J. Bangladesh Acad. Sci. 49(1); 21-29: June 2025 DOI:https://doi.org/10.3329/jbas.v49i1.76180

Journal of Bangladesh Academy of Sciences

Journal homepage: http://www.bas.org.bd/publications/jbas.html

Research Article

Influence of clybio as a Bio-stimulant on the growth and yield of ornamental sunflower

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ARTICLE INFO

Article History

Received: 17 September 2024
Revised: 14 January 2025
Accepted: 15 January 2025

Keywords: *Helianthus annuus* L., Bio-stimulant, *Lactobacilli*, Yeast fungi, *Bacillus natto*.

ABSTRACT

A field experiment was accomplished at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, from October to December 2021. The single-factor experiment was laid out in a randomized complete block design (RCBD) with three replications to evaluate the influence of Clybio as a bio-stimulant on ornamental sunflower growth and yield performance. The "Hybrid Ornamental Sunflower (F1)" (Vincent Choice) variety was utilized. The experiment consisted of three treatments, viz., C_1: No Clybio application (Control), C_2: Clybio @ 4 ml/L, and C_3: Clybio @ 6 ml/L, which were applied in a foliar manner to the leaves and surrounding soil, respectively. Each treatment exhibited notable variations based on data on various vegetative growth, flower yields, and quality standards. The study revealed that plants treated with C_3 (Clybio @ 6 ml/L) showed superior vegetative growth and yield-attributing performance, including the tallest plant height (110.0 cm), the maximum number of leaves per plant (33.2), highest SPAD value (34.4), maximum leaf area (140.6 cm^2), maximum flower head size (14.3 cm), maximum ray floret area (6.0 cm^2), maximum ray floret numbers per flower (31.8), long flower vase-life (8 days), and maximum number of secondary flowers (kid's flower) per plant (12.2). On the contrary, the shortest plant height (99.9 cm), the minimum number of leaves per plant (30.2), lowest SPAD value (32.0), minimum leaf area (127.4 cm^2), minimum flower head size (12.0 cm), minimum ray floret area (4.6 cm^2), minimum ray floret numbers per flower (29.9), short flower vase-life (5 days), and minimum number of secondary flowers (kid's flower) per plant (10.0) were observed in C_1 (Control) treatment. The most amazing aspect was that this ornamental sunflower also produced numerous commercially valuable secondary flowers (kid's flowers) with the application of C3 (Clybio @ 6 ml/L), which might have provided additional income to the producers. So, considering the aforementioned findings, the C_3 (Clybio @ 6 ml/L) treatment proved the best promising prospect for producing high-quality ornamental sunflowers.

Introduction

The sunflower (*Helianthus annuus* L.) is one of the most indispensable cut flowers in the world. It belongs to the family Asteraceae and is cultivated globally as annuals and perennials in various soil types (Kirtimala et al., 2018). According to Devecchi (2005), sunflowers signify the finest quality cut flowers with excellent economic value and are in great demand in the global floral industry. In Bangladesh, the production of ornamental sunflowers is a very new concept for an oil-seed sunflower

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farming country, so an experiment was tested here. Regrettably, the widespread use of chemical pesticides in sunflower cultivation is a common agricultural practice that deteriorates soil quality (Dwivedi and Dwivedi, 2019) by killing essential microbes. Soil microbes are vital indicators of soil quality, enhance soil texture, promote nutrient cycling, suppress pathogens, stimulate plant growth, and mineralize organic nutrients for sustainable plant growth and development. The destruction of beneficial soil microbes decreases the generation of plant fertilizer through the weathering of organic matter, which is detrimental to the ecosystem and agricultural yield. To alleviate this concern, a bio-stimulant called Clybio is a safer substitute. Clybio, a Japanese organic fungicide, is a composite mixer of *lactobacilli*, yeast fungus, and *bacillus natto*, which can help the soil microbes degrade organic matter and release readily available essential plant nutrients for superior quality sunflower production. The bio-stimulant Clybio greatly enhances ornamental sunflowers through reduced fertilizer use, stress protection, vase life extension, petal color enhancement, efficient growth, and lucrative secondary flower (kid's flower) production. Various research on horticultural crops has shown that beneficial microbes can enhance vegetative growth and crop production (Uddin et al., 2020; Rakibuzzaman et al., 2021). Clybio improves soil health and plant growth by harnessing the power of enzymes and the synergy of *Lactobacilli* (Shrestha et al., 2014), Yeast Fungi (El-Tarabily and Sivasithamparam, 2006), and *Bacillus Natto* (Tiwari et al., 2019). The study aimed to achieve superior-quality cut flowers by investigating how Clybio affects ornamental sunflower growth and yield attributes.

Material and Methods

The experiment was accomplished at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, from October to December 2021. The soil was non-calcareous dark grey, having a pH of 6.5. The study was comprised of three treatments of Clybio concentrations viz., C_1: Control (No Clybio application); C_2: Clybio @ 4 ml/L, and C_3: Clybio @ 6 ml/L were used in this experiment arranged in a randomized complete block design (RCBD) with three replications. The "Hybrid ornamental sunflower (F1)" (Vincent Choice) variety was used in this experiment, and seeds were collected from A R Malik Seeds Pvt. Ltd., Dhaka, Bangladesh. For planting them, the field was well prepared and divided into 9 plots spanning 3 m x 1.8 m with a plant spacing of (30 cm x 30 cm) and sunflower seeds were sown at 2 cm depth. In this experiment, Clybio was treated twice, 15 and 30 days after sowing at the studied concentrations of 4 and 6 ml/L. Earthen-up was applied twice at 15 and 35 days after sowing, and the standard management practices were followed during cultivation. Data were collected on vegetative growth, physiological, quality, and yield-attributing parameters with three plants randomly selected from each experimental plot per one replication and repeated three times. Data on plant height, leaf number, leaf area, SPAD value, stem diameter, internode length, head size, disc size, head weight, disc weight, ray floret area, ray floret numbers per flower, vase-life, ray floret's color measurement, and morphological features of secondary flowers (kid's flowers) were measured. The data were analyzed using Statistix-10 scientific analysis software to determine the significance of variations among treatments, and treatment means were compared using the LSD test at a 5% level.

Results and Discussion

Plant height

Vegetative growth is a crucial characteristic in ornamental sunflowers and is positively correlated with yield and growing conditions, with foliar application of Clybio causing significant differences in plant height. The tallest plant height (110.0 cm) was attained from C_3 (Clybio @ 6 ml/L), whereas the shortest plant height (99.9 cm) was recorded from C_1 (Control) treatment (Table 1). Clybio made of *Bacillus spp.* converts the complex form of essential nutrients, such as P and N, to a simple available form that enhances plant growth and development (Kang et al., 2015). Akter et al. (2021) observed that the plant height increases in spinach with the application of Clybio concentrations.

Number of leaves per plant

Growing ornamental sunflowers at different concentrations of Clybio resulted in a considerable discrepancy in the number of leaves. Plants with C_3 (Clybio @ 6 ml/L) treatment showed the maximum number of leaves (33.2), whereas the minimum (30.2) was found C_1 (Control) treatment (Table 1). It was revealed that with the increases in Clybio concentration, the number of leaves per plant increased in sunflowers.

Leaf area

Leaf area showed significant variation in different Clybio concentrations on ornamental sunflowers. The C_3 (Clybio @ 6 ml/L) showed the maximum leaf area (140.6 cm^2), while the minimum (127.4 cm^2) was observed from the C_1 (Control) treatment (Table 1 and Plate 1).

C_1 C_2 C_3

Plate 1. Leaf area of ornamental sunflower under different treatments. C_1: Control; C_2: Clybio @ 4 ml/L; C_3: Clybio @ 6 ml/L.

SPAD value

Chlorophyll significantly impacts plant growth with variations in leaf SPAD value across various treatments. The highest SPAD value (34.4) was observed from C_3 (Clybio @ 6 ml/L), whereas the lowest value (32.0) was from C_1 (Control) treatment (Table 1). Clybio, a yeast-based bio-stimulant, enhances growth and chlorophyll content by promoting essential amino acids, vitamins, and phytohormones (Taha et al., 2020). Chlorophyll content in leaves is linked to photosynthetic capacity, leaf N status, and RuBP carboxylase activity (Evans, 1998; Seemann et al., 1987), while lower content decreases growth and grain development. Clybio can affect a plant's ability to perceive light and its nutritional state.

Stem diameter

The performance of stem diameter showed significant variation across different Clybio concentrations. The maximum stem diameter (11.7 mm) was observed from C_3 (Clybio @ 6 ml/L), while the minimum diameter was noticed in the treatment of C1 (Control) (9.9 mm) (Table 1). Many studies proved that effective microorganisms (EMO) of Clybio enhance plant growth (Chowdhury et al., 1994; Javid, 2006; Khaliq et al., 2006).

Internode length

When subjected to different treatments, ornamental sunflower internode length varied significantly. The highest internode length (8.2 cm) was observed from C_3

Table 1. The effect of Clybio concentrations on growth attributing performance (Plant height, number of leaves per plant, SPAD value, leaf area, stem diameter, and internode length) of ornamental sunflower

Treatment*	Plant height (cm)	Leaf number	SPAD value	Leaf area (cm^2)	Stem diameter (mm)	Internode length (cm)
C_1	99.9^b	30.2^b	32.0^b	127.4^b	9.9^b	7.1^b
C_2	108.8^a	31.5^{ab}	33.4^a	132.9^{ab}	10.5^b	7.4^b
C_3	110.0^a	33.2^a	34.4^a	140.6^a	11.7^a	8.2^a
$LSD_{0.05}$	5.7	2.5	1.2	10.0	0.9	0.7
CV (%)	6.4	9.29	4.43	8.9	10.4	10.4

*C_1: Control; C_2: Clybio @ 4 ml/L; C_3: Clybio @ 6 ml/L

In a column means having a similar letter (s) are statistically identical, and those having a dissimilar letter (s) differ significantly as per a 0.05 level of probability.

(Clybio @ 6 ml/L), and the lowest (7.1 cm) was found from C_1 (Control) treatment (Table 1). This cost-effective biofertilizer enhances plant nutrition and vigor during the early growth phase (Lonhienne et al., 2014).

Head size

The application of different clybio concentrations significantly enhanced the yield attributes of ornamental sunflowers (Fig. 1). Among other concentrations, C_3 (Clybio @ 6 ml/L) showed better performance in enhancing the flower head size (14.3 cm) while the minimum (12.0 cm) was found from C_1 (Control) treatment.

Disc size

The study revealed significant variation in the flower disc size of ornamental sunflowers under various treatments (Fig. 1). The maximum flower disc size (7.1 cm) was observed from C_3 (Clybio @ 6 ml/L). In comparison, the minimum (6.1 cm) was found in the C_1 (Control) treatment.

Head weight

The various treatments resulted in significant differences in flower head weight (Fig.1). The maximum flower head weight (28.1 g) was observed in C_3 (Clybio @ 6 ml/L). In comparison, the minimum weight (24.3 g) was found in C_1 (Control) treatment. Clybio is a potent bio-stimulator that enhances the fruit weight of strawberries (Uddin et al., 2021), and tomatoes (Aman, 2016) through higher concentrations (@ 6ml/L).

Disc weight

The significant variation is noted by the application of different Clybio concentrations considering the disc weight (Fig. 1). As observed, the maximum flower disc weight (25.8 g) was observed in C_3 (Clybio @ 6 ml/L) treated plants. In comparison, the minimum (22.3 g) was found in C_1 (Control) treatment. Clybio concentrations (6 ml/L) enhance the strawberry plant's fruit weight (Bhuiyan, 2021).

Ray floret area

Significant variation in ray floret area was recorded among the treatments (Fig. 1). The maximum ray floret area (6.0 cm^2) was observed from C_3 (Clybio @ 6 ml/L), whereas the minimum (4.6 cm^2) was recorded from C_1 (Control) treatment.

Ray floret numbers per flower

In different treatments, ray floret numbers per flower showed significant variation. The maximum ray floret number (31.8) was observed in C_3 (Clybio @ 6

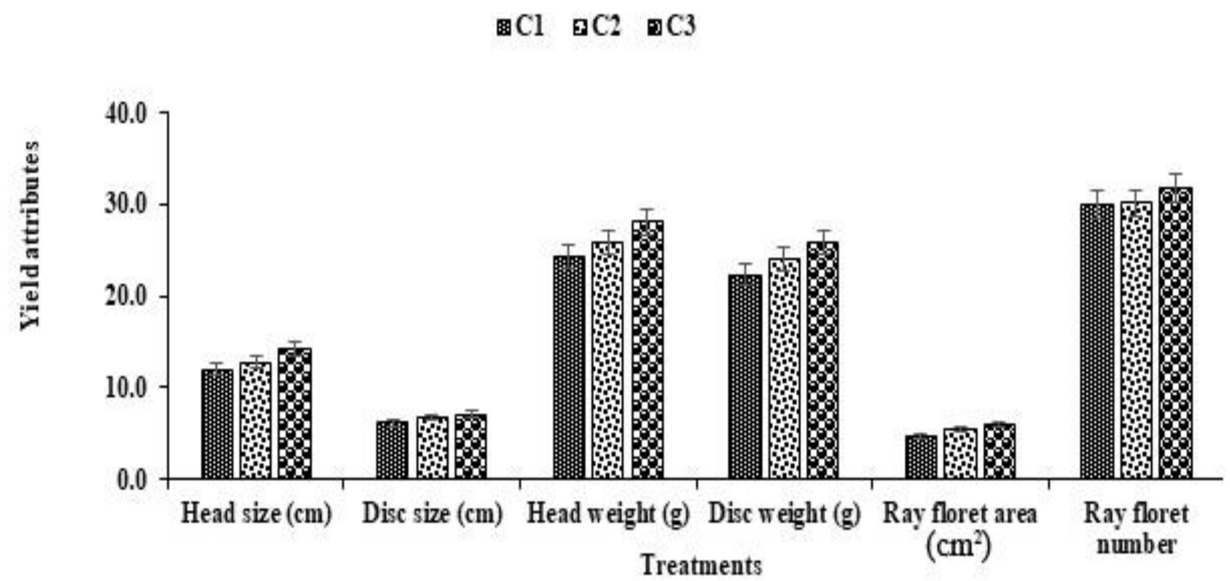


Fig. 1. Effect of Clybio concentrations on head size, disc size, head weight, disc weight, ray floret area, and ray floret numbers per flower on different days after sowing (C_1: Control; C_2: Clybio @ 4 ml/L; C_3: Clybio @ 6 ml/L).

ml/L), while the minimum (29.9) was found in the C_1 (Control) treatment (Fig. 1). The plant growth-influencing substances produced by microorganisms contributed to increased flowering, and enhanced flower quality (Arancon et al., 2008).

Vase life

To evaluate vase life, three stages of sunflowers viz., closed, intermediate, and completely open types were collected from C_3-treated plants. On the 5th day, sunflowers started blooming with the highest expansion in the closed stage (11.5 cm) and the intermediate stage (14.0 cm). After that, the ray florets gradually shrank and faded due to an increase in withering and abscission, but by the 9th day, they had stopped by achieving their smallest expansion. Fully open-stage flowers (15.5 cm) lost their ray florets more quickly and began to diminish after the first day (Fig. 2 and Plate 3).

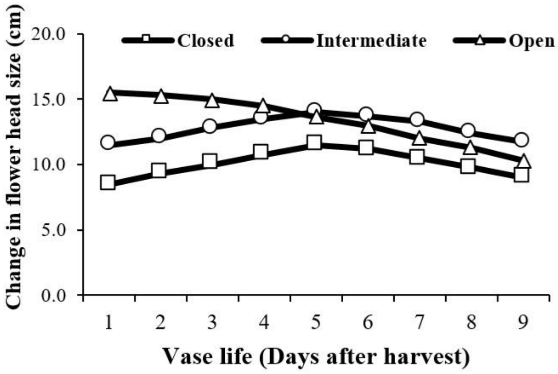


Fig. 2. The variation in flower head size (cm) from C_3 (Clybio @ 6 ml/L) treated plants at three stages (closed, intermediate, and fully open) for vase life as a function of days after harvest.

Some visual requirements determine the market value and higher acceptance to the customer, including the brightness of color, turgescence, proper flower head size, and proper petal number. Our result showed that the marketable appearance for cut sunflower head at the intermediate stage showed the maximum durability (7 days), whereas 7-8 days for the closed stage and 5-6 days for the open type. Though the closed stage had the maximum

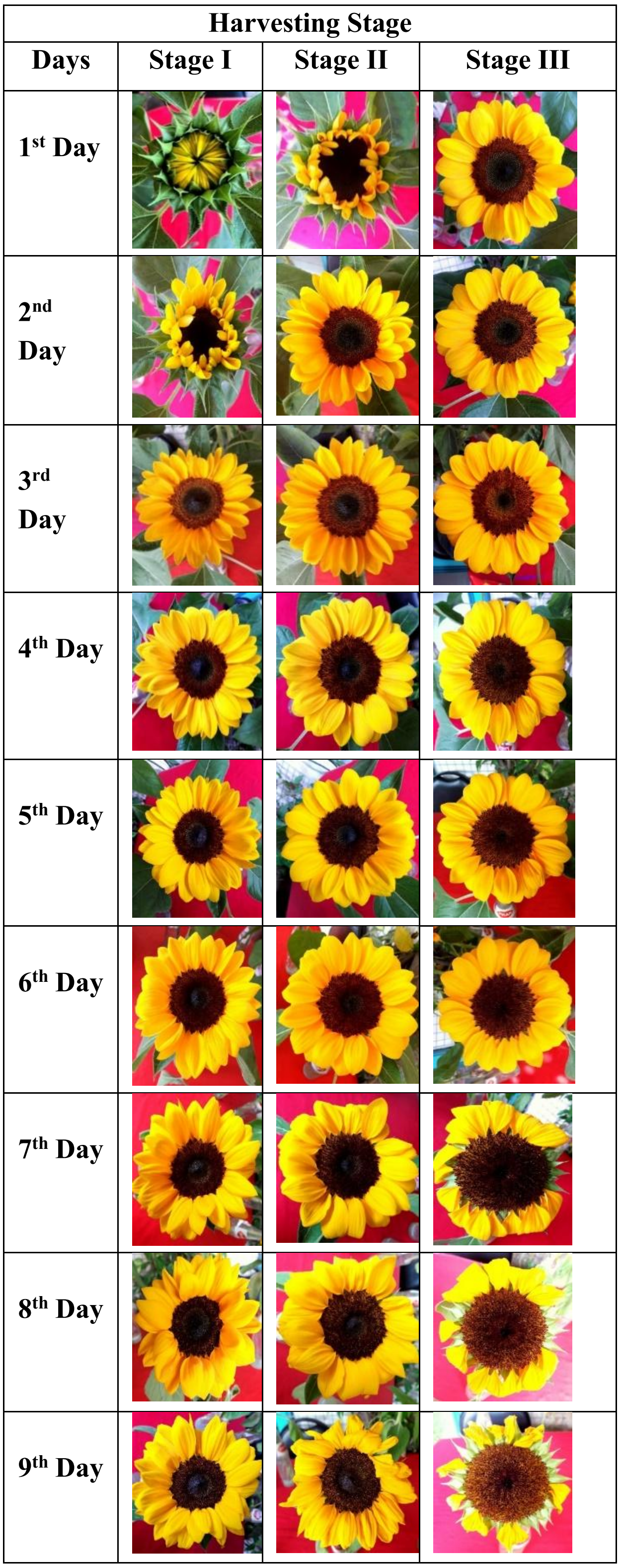


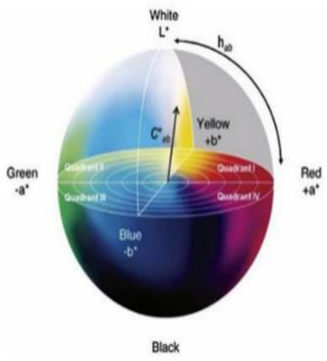
Plate 2: Vase life of ornamental sunflowers treated with C_3 (Clybio @ 6 ml/L) at three different stages (I. Closed, II. Intermediate, and III. Fully open) starting from days after harvest.

durability, its visual quality was less than that of the intermediate stage (Plate 2). The vase life of sunflowers varies between 5-13 days depending on the cultivar and is terminated by symptoms such as scape bending, abscission, and wilting of ray flowers (Gast, 1995; Sodi and Ferrante, 2005). Scape bending is thought to be correlated with genetic backgrounds (Ferrante et al., 2007); however, abscission and wilting of ray florets are known to be caused by senescence. Ethylene is a major factor in senescence, especially in ethylene-sensitive flowers (Tripathi and Tuteja, 2007). The vase life of a sunflower is indicated utilizing water uptake, the percent of change in flower head fresh weight, flower head diameter, and content in petals number (Amin, 2016). Increased longevity is another beneficial effect already observed in sunflowers (Gonzaga et al., 2003).

Colorimetric analysis of the sunflower under investigation with CIELab

The color of the ornamental sunflower's ray florets was measured using a precision colorimeter IWAVE WF32 (Shenzhen Wave), L* (lightness), a* and b* (two Cartesian coordinates), including c*, and h_{ab} (chroma & hue angle), based on the CIELab scale with standard observer 100 and standard illumination D65 (CIE, 1986; McGuire, 1992). The color values of the ray florets were recorded using the CIE L*, a*, and b* scales (Jamal Uddin et al., 2017). a*, and b* were further used to calculate chroma [C*= (a*2+b*2) 1/2] and hue angle (hº= tan-1 b*/a*). Chroma (C*) refers to color intensity, while hue angle represents red-purple (0º), yellow (90º), bluish-green (180º), and blue (270º) (Plate 3).

Treatment*	Color coordinates		Sunflower
C_1	L*	73.69	
	a*	19.73	
	b*	92.70	
	c*	94.77	
	h_{ab}	77.99	
C_2	L*	70.40	
	a*	20.83	
	b*	103.51	
	c*	105.59	
	h_{ab}	78.62	
C_3	L*	65.88	
	a*	20.43	
	b*	106.23	
	c*	108.18	
	h_{ab}	79.12	



(a)

(b)

Plate 3. Impact of Clybio concentrations on ray-floret color attributes of ornamental sunflowers: (a) CIELab Colorimetric Coordinates; (b) IWAVE WF32 (Shenzhen Wave) Colorimeter.

Morphological features of secondary flower (Kid's flower)

The intriguing part was that this ornamental sunflower produced numerous secondary flowers known as the "kid's flower," which addressed a market value for the commercial cut flower industry, and provided additional benefits to the producers. The application of different Clybio concentrations on ornamental sunflowers showed significant variations in the production of secondary flowers (kids flowers). The maximum flower head size (8.4 cm), maximum flower disc size (3.1 cm), the highest stalk length (10.8 cm), the maximum number of flowers per plant (12.2), and maximum ray floret numbers per secondary flower (17.3) were found in the C_3 (Clybio @ 6 ml/L) treatment. While the minimum flower head size (7.3 cm), minimum flower disc size (2.5 cm), the lowest stalk length (8.5 cm), the minimum number of flowers per plant (10.0), minimum ray floret numbers per secondary flower (15.4) were observed from C_1 (Control) treatment (Table 2). The application of Clybio showed higher performance in strawberry yield and yield components, which enhances the maximum number of flowers and fruits per plant (Uddin et al. 2021).

Table 2. The effect of Clybio concentrations on the performance of secondary flower (kid's flower) head size (cm), disc size (cm), stalk length (cm), flower numbers per plant, and ray floret numbers per secondary flower in ornamental sunflower.

Treatment*	Head size (cm)	Disc size (cm)	Stalk length (cm)	Flower numbers/plant	Ray floret numbers/flower
C_1	7.3^b	2.5^b	8.5^b	10.0^b	15.4^b
C_2	7.5^b	2.7^b	8.7^b	10.7^b	16.1^b
C_3	8.4^a	3.1^a	10.8^a	12.2^a	17.3^b
$LSD_{0.05}$	0.6	0.3	0.9	1.1	1.0
CV (%)	8.8	12.9	11.2	12.3	6.9

* C_1: Control; C_2: Clybio @ 4 ml/L; C_3: Clybio @ 6 ml/L

In a column means having a similar letter (s) are statistically identical, and those having a dissimilar letter (s) differ significantly as per a 0.05 probability level.

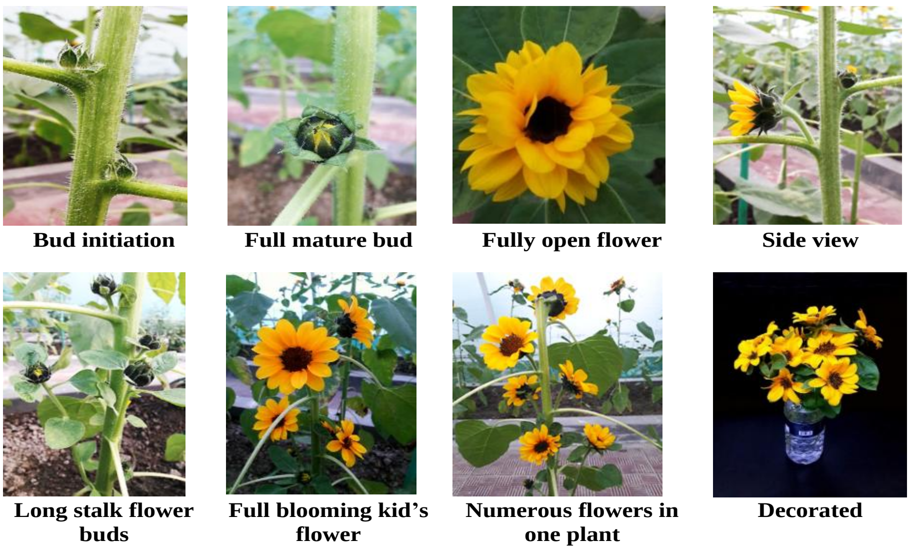


Plate 4. Different features of the secondary flowers (Kid's flower) of ornamental sunflower.

Conclusion

Based on the overall results, the study suggested utilizing Clybio at a concentration of 6 ml/L to increase the production of ornamental sunflowers. This bio-stimulant dosage provided potential growth, high-quality yield, long vase life, excellent color, and desired industrial attributes. Farmers will be encouraged to cultivate ornamental sunflowers due to their year-round availability for commercial use.

Acknowledgment

The authors would like to express gratitude to A R Malik Seeds Co Ltd. for providing seeds.

Author contributions

AFM Jamal Uddin: Experimental designing, data analysis, manuscript correction, Shanzida Akhter Ripa: Experiment conducting, data collection, writing, Fatema Tuz Juhora Chaitee: Data collection, data compiling, writing, Tamima Dastagir: Data collection, writing, Mst. Asmaul Husna: Data analysis, writing.

Declaration of conflicting interests

The authors declare that they have no conflicts of interest regarding the publication of this article.

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