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ORIGINAL ARTICLE

Growth response of *Tabernaemontana pandacaqui* Poir. in soil from a former mining site in Marinduque, Philippines

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Potential hazards and immediate rehabilitation of inactive/abandoned mines are concerns in Mogpog, Marinduque. Efforts have been initiated in the area through the use of species such as *Acacia auriculiformis* Benth*.* The study focused on assessing and mass-producing endomycorrhizal fungi from plants collected in the area and investigating the growth response of indigenous *Tabernaemontana pandacaqui* Poir. with *A. auriculiformis,* endomycorrhizas, and biofertilizers in soil from mine tailings. Soil and plant collection was done in three major ecosystems: agroforestry, *A. auriculiformis* stand, and natural forest. Associated endomycorrhizas were isolated and prepared as an inoculant to test the response of *T. pandacaqui*. Field endomycorrhizal spores from *T. pandacaqui* were: *Acauslospora* (50), *Glomus* (16), and *Gigaspora* (2), which sporulated (422/75g dry soil) as inoculant. Height and diameter of *T. pandacaqui* were significantly ($p < 0.01$) better with *A. auriculiformis,* possibly due to its N-fixing property, though host mortality was higher brought by competition. Soil inoculants promoted the growth of *T. pandacaqui* but MykoVAM performed significantly ($p < 0.05$) in height than control. To facilitate the ecological succession in old mining, the *A. auriculiformis* could be maintained as a nurse crop for threatened species like *T. pandacaqui* and consider the potential of local endomycorrhizal isolates encouraging growth and survival.

Keywords: mining site, mycorrhiza, *Acacia auriculiformis*, *Tabernaemontana pandacaqui*, growth response.

Introduction

Mineral resources are one of the Philippines' acknowledged rich assets that significantly contribute to the country's economic development.Marinduque is among the mineral-rich provinces with large copper deposits discovered by mining prospectors in the late 1960s. It became the home to one of the most significant mining operations in the country. However, a disastrous mine tailing accident in 1996 affected the provincial island (Gregory, 2004)). Republic Act 7942 or the Philippine Mining Act of 1995 states that "*Contractors and permittees shall technically and biologically rehabilitate the excavated, mined-out tailings covered, and disturbed areas to the condition of environmental safety, as may be provided in the implementing rules and regulations of this Act*". In this regard, the Department of Environment and Natural Resources (DENR) requires responsible rehabilitation of mined areas (Teves, 2018). At least 22 inactive and abandoned mine sites need a plan for rehabilitation like the case of an inactive copper mining site in Mogpog, Marinduque. The government and mining companies are making efforts to restore mined-out areas and convert areas as productive assets (Felix, 2004). The mountain situated in the area was flattened and replaced with a mountain of dogged unprocessed copper-rich soil that reached adjacent rice fields, agricultural areas, and mangrove forest. The mining activities led to several environmental problems, such as deforestation, leaving only less than 30% of the forest cover in 2001 (Medianista & Labay, 2017). Such events caught the Philippine government's attention and made them prioritize the rehabilitation and remediation of the inactive mine sites to enhance its current state. Thus, improving biodiversity can help in natural ecological processes and human consumption (Coracero & Malabrigo, 2020).

Phytoremediation is an emerging biological approach in the cleanup of contaminated sites. According to Sigurdur Greipsson (2011), phytoremediation is a recently developed technology that offers a cost-effective solution by using plants, and associated soil microbes, to reduce the content, or toxic effects, of contaminants in the environment. It is a remediation technique that takes advantage of the biological processes of plants. Plants alter the soil through theshoot and root growth, water and mineral acquisition, senescence, and eventual decay. The following series of actions directly or indirectly absorb, sequester, and/or degrade contaminants. Planting and growing compatible species either remove the pollutant or physically or chemically convert contaminants into a component that no longer poses a threat to the surrounding environment (Cunningham & Ow, 1996).

In the Philippines, DENR uses phytoremediation species such as *Acacia auriculiformis* Benth., *Acacia mangium* Willd., *Pterocarpus indicus* Willd., *Casuarina equisitifolia* Forst., and *Chrysopogon zizanioides* (L.) Roberty. The Philippine governmentPhilippine government supported the department's initiative supported the department's initiative, thus issuing Executive Order 270 entitled "National Policy Agenda in Revitalizing Mining in the Philippines" on January 16, 2001 and stated in Section 2-I, that "Remediation and Rehabilitation of abandoned mines shall be accorded top priority to address the negative impacts of past mining projects." (DENR, 2014). One of major steps under the executive order was the formation of the Bioremediation Research Team through National Academy for Science and Technology to conduct studies and research to develop technologies that can help in the remediation of toxic mining wastes (Raymundo et al., 2006).

The DENR initiated to plant *A. auriculiformis*, also known as earleaf acacia, in the area in 2009. According to the ERDB Director Dr. Henry A. Adornado, 42 ha of mined-out areas were successfully planted. It was found that *A. auriculiformis* and other species that were planted for the phytoremediation, such as *Pterocarpus indicus* Willd. were most efficient in the absorption of metals (Sarian, 2017). There were no records on the number of individuals of these species the DENR have planted throughout the years. Nevertheless, patches of *A. auriculiformis* can be observed and have dominated the area. Earleaf Acacia is a fast-growing introduced species that can grow on poor quality soil. Earleaf acacia has dense roots and tolerates seasonally waterlogged soil and produces a heavy leaf litter, making it suitable for rehabilitation of degraded land (Contu, 2012). These characteristics make the species compatible for stabilizing eroded land. It is popular in stabilizing and revegetating mined-out areas because *A. auriculiformis* can grow in infertile soil and tolerate both highly acidic and alkaline soils capable of nitrogen fixing (Orwa et al., 2009).

This study aims to assess the effects of *A. auriculiformis* to the growth of Philippine native species in Mogpog, Marinduque, such as *Tabernaemontana pandacaqui* (Pandakaki). Specifically, the study aims to assess and mass produce endomycorrhizal fungi of plants in the area and determine the growth behavior and response of Pandakaki with Earleaf Acacia, endomycorrhizas, and soil amendments (inclusion of biofertilizers).

Methodology

Study site

Inventory and samples were gathered in Brgy. Capayang, Mogpog, Mariduque and is located at 13°30'16"N 121°51'42"E (Fig. 1a). Post mine areas have soils contaminated with acid mine drainage (Medianista & Labay, 2017).

The province was known for its rich mineral deposits such as gold, copper, and iron. During the 1960s up to the late 1990s, mining activities were operated on-site in open-pit mining. All mining activities on the island were ceased when the 1.5-3 million cubic meters of sulfidic tailings slurry from the Tapian Pit storage area into the Makulapnit River, Boac River, and eventually the ocean west of the island (Plumlee et al., 2000). The mining caused several environmental issues brought about by improper mining activities, such as mine tailings being dumped in rice fields, mangroves, agricultural lands, and forest lands.

The mine site in Brgy. Capayang, Mogpog, Mariduque is a copper-rich soil. Soil analysis from the study of Tulod et al. (2012) showed a high concentration of copper and low content of other toxic contaminants (Table 1). The soil in the area was a loam type soil with 62% sand, 36% silt, and 2% clay and highly acidic (pH = 4.4) with deficient nitrogen (100 ppm), potassium (101.4 ppm), phosphorus (81 ppm), OM content (0.24%), and CEC (17.7 cmol/kg).

Table 1. Heavy metals present in the soil of inactive/abandoned mining sites (Tulod et al., 2012).

Metal Elements	Concentration (ppm)
Lead	4
Arsenic	13
Mercury	3
Zinc	85
Copper	2.603
Nickel	52
Chromium	55
Cadmium	2.69

The growth of vegetation in this kind of condition is nearly unattainable. Excess levels in minerals in soil lead to abnormality in plant growth. The ideal pH for plant growth ranges from 5.5 to 7.0, although plants have adapted to withstand pH beyond this range. It is significant in many chemical processes in the soil, such as plant nutrient availability (The Mosaic Company, 2020). To address the condition in the inactive/abandoned mining site, the DENR initiated the site's rehabilitation, and one of their actions was planting *A. auriculiformis*. The area is now covered with clumps of *A. auriciformis,* and other plant species are starting to thrive in the copper-rich soil (Fig. 1b).

Selection of species for the experiment

The vegetation composition of the area was considered in selecting the appropriate species to test. Thus, vegetation assessment was conducted in the area. Three types of ecosystems were surveyed: the natural forest, agroforestry ecosystem, and the Earleaf Acacia stand. *Acacia auriculiformis* A. Cunn. ex Benth. and *Tabernaemontana pandacaqui* Poir. were found to be the most populous for trees and understorey species, respectively. *A. auriculiformis* is an introduced species used in the rehabilitation and phytoremediation, while *T. pandacaqui* is a Philippine native species thriving in the area. The effect of their coexistence in an ecosystem is an interesting aspect to study. Therefore, these two species were used as the subjects of the experiment.

Isolation, characterization, and mass production of endomycorrhizas

Eight (8) abundant wildling species were collected still attached to its soil at the rhizosphere layer. These species were *Trema orientalis* (L.) Blume*, Guioa koelreuteria* (Blanco) Merr.*, Antidesma ghaesembilla* Gaertn.*, Morinda citrifolia* L.*, T. pandacaqui, Pittosporum pentandrum* (Blanco) Merr.*, Alstonia macrophylla* Wall. ex G. Don*,* and *A. auriculiformis.* For each sample, 25 grams of soil closest to the host plant's fine roots was subjected under the sieving and centrifugation method as stated in the book of Brunrdett et al. (1996). The soil is then continuously washed with water and sieved in 150 μm and 45μm mesh. After sieving, the remaining soils were placed in centrifuge tubes labeled with the corresponding mesh size, and then water was added. These tubes were placed in a centrifuge for 5 minutes at 2000 revolution per minute (RPM). Next, floating sediments were drawn off. Then, sucrose was poured into the centrifuge tubes. Once again, the tubes were placed in the centrifuge and were left to spin for 2 minutes at 2000 RPM. Following this step, floating sediments were sieved, and soils that settled at the bottom of the centrifuge tubes were discarded. Sieved soils were then placed in a petri dish. All were examined under the microscope and were isolated. The genus and individual count of the spores were recorded. The same procedures were done for each soil sample for the plant species collected. All isolated spores were then mass produced at UPLB BIOTECH laboratory and harvested with *Centrosema pubescens* Benth. as host plants.

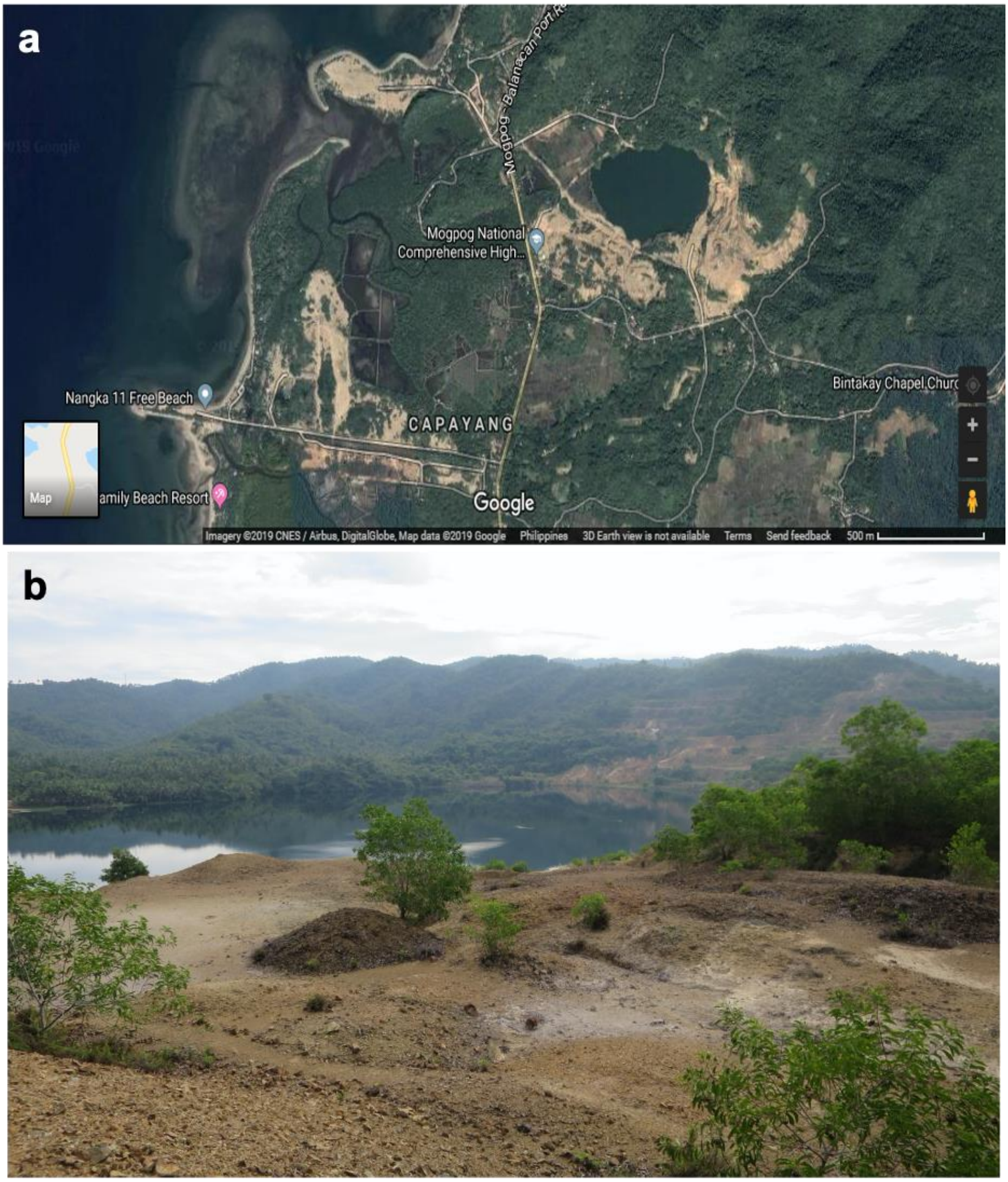


Fig. 1. Satellite image of mining area (a) (Google Earth, 2019) and the study site (b) in Brgy. Capayang, Mogpog, Marinduque.

Experimental set-up and observation

The interaction of *T. pandacaqui* and *A. auriculiformis* under different soil amendments were observed from January 4, 2018, to April 4, 2018. To have an in-depth observation of the influence of *A. auriculiformis* to *T. pandacaqui*, some 50 *T. pandacaqui* were planted individually in 4 x 8 cm polyethylene bags, and 50 T. pandacaqui with A. auriculiformis were planted together in 4 x 8 cm polyethylene bags (Fig. 2). Sterilized soils collected from the old mining site were used as planting media. Both *T. pandacaqui* with and without *A. auriculiformis* were subjected to five soil amendments with ten replicates for each amendment. The following treatments were done, and all arranged based on CRD:

a. **Control** – sterilized soil only
b. **Marinduque inoculants** – sterilized soil plus the mass-produced endomycorrhizal inoculant
c. **MykoVAM** – sterilized soil plus MykoVAM biofertilizer (a biofertilizer produced by UPLB Biotechnology which aims to aid in nutrient and water absorption)
d. **BIO-N** – sterilized soil plus BIO-N biofertilizer (a biofertilizer produced by UPLB Biotechnology which has effective nitrogen supplement for plants)
e. **The combination of all treatments** – sterilized soil plus massed produced endomycorrhizal inoculant, MykoVAM, and BIO-N.

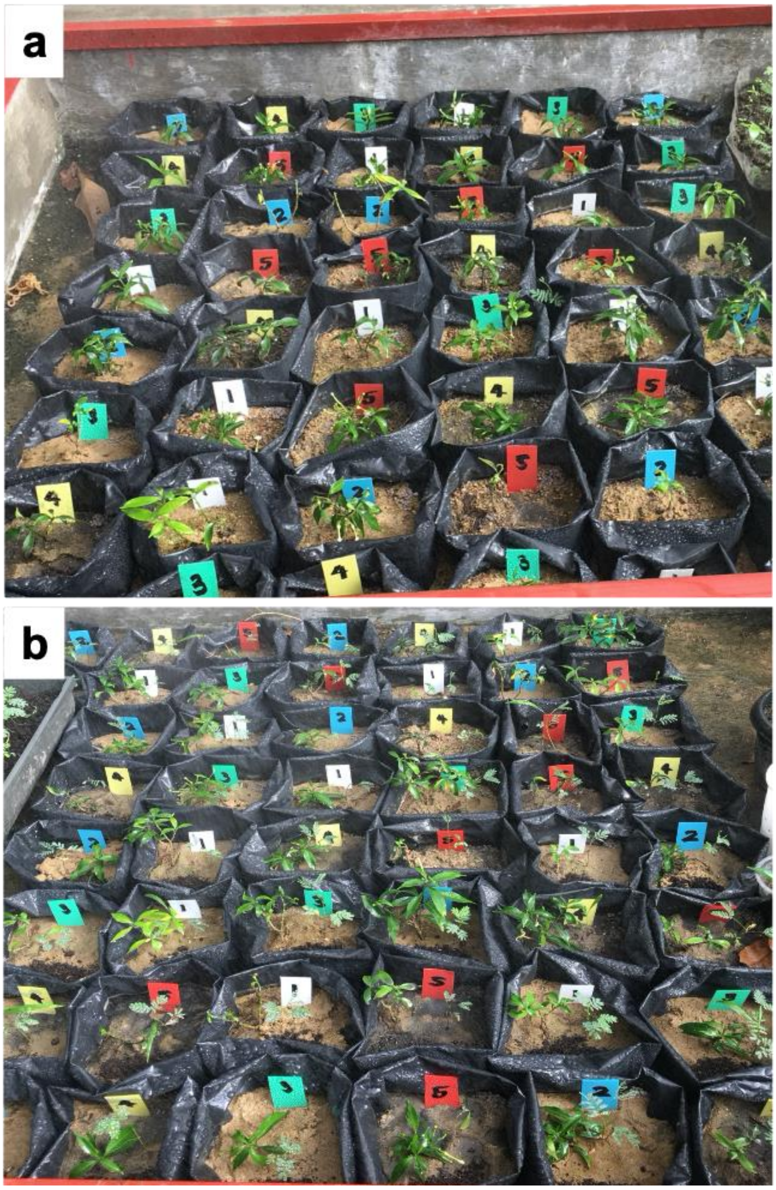


Fig. 2. Experimental layout showing *T. pandacaqui* (a) and set up with *A. auriculiformis* () **(Note for colored tags: Control (white), Marinduque inoculants (blue), MykoVAM (green), Bio-N (yellow), and combination (red))*

Initial root, height, and diameter were recorded for all plants. The plant's height and diameter were measured in the level of the half of the height of polyethylene bag at least once a week. Changes in the diameter, height, and distinguishable physiological differences of the plants were recorded.

Results and discussion

Endomycorrhizal assessment and mass production

The endomycorrhizal fungi isolated from sampled ecosystem were characteristically microscopic that ranged from 45-150 µm. Their colors were transparent to white, pale to dark yellow, orange to dark orange, or brownish to dark brown. Some spores were double-walled with shapes characteristically spherical, oblongate, ellipsoidal, or irregular. Other endomycorrhizal isolates exhibited hyphal attachments that were connected to fine roots (Table 2 & Fig. 3).

There were only three genera of endomycorrhizal fungi that were analysed in terms of spore density across all plots. Endomycorrhizal spores consisted of 228 *Acaulospora* (54%), 116 *Acaulospora* with hyphae (28%), 73 *Glomus* (17%), and 5 *Gigaspora* (1%) (Fig. 4). *Acaulospora* was the most prevalent among the AMF isolated.

Based on host plants, species *A. auriculiformis* had the highest spore count among the few soil samples of various plant species. A total of 101 individual counts of endomycorrhizal fungi were isolated from the rhizosphere of *A. auriculiformi*s (Fig. 5). The spores per 75 g of dry soil comprised of *Acaulospora* (71) and *Glomus* (30). The number of spores isolated from rhizosphere per 75 g of dry soils of T. pandacaqui comprised of *Acaulospora* (50), *Glomus* (16), and *Gigaspora* (2).

Table 2. Morphological description of endomycorrhizal genera isolated from different host plants and vegetative genera.

Host Plants	Endomycorrhiza Genera	Morphological Description of Endo- mycorrhizal Spores
T. orientalis	*Acaulospora*	Spores collected were relatively small; have colors ranging in yellow, orange, and brown; double-walled; shapes observed were spherical and oblongate, and some have hyphal attachments.
	Gigaspora	It was dark orange and spherical.
	Glomus	Collected Glomus was transparent to dark brown; double-walled, and have spherical and irregular shapes.
G. koelreuteria	*Acaulospora*	Spores were yellow to orange in color; ellipsoidal, and some of them has hyphal attachment.
A. ghaesembilla	*Acaulospora*	These were yellow to dark yellow colored, transparent, and some have hyphal attachment.
M. citrifolia	*Acaulospora*	Some spores were pale yellow to dark yellow, and others were dark orange; shapes observed were ellipsoidal and spherical, and some have hyphal attachments.
	Gigaspora	These were dark orange and spherical.
T. pandacaqui	*Acaulospra*	Most were yellow, some are dark orange, and some were brownish to dark brown in color; double-walled; have an ellipsoidal and spherical shape, and some have hyphal attachment.
	Glomus	Spores were transparent to dark brown, double-walled, and spherical, and irregular in shape.
P. pentandrum	*Acaulospra*	These were pale yellow to dark yellow, and some were brownish; transparent; double-walled; spherical, and oblongate; and some have hyphal attachments.
	Glomus	Color ranges from light brown to dark brown and relatively small.
A. macrophylla	*Acaulospra*	Spores were in different shades of yellow, orange, and brown; double-walled; spherical, ellipsoidal, and oblongate in shape; and some have hyphal attachments.
	Glomus	Observed spores were transparent to dark brown; double-walled; spherical, and irregular in shape.
A. auriculiformis	*Acaulospra*	Transparent to dark yellow, orange, and brownish in color; double-walled; with shapes spherical, oblongate, and irregular; some have a hyphal attachment.
	Glomus	Observed spores were transparent to dark brown; double-walled; spherical, and irregular in shape.

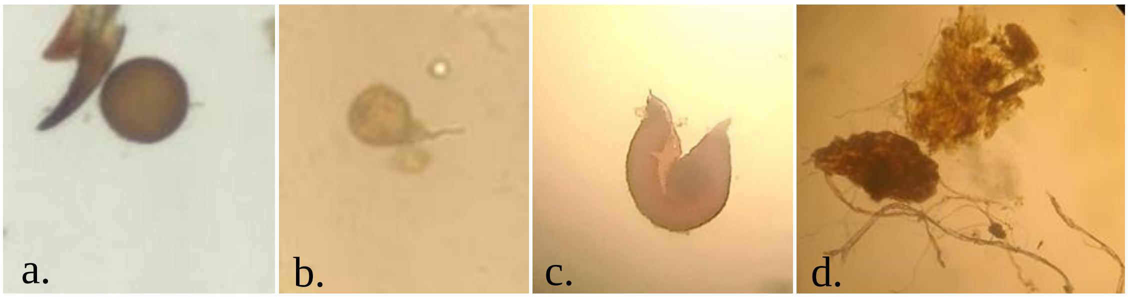


Fig. 3. Samples of (a) *Acaulospora,* (b) *Gigaspora,* (c) *Glomus,* and (d) hyphal attachment of endomycorrhizal isolates from rhizosphere in inactive/abandoned mining site in Brgy. Capayang, Mogpog, Marinduque.

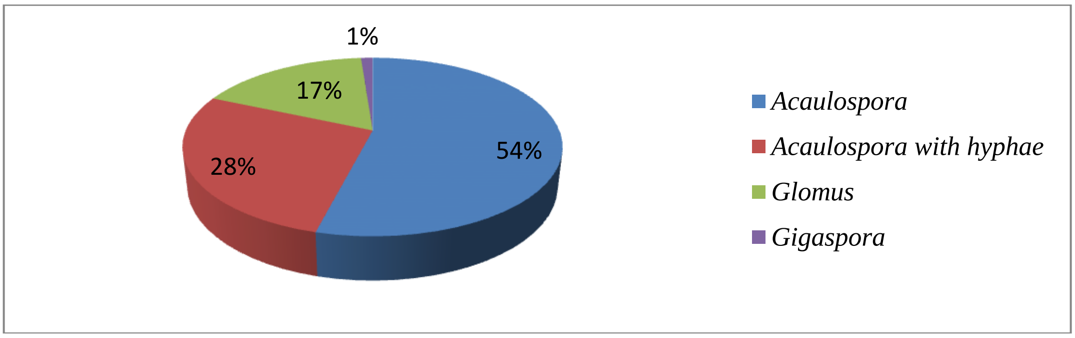


Fig. 4. The proportion of endomycorrhizal genera of spores isolated from rhizosphere across vegetation formerly mined from Brgy. Capayang, Mogpog, Marinduque.

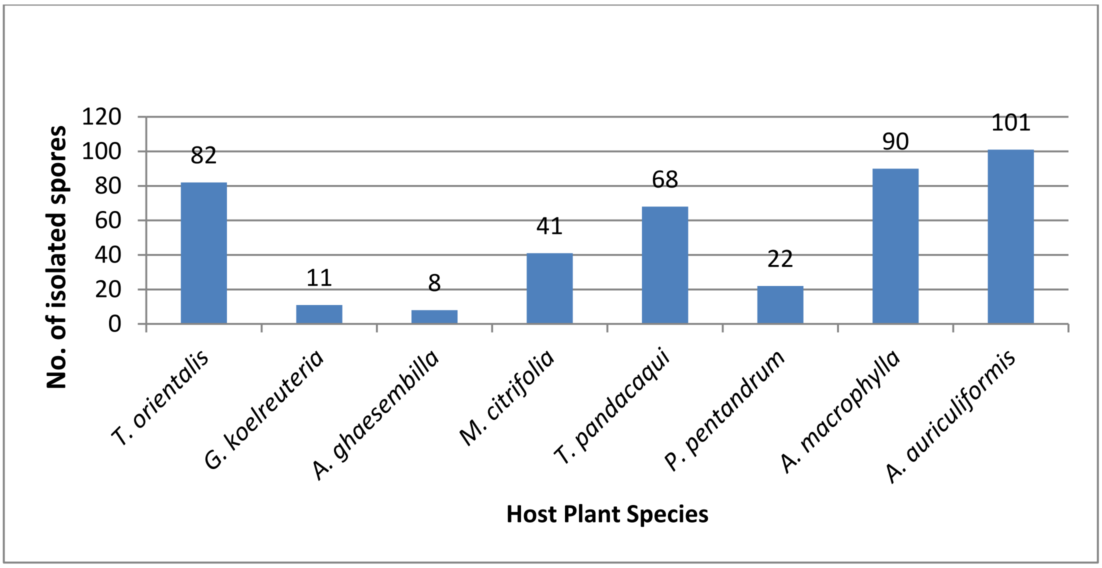


Fig. 5. Total number of isolated spores (per 75 g dry soil) from different host plants growing within ecosystems in inactive/abandoned mine

The diversity of endomycorrhizal fungi is significantly influenced by nutrient composition and soil texture varying in capacity and tolerance (Zhao, et al., 2017). Certain species are drought resistant, tolerant to pathogens, and could endure extreme soil temperature (Aggangan et al., 2015). *Acaulospora, Glomus*, and *Gigaspora* are particularly tolerant to acidic and/or alkaline soils (Clark, 1997). These species are resistant to harmful soil conditions like the mine site left in Brgy. Capayang, Mogpog, Marinduque.
According to Lovelock et al. (2003), endomycorrhizal fungi are interdependent with plant roots. Growth rates or productivity could influence the sporulation of fungal symbionts. A greater volume of spores was observed with fast-growing species. *A. auriculiformis* is a fast-growing species, and its physiological characteristics affected the abundance of endomycorrhizal fungi.
After mass production, the number of isolated spores gathered from the rhizosphere of 75 g dry soil of *T. pandacaqui* was comprised of 212 spores in 150 µm sieve, and 228 in 45 µm sieve collected and were utilized as soil amendments for the experimental setup. Spores that were isolated were relatively large, have a range of colors from yellow to dark orange and light to dark brown, and the shapes were oblongate and ellipsoidal (Table 3), and the majority were *Acaulospora* species.

Table 3. Endomycorrhizal fungi (spore/ 25 g dry soil) isolated from rhizosphere of *T. pandacaqui* with sizes and description.

Soil Sample	Endomycorrhizal genera	45 µm	150 µm	Description
Replicate 1	*Acaulospora*	52	41	Double-walled, light yellow to dark brown, oblongate, transparent
	Glomus	36	32	Spores were transparent to dark brown, double-walled, and spherical, and irregular in shape
Replicate 2	*Acaulospora*	43	62	Mostly yellow, others transparent to white, ellipsoidal
Replicate 3	*Acaulospora*	64	56	Yellow to dark brown, oblongate, double-walled
	Glomus	33	21	Transparent to dark brown; double-walled; spherical and irregular in shape
	Total	228	212	
	Mean	46	42	

There were no reports and/or studies found discussing the symbiotic relationship between *T. pandacaqui* and endomycorrhizae. It is possible that the two have no special interaction, and it only exhibits only typical association of plants and endomycorrhizae. Nevertheless, the isolated spore may be accounted for *T. pandacaqui* to in such an environment as the copper-rich soil of the former mining site in Mogpog, Marinduque. Aggangan and Cortes (2018) stated that endomycorrhizal fungi enhance plant tolerance to heavy metal contamination and induced Cu retention in the roots of the seedlings. Indigenous endomycorrhizal fungi have the potential in the rehabilitation of mined-out areas in the Philippines.

Growth Response of T. pandacaqui

Growth and survival of *T. pandacaqui* with *A. auriculiformis* and amendments were not significant in height and diameter at first month. Development of *T. pandacaqui* at 60 days after inoculation showed 50% mortality when planted with *A. auriculiformis.* Growth of *T. pandacaqui* was improved when applying MykoVAM than all other amendments. At harvest, the growth of *T. pandacaqui* was better with *A. auriculiformis.* The height of *T. pandacaqui* with and without *A. auriculiformis* was significantly different ($p < 0.01$). Height measurement of *T. pandacaqui* with *A. auriculiformis* in the control was the highest and grew by 21%. And the highest height value of *T. pandacaqui* without *A. auriculiformis* was MykoVAM by 11% among all treatments (Fig. 6).

Height increment every 30 days of *T. pandacaqui* with *A. auriculiformis* applied with MykoVAM had the highest increment and by the end of the observation, it grew by 22% (Fig. 7a). Control and MykoVAM was significantly different ($p < 0.05$). The response of *T. pandacaqui* without *A. auriculiformis* to Bio-N yielded the highest growth increment in height by 14% after 30 days, and from the second month until harvest, control had the highest growth increment by 11% (Fig. 7b). Both Bio-N and Control have significant differences ($p < 0.05$).

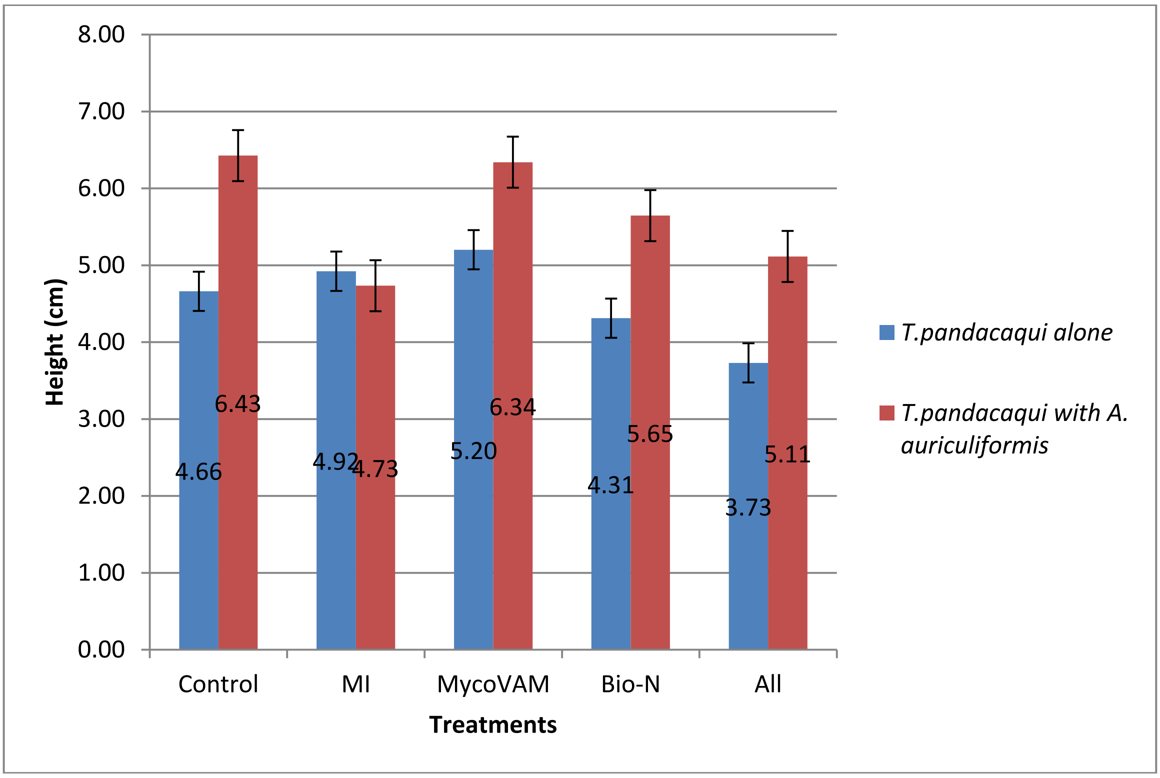
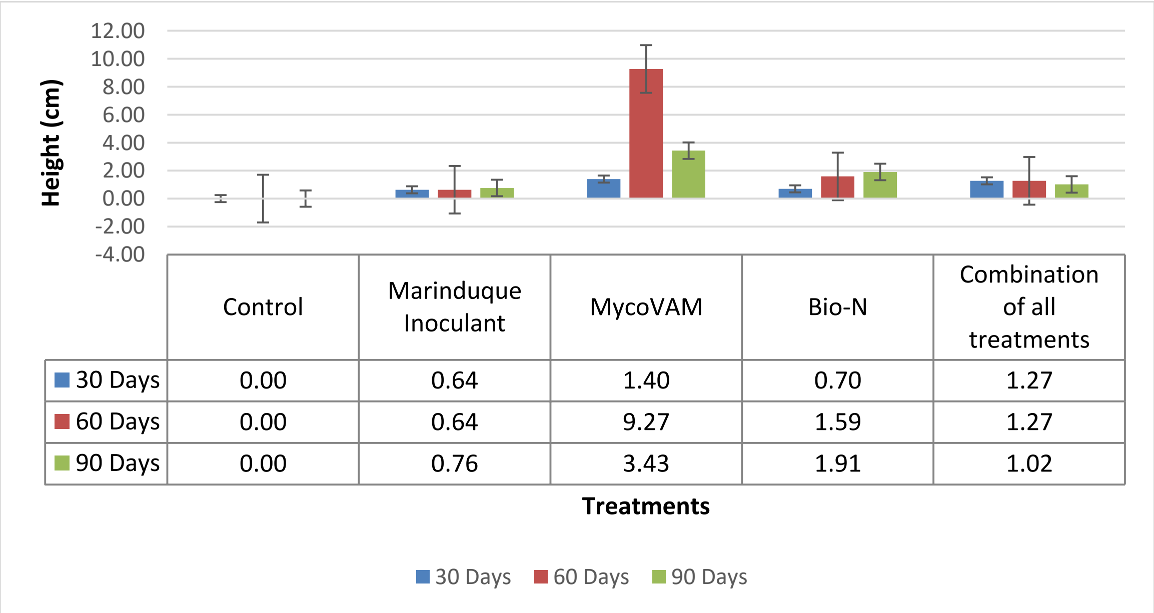


Fig. 6. Final Height of *T. pandacaqui* in response to inoculation of amendments when planting with or without *A. auriculiformis* (Note: MI- refers to Marinduque inoculants; All-means combination of all treatments)

(a)



	Control	Marinduque Inoculant	MycoVAM	Bio-N	Combination of all treatments
30 Days	0.00	0.64	1.40	0.70	1.27
60 Days	0.00	0.64	9.27	1.59	1.27
90 Days	0.00	0.76	3.43	1.91	1.02

(b)

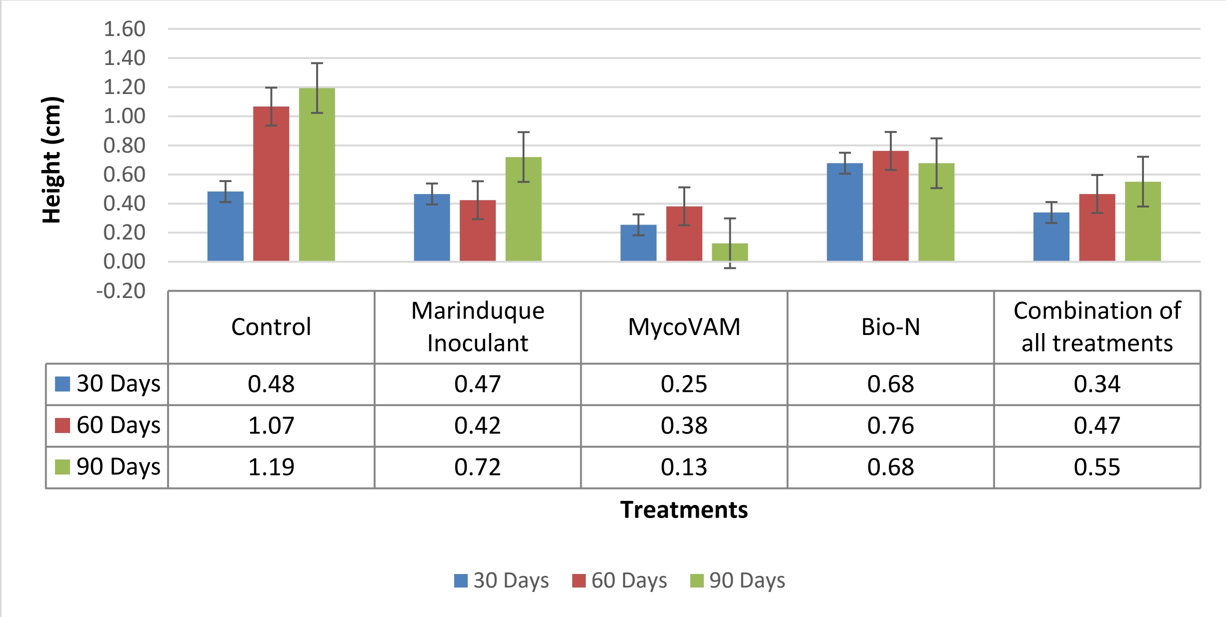


Fig. 7. Height Increment of *T. pandacaqui* in response to inoculation of amendments when planting (a) with and (b) without *A. Auriculiformis.*

On the contrary, *T. pandacaqui* without *A. auriculiformis* had a superior final diameter measurement and was significantly different from *T. pandacaqui* with *A. auriculiformis* ($p < 0.05$). Both Control and MykoVAM were the top treatments for the diameter (Fig. 8). In *T. pandacaqui* with *A. auriculiformis*, MykoVAM had the highest increment for 2 months by 6%, and after 90 days, Marinduque inoculant had the highest diameter increment valuing 38% from the initial (Fig. 9a). The diameter of *T. pandacaqui* alone, Bio-N has the highest diameter increment in 30 days by 14%, in 60 days, Marinduque inoculants had the highest increment valuing 34%, and combination of all treatments after 90 days with 60% increment from the initial planting; no significant difference was observed (Fig. 9b).

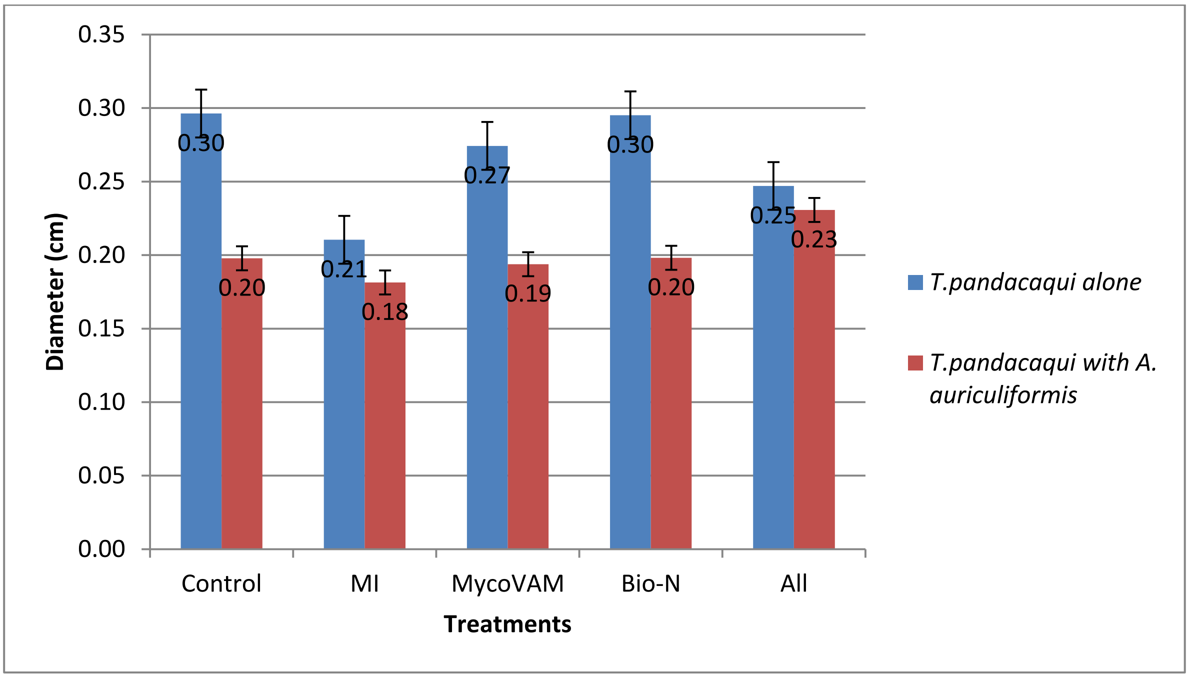
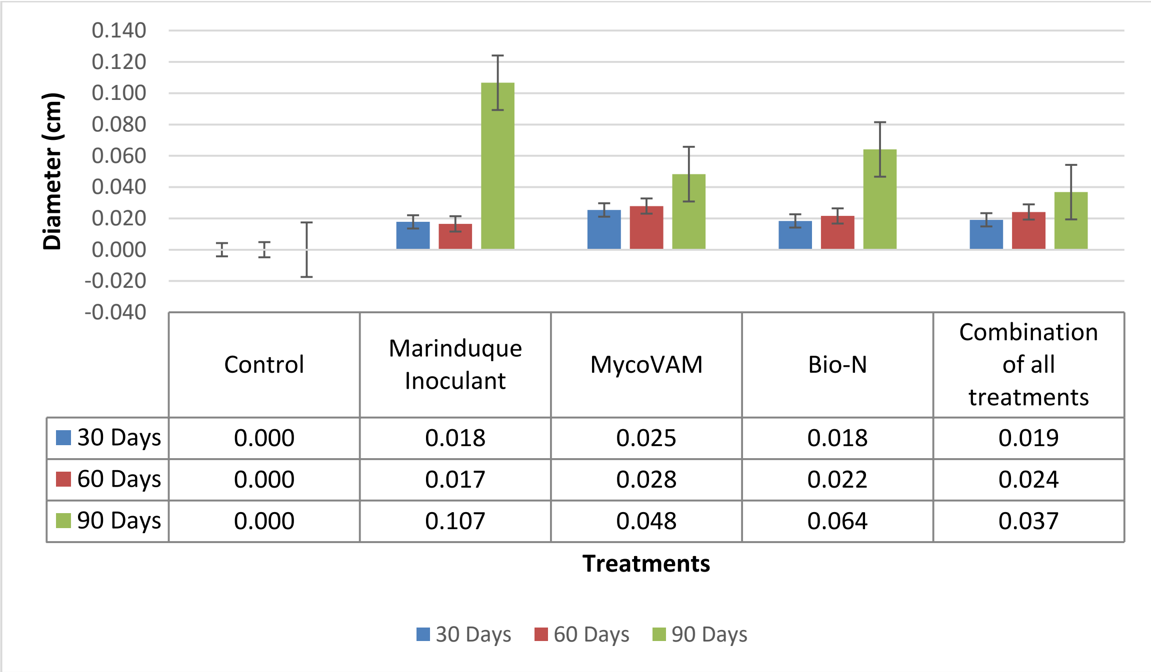
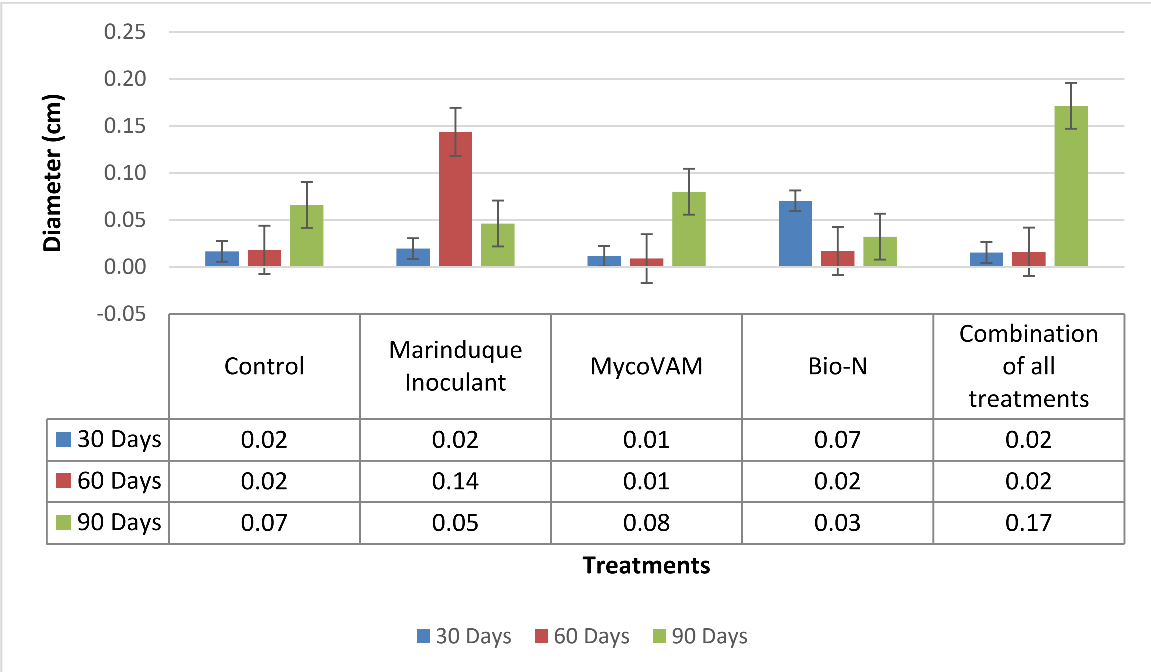


Fig. 8. The final diameter of *T. pandacaqui* in response to inoculation of amendments when planting with or without *A. auriculiformis* (Note: MI- refers to Marinduque inoculants; All- means the combination of all treatments)



(a)



(b)

Fig. 9. Diameter increment of *T. pandacaqui* in response to inoculation of amendments when planting (a) with and (b) without *A. auriculiformis*

Based on the results, the application of MykoVAM has standout among other treatments; a fungi-based bio-fertilizer developed by UPLB- BIOTECH. It contains spores, infected roots, and propagules of endomycorrhizal fungi that are estimated to replace 60%-85% of plants' chemical fertilizer requirement and absorb water and nutrients, particularly phosphorus. Also, the inoculants aid the plant in the resistance of pathogens in the roots and increase drought and heavy metal tolerance (de la Cruz, 2012). Phosphorus is vital to the growth of new tissue and the division of cells. It provides the plant the ability to resist disease due to stimulating the fast growth and development of plants (Tajer, 2016). Similar to this amendment was Marinduque inoculant. However, it did not perform well like the MykoVAM. It may be possible that the spores that can be found in the Marinduque soil that were collected had reduced due to the long storage before it was isolated.

Moreover, it was also observed that Bio-N provided a positive impact on the growth of *T. pandacaqui.* Bio-N is a microbial-based fertilizer that converts nitrogen gas into its available form and reduces the nitrogen requirements of crops up to 50%. It was developed by Dr. Mercedes Umali-Garcia and Teofila S. J. Santos of the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), in 1985 to lessen the cost of fertilizers (Calibo, n.d.). Nitrogen is a substantial food for plants that promotes the vegetative part's growth and development and prompts the uptake and utilization of nutrients, including potassium, phosphorous, and controls overall plant growth (Leghari *et al.*, 2016).

15 replicates out of 50 who survived in the experimental setup for *T. pandacaqui* with *A. auriculiformis* and only 5 out of 50 were left in the other set up. Length of the roots and number of leaves were higher in *T. pandacaqui* with *A. auriculiformis* (Fig. 10 & 11). Root colonization of the ten root samples of *T. pandacaqui* without *A. auriculiformis* showed 33% of colonization. However, *T. pandacaqui* with *A. auriculiformis* exhibited higher root colonization having 45% infection. Only the hyphae were accounted for root colonization because other parts of the roots were disfigured during the staining.

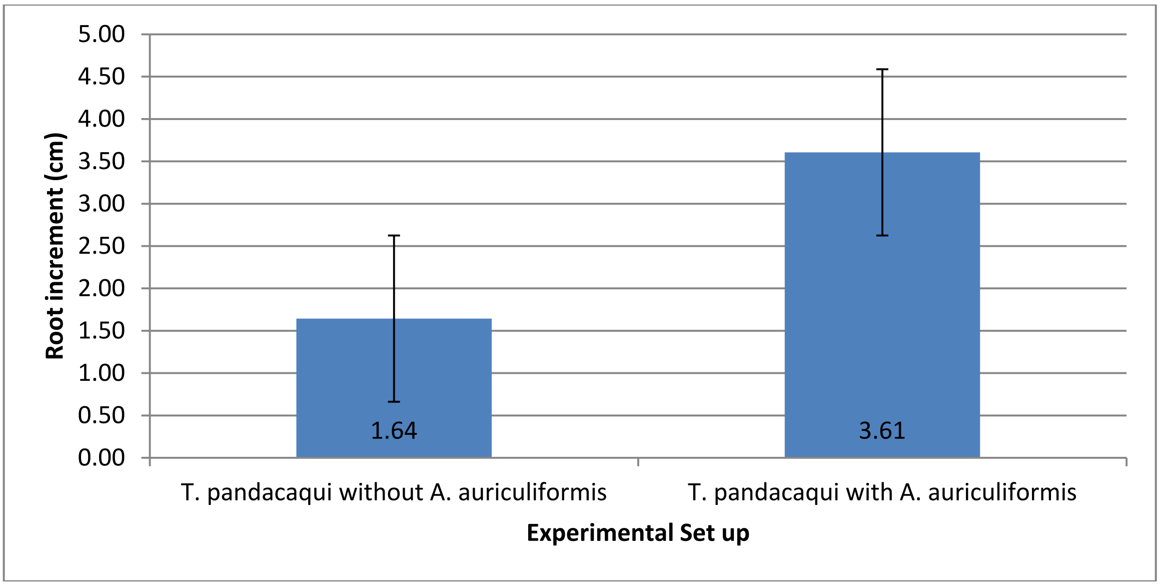


Fig. 10. Root development of *T. pandacaqui* in response to planting with and without *A. auriculiformis* (n=20).

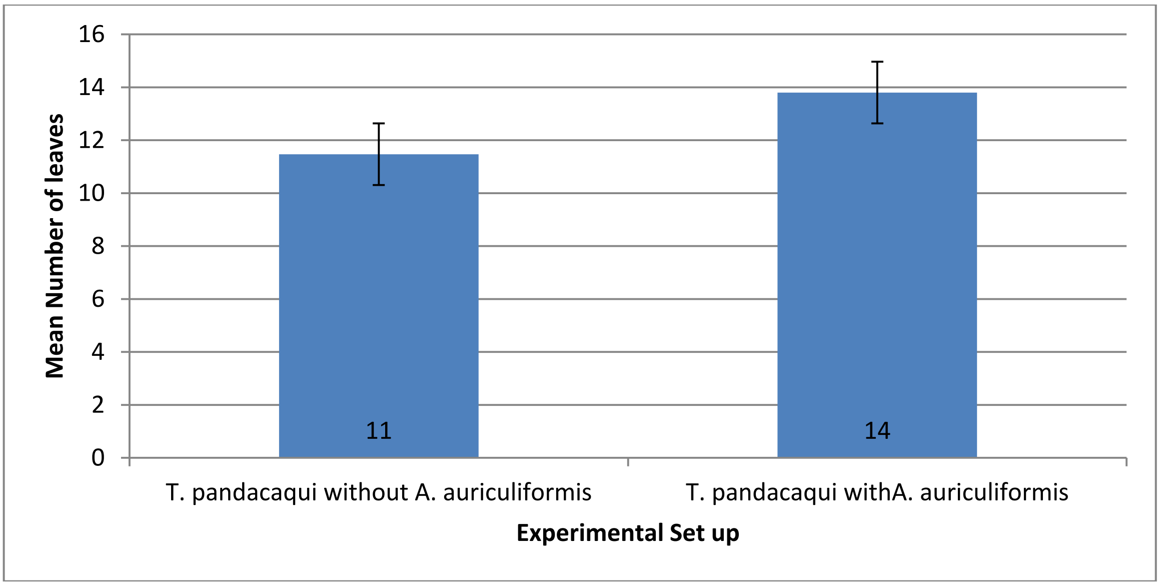


Fig. 11. Foliage count of *T. pandacaqui* in response to planting with and without *A. auriculiformis* (n=20).

The growth performance of the indigenous species, *T. pandacaqui*, in terms of root and leaf development was better with *A. auriculiformis*. The introduced species' ability to modify soil composition may have aided the growth of *T. pandacaqui* in the copper-rich soil that came from the former mine site in Brgy. Capayang, Marinduque. *A. auriculiformis* has a symbiosis in rhizobia and can easily enteran n easily enter relation with new rhizobia species, leading to increased nitrogen content in soil. The association of *A. auriculiformis* with both ecto- and endo-mycorrhizal fungi may have influenced the percentage of root colonization of *T. pandacaqui*. *A. auriculiformis* has a symbiotic relationship with rhizobial bacteria because it provides nitrogen for the species to grow on low nitrogen soils (Duponnois & Planchette, 2003). Treatments with high root colonization were microbial- and fungi-based fertilizers. The species can also significantly raise the soil organic carbon and the cation exchange capacities (Schmerbeck & Naudiyal, 2014). Several countries are using *A. auriculiformis*. for phytoremediation. It's popular for revegetating mine spoils due to its rapid growth and regeneration that result in abundant litter fall, improving the physio-chemical properties of soilin abundant litter fall, improving the of soilin abundant litter fall, which improvesin abundant litter fall improving soil's physio-chemical properties. The species' phyllodes provide good, long-lasting mulch (Orwa *et al*., 2009).

Conclusion

Acaulospora species were prevalent from sampled rhizosphere across vegetation types compared with *Glomus* and *Gigaspora*, but when mass-produced *Acaulospora* and *Glomus* prevailed as an inoculant for *T. pandacaqui*. Further improvement of endomycorrhizal production is necessary to ensure soil inoculant quality for *T. pandcaqui* and other indigenous plants. The height and diameter of *T. pandacaqui* were significant with *A. auriculiformis* brought by its N-fixing property, though host mortality was higher due to competition. Soil inoculants promoted the growth of *T. pandacaqui,* but MykoVAM performed significantly in height than control. *A. auriculiformis* could be maintained as a nurse crop for threatened species like *T. pandacaqui* and consider the potential of local endomycorrhizal isolates to encourage growth and survival.

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