BIBLIOGRAPHY

ESTACIO, MICHAEL JAVIER. MAY 2011. Coconut Water as a Liquid Medium Base for *Lentinula*. Benguet State University, La Trinidad, Benguet.

Adviser: Dr. Bernard S. Tad-awan, Ph.D

ABSTRACT

The study was conduced at the Plant Pathology Department of College of Agriculture at Benguet State University, La Trinidad, Benguet from April 2011- June 2011. The study aimed to determine the effect of coconut water as culture medium base for liquid culture of *Lentinula sp.*

Coconut water added with different amounts of molasses and rice bean were evaluated on its effect on mycelia weight of *Lentinula sp.*

Coconut water added with 5 grams powdered rice bean and 20 grams molasses as carbon source gave the highest mycelial yield compared with plain coconut water, rice bean and molasses.

Use of Potato Dextrose Broth supplemented with 1% urea, 0.3% KH_2PO_4 and $MgSO_4$ has the highest mycelia yield compared to the use of organic media.

INTRODUCTION

Mushrooms have long been regarded as effective medicines for the treatment of various human diseases. The medicinal properties are due to various cellular components and secondary metabolites, which can be isolated and identified from the fruiting- body, culture mycelium and culture broth of mushrooms (Tang, 2007).

Mushrooms also show potential for use in waste management. However, as fungi, mushrooms have life cycles very different from those of green plants (Stamets and Chilton, 1983).

The substrate material is also important. The substrate material is also very important. The substrate serves mainly as the vehicle that carries the mycelium of the mushroom. The growth pattern of the mycelium may be influenced by the substrate, as seen by more rapid growth (Chang and Miles, 2004).

Coconut water is a healthy and one of the best drinks to hydrate. It helps to remove toxins from the body and aid digestion, coconut have anti-viral, anti-fungal and anti-microbial properties. Once exposed to air, the liquid rapidly loses most of its nutritional characteristics and begins to ferment (Anonymous, 2007). Coconut water contains a variety of nutrients including vitamins, minerals, anti-oxidants, amino acids, enzymes, growth factors and other nutrients. Over the past two decades, it's been used extensively as treatment for cholera, influenza and other infectious diseases that promote dehydration (Lee, 2008). Coconut water is a natural water filter that takes almost a month to filter each liter of water. The water travels through many fibers being purified where it is stored away sterile itself (Safron, undated).

Indoor mushroom production demands a much higher level of knowledge, continuous monitoring and timely manipulation of environmental conditions. Early production methods

involving the use of solid culture medium such as grain and manure have been reported the growth of vegetative mycelia. These methods require an undesirable extended period of time for the mycelium to attain a suitable developmental stage and are labor- intensive requiring manual transfers of aseptic materials as production is scaled up (Holtz and McCulloch, 1994).

A submerged fermentation process using liquid media reduces the time required to produce mushroom mycelia. These submerged culture technique, however is a not suitable for commercial mushroom production. The process is characterized by an increase in broth viscosity with time which can be due to one or more factors, including increase in cell concentration, changes in growth morp0hology or the production of extracellular products that are alter the character of the culture fluid (Tang, 2007).

Mycelium can be cultured in a liquid medium (as opposed to a solid like vermiculite/ brown rice flour). In using liquid medium, it is easier to inoculate substrate and start mycelial growth much faster than using spore (Biotronics, 2000).

Liquid cultures are used to expand mycelium into a liquid solution to inoculate the chosen substrate. This is similar in using a multi- spore syringe, except the spores have germinated into network. Since the spores are already germinated, colonization times are substantially faster and inoculated substrates have an edge over contamination with speed (Frequently Asked Questions, 2004). Liquid cultures can be economical, as 1cc from spore syringe can supply with a volume of liquid inoculants which can be used on many jars or bags (Frequently Asked Questions, 2004).

Information generated in the study will help future producers of liquid culture utilizing coconut water as based substrate.

The study aimed to determine the effect of coconut water as culture medium base for liquid culture of *Lentinula sp.*

The study was conducted at the Plant Pathology Department of College of Agriculture at Benguet State University, La Trinidad, Benguet from April 2011- June 2011.

REVIEW OF LITERATURE

Chemical Constituents of Coconut Water

Numerous medicinal properties of tender coconut water were noted (Veekey, undated). Coconut water was good for feeding infants suffering from intestinal disturbances, oral rehydration medium, contains organic compounds possessing growth promoting properties, presence of saline and albumine make it a good drink in cholera cases and many more.

Pradera (1946) also noted the tender coconut water contains sugar which is steadily increases from 1-5% to about 5-5.5% in the early months of maturation and then slowly falls reaching about 2% at the stage of the full maturity of the nut.

The tender coconut water contains most of the minerals such as potassium, sodium, phosphorus, iron, copper, sulfur and magnesium

Coconut water contains amino acids. These composed of 2.41% alanine, 10.75% ariginine,3.60% aspartic acid,0.97%x 1.17% cystine, 9.76 x 14.5% glutamic acid, 1.95 x 4.18% leucine,1.95 x 4.57 lysine, 1.21 x 4.1 % proline,1.23% phenylalanine,0.59 x 0.91 % serene, 2.83 x 3.56 % tyrosine (Pradera, 1946).

Tender coconut water contains both ascorbic acids and vitamin of B group. The concentration of ascorbic acid ranges for 2.2 to 3.7 mg/ml, which gradually diminishes as the kernel surrounding the water begins to harden (Pradera, 1946). Vitamin of B group in coconut water containing 0.64 mg/ml Nicotinic acid, 0.52 mg/ml Panthothenic acid, 0.02 mg/ml Biotin,0.01 mg/ml Riboflavin, 0.003 mg/ml Thiamine (trace), Pyridoxine (trace).

Coconut water composed of 95.5% water, 0.05% nitrogen. 0.56% Phosphoric acid, 0.25% Potassium, 0.69% Calcium oxide, 0.59% Magnesium oxide, 0.5% Iron, 4.71 of total solid, 0.80 reducing sugar, 2.08 total sugars and 0.62 ash (Pandatal, 1958).

Quality of Coconut Water

Coconut water of drinking quality is clear and colorless, with pH of 5 to 5.4 and a Brix level (a measurement of sugar concentration) of 5 to 6.5. Per milliliter, it should have a totlal microbiological count of less than 5,000, less than 10 of coliform bacteria and zero fecal coliform. A rancid odor indicates that the small quantity of fats in the liquid has oxidized. Bacteria and yeast are the main microorganisms that threaten coconut water (Food and Agriculture Organization, 2007). Young coconut water alone contains candida (known as yeast infection), a single-cell organisms, candida reproduces asexually and thrives on some of the body's by- products; dead tissue and sugars from food.

Mushroom Description

According to Stamets (1998), the genus *Lentinula* was originally conceived by Earle in 1990`s and resurrected by Pegler in the 1970`s to better define members formerly placed in Lentinus. Both genera are characterized by white spores, centrally to eccentrically attached stems, gills edges which are often serrated and distinct preference for woodland environment. Genus *Lentinula* have flesh composed of dimitic hyphae or irregular or interwoven cells in the gill trama.

The cap measures 3-6 cm broad, hemispherical then later expanded, light brown to deep brown, not viscid, margin inflexed. Lamella is white, adnexed, sinuate to adnate then later separated to stipe. The stipe is cylindrical, 2-5.5 cm long, 10-18 mm thick at the apex and fibrous. Spores ellipsoid, 5-7 x 2-3.5µm, smooth, hyaline and inamyloid (Chang and Quimio, 1982).

Substrate on Mycelial Production

Wasser *et al*. (2000), reported that the growth of pure mushroom cultures in submerged conditions like in a liquid medium, permits the acceleration in growth resulting to biomass yield. The several optimization of the culture medium composition allows the regulation of mushroom metabolism in order to obtain high yield of mycelia biomass.

Sornprosent and Sangsuwan (2004), also found out that culture mycelia of *Ganoderma lucidum* in Potato Dextrose Agar (PDA) was stationary culture for 52 days while in liquid medium gave the highest dry weight of mycelia of 0.91g/ 1000ml was produced in 25 days.

Carbon and Nitrogen Ratio

The effect of nitrogen is less specific than that of carbon. The carbon- nitrogen ratio (C:N ratio) is important in fruiting body formation. The C: N ratio obtained by chemical analysis of fungal cell is approximately 10:1, but substrate carbon is also used for energy and is respired as CO_2.Thus it is, estimated that an amount is converted to cellular material that is similar to the amount of CO_2; consequently, for growth, a C:N ratio of 20:1 is suitable (Miller,2000).

Medicinal Importance of Mushroom

In oriental folk medicine, *Lentinula sp* is useful in normalizing high blood pressure and lowering blood cholesterol level. It is also valuable in stimulating immune system to increase the body's ability to ward off cancerous tumors, viral infections and chronic fatigue syndrome. *Lentinus sp* is the source of a well known anti tumor polysaccharide, lentinan, from the fruiting bodies or mycelia (Chihara, 1992).

pH of Culture Medium

Several environmental factors affect the growth of mycelia under submerged culture such as pH and temperature. In submerged culture, the pH of the medium begins to fall rapidly after commencement of the culture, irrespective of the initial pH range from 3-6 and becomes about 3.0 within several days. The optimal pH in liquid culture is 5-6 (Chang and Hayes, 1978).

Nutritional Requirement of Fungi

Essential elements required for the growth of fungi includes macro elements: potassium, sulfur, magnesium, and calcium; micro elements; iron, copper, manganese, zinc and molybdenum (Griffin, 1993). Nitrogen promotes enzyme activity and ATP production. Fungi have natural deficiencies on vitamins (Griffin, 1993).

Carbon source provides structural and energy requirements of the fungal cell concentration of carbon should probably not exceed 2% (Chang and Miles, 2004). Nitrogen is essential in the synthesis of amino acids, nucleotides and vitamins (Griffin, 1993). Potassium is commonly supplied as potassium phosphate. A concentration of potassium at around 10^{-3} M satisfies the requirement of fungi. Potassium has a role as a co-factor in some enzyme systems involving carbohydrate metabolism and important in the maintenance of ionic balance (Griffin, 1993; Chang and Miles, 2004).

Among all metabolic minerals, potassium at 0.004 M concentration is adequate for most of all the fungi (Belgrami, 1978; Griffin, 1993; Chang and Miles, 2004). Magnesium is essential to all fungi (Chang and Miles, 2004) and its function as enzymes activation and ATP metabolism statics at 10^{-3} M (Griffin, 1993: Chang and Miles, 2004).

MATERIALS AND METHODS

The substrates used in the study was sterilized at 20 psi for 1- 2 hours and laid out following the Complete Randomized Design (CRD). The treatments are replicated 3 times.

Treatments

T_{01} = Potato Dextrose Broth + 1% urea + 0.3%KH_2PO_4 + 0.2%$MgSO_4$

T_{02} = Coconut water

T_1 = Coconut water + rice bean

T_2 = Coconut water + molasses

T_3 = Coconut water + rice bean + molasses

The rice bean was soaked overnight, cooked and then dried. After drying, the rice bean was macerated to powder form.

Source of Inoculum

A parent culture of *Lentinula sp* was subcultured by cutting one centimeter square and then transferred to petri dishes with PDA. The culture was incubated until the mycelia reaches 1cm from inside the peripheral side of the petri dish.

Introduction of Inoculum

Pure culture of *Lentinula sp* was sectioned into quadrants with a heat sterilized scalpel and aseptically transferred into the blender containing the substrate sterilized previously.

The blender was placed in power and stirred in 3 seconds burst and pausing for 5 seconds so that the surviving chunks of agar would fall downwards into the blades. Another 3 seconds burst decimates these pieces. This was followed by one more 5 seconds pause followed

by the last 3 seconds. Mycelium blend was pour into a 230 ml jars previously sterilized with equal volume. The jars were placed in a shaker for 2 hours. After shaking, the jars were placed in water bath overnight and maintained 27-28^{0}C. These were incubated for 7 days to allow the substrate to be fully colonized with mycelia.

Harvesting Mycelium

Mycelia were filtered using the filter paper allowing the entire liquid medium to pass through. The mycelial filtrate on the filter paper was dried in the electric stove at low heat for 15 minutes. The mycelial filtrate was transferred onto the aluminum foil at known weight, taken from the fresh weight, oven dried at 80^{o}C for 48 hours to get the oven dried weight (Aspiras and Tad- awan, 2006).

Data Gathered

1. Incubation period (days) - from inoculation to full mycelial colonization of the substrates.

2. Mycelial fresh weight (g). Mycelia were filtered using filter paper allowing the entire liquid pass through. The mycelia filtrate on the filter paper was dried at electric stove at low heat for 15 minutes

3. Mycelial dry weight (g). The mycelia filtrate obtained in data 2 was transferred in a aluminum foil with known weight and oven dried at 80^{0}C for 48 hours and get the oven dried weight.

RESULTS AND DISCUSSIONS

Effect of Coconut Water with Molasses and Rice Bean

Among of all the media used in the study, control treatment containing the addition of 1 % urea, 0.3% KH_2PO_4 and 0.2% $MgSO_4$ gave the highest mean weight of 0.12 g (Table 1, Figure 1). However, there was no significant difference between the above treatment with that of coconut water also gave a good mycelia yield.

Coconut water contains elements such as zinc, selenium, iodine, sulfur, manganese, boron, molybdenum and others (Lee, 2008). Nutritional facts also cited that it is composed of 95.5% water, 0.05% nitrogen. 0.56% Phosphoric acid, 0.25% Potassium, 0.69% Calcium oxide, 0.59% Magnesium oxide, 0.5% Iron, 4.71 of total solid, 0.80 reducing sugar, 2.08 total sugars and 0.62 ash (Pandatal,1958). However, addition of 20 grams molasses and 5 grams powdered rice bean into coconut water increased mycelial weight (Figure 2).

Figure 3a, shows the mycelia of *Lentinula sp* after oven drying and Figure 3b shows the mycelia of *Lentinula sp* taken from the dried mycelia. The mycelium of the dried *Lentinula sp* is hyaline.

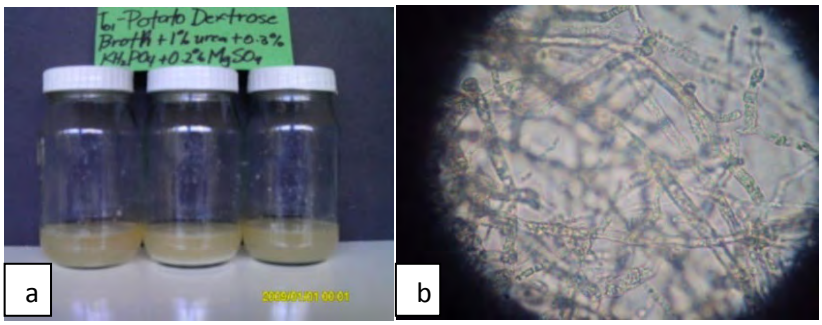

Figure 1.a) T_{01}- Potato Dextrose Broth + 1% urea + 0.3% KH_2PO_4 + 0.2 % $MgSO_4$; (b) mycelia of *Lentinula sp*

Table 1. Mean mycelia fresh and dry weight (g) of *Lentinula sp* at 7 days after inoculation

TREATMENT	MEAN WEIGHT FRESH	DRY
Dextrose Broth + 1% urea + 0.3% KH_2PO_4 + 0.2 % $MgSO_4$ + Potato	0.47[a]	0.12[a]
Coconut Water	0.28[a]	0.05[b]
Coconut Water + 5grams Rice bean	0.19[a]	0 .02[b]
Coconut Water + 20 grams Molasses	0.26[a]	0.05[b]
Coconut Water + 5 grams rice bean + 20 grams molasses	0.24[a]	0.11[a]

Means in a column followed by a common letter are not significantly different from each other at 5% DMRT

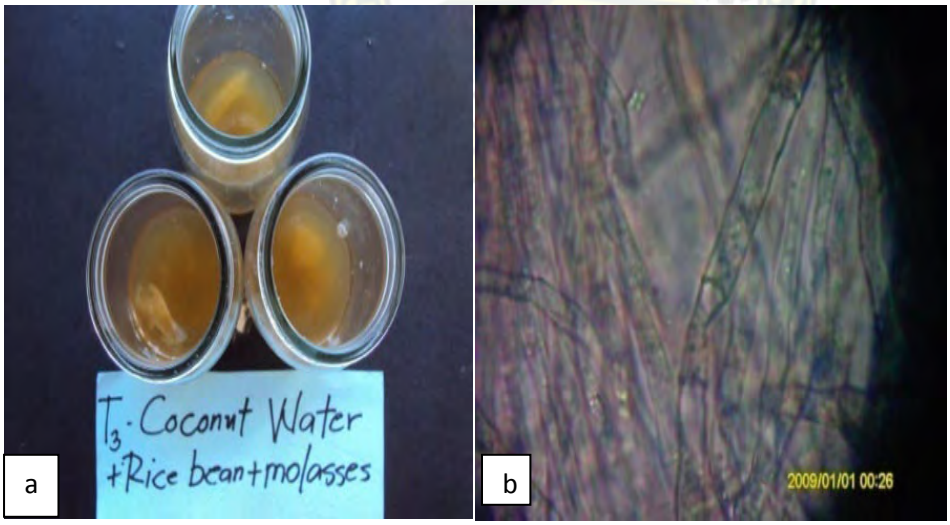

Figure 2.a) T_3- Coconut water + 20 grams molasses + 5 grams rice bean;
(b) mycelia of *Lentinula sp*

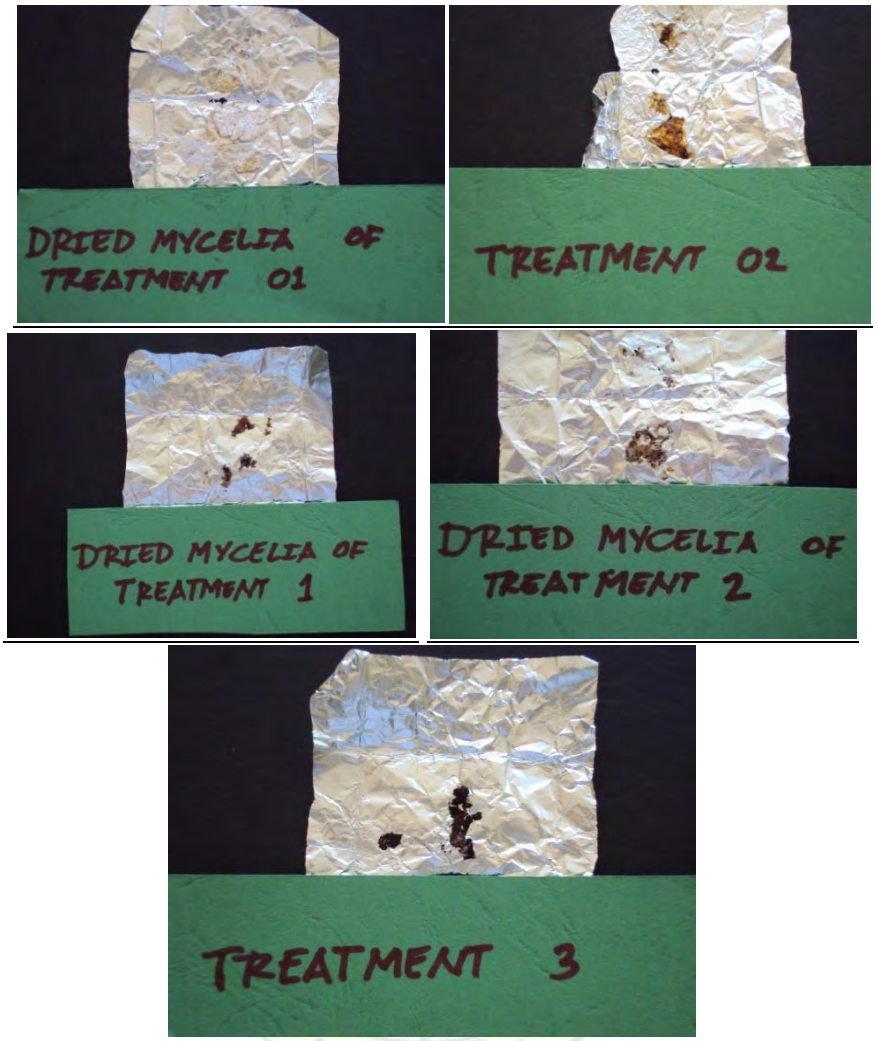

Figure 3a. dried mycelia of *Lentinula sp*

Figure 3b. Mycelia of *Lentinula sp.* after drying

pH of the Substrate

The addition of nutrients and fermentation of the substrate significantly lowered the pH. This is observed after harvesting the mycelia. Decrease of pH is observed on the treatments with rice bean, which might have fermented on the process and maybe also the coconut water begins to ferment.

Based on findings of Chang and Hayes, 1978, pH of the submerged culture begins rapidly fall after commencement of the culture, irrespective of the initial pH. The optimum pH of the culture media is 5-6.

The lower the decrease of pH the higher the yield of the mycelia. However, there was no significance difference between the pH of the treatment (Table 2).

Table 2. Mean ph of the substrate before inoculating and after harvesting

TREATMENTS	pH
Potato Dextrose Broth + 1% urea + 0.3 % KH_2PO_4 + 0.2% $MgSO_4$	0.80[b]
Coconut Water	0.96[b]
Coconut water + 5 grams rice bean	1.27[a]
Coconut water + 20 grams molasses	0.89[b]
Coconut water + 5 grams rice bean + 20 grams molasses	1.03[a]

Means in a column followed by a common letter are not significantly different from each other at 5% DMRT

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

Among all the media used, Potato Dextrose Broth with 1% urea, 0.2% KH_2PO_4 and 0.3% $MgSO_4$ has the highest mycelial yield.

There is no difference in the dry weight between T_{01} which is the Potato dextrose Broth with 1% urea, 0.2% KH_2PO_4 and 0.3% $MgSO_4$ and T_3 which is the Coconut water with 20g molasses and 5g rice bean.

Favorable environment, such as pH is used for the growth of the mycelia. Achieving the optimum pH will help the inoculum grow faster. Shaking also help for the production of the oxygen in an anaerobic condition which is needed by the *Lentinula* in order to grow.

Conclusion

Coconut water has the potential as a liquid culture medium based for *Lentinula sp* with or without the addition of molasses and rice bean. Coconut water contains vitamins and minerals that can supply the requirements of the mushroom.

Recommendations

Based on the findings, it is recommended:

Further studies in use of coconut water as liquid medium base for the other species of mushroom and improve the process in the assessment of the purity of the liquid culture.

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APPENDICES

Appendix Table 1. Mycelial fresh weight (g), of Lentinula sp 7 days after inoculation

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Potato dextrose Broth 1% urea + 0.3 % KH_2PO_4 + 0.2 % MgSO	0.38	0.15	0.87	1.40	0.47
Coconut Water	0.14	0.48	0.23	0.85	0.28
Coconut Water + 5g Rice bean	0.11	0.08	0.39	0.58	0.19
Coconut water + 20g Molasses	0.21	0.19	0.37	0.77	0.26
Coco nut Water + rice Bean + molasses	0.05	0.10	0.57	0.72	0.24
GRAND TOTAL				4.32	
GRAND MEAN					0.28

ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	COMPUTED F	TABULAR F .05	.01
Treatment	4	0.13	0.03	0.60^{ns}	3.11	5.99
Experimental Error	10	0.58	0.05			
Total	14	0.71				

ns= Not significant cv= 79.68

Appendix Table 2. Mycelial dry weight(g), of Lentinula sp 7 days after inoculation

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Potato dextrose Broth + 1% urea + 0.3 % KH_2PO_4 + 0.2 % $MgSO_4$	0.05	0.04	0.27	0.36	0.12
Coconut Water	0.04	0.07	0.05	0.16	0.05
Coconut Water + 5g Rice bean	0 .03	0 .04	0.12	0.19	0.02
Coconut water + 20g Molasses	0.03	0.04	0.09	0.16	0.05
Coco nut Water + rice Bean + molasses	0.01	0.03	0.31	0.36	0.11
GRAND TOTAL				1.21	
GRAND MEAN					0.07

ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	COMPUTED F	TABULAR F .05	.01
Treatment	4	0.02	0.005	5.0*	3.11	5.99
Experimental Error	10	0.01	0.001			
Total	14	0.71				

ns= Not significant cv= 39.52%

Appendix Table 3. Initial pH of the liquid media before inoculation

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Potato dextrose Broth + 1% urea + 0.3 % KH_2PO_4 + 0.2 % $MgSO_4$	7.5	7.2	7.3	22.0	7.33
Coconut Water	6.2	5.9	6.3	18.40	6.13
Coconut Water + 5g Rice bean	6.3	6.0	6.0	18.30	6.10
Coconut water + 20g Molasses	5.9	6.0	6.0	17.90	5.96
Coco nut Water + rice Bean + molasses	5.9	5.8	5.7	17.40	5.80
GRAND TOTAL				94.0	
GRAND MEAN					6.26

ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	COMPUTED F	TABULAR F .05	.01
Treatment	4	4.47	1.12	14**	3.11	5.99
Experimental Error	10	0.81	0.08			
Total	14	0.71				

**= highly significant cv= 4.52%

Appendix Table 4.Final pH of the liquid media after harvesting

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Potato dextrose Broth + 1% urea + 0.3 % KH_2PO_4 + 0.2 % $MgSO_4$	6.9	6.8	6.5	20.2	6.73
Coconut Water	5.1	5.5	4.9	15.5	5.17
Coconut Water + 5g Rice bean	4.8	4.8	4.9	14.5	4.83
Coconut water + 20g Molasses	4.9	5.2	5.1	15.2	5.07
Coco nut Water + rice Bean + molasses	4.7	4.8	4.8	14.3	4.77
GRAND TOTAL				79.7	
GRAND MEAN					5.31

ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	TABULAR F .05	.01
Treatment	4	7.89	1.97	59.69**	3.11	5.99
Experimental Error	10	0.33	0.033			
Total	14	0.71				

**= highly significant cv= 3.42%

CA-UR FORM 9

Benguet State University
COLLEGE OF AGRICULTURE
La Trinidad, Benguet

Date_____

APPLICATION FOR MANUSCRUPT ORAL DEFENSE

Name: MICHAEL J. ESTACIO_____

Degree (Major Field): Bachelor of Science in Agriculture (Plant Pathology) _____

Title of Research: COCONUT WATER AS LIQUID CULTURE MEDIUM BASE FOR *LENTINULA* .

Date and Time of Defense: JUNE 3, 2011

Place of Defense: AC 107 Building, Benguet State University

Endorsed:

BERNARD S. TAD-AWAN
Adviser, Advisory Committee
(Printed Name and Signature)

APPROVED

ASUNCION L. NAGPALA
Member, Advisory Committee
(Printed Name and Signature)

JOCELYN C. PEREZ
Member, Advisory Committee
and Department Chairperson
(Printed name and Signature)

RESULT OF MANUSCRIPT DEFENSE

Name and Signature	Remarks (Passed/Failed)
BERNARD S. TAD-AWAN Adviser, Advisory Committee	_____
ASUNCION L. NAGPALA Member, Advisory Committee	_____
JOCELYN C. PEREZ Member, Advisory Committee and Department Chairperson	_____