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Optimizing Foliar Application Strategies of Humic Substance Based Biostimulants for Enhancing Okra Productivity and Fruit Quality

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Authors' contributions

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

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Abstract

Okra production may benefit from appropriately timed foliar applications of humic- and fulvic-acid-based biostimulants. A field experiment was conducted during the 2024 summer season at the Agricultural and Horticultural Research Station, Honnavile, Shivamogga, Karnataka, India, on red sandy loam soil to assess crop growth, flowering, yield, fruit quality and phytotoxicity. Treatments involving a water-sprayed control and varying biostimulant concentrations applied at the active vegetative stage alone or at both the active vegetative and early flowering stages were evaluated with three replications using okra cv. Arka Anamika. Foliar application at 4 ml L^{-1} at both stages (4AV4EF) produced the greatest plant height (135.32 cm), the highest number of leaves per plant (9.38) and the earliest 50% flowering (37 days). The same treatment recorded 22.24 fruits per plant, a fruit length of 13.27 cm and the highest total fruit yield (15.24 t ha^{-1}), compared with 11.52 t ha^{-1} in the water-sprayed control. The highest SPAD reading

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(30.85) and crude fibre value (34.86) occurred under 6AV6EF, whereas 4AV4EF recorded corresponding values of 30.65 and 33.41. Mild phytotoxic symptoms observed in 6AV and 6AV6EF disappeared by nine days after spraying. Under the tested field conditions, application at 4 ml L^{-1} at both developmental stages improved okra productivity without persistent phytotoxicity.

Keywords: Crude fibre; fulvic acid; humic acid; ladies finger; SPAD.

1. Introduction

Vegetables play a vital role in Indian agriculture by contributing to nutritional security, livelihood generation and employment opportunities. Among the major vegetable crops, okra (*Abelmoschus esculentus* L.), commonly known as lady's finger or bhindi, belongs to the family Malvaceae and is widely cultivated in tropical and subtropical regions. India is the largest producer of okra globally (Agricultural and Processed Food Products Export Development Authority, 2026). Its adaptability to warm climatic conditions, relatively short crop duration and economic importance make it an essential vegetable crop. Okra is primarily cultivated for its immature green fruits, which are valued for their nutritional and functional properties. The fruits are rich in dietary fibre, proteins, minerals, vitamins C, K and B-complex, and bioactive compounds such as flavonoids and phenolics (Kwok *et al.*, 2025). The mucilage present in okra also has potential applications in pharmaceutical and food-processing industries (Dantas *et al.*, 2021). Furthermore, its antioxidant properties, associated with vitamin C, carotenoids and phenolic compounds, enhance its nutritional importance (Kumari *et al.*, 2020; Wu *et al.*, 2020). Therefore, improving okra productivity and fruit quality is essential to meet increasing consumer demand and enhance farmers' income.

Sustainable okra production requires balanced nutrient management and improved crop production practices. Although the application of essential nutrients, particularly nitrogen, phosphorus and potassium, is fundamental to crop growth and yield formation, conventional fertilisation alone may not always ensure optimum nutrient-use efficiency and physiological performance. Consequently, plant biostimulants have gained considerable attention as complementary inputs for improving crop growth, nutrient acquisition and productivity. Biostimulants are substances or microorganisms that stimulate plant nutrition processes independently of their direct nutrient contribution, thereby improving nutrient-use efficiency, stress tolerance and crop quality (du Jardin, 2015). A recent lettuce study likewise documented effects of foliar and root applications of leonardite-derived humic substances on phosphorus-use efficiency and chlorophyll status under differing phosphorus supplies (Atero-Calvo *et al.*, 2026).

Among various categories of plant biostimulants, humic substances, particularly humic acid and fulvic acid, have gained considerable attention owing to their beneficial effects on plant growth and development (Abou-Hussein *et al.*, 2025). These substances influence root architecture, nutrient availability, membrane transport, enzymatic activities and plant metabolic processes, thereby enhancing overall crop performance (Canellas & Olivares, 2014; Nardi *et al.*, 2016). Their beneficial effects have also been documented in several vegetable crops. For instance, humic acid application improved fruit productivity and nutrient uptake in brinjal (Paramasivan, 2015), while enhanced yield attributes and chlorophyll content were reported in chilli (Pavani *et al.*, 2022). Collectively, these findings highlight the potential of humic acid- and fulvic acid-based formulations as effective biostimulants for improving the growth, productivity and physiological performance of vegetable crops. In okra, compost-derived humic-acid foliar treatments produced different growth and yield responses according to the source and concentration used (Anshida *et al.*, 2026). Humic-acid treatments have also been associated with flowering and fruit-yield responses in strawberry (Bharat *et al.*, 2026). In tomato, the effects of humic- and fulvic-acid biostimulants varied with application rate, frequency and method (Quijia Pillajo *et al.*, 2024).

Despite these encouraging findings, the effectiveness of biostimulants depends on their formulation, concentration, frequency and timing of application, as well as crop developmental stage. Moreover, improved crop performance alone does not establish the safety of a formulation at different application concentrations. Systematic evaluation of bio-efficacy and phytotoxicity is therefore essential to identify effective application schedules and ensure crop tolerance. Although previous studies have investigated humic acid and combined humic–fulvic acid applications in okra, information remains limited regarding their comparative effectiveness under different application schedules, particularly at critical vegetative and early flowering stages, along with systematic phytotoxicity assessment.

Considering these aspects, the present investigation was undertaken to evaluate the bio-efficacy of humic acid- and fulvic acid-based biostimulants applied at different crop growth stages for improving vegetative growth, flowering behaviour, yield attributes, fruit yield and quality of okra. The study also aimed to assess their phytotoxic effects under field conditions to identify effective and crop-safe application schedules for enhancing okra productivity.

2. Materials and Methods

2.1 Experimental Site

The experiment was conducted at the Agricultural and Horticultural Research Station, Honnavile, Shivamogga, Karnataka, during 2024. The experimental field had uniform topography and adequate irrigation and drainage facilities. The soil was classified as red sandy loam, with medium organic carbon content (0.67%), slightly acidic pH (5.66), and medium levels of available nitrogen (266.28 kg ha^{-1}), phosphorus (54.62 kg ha^{-1}), and potassium (172.30 kg ha^{-1}).

2.2 Experimental Details

The experiment was conducted in a randomised block design (RBD) comprising nine treatments, each replicated three times. The treatments were randomly allocated to individual plots measuring 6.0 m × 4.8 m (27 m²). The treatments included water control (WC), 2 ml L^{-1} at the active vegetative stage (2AV), 3 ml L^{-1} at active vegetative stage (3AV), 4 ml L^{-1} at active vegetative stage (4AV), 6 ml L^{-1} at active vegetative stage (6AV), 2 ml L^{-1} at active vegetative stage and 2 ml L^{-1} at the early flowering stage (2AV2EF), 3 ml L^{-1} at active vegetative stage and 3 ml L^{-1} at early flowering stage (3AV3EF), 4 ml L^{-1} at active vegetative stage and 4 ml L^{-1} at early flowering stage (4AV4EF), 6 ml L^{-1} at active vegetative stage and 6 ml L^{-1} at early flowering stage (6AV6EF). Each treatment was replicated three times.

2.3 Crop Husbandry

The experimental field was thoroughly ploughed using a mouldboard plough, followed by repeated cultivation to achieve a fine tilth by breaking soil clods. Farmyard manure (FYM) was incorporated into the soil at 20 t ha^{-1} during the final ploughing. Subsequently, plots measuring 6.0 m × 4.8 m (27 m²) were prepared, and seeds of okra (*Abelmoschus esculentus L.*) cv. Arka Anamika were directly sown manually at a spacing of 60 cm × 30 cm. The recommended fertiliser dose of 125:75:62.5 kg $N:P_2O_5:K_2O$ ha^{-1} was applied, with 50% of the nitrogen, the full dose of phosphorus and 50% of the potassium applied as basal fertilisers before sowing. The remaining 50% of nitrogen and potassium was top-dressed in two equal splits at 30 and 60 days after sowing (DAS). Urea, diammonium phosphate (DAP) and muriate of potash (MOP) were used as the respective fertiliser sources. The crop was maintained under protective irrigation at 15-day intervals. Weed management was achieved through the pre-emergence application of pendimethalin (30 EC) at 1 L ha^{-1}, applied to moist soil one day after sowing, followed by two hand-weeding operations at 30 and 60 DAS. Plant protection measures were undertaken as required to prevent pest and disease incidence, using monocrotophos, chlorpyrifos and carbendazim at their respective recommended doses as prophylactic treatments. The crop was harvested manually at marketable maturity by picking the tender okra pods, which were subsequently stored in a cool place until marketing.

2.4 Imposition of Treatment

The biostimulant was diluted in water with an appropriate emulsifier and applied as a foliar spray at the respective crop growth stages according to the assigned treatments. Spraying was carried out during the early morning hours to facilitate effective foliar absorption under favourable environmental conditions.

2.5 Data Collection

Plant height and number of leaves were recorded at the grand growth stage. Fruit yield per plant, number of fruits per plant, fruit length, individual fruit weight, and total fruit yield were recorded at harvest. Crude fibre content was determined using the conventional acid–alkali digestion method. Leaf chlorophyll status was assessed non-destructively using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Japan).

2.6 Statistical Analysis

Data recorded from five randomly selected plants in each experimental unit were averaged and subjected to analysis of variance (ANOVA). The significance of treatment effects was evaluated using the F-test at a 5% probability level. Whenever the F-test indicated significant treatment differences, the critical difference (CD) was calculated at the 5% significance level to compare treatment means.

3. Experimental Results

3.1 Growth Attributes

Biostimulant application significantly influenced the vegetative growth and flowering behaviour of okra (Table 1). Among the evaluated treatments, 4AV4EF recorded the maximum plant height (135.32 cm) and number of leaves per plant (9.38), followed by 6AV6EF (131.26 cm and 9.25 leaves per plant), with both treatments being statistically comparable. In general, biostimulant application at both the active vegetative and early flowering stages resulted in superior growth compared with application exclusively at the active vegetative stage. Among the single-stage applications, 6AV exhibited the highest plant height (115.46 cm) and number of leaves per plant (8.05), followed by 4AV. Nevertheless, all biostimulant treatments significantly improved vegetative growth compared with the untreated control (89.31 cm and 7.14 leaves per plant). A similar trend was observed for flowering behaviour, with biostimulant application significantly reducing the time required to attain 50% flowering. The earliest flowering was recorded under 4AV4EF (37.00 days), followed by 3AV3EF (39.33 days), whereas the untreated control required the longest duration (53.00 days) to reach 50% flowering.

3.2 Yield Attributes

The timing of biostimulant application significantly influenced the yield attributes of okra (Table 2). Among the treatments, 4AV4EF recorded the highest values for fruit number per plant (22.24), fruit length (13.27 cm), and individual fruit weight (12.64 g), followed by 3AV3EF, which exhibited corresponding values of 20.78, 12.95 cm, and 12.38 g, respectively. Among the single-stage applications, 6AV (17.55 fruits per plant, 10.95 cm, and 11.85 g) and 4AV (16.28 fruits per plant, 10.48 cm, and 11.36 g)

showed comparable performance. In contrast, the water-sprayed control consistently recorded the lowest values for all three yield attributes (14.67 fruits per plant, 8.56 cm, and 10.85 g).

Table 1. Effect of humic acid and fulvic acid bio-stimulant on growth attributes of okra

Treatments	Plant height (cm)	Number of leaves per plant	Days to 50% flowering
WC	89.31±1.55	7.14±0.26	53±0.5
2AV	98.27±1.63	7.32±0.06	51.66±0.57
3AV	102.84±2.53	7.45±0.23	49.33±0.4
4AV	110.29±0.32	7.91±0.07	47.33±0.91
6AV	115.46±0.79	8.05±0.15	44.66±1.35
2AV2EF	125.40±1.04	8.27±0.52	40.66±0.36
3AV3EF	129.68±1.65	9.11±0.8	39.33±0.35
4AV4EF	135.32±1.69	9.38±0.01	37±1.33
6AV6EF	131.26±1.13	9.25±0.16	42.33±0.84
SEm±	1.06	0.25	0.49
CD (P=0.05)	3.17	0.76	1.48

Table 2. Effect of humic acid and fulvic acid bio-stimulant on yield attributes of okra

Treatments	Number of fruits per plant	Fruit length(cm)	Individual Fruit weight(g)
WC	14.67±0.82	8.56±0.89	10.85±1.15
2AV	15.47±0.67	9.63±0.23	11.09±0.26
3AV	15.89±0.57	10.12±0.89	11.21±0.25
4AV	16.28±0.71	10.48±0.28	11.36±1.05
6AV	17.55±0.45	10.95±0.34	11.85±0.27
2AV2EF	19.37±0.99	12.44±0.43	12.18±0.15
3AV3EF	20.78±0.61	12.95±0.31	12.38±0.76
4AV4EF	22.24±0.23	13.27±0.44	12.64±0.11
6AV6EF	20.81±1.54	12.88±1.57	12.01±0.92
SEm±	0.59	0.53	0.49
CD (P=0.05)	1.77	1.60	1.48

3.3 Okra Yield

Biostimulant application at two growth stages significantly enhanced fruit yield per plant and total fruit yield of okra (Table 3). Among the treatments, 4AV4EF recorded the highest fruit yield per plant (240.32 g) and total fruit yield (15.24 t ha^{-1}), significantly outperforming the water-sprayed control (164.66 g $plant^{-1}$ and 11.52 t ha^{-1}) as well as the single-stage applications. Nevertheless, single-stage applications also resulted in significant yield improvements over the control, with 6AV recording the highest fruit yield per plant (196.35 g) and total fruit yield (12.29 t ha^{-1}), which were statistically comparable to 4AV (196.11 g $plant^{-1}$ and 12.05 t ha^{-1}).

Table 3. Effect of humic acid and fulvic acid bio-stimulant on yield of okra

Treatments	Fruit yield per plant (g $plant^{-1}$)	Total fruit yield (t ha^{-1})
WC	164.66±9.91	11.52±0.29
2AV	175.29±10.45	11.68±0.12
3AV	189.5±13.01	11.84±0.36
4AV	196.11±11.45	12.05±0.43
6AV	196.35±7.37	12.29±0.28
2AV2EF	212.22±8.15	14.46±0.43
3AV3EF	218.87±10.28	14.96±0.42
4AV4EF	240.32±8.69	15.24±0.64
6AV6EF	234.83±13.14	14.15±0.37
SEm±	7.04	0.29
CD (P=0.05)	21.11	0.87

3.4 Chlorophyll Content

Biostimulant application significantly enhanced the chlorophyll status of okra, as reflected by the SPAD readings (Fig. 1). Among the treatments, 6AV6EF recorded the highest SPAD value (30.85), closely followed by 4AV4EF (30.65), with both treatments exhibiting significantly higher chlorophyll status than the single-stage applications and the water-sprayed control (18.63). Among the single-stage applications, 6AV recorded the highest SPAD value (25.62), significantly outperforming the other treatments within this application category.

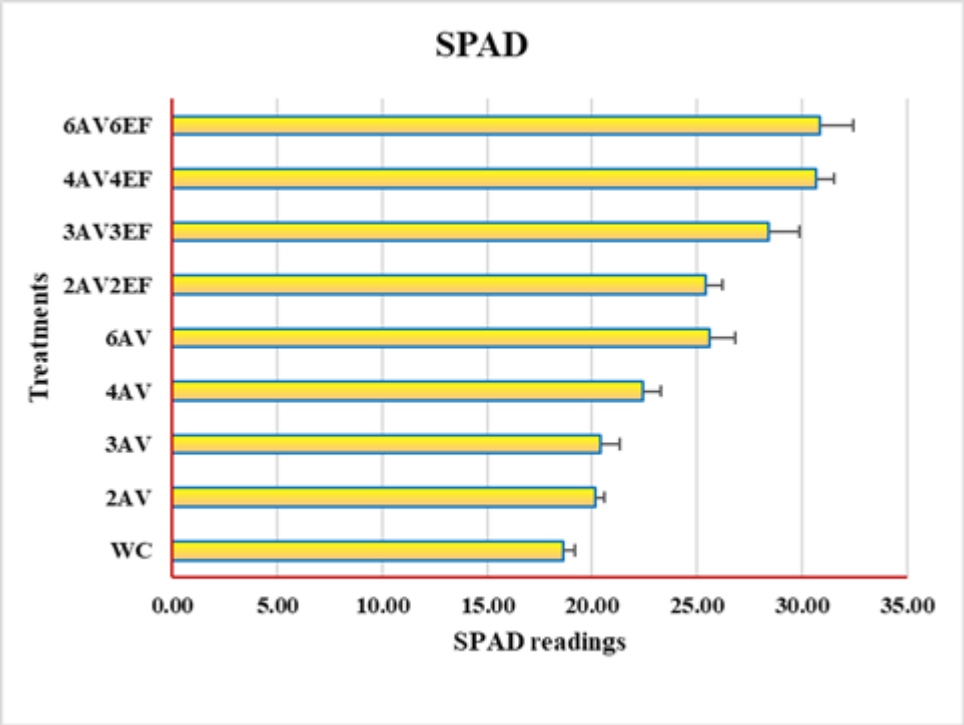

Fig. 1. Effect of biostimulant on SPAD values of okra

3.5 Crude Fibre Content

Biostimulant application significantly influenced the crude fibre content of okra fruits (Fig. 2), with values ranging from 25.63 to 34.86. Among the treatments, 6AV6EF recorded the highest crude fibre content (34.86), followed by 4AV4EF (33.41) and 3AV3EF (31.63). Among the single-stage applications, 4AV exhibited the highest crude fibre content (30.41), followed by 6AV (29.63). In contrast, the water-sprayed control (WC) recorded the lowest crude fibre content (25.63).

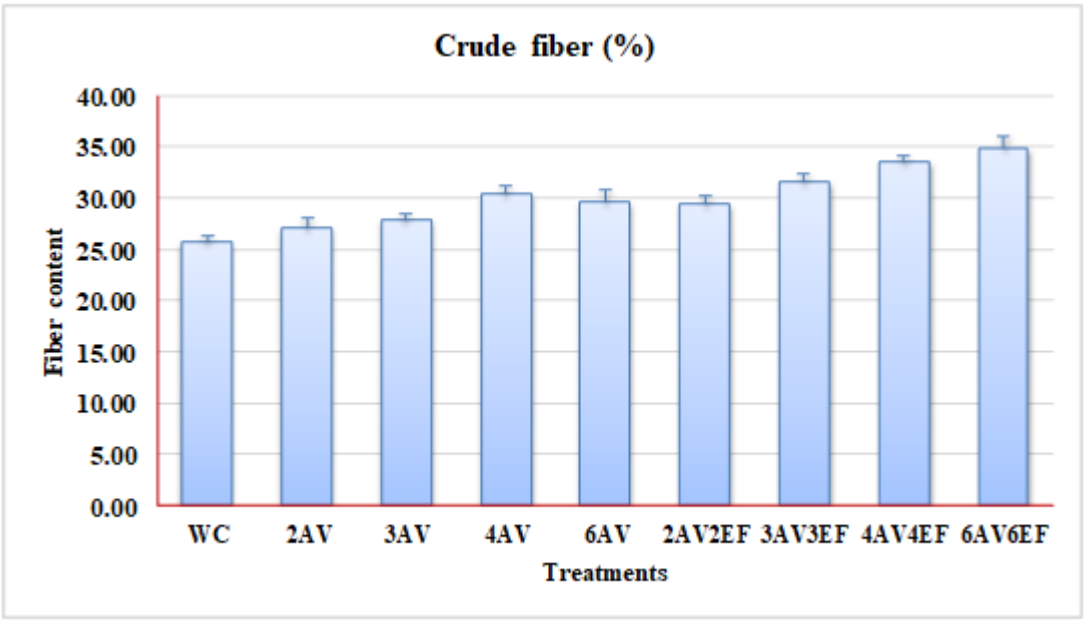

Fig. 2. Effect of biostimulant on crude fiber content of okra

3.6 Phytotoxicity

Phytotoxicity assessment revealed no visible phytotoxic symptoms in most of the biostimulant treatments throughout the observation period (Fig. 3). However, mild phytotoxicity (score 1) was observed in 6AV and 6AV6EF at 3 and 6 days after spraying (DAS), which subsequently disappeared by 9 DAS. No phytotoxic symptoms were recorded in either treatment at 9 or 12 DAS. All other treatments, including the water-sprayed control, consistently exhibited a phytotoxicity score of zero throughout the assessment period.

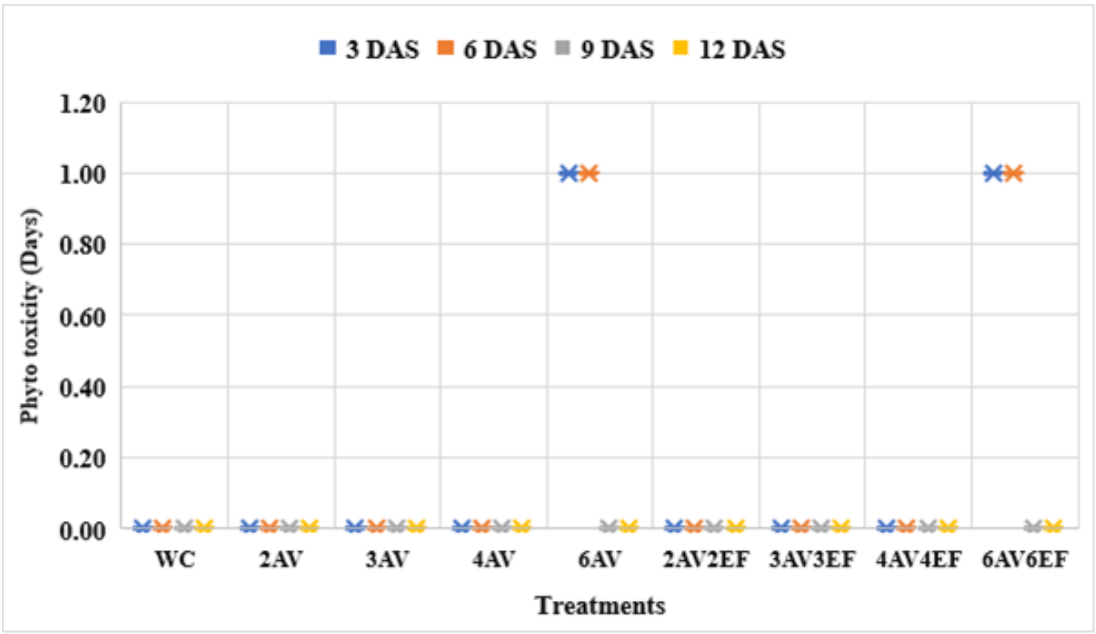

Fig. 3. Phytotoxicity effect of biostimulants an okra

4. Discussion

Various biostimulants, including seaweed extracts, humic substances, plant-derived extracts, microbial formulations and polysaccharides, are widely employed in vegetable production to improve crop productivity and fruit quality (Drobek *et al.*, 2019; Shahrajabian *et al.*, 2021). Among these, humic substances containing humic and fulvic acids have gained considerable attention owing to their potential to regulate plant growth and physiological processes (Canellas *et al.*, 2015). In this context, the present study evaluated the efficacy of humic acid- and fulvic acid-based biostimulants in okra, demonstrating their substantial influence on crop growth, reproductive development and overall productivity.

The combined application at the active vegetative and early flowering stages, particularly 4AV4EF, consistently improved crop performance compared with single-stage applications and the water-sprayed control. This response highlights the importance of synchronising biostimulant application with critical crop developmental stages to maximise their agronomic benefits. The enhanced vegetative growth may be attributed to the multifunctional effects of humic substances on root development, nutrient acquisition, cellular metabolism and hormonal regulation (Bhatt & Singh, 2022; Meena *et al.*, 2023). Humic substances are known to exhibit auxin- and cytokinin-like activities, stimulate root growth and improve nutrient uptake, thereby supporting plant development (Canellas & Olivares, 2014; Nardi *et al.*, 2016).

The improved SPAD readings under repeated applications further indicate a favourable influence on leaf physiological status. Humic and fulvic acids may facilitate the acquisition and assimilation of essential nutrients, particularly nitrogen, magnesium and iron, which are directly involved in chlorophyll biosynthesis and photosynthetic metabolism (Nardi *et al.*, 2002; van Tol de Castro *et al.*, 2021). Enhanced chlorophyll status may consequently support photosynthetic activity and assimilate production, providing a favourable physiological environment for reproductive development (Smith *et al.*, 2018). These responses are consistent with the established role of humic substances in regulating plant metabolism, nutrient transport and photosynthetic efficiency (Canellas *et al.*, 2015; Nardi *et al.*, 2002).

The improvements in vegetative growth and physiological status were also reflected in reproductive development and fruit productivity. The earlier attainment of 50% flowering under 4AV4EF suggests that biostimulant application may have facilitated a timely transition towards reproductive growth. Furthermore, the greater fruit development and yield under repeated applications may be associated with improved assimilate production and partitioning towards reproductive sinks. The highest fruit yield under 4AV4EF demonstrates the advantage of maintaining biostimulant availability across successive developmental stages. Similar findings have been reported in tomato, green chilli, and brinjal, where humic and fulvic acid applications improved fruit yield (Disciglio *et al.*, 2024; Kolape *et al.*, 2024; Paramasivan, 2015).

The higher crude fibre content under repeated applications indicates that these treatments also influenced fruit compositional characteristics. This response may be associated with enhanced structural development and deposition of cell-wall constituents, including cellulose and hemicellulose, as well as with the stimulation of secondary and phenolic metabolism by humic substances (Conselvan *et al.*, 2017; Nardi *et al.*, 2017). However, the implications of increased fibre content should be considered in relation to fruit maturity and market preferences.

The differential responses between single-stage and repeated applications emphasise the importance of application frequency and timing in determining biostimulant effectiveness. Although single-stage applications, particularly 6AV, improved crop performance over the control, repeated applications generally produced greater benefits, possibly by supporting plant growth during both vegetative and reproductive development. Importantly, phytotoxicity assessment revealed no persistent adverse effects. The mild symptoms observed under 6AV and 6AV6EF disappeared by 9 days after spraying, indicating good crop tolerance under the tested conditions. Overall, the findings establish the potential of appropriately timed humic acid and fulvic acid applications to improve the growth, physiological performance and productivity of okra without causing persistent phytotoxic effects.

5. Conclusion

The present study demonstrated the effectiveness of humic acid- and fulvic acid-based biostimulants in improving the growth, flowering behaviour, yield attributes and productivity of okra. Application at both active vegetative and early flowering stages was generally more effective than single-stage application, with 4AV4EF consistently exhibiting superior performance in vegetative growth, earliness of flowering and fruit yield. The highest total fruit yield (15.24 t ha^{-1}) was recorded under 4AV4EF, representing a substantial improvement over the water-sprayed control. Repeated applications also favourably influenced chlorophyll status and crude fibre content, while no persistent phytotoxic effects were observed. Overall, the findings highlight the importance of synchronising biostimulant application with critical crop growth stages and establish 4AV4EF as a promising application schedule for enhancing okra productivity under the tested conditions.

6. Limitations

The investigation was conducted during one summer season at a single research station on red sandy loam soil, using okra cv. Arka Anamika. The findings therefore relate to the tested cultivar, location, biostimulant concentrations and foliar application schedules. Fruit-quality evaluation focused on crude fibre content, while leaf chlorophyll status was assessed using SPAD readings. Phytotoxicity was assessed during the reported observation period. Additional field evaluations across seasons, soils and cultivars could establish the consistency of the observed treatment responses under other production conditions.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. ChatGPT, Claude and Perplexity were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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