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## Effect of rootstock and growing media on seedling growth and success of micro budding technique of Mandarin (*Citrus reticulata* Blanco)

**Bharti Choudhary, TR Sharma, SK Pandey and VK Paradkar**

### Abstract

Rootstocks modulate several characteristics of citrus trees, including vegetative growth, yield, quality and resistance or tolerance to different biotic and abiotic stresses. Rough lemon (*Citrus × jambhiri*) and Rangpur lime (*Citrus limonia* Osbeck) is the most promising rootstock for mandarin. Good growing medium support to the plant, serves as reservoir for nutrients and water allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad *et al.*, 2002). Micro-budding is a new propagation technique and standardized in Citrus species which could revolutionize the commercial citrus industry by saving grower's time, space and money. The experimental was consisted in Asymmetric Factorial Completely Randomized Design with three replication. Treatment includes three factors i.e. Rootstock (rough lemon & rangpur lime), growing media (Soil + Vermicompost (1:1), Soil + Azotobacter (1:1), Soil + Vermicompost + Azotobacter (1:1:1), Cocopeat + Vermicompost (1:1)) and age of rootstock (4, 5 and 6 month). On the basis of results found in the present investigation it is concluded that Cocopeat + Vermicompost (1:1) with rough lemon seedling found superior in seedling height (cm), stem diameter (mm), and survival % after transplanting in different growing media followed by Soil + Vermicompost + Azotobacter (1:1:1) with Rough lemon. Micro budding done at six month of rough lemon seedling recorded highest success percent in micro budding, sprout length (cm) at 60 days after micro budding of seedling and minimum days taken to first sprout after micro budding. In combined effect of rootstock, growing media and age of rootstock, rough lemon with Cocopeat + Vermicompost (1:1) at six month age of rough lemon rootstock seedling found superior in seedling height (cm), stem diameter (mm), and survival %, micro budding success %, sprout length (cm) at 60 days after micro budding of seedling and minimum days taken to first sprout after micro budding followed by Soil + Vermicompost + Azotobacter (1:1:1) at five month age of rootstock. Maximum survival percent of seedling was recorded in four month age of seedling.

**Keywords:** Micro budding, cocopeat, vermicompost and rootstock

### Introduction

Citrus fruit belongs to Rutaceae family and it is one of the major horticultural crops grown in more than 100 countries worldwide. Citrus is the third most important fruit crop of India after mango and banana since it is grown almost all the tropical and subtropical region of the country. Mandarins are easily peelable fruits and segments are conveniently consumed by hands. Among mandarins, 'Nagpuri' or 'Nagpur' mandarin is cherished for its unique thirst quenching sweet and sour taste.

Rootstocks modulate several characteristics of citrus trees, including vegetative growth, yield, quality and resistance or tolerance to different biotic and abiotic stresses. Rough lemon (*Citrus × jambhiri*) and Rangpur lime (*Citrus limonia* Osbeck) is the most promising rootstock for mandarin.

Rough lemon is a cold-hardy citrus and commonly known as Jamberi is widely used rootstocks for propagating mandarin in India and it is faster growing rootstock as compared to other rootstocks as well as having better success rate. Rough lemon are an ancient hybrid variety, it is a cross between a mandarin and a citron. Rangpur, *Citrus reticulata × medica*, sometimes called the rangpur lime, mandarin lime or lemandarin. It is a hybrid between the mandarin orange and the citron. It is the most utilized rootstock for its specific qualities i.e. due to its heavy and early bearing, and drought resistance.

A good growing medium support to the plant, serves as reservoir for nutrients and water allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad *et al.*, 2002) [1].

The potting mixture should have enough nutrients, good water holding capacity and drainage to ensure better growth of seedlings. Growing media components are either organic or inorganic. Organic components include peat moss, bark, coconut coir, rice hulls, wood fiber, etc. and inorganic components include perlite, pumice, vermiculite, sand, hydrogel etc.

Presently, there is huge demand for quality planting material and it is unable to meet the demand through conventional budding. Rapid germination, maximum germination percentage and healthy seedlings stock are very much essential to fulfil the growing demands for budded and grafted quality planting material. Seed without use of growth regulators showed poor response to germination and growth (Dongre, 2007) [8]. Hence, there is an urgent need for alternate rapid multiplication technique which fastens the planting material production by reducing nursery phase. In this way micro budding and micro grafting both the techniques are very useful for multiplication. *In vitro* micro grafting and the plant material derived from it can be further multiplied in tissue culture conditions or acclimatized to outdoor conditions. The technique has been used in woody species, especially fruit trees (Choudhary and Dongre, 2020) [6]. Micro-budding is a new propagation technique and standardized in Citrus species which could revolutionize the commercial citrus industry by saving grower's time, space and money. Skaria and Zhang (2000) [15] first developed this technique and later this was standardized in Nagpur mandarin, sweet orange and grapefruit (Alam *et al.*, 2006) [3]. Micro budding technique was standardized in *Citrus reticulata* Blanco cv. Nagpur mandarin which is also applicable for other Citrus species for fast and year round multiplication of disease free planting material and also commercial citrus propagation for getting the marketable budded plants earlier with reduced cost (Vijayakumari and Singh, 2003) [17]. In the view of above facts the current research work was conducted to evaluate suitable rootstock and growing media on seedling growth and success of micro budding.

### Material and Method

The experiment was carried out at Zonal Agricultural Research Station, Chhindwara (M.P.) India under Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. The experimental was consisted in Asymmetric Factorial Completely Randomized Design with three replication. Treatment includes three factors i.e. Rootstock (rough lemon & rangpur lime), growing media (Soil + Vermicompost (1:1), Soil + Azotobacter (1:1), Soil + Vermicompost +Azotobacter (1:1:1), Cocopeat + Vermicompost (1:1)) and age of rootstock (4, 5 and 6 month) with 24 treatment combination i.e. T<sub>1</sub>- Rough lemon + Soil + Vermicompost (1:1) + Micro budding at 4 month ARS, T<sub>2</sub>- Rough lemon + Soil + Vermicompost (1:1) + Micro budding at 5 month ARS, T<sub>3</sub> - Rough lemon + Soil + Vermicompost (1:1) + Micro budding at 6 month ARS, T<sub>4</sub>- Rough lemon + Soil + Azotobacter (1:1) + Micro budding at 4 month ARS, T<sub>5</sub> - Rough lemon + Soil + Azotobacter (1:1) + Micro budding at 5 month ARS, T<sub>6</sub>- Rough lemon + Soil + Azotobacter (1:1) + Micro budding at 6 month ARS, T<sub>7</sub> -Rough lemon + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 4 month ARS, T<sub>8</sub> - Rough lemon + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 5 month ARS, T<sub>9</sub> -Rough lemon + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 6

month ARS, T<sub>10</sub> -Rough lemon + Cocopeat + Vermicompost (1:1) + Micro budding at 4 month ARS, T<sub>11</sub> -Rough lemon + Cocopeat + Vermicompost (1:1) + Micro budding at 5 month ARS, T<sub>12</sub> -Rough lemon + Cocopeat + Vermicompost (1:1) + Micro budding at 6 month ARS, T<sub>13</sub> - Rangpur lime + Soil + Vermicompost (1:1)+Micro budding at 4 month ARS, T<sub>14</sub> - Rangpur lime + Soil + Vermicompost (1:1)+ Micro budding at 5 month ARS, T<sub>15</sub> - Rangpur lime + Soil + Vermicompost (1:1) + Micro budding at 6 month ARS, T<sub>16</sub> -Rangpur lime + Soil + Soil + Azotobacter (1:1) + Micro budding at 4 month ARS, T<sub>17</sub> -Rangpur lime + Soil + Azotobacter (1:1)+ Micro budding at 5 month ARS, T<sub>18</sub> -Rangpur lime + Soil + Azotobacter (1:1) + Micro budding at 6 month ARS, T<sub>19</sub> - Rangpur lime + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 4 month ARS, T<sub>20</sub> -Rangpur lime + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 5 month ARS, T<sub>21</sub> - Rangpur lime + Soil + Vermicompost + Azotobacter (1:1:1) +Micro budding at 6 month ARS, T<sub>22</sub> - Rangpur lime + Cocopeat + Vermicompost (1:1) + Micro budding at 4 month ARS, T<sub>23</sub> -Rangpur lime + Cocopeat + Vermicompost (1:1) + Micro budding at 5 month ARS, T<sub>24</sub> - Rangpur lime + Cocopeat + Vermicompost (1:1) + Micro budding at 6 month ARS. 15 seedlings of each rootstock are transplanted in polybags which contain different growing media as per the treatments wise and filled up polythene bags (20 X 30 cm). Growing media were used in different ratio comprising G<sub>1</sub> - Soil + Vermicompost in the ratio of 1:1, G<sub>2</sub> - Soil + Azotobacter in the ratio of 1:1, G<sub>3</sub> - Soil + Azotobacter + Vermicompost in the ratio of 1:1:1 and G<sub>4</sub> - Cocopeat + Vermicompost (1:1). Observations were recorded on physiological parameter such as seedling height (cm), stem diameter (mm), survival of seedlings (%) and post budding parameter i.e. days taken to first sprout, Micro budding success (%), length of micro budded sprout (cm) at 30, 60 and 90 days after micro budding influenced by different growing media, rootstocks, age of root stock and its interactions.

### Planting material for Micro budding

For micro budding two different rootstocks (Rough lemon and rangpur lime) were transplanted in various growing media. Bud sticks of Mandarin cv. Nagpur Santra were taken in micro budding. Bud stick was collected from mother blocks maintained at Zonal Agricultural Research Station, Chhindwara. Thirty rootstock seedlings were maintained in each age in three replications. Micro budding was done on different age groups (4, 5 and 6 months old) rootstock seedlings with Nagpur Santra scion under different growing media.

### Preparation of bud for microbudding

Healthy and elite mother trees with sufficient new flush of Mandarin cv. Nagpur santra were selected in the mother block of Zonal Agricultural Research Station, Chhindwara and the trees were tagged for collection of bud. The terminal shoots of current season growth with 15-20 cm length and nodal buds having pencil thickness were selected. Pre-curing such as removal of leaves was done with sterilized secateurs on the same day of micro budding. The scion shoots were collected from mother trees early in the morning on the day of budding. Immediately after separation of scions from mother trees, they were wrapped in moist cloth and carried in polythene cover to the site of budding.

### Procedure of micro budding

The leaf petiole is cut off just above the bud, and the bud is removed from the bud stick with a razor-sharp knife. A flat cut is made just underneath the bud. Only the bud and a small piece of wood under it are used. Selected rootstock seedling is beheaded leaving 15-20 cm. On beheaded portion a wedge-shaped cut (2-2.5 cm) is given downward with a sharp knife and tender scion bud (3-4 mm width and 1 to 1.5 cm length) inserted on the de topped root stock in a wedge cut and immediately protected by covering with a micropipette tip to achieve close physical contact between scion and stock tissues was placed with slight pressure over top of bud union. After a week, micro buds are observed -and then micro tips are removed after their sprouting within 12-14 days. The plant growth of mandarin budlings were periodically assessed at monthly intervals up to 90 days after Microbudding (DAM).

### Result and Discussion

The results of the present investigation show that different physiological parameter such as seedling height (cm), stem diameter (mm), survival of seedlings (%) and post budding parameter i.e. days taken to first sprout, Micro budding success (%), length of micro budded sprout (cm) at 60 days after micro budding were significantly influenced by the Growing media, rootstocks, age of rootstock and its interaction.

#### Physiological parameter

##### Growing Media

Data revealed that, maximum seedling height (17.58 cm), stem diameter (2.95 mm), and survival percent of seedling (81.02%) at the time of microbudding was recorded under Cocopeat + Vermicompost (G4) growing media in the ratio of 1:1.

Vermicompost stimulates to influence the microbial activity of soil, increases the availability of oxygen, maintains normal soil temperature, increases soil porosity and infiltration of water, improves nutrient content and increases growth, yield and quality of the plant. Coco peat have high water holding capacity, high cation exchange capacity and increases the porosity of the potting mix. High Cation Exchange Capacity allowing nutrients to be absorbed and released to the plants according to their need due to in that's qualities the G4 growing media promotes better growth of seedlings all over all respective growth parameters than the rest of growing media.

The result was supported with the finding of Bhagat *et al.* (2013) [5] proved that Soil+ FYM+ cocopeat (2:1:1). Nearby result also found by Dongre *et al.* (2014) [9] that soil+ vermicompost was found suitable for early germination and faster growth.

##### Root stock

Rootstocks showed significant effect on different parameters. Maximum seedling height (15.95 cm), stem diameter (2.74 mm), and survival percent of seedling (80.42%) at the time of microbudding was recorded under rough lemon seedling (R1).

Rough lemon is the quickest growing and high vigour rootstocks of mandarin. Due to fast and early germination character of rough lemon gives seedling growth quickly and attain maximum height of shoot & root and number of leaves. Due to all these properties in rough lemon rootstock seedling were observed maximum growth in all respective

physiological parameters as compared to rangpur lime rootstock seedlings. The result was confirmed with finding of Qadri *et al.*, 2021 [12] and Sharma *et al.* (2013) [14].

##### Age of Rootstock

Height of seedling at different age ultimately showed significant effect observed that the maximum seedling height (18.76 cm), stem diameter (3.26 mm), was under 6 month age of rootstock (A<sub>3</sub>) at the time of microbudding. The maximum survival percent of seedling (89.52%) was found in 4 month age of rootstock (A<sub>1</sub>) at the time of microbudding.

As the age of the plant age increases, the mortality in the plant increases due to different biotic and abiotic factors, the mortality in the initial is less, so the survival percent in four month age of seedling is higher.

At six month old seedlings, as the period increases of sowing or age of rootstock, the rootstock seedlings become extra time so that they can take more nutrients from the soil and produce more and more food through photosynthesis and all other physiological process. The results found in present investigation are in close conformity with the results of Jadhav (2003) [10] in Rangpur lime, Jaiswal, *et al.* (2018) [11] in Kagzi lime as increase the age of sowing or transplanting enhance growth parameters.

##### Interaction (Growing media and Rootstock)

As regards the interaction of growing media and rootstock had shown significant effect on different physiological parameters of seedlings. The maximum seedling height (17.84 cm), stem diameter (2.95 mm), and maximum survival percent of seedling (83.31%) was recorded under Rough lemon seedling transplanted in cocopeat +Vermicompost growing media in combination of 1:1 ratio (R<sub>1</sub>G<sub>4</sub>) at the time of micro budding.

Combined application of vermicompost and cocopeat showed significant effect on seedling growth parameters and plant Biomass probably due to the synergistic combination of both the factor is improvement of physical condition of the media and nutritional factor. The result was closely supported with the finding of Bhagat *et al.* (2013) [5] proved that Soil+ FYM+ cocopeat (2:1:1) best media for the growth observed greatest seedling height, stem diameter, number of leaves, leaf area, tap root length and tap root diameter in rough lemon seedlings.

##### Interaction (Age of rootstock and Rootstock)

The interaction of age of rootstock and rootstocks had shown significant effect on different physiological parameters of seedlings. The maximum seedling height (19.34 cm), stem diameter (3.44 mm) was recorded under A3R1 at the time of micro budding. While the maximum survival percent of seedling (92.02%) was found in 4 month age of rough lemon rootstock seedlings (R1A<sub>1</sub>) at the time of microbudding. Rough lemon seedlings are quick and fast growing, at six month age get extra duration for all physiological process responsible to growth, due to which the seedlings found maximum length of seedlings, maximum stem diameter, and maximum survival percent in six four month age of seedling.

##### Interaction (Age of rootstock and Growing media)

The interaction effect of rootstocks age and growing media had shown significant effect on seedling height at the time of micro budding. The maximum seedling height (20.57 cm),



stem diameter (3.44 mm), fresh weight of shoot (2.19 g), dry weight of seedling (0.77), fresh weight of root (1.56), dry weight of root (0.73 g) and maximum leaf area index (1.1) was recorded under age of six month seedling in cocopeat + Vermicompost growing media in combination of 1:1 ratio (A3G4) at the time of micro budding. While the maximum survival percent of seedling (93.16%) was found in age of 4 month seedling in cocopeat + Vermicompost growing media in combination of 1:1 ratio (A1G4) at the time of microbudding.

Genetically, rough lemon has a quick & fast-growing seedling in nursery stage, that's why when it is transferred to media containing cocopeat and vermicompost, as time progresses, it gets excellent growth in all physiological parameters as compared to other treatments.

## Post budding parameters

### Growing media

Growing media significantly influenced the survival and growth of mandarin after microbudding. The minimum days taken to first sprout after micro budding (16.12), maximum microbudding success (62.41%), length of micro budded sprout (6.10 cm) at 60 day after micro budding was noticed in budlings transplanted in cocopeat + vermicompost (1:1) growing media.

Combined application of vermicompost and cocopeat showed significant effect on seedling growth parameters and plant biomass probably due to the synergistic combination of both the factor is improvement of physical condition of the media and nutritional factor. Cocopeat and vermicompost improved the growth, buddability and budding success. The obtained was closely conformed with the finding of Bhagat *et al.* (2013) <sup>[5]</sup> found highest proportion of early buddable seedlings and budding success in Soil+ FYM+ cocopeat (2:1:1) growing media and Malakar *et al.* (2019) <sup>[19]</sup> the minimum days taken to sprout in the Cocopeat + G1 IBA 500 ppm better growth and buddability seedlings.

### Rootstock

Rootstocks significantly influenced the survival and growth of mandarin after microbudding. The minimum days taken to first sprout after micro budding (17.16), maximum microbudding success (57.15%), length of micro budded sprout (5.98 cm) at 60 day after micro budding was recorded in Rough lemon rootstock seedlings.

Rootstocks play a crucial role in grafting and budding. Success of a budding operation depends on combining anatomical structures of the stock and scion. A "de novo" formed meristematic area must develop between scion and rootstock for a successful bud union. Similar result was found by Alam *et al.*, (2006) <sup>[3]</sup> highest success of microbudding and number of leaves and Abbas (2002) <sup>[2]</sup> maximum number of leaves was observed on rough lemon rootstock.

### Age of Rootstock

Age of rootstock seedlings significantly influenced the survival and growth of mandarin after microbudding. The minimum days taken to first sprout after micro budding (17.01), maximum microbudding success (64.01 %), length of micro budded sprout (6.24 cm) at 60 day after micro budding was recorded in six month old age of rootstock.

Highest sprouting percentage as the age of the rootstocks has a relationship with regenerating ability of a plant which is found to be higher in younger rootstocks because of higher activity of meristematic cells resulting in faster formation of callus and quick healing of bud union. Length of sprout could be due to vigorous nature of the younger rootstocks resulting from the faster multiplication of meristematic cells in the juvenile rootstocks, (Archana, *et al.*, 2018) <sup>[4]</sup>.

The highest length of the initial sprout was observed by microbudding on six months old root stock seedlings. The highest length of the initial sprout, highest success rate of microbudding, and plant height at different intervals was observed by microbudding on six months old root stock seedlings Teja *et al.* (2016) <sup>[16]</sup>. Vijayakumari *et al.* (2003) <sup>[17]</sup> and Vijayakumari (2019) <sup>[18]</sup>.

## Interaction (Growing media and Rootstock)

With regards to interaction between different growing media and root stock, seedlings of Rough lemon rootstock along with Cocopeat + Vermicompost (1:1) (R1G4) recorded significantly the lowest number of days for the emergence of first sprout (16.73), maximum microbudding success (63.63 %), length of micro budded sprout (6.13 cm) at 60 day after micro budding.

Similar result was observed by Bhagat *et al.* (2013) <sup>[5]</sup> found highest proportion of early buddable seedlings and budding success in rough lemon with Soil+ FYM+ cocopeat (2:1:1) growing media.

## Interaction (Rootstock and age of rootstock)

Interaction between rootstock and different age of root stock, six months old rough lemon rootstock (R1A3) recorded the lowest number of days for the emergence of first sprout (15.51), maximum microbudding success (67.52 %), length of micro budded sprout (6.33 cm) at 60 days after micro budding was recorded in Rough lemon rootstock seedling.

This could be due to the relationship between age of the rootstock and regenerating ability of the plant and higher activity of meristematic cells, maximum regeneration and callus forming capacity and better union of the bud scion. The result was confirmed with Vijayakumari N. (2019) <sup>[18]</sup> recorded minimum days taken to first sprout and high successes percent of microbudded plant found in six month old rough lemon seedling.

## Interaction (Growing media and age of Rootstock)

Similarly the interaction of rootstocks age and growing media had shown significant effect. The minimum days taken to first sprout after micro budding (14.38), maximum microbudding success (74.66%), length of micro budded sprout (6.55 cm) at 60 days after micro budding was recorded in six month root stock seedlings with growing media cocopeat + vermicompost (1:1) G<sub>4</sub> growing media.

Vermicompost improved seedling growth and cocopeat was hypothesized that supplementation of growing media with organic amendments will promote growth and buddability or rootstock seedlings. The result was closely related with the findings of Bagat *et al.* 2013 <sup>[5]</sup> observed highest proportion of buddable seedling of rough lemon in growing media soil +FYM +cocopeat (2:1:1).

**Table 1:** Effect of growing media, age of rootstock on seedling height (cm) of different rootstocks at the time of micro budding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
G1	Soil + Vermi. (1:1)	13.79	13.41		13.60	
G2	Soil + Azato. (1:1)	15.84	13.18		14.51	
G3	Soil + Vermi + Azato. (1:1:1)	16.30	16.30		16.30	
G4	Cocopeat + Vermi. (1:1)	17.84	17.32		17.58	
Mean		15.94	15.05			
		Growing media		rootstocks	R X G	
SE(m)±		0.078	0.110		0.155	
CD 5%		0.224	0.316		0.447	
		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
A1	4 month ARS	11.56	11.69		11.62	
A2	5 month ARS	16.88	15.34		16.11	
A3	6 month ARS	19.38	18.13		18.76	
Mean		15.94	15.05			
		Age of rootstocks		Rootstocks	A X R	
SE(m)±		0.078	0.095		0.135	
CD 5%		0.224	0.274		0.387	
		G1	G2	G3	G4	Mean
A1	4 month ARS	9.20	9.78	14.26	13.25	11.62
A2	5 month ARS	14.61	15.67	15.23	18.92	16.11
A3	6 month ARS	16.98	18.08	19.40	20.57	18.76
Mean		13.60	14.51	16.30	17.58	
		Age of rootstocks		Growing media		A X G
SE(m)±		0.095		0.110		0.190
CD 5%		0.274		0.316		0.548

**Table 2:** Effect of growing media, age of rootstock on stem diameter (mm) of different rootstocks at the time of micro budding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
G1	Soil + Vermi. (1:1)	2.46	2.35		2.40	
G2	Soil + Azato. (1:1)	2.76	2.42		2.59	
G3	Soil + Vermi + Azato. (1:1:1)	2.76	2.74		2.75	
G4	Cocopeat + Vermi. (1:1)	2.99	2.91		2.95	
Mean		2.74	2.61			
		Growing media		rootstocks	R X G	
SE(m)±		0.010	0.014		0.020	
CD 5%		0.029	0.042		0.059	
		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
A1	4 month ARS	2.11	2.20		2.15	
A2	5 month ARS	2.85	2.59		2.72	
A3	6 month ARS	3.26	3.03		3.14	
Mean		2.74	2.61			
		Rootstocks		Age of Rootstocks	A X R	
SE(m)±		0.010	0.013		0.018	
CD 5%		0.029	0.036		0.051	
		G1	G2	G3	G4	Mean
A1	4 month ARS	1.95	2.04	2.43	2.18	2.15
A2	5 month ARS	2.42	2.64	2.61	3.23	2.72
A3	6 month ARS	2.84	3.09	3.21	3.44	3.14
Mean		2.40	2.59	2.75	2.95	
		Age of rootstocks		Growing media		A X G
SE(m)±		0.013		0.014		0.025
CD 5%		0.036		0.042		0.072

**Table 3:** Effect of growing media, age of rootstock on survival of seedlings (%) of different rootstocks at the time of micro budding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)		Mean
G1	Soil + Vermi. (1:1)	77.36	72.97		75.17
G2	Soil + Azato. (1:1)	79.85	74.59		77.22
G3	Soil + Vermi + Azato. (1:1:1)	81.16	76.61		78.89
G4	Cocopeat + Vermi. (1:1)	83.31	78.72		81.02
Mean		80.42	75.72		
		Growing media		Rootstocks	R X G
SE(m)±		0.135	0.191		0.270
CD 5%		0.388	0.549		0.776
		Rough lemon (R1)	Rangpur Lime (R2)		Mean

A1	4 month ARS	92.02	87.03	89.52		
A2	5 month ARS	79.77	75.70	77.74		
A3	6 month ARS	69.48	64.44	66.96		
Mean		80.42	75.72			
		Age of rootstocks	Rootstocks	A X R		
SE(m)±		0.165	0.135	0.23		
CD 5%		0.475	0.388	0.672		
		G1	G2	G3	G4	Mean
A1	4 month ARS	63.36	64.36	72.87	69.98	67.64
A2	5 month ARS	74.66	77.50	76.17	89.08	79.35
A3	6 month ARS	81.28	85.59	88.87	93.16	87.22
Mean		73.10	75.82	79.30	84.07	
		Age of rootstocks	Growing media		A X G	
SE(m)±		0.165	0.191		0.330	
CD 5%		0.475	0.549		0.950	

**Table 4:** Effect of growing media, age of rootstock on days taken to first sprout of rootstocks after microbudding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)	Mean		
G1	Soil + Vermi. (1:1)	19.46	19.50	19.48		
G2	Soil + Azato. (1:1)	17.94	19.50	18.72		
G3	Soil + Vermi + Azato. (1:1:1)	18.04	18.44	18.24		
G4	Cocopeat + Vermi. (1:1)	16.73	17.28	17.01		
Mean		18.04	18.68			
		Rootstock	Growing media	R X G		
SE(m)±		0.049	0.069	0.097		
CD 5%		0.140	0.198	0.279		
		Rough lemon (R1)	Rangpur Lime (R2)	Mean		
A1	4 month ARS	20.53	20.24	20.39		
A2	5 month ARS	18.09	19.04	18.57		
A3	6 month ARS	15.51	16.76	16.13		
Mean		18.04	18.68			
		Rootstocks	Age of Rootstocks	A X R		
SE(m)±		0.049	0.059	0.084		
CD 5%		0.140	0.171	0.242		
		G1	G2	G3	G4	Mean
A1	4 month ARS	20.90	20.75	19.58	20.32	20.39
A2	5 month ARS	19.75	18.75	19.45	16.32	18.57
A3	6 month ARS	17.78	16.67	15.70	14.38	16.13
Mean		19.48	18.72	18.24	17.01	
		Age of rootstocks	Growing media		A X G	
SE(m)±		0.059	0.069		0.119	
CD 5%		0.171	0.198		0.342	

**Table 5:** Effect of growing media, age of rootstock on micro budding success (%) of rootstocks at the time of micro budding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)	Mean		
G1	Soil + Vermi. (1:1)	49.42	49.28	49.35		
G2	Soil + Azato. (1:1)	55.84	47.75	51.80		
G3	Soil + Vermi + Azato. (1:1:1)	55.99	53.86	54.93		
G4	Cocopeat + Vermi. (1:1)	63.63	61.20	62.41		
Mean		56.22	53.02			
		Rootstock	Growing media	R X G		
SE(m)±		0.153	0.216	0.305		
CD 5%		0.439	0.621	0.878		
		Rough lemon (R1)	Rangpur Lime (R2)	Mean		
A1	4 month ARS	45.95	46.33	46.14		
A2	5 month ARS	55.19	52.24	53.71		
A3	6 month ARS	67.52	60.50	64.01		
Mean		56.22	53.02			
		Rootstocks	Age of Rootstocks	A X R		
SE(m)±		0.153	0.187	0.264		
CD 5%		0.439	0.538	0.760		
		G1	G2	G3	G4	Mean
A1	4 month ARS	44.19	43.10	48.70	48.57	46.14
A2	5 month ARS	48.79	52.41	49.66	64.00	53.71
A3	6 month ARS	55.07	59.88	66.42	74.66	64.01
Mean		49.35	51.80	54.93	62.41	

	Age of rootstocks	Growing media	A X G
SE(m)±	0.187	0.216	0.374
CD 5%	0.538	0.621	1.075

**Table 6:** Effect of growing media, age of rootstock on length of sprouted shoot (cm) at 60 days of rootstocks at the time of micro budding

Treatment		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
G1	Soil + Vermi. (1:1)	5.60	5.64		5.62	
G2	Soil + Azato. (1:1)	5.94	5.55		5.74	
G3	Soil + Vermi + Azato. (1:1:1)	5.87	5.84		5.86	
G4	Cocopeat + Vermi. (1:1)	6.13	6.07		6.10	
Mean		5.89	5.77			
		Rootstock	Growing media		R X G	
SE(m)±		0.062	0.088		0.124	
CD 5%		0.178	0.252		0.357	
		Rough lemon (R1)	Rangpur Lime (R2)		Mean	
A1	4 month ARS	5.43	5.42		5.42	
A2	5 month ARS	5.90	5.75		5.82	
A3	6 month ARS	6.33	6.16		6.24	
Mean		5.89	5.77			
		Rootstocks	Age of Rootstocks		A X R	
SE(m)±		0.062	0.076		0.107	
CD 5%		0.178	0.218		0.309	
		G1	G2	G3	G4	Mean
A1	4 month ARS	5.30	5.35	5.58	5.46	5.42
A2	5 month ARS	5.59	5.78	5.63	6.29	5.82
A3	6 month ARS	5.97	6.10	6.36	6.55	6.24
Mean		5.62	5.74	5.86	6.10	
		Age of rootstocks	Growing media		A X G	
SE(m)±		0.076		0.088	0.152	
CD 5%		0.218		0.252	0.437	

## Conclusions

On the basis of results found in the present investigation it is concluded that Cocopeat + Vermicompost (1:1) with rough lemon seedling found superior in seedling height (cm), stem diameter (mm), and survival % after transplanting in different growing media followed by Soil + Vermicompost + Azotobacter (1:1:1) with Rough lemon.

Micro budding done at six month of rough lemon seedling recorded highest success percent in micro budding, sprout length (cm), at 60 days after micro budding of seedling and minimum days taken to first sprout after micro budding.

In combined effect of rootstock, growing media and age of rootstock, rough lemon with Cocopeat + Vermicompost (1:1) at six month age of rough lemon rootstock seedling found superior in seedling height (cm), stem diameter (mm), and survival %, micro budding success %, sprout length (cm) at 60 days after micro budding of seedling and minimum days taken to first sprout after micro budding followed by Soil + Vermicompost + Azotobacter (1:1:1) at five month age of rootstock. Maximum survival percent of seedling was recorded in four month age of seedling.

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