The Use of Sawdust Mixed with Ground Branches Pruned from Wax Apple or Indian Jujube as Substrate for Cultivation of King Oyster Mushroom (*Pleurotus eryngii*)

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Abstract. The king oyster mushroom [*Pleurotus eryngii* (DC.:Fr.) Quél.] is gaining popularity across the world due to its excellent taste, high nutritional quality, medicinal value, and long shelf life. Conventional substrates for king oyster mushroom cultivation consist of sawdust derived from various tree species. Sawdust demand is increasing worldwide, creating a need for alternative materials that can at least partially replace sawdust as substrate for king oyster mushroom. In Taiwan, as in other countries that grow fruit trees, pruned fruit tree branches are an expensive agricultural waste, particularly if they are not recycled or reused. In the present study, we evaluated substrates containing sawdust and different proportions of material ground from pruned wax apple or Indian jujube branches for cultivation of king oyster mushroom. Our results suggested that among all five substrate mixes tested, the best substitute for conventional sawdust (100% sawdust) was a substrate that contained 75% sawdust mixed with 25% materials ground from trimmed wax apple branches (Wax apple 25%). Furthermore, determination of mineral element content, pH, and electrical conductivity (EC) levels of the substrates both before spawn inoculation and after harvesting revealed no significant changes in mineral content, a slight reduction in pH value, and a minor increase in EC levels after cultivation. Taken together, results from this study suggest that agricultural wastes from pruned fruit tree branches can partially replace sawdust as the cultivation substrate for king oyster mushroom.

The king oyster mushroom (*Pleurotus eryngii*) is classified as a white rot fungus capable of digesting lignocellulose (Sharma and Arora, 2015). Currently, lignocellulosic materials such as sawdust obtained from various tree species are widely used for commercial production of this mushroom (Peng, 1997; Yamanaka, 2011). Due to the crisis of global warming, the role as carbon sink of forest trees has received much attention, with the aim of reducing use of raw tree materials. Thus, many research efforts have focused on finding lignocellulosic wastes suitable for mushroom production to replace sawdust, which is often associated with deforestation (Baysal, 2003; Kurt and Buyukalaca, 2010; Obodai et al., 2003; Petre and Teodorescu, 2012; Rani et al., 2008; Yildiz et al., 2002; Zervakis, 2005).

According to Poppe (2000), at least 200 agroforestry wastes can be used for oyster mushroom production. However, numerous studies have indicated that individual

mushroom species exhibit different growth responses to various cultivation substrates derived from particular agricultural residues. For example, Sherief et al. (2010) compared the growth and fruiting of a commercial strain of oyster mushroom cultivated on the two most commonly used substrates, namely rice straw and sawdust, and found that fruiting was earlier on rice straw. Obodai et al. (2003) evaluated eight lignocellulosic residues for growing the oyster mushroom *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer. They concluded that rice straw may be the best choice for oyster mushroom cultivation. Therefore, scientific evaluation of mushroom growth responses should be performed before any of the lignocellulosic wastes are recommended as alternative substrates for mushroom cultivation.

Current statistical data in Taiwan indicated that mushroom growers depend largely on imported sawdust: ≈5500 t annually at an average price of NT\$15/kg (Customs Administration, R.O.C., 2014). Due to both the desire to limit deforestation and the increased demands for raw products, the free market supply of sawdust has decreased, creating an urgent need to reduce the reliance of mushroom growers on sawdust, particularly in

markets that rely on imports. On the other hand, horticultural wastes, such as the trimmed branches of fruit trees, are a good source of lignocellulose. However, it is unknown whether pruned fruit tree branches can serve as an alternative substrate for king oyster mushroom growth. In Taiwan, around 5113 ha of land are devoted to wax apple [*Syzygium samarangense* (Blume) Merrill & Perry] cultivation, with 70% to 80% of wax apple tree branches pruned annually to achieve off-season production. Additionally, around 2048 ha of land in Taiwan are used for Indian jujube (*Ziziphus mauritiana* Lam.) production (Council of Agriculture–Taiwan, 2013), with annual tree pruning a common practice among growers to obtain higher yields. These pruned fruit tree branches are a horticultural waste and their removal from the orchard are an expense. In the present study, we investigated the potential of ground branches pruned from wax apple or Indian jujube trees to partially substitute sawdust in king oyster mushroom cultivation.

Materials and Methods

Preparation of mushroom growing materials and spawn inoculation. The spawn of a commercial king oyster mushroom strain (Ruifeng-6) and sawdust were provided by a local farm located in Taichung, Taiwan. Pruned wax apple and Indian jujube tree branches were collected from the Horticulture Research Station at the College of Agriculture and Natural Resources, National Chung Hsing University, Taichung, Taiwan. Pruned fruit tree branches including leaves were first completely dried in a 70 °C oven for 2–3 d and then ground in a small batch pulverizing machine to 25-mesh size (Model RT-01A; Rong Tsong Precision Technology Co., Taiwan). In this study, five mixes were prepared: sawdust as the control (Sawdust 100%), 25% replacement of sawdust with materials ground from pruned wax apple (Wax apple 25%) or Indian jujube (Indian jujube 25%) tree branches, and 50% replacement of sawdust with materials ground from pruned wax apple (Wax apple 50%) or Indian jujube (Indian jujube 50%) tree branches. The water content of the five sawdust-based mixes was adjusted to 60% by weight with tap water. The final substrates contained standard ingredients in a ratio (w/w) of 89% wet sawdust mix, 5% dried rice bran, 5% dried wheat bran, and 1% calcium carbonate and were packed into a polyethylene (PE) bag (36 cm × 9 cm). The overall water content of the mixed substrate inside the PE bag was then adjusted to 62% using tap water. A cotton plug was inserted into the bag and a ring wrapped around the neck to seal the PE bag. The finished PE bags containing 980 g of cultivation substrate were autoclaved at 100 °C for 8 h, cooled to 30 °C at room temperature, and then inoculated with 15–18 g/bag of Ruifeng-6 king oyster spawn.

Evaluation of mycelium growth and mushroom fruiting conditions. After spawn inoculation, the PE bags were kept in the dark

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at 25 °C. Ten culture bags were prepared for each of the five substrates. For each replicate, mycelium length, mycelium extension rate, mycelium density, mycelium color, and mycelium growth period (MGP) were monitored. The length of mycelium was recorded every 3 d. The daily growth rate was then calculated as centimeters per day. A digital index was used to rate mycelium density, with 5 indicating the highest density and 1 the lowest. A color indexing system based on the mycelium color observed in 100% sawdust substrate was developed to describe mycelium color, with 5 representing pure white color and a yellowish-white color designated by 1. The mycelium growth period was defined as the days after inoculation for the substrate to be fully colonized with mycelium.

After mycelium fully colonized the substrate, the PE bags were moved into a cropping room with a temperature of 15 °C, a relative humidity (RH) of more than 95%, and a CO₂ concentration below 3000 ppm to promote fruiting body development. For all five substrates tested, the yield (total fresh weight of fruiting body/bag), the average length of fruiting body, the number of marketable fruiting bodies (with normal stipe, pileus, and a fresh weight of more than 15 g), and the biological efficiency (fresh weight of mushroom/dry weight of substrate*100) were measured. Furthermore, average days after inoculation for the mushroom primordia to be first visible (denoted DPV) and average days from inoculation to harvest (denoted DIH) were also recorded. All measurements were repeated 10 times.

Measurement of substrate mineral elements. Substrate samples were first blanched in a 100 °C oven for 1 h and then placed in a 70 °C oven until completely dry. The dried substrate samples were ground into powder. Powder (0.5 g) was weighed into a crucible and heated in a muffle furnace at 200 °C for 2 h, followed by 400 °C for another 2 h, and finally heated to ash at 550 °C for 2 h. After cooling, 5 mL of 2N HCl (Merck, Germany) was added to completely dissolve the ash. The ash solution was filtered through a filter paper (Whatman #42, USA) and the filtrate was diluted to 25 mL with deionized water in a PE plastic bottle. The content of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were quantitatively measured using an Atomic Absorption Spectrophotometer (Model Z-2300, Hitachi, Japan). After proper dilution of the filtrate, potassium (K) and magnesium (Mg) were likewise quantitatively determined. The same device was used to quantify calcium (Ca) with the substrate sample prepared by taking 0.1 g of filtered ash solution and mixing with 3.9 mL of deionized water and 1 mL of lanthanum oxide.

A vanadate/molybdate method was adopted to determine phosphorus (P) content. Briefly, 1 mL of filtered ash solution was added to 3 mL of deionized water and 1 mL of vanadate/molybdate reagent, thoroughly mixed, and incubated for 10 min.

The sample was subjected to spectrophotometric determination using the Hitachi U-2000 Spectrophotometer.

The micro-Kjeldahl method was used to analyze nitrogen (N). Briefly, 1 kg of substrate sample was ground and dried in a 70 °C oven for one night. Then 0.2 g of dried sample was wrapped with filter paper (Whatman #1) and placed into a digestion tube together with 1 g of catalyst (Merck 8030) and 4.5 mL of concentrated sulfuric acid. The mixture was placed in the digestion vessel and heated at 410 °C for 2.5–3 h until the sample became clear or light green in color. The digested sample was then poured into the Kjeldahl flask with addition of 20 mL 12 N NaOH. After reaction, 50 mL of the converted ammonia was collected in a plastic beaker containing 20 mL of 2% boric acid mixed with 19 µM bromocresol green and 25 µM methyl red indicator. The reaction was then titrated with 1/14 N sulfuric acid to determine the percentage of ammonia in the substrate sample.

Determination of substrate pH and EC values before inoculation and after harvesting. For each substrate evaluated, two PE bags were sampled (100 g) before inoculation and after harvesting to determine the pH and EC of the substrate. The two samples were thoroughly mixed in a plastic container, from which 100 g of well-mixed substrate was sampled, air-dried for 2–3 d, and then stored in a sulfuric acid paper bag before measurement of pH and EC. To determine the pH and EC values, 5 g of dried substrate sample was homogenized with 40 mL of purified water, incubated for 2 h, and then filtered through gauze. The pH and EC of the liquid was determined with a pH meter (SP-701; Sontex, Taiwan) and an EC meter (SC-170; Sontex), respectively. In this analysis, three repeats were performed for each substrate tested.

Statistical analysis. Calculation of sample means, analysis of variance (ANOVA), and least significant difference (LSD) were

performed using SAS ver. 9.0 (SAS Institute, Cary, NC).

Results and Discussion

Substrate effects on mycelium growth and mushroom fruiting. Substrate mixes containing various ratios of sawdust and wax apple or Indian jujube grindings were prepared and used to cultivate king oyster mushrooms. The four mixes showed no significant differences ($P < 0.05$) in mycelium length or mycelium extension rate at 14 or 42 d after spawn inoculation relative to control (Sawdust 100%) (Tables 1 and 2). However, at 28 d after spawn inoculation, the mycelium length and mycelium extension rate values were significantly smaller in the substrate mixes with 25% or 50% ground Indian Jujube than in control ($P < 0.05$; Tables 1 and 2). The reason for this is not clear and requires further analysis.

The densest mycelium was observed in the Wax apple 25% and Wax apple 50% substrates (Table 3). The mycelium color appeared white in all substrates tested (Table 3). The shortest MGP was noted for Sawdust 100% and Wax apple 25% substrates, with values of 40.7 and 40.0 d, respectively. In contrast, the other substrates were fully colonized with mycelium after more than 50 d (Table 3). Similarly, the earliest primordia were observed in Sawdust 100% and Wax apple 25% substrates, with average DPV values of 56.5 and 55.4 d, respectively (Table 3). These substrates also resulted in an earlier harvest (DIH, Table 3). The earliest harvest, at 68.0 d after inoculation, was obtained when king oyster mushroom was cultivated in Wax apple 25% substrate (Table 3). These results suggested that up to 25% of the sawdust can be replaced with wax apple grindings.

The yield of mushroom in the Indian jujube 25% substrate was significantly lower ($P < 0.05$) than in the control (Sawdust 100%) (Table 4). The highest yield was

Table 1. The effect of different substrate base mixes on mycelium length (centimeters).

Substrate Bases	Days after inoculation			
	0	14	28	42
Sawdust 100%	0.00 a ^z	1.31 a	6.58 a	15.23 a
Wax apple 25%	0.00 a	1.17 a	5.00 ab	12.97 a
Wax apple 50%	0.00 a	1.30 a	5.13 ab	12.95 a
Indian jujube 25%	0.00 a	1.23 a	4.55 b	12.88 a
Indian jujube 50%	0.00 a	1.85 a	3.20 b	11.90 a

^zMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

Table 2. The effect of different substrate base mixes on mycelium extension rate (centimeter per day).

Substrate Bases	Days after inoculation			
	0	14	28	42
Sawdust 100%	0.00 a ^z	0.09 a	0.39 a	0.62 a
Wax apple 25%	0.00 a	0.08 a	0.29 ab	0.57 a
Wax apple 50%	0.00 a	0.09 a	0.28 ab	0.56 a
Indian jujube 25%	0.00 a	0.09 a	0.25 b	0.55 a
Indian jujube 50%	0.00 a	0.13 a	0.23 b	0.62 a

^zMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

observed in Wax apple 25% substrate, with a value of 241.84 g/bag (Table 4). No significant difference ($P < 0.05$) was recorded for the average length of fruiting body among the different substrates tested, with values ranging from 9.78 to 11.00 cm (Table 4). The number of marketable fruiting bodies was significantly ($P < 0.05$) smaller in Indian jujube 50% substrate (1.00) relative to control (3.18). No significant differences ($P < 0.05$) were detected among the other substrates, with values ranging from 1.88 to 3.18 (Table 4). Similar to the mycelium length and mycelium extension rate recorded at 28 d after spawn inoculation, biological efficiency was higher in Sawdust 100%, Wax apple 25%, and Wax apple 50% substrates, with efficiency values of more than 60% and no significant difference ($P < 0.05$) among them (Table 4).

These results suggest that a substrate containing 25% wax apple grindings may serve as a suitable alternative substrate for king oyster mushroom cultivation.

Low temperature effects on fruiting body yield and biological efficiency. Juang et al. (2012) reported that treatment of fully colonized PE bags with lower temperatures, such as 12 °C for 3–5 d or 15 °C for 5 d, before shifting them to 17 °C (to induce primordia and fruiting body development) yielded 224–229 g/bag and a biological efficiency between 54.7% to 56.4%, both significantly ($P < 0.05$) higher than bags cultivated at a constant temperature of 17 °C. In the present study, a lower temperature of 15 °C was constantly applied during the period of primordia induction and fruiting body development, which may in turn have resulted in the higher fruiting body yield and biological efficiency relative to those reported by Juang et al. (2012). Further detailed studies on temperature effects on fruiting body yield and biological efficiency should be conducted and include different periods of low temperature treatment.

Nutrient status of different substrates before inoculation and after harvesting. The mineral content of the substrate mixes and any

changes in content caused by king oyster mushroom cultivation were determined. In all substrates analyzed, the amounts of N and K remained more or less constant before inoculation and after harvesting. In contrast, phosphorus levels doubled, and Mg and Ca were slightly increased in the postharvest substrates (Tables 5 and 6). Since the measurements are based on dry weight analysis, the increased P, Ca and Mg contents in postharvest substrates may be explained by the concentration effect, caused by the reduction in substrate dry weight due to lignocellulosic digestion during mycelium growth and fruiting body development. Interestingly, the presence of lignocellulose digestion enzymes such as ligninase and cellulase has been reported in the spent substrate of *Pleurotus eous* and *Pleurotus ostreatus* (Koshy and Nambisan, 2012).

Similar to what we observed, Lee et al. (2009) found that the mineral nutrients in the substrates after cultivation of three edible mushrooms, including *Pleurotus eryngii*, are more abundant than the levels in the substrates before inoculation. They suggested that the increase in minerals can be attributed to the water supplied during cultivation, which may also apply in our case since tap water was used to adjust the water content of the substrates. The steady levels of N and K in this study (Tables 5 and 6) may be attributed to the rapid and high level of delivery of these two nutrients from the substrate to the fruiting body, which would offset the concentration effect. Interestingly, Lee et al. (2009) observed rapid uptake of K from the substrate to the fruiting body in the three edible mushrooms tested.

On the other hand, most of the micro-nutrient levels (such as Mn, Zn, and Cu) were unchanged in all the substrates from before inoculation to after harvesting (Tables 5 and 6). The Fe content was lower in the postharvest substrates (Tables 5 and 6), suggesting that king oyster mushroom may take up more Fe than other micronutrients during growth and development. There was still a fair amount of Fe left in all five postharvest substrates analyzed (Table 6).

Overall, our results showed that no potentially limiting reductions in mineral nutrient content occurred in the substrates through

Table 3. Characteristics of mycelium growth in different substrates.

	Density index ^z	Color index ^y	MGP ^x	DPV ^w	DIH ^v
Sawdust 100%	4 c ^u	5 a	40.7 b	56.5 c	75.8 c
Wax apple 25%	5 a	5 a	40.0 b	55.4 c	68.0 d
Wax apple 50%	5 a	5 a	51.8 a	65.6 b	82.3 b
Indian jujube 25%	4 c	5 a	53.3 a	66.1 b	84.8 b
Indian jujube 50%	4.3 b	5 a	57.1 a	75.5 a	93.6 a

^zThe number 5 indicates the highest density and 1 indicates the lowest density.

^yThe number 5 represents pure white color and 1 indicates a yellowish white color.

^xMGP: average days after inoculation for the substrate to be fully colonized with mycelium.

^wDPV: average days after inoculation for the mushroom primordia to be first visible.

^vDIH: average days from inoculation to harvest.

^uMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

Table 4. Characteristics of fruiting body development in different substrates.

	Yield (g/bag)	Length (cm)	Number of marketable fruiting bodies ^z	Biological efficiency (%)
Sawdust 100%	240.11 a ^y	10.52 a	3.18 a	64.42 a
Wax apple 25%	241.84 a	10.13 a	3.00 ab	64.88 a
Wax apple 50%	231.09 a	10.83 a	2.67 ab	61.97 a
Indian jujube 25%	165.20 b	9.78 a	1.88 ab	44.30 b
Indian jujube 50%	193.61 ab	11.00 a	1.00 b	51.90 ab

^zFruiting body with normal stipe, pileus and a fresh weight of more than 15 g.

^yMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

Table 5. Nutrient content in different substrates before inoculation.

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Sawdust 100%	0.86 bc ^z	0.13 b	0.39 c	1.01 c	0.16 c	754.65 a	95.29 a	25.32 d	3.33 ab
Wax apple 25%	0.92 bc	0.15 ab	0.48 b	1.00 c	0.17 c	657.23 b	86.47 b	24.99 d	3.17 b
Wax apple 50%	1.05 a	0.17 ab	0.54 b	1.02 c	0.17 c	568.20 c	62.93 c	27.30 c	3.50 ab
Indian jujube 25%	0.85 bc	0.14 b	0.52 b	1.39 b	0.20 b	827.33 a	88.15 b	50.32 a	3.50 ab
Indian jujube 50%	0.77 c	0.19 a	0.70 a	1.92 a	0.27 a	496.00 c	82.42 b	44.96 b	4.50 a

^zMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

Table 6. Nutrient content in different substrates after harvesting.

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Sawdust 100%	0.86 b ^z	0.28 b	0.39 d	1.43d	0.22 d	340.86 b	73.97 a	23.33 d	3.17 d
Wax apple 25%	0.97 b	0.33 a	0.48 c	1.63bc	0.25 c	374.74 b	73.45 ab	25.82c	3.83 cd
Wax apple 50%	1.23 a	0.34 a	0.61 b	1.71ab	0.27 b	441.91 a	70.49 ab	28.16 b	4.83 b
Indian jujube 25%	0.82 b	0.32 a	0.52 c	1.51cd	0.25 bc	275.95 c	69.65 b	27.33 bc	4.33 bc
Indian jujube 50%	0.84 b	0.34 a	0.77 a	1.83a	0.31 a	226.11 c	61.98 c	36.49 a	7.17 a

^zMeans with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

Table 7. The pH and electrical conductivity (EC) of different substrates before inoculation and after harvesting.

	Before inoculation		After harvesting	
	pH	EC (dS/m)	pH	EC (dS/m)
Sawdust 100%	6.25 a ²	1.12 d	4.91 b	1.89 d
Wax apple 25%	6.06 a	1.64 c	4.96 b	2.18 c
Wax apple 50%	5.66 b	2.07 b	5.23 a	2.42 b
Indian jujube 25%	5.45 b	2.11 b	4.96 b	2.15 c
Indian jujube 50%	5.58 b	2.61 a	5.31 a	2.92 a

²Means with the same letter are not significantly different (Fisher's least significant difference test at 5% level).

mushroom cultivation, results that are consistent with Lee et al. (2009).

pH and EC values of substrates before inoculation and after harvesting. To determine the effect of substrate pH and EC values on king oyster mushroom growth, the pH and EC levels of different substrates were determined before inoculation. To determine how mycelium growth affects pH and EC, the values were measured after harvesting. The initial pH values in Sawdust 100% and Wax apple 25% substrates before inoculation were 6.25 and 6.06, respectively. These two pH values were significantly ($P < 0.05$) higher than those in the other substrates (Table 7). In contrast, the initial EC levels in Sawdust 100% and Wax apple 25% substrates before inoculation were 1.12 and 1.64 dS/m, respectively, which were significantly ($P < 0.05$) lower than those in other substrates (Table 7). Results from this study suggested that greater yield and growth occurred in substrate with a higher pH value (Tables 4 and 7). After harvesting, the pH was acidified, with all substrate showing pH values less than 5.31. The EC levels were greater after mycelium growth in all five substrates tested (Table 7). Consistent with our findings, Khan et al. (2013) suggested that pH plays a pivotal role in oyster mushroom production and that most mushrooms grow best with a near-neutral or slightly basic pH.

Conclusion

In this study, the possibility of using sawdust mixed with ground fruit tree

branches as substrate for cultivation of king oyster mushroom was evaluated. The results indicated that the best substitute for conventional sawdust was a substrate that contained 75% sawdust mixed with 25% ground wax apple branches. Furthermore, analyses revealed that mycelium and fruiting did not significantly change mineral content, slightly reduced pH, and somewhat increased EC of the spent substrates. Overall, results from this study suggested that the pruning waste of fruit trees should be tested for potential utilization in king oyster mushroom production.

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