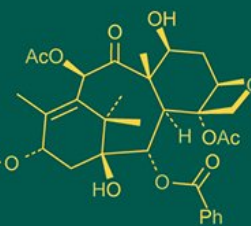
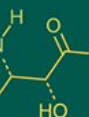
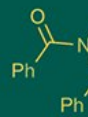


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Samiksha Singh Dewangan,
Ph.D. (Horticulture)
Department of Vegetable
Science Indira Gandhi Krishi
Vishwavidyalaya Raipur,
Chhattisgarh, India

Dr. Deo Shankar, Professor
Department of Vegetable
Science Indira Gandhi Krishi
Vishwavidyalaya
Raipur, Chhattisgarh, India

**Dr. Nisha Jangre, Assistant
Professor**
Department of Vegetable
Science Indira Gandhi Krishi
Vishwavidyalaya
Raipur, Chhattisgarh, India

Dr. Rakesh Banwasi, Scientist
Department of Soil Science
Indira Gandhi Krishi
Vishwavidyalaya Raipur,
Chhattisgarh, India

Dr. Dharmendra Khokhar,
Scientist
Department of Plant
Physiology Indira Gandhi
Krishi Vishwavidyalaya
Raipur, Chhattisgarh, India

Corresponding Author:
Samiksha Singh Dewangan,
Ph.D. (Horticulture)
Department of Vegetable
Science Indira Gandhi Krishi
Vishwavidyalaya
Raipur, Chhattisgarh, India

Effect of biostimulants on vegetative growth parameters of broccoli (*Brassica Oleracea* L. Var *Italica*) under protected structure

Samiksha Singh Dewangan, Deo Shankar, Professor, Nisha Jangre, Assistant, Rakesh Banwasi, Scientist and Dharmendra Khokhar

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Abstract

The present investigation entitled “Effect of biostimulants on growth of broccoli under protected structure” was conducted at the Centre of Excellence on Protected Cultivation and Precision Farming, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, during the winter seasons of 2024-25 and 2025-26. The experiment was conducted to evaluate the effect of individual and combined applications of humic acid, seaweed extract, Jeevamrit and novel organic liquid nutrient on the growth of broccoli under protected conditions. The combined application of humic acid at 5 milliliters per litre, seaweed extract at 3 milliliters per litre, Jeevamrit at 3 per cent and novel organic liquid nutrient at 1.5 per cent recorded the maximum plant height at 60 days after transplanting (67.64 cm) and at harvest (76.84 cm), as well as the maximum stem diameter at harvest (3.35 cm). The combination of Jeevamrit at 3 per cent, humic acid at 5 milliliters per litre and novel organic liquid nutrient at 1.5 per cent recorded the maximum number of leaves at 15 days after transplanting (5.60) and at harvest (22.28). The maximum leaf width and leaf area at 30 days after transplanting were recorded with the combination of Jeevamrit at 3 per cent, seaweed extract at 3 milliliters per litre and novel organic liquid nutrient at 1.5 per cent, with values of 11.01 cm and 129.28 square centimeters, respectively. At 60 days after transplanting, seaweed extract at 3 milliliters per litre combined with novel organic liquid nutrient at 2.0 per cent recorded the maximum leaf width (18.75 cm), leaf area (365.55 square centimeters) and plant spread in the north-south (71.49 cm) and east-west (74.56 cm) directions. Overall, combined applications of biostimulants improved different growth attributes of broccoli under protected conditions.

Keywords: Broccoli, biostimulants, growth, humic acid, seaweed extract, protected cultivation

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*, $2n = 2x = 18$) is an important cole vegetable belonging to the family Brassicaceae and is valued for its high nutritional and health-promoting properties. It is a rich source of vitamins, minerals, dietary fibre and bioactive compounds, particularly glucosinolates, flavonoids and carotenoids (Talalay and Fahey, 2001; Martins *et al.*, 2022) [12, 18]. The increasing demand for nutritious and functional vegetables has enhanced the commercial importance of broccoli. Growth and productivity of broccoli are greatly influenced by environmental conditions, particularly temperature, light, humidity and soil moisture. Protected cultivation provides a favorable and relatively controlled environment for crop growth by regulating these factors and improving resource-use efficiency (Singh and Sirohi, 2006) [16]. However, optimum vegetative growth under protected conditions also depends on proper nutrient management. Excessive dependence on synthetic fertilizers may contribute to increasing production costs, soil degradation and environmental pollution (Calvo *et al.*, 2014) [3]. Therefore, sustainable nutrient-management approaches are required to maintain crop productivity and soil health. Biostimulants have emerged as promising inputs for sustainable crop production owing to their ability to stimulate plant growth, improve nutrient uptake and utilization, enhance physiological activity and increase tolerance to environmental stresses (Du Jardin, 2015; Roupael and Colla, 2020) [6, 14]. Their application can promote root development, chlorophyll antibacterial [17, 18] activities, synthesis, photosynthetic activity and overall vegetative growth,

thereby improving plant performance (Nardi *et al.*, 2002)^[13]. Among various biostimulants, seaweed extracts contain naturally occurring plant growth regulators, macro- and micronutrients that can promote plant growth and improve tolerance to environmental stresses (Durand *et al.*, 2003; Sahoo, 2000; Arioli *et al.*, 2015)^[1, 7, 15]. Humic acid can improve nutrient availability, nutrient uptake and root development, thereby supporting vigorous plant growth (Susic, 2016)^[17]. Similarly, Jeevamrit, a fermented organic preparation rich in beneficial microorganisms, may enhance microbial activity and nutrient mineralization, improving nutrient availability to plants (Devakumar *et al.*, 2014)^[5]. Novel Organic Liquid Nutrient (NOLN), prepared from banana pseudo stem sap and organic inputs, also has potential as an organic plant-growth-promoting input (Desai *et al.*, 2016)^[4].

Although biostimulants have shown beneficial effects in several horticultural crops, limited information is available on the combined use of humic acid, seaweed extract, Jeevamrit and NOLN for improving the vegetative growth of broccoli under protected conditions. Therefore, the present investigation was undertaken to evaluate the effect of different biostimulants on vegetative growth parameters of broccoli (*Brassica oleracea* L. var. *italica*) under protected structure and to identify suitable biostimulant treatments for vigorous crop growth.

Materials and Methods

Healthy and uniform seedlings of broccoli (*Brassica oleracea* L. var. *italica*) variety Saki were used for conducting the investigation. The experiment was carried out during the *Rabi* seasons of 2024-25 and 2025-26 at the Centre of Excellence on Protected Cultivation and Precision Farming (COE on PCPF), Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh. The experiment was laid out in Randomized Block Design (RBD) with 20 treatments and three replications. The experimental plot measured 4.6 m × 1.6 m, and the crop was planted at a spacing of 120 cm × 45 cm × 45 cm in zig-zag manner.

Humic acid (5 mL L⁻¹)

For preparation of humic acid solution, 5 mL of humic acid was diluted with water and the volume was made up to 1 L. The prepared solution was applied as foliar spray two times. The first spray was given at 25 days after transplanting (DAT) and the second spray at 45 DAT. The solution was uniformly sprayed over the foliage until the leaves were adequately wetted using a hand/knapsack sprayer.

Seaweed extract (3 mL L⁻¹)

For preparation of seaweed extract solution, 3 mL of seaweed extract was diluted with water and the volume was made up to 1 L. The prepared solution was applied as foliar spray two times. The first spray was given at 25 DAT and the second spray at 45 DAT. The solution was uniformly sprayed over the foliage using a hand/knapsack sprayer.

Jeevamrit (3%)

Jeevamrit was prepared and diluted to obtain a 3 per cent solution. It was applied through both foliar spray and soil drenching in the treatments where it was included. For foliar application, the prepared solution was uniformly sprayed over the foliage, whereas for soil application, the solution

was applied as a drench near the root zone. The application was carried out according to the treatment schedule. Fresh Jeevamrit solution was prepared before each application.

Novel organic liquid nutrient (NOLN)

NOLN was applied at 1.5 and 2.0 per cent concentrations. For preparation of 1 L of solution, 15 mL and 20 mL of NOLN were diluted with water to obtain 1.5 and 2.0 per cent concentrations, respectively. The prepared solutions were applied as foliar sprays according to the treatment schedule. Fresh solutions were prepared immediately before spraying.

The treatment combinations were as follows: T₁ - Control (water spray); T₂ - Humic acid @ 5 mL L⁻¹; T₃ - Seaweed extract @ 3 mL L⁻¹; T₄ - Jeevamrit @ 3%; T₅ - NOLN @ 1.5%; T₆ - NOLN @ 2.0%; T₇ - Humic acid @ 5 mL L⁻¹ + NOLN @ 1.5%; T₈ - Humic acid @ 5 mL L⁻¹ + NOLN @ 2.0%; T₉ - Seaweed extract @ 3 mL L⁻¹ + NOLN @ 1.5%; T₁₀ - Seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%; T₁₁ - Jeevamrit @ 3% + NOLN @ 1.5%; T₁₂ - Jeevamrit @ 3% + NOLN @ 2.0%; T₁₃ - Humic acid @ 5 mL L⁻¹ + seaweed extract @ 3 mL L⁻¹ + NOLN @ 1.5%; T₁₄ - Humic acid @ 5 mL L⁻¹ + seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%; T₁₅ - Jeevamrit @ 3% + seaweed extract @ 3 mL L⁻¹ + NOLN @ 1.5%; T₁₆ - Jeevamrit @ 3% + seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%; T₁₇ - Jeevamrit @ 3% + humic acid @ 5 mL L⁻¹ + NOLN @ 1.5%; T₁₈ - Jeevamrit @ 3% + humic acid @ 5 mL L⁻¹ + NOLN @ 2.0%; T₁₉ - Humic acid @ 5 mL L⁻¹ + seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 1.5%; and T₂₀ - Humic acid @ 5 mL L⁻¹ + seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 2.0%. The seedlings were transplanted at the 3-4 true-leaf stage on raised beds. Farmyard manure and recommended basal fertilizers were incorporated into the soil, while water-soluble fertilizers were subsequently applied through fertigation. The crop was maintained under uniform cultural practices throughout the experimental period. Irrigation was provided through drip irrigation, and necessary intercultural and plant protection measures were followed uniformly in all treatments.

Five healthy plants were randomly selected and tagged from each experimental plot for recording observations on vegetative growth. The observations recorded included plant height at 15, 30 and 60 DAT and at harvest; number of leaves per plant at 15, 30 and 60 DAT and at harvest; stem diameter at 30 DAT and at harvest; leaf width at 30 and 60 DAT; leaf length at 30 and 60 DAT; leaf area at 30 and 60 DAT; plant spread in north-south direction at 30 and 60 DAT; and plant spread in east-west direction at 30 and 60 DAT. The recorded observations were subjected to statistical analysis to assess the effect of different biostimulant treatments on vegetative growth of broccoli under protected conditions.

Observations recorded on vegetative growth parameters

Five healthy and representative plants were randomly selected and tagged from each experimental plot for recording observations on vegetative growth. The following eight vegetative growth parameters were recorded at the specified stages of crop growth:

1. **Plant height (cm):** Plant height was recorded at 15, 30 and 60 days after transplanting (DAT) and at harvest. The height was measured from the base of the plant to

- the growing tip using a measuring scale and expressed in centimetres.
- Number of leaves per plant:** The total number of fully developed leaves on each tagged plant was counted at 15, 30 and 60 DAT and at harvest, and the mean was calculated and expressed as number of leaves per plant.
 - Stem diameter (cm):** Stem diameter was measured at 30 DAT and at harvest using a digital/vernier caliper and expressed in centimetres.
 - Leaf width (cm):** The maximum width of a fully expanded representative leaf was measured at 30 and 60 DAT using a measuring scale and expressed in centimetres.
 - Leaf length (cm):** The length of a fully expanded representative leaf was measured from the base of the leaf to the tip at 30 and 60 DAT and expressed in centimetres.
 - Leaf area (cm²):** Leaf area was recorded at 30 and 60 DAT using the adopted leaf-area measurement method/instrument and expressed in square centimetres.
 - Plant spread (cm) - North-South (N-S):** Plant spread in the north-south direction was measured at 30 and 60 DAT from the maximum horizontal spread of the plant and expressed in centimetres.
 - Plant spread (cm) - East-West (E-W):** Plant spread in the east-west direction was measured at 30 and 60 DAT from the maximum horizontal spread of the plant and expressed in centimetres.

Results and Discussions

Plant height (cm)

The data on plant height (cm) were recorded at 15, 30 and 60 days after transplanting (DAT) and at harvest and statistically analyzed.

At 15 DAT, plant height differed non-significantly among the treatments during 2024-25, 2025-26 and in the pooled analysis. However, numerically higher plant height was recorded in T5 (NOLN @ 1.5%), with values of 8.48, 9.45

and 8.97 cm during 2024-25, 2025-26 and pooled analysis, respectively. The lowest plant height was recorded in T1 (Control), with corresponding values of 7.38, 7.56 and 7.47 cm, respectively.

At 30 DAT, plant height also differed non-significantly among the treatments during 2024-25, 2025-26 and in the pooled analysis. However, numerically higher plant height was recorded in T19 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 1.5%), with values of 33.02, 40.83 and 36.92 cm during 2024-25, 2025-26 and pooled analysis, respectively. The lowest plant height was observed in T1 (Control), with corresponding values of 32.93, 34.42 and 33.67 cm, respectively.

In the pooled analysis at 60 DAT, the maximum plant height (67.64 cm) was recorded in T19, which was statistically at par with T20 (66.40 cm), T16 (64.28 cm), T18 (60.75 cm) and T14 (60.21 cm). The minimum pooled plant height (50.32 cm) was recorded in T1 (Control).

In the pooled analysis at Harvest, the maximum plant height (76.84 cm) was recorded in T19, which was statistically at par with T10 (75.62 cm). The minimum pooled plant height (58.25 cm) was recorded in T1 (Control).

The non-significant differences observed at 15 and 30 DAT may be attributed to the relatively early stage of crop growth, when the effects of biostimulant application were not sufficiently pronounced. The significant differences observed at 60 DAT and harvest indicate that the beneficial effects of biostimulants became more evident with advancement of crop growth. The superior performance of T19, comprising humic acid, seaweed extract, Jeevamrit and NOLN, may be attributed to their complementary effects on nutrient availability, root activity, cell division and elongation, and overall plant growth. Humic substances are known to stimulate root development and improve nutrient uptake, thereby promoting vegetative growth. Similar positive effects of biostimulants on vegetative growth and plant height in broccoli have been reported by Baldaniya *et al.* (2023) [2], and Ismael *et al.* (2024) [10].

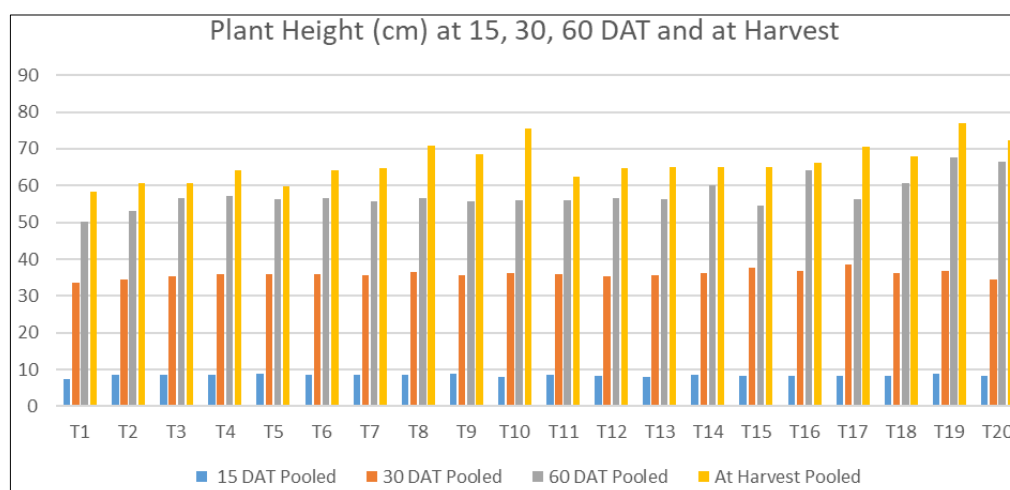


Fig 1: Plant Height (cm) at 15, 30, 60 DAT and at Harvest as influenced by different doses of Biostimulants application

Table 1: Effect of different doses of biostimulants application on plant height (cm) at 15, 30, 60 DAT and at Harvest

Treatments	Plant Height at 15 DAT			Plant Height at 30 DAT			Plant Height at 60 DAT			Plant Height at Harvest		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	7.38	7.56	7.47	32.93	34.42	33.67	49.23	51.42	50.32	53.64	62.33	58.25
T2	7.39	9.42	8.40	33.56	35.51	34.54	51.58	54.39	52.98	54.52	67.07	60.80
T3	8.58	8.66	8.62	34.85	35.83	35.34	56.64	56.43	56.54	54.92	66.59	60.75
T4	8.96	8.05	8.51	35.33	36.63	35.98	57.35	57.21	57.28	55.03	73.33	64.18
T5	8.48	9.45	8.97	35.67	36.38	36.02	57.84	54.69	56.26	54.17	66.13	59.88
T6	8.23	8.61	8.42	33.90	38.09	36.00	57.78	55.34	56.56	58.83	69.57	64.20
T7	8.41	8.66	8.54	32.98	38.13	35.55	57.27	54.17	55.72	62.09	67.38	64.74
T8	8.16	8.91	8.54	33.09	39.66	36.38	58.00	55.03	56.52	63.98	77.53	70.76
T9	8.56	8.96	8.76	33.08	38.27	35.67	57.68	53.64	55.66	69.88	66.90	68.39
T10	8.12	7.97	8.05	33.29	39.18	36.23	57.69	54.53	56.11	75.48	75.76	75.62
T11	8.11	8.79	8.45	33.59	38.04	35.82	57.45	54.52	55.99	60.24	64.37	62.31
T12	7.94	8.61	8.27	31.00	39.72	35.36	58.11	54.92	56.52	61.98	67.64	64.81
T13	8.06	7.99	8.03	34.09	37.09	35.59	57.33	55.54	56.44	63.76	66.16	64.96
T14	8.62	8.27	8.44	35.39	36.95	36.17	65.03	55.38	60.21	63.25	67.02	65.14
T15	8.15	8.41	8.28	37.09	38.10	37.60	54.95	54.22	54.58	62.05	68.26	65.15
T16	8.36	8.33	8.35	35.87	37.64	36.76	64.37	64.18	64.28	64.29	68.20	66.24
T17	8.48	8.22	8.35	37.14	39.89	38.52	57.44	55.28	56.36	66.54	74.47	70.50
T18	8.31	8.21	8.26	35.49	36.94	36.22	58.01	63.49	60.75	67.34	68.69	68.01
T19	8.37	9.23	8.80	33.02	40.83	36.92	67.32	67.95	67.64	77.96	75.71	76.84
T20	8.25	8.50	8.37	32.89	36.13	34.51	65.17	67.63	66.40	76.45	68.02	72.23
SEm ($\pm$)	0.39	0.49	0.25	1.82	1.20	1.26	3.08	2.47	2.23	2.34	1.81	1.50
CD ($p=0.05$)	NS	NS	NS	NS	NS	NS	9.16	7.50	6.69	6.70	5.20	4.30
CV (%)	8.13	9.86	5.23	9.20	5.34	6.07	8.83	7.07	6.37	6.41	4.55	3.93

Number of leaves plant⁻¹

The data on the number of leaves plant⁻¹ were recorded at 15, 30 and 60 days after transplanting (DAT) and at harvest and statistically analyzed. In the pooled analysis at 15 DAT, the differences among treatments were non-significant. The maximum number of leaves plant⁻¹ (5.60) was recorded in T17 (Jeevamrit @ 3% + Humic acid @ 5 mL L⁻¹ + NOLN @ 1.5%), followed by T19 (5.57), whereas the minimum (4.97) was recorded in T1 (Control). In the pooled analysis at 30 DAT, the differences among treatments remained non-significant. The maximum number of leaves plant⁻¹ (8.90) was recorded in T14 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%), followed by T17 (8.84) and T15 (8.81). The minimum (7.32) was recorded in T1 (Control). In the pooled analysis at 60 DAT, the differences among treatments were non-significant. The

maximum number of leaves plant⁻¹ (18.23) was recorded in T8 (Humic acid @ 5 mL L⁻¹ + NOLN @ 2.0%) and T20 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 2.0%), followed by T10 (18.15). The minimum (16.43) was recorded in T12 (Jeevamrit @ 3% + NOLN @ 2.0%). In the pooled analysis at harvest, the differences among treatments remained non-significant. The maximum number of leaves plant⁻¹ (22.28) was recorded in T17, followed by T20 (22.00) and T8 (21.30). The minimum (17.30) was recorded in T1 (Control). Overall, the number of leaves plant⁻¹ showed non-significant differences among the treatments at all growth stages and in both individual years as well as pooled analysis. Similar observations regarding the influence of biostimulants on vegetative growth attributes in broccoli have been reported by Mahmood *et al.* (2023).

Table 2: Effect of different doses of biostimulants application on number of leaves per plant at 15, 30, 60 DAT and at Harvest

Treatments	Number of leaves at 15 DAT			Number of leaves at 30 DAT			Number of leaves at 60 DAT			Number of leaves at Harvest		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	4.07	5.80	4.97	7.90	6.73	7.32	14.80	17.03	15.92	17.43	17.17	17.30
T2	4.53	6.13	5.33	8.27	7.27	7.77	16.40	18.27	17.33	19.50	19.73	19.62
T3	4.73	6.07	5.40	8.27	7.27	7.77	15.80	19.63	17.72	20.83	21.03	20.93
T4	4.87	6.07	5.47	8.13	7.33	7.73	17.00	18.87	17.93	20.17	20.33	20.25
T5	4.73	6.00	5.37	8.13	7.73	7.93	16.20	18.50	17.35	19.70	19.87	19.78
T6	4.53	6.00	5.27	8.53	7.74	8.14	17.20	18.47	17.83	19.83	19.97	19.90
T7	4.40	6.00	5.20	8.20	7.78	7.99	16.00	19.07	17.53	20.27	20.43	20.35
T8	4.33	5.87	5.07	7.93	8.00	7.97	16.80	19.67	18.23	21.13	21.47	21.30
T9	4.73	6.07	5.40	7.93	7.47	7.70	16.00	18.87	17.43	20.40	20.73	20.57
T10	4.67	6.13	5.40	8.33	7.57	7.95	17.23	19.07	18.15	20.67	20.93	20.80
T11	4.40	6.53	5.47	8.27	7.40	7.83	17.00	16.27	16.63	18.13	17.70	17.92
T12	4.73	6.33	5.53	9.27	7.53	8.40	17.20	15.67	16.43	16.83	18.27	17.55
T13	4.53	6.20	5.37	9.07	7.57	8.32	15.40	18.27	16.83	19.53	19.80	19.67
T14	4.60	6.13	5.37	9.47	8.33	8.90	15.80	18.27	17.03	19.73	20.07	19.90
T15	4.60	6.40	5.50	9.27	8.35	8.81	15.60	17.87	16.73	19.03	19.33	19.18
T16	4.80	6.20	5.50	9.00	7.40	8.20	16.00	18.27	17.13	19.57	19.83	19.70
T17	4.73	6.47	5.60	9.60	8.07	8.84	15.40	20.70	18.05	22.13	22.43	22.28
T18	4.53	6.20	5.37	9.13	7.80	8.47	16.80	18.07	17.43	19.23	19.50	19.37
T19	4.87	6.27	5.57	9.20	7.73	8.47	17.00	18.87	17.93	20.27	20.53	20.40
T20	4.73	6.13	5.43	9.47	7.33	8.40	16.00	20.47	18.23	21.87	22.13	22.00
SEm ($\pm$)	0.16	0.25	0.14	0.48	0.39	0.33	1.46	1.30	1.19	1.50	1.35	1.42
CD ($p=0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	5.92	6.94	4.66	9.62	8.88	7.12	15.56	12.13	11.88	13.14	11.69	12.38

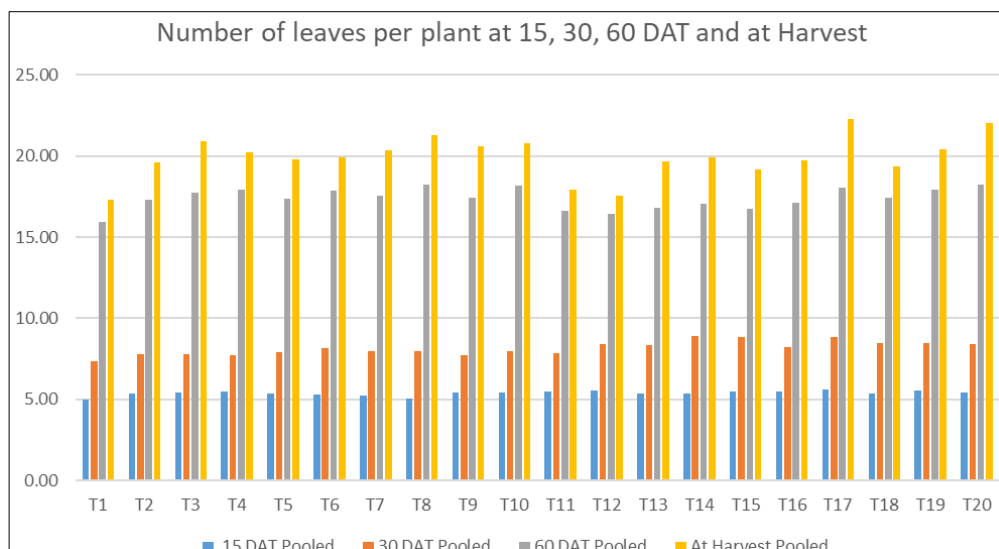


Fig 2: Number of leaves per plant at 15, 30, 60 DAT and at Harvest as influenced by different doses of Biostimulants application

Stem Diameter (cm)

The Stem Diameter data was noted at growth stages *i.e.*, 30 DAT and at Harvest and statistically evaluated. The pooled mean data at 30 DAT, showed non-significant difference for this trait. The data revealed that maximum stem diameter (5.60) was recorded under the treatment T20 (Humic Acid @ 5 ml/lit + Seaweed Extract @ 3 ml/lit + Jeevamrit @ 3 % + NOLN @ 2.0 %). Moreover, the treatment T1 (control) was recorded minimum stem diameter (0.97). On the basis of pooled mean, the stem diameter data differed non-significantly. The data showed that the treatment T19 (Humic Acid @ 5 ml/lit + Seaweed Extract @ 3 ml/lit + Jeevamrit @ 3 % + NOLN @ 1.5 %) was recorded for highest stem diameter (3.35). However, lowest stem

diameter (2.98) was observed under the treatment T₁ (control). The non-significant variation in stem diameter at 30 DAT across the treatments indicates that the applied biostimulants did not produce a sufficiently pronounced response in stem thickening during the early vegetative stage. This may be attributed to the variation in response according to the type and concentration of the biostimulant and the prevailing growing conditions. Similar findings were observed in broccoli, where lower concentrations of Isabion and seaweed extract did not significantly increase stem diameter, whereas higher concentrations produced greater stem diameter, indicating that stem thickening may depend on the concentration of the applied biostimulant (Iqbal *et al.*, 2023) ^[9].

Table 3: Effect of different doses of biostimulants application on stem diameter at 30 DAT and at Harvest

Treatments	Stem Diameter at 30 DAT			Stem Diameter at Harvest		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	0.95	0.98	0.97	2.95	3.02	2.98
T2	0.98	0.99	0.99	3.00	3.05	3.03
T3	0.98	0.99	0.99	3.06	3.07	3.06
T4	1.01	1.06	1.04	3.05	3.11	3.08
T5	0.86	0.99	0.93	3.04	3.24	3.14
T6	1.03	1.04	1.04	3.05	3.05	3.05
T7	1.06	1.02	1.04	3.19	3.29	3.24
T8	1.05	0.99	1.02	3.19	3.37	3.28
T9	1.06	1.03	1.05	3.18	3.09	3.13
T10	1.03	1.02	1.03	3.19	2.79	2.99
T11	0.96	0.99	0.98	3.10	3.01	3.06
T12	0.95	0.99	0.97	3.07	3.09	3.08
T13	1.05	1.02	1.04	3.31	3.36	3.33
T14	1.08	1.07	1.08	3.32	3.09	3.21
T15	1.01	1.09	1.05	3.12	3.09	3.11
T16	1.08	1.06	1.07	3.19	3.43	3.31
T17	1.03	1.01	1.02	3.22	3.18	3.20
T18	1.05	1.02	1.04	3.37	3.24	3.31
T19	1.06	1.03	1.05	3.37	3.33	3.35
T20	1.09	1.05	1.07	3.27	3.39	3.33
SEm (±)	0.05	0.05	0.04	0.15	0.18	0.13
CD (p=0.05)	NS	NS	NS	NS	NS	NS
CV (%)	7.74	8.36	6.00	8.44	10.04	7.06

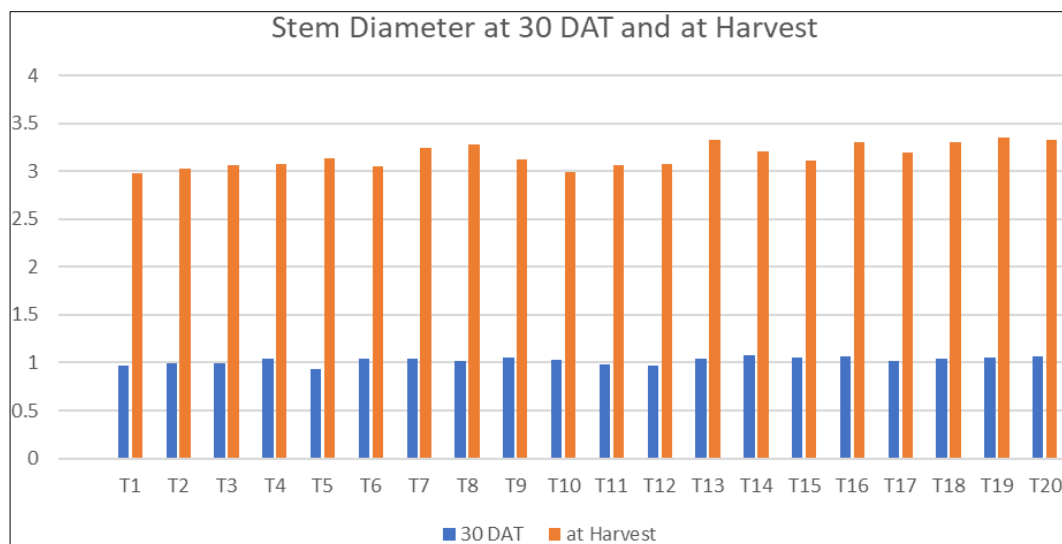


Fig 3: Stem Diameter at 30 DAT and at Harvest as influenced by different doses of Biostimulants application

Leaf Width (cm)

In pooled data, the maximum Leaf width (11.01 cm) was again noted in T15, which was statistically at par with T4 (10.64 cm), T13 (10.59 cm), T14 (10.54 cm), and T12 (10.53 cm), thereby indicating the consistent superiority of these treatments. The lowest pooled Leaf width (8.09 cm) was recorded in T1 (control).

In pooled data, the maximum Leaf width (18.75 cm) was noted in T10 (Seaweed Extract @ 3 ml/lit + NOLN @ 2.0 %), which was statistically at par with T7 (18.60 cm), T8 (17.91 cm), T19 (17.83 cm), thereby indicating the consistent superiority of these treatments. The lowest pooled Leaf width (12.41 cm) was recorded in T1 (control).

The comparatively greater leaf width under the superior

treatments may be attributed to the combined influence of biostimulants on nutrient availability, nutrient uptake and physiological activity, which favoured better leaf expansion and vegetative development. Seaweed extracts contain bioactive compounds and nutrients that can support plant growth, while organic nutrient sources may further contribute to improved nutrient supply. The variation in the superior treatment between the two years may be associated with differences in environmental conditions and the interaction between the applied biostimulants and crop growth conditions. Similar enhancement of vegetative growth and leaf development through biostimulant application has been observed by Iqbal *et al.*, 2023 ^[9]. in broccoli.

Table 4: Effect of different doses of biostimulants application on Leaf Width(cm) at 30 DAT and at Harvest

Treatments	Leaf Width at 30 DAT			Leaf Width at 60 DAT		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	0.95	0.98	0.97	2.95	3.02	2.98
T2	0.98	0.99	0.99	3.00	3.05	3.03
T3	0.98	0.99	0.99	3.06	3.07	3.06
T4	1.01	1.06	1.04	3.05	3.11	3.08
T5	0.86	0.99	0.93	3.04	3.24	3.14
T6	1.03	1.04	1.04	3.05	3.05	3.05
T7	1.06	1.02	1.04	3.19	3.29	3.24
T8	1.05	0.99	1.02	3.19	3.37	3.28
T9	1.06	1.03	1.05	3.18	3.09	3.13
T10	1.03	1.02	1.03	3.19	2.79	2.99
T11	0.96	0.99	0.98	3.10	3.01	3.06
T12	0.95	0.99	0.97	3.07	3.09	3.08
T13	1.05	1.02	1.04	3.31	3.36	3.33
T14	1.08	1.07	1.08	3.32	3.09	3.21
T15	1.01	1.09	1.05	3.12	3.09	3.11
T16	1.08	1.06	1.07	3.19	3.43	3.31
T17	1.03	1.01	1.02	3.22	3.18	3.20
T18	1.05	1.02	1.04	3.37	3.24	3.31
T19	1.06	1.03	1.05	3.37	3.33	3.35
T20	1.09	1.05	1.07	3.27	3.39	3.33
SEm (±)	0.05	0.05	0.04	0.15	0.18	0.13
CD (p=0.05)	NS	NS	NS	NS	NS	NS
CV (%)	7.74	8.36	6.00	8.44	10.04	7.06

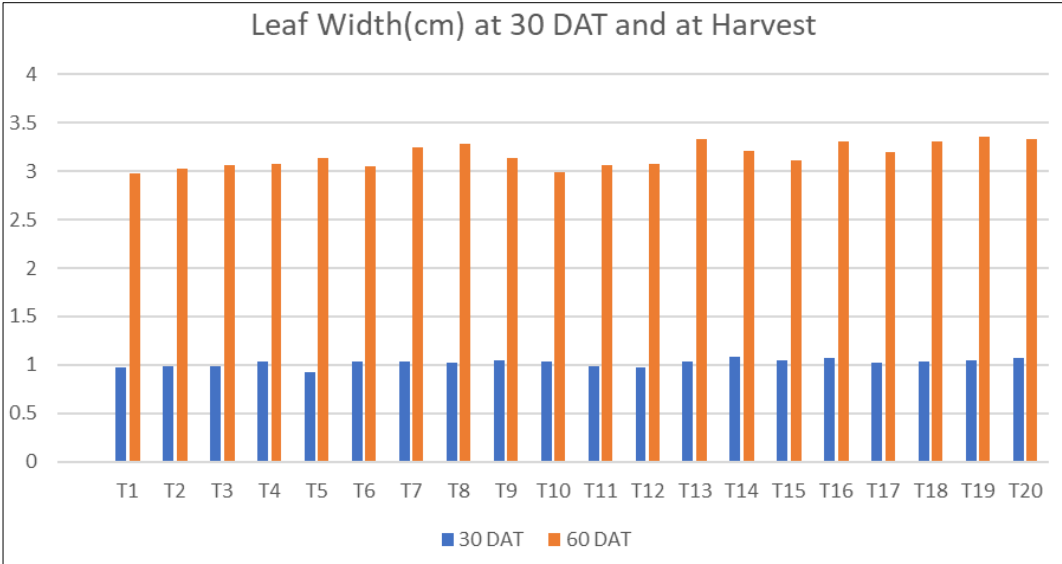


Fig 4: Leaf Width (cm) at 30 DAT and at 60 DAT as influenced by different doses of Biostimulants application

Leaf Length (cm)

The Leaf length data was noted at different growth stages *i.e.*, 30 and 60 DAT and statistically evaluated. In pooled data, the maximum Leaf length (15.63 cm) was again noted in T14, which was statistically at par with T5 (15.23 cm), T15 (15.20 cm), T18 (14.91 cm), and T13 (14.53 cm), thereby indicating the consistent superiority of T14. The lowest pooled Leaf length (12.02 cm) was recorded in T1 (control). In pooled data, the maximum Leaf length (26.75 cm) was noted in T20 (Humic Acid @ 5 ml/lit + Seaweed Extract @ 3 ml/lit + Jeevamrit @ 3% + NOLN @ 2.0%), which was statistically at par with T14 (26.66 cm), T19 (26.65 cm), T10 (25.96 cm), and T7 (25.84 cm), thereby indicating the consistent superiority of these treatments. The lowest pooled Leaf length (21.73 cm) was recorded in T1 (control). The greater leaf length recorded under T14 during the first

year and T20 during the second year may be attributed to the favorable influence of the combined biostimulants on nutrient availability, nutrient uptake and vegetative growth. The superior performance of T20, comprising humic acid, seaweed extract, Jeevamrit and NOLN, may be associated with the complementary effects of these inputs in supporting plant nutrition and physiological activity, thereby favoring greater leaf expansion. Humic substances can influence nutrient uptake and plant growth, while seaweed-based biostimulants may support physiological processes associated with vegetative development. The variation in the best-performing treatment between the two years may be related to differences in environmental conditions and the interaction between the biostimulant treatments and crop growth. Similar enhancement of vegetative growth and leaf development through biostimulant application has been observed Iqbal *et al.*, 2023 ^[9]. in broccoli.

Table 5: Effect of different doses of biostimulants application on Leaf Length (cm) at 30 DAT and at 60 DAT

Treatments	Leaf Length at 30 DAT			Leaf Length at 60 DAT		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	12.19	11.84	12.02	21.5	21.96	21.73
T2	13.77	13.64	13.71	24.92	24.28	24.6
T3	13.44	12.91	13.18	23.98	23.95	23.97
T4	13.83	13.78	13.81	24.95	24.22	24.59
T5	15.09	15.36	15.23	24.91	24.18	24.54
T6	13.89	13.77	13.83	26.11	24.24	25.18
T7	13.89	13.78	13.84	24.92	26.75	25.84
T8	12.95	13.22	13.09	24.84	23.62	24.23
T9	12.61	14.72	13.67	24.98	24.12	24.55
T10	13.58	12.31	12.95	24.91	27.01	25.96
T11	13.89	13.78	13.83	23.75	22.91	23.33
T12	13.88	13.76	13.82	22.84	22.73	22.79
T13	15.31	13.76	14.53	24.38	23.84	24.11
T14	15.72	15.54	15.63	26.78	26.54	26.66
T15	15.47	14.92	15.2	24.96	24.15	24.55
T16	13.87	13.75	13.81	24.93	24.05	24.49
T17	13.85	13.69	13.77	23.81	23.98	23.9
T18	14.88	14.93	14.91	26.15	24.27	25.21
T19	13.11	12.82	12.97	26.35	26.94	26.65
T20	13.06	12.91	12.99	26.77	26.72	26.75
SEm (±)	0.48	0.59	0.42	0.56	0.59	0.4
CD (p=0.05)	1.38	1.69	1.21	3.88	4.18	2.78
CV (%)	5.98	7.43	5.31	1.59	1.7	1.13

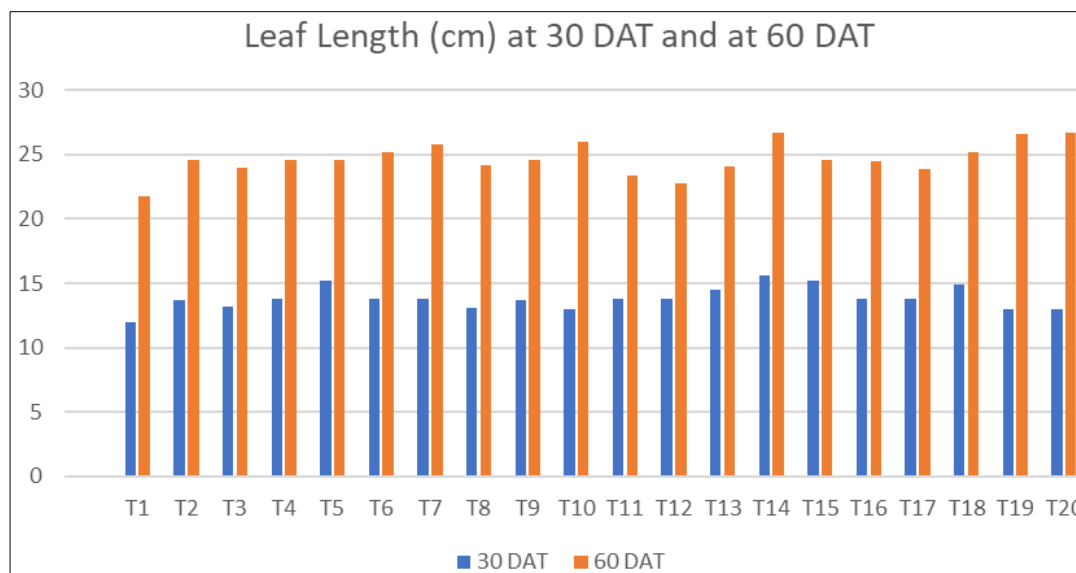


Fig 5: Leaf Length (cm) at 30 DAT and at 60 DAT as influenced by different doses of Biostimulants application.

Leaf Area (cm²)

In pooled data, the maximum Leaf area (129.28 cm²) was again noted in T15, which was statistically at par with T14 (126.57 cm²), thereby indicating the consistent superiority of these treatments. The lowest pooled Leaf area (75.35 cm²) was recorded in T1 (control).

In pooled data, the maximum Leaf area (365.55 cm²) was noted in T10, which was statistically at par with T7 (360.85 cm²) and T19 (356.83 cm²), thereby indicating the consistent superiority of these treatments. The lowest pooled Leaf area (202.75 cm²) was recorded in T1 (control).

The enhanced leaf area under the superior treatments may be attributed to improved nutrient availability and uptake along with sustained physiological activity, which favored leaf expansion and development of a larger photosynthetic surface. The presence of bioactive compounds and nutrients in seaweed extracts may further contribute to improved vegetative growth and leaf development. Similar enhancement of leaf area and vegetative growth through biostimulant application has been observed in broccoli at harvest (Baldaniya *et al.*, 2023) ^[2].

Table 6: Effect of different doses of biostimulants application on Leaf Area (cm²) at 30 DAT and at 60 DAT

Treatments	Leaf Area at 30 DAT			Leaf Area at 60 DAT		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	75.87	74.82	75.35	202.49	203.01	202.75
T2	99.87	99.51	99.69	278.89	278.29	278.59
T3	94.99	95.38	95.19	287.09	289.81	288.45
T4	113.82	112.50	113.16	291.20	285.70	288.45
T5	107.38	119.68	113.53	291.61	294.50	293.06
T6	100.55	106.84	103.70	309.23	300.75	304.99
T7	96.52	107.26	101.89	343.09	378.62	360.85
T8	83.43	93.56	88.49	328.95	321.85	325.40
T9	85.30	100.38	92.84	293.14	292.49	292.82
T10	84.43	93.10	88.77	349.14	381.95	365.55
T11	97.67	112.34	105.00	249.56	246.53	248.05
T12	116.31	106.98	111.65	245.09	246.48	245.79
T13	124.22	112.13	118.17	291.38	243.96	267.67
T14	124.91	128.24	126.57	258.51	327.00	292.76
T15	131.01	127.55	129.28	324.82	236.85	280.84
T16	97.29	104.98	101.13	271.35	322.69	297.02
T17	97.81	107.81	102.81	289.79	268.89	279.34
T18	106.36	112.60	109.48	309.20	304.75	306.98
T19	82.20	93.32	87.76	358.42	355.23	356.83
T20	89.53	87.78	88.66	306.20	316.03	311.11
SEm (±)	7.42	4.78	5.00	15.95	17.06	12.22
CD (p=0.05)	21.23	13.69	14.32	45.67	48.84	34.98
CV (%)	12.79	7.90	8.44	9.40	10.02	7.19

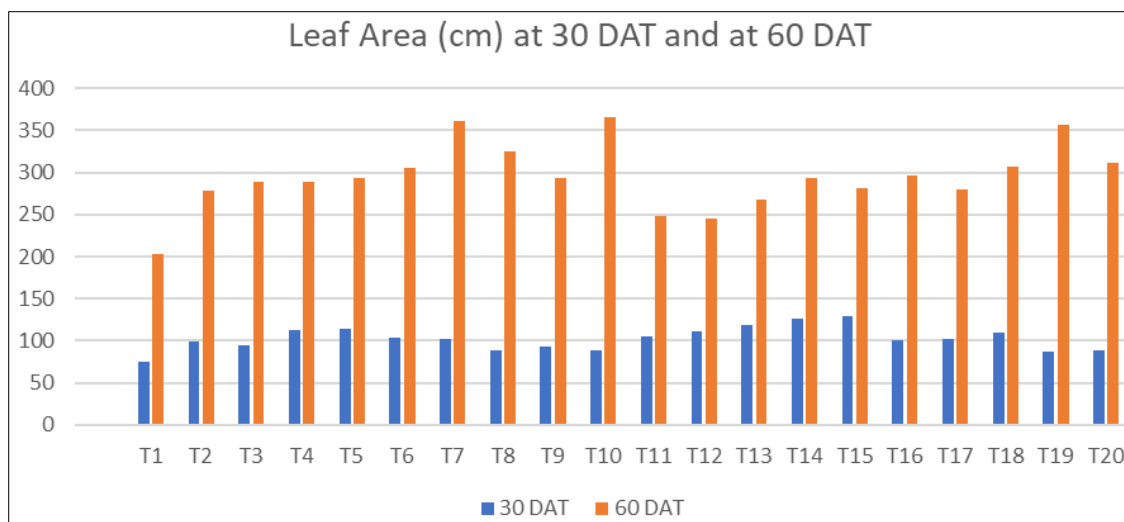


Fig 6: Leaf Area (cm) at 30 DAT and at 60 DAT as influenced by different doses of Biostimulants application.

Plant Spread (cm) (N-S)

The pooled mean data at 30 DAT, showed non-significant difference for this trait. The data revealed that maximum plant spread (42.14) was recorded under the treatment T13 (Humic Acid @ 5 ml/lit + Seaweed Extract @ 3 ml/lit + NOLN @ 1.5 %). Moreover, the treatment T1 (control) was recorded minimum plant spread (37.05 cm).

In pooled data, the maximum Plant Spread (71.49 cm) was again noted in T10, which was statistically at par with T17 (67.96 cm) and T20 (67.39 cm), thereby indicating the consistent superiority of these treatments. The lowest pooled Plant Spread (45.33 cm) was recorded in T1 (control).

The non-significant response at 30 DAT may be attributed to the comparatively limited influence of the applied biostimulants on lateral vegetative growth during the early stage of plant development. The greater plant spread under the superior treatments may be attributed to improved nutrient availability which promoted lateral growth and overall canopy development. Similar enhancement of vegetative growth through biostimulant application has been observed in broccoli, where seaweed extract and Jeevamrit treatments improved growth attributes (Baldaniya *et al.*, 2023) [2].

Table 7: Effect of different doses of biostimulants application on Plant Spread (cm) (N-S) at 30 DAT and 60 DAT

Treatments	Plant Spread (N-S) at 30 DAT			Plant Spread (N-S) at 60 DAT		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	37.06	37.04	37.05	44.35	46.31	45.33
T2	38.83	38.41	38.62	59.32	59.94	59.63
T3	38.30	38.30	38.30	68.85	59.87	64.36
T4	38.55	38.93	38.74	53.98	57.36	55.67
T5	41.70	39.72	40.71	61.66	59.95	60.80
T6	41.21	43.81	42.51	62.46	59.42	60.94
T7	40.33	38.81	39.57	62.56	58.98	60.77
T8	42.36	40.92	41.64	61.02	59.74	60.38
T9	40.66	41.84	41.25	51.05	50.78	50.92
T10	43.35	39.73	41.54	73.41	69.58	71.49
T11	41.41	38.15	39.78	59.98	59.68	59.83
T12	43.10	38.60	40.85	64.95	59.82	62.39
T13	45.56	38.72	42.14	63.39	59.96	61.68
T14	40.02	39.88	39.95	57.01	56.99	57.00
T15	39.22	38.80	39.01	64.82	60.18	62.50
T16	39.09	38.37	38.73	56.98	57.02	57.00
T17	39.27	39.51	39.39	68.00	67.92	67.96
T18	40.76	39.68	40.22	58.96	59.05	59.00
T19	39.37	38.29	38.83	64.95	60.32	62.64
T20	40.41	39.33	39.87	67.42	67.36	67.39
SEm (±)	1.80	1.93	1.33	2.49	2.36	1.77
CD (p=0.05)	NS	NS	NS	7.14	6.76	5.05
CV (%)	7.70	8.48	5.76	7.05	6.88	5.06

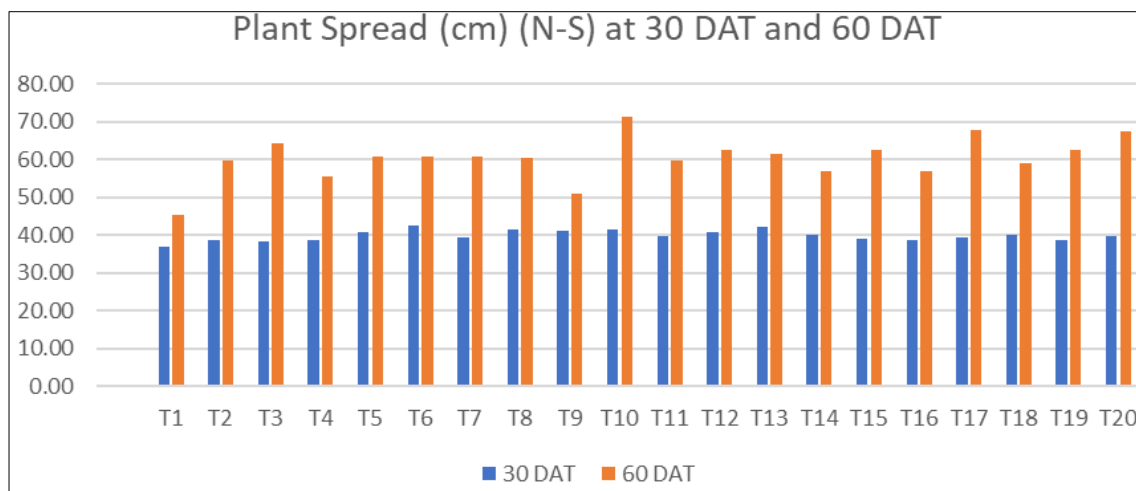


Fig 7: Plant Spread (cm) (N-S) at 30 DAT and 60 DAT as influenced by different doses of Biostimulants application.

Plant Spread (cm) (E-W)

In pooled data, the maximum Plant Spread (44.20 cm) was noted in T18, while the lowest pooled Plant Spread (36.40 cm) was recorded in T1 (control). However, the differences were non-significant, indicating that no treatment was statistically superior to the control for this parameter.

In pooled data, the maximum Plant Spread (74.56 cm) was noted in T6 (Novel Organic Liquid Nutrient @ 2.0%), which was statistically at par with T7 (72.46 cm), T8 (70.42

cm), and T17 (70.13 cm), thereby indicating the consistent superiority of these treatments. The lowest pooled Plant Spread (46.36 cm) was recorded in T1 (control).

The greater plant spread under NOLN @ 2.0% may be attributed to improved nutrient availability and physiological activity, which favored lateral vegetative growth and canopy expansion. The significant increase in E-W plant spread indicates the positive effect of biostimulants on lateral growth and canopy development.

Table 8: Effect of different doses of biostimulants application on Plant Spread (cm) (E-W) at 30 DAT and at 60 DAT

Treatments	Plant Spread (E-W) at 30 DAT			Plant Spread (E-W) at 60 DAT		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
T1	36.64	36.16	36.40	46.60	46.12	46.36
T2	38.10	39.71	38.91	53.15	62.86	58.01
T3	37.14	36.07	36.61	58.81	66.44	62.63
T4	41.46	39.54	40.50	57.69	72.22	64.96
T5	36.84	37.28	37.06	61.74	65.85	63.80
T6	37.40	39.40	38.40	66.08	83.03	74.56
T7	38.28	38.72	38.50	58.65	86.26	72.46
T8	38.05	37.62	37.84	61.41	79.43	70.42
T9	38.97	37.03	38.00	50.93	70.04	60.49
T10	39.83	37.77	38.80	69.76	64.82	67.29
T11	38.30	39.10	38.70	60.22	65.24	62.73
T12	37.25	38.83	38.04	64.77	63.41	64.09
T13	37.17	38.42	37.80	61.39	68.03	64.71
T14	37.37	38.31	37.84	60.51	57.22	58.87
T15	38.08	40.26	39.17	66.91	68.43	67.67
T16	38.82	38.78	38.80	63.79	61.03	62.41
T17	40.53	38.67	39.60	67.84	72.41	70.13
T18	43.33	45.07	44.20	62.70	73.22	67.96
T19	41.63	40.26	40.95	66.72	70.43	68.58
T20	38.22	37.62	37.92	67.48	60.07	63.78
SEm ($\pm$)	1.90	1.80	1.50	2.36	2.91	1.76
CD ($p=0.05$)	8.53	8.04	6.70	6.74	7.44	4.75
CV (%)	NS	NS	NS	6.75	8.34	5.05

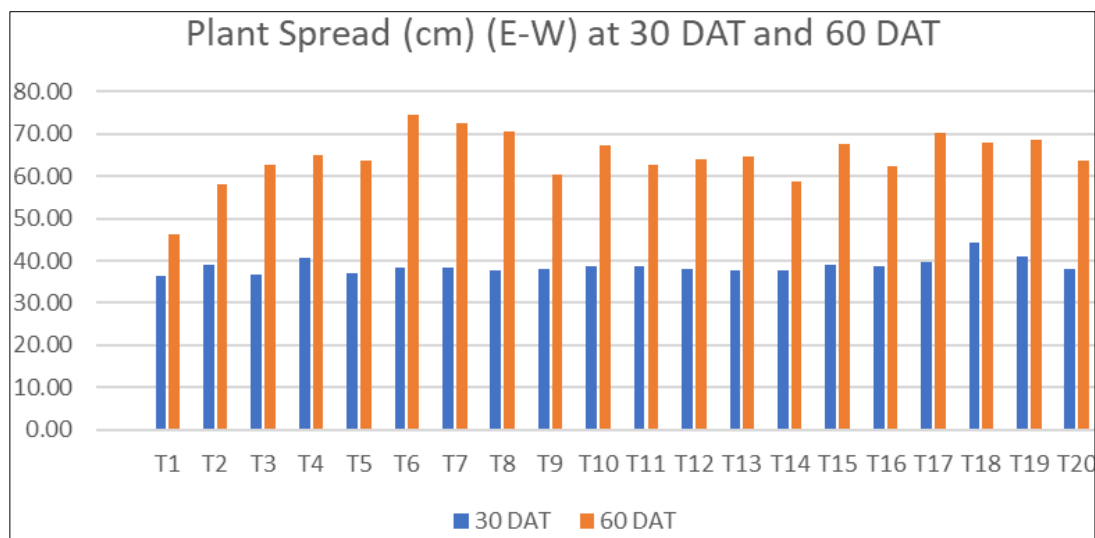


Fig 8: Plant Spread (cm) (E-W) at 30 DAT and 60 DAT as influenced by different doses of Biostimulants application.

Conclusion

The present investigation demonstrated that the application of biostimulants, particularly their combined application, influenced several vegetative growth attributes of broccoli under protected conditions. Based on the pooled analysis of the two years, T19 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 1.5%) recorded the maximum plant height at 60 DAT (67.64 cm) and at harvest (76.84 cm), indicating its superior performance for plant growth. At 60 DAT, T19 was statistically at par with T20, T16, T18 and T14, whereas at harvest it was statistically at par with T10.

The differences in number of leaves per plant and stem diameter were non-significant at the respective stages of observation. Nevertheless, numerically, T17 (Jeevamrit @ 3% + Humic acid @ 5 mL L⁻¹ + NOLN @ 1.5%) recorded the maximum number of leaves at 15 DAT (5.60) and at harvest (22.28), while T20 recorded the maximum stem diameter at 30 DAT (1.07 cm) and T19 recorded the maximum stem diameter at harvest (3.35 cm).

For leaf growth attributes, T15 (Jeevamrit @ 3% + Seaweed extract @ 3 mL L⁻¹ + NOLN @ 1.5%) recorded the maximum pooled leaf width (11.01 cm) and leaf area (129.28 cm²) at 30 DAT. At 60 DAT, T10 (Seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%) recorded the maximum leaf width (18.75 cm) and leaf area (365.55 cm²). The maximum leaf length at 30 DAT (15.63 cm) was recorded in T14 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + NOLN @ 2.0%), while at 60 DAT the maximum leaf length (26.75 cm) was recorded in T20 (Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 2.0%).

For plant canopy development, the maximum pooled plant spread in the N-S direction at 60 DAT (71.49 cm) was recorded in T10, which was statistically at par with T17 and T20. Similarly, the maximum pooled plant spread in the E-W direction at 60 DAT (74.56 cm) was recorded in T6 (NOLN @ 2.0%), which was statistically at par with T7, T8 and T17. At 30 DAT, plant spread in both N-S and E-W directions remained non-significant.

Overall, the findings indicated that combined application of biostimulants was more effective in promoting vegetative growth and canopy development of broccoli under protected conditions than individual applications. Among the

treatments, combinations involving seaweed extract, humic acid, Jeevamrit and NOLN performed consistently well across different growth parameters. Therefore, T19, comprising Humic acid @ 5 mL L⁻¹ + Seaweed extract @ 3 mL L⁻¹ + Jeevamrit @ 3% + NOLN @ 1.5%, emerged as the most promising treatment for overall plant growth, particularly with respect to plant height, while T10, T15, T14, T20 and T6 were superior for specific leaf and canopy attributes.

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