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Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*)

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Abstract

The possibility of using hazelnut husk (HH) as a new basal ingredient for substrate preparation in *Lentinula edodes* cultivation was investigated. Some chemical properties of the substrates prepared by HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios were compared, and their effects on spawn run time, days to first harvest (earliness), yield and biological efficiency (BE) were determined. The N content of the substrate prepared from HH alone was very high (0.82%), and thus the C:N ratio of substrates decreased with an increase in the rate of HH in the mixtures. Yield and BE in the HH alone substrate was considerably low compared with the controls (80BWC:10WS:10M and 60BWC:20WS:20WB), and decreased with an increase in the rate of HH in the mixtures. However, when the HH content in the mixtures was kept below 50%, the yield was relatively high (50HH:50WS and 50HH:50BWC). Even when the HH content increased to 75% in the mixture, the comparable yield and BE to the controls could be obtained by adding 10% of WB as nutrients (75HH:15WS:10WB and 75HH:15BWC:10WB). The results revealed that HH could be used as a new basal ingredient for substrate preparation in *L. edodes* cultivation.

Keywords: *Lentinula edodes*; Hazelnut husk; Substrate; Chemical properties; Yield; Biological efficiency; Correlation

1. Introduction

Lentinula (*Lentinus*) *edodes* (Berk.) Pegler (shiitake) is the second most popular edible mushroom in the world because of its flavor, taste and quality (Jong and Birmingham, 1993). In addition, shiitake is one of the best known and the best characterized mushrooms used for medicinal purposes (Royse, 1996; Ooi, 2000).

Shiitake can be grown on synthetic logs as well as natural logs. Sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing *L. edodes*. Different substrate formulas have been developed in different countries, depending on their readily available raw material. Growers typically select the best and the least expensive, locally available substrate materials. Agricultural wastes such as oak, hornbean, sweetgum, poplar, alder, ironwood, beech, willow, pine, maple and birch sawdust, cereal straw, corn cobs, sugarcane bagasse, tea waste, sunflower seed hulls, peanut shells, cotton straw and seed hulls can be used alone or in combination with other wastes in shiitake cultivation (Diehle and Royse, 1986; Miles and Chang, 1989; Salmones et al., 1999; Morais et al., 2000; Zhao et al., 2001; Pire et al., 2001; Zervakis et al., 2001; Curvetto et al., 2002; Philippoussis et al., 2003; Rossi et al., 2003). Starch based supplements such as wheat and rice bran, sugarcane molasses, millet, rye, maize, and corn flour are added to the mix (İlbay, 1994; Royse, 1996; Royse, 1997; Fomina et al., 1999; Kalberer, 2000; Rossi et al., 2003).

A widely used "standard" substrate formula is 80% hardwood sawdust and 20% supplements on a dry weight basis (Royse, 1985; Miller and Jong, 1987). Some formulations, with all ingredients based on oven dry substrate weight, consisting of 80% sawdust and 20% bran in Asia

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(Lizuka and Takeuchi, 1978); 80% sawdust, 10% bran and 10% wheat or millet in the USA (Royse, 1985; Miller and Jong, 1987); and 84% sawdust, 5% rice bran, 5% wheat bran, 3% soybean and 3% lime in Taiwan (Kalberer, 1987) are commonly used for *L. edodes* cultivation as standard substrates. Swiss researchers reported that the mixture of 75% spruce sawdust, 24% wheat bran and 1% lime could be used for the successful cultivation of *L. edodes* (Kalberer, 1987).

Hazelnut is one of the major agricultural products of the Black Sea region in Turkey. The production of hazelnut in 2004 was about 425 Mt (FAO, 2005). Hazelnut processing produces large quantities of husk and shell waste. They are burnt in stoves for house heating, also causing pollution. It has been thought that these agricultural wastes could be used for *L. edodes* cultivation.

The objective of this study was to investigate the possibility of using hazelnut husk as a new basal ingredient for substrate preparation in *L. edodes* cultivation. Some characteristics of substrates prepared by hazelnut husk alone, and its mixtures with wheat straw, beech wood-chip and wheat bran in different ratios were compared, including their effects on spawn run time, days to first harvest (earliness), yield and biological efficiency.

2. Methods

Hazelnut husk (HH) was supplied by hazelnut growers in the vicinity of Samsun province in Turkey. Air-dried HH in 3.0–3.5 cm size was used to prepare substrates without chopping into pieces. Particle size of wheat straw (WS) was less than 10 cm and beech wood-chip (sawdust) (BWC) was 0.5–4.0 cm long. Homogeneous substrate mixtures were prepared by mixing component materials based on their dry weight (w/w) (Table 1).

The substrate mixtures were wetted for two days to raise their moisture content to 60–65%. The mixed substrate (1 kg wet wt) was put in unused heat resistant polypropylene bags (28×42 cm) and sterilized in an autoclave at 121 °C for 1.5 h. After sterilization, moisture, pH, ash, carbon (C) and total nitrogen (N) content of substrates were determined (Kacar, 1994) and C:N ratios were calculated. Mineral (K, P, Ca, Mg, Fe, Cu, Mn and Zn) contents of substrates were also determined in ash by atomic absorption spectrophotometer (Kacar, 1994). Spawn of *L. edodes* (Berk.) Pegler used in the experiment were supplied by Agromycel Company, Denizli, Turkey. The sterilized substrates were inoculated with 0.8% (w/w) spawn, and incubated at 22 ± 2 °C in darkness. After the mycelium colonization was completed, the bags were exposed to daylight for a 10 h photoperiod to promote mushroom formation at 18 ± 2 °C room temperature and 80–90% relative humidity in a controlled room. After each flush of mushrooms was harvested, synthetic logs were re-soaked to increase log weight to 0.9 kg. Spawn run time, earliness (days to first mushroom harvest) and total mushroom yield were also determined. Mushroom yields were obtained from two or three flushes in a harvest period of 120 days. The biological efficiency percentage (BE) was calculated using the substrate dry weights as follow: ([weight of fresh mushrooms harvested/substrate dry matter content] $\times$ 100) (Royse, 1985).

The experiments were designed in a Completely Randomized Plots with six replications. The data obtained from the experiment were subjected to analysis of variance and means showing statistical significance were compared by Duncan's multiple range tests using the MSTATC statistical computer program. Correlation analyses were carried out to determine the relationships among chemical constituents of the substrates. Correlation coefficients (R) between chemical constituents of the substrates and spawn run time, earliness, yield and BE were also computed.

3. Results

Moisture content, some chemical properties of substrates such as, pH, ash, C and N contents, and C:N ratios are given in Table 2. Substrates varied for moisture content and pH values ($P < 0.05$). The highest moisture content was determined in 60BWC:20WS:20WB, followed by 75HH:25WS. Significant differences ($P < 0.01$) were found among substrates regarding ash, C and N contents, and C:N ratio. In general, ash content of substrates prepared by the mixtures of HH with BWC was lower than the

Table 1
Materials used for substrate prepared and their mixing ratios

Substrates and mixing ratio	Symbol
Hazelnut husk	HH
25% hazelnut husk:75% wheat straw	25HH:75WS
50% hazelnut husk:50% wheat straw	50HH:50WS
75% hazelnut husk:25% wheat straw	75HH:25WS
75% hazelnut husk:15% wheat straw:10% wheat bran	75HH:15WS:10WB
25% hazelnut husk:75% beech wood-chip	25HH:75BWC
50% hazelnut husk:50% beech wood-chip	50HH:50BWC
75% hazelnut husk:25% beech wood-chip	75HH:25BWC
75% hazelnut husk:15% beech wood-chip:10% wheat bran	75HH:15BWC:10WB
80% beech wood-chip:10% wheat bran:10% millet (control-1)	80BWC:10WB:10M
60% beech wood-chip:20% wheat straw:20% wheat bran (control-2)	60BWC:20WS:20WB

Table 2
Moisture content and some chemical properties of the substrates prepared by HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios

Substrates	Moisture (%)	pH	Ash (%)	C (%)	N (%)	C:N (%)
HH	65.56bc*	6.80bc*	8.75a–d**	45.63a–d**	0.82ab**	58.59c**
25HH:75WS	65.46bcd	6.93ab	12.87ab	43.57cd	0.58bcd	75.38bc
50HH:50WS	64.80bcd	6.78bc	11.85abc	44.08bcd	0.71abc	62.48c
75HH:25WS	66.47ab	6.88ab	9.79abc	45.11bcd	0.90a	50.43c
75HH:15WS:10WB	63.09bcd	6.88ab	8.99a–d	45.51a–d	0.90a	50.60c
25HH:75BWC	63.05bcd	6.85bc	4.58d	47.72a	0.41d	117.82a
50HH:50BWC	62.37cd	6.75bc	4.71d	47.65a	0.48cd	99.43ab
75HH:25BWC	64.31bcd	6.65c	8.57bcd	45.72abc	0.76abc	61.51c
75HH:15BWC:10WB	61.93d	7.08a	7.50cd	46.26ab	0.86ab	53.79c
80BWC:10WB:10M	62.22cd	6.90ab	13.37a	43.32d	0.89a	48.82c
60BWC:20WS:20WB	69.94a	6.73bc	7.79cd	46.11ab	0.50cd	93.19ab

* Significant at 0.05 level.
** Significant at 0.01 level (means followed by the same letters within the same column are not significantly different according to Duncan's Multiple Range Test).

others. The C contents of 80BWC:10WB:10M (control-1) and the mixtures of HH with WS were lower when compared with that of the other substrates. The N content of the substrate prepared from HH alone was 0.82%. N contents in the substrates increased with increasing rate of HH in the mixtures. C:N ratios of substrates varied between 48.82% and 117.82%. The mixture of 25HH:75BWC presented the highest C:N ratio, followed by 50HH:50BWC and 60BWC:20WS:20WB (Table 2).

Significant differences were found among K, P, Ca, Mg and Mn contents of the substrates ($P < 0.01$), while Fe, Cu and Zn contents of the substrates were not different. Increasing rate of HH in the mixtures increased K content of the substrates. P content in both control substrates was higher than that in the others. Increasing HH contents in the substrates caused increases in Ca contents in HH–BWC mixtures and decreased Ca contents in HH–WS mixtures. The highest and the lowest Mg contents were obtained from HH used alone and the mixture of 25HH:75BWC, respectively (Table 3).

Spawn run times were shorter in control substrates and mixtures of HH with BWC when compared with the mixtures of HH with WS and HH alone. Substrates differed for earliness, which ranged from 77.00 to 129.00 days ($P < 0.01$). Yields varied between 150.77 and 233.92 g kg^{-1} substrate. The highest yield was obtained from the mixture of 60BWC:20WS:20WB (control-2). Yields from HH, 75HH:25BWC, 75HH:25WS and 25HH:75BWC were lower. BE was affected by the substrate mixtures ($P < 0.01$) and varied between 43.73% in HH alone and 87.73% in 60BWC:20WS:20WB (control-2) (Table 4).

Correlations between ash and C content, ash and C:N ratio, and N content and C:N ratio were negative and significant ($P < 0.01$). There was a positive relationship between N and ash content, N and Mg content, N and Mn content, C and Cu, P and Fe content, K and Mn content ($P < 0.05$). Positive and strong relationships were found between C content and C:N ratio, K and Mg content, P and Zn content, and Mg and Mn content ($P < 0.01$) (Table 5).

Negative relationships were found between Zn content of substrates and spawn run time, Mg content and earliness ($P < 0.05$). Mushroom yield was positively correlated with P and Zn content ($P < 0.01$). The highest P and Zn con-

Table 3
Chemical composition of the substrates prepared by HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios

Substrates	K (%)	P (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
HH	3.381a**	0.005d**	0.731b**	0.168a**	438.208	8.138	83.690cd**	10.273
25HH:75WS	1.700e	0.007c	0.514bc	0.109f	374.095	7.951	77.033d	11.231
50HH:50WS	2.270c	0.005d	0.533bc	0.112e	573.894	9.932	68.108d	9.932
75HH:25WS	2.187cd	0.003d	0.216d	0.105g	466.226	11.060	80.558cd	9.532
75HH:15WS:10WB	2.537bc	0.008c	0.610b	0.150b	587.237	9.924	124.956b	12.800
25HH:75BWC	0.887fg	0.003d	0.302cd	0.061i	425.717	11.154	35.130e	14.501
50HH:50BWC	1.150f	0.004d	0.536bc	0.075h	462.454	11.361	77.242d	12.033
75HH:25BWC	1.825de	0.008c	0.637b	0.118d	728.618	8.591	142.562a	11.491
75HH:15BWC:10WB	2.723b	0.009c	0.556bc	0.148c	450.659	13.099	142.855a	13.150
80BWC:10WB:10M	0.165h	0.011b	1.371a	0.109f	455.308	6.397	95.507c	12.844
60BWC:20WS:20WB	0.606g	0.019a	0.581b	0.116d	861.282	12.179	40.067e	17.319

** Significant at 0.01 level (means followed by the same letters within the same column are not significantly different according to Duncan's Multiple Range Test).

Table 4
Spawn run time, earliness, yield, and biological efficiency (means of six replicates) of the substrates prepared by HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios

Substrates	Spawn run time (days)	Earliness (days)	Yield (g kg^{-1} substrate)	BE (%)
HH	51.83ab**	78.83b**	169.24b**	43.73c**
25HH:75WS	56.00a	92.17b	188.15ab	62.24b
50HH:50WS	57.00a	85.17b	180.80ab	51.46bc
75HH:25WS	59.00a	86.67b	153.33b	51.29bc
75HH:15WS:10WB	53.67a	77.00b	178.75ab	48.72bc
25HH:75BWC	44.83bc	129.00a	150.77b	55.91bc
50HH:50BWC	46.00bc	87.17b	197.45ab	54.25bc
75HH:25BWC	45.83bc	82.83b	154.02b	50.32bc
75HH:15BWC:10WB	45.00bc	82.67b	202.96ab	56.41bc
80BWC:10WB:10M	38.83c	95.33b	176.78ab	53.67bc
60BWC:20WS:20WB	45.67bc	80.33b	233.92a	87.73a

** Significant at 0.01 level (means followed by the same letters within the same column are not significantly different according to Duncan's Multiple Range Test).

Table 5
Correlations (R) among chemical constituents of the substrates prepared by HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios

Properties	pH	Ash	C	N	C:N	K	P	Ca	Mg	Fe	Cu	Mn	Zn
Moisture	−0.242	0.197	−0.197	−0.071	0.030	−0.043	0.468	−0.002	0.105	0.570	0.125	−0.485	0.213
pH	–	0.166	−0.165	0.327	−0.291	0.214	−0.089	−0.254	0.233	−0.619*	0.161	0.250	−0.031
Ash	–	–	−1.000**	0.492*	−0.621**	0.020	0.162	0.121	0.306	−0.104	−0.679*	0.152	−0.394
C	–	–	–	−0.491*	0.620**	−0.020	−0.163	−0.122	−0.306	0.104	0.680*	−0.152	0.394
N	–	–	–	–	−0.964**	0.505	−0.041	0.171	0.696*	−0.106	−0.297	0.700*	−0.474
C:N	–	–	–	–	–	−0.502	−0.029	−0.262	−0.720*	0.055	0.370	−0.695*	0.524
K	–	–	–	–	–	–	−0.385	−0.290	0.679**	−0.157	0.107	0.452*	−0.466*
P	–	–	–	–	–	–	–	0.399	0.252	0.469*	0.165	−0.028	0.686**
Ca	–	–	–	–	–	–	–	–	0.309	−0.054	−0.458*	0.251	0.103
Mg	–	–	–	–	–	–	–	–	–	0.073	−0.058	0.560**	−0.095
Fe	–	–	–	–	–	–	–	–	–	–	0.124	−0.034	0.222
Cu	–	–	–	–	–	–	–	–	–	–	–	−0.043	0.370
Mn	–	–	–	–	–	–	–	–	–	–	–	–	−0.198

* Significant at 0.05 level.
** Significant at 0.01 level.

tents were determined in 60BWC:20WS:20WB, which gave the highest yield (Table 3 and Table 4). BE showed positive significant correlations with moisture, P and Zn contents of substrates ($R = 0.638^{*}$, 0.784^{**} and 0.776^{**}, respectively) (Table 6).

Table 6
Correlations (R) between chemical constituents of the substrates and spawn run time, earliness, yield and BE

Properties	Spawn run time (days)	Earliness (days)	Yield (g kg^{-1} substrate)	BE (%)
Moisture	0.355	−0.278	0.193	0.638*
pH	0.034	0.114	−0.028	−0.109
Ash	0.298	−0.278	0.003	−0.084
C	−0.298	0.279	−0.003	0.084
N	0.189	−0.528	−0.221	−0.527
C:N	−0.268	0.630*	0.139	0.450
K	0.600	−0.491	−0.180	−0.547
P	−0.425	−0.340	0.581**	0.784**
Ca	−0.237	−0.618	0.098	−0.038
Mg	0.203	−0.732*	0.089	−0.211
Fe	−0.147	−0.394	0.320	0.556
Cu	0.021	0.007	0.346	0.385
Mn	−0.089	−0.508	−0.114	−0.467
Zn	−0.619*	0.231	0.455**	0.776**

* Significant at 0.05 level.
** Significant at 0.01 level.

4. Discussion

The influence of physical and chemical properties of the substrates on mushroom production characters and especially on mycelium growth, yield and BE has been emphasized in recent studies (Philippoussis et al., 2001, 2002; Rossi et al., 2003; Obodai et al., 2003). In the framework of the present study, moisture contents determined in the mixtures of HH with WS were higher than that in the mixtures of HH with BWC. This can be attributed to the greater water holding capacity of WS compared with BWC. Water availability during shiitake cultivation is a very important factor influencing growth and fruit body production (Ohga, 1999a). The optimum moisture content was suggested between 55% and 70% before heat treatment (Royse, 1985; Miller and Jong, 1987; Przybylowicz and Donoghue, 1988). In this study, the moisture contents of substrates were in agreement with this range.

Shiitake mycelium grew best at pH 4.5–6.0 (Liao, 1993). Although pH values in this study were higher than the mentioned pH ranges, mycelium growth was not affected by high pH level. The pH values of sawdust based cultures varied from 6.3 (before inoculation) to 4.0 (during the fruiting stage) (Ohga, 1999b). Philippoussis et al. (2003) reported that the initial pH values ranged from 6.2 to 6.91 in substrates of oak wood sawdust, wheat straw and corn cobs. Our pH values were in comparable to those previously reported (Ohga, 1999b; Philippoussis et al., 2003).

As concerns nutrition, *L. edodes* demonstrates biodegradation ability on cellulose and lignin, but the degree of benefit from them varies depending on carbohydrate content of organic materials (Royse, 1985). N contents of the mixtures of HH with WS were higher than that of HH with BWC mixtures due to initial N content of ingredients used for substrate preparation. For the present study, initial N content of WB, HH, WS and BWC were 2.17%, 0.78%, 0.59% and 0.11%, respectively (not shown in Tables). Boyle (1998) reported that N availability limits growth rate of wood decaying fungi, while Philippoussis et al. (2002, 2003) demonstrated that low N content of the waste substrates is a growth depressing factor in shiitake cultivation. However, in *L. edodes* cultivation on synthetic media, supplementary nutritive materials should be used at low rates. Otherwise, the risk of infection in the growing media increases (Schunemann, 1988). In a relevant experiment, İlbay (1994) detected more contamination in the substrates containing N at the rate of 1.5–2.0% than at the rate of 1%.

The C:N ratio decreased when the amount of HH increased in the mixtures of HH with WS and BWC (Table 2). This could be explained by increases in the N content of substrates. The C:N ratio depends on the availability and the concentration of both the C and N sources (Kalberer, 2000). C:N ratio influenced the beginning of fructification and the mushroom yield on synthetic media in *Lentinus tigrinus* cultivation (Eul and Schwantes, 1984).

Spawn run time varies depending on mushroom genotype and C:N ratio of substrates (Philippoussis et al., 2001, 2002; Obodai et al., 2003). C:N ratios determined in the present study were higher than those of Oei (1991). The obtained data indicating that spawn run time varies depending on materials used in the substrates mixtures were consistent with previous relevant studies (Zervakis et al., 2001). Plant materials with low C:N ratios decayed more rapidly than those with high C:N ratios, indicating that mycelium extension rate is related to the bioavailability of nitrogen (Carreiro et al., 2000; Philippoussis et al., 2003). Spawn run time for the mixtures of HH with WS and HH alone was longer than that for the others. In a previous work, Philippoussis et al. (2002) reported that substrate type and porosity exercises considerable influence on spawn run time. From our results it is reasonable to assume that the prolongation of spawn run time could be resulted from the larger particles of HH and WS wastes compared with BWC. Han et al. (1981) determined that *L. edodes*, a wood-decaying fungus, obtains its nutrients from compounds in cell walls, but the time of cell wall break down by enzymes and the degree of enzyme destruction varied among tree species. The length of this time is also influenced by the shiitake strain used, the substrate formula, the amount of substrate available, the spawning rate, the spawn distribution and the temperature during incubation (Royse and Bahler, 1986; Philippoussis et al., 2003). Some researcher informed that spawn run time varied between 40 and 90 days (Kaur and Lakhanpal, 1995; Kawai et al., 1997; Rossi et al., 2003).

Days to first harvest found in this study (77–129 days) were similar to or longer than the values reported by Balazs and Kovacs-Gyenes (1993) (60–70 days) and Gaitan-Hernandez and Mata (2004) (81–117 days), while they were shorter than values found by İlbay (1994) (100–171 days). Successful fruiting of *L. edodes* on artificial media and on amended hardwood sawdust has been achieved 3–4 months after substrate inoculation (Tan and Moore, 1992; Pacumbaba and Pacumbaba, 1999).

Fomina and Lysenkova (1989) found that oak sawdust + rice husks (4:1) and oak sawdust + chaff (10:1) were the most suitable substrates, and strain 47 cultivated on oak sawdust + rice husks gave 50–60 g fruiting bodies per 100 g of substrate. A yield of 540 g mushroom kg^{-1} dry substrate was obtained from a mixture of oak sawdust with wheat bran at a ratio of 12.5% (Morales and Martines, 1990). The mushroom yields obtained from the present study were similar to or lower than those reported by Balazs and Kovacs-Gyenes (1993) (200–250 g kg^{-1}). At the end of the 120 days harvest period, mushroom yields obtained from the substrates prepared from different tree sawdust and wheat straw varied between 5.5 and 250.5 g kg^{-1} substrate. Substrates of oak sawdust, beech sawdust and wheat straw with supplemental wheat bran gave 115.5–322.0 g kg^{-1} substrate at the end of the 150 days harvest period (İlbay, 1994). Kaur and Lakhanpal (1995) determined that eucalyptus + populus sawdust gave the highest yield (360 g kg^{-1} dry substrate) and BE (36%), followed by populus sawdust (60 g kg^{-1}, BE 6%).

BE was significantly affected by the interaction between genotype, spawn run time, and substrate formulation (Royse and Bahler, 1986). The biodegradation efficiency of different substrates varies for *L. edodes* mushroom varieties. BE of substrates ranged from 46% to 100% in many studies (Royse, 1985; Przybylowicz and Donoghue, 1988; Kalberer, 1989; Triratana and Tantikanjana, 1989; Oei, 1991; Levanon et al., 1993a,b; İlbay, 1994; Sobal et al., 1997; Leifa et al., 1999; Royse and Sanchez-Vazquez, 2003). BE values of shiitake strain IE-40 cultivated on pineapple crown bracts, sugarcane leaves and sugarcane bagasse were 37.5%, 82.7% and 130.2%, respectively (Salmones et al., 1999). Pire et al. (2001) informed that the BE was between 2.0% and 60.4%, using sterilized and supplemented wood shavings with incubation periods of 30–90 days. The BE values obtained by Morais et al. (2000) (18.9–59.5%) and Gaitan-Hernandez and Mata (2004) (24.8–55.6%) were lower than reported in this study. The yield and BE values found in the present study were acceptable when compared with those mentioned above.

A positive and significant correlation was found between days to first harvest (earliness) and C:N ratio of the substrates. This was confirmed by Philippoussis et al. (2001) stated that a positive correlation of mycelial growth to low C:N ratio values of substrates used for the cultivation of *Pleurotus* spp. has been demonstrated as well. Minerals such as Mg, Ca, Fe, Cu, Mn, Zn and Mo are required by fungi for growth (Jenning, 1995). Study results revealed that P and Zn contents of the substrates were strongly related with BE.

5. Conclusions

Mushroom yield and BE in the HH alone substrate was considerably lower than in the controls (80BWC:10WB:10M and 60BWC:20WS:20WB), and decreased with the increase in the rate of HH in the mixtures. In contrast, most of the mixtures including HH were not different from the controls and gave high yield when they compared for yield. Study results showed that mixtures of HH with WS or BWC could be used as a new, practical and abundantly available substrate material in *L. edodes* cultivation. Nevertheless, different mixture combinations and mixing ratios of HH and WS or wood-chip or sawdust, and additive materials should be investigated in more detail to increase the mushroom yield and quality. Furthermore, examining the effect of HH particle size on water holding capacity of substrate, spawn run time, yield and BE will be useful.

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