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Full Length Research Paper

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

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This present investigation was carried out at the Fruits Research Farm, College of Horticulture and 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: *Azotobater,* 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.

Growing media play an important role in germination of seeds and for further growth and development of seedling. Among different media used Vermicompost provide simultaneously sufficient levels oxygen and water to the roots, adequate storage of water and nutrients for the plant, balancing of physical, chemical and biological requirements for good plant growth, lightweight and to produce uniform plant growth (Atefe et al., 2012), cocopeat improve moisture retention capacity and increase available nutrient content, infiltration rate, total porosity, and hydraulic conductivity of that soil (Savithri and Khan, 1993), 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 and vermiculite improves soil aeration and moisture retention. When vermiculite is mixed with peat or other composted materials, such as pine bark, the resulting product provides a good growing medium for plants and helps them to propagate. As a soil conditioner, exfoliated vermiculite can improve the aeration of “sticky” soils (containing clay) and the water-holding characteristics of sandy soils. This allows for easier watering and reduces the likelihood of compaction, cracking and

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crusting of the soil (Michael, 2008). All these media 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 has 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 to 2010 at the Fruits Research Farm, College of Horticulture and 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% 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 to 48°C and minimum 1 to 2.6°C during winter. The meteorological data during this 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 treatment.

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 area was observed in medium soil + vermicompost -1:1, that is, 1.18 cm^2. The leaf size and chlorophyll content were maximum in *Azotobacter* treatment, 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)

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 + Cocopeat (1:1:1:1:1)	13.08	13.75	20.46	22.46	3.12	3.35	2.47	2.57	1.35	1.43
Mean	11.04	11.97	18.67	18.84	2.73	2.89	1.90	2.12	1.19	1.35

	Height (cm)			Number of leaves per plant			Diameter of stem (mm)			Root/shoot ratio			Leaf area (cm^2)		
	M	A	M × A	M	A	M × A	M	A	M × A	M	A	M × A	M	A	M × A
SEm ±	0.268	0.119	0.379	0.459	NS	0.650	0.079	0.035	0.112	0.055	0.024	0.078	0.033	0.015	NS
CD at 5 %	0.782	0.350	1.107	1.342	NS	1.898	0.231	0.103	0.327	0.161	0.072	0.228	0.099	0.044	NS

M = Media, A = *Azotobacter* and M × A = Interaction of Media and *Azotobacter*.

and root/shoot ratio (1.31) were recorded in soil without *Azotobacter* (Table 2).

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

Table 2. 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 + Vermiculite (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 + Cocopeat (1:1:1:1:1)	37.66	40.66	19.06	19.73	2.56	2.77	1.11	1.18	2.86	2.95
Mean	34.83	35.68	17.05	18.03	1.99	2.24	0.69	0.78	2.54	2.63

	Number of secondary roots			Length of the longest tap root (cm)			Fresh weight (g)			Dry weight (g)			Diameter of tap root (mm)		
	M	A	M × A	M	A	M × A	M	A	M × A	M	A	M × A	M	A	M × A
SEm ±	0.987	NS	NS	0.495	0.221	0.701	0.057	0.025	0.081	0.019	0.008	0.027	0.070	0.031	0.100
CD at 5 %	2.883	NS	NS	1.447	0.647	2.046	0.167	0.074	0.236	0.056	0.025	0.079	0.206	0.092	0.292

M = Media; A = *Azotobacter*; M ×A = Interaction of Media and *Azotobacter*.

and Choudhuri, 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

Table 3. 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*	Chlorophyll-a	Chlorophyll-b	Tatal chlorophyll	Chlorophyll-a	Chlorophyll-b	Total chlorophyll
T_0 Control (soil)	1.30	1.43	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

	Percent nitrogen content			Chlorophyll content								
				Chlorophyll-a			Chlorophyll-b			Total chlorophyll		
	M	A	M × A	M	A	M × A	M	A	M ×A	M	A	M × 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

M = Media; A = *Azotobacter;* M x A = Interaction of Media and *Azotobacter.*

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 percent 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).

Summary

This study was conducted to see the effect of different media combination with or without *Azotobacter* on growth and development of acid

Table 4. Mean weekly weather data during September 2009 to February 2010.

Standard week number	Duration	Temperature (ºC)		Relative humidity (%)		Total rainfall during week (mm)
		Maximum	Minimum	Maximum	Minimum	
37	10 -16 September	33.7	23.7	89	63	58
38	17-23 September	36.6	23.1	85	48	0
39	24-30 September	38.1	23.6	70	44	0
40	1-7 October	36.9	21.3	93	42	98
41	8-14 October	34.7	19.7	86	44	0
42	15-21 October	34.5	17.5	81	36	0
43	22-28 October	32.5	14.3	83	31	0
44	29-4 November	33.5	15.5	79	31	0
45	5-11 November	32.3	16.5	81	41	0
46	12-18 November	28.5	14.7	80	41	36
47	19-25 November	26.7	9.5	95	38	0
48	26-2 December	27.7	11.3	84	32	0
49	3-9 December	28.7	11.5	93	40	0
50	10-16 December	28.7	14.5	89	40	0
51	17-23 December	24.7	10.3	91	41	21
52	24-31 December	25.3	9.9	85	37	0
1	1-7 January	24.9	6.9	87	33	0
2	8-14 January	23.5	7.3	87	35	0
3	15-21 January	26.9	7.3	95	35	5
4	22-28 January	29.9	9.3	85	29	0
5	29-4 February	30.1	10.9	86	31	0
6	5-11 February	32.25	13.71	82	25	0
7	12-18 February	32.04	14.77	78	25	0

Source: Irrigation department government of Rajasthan, Jhalawar.

lime seedling. The maximum height of seedling (13.75 cm), number of leaves per seedling (22.46), diameter of stem (3.35 mm), leaf area (1.43 cm^2), fresh weight of seedling (2.77 g) and dry weight of seedling (1.18 g), 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), maximum total chlorophyll content in leaf (5.44 mg/g) and nitrogen content in leaf (1.86%) were observed at treatment com-bination (soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with *Azotobacter*), whereas, the minimum were observed at control.

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