

**Bio-stimulant: Its Effect on the growth and survival of asexually propagated mango (*Mangifera indica* L.) Cv. Alphonso**

**Abstract:** The Konkan belt of Maharashtra, India is home to over 400 nurseries producing more than 1 million high quality mango planting materials annually. To enhance the survival rate and growth of mango grafts at the nursery stage, a study was conducted as “Bio-stimulant: Its Effect on the growth and survival of asexually propagated mango (*Mangifera indica* L.)” Cv. Alphonso during August 2023 to April 2024 at Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India. The experiment, designed in a factorial randomized block design, tested 26 treatment interactions in which NATCA (10.00%) + folic acid (0.2%) combination @ 1ml/Lat 60 days interval) performed best in terms of plant growth parameters and also reduced cost of production of mango grafts. Cv. Alphonso followed by Chitosan @ 150ppm at 60 days interval also showed maximum B:C (1.49) and maximum net profit.

**Keywords:** mango graft, Alphonso, bio-stimulant, application interval, survival

## Introduction

Mango, botanically known as *Mangifera indica* L. and belonging to Anacardiaceae family, is widely regarded as the King of Fruits (Yadav and Singh, 2017). It is extensively cultivated in tropical and subtropical regions of India, which is also home to diverse range of mango varieties. Notably, the Coastal Konkan belt of Maharashtra is recognized as one of India's premier mango-growing regions. India is shifting towards high-density mango plantations and has created a surge in demand for premium planting material. Fortunately, the Konkan region is well-equipped to meet this demand, with over 400 nurseries producing more than 1 million high-quality mango plants annually. Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth has played a pivotal role in this success, pioneering the innovative stone grafting technique for mango cultivation. Through its comprehensive training programs, the university has also empowered a skilled workforce to support the expansion of mango cultivation and meet the increasing demand for quality plants.

Bio-stimulants are increasingly recognized for their beneficial effects on plant growth, enhanced productivity and ability to mitigate both biotic and abiotic stresses, leading to their growing importance in agriculture. In Konkan region, previous research focused on nutrient enrichment through media to improve graft growth in nurseries, but investigations on the application of bio-stimulants and growth regulators in this context were limited. To address this knowledge gap, a study was conducted to evaluate the effect of various bio-stimulant concentrations, including CPPU, salicylic acid, NATCA (10%) + folic acid (0.2%) and chitosan applied at monthly and bimonthly intervals on the performance of mango grafts Cv. Alphonso.

Biostimulant offer a cost-effective solution, as they do not increase production costs for grafts. By improving graft survival rates with reduced concentrations or bimonthly applications, nurseryman can potentially increase profits. This may help the nursery operator to hasten the asexual propagation process of mango grafts enhancing survival rates of mango grafts. It is environment friendly and practical to the part of nursery operators and farmers, thereby producing safe and quality planting materials. It also emphasizes the importance of biostimulant which aids in growth and development of mango grafts. Moreover, this can also lead to lower costs of grafts for farmers. Certain wild plants native to the Konkan region such as moringa, custard apple, neem, karnaj and ghaneri etc. exhibit growth-promoting properties in their leaves, which could be explored for future applications.

**Material and Methods:** The experiment was performed at nursery No 10. Department of Fruit Science, College of Horticulture, Dapoli, from August 2023 to April 2024. The experiment was carried out by factorial randomized block design with 26 treatment combination which were replicated by two times. Factor A represents different concentration of biostimulant and factor B represents application interval.

**Factor A: Different concentration of biostimulant**

T <sub>1</sub> -CPPU@15 ppm	T <sub>6</sub> -Salicylic acid@500ppm	T <sub>11</sub> -Chitosan@125ppm
T <sub>2</sub> -CPPU@20 ppm	T <sub>7</sub> -NATCA(10%)+folic acid(0.2%)@ 0.5ml	T <sub>12</sub> -Chitosan@150 ppm
T <sub>3</sub> -CPPU@25 ppm	T <sub>8</sub> -NATCA(10%)+folic acid(0.2%)@ 1 ml	T <sub>13</sub> -Control(without any spray)
T <sub>4</sub> -Salicylic acid@100ppm	T <sub>9</sub> -NATCA(10%)+folic acid (0.2%)@ 1.5 ml	
T <sub>5</sub> -Salicylic acid@250ppm	T <sub>10</sub> -Chitosan@100ppm at 30	

**Factor B: Spraying interval**

S <sub>1</sub> -Spraying at 30 days interval (DI)	S <sub>2</sub> -Spraying at 60 days interval (DI)
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Initially, epicotyl grafted 50 grafts were selected. Application of biostimulant was conducted by spraying from 30 days after grafting up to 6 months after grafting and the observation was recorded at 30 days interval up to 270 days after grafting and in values mentioned in table was at end of the experiment. Observation of all vegetative growth parameter (height, girth, leaf area) recorded at monthly interval, whereas root length, dry root weight of mango graft was noted at the end of experiment. The grafts of plants were handed over for experimentation after 30 days, marking the beginning of the trial. Initially, a small

number of grafts were found to have died by day 30. These were recorded before the application and the survival (%) was determined from the surviving grafts at the end of the 30 days study. Economics was calculated on the basis of cost of production of mango grafts and interval of application of biostimulant. The data after investigation statistically analysed by the method suggested by Panse and Sukhatme (1995).

### **Result and discussion**

At the end of experiment at 270 days after grafting, it was observed that all the growth parameter, survival and B:C was significantly influenced by biostimulant and its interval of application.

#### **Effect of biostimulant and its interval of application on height of mango grafts Cv.**

##### **Alphonso**

The data related to effect of biostimulant and its interval of application on height of mango grafts have shown in Table 1. The interaction effect at 270 DAG, on height of mango graft was noted significant and T<sub>11</sub>S<sub>1</sub> (Chitosan @ 125 at 30 DI) recorded maximum height (45.15 cm) at par with T<sub>8</sub>S<sub>2</sub> (NATCA + folic acid @ 1 ml at 60 DI) (43.73 cm) and lowest height (31.38 cm) and (31.75 cm) was noted in T<sub>13</sub>S<sub>2</sub> (Control without any spray) and T<sub>13</sub>S<sub>1</sub> (Control without any spray) treatment.

Monthly application of chitosan at 125 ppm boosted plant growth by stimulating hormones and making minerals more available. Dove *et al.* (2023) Chitosan application helps to synthesis of auxin which known for its apical dominance which might help to plant to grow. Bimonthly application of T<sub>8</sub>S<sub>2</sub> (NATCA + folic acid @ 1 ml at 60 DI) also produced similar results to chitosan application. NATCA + folic acid is also the great source of amino acid, folic acid also helps to synthesis of various growth hormones (GA<sub>3</sub>, auxin and cytokinin) Hota *et al.* (2019) which might be helped for increased height grafts. Similar results reported by El-Tanahy *et al.* (2012) in cowpea, noted maximum height (55.01 cm) by the application of chitosan and El-miniawy *et al.* (2013) in strawberry noted maximum height (23.13 cm) and (23.83 cm) during two respective seasons.

#### **Effect of biostimulant and its interval of application on girth of mango grafts Cv.**

##### **Alphonso**

Data related to girth was given in Table 1. At 270 DAG, the maximum girth (11.66 mm) was reported in T<sub>6</sub>S<sub>1</sub> (Salicylic acid @ 500 ppm at 30 DI) at par with T<sub>10</sub>S<sub>2</sub> (Chitosan @ 100 ppm at

60 DI)(11.64 mm) and T<sub>11</sub>S<sub>1</sub>(11.38 mm) and the lowest girth (8.60 mm) and (8.67 mm) was noted in T<sub>13</sub>S<sub>2</sub> and T<sub>13</sub>S<sub>1</sub>(Control) treatment.

During the investigation period it was observed that at the end T<sub>6</sub>S<sub>1</sub>(salicylic acid @ 500 ppm at 30 DI) showed best results. Salicylic acid plays a key role in plant defence and regulates various physiological processes. It also contributes to the synthesis of growth hormones, including auxin and gibberellin which might be increasing stem diameter through stem elongation. Similar results found by Akram *et al.* (2022) in *Antirrhinum majus* and noted maximum height (91.33 cm) by the application of salicylic acid

### **Effect of biostimulant and its interval of application on leaf area (cm<sup>2</sup>) of mango grafts Cv. Alphonso**

Data despite in Table 1. At 270 DAG, the maximum leaf area (818.57 cm<sup>2</sup>) was recorded in T<sub>10</sub>S<sub>1</sub>(Chitosan @ 100 ppm at 30 DI) followed by treatment T<sub>8</sub>S<sub>2</sub>(760.35 cm<sup>2</sup>) and minimum leaf area (481.40 cm<sup>2</sup>) and (487.72 cm<sup>2</sup>) was noted in T<sub>13</sub>S<sub>1</sub> and T<sub>13</sub>S<sub>2</sub> control treatments.

The study found that the biostimulant interaction effect and application interval had a significant impact on the leaf area of mango grafts. Application of chitosan 100 ppm on a monthly basis gave best results followed by NATCA + folic acid on bimonthly basis application also gave significant results in terms of leaf area of mango grafts. Chitosan applied monthly enhanced amino acid and nitrogen production, leading to increased synthesis of auxin and cytokinin hormones. This, in turn, stimulated growth, producing more leaves with increased length, width and expanded leaf area. Similar reported by Abul-magdet *et al.* (2023) in *Aglaonema commutatum*) and noted that chitosan application gave maximum leaf area (36.38 cm<sup>2</sup>) and Hota *et al.* (2024) in apricot plant noted that NATCA in combination with CPPU or sole increased leaf area of mango grafts.

### **Effect of biostimulant and its interval of application on root length of mango grafts Cv.**

#### **Alphonso**

Data related to roots and survival was given in Table 2. All the root observations were recorded at the end of experiment at 270 DAG. Interaction effect of biostimulant and its application interval showed significant effect on root length of mango graft. The maximum root length (18.75 cm) was found in T<sub>8</sub>S<sub>2</sub> at par with T<sub>10</sub>S<sub>2</sub>(18.50 cm) and T<sub>11</sub>S<sub>1</sub>(17.55 cm) treatments while, the minimum root length (12.50 cm) and (13.70 cm) was noted in T<sub>13</sub>S<sub>1</sub> and T<sub>13</sub>S<sub>2</sub> control treatment.

Amino acid in NATCA helped to synthesis of hormones like GA and IAA. IAA was well known for its ability to form new roots and promote root growth, which might increase the root length of mango grafts.

### **Effect of biostimulant and its interval of application on dry weight of root of mango grafts**

#### **Cv. Alphonso**

The significant result was observed in interaction effect and maximum dry weight (12.92g) was noted in T<sub>8</sub>S<sub>2</sub> treatment at par with T<sub>6</sub>S<sub>1</sub> (12.90g), T<sub>10</sub>S<sub>2</sub> (12.80g), T<sub>11</sub>S<sub>1</sub> (12.65g), T<sub>1</sub>S<sub>1</sub> (CPPU @ 15ppmat30DI) (12.62g), T<sub>5</sub>S<sub>1</sub> (Salicylic acid @ 250ppmat30DI) (12.60g) and T<sub>12</sub>S<sub>2</sub> (Chitosan @ 150ppmat60DI) (12.00) however the minimum dry weight (6.85g) and (7.05g) was noted in T<sub>13</sub>S<sub>1</sub> and T<sub>13</sub>S<sub>2</sub> control treatment.

T<sub>8</sub>S<sub>2</sub> (NATCA + folic acid @ 1ml at 60DI) which gives superior results, even it applied at 60 days interval of application. NATCA acid contained hormones such as IAA and GA, with IAA stimulating root formation and promotes healthy growth of roots which might increase the dry weight of root. Elbohy *et al.* (2018) in *Zinnia elegans* plant and noted maximum dry weight (8.66g) and (8.44g) by the application of salicylic acid meanwhile Ebrahim poure *et al.* (2020) also reported in pistachio seedling that application salicylic acid increased dry weight of roots up to 5 % to 33 %

### **Effect of biostimulant and its interval of application on survival of mango grafts of root of mango grafts Cv. Alphonso**

Maximum survival (86.05%) of mango grafts was reported in T<sub>6</sub>S<sub>1</sub> at par with T<sub>9</sub>S<sub>1</sub> (NATCA + folic acid @ 1.5 ml at 30 DI) (85.89 %) while minimum survival (49.32 %) and (51.89 %) was noted in T<sub>13</sub>S<sub>1</sub> and T<sub>13</sub>S<sub>2</sub> control treatment. Salicylic acid increased the grafts union also related grafting stress and aids in recovery and establishment. This all might produce healthy plant growth which might increase survival (%) mango graft. Thorat *et al.* (2018) in mango Cv. Kesar that application of salicylic acid 500 to 1000 mg/L increased the survival percentage of plant by (73.33 %) to (71.67 %).

### **Effect of biostimulant and its interval of application on cost of production (B:C) of mango grafts of root of mango grafts Cv. Alphonso**

The cost of production application was shown in Table 3. The highest B:C (1.51) was noted in treatment T<sub>8</sub>S<sub>2</sub> (NATCA + folic acid @ 1 ml at 60 DI) treatment with 1692.62 Rs net profit followed by T<sub>12</sub>S<sub>2</sub> (Chitosan @ 150ppmat60DI) and reported maximum B:C (1.49)

with 1829.60Rs net profit but T<sub>8</sub>S<sub>2</sub>treatment required lowest cost of production compared to all the treatment at 30- or 60-days application interval. However, the lowest B:C (1.10) and (1.06) was noted in T<sub>13</sub>S<sub>1</sub> and T<sub>13</sub>S<sub>2</sub> treatment.

**Table1.Effectofbiostimulantanditsintervalofapplicationonheight(cm),girth(mm) and leaf area (cm<sup>2</sup>) of mango grafts Cv. Alphonso**

Treatments	Height(cm)ofmango graft at 270 DAG			Girth(mm)ofmango graft at 270 DAG			Leafarea(cm <sup>2</sup> )ofmango graft at 270 DAG		
	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean
T <sub>1</sub>	40.55	36.25	38.40	10.92	10.29	10.61	651.78	572.02	611.90
T <sub>2</sub>	35.70	36.13	35.92	10.24	9.57	9.90	727.70	530.35	629.03
T <sub>3</sub>	37.50	33.00	35.25	10.47	9.70	10.09	697.17	578.52	637.85
T <sub>4</sub>	36.45	35.00	35.73	10.47	9.68	10.07	576.52	612.79	594.66
T <sub>5</sub>	38.50	38.00	38.25	10.38	10.93	10.65	582.77	656.02	619.40
T <sub>6</sub>	37.60	37.30	37.45	<b>11.66</b>	11.07	11.36	673.08	735.53	704.31
T <sub>7</sub>	35.25	39.05	37.15	10.14	10.45	10.29	513.71	687.54	600.62
T <sub>8</sub>	37.00	43.73	40.37	10.11	10.60	10.35	605.42	760.35	682.89
T <sub>9</sub>	35.40	37.23	36.32	10.58	10.13	10.35	625.20	697.77	661.48
T <sub>10</sub>	42.93	39.08	<b>41.01</b>	11.28	11.64	<b>11.46</b>	<b>818.57</b>	683.58	<b>751.08</b>
T <sub>11</sub>	<b>45.15</b>	35.28	40.22	11.38	10.78	11.08	735.46	552.97	644.22
T <sub>12</sub>	40.45	35.20	37.83	10.33	10.36	10.34	641.59	599.50	620.55
T <sub>13</sub>	31.75	31.38	31.56	<b>8.60</b>	8.67	8.64	481.40	487.72	484.56
<b>Mean</b>	<b>38.02</b>	36.66	-	<b>10.50</b>	10.29	-	<b>640.80</b>	627.28	-
-	S. Em±	C.D at5%	Result	S. Em±	C.Dat 5%	Result	S. Em±	C.Dat 5%	Result
<b>T</b>	0.29	0.85	SIG	0.06	0.17	SIG	0.30	0.86	SIG
<b>S</b>	0.04	0.13	SIG	0.01	0.03	SIG	0.05	0.13	SIG
<b>T×S</b>	0.58	1.70	SIG	0.12	0.34	SIG	0.59	1.73	SIG

**Table 2. Effect of biostimulant and its interval of application on root length (cm), dry weight of root (g) and survival (%) of mango grafts Cv. Alphonso**

Treatment	Root length of mango grafts at 270 DAG (cm)			Dry weight of root of mangograft at 270 DAG (g)			Survival (%) of mango grafts at 270 DAG		
	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean
<b>T<sub>1</sub></b>	16.85	14.00	15.43	12.62	7.90	10.26	73.63	67.54	70.59
<b>T<sub>2</sub></b>	14.10	15.50	14.80	8.00	11.20	9.60	71.82	77.50	74.66
<b>T<sub>3</sub></b>	14.20	13.80	14.00	8.10	7.80	7.95	73.91	70.89	72.40
<b>T<sub>4</sub></b>	14.80	14.30	14.55	9.20	8.20	8.70	80.00	71.76	75.88
<b>T<sub>5</sub></b>	16.40	14.40	15.40	12.60	8.40	10.50	71.88	69.37	70.63
<b>T<sub>6</sub></b>	16.15	14.50	15.33	12.90	8.60	10.75	<b>86.05</b>	69.57	77.81
<b>T<sub>7</sub></b>	14.90	15.90	15.40	9.20	11.50	10.35	74.00	78.00	76.00
<b>T<sub>8</sub></b>	14.60	<b>18.75</b>	16.68	8.90	<b>12.92</b>	10.91	79.35	70.50	74.92
<b>T<sub>9</sub></b>	15.10	15.80	15.45	9.40	11.40	10.40	85.89	70.73	<b>78.31</b>
<b>T<sub>10</sub></b>	15.30	18.50	<b>16.90</b>	9.60	12.80	<b>11.20</b>	73.00	71.47	72.24
<b>T<sub>11</sub></b>	17.55	15.20	16.38	12.65	9.50	11.08	74.02	77.22	75.62
<b>T<sub>12</sub></b>	15.40	16.25	15.83	9.98	12.00	10.99	72.62	74.17	73.40
<b>T<sub>13</sub></b>	12.50	13.70	13.10	7.05	6.85	6.95	51.89	49.32	50.60
<b>Mean</b>	15.22	15.43	-	10.01	9.93	-	<b>74.47</b>	70.62	-
-	S. Em±	C.D	Result	S. Em±	C.D	Result	S. Em±	C. Dat 5%	Result
<b>T</b>	0.24	0.71	SIG	0.19	0.56	SIG	0.97	2.81	SIG
<b>S</b>	0.04	0.11	NS	0.03	0.09	NS	0.15	0.43	SIG
<b>T×S</b>	0.49	1.42	SIG	0.38	1.12	SIG	1.93	5.62	SIG

**Table3. Effect of biostimulant and its interval of application on cost of production (B:C) of mango grafts of root of mango grafts Cv. Alphonso**

Sr.No	Treatment	Total number of grafts survived	Total cost (Rs)	Gross return (Rs. 80× survive graft)	Net Profit (Rs)	B:C ratio
1.	T <sub>1</sub> S <sub>1</sub>	61	3833.77	4880	1046.23	1.27
2	T <sub>2</sub> S <sub>1</sub>	61	3987.31	4880	892.69	1.22
3	T <sub>3</sub> S <sub>1</sub>	68	4094.44	5440	1345.56	1.32
4	T <sub>4</sub> S <sub>1</sub>	68	3677.12	5440	1763.01	1.47
5	T <sub>5</sub> S <sub>1</sub>	64	3624	5120	1496	1.41
6	T <sub>6</sub> S <sub>1</sub>	67	3664.35	5360	1695.65	1.46
7	T <sub>7</sub> S <sub>1</sub>	58	3544.71	4640	1095.29	1.30
8	T <sub>8</sub> S <sub>1</sub>	65	3639.1	5200	1560.9	1.42
9	T <sub>9</sub> S <sub>1</sub>	61	3586.82	4880	1293.18	1.36
10	T <sub>10</sub> S <sub>1</sub>	62	3597	4960	1363	1.37
11	T <sub>11</sub> S <sub>1</sub>	61	3583.67	4880	1296.33	1.36
12	T <sub>12</sub> S <sub>1</sub>	60	3570.35	4800	1229.65	1.34
13	T <sub>13</sub> S <sub>1</sub>	47	3396.99	3760	363	1.10
14	T <sub>1</sub> S <sub>2</sub>	56	3642.22	4480	837.78	1.22
15	T <sub>2</sub> S <sub>2</sub>	59	3723.97	4720	996.03	1.26
16	T <sub>3</sub> S <sub>2</sub>	56	3725.71	4480	754.29	1.20
17	T <sub>4</sub> S <sub>2</sub>	61	3583.72	4880	1296.28	1.36
18	T <sub>5</sub> S <sub>2</sub>	59	3583.82	4880	1296.18	1.36
19	T <sub>6</sub> S <sub>2</sub>	59	3557.33	4720	1162.67	1.32
20	T <sub>7</sub> S <sub>2</sub>	61	3584.18	4880	1295.82	1.36
21	T <sub>8</sub> S <sub>2</sub>	62	3267.38	4960	1692.62	<b>1.51</b>
22	T <sub>9</sub> S <sub>2</sub>	58	3545.24	4640	1094.76	1.30
23	T <sub>10</sub> S <sub>2</sub>	58	3545.24	4640	1094.76	1.30
24	T <sub>11</sub> S <sub>2</sub>	67	3663.8	5360	1696.2	1.46
25	T <sub>12</sub> S <sub>2</sub>	69	3690.34	5520	1829.66	1.49
26	T <sub>13</sub> S <sub>2</sub>	45	3370.33	3600	229.67	1.06

## Conclusion

From the present study it can be concluded that application of NATCA(10 %) +folic acid (0.2 %) @ 1 ml/L at 60 days interval for 180 days to mango grafts of Alphonso and obtained superior results in plant growth parameter like (height, girth, root length and dry weight of root) by reducing cost of production with highest B:C (1.51) over all other application of biostimulant at 30- or 60- days interval followed by Chitosan @ 150 ppm at 60 days interval also showed maximum B:C (1.49) and maximum net profit.

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