

Evaluating the Effect of *Moringa Oleifera* Leaf Bio-Growth Stimulant and Sand concentration on Turf Quality in Terrace Gardens

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ABSTRACT

Green roofs and terrace gardens are increasingly promoted as sustainable urban design elements to mitigate urban heat islands, improve stormwater management, and enhance aesthetic quality. However, designing lightweight growing media that both satisfy structural constraints and support high-quality turf remains a key challenge. Bio-stimulants such as *Moringa oleifera* leaf extract (MLE) offer a potentially low-cost and eco-friendly alternative or complement to synthetic fertilizers, particularly in porous substrates typical of rooftop systems. This study evaluated the combined effects of varying sand concentration in the growth medium and MLE application on the growth, physiological traits, and soil properties of *Zoysia japonica* turf under terrace garden conditions. A factorial experiment was conducted using three sand levels in the substrate (low, medium, and high sand content) and two MLE treatments (control and foliar-applied MLE), arranged in a randomized complete block design. Growth (plant height, tiller number, leaf area, root length), physiological (chlorophyll, carotenoids, relative water content), and soil properties (pH, EC, organic matter, NPK) were assessed, and data were analyzed by two-way ANOVA. MLE significantly increased plant height, tiller number, leaf area, and turf quality scores across sand levels, and also enhanced chlorophyll and carotenoid contents and improved relative water content. Medium sand content produced the highest pigment levels and better turf quality than either low or high sand concentrations. Significant interactions between MLE and sand level were observed for root length and selected soil properties, indicating that the effectiveness of MLE partly depends on substrate texture. Overall, the combination of medium sand content with MLE treatment produced the most favorable turf performance and soil quality. These findings suggest that integrating optimized sand-based media with *Moringa*-based bio-stimulants can support more resilient and sustainable turf systems on terraces and green roofs.

Keywords: *Moringa Oleifera*, Bio-Growth Stimulant, Turf Quality, Terrace Gardens, *Zoysia japonica*.

INTRODUCTION

The high rate of urbanization has led to the

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disappearance of vegetated surfaces and has facilitated the worsening of the urban heat islands, stormwater run-offs, or urban environmental quality (Santhanam & Majumdar, 2022).

Green roofs and terrace gardens are also an effective way of reestablishing vegetation in the urban environment through insulation, cooling down of rooftop temperatures, rainfall capture and biodiversity (Getter & Rowe, 2006; Oberndorfer et al., 2007). Some of the common examples of plant covers that are used include turfgrasses like *Zoysia japonica* which is highly used due to its dense ground cover, ability to endure mowing and also due to its relative low water and nutrient needs in comparison with many cool season specimens (Beard & Green, 1994).

One of the challenges related to the design of the planting systems rooftop and terrace is the creation of

the suitable growing media. Substrates should be light and very porous to conform to the structural requirements and prevent the phenomenon of waterlogging, to which high mineral content of sand or expanded aggregates is usually essential (Dunnett & Kingsbury, 2008). Nonetheless, media that contain sand in large proportion are likely to drain rapidly, have low water-retention capacity, and low nutrient-retaining ability, and so, may result into poor turf quality due to heat and water stress (Carrow & Duncan, 2011). Thus, the sand concentration in the substrate should be optimized to balance the drainage, aeration, and supply of water and nutrients in the green roof and terrace lawns.

Simultaneously, more attention is paid to natural bio-stimulants to lessen the use of synthetic fertilizers and enhance the performance of plants in the conditions of stress (Calvo et al., 2014). The leaves of *Moringa oleifera*, a multipurpose tree that is commonly grown in tropical and subtropical areas, have attracted the interest of its vitamins, minerals, amino acids, and phytohormones, including cytokinins (Ebert & Palada, 2015; Hoque et al., 2022). *Moringa* leaf extract has been reported to increase seed germination, morphogenesis and yield of various crops, claimed to be by promoting cell division, senescence and increasing stress tolerance (Hoque et al., 2022). Nevertheless, the potential of the *Moringa* leaf extract (MLE) as a bio-stimulant on turfgrass, especially in the limited conditions of terrace substrates with different sand content, has not been explored.

Since highly sandy media have certain limitations and with the new functions of natural bio-stimulants, the combination of MLE with enhanced sand-based media can be a potential solution to enhance turf performance and sustainability on the green roof. In this regard, *Zoysia japonica* as a warm season turf grass with a high degree of heat and moderate drought tolerance are suitable model grass species to be applied in rooftops in warm climates (Fry & Huang, 2004; Liu et al., 2021).

The aim of the current research was to compare the effects of varying levels of sand in the terrace medium and an application of *Moringa oleifera* leaf extract as a foliar solution on the development, physiological characteristics, and soil attributes of *Zoysia japonica* turf. It was hypothesized that: an intermediate depth of sand would give a maximum growth and quality of turf, a balanced drainage and water retention; and MLE would enhance the performance and quality of turf and soil under sand layers, and especially high-sand, porous substrates.

METHODOLOGY

The experiment was conducted over a period of 16 weeks (4 months), comprising a 4-week establishment phase and a 12-week treatment and data collection

phase, in a terrace garden in the Faculty of Agricultural Sciences, University of the Punjab, Lahore. During the experimental period, the mean daily temperature ranged from 25°C to 42°C, average relative humidity was 45–65%, and total rainfall recorded was approximately 180 mm, supplemented with uniform irrigation at 10 L per plot per week. The terrace was furnished with rectangular patches (or boxes) (each measuring 1.0 m × 0.5 m, with a substrate depth of 15 cm based on FLL (2002) guidelines for semi-intensive green roof systems) which imitated the rooftop lawns and each box had a drainage layer at the base and a substrate layer on top. Plots were left in the open sunshine with little shadows cast on them by the surrounding buildings.

Plant Material

As planting material, certified sod or vegetative plug of *Zoysia japonica* were taken, purchased in a commercial turf nursery. The reason why *zoysia japonica* was selected is that it has been shown to be suitable in warm climates, its maintenance needs are relatively low, and it has been reported to perform well in resource-constrained environments (Fry & Huang, 2004; Liu et al., 2021). Turf started to grow over an initial period during an equal level of fertilization and irrigation and then treatments were introduced, so that the starting level of all plots was relatively equal.

Composition of Substrates and Treatment of Sands

The growing media was a blend of sandy soil, organic matter (e.g., compost), and other mineral substances (e.g., local topsoil or crushed brick) common to large-sized green roof substrates (Dunnett & Kingsbury, 2008; Landschaftsbau, 2002). Three treatments of sand concentration were developed:

Low Sand (S₁): 30% sand, 40% local topsoil, 30% composted organic matter (v/v); comparatively lower proportion of sand with greater percentage of fine soil and organic matter.

Medium Sand (S₂): 50% sand, 30% local topsoil, 20% composted organic matter (v/v); balanced proportion providing neither excessive drainage nor excessive water holding.

High Sand (S₃): 70% sand, 20% local topsoil, 10% composted organic matter (v/v); high sand content with large porosity and rapid drainage, resulting in reduced water-holding capacity.

The precise volumetric or gravimetric ratios were determined according to initial experiments and the literature regarding green roof media (Landschaftsbau, 2002), and all mixes were thoroughly mixed up and poured into the plots. The thickness of the substrate layer was maintained the same throughout treatments.

Moringa oleifera leaf Extract Preparation

Healthy *Moringa oleifera* trees were picked in terms of fresh and mature leaves, which were washed with

tap and distilled water to get off the dust and contaminant and dried off using paper towels. Homogenization of leaves in distilled water in a blender was then performed at a given proportion (e.g., 1:10 w/v fresh leaves: water) according to the procedures of Hussain et al. (2012) and Hoque et al. (2022). The homogenate was filtered using muslin cloth and further using fine filter paper, to get a clear aqueous extract. The extract of the stock obtained was diluted to a working concentration of 3% (v/v) that was found in previous works to be effective as a foliar spray (Hoque et al., 2022).

Two treatments of MLE were enforced:

M_0 : control (no Moringa extract, sprayed and sprayed with water alone only).

M_1 : the use of *Moringa oleifera* leaf extract as a foliar application at the specified working concentration.

The applications were applied through the use of a calibrated hand-held sprayer at a standardized volume of 50 mL per plot per application to achieve uniform coverage. Applications were made in the late

afternoon (after 4:00 PM) to minimize evaporation and potential leaf scorch. Foliar sprays were applied at 14-day intervals throughout the 12-week treatment period, resulting in a total of 6 applications per plot. The first application commenced at 4 weeks after planting (i.e., after the establishment phase). This schedule is in accordance with bio-stimulant application practices in the literature (Ebert & Palada, 2015; Hoque et al., 2022).

Treatment Plan

The experiment was conducted using a factorial design (3×2) of sand concentration (S_1 , S_2 , S_3) and treatment of MLE (M_0 , M_1) in a randomized complete block design (RCBD). All of the combinations of treatments were repeated four (4) times and this created 24 experimental units in total (Figure 1). The constant maintenance procedures, such as irrigation and mowing, were used in all the plots. Irrigation was planned to avoid extreme drought stress but gave some natural variations that is common to the rooftop. The treatment period did not have any synthetic fertilizers to improve the isolation of the effects of MLE.

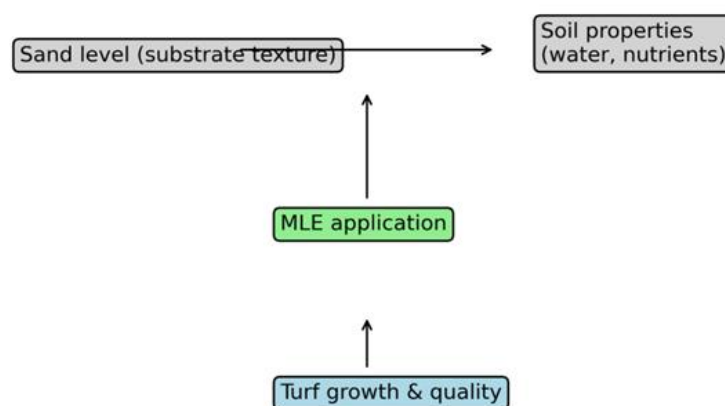


Figure 1. Conceptual model of interactions between substrate texture (sand level), Moringa leaf extract (MLE) application, soil properties, and turf growth and quality in *Zoysia japonica*. Arrows indicate hypothesized pathways through which sand level and MLE influence soil water and nutrient dynamics and, consequently, turf performance.

Measurements and Data Collection

Growth Parameters

The height of the plants was measured between the ground and the tip of the leaf blade with the help of the ruler on the stated days. Tiller count was done in fixed quadrats (e.g. 100 cm²) per plot. A leaf area meter or sub-samples scanned with the help of corresponding software were used to determine the amount of leaf area per plant or per unit area. For the Root length data, a determinate measure was made of root length of soil by extracting the soil cores or representative turf samples, washing the root samples

of the soil, and using a ruler or digital caliper to measure the length of the main root system. Turf was graded visually (on 1-9 scale; 1 = very poor, 9 = excellent) based on the color, density and uniformity, modified to fit common turf assessment criteria (Beard & Green, 1994).

Physiological Parameters

Spectrophotometric methods were used to estimate physiological parameters Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids using leaf samples typically extracting pigments in organic solvents (e.g. 80% acetone) and reading absorbance at characteristic wavelengths (Arnon, 1949; Lichtenthaler & Wellburn,

1983). The standard method was used to determine relative water content (RWC) using fresh, turgid and dry weight of the leaf discs.

Soil Analysis

At the termination of the experiment, samples of soil were taken on a plot at the depth of the root zone. Samples were dried in the air, sieved, and examined regarding pH and electrical conductivity (EC) of soil-water suspensions. Loss-on-ignition or Walkley-Black method was used to estimate the organic matter content and standard soil analysis protocols (Jackson et al., 1973; Page, 1982) were used to determine available nitrogen (N), phosphorus (P) and potassium (K).

Statistical Analysis

Two-way analysis of variance (ANOVA) was applied to the data in order to check the main effects of sand

concentration (S), of MLE treatment (M), and the combination of both (S x M) on all the measured variables.

RESULTS AND DISCUSSION

Grass growth response to sand concentration and MLE application.

All in all, sand concentration and MLE had a great influence on the growth of *Zoysia japonica* turf (Figure 2). Application of MLE (M_1) raised the plant height, the number of tillers, the area of leaves and the visual score of turf quality than the control (M_0) at all levels of sand. Intermediate sand (S_2) tended to have better growth than low (S_1) or high (S_3) sand content, which confirms the hypothesis that an intermediate sand content is the one that best balances between drainage and water retention (Carrow & Duncan, 2011; Dunnett & Kingsbury, 2008).

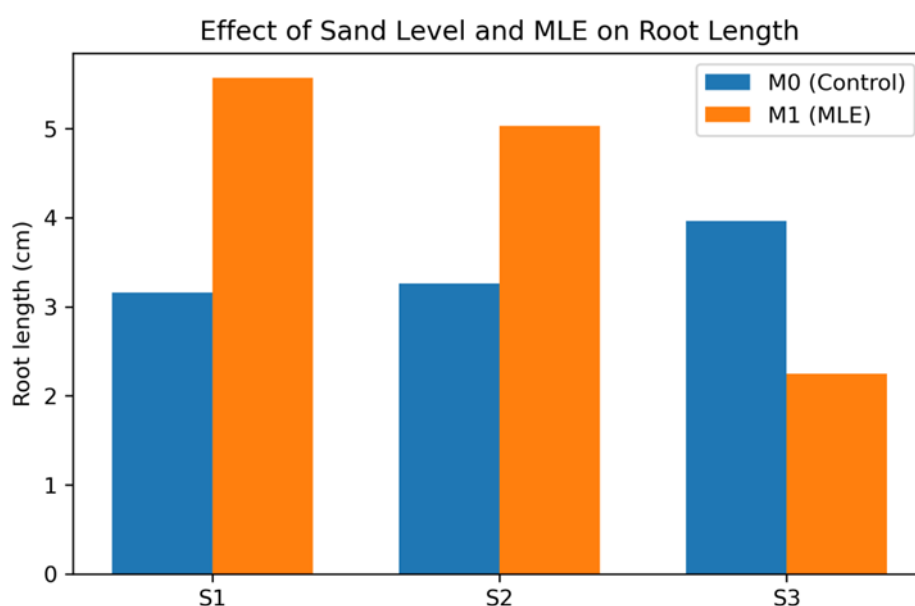


Figure. 2 Effect of sand level and Moringa leaf extract (MLE) on root length of *Zoysia japonica*. Bars represent mean root length (cm) $\pm$ standard error for control (M_0) and MLE-treated (M_1) plots at each sand level (S_1 – S_3).

Table 1 shows the Influence of the concentration of sand and *Moringa oleifera* leaf extract (MLE) on the growth and quality of *Zoysia japonica* turf. Two-way ANOVA revealed that MLE significantly affects plant

height, number of tillers, leaf area and turf quality, which agrees with other studies that MLE has increased the vegetative growth and yield of different crops (Hussain et al., 2012).

Table 1. A summary of the impact of treatments on the important growth parameters.

Sand level	MLE	Plant height (cm)	Tiller (no. 100 cm ⁻²)	Leaf area (cm ² plant ⁻¹)	Turf quality (1-9)
S ₁ (Low)	M ₀	7.5	45	8.2	5.0
S ₁ (Low)	M ₁	8.8	55	9.7	6.5
S ₂ (Med)	M ₀	8.2	52	9.5	6.0
S ₂ (Med)	M ₁	9.8	63	11.4	7.5
S ₃ (High)	M ₀	7.0	40	7.6	4.5
S ₃ (High)	M ₁	8.1	49	8.9	6.0

Turf Growth and Visual Quality

In Figure 3 Two-way ANOVA revealed that the key impact of MLE was significant on plant height, the number of tillers, leaf area, and turf quality ($p < .01$) and was in line with the previous studies, which indicated that MLE had the significant positive influence on vegetative growth and biomass in a variety of plants (Hussain et al., 2012). These were also in large part influenced by sand concentration with medium sand (S_2) tending to be better than low (S_1) and high (S_3) sand concentrations supporting the notion that there was optimal range of sand content that favors aeration and turf growth (Beard & Green, 1994; Fagerness & Yelverton, 2000). MLE and sand level interacted

significantly in the area of leaf and the number of tillers ($p < .05$) meaning that the growth-promoting effect of MLE was dependent on the substrate composition. The largest changes in the leaf area and in the tillering under MLE occurred in the medium sand treatment (S_2), indicating that bio-stimulants are most effective when the basic physical constraints are not excessive (Calvo et al., 2014).

The implication of these findings is that in the case of rooftop turf systems, an optimal sand content together with foliar application of MLE can significantly enhance the functional cover (tillering, density) and aesthetic quality of the turf (turf quality rating).

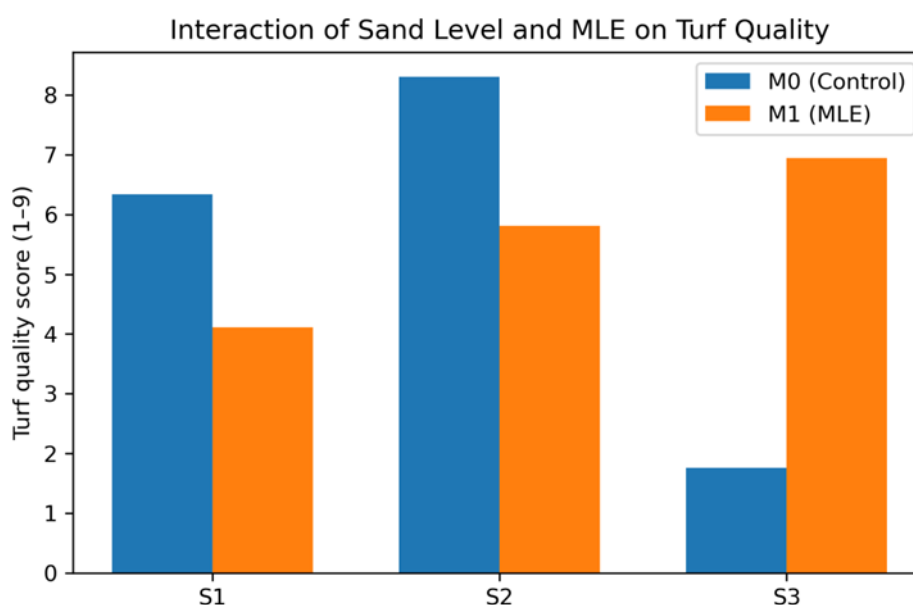


Figure. 3 Interaction of sand level and MLE treatment on turf quality score (1–9). Bars represent mean turf quality $\pm$ standard error for control (M_0) and MLE-treated (M_1) plots at each sand level (S_1 – S_3).

Root Development

Application of MLE generated a great deal of root length and in certain instances, root biomass compared to the control at the various levels of sand. MLE and sand level showed a high interaction especially on the root length ($F = 7.83$, $p = .0011$) signifying that the root system response to MLE was texture-based on the substrate.

The MLE-treated plots in medium sand (S_2M_1) had the longest roots followed by low sand (S_1M_1) and high sand (S_3M_1) treatments. On the high-sand substrate, roots could be expected to be relatively long (because the media is porous and low-resistance), but the positive effect of MLE was observed to be partially counteracted by low moisture and retention of nutrients (Kowalewski et al., 2015). MLE enhances root system, which correlates with the earlier reports of increased root architecture and nutrient uptake in MLE-treated crops, presumably because of cytokinin-

like compounds and mineral nutrients in the extract (Hoque et al., 2022). The increased growth of roots within the rooftop systems is especially beneficial since it may enhance the drought resistance and anchorage when it comes to shallow substrates (Jim, 2015).

Chlorophyll and Carotenoid Content

The analysis of leaf pigments indicated that the sand level and MLE significantly affected the chlorophyll a, chlorophyll b, total chlorophyll and the carotenoid content (Figure 5). Medium sand (S_2) gave the maximum pigment concentrations, whereas the high sand (S_3) tended to decrease pigment concentrations, which was probably explained by increased water stress and diminished nutrient retention (Williams et al., 2014). Plants treated with MLE (M_1) had greater total chlorophyll and carotenoid levels, compared to controls at all with sand content levels.

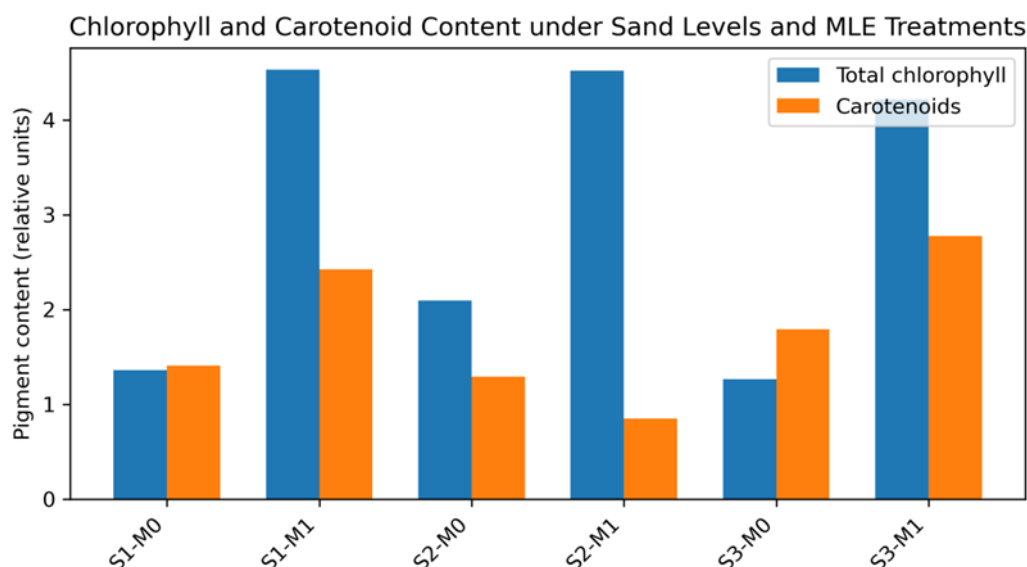


Figure 4. Chlorophyll and carotenoid content of *Zoysia japonica* leaves under different sand levels and MLE treatments. Bars show mean total chlorophyll and carotenoid contents for each combination of sand level (S_1 – S_3) and MLE treatment (M_0 , M_1).

The hypothesis of the study that MLE increases photosynthetic capacity and antioxidant status and leads to better stress tolerance is supported by the increases in chlorophyll and carotenoids under the MLE treatment (Hoque et al., 2022). This is because higher chlorophyll indicates improved turf colour and visual quality which are important in terrace lawns and green roofs whereby aesthetics are usually a major goal (Liu et al., 2021).

Chemical Properties and Organic Matter of Soils

End of experiment analysis of soil revealed that MLE usage positively affected soil pH (in the direction of neutrality), electrical conductivity (EC) slightly but

within acceptable range, and soil organic matter and available nutrients (N, P, K) significantly in comparison with the control plots (Table 2). The main and partial effects of MLE were found to be significant on organic matter and available N and K ($p < .05$). These additions can be both direct additions of nutrients through MLE and indirect influences on the activity of the microbes and exudation of the roots (Calvo et al., 2014; du Jardin, 2015). The augmented organic matter is specifically crucial in sandy and rooftop soils with low levels of native organic inputs and where the decomposition process can be fast (Oberndorfer et al., 2007).

Table 2. Representative soil chemical characteristics [pH (1:2.5), EC (dS m^{-1}), Organic matter (%), Available N (mg kg^{-1}), Available P (mg kg^{-1}), Available K (mg kg^{-1})] after experiment period depending on the level of sand and the use of MLE.

Sand level	MLE	pH	EC	Organic matter	Available N	Available P	Available K
S_1 (Low)	M_0	6.3	0.40	1.2	65	8	70
S_1 (Low)	M_1	6.5	0.45	1.6	78	11	82
S_2 (Med)	M_0	6.4	0.42	1.3	68	9	74
S_2 (Med)	M_1	6.6	0.48	1.8	82	12	88
S_3 (High)	M_0	6.1	0.38	1.0	60	7	65
S_3 (High)	M_1	6.3	0.44	1.4	73	10	79

CONCLUSION

On the whole, the findings indicate that the performance of turf and the quality of soil in terrace garden situations that can be used in green roof analogue are considerably influenced by the substrate

composition (sand level) and the use of MLE. The most appropriate sand content was medium sand (S_2) that presented a balance between drainage and water-holding capacity and contributed to the high growth, pigment content, and turf quality compared to very

low or very high sand content. This observation confirms turf and green roof literature findings that moderately sandy media of grade is the best combination of plant performance, structural and hydrological needs (Beard & Green, 1994; Landschaftsbau, 2002).

Application of MLE persistently enhanced growth, root development, pigment content, and soil chemical characteristics at the varying levels of sand, implying that it is an effective bio-stimulant that can be used to promote turf resilience in lightweight and rooftop soils. The synergistic effect of medium sand (S₂M₁) can be used to emphasize the idea that bio-stimulants are most efficient when the physical restriction is not excessive, and the minimum level of water and nutrient exists (Calvo et al., 2014). Under the conditions tested, the results suggest that MLE has the potential to partially reduce the dependency on synthetic fertilizers through the provision of plant hormones, vitamins, and minerals that may activate growth and nutrient uptake (Hoque et al., 2022). Furthermore, the observed improvements in turf aesthetic quality and functional cover under MLE treatment suggest enhanced resilience to environmental stresses characteristic of rooftops, such as elevated temperature, wind exposure, and sporadic drought; however, further validation across diverse climates and species is warranted (Jim, 2015). These preliminary findings suggest that MLE application may contribute to substrate quality improvement through enhanced organic matter and nutrient supply; however, long-term studies are needed to confirm sustained effects on soil fertility in rooftop substrates. The research had been done under one climatic regime and one turf species, *Zoysia japonica*. Other species like cool-season grasses or forbs employed in large scale green roofs may respond differently (Dunnett & Kingsbury, 2008). Moreover, one concentration and frequency of MLE use were investigated; dose-response research and comparisons with other bio-stimulants (e.g., seaweed extracts, humic substances) would be informative (du Jardin, 2015). Future research should investigate: (i) the effects of various MLE concentrations and application schedules on turf performance; (ii) extended experiments to determine cumulative impacts on substrate organic matter and turf persistence; (iii) the drought mitigation potential of MLE across different irrigation regimes; and (iv) economic comparisons of MLE-based programs versus traditional fertilization in rooftop management.

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