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FULL PAPER

Inoculation of Arbuscular Mycorrhizal Fungi and Nitrogen-fixing Bacteria Affects Growth and Development of *Melia dubia* Seedlings Under Different Ni Concentrations

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Two experiments were established in the screenhouse of the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños (BIOTECH-UPLB), to determine the effectiveness of new isolates of arbuscular mycorrhizal fungi (AMF) (NMYC) and nitrogen fixing bacteria (NNFB) from rhizosphere soil and roots of ferns and grasses growing in a more than three-decade barren copper mine tailing dumpsite in Barangay Capayang, Mogpog, Marinduque, on the growth and development of bagalunga (*Melia dubia)* belonging to the family Meliaceae. These were compared with those commercially available AMF inoculants (MYKOCAP® and MYKORICH®). The first experiment was established where seedlings were subjected to two factors (inoculation and nickel (Ni) level for a period of six months, following a Randomized Complete Block Design in four blocks with five treatments. The treatments were: control, MYKORICH®, MYKOCAP®, NewMYC (NMYC), and NewNFB (NNFB). Ni levels were: 0, 6, and 12mg kg^{-1} soil (coded as Ni-0, Ni-6 and Ni-12). Experiment 2 had similar inoculation treatment as Experiment 1 but the Ni rates were higher: 0, 3, and 6 g kg^{-1} soil (coded as Ni-0, Ni-3 and Ni-6). Results showed that in Experiment 1, the highest height and stem diameter increments were obtained in plants inoculated with MYKORICH® and grown in Ni-6. In Experiment 2, NMYC in Ni-0 produced the tallest height after six months whereas in Ni-6, MYKOCAP® gave the biggest stem diameter. The heaviest total biomass was obtained in MYKORICH® with Ni-6 and Ni-0 in Experiments 1 and 2, respectively. Moreover, in both trials, MYKOCAP® and MYKORICH®, exhibited highest root colonization and mycorrhiza spore counts, while isolates from the Cu mine dumpsite gave the highest NFB count. Inoculation with AMF including the isolates from the Cu mine site presented positive effect on growth and development of bagalunga seedlings grown in soil with Ni in the first experiment, and in soil without Ni in the second experiment. The study demonstrated better growth performance, soil microbial community, soil nutrient and heavy metal (HM) uptake due to inoculation with microbial biofertilizers including those Cu mine isolates. It is recommended that bagalunga be tested under field conditions specifically in Ni mined areas and determine the long-term impact of microbial inoculants on its growth and its capability as a bioremediation tree species.

Keywords: Arbuscular mycorrhizal fungi, bioremediation, mined area, Ni, nitrogen-fixing bacteria

INTRODUCTION

The mining industry has helped to boost the Philippine economy through mineral production as well as the provision of immediate employment (Nelson et al. 2020). However, mining extraction and operations are associated with negative impacts on human health and the environment, including HM contamination in soil, surface water and groundwater; deforestation and erosion; acid mine drainage; and an increase in dust and emission (Samaniego et al. 2020). For instance, Ni ore is extracted via strip mining which results in large opencast areas resulting in sediments collecting in mining catchments, which are significant water resources for domestic and agricultural use (Gunkel-Grillon et al. 2014). Begum et al. (2022) mentioned that Ni contributes to contamination of the environment through its manufacture and use, especially with the increasing demand for Ni-containing products. Thus, it is crucial to rehabilitate mined-out areas to reduce the adverse human health and environmental impacts of mining waste and pollutants.

Many HM are considered micronutrients (i.e., Cu, Mn, Ni, Fe, and Zn) and are essential for the normal growth of plants and animals. However, they become toxic at concentrations higher than the required (Ngole-Jeme and Fantke 2017). Ni toxicity compromises the overall photosynthetic efficiency of the plants as well as the net photosynthetic productivity and carbon fixation, therefore affecting the growth of plants. The stress conditions led to the loss of accessory pigments and chlorophyll as well as the inhibition of numerous Krebs cycle enzymes. Additionally, Ni toxicity hinders plant uptake and transfer of vital nutrients as well as water transport (Banerjee and Roychoudhury 2020; Ngole-Jeme and Fantke 2017).

Microbial fertilizers, on the other hand, are products that contain beneficial microorganisms, such as bacteria, fungi, and algae, which can enhance soil fertility and plant nutrient uptake (Stamenković et al. 2018). These microorganisms play vital roles in nutrient cycling, organic matter decomposition, and nutrient availability in the soil-plant system (Sun et al. 2017). Nitrogen-fixing bacteria (NFB), on the other hand, are microorganisms that can form symbiosis, acting as plant-growth-promoting bacteria that can enhance the soil's fertility (Kawaka 2022).

Melia dubia, known as Bagalunga in the Philippines, is a fast-growing deciduous tree species belonging to the family Meliaceae. As with any introduced species, it is important to consider the potential ecological impacts and manage its cultivation responsibly to avoid invasive behavior or negative effects on native ecosystems. Chavan et al. (2013) and Dhanya et al. (2019) describe studies on the effect of different types of fertilizers, including biofertilizers, on the growth and establishment of *M. dubia* but this is the first study conducted in the Philippines. Furthermore, previous studies have proven the suitability and phytoremediation ability of *M. dubia* against HMs, specifically lead, cadmium, and chromium, wherein the species' bio absorption of HMs was found to be more than 1, except for the translocation factor, which was less than 1. This suggests the bioaccumulation of HMs in the root system and a diminished translocation from the root to the shoot system, making it suitable for the rehabilitation of HM-contaminated soils (Uma Gowrie et al. 2020; Sezhian et al. 2023).

Despite the literature on bagalunga's phytoremediation potential, no study specifically focusing on bagalunga for Ni remediation has been conducted. Thus, this paper determines the effectiveness on the growth and development of bagalunga in the nursery condition grown in soil contaminated with different levels of Ni and inoculated with commercially available biofertilizers and compare with those isolated from mined areas.

METHODOLOGY

Experimental design

The experiment was established in a screenhouse at the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños (BIOTECH-UPLB). The experiment was laid out in a Randomized Complete Block design in four blocks using two factors (inoculation and Ni level). The five treatments were: Control, MYKORICH®, MYKOCAP®, NMYC and NNFB (isolates from a copper mine dumpsite in Barangay Capayang, Mogpog, Marinduque). Sixty (60) seedlings were subjected to increasing levels of Ni: 0, 6, and 12 mg kg^{-1} and another 60 pots for Ni: 0, 3, and 6 g kg^{-1}.

Seedling preparation and inoculation

Bagalunga seeds were germinated in trays filled with sterilized garden soil. After two months, the seedlings were transferred into individual black polybags (4" x 8") filled with oven-sterilized garden soil. Two capsules of MYKORICH®, and 5 g each of MYKOCAP®, NMYC, and NNFB were inoculated into seedling bags assigned to the treatments. Each plant was inoculated with 100 to 200 spores with chopped roots with 100% colonized by AMF and 10 x 10^5 CFU per plant for those inoculated with NNFB. The inoculated seedlings were incubated for two weeks.

MYKOCAP® is a soil based while MYKORICH® is a sand based biofertilizer both containing 15 species of AMF. AMF were isolated from rhizosphere soil of plants sourced in stressed environment like grasslands and abandoned mine tailing areas. These species belong to the genera *Glomus, Gigaspora, Acaulospora, Scutellospora*, and *Entrophospora* (Aggangan et al. 2019).

Two Ni solutions were prepared: the first had 6mg and 3g of Ni diluted in 5mL water while the second had 12mg and 6g of Ni diluted in 10mL water. The solutions were then applied separately to 1kg garden soil inside a plastic pot lined with poly bags which extended beyond the pot rim. The setup was left on benches to dry, and upon drying the soil was mixed by hand. After mixing, the soil-Ni mixture was watered to 30% field capacity. The poly bags were then tied at the top to minimize water loss due to evaporation and then incubated for two weeks.

After incubation, inoculated seedlings were transferred to pots with different levels of Ni. The pots were then filled with garden soil and watered three times a week. There were 20 pots each for Ni-0, Ni-6, and Ni-12 mg kg^{-1} soil as well as for the Ni-0, Ni-3, and Ni-6 g kg^{-1} soil.

Parameters gathered

Plant growth (**Plant height and stem diameter)**. Measurements were recorded monthly over a six-month period. Plant height (cm) was measured with a meter stick, positioned vertically from one inch above the base of the stem to the apical (terminal) bud of the plant. Stem diameter (mm) was measured using a manual vernier caliper, placed perpendicular to the stem approximately one inch above the soil surface.

Plant biomass. The plants were harvested after six months to evaluate their biomass. Root, stem, and leaf samples were thoroughly washed and subsequently placed in a drying oven at 60°C for a duration of three days. After drying, the samples were weighed using an analytical balance.

Microbial Population Count

A. Mycorrhizal root colonization. To assess mycorrhizal root colonization, three samples of fine roots were collected from each treatment. The collected fine roots were washed in running water to remove sediments and soil, then placed in test tubes with labels. Fifty percent ethyl alcohol was poured into each test tube, ensuring the roots were completely submerged to prevent mold growth while the samples were not yet being processed. The roots were then washed again in running water to remove any remaining sediments and alcohol. After rinsing, 10% KOH was added to each test tube (Vierheilig et al. 2005). Soaking is necessary before heat treatment in a water bath. The test tubes were placed in a test tube rack and soaked in the water. After several hours of soaking, the roots were removed from

the water bath. The roots were then rinsed again in tap water, and 5% HCl was added. After soaking for minutes, the HCl was removed, and trypan blue stain (0.5%) was added. Finally, AMF infected roots were detected using a stereo microscope.

B. Mycorrhizal spore count. To evaluate the mycorrhizal spore count, three samples of 50g soil samples were collected from each treatment potting medium. Each 50g sample was placed into a labeled Petri dish. Mycorrhizal spores were extracted using the wet sieving and decanting method (Jenkins 1964). Soil samples were suspended in water, stirred, and subsequently passed through sieves with mesh sizes of 0.5, 100, and 325 µm, performed this process three times. The soil collected from the finest sieve was transferred into a centrifuge tube containing water. After the first centrifugation, the supernatant was discarded, and 60% sucrose was added to the tube. A second centrifugation was performed, and the resulting spores in the sucrose solution were filtered through fine mesh, rinsed with tap water, and placed into a petri dish with grid lines. Spores were then counted under a stereo microscope and reported as the number of spores per 50g of soil sample.

C. Nitrogen-fixing bacteria count. To determine the nitrogen-fixing bacteria (NFB) count, three 10g soil samples were collected from each treatment and placed on aluminum foil. Döbereiner nitrogen-free agar plates (Döbereiner 1988) were used as the growth medium. To isolate the bacteria, the 10g soil samples were suspended in 90mL of water (first serial dilution). A micropipette was used to transfer 1mL of the suspension into vials containing 9mL of water for subsequent serial dilutions (2^{nd}–5^{th} dilution). After preparing the dilutions, 0.1mL of the last three suspensions were spread onto the agar plates. The plates were incubated for 3 to 5 days, and those that exhibited a blue coloration were indicative of bacterial growth. NFB colonies were then counted, and the colony-forming units (CFU) per gram of soil were calculated to estimate the NFB population.

Soil Nutrient Status and Ni analysis

To evaluate changes in soil nutrients and Ni content, approximately 250g of soil were collected from each treatment. The samples were air-dried and then ground into a fine powder. The pulverized samples were subsequently sent to the Central Analytical Service Laboratory at UPLB-BIOTECH for analysis of nitrogen (N), phosphorus (P), and nickel (Ni) concentrations.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) in RCBD. Treatment means were compared using the Least Significant Difference (LSD) and Tukeys' test at significance levels of $p<0.001$, $p<0.01$ and $p<0.05$.

RESULTS AND DISCUSSION

Plant Growth

The current study revealed that bagalunga can tolerate Ni in soil, and attain the highest height and stem diameter, as well as biomass of roots and shoots for a period of six months, when inoculated with MYKORICH®. Notably, AMF has a significant impact in reducing Ni transport from roots to shoots, which alleviates Ni-induced stress in plants (Shabani et al. 2015).

Experiment 1. The baseline data for plant height and stem diameter were measured after transplanting seedlings to pots. During the first month following transplantation, bagalunga seedlings treated with MYKORICH® under Ni-12mg exhibited the biggest height increment. From the second to the sixth month, bagalunga seedlings treated with MYKORICH® under Ni-6 consistently were the tallest height, reaching a maximum of 51.95 cm over the period. In the second and third months, while the bagalunga seedlings treated with MYKORICH® were the tallest, they were not significantly different from the MYKOCAP®- and NMYC-treated seedlings under Ni-6mg, the NMYC-treated seedlings under Ni-12mg, and from the Control treatment under Ni-0mg. In the fourth month of treatment, the MYKORICH®-treated seedlings were significantly taller than all the other treatments. In the 5^{th} and 6^{th} months, the MYKORICH® seedlings were the tallest but not significantly different from the control treatment under the Ni-0mg level. The absence of Ni contamination in the soil eliminates the potential toxicity that could hinder plant growth. The lack of contamination has likely enhanced the physiological process of the plant, creating an environment in which the mycorrhizal fungi could function optimally. In turn, this symbiosis would have contributed to improved nutrient uptake and increased photosynthetic activity, further promoting healthy growth (Chen et al. 2017).

In terms of stem diameter, MYKOCAP® treatment under Ni-6 produced the widest stem diameter increment during the 1^{st} month. On the second to the 4^{th} months, there were no significant differences in stem diameter between and among all treatment combinations. In the 5^{th} and 6^{th} months, the biggest increment in stem diameter was observed in seedlings treated with MYKORICH® under Ni-6. They were significantly wider than all the other seedlings treated with the different treatment combinations. Moreover, the lowest height and stem diameter increment was recorded in control under Ni-12 with 14.25 cm and 0.29 mm, respectively. This demonstrates the detrimental effect of Ni at higher concentrations. Lešková et al. (2019) highlighted that excess Ni inhibits root elongation and disrupts gravitropic responses of plants, thereby reducing overall plant growth.

Experiment 2. Except for month 2, NMYC-treated plants in Ni-0g were consistently the tallest throughout the duration of the nursery experiment. This finding aligns to Orłowska et al. 2011 wherein inoculant derived from indigenous AMF strain like NMYC have demonstrated that AMF colonization improved plant growth and survival, especially in the absence of stressors like Ni contaminants. They were significantly different from all the other treatment combinations, except in month six when the NMYC treatment did not differ from the MYKOCAP®- and NNFB-treated seedlings in month 2, the MYKOCAP®-treated seedlings in Ni-0g had the biggest height increment. On the other hand, MYKORICH®-treated seedlings in Ni-6g consistently had

Table 1. Periodic height (A) and stem diameter (B) increments (cm) of bagalunga seedlings due to inoculation and grown in soil with Ni-0, 6, and 12 mg kg^{-1}.

	Ni level (mg)	Inoculation Treatment	1 month	2 months	3 months	4 months	5 months
A	0	Control	11.55 ± 0.35 h	34.35 ± 0.45 ab	37 ± 0.8 abc	47.75 ± 5.17 b	48.6 ± 4.61 a
		MYKORICH®	15.2 ± 0.2 e	21.5 ± 2.5 bc	27 ± 1 bc	29.25 ± 0.75 h	29.5 ± 0.5 h
		MYKOCAP®	20.75 ± 0.25 b	23.83 ± 0.85 bc	27.85 ± 0.65 bc	33.93 ± 3.27 e-h	34.1 ± 3 def
		NMYC	12.9 ± 0.9 g	25.97 ± 0.91 b	30.63 ± 1.46 b	33.3 ± 2.17 def	33.8 ± 2.23 d-g
		NNFB	19.9 ± 0.1 c	23.27 ± 0.64 bc	25.4 ± 1.6 bc	33.5 ± 1.5 def	33.25 ± 1.75 efg
	6	Control	4.75 ± 0.75 j	18.83 ± 1.76 bc	24.25 ± 0.25 bc	28.75 ± 1.75 h	28.75 ± 1.75 h
		MYKORICH®	16.95 ± 0.25 d	43.6 ± 4.6 a	44.6 ± 4.6 a	51.45 ± 4.32 a	51.45 ± 1.75 a
		MYKOCAP®	19.75 ± 0.25 c	31.85 ± 0.15 ab	39.85 ± 0.85 ab	33.75 ± 175 e-h	33 ± 3 e-h
		NMYC	21.25 ± 0.25 b	29.25 ± 0.15 ab	36.75 ± 0.25 ab	36.33 ± 2.31 de	36.33 ± 2.31 de
		NNFB	9.75 ± 0.88 i	23.33 ± 0.76 bc	28 ± 1.32 bc	31.33 ± 1.26 f	32 ± 0.87 fgh
	12	Control	4.25 ± 0.25 j	9.25 ± 0.25 c	10.75 ± 1.25 c	12 ± 0.5 i	13 ± 1 i
		MYKORICH®	22 ± 0.5 a	25.17 ± 2.08 b	36.5 ± 0 b	45 ± 2 b	44.17 ± 2.75 b
		MYKOCAP®	17.5 ± 0.5 d	27.5 ± 0.5 b	37.5 ± 1.5 b	35.75 ± 1.75 cd	37.83 ± 3.75 cd
		NMYC	10 ± 0.5 i	31.33 ± 0.29 ab	37 ± 1.5 ab	39.83 ± 1.61 c	41.17 ± 0.76 bc
		NNFB	14 ± 0.5 f	21.5 ± 1 bc	26.5 ± 2 bc	28.83 ± 2.89 gh	30.33 ± 2.02 fgh
B	0	Control	0.22 ± 0 fg	0.37 ± 0.01 a	0.57 ± 0 a	0.7 ± 0.01 a	0.75 ± 0.03 a
		MYKORICH®	0.23 ± 0 fg	0.31 ± 0.01 a	0.35 ± 0.04 a	0.43 ± 0.03 a	0.44 ± 0.04 g
		MYKOCAP®	0.30 ± 0 bc	0.37 ± 0 a	0.54 ± 0.04 a	0.44 ± 0.01 a	0.46 ± 0.02 g
		NMYC	0.22 ± 0 fg	0.39 ± 0.01 a	0.56 ± 0.01 a	0.64 ± 0.02 a	0.65 ± 0.05 c
		NNFB	0.225 ± 0 fg	0.34 ± 0 a	0.35 ± 0.03 a	0.53 ± 0.02 a	0.55 ± 0.02 ef
	6	Control	0.22 ± 0 fg	0.27 ± 0.02 a	0.47 ± 0.04 a	0.51 ± 0.01 a	0.53 ± 0.03 f
		MYKORICH®	0.29 ± 0.02 b	0.41 ± 0.03 a	0.52 ± 0.01 a	0.76 ± 0.05 a	0.78 ± 0.06 a
		MYKOCAP®	0.36 ± 0.02 a	0.47 ± 0.01 a	0.58 ± 0.02 a	0.66 ± 0.01 a	0.7 ± 0 b
		NMYC	0.28 ± 0 cd	0.45 ± 0.03 a	0.56 ± 0.03 a	0.65 ± 0.04 a	0.66 ± 0.01 bc
		NNFB	0.23 ± 0 fg	0.39 ± 0.03 a	0.48 ± 0 a	0.59 ± 0.02 a	0.58 ± 0 de
	12	Control	0.26 ± 0 de	0.26 ± 0 a	0.25 ± 0.03 a	0.28 ± 0.03 a	0.28 ± 0.01 h
		MYKORICH®	0.22 ± 0 fg	0.37 ± 0.02 a	0.53 ± 0.01 a	0.60 ± 0.01 a	0.62 ± 0.01 cd
		MYKOCAP®	0.28 ± 0 cd	0.37 ± 0.02 a	0.43 ± 0.04 a	0.46 ± 0.02 a	0.47 ± 0 g
		NMYC	0.24 ± 0 ef	0.41 ± 0.03 a	0.53 ± 0.04 a	0.56 ± 0.01 a	0.57 ± 0 e
		NNFB	0.2 ± 0 g	0.32 ± 0.02 a	0.46 ± 0.01 a	0.56 ± 0.03 a	0.46 ± 0.04 g

Columns in A or B with the same letters are not significantly different from each other.

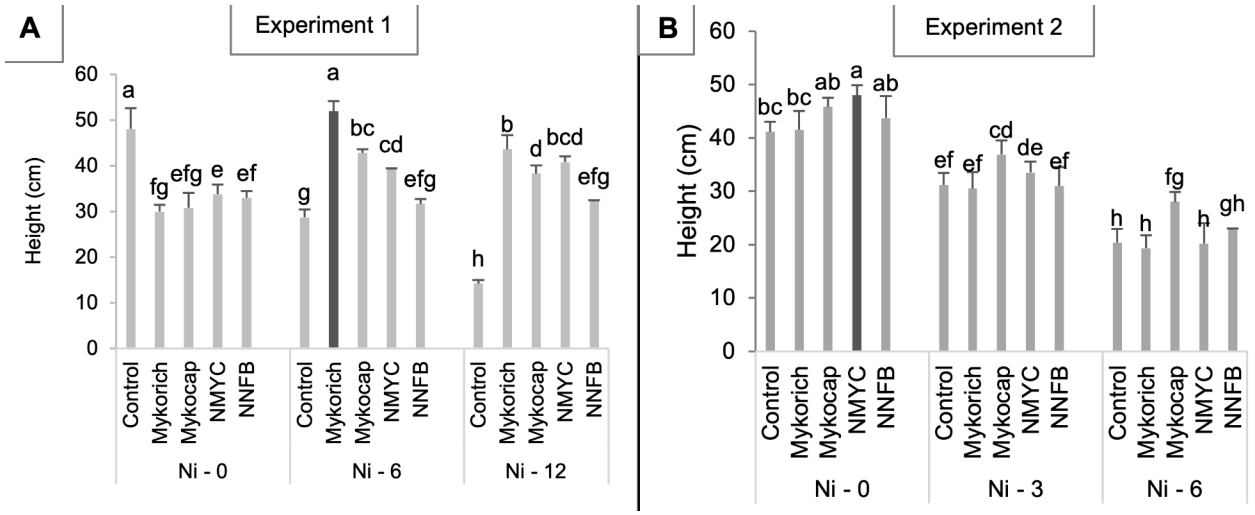

Figure 1. Height increment of bagalunga six months after inoculation and grown in soil with 0, 6, and 12mg kg^{-1} soil (A) and 0, 3, and 6g kg^{-1} soil (B).

the lowest height increment throughout the experiment except in month 1. However, this was not significantly different from most of the treatments in the Ni-6g, especially from NNFB- and NMYC-treated seedlings. In the first month, the NNFB-treated seedlings significantly had the lowest height increment.

In the first three months after treatment, NNFB-treated seedling in Ni-0 had the biggest stem diameter increment that was significantly different from all other treatment combinations. In months 4 to 6 however, this treatment did not differ from the other treatments within the Ni-3g level. Additionally in month 6, it also did not differ significantly from the control and MYKORICH® treatments in the Ni-6g level, and from the MYKOCAP® treatment in the Ni-6g level, and from the MYKOCAP® treatment in the Ni-0 level. The seedlings that had the least increment in stem diameter were all at the Ni-6g level. In the first month, however, the NNFB-treated seedlings had the lowest stem diameter increment that was significantly different from all other treatments.

Table 2. Periodic height (A) and stem diameter (B) increments (cm) of bagalunga seedlings due to inoculation and grown in soil with Ni-0, 3, and 6 g kg^{-1}.

	Ni level (g)	Inoculation Treatment	1 month	2 months	3 months	4 months	5 months
A'	0	Control	10.5 ± 0.70 c	37.67 ± 0.94 bc	40 ± 0.82 bc	39.5 ± 1.08 bc	42.33 ± 1.70 b
		MYKORICH®	8.17 ± 0.47 d	35.83 ± 1.25 c	37.17 ± 0.85 c	37.67 ± 1.03 cd	38.5 ± 0.82 cd
		MYKOCAP®	15.5 ± 0.5 b	44.83 ± 2.78 a	40.33 ± 1.25 b	41.83 ± 1.65 b	40.5 ± 1.22 bc
		NMYC	18 ± 0 a	40.83 ± 0.85 b	44 ± 1.22 a	46 ± 0.41 a	46.67 ± 1.31 a
		NNFB	15.17 ± 0.47 b	32.33 ± 1.43 d	33.33 ± 0.62 d	34.67 ± 0.62 d	35.5 ± 0 de
	3	Control	8.5 ± 0.41 d	27.67 ± 1.89 e	29.83 ± 1.70 e	28.17 ± 2.46 ef	32.83 ± 1.65 ef
		MYKORICH®	5.17 ± 0.62 ef	23.67 ± 0.85 f	29.33 ± 2.01 ef	30.5 ± 3.08 e	30.5 ± 2.68 fg
		MYKOCAP®	8.17 ± 0.62 d	24.83 ± 2.49 ef	26 ± 1.47 gh	27.83 ± 0.62 ef	29.67 ± 1.03 fg
		NMYC	9.33 ± 1.25 cd	24.67 ± 3.01 ef	26.67 ± 2.62 fg	28 ± 0.71 ef	32.67 ± 2.36 ef
		NNFB	9.83 ± 0.62 c	24 ± 1.78 f	23.17 ± 1.70 h	26.83 ± 2.01 f	28 ± 2.55 g
	6	Control	4.33 ± 0.47 f	13.67 ± 1.25 gh	13.33 ± 0.94 j	17.33 ± 1.70 h	18.17 ± 0.62 hi
		MYKORICH®	4.83 ± 1.43 f	11.67 ± 0.47 h	12.33 ± 0.47 j	13.67 ± 0.62 i	14.17 ± 1.65 j
		MYKOCAP®	6.17 ± 0.24 e	15.67 ± 0.85 g	16.67 ± 1.31 i	21 ± 1.22 g	20.67 ± 1.18 h
		NMYC	6.44 ± 0.24 ef	12.33 ± 1.65 gh	12.67 ± 2.39 j	15 ± 3.24 hi	16 ± 3.89 ij
		NNFB	3 ± 0 g	13 ± 0 gh	14 ± 0 ij	17 ± 0 hi	17 ± 0 ij
B	0	Control	0.23 ± 0 cd	0.36 ± 0.04 b	0.40 ± 0.05 ab	0.52 ± 0.02 a	0.53 ± 0.06 a-d
		MYKORICH®	0.22 ± 0 de	0.30 ± 0.02 cd	0.38 ± 0.02 bc	0.40 ± 0.02 ef	0.47 ± 0.01 c-f
		MYKOCAP®	0.31 ± 0.02 b	0.34 ± 0.05 bc	0.40 ± 0.02 ab	0.44 ± 0.01 de	0.48 ± 0.01 b-f
		NMYC	0.25 ± 0 c	0.35 ± 0.02 b	0.35 ± 0.02 cd	0.38 ± 0.04 f	0.46 ± 0.03 ef
		NNFB	0.35 ± 0.02 a	0.43 ± 0.01 a	0.43 ± 0.01 a	0.53 ± 0.02 a	0.53 ± 0.02 abc
	3	Control	0.19 ± 0.02 fg	0.26 ± 0.03 ef	0.37 ± 0.05 bc	0.51 ± 0.02 ab	0.45 ± 0.02 ef
		MYKORICH®	0.16 ± 0.01 fg	0.24 ± 0.01 f	0.39 ± 0.02 bc	0.50 ± 0.01 ab	0.53 ± 0.01 ab
		MYKOCAP®	0.20 ± 0.01 ef	0.25 ± 0.03 f	0.39 ± 0.01 bc	0.51 ± 0.02 ab	0.55 ± 0.04 a
		NMYC	0.20 ± 0.02 ef	0.36 ± 0.05 b	0.38 ± 0.01 bc	0.49 ± 0.03 abc	0.55 ± 0.04 a
		NNFB	0.22 ± 0 de	0.31 ± 0.01 cd	0.37 ± 0.04 bcd	0.46 ± 0.02 bcd	0.50 ± 0.02 a-e
	6	Control	0.18 ± 0 fgh	0.31 ± 0.01 cd	0.37 ± 0.04 bc	0.41 ± 0.04 def	0.48 ± 0.03 b-f
		MYKORICH®	0.15 ± 0 hi	0.30 ± 0.02 de	0.37 ± 0.01 bc	0.45 ± 0 cde	0.49 ± 0.04 b-f
		MYKOCAP®	0.13 ± 0.03 i	0.26 ± 0.02 ef	0.37 ± 0.02 bc	0.37 ± 0.01 f	0.46 ± 0.02 ef
		NMYC	0.18 ± 0.02 fg	0.30 ± 0.05 de	0.35 ± 0.04 cd	0.39 ± 0.06 f	0.44 ± 0.04 f
		NNFB	0.17 ± 0 fgh	0.28 ± 0 def	0.33 ± 0 d	0.4 ± 0 ef	0.47 ± 0 def

Columns A or B with the same letters are not significantly different from each other.

Table 3 presents the partitioned biomass and total dry weight of bagalunga seedlings with Ni-0, 6, and 12 mg/kg soil after six months in the nursery. Dry weights of root, stem, and leaves attained the highest weight consistently under MYKORICH® treatment in Ni-6mg throughout the six months duration. Thus, attaining the highest total dry weight of 26.15 g $plant^{-1}$ at the end of the six months observation. The seedlings in this treatment significantly differed neither from the other treatments in the Ni-6mg level nor from the control treatment in Ni-0mg.

Research has demonstrated that inoculation with AMF can significantly enhance plant biomass and nutrient uptake. For instance, a meta-analysis comprising of various studies found that AMF inoculation increases aboveground and belowground biomass by 47 and 59%, respectively (Wu et al. 2024). Furthermore, research on *Alyssoides utriculata*, another Ni-hyperaccumulator, revealed that inoculation with plant-growth-promoting microorganisms, including AMF, resulted in a 4- to 5-fold increase in aboveground biomass (Prianore et al. 2023). These studies highlighted the efficacy of AMF inoculant in improving pant biomass production under Ni stress.

The lowest dry weights, on the other hand, were observed in the control treatments under Ni-12mg. Like the highest dry weights, these lowest weights did not significantly differ from other treatments. Unlike those weights however, the variances were with different treatments. For the root dry weight, the control treatment under Ni-12mg did not significantly differ from the control treatment under Ni-6mg, and from the NNFB treatments under Ni-12mg and under Ni-0mg. For the stem dry weight, the control treatment under Ni-12mg did not significantly differ from the control under Ni-6mg, the NNFB, MYKORICH® and MYKOCAP® treatments under Ni-0mg, and the NNFB treatment under Ni-12mg. Finally for the leaf dry weight, the control treatment under Ni-12mg did not significantly differ from all the other treatments under Ni-12mg level and the control and NNFB treatments under Ni-6mg.

In the second experiment (Ni-0, 3, and 6 mg kg^{-1} soil), MYKORICH® and NMYC in Ni-3 soil had the highest root and stem dry weights (Table 4), with leaf dry weight being the highest similarly in untreated seedlings and those treated with NMYC under Ni-6, and in MYKORICH®-treated seedlings under Ni-0g. Stem dry weight was highest in NMYC treatment under Ni-3g. However, this was not significantly different from the MYKORICH®, MYKOCAP® and NMYC treatments under Ni-0g. Root dry weight, on the other hand, was highest in NMYC-treated seedlings under Ni-6g. However, it was not significantly different from the following treatments: MYKORICH® and NNFB under Ni-3g, and MYKORICH® under Ni-0g.

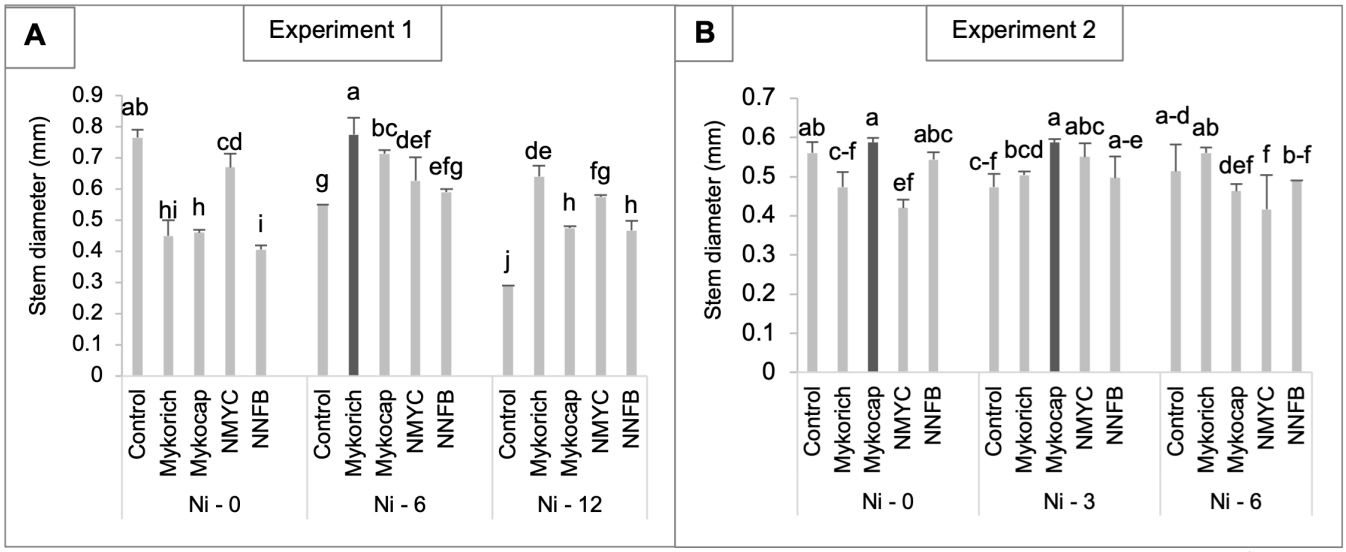

Figure 2. Stem diameter increment six months after inoculation and grown in soil with 0, 6, and 12mg kg^{-1} soil (A) and 0, 3, and 6g kg^{-1} soil (B).

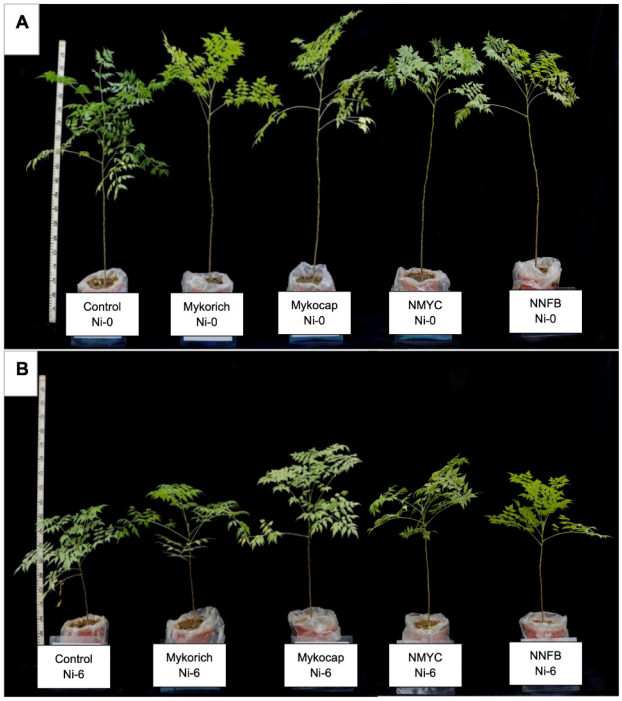

Figure 3. General appearance of bagalunga seedlings (Experiment 2) six months after inoculation and grown in soil with 0 (A) and 6g kg^{-1} (B) of Ni.

The total dry weights were highest under Ni-0 with MYKORICH® with 17.42 g $plant^{-1}$. However, this was not significantly different from the following treatments: NMYC under Ni-0g; MYKORICH®, NMYC and NNFB under Ni-3g; and NMYC under Ni-6g. In contrast, the lowest total dry weight of 6.95 g $plant^{-1}$ was recorded in Ni-6 with NNFB treatment.

Biomass produced when inoculated with AMF-MYKORICH® in the first experiment, and MYKORICH® and NMYC in the second experiment, was significantly higher compared to uninoculated seedlings. This was also seen in Solis-Dominguez et al. (2011), wherein AMF-inoculated treatment, specifically *Glomus intraradices* inoculated seedlings was found to have the highest biomass production, hence, effective in reducing the use of compost to achieve a certain level of biomass production in mine tailings.

A group of metal-resistant plant growth-promoting rhizobacteria (PGPR), cited in Enebe and Babalola (2018), were noted to have a great potential to promote the growth of various plants in the metal-contaminated environments. These include *Pseudomonas, Arthrobacter, Agrobacterium, Bacillus, Azoarcus, Azospirillum, Azotobacter, Burkholderia, Klebsiella, Alcaligenes, Serratia, Rhizobium*, and *Enterobacter* species. PGP rhizobia can enhance plant growth and increase plant tolerance to Ni by improving different physiological and biochemical mechanisms (Sujkowska-Rybkowska et al. 2022; Kamran et al. 2016; Akhtar et al. 2018). For instance, in Akhtar et al. (2018), two PGPR – *Bacillus* sp. and *Stenotrophomonas* sp. were inoculated in soil with different Ni concentrations (0, 50, 100, and 150 mg.kg-1) to test their potential in the growth of radish (*Raphanus sativus*). *Bacillus* sp. was found to increase the root and shoot length, root and shoot dry biomass, root girth, total chlorophyll, and shoot nitrogen contents of radish plants in Ni-contaminated and non-contaminated soil. Aside from that, inoculation of the bacterial strain shows plant tolerance mechanism to Ni stress.

Microbial population count

MYKOCAP®-treated seedlings under Ni-12mg had significantly higher significant difference noted in mycorrhizal root colonization in Ni-12 with MYKOCAP® treatment in experiment 1 after six months, with 50.5% of roots showing the highest colonization (Figure 4A). However, this was not significantly different from MYKOCAP®-treated seedlings under Ni-0mg. The lowest colonization (4%) was observed in control under the Ni-12mg , which was not significantly different from the NNFB treatments under Ni-0mg. Moreover, in both experiments, there were no significant differences seen in treatments NNFB under Ni-0, Control under Ni-6, and Control under Ni-12. The lack of significant difference between control and NNFB treatments in both experiments may suggest that while indigenous NFB can promote plant growth and produce increased plant

Table 3. Partitioned plant biomass and total dry weight (g) of bagalunga seedlings six months after inoculation and grown in soil with Ni-0, 6 and 12 mg kg^{-1} soil.

Ni level	Inoculation Treatment	Dry weight (g $plant^{-1}$)			Total biomass
		Root	Stem	Leaves	
Ni – 0 mg	Control	14.07 ± 2.35 a	8.16 ± 0.74 ab	1.52 ± 0.13 abc	23.75 ± 3.11 ab
	MYKORICH®	6.04 ± 0.89 def	2.43 ± 1.12 d-g	1.86 ± 1.92 abc	10.33 ± 1.68 efg
	MYKOCAP®	8.09 ± 3.24 cd	2.99 ± 1.96 d-g	2.86 ± 3.45 a	13.94 ± 4.77 de
	NMYC	10.34 ± 1.68 bc	5.89 ± 1.23 bc	1.47 ± 0.35 abc	17.70 ± 3.09 cd
	NNFB	4.77 ± 2.73 d-g	2.23 ± 2.36 efg	1.24 ± 0.76 abc	8.25 ± 4.54 fgh
Ni – 6 mg	Control	3.17 ± 2.31 fg	1.86 ± 1.07 fg	0.67 ± 0.22 bc	5.7 ± 3.48 gh
	MYKORICH®	14.31 ± 0.85 a	9.38 ± 0.92 a	2.46 ± 0.63 ab	26.15 ± 2.13 a
	MYKOCAP®	13.43 ± 1.95 ab	7.81 ± 1.03 ab	1.89 ± 0.59 abc	23.12 ± 1.82 abc
	NMYC	12.30 ± 2.53 ab	7.50 ± 0.76 ab	1.65 ± 0.41 abc	21.45 ± 3.26 abc
	NNFB	6.86 ± 1.93 de	4.63 ± 2.78 cde	1.01 ± 0.28 bc	12.50 ± 3.81 def
Ni – 12 mg	Control	1.75 ± 0.12 g	0.83 ± 0.24 g	0.82 ± 0.63 bc	3.4 ± 0.71 h
	MYKORICH®	12.34 ± 1.93 ab	7.75 ± 2.1 ab	0.23 ± 0.23 c	20.32 ± 2.37 bc
	MYKOCAP®	6.29 ± 1.47 def	3.43 ± 0.67 c-f	0.52 ± 0.45 c	10.24 ± 1.58 efg
	NMYC	6.96 ± 3.03 de	4.74 ± 1.78 cd	0.67 ± 0.11 bc	12.37 ± 4.62 def
	NNFB	4.32 ± 1.38 efg	3.04 ± 0.84 d-g	0.81 ± 0.13 bc	8.17 ± 2.33 fgh

Columns A or B with the same letters are not significantly different from each other.

Table 4. Partitioned plant biomass and total dry weight (g) of bagalunga seedlings six months after inoculation and planted in soil with Ni-0, 3 and 6 g kg^{-1} soil.

Ni level	Inoculation Treatment	Dry weight (g $plant^{-1}$)			Total biomass
		Root	Stem	Leaves	
Ni – 0 mg	Control	6.65 ± 1.57 c-g	5.18 ± 1.32 c	1.34 ± 0.39 cde	13.18 ± 3.24 c-f
	MYKORICH®	8.67 ± 0.32 abc	6.72 ± 0.46 ab	2.03 ± 0.44 a	17.42 ± 0.88 a
	MYKOCAP®	6.36 ± 1.09 d-g	6.63 ± 0.8 ab	1.41 ± 0.25 b-e	14.40 ± 0.86 b-e
	NMYC	7.8 ± 0.35 b-f	6.59 ± 0.53 ab	1.13 ± 0.22 ef	15.52 ± 0.66 abc
	NNFB	5.69 ± 0.78 fgh	5.55 ± 1.15 bc	1.05 ± 0.02 ef	12.29 ± 1.83 d-g
Ni – 3 mg	Control	6.79 ± 0.86 c-g	5.07 ± 0.6 c	1.45 ± 0.21 b-e	13.30 ± 0.23 cde
	MYKORICH®	9.61 ± 1.15 ab	5.14 ± 0.65 c	1.95 ± 0.67 ab	16.7 ± 1.24 ab
	MYKOCAP®	7.03 ± 0.89 c-g	5.21 ± 0.24 c	1.3 ± 0.23 cde	13.54 ± 0.92 cde
	NMYC	7.95 ± 1.68 b-e	7.58 ± 1.3 a	1.71 ± 0.34 a-d	17.23 ± 3.21 ab
	NNFB	8.22 ± 1.42 a-d	5.22 ± 0.62 c	1.21 ± 0.17 de	14.65 ± 0.93 a-d
Ni – 6 mg	Control	4.80 ± 0.61 gh	2.71 ± 0.5 d	2.03 ± 0.20 a	9.55 ± 0.57 gh
	MYKORICH®	6.62 ± 1.35 c-g	3.34 ± 0.46 d	1.83 ± 0.32 abc	11.78 ± 1.49 efg
	MYKOCAP®	5.82 ± 0.52 e-h	3.26 ± 0.98 d	1.31 ± 0.68 cde	10.38 ± 1.3 fg
	NMYC	8.04 ± 0.89 a	4.76 ± 0.12 c	2.40 ± 0.25 a	15.19 ± 0.76 ab
	NNFB	3.87 ± 0 h	2.46 ± 0 d	0.62 ± 0 f	6.95 ± 0 h

Columns A or B with the same letters are not significantly different from each other.

biomass (Aggangan and Anarna 2019), in this specific case, their effectiveness may have varied based on environmental conditions and plant-microbe compatibility. NNFB strains may have found difficulty in adapting to the Ni contamination as it is adapted in copper-contaminated soils, making it difficult to form symbiotic relationship with the plant species.

Moreover, in experiment 2, MYKOCAP®-treated seedlings grown in soil without Ni and MYKORICH®-treated seedlings in 6 g Ni exhibited the highest % root colonization with 72.5% and 69.33%, respectively. They did not differ from each other, but both were significantly higher than all other treatments. The lowest colonization (3%) was observed in uninoculated and NNFB-treated seedlings in Ni-6g. However, this was not significantly different from control and NNFB-treated seedlings in Ni-0 and Ni-3, respectively, as well as from the control seedling in Ni-6 g Ni in soil. The roots of bagalunga plants were well colonized by AMF, with MYKOCAP® treatment with the highest colonization in both Ni-contaminated (Experiment 1) and non-contaminated soil (Experiment 2). Hodge et al. (2010) noted the positive impact of mycorrhizal colonization is evident in the supply of nitrogen, phosphorus, and water directed toward the fungi's growth or translocated to plants. This, in turn, promotes growth, starting with the contribution of carbohydrates from the photosynthetic organ and the supply of water and a higher quantity of nutrients by roots for the synthesis of plant biomass.

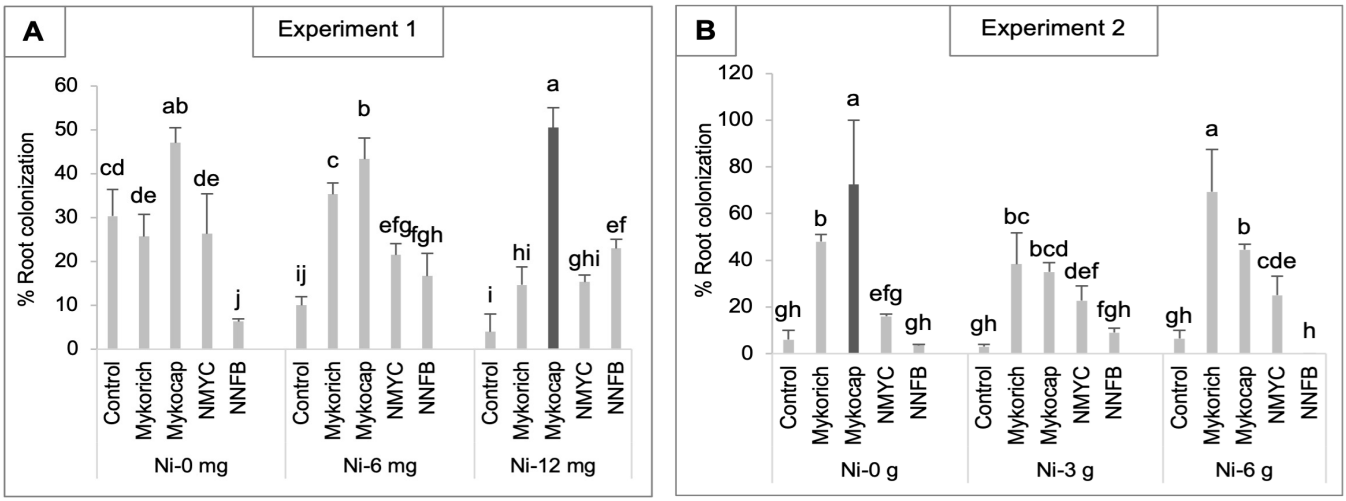

Figure 4. Mycorrhizal root colonization (%) on bagalunga seedlings six months after inoculation and grown in soil with Ni-0, 6 and 12 mg kg^{-1} soil (A) and Ni-0, 3 and 6 g kg^{-1} soil (B).

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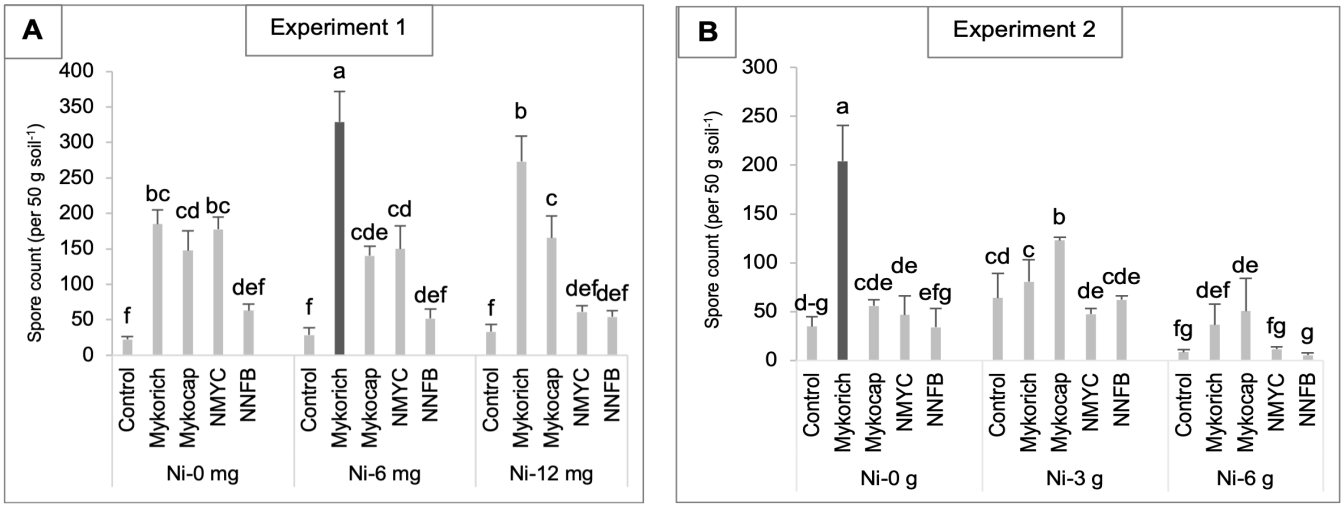

Figure 5. Mycorrhizal spore count on bagalunga seedlings six months after inoculation and grown in soil with Ni-0, 6 and 12 mg kg^{-1} soil (A) and Ni-0, 3 and 6 g kg^{-1} soil (B).

Bars with the same letters are not significantly different from each other.

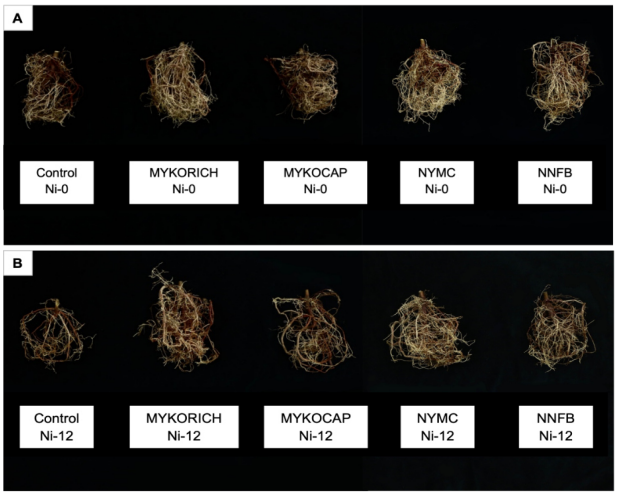

Figure 6. General appearance of bagalunga roots six months after inoculation and grown in soil with Ni-0 (A) and Ni-12 mg kg^{-1} (B).

Bars with the same letters are not significantly different from each other.

In terms of mycorrhizal spore count, MYKORICH®-treated seedlings in Ni-6mg gave the highest count after six months in the first experiment (Figure 5A). The lowest count was recorded in control plants grown in all Ni levels. However, these were not significantly different from the NNFB treatments in all Ni levels. In experiment 2, a high significant difference among all other treatments was recorded, with MYKORICH®-treated seedlings grown in soil without Ni had significantly the highest count of mycorrhizal spores (204 per 50 g $soil^{-1}$). The lowest spore counts were in the control, NMYC and NNFB treatments under Ni-6g, and the NNFB treatment in the Ni-0g level. MYKORICH® constitutes 12 AMF species belonging to genera *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, and *Entrophospora* (Aggangan et al. 2019). The diverse AMF species found in MYKORICH® may have affected the high spore production in bagalunga seedlings in moderate Ni contamination. In Magsayo et al. 2024, these AMF species have demonstrated resilience and adaptability to Ni-contaminated soils, effectively colonizing plant roots and producing spores even under metal stress.

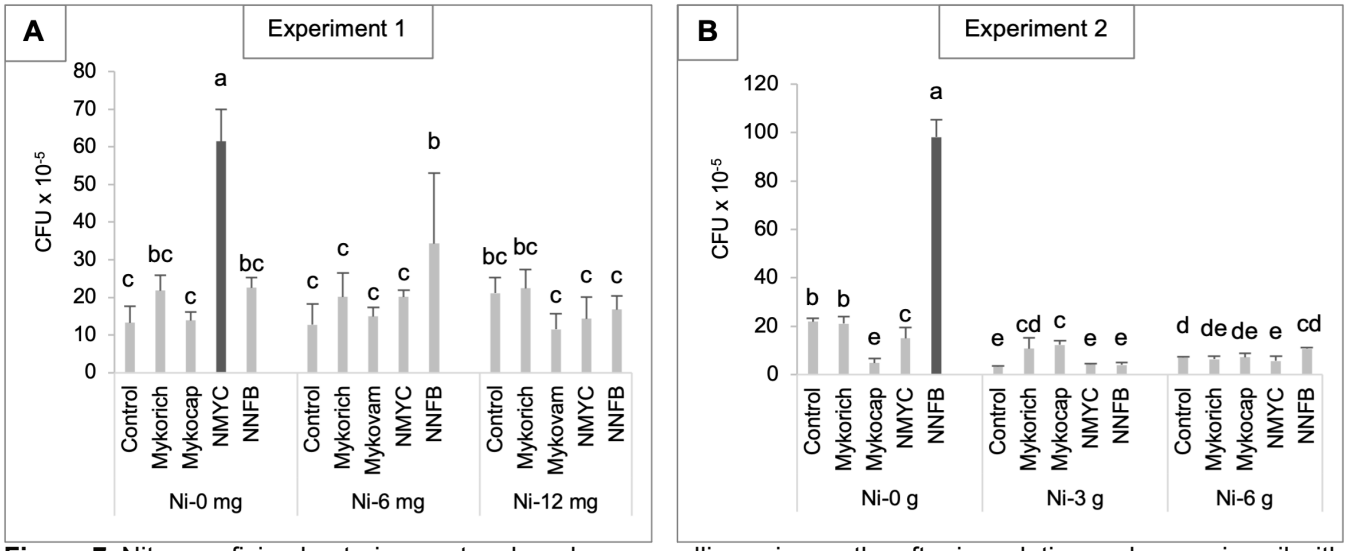

Figure 7. Nitrogen-fixing bacteria count on bagalunga seedlings six months after inoculation and grown in soil with Ni-0, 6 and 12 mg kg^{-1} soil (A) and Ni-0, 3 and 6 g kg^{-1} soil (B).

Bars with the same letters are not significantly different from each other.

Table 5. Main effects of inoculation and Ni level on N, P, and Ni concentration in bagalunga six months after inoculation and planted in soil with 0, 3 and 6 g Ni kg^{-1} soil.

Source of Variation	N (ppm)	P (ppm)	Ni (ppm)
Inoculation (Factor A)	***	**	ns
Control	0.048 c	154.125 a	255.508 a
MYKORICH®	0.048 c	142.278 ab	241.943 a
MYKOCAP®	0.047 d	148.263 a	234.65 a
NMYC	0.052 b	116.867 c	256.685 a
NNFB	0.055 a	127.078 bc	263.317 a
Ni level (Factor B)	***	**	***
Ni-0	0.056 a	149.4 a	15.39 c
Ni-3	0.046 c	158.7 a	267.1 b
Ni-6	0.048 b	105.1 b	468.8 a

***,**, ns = significant at p<0.001, p<0.01 and not significant, respectively.
Columns with the same letters are not significantly different from each other.

In terms of NFB count, NMYC-treated seedlings in Ni-0mg notably had the highest count (61 CFU x 10^5) in the first experiment. Whereas the lowest count (12.7 CFU x 10^5) was obtained from uninoculated seedlings under Ni- 6mg. All other treatments were not significantly different from this treatment and from each other (Figure 7). This trend was similar in Victoria and Aggangan (2019) in which an indigenous AMF isolated from Surigao, Mindanao coupled with an NFB biofertilizer produced the highest NFB count in *Acacia mangium* and *Eucalyptus urophylla*. In experiment 2, NNFB in Ni-0 resulted in a high NFB count (98.12 CFU x 10^5) that was highly significant from all other treatments. The lowest count was obtained from seedlings under the control, NMYC and NNFB treatments, MYKOCAP® under Ni-0g, and the MYKORICH®, MYKOCAP® and NMYC treatments under Ni-6g. NMYC and NNFB, as indigenous isolates, demonstrated the potential to produce high NFB populations, but this was observed in soils without contaminants. This raises uncertainty about whether these microbes can promote high NFB counts in Ni-contaminated soils, where environmental stressors may alter microbial activity and efficacy. Therefore, continual monitoring is required, or further studies utilizing the same species and microorganism inoculants must be carried out.

Soil nutrient and Ni analysis

Table 5 presents the effect of the two factors, treatments and Ni level, on the N, P and Ni uptake of bagalunga seedlings after six months using the ANOVA. The inoculation of NNFB effected the highest N (0.055 ppm), which also resulted in a highly significant difference among all other treatments. Introducing indigenous NFB may positively influence the rhizosphere microbiome, promoting nutrient uptake and plant health. On the other hand, control plants obtained the highest P (154.125 ppm) comparable with those inoculated with MYKORICH® and MYKOCAP®. In terms of Ni concentration, there was no significant difference observed among treatments. Further, there was a highly significant difference observed in N and Ni level as influenced by Ni applied to the soil. The highest N was obtained in soil without Ni, while Ni level expectedly was highest in Ni-6. The highest P was obtained in Ni-3 comparable to those without Ni. Recently, Akib et al. (2019) found that *Acaulospora* promoted the highest Ni accumulation in the roots of *Canavalia ensiformis*. Decreasing concentrations were noted in the stem, leaf and pod, seeds, and flowers of the plant. For their part, Magsayo et al. (2022) revealed the fast growth and development of narra seedlings when inoculated with AMF.

Corkidi et al. (2011) observed enhanced growth and nutrient uptake, or a reduction in nutrient leaching, in *Encelia californica* and *Rhus integrifolia* because of mycorrhizal colonization. In the present study, the highest levels of N and Ni in soil were obtained through the use of NNFB inoculation, whereas the concentration of P was greatest in the control group. Nonetheless, in the specific case studied, NNFB facilitated the highest uptake of N and Ni. This aligns with the findings of Akhtar et al. (2018), where a PGPR, *Bacillus* sp. Strain was reported to have

increased uptake and accumulation of N and Ni in radish in soil with Ni contamination. Bacterial and fungal inoculants are responsible for increased nitrogen fixation and mineral nutrient availability under HM stress (Rashid et al. 2016). In a review by Ma et al. (2016), bacterial inoculants can alter HM bioavailability and translocation in plants which modify metal toxicity and plant metal uptake and secretion of metabolites, such as citric, acetic acid, oxalic, among others.

CONCLUSION

The study assessed the growth performance, soil microbial community, soil nutrient dynamics, and HM uptake in Bagalunga (*Melia dubia*), emphasizing its potential to thrive in contaminated soils, particularly when supplemented with microbial inoculants. Both commercially available biofertilizers and indigenous isolates from a copper mine dumpsite were tested. After six months, results from two experiments revealed notable findings. In Experiment 1, bagalunga seedlings grown in Ni-6 soil with MYKORICH® showed the highest height and stem diameter increment. In Experiment 2, the tallest seedlings were observed in soil without Ni (Ni-0) with NMYC, while the highest stem diameter was recorded in Ni-6 with MYKOCAP®. MYKORICH® also produced the heaviest biomass in both experiments, specifically in Ni-6 (Experiment 1) and Ni-0 (Experiment 2). In terms of microbial population count, MYKOCAP® resulted in the highest root colonization in Ni-12 (Experiment 1) and Ni-0 (Experiment 2). MYKORICH® gave the highest mycorrhizal spore count in Ni-6 (Experiment 1) and Ni-0 (Experiment 2). Isolates from the Cu mine dump site gave the highest: NMYC in Ni-0 (Experiment 1), and NNFB in Ni-0 (Experiment 2). In terms of nutrient uptake, NNFB significantly enhanced N and Ni absorption, while P uptake was highest in control treatments. The findings showcase the importance of conducting both nursery and field trials to assess plant-microbe compatibility. Additionally, the study highlighted the optimized use of AMF and NFB inoculants in potential forest rehabilitation efforts for mined areas. These findings could assist forest managers in incorporating sustainable practices into forest rehabilitation strategies. Moreover, the plant's ability to absorb various heavy metals, supported by both this study and prior research, further reinforces its suitability for remediation applications. The study recommends additional analysis of plant and soil microbial parameters, along with the inclusion of other growth metrics such as nutritional and heavy metal dynamics in different plant parts under both nursery and field conditions, using the microbial inoculants tested in this study.

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