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EFFICACY TRIAL OF INNOVATIVE LIQUID NUTRIENT FORMULATIONS FOR KALE (*Brassica oleracea* var. *acephala* L.) PRODUCTION UNDER AGGREGATE HYDROPONIC SYSTEM

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ABSTRACT – This study was conducted to investigate the effect of liquid nutrient formulations on the horticultural characteristics, yield, chlorophyll content, total carotenoids, free radical scavenging activity, and oxidation-reduction potential of kale under aggregate hydroponic production system. The efficacy of seven nutrient solutions was evaluated using kale as test plants in a split plot randomized complete block design with the different nutrient solutions as main plot and the two varieties of kale (Kailaan and Toscana) as sub-plot. The seven treatments were composed of T1 = Visayas State University-Liquid Nutrient Formulation (VSU-LNF), T2 = Fermented Acacia (FAC), T3 = Fermented Malunggay (FMY), T4 = (T1 + T2), T5 = (T1 + T3), T6 = T4 + Effective Microorganism (EM), and T7 = (T5 + EM) replicated four times. The aggregates were composed of river sand and coconut coir in a ratio of 3:1 by volume. The pigment composition and free radical scavenging activity were done through an ultraviolet-visible spectrophotometer. Results have indicated that kale grown on VSU-LNF produced the best horticultural characteristics, yield performance, and *chlorophyll a* and *b*. The total carotenoids and free radical scavenging activity of kale were enhanced by the application of FAC and FMY. The combined application of VSU-LNF with FMY significantly influenced the free radical scavenging activity of kale. The incorporation of EM in VSU-LNF with either FAC or FMY has significantly improved oxidation-reduction potential of kale. Nevertheless, Kailaan variety of kale exhibited better horticultural and yield characteristics while Toscana indicated better postharvest qualities in an aggregate hydroponic system under Visca agro-climatic condition. These results indicate the potential of these nutrient solutions for innovative nutrient management strategy for productive and quality kale production under an aggregate hydroponic system which can be helpful in community resilience and preparedness program.

Keywords: aggregate hydroponics, free radical scavenging activity, Kale, liquid nutrient formulations, oxidation-reduction potential, pigment composition, yield

INTRODUCTION

Vegetables are short duration crops which are easily adjusted in crop diversification (Bautista and Esguerra, 2007), provide higher yield and return to resource-poor farmers. In the Philippines, vegetables contribute 8% of the total agricultural input while utilizing only 5% of the agricultural area (BAS, 2006). This means that more income can be derived from vegetables per unit area and time compared to other crops like cereals and plantation crops. The vegetable industry has shown growth

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by 13.3% in the last seven years which is about 2% per year. The total exports derived from vegetables reached about 31 M USD which include crucifers among others. However, the aggregate domestic demand for vegetables in the region is greater than the reported consumption (BAS, 2015). The problems of the local and national vegetable industry relate to production, postproduction, marketing and infrastructure. Often cited under production problem is the lack of novel information to sustain profitable yield (AARNET, 2006) under various production systems.

Kale (scientifically known as *Brassica oleraceae var acephala* L.) is one of the most important promising vegetables in the Philippines and in Asia for health and wellness. It is rich in nutrients such as vitamins and minerals (Palada and Ali, 2007), and other phytochemicals (Marxen et al., 2007; Srinivasan, 2010). It is also considered an important dietary component because it contains phytochemicals and antioxidants (Prakash, 2007; Salas et al., 2015) that reduce the risks associated with cancers, dementia, hypertension and other neurodegenerative diseases (Fang et al., 2002). These nutrients and phytochemicals play a vital role in providing nutritional security and health benefits which can alleviate hunger and malnutrition. Farmers plant vegetables as an important cash crop or for subsistence (Bautista and Esguerra, 2007). The major quality factor in kale is the roughage content or its dietary fiber which could be maintained by keeping the foliage healthy with good fertilizer and irrigation program. In addition, it adds pleasing aromatic flavor when cooked or stewed together with meat products. Moreover, vegetables are high in protein per calorie but low in calories by volume which is important for health and wellness (Grubben and Denton, 2004). Preliminary studies conducted at the Visayas State University have shown that kale can be grown in the lowlands of the Visayas region in the Philippines. Some indigenous materials such as Acacia and Malunggay (Salas et al., 2016) have been found applicable as nutrient solutions in some horticultural crops and vegetables. However, it is really difficult to grow vegetables in problematic soils. On the other hand, it has also been shown that aggregate hydroponics is capable of producing high quality and safe vegetables (Salas and Salas, 2014) particularly in areas where soil borne diseases are prevalent and obnoxious. This study aimed to (1) determine the nutrient profile of ferments derived from fermented Acacia and Malunggay, (2) investigate the horticultural characteristics and yield performance of kale applied with different nutrient solutions under aggregate hydroponic system, (3) evaluate the postharvest quality of kale in terms of its pigment composition (*chlorophyll a, chlorophyll b*, and total carotenoids), free radical scavenging activity (FRSA), and oxidation-reduction potential (ORP); and (4) assess which of the two varieties of kale would give better horticultural characteristics, yield performance, and postharvest qualities on different nutrient solutions in an aggregate hydroponic system under Visca agro-climatic condition.

MATERIALS AND METHODS

Preparation and fermentation of Acacia and Malunggay Leaves

Leaves of Acacia (*Samanea saman* (Jacq.) Merr.) and Malunggay (*Moringa oleifera* L.) were collected within the vicinity of the Visayas State University main campus, Visca, Baybay City, Leyte. The leaves were subjected to fermentation process with molasses and water in a 1:1:1 ratio by weight inside a plastic drum covered with cheesecloth. The ferments were harvested after a month and were subjected for chemical analysis. Nitrogen was analyzed through the Kjeldahl method (Black, 1965), phosphorus (Nierves and Salas, 2015) and sulfur (Chauddry and Cornfield, 2006) through an ultraviolet-visible spectrophotometry, potassium, calcium, and magnesium through an atomic absorption spectrophotometry. The ferments were diluted with water at a ratio of 1:16 by volume prior to its application as nutrient solution for kale production.

Evaluation of formulated nutrient solution

The different formulated nutrient solutions were tested on kale under an aggregate hydroponic system. The aggregate was composed of river sand and coconut coir at a ratio of 3:1 by volume. The experimental evaluation was conducted following a split plot randomized complete block design with the following treatments: T1 = VSU-LNF, T2 = fermented Acacia (FAC), T3 = fermented Malunggay (FMY), T4 = T1 + FAC, T5 = T2 + FMY, T6 =. T4 + Effective Microorganism (EM), and T7 = T5 + EM. Horticultural characteristics (such as number of leaves, length and width of leaves, and plant height) and yield performance (such as length and weight of roots, plant biomass, and total yield per plot) of kale were gathered as well as the postharvest quality in terms of its pigment composition, free radical scavenging activity, and oxidation-reduction potential.

Pigment composition of kale

About a gram of each representative sample of kale was weighed in an analytical balance, cut into small pieces, placed in glass tubes and soaked in 10 milliliter of 80% acetone for two hours. The absorbance readings of the filtrates were taken at 662, 645, and 470 nm wavelengths using an ultraviolet-visible spectrophotometer. *Chlorophylls a, b* and total carotenoids were calculated according to Dere et al. (1998).

Free radical scavenging activity of kale

A gram of each representative sample of kale was blended with 10 milliliters of 95% ethanol for a minute. The extracts were filtered and tested for its free radical scavenging activity by reacting it with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical for an hour under dark condition using an ultraviolet-visible spectrophotometer set at 517 nm wavelength (Boko and Salas, 2015). The result of the assay was expressed in percent scavenging based on the initial absorbance of DPPH.

Oxidation-Reduction potential of kale

A gram of each representative sample of kale was blended with ten milliliters of distilled water for a minute and then sonicated for another minute. The mixture was filtered and the filtrate was tested for oxidation-reduction potential using an Oakton multimeter expressed in millivolts.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the significance of results and the differences between treatments were assessed using the Tukey's Honestly Significant Difference (HSD) at 5 % level.

RESULTS AND DISCUSSION

Chemical analysis of nutrient solutions for kale production under aggregate hydroponics

Table 1 shows that the ferments derived from the leaves of acacia and malunggay were rich in most macronutrients such as nitrogen, phosphorus, potassium, magnesium and calcium. The fermented acacia, nonetheless, showed a better sulfur content than the fermented malunggay. This finding supportsthe utilization of these underutilized materials as a fertilizer source for vegetable production. Marginal farmers have to consider other ways or strategies to sustain vegetable production and profitability without spending so much on commercial fertilizer inputs. In as much as these ferments can stand alone as nutrient solution based on its chemical analysis, efficacy trial for hydroponic system of crop production is noteworthy to consider. Inorganic fertilizers, although of relatively high nutrient content, are expensive and can negatively affect our environment by destructing and acidifying the soil if managed poorly (Llegunas Jr and Salas, 2015).

Table 1. Chemical analysis of Visayas State University-Liquid Nutrient Formulation (VSU-LNF) and the ferments derived from the leaves of Acacia (FAC) and Malunggay (FMY) .

NUTRIENT SOLUTION	**CHEMICAL ANALYSIS (ppm)**					
	Nitrogen	**Phoshorus**	**Potassium**	**Calcium**	**Magnesium**	**Sulfur**
VSU-LNF	175	55.0	185	175	40	65
Fermented acacia (FAC)	6530	1220.7	348	920	710	105
Fermented malunggay (FMY)	1106	7230.0	420	515	105	57

Numerical entries of the different chemical analyses were taken from average values.

The application of some fermented plants as nutrient solutions was already noticed to enhance yield and quality of some vegetables as well as some ornamental plants (Mante and Mante Jr, 2016). Several studies had already been conducted on plant ferments to supply needed nutrients to plants and improve yield and quality specifically on tomato, sweet pepper and eggplant (Pagluanan and Anical, 2010), lettuce, melons, pakchoi and pechay (Barcenas Jr, 2015; Llegunas Jr and Salas, 2015; Salas and Salas, 2014). With these positive effects on plants, these two ferments can be used as organic nutrient solutions to pursue organic system of production. In a world full of choices, it is a serious matter to opt for organic ways that enable us to move forward in achieving sustainable agriculture without compromising our health and environment.

Horticultural characteristics of kale grown on different nutrient solutions under an aggregate hydroponic system

Table 2 shows the horticultural characteristics of kale grown on different nutrient solutions under aggregate hydroponic system. Kale receiving VSU-LNF responded the most number of leaves, widest leaves and tallest height. The leaves are primarily responsible for the photosynthetic activity of the plants. The development of greater number of leaves indicates higher photosynthetic efficiency for the growth of the plants. The VSU-LNF contains magnesium ion which is essential for the structural build-up of chlorophyll. The absorption of light is facilitated by this pigment and thus there is a strong relationship between leaf development and magnesium nutrition of the plants (Marschner, 1995). A wider leaf could intercept greater amount of light and taller plants have greater opportunity to produce more number of leaves. These results are desirable horticultural characteristics for crop production. Nevertheless**,** Toscana variety exhibited significantly greater number and longer leaves while Kailaan variety showed wider leaves at harvest. Both length and width affect the overall leaf size. From these values of leaf length and width, it appeared that those applied with VSU-LNF exhibited the greatest leaf sizes followed by those applied with fermented malunggay and combination of VSU-LNF and fermented acacia. This implies that the VSU-LNF and FMY were able to supply nutrients necessary for the vegetative growth and development of kale. This is the reason why plants grown on VSU-LNF significantly exhibited the greatest leaf area.

Leaves with greatest surface areas tend to have the best interception of light from the sun which is an important consideration for photosynthetic activity of the plant. On the other hand, FMY is a potential source of liquid nutrient fertilizer for kale production under an aggregate hydroponic system. This finding also demonstrated the utilization of cheap, local and indigenous resource as important alternative for organic fertilization.

Table 2. Horticultural characteristics of kale grown on different nutrient solutions under an aggregate hydroponic system.

TREATMENTS	PLANT HEIGHT (cm)	NUMBER OF LEAVES	LEAF LENGTH (mm)	LEAF WIDTH (mm)
Nutrient Solutions:				
T1 (*VSU-LNF*)	39.2^{a}	11.6^{a}	226.2	181.7
T2 (*FAC*)	28.3^{cd}	9.2^{bc}	183.6	118.6^{d}
T3 (*FMY*)	34.1^{ab}	10.9^{abc}	243.5	127.6^{c}
T4 (T1 + T2)	35.0^{ab}	11.0^{ab}	229.7	137.7^{b}
T5 (T1 + T3)	33.4^{bc}	10.3^{abc}	218.6	132.6^{b}
T6 (T4 + *EM*)	34.6^{ab}	10.6^{abc}	234.1	124.1^{c}
T7 (T5 + *EM*)	26.7^{d}	10.8^{abc}	222.7	133.1^{b}
CV (%)	8.0	6.1	9.7	12.6
Varieties of Kale:				
Kailaan	31.4	6.9^{b}	179.8^{b}	160.1^{a}
Toscana	35.1	13.9^{a}	252.2^{a}	102.2^{b}
CV (%)	8.5	11.3	9.1	7.6

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (HSD). VSU-LNF refers to Visayas State University-Liquid Nutrient Formulation; FAC refers to solution derived from fermented acacia leaves; FMY refers to solution derived from fermented malunggay leaves; *EM* refers to the commercial Effective Microorganism as organic supplement. Numerical entries of the different parameters were taken from average values.

Yield performance of kale grown on different nutrient solutions under an aggregate hydroponic system

Table 3 shows the yield characteristics of kale grown on different nutrient solutions under an aggregate hydroponic system. Kale grown with VSU-LNF significantly gave the longest and heaviest roots, plant biomass and consequently the highest yield per plot. This is supported by the horticultural characteristics of kale applied with VSU-LNF. A well-developed root system could afford better absorption of nutrients for plant growth and development such as number of leaves and leaf sizes. The development of vegetative parts should translate into yield characteristics. Kailaan variety of kale exhibited heaviest roots, biggest plant biomass and highest yield per plot which can be attributed to its wider leaves for a better light interception for photosynthesis. As a result, this variety of kale significantly yielded better than Toscana. In other words, Kailaan responded well to the application of inorganic nutrient solution (VSU-LNF) under an aggregate hydroponic system.

Pigment composition, free radical scavenging activity, and oxidation-reduction potential of kale grown on different nutrient solutions under an aggregate hydroponic system

Table 4 shows the average pigment composition, free radical scavenging activity, and oxidation-reduction potential of kale grown on different nutrient solutions under an aggregate hydroponic system. Highest *chlorophyll a* content was found in kale cultivated with fermented malunggay although significantly comparable with VSU-LNF, fermented acacia, combination of VSU-LNF and fermented

Table 3. Yield performance of kale grown on different nutrient solutions under an aggregate hydroponic system.

TREATMENTS	PLANT WEIGHT (g)	YIELD PER PLOT (g)	ROOT LENGTH (mm)	ROOT WEIGHT (g)
Nutrient Solutions:				
T1 (*VSU-LNF*)	153.0[a]	611.9[a]	171.0[a]	49.8[a]
T2 (*FAC*)	81.8[c]	327.1[c]	131.4[c]	34.8[ab]
T3 (*FMY*)	109.0[b]	435.9[b]	182.6a	44.6[ab]
T4 (T1 + T2)	109.3[b]	437.3[b]	163.9[b]	44.1[ab]
T5 (T1 + T3)	115.0[b]	459.9[b]	167.9[b]	43.3[ab]
T6 (T4 + *EM*)	113.4[b]	453.7[b]	158.6[b]	39.2[ab]
T7 (T5 + *EM*)	116.5[b]	469.5[b]	165.5[b]	47.2[ab]
CV (%)	10.2	9.9	12.9	14.7
Varieties of Kale:				
Kailaan	133.4[a]	509.6[a]	161.3	46.5[a]
Toscana	88.0[b]	342.9[b]	152.4	37.2[b]
CV (%)	10.3	13.3	16.9	12.9

Numerical entries of the different parameters were taken from average values. Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (HSD). VSU-LNF refers to Visayas State University-Liquid Nutrient Formulation; FAC refers to solution derived from fermented acacia leaves; FMY refers to solution derived from fermented malunggay leaves. *EM* refers to the commercial Effective Microorganism as organic supplement.

malunggay, and combination of VSU-LNF, fermented acacia and Effective Microorganism (VSU-LNF + FAC + EM). This means that the nutrients in malunggay ferment were made available for kale uptake particularly magnesium which is an important consideration for chlorophyll development. This observation also harmonizes with the result on horticultural characteristics of kale cultivated with VSU-LNF. The significant chlorophyll development of kale grown on inorganic nutrient solution could explain its yield performance. No significant difference in *chlorophyll a* between the two varieties of kale was noted. Moreover, significantly highest *chlorophyll b* was observed on kale cultivated with VSU-LNF, however, Toscana gave higher *chlorophyll b* content than Kailaan variety of kale. *Chlorophyll b* facilitates photosynthetic activity by supporting light interception by *chlorophyll* a. Toscana variety possesses curlleaves and some surfaces had been hidden or improperly aligned for optimum exposure to sunlight. This possibly explains why Toscana had lower yield than Kailaan variety of kale. The development of *chlorophyll b* is possibly a response mechanism to balance photochemical reactions that are inadequately handled by *chlorophyll a*. *Chlorophyll b* primarily absorbs blue light and is used to complement the absorption spectrum of *chlorophyll a* by extending the range of light wavelengths a photosynthetic organism is able to absorb. Both of these types of chlorophyll work in concert to allow maximum absorption of light in the blue to red spectrum. These chlorophyll molecules capture light energy, transfer it to the reaction center of the cell where electrons are passed from this absorbed light energy to water molecules for the conversion of carbon dioxide into a carbohydrate. Meanwhile, significantly highest total carotenoids were exhibited by kale cultivated with FAC and FMY and this indicates superior quality of the harvested vegetable. Acacia and malunggay plants had been found to contain tannins and terpenoids (Prasad *et al*., 2008) which could have triggered formation of carotenoids in kale. It was also noticed that kale with lower *chlorophyll b* has higher total carotenoids particularly on Kailaan variety. This suggests some important role of carotenoid in the growth and development of plants. It acts as accessory light-harvesting pigment, effectively extending the range of light absorbed by the photosynthetic apparatus.

Carotenoids perform an essential photoprotective role by quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen species formed within the chloroplast. In addition, a protective role on plants had been proposed involving the dissipation of harmful excess excitation energy (Young, 1991) under stress conditions.

Table 4. Average pigment composition, free radical scavenging activity and oxidation-reduction potential of kale grown on different nutrient solutions under an aggregate hydroponic system.

TREATMENTS	CHLOROPHYLL		TOTAL CAROTENOIDS (ppm)	RADICAL SCAVENGING (%)	REDOX POTENTIAL (Mv)
	A	B			
Nutrient Solutions:					
T1 (*VSU-LNF*)	32.6^{a}	37.7^{a}	3.8^{c}	59.5^{b}	2.27^{a}
T2 (*FAC*)	32.5^{a}	23.3^{d}	8.5^{a}	75.8^{a}	2.07^{a}
T3 (*FMY*)	32.7^{a}	24.6^{d}	8.2^{a}	75.3^{a}	2.08^{a}
T4 (T1 + T2)	28.4^{ab}	28.3^{c}	3.9^{c}	66.0^{b}	1.88^{a}
T5 (T1 + T3)	32.1^{a}	31.6^{b}	5.4^{b}	72.5^{a}	1.92^{a}
T6 (T4 + *EM*)	31.8^{a}	24.9^{d}	6.8^{b}	63.5^{c}	1.48^{b}
T7 (T5 + *EM*)	25.6^{b}	20.7^{e}	5.2^{b}	66.5^{b}	1.45^{b}
CV (%)	0.3	0.2	0.6	0.5	17.92
Varieties of Kale:					
Kailaan	29.56	19.42^{b}	7.39^{a}	65.8^{b}	2.21^{a}
Toscana	32.01	35.19^{a}	4.56^{b}	71.1^{a}	1.55^{b}
CV (%)	0.26	0.18	0.71	0.5	15.82

Means followed by the same letter do not differ significantly from one another at 0.05 level of significance (HSD). VSU-LNF refers to Visayas State University-Liquid Nutrient Formulation; FAC refers to solution derived from fermented acacia leaves; FMY refers to solution derived from fermented malunggay leaves. *EM* refers to the commercial Effective Microorganism as organic supplement.

Significantly highest free radical scavenging activity was found on kale applied with fermented acacia, fermented malunggay, and combination of VSU-LNF and fermented malunggay. The antioxidant principles in the leaves of plants (Reejo et al., 2014) might have promoted the free radical scavenging activity of kale. Protective effects such as radical scavenging can be induced by phytochemicals to maintain stability, growth and development of test plant. This had been clearly observed on Toscana variety of kale which significantly exhibited higher free radical scavenging activity. Phytochemicals such as alkaloids, flavonoids, polyphenolics, tannins and terpenoids in plants might have induced the production of antioxidant components in kale as a self-defensive mechanism against prevailing stress. Secondary metabolites and phytochemicals associated with the fermented materials had already been reported (Salas et al., 2014). This illustrated the advantage of organic ferments on this particular postharvest quality over inorganic and synthetic inputs.

Kale applied with combination of VSU-LNF, fermented acacia and effective microorganism (VSU-LNF + FAC + EM) and VSU-LNF, fermented malunggay and effective microorganism (VSU-LNF + FMY + EM) possessed the best redox potential to indicate best shelf-life and storability. The addition of effective microorganism clearly influenced the oxidation-reduction potential of harvested kale. Degradation of harvested products had been known to be affected by decomposer microbes (Benada, 2008). However, the microbial composition of EM may have balanced the various equilibria occurring in the plant system of kale. This is a very positive finding as the storability and shelf-life of harvested vegetable greatly influenced the marketability and profitability of the food commodity. Between the two varieties, Toscana had a better ORP value which means that it can afford greater opportunity to be sold in the market although it yielded lesser. This is beneficial to farmers and vegetable entrepreneurs at times when prevailing circumstances may not be favorable for equitable transaction.

The overall result of the study simply implied that horticultural characteristics and yield were determined by the nutrient availability for plant uptake. However, postharvest qualities might be influenced by other components inherent to the organic nutrient solutions from where they were derived. It is possible that the best yield and quality of kale can be produced with combinatorial formulation and application of different nutrient solutions such as the VSU-LNF, FAC and FMY.

CONCLUSION

Based on the results of the study, the following conclusions can be drawn:

1. Acacia and Malunggay ferments were rich sources of macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulfur;
2. The application of VSU-LNF produced the best horticultural and yield characteristics, *chlorophyll a* and *b*, electrical conductivity, and total dissolved solids of kale cultivated under an aggregate hydroponic system;
3. Fermented acacia and malunggay enhanced the formation of total carotenoids and free radical scavenging activity of kale;
4. The combination of VSU-LNF and FMY significantly influenced the free radical scavenging activity of kale;
5. The incorporation of EM in VSU-LNF with acacia (FAC) and malunggay (FMY) ferments significantly improved oxidation-reduction potential of Kale; and
6. Kailaan variety of kale exhibited better horticultural and yield characteristics while Toscana indicated better postharvest qualities in an aggregate hydroponic system under Visca agro-climatic condition.

RECOMMENDATIONS

The following recommendations are hereby suggested for future directions:

1. The utilization of VSU-LNF as nutrient solution for hydroponic kale production should be promoted for technological adoption;
2. Organic nutrient solutions such as EM, FAC and FMY can be incorporated with inorganic nutrient solution like VSU-LNF to improve shelf-life, storability, free radical scavenging activity, and total carotenoids;
3. Combinatorial formulation and application of VSU-LNF, FAC, FMY and EM should be done on horticultural vegetables for optimum yield and postharvest quality; and
4. Standardization and development of low-cost and environment-friendly innovative nutrient solutions be made available in the market.

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STATEMENT OF AUTHORSHIP

The first author initiated the concept, prepared the conceptual framework, identified thematic points, analyzed postharvest qualities of kale, formulated recommendations, and undertook the writing up. The other authors identified some issues, did literature search, conducted the field experiment, gathered data on horticultural characteristics and yield components, formulated recommendations, and reviewed the paper.

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