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Effect of media composition on growth and development of acid Lime (*Citrus aurantifolia* Swingle) seedling with or without Azotobacter

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Abstract

The present investigation was carried out at the Fruits Research Farm, College of Horticulture & Forestry Jhalarapatan, Jhalawar during the year 2010. In this experiment, freshly extracted acid lime seeds were sown into different media with or with out Azotobacter to study their effect on growth and development acid lime seedlings. The results indicated that the medium combination soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter had given significantly better result among different combinations. Under this treatment the height of seedling (13.75 cm), number of leaves per seedling (22.46), diameter of stem (3.35 mm), fresh weight (2.77 g) and dry weight of seedling (1.18 g) were recorded significantly superior over other treatments used. Further it was also found superior with relation to length of longest tap root (19.76 cm), diameter of tap root (2.95 mm), number of secondary roots (40.66), root/shoot ratio (2.57), nitrogen content in leaf (1.86 %), chlorophyll content in leaf (5.44 mg/g) and leaf area of seedlings (1.43 cm^2).

Key Words: *Azotobacter, media, acid lime seedling, vermicompost*

Introduction

Acid lime is an important Sub-tropical fruit crop of the world. It is native of India and South-Eastern China. The trees medium sized, hardy and semi-vigorous, growth upright with an irregular and loose crown, foliage not dense, light green, thorns numerous, fruit round and oblong, greenish yellow in colour and juice is highly acidic and its seeds are highly polyembryonic in nature. Hence it is still commercially propagated by seed.

Propagation media play an important role in germination of seeds and for further growth and development of seedling. Among different media used vermicompost, cocopeat, sphagnum moss are organic in nature and vermiculite, perlite and sand are inorganic in nature. Many organic media decompose readily, get compact easily and thus decreases pore space and aeration in soil. Use of some coarse minerals component has been found useful in increasing aeration and improving drainage. Sand, vermiculite and perlite are useful in this regard. In addition to this, Azotobacter, a heterotrophic aerobic bacterium capable of fixing nitrogen as non-symbiotic is of wide occurrence in rhizosphere of many plants. There has been rise in the use of Azotobacter as biofertilizer as the ability of it to produce biologically active substances was ascertained, its effect on plants was associated not only with the process of nitrogen fixation and improving nitrogen of plants, but also with the supply of biologically active compounds such as vitamins and gibberellins. Therefore an attempt have been made to utilize the effect of different medium combination with or without Azotobactor for growth and development of acid lime seedlings.

Materials and Methods

This experiment was carried out to evaluate the effect of media on growth and development of acid lime (*Citrus aurantifolia* Swingle) seedling with or without *Azotobacter* during the year 2009-10 at the Fruits Research Farm, College of Horticulture & Forestry Jhalarapatan, Jhalawar (Rajasthan) India. District Jhalawar extends on 6.32 Lac hectare land among 23°4' to 24°52' N latitude and 75°29' to 76°56' E Longitude in South Eastern Rajasthan. Agro climatically, the district falls in zone V (Humid South Eastern Plain). About 84.22 per cent population is rural whose main occupation is agriculture and its related activities. Average rainfall is 954.7 mm. Maximum temperature range in summer is 43°-48°C and minimum 1°-2.6°C during winter. The meteorological data during the study are presented in table 4. The treatments consisted of five media (soil, sand, vermicompost, vermiculite, and cocopeat) and their combinations with or without Azotobacter with three replications. For this experiment freshly extracted seeds of acid lime variety "Kagzi gol" were sown in different media mixture filled in the pro-trays (9×7 cm sized). These portrays, after seed sowing were placed in open nursery, watered regularly with the help of watering rose can to keep medium moist and observations were recorded as per study schedule. Periodic observation on height of seedling was measured with the help of meter scale from ground level to growing tip, number of leaves per seedling were counted every month up to 150 days, diameter of stem was measured with the help of digital

verniear calliper, fresh and dry weight of seedling was measured by electronic balance and average weight calculated, length of longest tap root was measured from the point of initiation of roots to the tip of the root with the help of a meter scale, after washing the soil ball total number of secondary roots were counted, diameter of tap root was measured near the point of initiation of root with the help of verniear calliper. For estimation of nitrogen the powder of 10 fully grown leaves was used in laboratory and subjected to "Wet Digestion Method (Snell and Snell, 1955)" while chlorophyll content of leaves was measured as per method suggested by Sadasivam and Manickam (1997). Average leaf area was calculated with the help of non-destructive type of Laser leaf area meter Model No. CI-203, CID-INC, USA by taking randomly 10 fully grown and physiologically matured leaves in each treatmen.

Results and Discussion

Shoot parameters

Application of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter treatment had given significantly maximum number of leaves per seedling (22.46), diameter of stem (3.35 mm), height of seedling (13.75 cm), fresh weight of seedling (2.77 g), and dry weight of acid lime seedling (1.18 g) after 150 days of sowing. However, minimum number of leaves per seedling (15.73), diameter of stem (2.18 mm), height of seedling (8.82 cm), fresh weight of seedling (1.35 g), and dry weight of seedling (0.45 g) were observed in medium soil without Azotobacter (Table 1). The increase in the shoot growth parameters due to application of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with *Azotobacter* could be attributed to the conducive effect of this medium mixture on water holding capacity, porosity, soil aeration and supplying substantial amount of nutrient specially nitrogen and micro nutrients for good root and shoot growth over control (Chopde *et al.* 1999). Increase in number of leaves might be mainly due to corresponding increase in plant height (Govind and Chandra, 1993). This treatment also has higher leaf chlorophyll content which might certainly improved the photosynthetic rate, dry matter production and their by more fresh and dry weight of shoot. The increase in height of seedling with inoculation of Azotobacter may be due to fact that it stimulates nutrient uptake especially nitrogen which has role in the assimilation of numerous amino acids that are subsequently incorporated in proteins and nucleic acid, which provides framework for chloroplast, mitochondria and other structures in which the most of the biochemical reactions occurs (Awasthi *et al.* 1996).

The application of different media combination had significant effect on leaf area (cm^2) of acid lime seedling. The medium consisting of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) showed maximum leaf area (1.39 cm^2) and minimum leaf

Table 1. Effect of media with or without *Azotobacter* on shoot parameters of acid lime seedlings

Treatments	Height (cm)		Number of leaves per plant		Diameter of stem (mm)		Root/shoot ratio		Leaf area (cm^2)	
	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter
T_0 Control (soil)	8.82	10.27	15.73	16.40	2.18	2.46	1.31	1.93	1.12	1.30
T_1 Soil + Sand (1:1)	8.83	10.94	18.80	15.93	2.78	2.75	1.69	1.97	1.14	1.31
T_2 Soil + Vermicompost (1:1)	11.98	11.54	19.53	19.00	2.32	3.03	1.98	2.03	1.09	1.28
T_3 Soil + Vermiculite (1:1)	11.95	12.43	19.33	19.80	2.47	2.76	1.49	1.99	1.17	1.32
T_4 Soil + Cocopeat (1:1)	11.86	11.03	20.00	17.00	2.97	2.79	2.03	2.01	1.13	1.38
T_5 Soil + Vermicompost + Vermiculite (1:1:1)	10.96	10.56	19.93	17.13	3.11	3.09	2.04	2.06	1.18	1.37
T_6 Soil + Vermicompost + Cocopeat (1:1:1)	11.66	12.22	19.53	19.33	3.06	2.93	1.89	2.07	1.26	1.36
T_7 Soil + Vermicompost + Vermiculite + Cocopeat (1:1:1:1)	11.32	13.31	16.46	20.53	2.39	2.45	1.87	2.28	1.23	1.37
T_8 Soil + Sand + Vermicompost + Vermiculite (1:1:1:1)	10.01	13.68	17.00	20.86	2.90	3.29	2.30	2.35	1.27	1.39
T_9 Soil + Sand + Vermicompost + Vermiculite	13.08	13.75	20.46	22.46	3.12	3.35	2.47	2.57	1.35	1.43

Table 2. Effect of media with or without *Azotobacter* on bio -chemical parameters of acid lime seedlings

Treatments	Per cent nitrogen content		Chlorophyll content (mg/g)					
	Without Azotobacter	With Azotobacter	Without Azotobacter			With Azotobacter		
T_0 Control (soil)	1.30	1.43	Chlorophyll-a	Chlorophyll-b	Tatal chlorophyll	Chlorophyll-a	Chlorophyll-b	Total chlorophyll
			1.42	0.69	2.11	1.59	1.70	3.29
T_1 Soil + Sand (1:1)	1.31	1.46	1.41	0.70	2.12	1.54	2.02	3.55
T_2 Soil + Vermicompost (1:1)	1.28	1.40	1.42	0.73	2.16	1.64	2.01	3.65
T_3 Soil + Vermiculite (1:1)	1.36	1.53	1.42	0.80	2.22	1.62	2.11	3.73
T_4 Soil + Cocopeat (1:1)	1.38	1.65	1.44	0.91	2.35	1.70	2.03	3.73
T_5 Soil + Vermicompost + Vermiculite (1:1:1)	1.33	1.60	1.44	0.80	2.25	1.83	1.85	3.68
T_6 Soil + Vermicompost + Cocopeat (1:1:1)	1.48	1.66	1.45	1.06	2.51	2.00	2.04	4.04
T_7 Soil + Vermicompost + Vermiculite + Cocopeat (1:1:1:1)	1.51	1.70	1.49	0.79	2.28	2.29	2.06	4.63
T_8 Soil + Sand + Vermicompost + Vermiculite (1:1:1:1)	1.57	1.76	1.40	1.79	3.19	2.44	2.63	5.07
T_9 Soil + Sand + Vermicompost + Vermiculite + Cocopeat (1:1:1:1:1)	1.65	1.86	1.59	1.88	3.47	2.54	2.90	5.44
Mean	1.41	1.60	1.44	1.01	2.46	1.91	2.13	4.08

M = Media, A = Azotobacter and M x A = Interaction of Media and Azotobacter

	Per cent nitrogen content			Chlorophyll content								
				Chlorophyll-a			Chlorophyll-b			Total chlorophyll		
	M	A	M x A	M	A	M x A	M	A	M x A	M	A	M x A
SEm ±	0.040	0.018	NS	0.043	0.019	0.061	0.036	0.016	0.051	0.080	0.035	0.113
CD at 5 %	0.118	0.052	NS	0.127	0.056	0.179	0.106	0.047	0.150	0.234	0.104	0.331

Table 3. Effect of media with or without *Azotobacter* on root parameters of acid lime seedlings

Treatments	Number of secondary roots		Length of the longest tap root (cm)		Fresh weight (g)		Dry weight (g)		Diameter of tap root (mm)	
	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter	Without Azotobacter	With Azotobacter
T_0 Control (soil)	30.93	31.13	12.60	17.26	1.35	1.53	0.45	0.52	2.14	2.21
T_1 Soil + Sand (1:1)	32.46	32.00	16.73	17.43	1.68	1.99	0.53	0.57	2.16	2.40
T_2 Soil + Vermicompost (1:1)	36.26	35.93	17.16	16.66	1.93	2.25	0.59	0.60	2.70	2.83
T_3 Soil + Vermiculite (1:1)	34.93	33.86	18.03	18.70	1.97	2.02	0.57	0.58	2.57	2.60
T_4 Soil + Cocopeat (1:1)	36.13	34.33	18.46	17.60	2.16	2.08	0.62	0.70	2.66	2.60
T_5 Soil + Vermicompost + Vermiculit e (1:1:1)	34.06	37.66	16.70	17.20	1.69	2.06	0.63	0.76	2.83	2.35
T_6 Soil + Vermicompost + Cocopeat (1:1:1)	34.86	33.73	16.16	17.06	2.39	2.43	0.78	0.92	2.71	2.63
T_7 Soil + Vermicompost + Vermiculite + Cocopeat (1:1:1:1)	37.20	38.53	18.00	19.30	2.36	2.64	0.83	1.00	2.36	2.89
T_8 Soil + Sand + Vermicompost + Vermiculite (1:1:1:1)	33.86	39.06	17.66	19.36	1.87	2.67	0.87	1.03	2.43	2.91
T_9 Soil + Sand + Vermicompost + Vermiculite	37.66	40.66	19.06	19.73	2.56	2.77	1.11	1.18	2.86	2.95

area was observed in medium soil + vermicompost -1:1 i.e. 1.18 cm^2.The leaf size and chlorophyll content were maximum in *Azotobacter* treatment (Table 2), it may be because of synthesis of chlorophyll and the higher absorption of nutrients especially nitrogen as a result of inoculation with Azotobacter (Joolka *et al.* 2004).

Root parameters

The length of longest tap root, diameter of tap root, number of secondary roots and root/shoot ratio increased significantly due to application of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter. Likewise, at 150^{th} day of sowing the length of longest tap root (19.73 cm), diameter of tap root (2.95 mm), number of secondary roots (40.66) and root/shoot ratio (2.57) were found maximum at medium treatment T_9 consisting of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter. Whereas, the minimum length of longest tap root (12.60 cm), diameter of tap root (2.14 mm), number of secondary roots (30.93) and root/shoot ratio (1.31) were recorded in soil without *Azotobacter* (Table 3).

The beneficial effect on root growth parameters due to application of the medium treatment consisting of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter might be due to improved soil texture, structure, porosity, water holding capacity, activity of useful soil micro fauna and flora, maintained soil temperature and improved soil health and nutrient status of medium (Hartmann and Kester, 1997). Further the vermicompost also provides close contact between seed and media, increases steady moisture supply facilitates root respiration and encourages overall root growth (Chatterjee and Choudhari, 2007).

Biochemical analysis

The nitrogen content in leaves of acid lime as affected by different rooting media reveals that it had significant effect on nitrogen content in leaves of acid lime (Table-3). The medium consisting of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) had estimated significantly maximum nitrogen content (1.75%) in leaves of acid lime seedling and minimum nitrogen content was observed in control (1.36%). The increase in nitrogen content of leaves in acid lime seedling might be due to application of Azotobacter along with suitable media mixture had fixed sufficient quantity of atmospheric nitrogen for which it is known.

These results are in line with the findings of Joolka *et al.* (2004) in pecan and Rao and Dass, (1989) in fruit plants, they reported increased per cent nutrient content particularly nitrogen in the leaves of plants by inoculation of *Azotobacter.*

Similarly, the this medium treatment had estimated maximum Chlorophyll-a (2.06 mg/g), Chlorophyll-b (2.39 mg/g) and thereby total chlorophyll (4.45 mg/g) content of acid lime seedling leaves which were significantly superior over all other their respective treatments including control. However, minimum Chlorophyll-a (1.47 mg/g) content was estimated in treatment soil + sand (1:1) which was statically at par with control (soil) while minimum Chlorophyll-b (1.19 mg/g) and total chlorophyll (2.70 mg/g) content of acid lime seedling leaves were recorded in medium soil (control). The increase in chlorophyll content in leaves of seedling with application of medium combination along with vermicompost and Azotobacter may be due to stimulated nutrient uptake specially nitrogen and synthesis of chlorophyll which have role in the assimilation of numerous amino acids that are subsequently incorporated in proteins and nucleic acid, which provides framework for chloroplast results into better Chlorophyll content in leaves of treated plant (Awasthi *et al.*, 1996).

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