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#### **Research Paper**

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# GROWTH PERFORMANCE OF WATER-STRESSED BETEL NUT (Areca catechu Linn.) INOCULATED WITH VESICULAR ARBUSCULAR MYCORRHIZA

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#### ABSTRACT

The study revealed that significance differences were obtained on the number of Betel nut at 150 days after planting. At 60 days watering of plants was gradually reduce to at least twice a week and after 90 days it was only watered once a week. A highly significant difference in the length of the shoot at 90 days after planting and at 120 days, there was a significant difference in the length of shoots and roots among treatment means. Result indicated that inoculated Betel Nut (*Areca catechu* Linn.) produce the longest roots with mean length of 124.06 cm. Inoculated seedling significantly increases the total height number of days after water was withheld. It shows that inoculated seedlings contributed to the body resilience adopted in a water-stress condition. The analysis of variance shows significant differences among treatment which implies that mean length of the shoots was influences by Vesicular Arbuscular Mycorrhiza (VAM). Result showed further from 90 to 150 days after planting to plants growth were slowed down while plant inoculated with Vesicular Arbuscular Mycorrhiza (VAM) outgrew other treatments. Slow growth may be attributed to water-stressed condition. Highly significant difference was recorded between treatment means from 90 to 150 days after planting.

Keywords: Arbuscular, Influence, Mycorrhiza, Vesicular, Water-stressed

Introduction

The Philippines agriculture remains of crucial important in the economy. Its relative contribution to gross domestic product (GDP) has been declining over the years while the gross value added has been growing. The declining contribution of agriculture to gross domestic product is due to structural transformation as industry and services grew relatively faster, in which accounting for increasing contribution to the growing economy (Amadore, 2005). In a subtropical forest experimental system, Singavarapu *et al.* (2024) clarified how coexistence of arbuscular

mycorrhizal and ectomycorrhizal trees shapes soil fungal communities and coexistence of fungi of different mycorrhizal types.

High productivity growth in the agricultural sector in most countries is a way of structural transformation in promoting economic growth. Historically, the Philippines has a low agricultural productivity growth that creates hindrance for economic growth and employment (BIOTECH, 2020). The study of Peng *et al.*'s (2024) revealed that comparative examination of low-input and conventional farming methods illuminates their varying effects on arbuscular mycorrhizal symbiosis and soil ecosystem functions.

Climate Change has been identified as the most consequential challenge facing the world and it is particularly more serious in developing countries due to its geographic exposure, low incomes, greater reliance on climate-sensitive sectors and the weak capacity in adapting the changing climate (Birkmann, *et al* 2011). Through the established infrastructure, local farming practice and individual experience, farmers learned to adapt with the weather and year to year climate variability causes by climate change. The dynamics of coexisting plants of different mycorrhizal types can have important implications for ecosystem function, including the reduced recalcitrance of roots and leaves of both arbuscular and ectomycorrhizal plants (Xia *et al.*, 2024).

It also become an important challenge because of its adverse impacts such as increased flooding incidence, drought, soil degradation, water shortages and increased pests and diseases constantly it threatens agricultural output and productivity (Butola, *et al.*, 2004). The implications of this study for ecosystem management and climate change mitigation are significant (Francis *et al.*, 2024). The study of D. Wang *et al.* (2024) validation of metabarcoding data for quantitative assessments of orchid mycorrhizal fungal communities provides a methodological framework for analyzing the role of these poorly known associations, with implications for biodiversity conservation and ecosystem restoration.

The chemical fertilizer was traditionally used to enhance survival and growth of forest tree species that causes tremendous harm to the environment such as pollution and contamination. Nowadays, bio-organic fertilizer was discovered as an alternative solution to this problem. Vesicular

Arbuscular Mycorrhiza (VAM) and Bio fertilizers are bacteria, fungi and cyanobacteria (Cruz *et al.*, 2017). The most striking symbiotic relationship both partner derives the benefit from each other. Today, mycorrhiza is now recognized as a versatile fungi associated with plant roots which have more than 500 known species Vesicular Arbuscular Mycorrhiza worldwide (Davis, et al., 1978).

Betel nut (*Areca catechu* Linn.) is traditionally chewed by Ifugao people as part of their culture for hundred years. Nowadays it is not only the Ifugao or upland people chewed betel nut it became very popular even to lowland community. Due to growing demand for betel nut the price is sky rocketing yet the technology for the propagation of these plants is limited, it is observed that this slow growing plant, high mortality rate and high water demand are few of the several problems that arises. It is also used as an interior landscaping species. Often used in large indoor areas such as malls and hotels.

Mycorrhizal fungi are known to affect growth of most plant species through various ways (Ebora, *et al.*, 2018). It has also the capacity of genetic affectivity in the host plant up to the next two final generations which has been observed to increase fecundity, seed quality and offspring vigor (Forseca *et al.*, 1992). One of the central themes of mycorrhizal research is nutrient cycling; mycorrhizas play a significant role in mineralizing nutrients and transporting carbon across the soil profile (Mahmood *et al.*, 2024)

While the Vesicular Arbuscular Mycorrhiza (VAM) when inoculated to seedlings will infect the roots, help in the absorption of water and nutrients particularly phosphorus, prevent root infection by pathogens and can increase plant tolerance to drought and heavy metals (Hamel *et al.*, 1992). Vesicular Arbuscular Mycorrhiza (VAM) is easier and less expensive.

The increase of price in farm inputs puts heavy burden to farmers in order that they may profit alternative soil fertilizer is being recommended this include Vesicular Arbuscular Mycorrhiza (VAM). Most farmers are considered as low earners because of the high production cost, low quality yield, unstable prices due to urbanization and rapid population growth which caused decrease in the land area tilled by the farm family. He further added that most former are dependent to their farm output (Harvey et al., 1983).

Since agriculture is almost completely dependent on the environment, climate change will only continue to threaten agricultural practices. Techniques that farmers had learned for the past years will no longer be applied to the changing environment. According to Giovannetti *et al.* (2024) sheds light on the intricate communication networks that exist between plants and arbuscular mycorrhizal fungi during their symbiotic interactions.

## **Materials and Method**

The study was conducted inside a screen house for 150 days after transplanting. Vesicular – Arbuscular Mycorrhiza (MycoVAM) inoculants were obtained at Ecosystem Research Development Services (ERDS), Loacan, Baguio City. Seed of Areca catechu Linn shown in Figure 1 were pre-treated with fungicide to prevent contamination of other living microorganism prior to germination treatment. Each treatment consisted of four 20 cm polyethylene pots.

The experiments were laid out in a Randomized Complete Block Design (RCBD) with four treatments and three replications. A total of 120 plants were grown. Data on total survival rate, shoot and root increment was analysed using Analysis of Variance (ANOVA). The F-test at 5% and 1% will be used to test the level of significance among treatment.

The different treatment were as follows:

- T1 Watered once a week inoculated with MykoVam
- T2 Watered once a week uninoculated mixed with organic soil
- T3 Watered once a week uninoculated mixed with chicken dung
- T4 Watered once a week uninoculated mixed with sand and soil

The seedlings were placed on top and covered gently with soil, fully covered. The pots were watered after inoculation inside the screen house. Seedlings were maintained inside screen house. Necessary cultural management practices such as weeding and application of pesticide when necessary were undertaken.

To measure the effect of Vesicular Arbuscular Mycorrhiza (VAM) on survival rate and drought resistance of Betel Nut, uninoculated and inoculated plants was raised for five months. Water regime are once every other day until 60 days. Upon reaching 60 days, watering was withdrawn gradually (once in every 5 days was applied. This is the beginning of water stressed observation to know the effect of Vesicular Arbuscular Mycorrhiza (VAM)).

## **Results and Discussion**

### Survival Rate of inoculated and uninoculated Betel Nut

Study result shows in Table 1 that survival rate was high in inoculated Betel Nut until full development. This might be attributed by the inoculation of mycorrhiza in the roots. Mycorrhizal inoculated seedling produces 8 times as much root and hyphal surface than ordinary uninoculated plants (Javier et al., 2009). The study further revealed the ability of mycorrhiza to assist the Betel nut (*Areca catechu* Linn.) in absorbing water especially in water stressed condition. Resource exchange between plants and fungal symbionts is crucial for nutrient cycling (Zhao *et al.*, 2024)

In the period of the first 30 days after planting, there was no significant increase shoot height between the inoculated and uninoculated seedlings. Study illustrated that this may have resulted from "lag phase" effect of Vesicular Arbuscular Mycorrhiza inoculation. After 150 days after planting there was highly significant effect in inoculated seedlings as compared to uninoculated seedlings (Kormanik, 1985). The study of Plett *et al.* (2024) found that nitrogen transfer from ectomycorrhizal fungi to plants is correlated with free amino acids in fungal hyphae, which may be consistent with a surplus of resources driving exchange, while direct C for N exchange was not supported.

Treatment	REPLICATION				
	Ι	II	III	Total	Mean
T1	10.41	10.36	11.23	32	10.7
T2	11.53	11.42	11.79	34.74	11.6
T3	8.29	8.39	8.74	25.42	8.47

Table 1: Survival rate of Betel Nut at 150 days after planting

T4	8.76	8.92	9.18	26.86	8.95
Treatment for	or comparison	Mean Diffe	erence	Statistical Signific	cance
1 vs 2		0.9		*	
1 vs 3		2.23		**	
1 vs 4		1.75		**	
2 vs 3		3.13		**	
2 vs 4		2.65		**	
3 vs 4		0.48		*	

Note: \*\* = Significant at 1 % level

\* = Significant at 5 % level

ns = Not significant

## Length of the Shoots developed (cm)

Table 2 shows the result in height increment, the highest mean length with mean differences of 130 cm obtained by inoculated Betel Nut after 150 days after planting could be the effect of mycorrhiza in the roots absorption and retention of water in root collar as there is a symbiosis between the organism and the plants. Lekberg *et al.* (2024) argue that the use of a whole-community soil inoculum can explain differences in their observations compared with previous, more controlled, single-fungus experiments.

This affirms the result of study that Vesicular Arbuscular Mycorrhizae may influence development of root and growth of plants (Kung *et al.*, 2008). The analysis of variance shows significant differences among treatment which implies that mean length of the shoots was influences by Vesicular Arbuscular Mycorrhiza (VAM). Plant communities, particularly in terms of mycorrhizal strategies, also play key roles in nutrient cycling (Francis *et al.*, 2024).

Result showed further from 90 to 150 days after planting to plants growth were slowed down while plant inoculated with Vesicular Arbuscular Mycorrhiza (VAM) outgrew other treatments. Slow growth may be attributed to water-stressed condition. Highly significant difference was recorded

between treatment means from 90 to 150 days after planting. Bönisch *et al.* (2024) show that having multiple mycorrhizal strategies is a key driver of plant diversity effects.

Treatment	Mean length	Mean Length	
	60 days after planting	150 days after planting	
T1	15.6	130	
T2	17.4	128.9	
Т3	22.1	115	
T4	23.41	112	

Table 2: Length of Developed Shoots at 60 and 150 days after planting

Treatment for	Mean Difference	Statistical	Mean	Statistical
comparison	60 days after	Significance	Differences	Significance
	planting			
1 vs 2	1.8	**	1.1	ns
1 vs 3	6.56	**	15	**
1 vs 4	7.81	**	18	**
2 vs 3	4.7	**	13.9	**
2 vs 4	6.01	**	16.9	**
3 vs 4	1.31	**	3	ns

\*\* Significant at 1 %

ns- not significant

## Length of the Roots Developed

Table 3 shows that inoculated Betel Nut with Vesicular Arbuscular Mycorrhiza (VAM) significantly enhanced its resistance to water stresses condition. Result indicated that inoculated Betel Nut (*Areca catechu* Linn.) produce the longest roots with mean length of 124.06 cm. Inoculated seedling significantly increases the total height number of days after water was withheld. It shows that inoculated seedlings contributed to the body resilience adopted in a water

stress condition. Gille *et al.* (2024) shed light on the intricate interplay between symbiotic and nonsymbiotic plants in terrestrial ecosystems.

This shows the effect contributed by Vesicular Arbuscular Mycorrhiza (VAM) in the development of seedlings. Mycorrhizal inoculation had an effect on stem weight and percentage colonization (Lambert et al., 1979). Zhang et al. (2024) conducted a study to examine the molecular mechanisms associated with mycorrhiza-assisted iron processing in plants and discovered that there are trade-offs between symbiosis and plant growth. Perotto & Balestrini (2024) examined the mechanisms of nutrient transfer in arbuscular mycorrhizal and orchid mycorrhizal associations, which led the identification of conserved traits that underlie symbiotic to interactions. Wu et al. (2024) proposed a conceptual framework that elucidates four pathways through which arbuscular mycorrhizal fungi influence soil organic matter dynamics.

Treatment	REPLICATION				
	Ι	II	III	Total	Mean
T1	123.1	124	125.1	372.2	124.06
T2	120.8	121.2	120.32	362.32	120.77
Т3	103.3	109.61	106.23	319.14	106.38
T4	102.9	94.42	92.8	290.12	96.70

Table 3: Length of Developed roots (cm) at 150 days after planting

Treatment for comparison	Mean Difference	Statistical Significance
1 vs 2	3.29	ns
1 vs 3	17.68	**
1 vs 4	27.36	**
2 vs 3	14.39	**
2 vs 4	24.70	**
3 vs 4	9.68	*

Note: \*\* = Significant at 1 % level

\* = Significant at 5 % level

Page 6998 of 14

ns = Not significant

Moreover, many study reported that the beneficial effects of mycorrhizae on alfalfa production are associated with a better nutrient balance (Rimando, 2004). Inoculation enhanced the growth, 7-8 times as compared to uninoculated system for flat pad crown vetch ad lotus, the colonization of arbuscular mycorrhizae increased the dry mater production (Rosegrant *et al.*, 2016). Auer *et al.*'s (2024) study elucidated the role of fungal guilds in soil functioning, carbon stabilization, and overall ecosystem resilience, highlighting the delicate balance within soil microbial communities and their implications for ecosystem functioning and stability.

The main influence of Vesicular Arbuscular Mycorrhiza relationship is to increase the source of mineral nutrients to the plant (Shamshiri et al., 1980). The study revealed that Areca catechu when inoculated with Vesicular Arbuscular Mycorrhiza enhances the drought tolerance and growth development. After 150 days under water stressed condition there is highly significant effect in shoot and root growth. L. Wang *et al.* (2024) summarized how the mycorrhizal core microbiome matters for mycorrhizal nutrient cycling and operation of the mycorrhizal holobiont.

## Conclusion

Highest survival rate was recorded on uninoculated Betel Nut (*Areca catechu* Linn.) planted in soil media mixed with organic soil however after inoculated plants obtained highest growth increments after 90 days and under water-stressed condition. Mean length of the shoots was influenced by the MykoVam Inoculation at 90-150 days after planting, inoculated plants outgrown other treatments. The inoculated plants under waters-stressed condition produced longest roots.

The result in height increment could be the effect of mycorrhiza in the roots absorption and retention of water in root collar as there is a symbiosis between the organism and the plants. This shows the effect contributed by VAM in the development of seedlings.

It is recommended that Vesicular Arbuscular Mycorrhiza (MykoVam) should be used to enhance growth of forest plant and improve the tolerance under certain stress such as drought and nutrient uptake. Since the study result are limited to the study area microclimate, soil condition and other factor that affect the plant; in depth study may be further undertaken to validate the result of the study. Root mycorrhizal colonization study in this species is recommended.

To summarized the climate change impacts that have occurred in the Philippines are mostly manifested through the occurrence of severe El Niño and La Niña events, as well as deadly and damaging typhoons, and other severe storms, floods, landslides, drought, forest fires, etc. These extreme events have one thing in common- persistent torrential rains, that causes landslides and flash floods, killing people and destroying properties and the environment along its path (Smith *et al.*, 1997). The Philippines' future is currently uncertain. The alarming threat of the adverse effects of climate change in people's lives pushes people and the government to make a comprehensive strategic plan to help on the part of policy and decision making. The country faces grave challenges that teamwork and unity is a big help.

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