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Effects of Seed Soaking and Growing Media on Germination and Survivability of Acid Lime (*Citrus aurantifolia* Swingle)

Radhika J. Lunagariya [a++*], R. K. Jat [a#] and Sandhya M. Solanki [a]

[a] Department of Fruit Science, College of Horticulture, S. D. Agricultural University, Jagudan, Mehsana, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Seed germination is the most important aspect for raising the nursery for successful seedling production of acid lime. The present study was carried out to explore the effect of different seed soaking treatment with growing media on seed germination and survivability of acid lime (*Citrus aurantifolia* Swingle) under net house condition. The experiments were laid out as a Completely Randomized Design with Factorial concept comprising twenty four treatment combinations with three replicates. The seeds of acid lime were treated with different seed soaking: water, GA_3 @ 50 ppm, GA_3 @ 100 ppm, cow urine @ 25%, cow urine @ 50% and cow dung slurry (1:1, cow dung:water w/w) and sowed in different growing media combinations: Sand + Vermicompost (1:1 v/v), Sand + Farmyard manure (1:1 v/v), Sand + Vermicompost + Cocopeat (1:1:1 v/v/v), Sand + Farmyard manure + Cocopeat (1:1:1 v/v/v). The result indicated that GA_3 @ 100 ppm was found to be the most effective for better germination parameters, growth parameters and survival percentage of acid lime seedlings. Among the different growing media, Sand + Vermicompost + Cocopeat (1:1:1 v/v/v) recorded higher values for germination parameters, growth parameters and survival percentage of acid lime seedlings. The treatment combination of GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v) showed better germination parameters, growth parameters and survival percentage. In conclusion, the combination of GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v) was found most suitable for better growth of acid lime seedling.

[++] *Ph.D. Student;*
[#] *Assistant Professor;*
**Corresponding author: E-mail: radhikalunagariya8@gmail.com;*

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1. INTRODUCTION

Citrus is one of the most important fruit crops of tropical and subtropical regions (Jaiswal and others 2018). It occupies 3rd rank after mango and banana in India [1]. It is grown in over 100 countries and often regarded as golden fruit. The total area under cultivation of acid lime and lemon in India is 316 thousand hectares with production of around 3628 thousand MT [2]. In Gujarat, area under citrus cultivation is 48503 hectares with 625833 MT productions covering districts of Ahmedabad, Kheda, Mehsana, Bhavnagar, Gandhinagar and Junagadh districts of Gujarat [2].

Propagation of acid lime is generally done by grafting, budding, air layering and by seed [3]. The commercial practice of propagation is by seed [3]. It produces true to type, because of high degree (39-60%) of nucellar embryony [4]. The resultant seedlings are known to be free from tristeza virus and thus perform better [4]. Some of the problems faced by acid lime growers are slow, erratic and incomplete germination with high initial seedling mortality [5]. The possible reason of slow germination is presence of the growth inhibitors and physical resistance of seed coat to radical protrusion [6]. The growth of acid lime seedling is very slow in nursery as well as in the field. The increasing germination percentage and producing healthier seedling are major challenges for farmers.

So, the seeds of acid lime cv. Kagzi Lime are soaked with plant growth regulator such as gibbrellic acid (GA_3) as well as with organic substance such as cow urine, cow dung slurry and water for better seed germination. "GA_3 controls mobilization of starch which acts as a respiratory substrate leading to immediate enhancement in cell elongation" [7]. "Gibberellins also help in enhancing the availability of reserved mineral elements which promote the germination process" [7]. "Cow urine contains iron, urea, uric acid, estrogens and progesterone which affect the inhibitory responses to seed germination, shoot growth and seedling vigour" [7]. "Cow dung slurry also contains some growth promoting substances (auxin), N, P, K, micronutrients and biodigestable enzymes which have been cause for softening of seed coat and thereby enhances seed germination and growth of seedlings" [8]. "Water soaking of seeds is done to modify hard seed coats, remove inhibitors, soften seeds and reduce the time of germination" [9].

"Media not only act as a growing place but also as a source of nutrient for plant growth" [10]. "It is a substrate that provides the required elements and physical support to the growing plants" [10]. "Vermicompost provides sufficient levels of oxygen to roots, adequate storage of water and nutrient for the plants" [11]. "FYM is having good water holding capacity as well as enough porosity" [12]. "Cocopeat provides excellent pore space (25-30%) and fine structure required for proper growth" [13]. "It is a rich source of nutrients and can easily mixed with other growing media used in seed germination and growth of seedling" [13]. "Sand is used as a rooting media or adding the coarser texture needed to induce proper drainage and aeration" [14].

Producing plants from seeds are the most important propagation method. Seedling propagation involves careful management of seeds, seed storage period, germination conditions and knowledge of requirements of seed for germination as well as the overall growth. Growing media and seed soaking both are an integral part for most of the horticultural production system. Keeping in view above, the aims of the study was to identify suitable treatment combinations for better seed germination and seedling growth of acid lime.

2. MATERIALS AND METHODS

The experiment was carried out under a shade net house at College Farm, College of Horticulture, Sardarkrushinagar Dantiwada Agricultural University, Jagudan, District Mehsana, Gujarat during July, 2021 to December, 2021. The experiment was laid out by Completely Randomized Design with Factorial concept (FCRD), which included 24 treatments with 3 replicates, there were two factors which comprised six levels of seed soaking: S_1 (Water), S_2 (GA_3 @ 50 ppm), S_3 (GA_3 @ 100 ppm), S_4 (Cow urine @ 25%), S_5 (Cow urine @ 50%) and S_6 (Cow dung slurry; 1:1 w/w) and four levels of different growing media: G_1 (Sand + Vermicompost), G_2 (Sand + FYM), G_3 (Sand + Vermicompost + Cocopeat) and G_4 (Sand + FYM + Cocopeat) which were used in 1:1 proportion on volume basis. The seeds of acid lime were soaked before sowing in different seed soaking

treatments for 12 hrs in beaker. The seeds were dried for 10 minutes in shade after soaking. The dried seeds were immediately sown in the polythene bags at 1.2 cm depth. The polythene bags were watered artificially.

The days taken for germination of 50 % seeds from the date of sowing were observed daily. The number of days was counted from the date of initiation of germination up to the date of germination of 50 % seeds. Germination percentage was calculated by dividing the total number of seeds germinated by the total number of seeds sown and multiplying it by 100. Observations were recorded with respect to seedling height, stem diameter and number of leaves at 90, 120 and 150 days after sowing. Seedling height was measured from base of seedling to highest tip of plant. Stem diameter was measured 1 cm above from the base of the stem using Vernier caliper. Fresh and dry weight of shoot as well as fresh and dry weight of root was measured by destructive method of uprooting the plant and taking measurement by standard method.

Germination percentage was recorded by using following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated}}{\text{Total number of seedlings}} \text{x } 100$$

Survival per cent was recorded by using following formula:

$$\text{Survival (\%)} = \frac{\text{Total number of survived seedlings}}{\text{Total number of seedlings}} \text{x } 100$$

2.1 Data Analysis

The recorded data were analyzed statistically using various techniques as described by Panse and Sukhatme [15]. The treatment means were compared with C.D. at 5 per cent level.

3. RESULTS AND DISCUSSION

As per the results, the significant different was observed between the treatment on seed germination and seedling growth attributes.

3.1 Germination Parameters

The germination parameters were significantly influenced by application of different seed soaking treatments and growing media combinations as shown in Figs. 1-2. In Fig. 1, the minimum number of days taken to 50% germination (16.09) as well as maximum germination percentage at 30 DAS (87.50%) were recorded with treatment S_3 (GA3 @ 100 ppm). The minimum number of days taken to 50% germination (16.49) as well as maximum germination percentage at 30 DAS (85.56%) were observed when seeds of acid lime were sown in growing media G_3 [Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded minimum number of days taken to 50% germination (15.00) as well as maximum germination percentage (93.33%) at 30 DAS.

"This might be due to the promising effect of GA_3 on seed germination owing to its participation in the activity of alpha-amylase which catalyzed the starch conversion into simple carbohydrates and chemical energy was liberated used in the activation of embryo. Growing media have appropriate cation exchange capacity for retention of nutrients and have the properties of good water holding capacity as well as being sufficient porous for permitting adequate moisture and exchange of gases between media and embryo as reported" by Ramteke and others [16] in papaya. Similar results were also found by Parasana and others [17] in mango and Khatana and others [3] in Kagzi Lime.

3.2 Growth Parameters

The growth parameters were significantly influenced by application of different seed soaking treatments and growing media combinations as shown in Tables 1-3. The various growth parameters such as seedling height, stem diameter, number of leaves, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root and survival percentage of seedling were found significant among different treatments.

The height of seedling (cm) at 90, 120 and 150 DAS as shown in Table 1.

Among seed soaking treatments, maximum height of seedling (16.68 cm, 23.40 cm and 27.81 cm) at 90, 120 and 150 DAS, respectively was observed in S_3 (GA_3@ 100 ppm). Among growing media, maximum height of seedling (15.79 cm, 22.19 cm and 26.76 cm) at 90, 120 and 150 DAS, respectively was observed in G_3

[Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum seedling height (18.30 cm, 26.13 cm and 30.50 cm) at 90, 120 and 150 DAS, respectively.

"This might due to the fact that the effect of gibberellic acid in increasing the osmotic uptake of nutrients, and thereby causing cell multiplication and cell elongation reflects in greater intermodal length, ultimately resulting in increase in plant height as reported" by Deb and others (2014) in papaya. Conducive effect of the media composition have low bulk density and higher water holding capacity resulting in better drainage of water and aeration which might have sustained good root and shoot growth leading to increase in seedling height (Abirami and others [18] in nutmeg). Synergistic effect of both factors which help in increasing seedling height.

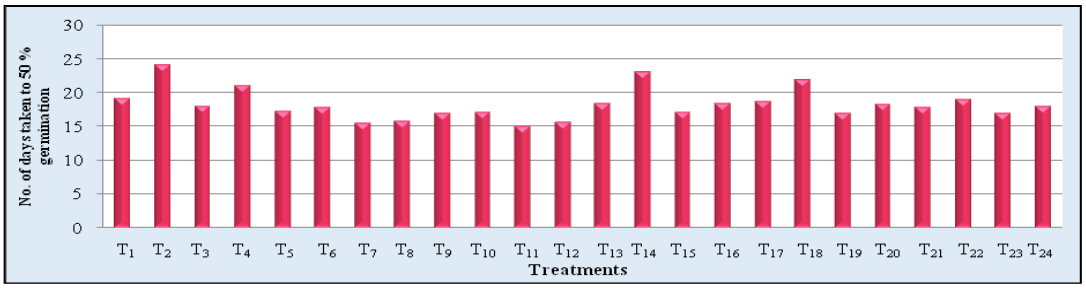

Fig. 1. Effect of seed soaking and growing media on number of days taken to 50% germination of acid lime

Table 1. Effect of seed soaking and growing media on number of days taken to 50% germination, germination percentage at 30 DAS and seedling height (cm) of acid lime seedling

Treatments	Number of days taken to 50% germination	Germination percentage at 30 DAS	Seedling height (cm)		
			90 DAS	120 DAS	150 DAS
Factor A: Seed soaking (s)					
S_1: Water	20.47	65.83	9.46	14.69	19.20
S_2: GA_3 @ 50 ppm	16.49	84.58	14.35	20.35	25.11
S_3: GA_3 @ 100 ppm	16.09	87.50	16.68	23.40	27.81
S_4: Cow urine @ 25 %	19.16	71.67	10.98	16.49	21.31
S_5: Cow urine @ 50 %	18.86	73.33	11.35	17.12	21.72
S_6: Cow dung slurry (1:1 w/w)	17.83	75.00	12.16	17.94	22.67
S.Em±	0.25	1.09	0.16	0.23	0.31
C.D. at 5 %	0.70	3.10	0.46	0.65	0.89
Factor-B: Growing media (g)					
G_1: Sand + Vermicompost (1:1 v/v)	17.94	74.72	11.94	17.52	22.30
G_2: Sand + FYM (1:1 v/v)	20.41	67.78	10.04	15.51	20.12
G_3:Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)	16.49	85.56	15.79	22.19	26.76
G_4: Sand + FYM + Cocopeat (1:1:1 v/v/v)	17.76	77.22	12.22	18.11	22.69
S.Em±	0.20	0.89	0.13	0.19	0.25
C.D. at 5 %	0.57	2.53	0.38	0.53	0.73
Interaction (S x G)					
S.Em±	0.49	2.18	0.33	0.45	0.62
C.D. at 5 %	1.41	6.20	0.93	1.29	1.78
C.V. %	4.72	4.94	4.51	4.30	4.71

The stem diameter (mm) at 90, 120 and 150 DAS as shown in Table 2.

Among seed soaking treatments, maximum stem diameter (16.68 cm, 23.40 cm and 27.81 cm) at 90, 120 and 150 DAS, respectively was observed in S_3 (GA_3 @ 100 ppm). Among growing media, maximum stem diameter (15.79 cm, 22.19 cm and 26.76 cm) at 90, 120 and 150 DAS, respectively was observed in G_3 [Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum stem diameter (2.09 mm, 2.90 mm and 3.49 mm) at 90, 120 and 150 DAS, respectively.

This might be because the GA_3 induced more cell elongation and division in the stem part, increasing the stem diameter.The above results are conformity with Meena and Jain [19] and Anjanawe and others [12] in papaya, Parvin and others [20] in black walnut seeds. Synergistic combinations of these growing media attributed proper aeration, high water holding capacity and better nutrient availability that enhance the physical condition of growing media leading to higher production of photosynethetically functional leaves in this treatment resulting in better diameter of seedling (Borah and others [21] in silk cotton). These findings are in close accordance with the result of Ramteke and others [16] in papaya, Rahangdale [22] in custard apple.

The number of leaves at 90, 120 and 150 DAS as shown in Table 2.

Among seed soaking treatments, maximum number of leaves (17.82, 24.10 and 28.78) at 90, 120 and 150 DAS, respectively was observed in S_3 (GA_3 @ 100 ppm). Among growing media, maximum number of leaves (16.94, 23.49 and 27.66) at 90, 120 and 150 DAS, respectively was observed in G_3 [Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum number of leaves (19.87, 27.07 and 32.27) at 90, 120 and 150 DAS, respectively.

Table 2. Effect of seed soaking and growing media on seedling diameter (cm) and number of leaves of acid lime seedling

Treatments	Seedling diameter (cm)			Number of leaves		
	90 DAS	120 DAS	150 DAS	90 DAS	120 DAS	150 DAS
Factor A: Seed soaking (s)						
S_1: Water	1.44	1.87	2.27	10.15	15.52	19.42
S_2: GA_3 @ 50 ppm	1.80	2.45	2.79	15.63	21.58	25.83
S_3: GA_3 @ 100 ppm	1.94	2.66	3.11	17.82	24.10	28.78
S_4: Cow urine @ 25 %	1.53	2.03	2.40	12.18	17.55	21.87
S_5: Cow urine @ 50 %	1.60	2.10	2.44	12.63	18.07	22.45
S_6: Cow dung slurry (1:1 w/w)	1.66	2.18	2.56	13.43	19.13	23.33
S.Em±	0.02	0.03	0.03	0.19	0.20	0.24
C.D. at 5 %	0.06	0.08	0.09	0.54	0.56	0.67
Factor-B: Growing media (g)						
G_1: Sand + Vermicompost (1:1 v/v)	1.63	2.14	2.48	13.08	18.52	22.94
G_2: Sand + FYM (1:1 v/v)	1.47	1.95	2.33	11.20	16.24	20.41
G_3: Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)	1.90	2.57	3.02	16.94	23.49	27.66
G_4: Sand + FYM + Cocopeat (1:1:1 v/v/v)	1.64	2.20	2.55	13.34	19.04	23.44
S.Em±	0.02	0.02	0.02	0.16	0.16	0.19
C.D. at 5 %	0.05	0.07	0.07	0.44	0.46	0.55
Interaction (S x G)						
S.Em±	0.04	0.06	0.06	0.38	0.40	0.47
C.D. at 5 %	0.12	0.16	0.18	1.08	1.13	1.34
C.V. %	4.35	4.50	4.14	4.84	3.55	3.45

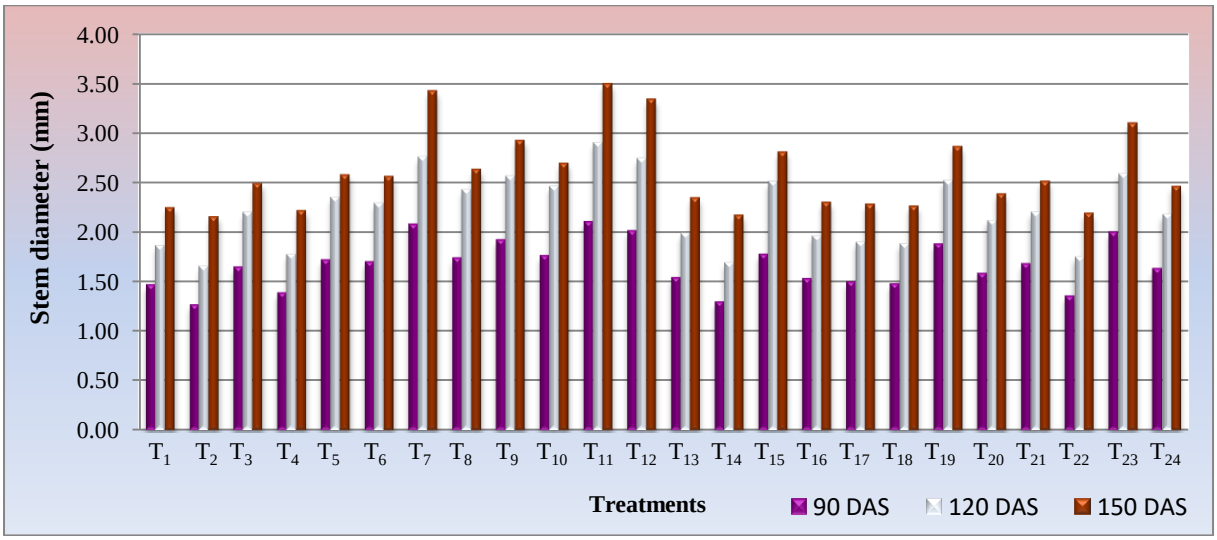

Fig. 2. Effect of seed soaking and growing media on stem diameter (mm) at 90 DAS, 120 DAS and 150 DAS of acid lime

"This might be due to the activity of GA_3 at apical meristem resulting in more synthesis of nucleoprotein which is responsible for increasing leaf number and expansion. An appropriate media mixture provides better root environment to the plant leading to better nutrient availability to the photo synthetically functional leaves that ultimately utilized for more number of leaves" [16]. Similar findings were also reported by Meena and others [19] and Anjanawe and others [12] in Papaya and Parasana and others (2014) in mango.

The fresh and dry weight of shoot (gm) at 150 DAS as shown in Table 3.

Among seed soaking treatments, maximum fresh and dry weight of shoot (6.90 g and 2.60 g, respectively) at 150 DAS was observed in S_3 (GA_3 @ 100 ppm). Among growing media, maximum fresh and dry weight of shoot (6.60 g and 2.57 g, respectively) at 150 DAS was observed in G_3 [Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum fresh and dry weight of shoot (7.57 g and 3.05 g, respectively) at 150 DAS.

"This might be due to the combination containing many macro and micronutrients, humid acid, which maintaining proper aeration and porosity and GA_3, which increases cell division and uptake of nutrients, thus increasing the growth of seedling. The increase in fresh weight and dry weight might be because the combination of GA_3 and media increases the water and nutrient transportation to aerial parts, which leads to production of photosynthetic product and translocation of various plant parts, resulting in a higher fresh and dry weight of shoot" [23]. Similar findings were obtained by Kumawat and others [24] and Dayeshwari and others [25] in papaya.

The fresh and dry weight of root (gm) at 150 DAS as shown in Table 3.

Among seed soaking treatments, maximum fresh and dry weight of root (1.90 g and 0.62 g, respectively) at 150 DAS was observed in S_3 (GA_3 @ 100 ppm). Among growing media, maximum fresh and dry weight of root (1.83 g and 0.59 g, respectively) at 150 DAS was observed in G_3 [Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum fresh and dry weight of root (2.59 g and 0.81 g, respectively) at 150 DAS.

The increased root growth is attributed to the fact that the combination of GA_3 and media provides a better root environment for the plant, resulting in better nutrient availability to the photo synthetically functional leaves and it also increases the translocation of food into the root zone, which leads to increase in fresh weight of root thereby increased dry weight of root [26,27]. A similar result was recorded by Ramteke and others [16] in papaya, Kaur [28] in mango, Parab and others [29] in papaya.

Table 3. Effects of seed soaking and growing media on fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root and survival percentage of acid lime seedling

Treatments	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Survival percentage
Factor A: Seed soaking (s)					
S_1: Water	4.33	1.54	0.84	0.21	62.50
S_2: GA_3 @ 50 ppm	6.02	2.33	1.61	0.52	80.83
S_3: GA_3 @ 100 ppm	6.90	2.60	1.90	0.62	88.75
S_4: Cow urine @ 25%	4.92	1.83	1.00	0.29	69.58
S_5: Cow urine @ 50%	5.14	1.99	1.06	0.31	71.67
S_6: Cow dung slurry (1:1 w/w)	5.22	2.02	1.18	0.36	73.75
S.Em±	0.06	0.02	0.01	0.01	0.95
C.D. at 5%	0.18	0.06	0.04	0.01	2.70
Factor-B: Growing media (g)					
G_1: Sand + Vermicompost (1:1 v/v)	5.24	2.01	1.14	0.34	73.33
G_2: Sand + FYM (1:1 v/v)	4.59	1.59	0.91	0.25	65.00
G_3:Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)	6.60	2.57	1.83	0.59	86.11
G_4: Sand + FYM + Cocopeat (1:1:1 v/v/v)	5.26	2.04	1.19	0.37	73.61
S.Em±	0.05	0.02	0.01	0.00	0.77
C.D. at 5%	0.15	0.05	0.03	0.01	2.20
Interaction (S x G)					
S.Em±	0.13	0.04	0.03	0.01	1.89
C.D. at 5%	0.36	0.12	0.08	0.03	5.39
C.V. %	4.10	3.48	3.96	4.35	4.40

The survival percentage of seedling at 150 DAS as shown in Table 3.

Among seed soaking treatments, maximum survival percentage of seedling (88.75%) at 150 DAS was observed in S_3 (GA_3 @ 100 ppm). Among growing media, maximum survival percentage (86.11%) at 150 DAS, respectively was observed in G_3 [Sand + Vermicompost + Cocopeat (1:1:1)]. Interaction of S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] recorded maximum survival percentage of seedling (96.67%) at 150 DAS.

This may be due to GA_3 helps in cell expansion and its elongation resulting better root and shoot growth, which supports and encourage better survival of the seedlings [22] and media containing vermicompost and cocopeat as most of the constituents provided a start for establishment of seedlings which further got supplemented by PGPR's. Good physical and biological conditions in media combination had positive effect on root and shoot growth which also helps in better survival. Similar results were obtained by Ramteke and others [16] for papaya. These findings are in agreement with the results obtained by Gupta [30] and Khatana and others [3] in Kagzi Lime.

4. CONCLUSION

The current investigation has led to the conclusion that seed soaking with GA_3 100 ppm and growing media Sand + Vermicompost + Cocopeat (1:1:1) significantly influenced the seed germination, seedling growth and survivability of acid lime. The treatment combination S_3G_3 [GA_3 @ 100 ppm and Sand + Vermicompost + Cocopeat (1:1:1 v/v/v)] was found superior and most effective for better physiological development of acid lime seedlings over the rest of the treatment combinations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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