Corn Husk as Lignocellulosic Agricultural Waste for the Cultivation of *Pleurotus florida* Mushroom

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The growth and yield of *Pleurotus florida* mushroom were evaluated in media with corn husk and wood sawdust substrates. Five formulations of substrates, namely 0%, 25%, 50%, 75%, and 100% of wood sawdust substituted with corn husk, were tested with 0% corn husk or 100% wood sawdust serving as the control. The total number of fruiting bodies, the number of effective fruiting bodies, the total fresh weight and dry weight of the mushroom, and biological efficiency were significantly increased with as low as 25% corn husk in the substrate, and they showed a significant increasing trend as the composition of corn husk in the substrate increased. Superior yield was produced by *P. florida* cultivated in 100% corn husk, where the total number of fruiting bodies, the number of effective fruiting bodies, the total fresh weight, and the total dry weight of the mushroom were 4.8 times, 5.4 times, 4.6 times, and 5.4 times greater than the control, respectively. The biological efficiency of *P. florida* increased gradually from 8.8% in the control to 51.37% in the 100% corn husk substrate. Therefore, corn husk could be exploited as a substitute or alternative substrate to wood sawdust for more sustainable production of *P. florida*.

Keywords: Agricultural waste; Biological resource; Mushroom substrate; Regression analysis; Sustainable mushroom production; Trend analysis; White oyster mushroom

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INTRODUCTION

The global market for edible mushrooms is estimated to be worth US $42 billion per year, where at least 350 species of fungi are edible, and these edible species come from 18 orders of fungi (Prescott *et al.* 2018). Oyster mushroom (*Pleurotus* species) is one of the most popular and widely cultivated edible mushrooms throughout the world, especially in Asia, America, and Europe due to their simplicity in cultivation and low cost of production (Hoa *et al.* 2015; Royse *et al.* 2017). In addition, *Pleurotus* species can be cultivated on a wide range of organic substrates and temperatures (Sánchez 2010).

Cropping activities generate large volumes of organic by-products or wastes rich in lignocellulosic composition. The biomasses cause disposal problems and pollute the environment (Tesfaw *et al.* 2015). Using these lignocellulosic biomasses as substrate to cultivate oyster mushrooms is a more sustainable solution, which could increase productivity and conserve the environment (Sözbir *et al.* 2015; Myronycheva *et al.* 2017). There are many agricultural wastes used for cultivation of mushrooms, such as corn straw, corn cob, palm kernel, cotton, rice straw, sugarcane bagasse, wheat straw, barley straw, and oil palm empty fruit bunch (Hoa *et al.* 2015; Marlina *et al.* 2015; Tesfaw *et al.* 2015; Adedokun 2014; Chukwurah *et al.* 2013). Corn husk is an abundant agricultural waste that also widely used for cultivation of mushrooms (Adjapong *et al.* 2015; Adedokun 2014),

and it contains cellulose, hemicellulose, and lignin as carbohydrate sources for fungi (Chitra and Vasanthakumari 2012).

Wood sawdust is a common substrate used for the commercial cultivation of mushrooms (Hoa *et al.* 2015). Unfortunately, the sawdust supply will reach a limit, as forest protection has become a global issue (Shah *et al.* 2004). The cultivation of mushroom requires softwood sawdust rather than hardwood, as the decomposition ability of mushroom is limited (Pathmashini *et al.* 2008). Low hemicellulose content and high lignin content in wood sawdust (Badu *et al.* 2011) justify more reasons for exploration of an alternative substrate without those limitations. Strugstad and Despotovski (2012) reported that the dust from black walnut tree contains a toxic substance, juglone, which may become transmitted to the fruitbodies during cultivation. Furthermore, the use of wood sawdust from a sawmill requires thorough washing to remove fungicide treatments on the woods (Levin *et al.* 1976). Therefore, alternative substrate materials are required to replace sawdust and move towards more sustainable mushroom production.

Corn husks are the thin cellulose-rich leafy sheaths that cover the corn cobs. They contain high cellulose content (Mahalaxmi *et al.* 2010; Badu *et al.* 2011) and could be exploited for various applications such as mushroom production. This study evaluated the growth and yield of white oyster mushroom (*P. florida*) cultivated with gradually increased composition of corn husk over wood sawdust substrates.

EXPERIMENTAL

Preparation of Pure Culture

Fresh samples of *P. florida* fruit basidiocarps were obtained from a mushroom farm located in Kundasang, Sabah, Malaysia (6° 1'19.11"N, 116°36'18.57"E), and then isolated using potato dextrose agar (PDA). The PDA was prepared according to Mondal *et al.* (2010) with slight modification, in which potato dices (200 g L^{-1}), dextrose powder (20 g L^{-1}), and agar powder (20 g L^{-1}) were mixed and sterilized in an autoclave at 121 °C and 15 p.s.i. (103.4 kPa) pressure for 15 min. The molten PDA (approximately 45 °C) was poured into a standard sterilized 9 cm Petri dish and solidified. The basidiocarp was split into half by hand in the longitudinal direction, a small piece of tissue was cut from the middle where the stem joined with the underside of the cap using a scalpel, and the tissue was then transferred onto a PDA plate (Ogden and Prowse 2004). It was then incubated in darkness at room temperature (25 ± 2 °C) for 5 days. An active, healthy, and uncontaminated mycelia plug (8 mm) was sub-cultured onto a fresh PDA plate to obtain purified cultures. The procedures for the preparation of the pure culture were performed in aseptic conditions. Working cultures were maintained on PDA plates, while stock cultures were kept in PDA slants at 5 to 7 °C.

Preparation of Substrates

Fresh corn husks were obtained from a local market, cleaned by trimming the rotten parts and removing the silk, washed with tap water, and sun-dried. The sun-dried corn husks were chopped to an approximately 1 cm long filaments and kept until further use. Wood sawdust was obtained from a local sawmill and cleaned by multiple soaking and rinsing with tap water until the tap water became clear. Excess water was drained-out and sun-dried for 5 days and kept until further use.

The mushroom cultivation substrate bags were comprised of basal substrate

(contained 65% water), rice bran, and calcium sulphate at a ratio of 100:10:1 (Islam *et al.* 2017). The basal substrates were prepared by mixing the corn husk and wood sawdust to five formulations, namely 0%, 25%, 50%, 75%, and 100% of wood sawdust substituted with corn husk. The substrates were tested with 0% corn husk or 100% wood sawdust serving as the control (conventional practice). One kilogram (wet basis) of the substrate mixture were filled into heat resistant polypropylene bags (9 cm x 35 cm) with a 3 cm diameter opening. The filled bags were sterilized in an autoclave at 121 °C and pressure 15 p.s.i. for 15 min and cooled to room temperature before inoculation with mushroom spawn. Each formulation had 10 replications, resulting in a total of 50 experimental units.

Preparation of Spawn, Inoculation, and Cultivation

The mushroom spawn was prepared using rice grains according to Pathmashini *et al.* (2008) and Kinge *et al.* (2016). After rice grains were soaked overnight in tap water, the water was drained, and calcium sulfate (0.5%), calcium carbonate (0.2%), and magnesium sulfate (0.2%) were added. The mixture was sterilized in an autoclave at 121 °C and pressure 15 p.s.i. for 15 min. The grains were cooled to room temperature, inoculated with mycelia plug (8 mm) of 7-day-old *P. florida* pure culture aseptically, sealed, and incubated in darkness at room temperature (25 ± 2 °C) for 20 days. Fully colonized grains served as spawn for the mushroom cultivation.

All mushroom bags were inoculated aseptically with 10 g spawn per bag. The inoculated substrates were arranged in a completely randomized design (CRD) and kept in an incubation room at 28 °C temperature and 60 to 70% relative humidity under dark condition for 33 days for colonization. Fully colonized substrate bags were moved to a mushroom cultivation facility to initiate formation of primordia and basidiocarps. The temperature of the mushroom cultivation facility room was maintained at 25 ± 2 °C, and relative humidity was maintained at 80% to 85% by spraying with water (Nguyen 2004). However, spraying at the spout area of the bags was avoided to prevent accumulation of water droplets and infestation of pests and diseases. The substrate bags were exposed to 12 h of dimmed light (50% intensity) during a day, and 12 h darkness during a night. The temperature and relative humidity were recorded and monitored using a data logger.

Data Collection

The growth of *P. florida* was evaluated based on the duration of complete mycelia run, duration of primordia initiation, duration from primordia until fruiting body, total duration from incubation until harvest, diameter of pileus (average diameter of the longest and shortest cap), and length of stipe. The duration of complete mycelia run, duration of primordia initiation, and duration from primordia until fruiting body were recorded based on daily observation. The total duration from incubation until harvest was calculated by adding the days required from inoculation until harvest of first flush, while the diameter of pileus and length of stipe were measured using a digital caliper after the fruiting body was harvested.

The yield performance of *P. florida* was evaluated based on the total number of fruiting bodies, total number of effective fruiting bodies, total fresh weight, total dry weight, and moisture content of the mushrooms produced in the first flush. The effective (marketable) and non-effective (non-marketable) fruiting bodies were differentiated based on their size. The fresh weight was measured immediately after harvesting, whereas dry weight was measured prior to oven-drying at 60 °C for 3 days until constant weight. The weight was measured using an electronic balance. Moisture content and biological

efficiency were calculated according to Das and Mukherjee (2007).

Data Analysis

One-way analysis of variance (ANOVA) was performed, and the treatment means were compared by Least Significant Difference (LSD) test at $p \leq 0.05$. The data were subjected to Pearson's correlation analysis to evaluate the relationship between the variables, and regression analysis to evaluate the trend across the substrates. The trends were either fitted to linear, quadratic, or cubic models. All statistical analysis were performed using the Statistical Analysis Software Version 9.4 (SAS 9.4).

RESULTS AND DISCUSSION

All growth variables recorded in this study were significantly affected by the different formulation of substrates, except the duration from primordia until fruiting body and the diameter of pileus (Table 1). The fastest mycelia colonization of the substrate was recorded at 33 days by the control (100% wood sawdust), whereas mixing the substrate with corn husk resulted in slower mycelia colonization, where they required an additional 8 days to fully colonize the substrates with 75% and 100% corn husk as compared to the control. Mondal *et al.* (2010) reported that the right proportion of alpha-cellulose, hemicellulose, lignin, and a suitable carbon-nitrogen ratio might be responsible for the mycelia growth. Primordia initiation after the substrate fully colonized was fastest in 75% and 100% corn husk with a range of 1.4 days to 2.4 days, and this was 8 days faster as compared to the control. Primordia initiation is the second stage of mycelia growth in mushroom cultivation (Buah *et al.* 2010). The present finding does not align with other studies, which reported longer times to initiate primordia. Mondal *et al.* (2010) reported primordia initiates 6 to 8 days after substrates mixed with rice straw or banana leaves fully colonized while Patel *et al.* (2019) found that oyster mushrooms require 16 to 20.33 days for full colonization of substrate and 20.33 to 25.33 days for primordia initiation in wheat straw substrate, which takes 4.33 days to 5 days of primordia initiation after fully colonization of substrate. There was no significant difference in the duration from primordia initiation until the formation of fruiting body, which ranged from 4.0 to 4.2 days. Therefore, it was found that the total duration from inoculation of spawn to substrate until harvest did not significantly differ in all formulations, except in 25% corn husk, which resulted in a total of 51.4 days. This was attributed to slower mycelia run and primordia initiation as compared to other treatments. This possibly related to the undesirability of the mushroom to the carbon-nitrogen ratio in the substrate (Hoa *et al.* 2015).

Although the diameter of pileus was not significantly affected by the substrate, the mushrooms cultivated on 100% wood sawdust had a longer stipe (2.11 cm), while the mushrooms cultivated with a mixture of sawdust and corn husk had shorter stipe length between 1.28 cm to 1.64 cm. This is comparable with other similar studies. Hoa *et al.* (2015) reported that *P. ostreatus* and *P. cystidiosus* grown on 100% sawdust substrates resulted in longer stipe lengths as compared to being grown on substrates mixed with sugarcane bagasse and corn cob. Ajonina and Tatah (2012) also reported that oyster mushroom cultivated on 100% sawdust substrate had a longer stipe length compared to other substrates with mixture of corn cobs and palm cones.

In terms of the yield, the mushrooms cultivated on 25% to 100% corn husk produced 3 to 4.8 times (significantly) greater number of fruiting bodies as compared to the control, where it produced 7.4 fruiting bodies on average. Similarly, higher number of

effective fruiting bodies was produced by *P. florida* cultivated on substrates mixed with corn husk, especially the 100% corn husk which recorded a total of 26 effective fruiting bodies, which was 5.4 times more than the control. In addition, mushrooms cultivated on 100% corn husk substrate produced 72.62% of effective fruiting bodies out of the total number of fruiting bodies, which was 7.75% to 21.93% better than other formulations. Moreover, it was found that *P. florida* cultivated on 100% corn husk produced the heaviest fruiting bodies in terms of total fresh and total dry weight, which were 76.05 g and 9.93 g, respectively.

Table 1. Growth of *Pleurotus florida* Cultivated in Different Compositions of Corn Husk Substrate

Composition of corn husk in substrate (%)	Duration to complete mycelia run (day)	Duration of primordia initiation (day)	Duration from primordia until fruiting body (day)	Total duration from inoculation until harvest (day)	Diameter of pileus (cm)	Length of stipe (cm)
0	33.20c	11.20a	4.20a	48.60b	4.34a	2.11a
25	40.00ab	7.20b	4.20a	51.40a	4.48a	1.64ab
50	39.40b	4.20c	4.00a	47.60b	4.40a	1.28b
75	41.00a	2.40d	4.00a	47.40b	3.85a	1.41b
100	41.20a	1.40d	4.00a	46.60b	4.24a	1.46b
p-value	<0.0001	<0.0001	0.9800	0.0040	0.8660	0.0326

Means followed with different letters within columns were significantly different at $p \leq 0.05$ by least significant difference (LSD).

By contrast, the use of 100% sawdust produced the least yield in terms of total fresh and total dry weight, which were 16.51 g and 1.83 g, respectively. Meanwhile, the moisture content in the mushroom was not affected by the substrate, where it ranged from 83.87% to 87.38% (Table 2). Viziteu (2004) stated that corn cob has a higher level of assimilable nitrogen, cellulose, and hemicellulose. This statement is supported by Rambey *et al.* (2019), who reported *P. ostreatus* grows best in sawdust substrate mixed with 30% corn cob. The addition of corn residue in the present study affected chemical composition of the substrate which resulted in better mushroom yield as compared to substrate with solely sawdust.

The Pearson's correlation analysis (Table 3), revealed that faster colonization of substrate led to later initiation of primordia (R = -0.90), with a smaller number of total fruiting bodies (R = 0.94). Particle size of substrate may have influenced the duration of complete mycelia run. Although substrates for the control and the other treatments had the same mass, the difference in volume was due to particle size, porosity, and compaction of substrate. Sawdust substrate has a smaller particle size, which creates a more compact substrate, as it has lower porosity. Hence, the volume will be smaller than substrate with corn husk mixture. The larger volume substrate could be the reason for slower mycelia colonization. According to Mihilall *et al.* (2011), substrate with finer particles tends to be more compact, which causes the substrate nutrient to be less accessible, impairing spreading and permeation of mycelia through the substrate. This statement is supported by the present study, in which later primordia initiation and lesser fruiting bodies were found in more compacted substrate.

Table 2. Yield of *Pleurotus florida* cultivated in Different Compositions of Corn Husk

Composition of corn husk in substrate (%)	Total number of fruiting bodies	Total number of effective fruiting bodies	Total fresh weight (g)	Total dry weight (g)	Moisture content (%)
0	7.40b	4.80c	16.51c	1.83c	87.38a
25	22.20a	11.40bc	45.79b	7.34b	83.87a
50	24.20a	14.40b	51.92b	7.98ab	84.23a
75	28.80a	14.60b	51.06b	7.85ab	84.10a
100	35.80a	26.00a	76.05a	9.93a	86.43a
p-value	0.0084	0.0002	<0.0001	<0.0001	0.6406

Means followed with different letters within columns were significantly different at $p \leq 0.05$ by least significant difference (LSD).

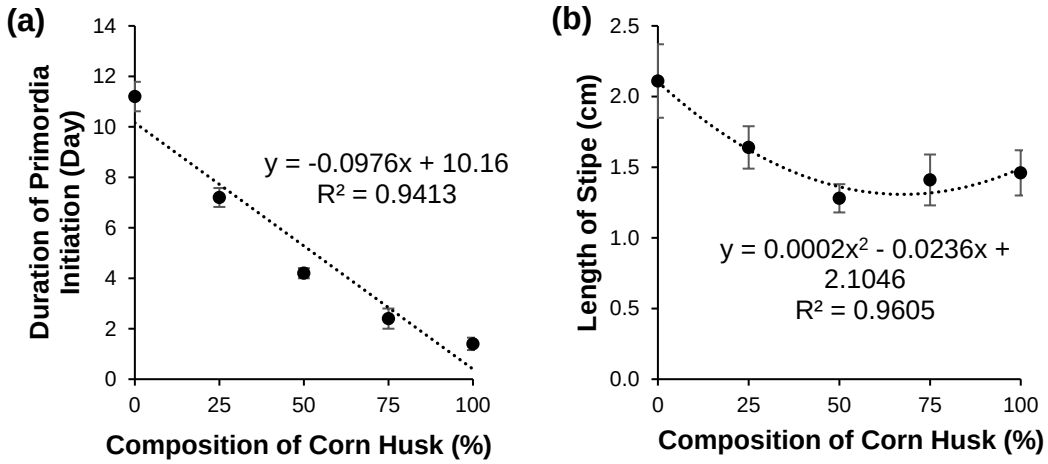
Furthermore, slower initiation of primordia was correlated to the slower duration from primordia until fruiting body formation (R = 0.90), and the mushrooms produced were longer in the stipe length (R = 0.88), with less total number of fruiting bodies (R = -0.97). In contrast, Kortei *et al.* (2018) and Chukwurah *et al.* (2013) reported that the pileus diameter and stipe were positively correlated, and both variables were also positively correlated with biological efficiency of the mushroom. The differences in the total fresh and total dry weight were mainly due to the difference in total number of fruiting bodies produced by respective formulation of substrate as shown by Pearson's correlation analysis in Table 4, where the total number of fruiting bodies was highly correlated with the total fresh weight (R=0.98), and total dry weight (R=0.97).

Table 3. Pearson's Correlation Analysis between Variables Evaluated for Growth and Yield of *Pleurotus florida* Cultivated in Different Compositions of Corn Husk

Variable	CMR	PRI	FFB	TD	PD	SL	TFB	EFB	TFW	TDW
PRI	-0.90*									
FFB	-0.65	0.90*								
TD	-0.18	0.60	0.82							
PD	-0.32	0.51	0.55	0.55						
SL	-0.88	0.88*	0.83	0.38	0.27					
TFB	0.94*	-0.97**	-0.77	-0.45	-0.38	-0.83				
EFB	0.77	-0.89*	-0.73	-0.56	-0.23	-0.67	0.94*			
TFW	0.89*	-0.92*	-0.73	-0.44	-0.20	-0.80	0.98**	0.97**		
TDW	0.97**	-0.93*	-0.72	-0.31	-0.20	-0.89*	0.97**	0.89*	0.97**	
MC	-0.66	0.39	0.24	-0.33	0.11	0.68	-0.37	-0.06	-0.28	-0.51

CMR, duration to complete mycelia running; PRI, duration of primordia initiation; FFB, Duration from primordia until fruiting body; TD, total duration from inoculation until harvest; PD, diameter of pileus; SL, length of stipe; TFB, total number of fruiting bodies; EFB, total number of effective fruiting bodies; TFW, total fresh weight; TDW, total dry weight, MC, moisture content.
*Significantly correlated at $p \leq 0.05$, and **Significantly correlated at $p \leq 0.01$.

The duration of primordia initiation showed strongly significant regression (R^2 = 0.9413) with a linear declining trend across composition of corn husk in the substrate (Fig. 1a). The length of stipe significantly declined as the composition of corn husk in the substrate increased, until the minimum length of stipe at 1.41 cm was achieved with 59% corn husk in the substrate according to the trend's equation (Fig. 1b). The total number of fruiting bodies, the total number of effective fruiting bodies, and the total fresh weight were fitted to a linear positive trend as the composition of corn husk was increased in the substrate (Fig. 1c, 1d, and 1e). The total dry weight showed significant regression (p = 0.0280) when it was fitted in a cubic model (R^2 = 0.9995) (Fig. 1f). The biological efficiency of *P. florida* gradually increased according to a linear model (R^2 = 0.9672), where 8.8% biological efficiency was recorded in the control, and up to 51.37% biological efficiency in 100% corn husk substrate, which was 5.8 times higher as compared to the control (Fig. 2). Meanwhile, other variables evaluated in this study showed no signification regression (p > 0.05). According to Hoa *et al.* (2015), differences in terms of mushroom yield and biological efficiency are due to the physical and chemical composition of the substrate such as cellulose, lignin, mineral content, pH, electronic conductivity, and carbon-nitrogen ratio. Generally, substrates with higher yield are associated with higher biological efficiency. According to Mendes *et al.* (2015), corn husk has a chemical composition of 31 to 39% alpha-cellulose, 34 to 41% hemicellulose, and a lower lignin content which is 2 to 14%. Badu *et al.* (2011) reported wood sawdust has 44.8 to 46.8% cellulose, 15.3 to 16.3% hemicellulose, and 27.6 to 34.1% lignin, where higher lignin content was noted as a reason for the lesser yield of mushroom. Significant amounts of cellulose, hemicellulose, and nitrogen are the main nourishment source for fruiting body formation of oyster mushrooms, whereas lignin is rarely used for fruiting body formation (Buah *et al.* 2010). Higher hemicellulose and lower lignin in the corn husk as compared to wood sawdust could be attributed the superior mushroom yield and biological efficiency in substrates mixed with corn husk.



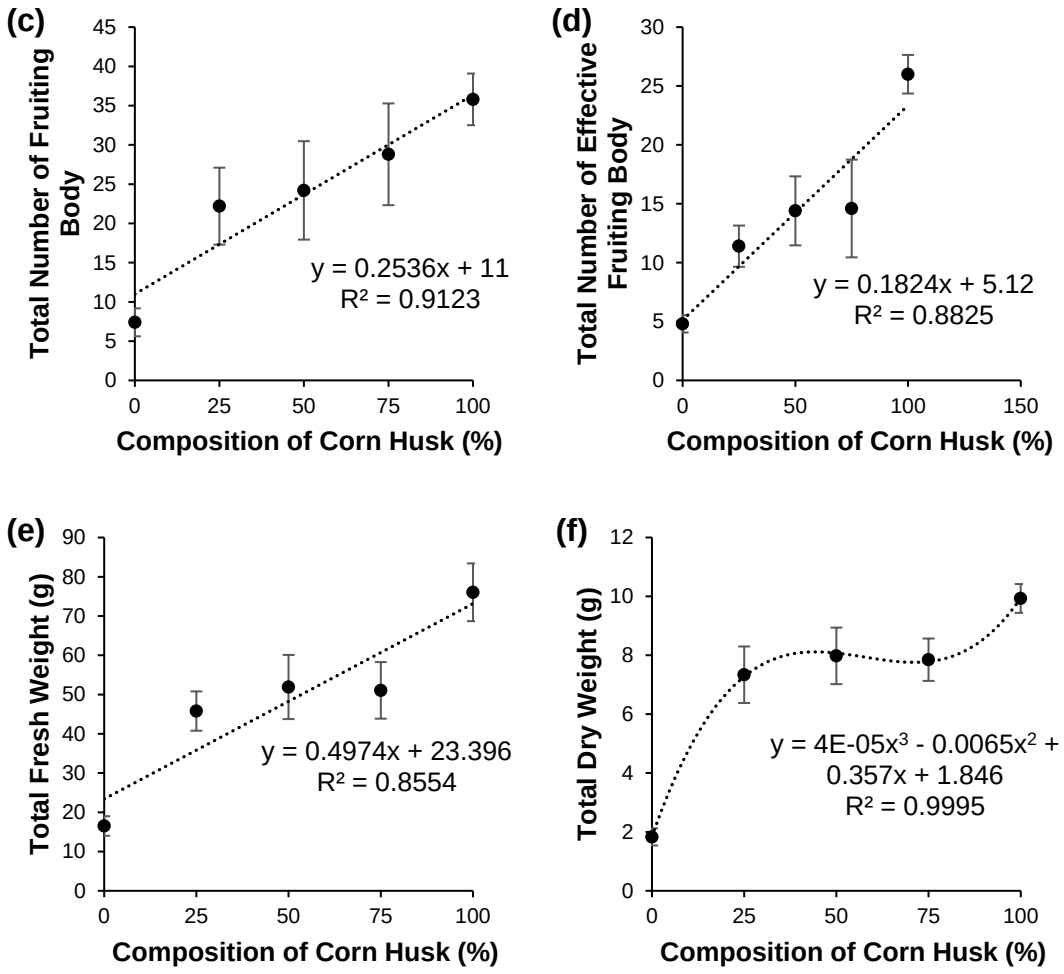


Fig. 1. Growth and yield variable (±standard error) trends of *Pleurotus florida* as the percentage of corn husk in the substrate gradually increases: (a) duration of primordia initiation fitted to linear model, (b) length of stipe fitted to quadratic model, (c) total number of fruiting bodies fitted to linear model, (d) total number of effective fruiting bodies fitted to linear model, (e) total fresh weight fitted to linear model, and (f) total dry weight fitted to cubic model

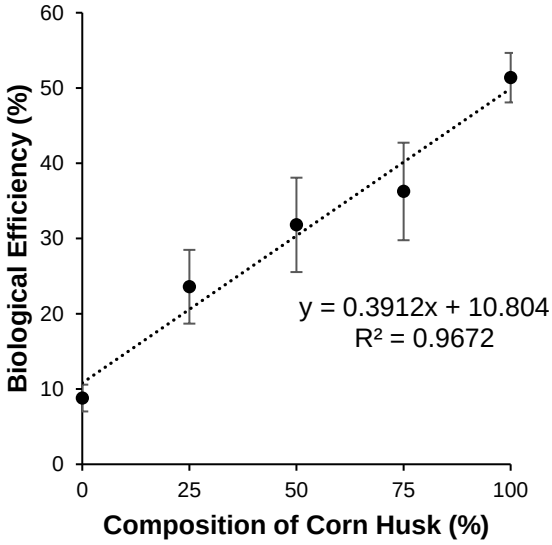


Fig. 2. Biological efficiency (±standard error) trend of *Pleurotus florida* as the percentage of corn husk in the substrate gradually increases fitted to linear model

CONCLUSIONS

1. Agronomically, *P. florida* cultivated on corn husk substrates exhibited better growth and yield as compared to the control, which was 100% wood sawdust substrate.
2. The yield and the biological efficiency of the mushroom were significantly increased with as low as 25% corn husk in the substrate, and they showed a significant increasing trend as the composition of corn husk in the substrate gradually increased.
3. Superior yield was produced by *P. florida* cultivated in 100% corn husk, where the total number of fruiting bodies, the number of effective fruiting bodies. the total fresh weight, and the total dry weight of the mushroom were 4.8 times, 5.4 times, 4.6 times, and 5.4 times greater than the control, respectively.

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