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EFFECTS OF FERTILISATION AND MYCORRHIZAL INOCULATION ON THE EARLY GROWTH OF SELECTED PHILIPPINE INDIGENOUS FRUIT TREE SPECIES

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The global need for food security has sparked interest in the potential of our indigenous food systems, including indigenous fruits and other wild edible flora and fauna. However, majority of these indigenous food resources remain underutilised despite its potentially high nutritional and economic benefits. This is largely due to the inadequacy of technical and practical information regarding the availability, propagation, and overall utility of the species. In this regard, the present study investigated the early growth responses of three selected indigenous fruit tree species, namely, Kalumpit (*Terminalia microcarpa*), Libas (*Spondias pinnata*) and Tibig (*Ficus nota*) to fertilisation and mycorrhizal inoculation following a Completely Randomized Design with treatments being Control, 2.5-g complete fertiliser (14:14:14), MYKOVAM, and Fertilisation + MYKOVAM. After 24 weeks (6 months), it was found that the selected indigenous fruit tree species can grow equally well without fertiliser or mycorrhizal inoculation. However, when necessary, MYKOVAM can be a better option than complete fertiliser. Findings showed that *S. pinnata* and *F. nota* seedlings inoculated with MYKOVAM yielded higher height increments than the fertiliser group. MYKOVAM inoculated *F. nota* seedlings also yielded higher diameter growth and total biomass than those treated with fertiliser. No significant variation in root volume was recorded for all selected indigenous fruit tree species among all treatments. Early growth of *T. microcarpa* was not enhanced by any of the treatments. The study, therefore, revealed relevant information that could enhance the mass propagation of these species in support of food security.

Keywords: *Spondias, Terminalia, Flacourtia*, MYKOVAM, complete fertiliser, root colonisation

INTRODUCTION

Indigenous fruit tree species are edible fruit-bearing trees that are native in the Philippines. Coronel (2011) estimated around 170 indigenous fruit tree species in the country, but most of which are underutilised. Economic interest for indigenous fruit tree species is limited primarily because public awareness regarding the same is also limited (Miranda et al. 2018). This lack of awareness is exacerbated by the high seasonality of indigenous fruits and the prevailing perspective that indigenous fruit tree species are "minor" produce, which are incapable of competing with the more widely accepted varieties. Among such underutilised Philippine indigenous fruit tree species are Kalumpit (*Terminalia microcarpa* Decne), Libas (*Spondias pinnata* (Linn.F.) Kurz.) and Tibig (*Ficus nota* (Blanco) Merr).

Terminalia microcarpa or Kalumpit in Tagalog is a widely distributed fruit-bearing tree species in the Philippines. It has been a subject of research across various fields, from forestry and environmental sciences to food and medical sciences. One of the earliest literatures on the utilisation of *T. microcarpa* is Sanchez et al. (1976), where they demonstrated the

compatibility of the indigenous fruit tree species for wine, preserved fruit, and jam making. Studies reveal the abundance of nutrients and the promising antioxidant activity from *T. microcarpa* fruits, thereby promoting the same as a good nutrient-dense food option (Santiago et al. 2021). Among the limited investigations on *T. microcarpa* early growth is Gapasin et al. (1997), where the association of the said species, albeit poorly, with vesicular-arbuscular mycorrhiza (VAM) was demonstrated. *T. microcarpa* was further documented to survive well in mined out sites, and its growth was found to be better than the widely planted non-native, *Swietenia macrophylla* (Schneider et al. 2014, Varela et al. 2016). This made *T. microcarpa* a desirable native species for reforestation and rehabilitation.

The other indigenous fruit tree species, *Ficus nota*, is a very common riparian species in the country. Mapatac (2015) reported that leaf and fruit extracts from this plant can be a potential source of antibiotic against certain bacterial strains including *Escherichia coli* and *Salmonella typhimurium*. Moreover, *F. nota* leaves were also found to be a beneficial addition to ruminant feed due to its tanniniferous nature (Asaad et al. 2006). Apart from these few investigations, *F. nota* and its fruits are not known to have any valuable uses, especially in the Philippines. Understanding and value of the species is currently confined to its ecological importance as a host plant for insects and birds (Reyes et al. 2020). Cultivation studies for *F. nota* are also scant compared to other *Ficus* species, like *F. benjamina*, which is a common ornamental tree.

Among the three selected indigenous fruit tree species for this study, *Spondias pinnata* is by far the most studied overseas, but the least explored locally. *S. pinnata*, known as "libas" or "lubas" in the Philippines, is also native to other tropical Asian countries like India, Myanmar, Indonesia, South China and Thailand (Florido & Cortiguerra 2003). Badoni & Bisht (2009) cited the diverse uses of almost all *S. pinnata* plant parts to support its potential economic importance and to reverse its underutilisation. In Bicol Region of the Philippines, it is widely known as a souring agent. Its leaves are a coveted ingredient for local delicacies, while its fruits are usually enjoyed raw, jellied, or juiced (Florido & Cortiguerra 2003). Studies show that *S. pinnata* fruits, fruit extracts and leaf oils have high nutritive and pharmaceutical potential (Andola & Purohit 2010, Attanayake 2015, Ghate et al. 2014, Jyothi et al. 2022, Muhammad et al. 2011, Sameh et al. 2019). On the propagation and utilization of *S. pinnata*, Badoni & Bisht (2009) emphasised that mass propagation and commercial exploitation of *S. pinnata* is both a challenge and an opportunity. In India, extensive research has described the germination and seedling behavior of *S. pinnata* relative to different pre-sowing treatments but were limited in terms of describing seedling responses in relation to possible soil amendments (Dey et al. 2016, Dulay et al. 2022, Kumar 2016, Panda et al. 2022). Specifically, no recent studies on the effect of fertiliser or mycorrhizal inoculation have been conducted for *S. pinnata*.

Research in the past decade has shed light on a variety of equally important aspects of the selected indigenous fruit tree species but has fallen short on tackling the propagation and cultivation of the selected species. While there are a few noteworthy studies, such as the germination study conducted by Dulay et al. (2022) on the same selected indigenous fruit tree species, and the extensive identification-to-cultivation initiative led by Miranda et al. (2018) for other indigenous fruits in the country, recent research on the propagation of the selected indigenous fruit tree species remains insufficient. This research gap hampers the widespread adoption of these species, not only for reforestation and conservation; but even more so, for commercial fruit production. Furthermore, when examining the available nursery and cultivation studies, it becomes evident that most of them have been conducted in other countries. This highlights the dearth of consistent local exploration into the growth and propagation of these specific indigenous fruit tree species within our region.

Ultimately, the present study aimed to document the early growth of three of the underutilized indigenous fruit tree species, namely Kalumpit (*Terminalia microcarpa*), Libas (*Spondias pinnata*), and Tibig (*Ficus nota*) and to assess the effectiveness of fertilisation and mycorrhizal inoculation on the early growth of the same species.

MATERIALS AND METHODS

Experimental setup

The experiment was conducted for 24 weeks from March to September 2022 in the Forest-Agroforest Nursery Learning Laboratory of the Institute of Renewable Natural Resources, College of Forestry and Natural Resources, University of the Philippines Los Baños. Pre-germinated seedlings of more or less uniform size were transplanted in black polyethylene bags (4" × 6" × 0.002") filled with heat-sterilised garden soil. The garden soil has optimum pH of 6.5, 1.73% organic matter (OM) content, 0.09% N, ~84 mg kg^{-1} available P, and 2.54 $cmol_c$ kg of $soil^{-1}$ exchangeable K. Separate set ups were made for every species. The experiment was laid out separately for each species, following a Completely Randomized Design with the following treatments; Control consisted of sterilised garden soil without any fertilisers, Complete Fertiliser consisted of 2.5 grams of complete (14:14:14) fertiliser applied through top dressing to every seedling after 2 weeks of potting, MYKOVAM consisted of 5 grams or 1 soda bottle cap-full of mycorrhizal inoculant developed by the National Institute of Molecular Biology and Biotechnology placed at the bottom of the dibble or planting hole prior to seedling insertion during potting, and Complete Fertiliser with MYKOVAM consisted of the combination of the 2.5 grams of complete (14:14:14) fertiliser and mycorrhizal inoculation applied following the description for FE and MY treatments.

Three replications were made per treatment with ten plants comprising each replicate for a total of 120 seedlings per species. Figure 1 displays a representative seedling of each species. Plants were watered as needed but done so across the whole setup, and monitored for survival and possible pest and disease incidences. All diseased plants were immediately removed from the nursery to prevent potential damage to the other plants in the setup.

Height and diameter measurement

Measurements of height and diameter commenced two weeks after transplanting and were done every other week thereafter until the experiment was terminated on the 24th week. Root collar diameter (mm) was measured using a digital vernier caliper. Plant height (cm) was determined using a ruler or a meter stick, based on the suitability for the plants at the time of observation. Height was measured from the root collar mark at 0.5 cm from the ground. The root collar was marked with white paint to serve as reference to ensure consistent measurement.

Figure 1 Kalumpit (*Terminalia macrocarpa*), Libas (*Spondias pinnata*) and Tibig (*Ficus nota*) seedlings at 2–4 weeks of observation

Biomass determination

On the 24th week, termination of the pot experiment and destructive sampling of three seedlings per treatment for a total of 12 seedlings per species was done for aboveground and belowground biomass determination. Above ground part was separated from the belowground by cutting the plant at the root collar mark. The roots (belowground) were cleaned of all soil particles through rinsing in continuous flowing water, before root volume determination and oven drying. Root volume was determined using a graduated cylinder partially filled with water, with the root volume estimated through the water displacement method. Thereafter, the roots and shoots (aboveground) were oven dried at 80°C for 24 hours and weighed.

Assessment of mycorrhizal root colonisation

Separate root samples were collected from the remaining experimental plants for assessment of root colonisation by mycorrhiza. For each treatment, three individual plants were used. Prior to analysis, roots were preserved in 50% ethanol. During analysis, the preserved roots were rinsed with distilled water to remove the ethanol, and were then cleared and stained using Trypan blue, following the protocols developed by Brundrett et al. (1994). The assessment of the frequency of mycorrhiza in the root system (%F) followed the Trouvelot method, as cited by Kokkoris et al. (2019) and Hart & Forsythe (2012). The roots were divided into 1-cm segments, and subsequently, 30 segments were randomly selected. The root segments were observed under a compound microscope 10× and 40× magnification, and were inspected for signs of mycorrhizal colonisation, i.e., presence of arbuscules, hyphae, vesicles and spores. Positive identification of a segment was made when at least two out of the four signs were observed, following the requirement set by Hart & Forsythe (2012). Thereafter, the frequency of mycorrhiza in the root system (%F) was calculated using the equation:

$$\%F = \frac{\text{No. of fragments positive of mycorrhiza}}{\text{Total no. of fragments}} \times 100\%$$

Statistical analysis

One-way Analysis of Variance (α = 0.05) was performed to test whether significant differences occurred across treatments for all parameters namely change in height in cm, change in diameter in mm, total biomass in grams, root volume in ml, and %root colonisation. Tukey's HSD Test (α = 0.05) was further conducted as a post-hoc analysis for treatment mean comparison.

RESULTS AND DISCUSSION

The study revealed that different native fruit tree species respond differently to fertilisation and mycorrhizal inoculation. While MYKOVAM consistently performed better than fertiliser, it only did so equally with the control for two of the selected indigenous fruit tree species. None of the treatments was found to encourage favorable growth of *T. microcarpa* more than the control group. In terms of mycorrhizal frequency, values indicate statistical differences, with MYKOVAM-inoculated seedlings having the highest mycorrhizal frequency for all species. Root volume, on the other hand, showed no significant variation across all treatments for all selected species.

Ficus nota

Out of the three selected native fruit trees, only *Ficus nota* yielded significant growth for both diameter and height, as well as total biomass (Table 1). Findings show that MYKOVAM performed equally with control, and that the control group yielded statistically higher height and diameter growth than fertiliser–treated group. Average height and diameter increment of seedlings in MYKOVAM-fertiliser group did not differ from fertiliser-treated seedlings. This suggests the favorable growth of the species, even without mycorrhizal inoculation or fertilisation in soils of similar status to that used in this study.

The average change in diameter of *F. nota* seedlings was significantly higher in MYKOVAM than both fertiliser and MYKOVAM-fertiliser groups. The same is true for *F. nota* biomass: MYKOVAM and the control groups had no significant difference, but MYKOVAM

yielded higher biomass than both fertiliser and MYKOVAM-fertiliser groups. For height, MYKOVAM also showed higher increments than fertiliser group, but were only statistically equal to MYKOVAM-fertiliser group. Hence, findings suggest that the use of MYKOVAM inoculation alone is the more practical option for promotion of higher height and diameter growth, as well as higher total biomass of *F. nota*. This is especially applicable in nutrient-poor soils that require improvements, such as mine out soils, as demonstrated by several studies (Belarmino et al. 2021, Aggangan & Anarna 2019). Tarranco-Castañeto & Follosco-Edmiston (2003) were also unable to demonstrate the effectiveness of combining MYKOVAM and Biocore, an organic fertiliser produced from carefully decomposed agricultural wastes. In their investigation, *Tectona philippinensis* seedlings treated with combined MYKOVAM and Biocore did not yield significant differences in height and diameter. In addition, Ortas (2018) was able to document the reduction of mycorrhizal efficiency under high fertiliser and soil fertility conditions in his study with fruit crops in Turkey. Therefore it provides a possible explanation why the combination of MYKOVAM and complete fertiliser failed to demonstrate any synergistic effects on the growth of selected indigenous fruit tree species such as *F. nota* and *S. pinnata* in this study.

The mycorrhizal frequency for *F. nota* seedlings is highest in treatments with MYKOVAM (MYKOVAM and MYKOVAM-fertiliser groups). Values were significantly higher than fertiliser group, but did not differ with the control group. Despite being not inoculated with MYKOVAM and the use of sterilised potting medium, signs of mycorrhizal colonisation were still observed from the control group. Detection of mycorrhizal frequency in non-inoculated seedlings is not uncommon in literature. For instance, Jeyanny et al. (2011) detected 0.40% to 24.4% root infection in non-inoculated *Acacia mangium* seedlings in their study. This occurrence may be attributed to the wide potential dispersal vectors of mycorrhizal fungi. Bueno & Moora (2019) elucidated the possibility of mycorrhizal dispersal through water and air that enable the cosmopolitan distribution of mycorrhizal fungi all over the world.

Spondias pinnata

Treatments statistically improved only the height increments of *S. pinnata*. Total biomass, root volume and diameter growth were all found to have no significant variations (Table 2). Similar to the results for *F. nota*, control seedlings of *S. pinnata* had significantly higher height growth than the seedlings with complete fertiliser. Although MYKOVAM did not improve the height of the control group, seedlings inoculated

Table 1 Growth responses of *Ficus nota* to different fertilisation regimes in terms of average diameter change, average height change, total biomass, root volume, and mycorrhizal frequency

Treatment	Change in diameter (mm)**	Change in height (cm)*	Biomass (g)*	Root volume (ml)	Mycorrhizal frequency (%F)**
Complete fertiliser + MYKOVAM	$4.04^{bc} \pm 0.20$	$11.36^{bc} \pm 27.64$	$5.58^{b} \pm 3.12$	8.33 ± 4.16	$82.22^{a} \pm 5.09$
MYKOVAM	$5.98^{a} \pm 0.96$	$25.29^{ab} \pm 55.06$	$13.71^{a} \pm 5.29$	12.00 ± 4.36	$88.89^{a} \pm 6.94$
Complete fertiliser	$3.27^{c} \pm 0.65$	$9.28^{c} \pm 24.34$	$3.95^{b} \pm 1.26$	5.67 ± 2.52	$27.78^{b} \pm 10.18$
Control	$5.37^{ab} \pm 0.49$	$28.12^{a} \pm 101.79$	$9.98^{ab} \pm 1.90$	10.33 ± 1.53	$56.67^{ab} \pm 26.67$
CV (%)	26.44	51.68	53.13	30.00	43.50

Asterisks indicate statistical significance determined through one-way ANOVA; * = significance at $\alpha = 0.05$, ** = significance at $\alpha = 0.01$; Mean values followed with a common letter in a column do not significantly differ as per Tukey's HSD at $\alpha = 0.05$

Table 2 Growth responses of *Spondias pinnata* to different fertilisation regimes in terms of average diameter change, average height change, total biomass, root volume and mycorrhizal frequency

Treatment	Change in Diameter (mm)	Change in Height (cm)*	Biomass (g)	Root Volume (ml)	Mycorrhizal Frequency (%F)**
Complete fertiliser + MYKOVAM	1.37 ± 0.34	8.19^{ab} ± 3.09	3.97 ± 1.02	5.30 ± 1.15	72.22^{ab} ± 11.71
MYKOVAM	1.66 ± 0.33	15.83^{a} ± 6.01	3.38 ± 0.90	4.67 ± 1.53	87.78^{a} ± 6.94
Complete fertiliser	1.04 ± 0.58	5.22^{b} ± 2.28	3.45 ± 0.90	4.00 ± 1.00	41.11^{bc} ± 18.95
Control	1.47 ± 0.07	16.11^{a} ± 1.44	3.90 ± 0.88	5.00 ± 1.00	22.22^{c} ± 1.92
CV (%)	18.74	48.39	8.24	11.76	53.09

Asterisks indicate statistical significance determined through one-way ANOVA; * = significance at α = 0.05, ** = significance at α = 0.01; Mean values followed with a common letter in a column do not significantly differ as per Tukey's HSD at α = 0.05

with only MYKOVAM exhibited significantly higher height increments than those treated with complete fertiliser. The combination of MYKOVAM and complete fertiliser did not induce significant improvements compared to control, fertiliser and MYKOVAM groups. Mycorrhizal frequencies for *S. pinnata* varied significantly. The highest mycorrhizal frequency for *S. pinnata* was observed from MYKOVAM, followed by the MYKOVAM-fertiliser group. Around 22% and 41% of mycorrhizal frequency were detected for non-inoculated treatments in control and fertiliser, respectively.

Yambagon et al. (2018) also had a similar observation when their organic concoctions increased the height of *Litsea philippinesis* seedlings more than the complete fertiliser group. The above-cited study also documented the benefits of using organic and inorganic fertilisers in combination. Unlike other research on combined vesicular arbuscular mycorrhiza inoculation and NPK fertilisation on tree species, such as Jeyanny et al. (2011) for *Acacia mangium*, the present study did not find any signs of MYKOVAM and complete fertiliser compatibility. This does not necessarily mean that mycorrhizal inoculation and complete fertiliser may no longer be used in combination. Effectiveness of mycorrhizal fungi also tends to vary depending on the species, soil status and nutrient availability, microbial activity and timing of application (Ortas 2018, Qin et al. 2015, Willis et al. 2013). These factors, among others, could have affected the performance of the mycorrhizal inoculum. Mycorrhizal fungi are known to contribute to inorganic phosphorus assimilation, particularly in nutrient-poor environments (Willis et al. 2013). In contrast, this greenhouse experiment used a potting medium that contained an optimum level of nutrients. The use of complete fertiliser could have potentially made the soil less optimum for mycorrhization, due to the addition of nutrients on top of the inherent NPK in the potting medium. It is also worth noting that the mycorrhizal inoculum and soil nutrients may not be the sole sources of incompatibility. The type and concentration of the mineral fertiliser used in the present study could have also contributed to the lack of synergistic effects. This identified gap serves as a relevant focal point for future studies.

Terminalia microcarpa

The result for *T. microcarpa* showed that the average change in height, average change in diameter, average biomass, and average root volume were not statistically different (Table 3). All treatments were found to have equal effect on the growth of *T. microcarpa* seedlings after the 24-week growing period. Only the mycorrhizal frequency varied significantly across treatments. The mycorrhizal frequency of

Table 3 Growth responses of *Terminalia microcarpa* to different fertilisation regimes in terms of average diameter change, average height change, total biomass, root volume and mycorrhizal frequency

Treatment	Change in diameter (mm)	Change in height (cm)	Biomass (g)	Root volume (ml)	Mycorrhizal frequency (%F)**
Complete + MYKOVAM	1.49 ± 0.17	5.16 ± 0.36	1.96 ± 0.29	1.50 ± 0.50	81.11^{a} ± 3.46
MYKOVAM	1.45 ± 0.09	5.94 ± 0.51	1.84 ± 0.09	1.17 ± 0.29	83.33^{a} ± 0.00
Complete Fertiliser	1.27 ± 0.14	4.41 ± 0.36	1.56 ± 0.34	1.00 ± 0.00	47.78^{b} ± 10.79
Control	1.13 ± 0.31	4.76 ± 1.23	1.56 ± 0.25	0.67 ± 0.29	32.22^{b} ± 15.01
CV (%)	12.50	12.97	11.69	31.88	41.25

Asterisks indicate statistical significance determined through one-way ANOVA; * = significance at α = 0.05, ** = significance at α = 0.01; Mean values followed with a common letter in a column do not significantly differ as per Tukey's HSD at α = 0.05

MYKOVAM-inoculated *T. microcarpa* seedlings (MYKOVAM and MYKOVAM-fertiliser groups) were significantly higher than the mycorrhizal frequency of non-inoculated ones (control and fertiliser groups). Gapasin et al. (1997) were also unable to demonstrate significantly different height and diameter growth in mycorrhiza inoculated and non-inoculated *T. microcarpa* seedlings. Therefore, this study supports the potentially poor association of *T. macrocarpa* with vesicular arbuscular mycorrhiza, as demonstrated by Gapasin et al. (1997).

The absence of variance in all growth parameters for *T. microcarpa* also merits further elaboration. Since there is little information on the growth and biomass of *T. macrocarpa*, that of other *Terminalia* species may serve as a good baseline for discussion. Zafar et al. (2020) documented that the average plant height and average stem diameter for 6-month-old control *Terminalia arjuna* seedlings was 38.3 cm and 4.28 mm, respectively. In the present study, the highest average height at 9.46 cm was attained by the MYKOVAM-treated seedlings, and the highest average diameter at six months was just 2.38 mm. The highest average diameter was obtained from seedlings treated with a combination of MYKOVAM and complete fertiliser. Since the study of Zafar et al. (2020) and the present study were conducted in entirely different soil and climatic conditions, direct comparison between these two sets of findings is not suitable. Even so, this still provides evidence that growth rates among *Terminalia* species vary and are, therefore, worthy of further research.

CONCLUSIONS AND RECOMMENDATIONS

The present study provided sufficient demonstration of the early growth and responses of the selected indigenous fruit tree species to fertilisation and mycorrhizal inoculation. Findings revealed that *F. nota*, *T. microcarpa,* and *S. pinnata* can potentially grow well with or without the implemented treatments in soils of similar status. This is especially evident for *T. microcarpa,* where all variables were statistically equal across treatments. Although the performance of the MYKOVAM did not exceed the control group, it is still important to note that MYKOVAM outperformed both the complete fertiliser and the combination in terms of height and diameter growth for some of the indigenous fruit tree species. Therefore, when soil improvement is required, using MYKOVAM alone may be a more effective option.

The use of other growth quality parameters but not limited to photosynthetic parameters and root architectural traits, are recommended for future research endeavors. Additionally the conduct of field trials are viable ways forward since the manifestation of the effects of the treatments on the growth of the species may also be observed beyond its early growth and after the seedlings have been out planted and exposed to the external environments.

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