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Effect of humic acid enriched cotton waste on growth, nutritional and chemical composition of oyster mushrooms (*Pluerotus ostreatus* and *Lentinus sajor-caju*)

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ABSTRACT

Humic acid (HA) is natural product obtained by plant decomposition. It improves systematic resistance in plants and the shelf life of food products. Oyster mushrooms occupy important place in human food due to their palatability and nutritional enrichment. Little is known about the impacts of HA on mushrooms yield. Therefore, a trial was conducted to study the role of HA improving growth, nutritional and chemical composition of two oyster mushroom strains (*Pleurotus ostreatus, Lentinus sajor-caju*). Pure cotton waste amalgamated with five levels of HA, i.e., 2, 4, 6, 8 and 10 mM/L was used as growth media. The responses of oyster mushroom to HA were recorded in various traits i.e. time to spawn initiation, time to mycelium growth initiation, time to maturity of flushes, time to initiation of pinheads, yield, biological efficiency (BE), minerals (N, P, K, and ascorbic acid, Zn, Cu, Mg, Mn, Fe, Na and Ca), sugars (total sugars, reducing and non-reducing sugars), proximate, total soluble solids (TSS), acidity, and Fourier-transform infrared spectroscopy (FTIR). The HA amalgation notably improved the growth, nutritional and chemical composition of oyster mushroom; however, strains differences were non-significant (>0.05) to various level of HA on dry weight basis TSS ranged from 6 to 6.8 °Brix, total sugar was 5.8–11.9%, reducing sugar was 2.6–3%, non-reducing sugar was 9.2–9.6%, ascorbic acid was 35.9–43 mg/100 g, carbohydrates were 68–74%, crude protein was 62–69%, crude fiber was 22–37%, fat contents were 2.5–17%, ash content was 9–11%. These results suggest that HA is an innovative substrate for valuable and high-quality production of the oyster mushroom.

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1. Introduction

Mushroom is a macro-fungus with epigeous or hypogenous fruiting structure, visible with naked eye (Chang, 1991). Oyster mushroom (class: Basidiomycetes) has edible flushy fruiting body and is consumed worldwide. White oyster (*Pleurotus ostreatus*) and Phoenix oyster (*Lentinus sajor-caju*) are the two most important types of oyster mushroom (Ayodele and Akpaja, 2007).

Abbreviations: BE, biological efficiency; HA, humic acid; TSS, total soluble solids; PDA, potato dextrose agar; HIV, human immunodeficiency virus.

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The name oyster originated from oyster shell like appearance of pileus i.e. the cap of fruiting body (Wood and Smith, 1988). Oyster mushroom has wide fan like fruiting body, which has white to creamy lamella or gills. *Pluerotus* species are widely distributed in wild. Moreover, different agro-industrial waste is used as growth media for domestic and commercial production of oyster mushroom (Jonathan and Adeoyo, 2011). Production and quality of mushroom is directly influenced by type of growth media. The most common type of substrate used are waste cotton, paper waste, sugar mills waste, cereals straws and crop leftovers (wheat, rice, millet and maize etc) (Jonathan and Esho, 2010; Fasidi et al., 2008). Cotton waste is most suitable substrate for oyster mushrooms (Sardar et al., 2017). Non-conventional substrate is the most important factor of mushroom's production (Muhammad et al., 2019). Oyster mushrooms grow well at temperature range of 22–28 °C and > 85% humidity (Onyango et al., 2011).

Oyster mushroom is a low calory healthy food rich in protein, vitamins and minerals (Kalmıs et al., 2008). Oyster mushrooms regulate the immune system, lower blood sugar & lipid levels and have antiseptic, anticarcinogenic, anti-human immunodeficiency virus (HIV) and anti-inflammatory properties (Gunde-Cimerman, 1999; Zervakis, 2005).

HA is gaining popularity as low-cost organic fertilizer in agriculture. Previous studies showed that humic substances increase the root, shoot and leaf growth; also boost the germination of different crops (Piccolo et al., 1993). The HA plays important role in uptake of minerals (S, P, K and N) (Arslan and Pehlivan, 2008). Moreover, the HA has positive morphological and physiological effects on growth of higher plants (Trevisan et al., 2010) like pepper (Karakurt et al., 2009) and tomato (Adani et al., 1998). The HA plays imporatnt role in biosynthesis of various proteins and enzymes (Nardi et al., 2007). In addition, HA is also beneficial to increase yield and quality of oyster mushrooms (Prakash et al., 2010).

However, role of humic acid in growth and quality of oyster mushroom is not well studied. Therefore, an experiment was conducted to study the effect of HA amalgamated with cotton waste substrate on growth, yield and quality of oyster mushroom.

2. Materials and methods

2.1. Research material

Two commercial strains of oyster mushroom that is white tree oyster (V_1P_1) and Phoenix oyster (V_2P_3) were cultured on potato dextrose agar (PDA) (PanReac AppliChem, Spain) media at Medicinal and Mushroom lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

2.2. Spawn preparation

The spawn was prepared by mixing boiled wheat grains with animal waste manure 2% $CaCO_3$ and 2% $CaSO_4$ as described by (Khan et al., 2019). Firstly, wheat grains were boiled until softness and then mixed with other constituents. The substrate was autoclaved at 121 °C for 20 min followed by overnight cooling and inoculation with mycelium on PDA. At the 17th day of inoculation, the spawn was shaken for even distribution of wheat grains and re-incubated for eight days until grains were fully impregnated with mycelium.

2.3. Preparation of substrate and bag filling

Cotton waste was used as a substrate. It was soaked in water and pH was maintained by adding two percent lime. After that substrate was wrapped with polythene and placed in open for 5 days to allow fermentation. Excessive water from substrate, evaporated by spread on floor. The substrate was amalgamated with different level of humic acid solutions of 0 (Control), 2, 4, 6, 8, 10 mM/L designated as T_1, T_2, T_3, T_4, T_5, and T_6 respectively. The trial was conducted in triplicated CRD design. Each replication had 12 bags (each bag has 800 g of substrate), one for each combination of variety and treatment (Rodriguez Estrada and Royse, 2007).

2.4. Agronomic features

In the presence of light, the bags were incubated at 24 ± 2 °C and 80% R.H. for mycelia growth. After the completion of colonization, bags were shifted in cropping room at the temperature of 18 ± 2 °C with 80–90% R.H. in order to increase the fructification. Different agronomic features were measured such as (i) time to mycelium growth initiation, (ii) time to completion of 1st, 2nd and 3rd flushes, (iii) time to pinhead formation, (iv) total yield and (v) BE.

2.5. Determination of sugar contents

Reducing, non-reducing and total sugars along with total soluble solids (Brix) were estimated in mushroom extract as described by (Hortwitz, 1960).

2.6. Nitrogen, phosphorus, potassium, and ascorbic acid contents

Fruiting bodies of oyster mushrooms were oven dried at 60 °C for 48 h and grinded to pass through 1 mm sieve. One gram of grinded sample was digested in HNO_3 (0.6 mol/L). Finally, this prepared solution was used for the determination potassium by flame photometer, phosphorus by spectrophotometer and nitrogen by kjeldhal's method (Mapya, 1998). Ascorbic acid was measured quantitively with titration method using 2, 6- dichloro-indophenol dye Tillmans reagent (Tillman's method).

2.7. Estimation of minerals contents

One gram of fruiting body powder was burnt for 15 h at 560 °C in a muffle furnace (Atila et al., 2017). After that, ash was digested with HNO_3 (0.6 mol/L). Mn, Cu, Zn and Fe measured by atomic absorption spectrometer (230ATS), while Ca, Mg and Na measured by flame photometer (Mapya, 1998).

2.8. Estimation of proximate

Protein, fat, ash and total carbohydrates were estimated as described by AOAC (1995). The crude fiber was measured with the procedure that was recommended by Ranganna (1986).

2.9. Molecular structure

Structural changes at molecular level in both strains of oyster mushroom were studied through Fourier-transform infrared spectroscopy (FTIR) (Agilent 680, Department of Chemistry, University of Engineering and Technology Lahore, Faisalabad, Pakistan). 1 mg of grounded sample was mixed in 100 mg KBr and compressed to form tablets. Spectra were recorded from 650 to 4000 per cm (Muhammad et al., 2019).

2.10. Statistical analysis

Experiment was conducted in completely randomized design (CRD). Data was subjected to analysis of variance (one way). The data were analyzed with the software of Statistix 8.1. Significance

Table 1
Effect of different concentration of humic acid enriched with cotton waste on time to spawn initiation, time to mycelium growth initiation, time to initiation of pinheads, time to maturity of flushes, yield and biological efficiency (BE) in two strains of oyster mushroom.

Treatments	Time to spawn initiation (days)		Time to mycelium growth initiation (days)		Time to Initiation of pinhead's (days)		Time to maturity of flushes (days)				Yield		Biological efficiency (BE)	
	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)100\%$	$V_2(P_3)100\%$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)1st$ flush	$V_2(P_3)1st$ flush	$V_1(P_1)$ 2nd flush	$V_2(P_3)2nd$ flush	$V_1(P_1)3rdflush$	$V_2(P_3)3rd$ flush	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$
T_1	2.0 ± 0.1	1.4 ± 0.5	48.7 ± 3.5^{a}	52.5 ± 1.7^{a}	65.0 ± 2^{a}	67.0 ± 2^{a}	90.3 ± 1^{a}	87.0 ± 2^{a}	100.3 ± 5^{a}	97.0 ± 2^{a}	110.3 ± 3^{a}	107.0 ± 2.5^{a}	184.0 ± 5^{d}	245.3 ± 5^{e}	45.1 ± 2^{f}	61.7 ± 2^{d}
T_2	2.0 ± 1.0	1.6 ± 1.1	48.0 ± 2^{a}	49.7 ± 2.2^{a}	63.3 ± 1^{a}	62.0 ± 2^{b}	84.0 ± 2^{b}	83.3 ± 2^{ab}	94.0 ± 2^{b}	95.3 ± 2^{ab}	104.0 ± 3.5^{bc}	105.3 ± 1.1^{ab}	248.0 ± 7^{c}	338.3 ± 8^{d}	62.2 ± 2^{e}	84.4 ± 1^{c}
T_3	1.3 ± 0.5	2.0 ± 1.0	46.7 ± 2^{ab}	45.5 ± 2^{b}	64.0 ± 3^{a}	61.3 ± 1^{b}	83.6 ± 2^{b}	80.3 ± 2^{bc}	93.6 ± 2^{b}	91.3 ± 1.1^{bc}	106.6 ± 3^{ab}	101.3 ± 1.5^{cd}	301.0 ± 12^{b}	438.3 ± 8^{b}	67.2 ± 2^{d}	86.3 ± 1^{c}
T_4	1.3 ± 0.5	2.0 ± 1.0	44.0 ± 3.5^{ab}	43.0 ± 2^{bc}	63.6 ± 3^{a}	62.6 ± 2^{b}	82.0 ± 2^{bc}	85.0 ± 2^{a}	94.0 ± 2.5^{b}	93.0 ± 2^{bc}	101.0 ± 3^{cd}	99.0 ± 1^{dc}	259.6 ± 9^{c}	340.0 ± 5^{d}	74.3 ± 2^{c}	85.4 ± 3^{c}
T_5	1.0 ± 0	2.3 ± 1.5	43.2 ± 2^{bc}	40.2 ± 1.5^{cd}	62.0 ± 1^{a}	60.0 ± 2^{bc}	83.3 ± 2^{b}	83.6 ± 2^{ab}	92.3 ± 1.1^{b}	94.6 ± 3^{ab}	102.3 ± 2^{bcd}	104.6 ± 2^{bc}	320.0 ± 26^{b}	405.3 ± 6^{c}	109.2 ± 3^{b}	105.5 ± 3^{b}
T_6	1.0 ± 0.1	1.0 ± 0	39.1 ± 2^{c}	39.1 ± 2^{d}	53.0 ± 1^{b}	57.0 ± 2^{c}	79.6 ± 1^{c}	78.0 ± 1^{c}	91.6 ± 2.5^{b}	90.0 ± 2^{c}	99.6 ± 1.5^{d}	98.0 ± 2^{e}	547.6 ± 15^{a}	580.0 ± 5^{a}	137.6 ± 2^{a}	146.3 ± 1^{a}

Mean value (n = 3) in the same column with the same following letter do not significantly differ ($p < 0.05$). (T_1 = Control, T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L); V_1 (P_1) = White oyster mushroom; V_2 (P_3) = Phoenix oyster mushroom.

of differences among treatments means were tested using LSD test $\alpha = 0.05$ (Steel and Torrie, 1980).

3. Results

3.1. Time to spawn initiation, 100% mycelium growth and pinhead formation

Analysis of variance revealed no significant differences in time to spawn initiation among both varieties of oyster mushrooms subjected to various levels of humic acid. However significant differences were observed in time taken for full mycelium growth and pinhead formation in response to different levels of humic acid. Out of six humic acid levels, least time for complete mycelium growth was given by T_6 (39.1 days) whereas max days (48.7, 48.0 days) were observed in T_1 and T_2 for white oyster mushroom. Same trend was observed for mycelial growth in Phoenix oyster mushroom i.e maximum days for mycelial growth was observed in T_1 (52.5 days) and minimum days were observed in T_6 (39.1 days). Moreover, raw cotton substrate complemented with humic acid exhibited fast mycelium growth. It is depicted that different levels of humic acid and strains of oyster mushroom significantly affected the time to pinhead formation. Minimum time (53.0 days) was shown by T_6 and maximum time (65.0 days) in T_1 by variety white oyster. However, in phoenix oyster, treatments T_6 (57.0 days) and T_1 (67.0 days) significantly affected the days taken to pinhead formation as compare with others (Table1).

3.2. Time to maturity of flushes and yield (g)

The different concentrations of humic acid exhibited significant effects on days taken to harvest ready flushes in both strains of oyster mushroom. Less time was taken by treatment T_6 and maximum was taken by T_1. In both strains of oyster mushroom treatment T_6, T_5, and T_3 showed min days for the maturity of 1st and 2nd flushes as compared to control T_1. Moreover, minimum time taken for the completion of 3rd flush by the treatments T_6 and T_4 than control T_1. The yield of mushroom obtained from cotton waste supplemented with humic acid was significantly higher in treatments T_6, T_5, and T_3 in both varieties as compared to control T_1 (Table 1).

3.3. Reducing, non-reducing and total sugars and total soluble solids (TSS)

Humic acid significantly increased total soluble solids (^{0}Brix). Among different treatments of HA, max total soluble solids were noticed in treatment T_6, following T_5 and minimum TSS was recorded in T_1. The highest value of TSS was regarded for T_6 as compared to T_1 in both varieties. Maximum TSS was observed in white oyster as compared to phoenix oyster. Cotton waste enriched with HA showed the significant increase in the total sugar contents. HA significantly boosted the reducing and non-reducing sugars. Maximum reducing and non-reducing sugars were seen in treatment T_6 and min was observed in T_1 in both varieties (Table 2).

3.4. Ascorbic acid and K, P, N contents of mushroom (mg/100 g)

Humic acid significantly increased the ascorbic acid contents of fruiting body. Highest concentration of ascorbic acid was recorded in T_6 and T_5 in white oyster and phoenix oyster. Concentration of K, P and N was higher in T_6. The highest amount of potassium (266.6 mg/100 g), phosphorus (122.3 mg/100 g) and nitrogen (84.3 mg/100 g) were noticed treatment T_6 in white oyster

Table 2
Change in sugar contents of two strains of oyster mushroom in response to different concentrations of humic acid.

Treatment s	Total soluble solids (°Brix)		Total sugars (%)		Reducing sugars (%)		Non-reducing sugars (%)	
	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$
T_1	4.2 ± 0.2^{c}	4.5 ± 0.9b	5.8 ± 0.3^{e}	6.8 ± 0.3^{e}	0.4 ± 0.02^{d}	1.1 ± 0.1^{c}	5.5 ± 0.2^{d}	5.8 ± 0.2^{e}
T_2	4.7 ± 0.2^{c}	5.1 ± 1.2b	9.1 ± 0.3^{d}	8.6 ± 0.3^{d}	1.2 ± 0.2^{c}	1.4 ± 0.3^{c}	7.9 ± 0.3^{c}	7.1 ± 0.2^{d}
T_3	5.4 ± 0.4^{b}	5.4 ± 1.2^{b}	9.8 ± 0.3^{c}	10.1 ± 1.3^{c}	1.6 ± 0.3^{c}	2.4 ± 0.3^{b}	8.2 ± 0.2^{c}	7.6 ± 0.3^{c}
T_4	5.5 ± 0.4^{b}	5.8 ± 1.3^{a}	11.1 ± 0.2^{b}	11.1 ± 0.1^{b}	2.1 ± 0.2^{b}	3.1 ± 0.1^{a}	8.8 ± 0.2^{b}	7.8 ± 0.1^{c}
T_5	6.2 ± 0.4^{a}	6.2 ± 1.1^{b}	11.4 ± 0.3^{b}	11.8 ± 0.2^{a}	2.4 ± 1.3^{ab}	3.1 ± 0.1^{a}	9.1 ± 0.3^{b}	8.5 ± 0.2^{b}
T_6	6.4 ± 0.4^{a}	6.3 ± 1^{b}	12.1 ± 0.3^{a}	12.1 ± 0.3^{a}	2.6 ± 0.2^{a}	3.2 ± 0.3^{a}	9.6 ± 0.1^{a}	9.0 ± 0.1^{a}

Mean value (n = 3) in the same column with the same following letter do not significantly differ ($p < 0.05$). (T_1 = Control, T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L); $V_1(P_1)$ = White oyster mushroom; $V_2(P_3)$ = Phoenix oyster mushroom.

Table 3
Effect of various concentration of humic acid enriched cotton waste on ascorbic acid, potassium (K), phosphorus (P), and nitrogen (N) contents of mushroom in two strains of oyster mushroom.

Treatments	Ascorbic acid (mg/100 g)		K (mg/100 g)		P (mg/100 g)		N (mg/100 g)	
	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$
T_1	32.1 ± 2.1^{c}	32.1 ± 2.1^{c}	150.3 ± 2.5^{f}	20.3 ± 3.5 ^{cd}	41.3 ± 3.2^{e}	24.6 ± 3.5^{e}	49.6 ± 2.5^{e}	40.0 ± 2^{e}
T_2	33.5 ± 0.4^{bc}	33.4 ± 1.5^{bc}	180.6 ± 3.0^{e}	18.3 ± 3^{d}	64.6 ± 3.5^{d}	27.6 ± 2.5^{e}	60.3 ± 3.5^{d}	45.0 ± 3de
T_3	33.8 ± 0.1^{bc}	33.5 ± 0.4^{bc}	200.3 ± 5.5^{d}	30.3 ± 3.5^{bc}	80.0 ± 3^{c}	33.3 ± 2.0^{d}	60.5 ± 3 ^{cd}	50.0 ± 3 ^{cd}
T_4	34.3 ± 0.4^{b}	34.0 ± 0.4^{abc}	220.3 ± 3.5^{c}	40.0 ± 4^{ab}	95.3 ± 2.5^{b}	41.3 ± 3.2^{c}	70.3 ± 2.5^{bc}	54.6 ± 3.5^{c}
T_5	44.4 ± 0.4^{a}	34.6 ± 0.2^{ab}	245.3 ± 4.5^{b}	45.3 ± 2.5^{a}	118.0 ± 3^{a}	57.2 ± 2.3^{b}	75.0 ± 3^{b}	62.3 ± 1.5^{b}
T6	44.8 ± 1.5 ^{a}	35.6 ± 0.3^{a}	266.6 ± 3.0^{a}	48.2 ± 2.0^{a}	122.3 ± 1.5^{a}	62.3 ± 2.0^{a}	84.3 ± 3.5^{a}	78.6 ± 3.5^{a}

Mean value (n = 3) in the same column with the same following letter do not significantly differ ($p < 0.05$). (T_1 = Control, T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L); V_1 (P_1) = White oyster mushroom; V_2 (P_3) = Phoenix oyster mushroom.

mushroom. The highest amount of potassium (48.2 mg/100 g), phosphorus (62.3 mg/100 g) and nitrogen (78.6 mg/100 g) were noticed in phoenix oyster cultivated on cotton waste substrate supplemented with 10 mM humic acid (Table 3).

3.5. Biological efficiency (%)

Biological efficiency (BE) is the ratio of fresh edible weight of mushroom to dry substrate weight expressed in %. Humic acid significantly increased the biological efficiency in two strains of oyster mushroom. Highest biological efficiency was observed in T_6 and T_5 followed by T_1. Highest BE was recorded in phoenix oyster as compared to white oyster mushroom (Table 1).

3.6. Proximate analysis (%)

Carbohydrate concentrations of fruiting body were examined between 46% and 72%, depending upon the diverse treatments of HA in substrate. The highest amount of crude protein noted in treatment T_6 as compared to control (Fig. 1) By the application of humic acid enriched with cotton waste low level of fat (2.66%) was noticed in T_6 in variety V_2P_3 and in case of variety (V_1P_1) 16% fats were observed (Fig. 1) Maximum ash content (10.8%) was noticed in T_6 in V_2P_3, while in variety (V_1P_1), T_6 and T_5 gave the highest ash content as compared to control. The carbohydrates, fibers, crude protein, fat, ash contents of mushroom were significantly altered by different concentrations of HA (Fig. 1).

3.7. Mineral contents in mushroom (mg/kg)

Cotton waste enriched with various level of HA significantly affected Zn, Cu, Mg, Mn, Na, Fe and Ca in fruiting body of mushroom (Table 4) Different concentrations of HA on cotton waste substrate increased the mineral contents of fruiting body in both varieties of oyster mushroom. The highest value for mineral contents was observed in T_5 and T_6, including Zn (28.0 mg/kg), Cu (74.0 mg/kg), Mg (203.4 mg/kg), Mn (28.1 mg/kg), Na (77.6 mg/kg) Fe (550.8 mg/kg) and Ca (362.0 mg/kg) (Table 4) on dry weight basis.

3.8. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is vastly technique that gives the detailed information about organic compounds and functional group present in the mushrooms, that can be observed by wave number of bands. Different FTIR absorption peaks distinguished variation in nutritional contents of white and phoenix oyster mushroom in response to various levels of HA. Considerable variation was observed in absorption spectrum of both oyster mushroom varieties for important nutritional components i.e. carbohydrates, fatty acids, proteins, alkanes, alkynes and hydroxyl group. The FTIR spectral area of both verities of oyster mushroom demonstrated characteristics features in two regions. The first region range, between (4000 – 1800 cm^{-1}). Important spectral peaks in white oyster (V1P1) were 3302, 3289, 2920, 2918, 2853, 2151, 2119 cm-1, that may be allocated to O–H, CH_3, - CH_2 lipids, C≡C and C–H group. The second region, between (1500–750 cm^{-1}) is consisted of carbonyl group and C=C double bond. Differentiated bands were observed in spectrum of white oyster s(V1P1) as compared to control, 3289 and 3202 (O–H and C–H), 1617 (protein), 1146 and 1015 cm^{-1} (C-O bond, β (1 → 3) glucan, cell wall, polysaccharide) (Table 5). Highest peaks value in spectra observed in ($V_1P_1T_6$ and $V_2P_3T_6$) (Table 5); Supplementary figures S2 (A-L).

4. Discussion

4.1. Time to spawn initiation, 100% mycelium growth and pinhead formation

There was no significant effect of different level of HA on days to spawn growth initiation. According to Baysal et al. (2003) humic acid is the source of nitrogen, both high and low nitrogen contents reduce the growth of mycelia growth. The high C/N ratio in the substrate helps to increase the mycelium growth (Hoa et al.,

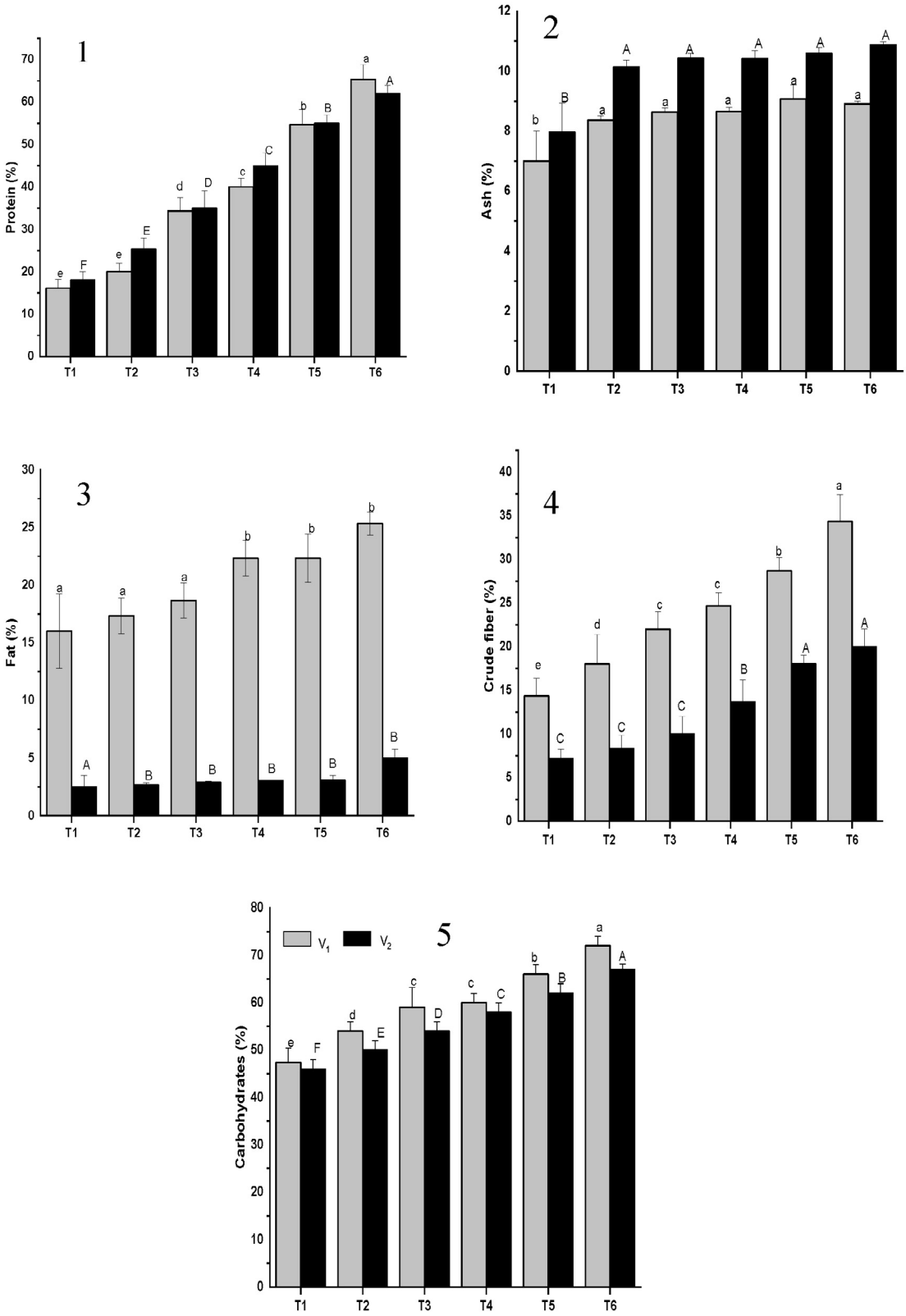

Fig. 1. Protein (%) (1); Ash content (%) (2); Fat (%) (3); Crude Fiber (%) (4); Carbohydrates (%) (5) of two strains of Oyster mushroom cultivated on different concentration of humic acid enriched with cotton waste. (T_1 = Control (100% cotton waste only), T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L); V_1 (P_1) = White oyster mushroom; V_2 (P_3) = Phoenix oyster mushroom.

2015). Mushroom growth mainly depends upon the different factors like nature of substrate, spawn rate, fertility of substrates, distribution of spawn and abiotic factors like humidity, light intensity, temperature, level of oxygen and carbon dioxide and moisture percentage during incubation period (Hassan et al., 2010). Fertile substrate enriched with macro and micro nutrients causes early

Table 4
Effects of various concentration of humic acid enriched cotton waste on mineral contents of two strains of oyster mushroom.

Treatments	Zn (mg/kg)		Cu (mg/kg)		Mg (mg/kg)		Mn (mg/kg)		Na (mg/kg)		Fe (mg/kg)		Ca (mg/kg)	
	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$	$V_1(P_1)$	$V_2(P_3)$
T_1	6.0 ± 1^{e}	10.0 ± 2^{d}	47.2 ± 2^{e}	16.6 ± 2.5^{d}	90 ± 5^{f}	150.3 ± 2.5^{e}	13.6 ± 1.5^{d}	13.6 ± 1.5^{d}	50.3 ± 2.5^{d}	50.3 ± 2.5^{d}	300 ± 5^{f}	251.3 ± 7^{e}	249.3 ± 4.0^{f}	150 ± 5^{e}
T_2	9.3 ± 1.5^{d}	13.3 ± 2.5^{d}	52.3 ± 2.5^{d}	20.6 ± 2^{d}	102.6 ± 6.4^{e}	159.3 ± 4^{d}	17.0 ± 2^{cd}	17.0 ± 2^{cd}	59.6 ± 2.5^{c}	59.6 ± 2.5^{c}	350 ± 3^{e}	264.6 ± 4.5^{d}	274.3 ± 4.0^{e}	174.3 ± 4.0^{d}
T_3	12.3 ± 2.1^{bc}	17.6 ± 2.1^{c}	59.6 ± 2.5^{c}	28.3 ± 3.5^{c}	115 ± 5^{d}	181 ± 3.6^{c}	18.6 ± 1.5^{c}	18.6 ± 1.5^{c}	65.3 ± 2.5^{bc}	65.3 ± 2.5^{bc}	401 ± 3.6^{d}	271 ± 3.6^{d}	300 ± 10^{d}	198.0 ± 8.1^{c}
T_4	10.3 ± 1.5^{cd}	22.0 ± 2.6^{b}	63.3 ± 2.5^{bc}	37.0 ± 2^{b}	127.6 ± 2.5^{c}	190 ± 3^{b}	20 ± 2^{bc}	20 ± 2^{bc}	68.6 ± 3.5^{ab}	68.6 ± 3.5^{ab}	451 ± 3.6^{c}	291 ± 3.6^{c}	316 ± 7.6^{c}	211.6 ± 7.6^{b}
T_5	13.0 ± 1^{b}	24.6 ± 3.0^{ab}	65 ± 2^{b}	40.0 ± 3^{ab}	149.3 ± 4^{b}	192.3 ± 3.05^{b}	22.3 ± 1.5^{b}	22.3 ± 1.5^{b}	77.0 ± 9.6^{a}	77.0 ± 9.6^{a}	501 ± 3.6^{b}	308 ± 2.6^{b}	340 ± 10^{b}	225 ± 5^{a}
T_6	15.6 ± 1.5^{a}	28.0 ± 2^{a}	74 ± 2.8^{a}	44.6 ± 3.5^{a}	165.9 ± 2.6^{a}	203.4 ± 4.1^{a}	28.1 ± 3^{a}	28.1 ± 3^{a}	75.7 ± 3^{a}	75.7 ± 3^{a}	550.8 ± 3.3^{a}	332.1 ± 2.2^{a}	362 ± 4.3^{a}	227.1 ± 7.0^{a}

Mean value (n = 3) in the same column with the same following letter do not significantly differ ($p < 0.05$). (T_1 = Control, T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L); V_1 (P_1) = White oyster mushroom; V_2 (P_3) = Phoenix oyster mushroom.

initiation of pinhead and reduces the time to harvestable produce (Singh et al., 2011). Our results declared that HA treatment took less for pinheads' formation as compared to control.

4.2. Time to maturity of flushes and yield (g)

The total yield obtained by first flush is higher than subsequent flushes. In the second and third flush, mushroom quality is lower as compared to the first flush. Increasing in the number of flushes decreases the yield acquired from substrate due to the less nutrient's accessibility in the substrate (Rizki and Tamai, 2011). In tomato, humic acid is reported to enhance vegetative growth, fruit yield and quality (Kazemi, 2014). Our results correlate with Prakash et al. (2010) reveal that supplementation of substrate with HA increases the yield in white oyster mushroom.

4.3. Reducing, non-reducing and total sugars and total soluble solid

In present study, humic acid significantly increased the TSS of fruiting body (Kazemi, 2014). The fruit quality, sweetness and taste are bounded to sugar contents like glucose, sucrose and sorbitol in fruit. These factors determined the fruit quality and its market value. Moreover, sucrose plays important role in activation anti-oxidization system, regulation of osmotic pressure, cell membrane stabilization and other metabolic pathways (Nishizawa et al., 2008). Increasing humic acid concentration significantly increases the total, reducing and non-reducing sugars in cucumber plant (Unlu et al., 2011). We observed similar increasing trend in our study.

4.4. Nitrogen, phosphorus, potassium and ascorbic acid contents of mushroom

Different levels of HA notably affected mycelia growth rate spawn growth, pinhead's and fruiting body formation, protein contents and flush yield (Elhami et al., 2008). These results correlate that nitrogen content of lettuce plant increase in response to HA application (Haghighi et al., 2010).

For the completion of biochemical reaction within cells of mushrooms phosphorus works as a co-factor (Khan et al., 2007). Quality of mushroom depends upon the phosphorus availability (Beyer and Muthersbaugh, 1996). The HA considered to increase the uptake of nutrients like P, Ca, and Mg making it more mobile and available to plant root system (Wang et al., 1997).

Potassium plays the important role in different mechanism such as growth, carbohydrates metabolism, ionic balance, enzyme activity and cap and gills discrimination (Griffin, 1996). Humic acid considered to increase the K uptake. The HA increases the N and K concentrations in the roots of tomatoes (Turkmen et al., 2004). Our results are in accordance with Kazemi (2014) who studied and found the increase in TSS and Vitamin C in tomato plant as a result of HA application.

4.5. Biological efficiency (%)

Bhattacharjya et al. (2014) reported that BE of mushroom increases if substrate is supplemented with different chemicals. Similar results were observed by Kirbag and Akyuz (2008) regarding an increase in BE of oyster mushroom at various biological structures of substrate along with supplementation of different chemicals.

4.6. Proximate analysis (%)

Karakurt et al. (2009) investigated that different applications of HA influenced total yield and carbohydrate contents of pepper.

Table 5
Frequency bands assessments of the Fourier transform infrared (FTIR) spectrum of two strains of oyster mushrooms i.e. $V_1(P_1)$ and $V_2(P_3)$.

$V_1(P_1)T_1$		$V_1(P_1)T_2$		$V_1(P_1)T_3$	
Frequency	Band assessment	Frequency	Band assessment	Frequency	Band assessment
2922	C–H	2922	C–H	2924	CH_3
2186	CH_3, - CH_2 lipids	2357	CH_3, - CH_2 lipids	2853	CH_3, - CH_2 lipids
1958	O–H bond	2273	CH2 Lipids	2163	C≡C
1943	O–H bond	2249	CH_3	2037	O–H bond
1584	Amide I, chitosan	1703	Ester group, C-O	1979	CH_2, Fatty acid
1518	Protein	1625	Carbonyl group	1578	Amide I, Protein
1459	CH_2	1597	Amide I, chitin	1522	Amide II, protein
1405	C-O bond	1459	CH_2	1457	CH_2
1375	polysaccharide	1401	C-O	1399	C-O bond, β (1 → 3) glucan, cell wall, polysaccharide
1142	C-O	1364	Polysaccharides	1203	C-O bond
1015	C-O Protein	1148	C-O bond, β (1 → 3) glucan, cell wall, polysaccharide	1146	C-O bond, β (1 → 3) glucan, cell wall, polysaccharide
		1013	C-O Protein	1012	C-O
				846	α-Glycosides

$V_1(P_1)T_4$		$V_1(P_1)T_5$		$V_1(P_1)T_6$	
Frequency	Band assessment	Frequency	Band assessment	Frequency	Band assessment
3263	O–H AND C–H	3356	O–H AND C–H	3481	O–H AND C–H
2920	C–H	2920	C–H	3408	O–H bond
2853	CH_3	2135	C≡C alkyne	3375	O–H AND C–H
2160	CH_2	1899	C=O carbonyl stretching of saturated aliphatic esters	3352	O–H AND C–H
2833	CH_3 bond	1578	Amide I, Protein	3339	O–H AND C–H
1779	C=O carbonyl stretching of saturated aliphatic esters	1507	Amide I, protein	2924	CH_3
1617	Amide II, Protein	1399	C=C bond	2857	CH_3, - CH_2 lipids
1397	C=C bond	1146	C-O bond, β (1 → 3) glucan, cell wall, polysaccharide	2135	C≡C alkyne
1364	C=C bond	1021	C-O bond, β (1 → 3) glucan, cell wall, polysaccharide	1578	Amide II, chitosan

$V_2(P_3)T_1$		$V_2(P_3)T_2$		$V_2(P_3)T_3$	

T_1= Control (100% cotton waste only), T_2 = 2 mM/L humic acid, T_3= 4mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L humic acid; $V_1(P_1)$) = White oyster mushroom.

Frequency	Band assessment	Frequency	Band assessment	Frequency	Band assessment
2918	C–H	2918	C–H	2920	C–H
2853	CH3, - CH2lipids	2853	CH3, - CH2lipids	2855	CH3, - CH2lipids
2169	C≡C	2078	O–H bond	2156	C≡C
2143	C≡C	1817	C=O carbonyl stretching of saturated aliphatic esters	1929	Amide II, chitosan
1617	Amide II, chitosan	1459	Amide II, chitosan	1891	C=O carbonyl stretching of saturated aliphatic esters
1578	Amide II, chitosan	1399	Symmetric bending of aliphatic CH3, triterpene compounds (CH2=CH-CH3)	1604	Amide I, chitosan
1146	C-O-C glycoside	1375	Symmetric bending of aliphatic CH3, triterpene compounds (CH2=CH-CH3)	1399	Symmetric bending of aliphatic CH3, triterpene compounds (CH2=CH-CH3)
1015	C-O bond, β (1→3) glucan, cell wall, polysaccharide	1146	C-O-C glycoside	1366	Symmetric bending of aliphatic CH3, triterpene compounds (CH2=CH-CH3)
		1015	C-O bond, β (1→3) glucan, cell wall, polysaccharide	1146	C-O-C glycoside
				1032	C-O bond, β (1→3) glucan, cell wall, polysaccharide
				1017	C-O bond, β (1→3) glucan, cell wall, polysaccharide

$V_2(P_3)T_4$		$V_2(P_3)T_5$		$V_2(P_3)T_6$	
Frequency	Band assessment	Frequency	Band assessment	Frequency	Band assessment
2920	C–H	3289	O–H AND C–H	3302	O–H AND C–H
2853	C–H	2922	C–H	2924	C–H
2197	C≡C	2853	CH3, - CH2lipids	2851	C–H
2137	C≡C	2096	O–H bond	2119	C≡C alkyne
2050	O–H bond	1625	Amide I, chitin	2094	O–H bond
1610	Amide I, chitin	1399	Amide I, chitin	1636	Amide I, chitin
1399	Polysaccharide	1015	C-O bond, β (1→3) glucan, cell wall, polysaccharide	1541	Amide II, chitosan
1375	βglucan				
1252	Lipids, Protein				
1015	C-O bond, β (1→3) glucan, cell wall, polysaccharide				

T_1 = Control (100% cotton waste only), T_2 = 2 mM/L humic acid, T_3 = 4 mM/L humic acid, T_4 = 6 mM/L humic acid, T_5 = 8 mM/L humic acid, T_6 = 10 mM/L humic acid); $V_2(P_3)$ = Phoenix oyster mushroom

Organic fertilizer like humic acid increases the crude protein in pumpkin seed (Jariene et al., 2007). The HA is rich source of nitrogen contents which helps to decrease the fat contents in pumpkin seed. Our results correlate with Jariene et al. (2007) stating that supplementation of substrate with HA increases the crude fiber, decrease fat in pumpkin seed. Our results of ash content are similar to Prakash et al. (2010).

4.7. Mineral contents in mushroom (mg/kg)

Atiyeh et al. (2002) demonstrated that nutrints uptake in tomato plants is significantly improved through HA application. Lime soil treatment with HA enhances absorption of Zn, Cu and Mn in maize crop (Hakan et al., 2010). Nikbakht et al. (2008) revealed that applying the humic compound in cut gerbera flowers increases the uptake of Ca. The HA application improved absorption of minerals (N, P, K, Mg, Fe and Ca) in cucumber and gerbera plants (Behzad, 2014; Nikbakht et al., 2008). We also noted similar increasing trends in our study's results.

4.8. FTIR characterization

The presence of several functional groups of different biochemical compounds was noticed by FTIR spectroscopy (Supplementary Figs. S2 (A-L). Oyster mushrooms contained high nutritional profile like as, proteins, carbohydrates, macro and microelements with less fat. Absorption bands and spectrum formed by FTIR could properly explained phytochemical analysis relied on functional groups (Muhammad et al., 2019; Ibrahim et al., 2019: Ghramh et al., 2019b). Alcohols, alkyne, and ketone were detected by FTIR spectroscopy (Ghramh et al., 2019a). Moreover, FTIR spectra are proved to be suitable means for estimation of tiny structures in interactions between metallic nanoparticles and biomolecules (Ghramh et al., 2019c). The importance of FTIR is capability of particular characterization of starch, sugars, fats, proteins, nucleic acid and other functional groups (O'Gorman et al., 2010). Some previous work described chemical compositions for various species of Amanita, king oyster, truffles and *Agaricus bisporus* by FTIR spectra (Zhao et al., 2006; Khan et al., 2019; Muhammad et al., 2019). The present study illustrated that various concentrations of HA highly influenced the nutritional quality of both strains of oyster mushroom.

5. Conclusion

This present study demonstrated that oyster mushroom cultivated on cotton waste enriched with humic acid; provided a favorable media for mushroom growth with significant increase in macro & micro nutrients, Reducing and non-reducing sugar, TSS, Vitamin C, carbohydrates, crude protein, ash and fiber contents. Hence, addition of humic acid in substrate provided better results in yield, BE and mushroom quality. On the other hand, it provided maximum nutritional value and significantly decreased the production-cost. For future aspects, humic acid could be an effective substance for growing quality mushrooms on commercial scales, especially the oyster mushroom.

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Appendix A. Supplementary data

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