaken to assess the growth of *P. ostreatus* on the *Grevillea* leaves of solid waste disposed in order to reduce environmental pollution and obtain a bioconversion of this waste into health food.

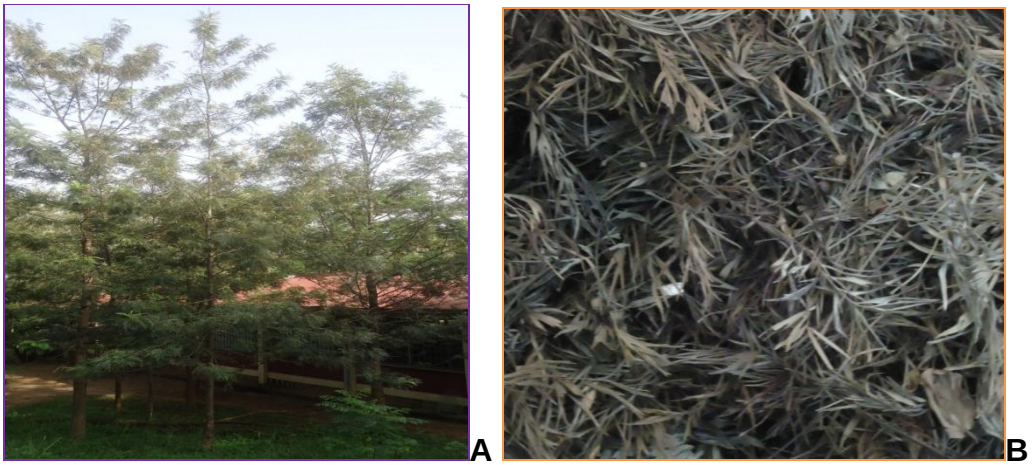

Figure 1. A, *Grevillea robusta* plant; B, its leaves after dry.

Figure 2. A, composting area and observation; B, filling compost substrate (*G. robusta* leaves) into plastic bags for sterilization.

MATERIALS AND METHODS

Pure culture collection and maintain

P. ostreatus was obtained from Mycology Laboratory, Department of Biology from Addis Ababa University, where it was brought from China. The pure culture of *P. ostreatus* was inoculated onto malt extract agar. The pure culture was maintained on malt extract agar slants at -4°C for one month, then sub-cultured subsequently after one month and transferred (inoculated) onto fresh slant of malt extract agar.

Substrate collection

G. robusta leaves used as substrate for composting were collected in Dilla University from Main Campus from in October -2014 April as shown in Figure 1. Other nutrient supplement such as wheat bran and wood ash was obtained from the Dilla Town. Beside this, cow dung and chicken manure were obtained from Allege Research Centre.

Compost preparation

The compost was prepared by outdoor single-phase solid-waste fermentation (Nair and Price, 1991). In order to prepare aerobic composted substrate, about 80% of *G. robusta* leaves were chopped manually into small pieces by using hammer mill. After chopping, the chopped *Grevillea* leaves with wood ash, wheat bran, cow dung and chicken manure were mixed, then water was added until moisture content was between 40-60% (Figure 2). This is usually being determined by the 'rule of thumb' method (Buswell, 1984). Then supplemented with 20% of three different supplements on 80% of *Grevillea* leaves as follows: Substrate A, 80% of *Grevillea* leaves with 10% chicken manure, 8% wheat bran and 2% wood ash; Substrate B, 80% of *Grevillea* leaves with 10% cow dung, 8% wheat bran and 2% wood ash; Substrate C, 80% of *Grevillea* leaves with 18% wheat bran and 2% wood ash; Substrate D, 80% of *Grevillea* leaves with 18% cow dung and 2% wood ash;

Figure 3. A, Weighing of sorghum; B, adding of tap water for overnight soaking; C, supplement of wheat bran and pH adjustment; D, addition of formulated sorghum into glass bottle.

Substrate E, 80% of *Grevillea* leaves with 18% chicken manure and 2% wood ash on dry weight basis with some modification of Dawit (1998). The substrates were then added into hole of about 1.5 m wide, 1.5 m high and 1.5 m long which was under shadow area at Dilla University. This was covered with banana leaves and left for 2 weeks with turning and restacking every 3-4 days to produce homogenous compost.

Spawn production

Spawn is the vigorous mycelia growth of a single fungus on a chosen substrate material (liquid media, grains, saw dust substrate, wooden sticks (Jiskani, 2000). Sorghum was used for mother spawn. About 20 kg of sorghum was washed and dead floating sorghum removed then soaked overnight in 15 L water and rinsed three times in distilled water. The excess water was drained off and 20% wheat bran, 12% gypsum ($CaSo_4$. $2H_20$), and 3% limes (CaCO3) were added as shown in Figure 3. The ingredients were thoroughly mixed; moisture was maintained at the level of 55%, and distributed equally in to 500 ml glass bottle at the rate 370.66 g seed per bottles and autoclaved for 121°C to 1 h. After cooling, each bottle was inoculated with 7 day old cultures grown on malt extract agar and incubated for 25 days at 25°C until the substrate was fully colonized; at 10 days interval mycelia invasion and contamination were recorded.

Sterilization of substrates and cultivation of mushrooms

After two weeks of composting, these substrates were distributed equally into plastic bags of 40 x 60 cm size at the rate of 3.5 kg substrate in triplicates and sterilized for three hours in barrel by fire. After cooling, they were inoculated with the spawn (one glass bottle per bag) and mixed thoroughly to facilitate rapid and uniform mycelia growth. The mouth of the bags was tied using a cotton plug and thread and holes were made over the polythene bags for aeration. Then, they were incubated in the dark at 27°C and mycelia development in the bag was observed and noted within 5 days.

Cultivation conditions

The bags were subsequently placed, long side down, into a spawn running room at 20 - 23°C in the dark and 65 - 70% relative humidity until completion of spawn running. After completion of spawn running, the temperature and relative humidity was changed to 19 to 20°C and 80 - 90% RH, respectively. The bags were slit and the cut portions folded back. Water was sprayed for maintaining

moisture up to the desired level in the form of fine mist from a nozzle.

Watering

Each cultivating bags were irrigated using tap water every morning and evening until 2 flushes of *P. ostreatus* fruiting bodies appears.

Harvesting of mushroom

The first primordia appear 2-4 days after scratching depending upon types of substrate, which were recorded. The harvesting date also varied depending upon types of substrate. Matured mushroom identified by curl margin of the cap was harvested by twisting to uproot from the base. Mushroom matured generally 48 h after appearance of the primordia. Data were recorded periodically during culture.

Biological efficiency

The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following the formula of Chang et al. (1981):

$$\mathbf{B.E}\ (\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \mathbf{x100}$$

Moisture content

The moisture content of mushroom was also expressed in percent and calculated by the formula:

$$\mathbf{Moisture\ content}\ (\%) = \frac{\text{Weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \mathbf{x100}$$

Data analysis

The data of actively mycelium growth during spawn making and formation of full morphology of oyster mushroom and fruiting body were observed during cultivation on substrate. Analysis was performed for all data with triplicates for each. The data were expressed qualitatively in the form of picture as well as quantitatively. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows 16.0.

RESULTS

P. ostreatus cultured on malt extract agar for seven days at 28°C and mycelium covered the medium. Full mycelium invasion of *P. ostreatus* on culture plate took seven days. It was fully grown on plates as shown in Figure 4. It was ready to be used for the inoculation into sorghum for spawn preparation.

Spawn production

Sorghum is important cereals for spawn production of mushroom species (*P. ostreatus*). Sorghum based spawn took 25 days to colonize the substrate completely (Figure 5). The moisture content of the sterile moist sorghum (55-60%) was found to be suitable. It was ready to be used for the inoculation of the solid substrate.

Substrate sterilization and spawn inoculation

The substrate was sterilized by soaking into the boiled water for three hours in the barrel. Mycelium running rate on the substrates was observed after seven days inoculation of spawn (Figure 6). Therefore, mycelium running required high humidity and cultivation room should be dark.

Primordia formation of *P. ostreatus*

The first primordia appeared 20 days after scratching depending on types of substrate. The primordia formation and number of primordia per plastic bag (substrate) was affected by humidity and the substrate itself. The supplements such as wheat bran and manure also caused either high or low number of primordial formation as indicated in Figure 7.

Fruiting body production

The effect of supplemented ingredients on substrates (*G. robusta* leaves) were investigated and found to influence the number of fruit body and size of fruit body. Fruiting body is the edible part of mushroom *P. ostreatus*. On the substrates that contains wheat bran and manure as supplements, the number and size of fruit bodies was higher and larger than that in the substrate alone (*G. robusta* leaves only) (Figure 8).

Biological efficiency

Considerable variation was found in yield of oyster mushroom using different supplements on *G. robusta* leaves. The maximum biological yield (555 g/3.5 kg) was found with supplements of 18% cow dung on substrate which gives 15.86% of biological efficiency. The maximum moisture content was found with supplement of 18% chicken manure (94.05%) and the lowest was found with supplements of 10% cow dung and 8% wheat bran (91.88%) (Table 1).

DISCUSSION

The harvesting date of mature fruit body varied depending upon types of substrate. Oyster mushroom (*P.*

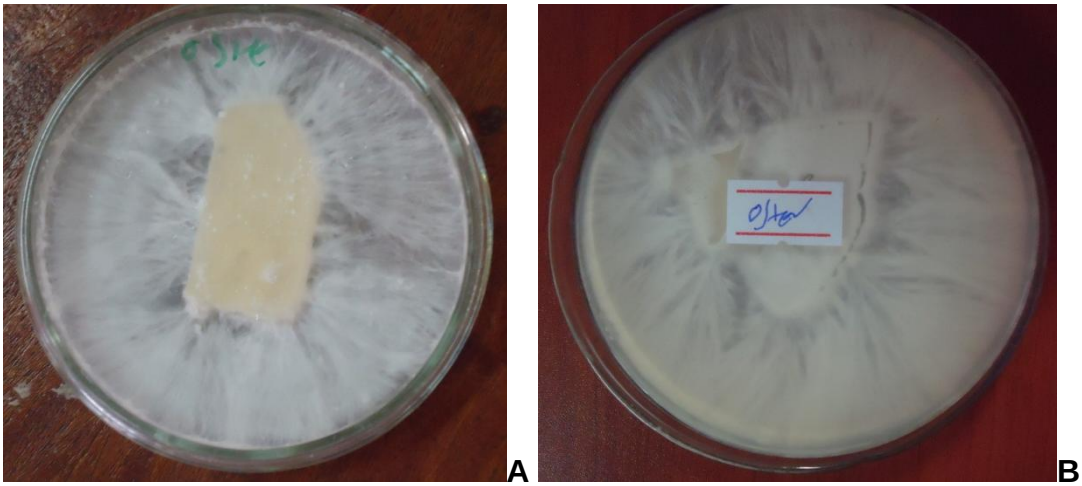

Figure 4. *P. ostreatus* mycelium grown on malt extract agar: A, Front observation of mycelium growth on plate; B, back observation of mycelium growth on plate.

Figure 5. Spawn preparation on sorghum: A, inoculation of old culture (seven days) *P. ostreatus* on the sorghum; B, fully colonized sorghum by *P.ostreatus* mycelium after 25 days.

ostreatus) is an edible mushroom having excellent fragrant and taste and its cultivation on crop residues is considered as potential source of income, an alternative food production, provision of employment, and for recycling of agricultural wastes (Oseni et al., 2012). The market for mushrooms has been reported to be on a continuous growth due to the interest in their culinary, nutritional, health benefits and their potential for use in waste management (Beetz and Kustidia, 2004).

Shah et al. (2004) reported that oyster mushrooms are one of the most delicious foods due to their high nutritional value, very good taste and medicinal value. Several different polysaccharide anti-tumor agents have been developed from the fruiting body, mycelia and culture medium of various medicinal mushrooms: *Lentinus edodes, Ganoderma lucidum, Schizophyllum commune, Trametes versicolor, Inonotus obliquus,* and *Flammulina velutipes*. Both cellular components and secondary metabolites of a large number of mushrooms have shown an effect on the immune system of the host and can be used to treat a variety of disease states (Wasser, 2002).

Mushrooms matures generally 48 h after primordial appearance. Mushrooms not only can convert these huge lignocelluloses biomass wastes into human food, but can also produce notable immune enhanced products, which

Figure 6. Sterilization and inoculation spawn: A, sterilization of substrate; B, inoculation of *P. ostreatus* spawn on *G. robusta* leaves substrate; C, mycelium colonization of the *G. robusta* leaves.

have many health benefits (Chang et al., 1993). In Ethiopia, hunger and malnutrition are devastating problems, particularly for the poor and unprivileged society. The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), iodine; vitamin A deficiency disorders (Edris, 2004).

The mature fruit bodies became curl margin of the cap of *P. ostreatus* as shown in Figure 8. *Pholiota nameko* is one of the hygrophilous fungi and needs more moisture for fruiting as compared to other cultivated mushrooms, such as *L. edodes, F. velutipes* and *P. ostreatuse* (Chang and Hayes, 1978). Aeration also plays an important role in fructification. The fruiting body formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room (Stamets, 2000). The appearance of fruiting bodies varies according to the species, but all have a vertical stalk (stipe) and a head (pileus or cap). This mushroom produces a cluster of yellowish and creamy fruit bodies, also cinnamon brown spores. Fructification requires 30 days (Marshall and Nair, 2009).

Highest biological efficiency was found with supplements of 18% cow dung (15.86%) and the lowest biological efficiency was found without the supplements (11.94%). The yield of mushrooms was affected by different supplements (Tikdari and Bolandnazar, 2012). On another study, Alam et al. (2007) observed that the biological efficiency ranged from 45.21 to 125.70% in the case of oyster mushroom. As reported by Islam et al. (2009), the maximum biological yield (150 g) was found in mango sawdust based substrate. Coconut sawdust based substrates gave the minimum yield (83 g). The fresh mushroom yield or biological efficiency of a species is directly related to strain, substrate nutrition and growth

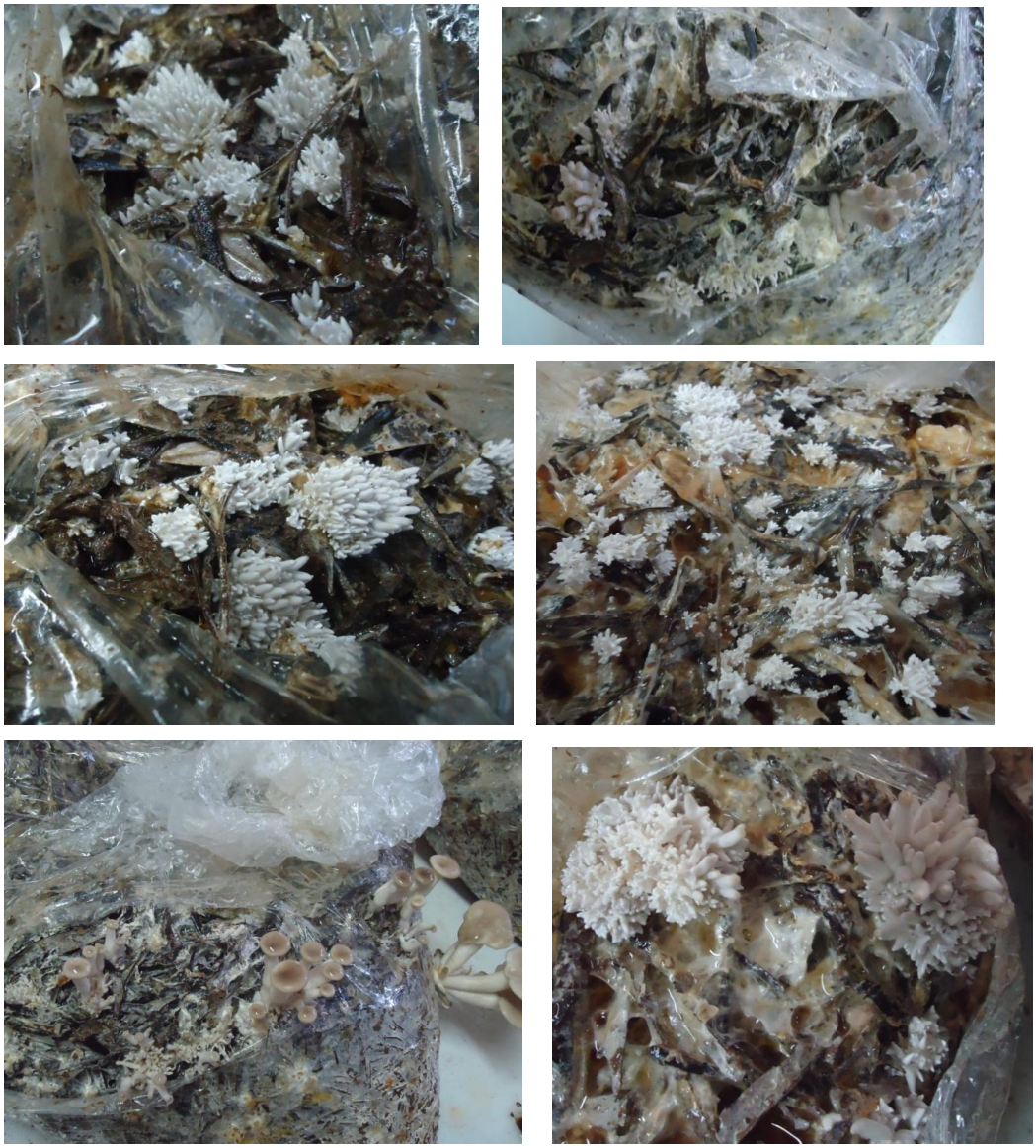

Figure 7. Primordial formation on the *G. robusta* leaves which contain wheat bran and manure.

conditions (Upadhyay et al., 2002).

Conclusion

Mushroom cultivation needs knowledge as well as experience to grow fungi on plant solid waste materials that are not necessarily consumed by humans. Oyster mushrooms can convert these wastes into protein and vitamin rich food. Commercial production of oyster mushrooms is largely determined by the availability and utilization of cheap solid waste products, which are agricultural and industrial waste that are the most promising substrates for cultivation. Therefore, this study demonstrate the feasibility to cultivate *P. ostreatus* on *G. robusta* leaves supplemented with wheat bran manure as a way to solve the food supply scarcity and quality problem and also remove solid pollutant from the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Department of Biology, College of Natural and Computational Sciences of Dilla University for the kind assistance in providing the laboratory facilities and all the required consumables and

Figure 8. Fruit body grown on the *Grevillea robusta* leaves and the mature fruit body.

equipment during the whole period of this research.

REFERENCES

Adejoye OD, Adebayo-Tayo BC, Ogunjobi AA, Olaoye OA, Fadahunsi FI (2006). Effect of carbon, nitrogen and mineral sources on growth of *Pleurotus florida*, a Nigeria edible mushroom. Afr. J. Biotechnol. 5:1355-1359.

Akycampong E, Hitimana E, Torquebiaus EM, Unyemana PC (1999). Multistrata agroforestry with beans; bananas and *Grevillea robusta* in the highlands of Burundi. Exp. Agric. 35:357-369.

Alam N, Amin SMR, Sarker NC (2007). Efficacy of five different growth regulators on the yield and yield contributing attributes of *Pleurotus ostreatus* (Jacquin ex Fr.) Kummer. Bang. J. Mush. 1 (1): 51-55.

Banik S, Nandi R (2004). Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. Industrial Crops and Products 20:311-319.

Beetz A, Kustidia M (2004). Mushroom Cultivation and Marketing. ATTRA Publication IP 087. http://attra.ncat.org/attra-pub/mushroom.html.

Buswell JA (1984). Potentials of spent mushroom substrates for bioremediation purposes. Compost 2:31-35.

Cannon JR, Chow PW, Fuller MW, Hamilton BH, Metcalf, BW, Power AJ (1973). Phenolic constituents of *Grevillea robusta* (Proteaceae)-structure of robustol, a novel macrocyclic phenol. Aust. J. Chem. 26:2257-2275.

Chang ST (1999). World production of cultivated and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. In China. Int. J. Med. Mushrooms 1:291-300.

Chang ST, Buswell T, Chiu SW (1993). Mushroom Biology and Mushroom Products. The Chinese University Press, Hong Kong. pp: 45-57.

Chang ST, Hayes WA (1978). The Biology and Cultivation of Edible Mushrooms. Harcourt brace Jovanovich publishers. pp:475-493.

Chang ST, Lau OW, Cho KY (1981). The cultivation and nutritive value of *Pleurotus sojar* caju. European J. Appl. Microbiol. Biotechnol. 12:58-62.

Dawit A (1998). Mushroom cultivation; a practical approach. Addis Ababa: Berhanena Selam Printing Enterprise.

Edris M (2004). Textbook of Food and Nutrition. University of Gondar. pp: 42-43.

FAO (2001). Protecting plantations from pests and diseases. Reports based on the works of W.M Ciesla. Forest plantation thematic papers working paper 10. Forest Resources Development Service, Forest Resources Division. FAO, Rome.

FAO (2011). Available on: www. FAO. com.

Gregori A, Vagelj MS, Pohleven J (2007). Cultivation techniques and medicinal properties of Pleurotus spp. Food Technology and Biotechnology 45: 238-249.

Gunde-Cimmerman N (1999). Medicinal value of the genus Pleurotus (Fr.) P. Kaest. (Agaricales S.l., Basidiomycetes). Int. J. Med. Mushrooms 1: 69-80.

Hayes WA, Haddad SP (1976). The nutritive value of mushrooms. Mush. J. 30:204.

Holding C, Carsan S, Nijuguna P (2006). Small holder timber and firewood marketing in the coffee and cotton/ tobacco zones of eastern Mount Kenya in proceedings of IUFRO conference hosted by Galway-Mayo institute of technology, Galway Ireland.

Islam MZ, Rahman, MH, Hafiz F (2009). Cultivation of Oyster Mushroom (*Pleurotus flabellatus*) on Different Substrates. Int. J. Sustain. Crop Prod. 4(1):45-48

Jiskani MM, Wagan KH, Pathan MA, Jamro GH (2000). Spawn growth of oyster mushroom as affected by different temperatures and grain media. J. Farm Scie. 6 (10):33-35.

Kurtzman RH (1976). Nitration of *Pleurotus sapidus* effects of lipid. Myco. 68:268-295.

Kurtzman RH (Jr) (2005). A review mushrooms: sources for modern western medicine. Micologia Aplicada International. 17:21-33.

Marshall E, Nair NG (2009). Make money by growing mushroom. Rural Infrastructure and Agro-Industries Division Food and Agriculture Organization of the United Nations. Diversification booklet number 7, p. 42.

McKinley VL, Vestal JR (1984). Biokinetic Analysis and Succession of Microbial Activity in Decomposition of Municipal Sewage Sludge. Appl. Environ. Microbiol. 47:933-941.

Muchiri S, Pukkala T, Miina J (2002). Modeling tress effect on maize in the *Grevillea robusta* + maize system in Central Kenya. Agro Forestry System 55:113-123.

Nair NG, Price G (1991). A Composting process to minimize odour pollution. Mushroom Sci. 13:205-206.

Neyrinck AM, Bindels LB, De Backer F, Pachikian BD (2009). Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid lowering action. Int. Immunopharmacol. 9:767-773.

Oseni TO, Dube SS, Wahome PK, Masarirambi M T, Earnshaw DM (2012). Effect of wheat bran supplement on growth and yield of oyster mushroom (*Pleurotus ostreatus*) on fermented pine sawdust substrate. Experimental Agriculture and Horticulture. pp. 30-40.

Ritchie E, Taylor WC, Vautin STK (1965). Chemical studies of the Proteaceae. I. *Grevillea robusta* A. Cunn, and Orites excelsa R. Br. Aust. J. Chem. 18:2015-2020.

Royse DJ (2002). Influence of spawn rate and commercial delayed release of nutrient levels on *Pleurotus conocopiae* yield, size and time to production. Appl. Microbiol. Biol. 17:191-200.

Shah ZA, Ashraf M, Ishtiaq CH (2004). Comparative study on cultivation and yield performance of Oyster mushroom (*Pleurotus ostreatus*) on different substrates wheat straw, leaves, saw dust. Pakistan J. Nutr. 3: 158-160.

Shelly NJ, Ruhul Amin SM, Nuruddin MM, Ahmed KU, Khandakar J (2009). Comparative study on the yield and yield related attributes of some newly introduced strains of *Pleurotus cystidiosus* with *Pleurotus ostreatus* (PO2). Bang. J. Mush. 3(1):67-72.

Stamets P (2000). Growing gourmet and medicinal mushrooms 3rd ed. Ten Speed Press, Berkeley. pp: 574.

Straatsma G, Gerrits PG, Thissen TN, Amsing GM, Loeffen H, Vab Griensven JLD (2000). Adjustment of the composting process for mushroom cultivation based on initial substrate composition. Bioresour. Technol. 72(1):67-74.

Tikdari MM, Bolandnazar S (2012). Application of organic nitrogen supplementations increases the yield of oyster mushroom (*Pleurotus florida*). Research in Plant Biology 2(3):10-15.

Tyndall B (1996). The socio-economics of *Grevillea robusta* within the coffee land use system of Kenya. AFRENA report No 109 p. 71.

Upadhyay RC, Verma RN, Singh SK, Yada MC (2002). Effect of organic nitrogen supplementation in *Pleurotus* species. Mushroom Biology and Mushroom Products 105(3):225-232.

Wasser SP (2002). Medicinal mushrooms as a source of antitumor and immune-modulating polysaccharides. J. Appl Microbiol. Biotechnol. 60: 258-274.

Yildiz A, Karakaplan M, Aydın F (1998). Studies on *P. ostreatus* (Jacq.ex Fr.) Kum. var. salignus (Pers. ex Fr.) Konr. et Maubl.: cultivation, proximate composition, organic and mineral composition of carpophores. Food Chem. 61:127-130.

Zadrazil F (1978). Cultivation of *Pleurotus*: In the biology and cultivation of edible mushrooms.(Eds) Chang, S. T., and Hayes, W. A., Academic Press, New York. pp. 521-558.

Zied DC, Savoie J-M, Pardo-Gimé nez A (2011). Soybean the Main Nitrogen Source in Cultivation Substrates of Edible and Medicinal Mushrooms, Soybean and Nutrition.

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Full Length Research Paper

Polycyclic aromatic hydrocarbons and small related molecules: Effects on *Schizosaccharomyces pombe* morphology measured by imaging flow cytometry

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Effects of polycyclic aromatic hydrocarbons and small related molecules on the morphology of fission yeast (*Schizosaccharomyces pombe*) are described. Polycyclic aromatic hydrocarbons are important environmental pollutants that act as carcinogens via several mechanisms of action. Fission yeast is a useful model organism for revealing the mechanisms by which these molecules affect the cell. None of the molecules studied affected cell length of wildtype or a rad26Δ mutant yeast relative to control, indicating that none of these operate like known genotoxins that lengthen the cell. Five compounds are shown to decrease cell width in wildtype fission yeast, but not in the rad26Δ strain. These results indicate that machinery controlling the cell's width is affected by these molecules, and that this change is not detected when the rad26 protein is absent. These observations were made using imaging flow cytometry, which captures tens of thousands of two-dimensional cell images in a short time and provides statistically rigorous data on large cell populations.

Key words: Fission yeast, *Schizosaccharomyces pombe*, hydrocarbon, polycyclic aromatic hydrocarbon (PAH), morphology, imaging flow cytometry.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are an important group of persistent environmental pollutants generated by combustion and industrial processes and found in air, soil and water. Several PAHs have been identified as priority pollutants by the United States Environmental Protection Agency (USEPA, 2014). They have also been found in tobacco smoke and its extract (Talhout, 2011).

PAHs have been shown to act as carcinogens, and they operate by a variety of mechanisms. One of the importance of these is that they form DNA-adducts, in which the PAH covalently binds to DNA, affecting its replication (Beland, 1994). Another pathway is the activation of the aryl hydrocarbon signaling receptor.

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While this step is usually the initial activating factor that makes a PAH available to form a DNA adduct, this step also has been shown to have other effects on cellular growth and proliferation (Chramostova, 2004). A third pathway is the generation by PAHs of reactive oxygen species that cause oxidative stress (Munoz, 2011). Often these mechanisms work synergistically (Rubin, 2001) or against each other, and separating the threads of causality is an ongoing focus of research.

Examining PAH mechanisms of action has been done in several cell types and organisms, including *Saccharomyces cerevisiae* (Siebert, 1981; Deng, 2010; Alnafisi, 2007). As well, the effects of whole tobacco smoke extract, which contains several PAHs, on fission yeast (*Schizosaccharomyces pombe*) have been characterized in detail (Chaudhuri, 2005; Sundaram, 2008a, b). However, there is no systematic study of PAHs in fission yeast in the literature.

Fission yeast is a simple and useful model to study cellular processes. It possesses many genes similar to human genes, and processes affecting many types of human cells and instrumental in disease have been studied in fission yeast and insights made toward understanding those diseases (Wixon, 2002). It is a cylindrical cell that grows in a lengthwise direction and divides by splitting in the middle into two daughter cells, and its morphology and morphogenesis are generally well understood (Brunner, 2000; La Carbona, 2006). The width of *S. pombe* has been found to be controlled by the proteins Cdc42 (Kelly, 2011), Rga2 (Villar-Tajadura, 2008) and Rga4 (Das, 2007).

Also, many mutants of fission yeast have been generated to achieve a wide variety of purposes (Nasmyth, 1981; Forsburg, 2006; NBRP, 2014). Some of these include cell cycle checkpoint mutants that do not conduct the usual checks a cell performs in order to proceed successfully into the next stage of the cell cycle. One example is the Rad26 deletion (rad26Δ) strain designated 1001, which does not produce the Rad26 protein. Rad26 serves to respond to structural abnormalities of DNA and the microtubule cytoskeleton (al-Khodairy, 1994; De Souza, 1999; Baschal, 2006; Herring, 2010).

Many properties of fission yeast can be observed in order to understand toxin effects, including structural properties (Blasko, 2013). Cell size and morphology are easily measured properties that can reveal much about a cell's behavior, and morphology has been a useful indicator of responses to external stimuli (Mos, 2013). Fission yeast studies on common toxins have shown morphological changes consistent with expectations (Pyati, 2011). In particular, significant lengthening of cells has been observed upon exposure to phleomycin and hydroxyurea, two known drugs that damage DNA. As well, latrunculin A treatment has produced cells with decreased aspect ratio (width divided by length). Fission yeast studies have also revealed morphological effects of a variety of natural product toxins found in plants and other organisms (Heisler, 2014).

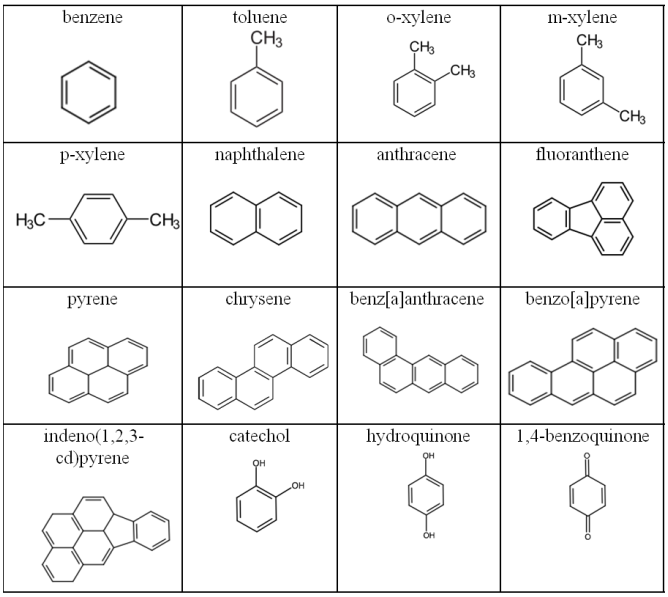


Figure 1. PAHs and related compounds studied in this work.

In this work, we reported the morphological effects on wildtype and rad26Δ fission yeast of several PAHs and related small molecules, measured by IFC. These molecules shown in Figure 1 include small molecule building blocks of PAHs, as well as related compounds found in tobacco smoke. The operating hypothesis of this study is that PAHs and related compounds affect the cells by either damaging the DNA or the cytoskeleton, and that the wildtype DNA detects this damage, whereas the rad26Δ strain does not.

This study was conducted using imaging flow cytometry. IFC enables the study of large populations of cells by acquiring tens of thousands of 2-D images in a few minutes using automated image capture and analysis. This allows a statistically rigorous analysis of populations than does traditional counting in a hemocytometer. In the IFC instrument used in this study, cells pass through a 50-mm flow cell and are observed and photographed through a 20x objective. Image analysis software measures and calculates parameters, such as cell length and width, for each image. It has been used to study other organisms such as cyanobacteria (Wert, 2013) and protozoa (Day, 2012).

MATERIALS AND METHODS

Chemicals

Anthracene (Matheson Coleman Bell, USA), benz[a]anthracene (Supelco, USA), benzene (Acros, USA), benzo(a)pyrene (Supelco, USA), 1,4-benzoquinone (Sigma-Aldrich, USA), catechol (Matheson Coleman Bell), chrysene (Sigma-Aldrich), fluoranthene (Supelco),

hydroquinone (Fisher, USA), indeno[1,2,3-cd)pyrene (Supelco), naphthalene (Fisher), pyrene (Sigma-Aldrich), toluene (Fisher), o-xylene (Fisher), m-xylene (Fisher) and p-xylene (Fisher) were used. Stock solutions of each toxin were prepared in dimethylsulfoxide (DMSO, Burdick & Jackson, USA). YE5S broth media was prepared from yeast extract (Becton Dickinson Bacto, USA), dextrose (anhydrous, BDH, USA), adenine hemisulfate dehydrate, (MP Biomedicals, Solon, Ohio, USA), L-histidine free base (MP Biomedicals), L-leucine (MP Biomedicals) and uracil (MP Biomedicals).

Strains and cell culture

S. pombe wild-type fission yeast with genotype leu1-32, ura4-d18,h, designated 236, and mutant strain with genotype rad26::ura^{+}, ura4-D18, leu1-32, ade6-704, h^{-}, designated 1001, were stored on YE5S agar slant media at 4°C. Reanimation of the yeast was accomplished in YE5S media broth with 24 h incubation at 30.5°C and a 120 rpm rotational shake.

Cell cultures were grown in a VWR Incubator (Sheldon Manufacturing, Cornelius, OR) at 30.5°C and a 120 rpm rotational shake function for liquid solutions. Optical densities at 595 nm (OD_{595}) were recorded with a blank of YE5S. Following starter culture, transfer volumes and growth times for final samples were controlled in order to obtain OD_{595} values between 0.2 and 0.9. The transfer volume ($V_{transfer}$) was calculated using the OD_{595} obtained from the starter culture and the formula below:

$$V_{transfer} = \frac{0.3}{OD_{actual}} \frac{1}{2^{N-1}} * V_{final} \quad where\ N = \frac{t_{incub}}{t_{gen}}$$

The calculated $V_{transfer}$ was added into 5 mL of YE5S media and incubated at 30°C and 120 rpm for a minimum of 12 h. Following this growth period, OD_{595} was measured again. If OD_{595} exceeded 0.3, the sample was diluted with YE5S in order to achieve an OD_{595} of 0.3, to ensure that plateau growth and senescence did not occur during the 6-hour period of toxin exposure. Each individual toxin was then added to a triplicate group of cultures to yield a final toxin concentration of 10 µM. Control samples did not have any toxin added but underwent the same OD_{595} measurement, dilution and 6-hour growth period. OD_{595} was measured once more; all final OD_{595} values were between 0.2 and 0.9 and were higher than the OD_{595} measured before toxin incubation. The samples were centrifuged at 5000 rpm for 2 min, washed in phosphate buffered saline (PBS, 0.2 M phosphate, 1.5 M NaCl), aspirated, centrifuged again and resuspended in 70% cold ethanol to preserve samples. The samples were stored at 4°C until ready for flow cytometric analysis.

Imaging flow cytometry

Samples in ethanol were resuspended in phosphate buffered saline (PBS) prior to IFC analysis. Samples underwent two cycles of the following: centrifugation at 5,000 rpm for 2 min, removal of supernatant, dilution in PBS, aspiration and vortexing to break up cell clumps. Final samples in PBS were allowed to incubate for 30 min prior to flow cytometric analysis.

Two-dimensional fission yeast cell images were collected with a FlowCAM Imaging Flow Cytometer (IFC), 20x microscope objective, and 50 mm flow cell (Fluid Imaging Technologies, Yarmouth, ME). Eight to ten drops of the sample solution was placed into the opening of the FlowCAM IFC and 75,000 images were collected using FlowCAM VisualSpreadsheet software version 3.4.5. A post capture filter, a filter that contains ranges of five parameters that reflect single cell images, was used to filter the raw image files of all the PAHs and control samples. The postcapture filter was validated using established observations and consisted of the following: Circle Fit 0.2 - 2.0, Circularity 0.4 - 4.0, Fiber Curl 0.0 - 0.2, Fiber Straightness 0.7 - 4.0 and Symmetry 0.75 - 2.0. FlowCAM software eliminated images that did not have values within those parameters set by the filter, resulting in a refined set of predominantly acceptable images. All samples have a minimum of 10,000 acceptable images.

Data analysis

A table containing parameters for each cell was exported to Microsoft Excel. The two parameters of interest in this work were length and width; mean values of these were calculated in Excel. Triplicate trials of each experiment yielded average values and standard deviations for both mean length and width. Error for a set of triplicate was calculated using a partial derivative formula (Mortimer, 1981).

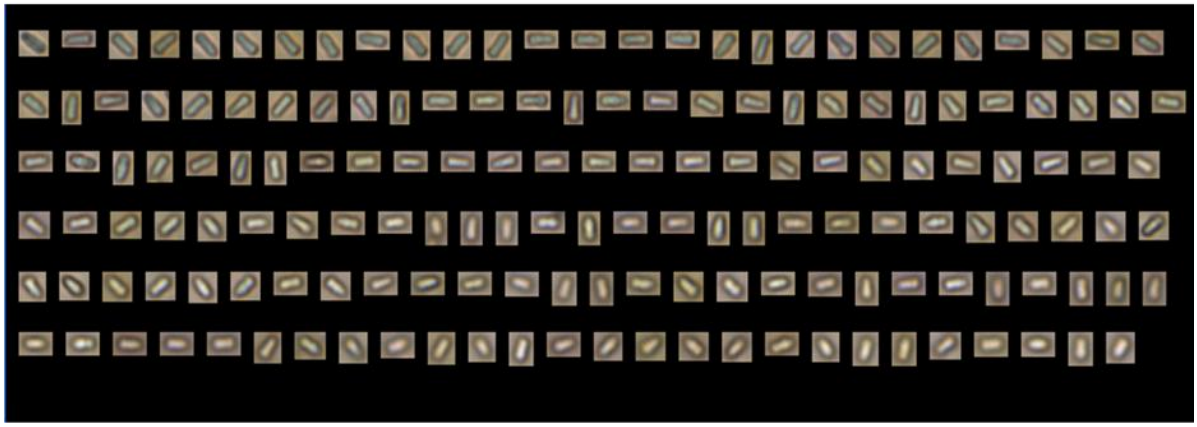
RESULTS AND DISCUSSION

Figure 2 shows a representative sample of images. For comparison purposes, each panel is drawn from images having approximately the same length range. Panel A shows images in the 6 mm range from strain 236 exposed to chrysene, and Panel B shows images in the same length range for untreated cells as a control. The width values are lower in the chrysene images than in the control images, in this sample set of images. While it is difficult to visually estimate changes in length or width, this image is a very small representative sample of the full data set of 10,000 images for each experiment. The comprehensive analysis presented in Table 1 includes the full data set.

For the group of experiments as a whole, mean absolute length values fell between 5.1 and 6.3 µm. Mean absolute width values fell between 2.3 and 3.2 µm. Absolute values for length and width are subject to day-to-day variations in growth behavior, so all length and width data is presented relative to a control experiment growing under the same conditions with no toxin. Table 1 shows the results of the mean for three trials for L/Lc, where Lc stands for Lcontrol, and W/Wc, where Wc stands for W control, in percentages. Results for wildtype 236 for known drugs are also included (Pyati, 2011). A result of 100% means that no change from control is observed.

Several observations are clear from the results in Table 1. First, for all toxins, L/Lc is at or very close to 100%, meaning that none of the treatments served to lengthen the cells relative to control. This is a marked difference from treatment by phleomycin and hydroxyurea, two known genotoxins that have been shown to lengthen yeast cells (Pyati, 2011; Belenguer, 1995). Each of these operates in its own manner: phleomycin acts to create DNA breaks, (Sleigh, 1977) whereas hydroxyurea prevents DNA synthesis (Bianchi, 1986). Nevertheless, the morphological effects of phleomycin and hydroxyurea are not observed upon exposure to this set of PAHs, indicating that the mechanism of action for these PAHs in

A. Chrysene-exposed 236 yeast cell images.



B. Control 236 yeast cell images.

Figure 2. Images collected with FlowCAM IFC. Panels contain images in the same length range from each representative sample, for comparison. (a) *S. pombe* 236 strain cells subjected to chrysene, lengths ranging from 6.05-6.12 mm and widths ranging from 1.46- 3.87 mm. (b) S. pombe 236 strain cells subjected to no toxin as a control, lengths from 6.05-6.11 mm and widths from 1.70-4.11 mm.

fission yeast is different from those of phleomycin and hydroxyurea.

Second, a clear decrease in cell width for wildtype 236 yeast is observed upon exposure to the following group of five toxins: chrysene, fluoranthene, o-xylene, indeno-(1,2,3-cd)pyrene, and naphthalene. These cells exhibit essentially the same length as control, but their widths are lowered. Chrysene exhibits the lowest width of all: 73.1% of control. This suggests a mechanism whereby these toxins affect cytoskeletal structures that determine the width of a cell. This decrease in width is observed only in the wildtype 236 strain. The 1001 strain does not detect the problem and continues replicating with an impairment.

Cell width has been shown to be controlled by small GTPases such as Cdc42, Rga2, and Rga4. Cdc42 has been shown to have multiple effects on cell width (Kelly, 2011). Via one pathway, deletion of Cdc42 guanine nucleotide exchange factors Scd1 and Scd2 has been shown to reduce cellular levels of Cdc42 and yield wider cells. Via a separate pathway, deletion of Cdc42 GTPase activating protein Rga4 resulted in increased Cdc42 and produced wider cells. So this work demonstrated that while both increased and decreased Cdc42 levels resulted in wider cells, each of these two mechanisms worked separately in different parts of the cell. Factors that cause reduced cell width was not observed in this work, although reference was made to other studies in which cell width was reduced. These studies have included other small GTPases, particularly Rga2 and Rga4. Reduced cell width has resulted from over-expression of Rga4, whereas deletion of rga4 yields

Table 1. Mean length and width relative to control of wildtype 236 and rad26Δ 1001 mutant fission yeast exposed to PAHs and related compounds.

Toxin	Yeast strain	L/Lc (%)[1]	W/Wc (%)[2]
Effects on 236 but not 1001			
236 less wide			
Chrysene	236	100.7 ± 11.4	73.1 ± 8.7
	1001	107.0 ± 4.4	103.9 ± 4.0
Fluoranthene	236	100.0 ± 6.1	75.3 ± 9.0
	1001	101.5 ± 8.4	102.1 ± 11.6
O-xylene	236	97.7 ± 8.6	77.5 ± 8.8
	1001	102.9 ± 4.4	100.8 ± 5.9
Indeno-(1,2,3-cd)pyrene	236	94.6 ± 6.9	79.4 ± 14.5
	1001	105.4 ± 5.4	98.2 ± 3.2
Naphthalene	236	103.3 ± 5.3	85.6 ± 10.4
	1001	102.9 ± 4.2	103.3 ± 2.2
236 wider			
Benzo(a)pyrene	236	101.0 ± 5.4	106.7 ± 2.6
	1001	99.1 ± 1.9	98.9 ± 2.8
P-xylene	236	105.3 ± 20.9	107.5 ± 6.6
	1001	101.2 ± 4.4	98.2 ± 3.6
No effects			
Anthracene	236	93.3 ± 13.7	98.7 ± 9.5
	1001	104.2 ± 4.7	104.5 ± 1.9
Benz(a)anthracene	236	97.7 ± 10.5	99.0 ± 13.0
	1001	103.0 ± 4.7	100.5 ± 2.4
1,4-Benzoquinone	236	102.1 ± 9.8	100.0 ± 5.3
	1001	105.1 ± 4.7	96.8 ± 3.7
Hydroquinone	236	100.4 ± 9.6	100.8 ± 6.3
	1001	103.4 ± 4.5	101.6 ± 2.3
Toluene	236	101.1 ± 20.9	101.2 ± 9.6
	1001	103.5 ± 4.3	98.1 ± 6.2
M-xylene	236	103.9 ± 20.8	101.3 ± 7.8
	1001	101.5 ± 4.4	101.1 ± 2.8
Benzene	236	103.9 ± 20.6	101.8 ± 8.6
	1001	97.6 ± 7.0	95.8 ± 6.7
Pyrene	236	97.4 ± 18.5	105.6 ± 7.7
	1001	105.8 ± 5.2	100.5 ± 3.8
Catechol	236	110.2 ± 23.5	105.9 ± 8.7
	1001	98.7 ± 4.6	95.6 ± 4.2
236 with known drugs			
Phleomycin		156.0	96.2
Hydroxyurea		109.3	104.7
Latrunculin A		115.4	110.5

[1]L/Lc = mean length divided by mean length of control, in percent form;
[2]W/Lc = mean width divided by mean width of control, in percent form.

wider cells (Das, 2007). Conversely, thinner, longer cells have resulted from deletion of rga2, whereas Rga2 overexpression produced shorter, broader cells (Villar-Tajadura, 2008). Clearly small GTPases play an essential

role in control of cell width. It is possible that the five hydrocarbons causing marked reductions in cell width in this study may be playing a role regarding the expression, function, or activation of Cdc42, Rga2, or Rga4.

Another possibility is that the effects of these toxins accompany formation of the contractile actinomyosin ring that is formed in early anaphase. This bears an interesting similarity to the toxin latrunculin A, a drug known to depolymerize actin structures (Liu, 2000; La Carbona, 2006). Previous experiments on latrunculin A effects on fission yeast have shown that the aspect ratio decreases relative to control for strain 236. The latrunculin A results in Table 1 indicate increases in both length and width relative to control, but width decreases to a greater extent. This yields a decrease in aspect ratio of 9.2% (Pyati, 2011), or a narrowing of the cell. This narrowing is comparable to the behavior of this set of PAH toxins. This suggests that this group of PAH toxins may serve to depolymerize actin, like latrunculin A, thus limiting the size of the contractile actinomyosin ring, among other effects.

In addition, there are some slight effects observed in some other toxins. Benzo(a) pyrene and p-xylene show a slightly wider 236 cell, at 106.7 and 107.5% of control respectively, although not to the extent observed in the first group of less wide cells. Anthracene shows a wider cell in 1001, at 104.5% of control, but again to a very small extent.

The lack of significant effects exerted by benzo(a)pyrene is a significant finding, because benzo(a)pyrene is one of the most toxic PAHs (Crosby, 1998). This suggests a mechanism distinct from the usual mechanism of benzo(a)pyrene toxicity: formation of a diol-epoxide and consequent formation of a DNA adduct (Beland, 1994). This finding lends strength to the argument that changes to fission yeast morphology reported here do not occur via DNA damage, but rather, by another mechanism.

As well, the chemical structural requirement for this mechanism, referred to as a “bay region,” appears not to be a requirement for the mechanism of cellular change observed in this study. This bay region, illustrated in Figure 3, is known to increase the biochemical reactivity of PAHs by facilitating the formation of the diol-epoxide (Baird, 2008). Therefore, other species with bay regions may not necessarily show changes in cell width in this study.

Several toxins have no effect on morphology: these are benz(a)anthracene, 1,4-benzoquinone, hydroquinone, toluene, m-xylene, benzene, pyrene and catechol. Some of these are quite water-soluble, such as catechol and hydroquinone, and are likely excreted rapidly by cells. Others, like toluene, m-xylene, pyrene and 1,4-benzoquinone, are not known to possess high toxicity or carcinogenicity. The most surprising among these are benz(a)anthracene and benzene, both of which are known carcinogens. Clearly, these compounds must address pathways separate from morphology that produce cancerous phenomena. Benz(a)anthracene does possess a bay region, but this structural feature is not essential in the activity of PAHs found in this study.

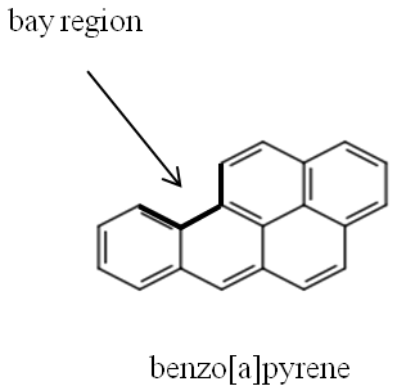


Figure 3. Benzo(a)pyrene with bay region illustrated by dark lines.

Finally, the rad26D mutant 1001 also displays no effects caused by these molecules. Rad26 is known to be essential to both DNA checkpoint signaling and to microtubules, so use of this mutant enables the study of whether the DNA checkpoint or the microtubules are affected. In terms of DNA, a Rad26/Rad3 complex detects DNA damage by a mechanism not fully understood, and then the Rad26 participates in the cell cycle arrest and repair of DNA (Wolkow, 2003). Also, Rad26 plays a role regarding microtubules: rad26Δ cells are unable to correctly complete two processes that depend on microtubules: chromosome segregation and morphogenesis (Baschal, 2006). The lack of effects by these compounds on the rad26Δ mutant adds support to the idea that they are not affecting DNA structure or microtubules.

Conclusions

This study shows that the cell width of wildtype fission yeast is significantly reduced by five toxins: chrysene, fluoranthene, o-xylene, indeno-(1,2,3-cd)pyrene and naphthalene. The cause of this bears further investigation and likely involves small GTPases such as Cdc42, Rga2, and Rga4, as well as the cell’s actin ring. The chemical structures of these molecules possess some similarities but are quite similar to other toxins exhibiting no effect in this work. Clearly, the bay region is not a critical structural element to this effect.

It is even possible that there are some causality in the other direction, that is, changes in cell morphology are part of the cause of carcinogenesis, instead of morphology’s simply being a second symptom of a primary pro-

blem induced by the toxin. This causal link between morphology change and cancer development has been found in another work (Hall, 2009).

Conflict of Interest

The authors have no financial or other interest that would influence the results of the study.

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REFERENCES

al-Khodairy F, Fotou E, Sheldrick KS, Griffiths DJ, Lehmann AR, Carr AM. (1994). Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast. Mol. Biol. Cell. 1994 Feb; 5(2):147-60.

Alnafisi A., Hughes J, Wang G, Miller CA 3rd. (2007) Evaluating polycyclic aromatic hydrocarbons using a yeast bioassay. Environmental Toxicology and Chemistry 26(7):1333-1339.

Baird C, Cann M. (1998). Environmental Chemistry 4th Edition, WH Freeman: New York. pp. 514-515.

Baschal EE, Chen KJ, Elliott LG, Herring MJ, Verde SC, Wolkow TD (2006). The fission yeast DNA structure checkpoint protein RAD26ATRIP/LCDI/UVSD accumulates in the cytoplasm following microtubule destabilization. BMC Cell Biol. 7:32.

Beland FA, Poirier MC. (1994). DNA adducts and their consequences. Methods to assess dna damage and repair: interspecies comparisons. Tardiff RD, Lohman PHM, Wogan GN ed. Scope-John Wiley and Sons: 29-55.

Belenguer P, Oustrin M, Tiraby G, Ducommun B (1995). Effects of phleomycin-induced DNA damage on the fission yeast *Schizosaccharomyces pombe* cell cycle. Yeast 11:225-231. PMID:7785323.

Bianchi V, Pontis E, Reichard P (1986). Changes of deoxyribonucleoside triphosphate pools induced by hydroxyurea and their relation to DNA synthesis. J. Biol. Chem. 261:16037-16042.

Blasko A, Mike N, Grof P, Gazdag Z, Czibulya Z, Nagy L, Kunsagi-Mate S, Pesti M (2013). Citrinin-induced fluidization of the plasma membrane of the fission yeast *Schizosaccharomyces pombe.* Food Chem. Toxicol. 59:636-642.

Brunner D, Nurse P (2000). New concepts in fission yeast morphogenesis. Phil. Trans. R.Soc. Lond B 355, 873-877.

Chaudhuri SP, Sundaram G., Bhattacharya A, Ray P, ray A, Chatterjee IB, Chattopadhyay D (2005). Activation of S phase checkpoint by cigarette smoke extract in *Schizosaccharomyces pombe*. Yeast 22:1223-1228.

Chramostova K, Vondracek J, Sindlerova L, Vojtesks B., Kozubik A, Machala M (2004). Polycyclic aromatic hydrocarbons modulate cell proliferation in rat hepatic epithelian stem-like WB-F344 cells. Toxicol. Appl Pharmacol. 196:410-421.

Crosby DG (1998). Environmental Toxicology and Chemistry. Oxford University Press: New York. p. 254.

Das M, Wiley DJ, Medina S, Vincent HA, Larrea M, Oriolo A, Verde F (2007). Regulation of cel diameter, For3p localization, and cell symmetry by fission yeast Rho-GAP Rga4p. Mol. Biol. Cell 18:2090-2101.

Day JG, Thomas NJ, Achilles-Day UE, Leakey RJ (2012). Early detection of protozoan grazers in algal biofuel cultures. Bioresour. Technol. 114:715-719.

De Souza CP, Ye XS, Osmani SA (1999). Checkpoint defects leading to premature mitosis also cause endoreplication of DNA in *Aspergillus nidulans*. Mol. Biol. Cell 10:3661-3674.

Deng Y, Zhang Y, Hesham A, Liu R, Yang M (2010). Cell surface properties of five polycyclic aromatic compound-degrading yeast strains. Appl Microbiol Biotechnol 86(6):1933-9.

Forsburg S, Rhind N. (2006). Basic methods for fission y east. Yeast 23:173-180. http://dx.doi.org/10.1002;yea.1347.

Hall A. (2009). The cytoskeleton and cancer. Cancer Metastasis Rev. 28:5-14.

Heisler J, Elvir L, Barnouti F, Charles E, Wolkow TD, Pyati R (2014). Morphological effects of natural products on schizosaccharomyces pombe measured by imaging flow cytometry. Nat. Prod. Bioprospecting. 4(1):27-35.

Herring M, Davenport N, Stephan K, Campbell S, White R, Kark J, Wolkow TD (2010). Fission yeast RAD26ATRIP delays spindle-pole-body separation following interphase microtubule damage. J. Cell Sci. 123(9):1537-45.

Kelly FD, Nurse P (2011). , Spatial control of Cdc42 activation determines cell width in fission yeast. Mol. Biol. Cell 22:3801-3811.

La Carbona S, Le Goff C, LeGoff X. (2006). Fission yeast cytoskeletons and cell polarity factors: connecting at the cortex. Biol. Cell 98:619--631.

Liu J, Wang H, Balasubramanian , MK .(2000). A checkpoint that monitors cytokinesis in Schizosaccharomyces pombe. J. Cell Sci. 113:1223-1230.

Mortimer RG. (1981). Mathematics for Physical Chemistry; Macmillan Publishing: New York: 280.

Mos M, Esparza-Franco MA, Godfrey EL, Richardson K, Davey J, Ladds G (2013). The role of the RACK1 ortholog Cpc2p in modulating pheromone-induced cell cycle arrest in fission yeast. PLOS One 8(7): e65927. doi:10.1371/journal.pone.0065927.

Munoz B, Albores A. (2011). DNA damage caused by polycyclic aromatic hydrocarbons: mechanisms and markers, selected topics in DNA repair. Chen D ed. In Tech, http://www.intechopen.com/books/selected-topics-in-dna-repair/dna-damage-caused-by-polycyclic-aromatic-hydrocarbons-mechanisms-and-markers.

Nasmyth K, Nurse P (1981). Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast Schizosaccharomyces pombe. Mol Gen Genet 182(1):119-24. http://dx.doi.org/10.1007/BF00422777.

NBRP National BioResource Project (Yeast), (2014). http://yeast.lab.nig.ac.jp/nig/index_en.html, accessed May 29, 2014.

Pyati R, Elvir LL, Charles, CE, Seenath U, Wolkow TD. (2011). Imaging flow cytometric analysis of *Schizosaccharomyces pombe* morphology. J. Yeast Fungal Research 2(7):106-112.

Rubin H (2001). Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. Carcinogenesis 22(12):1903-1930.

Siebert D, Marquardt H, Friesel H, Hecker E (1981). Polycyclic aromatic hydrocarbons and possible metabolites: convertogenic activity in yeast and tumor initiating activity in mouse skin. Cancer Res. Clin. Oncol. 102(2):127-139.

Sleigh MJ, Grigg GW. (1977). Sulphydryl-mediated DNA breakage by phleomycin in *Escherichia coli*. Mutat. Res. 42:181-190.

Sundaram G, Palchaudhuri A, Chaudhuri S, Karunanithi S., Chattopadhyay D. (2008a). Characterization of Sro1, a novel stress responsive protein in *Schizosaccharomyces pombe*. FEMS Yeast Res. 8:564-573.

Sundaram G, Palchaudhuri S, Dixit S, Chattopadhyay D. (2008b). MAPK mediated cell cycle regulation is associated with Cdc25 turnover in S. pombe after exposure to genotoxic stress. Cell Cycle 7(3):365-372.

Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperjuizen A (2011). Hazardous compounds in tobacco smoke. Int. J. Environ. Res. Public Health 8:613-628.

USEPA (2014). Priority Pollutants, United States Environmental Protection Agency. http://water.epa.gov/scitech/methods/cwa/pollutants.cfm. Accessed May 27, 2014.

Villar-Tajadura MA, Coll PM, Madrid M, Cansado J, Santos B, Perez P (2008). Rga2 is a Rho2 GAP that regulates morphogenesis and cell integrity in *S. pombe*. Mol. Microbiol. 70(4):867-881.

Wert EC, Dong MM, Rosario-Ortiz FL (2013). Using digital flow cytometry to assess the degradation of three cyanobacteria species after oxidation processes. Water Res. 47(11)3752-3761.
Wixon J. (2002). Featured organism: schizosaccharomyces pombe, the fission yeast. Comparative and Functional Genomics 3(2):194-204.
Wolkow TD, Enoch T (2003). Fission yeast Rad26 responds to DNA damage independently of Rad3. BMC Genetics 4:6-16.

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