Leaflet No.14/2019

The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation
Forest Department

Study on the Cultivation of *Agaricus blazei* (Almond Mushroom) Grown on Compost Mixed with Selected Agro-residues

Tun Tun Win, Staff Officer,
Forest Research Institute
and
Shoji Ohga, Professor, Forest Production Control Laboratory, Kyushu University

December, 2019

Contents

ဆေးဖက်ဝင်လှသော အပူပိုင်းဒေသ မှိုတစ်မျိုးဖြစ်သည့် *Agaricus blazei* (Almond Mushroom) အား မြေဆွေး၊ သစ်တောစိုက်ပျိုးရေးစွန့်ပစ်ပစ္စည်းများ အသုံးပြု၍ စိုက်ပျိုးနိုင်မည့်နည်းလမ်းများအား ဖော်ထုတ်လေ့လာခြင်း

ထွန်းထွန်းဝင်း၊ ဦးစီးအရာရှိ၊ သစ်တောသုတေသနဌာန
Shoji Ohga, Professor, Forest Production Control Laboratory, Kyushu University

စာတမ်းအကျဉ်း

ဤသုတေသနစာတမ်းသည် ဂျပန်နိုင်ငံ၊ ကျူရှူးတက္ကသိုလ်၊ Forest Production Control Laboratory မှ *Agaricus blazei* (KUMB 1221) မှိုမျိုးကို သစ်တော စိုက်ပျိုးရေး စွန့်ပစ်ပစ္စည်းများဖြစ်သည့် သစ်တိုသစ်စများ၊ လွှာစာမှုန့်၊ ပြောင်းဖူးရိုးများနှင့် မြေဆွေးကို ၃:၁၊ ၁:၁၊ ၁:၃နှင့် ၁:၀ အချိုးများအလိုက် ရောစပ်ကာစိုက်ပျိုးအသုံးပြုနိုင်မည့် နည်းလမ်းများကို ဖော်ထုတ်လေ့လာထားသည့် စာတမ်းဖြစ်သည်။ မှိုမျှင်ကြီးထွားလာမှုများကို တိုင်းတာခြင်းနှင့် ကြာချိန်မှတ် သားခြင်း၊ မှိုများစတင်ထွက်ရှိသည့် အချိန်ကိုမှတ်သားခြင်း၊ မှိုမျှင် အရောင်ခွဲခြား တိုင်းတာခြင်း၊ မှိုရိတ်သိမ်းနိုင်သည့် အချိန်ကိုမှတ်သားခြင်း၊ မှိုအရေအတွက်၊ အရွယ်အစားနှင့် အလေးချိန် (အစို/အခြောက်)များအားမှတ်သားခြင်း၊ ဇီဝဆိုင်ရာ ထိရောက်မှုအား တွက်ချက် ခြင်းနှင့် မှိုထုတ်လုပ်နိုင်မှုအား တွက်ချက်ခြင်းများကို စမ်းသပ်လေ့လာ ဆောင်ရွက်ခဲ့ပါသည်။ မှိုများစတင် ထွက်ရှိသည့်ကြာချိန်နှင့် မှိုရိတ်သိမ်းနိုင်သည့် အဆင့်ထိ ရောက်ရှိကြာချိန်များကို နှိုင်းယှဉ်လေ့လာရာ၌ လွှာစာမှုန့်နှင့် မြေဆွေး ရောစပ်ထားသည့် စမ်းသပ်မှုများသည် သစ်တို သစ်စ၊ ပြောင်းဖူးရိုးနှင့် မြေဆွေးရောစပ်ထားသည့် စမ်းသပ်မှုများထက် နှေးကွေးကြောင်းကို တွေ့ရှိခဲ့ရပါသည်။ မြေဆွေး အချိုးအစားများစွာ ပါဝင်သည့် substrates များသည် ပိုမို ကောင်းမွန်သော အထွက်နှုန်းကို ပေးစွမ်းနိုင်ကြောင်းတွေ့ရှိရသည့် အပြင်အခြားသော စွန့်ပစ် ပစ္စည်းများဖြင့် အသုံးပြုထားသော စမ်းသပ်မှုများသည်လည်း ထိုက်သင့်သော အထွက်နှုန်းများကို ဖြစ်ပေါ်စေကြောင်း တွေ့ရှိခဲ့ရပါသည်။ ယင်းကဲ့သို့ ရလာဒ် များ ကွဲပြားခြားနားနေခြင်းမှာ အသုံးပြုသော substrates အမျိုးမျိုး၏ ရူပဓါတု ပါဝင်မှုများ ကွဲပြားခြင်းကြောင့် သော်လည်း ကောင်း၊ ကာဗွန် နိုက်ထရိုဂျင် အချိုး အစား ကွဲပြားမှု၊ pH နှင့် အခြားသော ပတ်ဝန်းကျင်ဆိုင်ရာ အကြောင်းအရာများကြောင့် ဖြစ်နိုင်ကြောင်း လေ့လာတွေ့ရှိခဲ့ရပါသည်။ သို့ဖြစ်ပါ၍ ယခု စာတမ်းတွင် အသုံးပြုသောမြေအမျိုး အစားဆိုင်ရာ သုတေသနများ၊ အခြားသော စွန့်ပစ် ပစ္စည်းများဖြင့် အသုံးပြုခြင်း ဆိုင်ရာသုတေသနများ၊ စမ်းသပ်မှုများပြင်ဆင်ရာတွင် ထည့်ဝင်သော ပင်ပိုင်းပစ္စည်း အချိုးအစားဆိုင်ရာ နှင့် မှိုမျိုးစိတ် (strain) ရွေးချယ်ခြင်းဆိုင်ရာ သုတေသနလုပ်ငန်းများကို ဆက်လက်ဆောင်ရွက်ရန် လိုအပ်နေသေးကြောင်း အကြံပြု သုံးသပ် တင်ပြထားပါသည်။

Study on the Cultivation of *Agaricus blazei* (Almond Mushroom) Grown on Compost Mixed with Selected Agro-residues

Tun Tun Win, Staff Officer,

Forest Research Institute

and

Shoji Ohga, Professor, Forest Production Control Laboratory, Kyushu University

Abstract

The *Agaricus blazei* strain (KUMB 1221) from Forest Production Control Laboratory, Kyushu University was grown on the basal media of compost mixed with selected agro-residues (sawdust, woodchips and corncob) in the ratio of 25, 50, 75 and 100% (by weight). The data was collected on the colony diameter of mycelial growth, days required for spawn run (colonization), days required for primordial formation, whiteness measurement, days required for fruit body formation, number, size and fresh weight of mushrooms, biological efficiency (BE) and mushroom production (MP). It was observed that the mycelial growth showed two types of mycelia according the level of mycelial density; compact (C) and somewhat compact (SC). The fastest spawn run (17 days) was found in woodchips (50%) among all the different treatments while the least spawn run took 26 days in corncob (75%). Sawdust substrates promoted longer days for primordial formation and fruit body development, if compared with woodchips substrates and corncob substrates. With regard to yield, 100%, 75% and 50% mixture with compost were superior to 25% mixture with compost in each group of selected agro-residues and it indicated that adding more compost gave the increased yield. Interestingly, it was noted that mushroom size on compost (100%) was double to the mushroom size of other treatments. In conclusion, it was clearly showed that compost (100%), woodchips (25%) and corncob (25%) could produce better yields among all treatments, and it was also possible to obtain acceptable yields of good quality almond mushroom using main substrates of compost mixed with different agro-residues at various concentrations. Despite the observed differences among substrates, the mushroom yield obtained is still low to be economically practical in this study. The yield of the production should be improved to reach a commercial scale. Attempts to improve the yield depend on several factors such as casing soil, casing technology, optimal substrate formulations, particle size, spawn type, supplement type and the inoculum rate, the environmental factors and selection of the strains.

Keywords: *Agaricus blazei*, Agro-residues, Compost, Mushroom cultivation, Substrate

Study on the Cultivation of *Agaricus blazei* (Almond Mushroom) Grown on Compost Mixed with Selected Agro-residues

1. Introduction

Mushrooms are an important product in global trade with an estimated production of 3.4 million metric tons in 2007 (Linde et al., 2014). They have attracted the attention of the biotechnological industries as food supplements or materials for developing medicines (Mizuno et al., 2002). The mushroom species of *Agaricus blazei* (Cogumelo do sol), *Agaricus bisporus* (Champignon), *Pleurotus sajor-caju* (Oyster mushroom) and *Lentinula edodes* (Shiitake) are of important economic value on account of their exquisite flavor and proven medicinal properties (Silva et al., 2009). The *A. blazei* is naturally found on organic litter which has already been occupied by the first stage decomposers capable of digesting complex lignocellulosic components (Gregori et al., 2008). It has been referred to by various names, in China as Gee Song Rong or Brazilian mushroom, in Brazil as mushroom of God (Cogumelo de Deus) or mushroom of the Sun (Cogumelo do Sol) (Gregori et al., 2008) and Himematsutake in Japan (Stijve et al., 2004). Recently, it was discovered that both mycelium and fruiting bodies of almond mushroom unusually have high concentrations of beta glucans, immune-potentiating polysaccharides, which also inhibit the growth of malignant tumors (Stijve et al., 2004). Then, the popularity of this mushroom is increasing rapidly.

Nowadays it is also cultivated at the industrial level in Japan, China, Taiwan and Korea (Llarena-Harnández et al., 2014). With regard to its production level, it is still very low compared to *A. bisporus* (De Siqueira et al., 2009), thus, it is needed to develop substrate meeting requirements of almond mushroom and to produce higher biological efficiency. A cheap and stable source for commercial purpose is still sought. Stamets (1993) reported that each mushroom species thrives on a limited range of substrates and strains within a species are even more specific in their habitat requirements, temperature preferences, and their flushing intervals. Hence, it is still necessary to develop a proper technology and adapt its each species requirements for cultivation due to different ecological conditions of the countries. Even though horse manure with combination is used as basal media for cultivation, several researchers have modified the composition of the substrates by using different agro-residues compost to increase its yield. There is no standard formulation for substrate compositions and on the other hand, few scientific studies have still compared the effectiveness of different substrates on the productivity of the almond mushroom. Therefore, this study was conducted to investigate better commercial cultivation of *A. blazei* through revealing better biological efficiency and productivity by using main compost mixed with selected agro-residues.

2. Literature Review

Cultivation of edible mushrooms in the world started during 18^{th} century in France and white button mushroom (*A. bisporus)* is the first cultivated species. The cultivation of *A. blazei* is based on a technology which was adapted from the cultivation of button mushroom due to the fact that both mushrooms show a similar behavior (De Siqueira et al., 2011; Dias, 2010). Mushroom cultivation is usually performed in six main steps; phase I composting, phase II composting, spawning, casing, pinning, and harvesting. The final result of phase I

composting is a softened, dark brown product that smells strongly of ammonia. There are two main goals of phase II composting: 1) pasteurization to kill any harmful or competitive microorganisms that may affect growth of *A. blazei* and 2) conditioning of the substrate so that it becomes selective for the growth of *A. blazei* and free of gaseous ammonia. Typically, an average mushroom crop will have three distinct flushes, with mushroom production decreasing with each successive flush (Loehr, 2010). Culturing of almond mushroom requires the spawning growth of the mycelium on a solid culture medium, and subsequent casing of the mycelium with suitable soil to stimulate fruiting body formation. The composition of the culture medium, i.e., compost, provides nutrients for *A. blazei* mycelium growth and also effects the later production of the fruiting body (Chu et al., 2012). Cultivation of almond mushroom provides the opportunity to utilize renewable resources in the production of edible protein rich food that will sustain food security for people in the developing countries through bioconversion of lignocellulosic residues.

2.1 Composting

A. blazei is a secondary decomposer and cannot grow directly on cellulose and lignin present in the straw, requiring a previous degradation of the substrate through a composting process (Gern et al., 2010). Composting is a necessary process to obtain a selective and appropriate substrate for the cultivation of mushrooms that belong to the genus *Agaricus* (De Siqueira et al., 2011). In order to obtain good yields, it is not only necessary to consider the composition and condition of the compost, but also the quantity used in preparing the mushroom bed (Stoller, 1943). The composting process is time-consuming, energy-intensive. Compositing and substrate preparation may account for up to 40% of costs involved in mushroom production (Loehr, 2010). *A. blazei* has normally been produced in solid cultures using substrates such as grain, sawdust, or wood.

2.2 Supplementation

Most mushroom growers use supplements to increase production. Any nutrient added after phase I composting is termed as supplement (Loehr, 2010). Supplementing the substrate is a common method to increase productivity, which is evaluated by biological efficiency (Alam et al., 2010). *Agaricus* species need nitrogen rich humus like substrates obtained by composting (Zied et al., 2011). Lignocellulosic materials are generally low in protein, which is insufficient for commercially cultivating mushrooms. These materials require different supplements or additives with sufficient amounts of nitrogen, phosphate, potassium, and vitamins for better growth and yield of mushrooms (Alam et al., 2010). Since almond mushroom is a fungus, an organic source of carbon is needed to supply. Organic nitrogenous materials are preferable to inorganic because they serve also as a source of carbon, contain some K_2O and P_2O_5, and have a better heating capacity (Stoller, 1943). The optimum carbon to nitrogen (C/N) ratio is 27/1, the minimum is 16/1 and the maximum is 33/1 (Zied et al., 2011).

Many commercial mushroom supplements are available to growers today. Most modern supplements based on vegetable materials and agricultural wastes, such as soybean meal, are rich in protein, which is the most important ingredient. The practice of nutritionally supplementing compost for mushroom cultivation at the time of spawning or casing to

maximize crop yield is widely recognized and accepted but is in limited use worldwide (Zied, 2017). Rice bran is the most popular and available organic substrate additive for growing a number of edible mushrooms in Asia (Alam et al., 2010).

2.3 Casing

A. bisporus is grown in vegetable materials and *A. blazei* is grown in soil, which differs mainly according to the physical properties (Zied et al., 2011). Commercial productivity of the almond mushroom is closely related to both the quality of the compost and the choice of soil to be used as a casing layer (De Siqueira et al., 2009). The casing layer is one of the most important steps of *Agaricus* spp. for commercial production. "Casing" refers to the practice of covering spawned compost with some material such as soil, peat, spent mushroom substrate and coconut fiber. The casing technique has been used since the 17^{th} century and is essential for the formation and development of mushrooms. The absence of casing leads to a drastic reduction in mushroom yield and quality (Zied et al., 2011).

Casing is applied when the mycelium spreads into 75% of the compost (Dias, 2010). A casing layer provides the necessary environmental conditions to support physiological changes in mycelium behavior, so that the fungus goes from a vegetative growth state to a reproductive one, forming primordia (Zied et al., 2011). Although many different materials may function as a casing layer, peat is generally regarded as the most suitable. Because of its unique water holding and structural properties, it is widely accepted peat as an ideal for casing (Mami et al., 2013). The use of renewable raw materials rather than non-renewable materials is a global concern. Peat use causes the loss of non-renewable resources and participates in the greenhouse effect by the liberation of CO_2 through the aerobic decomposition of carbon, thus generating a worldwide demand for alternatives in agriculture (Largeteau et al., 2013). Table 5 shows various materials have been tested as a casing substrate throughout the world by different workers (Jarial et al., 2005).

Table 1. Commonly used casing materials in different countries

Country	**Type of casing used**
Denmark	Peat/chalk
France	Local sub-soil mixture
Holland	Black peat/ moss peat/ moss/ sand
India	Farmyard manure + loam soil/$CaCO_3$; Farmyard manure + 3 years old spent compost/ $CaCO_3$
Poland	Peat/chalk
Korea	Clay loam sub soil/hydrated lime
Russia	Peat/chalk
Taiwan	Clay loam sub soil/chalk
U.K	Moss peat/chalk
U.S.A	Local sub soil, peat/chalk

Four factors might be considered for a successful choice of casing layer: water-holding capacity, pH, structure and nutrients (Zied et al., 2011). It has been proven that sterile casing soil is not effective, so fructification is directly related with living biomass in the casing soil. However, sterile casing soil with addition of activated carbon promotes fruitification as non-sterile casing soil does (Calvo, 2010). The microflora present in the casing soil also play an important role in the mushroom fruit body initiation and development (Rattan, 2013). Jasi (2013) reported that the best casing should be from deeper horizons with a moisture content of 70-75%. Watering after casing should be done for commercial growth. Casing soil is hand watered daily with a rose-face hose attachment to maintain adequate moisture for mushroom production (Loehr, 2010).

2.4 Environmental factors

It has been reported that the mycelial growth is affected by a wide range of environmental parameters as well as the medium composition (Kim et al., 2004). The chamber is needed to provide with humidification, heating/cooling and recirculating/outside ventilation systems to allow automatic control of the relative humidity, temperature and carbon dioxide concentration. When the mycelium has a fluffy white appearance and has grown well into the upper layer of the casing soil, cooling down can start. This measure is taken in order to trigger the change from vegetative growth (mycelium) to generative growth (fruiting bodies) (Nieuwenhuijzen, 2007). *A. blazei* belongs to middle temperature mushrooms. The growth temperature for mycelium ranges from 15 to 35 º C and the optimum growth temperature ranges from 23 to 27 º C. The temperature for fruiting can be from 16 to 30 º C and the optimum is 18 to 25 º C. The preferred air humidity in a mushroom house is 60-70 % for mycelium growth and 70-85% for fruiting body formation and development. The optimum pH of the compost is 6.5-6.8 while the optimum pH of the casing soil is 7.0 (Chang, 2009). An important factor to be considered is the capacity for gas exchange of the substrate (Rossi et al., 2003).

The culture medium influences the growth of mycelium by the nutrients existing in tis composition (Ró et al., 2016). The ability of *A. blazei* to grow in a large range of nitrogen content (C:N ratios ranging from 10:1 to 50:1) allows to consider a possible mycelial growth in a large variety of raw material of agro-industrial by-products mainly with low nitrogen content (Mantovani et al., 2007). Almond mushroom is informed to have a high lacasse activity during vegetative phase (Matute et al., 2010).

2.5 Different substrate compositions

A substrate is an important substance for growing mushrooms. The production of the substrate to mushroom growth is recognized as the most critical stage of cultivation, having dramatic consequences on the yield and quality of the crop and consequently on the economic viability. The substrates are both a physical support and a source of nutrients for the mushrooms needed to complete their life cycle (vegetative and reproductive phases) (Zied et al., 2011). It is important as well to develop a substratum formulation from available local organic sources, fully taking advantage of regional by-products and also reducing transportation costs (Zaghi et al., 2010). The aim of the mushroom substrate preparation is to

produce a medium that is nutritionally sufficient and selective for the mushroom mycelium growth (Demirer et al., 2005).

The commercial production of mushrooms (*Agaricus* spp.) traditionally involves the use of a substrate obtained by composting cereal (wheat and barley) straw, the fermentation of which is activated by a nitrogen source (Pardo et al., 2007). Horse manure and its straw bedding is the standard medium in commercial mushroom culture today (Stoller, 1943). Most of the substrate formulations used in the state (of Santa Cartarina) are composed of corncobs, wheat and rice straws, or one of several other grasses (Dias, 2010). Jasi (2013) and Wang et al., (2013) reported some examples of different substrate for almond mushroom growing (Table 2).

A. blazei can be grown in good yield on recycled sawdust blocks from the end of the cultivation cycles of other mushrooms, e.g. Shiitake and *Pleurotus* (Stijve et al., 2004). Experiments performed in China showed the possible use of cottonseed hulls, rice hulls, asparagus straw and soybean cake (Largeteau et al., 2013). It is possible to obtain acceptable yields of good quality *A. blazei* using sunflower seed hulls as one of the substrate formula lignocellulosic components used to obtain compost (Matute et al., 2010).

Table 2. Examples of different substrates for almond mushroom cultivation

Iwade and Mizuno (1997) Huang (1997)	**Huang (1997)**	**Huang (1997)**
• Rice or wheat straw (357 kg) • Rice bran (10 kg) • Poultry dung (15 kg) • Calcium carbonate (8 kg) • Ammonium sulfate (10 kg) • Calcium phosphate (5 kg)	• Wheat straw (700 kg) • Cotton burrs (125 kg) • Dried cattle manure (150 kg) • Calcium sulfate (10 kg) Calcium phosphate (10 kg) • Urea (5 kg)	• Wheat straw (750 kg) • Sawdust (700 kg) • Ammonium sulfate (10 kg) • Calcium sulfate (30 kg) • Calcium phosphate (10 kg) • Urea (5 kg)
Oei (2003) Taiwan	**Oei (2003) Korea**	**Wang et al., (2013)**
• Rice chaff (850) kg • Urea (10 kg) • Ammonium sulfate (20 kg) • Calcium phosphate (30 kg) • Potassium sulfate (8 kg) • Calcium carbonate (25 kg)	• Rice chaff (850 kg) • Urea (0.5 kg) • Poultry dung (63 kg) • Calcium sulfate (3 kg) • Calcium phosphate (2 kg)	• Asparagus straw/ Maize straw (700 kg) • Cottonseed hull/ Bean straw/ Cotton straw/ Corncob (200 kg) • Soybean cake (100 kg) • Gypsum (20 kg)

3. Material and Methods

3.1 Mushroom strain / Fungal Culture

The *A. blazei* strain (KUMB 1221) was obtained from Forest Production Control Laboratory, Kyushu University. It was cultured on potato dextrose agar (PDA) previously autoclaved at 121°C for 15 min, kept at 25 °C ± 1°C in the dark and sub-cultured every three months. The inoculum was selected from the mycelium growth border with uniform appearance and with sectioning.

3.2 Compost

One-year fermented horse manure bedding compost was used as a basal ingredient for producing spawn as well as substrate preparation. The compost was directly obtained from a local livestock company (Japan Agriculture (JA), Itoshima).

3.3 Substrate Preparation, Inoculation, Incubation and Casing

The compost was mixed with selected agro-residues; sawdust (hardwood), woodchips (hardwood) and corncob, in the ratio of 25, 50, 75 and 100% (by weight) (e.g compost/sawdust 1:3, 1:1, 3:1, and 1:0). Rice bran and wheat bran were used as supplementary substances to increase mushroom yield and to achieve faster mycelial growth. Each mixture of the substrates was supplemented by 20% (w/w) while 100% compost substrate was supplemented by 50% and mixed thoroughly. To adjust moisture content (MC), pre-tests were conducted and it was found that 60% of MC was the best condition for mycelial growth. Thus, the prepared substrates were adjusted to 60% moisture with tap water and the pH of substrates was measured.

The wet substrates of 700 g were added into polypropylene bags having holes on one side of the upper part (breathing patches) and 100 g were set in the petri dish for mycelial growth measurement, then sterilized both at 121°C for 1 hr. After that, they were moved to the wind-stream of a laminar flow hood and allowed to cool to the desired inoculation temperature. 700 g of wet substrates were inoculated by inoculum (2 %) containing mother spawn and agar cultures while 5 mm disc of agar culture was inoculated for mycelium colony diameter in the petri dishes. Inoculum was placed in the middle of each petri dish and special care was taken to keep the cylinder mycelium in direct contact with each substrate.

The bags were marked by permanent marker and were kept on the shelves in the incubation room, where the temperature was maintained at 23°C, relative humidity at 80 %, CO_2 level at 1000 ppm by automatic aeration, and were allowed to compete the whitish mycelial growth. In case of the inoculated petri dishes, they were incubated at 25°C in the darkness. During this period, bags were shaken daily, so that fungal mycelia may uniformly be mixed and cover around the materials. After the substrates were completely colonized with the fungal mycelia, the bags were torn apart and cased with 3.5 - 4 cm of local soil over the surface of the substrate by hand and kept in a fruiting environment where the temperature was maintained at 18.5 - 21.5 °C, relative humidity at 85 % and having good ventilation. Casing soil was sieved (2 mm) to remove roots and stones, then it was used without heat treatment or chemical treatments. Daily moderate watering was done in order to keep the casing layer humid, not over-soaked until the end of cultivation. In order to stimulate cased substrate, the procedure of ruffling was conducted. Ruffling implies that the mycelium is mixed by lightly raking the casing soil layer, to get a more uniform mycelium growth, the ruffling procedure will break the mycelium in the casing soil layer, stimulating regrowth.

3.4 Evaluated Parameters

The data was collected on colony diameter of mycelial growth, days required for spawn run (colonization), days required for primordial formation, whiteness measurement, days required for fruit body formation, number, size and fresh weight of mushrooms, biological efficiency (BE) and mushroom production (MP).

3.4.1 Diameter Measurement of Mycelial Growth

The mycelial growth was measured after 7 days of growth for two weeks and recorded photographically at the end of experiment. Levels of mycelial density among the treatments were compared visually and categorized by T (thin), ST (somewhat thin), Co (compact) and SC (somewhat compact).

3.4.2 Spawn Run and Primordial Formation

The duration required for completion of hypha spreading varies in accordance with the volume of the medium is called spawn run and primordial formation is started from the day of casing to the day when the first pins are verified.

3.4.3 Whiteness Percentage

Color analysis is one of the most important factors to determine the mycelial quality of mushroom. Before casing, MINOLTA CR-200 was used to analyze the whiteness percentage of different substrates covered with completely colonized mycelia. Then, the data was calculated by the following formula;

$Z = (1-x-y) / y * Y$, then $Z / 1.18$

3.4.4 Mushroom Number, Size and Fresh Weight

The number, size and fresh weight were regularly determined during the harvesting phase. Average mushroom size was calculated as fresh weight of mushrooms (FWM) (g) divided by number of mushrooms per bag and expressed as g/mushroom. Mushrooms were harvested in the morning and later in the afternoon, when they reached the maximum size; the sides of the pileus were parallel and before the veil opened. After the uncut mushrooms were harvested, the base of each stipe was cleaned with a brush, so that they could be weighed without any soil pieces.

3.4.5 Biological Efficiency (BE) and Mushroom Production (MP)

Mushroom yields expressed as percentage of BE and MP. BE (%) was determined by the equation of

BE = FWM / Dry weight of substrate (DWS)*100, while MP (%) was determined by the equation of

MP = FWM / Fresh weight of substrate (FWS)*100.

3.4.6 Experimental Design and Statistical Analysis

A completely randomized design was adopted with three replicates per treatment and SATISTIX 8.1 was used to determine the differences among the averages by the analysis of variance, then the means were compared by Tukey's test ($p<0.05$).

4. Results

4.1 Effect of different substrate formulations on vegetative growth, mycelial density and whiteness percentage

The results obtained for the colony diameter measurement, mycelial density and whiteness percentage on different substrate formulations of compost mixed with selected agro-residues are given in Table 3. It was observed that the mycelial growth showed two types of mycelia according the level of mycelial density. While substrates with 75% and 50% of sawdust, 75% of corncob, and 100% of compost supported Co level of mycelial density, other remaining treatments supported a more vigorous mycelial density as SC. Healthy and outstanding mycelium is a vital part which reflects the overall growth and productivity.

At the end of experiments, the substrate containing sawdust (50%) (72.34 mm) was the best for mycelial extension followed by sawdust (75%) (62.41 mm), woodchips (25%) (62.11 mm), woodchips (75%) (61.17 mm) and woodchips (50%) (58.11 mm), sawdust (25%) (48.64 mm), compost (100%) (39.93 mm), corncob (50%) (34.28 mm), corncob (75%) (22.73 mm) and corncob (25%) (8.63 mm) respectively. There were not significant differences between woodchips and sawdust treatments, but they were significant difference from corncob treatments. Poor mycelial extension was observed in all the corncob treatments and they stood up below compost (100%).

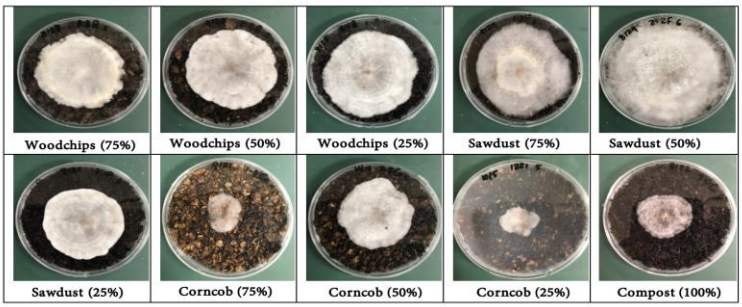

Figure 1. Comparison of mycelial growth of *A. blazei* on various concentrations of agro-residues with compost after two-week

Table 3. Mycelial growth, mycelial density and whiteness percentage of different substrate formulations

Types of Substrates	Colony Diameter (mm)		Mycelial Density	Whiteness (%)
	First Week	Second Week		
Woodchips (75%)	23.28 ± 2.23b	61.17 ± 9.20ab	SC	42.06 ± 4.17a
Woodchips (50%)	24.07 ± 1.61b	58.11 ± 12.95ab	SC	47.95 ± 3.81a
Woodchips (25%)	23.16 ± 2.82b	62.11 ± 10.12ab	SC	44.64 ± 5.22a
Sawdust (75%)	24.91 ± 3.22b	62.41 ± 7.04ab	Co	50.13 ± 4.88a
Sawdust (50%)	30.61 ± 2.31a	72.34 ± 13.94a	Co	51.06 ± 4.86a
Sawdust (25%)	25.64 ± 3.29b	48.64 ± 11.81bc	SC	47.09 ± 6.55a
Corncob (75%)	5.73 ± 0.91d	22.73 ± 5.85de	Co	43.70 ± 9.30a
Corncob (50%)	12.79 ± 5.09c	34.28 ± 18.62cd	SC	47.72 ± 8.95a
Corncob (25%)	5.10 ± 0.05d	8.63 ± 0.29e	SC	43.60 ± 5.79a
Compost (100%)	22.59 ± 2.11b	39.93 ± 8.99c	Co	50.26 ± 2.24a

Values are the mean ± standard deviation.

Means followed by the same letter in the same column are not significantly different according to Tukey's HSD.

All studied bags showed healthy white vigorous and rhizomorphic mycelia. With regard to different substrates' whiteness measurement, sawdust (50%), compost (100%) and sawdust (75%) supported the better whiteness percentage of 51.06, 50.26 and 50.13, but there were no apparent differences among all treatments (Figure 2).

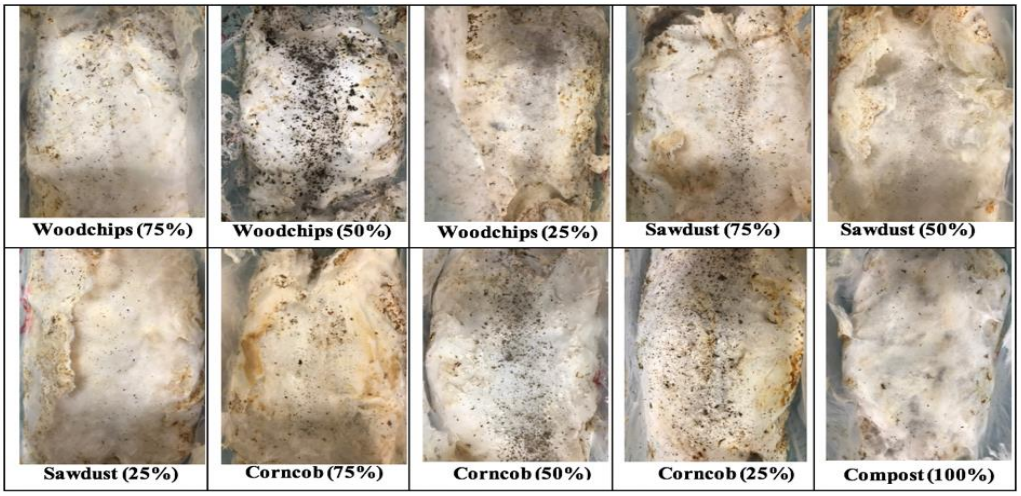

Figure 2. Physical appearance of different substrate formulations

4.2 Effect on spawn run, primordial formation and fruit body development

The duration required for spawn run, primordial formation and fruit body development depends on the substrates used and the results are presented in Table 4. The fastest spawn run (17 days) was found in woodchips (50%) among all the different treatments while the least spawn run took 26 days in corncob (75%). Except in sawdust (25%), sawdust (75%) and corncob (75%), all remaining treatments ran spawn faster than compost (100%) (22 days). Like mycelial colony diameter comparison, it was observed that substrates containing 50:50 mixture with compost (17 days in woodchips, 18 days in both sawdust and corncob) gave more rapid spawn run than other mixtures. It can be said that mycelial colony diameter was negatively correlated to spawn run in each treatment.

Table 4. Total day to spawn run, primordial formation, fruit body development on different substrates

Types of Substrates	Spawn run	Primordial formation	Fruit body development	Total day to first crop
Woodchips (75%)	21	12	18	39
Woodchips (50%)	17	14	20	37
Woodchips (25%)	20	10	15	35
Sawdust (75%)	25	19	26	51
Sawdust (50%)	18	28	35	53
Sawdust (25%)	23	28	34	57
Corncob (75%)	26	11	18	44
Corncob (50%)	18	16	22	40
Corncob (25%)	20	16	22	42
Compost (100%)	22	13	18	40

Values are the mean.

Means followed by the same letter in the same column are not significantly different according to Tukey's HSD.

Counting the total days required for primordial formation and fruit body development were started after transferring fully colonized bags into the growing incubator and casing with local soil. The primordial formation in terms of pin heads was observed in all treatments and the duration required for primordial formation was found to be variable for treatments. Sawdust substrates promoted longer days for primordial formation and fruit body development, if compared with woodchips substrates and corncob substrates. The shortest primordial formation was observed in woodchips (25%) at 10 days, then followed by 11 days, 12 days, 13 days, 14 days, 16 days, 19 days and 28 days with corncob (75%), woodchips (75%), compost (100%), woodchips (50%), corncob (50%), corncob (25%), sawdust (75%), sawdust (50%) and sawdust (25%) respectively. These results suggested that spawn run did not necessarily reflect faster primordial formation and fruiting body development. It took 5-7 days from primordial formation to fruit body development and was in the range of 35-57 days for total day to the first crop.

4.3 Effect on biological efficiency, mushroom production, weight and size

BE, MP and size of mushroom varied widely, depending on the mushroom, substrate, and conditions. Table 5 shows the results obtained for BE, MP, weight and size using different substrates in accordance with the experimental design for almond mushroom cultivation. During the reproductive stage of mushroom, two flushes were found and almost 90% yield has been harvested during the first flush. But not all bags produced in the second flush. Therefore, the yield data for only first flush was used to compare the effectiveness of different substrates in this study.

Among the conditions evaluated, the suitable substrates promoting the maximum yield in terms of weight, BE and MP were composted of compost (100%) (189.50g, BE of 62.15 %, MP of 27.07 %), woodchips (25%) (162.11g, BE of 53.17 %, MP of 23.16 %), corncob (25%) (138.40g, BE of 45.39 %, MP of 19.77 %), corncob (50%) (126.40g, BE of 41.45 %, MP of 18.06 %) and sawdust (25%) (115.20g, BE of 37.78 %, MP of 16.46 %), and they were statistically significant difference from others. Then it was followed by sawdust (50%), sawdust (75%), woodchips (50%), woodchips (75%) and corncob (75%) with a corresponding yield of 76.20g, 76.00g, 69.60g, 69.00g, and 61.00g respectively. In each group of selected agro-residues, 100%, 75% and 50% mixture with compost were superior to 25% mixture with compost and it indicated that adding more compost gave the increased yield.

Table 5. Total fresh weight, BE, MP and size on different substrates

Types of Substrates	Total fresh weight (g)	BE (%)	MP (%)	Size (g)
Woodchips (75%)	69.00 ± 2.40g	22.63g	9.86g	34.50 ± 1.20e
Woodchips (50%)	69.60 ± 3.81g	22.83g	9.94g	34.80 ± 0.75e
Woodchips (25%)	162.11 ± 4.34b	53.17b	23.16b	40.53 ± 1.09b
Sawdust (75%)	76.00 ± 2.40f	24.92f	10.86f	38.00 ± 1.20d
Sawdust (50%)	76.20 ± 2.60f	24.99f	10.89f	38.10 ± 1.30cd
Sawdust (25%)	115.20 ± 1.50e	37.78e	16.46e	38.40 ± 0.50c
Corncob (75%)	61.00 ± 2.60h	20.01h	8.71h	30.50 ± 1.30g
Corncob (50%)	126.40 ± 4.36d	41.45d	18.06d	31.60 ± 1.09f
Corncob (25%)	138.40 ± 6.56c	45.39c	19.77c	34.60 ± 1.64e
Compost (100%)	189.50 ± 3.97a	62.15a	27.07a	94.75 ± 1.98a

Means followed by the same letter in the same column are not significantly different according to Tukey's HSD.

Size of fruit bodies was significantly large on compost (100%) (94.75g) followed by woodchips (25%), sawdust (25%), sawdust (50%), sawdust (75%), woodchips (50%), corncob (25%), woodchips (75%), corncob (50%) and corncob (75%) with 40.53g, 38.40g, 38.10g, 38.00g, 34.80g, 34.60g, 34.50g, 31.60g and 30.50g respectively. Among groups, corncob gave the smallest size of mushroom and interestingly, it was noted that mushroom size on compost (100%) was double to the mushroom size of other treatments.

Figure 3. Primordial stage and fruit stage of almond mushroom

5. Discussion

Materials for composing a mushroom substrate are diverse and plentiful. The selection of the substrate components is more critical for growing gourmet mushrooms indoors than for growing outdoors (Stamets, 1993). Horm & Ohga (2008) and Pokhrel & Ohga, (2007) mentioned that substrates containing higher composts promoted better mycelial extension, but in our result, substrates containing 50% mixture with compost gave better mycelial extension when compared with other mixture of 25%, 75% and 100% except in woodchips treatments. High mineral nutrients, high moisture accumulation, accumulation of toxic products, changes in factors such as pH and rise in temperature inside the substrate could be the factors which affect mycelial growth. The particle size and pore of substrates were also responsible for the more mycelium growth rate. While the smaller particles stimulate quick growth, the larger particles encourage the mycelium to form thick and cord like strands. Substrates with larger particles present higher O_2 and lower CO_2 levels, and higher O_2 and CO_2 levels stimulate and inhibit growth, respectively (Lentinula & Spawn, 2003).

Suitable substrates lead to faster colonization and a higher density of mycelium, enhance primordial formation and fructification, and increase mushroom yield (Stamets, 1993). Mendonca et al., (2005) mentioned that spawn run usually takes 15-20 days depending on climate conditions and those results are partially similar to our findings. Pokhrel & Ohga (2007) presented that the fastest spawn run was 38 days in substrate containing 75:25 mixture (sawdust with compost) while Horm & Ohga (2008) took 31 days as the fastest spawn run in new sugarcane compost. Chu et al., (2012) and Pardo-Giménez et al., (2010) reported that the harvesting time of *A. blazei* is relatively long (approximately 60 days) when compared to other related *Agaricus* species, including *A. bisporus* (approximately 30 days). The Brazilian cultivar ABL-99/30 had a time to fruiting of 24.7-27 days after casing on sugarcane-based composts (Zied et al., 2012). Moreover, Satmets (2000) found that 18-24 days for primordial

formation after applying casing soil, and 4-8 days for fruit body development after primordial formation, which total day to the first flush was 50-72 days. Our result was supportive of those above mentioned findings, but it was longer growing cycle from casing to the first harvest than De Mendonca et al., (2005) who presented the growing cycle was around 20-21 days. Siqueira (2006) reported that 90% of mushrooms were harvested 91 days after casing. The delay fruiting is an economic problem for mushroom cultivation.

Substrates were enriched with higher content of nutrients by adding supplements, thus, it provided the maximum yield at the first flush. The compactness or poor aeration of the substrates after the first flush caused the reduction in yield. Both higher amounts of supplements and compost could provide better yield because it contained higher nutrient contents and water holding capacity (WHC). Our findings were similar to the results of Pokhrel & Ohga (2007), but opposite to the results of Horm & Ohga (2008) because they reported that higher concentrations of sugarcane compost as well as cattle compost produced lesser yield and BE. It had been reported that the BE of almond mushroom in rice or wheat straw compost was about 30-40% (24). Our BE (20.01% – 62.15%) have been lower than Pokhrel & Ohga (2007) (28.6% - 70.9%), Horm & Ohga (2008) (77.37% - 92.29 %) and Da Eira (2003) (a lower yield of 32.36%), while higher than Andrade et al.,(2007) (MP of 10.19% and BE of 33.63%). The differences in physical and chemical composition of substrate formulas, C:N ratio inside substrates, mineral contents, moisture content, pH and electrical conductivity (EC) of substrate caused the differences in terms of BE and MP on grown on different substrates. Not only sole compost with supplements but also suitable combinations of compost with agro-residues could provide acceptable yield in this study. The greatest number of fruit body and large size were seen where yield was the highest. It showed that number and size of fruit bodies and yield were positively correlated to each other. Different mushroom strain, less nutrients in compost, lower moisture in casing or compost and other environmental factors could be the reason for mushroom's size differentiation.

6. Conclusion

The *A. blazei* is an important edible mushroom and an interesting alternative one to developing countries due to its multiple advantages such as food, medicine and cosmetic uses. It was clearly showed that compost (100%), woodchips (25%) and corncob (25%) could produce higher BE and MP than others. It was also possible to obtain acceptable yields of good quality almond mushroom using substrates in different concentrations with different agro-residues; woodchips, sawdust and corncob. Furthermore, the substrates used in this study can be considered practically and economically feasible because utilization of these agro-residues is constantly available throughout the year in large quantities with low cost. However, it is impossible to produce *A. blazei* on non-composted substrates. Despite the observed differences among substrates, the mushroom yield obtained is still low to be economically practical in this study. The yield of the production should be improved to reach a commercial scale. Attempts to improve the yield depend on several factors such as casing soil, casing technology, optimal substrate formulations, particle size, spawn type, supplement type and the inoculum rate, the environmental factors and selection of the strains.

References

- Alam, N., Amin, R., Khair, A., & Lee, T. S. (2010). Influence of Different Supplements on the Commercial Cultivation of Milky White Mushroom. The Korean Society of Mycology, 38(3), 184–188.
- Calvo, M. H. (2010). Development of a growth model system for *Agaricus bisporus*, (October). Plant Breeding Department, Plant Research International Wageningen University and Research Center, Dissertation.
- Chang, S. T. (2009). Overview of Mushroom Cultivation and Utilization as Functional Foods. Mushrooms as Functional Foods, Chapter 1, 1–33.
- Chu, J. N., Young, C. C., Tan, C. C., Wu, S. P., & Young, L. (2012). Improvement of productivity and polysaccharide‐protein complex in *Agaricus blazei*, (1), 96–102.
- Colauto, N. B., Da Eira, A. F., & Linde, G. A. (2012). Cryopreservation of Agarius blazei in liquid nitrogen using DMSO as cryoprotectant. *Bioscience Journal*, *28*(6), 1034–1037.
- Colauto, N.B., da Silveria, A.R., da Eira, A.F., & G. A. L. (2011). Production flush of *Agaricus blazei* on Brazilian casing layers, *1830*, 616–623.
- Da Eira, A. F. (2003). Cultivo do cogumelo medicinal *Agaricus blazei* (Murril) ss. Heinemann ou *Agaricus brasiliensis* (Wasser et al.). CPT. Retrieved from https://books.google.co.jp/books?id=WZFIAAAACAAJ
- De Andrade, M. C. N., Kopytowski Filho, J., Minhoni, M. T. D. A., Coutinho, L. N., & Figueiredo, M. B. (2007). Productivity, biological efficiency, and number of Agaricus blazei mushrooms grown in compost in the presence of Trichoderma sp. and Chaetomium olivacearum contaminants. *Brazilian Journal of Microbiology*, *38*(2), 243–247. https://doi.org/10.1590/S1517-83822007000200010
- De Mendonca, M., Kasuya, M.C., Cadorin, A., & A. J. V. (2005). *Shiitake Cultivation Part II Mushroom for Better Life*.
- De Siqueira, F. G. (2006). Compostagem para cultivo de *Agaricus blazei*.
- De Siqueira, F. G. De, Dias, E. S., Silva, R., Tokuda, E., & Rinker, D. L. (2009). Cultivation of *Agaricus blazei* ss. Heinemann Using Different Soils As Source of Casing Materials. Victoria, 66(December), 827–830.
- De Siqueira, F. G., Martos, E. T., da Silva, E. G., da Silva, R., & Dias, E. S. (2011). Biological efficiency of *Agaricus brasiliensis* cultivated in compost with nitrogen concentrations. Horticultura Brasileira, 29(2), 157–161.
- Demirer, T., Röck-Okuyucu, B., & Özer, I. (2005). Effect of different types and doses of nitrogen fertilizers on yield and quality characteristics of mushrooms (*Agaricus bisporus* (Lange) Sing) cultivated on wheat straw compost. Journal of Agriculture and Rural Development in the Tropics and Subtropics, 106(1), 71–77.
- Dias, S. (2010). Mushroom cultivation in Brazil : Challenges and Potential for growth. Cienc. agrotec., Lavras, 34 (4), 795–803.
- Gern, R. M. M., Junior, N. L., Patrício, G. N., Wisbeck, E., Chaves, M. B., & Furlan, S. A. (2010). Cultivation of *Agaricus blazei* on *Pleurotus spp*. spent substrate. Brazilian Archives of Biology and Technology, 53(4), 939–944.
- Gregori, A., Pahor, B., Glaser, R., & Pohleven, F. (2008). Influence of carbon dioxide, inoculum rate, amount and mixing of casing soil on *Agaricus blazei* fruiting bodies yield. *Acta Agriculturae Slovenica*, *91*(2), 371–378. https://doi.org/10.2478/v 10014-008-0017-2
- Horm, V., & Ohga, S. (2008). Potential of Compost with Some Added Supplementary Materials on the Development of *Agaricus blazei* Murill. *J. Fac. Agri., Kyushu University*, *53*(2), 417–422.

- Jarial, R. S., Shandilya, T. R., & Jarial, K. (2005). Casing in Mushroom Beds - Agri. Review, 26(4), 261–271.
- Kerrigan, R. W. (2005). *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. *Mycologia*, *97*(1), 12–24. https://doi.org/10.3852/mycologia.97.1.12
- Kim, H. H., Na, J. G., Chang, Y. K., Chun, G. T., Lee, S. J., & Jeong, Y. H. (2004). Optimization of submerged culture conditions for mycelial growth and exopolysaccharides production by *Agaricus blazei*. *Journal of Microbiology and Biotechnology*.
- Largeteau, L., Ferrer, N., Llarena-hern, C. R., Regnault-roger, C., & Savoie, J. (2013). Optimization of the cultivation conditions for mushroom production with European wild strains of *Agaricus subrufescens* and Brazilian cultivars. J Sci Food Agric.
- Lentinula, S., & Spawn, E. (2003). Supplementation of sugarcane bagasse with rice bran and sugarcane molasses for shiitake. *Brazilian Journal of Microbiology*, 34 : 61-65.
- Linde, G. A., Alessandra, T., Nunes, R., Domingues, G., Figueiredo, E. F., Santin, K. C., & Colauto, N. B. (2014). Mushroom Acceptability and Consumption Intention for the Main Mushrooms Produced in Brazil, 627–632.
- Llarena-Hernández, C. R., Largeteau, M. L., Ferrer, N., Regnault-Roger, C., & Savoie, J. M. (2014). Optimization of the cultivation conditions for mushroom production with european wild strains of *Agaricus subrufescens* and brazilian cultivars. *Journal of the Science of Food and Agriculture*, *94*(1), 77–84. https://doi.org/10. 1002/jsfa.6200
- Loehr, S. M. (2010). Minimally composted substrate for the production of *Agaricus Bisporus*. Plant Pathology Department, The Pennsylvania State University, Dissertation.
- Mami, Y., Peyvast, G., Ghasemnezhad, M., & Ziaie, F. (2013). Supplementation at casing to improve yield and quality of white button mushroom. Agriculture Sciences, 4(1), 27–33.
- Mantovani, T. R. D., Linde, G. A., & Colauto, N. B. (2007). Effect of the addition of nitrogen sources to cassava fiber and carbon-to-nitrogen ratios on *Agaricus brasiliensis* growth. Canadian Jouranl of Microbiology, 53, 139–143.
- Mizuno, T. (2002). Medicinal Properties and Clinical Effects of Culinary-Medicinal Mushroom *Agaricus blazei* Murrill (Agaricomycetideae) (Review). *International Journal of Medicinal Mushrooms* (Vol. 4). https://doi.org/10.1615/IntJMedMushr .v4.i4.30
- Nieuwenhuijzen, B. van. (2007). Small-scale mushroom cultivation - 2. Agrodok-Series No.41.
- Pardo-Giménez, A., Zied, D. C., & Pardo-González, J. E. (2010). Utilización de compost agotado de champiñón como capa de coberturas en nuevos ciclos de producción. *Pesquisa Agropecuaria Brasileira*, *45*(10), 1164–1171. https://doi. org/10.1590/S0100-204X2010001000016
- Pardo, A., Perona, M. A., & Pardo, J. (2007). Indoor composting of vine by-products to produce substrates for mushroom cultivation. Spanish Journal of Agricultural Research, 5(3), 417–424.
- Pokhrel, C. P., & Ohga, S. (2007). Cattle bedding waste used as a substrate in the cultivation of *Agaricus blazei* Murill. *Journal of the Faculty of Agriculture, Kyushu University*, *52*(2), 295–298.
- Rattan, D. (2013). Use of supplements for yield imporvement of *Agaricus bisporus* Lange (Sing .) Department of Microbiology, Punjab Agricultural University, Dissertation.
- Rossi, I. H., Monteiro, A. C., Machado, J. O., & Barbosa, J. C. (2003). Supplementation of sugarcane bagasse with rice bran and sugarcane molasses for shiitake (*Lentinula edodes*) spawn production. Brazilian Journal of Microbiology, 34(1), 61–65.

- Silva, C. F., Azevedo, R. S., Braga, C., Silva, R., & Dias, E. S. (2009). Microbial diversity in a bagasse-based compost prepared for the production of *Agaricus brasiliensis*. Brazilian Journal of Microbiology, 590–600.
- Stamets, P. (2000). Growing gournmet and medicinal mushrooms. Third Edition, 208-216. Ten Speed Press. Berkeley, California, USA.
- Stamets, P. (1993). Growing gourmet and medicinal mushrooms. *Ten Speed Press*. https://doi.org/10.1017/CBO9781107415324.004
- Stijve, B. T., Angela, M., Amazonas, L. D. A., & Florestas, E. (2004). the Royal Sun *Agaricus* or Himematsutake – an Update, *22*(85), 47–50.
- Stoller, B. B. (1943). Preparation of synthetic composts for mushroom culture, 397–414
- Wang, H., Fu, Z., & Han, C. (2013). The Medicinal Values of Culinary-Medicinal Royal Sun Mushroom (*Agaricus blazei* Murrill), *2013*.
- Wang, Q., Li, B. B., Li, H., & Han, J. R. (2010). Yield, dry matter and polysaccharides content of the mushroom *Agaricus blazei* produced on asparagus straw substrate. *Scientia Horticulturae*, *125*(1), 16–18. https://doi.org/10.1016/j.scienta.2010. 02. 022
- Zaghi, L. L., Linde, G. A., & Colauto, N. B. (2010). Carbon-to-nitrogen ratios for *Agaricus brasiliensis* on the axenic method. Acta Scientiarum - Agronomy, 32(1), 55–60.
- Zied, D. C. (2017). Supplementation of High Nitrogen *Agaricus* Compost : Yield and Mushroom Quality. Journal of Agriculture Science and Technology, 19, 1–13.
- Zied, D. C., Minhoni, M. T. A., Kopytowski-filho, J., Barbosa, L., & Andrade, M. C. N. (2011). Medicinal mushroom growth as affected by non-axenic casing soil. Pedosphere, 21(2), 146–153.
- Zied, D.C., Pardo-Giménez, A., de Almeida Minhoni, M. T., Villas Boas, R. L., Alvarez-Orti, M., & Pardo-González, J. E. (2012). Characterization, feasibility and optimization of *Agaricus subrufescens* growth based on chemical elements on casing layer. *Saudi Journal of Biological Sciences*, *19*(3), 343–347. https://doi.org/ 10.1016/j.sjbs.2012.04.002