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VARIATION IN THE PROXIMATE COMPOSITION OF WATER HYACINTH (*Eichhornia crassipes* (Mart.) Solms) DUE TO POTENTIAL ENZYMATIC ACTIVITIES OF ENDOPHYTIC FUNGI

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ABSTRACT

Water hyacinth is a free floating aquatic plant in fresh water ecosystem that has the potential of becoming one the most destructive ecological problems worldwide which threatens the existence of other aquatic organisms. The study primarily aimed to determine potentiality of the water hyacinth as substrate for various enzymatic activities of nine endophytic fungi which would further lead to the bioconversion of the aforementioned. The enzymatic activities of the fungal endophytes were measured through its effect on the proximate composition of water hyacinth (crude protein, crude ash, crude fat, crude fiber and moisture) after 20 days of solid state fermentation. Crude protein content water hyacinth was altered by the test fungi. *Aspergillus ochraceus* recorded the highest CPC with 6.96% while *Aspergillus niger* and *Fusarium* sp.1 -treated water hyacinth both recorded the least value with 5.60%. *Aspergillus niger* -treated water hyacinth had the most moisture content with 16.75% from the initial value of 9.86%. Moreover, degradation of crude fat from 0.51% to 0% was recorded while crude fiber content of the fermented water hyacinth was elevated from 23.87% to 30.71% (*Cladosporium cladosporioides*).

Keywords: Crude protein content, endophytic fungi, enzymatic activities, proximate composition, water hyacinth

INTRODUCTION

In the Philippines, water hyacinth (*Eichhornia crassipes* (Mart.) Solms) is considered as one of the most commonly known noxious aquatic weed thriving in almost all of the water ecosystems. It has been reported that water hyacinth plant can multiply at around 15% surface area per day (Majid, 1986). However, its abundance does not equate its utilization causing threat and destruction to other forms of aquatic organisms. Additionally, it has been proven to be of significant economic and ecological burden to many sub-tropical and tropical regions of the world (Jafari, 2010).

Several studies dealt on its utilization as feed and sources of organic compounds, however, low protein content, amino acid imbalance, presence of anti-nutritional factors, presence of crude fiber, cellulose and lignin imposes hindrance on its utilization as animal feeds (De Silva & Anderson, 1995; Wee, 1991). Accordingly, the utilization of water hyacinth as carbon source for enzymatic production.

Endophytic microorganisms are able to produce many enzymes (Firakova et al. 2007). Most of investigated endophytes utilize xylan and pectin, show lipolytic activity and produce non-specific peroxidases and laccases, chitinase and glucanase (Sieber et al. 1991; Leuchtmann et al. 1992; Li et al. 2004; Promputtha et al. 2005). They also produce extracellular hydrolases (pectinases, cellulases, lipases, laccase from the endophytic fungus *Monotospora* sp., xylanase, -1, 4-glucanlyase , phosphotases and proteinase) and hydrolytic enzymes (amylase, cellulase and laccase) to obtain nutrition from host (Patil et al. 2015; Lee et al. 2014; Sunitha et al. 2013). Furthermore, fungal enzymes are one of them which are used in food, beverages, onfectionaries, textiles, medical, pharmaceutical, agricultural and leather industries and often more stable than any other enzymes from plants and animals (Sunitha et al. 2013; Caroll &Petrini, 1983).

Hence, the conduct of the research to assess the effect of different enzymatic activities of endophytic fungi in the proximate composition of water hyacinth.

MATERIALS AND METHODS

Methodology was based from the works of Valentino et al. (2015) and Paynor et al. (2016). Water hyacinth was collected from Barangay San Roque, Lupao, Nueva Ecija.

Solid state fermentation of water hyacinth treated with fungal endophytes

Twenty (20) ml of the adjusted spore suspension (5.0 x 10^6 cells per ml) of different fungal endophytes was aseptically transferred to 100 grams of sterilized water hyacinth with a moisture content of 60-65% in a fermentation bottle. Then it was allowed to ferment for 20 days at room temperature.

Harvesting

Water hyacinth treated wuth endophttic fungi were harvested after 20 days of solid state fermentation and were stelirized at 15 psi for one hour. It was then air dried and pulverized.

Proximate Composition

Samples of water hyacinth tretd with fungal endophytes were sent to Lipa Quality Control Center – Bocaue, Bulacan, Philipines for proximate analysis of the nutritional content such as crude protein, crude fat, crude fiber, moisture and ash content.

Statistical analysis

Data was analyzed using Analysis of Variance (ANOVA) and Comparison Among Means by Duncan's Multiple Range Test (DMRT). All tests of significance were done at 5% and 1% probability levels.

RESULTS AND DISCUSSION

Proximate composition which includes ash, moisture, crude protein, crude fat and crude fiber content of the water hyacinth treated with endophytic fungi were determined and associated with the potential enzymatic activities of the fungal endophytes. Reduction in crude protein and content crude fat; elevation in the moisture and crude fiber was detected in the study.

After 20 days of solid state fermentation, remarkable alteration in the crude protein content (CPC) of the water hyacinth was observed. Among the treated water hyacinth only those treated with *Aspergillus ochraceus* had an increase of 2.65% which recorded the highest CPC with 6.96%. On the other hand, decrease in the CPCs was recorded in the eight remaining endophytic fungi treated water hyacinth. Among which, the CPCs of *Aspergillus niger* and *Fusarium* sp.1 -treated water hyacinth both recorded the least value with 5.60%, followed by *Penicillium citrinum*-treated water hyacinth with 5.82%. Correspondingly, *Aspergillus niger* and *Fusarium* sp1-treated water hyacinth had the highest % decrease in CPC of 17.41%, followed by *Penicillium citrinum* of 14.16%. Moreover, CPC of water hyacinth treated with *Aspergillus flavus, Aspergillus niger, Fusarium semitectum, Fusarium* sp1 and *Penicillium citrinum* were significantly lesser than the untreated water hyacinth which suggest the ability of the fungal endophytes to degrade the crude protein content of the substrate, thus the possible secretion of proteolytic enzymes by the fungal endophytes through solid state fermentation. Similarly, it also provides facts as to water hyacinth as a good inducer of protease production.

This is in line with various studies wherein fermentation with filamentous fungi led to production of proteases through solid state fermentation which cause reduction of CPC of the substrates (Jisha et al. 2013). Reduction in CPC is due to the utilization of sugar for fungal growth. The produced fungal proteases degrade the substrate's protein into amino acids to supplement fungal growth when carbon/ nitrogen sources are not available for bio conversion and itenable cell absoption and utilization of hydrolytic products (Colombatto & Beauchemin, 2009; Braaksma & Punt, 2009; Gupta et al. 2002).

Among the nine fungal endophytes tested, three species belonging to different genera were found superior (*Aspergillus, Fusarium* and *Penicillium*). Accordingly, Oseni & Ekperigin (2007), Oyeleke et al. (2010) and Sandhya et al. (2005) revealed that fungi of the genera *Aspergillus*, *Penicillium*, *Cephalosporium* and *Rhizopus* are especially used for producing proteases. Moreover, like *A. niger, A. terreus, A. parasiticus, A. fumigatus A. nidulans, A. glaucus and A. terreus* have higher proteases producing ability compared to other fungi when grown in various substrate (Kranthi et al. 2012; Kakde & Chavan 2011). Additionally, proteases of *Aspergillus* species, in particular, have been studied in detail since they are known for their capacity to secrete high levels of enzymes in their growth environment. Several of these secreted enzymes, produced in a large-scale submerged fermentation, have been widely used in the food and beverage industry for decades (Biesebeke et al., 2006). Whereas, Haq & Mukhtar (2007) and De Souza et al. (2015) showed the potential of *Penicillium species* for the production of proteases and other enzymes when solid state fermentation was carried out on a substrate containing wheat bran and soybean meal. Finally, Ahmad et al. (2014) and Ali & Vidhale (2013), revealed the protease production of *P. citrinum* and *Fusarium sp.*

Table 1: Mean percentage of crude protein composition of fungal enriched water hyacinth

TREATMENTS	Crude Protein*	% change in Crude Protein
Uninoculated Water Hyacinth	6.78[ab]	
Aspergillus flavus-treated water hyacinth	6.04[cde]	10.98
Aspergillus niger -treated water hyacinth	5.60[de]	17.41
Aspergillus ochraceus -treated water hyacinth	6.96[a]	2.65
Clasdosporium cladosporioides-treated water hyacinth	6.78[ab]	0
Fusarium semitectum -treated water hyacinth	5.86[cd]	13.57
Fusarium sp.1 -treated water hyacinth	5.60[de]	17.41
Fusarium sp. 2 -treated water hyacinth	6.42[abc]	5.31
Monascus ruber -treated water hyacinth	6.19[bc]	8.70
Penicillium citrinum-treated water hyacinth	5.82[cd]	14.16

***Treatment means in the column with the same letter are not significantly different at 5% level of significance**

Proximate composition of fungal treated dried water hyacinth revealed an increase in moisture, ash, and crude fiber of the fermented substrate (Table 2). Increase in moisture and crude fiber, reduction in crude fat was recorded in the fermented water hyacinth. Meanwhile, crude ash content was not affected upon solid state fermentation of the fungal endophytes.

Aspergillus niger -treated water hyacinth had the most moisture content with 16.75%, followed by *Penicillium citrinum* of 14.24% , *Fusarium* sp.1 with 13.97%, and *F. semitectum* -treated water hyacinth with 13.95%. Whereas the moisture content of the rest of fungal endophytes treated water hyacinth were comparable to the untreated water hyacinth ranging from 9.86 to 9.12%. For the % ash, statistically all fungal treated water hyacinth were comparable to the untreated substrate, thus indicating their inability to affect the % crude ash. *Monascus ruber*-treated water hyacinth being the highest of 12.66, followed by *Fusarium s*p2 of 12.63% and *Aspergillus ochraceus* of 12.55%, while the least was recorded in *Penicillium citrinum* of 8.33%.

Meanwhile, reduction in crude fat of water hyacinth is very evident from the initial reading of 0.51%. No traces of crude fat were noted in water hyacinth treated with *Aspergillus niger* and *Monascus ruber*. Additionally, *Aspergillus flavus* and *Cladosporium cladosporioides,* were significantly lower than the untreated one. Hence, the possible production of lipase by the fungal endophytes. The results of this study coincides with the reports of Khodanazary (2013), Nasseri et al. (2011), Oseni & Akindahunsi (2011), Kakde & Chavan (2011), wherein submerged/solid state fermentation of different substrate with filamentous fungi have resulted to reuced crude fat due to secretion of enzyme lipase.

Fungi such as *Aspergillus, Penicillium, Mucor, Rhizopus* grown in different types of agricultural wastes and substrate are widely recognized as the best lipase sources (Muralidhar et al. (2001), Sharma et al. (2001), Gordillo et al. (1998). Accordingly, lipases are the most versatile enzymes that play important roles in lipid metabolism in eukaryotes at various stages like fat digestion, reconstitution, adsorption, and lipoprotein metabolism (Bevilagua et al. 2004; Du et al. 2004).

Increment in crude fiber from 23.87% to 30.71% (*Cladosporium cladosporioides)* was obtained. Among all the treated water hyacinth, only *Fusarium* sp1 (24.85%) and *Penicillium citrinum* -treated water hyacinth (25.74%) are comparable to the untreated substrate. Consequently, the ability of the seven endophytes (*Aspergillus flavus, Aspergillus niger, A. ochraceus, Monascus ruber, Cladosporium cladosporioides, Fusarium semitectum and Fusarium sp 2*) in enhancing the crude fiber of the substrate was disclosed in the study. Results are in agreement with the findings of Paynor et al. (2016) using the same fungal endophytes, same methodology but different substrate and it could be attributed to fungal growth and physic-chemical composition of water hyacinth.

Table 2: Mean percentage (%) of proximate composition of the fungal enriched water hyacinth

TREATMENTS	%Moisture	%Ash	%Crude Fat	%Crude Fiber
Uninoculated Water Hyacinth	**9.12^{b}**	**11.43^{ab}**	**0.51^{a}**	**23.87^{e}**
***A. flavus*-treated water hyacinth**	**9.13^{b}**	**12.34^{a}**	**0.10^{cd}**	**26.50^{cd}**
***A. niger* -treated water hyacinth**	**16.75^{a}**	**11.53^{ab}**	**0.00^{d}**	**27.62^{bc}**
***A. ochraceus* -treated water hyacinth**	**8.79^{b}**	**12.55^{a}**	**0.44^{ab}**	**26.85^{c}**
***C. cladosporioides*-treated water hyacinth**	**9.60^{b}**	**12.26^{a}**	**0.17^{bcd}**	**30.71^{a}**
***F. semitectum* -treated water hyacinth**	**13.95^{a}**	**11.51^{ab}**	**0.38^{abc}**	**26.08^{cd}**
***Fusarium* sp.1 -treated water hyacinth**	**13.97^{a}**	**11.98^{ab}**	**0.25^{abcd}**	**24.85^{de}**
***Fusarium* sp. 2 -treated water hyacinth**	**9.27^{b}**	**12.63^{a}**	**0.34^{abc}**	**26.47^{bc}**
***M. ruber* -treated water hyacinth**	**9.86^{b}**	**12.66^{a}**	**0.00^{d}**	**28.09^{ab}**
***P. citrinum*-treated water hyacinth**	**14.24^{a}**	**8.33^{b}**	**0.52^{a}**	**25.74^{cde}**

***Treatment means in the column with the same letter are not significantly different at 5% level of significance**

CONCLUSION

Based from the results of the study, it can be concluded that potential enzymatic activities of the endophytic fungi could greatly affect the proximate composition of water hyacinth. Reduction in crude protein and crude fat is an indication of protease and lipase production.

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To God be the Highest Glory! Thy Will Be Done

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