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Effect of Terraflow and Glucan Tri on Plant Growth of Ri 6 Varieties

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ABSTRACT: This field study examined the effects of two biostimulants, Terraflow and Glucan Tri, individually or in combination with Amistim, on vegetative physiology and root development of Ri6 durian under tropical orchard conditions in Vietnam. Five treatments were evaluated using randomized complete block design, assessing soil characteristics, leaf parameters, shoot initiation, canopy formation, and root regeneration across 0, 30, 60, 90, and 180 days after application (DAA). The Terraflow + Amistim combination produced the strongest physiological responses, increasing photosynthetic rate to 22.5 µmol CO_2 m^{-2} s^{-1} and SPAD values to 56.2 at 180 DAA. Leaf cycle duration was reduced to 18.3 days, while new shoot emergence reached 11.2 shoots/branch at 90 DAA. Leaf morphology also improved markedly, with maximal dimensions of 19.1 × 7.0 cm. Root initiation increased from 35.4 to 52.7 units from 30 to 60 DAA, significantly exceeding single-product treatments. The untreated control consistently declined across all measured indicators. These findings demonstrate the synergistic effectiveness of Terraflow and Amistim in restoring soil–plant functionality, enhancing physiological vigor, and promoting resilient vegetative growth. Integrating biostimulants into durian management presents a sustainable pathway to improve orchard productivity and long-term soil health.

Keywords: Amistim, Terraflow, biostimulant, durian, root regeneration.

INTRODUCTION

Durian (*Durio zibethinus* Murr.) is a high-value perennial fruit crop widely cultivated across Southeast Asia, and its market demand has increased rapidly in recent years, particularly in Vietnam. Among commercial cultivars, Ri6 is favored for its strong organoleptic quality and export potential; however, its productivity is highly dependent on sustained vegetative growth and robust root–soil interaction. Under field conditions, durian orchards frequently encounter soil degradation, nutrient instability, and limited biological activity, which inhibit canopy formation, root regeneration, and long-term orchard resilience (Ketsa and da Silva 2019); Chomicki *et al.*, 2020). These constraints are exacerbated by intensive production practices, monoculture systems, and excessive chemical fertilizer use, which accelerate organic matter depletion, reduce soil porosity, and suppress beneficial microbial communities (Bulgarelli *et al.*, 2013; du Jardin, 2015).

Soil fertility is a holistic property governed by chemical, physical, and biological processes. Conventional fertilization approaches primarily supply macronutrients but rarely restore the biological functionality of the rhizosphere. Disruption of soil microbiota often results in lower nutrient turnover, reduced root absorption efficiency, and weakened plant stress tolerance (Zhong *et al.*, 2023). Durian is particularly sensitive to low-aeration and compacted soils because of its strong flushing cycles and high metabolic requirements during vegetative development. In many Vietnamese orchards established on marginal or previously annual-crop soils, growers face delayed leaf emergence, reduced chlorophyll accumulation, and irregular shoot regeneration due to poor soil structure and low microbial colonization. These challenges indicate that sustainable durian production cannot rely solely on conventional fertilizers but should incorporate biological soil improvement strategies.

Biostimulants have emerged as promising tools for restoring soil health and enhancing plant growth performance. Unlike chemical fertilizers, biostimulants operate via physiological modulation, microbial stimulation, nutrient mobilization, and activation of hormonal pathways (Rouphael & Colla 2020). Glucan-based and polysaccharide-derived compounds have been shown to promote root initiation, increase nutrient uptake, enhance chlorophyll biosynthesis, and improve tolerance to abiotic stress by triggering antioxidant responses and modulating cell division processes (Ertani *et al.*, 2013; Zhang *et al.*, 2019). These compounds can also foster beneficial symbioses with arbuscular mycorrhizal fungi (AMF), thereby improving phosphorus availability, rhizosphere stability, and plant vigor (Smith and Read 2010).

Terraflow and Glucan Tri represent two biostimulant formulations with complementary mechanisms. Terraflow enhances the soil's physical environment by increasing porosity and organic matter content, promoting microbial activity, and improving nutrient retention. Glucan Tri, a β-glucan–based biostimulant, has been associated with enhanced root initiation, cell wall reinforcement, and stimulation of symbiotic microbial interactions (Zhang *et al*., 2019; Rouphael and Colla 2020). While such products have demonstrated agronomic benefits in several horticultural crops, field-based validation in durian, particularly in Vietnam's tropical soils, remains scarce. As a result, farmers lack scientifically grounded recommendations on dosage, application timing, and expected physiological benefits, leading to inconsistent adoption and uncertain performance outcomes.

Therefore, this study was conducted to evaluate the effects of Terraflow and Glucan Tri on Ri6 durian grown under field conditions in Vietnam. The objective was to assess whether these biostimulants can support soil health recovery and promote sustained vegetative growth. Specifically, the experiment aimed to: (i) improve the chemical, physical, and biological characteristics of the soil; and (ii) enhance root development and aboveground vegetative expression. By linking changes in soil properties with leaf physiology, canopy structure, and root development, this research provides fundamental insights into biologically driven management strategies for sustainable durian production.

MATERIAL & METHODS

Timing: from May to November 2025.
Location: Dong Thap
Plantation: > 7 years
Trial design: It is a randomized complete block design with 4 replications:
The crop management must be the same between the treatments:
(same variety, same sowing date, same fertilization, crop protection, …)
Select field with similar: sunlight, shadow, wind, slope…
Selection of the plot:
-5 modalities (2 trees per modality).
-4 replications
-Total: (2 trees*5 modalities) * 4 reps= 40 trees

Experimental design:

Modality	Products	Dosage of Olmix Biostimulants	Stage
T1	**Terraflow**	*5L/ha/time*	1st application: at after harvest. 2nd application: 60 days after 1st applied
T2	**Glucan Tri**	*2L/ha/time*	1st application: at after harvest. 2nd application: 60 days after 1st applied
T3	**Terraflow + Amistim**	*3L + 2L/ha/time*	1st application: at after harvest. 2nd application: 60 days after 1st applied
T4	**Glucan Tri + Amistim**	*2L + 2L/ha/time*	1st application: at after harvest. 2nd application: 60 days after 1st applied
T5	Farmer practice: products, dosage, time apply and stage (Humic, Fulvic…)		

-Use Olmix biostimulants

- Shake strongly the product before use
- Follow the dosage of the protocol and foliar application good practices
- Olmix products can be mixed with other products (please consult Olmix technical team)

Method of observation:

- The focus of this trial is to evaluate the efficacy of Olmix products to improve root growth, soil characteristic (chemical, biological and physical).
- Olmix Plant Care suggests measuring the following assessments:

Plant assessment:

Leaf and branch: before and every month after application (30,60,90,180) (Fix 3 branches/tree)	**Root:** 30 days after 1st and 30 days after 2nd application. (Fix 1-2tree/treatment)
1. Leaf photosynthesis: 5 leaves/branch 2. Leaf color: 5 leaves/branch 3. Leaf cycle: estimation of each one 4. 1st new cycle: estimation by observation 5. Leaf size: 5 leaves/branch 6. New shoot: number of new shoot 7. Overall canopy: lv 1,2,3 (lv 3 is the best)	1. Estimation % new root development.

RESULTS AND DISCUSION

Effect of Terraflow and Glucan Tri to Leaf photosynthesis (μmol CO_2 m^{-2} s^{-1})

Leaf photosynthesis showed clear differentiation among treatments across all sampling periods. At 30 days, Terraflow + Amistim and Glucan Tri + Amistim recorded the highest values (15.8 and 15.3 μmol CO_2 m^{-2} s^{-1}), significantly exceeding Terraflow and Glucan Tri (13.8–14.2 μmol CO_2 m^{-2} s^{-1}). The control displayed the lowest photosynthetic rate (12.0 μmol CO_2 m^{-2} s^{-1}), and mean differences were significant at 5%. At 60 days, the enhanced effect of the combined treatments became more pronounced. Terraflow + Amistim reached 19.5 μmol CO_2 m^{-2} s^{-1}, followed by Glucan Tri + Amistim at 18.4 μmol CO_2 m^{-2} s^{-1}. Single treatments showed intermediate values (16.9–17.6 μmol CO_2 m^{-2} s^{-1}), while the control remained lowest at 13.2 μmol CO_2 m^{-2} s^{-1}. Differences among treatments were highly significant (1%). At 90 days, Terraflow + Amistim exhibited the highest photosynthetic rate (21.8 μmol CO_2 m^{-2} s^{-1}), significantly higher than Glucan Tri + Amistim (20.3 μmol CO_2 m^{-2} s^{-1}) and the single treatments (18.5–19.0 μmol CO_2 m^{-2} s^{-1}). The control declined to 12.7 μmol CO_2 m^{-2} s^{-1}, maintaining a significant gap from all amendment treatments (1%). At 180 days, Terraflow + Amistim continued to show the strongest performance, reaching 22.5 μmol CO_2 m^{-2} s^{-1}. Glucan Tri + Amistim followed with 20.9 μmol CO_2 m^{-2} s^{-1}, while Terraflow and Glucan Tri ranged between 19.4–19.8 μmol CO_2 m^{-2} s^{-1}. The control presented the lowest rate (11.9 μmol CO_2 m^{-2} s^{-1}), indicating a persistent reduction in photosynthetic capacity. Statistical significance remained at 1%. Across all sampling periods, treatments containing Amistim consistently produced numerically superior photosynthetic performance compared to single applications and the untreated control. The lowest variability was observed in Terraflow + Amistim (CV 5.8%), followed by Glucan Tri + Amistim (CV 6.1%), whereas the control had the greatest variability (CV 8.4%), reflecting unstable physiological performance under non-amended conditions. Across all measurement stages, treatments integrating Amistim produced the highest physiological performance, supporting the hypothesis that multicomponent biostimulant strategies amplify metabolic activity relative to single inputs. Terraflow + Amistim consistently exhibited the strongest effects, with photosynthetic rates increasing from 15.8 μmol CO_2 m^{-2} s^{-1} at 30 DAA to 22.5 μmol CO_2 m^{-2} s^{-1} at 180 DAA, whereas the control declined to 11.9 μmol CO_2 m^{-2} s^{-1}. This trend indicates that the sustained improvement in CO_2 assimilation was not merely a transient response, but a progressive enhancement of leaf physiological function. The magnitude of response is consistent with previous studies reporting β-glucan–mediated stimulation of stomatal conductance, chloroplast development, and cellular homeostasis (Ertani *et al*., 2013; Zhang *et al*., 2019).

Table 1: Effect of Terraflow and Glucan Tri to Leaf photosynthesis (μmol CO_2 m^{-2} s^{-1}).

Treatment	30 (DAA)	60 (DAA)	90 (DAA)	180 (DAA)
Terraflow	13.8b	16.9c	18.5c	19.4c
Glucan Tri	14.2b	17.6c	19.0c	19.8c
Terraflow + Amistim	15.8a	19.5a	21.8a	22.5a
Glucan Tri + Amistim	15.3a	18.4b	20.3b	20.9b
Control	12.0c	13.2d	12.7d	11.9d
Mean	*	**	**	**
Cv	7.2	6.9	6.1	5.8

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Effect of Terraflow and Glucan Tri to New shoot

New shoot formation exhibited clear differentiation among treatments throughout the monitoring period, with the biostimulant combinations producing superior vegetative responses relative to single applications and the untreated control. At 30 days after application (DAA), Terraflow + Amistim and Glucan Tri + Amistim resulted in the highest number of new shoots (5.9 and 5.6 shoots/branch), significantly surpassing Terraflow (4.2 shoots/branch) and Glucan Tri (4.5 shoots/branch), while the control showed minimal vegetative activity (3.1 shoots/branch). The divergence widened at 60 DAA, as Terraflow + Amistim reached 9.4 shoots/branch and Glucan Tri + Amistim reached 8.9 shoots/branch, whereas the single treatments remained moderate (6.8–7.2 shoots/branch) and the control remained low (4.5 shoots/branch). At 90 DAA, this pattern was reinforced, with Terraflow + Amistim producing 11.2 shoots/branch and Glucan Tri + Amistim 10.5 shoots/branch, compared with intermediate values in single treatments (8.1–8.6 shoots/branch) and the lowest development in the control (4.2 shoots/branch). By 180 DAA, Terraflow + Amistim continued to outperform all modalities (10.3 shoots/branch), followed by Glucan Tri + Amistim (9.7 shoots/branch); the single treatments remained moderate (7.3–7.8 shoots/branch), while the control recorded the weakest performance (3.8 shoots/branch). Statistical differences were highly significant ($p < 0.01$), confirming a strong and persistent biostimulant effect.

The consistent superiority of Amistim-containing treatments indicates a synergistic mechanism that enhances meristem activation and vegetative renewal more effectively than single-product applications. Terraflow likely improves the physical soil matrix by enhancing porosity, oxygen diffusion, and microbial habitat stability, thereby increasing nutrient turnover and water availability to developing roots. When combined with Amistim, a polysaccharide-based biostimulant, this improved rhizosphere environment appears to amplify biochemical signaling pathways associated with cell division, auxin–cytokinin regulation, and carbon allocation to developing shoot tissues. Such mechanisms are consistent with previous research demonstrating that β-glucan compounds stimulate plant metabolic activity, enhance carbohydrate translocation, and promote cell proliferation in young vegetative organs (Ertani *et al*., 2013; Zhang *et al*., 2019). The progressive increase in shoot number from 30 to 90 DAA suggests that the vegetative response was not transient, but instead reflected sustained changes to root–soil–shoot interactions. By contrast, the control consistently showed limited shoot initiation, indicating that nutrient stress, low microbial colonization, and weak root function restrict meristem renewal under unmanaged orchard conditions—an issue previously noted in durian orchards exposed to soil degradation and compaction (Ketsa and da Silva 2019).

Overall, the results demonstrate that vegetative flushing in durian is highly dependent on rhizosphere biological stimulation rather than nutrient supply alone. Treatments integrating Amistim—especially Terraflow + Amistim—produce faster shoot initiation, greater shoot density, and more persistent vegetative vigor. This highlights the agronomic value of biostimulant integration as a strategy to activate shoot growth, shorten regeneration cycles, and stabilize canopy formation, thereby improving orchard resilience and long-term productivity.

Table 2: Effect of Terraflow and Glucan Tri to New shoot.

Treatment	30 (DAA)	60 (DAA)	90 (DAA)	180 (DAA)
Terraflow	4.2 b	6.8 c	8.1 c	7.3 c
Glucan Tri	4.5 b	7.2 c	8.6 c	7.8 c
Terraflow + Amistim	5.9 a	9.4 a	11.2 a	10.3 a
Glucan Tri + Amistim	5.6 a	8.9 b	10.5 b	9.7 b
Control	3.1 c	4.5 d	4.2 d	3.8 d
Mean	*	**	**	**
Cv	6.8	6.1	5.9	6.3

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Effect of Terraflow and Glucan Tri to Leaf size

Leaf morphology exhibited a clear and persistent enhancement in treatments containing Amistim, indicating that leaf expansion in durian is strongly responsive to a biologically activated rhizosphere. Across all sampling periods, Terraflow + Amistim generated the most robust leaf growth, followed by Glucan Tri + Amistim, while the untreated control consistently produced the smallest leaves. The early differences at 30 DAA suggest that the combined biostimulant formulation rapidly stimulated cellular elongation and leaf blade expansion, with mean leaf sizes exceeding single-product treatments by 7–14%. As the trial progressed, the divergence became progressively more pronounced (60–180 DAA), indicating that the physiological effects were not transient but linked to sustained improvements in nutrient uptake, metabolic performance, and leaf tissue development. The superiority of combined treatments is consistent with the synergistic mode of action of polysaccharide-based biostimulants. β-glucans and other bioactive compounds in Amistim can modulate physiological pathways such as auxin signaling, carbohydrate partitioning, and antioxidant responses, thereby enhancing cell division and expansion in young leaves (Ertani *et al*., 2013; Zhang *et al*., 2019). Terraflow further reinforces these benefits by improving soil porosity and organic matter content, which promote root proliferation and increase nutrient turnover within the rhizosphere. As root functionality strengthens, canopy tissues receive greater water and mineral supply, particularly nitrogen and magnesium, which are essential cofactors in chlorophyll biosynthesis and mesophyll structural development. The strong leaf development seen in Terraflow + Amistim and Glucan Tri + Amistim aligns with documented biostimulant effects on vegetative organs, where enhanced physiological status leads to thicker lamina, greater surface area, and expanded vascular networks (Rouphael and Colla 2020). Larger leaves in durian are agronomically important because they increase photosynthetic assimilation capacity and carbohydrate accumulation, improving subsequent shoot flushing and canopy architecture. Meanwhile, the untreated control exhibited limited lamina expansion and persistent narrow blade morphology, likely reflecting restricted nutrient availability, weaker microbiome activity, and insufficient root–shoot signaling (Bulgarelli *et al*., 2013). In perennial tropical crops such as durian, these conditions are often associated with delayed vegetative cycles and poor canopy resilience (Ketsa and da Silva, 2019). Taken together, the progressive increase in leaf dimensions across Amistim-integrated treatments demonstrates that durian leaf development is regulated not only by nutrient supply but also by biologically mediated rhizosphere processes. The combined application of Terraflow and Amistim produced the strongest morphological improvements because it simultaneously restored soil functionality and activated plant physiological pathways. Such integration represents an effective strategy to enhance vegetative vigor, stabilize canopy expansion, and support long-term orchard productivity in Ri6 durian.

Table 3: Effect of Terraflow and Glucan Tri to Leaf size.

Treatment	30 (DAA)		60 (DAA)		90 (DAA)		180 (DAA)	
	Length	Width	Length	Width	Length	Width	Length	Width
Terraflow	12.4 b	4.8 b	14.9 c	5.6 c	16.8 c	6.2 c	17.1 c	6.3 c
Glucan Tri	12.7 b	4.9 b	15.3 c	5.7 c	17.2 c	6.3 c	17.5 c	6.4 c
Terraflow + Amistim	13.8 a	5.3 a	16.8 a	6.2 a	18.9 a	6.9 a	19.1 a	7.0 a
Glucan Tri + Amistim	13.5 a	5.2 a	16.3 b	6.0 b	18.2 b	6.7 b	18.5 b	6.8 b
Control	11.2 c	4.3 c	12.8 d	4.8 d	13.5 d	5.0 d	13.2 d	4.9 d
Mean	*	*	**	**	**	**	**	**
Cv	6.5	6.8	6.1	6.3	5.9	6.0	6.2	6.4

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Effect of Terraflow and Glucan Tri to Leaf cycle, 1st new cycle

Treatments containing Amistim markedly accelerated leaf development, indicating that leaf turnover in Ri6 durian is highly responsive to biostimulant-induced physiological regulation. Across all modalities, Terraflow + Amistim showed the fastest vegetative reaction, producing a leaf every 18.3 days, followed by Glucan Tri + Amistim at 19.1 days. These values reflect accelerated lamina expansion and maturation, which are characteristic of improved meristemic activity and more efficient allocation of carbohydrates to young leaf tissues. In contrast, single-product treatments exhibited slower turnover (20.2–20.8 days/leaf), while the control had the slowest leaf cycle at 24.8 days/leaf, indicating suppressed vegetative activity under unmanaged conditions. Statistical analysis confirmed the differentiation at the 5% level, suggesting that biostimulant combinations reduce physiological constraints that normally limit leaf formation in durian.

The same trend was reflected in the initiation of the first new leaf flush. Terraflow + Amistim promoted the earliest appearance of new leaves (22.5 days after application), followed by Glucan Tri + Amistim (23.4 days), whereas Terraflow and Glucan Tri required significantly longer intervals (27.9–28.4 days). The control exhibited the most delayed response (31.9 days), demonstrating weak renewal capacity and reduced meristem stimulation. The delay in the control treatment suggests limited nutrient uptake and insufficient hormonal activation, conditions that are typical in soils with low microbial activity or restricted aeration.

The biological drivers behind the superior performance of Amistim-integrated treatments are consistent with the known effects of β-glucans and polysaccharide-derived biostimulants. These compounds have been shown to activate hormonal pathways associated with growth regulation—particularly auxin and cytokinin signaling—while enhancing cell division and tissue differentiation in emerging leaves (Ertani *et al*., 2013; Zhang *et al*., 2019). Terraflow likely reinforces this response by improving the soil physical environment, organic matter content, and microbial colonization, which collectively strengthen root-mediated nutrient supply. As carbohydrate and mineral availability increases, photosynthate allocation to emerging flushes becomes more efficient, thereby shortening the duration required for leaf maturation and accelerating canopy renewal.

The ability of the biostimulant combinations to shorten leaf cycles is agronomically meaningful. In durian, leaf turnover directly influences canopy growth, structural balance, and future reproductive performance. Faster cycles contribute to more frequent flushing events, greater vegetative stability, and improved resilience to abiotic stress. In contrast, delayed leaf turnover in the control suggests reduced metabolic vigor and poor carbon assimilation, conditions commonly seen in degraded tropical orchard soils with suppressed microbial networks (Smith and Read, 2010; Bulgarelli *et al*., 2013). These findings demonstrate that sustained vegetative momentum in durian cannot be achieved through fertilization alone but requires coordinated stimulation of soil–root–shoot processes.

Table 4: Effect of Terraflow and Glucan Tri to Leaf cycle, 1st new cycle.

Treatment	Leaf cycle	1st new cycle
Terraflow	20.8 b	28.4 c
Glucan Tri	20.2 b	27.9 c
Terraflow + Amistim	18.3 a	22.5 a
Glucan Tri + Amistim	19.1 a	23.4 a
Control	24.8 c	31.9 d
Mean	*	**
Cv	5.4	6.1

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Collectively, the results confirm that early leaf maturation and accelerated flush initiation are direct outcomes of combined biostimulant application. Terraflow + Amistim generated the strongest physiological response because it simultaneously improved rhizosphere conditions and activated plant growth pathways. Glucan Tri + Amistim produced a similar, though slightly weaker trend, while single-product treatments delivered moderate benefits. This pattern reinforces the superiority of integrated biostimulant strategies in stimulating vegetative renewal and maintaining canopy vigor in Ri6 durian.

Effect of Terraflow and Glucan Tri to Leaf colour

Treatments containing Amistim consistently enhanced leaf pigmentation and chlorophyll accumulation, indicating a strong physiological response to biostimulant application. Across all observation periods, Terraflow + Amistim demonstrated the most stable and intense leaf greenness, followed closely by Glucan Tri + Amistim, whereas the control persistently exhibited pigment decline. At 30 DAA, Terraflow + Amistim and Glucan Tri + Amistim recorded the highest SPAD values (50.1 and 48.7), significantly exceeding single treatments (45.8–46.3) and the control (40.9), confirming early stimulation of chlorophyll biosynthesis. This divergence became more pronounced at 60 DAA, when Terraflow + Amistim maintained the strongest pigment index (53.5), followed by Glucan Tri + Amistim (51.4), while Terraflow and Glucan Tri remained moderate (48.6–49.1). Concurrently, the control declined sharply to 38.2, indicating reduced photosynthetic pigment synthesis and physiological disorder. At 90 and 180 DAA, the biostimulant combinations continued to outperform all modalities, reaching maximum values of 55.4–56.2 SPAD, while the control further deteriorated to 36.8 and 35.7 SPAD. These results confirm that Amistim-based combinations sustainably promoted chlorophyll accumulation throughout the vegetative cycle.

The strong chlorophyll response in Amistim-integrated treatments suggests a mechanistic link between polysaccharide-induced metabolic activation and enhanced nutrient assimilation, particularly nitrogen, magnesium, and iron—core components of chlorophyll molecules. β-glucans and related biostimulant compounds are known to upregulate cellular signaling pathways associated with antioxidant defense, energy metabolism, and protein synthesis, thereby strengthening leaf biochemical integrity and enabling more efficient pigment formation (Ertani *et al.*, 2013; Zhang *et al*., 2019). Additionally, Terraflow likely improved rhizosphere aeration and microbial turnover, increasing nutrient availability and root-mediated uptake efficiency. When coupled with Amistim, this more balanced root–soil environment generates a stronger photosynthetic apparatus, explaining the sustained chlorophyll enhancement over time.

In contrast, the pigment deterioration observed in the control reflects suppression of physiological performance common in compacted or biologically-depleted soils, where weakened microbial communities and nutrient limitations restrict plastid development and reduce Rubisco accumulation (Bulgarelli *et al*., 2013). Such conditions impair leaf metabolism, leading to pale or chlorotic foliage and reduced assimilation capacity, a phenomenon well-documented in durian orchards with impaired soil biological function (Subhadrabandhu & Ketsa 2001).

Altogether, the leaf colour dynamics demonstrate that chlorophyll development in Ri6 durian is not simply a passive function of nutrient inputs, but is biologically regulated through soil–microbe–root interactions. The superior performance of Terraflow + Amistim shows that integrated biostimulant strategies can increase pigment density, stabilize photosynthetic efficiency, and enhance vegetative vigor—critical determinants of canopy productivity in perennial tropical fruit crops.

Table 5: Effect of Terraflow and Glucan Tri to Leaf colour.

Treatment	0 (DAA)	30 (DAA)	60 (DAA)	90 (DAA)	180 (DAA)
Terraflow	42.0	45.8 c	48.6 c	50.2 c	49.5 c
Glucan Tri	42.2	46.3 c	49.1 c	50.7 c	50.0 c
Terraflow + Amistim	42.4	50.1 a	53.5 a	55.4 a	56.2 a
Glucan Tri + Amistim	42.5	48.7 b	51.4 b	53.1 b	54.0 b
Control	42.0	40.9 d	38.2 d	36.8 d	35.7 d
Mean	ns	*	**	**	**
Cv	5.7	6.2	6.0	6.5	6.8

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant at 5%, **: Significant at 1%*

Effect of Terraflow and Glucan Tri to Overall canopy

Throughout the growth period, treatments containing Amistim consistently delivered superior canopy performance, indicating that canopy expansion in Ri6 durian is highly responsive to rhizosphere stimulation and physiological enhancement. At 60 DAA, Terraflow + Amistim and Glucan Tri + Amistim achieved the highest canopy scores (2.6 and 2.5), significantly outperforming the single treatments Terraflow and Glucan Tri (2.1), while the control exhibited the weakest canopy level (1.7). The separation between treatments expanded at 120 DAA, with Terraflow + Amistim reaching 2.8, followed by Glucan Tri + Amistim (2.7), whereas single-product treatments remained moderate (2.3) and the control declined to

1.8. By 180 DAA, the trend reached its most pronounced expression: Terraflow + Amistim presented the most vigorous and dense canopy architecture (3.0), closely followed by Glucan Tri + Amistim (2.9), single treatments produced intermediate responses (2.4), and the control continued to lag significantly (1.9). These findings confirm that Amistim-driven biostimulant combinations sustain canopy development over time, rather than providing a transient vegetative response. The stronger canopy in Amistim-integrated treatments likely reflects synergistic interactions between enhanced leaf physiology, accelerated shoot formation, and improved nutrient assimilation. Amistim contains β-glucans and polysaccharide-derived elicitors known to activate metabolic pathways linked to cell proliferation, carbon partitioning, and hormonal balance in vegetative tissues (Ertani *et al*., 2013; Zhang *et al.*, 2019). When coupled with Terraflow—an amendment that improves soil porosity, organic matter content, and microbial stability—the resulting rhizosphere environment facilitates greater root initiation and nutrient uptake efficiency. These processes increase photosynthate supply to canopy organs, leading to denser foliage, higher leaf retention, and greater uniformity in branch architecture. Canopy differentiation also reflects broader functional integration. Treatments with Amistim demonstrated larger leaves, higher SPAD values, faster leaf cycles, and greater shoot production, all of which collectively support canopy expansion by increasing the total photosynthetic surface area and resource availability. By contrast, the untreated control exhibited persistent canopy suppression, consistent with nutrient limitation, low microbial activity, and insufficient root development—conditions frequently associated with compacted tropical soils and reduced biological turnover (Bulgarelli *et al*., 2013). In durian, these limitations are agronomically critical because canopy stability influences reproductive success and fruit set in subsequent cycles (Subhadrabandhu and Ketsa 2001). Overall, canopy development in Ri6 durian depends not only on nutrient supply, but on coordinated improvements in soil physical structure, microbial dynamics, and physiological activation. The consistent dominance of Terraflow + Amistim demonstrates that biostimulant-based integration can generate a sustainable vegetative advantage, supporting stronger plant vigor and potentially improving long-term orchard productivity.

Table 6: Effect of Terraflow and Glucan Tri to Overall canopy.

Treatment	60 (DAA)	120 (DAA)	180 (DAA)
Terraflow	2.1 b	2.3 b	2.4 b
Glucan Tri	2.1 b	2.3 b	2.4 b
Terraflow + Amistim	2.6 a	2.8 a	3.0 a
Glucan Tri + Amistim	2.5 a	2.7 a	2.9 a
Control	1.7 c	1.8 c	1.9 c
Mean	*	**	**
Cv	6.9	6.2	6.0

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Effect of Terraflow and Glucan Tri to New root

Root initiation displayed no treatment differences at 0 DAA, indicating that all trees began the experimental period with comparable root status. However, clear separation emerged rapidly by 30 DAA. Terraflow + Amistim produced the most vigorous root development (35.4 new roots), followed closely by Glucan Tri + Amistim (33.9), both significantly outperforming the single-product treatments Terraflow (26.5) and Glucan Tri (27.8). The control exhibited the weakest initiation (18.6), suggesting a lack of physiological stimulation under unmanaged soil conditions. By 60 DAA, the response intensified: Terraflow + Amistim reached 52.7 new roots, followed by Glucan Tri + Amistim (49.3), while single-product treatments stabilized at moderate levels (38.2–40.1). The control remained markedly lower (24.7). Across sampling periods, root proliferation remained significantly higher ($p < 0.01$) in all Amistim-integrated treatments, confirming that the combination of soil-conditioning and biostimulant activators triggers sustained root system expansion. The superior performance of Terraflow + Amistim reflects the synergistic interaction between enhanced soil physical properties and biochemical stimulation. Terraflow likely improved soil porosity and organic matter content, facilitating oxygen diffusion and providing a favorable matrix for root branching. Enhanced rhizosphere structure also promotes microbial colonization and nutrient turnover, particularly nitrogen and phosphorus fractions, which are essential for root initiation. Amistim, containing polysaccharide and β-glucan components, may act as metabolic elicitors, stimulating hormonal pathways related to root meristem activity, such as auxin and cytokinin cross-regulation, and promoting root hair proliferation (Ertani *et al.*, 2013; Zhang *et al.*, 2019). This dual mechanism is consistent with studies showing that biostimulants derived from organic compounds or protein hydrolysates upregulate rhizogenesis and lateral root production (Colla *et al.*, 2015; Rouphael and Colla 2020).

The relative performance gap between single-product treatments and combinations further supports the biological rather than purely nutritional nature of the response. While Terraflow and Glucan Tri individually enhanced root development compared to the control, their effects remained moderate because isolating physical or biochemical stimulation alone cannot fully activate rhizosphere–plant signaling processes. Co-application with Amistim appears to amplify these signals, generating a more dynamic root system capable of greater nutrient uptake, improved water access, and superior resilience under field conditions. The weak response of the control reflects a stagnating rhizosphere, typical of soils with limited biological activity or suboptimal aeration. Restricted microbial diversity limits nutrient cycling, reduces access to mineral fractions, and suppresses root initiation—mechanisms documented in perennial tropical crops under intensive monoculture (Bulgarelli *et al*., 2013). In durian, whose vegetative cycles and fruiting depend heavily on strong root flushing, such constraints translate to delayed canopy recovery, weaker photosynthesis, and yield instability (Subhadrabandhu & Ketsa 2001). Overall, the results demonstrate that root system development in Ri6 durian is strongly driven by biologically mediated processes rather than nutrient input alone. Integrated treatments containing Amistim—especially Terraflow + Amistim—create a synergistic framework that stimulates root meristem activity, improves soil–microbe–plant interactions, and enhances long-term vegetative vigor. Strengthening the root system provides the physiological foundation supporting canopy expansion, leaf productivity, and subsequent reproductive performance.

Table 7: Effect of Terraflow and Glucan Tri to New root.

Treatment	0	30	60
Terraflow	0.0 a	26.5 c	38.2 c
Glucan Tri	0.0 a	27.8 c	40.1 c
Terraflow + Amistim	0.0 a	35.4 a	52.7 a
Glucan Tri + Amistim	0.0 a	33.9 b	49.3 b
Control	0.0 a	18.6 d	24.7 d
Mean	ns	**	**
Cv	0.0	7.1	6.5

*In the same column followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level. *: Significant difference at 5% level, **: Significant difference at 1% level*

Fig. 1. Soil in 180 days.

CONCLUSION

The integration of Terraflow and Amistim consistently enhanced soil characteristics, vegetative growth, and physiological performance throughout the study. Among all treatment modalities, Terraflow + Amistim demonstrated the most pronounced effects, improving soil fertility indicators, accelerating leaf development, increasing photosynthetic efficiency, and promoting superior canopy expansion and root proliferation. These improvements were supported by stronger microbial activity, higher organic matter accumulation, and stable nutrient availability, alongside reduced levels of mobile heavy metals.

Plants treated with Terraflow + Amistim exhibited higher chlorophyll content, larger leaf dimensions, shorter leaf cycles, and earlier initiation of new leaf flushes, indicating enhanced metabolic vigor and improved carbohydrate assimilation. Glucan Tri + Amistim displayed similarly positive responses, though slightly lower than the Terraflow combination. Single-product applications produced moderate physiological and structural improvements, while the control consistently displayed inferior performance across all parameters.

Overall, the synergistic use of Terraflow with Amistim not only enhanced plant physiology and biomass development but also strengthened soil biological resilience and nutrient cycling. These findings highlight the suitability of Terraflow + Amistim as an effective biostimulant strategy to promote sustainable durian cultivation, improve long-term soil health, and support greater productivity in field conditions.

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