



Effect of Mycorrhiza on Vegetative Growth of Mandarin's (*Citrus reticulata* Blanco) Seedlings

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Abstract

Citrus is among the top fruits which are cultivated around the world, and among citrus, Mandarin (*Citrus reticulata* Blanco) comes first and belongs to the family Rutaceae. A field experiment was conducted in Gorkha, Nepal, from April 2021 to July 2022, to study the effect of different doses of Mycorrhiza in the vegetative growth seedlings. The experiment was set up in a Randomized Complete Block Design (RCBD) with five treatments and four replications. The treatments were named T1: Control, T2: 3g/seedling, T3: 6g/seedling, T4: 9g/seedling, and T5: 11g/seedling, respectively. Eighteen months old seedlings of local Mandarin were uprooted and transplanted in finely pulverized and prepared seedbeds, and Mycorrhiza was added subsequently. Data was collected for plant height, number of leaves, and stem diameter. Data analysis was done through Microsoft- Excel, R-Studio, and Analysis of Variance (ANOVA). Results of the study showed that plants treated with 9g of Mycorrhiza produced the highest number of measured parameters compared to plants treated with other doses. Control treatment (0g) produces the least number of plant leaves, Height, and stem diameter, and it can be concluded that a mycorrhizal dose of 9g is the best for the vegetative growth of Mandarin.

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Keyword

Mandarin;
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Introduction

The term citrus is believed to be originated from the Latin form of the Greek word 'Kedros,' which signifies trees like cedar, pine, and cypress. It originated from Southeast Asia, south China, north-eastern India, and Burma (Pinhas Spiegel-Roy & Eliezer E. Goldschmidt, 1996). It is one of the most economically important fruit crops in the world. It is grown in developed and developing countries as it is one of the main sources of vitamin C, along with the most significant number of carotenoids, vitamin E, etc. (Iglesias et al., 2007).

Mandarin orange (*Citrus reticulata* Blanco) is also considered a native of south-eastern Asia and the Philippines. It is most abundantly grown in Japan, southern China, India, and the East Indies (Morton, 1987). Depending on the variety, the mandarin tree may be much smaller than the sweet orange or equal in size. The *Citrus reticulata* tree generally has



thorns and thin twigs with broad, dark green, shiny leaves that are alternately arranged (Yeung, 1985). Mandarin fruit is oblate, the peel bright-orange or red-orange when ripe, loose, and separating easily from the segments (Chopra, 1986).

Nitrogen, potassium, phosphorus, magnesium, and six micronutrients are required for proper growth and often limit their production in various environments. Vesicular-arbuscular mycorrhizal fungi develop symbiotic associations with roots of citrus and help in the active uptake of these essential nutrients and overcome the stunting of some rootstocks resulting in remarkable stimulation of growth (Pinhas Spiegel-Roy & Eliezer E. Goldschmidt, 1996).

Mycorrhiza forms relationships in and on the root of a host plant in a symbiotic association with a fungus (Jeffries & Rhodoc, 1987). Based on function and structure, mycorrhizal fungi are primarily of four types, namely arbuscular Mycorrhiza (AM), ectomycorrhiza (EM), orchid mycorrhiza and ericoid Mycorrhiza (van der Heijden et al., 2015).

They are ecologically significant and provide an increased capacity to absorb water and nutrients from the soil, whereas plants provide soluble carbon sources to the respective fungus (Entry et al., 2002). Their inoculation doubled P concentration in the shoot and root and increased dry weight, especially of the shoot, irrespective of P levels. Under stress conditions, too, AM-fungi play a vital role in the promotion of the biological and chemical properties of plants (Mohammadi et al., 2011). They help plants to tackle pathogen-causing root diseases by protecting the root system or by direct attack. They probably induce resistance in plants against pathogens by improving nutrition uptake (Barea & Azcon-aguilar, 1982).

Materials and Methods

Experimental site

The research was conducted at Bindhabaasini Mistrit Falful Nursery, located at Nareshwor, Gorkha Municipality ward no. 3 of Gorkha district. The site lies in the mid-region of Gorkha district and is situated at 28.00°N 84.65°E. The altitude of the site is around 1200m above sea level.

Land Preparation

A nursery was selected at an appropriate place. The place was selected for seedbed preparation. The seedbed of 1.32 m² area was prepared by using finely pulverized soil, and FYM was added.

Soil Analysis

Before incorporating FYM soil sample of the nursery was taken for nutrient assessment. The physio-chemical analysis of soil was carried out before incorporating FYM at the Agriculture Knowledge Center, Gorkha, with the help of the soil kit method. The soil of the research field was loamy, slightly acidic (pH: 5.9), with low nitrogen content, high phosphorus content, and medium potash content.

Variety Selection

Eighteen months old seedlings of the same variety of mandarin having uniform Height were selected. The varieties used were local varieties that are resistant to root rot.

Transplantation and Intercultural Operation

Mycorrhiza of the required dose according to treatment was added by incorporating it in the soil during transplanting. After the addition of Mycorrhiza, seedlings were transplanted accordingly. Weeding was done weekly for a critical period. The data were taken first after 15 days of transplanting, and after that, the following data were taken at an interval of 30 days. The data were taken using a measuring Scale and a Vernier caliper.

Experimental Design

The experiment was laid out in One factorial Randomized Complete Block Design (RCBD) with five treatments, and each treatment was replicated four times. Each replication of treatment had a plot. A plot contained three rows, and a row contained 3 plants. Altogether there were 20 plots with 180 seedlings with spacing 5*2.5 cm²; thus, the total area of the research field was 1.32 m².

Treatment Details

Five treatments were prepared during the study based on the dose of Mycorrhiza per seedling. Treatment 1 (0g / seedling) was considered a control treatment, whereas T2 (3g/ seedling), T3 (6g/ seedling), T4 (9g/ seedlings), and T5 (11 gram/ seedling) were respective treatments.

Parameters

The observations were done on these parameters on various days after transplantation.

- Plant height
- Plant diameter
- Length of Internode
- No of leaves
- Pre-testing of soil

Statistical Analysis

The collected data were analyzed, and F-calculated was calculated and compared to the tabulated value of F at 1% and 5% significance levels. Results were analyzed using the RStudio software, where the LSD was calculated, and the Duncan Multiple Range test was used at a confidence level of 5%.

Results and Discussion

Mycorrhiza helps increase plants' vegetative growth mainly due to the enhancement of nutrient uptake by plants' roots. The primary function of Mycorrhiza is the active uptake and transport of nutrients. Mainly, it enhances the uptake of phosphorus, 76% from low P₄-containing areas and 79% from high P₄ containing areas (O, 2003).

The production of plant hormones gibberellins, cytokinin, and auxins by VAM could also be involved in plant growth. Gibberellins help increase leaf area and lateral root formation; cytokinin helps plant growth, whereas auxin controls root formation and elasticity of cell walls (Barea & Azcon-aguilar, 1982).

They can alleviate many anthropogenic stresses, such as the effects of metals, polychlorinated, and phenolic pollutants, which enhance the growth of plants (Mohammadi et al., 2011).

Mycorrhiza functions as conduits for the flow of energy and matter between plants and soils due to which they absorb nutrients from soils, and they are found between hyphal fungi and underground organs of terrestrial plants, ultimately helping in plant growth (Mohammadi et al., 2011).

Mycorrhiza enhances plant growth as it also helps the plants to tackle pathogen-causing root diseases by protecting the root system or by a direct attack (Barea & Azcon-aguilar, 1982).

A higher number of leaves were recorded from treatment number four, i.e., 9g, which was statistically significant than other treatments, whereas control gave the least number of leaves which is statistically insignificant. Data taken at 75DAP showed a significant increase in leaves number compared to other dates.

Table 1. Effect of Different Treatments on The Average Number of Leaves Per Plant in Gorkha, 2022

Treatments	Number of Leaves			
	15DAP	45DAP	75DAP	105DAP
Control	2.5 ^d	3.75 ^c	3.75 ^b	6.5 ^c
Mycorrhiza (3g)	3.25 ^{cd}	4.5 ^{bc}	4.5 ^b	7 ^{bc}
Mycorrhiza (6g)	4 ^{bc}	5 ^{bc}	5 ^{ab}	7.5 ^{bc}
Mycorrhiza (9g)	5.5 ^a	7.25 ^a	8 ^a	10.5 ^a
Mycorrhiza (11g)	4.5 ^{ab}	5.75 ^b	6.25 ^{ab}	8.5 ^b
LSD	1.09 ^{***}	1.33 ^{***}	3.19 [*]	1.882235 ^{**}
SEm (±)	0.16	0.19	0.47	0.28
F-Probability	<0.001	<0.001	<0.05	<0.01
CV %	18.49	16.98	38.91	15.77082
Grand Mean	3.95	5.25	5.5	8

Mean followed by a typical letter(s) within columns are non-significantly different based on DMRT P=0.05, *Significant at 0.05 level, **Significant at 0.01 P level, ***Significant at 0.001P SEM: Standard Error of Mean, CV: Coefficient of Variance.

More extended plant heights were recorded from the plants treated with 9g mycorrhiza, which is T4 and statistically significant. Shorter stem lengths were obtained from the control treatment, which is statistically insignificant. A significant increase in stem length was obtained from data taken at 15DAP, and the lowest changes in length occurred at 75DAP.

Table 2. Effect of Different Treatments on Height Per Plant in Gorkha, 2022

Treatments	Height (cm)			
	15DAP	45DAP	75DAP	105DAP
Control	2.05 ^c	2.96 ^c	3.3 ^d	4.06 ^d
Mycorrhiza (3g)	2.53 ^{bc}	3.21 ^{bc}	3.80 ^{cd}	4.40 ^{cd}
Mycorrhiza (6g)	2.81 ^{bc}	3.36 ^{bc}	4.09 ^{bc}	5.03 ^{bc}
Mycorrhiza (9g)	3.78 ^a	4.30 ^a	4.64 ^a	5.81 ^a
Mycorrhiza (11g)	3.07 ^{ab}	3.75 ^{ab}	4.30 ^{ab}	5.48 ^{ab}
LSD	0.84 ^{**}	0.67 ^{**}	0.49 ^{***}	0.75 ^{***}
SEm (±)	0.12	0.10	0.07	0.11
F-Probability	<0.01	<0.01	<0.001	<0.001
CV %	19.88	12.78	8.29	10.14
Grand Mean	2.84	3.51	4.02	4.95

Mean followed by a typical letter(s) within columns are non-significantly different based on DMRT P=0.05, **Significant at 0.01 P level, ***Significant at 0.001P SEM: Standard Error of Mean, CV: Coefficient of Variance.

Stem diameters were more significant in plants treated with a 9g mycorrhizal dose, which is statistically significant than others. Unlike, the control treatment produced a smaller stem diameter, which is also statistically insignificant in terms of stem diameter. There was more significant fluctuation in stem diameter at 105DAP than at 15DAP and 45DAP. T5 (11g) produced no significant number of leaves, height, and stem diameter compared to 9g, however, it which was a higher dose than T4.

Table 3. Effect of Different Treatments on Diameter Per Plant in Gorkha, 2022

Treatments	Diameter (cm)		
	15DAP	45DAP	105DAP
Control	0.13 ^b	0.15 ^b	0.18 ^c
Mycorrhiza (3g)	0.14 ^b	0.16 ^b	0.21 ^{bc}
Mycorrhiza (6g)	0.14 ^b	0.18 ^b	0.24 ^{bc}
Mycorrhiza (9g)	0.17 ^a	0.26 ^a	0.33 ^a
Mycorrhiza (11g)	0.15 ^b	0.20 ^{ab}	0.26 ^b
LSD	0.01 ^{**}	0.06 ^{**}	0.064 ^{**}
SEm (±)	0.002	0.008	0.009
F-Probability	<0.01	<0.01	<0.01
CV %	7.43	19.80	17.62
Grand Mean	0.15	0.19	0.24

Mean followed by typical letter(s) within columns are non-significantly different based on DMRT P=0.05, **Significant at 0.01 P level, ***Significant at 0.001P SEM: Standard Error of Mean, CV: Coefficient of Variance.

Conclusion

Citrus belongs to the family Rutaceae. Among citrus, mandarin is cultivated chiefly and is the preferred fruit. Grafted plants also show faster growth than non-grafted plants in the case of citrus species. For enhancing the growth of seedlings, Mycorrhiza can be used. VAM can increase the growth of plants. VAM helps plant growth by enhancing the plant's capacity to absorb more nutrients from the soil. Mainly Phosphorus intake is accelerated by Mycorrhiza.

The experiment was done in the nursery of Nareshwor, Gorkha municipality, Gorkha. Different doses of Mycorrhiza were considered as treatments for finding the optimum dose. The experiment was laid out in One factor RCBD (Randomized Complete Block Design) with four replications and five treatments. The treatments were (T1-0g, T2-3g, T3- 6g, T4-9g and T5-11g) plant⁻¹. The local variety of Mandarin was used in the experiment. Previously grown, uniform seedlings were uprooted and transplanted at a spacing of 5*2.5 cm².

Based on the study's results, it can be concluded that Mycorrhiza promotes the vegetative growth of mandarin seedlings. Using Mycorrhiza 9g, plant⁻¹ can increase the Height of the plant, stem diameter, and the number of leaves and can be considered as the optimum dose for treatment. Above mycorrhiza 9g plant⁻¹, there is no significant increase in vegetative growth of a seedling

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