BIBLIOGRAPHY

ALFREDO, NOEL VELASCO. APRIL 2011. Characterization of Two Mycorrhizal Mushroom Associated with Benguet Pine (*Pinus kesiya*). Benguet State University, La Trinidad, Benguet.

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

ABSTRACT

Boletus leonis and *Tuber sp.* were collected within pine-based forest of Tublay. At an altitude of 1,300 meters above sea level, both *Tuber sp*. and *Boletus leonis* were abundantly present. *Tuber sp*. has the higher number of basidiocarp at the whole surveyed altitude compared to *Boletus leonis. Boletus leonis* was not found at altitude 1,200 masl and 1,100 masl. However, the *Tuber sp.* was found at the altitude of 1,200 masl and 1,100 masl which it suggests a trend that the higher altitude, the higher the number of basidiocarp and the lower altitude, the lower the number of basidiocarp.

Soil sample of truffle *Tuber sp.* have pH 5.45, 41.56% moisture content, 4.85% organic matter, 1.88% Nitrogen, 10.2 ppm Phosphorus and11.5 ppm Potassium. The edible *Tuber sp.* occurs in cluster, scattered or gregarious on the ground, pathways or roads and it is more abundant during rainy season.

Boletus leonis mushroom has been found to grow on soil with pH of 5.4, 41.53% moisture content, 6.4% organic matter, 1.9% Nitrogen, 10.36 ppm Phosphorus and 12 ppm

Potassium. The *Boletus leonis* grows on areas covered with pine needles, mostly in clusters and is abundant during rainy season.

The cap of *Boletus leonis* is yellow-orange to orange-brown, 8.0 to 12.0 cm wide and slightly convex when young and flatly expanded when matured. The hymenophore is tubular with tubes that are yellowish-brown and attachment of tube in the stipe is adnexed. The stipe is 4.0 to 8.o cm long and basal part is thickening and tapering to a beet-like extension. Spore print is yellowish to orange.

The cystidium is cylindrical, basidium is unicellular holobasidium with two sterigmata, basidiospore is subglobose and colorless. The spores measure 4-7mm and 8.5 x 4µm.

The truffle *Tuber sp*. is 1.2-12 cm in diameter but could reach up to 15 cm. The wall is dirty yellow to brown. *Tuber sp.* is semicircular, solid, hard and has a rough texture.

The spore is circular with a dedicate ornamentation. The spore is darkyellow with a diameter of 10 mm and 10 x 5µm.

Stained roots of pine seedlings taken from their natural habitat in surveyed area in Tublay developed ectomycorrhizal relationship with the mushroom. Likewise, the inoculated pine tree seedlings developed ectomycorrhizal relationship as evidenced by ocular inspection through a microscope. On the other hand, pine seedlings without inoculation of suspension did not show such relationship.Pine seedlings taken from their natural habitat had significantly higher mean plant height than those inoculated pine seedlings with spore concentration of 6.7 x 10^4/ml and pine seedlings without inoculation.

RESULTS AND DISCUSSIONS

Presence of Mycorhizal Mushroom

At an altitude of 1,300 meters above sea level, both *"Bu-o"* and *"Dappanan"* were abundantly present (Table 1). *"Bu-o"* has the higher number of basidiocarp at the whole surveyed altitude compared to *"Dappanan"*which was not found at altitude of 1,200 and 1,100 m asl. However, the "*Bu-o*" was found at the altitude of 1,100 m asl to 1,300 m asl. At a higher altitude, there was higher number of basidiocarps compared to lower altitude.

Table1. Abundance of *"Dappanan"* and *"Bu-o"* in Tublay

ALTITUDE (masl)	NUMBER OF BASIDIOCARP WITH IN A 100 m² AREA (*"Dappanan"*)	(*"Bu-o"*)
1, 300	7	139
1, 200	0	39
1, 100	0	36
TOTAL	7	214

Characterization of *"Dappanan"* and *"Bu-o"* Mushroom

Habitat. Both *"Dappanan"* and "*Bu-o"* were found under pine trees associated with grasses (Figure 1).

The edible *"Bu-o"* occurs in cluster, scattered or gregarious on the ground, pathways or roads and it is more abundantly during rainy season (Figure 2).

The *"Dappanan"* grows on soil covered with pine needles, mostly in cluster and abundantly during rainy season (Figure 3).

Analysis of the soil samples of *"Bu-o"* revealed: pH 5.45, 41.56% moisture content, 4.85% Organic matter, 1.88% nitrogen, 10.2 ppm phosphorus and11.5 ppm potassium. Soil analysis of *"Dappanan*: pH 5.4, 41.53% moisture content, 6.4% Organic matter, 1.9% nitrogen, 10.36 ppm phosphorus and 12 ppm potassium. This mushroom grows from August to October.

Figure 1. Surveyed area for the presence of *"Dappanan"* and *"Bu-o"* mushroom in Tublay

Figure 2. Truffle *"Bu-o"* was specifically collected at the pine forest

Figure 3. *"Dappanan"* mushroom in its habitat

Physical Description of *"Dappanan"* mushroom. The cap of this mushroom is yellow-orange to orange brown, 8.0 to 12.0 cm wide and slightly convex when young, flatly expanded when matured. The hymenophore is tubular, tubes are yellowish-brown and attachment of tube in the stipe is adnexed. The stipe is 4.0 to 8.o cm long and basal part is thickening and tapering to a beet-like extension (Figure 4a). Spore print is yellowish to orange.

Microscopic Description. The Cystidium is cylindrical (Figure 4b), basidium is unicellular holobasidium with two sterigmata (Figure 4c), basidiospore is subglobose and colorless (Figure 4d). The spores measure 4-7mm and 8.5 x 4 *u*m (Figure 4-e).

Based on the above descriptions as well as its edibility as identified by local folks, the mushroom is identified as *Boletus leonis*. Physical and microscopic descriptions agree with the description of Surcek and Kubicka (1980) and Phillips (1994).

Figure 4a. Fruiting body of *Boletus leonis* found at the surveyed area

Figure 4b. Cystidia of *Boletus leonis* at 40x

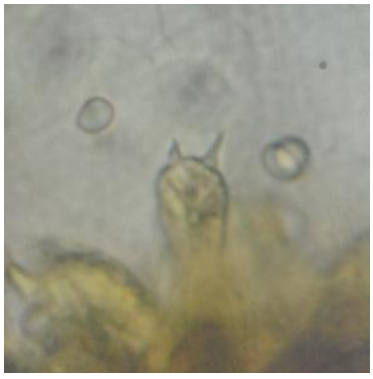

Figure 4c. Basidium of *Boletus leonis* at 40x

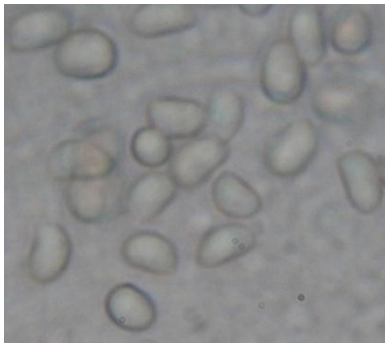

Figure 4d. Spores of *Boletus leonis* at 40x

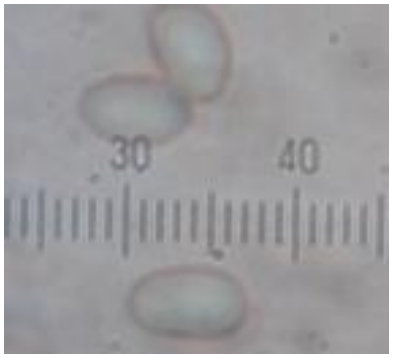

Figure 4e. Length of the spore of *Boletus leonis* with 7µm at 100x

Physical Description of *"Bu-o"* mushroom. Size of fruiting body is 1.2- 12 cm in diameter but could reach up to 15 cm. The wall is dirty yellow to brown; it is semicircular, solid, hard and ha a rough texture (Figure 5a).

Microscopic Description of *"Bu-o"* mushroom. The spore is circular with a dedicate ornamentation (Figure 5b). The spore is dark yellow and with a length of 10 mm, 10 x 5 μm (Figure 5c). This mushroom is edible and it was identified as *Tuber sp*. supported by information from Simpson (1989).

Figure 5a. Truffle *Tuber sp.* found at the surveyed area

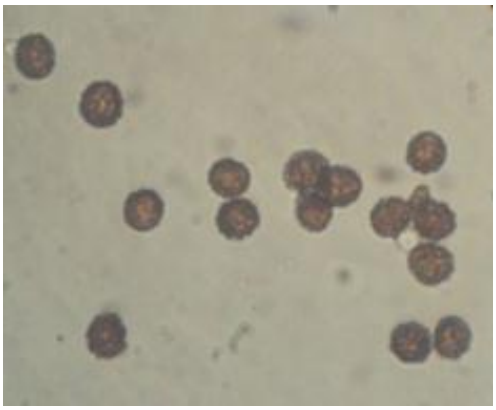

Figure 5b. Spores of *Tuber sp.* at 10x

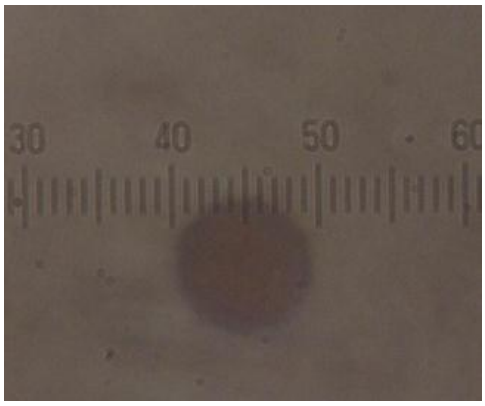

Figure 5c. Diameter of the spore of *Tuber sp.* with 10 µm at 100x

Test for Mycorrhizal Relationship
on Pine Tree Seedlings

Stained roots of pine seedlings at the surveyed area showed presence of mycelia of mycorrhizal mushrooms (Figure 6). Conversely, pine seedling without inoculum did not show any presence of mycelia (Table 2 and Figure 11). Likewise, inoculated pine seedlings showed the presence of mycelia of mycorrhizal relationship (Figures 7-10).

There were significant differences between pine seedlings inoculated with 25 ml, 75 ml and 100 ml compared to the pine seedlings taken from their natural habitat. Pine seedlings inoculated with 25 ml suspension with 167.5×10^4 ml spores showed the presence of mycelia indicating mycorrhizal relationship. Increasing the amount of inoculum resulted to a more indicative mycorrhizal relationship.

Table 2. Presence or Absence of Mycelia of *Tuber sp.*

TREATMENTS	MEAN
Pine seedling taken from their natural habitat	1^a
Pine seedling without inoculation of suspension	0^b
Pine seedling with 25 ml inoculation suspension	0.6^{ab}
pine seedling with 50 ml inoculation of suspension	0.1^b
pine seedling with 75 ml inoculation of suspension	0.4^{ab}
pine seedling with 100 ml inoculation of suspension	0.6^{ab}

*Significant differences exists at 0.05 level using DMRT CV = 23.85%

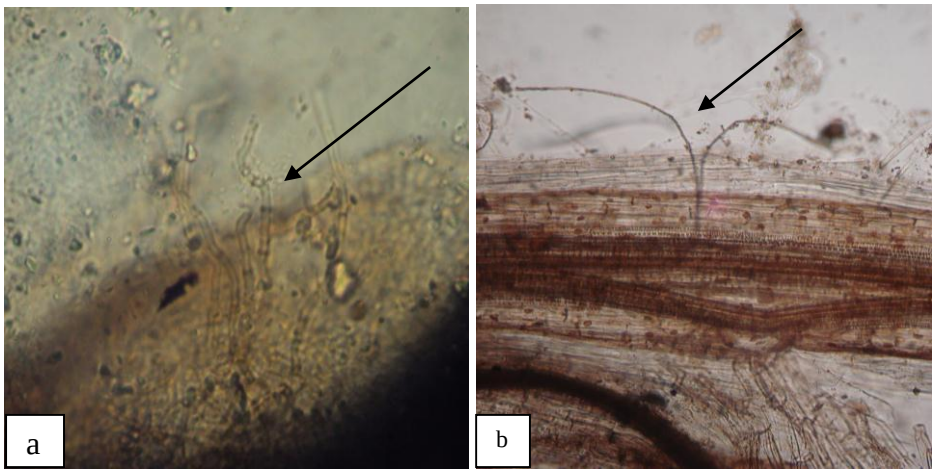

Figure 6. Stained roots of pine seedlings taken from their natural habitat at 40x(a) and 10x(b) magnification (arrow indicates the mycelia of *Tuber sp.*)

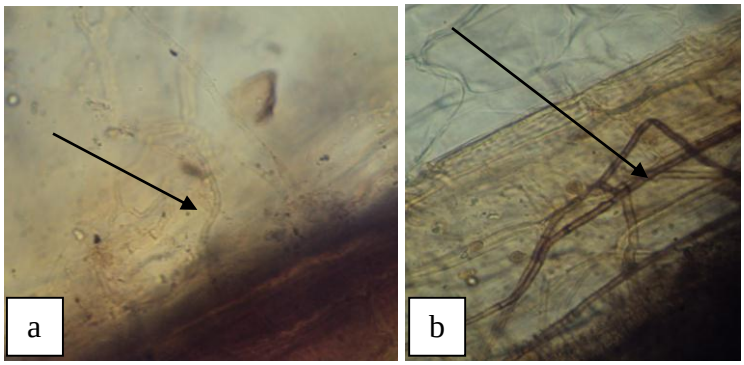

Figure 7. Stained roots of pine seedlings inoculated with (167.5×10^4) / ml spore suspension after 136 days at 40x magnification. (arrow indicates the mycelia of *Tuber sp.*)

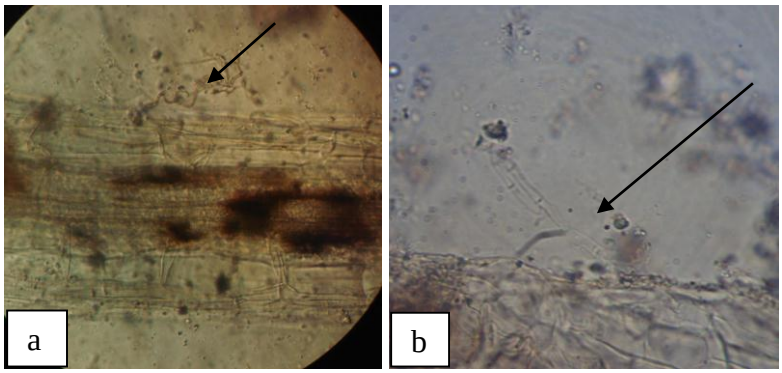

Figure 8. Stained roots of pine seedlings inoculated with (335×10^4) / ml spore suspension after 136 days at 10x and 40x magnification. (arrow indicates the mycelia of *Tuber sp.*)

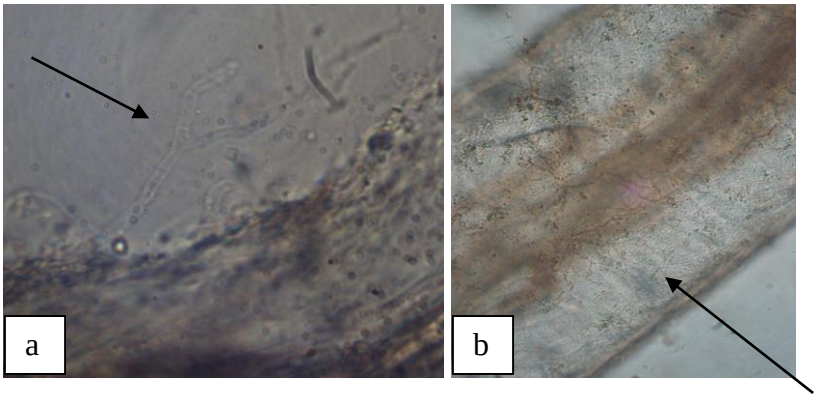

Figure 9. Stained roots of pine seedlings inoculated with (502.5 x 10^4) / ml spore suspension after 136 days at 40x and 10x magnification. (arrow indecates the mycelia of *Tuber sp.*)

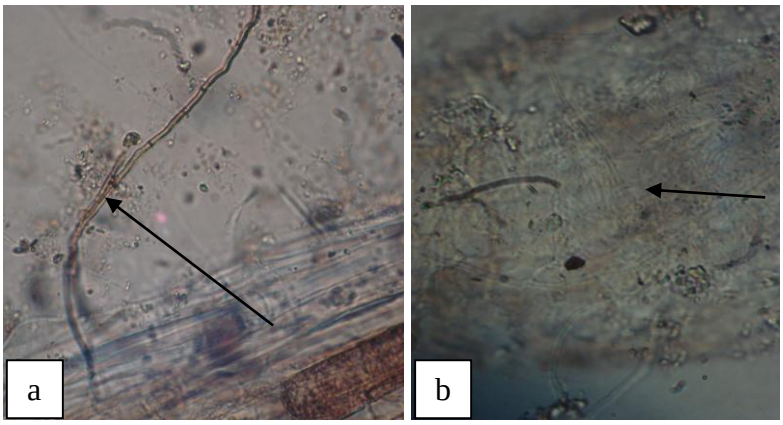

Figure 10. Stained roots of pine seedlings inoculated with (67 x 10^6) /ml suspension after 136 days at 10x magnification. (arrow indicates the mycelia of *Tuber sp.*)

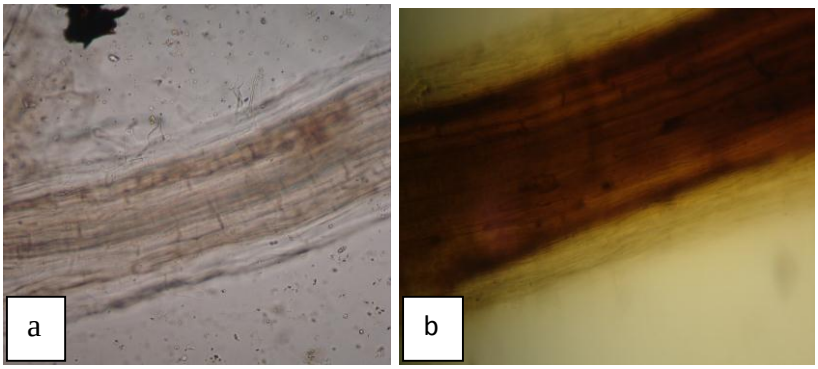

Figure 11. Stained roots of pine seedlings without inoculation

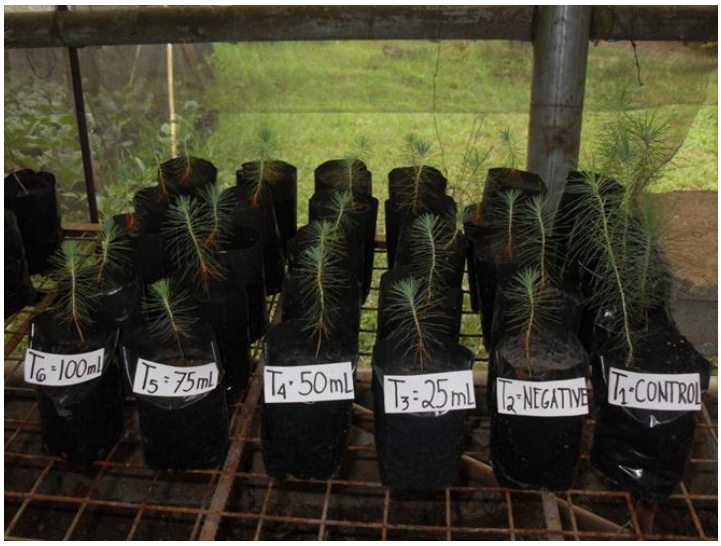

Figure 12. Pine (*Pinus kesiya*) seedling replicates for each treatments

Pine seedlings taken from their natural habitat had significantly higher mean plant height than the inoculated pine seedlings. Significantly taller pine seedlings were from those taken from natural habitat. This is followed by pine seedlings inoculated with 25 ml and 100 ml of suspension. The results suggest that pine seedlings taken from their natural habitat had significantly higher mean plant height than those inoculated regardless of amount of inoculum. These differences may be due to the maturity of the pine seedlings (figure 13).

Table 3. Height of Pine Seedlings

TREATMENTS	HEIGHT (cm)*[γ]
pine seedling taken from their natural habitat	11.75[a]
pine seedling without inoculation of suspension	8.98[b]
pine seedling with 25 ml inoculation of suspension	9.77[ab]
pine seedling with 50 ml inoculation of suspension	8.84[b]
pine seedling with 75 ml inoculation of suspension	8.78[b]
pine seedling with 100 ml inoculation of suspension	9.52[b]
TOTAL	57.64

*No significant differences at 0.05 level, significant differences at 0.10 level,

P-value=0.085
[y]Means with different letters signify significant differences using DMRT
CV=25.63%

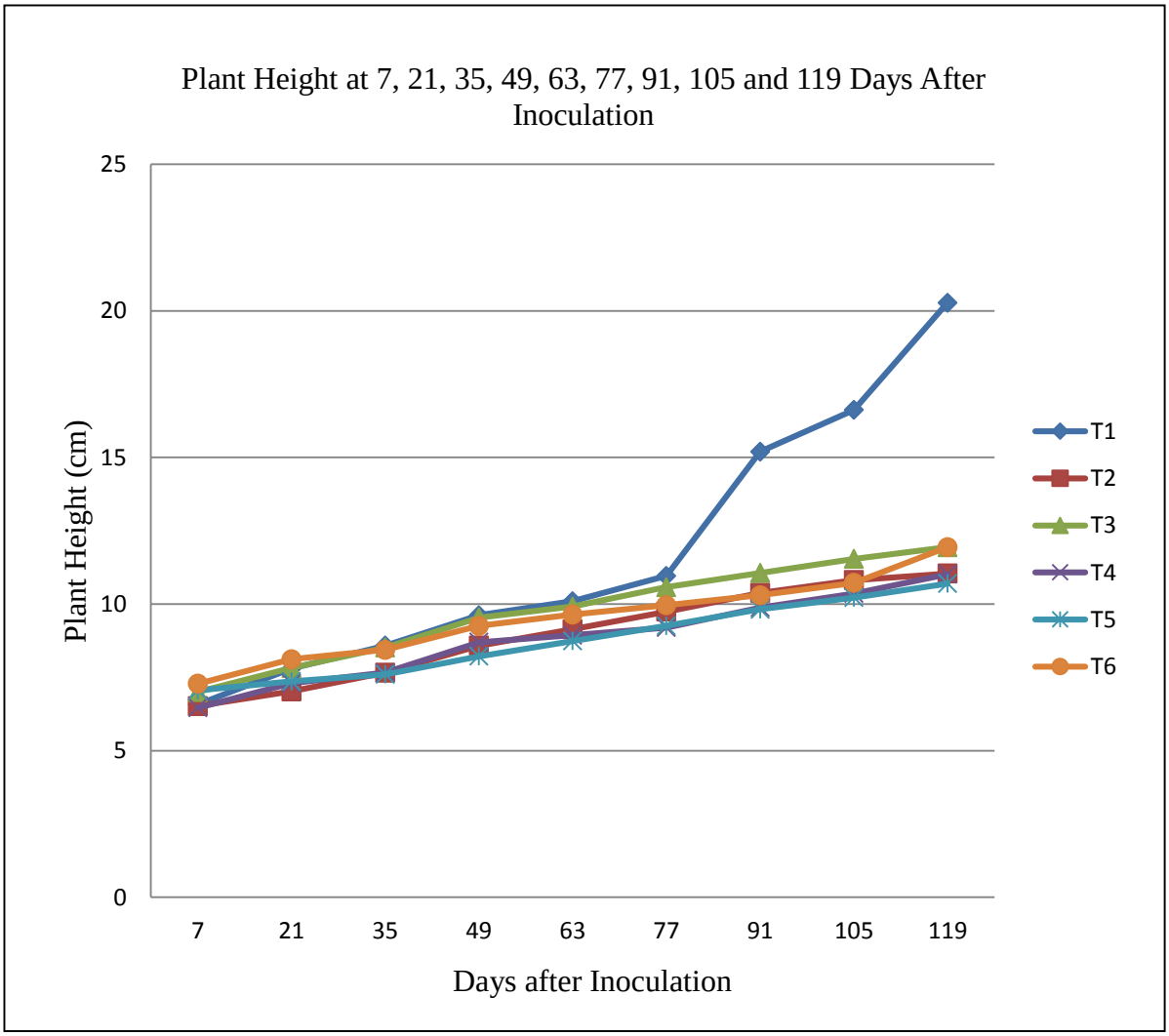

Figure 13. Plant Height at 7, 21, 35, 49, 63, 77, 91, 105 and 119 Days after inoculation (DAI)

Alexopoulus *et al*., (1996) reported that mycorhizal fungi benefit their host by increasing the plants ability to capture water and nutrients such as nitrogen, phosphorous, potassium and calcium from the soil, increasing tolerance to drought, high temperature and extremes of soil acidity and providing protection from certain plant pathogens.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study was conducted to characterized "*Bu-o*" and "*Dappanan*" mushroom based on: habitat (type of tree cover where found, altitude, temperature, moisture and soil pH) type of mycorrhizal relationship, morphological and anatomical features, abundance of each species with altitude, relative humidity and temperature and determined the mycorrhizal relationship between each mushroom and pine seedlings.

Microscopic studies were done at the Department of Plant Pathology, Benguet State University from October 2010 to October 2011. Soil analysis of mineral content was done at Department Soil Science, Benguet State University and Regional Soils Laboratory, San Fernando, La Union.

At an altitude of 1,300 meters above sea level, both "*Bu-o*" and *"Dappanan*" were abundantly present. "*Bu-o"* has the higher number of basidiocarp at this altitude, compared to "*Dappanan*". However at altitudes 1,200 to 1,100 m asl, only the "*Bu-o*" was found but at a lesser. This indicates that at higher altitudes, a higher number of basidiocarps were observed which decreased as altitude decreases.

The edible "*Bu-o*" occurs in cluster, scattered or gregarious on the ground, pathways or roads and it is more abundant during rainy season.

Soil samples of Truffle "*Bu-o*" revealed: pH 5.45, 41.56% moisture content, 4.85% Organic matter, 1.88% nitrogen, 10.2 ppm phosphorus and11.5 ppm potassium.

The "*Dappanan*" grows on covered with pine needles, mostly in cluster and abundantly during rainy season.

Soil analysis of "*Dappanan*" mushroom: pH 5.4, 41.53% moisture content, 6.4% Organic matter, 1.9% nitrogen, 10.36 ppm phosphorus and 12 ppm potassium.

The cap of "*Dappanan"* is yellow-orange to orange brown, 8.0 to 12.0 cm wide and slightly convex when young, flatly expanded when matured. The hymenophore is tubular with tubes that are yellowish-brown and attachment of tube in the stipe is adnexed. The stipe is 4.0 to 8.o cm long and basal part is thickening and tapering to a beet-like extension. Spore print is yellowish to orange.

The Cystidium is cylindrical, basidium is unicellular holobasidium with two sterigmata, basidiospore is subglobose and colorless. The spores measure 4-7mm and 8.5 x 4 μm.

The truffle "*Bu-o*" is 1.2- 12 cm in diameter but could reach up to 15 cm. The wall is dirty yellow to brown; it is semicircular, solid, hard and has a rough texture.

The spore is circular with a dedicate ornamentation. The spore is dark yellow with a length of 10 mm and 10 x 5 μm.

Based on physical and microscopic features, these mushrooms are edible. *"Dappanan*" is identified as *Boletus leonis* and truffle "*Bu-o*" is identified as *Tuber sp.*

Stained roots of pine seedlings at the surveyed area showed presence of mycelia of mycorrhizal mushrooms. Conversely, pine seedling without inoculum did not show any presence of mycelia. Likewise, inoculated pine seedlings showed the presence of mycelia of mycorrhizal relationship. Results of the analysis of variance test showed significant differences between pine seedlings inoculated with 25ml, 75 ml and 100 ml against the pine seedling taken from their natural habitat at the 0.05 level using DMRT.

Also, pine seedlings taken from their natural habitat had significantly higher mean plant height than those inoculated pine seedlings and pine seedlings without inoculation of suspension at the 0.05 level using DMRT. The results suggest that pine seedlings taken from their natural habitat had significantly higher mean plant height than those inoculated regardless of amount of inoculum.

Conclusion

"*Dappanan*" and "*Bu-o*" were found under pine-based areas associated with grasses in Tublay. Altitude, relative humidity, temperature, moisture content and soil pH provides a habitat which favors the growth and abundance of many truffles and mushrooms. Based on physical and microscope features, "*Dappanan*" is identified as *Boletus leonis* and truffle "*Bu-o*" is identified as *Tuber sp*. These mushrooms developed ectomychorrhizal relationship with its habitat.

Recommendation

It is recommended that further studies be conducted to identify other areas where truffle "*Bu-o*" and "*Dappanan*" may be found. In addition, studies to further understand the ectomycorrhizal relationship between the mushroom and pine tree roots should be done so that production could be explored. There is also a need to further verify the effects of inoculum on the growth and development of the pine tree seedlings.

LITERATURE CITED

ABAG, S. T. 2003. Survey, Characterization and Evaluation of "Lumsek" mushroom Effect on housefly (Musca domestica). BS Thesis (Unpub.). Benguet State University. La Trinidad Benguet. Pp 1-6.

ALEXOPOULUS, C. J., C. W. MIMS and M. BLACKWELL. 1996. Introductory Mycology. 4th Ed. John Wiley and Sons, Inc. New York, USA. Pp 514,524.

BALAWEN, M. 2003. Survey and Identification of Indigenous Mushrooms and Truffles in Ambasador, Tublay, Benguet. BS Thesis (Unpub.) Benguet State University. La Trinidad Benguet. Pp 1-4.

BRUNDRETT, M., N. BOUGHER, B. DELL, T. GROVE and N. MALAJCZUK. 1996. Working with mycorrhizas in forestry and agriculture. Australian Center for International Agricultural Research Monograph 32, Canberra. 374 pp. ISBN 1 86320 181 5. Retrieved July 2010 from http://www.ffp.csiro.au/research/mycorrhiza/.

CHANG, S. and M.G. PHILIP. 2004. Mushrooms Cultivation, Nutritional Value, Medical Effect, and Environmental Impact. 2nd Ed. Boca Raton, London, New York, Washington, D. C. Pp 2-3, 8, 11, 27-28.

DAMES, J.F., G.L. HAWLEY and A.F.S. TAYLOR. 2008. Ectomycorrhizas in association with *Pinus patula* in Sabie, South Africa. Retrieved August 2010 from http://www.scielo.org.za/scielo.php?pid=S003823532008000400011&script=sci_arttext.

DELL, B. 2002. Role of Mycorrhizal Fungi in Ecosystems. CMU. Journal Vol. School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia 6150. 1:1- 48. Retrieved December 2008 posted at http://cmuj.chiangmai.ac.th/full/2002/jan2002-3f.pdfrrhizal symbioses.

DHINGRA, O. D. 1985. Basic Plant Pathology Methods. CRC Press, Inc. Urbana-Champaign Urbana, Illinois. Pp. 279.

JONES, M.D., D.M. DURALL and P.B. TINKER. 1990. Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhizas at different soil phosphorus levels. New Phytologist 115: 259-267. Retrieved January 2007 from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.1990.tb00451.x/pdf

IOTTI, M., E. BARBIERI, V. STOCCHI and A. ZAMBONELLI. 2005. Morphological and Molecular characterization of mycelia of ectomycorrhizal fungi in pure culture.

Fungal Diversity 19: 51-68. Retrieved August 2009 from http://www.fungaldiversity.fdp/sfdp/19-3.pdf.

KAUL, T. N. 1997. Introduction to Mushroom Science (systematic). Science Publishers, Inc. Enfield, New Hampshire 03748, USA. P. 49.

KUO, M. 2004, December. *Scleroderma michiganense.* Retrieved October 2008 from the MushroomExpert.Com Web site: http://www.mushroomexpert.com/ scleroderma_michiganense.html.

LUMYONG, S., R. SANMEE, P. LUMYONG and B. DELL. 2010. In vitro cultivation and fruit body formation of the black bolete, Phlebopus portentosus, a popular edible ectomycorrhizal fungus in Thailand. Retrieved July 2010 from http://www.springerlink.com/content /2171221v43271823/fulltext.pdf.

PAOLOCCI, F., P. ANGELINI, E. CRISTOFARI, B. GRANETTI, and S. ARCIONI. 1995. Identification of *Tuber spp*. and corresponding ectomycorrhizae through molecular markers. J Sci Food Agric 69:511–517. Retrieved December 2009 from http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740690416/pdf

PHILIPS, R.1994. Mushrooms and other Fungi of Great Britain and Europe.London. Macmillan Reference. Pp. 16-17, 35, 105, 203, 215.

QUIMIO, T.H. 2002. Tropical Mushroom Cultivation. Cacho Hermanos, Inc. Pines cor. Union Sts. Mandaluyong City. P. 9.

SIMPSON J, W.E. 1989. Oxford English Dictionary. 2nd Edetion. Clarendon press, ISBN. Plant Industry, Canberra ACT 2601, Australia. Retrieved March 2011 from http://alan.richardson@csio.au.

STAMETS, P. 1993. Crowing Gourmet & Medical Mushrooms. Ten Speed Press. P.O. Box 7123, Berkeley, CA 94707. Pp 1-7.

SURCEK, M. and J. KUBICKA.1980. A Field Guide in Colour to Mushrooms and Other Fungi. London. Octopus Books Limited. Pp 12-15, 23-25.