THE EFFECTS OF SOIL APPLICATION OF BIOFERTILIZERS ON THE EARLY GROWTH OF BEAUTY LEAF (*Calophyllum inophyllum* L.)

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ABSTRACT

This study was carried-out to determine the early growth response of Beauty Leaf (*Calophyllum inophyllum* L.) to five biofertilizers namely Azotobacter, Bio Green, MyKo Vam, Vam Root Inoculant, and Bio N when compared with the Untreated control. Treatments were laid-out in a Randomized Complete Block Design, each replicated three times. Results showed that plant height and root length did not significantly vary among plants fertilized with Azotobacter, Bio Green, MykoVam, and Vam Root but significantly differ from those applied with Bio N and Untreated-control. Plants in all biofertilizers had comparable shoot dry matter weight but were significantly better from the Untreated control. Significantly highest root dry weight was registered by the Azotobacter-fertilized beauty leaf. Final leaf count, shoot base diameter, fresh shoot and root biomass, however, did not differ significantly among all treatments. Based on the results, Azotobacter, Bio Green, MyKoVam, and Vam Root Inoculant are potential biofertilizers for Beauty leaf under a screen house condition.

Keywords: Biofertilizers, Azotobacter, Bio Green , Bio N, MyKoVam. Vam Root Inoculant, Beauty leaf, *Calophyllum inophyllum* L.

INTRODUCTION

Rationale of the Study

Beauty leaf or Alexandrian laurel (*Calophyllum inophyllum* L.) of Family *Clusiaceae*, is a threatened (Stevens. 2006; Species Survival Commission, 2007) and emerging energy tree in the world. The alarming continuous use of fossil fuels that destroys the ozone layer has now become a major global concern and that search for alternative biofuel sources has been gaining ground of which Beauty leaf is one highly recommended plant species. Its mature, dried seeds yield about 65% oil from its dry weight and has the highest per tree oil yield of the 75 plant species evaluated (Hathurusingha & Ashwath, 2007). The average oil yield is 11.7 kg/tree or 4680 kg/hectare (Wikepedia, 2008) whose retail price in 2005 was already over US$ 450/l. Its seed oil contains up to 30% resinous materials and used as raw material for soap production, carrier oil, skin moisturizer, hair oil in cosmetics and also in aromatherapy (Lemmens, 2005).

The fatty-acid methyl ester component of seed oil meets all of the major bio-diesel requirements in the USA (ASTM D 6751), and contains benzoi*c* acid responsible for its cicatrizing properties which has a wide range of skin problems therapy. It is reportedly a good antiseptic, disinfectant, and astringent and has anti-inflammatory, antifungal, antibacterial and insecticidal activity (Quisumbing, 1978). It was discovered to have anti HIV and anti cancer agent called Calocoumarin A whose efficacy in treating the dreaded diseases is under investigation (Hathurusingha & Ashwath, 2007). Locally known as Dankalan or Bitaog, it is a durable multi-purpose timber but is rarely available in large quantities. The wood is resistant to termites while its heartwood is resistant to preservative treatment and is a prized timber for carving, cabinet making, boat building, and is traditionally used for food vessels (Hathurusingha & Ashwath, 2007). Despite its enormous uses, Beauty leaf current status is under threat and any conservation effort should be put in place to protect it.

One major step of protecting this threatened tree species is by *ex situ* conservation and mass propagation either by seeds or cuttings. It takes 5 to 8 months before seedlings can be planted in the field to ensure their vigorous and healthy condition to attain the desired survival rate. Beauty leaf can be moderately difficult to propagate because its initial growth is slow. Seeds germinate gradually but shelled seeds germinate faster than seeds in their shells. Germination rates for fresh seeds are greater than 90% (Elevitch, 2006). However, to ensure fast and vigorous growth of seedlings in the

nursery before establishing them in the field, one biotechnology that can be adopted with reportedly high efficacy is the application of biofertilizers (Elefan, 2008; Padilla, 2006),

Biofertilizers are natural fertilizers of symbiotic and asymbiotic beneficial microbial inoculants of bacteria, algae, and fungi alone or in combination. Their preference over chemical fertilizers offers economic and ecological benefits by way of soil health and fertility to farmers and reduced application of chemical inorganic fertilizers that pollute the environment (Gandanegara, 2007; Ozturk & Caglar, 2003). These can accelerate the decomposition of organic residues that release soil nutrients through mineralization and facilitate the uptake of plant nutrients like nitrogen and phosphorus (Tawfik, et al, 2006; Topolianits, et al, 2002; Galal, *et a*l. 2000; Rao, S. & Reddy, S., 2002); improve plant growth vigor by providing growth regulating substances (Bashan, et al, 2004; Chiu-Chung Young, 1994; Parr, Hornick, and Kaufmann, 2006; Mrkovacki & Milic, 2001; Verkade, et al, 2008) that help the host plant suppress pathogens and tolerate environmental stress (Li, 2007), and improve morpho-physiological characters that increased biomass and crop yield (Tawfik, *et al*, 2006; Cano, *et al*, 2007; Casta, 2001; Gandanegara, 2007; Nuntagij, 2007; Wu , et al, 2005; Nuruzzaman, *et al*., 2003; Ozturk, et al., 2003; Galal, *et al*, 2000; and Gupta & Baig, 2001).

Several biofertilizer researches on agro-forestry tree species and annual agricultural crops were conducted worldwide but no similar study had been done with beauty leaf. In the Philippines, few biofertilizer studies had been done and these were limited only to rice, corn, sugarcane, certain legumes, vegetables, fruit trees, and *Jatropha* (Dalmacio, 2008; Padilla 2006, Elefan, 2008; Sugar Regulatory Administration, 2007). So far, no published scientific report on the application of the biotechnology to Beauty leaf is available. Hence, the researcher finds it imperative to undertake this pioneering study to determine the early growth response of this threatened indigenous tree to the soil application of locally-available biofertilizers and arrive at recommended material for use.

Objectives of the Study

Generally, the study aimed to evaluate the early growth response of Beauty leaf (*Calophylllum inophyllum* L.) to biofertilizer application under a screen house condition based on seedling height, stem base diameter, leaf count, root length, shoot weight (fresh and dry), and root weight (fresh and dry).

Scope and Limitation of the Study

This pot experiment was conducted for five months to evaluate the early growth response of Beauty leaf (*Calophyllum inophyllum* L.) to the five biofertilizers namely *Azotobacter*, Bio Green, Bio N, Mykovam, and Vam Root inoculant which were applied only once at planting and then compared to the Untreated control. Although published work pointed out the effectiveness of Bio N in annual crops like corn and rice and less effectiveness in some tree species like *Jatropha curcas* (Padilla,2006), its inclusion in the study was based on the premise that different plant species would always differ in their physiologic response to the various kinds of biofertiflizers and this study was a pioneering one in Beauty leaf. Normally, in 5 to 8 months, the tree seedlings already acquire the desired shoot and root development while in pots that will allow them to be able to adapt in the field after they are transplanted (Elevitch, 2006). Early growth response herein was limited only to seedling height, leaf count, shoot base diameter, root length, shoot weight (fresh and dry), and root weight (dry) of plants. The planting media used was a mixture of clay-loam soil and river sand in a 1:2 ratio.

METHODOLOGY

This pot experiment was conducted in a screen house of the Life Sciences Department, Central Philippine University, Iloilo City Philippines in order to evaluate the effects of soil application of five locally produced biofertilizers namely Azotobacter, Bio Geen, Bio N, MykoVam, and Vam Root Inoculant on the early growth of Beauty leaf (*Calophyllum inophyllum* L.) which were compared to the Untreated Control. It was carried out in a randomized complete block design (RCBD) with three replications. The planting medium used was a mixture of 1 part clay-loam soil and 2 parts clean river sand.

The planting media were first oven-heated at 80°C before potting them. Five pots were allocated per replicated treatment or a total of 90 pots for the entire experiment. Biofertilizers were applied directly into the soil before sowing the seeds based on the manufacturers' recommended rates. Brown fruits of wrinkled skin collected from Dancalan, Negros Occidental, Philippines in December 2008 were soaked overnight to remove the skin. Just prior to planting, seeds were shelled entirely by cracking the shell using a

pair of pliers. Three seeds were sown per pot. One seedling per pot was maintained after thinning which was done a week after seedling emergence.

Initial plant height measurement and leaf count were done a week after seedling emergence and at 2-week intervals thereafter for 19 weeks. Shoot base diameter, root length, shoot biomass (fresh and dry), and root biomass (fresh and dry) were measured at the termination of the study. Data collected were statistically-analyzed using the analysis of variance ANOVA for RCBD. Significant differences among treatment means were analyzed using the Duncan's multiple range test at the 5% level of probability.

RESULTS AND DISCUSSION

Plant height. Average periodic plant height measurements significantly differed at 5, 7, 9, and 15 but not at 3, 11, 13, 17, and 19 weeks after seedling emergence. Final height in Azotobacter, Vam Root Inoculant, MyKoVam, and Bio Green- applied plants were about the same but were significantly taller than the Untreated control and Bio N treated plants. Generally, of the five biofertilizers used, Bio N appeared to be less effective for Beauty leaf. The fast growth rate of plants applied with biofertilizers, except Bio N, can be attributed to the ability of these beneficial microorganisms to effectively associate with higher plants. Similar published works (Chiu-Chung Young, 1994; Mrkovacki & Milic, 2001; Parr, Hornick, & Kaufmann, 2006) have shown that biofertilizers induced the production of phytohormone-like substances like auxins that alter plant growth and morphology which consequently improved the total vegetative growth of the experimental plants. The less efficacy of Bio N to induce significant growth in Beauty leaf can be also be due to the physiologic inability of the microbial inoculum to infect the root system that could have stimulated the synthesis of growth hormone and enhanced the absorption of vital nutrients from the environment.

Leaf count. Data in Table 2 shows that leaf count differed significantly among treatment means 5, 7, 9, and 15 weeks but not significant at 1, 3, 11, 13, 17, and 19 weeks after seedling emergence. On the first three weeks of growth, biofertilizer application did not significantly affect leaf count in which period, sustained growth of seedlings could have been dependent on the food stored in the large seed itself. Significant differences, however, were observed on the 5^{th}, 7^{th}, and 9^{th} week after seedling emergence. The five

Table.1. Average Periodic Plant Height (cm) of Beauty leaf Measured at Two-Week Intervals

Biofertilizers	Weeks After Seedling Emergence									
	1	3	5	7	9	11	13	15	17	19
Azotobacter	10.95^{ab}	17.33^{ab}	21.95^{a}	23.10^{a}	25.10^{a}	27.67^{a}	29.33^{a}	30.01^{a}	31.63^{a}	31.99^{a}
Bio Green	9.10^{cde}	15.66^{bc}	18.30^{c}	20.93^{cd}	22.90^{bc}	24.87^{bc}	25.50^{bcd}	26.47^{bcd}	27.7^{bc}	29.27^{a}
Bio N	9.77^{bc}	16.10^{b}	19.58^{cd}	20.76^{cd}	22.17c	24.57^{cd}	24.93^{cd}	26.17^{cd}	26.89^{bc}	28.37^{b}
MykoVam	7.79^{e}	15.90^{c}	20.07^{bc}	21.53^{bc}	24.29^{ab}	25.43^{abc}	26.43^{bc}	27.57^{bc}	28.63^{b}	29.73^{a}
Vam Root Inoculant	11.89^{a}	17.56^{a}	21.11^{ab}	22.28^{ab}	24.29^{ab}	26.18^{ab}	27.00^{b}	28.08^{b}	28.74^{b}	30.75^{a}
Control	9.70^{c}	15.23^{c}	18.58^{dc}	19.87^{d}	21.30^{c}	23.37^{d}	24.43^{d}	25.10^{d}	25.90^{c}	26.69^{b}

^{abcde} Treatment means followed by the same letter superscript are not significantly different at the 5 % level of probability

biofertilizers had no significant effects in terms of leaf count having registered a leaf count range of 9.06 to 10.00 per plant on the 9th week but had significantly more leaves than the Untreated Control plants. Bio Green and MyKo Vam were consistently most promising. The significantly higher leaf counts during this period can be attributed to enhanced plant uptake of available soil nutrients such as nitrogen and phosphorus as a result of their effective plant root colonization and consequently improved rooting ability especially during the early stage of plant growth (Sahay & Varma, 2000).

Table 2. Average Periodic Leaf Count in Beauty Leaf Measured at Two-week Intervals.

Biofertilizers	Weeks After Seedling Emergence									
	1	3	5	7	9	11	13	15	17	19
Azotobacter	5.60^{ns}	6.13^{ns}	8.00^{a}	8.53^{a}	9.40^{ab}	10.53^{ns}	11.07^{ns}	12.06^{ab}	12.40^{ns}	12.47^{ns}
Bio Green	5.40	6.00	7.47^{ab}	8.73^{a}	10.00^{a}	10.93	11.47	12. 30^{a}	12.53	12.60
Bio N	5.06	6.13	8.00^{a}	8.47^{a}	9.40^{ab}	10. 53	10.80	11.40^{bc}	11.40	11.40
MykoVam	5.06	6.53	8.00^{a}	9.00^{a}	9.80^{a}	10. 47	11.07	11.73^{abc}	12.07	12.13
VamRoot Inoculant	4.87	5.87	7.33^{bc}	8.33^{ab}	9.06^{bc}	10. 26	10.86	11.80^{ab}	12.20	12.26
Control (Untreated)	5.13	5.87	6.90^{c}	7.60^{b}	8.60^{c}	9.73	10.27	10.87^{c}	11.47	11.47

^{abc} Treatment means followed by the same letter superscripts are not significantly different from each other by DMRT.

^{ns} Not significantly different at the 5% level of probability

Shoot base diameter and root length. No significant differences on shoot base diameter were observed among treatment means indicative of the lack of influence of the materials on shoot base growth within the 19-week growth period (Table 3). Root growth, however, was influenced by biofertilizers as

shown by the significant differences in root length when compared with the Untreated control. Vam Root Inoculant induced a significantly greatest root elongation, however, MyKo Vam, Bio Green, and Azotobacter can be suitable alternative biofertilizers to use for Beauty leaf. These biofertilizers have shown to be compatible with Beauty leaf having influenced root development by possibly enhancing root meristematic activity, N-fixation, and P-solubilization (Young, et al., 1988). Ecologically, having longer roots becomes an advantage to beauty leaf for it will enhance uptake of water and nutrients (Wu, et al 2006) that may be available in the soil to sustain its growth and development.

Shoot and root biomass weight. Significant differences in the shoot and root dry biomass weights among treatment means were observed in 19 weeks of seedling growth but not the shoot and root fresh biomass weights (Table 3). .Azotobacter, MykoVam, and Vam Root were comparable in their shoot dry biomass and proved to be significantly better biofertilizers than Bio Green and Bio N which were comparable with the Untreated control. Azotobacter registered a significantly highest dry root biomass weight that can be attributed to its ability to effectively colonize Beauty leaf and enhance its production of organic material. Generally, Azotobacter showed to be the most compatible biofertilizer considering its consistently significant influence on shoot and root biomass dry weights.

Table 3. Average Shoot Base Diameter, Root Length, Shoot and Root Biomass Weight of Beauty 19 Weeks After Soil Application of Biofertilizers.

Biofertilizers	Shoot Base Diameter	Root Length	Shoot Biomass Weight		Root Biomass Weight	
			Fresh	Dry	Fresh	Dry
	(cm)	(cm)	(g)	(g)	(g)	(g)
Azotobacter	0.78^{ns}	23.92^{ab}	11.31^{ns}	4.63^{a}	4.06^{ns}	1.26^{a}
Bio Green	0.73	24.00^{ab}	10.87	4.28^{ab}	3.36	0.96^{bc}
Bio N	0.74	23.11^{bc}	10.51	4.27^{ab}	3.07	0.95^{bc}
MykoVam	0.74	24.74^{ab}	11.27	4.57^{a}	3.84	1.06^{b}
Vam Root Inoculant	0.76	26.12^{a}	11.00	4.63^{a}	3.29	0.91^{bc}
Control (Untreated)	0.70	20.83^{c}	10.00	3.71^{b}	3.11	0.89^{c}

^{abc} Treatment means followed by the same letter superscripts are not significantly different from each other.

^{ns} Not significant at the 5% level of probability.

CONCLUSION AND RECOMMENDATION

Basing on the various growth response parameters to evaluate the performance of Beauty leaf when applied with biofertilizers at planting, generally, Azotobacter, Bio Green, MyKoVam, and Vam Root Inoculant, but not Bio N proved to be potential recommended alternative biofertilizers for growing this biofuel tree under a screen house condition. A field study, however, is suggested for further performance evaluation of these materials.

REFERENCES

Bashan, Y., Holguin, G., & de-Bashan,L. (2004). *Azospirillum*-plant relationships: Physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of Microbiology*, *50*, 521-577.

BIOTECH-UPLB. (2008). *Organic inputs and technology*. Retrieved September 3, 2008 from http://www.biotech/tech.profile.html Bingquan, F. (2007). *Application of Biofertilizers and Plans for Phase II in China* FNCA 2007 Workshop on Bioferilizer Project. KualaLumpur,Malaysia25-29February2008.

Cano, A., Rodriguez-Romero, A. & Jaizme-Vega, M. (2007). Application of the new *in vitro* mycorrhizal inoculants Glomygel on micropropagated and seedbed-raised plants of commercial interest. Proceedings of the 2nd International Bio Micro World Conference, Seville, Spain, Nov. 28-Dec. 1, 2007.

Casta, Y. (2001). Growth response of molave (*Vitex parviflora* Juss.) seedlings to Biocore and Mykovam *Philippine Journal of Soils, 130 (*2), 52-56.

Chiu-Chung Young (1994). *Selection and application of biofertilizers in Taiwan* a*griculture*. Paper presented at the FFT International seminar workshop on the use of microbial and organic fertilizers in crop production held in Suweon, South Korea on June 13-18 1994.

Dalmacio, Ida F. (2008). *Biotechnology for sustainable agriculture*. Retrieved September 3, 2008 from www.agnet.org/library/bc/44009/bc44009.pdf

Elefan, Ernesto S. (2007). Biofertilizers *for Jatropha curcas* L. (Euphorbiaceae) grown in different planting media. *Proceedings of the International Conference on Environmental Research and Technology*, Penang, Malaysia, May 28-30, 2008. pp. 308-312.

Elevitch, C. R.(Ed.) (2006). Species Profile for Pacific Island Agroforestry. Retrieved September 5, 2008 from www.traditional Tree.org.

Galal, Y.G.M., El-Ghandour, I.A., Ally, S.S., Soliman, S. & Gadalla, A. (2000). Non-isotopic method for the quantification of biological nitrogen fixation and wheat production under field conditions. Biology and Fertility of Soils. 32(1):47-51.

Gandanegara, S. (2007). *Development of Azora, PGPR Biofertilizer for Corn*. FNCA 2007 Workshop on Bioferilizer Project. Kuala Lumpur, Malaysia 25-29 February, 2008.

Gupta, N. & Baig, S. (2001). Evaluation of synergistic effect of phosphate solubilizing *Penicillum* spp, AM fungi and rock phosphate on growth and yield of wheat. *Philippine Journal of Soils*. 130: 2, 21-23.

Hathurusingha, S. & Ashwath, N. (2007*). Beauty leaf (Calophyllum inophyllum* L.): a *tree with great economic potential.* Proceedings of the 12th International Symposium on Forestry and Environment, University of Sri Jayewardenepura, Nugegoda, Sri Lanka, November 30-December 1, 2007.

Lemmens, R.H.M.J.(2003). *Calophyllum inophyllum* L. In: Louppe, D. (Ed.). Prota Timbers/Bois d'œuvre. [CD-Rom]. PROTA, Wageningen, Netherlands. Lim, S.C. & Lemmens, R.H.M.J. (2003). *Calophyllum L*. In: Lemmens, R.H.M.J. & Bunyapraphatsara, N. (Eds.). *Plant Resources of South-East Asia No 12(3). Medicinal and poisonous plants* 3. Backhuys Publishers, Leiden, Netherlands. pp. 102106.

Li, Ru. (2007). *Plant growth-promoting Rhizobacteria.* Retrieved December 5, 2008 from *http//www.*umanitoba.ca/faculties/Plant Growth-Promoting Rhizobacteria.

Mrkovacki. N. & Milic, V. (2001). Use of *Azotobacter chroococcum* as potentially useful in agricultural application. *Annals of Microbiology*, 51.145-158.National Institute of Industrial Research Board (2006). *Biofertilizers and organic farm*. Retrieved July 16, 2009 from http://www.niir.org/books/book/zb.

Nuntagij, A.(2007) . Biofertilizer in Thailand. FNCA2007 Workshop on Bioferilizer Project.Kuala Lumpur, Malaysia , 25-29 February 2008.

Nuruzzaman, M., Ashrafuzzaman, M., Islam, M. & Islam, M. (2003). Field efficiency of biofertilizers on the growth of okra (*Abelmoschus esculentus* (L.) Moench]). *Journal of Plant Nutrition and Soil Science, 166* (6): *764-770*

Ozturk, A. & Caglar, O. (2003). Yield response of wheat and barley to inoculation of plant growth -promoting *Rhizobacteria* at various levels of nitrogen fertilization. *Journal of Plant Nutrition and Soil Science*, *166* (2), 262-266.

Padilla, V. (2006). *Microbial inoculant for growth and development of Physic nut (Jatropha curcas* L.). Paper presented during the 5th Asia-Pacific Biotechnology Congress held in Tagbilaran City, Bohol, Philippines on Mayh 10-13, 2007.

Parr, J.F., Hornick, S.B. & Kaufmann, D.D. (1994.). *Use of microbial inoculants and organic fertilizers in agricultural production*. Retrieved July 17, 2009 from http://www.agent.org./library/articlehtml.

Quisumbing, E. (1978). *Medicinal plants of the Philippines*. Quezon City, Philippines: Katha Publishing Co,, Inc.

Rao, S. & Sami Reddy, K. (2002). Integrated nutrient management *vis a vis* crop production, nutrient balance, farmer livelihood and environment : India, Retrieved November 22, 2008 from http://www.fao.org./docrep/010/ag120e/AG120E09.

Species Survival Commission (2007). IUCN Red List Categories. Retrieved September 3, 2008 from www.unep wcmc.org/species/tree.

Stevens , P. F. (2006). *Calophyllum inophyllum. 2006 IUCN Red List of Threatened Species.* Retrieved May 12, 2009 from http://www.iucnredlist.org/search/details.php/33196/all

Sugar Regulatory Administration. (2007). A Pamphlet on Biofertilizers for Sugarcane.4 pp.

Tawfik, M., Amany, M., Bahr, A., & Salem, A.K. (2006). Response of Kaller Grass (Leptochloa fusca L.) to biofertilizer inoculation under different levels of seawater irrigation. *Journal of Applied Sciences Research*, *2*(12), 1203-1211.

Topolianitz,S., Ponge, J.F., Arrouays, D., Ballof, S. & Lavelle, P. (2002). Effect of Organic Manure and the Endogenic Earthworm Pontoscolex corethrurus (Oligohaeta: Glossoscolecidae) on Soil Fertility and Bean Production. *Biology and Fertility of Soils*, *36*(4), 313-319.

Verkade, S. D., Hamilton, D.F. & Elson, L.C. (2008*). Effect of endomychorrhizal inoculation during propagation on growth following transplanting of Cornus sericea cuttings and seedlings.* Retrieved August 27, 2008 from http://www.actahort.org/books.

Wikepedia. (2008). *Calophyllum.* Retrieved October 10, 2008 from http://en.wikipedia.org/wiki/Calophyllum.

Wu, S. C., Cao, Z.H., Li, K., Cheung, C. & Wong, M.H. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers in a greenhouse trial. *Geoderma, 125* (1/2),155-166.

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