

Influence of Media, VAM (Vesicular Arbuscular Mycorrhizae) and Supplementary Nutrients on Growth of Khasi Mandarin Seedlings (*Citrus reticulata* Blanco)

Tumula Rushi Kumar¹, N. Devachandra², P.K. Nimbolkar², A.S. Mailappa³, Arwankie Shadap⁴,
N.T. Chanu⁵ and L. Wangchu^{6*}

¹M.Sc. Scholar, Department of Fruit Science,
College of Horticulture and Forestry (CAU), Pasighat (Arunachal Pradesh), India.

²Assistant Professor, Department of Fruit Science,
College of Horticulture and Forestry (CAU), Pasighat (Arunachal Pradesh), India.

³Associate Professor, Department of Soil Science,
College of Horticulture and Forestry (CAU), Pasighat (Arunachal Pradesh), India.

⁴Assistant Professor, Department of Vegetable Science,
College of Horticulture and Forestry (CAU), Pasighat (Arunachal Pradesh), India.

⁵Assistant Professor, Department of Microbiology,
College of Horticulture and Forestry (CAU), Pasighat (Arunachal Pradesh), India.

⁶Professor, Department of Fruit Science, College of Horticulture and Forestry,
Central Agricultural University, Pasighat (Arunachal Pradesh), India.

(Corresponding author: L. Wangchu*)

(Received: 25 November 2022; Revised: 19 December 2022; Accepted: 30 December, 2022; Published: 16 January, 2023)

(Published by Research Trend)

ABSTRACT: Khasi mandarin is commercially the most important citrus fruit in North Eastern Region of India. While significance of vegetative propagation had been established; its adoption by the nurserymen and preference by growers in this region is limited. Still, they are continuing plantation of seedlings. Prevalence of nucellar seedlings in Khasi Mandarin can be effectively explored in this aspect. Nucellar seedlings perform consistently and reproduce similarly to their mothers. Enhancing the growth of seedlings becomes essential in Khasi mandarin. The present investigation was carried out at Fruit nursery, Department of Fruit Science, College of Horticulture and Forestry, Pasighat during 2021-22 to study the Influence of media, VAM (Vesicular Arbuscular Mycorrhizae) and supplementary nutrients on growth of Khasi Mandarin seedlings (*Citrus reticulata* Blanco) in the nursery. The experiment was planned in two factorial CRD and replicated thrice, consisted of 12 treatments with different potting media, VAM and supplementary nutrients, and their effect was studied after transplanting to polybags till one-year of age. The results indicated that, seedlings were found to better with respect to vegetative growth parameter viz. plant height, number of leaves, stem diameter and physiological growth parameter like fresh and dry weight of leaves, stem and shoot; length of the longest root, number of primary roots, root volume. Biochemical parameters like chlorophyll 'a', 'b' and total chlorophyll; leaf carbohydrate and leaf protein. The medium combination of (M₂) soil + vermicompost (2:1) with (V₃)VAM (*Glomus mosseae*) @ 5gm and NPK 19:19:19 @ 1% had given significant results among different combination in 12 months old seedlings. This would ensure raising of seedlings more productively in shorter cycle of nursery production.

Keywords: Khasi Mandarin, Media, VAM, Nucellar seedlings.

INTRODUCTION

Khasi Mandarin (*Citrus reticulata* Blanco) is major commercial fruit crop in North-East India and extensively cultivated. Most of the taxonomists agree that Indo-Chinese peninsula is the birth place of Khasi Mandarin (Tolkowsky, 1938). The name of the Khasi Mandarin is derived from the Khasi hills of Meghalaya, which are excellent in quality. It is slow growing, hardy plant and the fruiting season usually starts from November to February. Fruits that are grown at higher elevation (1000-1500m) are more acidic and deep orange in colour, while growing at lower elevations,

they remain yellowish-green. In India mandarin is grown over 4, 62,000 Ha and the production is 60, 26,000 MT (Anonymous, 2020). The different species of Mandarin are Satsuma Mandarin (*C. unshiu*), King Mandarin (*C. nobilis*), Khasi Mandarin (*C. reticulata* Blanco), Willow leaf Mandarin (*C. deliciosa*), Cleopatra Mandarin (*C. reshmi*) etc. They are loose skinned and mostly grown in rainfed areas.

Media is a substrate that supplies the necessary elements and provide physical support to the growing plants. It is crucial for the growth and development of seedlings as well as for germination of seeds. Low bulk

density, ideal water conductivity, adequate aeration, and drainage are the medium's most important physical characteristics for applicability (Cabrera, 2003). Mixed potting soils increase the roots' ability to get air, as well as more nutrients, moisture, and oxygen for healthy growth. The characteristics of various substances employed as growth substrates demonstrate indirect and direct effects on plant physiology and productivity (Verdonck *et al.*, 1981). Typically, soil is employed as a basic, inexpensive and easily obtainable medium. It has good physical characteristics like CEC (Cation Exchange Capacity), porosity, fertility, and water holding capacity, all of which contribute to keep the nitrogen to carbon ratio in balance. Vermicompost is an even finer, coarser organic material that when mixed with clay soil, allows air to enter while also loosening the soil. It includes organic carbon, which permits nutrients to enter the system gradually and steadily while allowing plants to absorb them. Vermicompost regularly applied to the soil enhances the biological, chemical and physical characteristics of the soil (Gupta *et al.*, 2014). Vermicompost has been discovered to be extremely effective for enhancing all of the physical characteristics of the plants (Rajamanickam *et al.*, 2010). It is a peat-like material with high aeration, porosity, drainage, microbial activity and water holding capacity, which make it an excellence soil conditioner (Edwards, 2009).

Due to the numerous benefits that Vesicular Arbuscular Mycorrhizae (VAM) fungus provide to the host plant in addition to helping it absorb more water and nutrients, they are now widely regarded as a type of biofertilizer (Bohra *et al.*, 2007). When compared to non-mycorrhizal plants, VAM promotes plant growth and improves phosphate uptake. Additionally, it affects how phyto-mass is distributed between roots and shoots. Instead of adding a lot of phosphate fertilizer to the soil to replace the deceased local mycorrhizae, VAM inoculation can be done for the severely stunted citrus seedlings in the nurseries. Additionally, under conditions of stress like soil salinity, dehydration, nutrient deficiency, and soil disturbance, VAM is crucial to the plant's survival. It improves plant growth and nutrition absorption (Somani, 2005). Brazilian sour orange seeds are inoculated with *Glomus mosseae* to boost growth by up to 150% (Hattingh and Gerdemann 1975).

The most crucial nutrients for plant growth and development are nitrogen, phosphorus, and potassium. Among all nutrients, N, P, and K are the three most crucial ones that directly affect plant metabolism and support healthy plant growth and productivity (Shanmugavelu, 1988). As a fundamental component of all living things, nitrogen, phosphorus and potassium are the most important and indispensable of all mineral nutrients for the development and growth of the plant (Throughton *et al.*, 1974). Foliar application of fertilizer is beneficial over soil application. It helps in uniform supply of fertilizers, low application rates and rapid response to applied nutrients (Kumar *et al.*, 2015).

MATERIALS AND METHODS

The experiment was conducted at Fruit nursery, Department of Fruit science, College of Horticulture and Forestry, CAU, Pasighat during the year 2021-22. The experiment was laid out in a two factorial Complete Randomized Design (CRD) with three replications and 12 treatments and the methodology of the experiment was carried out on 3-month-old Khasi Mandarin seedlings by using different combinations of growing media and different strain of VAM (Vesicular Arbuscular Mycorrhizae). NPK 1% 19:19:19 is sprayed among all treatments in common.

Treatment details:

Factor 1: Media: **M₀**: Soil, **M₁**: Soil + vermicompost (4:1), **M₂**: Soil + vermicompost (2:1)

Factor 2: VAM: **V₀**: Control, **V₁**: *Acaulospora laevis*, **V₂**: *Glomus bhagrayaj*, **V₃**: *Glomus mosseae*.

Treatment combinations:

T₁(M₀V₀), T₂(M₀V₁), T₃(M₀V₂), T₄(M₀V₃), T₅(M₁V₀), T₆(M₁V₁), T₇(M₁V₂), T₈(M₁V₃), T₉(M₂V₀), T₁₀(M₂V₁), T₁₁(M₂V₂) and T₁₂(M₂V₃).

Seedlings were raised on the nursery bed and transplanted in the polybags (10 × 21 cm) containing different media and VAM inoculum. NPK 1% 19:19:19 is sprayed among all treatments in common.

In each treatment five plants were selected from each replication for recording vegetative growth parameters. Observation on plant height was measured by scale, number of leaves were counted in each seedling at 3, 6, 9 and 12 months after transplanting. And stem diameter was measured in each seedling by Vernier calliper at 9 and 12 months after transplanting. For physiological growth parameters, one plant was selected from each replication for analysis of fresh and dry weight of leaves, stem and shoot; length of the longest root, number of primary roots and root volume was measured at 12 months after transplanting. Fresh and dry weight of leaves, stem and shoot was recorded in using calibrated balance. Length of the longest root was measured by scale; number of primary roots was counted and root volume was measured by Biovis scanner. For Biochemical parameters one plant was selected from each replication for Chlorophyll 'a', 'b' and total chlorophyll; leaf carbohydrate and leaf protein. Estimation of chlorophyll content was done as per method suggested by Arnon (1949). Leaf carbohydrate was determined by Anthrone method described by Hedge and Hofreiter (1969). Leaf protein was determined by Lowry's method (1951)

RESULTS AND DISCUSSION

(i) Plant height (cm). The influence of media on plant height was significant, the maximum plant height in the treatment M₂ at 3, 6, 9 and 12 months after transplanting was 11.59, 13.72, 20.19, and 29.57 cm respectively. The above result could be linked to better soil aeration, water holding capacity, porosity and the availability of nutrients, particularly nitrogen and micronutrients in the treatment of M₂, that have aided in better seedling growth. Similar outcomes were found by Srinivasulu *et al.* (2015); Patel *et al.* (2019) in acid lime

seedlings of the rangpur lime and Australian sour orange rootstock. Since, vesicular-arbuscular mycorrhiza (VAM) notably boosts phosphorus uptake and trace elements like Zn and Cu, which in turn significantly improve plant growth and yield. Among the different VAM species experimented, the maximum plant height at 3, 6, 9 and 12 months after transplanting was 16.79, 19.55, 28.69 and 39.03 cm respectively, in the treatment V₃. The above results were in line with Viyanak and Bagyaraj (1990) in trifoliate orange. The combination effect of media and VAM on plant height was noteworthy in the media M₂ with VAM V₃ at 3, 6, 9 and 12 MAT. In the treatment T₁₂ the maximum plant height was recorded at 3, 6, 9 and 12 months after transplanting (20.80, 24.17, 35.14 and 46.25 cm). The combined effect of media and VAM would lead to the genesis of compounds that promote growth. Further, VAM aids in the absorption of nutrients including P, Zn, Cu, and Fe. Adding to that, it helps soil retain its physical stability and facilitates water absorption (Chiranjeevi *et al.*, 2018).

(ii) Number of leaves. The nutrient-rich vermicompost medium's enhanced capacity for photosynthetic activity results in more leaves, which may have an impact on seedlings' leaf counts (Surakshitha and Kumar 2015). The number of leaves at 3, 6, 9 and 12 months after transplanting (12.69, 15.46, 22.48, and 28.97) was maximum in the media M₂. The Influence of VAM showed its maximum in the treatment V₃ (15.39, 18.89, 27.41 and 34.37 at 3, 6, 9 and 12 months after transplanting. VAM-treated seedlings yielded more leaves when compared with non-treated seedlings. The above results were in conformity with Chebet *et al.* (2021), who documented similar events in rough lemon seedlings. The influence of media coupled with VAM was considerable in the media M₂ and VAM V₃. The highest number of leaves at 3, 6, 9 and 12 months after transplanting (17.93, 21.13, 32.93 and 38.73) was recorded in the treatment T₁₂. An increase in the number of leaves might be due to an increase in plant height. Our results share similarities with the findings of Surakshitha and Kumar (2015) in Jamun seedlings.

(iii) Stem diameter (mm). In the media M₂ the maximum stem diameter recorded at 9 and 12 months after transplanting was 2.6mm and 3.29mm respectively. The results are in agreement with our expectations as the treatment with greater availability of nutrients, incites proliferation of photochemically active leaves which ultimately improves seedling girth. Similar results were evident in an experiment carried out by Patel *et al.* (2019) with diverse media on the growth of acid lime seedlings. Next to that, VAM treatment exhibited maximum stem diameter in V₃ at 9 (3.13mm) and 12 (3.83mm) months after transplanting in Khasi mandarin seedlings when compared to control. A comparable outcome was disclosed by Ortas and Ustuner (2014) in citrus seedlings. The combination effect of Media and VAM on stem diameter was significant in the treatment combination of media M₂ and VAM V₃ (3.66 mm and 4.41 mm) noted at 9 and 12 months after transplanting.

Physiological growth parameters

(i) Fresh and dry weight of leaves. It is plausible that bearing more leaves is positively correlated with an increased fresh and dry weight of leaf biomass. Among the different media assessed, the maximum fresh and dry weight of leaves (5.25 g and 1.79 g) was persistent in the media M₂. On the contrary, the maximum fresh weight (6.47g) and dry weight (2.23 g) of leaves were noted in the VAM treatment V₃, observed in 12 months after transplanting. Consequently, our findings indicate that VAM fungi can enhance the Khasi mandarin seedling's biomass accumulation on a fresh and dry weight basis of leaves. The above results seem to be consistent with the work of Chebet *et al.* (2021) who observed similar findings in rough lemon seedlings. The combination effect of media and VAM was significantly positive on the fresh (8.77g) and dry weight (2.98g) of leaves. It was recorded highest in the treatment combination T₁₂, comprising media M₂ with VAM V₃ at 12 months after transplanting. The possible explanation for this effect might be the potting media enriched with mycorrhizal fungi and vermicompost which aided seedlings growth. This increased the number of leaves that were produced, which ultimately resulted in higher fresh and dry leaf weights. These results are consistent with Naik *et al.* (2018) in acid lime seedlings, Wu and Zou (2010) in citrus seedlings and Bankar *et al.* (2009) in lime seedlings.

(ii) Fresh and Dry weight of stem (g). Among the different media tested, media M₂ yielded a maximum stem fresh weight of 3.21g and dry weight of 1.54g for the same. The growth of the chlorophyll reservoir in the leaves, which tends to accelerate the rate of photosynthetic production in terms of the fresh and dry weight of the stem, may be the cause. This may be expected with the addition of the proper substrate media at the proper stage and time. The effect of VAM was significant in treatment V₃, it revealed maximum fresh (4.38g) and dry weight (2.16g) at 12 months after transplanting. The findings of our study suggest that VAM fungus maximizes biomass accumulation of Khasi mandarin seedlings'. Our findings are comparable with the results of Chebet *et al.* (2021) who experimented with lemon seedlings and concluded that the combined effect of the treatment T₁₂ comprising media M₂ and VAM V₃ was quite influential on the fresh weight and dry weight of leaves, the values were 6.33g and 3.26g respectively at 12 months after transplanting.

(iii) Fresh and Dry weight of shoot (g). The maximum fresh weight of 8.46g and dry weight of 3.33g was recorded in the shoot 12 months after transplanting. It is a well-known fact that the physical characteristics of soil encourage better shoot and vegetative growth. Furthermore, the growing media combination has a substantial impact on the fresh and dry weight of shoots. The microorganisms in vermicompost-rich media secrete phytohormone and other physiologically active substances that affect plant growth. The ability of these substances to increase the fresh and dry weight of shoots is well established (Choudhary *et al.*, 2018). Since VAM has a positive impact on the biomass of

shoots. The fresh and dry weight of the shoot was notable in VAM-treated media in contrast to control. Among the different VAM species experimented with, the seedlings inoculated with V_3 treatment yielded maximum fresh and dry weight of shoot (10.85 g and 4.39 g) at 12 months after transplanting. Ojha *et al.* (2008) experiment seem relevant to ours, they reported significant results in VAM inoculated plants of custard apple with a higher fresh and dry weight of the shoot.

The compound effect of media and VAM was found significant vis-à fresh and dry weight of shoot at 12 months after transplanting. Accordingly, the maximum fresh and dry weight of the shoot was 15.10g and 6.24g. Because the weight of the shoots both fresh and dry is greatly influenced by the growth media and VAM. Citrus plants rely heavily on mycorrhizae, resultantly it is crucial to promote harmony between the growing medium and mycorrhizae (Ortas and Ustuner 2014)

(iv) Length of the longest root (cm). The longest root length at 12 months after transplanting was measured in the media M_2 (33.94 cm). The possible explanation for the above outcome might be the enhanced soil texture, structure, porosity, water-holding capacity, the activity of beneficial soil microfauna, maintenance of soil temperature, enhanced soil health and nutritional status of media. Additionally, the vermicompost encourages the association of plants and media, creates a consistent moisture supply, encourages improved root aeration, and subsequently enhances overall root growth.

In accordance with the present results, previous studies of Srinivasulu *et al.* (2015) in Rangpur lime and Australian sour orange seedlings depicted the effect of VAM on the length of the longest root. The longest root length noted by them was in treatment V_3 (42.79cm) recorded at 12 months after transplanting. The reason cited by them was VAM inoculation in that treatment, it enhances significant growth in the plant. As expected, the minimum root length was documented in the non-mycorrhizal seedlings. The above results are in agreement with Kamble *et al.* (2009) who noted similar findings in mango seedlings.

The composite effect of media and VAM was significant on the length of the longest root. The maximum length of the longest root was observed in the treatment T_{12} (50.77 cm) where media (M_2) was collated with VAM (V_3) recorded 12 months after transplanting. As per our existing findings, the favourable role played by VAM added to the media can be ascertained as a principal component in the treatment of M_2V_3 for increasing root length. Vermicompost enhances the availability of vital nutrients to plants for a longer duration. Besides that, it improves soil organic matter levels. The VAM associated with the roots aids in the seedlings' ability to absorb water and nutrients. These findings further support the idea of Naik *et al.* (2018) whose research in acid lime seedlings matched our findings.

(v) Number of primary roots. Highest number of primary roots was recorded in the media M_2 (1.75) at 12 months after transplanting, this may be the consequence of the complementary effects of media composition. It is an established fact that vermicompost exhibits

hormone-like activity and increases the number of roots, successively improving plant growth, development and nutrient intake.

Choudhary *et al.* (2018) experimented with papaya seedlings for the highest number of primary roots and recorded the same in the treatment V_3 (2.56) at 12 months after transplanting. Media M_1 (1.67) was at par with the highest value. On contrary, the lowest primary roots were in the V_0 (1.00) followed by V_1 (1.00) at 12 months after transplanting. This may be due to the inoculation of VAM in the media. The maximum number of primary roots were developed in the mycorrhizal associated seedlings. However, the least number of primary roots was in non-mycorrhizal seedlings.

The concerted effect of media and VAM was significant on primary root augmentation. The maximum number of primary roots was observed in the treatment T_{12} (3.00) at 12 months after transplanting. It was composed of media M_2 with VAM V_3 . Hence, the treatment combination of media and VAM (M_2V_3), yielded the maximum number of primary roots. Nevertheless, the treatment T_8 of M_1V_3 (2.67) was at par with the highest value (M_2V_3).

(vi) Root volume (mm^3). Among the different media tested, the maximum root volume ($6071.58 mm^3$) was noticed in the media M_2 at 12 months after transplanting. As the potting media was comprised of soil and vermicompost in the ratio of 2:1, that enhanced the root volume in the media M_2 . Furthermore, the Influence of VAM showed maximum root volume in treatment V_3 ($6817.44 mm^3$) at 12 months after transplanting. Coming to the combined effect of media and VAM, a significant number of primary roots were positively influenced by VAM treatment. Further, it is also registered that the increase in root volume was significantly more in mycorrhizal seedlings when compared to non-mycorrhizal seedlings. Wu and Zou (2010) discussed similar outcomes in citrus seedlings. Maximum root volume ($6973.00 mm^3$) was observed in the treatment T_{12} a combination of media M_2 and VAM V_3 at 12 months after transplanting. He inferred that media and VAM work together to boost root volume in mycorrhizal seedlings.

Biochemical parameters

(i) Chlorophyll "a", "b" and total chlorophyll (mg/g). The effect of media on chlorophyll "a", "b" and total chlorophyll is eminent. Highest chlorophyll "a" (1.03mg/g), "b" (0.97mg/g) and total chlorophyll (2.00mg/g) content was measured in the media M_2 at 12 months after transplanting. Next to that the cumulative effect of media and VAM was significant. The highest chlorophyll "a" (1.12mg/g), "b" (1.08mg/g) and total chlorophyll content (2.20mg/g) were observed in the T_{12} (M_2V_3) recorded at 12 months after transplanting. Application of media along with vermicompost stimulates nutrient uptake particularly nitrogen and chlorophyll synthesis take place which plays a substantial role in the assimilation of numerous amino acids, which subsequently get transformed into proteins and nucleic acids. Furthermore, they serve as a framework for chloroplast and may be considered

responsible for an increase in chlorophyll content of the leaves of seedlings. Our results are in consonance with Rakesh *et al.* (2012). They experimented acid lime seedlings with different media compositions. Among the different VAM species examined, the highest chlorophyll “a” (1.05mg/g), “b” (0.96mg/g) and total chlorophyll (2.01mg/g) content was reportedly observed in the treatment V₃ at 12 months after transplanting. In comparison with the control, mycorrhiza-treated seedlings exhibit a sharp rise in the rate of photosynthesis, which might be a cause of elevated total chlorophyll content. This study supports evidence from previous observations of Masri *et al.* (1998); Manoharan *et al.* (2008).

(ii) **Leaf Carbohydrate and protein (%)**. The maximum leaf carbohydrate and protein was 6.43% and 0.33% respectively in the media M₂ at 12 months after transplanting, the justification for this might be the media containing soil + vermicompost in the ratio of 2:1.

The influence of VAM exhibited maximum leaf carbohydrate (6.62%) and protein (0.36%) synthesis in treatment V₃ at 12 months after transplanting. Since high sugar content is evident in vesicular-arbuscular mycorrhizal Khasi mandarin seedlings. A possible explanation for enhanced carbohydrates might be the carbohydrate build-up as a result of enhanced photosynthesis. Conversely, fungi could accelerate the accumulation of soluble proteins and decrease the accumulation of soluble sugar and proline upon inoculating with VAM (Mathur and Vyas 1995). The above results reflect those of Yin *et al.* (2010), who discovered similar outcomes. The combination effect of media and VAM was significant for leaf carbohydrates and protein. Maximum leaf carbohydrate and protein (8.04% and 0.42%) were registered in the treatment T₁₂ which was a combination of media M₂ with VAM V₃ at 12 months after transplanting.

Table 1: Vegetative growth parameters.

Influence of media on plant and number of leaves and stem diameter										
Media	Plant height (cm)				Number of leaves				Stem diameter (mm)	
	3 MAT	6 MAT	9 MAT	12 MAT	3 MAT	6 MAT	9 MAT	12 MAT	9 MAT	12 MAT
M ₀	8.87	10.25	15.52	23.64	10.78	13.61	18.08	24.2	2.20	2.84
M ₁	10.01	11.33	16.48	25.98	11.2	14.23	19.74	26.49	2.34	3.05
M ₂	11.59	13.72	20.19	29.57	12.69	15.46	22.48	28.97	2.60	3.29
SE _M ±	0.10	0.11	0.14	0.30	0.10	0.09	0.17	0.13	0.03	0.01
CD at 5%	0.29	0.33	0.43	0.86	0.30	0.27	0.49	0.37	0.11	0.08
CV (%)	3.43	3.36	2.87	3.88	3.09	2.28	2.91	1.63	5.18	2.97
Influence of VAM on plant, number of leaves and stem diameter										
VAM	Plant height (cm)				Number of leaves				Stem diameter (mm)	
	3 MAT	6 MAT	9 MAT	12 MAT	3 MAT	6 MAT	9 MAT	12 MAT	9 MAT	12 MAT
V ₀	5.73	6.59	8.53	16.43	8.77	10.59	14.22	19.52	1.76	2.40
V ₁	8.31	9.23	14.60	22.58	10.47	12.93	17.77	24.17	2.18	2.86
V ₂	9.8	11.71	17.77	27.55	11.59	15.32	21.01	27.61	2.45	3.11
V ₃	16.79	19.55	28.69	39.03	15.39	18.89	27.41	34.37	3.13	3.83
SE _M ±	0.12	0.13	0.16	0.34	0.12	0.11	0.19	0.14	0.04	0.01
CD at 5%	0.34	0.39	0.48	1.00	0.34	0.32	0.58	0.42	0.12	0.09
CV (%)	3.43	3.36	2.87	3.88	3.09	2.28	2.91	1.63	5.18	2.97

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyradj*, V₃: *Glomus mosseae*; MAT - Months after transplanting

Table 2: Combination effect of media and VAM on plant height, number of leaves and stem diameter.

Treatment	Plant height (cm)				Number of leaves				Stem diameter (mm)	
	3 MAT	6 MAT	9 MAT	12 MAT	3 MAT	6 MAT	9 MAT	12 MAT	9 MAT	12 MAT
T ₁ -M ₀ V ₀	5.44	5.97	7.78	13.26	8.20	9.67	13.52	17.09	1.53	2.25
T ₂ -M ₀ V ₁	7.43	7.90	13.42	22.18	10.08	12.67	15.72	22.13	2.16	2.76
T ₃ -M ₀ V ₂	9.16	11.03	16.14	23.99	11.03	14.77	19.87	26.10	2.36	2.99
T ₄ -M ₀ V ₃	13.47	16.13	24.73	35.13	13.80	17.35	23.23	31.5	2.75	3.38
T ₅ -M ₁ V ₀	5.56	6.25	8.43	17.47	8.58	10.45	14.17	20.00	1.77	2.33
T ₆ -M ₁ V ₁	8.54	8.98	14.90	22.62	10.43	12.86	18.02	24.98	2.17	2.89
T ₇ -M ₁ V ₂	9.84	11.73	16.39	28.11	11.35	15.42	20.72	26.46	2.47	3.14
T ₈ -M ₁ V ₃	16.09	18.37	26.19	35.72	14.45	18.20	26.07	32.87	2.98	3.84
T ₉ -M ₂ V ₀	6.19	7.55	9.37	18.56	9.53	11.67	14.98	21.47	1.98	2.63
T ₁₀ -M ₂ V ₁	8.97	10.80	15.47	22.95	10.90	13.27	19.57	25.4	2.21	2.94
T ₁₁ -M ₂ V ₂	10.41	12.37	20.78	30.55	12.38	15.78	22.45	30.27	2.53	3.20
T ₁₂ -M ₂ V ₃	20.80	24.17	35.14	46.25	17.93	21.13	32.93	38.73	3.66	4.41
SE _M ±	0.20	0.23	0.28	0.59	0.20	0.19	0.34	0.25	0.07	0.03
CD at 5%	0.60	0.68	0.84	1.74	0.61	0.55	0.99	0.73	0.21	0.16
CV (%)	3.43	3.36	2.87	3.88	3.09	2.28	2.91	1.63	5.18	2.97

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyradj*, V₃: *Glomus mosseae*; MAT - Months after transplanting

Table 3: Physiological growth parameters.

Influence of media on fresh and dry weight of leaves, stem and shoot, length of the longest root, number of primary roots, root volume									
Media	Fresh and dry weight of leaves (g)		Fresh and dry weight of stem (g)		Fresh and dry weight of shoot (g)		Length of the longest root (cm)	Number of primary roots	Root volume (mm ³)
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight			
M ₀	3.67	1.28	2.09	0.91	5.76	2.19	28.33	1.33	5720.75
M ₁	4.15	1.42	2.37	1.03	6.52	2.45	30.33	1.67	5790.17
M ₂	5.25	1.79	3.21	1.54	8.46	3.33	33.94	1.75	6071.58
SE _M ±	0.05	0.03	0.05	0.03	0.07	0.05	0.26	0.07	4.80
CD at 5%	0.15	0.11	0.13	0.06	0.19	0.16	0.76	0.20	14.09
CV (%)	3.98	8.56	6.38	6.63	3.39	6.97	2.93	15.54	0.28
Influence of VAM on fresh and dry weight of leaves, stem and shoot, length of the longest root, number of primary roots, root volume									
VAM	Fresh and dry weight of leaves (g)		Fresh and dry weight of stem (g)		Fresh and dry weight of shoot (g)		Length of the longest root (cm)	Number of primary roots	Root volume (mm ³)
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight			
V ₀	2.28	0.85	0.96	0.39	3.24	1.24	23.78	1.00	5002.00
V ₁	3.83	1.29	2.09	0.88	5.92	2.17	26.81	1.00	5476.67
V ₂	4.86	1.62	2.80	1.21	7.66	2.83	30.09	1.78	6147.22
V ₃	6.47	2.23	4.38	2.16	10.85	4.39	42.79	2.56	6817.44
SE _M ±	0.06	0.03	0.05	0.03	0.08	0.06	0.30	0.09	5.55
CD at 5%	0.17	0.12	0.15	0.07	0.22	0.19	0.89	0.25	16.26
CV (%)	3.98	8.56	6.38	6.63	3.39	6.97	0.93	15.54	0.28

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyraj*, V₃: *Glomus mosseae*; MAT - Months after transplanting

Table 4: Combination effect of media and VAM on fresh and dry weight of leaves, stem, shoot, length of the longest root, number of primary roots, root volume.

Treatment	Fresh and dry weight of leaves (g)		Fresh and dry weight of stem (g)		Fresh and dry weight of shoot (g)		Length of the longest root (cm)	Number of primary roots	Root volume (mm ³)
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight			
T ₁ - M ₀ V ₀	1.67	0.66	0.80	0.29	2.47	0.95	22.41	1.00	4952.67
T ₂ - M ₀ V ₁	3.17	1.21	1.90	0.81	5.07	2.02	26.33	1.00	5321.67
T ₃ - M ₀ V ₂	4.70	1.45	2.47	1.00	7.17	2.45	28.13	1.33	5861.00
T ₄ - M ₀ V ₃	5.17	1.81	3.20	1.54	8.37	3.35	36.47	2.00	6747.67
T ₅ - M ₁ V ₀	2.37	0.86	0.87	0.39	3.24	1.25	23.63	1.00	4993.00
T ₆ - M ₁ V ₁	3.90	1.28	2.17	0.90	6.07	2.18	26.53	1.00	5457.00
T ₇ - M ₁ V ₂	4.87	1.65	2.83	1.16	7.70	2.81	30.01	2.00	5979.00
T ₈ - M ₁ V ₃	5.47	1.90	3.60	1.68	9.07	3.58	41.13	2.67	6731.00
T ₉ - M ₂ V ₀	2.80	1.03	1.20	0.50	4.00	1.53	25.30	1.00	5060.33
T ₁₀ - M ₂ V ₁	4.43	1.38	2.20	0.95	6.63	2.33	27.57	1.00	5651.33
T ₁₁ - M ₂ V ₂	5.00	1.77	3.10	1.47	8.10	3.24	32.12	2.00	6601.67
T ₁₂ - M ₂ V ₃	8.77	2.98	6.33	3.26	15.10	6.24	50.77	3.00	6973.00
SE _M ±	0.10	0.06	0.09	0.05	0.14	0.10	0.52	0.15	9.61
CD at 5%	0.30	0.21	0.27	0.13	0.40	0.31	1.53	0.42	28.18
CV (%)	3.98	8.56	6.38	6.63	3.39	6.97	2.93	15.54	0.28

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyraj*, V₃: *Glomus mosseae*

Table 5: Biochemical parameters.

Influence of media on Chlorophyll 'a', 'b' and total chlorophyll, leaf carbohydrate and protein					
Media	Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Total chlorophyll (mg/g)	Leaf carbohydrate (%)	Leaf protein (%)
M ₀	0.85	0.80	1.65	4.11	0.22
M ₁	0.98	0.88	1.86	5.31	0.29
M ₂	1.03	0.97	2.00	6.43	0.33
SE _M ±	0.01	0.02	0.03	0.07	0.12
CD at 5%	0.01	0.02	0.02	0.20	0.02
CV (%)	1.07	3.09	1.68	4.55	7.00
Influence of VAM on Chlorophyll 'a', 'b' and total chlorophyll, leaf carbohydrate and protein					
VAM	Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Total chlorophyll (mg/g)	Leaf carbohydrate (%)	Leaf protein (%)
V ₀	0.86	0.83	1.69	3.89	0.20
V ₁	0.93	0.86	1.79	4.95	0.27
V ₂	0.97	0.87	1.84	5.69	0.31
V ₃	1.05	0.96	2.01	6.62	0.36
SE _M ±	0.01	0.03	0.03	0.08	0.02
CD at 5%	0.01	0.03	0.03	0.24	0.01
CV (%)	1.07	3.09	1.69	4.55	7.00

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyraj*, V₃: *Glomus mosseae*

Table 6: Combination effect of media and VAM on Chlorophyll 'a', 'b' and total chlorophyll, leaf carbohydrate and protein.

Treatment	Chlorophyll 'a' (mg/g)	Chlorophyll 'b'(mg/g)	Total chlorophyll(mg/g)	Leaf carbohydrate (%)	Leaf protein (%)
T ₁ - M ₀ V ₀	0.74	0.72	1.46	2.54	0.17
T ₂ - M ₀ V ₁	0.84	0.80	1.64	4.17	0.20
T ₃ - M ₀ V ₂	0.86	0.83	1.69	4.26	0.23
T ₄ - M ₀ V ₃	0.98	0.83	1.81	5.48	0.30
T ₅ - M ₁ V ₀	0.92	0.88	1.80	4.49	0.25
T ₆ - M ₁ V ₁	0.95	0.87	1.82	4.80	0.28
T ₇ - M ₁ V ₂	0.98	0.82	1.80	5.65	0.31
T ₈ - M ₁ V ₃	1.05	0.97	2.02	6.34	0.35
T ₉ - M ₂ V ₀	0.91	0.90	1.81	4.64	0.17
T ₁₀ - M ₂ V ₁	1.01	0.92	1.93	5.87	0.32
T ₁₁ - M ₂ V ₂	1.06	0.97	2.03	7.18	0.38
T ₁₂ - M ₂ V ₃	1.12	1.08	2.20	8.04	0.42
SE _M ±	0.02	0.05	0.05	0.14	0.03
CD at 5%	0.01	0.04	0.05	0.41	0.03
CV (%)	1.07	3.09	1.68	4.55	7.00

Media: M₀: Soil, M₁: Soil + vermicompost (4:1), M₂: Soil + vermicompost (2:1); VAM: V₀: Control, V₁: *Acaulospora laevis*, V₂: *Glomus bhagyraraj*, V₃: *Glomus mosseae*

CONCLUSION

The study revealed that media (M₂) of soil + vermicompost (2:1) with VAM (V₃) of *Glomus mosseae* and NPK 1 % 19:19:19 (Pramukh) spraying are more preferable for reducing the nursery growth period of seedlings. Therefore, farmers or nursery man who are interested in mass multiplication Khasi mandarin through seedlings are advised to use these combination treatments (*Citrus reticulata* Blanco).

FUTURE SCOPE

Study the influence of other alternative potting media like cocopeat, vermiculite, perlite over the seedling growth and development of citrus species and other fruit crops. Identifying the different VAM species and locally available biofertilizer to enhance seedling growth. The study may be conducted to know the effect of different concentrations of NPK seedling growth. The investigation can be repeated with other seedlings and in different locations conform the results.

REFERENCES

Anonymous (2020). National Horticulture Board Database. *National Horticultural Board* (NHB).http://nhb.gov.in/StatisticsViewer.aspx?enc=FdhWKi1URASyNAM+4mV5hOpJ_DviTxMmPkSfD97hsCEQ+Z+J1IzLFolcG88JyPsUQ.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24 (1), 1.

Bankar, S. P., Indi, D. V. and Gud, M. A. (2009). Effect of VAM fungi and Azospirillum on growth and development of Kagzi lime (*Citrus aurantifolia* L.) seedlings. *Journal Maharashtra Agricultural University*, 34(2), 183-185.

Bohra, A., Mathur, N., Bohra, S., Singh, J. and Vyas, A. (2007). Influence of AM fungi on physiological changes in *Terminalia arjuna* L.: An endangered tree of Indian thar desert. *Indian Forester*, 133(11), 1558-1562.

Cabrera, R. I. (2003). Fundamentals of container media management: Part-I Physical properties. The state University of New Jersey Agricultural Experimental Station.

Chebet, D. K., FK, W. and Kariuki, W. (2021). Vesicular Arbuscular Mycorrhizal Inoculation Influences Growth, Nutrient Absorption and Hyphae Colonization of Rough Lemon (*Citrus limon*) Seedlings. *African Journal of Education, science and Technology*, 6(3), 76-85.

Chiranjeevi, M. R., Hongal, S., Vinay, G. M., Muralidhara, B. M. and Sneha, M. K. (2018). Influence of media and biofertilizers on seed germination and seedling vigour of aonla. *International Journal of Current Microbiology Applied Sciences*, 7(1), 587-593.

Choudhary, R. C., Kanwar, J., Chouhan, G. S., Sing, P. and Tanwar, D. R. (2018). Effect of ga3 and growing media on seedling growth of papaya (*Carica papaya* L.) cv. pusa Nanha. *International Journal of Chemical Studies*, 6(6), 1008-1012.

Edwards, C. A. (2009). The use of earthworm in the breakdown and management of organic waste. *Earthworm Ecology*, pp. 327-354.

Gupta, R., Yadav, A. and Garg, V. K. (2014). Influence of vermicompost application in potting media on growth and flowering of marigold crop. *International Journal of Recycling of Organic Waste in Agriculture*, 3(1), 1-7.

Hattingh, M. J. and Gerdemann, J. W. (1975). Inoculation of Brazilian sour orange seed with and endomycorrhizal fungus, *Phytopathology*, 65, 1013-1016.

Hedge, J. E. and Hofreiter, B. T. (1962). In RL Whistler & JN BeMiller. *Carbohydrate chemistry*, 17.

Kumar, J., Kumar, R, Rai, R and Mishra, D. S. (2015). Response of 'Pant Prabaht' guava trees to foliar sprays of zinc, boron, calcium and potassium at different plant growth stages. *The Bioscan*, 10(2), 495-498.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological Chemistry*, 193(1), 265-75.

Manoharan, P. T., Pandi, M., Shanmugaiah, V., Gomathinayagam, S. and Balasubramanian, N. (2008). Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. *African Journal of Biotechnology*, 7(19).

Masri, M., Azizah, H., Razi, I. M. and Mamat, A. S. (1998). Arbuscular mycorrhiza enhances growth and reduces nursery period of mangosteen (*Garcinia mangostana* L.) seedlings. *Journal of Tropical Agriculture and Food Science*, 26, 7-16.

- Mathur, N. and Vyas, A. (1995). I. Influence of VA mycorrhizae on net photosynthesis and transpiration of *Ziziphus mauritiana*. *Journal of plant physiology*, 147(3-4), 328-330.
- Naik, S. R., Nandini, M. L. N., Venkataramana, K. T. and Mukundalakshmi, L. (2018). Effect of organic amendments and bio-agents on growth of acid lime (*Citrus aurantifolia* Swingle) cv. Balaji seedlings in the nursery. *Journal of Pharmacognosy and Phytochemistry*, 7(6), 1611-1616.
- Ortas, I. and Ustuner, O. (2014). Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake. *Scientia Horticulture*, 166, 84-90.
- Patel, M., Parmar, B., Halpati, A., Parmar, A. M. and Pandey, A. (2019). Effect of growing media and foliar spray of organics on seedling growth and vigour of acid lime. *International Journal of Chemical. Studies*, 7, 1-4.
- Rajamanickam, C., Balasubramanyan, S. and Natarajan, S. (2010). Studies on Nursery Management in Papaya (*Carica papaya* L.) Var. Co2. *Acta Horticulturae*, 851.
- Shanmugavelu, K. G. (1988). Studies on the effect of organic v/s inorganic source of nitrogen on growth, Yield and quality of okra (*Abelmoschus esculentus*). *Indian Journal of Horticulture*, 45(3&4), 312-318.
- Rakesh, K. Y. (2012). Effect of media on growth and development of acid lime (*Citrus aurantifolia* Swingle) seedling with or without Azotobacter. *African Journal of Agricultural. Research*, 7(48), 6421-6426.
- Somani, L. L. (2005). Handbook of biofertilizers. *Agrotech Publishing Academy*. Udaipur. pp. 1054.
- Srinivasulu, A., Ramana, K. V., Lakshmi, L. M., Sudhakar, P., Nagaraju, R. and Gopal, K. (2015). Effect of potting media on the growth of Rangpur lime (*Citrus limonia* Osbeck) and Australian sour orange (*Citrus aurantium*) root stock seedlings. *Journal of Research. ANGRAU*, 43(1/2), 88-95.
- Surakshitha, N. and Kumar, M. S. (2015). Growing Media Supplemented with Vermicompost and Glomus fasciculatum Acts as Gestation Period Reducers in Jamun (*Syzygium cumini* L. Skeels) Seedlings. *Trends in Biosciences*, 8(7), 1666-1675.
- Throughton, J. H., Morrby, J. and Currie, B. G. (1974). Investigation of carbon transport in plants. *Journal of Experimental Botany*, 25, 684-694.
- Tolkowsky, S. (1938) *Hesperides: A History of the Culture and Use of Citrus Fruits*. John Bale, Sons and Curnow Ltd, London.
- Verdonck, O. D., De Vleeschauwer, D. and De Boodt, M. (1981). The influence of the substrate to plant growth. In *Symposium on Substrates in Horticulture other than Soils In Situ* 126(pp. 251-258).
- Viyanak, K. and Bagyaraj, D. J. (1990). Selection of efficient VA mycorrhizal fungi for trifoliolate orange. *Biological Agriculture and Horticulture*, 6(4), 305-311.
- Wu, Q. S. and Zou, Y. N. (2010). Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Scientia Horticulture*, 125(3), 289-293.
- Yin, B., Wang, Y., Liu, P., Hu, J. and Zhen, W. (2010). Effects of vesicular-arbuscular mycorrhiza on the protective system in strawberry leaves under drought stress. *Frontiers of Agriculture in China*, 4(2), 165-169.

How to cite this article: Tumula Rushi Kumar, N. Devachandra, P.K. Nimbolkar, A.S. Mailappa, Arwankie Shadap, N.T. Chanu and L. Wangchu (2023). Influence of Media, VAM (Vesicular Arbuscular Mycorrhizae) and Supplementary Nutrients on Growth of Khasi Mandarin Seedlings (*Citrus reticulata* Blanco). *Biological Forum – An International Journal*, 15(1): 294-301.