

Vrikshayurveda based biostimulant ‘herbal *kunapjala*’ enhanced the survival, growth and biochemical characteristics of litchi air layers

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The present experiment was conducted to study the effect of biostimulants like herbal *kunapjala*, seaweed extract and triacontanol on survival percentage, vegetative growth and biochemical parameters of litchi air layers. The results revealed that at 120 and 180 days after planting (DAP), respectively, maximum survival percentage (90% and 88.33%), better vegetative growth parameters like number of primary roots (19.15 and 23.48), relative growth rate (1.902 mg/day and 2.672 mg/day), as well as biochemical parameters like total chlorophyll (1.46 mg/g, 3.40 mg/g), total carotenoids (0.117 mg/g and 0.292 mg/g), phenols (36.1 mg/100 g and 48.62 mg/100 g) and proline (0.382 μ mol/g and 0.462 μ mol/g) were found in the plants applied with herbal *kunapjala* 2 at 100 mL/L. The same treatment also recorded maximum values for shoot length, root length, number of leaves, biochemical parameters and leaf nutrients. The other types and concentration of herbal *kunapjala* and seaweed extract also showed better results when compared to triacontanol and control.

Keyword: Air layering, Herbal *kunapjala*, Litchi propagation, Seaweed extract, Triacontanol

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Litchi is an important evergreen, subtropical fruit crop of the family Sapindaceae. Litchi is very exacting in its climatic requirement and requires soil with proper mycorrhizal build up¹. India is the second largest producer of litchi in the world after China². In India, the major litchi growing states are Bihar, Jharkhand, Chattisgarh and Assam³. The sweet aril is unique in taste and very much relished for its aroma and flavor. Litchi fruit is an important source of vitamin C and also contains many minerals and antioxidants. It has strong anticancer activity and free radical scavenging activity^{4,5}.

Large scale production of commercial cultivars is constrained by many factors and lack of high-quality planting material is a major reason behind its restricted expansion in other areas. Vegetative propagation methods like cutting, grafting, tissue culture have been found to be less productive for litchi plant multiplication due to various reasons⁶ while, air layering gives maximum success percentage⁷. Despite being the commercial propagation method in litchi, the

air layered plants show a high rate of mortality after their detachment from the mother plants and during their subsequent establishment in the nursery^{8,9}. Young litchi plants often struggle for growth during their initial establishment firstly due to the poor rooting potential of shoots and secondly due to the transplanting shock the layers are subjected to, in the nursery and orchard. The high humidity and temperature within the wrapping material may also create favorable conditions for pathogen growth, which can lead to mold and fungal infections, further damaging the wounded area and reducing the success rate of air layering.

Therefore, development of a strong and healthy root system in litchi air layers is of utmost importance for their better growth and establishment. Sustainable and holistic approaches like use of biostimulants which can increase the rooting percentage, survival and overall growth of air layers, could significantly help in the production of high-quality planting material in litchi. A biostimulant can be any substance or microorganism applied to a plant with the aim to enhance nutrient efficiency, abiotic stress tolerance and quality traits,

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regardless of its nutrient content¹⁰. They offer wide range of benefits by providing a balanced and eco-friendly solution to chemical application.

Vrikshayurveda, an ancient Indian science, holds significant importance in modern times due to its holistic and sustainable approach in various aspects of crop production. The term *Vrikshayurveda* has been described by Surpala and is also mentioned in Kautilya's book *Arthashastra*. It is a sub branch of Ayurveda that mentions ways for healthy production of plants through the use of traditional organic manure, 'herbal *kunapjala*'. It is a compendium of horticulture, plant protection, plant physiology and other relevant information related to healthy growth of trees. It focuses on solving the problems of tree cultivation through sustainable ways. Among the different aspects, *Vrikshayurveda* also deals with plant propagation, which is an important branch of horticulture. Herbal *kunapjala* is a traditional liquid fermented fertilizer prepared from locally available plant vegetation and some other products. It is reported to act as a natural plant growth booster, controls pests and diseases in plants, improves plant growth, flowering and fruiting¹¹. It is also a rich source of N, P, K and provides quick supply of macro and micro nutrients to the plants. The ancient *kunapjala* preparation involved the use of animal bones, flesh, and other residues, which raised ethical concerns. Therefore, herbal *kunapjala* which is more ethical, environmental friendly and effective is used as a practical alternative to *kunapjala*.

Seaweed extract, a popular biostimulant also offers numerous benefits and is currently widely used for reducing the use of chemical fertilizers¹². It is reported to increase crop yield, improve seed germination and enhance resistance against various stresses¹³. Seaweed extract contains plant hormones and bioactive compounds that stimulate root growth, leading to a more extensive and efficient root system¹⁴ which improves nutrient uptake and overall plant health. Seaweed extract is a powerful tool for sustainable plant propagation; however, its initial cost may be a little higher than chemical fertilizers.

Triacontanol is an endogenous plant growth regulator which is found in small amounts in various plant waxes and epicuticular lipids. It is a potent alleviator of stress and is reported to promote various metabolic activities like seedling development, photosynthesis, enzymatic activities etc. in plants. It has been found to improve plant growth and yield by

stimulating various physiological processes of the plants and better root establishment under stress conditions^{14,15}. Therefore, considering the various benefits of the above mentioned biostimulants, an effort was made in the current study to assess the efficacy of sustainable products like herbal *kunapjala*, seaweed extract and triacontanol, on survival per cent, vegetative growth and biochemical parameters of litchi air layers.

Materials and Methods

The experiment was carried out during September, 2021-March, 2022 at Horticulture Research Centre, Patharchatta, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar (Uttarakhand), India. It is situated in the foothills of the Himalayas (*Tarai* region) of Shivalik range and is geographically situated at 29°N and 79.30°E, at an altitude of 243.84 m above mean sea level. Seaweed extract was applied through the commercial product 'Sagarika' (IFFCO) while Triacontanol was applied through commercial product 'Miraculan' (Corteva). All three types of herbal *kunapjala* were prepared at our centre. The common ingredients used in all the three types of herbal *kunapjala* were cow urine (10 L), cow dung (10 kg), sprouted urad pulse (2 kg), jaggery (2 kg), mustard cake (2 kg), milk (1/2 L), curd (1/2 kg), paddy straw water (5 L) and extract of dung cake (02) in water. In herbal *kunapjala* 1, 20 kg of stinging nettle, in herbal *kunapjala* 2, 10 kg stinging nettle + 10 kg local weeds while in herbal *kunapjala* 3, only 20 kg of local weeds were used along with the common ingredients. The various treatments used in the experiment were: control (water), seaweed extract at 5 mL/L, seaweed extract at 10 mL/L, triacontanol at 5 mL/L, triacontanol at 10 mL/L, herbal *kunapjala* 1 at 50 mL/L, herbal *kunapjala* 1 at 100 mL/L, herbal *kunapjala* 2 at 50 mL/L, herbal *kunapjala* 2 at 100 mL/L, herbal *kunapjala* 3 at 50 mL/L and herbal *kunapjala* 3 at 100 mL/L.

Uniform air layers with healthy root system were detached from the mother plants on 4th September, 2021. The rooted portion of the layers were dipped in respective biostimulant dilutions for 10 min and were then immediately planted in root trainers containing potting media (soil, sand and FYM in the ratio of 2:1:1). For the preparation of potting media, the soil and sand were autoclaved for 15 min (121°C and 15 lb/inch²) before mixing with FYM. The root trainers with the air layers were kept under

shade net (75%) for the next 21 days. The layers were drenched with different biostimulant solutions at 7 days interval. At 21 DAP; as the new leaves started emerging, the plants were transferred to polybags of 1 kg capacity filled with same potting media followed by drenching with biostimulants. After planting in polybags, the plants were alternatively treated with foliar sprays and soil drenching of different biostimulants at an interval of 7 days. This process continued till the end of the experiment (180 days). In December (90 DAP), the plants were shifted from shade net to polyhouse to protect them from extremely low temperature prevailing outside.

The observations on survival percentage, relative growth rate, vegetative growth parameters (shoot length, number of leaves, number and length of primary root, fresh and dry weight of shoot and root, root: shoot ratio), biochemical parameters^{16,17} (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phenols, flavonoids and proline) were recorded both at 120 and 180 DAP. Data on percentage of saleable plants, protein, catalase enzyme and nutrient content of leaves was recorded at 180 DAP. As litchi is a slow growing plant, therefore, plants with more than 40 cm height are generally considered fit for sale. The experiment was laid out in completely randomized block design having eleven treatments with three replications¹⁸. Significant differences among treatments were noted using critical difference (C.D.) at 5% level of significance along with standard error of mean. Data analysis was done with the help of OPSTAT and WASP2.0 softwares. The weather data (Fig. 1) was obtained from the meteorological laboratory of GBPUA&T, Pantnagar.

Results and Discussion

Vegetative parameters

Shoot and root growth

Litchi is relatively a slow growing plant, when compared to other fruit crops. This characteristic is a varietal feature but can be influenced by many factors like genetic, environmental and cultural practices. The observations on shoot and root growth of litchi air layers reflected significant effect of type and concentration of different biostimulants. In the present study, the data (Table 1, Fig. 2) indicated that both at 120 and 180 DAP, respectively, maximum per cent increase in shoot length (30.38% and 43.29%), number of leaves (22.23 and 25.46), shoot fresh weight (28.2 g and 38.26 g) as well as shoot dry weight (12.23 g and 21.88 g) were obtained in the plants treated with herbal *kunapjala* 2 at 100 mL/L. Similarly, at 120 and 180 DAP, respectively, (Table 2), the maximum number (19.15 and 23.48) and length of primary roots (7.8 cm and 9.86 cm), fresh (3.34 g and 4.3 g) and dry weight of roots (1.35 g and 2.46 g) was also reported in the same treatment. The effect of herbal *kunapjala* 2 at 100 mL/L and herbal *kunapjala* 3 at 100 mL/L was found to be statistically *at par* with respect to root characters. Application of triacontanol at 5 mL/L or 10 mL/L, adversely affected the shoot and root growth and application of triacontanol at 10 mL/L recorded lowest values for all the above parameters. The vegetative growth in the control plants was even better than the triacontanol treatments. It has been reported that the effect of triacontanol is dependent upon the concentration at which it is used in a crop¹⁹ and it seems that in the present experiment, the

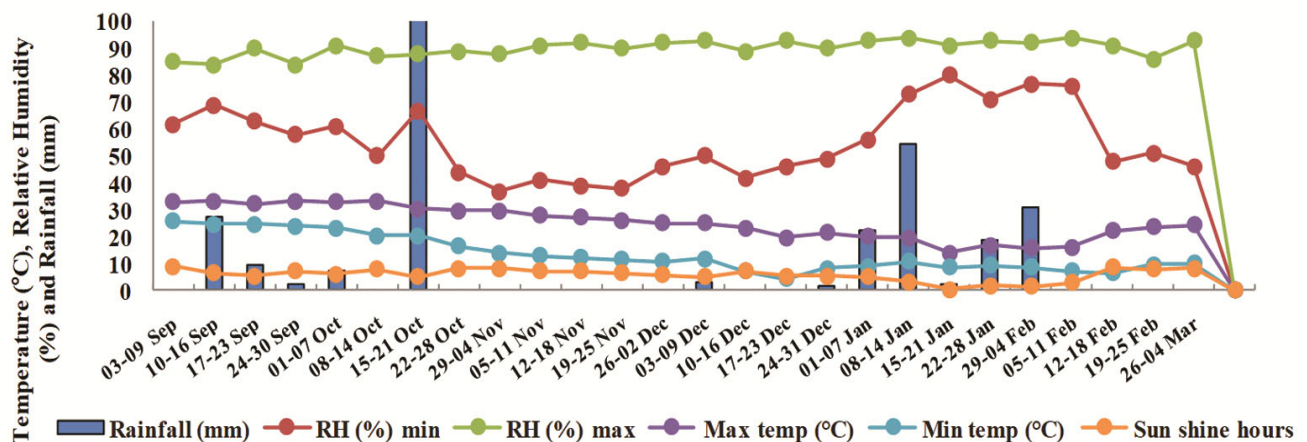


Fig. 1 — Meteorological data during crop growth period (weekly) 2021-2022

concentrations evaluated, adversely affected the shoot and root growth. The effect of seaweed extract, however, was found to be almost similar to that of herbal *kunapjala* 1 at 100 mL/L. Seaweed extract is known to contain a number of essential nutrients and can be effectively used in place of inorganic fertilizers for sustaining the crop growth. In the present experiment also, the effect of seaweed extract was found to be encouraging.

In general, all the three types of herbal *kunapjala* showed encouraging results on shoot and root growth

when compared with other treatments. The herbal *kunapjala* documented as the oldest fermented manure, contains a variety of nutrients and beneficial microorganisms²⁰. The fermentation process helps in converting the complex organic matter into simpler forms which are readily available to the plants. Besides, improving soil fertility, the fermented manures promote root growth and development, leading to production of more extensive and efficient root system which might have enhanced the plant's ability to absorb water and nutrients, indirectly



Fig. 2 — Effect of various biostimulants on vegetative growth of litchi air layers at 180 DAP

promoting shoot growth²¹. The herbal *kunapjala* is also a rich source of microorganisms which might have improved the nutrients availability resulting in optimum root growth. Positive impact of herbal *kunapjala* on vegetative growth parameters in different crops has been reported by many workers^{22,23}.

Relative growth rate (RGR), root: shoot ratio, survival and number of saleable plants

The relative growth rate is a crucial parameter in understanding the dynamics of plant growth. RGR helps to assess the resource use efficiency, stress responses and ecological interactions. A high RGR

can enhance the survival rate of plants in future although other factors like genetic diversity, rooting media, growth hormone, season and timing, wrapping material may also play a significant role. The relative growth rate (RGR) and survival percentage are two key metrics that can significantly enhance the production of saleable plants. Improving survival of air layers after their detachment from mother plants is a major challenge in litchi. The survival percentage is further reduced when the layers are subjected to low temperature in winters. Therefore, any treatment which can either increase or at least maintain RGR

Table 1 — Effect of biostimulants on shoot growth characteristics of litchi air layers

Treatments	Initial shoot length (cm)	Shoot length (cm)		Per cent increase in shoot length (%)		Number of leaves		Shoot fresh weight (g)		Shoot dry weight (g)	
		120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP
Control	31.45 ^{bcd}	36.12 ^{cde}	39.17 ^{ef}	14.85	24.54	15.44 ^c	17.53 ^c	18.63 ^f	27.03 ^f	7.02 ^e	10.21 ^e
Seaweed extract (5 mL/L)	30.67 ^d	36.41 ^{cde}	40.91 ^{de}	18.71	33.38	18.73 ^{cd}	21.53 ^{cd}	21.68 ^{de}	30.68 ^{de}	9.64 ^{bcd}	14.68 ^{cd}
Seaweed extract (10 mL/L)	31.04 ^{bcd}	36.15 ^{cde}	40.02 ^{de}	16.46	28.93	17.32 ^d	20.45 ^d	19.75 ^{ef}	28.75 ^{ef}	8.93 ^d	13.71 ^d
Triacantanol (5 mL/L)	32.08 ^{abc}	36.03 ^{de}	38.03 ^{fg}	12.3	18.55	9.7 ^f	11.56 ^f	16.27 ^g	23.57 ^g	5.84 ^f	7.52 ^f
Triacantanol (10 mL/L)	31.53 ^{bcd}	35.28 ^e	37.34 ^g	11.89	18.42	8.36 ^f	10.72 ^g	15.39 ^g	22.49 ^g	5.65 ^f	7.04 ^f
Herbal <i>kunapjala</i> 1 (50 mL/L)	31.94 ^{bcd}	37.57 ^{cd}	41.42 ^d	17.62	29.68	18.61 ^{cd}	20.26 ^d	21.74 ^{de}	30.74 ^{de}	9.57 ^{cd}	15.92 ^{bc}
Herbal <i>kunapjala</i> 1 (100 mL/L)	31.21 ^{bcd}	37.83 ^e	41.48 ^d	21.21	32.91	18.96 ^c	21.82 ^{cd}	22.44 ^{cd}	31.44 ^d	9.92 ^{bc}	16.01 ^{bc}
Herbal <i>kunapjala</i> 2 (50 mL/L)	32.02 ^{ab}	40.65 ^b	44.53 ^{bc}	26.95	39.07	20.09 ^{bc}	22.84 ^{bc}	26.86 ^{ab}	35.86 ^{bc}	10.43 ^b	17.76 ^b
Herbal <i>kunapjala</i> 2 (100 mL/L)	33.31 ^{ab}	43.43 ^a	47.73 ^a	30.38	43.29	22.23 ^a	25.46 ^a	28.2 ^a	38.26 ^a	12.23 ^a	21.88 ^a
Herbal <i>kunapjala</i> 3 (50 mL/L)	32.33 ^{abc}	39.65 ^b	43.51 ^c	22.69	32.58	19.01 ^c	22.19 ^{bc}	24.75 ^{bc}	33.75 ^c	10.38 ^{bc}	16.63 ^b
Herbal <i>kunapjala</i> 3 (100 mL/L)	32.52 ^{ab}	41.36 ^b	45.6 ^b	27.18	40.22	20.58 ^b	23.55 ^b	27.78 ^a	36.28 ^{ab}	11.54 ^a	20.18 ^a
C.D. (0.05)	1.338	1.724	1.784			1.495	1.576	2.313	2.257	0.847	1.873
SEm(±)	0.456	0.588	0.608			0.507	0.534	0.789	0.77	0.289	0.638

*Different lowercase letters within the same column indicate significant differences at 0.05 level of significance

Table 2 — Effect of biostimulants on root growth characteristics of litchi air layers

Treatments	Number of primary roots		Length of primary roots (cm)		Root fresh weight (g)		Root dry weight (g)	
	120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP
Control	11.53 ^c	13.05 ^g	5.07 ^f	6.17 ^{ef}	1.91 ^{fg}	2.75 ^{fg}	0.63 ^g	0.96 ^f
Seaweed extract (5 mL/L)	15.81 ^c	18.34 ^{de}	6.22 ^{cde}	7.52 ^{cd}	2.21 ^{de}	3.3 ^{de}	0.93 ^{de}	1.54 ^{de}
Seaweed extract(10 mL/L)	13.25 ^d	15.76 ^f	6.09 ^{de}	7.29 ^{de}	2.05 ^{ef}	3.05 ^{ef}	0.83 ^f	1.38 ^e
Triacantanol (5 mL/L)	9.54 ^f	10.85 ^h	4.39 ^{fg}	5.97 ^f	1.87 ^{fg}	2.58 ^g	0.45 ^h	0.67 ^f
Triacantanol (10 mL/L)	8.44 ^f	9.57 ^h	4.17 ^{fg}	5.89 ^f	1.73 ^g	2.41 ^g	0.41 ^h	0.65 ^f
Herbal <i>kunapjala</i> 1 (50 mL/L)	14.46 ^{cd}	17.94 ^e	5.97 ^e	7.87 ^{cd}	2.22 ^{de}	3.33 ^{de}	0.92 ^e	1.56 ^{de}
Herbal <i>kunapjala</i> 1 (100 mL/L)	15.86 ^c	19.23 ^{cde}	6.49 ^{bcd}	7.91 ^{cd}	2.38 ^d	3.49 ^{cd}	1.01 ^d	1.65 ^{cde}
Herbal <i>kunapjala</i> 2 (50 mL/L)	17.49 ^b	20.53 ^{bc}	7.02 ^{abc}	8.65 ^{bc}	2.95 ^b	3.95 ^{ab}	1.12 ^c	1.95 ^{bc}
Herbal <i>kunapjala</i> 2 (100 mL/L)	19.15 ^a	23.48 ^a	7.8 ^a	9.86 ^a	3.34 ^a	4.3 ^a	1.35 ^a	2.46 ^a
Herbal <i>kunapjala</i> 3 (50 mL/L)	15.57 ^c	19.73 ^{cd}	6.84 ^{bcd}	8.06 ^{cd}	2.66 ^c	3.77 ^{bc}	1.1 ^c	1.8 ^{cd}
Herbal <i>kunapjala</i> 3 (100 mL/L)	18.28 ^{ab}	22.02 ^{ab}	7.29 ^{ab}	9.59 ^{ab}	3.17 ^a	4.07 ^{ab}	1.23 ^b	2.22 ^{ab}
C.D. (0.05)	1.582	1.774	0.845	1.134	0.209	0.423	0.083	0.368
SEm(±)	0.539	0.605	0.288	0.387	0.588	0.608	0.507	0.534

*Different lowercase letters within the same column indicate significant differences at 0.05 level of significance

would significantly contribute in enhancing survival of plants.

The effect of herbal *kunapjala* 2 at 100 mL/L, (Table 3) at both 120 and 180 DAP outperformed other treatments in terms of RGR (1.902 and 2.672 mg/day), root: shoot ratio (0.110 and 0.112) and survival percentage (90% and 88.33%). At 180 DAP; highest production of saleable plants (76.67%) was also obtained in the same treatment. It was observed in general, that the mortality of plants was higher upto 120 DAP in all the treatments, but as the time advanced, the mortality rate during the next 60 days *i.e.*, upto 180 DAP significantly reduced. Interestingly the rate of mortality during this period (between 120-180 DAP) was relatively very low in the seaweed extract and herbal *kunapjala* treated plants. The plants in these treatments showed better resilience against the stress conditions.

Better root and shoot development in the herbal *kunapjala* treated plants helped them to efficiently utilize the available resources which might have improved their photosynthetic efficiency leading to better tolerance against low temperature stress that the plants went through in the subsequent winter months (November to February). Better vegetative growth, might have led to higher production of beneficial metabolites and antioxidants which helped the plants to overcome the adverse conditions. The increase in number of leaves, shoot length, number and length of primary roots per plant resulted in better dry matter accumulation, which increased the RGR. It has been documented that organically fermented fertilizers promote root system which facilitates better uptake of nutrients, eventually enhancing the RGR and net

assimilation rate²⁴. Application of fermented liquid fertilizers has been reported to increase RGR and survival of various plants^{23,25,26}.

Application of triacontanol, however at both the concentrations again showed negative impact on RGR, survival and production of saleable plants. At 180 DAP, lowest number of saleable plants were recorded with triacontanol (31.67 and 26.67% at 5 and 10 mL/L, respectively). The foliage of the triacontanol treated plants showed dull yellowish green colour with poor growth. Although, triacontanol, is considered to be a non-toxic compound due to its natural origin and has been widely used for promoting plant growth and enhancing stress tolerance but the present research indicated for thorough elucidation of its optimal concentration in the production of litchi air layers. Possibly the concentrations applied in this experiment were relatively higher and caused adverse effects.

Biochemical parameters

Chlorophyll, carotenoids, flavonoids and phenols

The biochemical parameters play crucial role in supporting the plant's growth and overall health. The influence of biostimulants application on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, flavonoids and phenols indicated significant variations among the treatments (Table 4). The treatments which enhanced vegetative growth were also found to be better in terms of biochemical parameters. Except for triacontanol treatments, all the biostimulants recorded higher values for these parameters over control. The application of herbal *kunapjala* 2 at 100 mL/L at 120 DAP and 180 DAP,

Table 3 — Effect of biostimulants on relative growth rate, root: shoot, survival and saleable plants of litchi air layers

Treatments	RGR (mg/day)		Root: Shoot		Survival (%)		Saleable plants (%)
	120 DAP	180 DAP	120 DAP	180 DAP	120 DAP	180 DAP	180 DAP
Control	0.870 ^g	1.484 ^d	0.090 ^d	0.094 ^{efg}	75 ^g	68.33 ^g	46.67 ^g
Seaweed extract (5 mL/L)	0.977 ^f	1.666 ^d	0.096 ^c	0.105 ^{abcd}	78.33 ^{ef}	76.67 ^e	55.00 ^{de}
Seaweed extract(10 mL/L)	0.918 ^g	1.646 ^d	0.093 ^{cd}	0.101 ^{cde}	76.67 ^{fg}	73.33 ^f	51.67 ^f
Triacontanol (5 mL/L)	0.217 ⁱ	0.755 ^e	0.077 ^c	0.089 ^g	61.67 ^h	51.67 ^h	31.67 ^h
Triacontanol (10 mL/L)	0.349 ^h	0.764 ^e	0.073 ^c	0.092 ^{fg}	55 ⁱ	48.33 ⁱ	26.67 ⁱ
Herbal <i>kunapjala</i> 1 (50 mL/L)	1.010 ^f	1.901 ^{cd}	0.096 ^c	0.098 ^{def}	80 ^{de}	78.33 ^{de}	53.33 ^{ef}
Herbal <i>kunapjala</i> 1 (100 mL/L)	1.200 ^e	1.955 ^{bcd}	0.102 ^b	0.103 ^{bcd}	81.67 ^{cd}	80 ^{cd}	56.67 ^d
Herbal <i>kunapjala</i> 2 (50 mL/L)	1.532 ^c	2.306 ^{abc}	0.107 ^a	0.110 ^{ab}	85 ^b	81.67 ^{bc}	65.00 ^c
Herbal <i>kunapjala</i> 2 (100 mL/L)	1.902 ^a	2.672 ^a	0.110 ^a	0.112 ^a	90 ^a	88.33 ^a	76.67 ^a
Herbal <i>kunapjala</i> 3 (50 mL/L)	1.429 ^d	1.977 ^{bcd}	0.101 ^b	0.108 ^{abc}	83.33 ^{bc}	80 ^{cd}	63.33 ^c
Herbal <i>kunapjala</i> 3 (100 mL/L)	1.638 ^b	2.440 ^b	0.107 ^a	0.110 ^{ab}	85 ^b	83.33 ^b	71.67 ^b
C.D. (0.05)	0.054	0.505	0.005	0.008	2.540	2.092	2.823
SEm(±)	0.018	0.172	0.002	0.003	0.866	0.713	0.963

*Different lowercase letters within the same column indicate significant differences at 0.05 level of significance

respectively, gave maximum values for chlorophyll a (0.845 mg/g and 1.681 mg/g), chlorophyll b (0.639 mg/g and 1.678 mg/g), total chlorophyll (1.46 mg/g and 3.40 mg/g), carotenoids (0.117 mg/g and 0.292 mg/g), flavonoids (32.66 mg/100 g and 42.89 mg/100 g) and phenol content (36.1 mg/100 g and 48.62 mg/100 g). Plants treated with triacontanol at 10 mL/L recorded lowest values for all these parameters. Encouraging effects of herbal *kunapjala* on biochemical parameters has been reported in various other crops also^{23,27}.

Better vegetative growth in the herbal *kunapjala* treated plants (Table 1) might have led to more synthesis of chlorophyll for maximizing photosynthetic efficiency. The increased photosynthetic activity in turn might have contributed to production of more number of leaves and higher biomass. Carotenoids and flavonoids strengthen the antioxidant defense system in plants, by neutralizing free radicals and preventing oxidative damage²⁸. In the present experiment, enhanced vegetative growth might have increased metabolic activity, which leads to generation of more ROS, necessitating production of more carotenoids required for protection. Flavonoids are reported to modulate auxin transport, which is crucial for the regulation of vegetative growth²⁹. This modulation

helps in fine-tuning auxin distribution, thereby influencing processes such as root development and overall vegetative growth. Phenols and polyphenolic compounds are potentially bioactive and protect the plants from oxidative damage, particularly during environmental stress. Phenols are also involved in the plant's defense mechanisms against pathogens and pests. The herbal *kunapjala* and seaweed extract treated plants, high level of these compounds were observed which might have helped the plants fight against pathogens developed inside wrapping material. A direct correlation between better biochemical parameters with root formation and shoot growth was observed.

Proline, protein and catalase activity

Proline, protein and catalase activity (Table 5) are all crucial biochemical parameters required for plant growth and root development. They play multifaceted roles in stress tolerance, antioxidant defense and hormonal regulation. At 120 and 180 DAP, respectively, maximum proline (0.382 $\mu\text{mol/g}$ and 0.462 $\mu\text{mol/g}$), at 180 DAP, highest protein (8.20%) and catalase activity (62.44 nmol/min/g) were observed in plants treated with herbal *kunapjala* 2 at 100 mL/L and was closely followed by herbal

Table 4 — Effect of biostimulants on chlorophyll, carotenoids, flavonoids and phenol content in the leaves of litchi air layers

Treatments	Chl a (mg/g)		Chl b (mg/g)		Total chlorophyll (mg/g)		Carotenoids (mg/g)		Flavonoids (mg/100 g)		Phenol (mg/100 g)	
	120	180	120	180	120	180	120	180	120	180	120	180
	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP	DAP
Control	0.532 ^e	0.745 ^g	0.393 ^c	0.815 ^f	1.063 ^{def}	1.66 ^h	0.023 ^h	0.078 ⁱ	12.2 ^f	19.83 ^g	18.68 ^f	25.98 ^g
Seaweed extract (5 mL/L)	0.684 ^d	1.09 ^d	0.484 ^d	0.873 ^{ef}	1.28 ^{abcd}	2.06 ^{de}	0.055 ^f	0.115 ^g	22.33 ^d	28.46 ^{ef}	24.03 ^e	35.32 ^{ef}
Seaweed extract (10 mL/L)	0.671 ^d	0.867 ^f	0.475 ^d	0.859 ^{ef}	1.1 ^{cde}	1.87 ^f	0.041 ^g	0.104 ^h	19.29 ^e	26.21 ^f	22.77 ^e	33.5 ^f
Triacontanol (5 mL/L)	0.313 ^f	0.496 ^h	0.213 ^f	0.313 ^g	0.927 ^{ef}	1.73 ^h	0.017 ⁱ	0.055 ^j	9.02 ^g	15.27 ^h	11.08 ^g	21.8 ^h
Triacontanol (10 mL/L)	0.234 ^g	0.478 ^h	0.195 ^f	0.24 ^g	0.83 ^f	1.58 ⁱ	0.012 ^j	0.043 ^k	8.87 ^g	13.39 ^h	10.23 ^g	20.73 ^h
Herbal <i>kunapjala</i> 1 (50 mL/L)	0.724 ^{cd}	0.987 ^e	0.557 ^c	0.967 ^{de}	1.167 ^{bcd}	2.02 ^e	0.057 ^f	0.125 ^f	24.36 ^{cd}	29.62 ^{de}	27.44 ^d	36.13 ^e
Herbal <i>kunapjala</i> 1 (100 mL/L)	0.733 ^{cd}	1.096 ^d	0.567 ^{bc}	1.005 ^d	1.3 ^{abcd}	2.09 ^d	0.064 ^e	0.134 ^e	25.37 ^c	31.71 ^d	28.01 ^d	38.94 ^d
Herbal <i>kunapjala</i> 2 (50 mL/L)	0.758 ^{bc}	1.279 ^c	0.584 ^{abc}	1.366 ^c	1.36 ^{ab}	2.50 ^c	0.082 ^c	0.218 ^c	29.52 ^b	38.05 ^b	32.92 ^b	43.29 ^{bc}
Herbal <i>kunapjala</i> 2 (100 mL/L)	0.845 ^a	1.681 ^a	0.639 ^a	1.678 ^a	1.46 ^a	3.40 ^a	0.117 ^a	0.292 ^a	32.66 ^a	42.89 ^a	36.1 ^a	48.62 ^a
Herbal <i>kunapjala</i> 3 (50 mL/L)	0.736 ^{cd}	1.236 ^c	0.582 ^{abc}	1.046 ^d	1.34 ^{abc}	2.46 ^c	0.071 ^d	0.189 ^d	28.44 ^b	34.78 ^c	30.18 ^c	42.92 ^c
Herbal <i>kunapjala</i> 3 (100 mL/L)	0.822 ^{ab}	1.539 ^b	0.627 ^{ab}	1.505 ^b	1.427 ^a	2.78 ^b	0.108 ^b	0.256 ^b	30.54 ^{ab}	39.71 ^b	32.17 ^b	45.32 ^b
C.D. (0.05)	0.073	0.085	0.062	0.110	0.259	0.046	0.002	0.005	2.263	2.448	1.674	2.184
SEm(±)	0.025	0.029	0.021	0.038	0.088	0.017	0.001	0.002	0.772	0.835	0.571	0.745

*Different lowercase letters within the same column indicate significant difference at 0.05 level of significance

kunapjala 3 at 100 mL/L and herbal *kunapjala* 2 at 50 mL/L. The lowest value of all these biochemical parameters was observed in plants treated with triacontanol at 10 mL/L. All the three types of herbal *kunapjala* at different doses showed significantly higher values for proline, protein and catalase activity. The effect of seaweed extract was better than control and triacontanol treated plants.

When the air layers are detached from mother plants, they suffer a lot of oxidative stress which limits their survival and establishment in the nursery. It is, therefore, necessary to increase their stress resilience for better survival. Proline is reported to mitigate oxidative stress by increasing water holding capacity of cells, protecting membrane integrity, stabilizing enzymes and proteins and acting as scavenger of free radicals^{30,31}. It is also a major source of energy and nitrogen during stress periods, thereby imparting tolerance to plants under adverse conditions^{31,32}. In our experiment, proline content was found to be higher in the herbal *kunapjala* and seaweed extract treated plants. The plant growth regulators, essential nutrients, microbial activities in the fermented herbal *kunapjala* solutions cause signalling through gene actions under stress stimuli eventually rising the proline content. The beneficial impact of herbal *kunapjala* on proline content has been reported in other crops also³². It also improves biotic stress resistance by promoting the production of antimicrobial compounds and beneficial microorganisms, which protect plants from diseases and pests.

Significantly higher protein content in herbal *kunapjala* treatments might be related to enhanced

vegetative growth and chlorophyll content in those plants. Proteins play a crucial role in growth and development of plants. The increase in soluble protein content can be well correlated to increase in photosynthetic pigments in the herbal *kunapjala* & seaweed treated plants. Catalase estimated in the leaves of treated plants is an enzyme that catalyzes the decomposition of hydrogen peroxide (H₂O₂) into water and oxygen and, thereby enhancing antioxidant properties of plants and protecting them from adverse situations³³. Better protection against oxidative damage provided by increased levels of catalase in the herbal *kunapjala* and seaweed treated plants must have supported root development and growth. Healthy roots are essential for the efficient uptake of water and nutrients, which are critical for overall plant growth and development. Herbal *kunapjala* has been found to enhance catalase and other enzymes activity. All the treatments where increase in the levels of these compounds and enzyme was obtained can be positively correlated with increased root length, root number and overall root biomass, ultimately improving the plant's establishment and growth.

Nutrient content of leaves

Application of biostimulants significantly affected the nutrient content in the leaves of litchi air layers (Table 5). The leaf nitrogen, phosphorus, potassium and zinc contents increased in all the herbal *kunapjala* and seaweed treated plants as compared to control except for triacontanol treatments. The highest value of leaf nitrogen (1.767%), phosphorus (0.266%), potassium (0.891%) and zinc (71.8 ppm) at 180 DAP were recorded in plants which received herbal *kunapjala* 2 at 100 mL/L which was closely

Table 5 — Effect of biostimulants on proline, protein, catalase activity and nutrient content of litchi air layers

Treatments	Proline ($\mu\text{mol/g}$)		Protein (%)	Catalase activity (nmol/min/g)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Zinc (ppm)
Control	0.084 ^g	0.147 ^g	4.55 ^f	14.57 ^h	0.981 ^e	0.148 ^g	0.440 ⁱ	32.8 ^h
Seaweed extract (5 mL/L)	0.112 ^e	0.226 ^e	5.78 ^e	36.10 ^f	1.246 ^{cd}	0.168 ^e	0.464 ^g	42.01 ^f
Seaweed extract(10 mL/L)	0.097 ^f	0.216 ^f	5.67 ^e	29.10 ^g	1.221 ^d	0.162 ^f	0.456 ^g	39.7 ^g
Triacontanol (5 mL/L)	0.061 ^h	0.102 ^h	3.77 ^g	9.05 ⁱ	0.821 ^{ef}	0.128 ^h	0.341 ⁱ	29.3 ⁱ
Triacontanol (10 mL/L)	0.056 ^h	0.097 ^h	3.35 ^h	8.55 ⁱ	0.721 ^f	0.111 ⁱ	0.302 ⁱ	28.2 ⁱ
Herbal <i>kunapjala</i> 1 (50 mL/L)	0.106 ^e	0.235 ^e	5.83 ^e	38.27 ^e	1.257 ^{cd}	0.164 ^f	0.496 ^f	43.1 ^{ef}
Herbal <i>kunapjala</i> 1 (100 mL/L)	0.145 ^d	0.28 ^d	6.18 ^d	44.16 ^d	1.333 ^{bcd}	0.168 ^e	0.536 ^e	43.4 ^e
Herbal <i>kunapjala</i> 2 (50 mL/L)	0.184 ^c	0.299 ^c	6.72 ^c	51.78 ^b	1.448 ^{bc}	0.187 ^c	0.569 ^e	59.4 ^c
Herbal <i>kunapjala</i> 2 (100 mL/L)	0.382 ^a	0.462 ^a	8.20 ^a	62.44 ^a	1.767 ^a	0.266 ^a	0.891 ^a	71.8 ^a
Herbal <i>kunapjala</i> 3 (50 mL/L)	0.178 ^c	0.28 ^d	6.24 ^d	47.21 ^c	1.344 ^{bcd}	0.171 ^d	0.552 ^d	56.7 ^d
Herbal <i>kunapjala</i> 3 (100 mL/L)	0.251 ^b	0.372 ^b	6.96 ^b	53.30 ^b	1.501 ^b	0.247 ^b	0.649 ^b	67.9 ^b
C.D. (0.05)	0.009	0.009	0.175	1.970	0.222	0.003	0.016	1.390
SEm(\pm)	0.003	0.003	0.06	0.672	0.076	0.001	0.006	0.474

*Different lowercase letters within the same column indicate significant difference at 0.05 level of significance

followed by herbal *kunapjala* 3 at 100 mL/L whereas, the lowest values of leaf nitrogen, phosphorus, potassium and zinc were recorded in plants treated with triacontanol at 10 mL/L. The addition of different kinds of local weeds along with stinging nettle in herbal *kunapjala* 2 seems to enhance its nutritive value which was well reflected in the nutrient content of treated plants. Nutritional analysis of leaves confirm that plants treated with all types of herbal *kunapjala* showed higher nutrients concentration, indicating better availability and mobilization of soil nutrients. The fermented fertilizers are reported to be a rich source of nitrogen fixing, phosphorus solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB) and other beneficial microbes, which improve the biological and physiochemical quality of soil ultimately enhancing the nutrient efficiently³⁴.

Conclusion

Herbal *kunapjala* followed by seaweed extract application in the present investigation established the fact that they enhanced nutrient uptake, antioxidant capacity and stress resilience of litchi air layers. The application of all the three types of herbal *kunapjala* improved survival and growth of litchi air layers. The ingredients required for the preparation of herbal *kunapjala* are cheap and easily available at the farm level. The method of preparation and application is easy which makes it a sustainable and affordable biostimulant. Seaweed extract also showed good results, however it needs to be procured from outside sources. The results of triacontanol, did not give satisfactory results in our experiment, hence more research on standardization of its doses need to be carried. Overall, application of traditional herbal *kunapjala* shows a ray of hope for sustainable production of litchi plants through air layering.

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Author Contributions

Conceptualization of research and designing of experiment (RR and STP), execution of experiment, data collection, data analysis and interpretation of results (SSM and RR), contribution of experimental

material (STP and P), preparation of manuscript (RR, HB, YPS, NP, GMG and TC).

Conflict of Interest

The authors declare that there is no conflict of interest in the publication of this paper.

Ethics Approval

The authors declare that all the guidelines in place pertaining ethical standards of plant research were followed.

Data Availability

The research data sheets associated with this study are available with corresponding author and will be shared on request.

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