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ARTICLE

Biostimulants improve growth, yield and quality of *Eustoma grandiflorum* L. and *Matthiola incana* L.

Os bioestimulantes afetam o crescimento, a produção e a qualidade de *Eustoma grandiflorum* L. e *Matthiola incana* L.

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Abstract: Lisianthus (*Eustoma grandiflorum* L.), a member of family Gentianaceae, and stock (*Matthiola incana* L.), a member of family Brassicaceae, are extensively grown as cut flowers globally and are also gaining popularity in Pakistani markets in recent years. This study was aimed at evaluating the efficacy of selected biostimulants on improving the growth, yield, and quality of lisianthus and stock at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, during 2022-2023. The experiment was laid out individually for each species to elucidate the effects of three different products, viz. Isabion (3 mL L^{-1}), Humic acid (0.4%) and Corteva XYZ (3 mL L^{-1}). Both experiments were laid out in randomized complete block design (RCBD) with three replications of 20 plants each. Biostimulants were applied at 3 mL L^{-1} Isabion and Corteva XYZ, while 0.4% humic acid, which were sprayed three times at fifteen days interval until runoff on each species starting after fifteen days of transplanting. Results demonstrated significant differences among treatments for both *Eustoma grandiflorum* L. and *Matthiola incana* L. Lisianthus utilized least production time (59 days) when sprayed with Corteva XYZ, while in stock Isabion application produced earlier flowers (75.6 days) compared to other tested treatments. Isabion application significantly increased plant height (60.3 and 79.6 cm), floret diameter (39.8 and 4.2 mm), stem diameter (4.0 and 6.1 mm), stem fresh weight (77.6 and 86.1 g), stem dry weight (16.9 and 15.1 g) and vase life (8.8 and 7.5 days) of lisianthus and stock, respectively. Greatest leaf area (27.7 and 32.9 cm^2) was recorded when plants were supplied with Corteva XYZ for lisianthus and stock, respectively. Greatest chlorophyll contents were recorded (75.3 SPAD) in lisianthus, while (81.6 SPAD) in stock, when sprayed with Isabion and humic acid, respectively. Isabion consistently yielded best results indicating its potential as an effective biostimulant for promoting growth, yield, and quality attributes, and may be used by the growers to enhance yield and quality of selected specialty cut species.
Keywords: antioxidants, foliar spray, humic acid, Isabion, postharvest longevity, stem elongation.

Resumo: O lisianthus (*Eustoma grandiflorum* L.), membro da família Gentianaceae, e o goivo (*Matthiola incana* L.), membro da família Brassicaceae, são amplamente cultivados como flores de corte e estão ganhando popularidade nos mercados paquistaneses nos últimos anos. Este estudo teve como objetivo avaliar a eficácia de bioestimulantes selecionados na melhoria do crescimento, rendimento e qualidade de lisianthus e goivo na Floriculture Research, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, durante os anos de 2022-2023. O experimento foi estabelecido individualmente para cada espécie para elucidar os efeitos de três produtos diferente, sendo eles: Isabion (3 mL L^{-1}), ácido húmico (0,4%) e Corteva XYZ (3 mL L^{-1}). Ambos os experimentos foram dispostos em delineamento de blocos casualizados completos (RCBD) com três repetições de 20 plantas cada. Os bioestimulantes foram aplicados na dose de 3 mL L^{-1} de Isabion e Corteva XYZ e de 0,4% de ácido húmico, os quais foram pulverizados três vezes em intervalos de quinze dias até a colheita de cada espécie, começando quinze dias após o transplantio. Os resultados demonstraram diferenças significativas entre os tratamentos para *Eustoma grandiflorum* L. e *Matthiola incana* L. Lisianthus obteve o menor tempo de produção (59 dias) quando pulverizado com Corteva XYZ, enquanto a aplicação de Isabion em goivo produziu flores mais cedo (75,6 dias) em comparação com outros tratamentos testados. A aplicação de Isabion aumentou significativamente a altura da planta (60,3 e 79,6 cm), diâmetro da flor (39,8 e 4,2 mm), diâmetro do caule (4,0 e 6,1 mm), peso fresco do caule (77,6 e 86,1 g), peso seco do caule (16,9 e 15,1 g) e vida de vaso (8,8 e 7,5 dias) de lisianthus e goivo, respectivamente. A maior área foliar (27,7 e 32,9 cm^2) foi registrada quando as plantas foram fornecidas com Corteva XYZ para lisianthus e goivo, respectivamente. Os maiores teores de clorofila foram registrados (75,3 SPAD) em lisianthus, enquanto (81,6 SPAD) em goivo, quando pulverizado com Isabion e ácido húmico, respectivamente. Isabion consistentemente produziu os melhores resultados, indicando seu potencial como um bioestimulante eficaz para promover o crescimento, o rendimento e os atributos de qualidade, e pode ser usado pelos produtores para aumentar o rendimento e a qualidade de espécies de corte especiais e selecionadas.
Palavras-chave: ácido húmico, alongamento do caule, antioxidantes, isabion, longevidade pós-colheita, pulverização foliar.

Introduction

Floriculture is experiencing rapid growth and intense competition on a global scale. (Yildrim et al., 2021). Nowadays, floriculture has gained recognition as a lucrative business, shifting the focus of growers towards high-value flower crops. Europe dominates in sales of cut flowers and ornamental potted plants with 31.0% share followed by China (18.6%), and the United States (12.5%) (Darras, 2021).

The lisianthus (*Eustoma grandiflorum* L.) is a native of the warm regions of the Southern United States, where it is primarily grown as a cut flower (Harbaugh, 2006). Cultivating lisianthus can be a rewarding but challenging endeavor as the plant has specific requirements for optimal growth. Lisianthus thrives in full sun or partial shade and can be grown both outdoors and indoors. Stock (*Matthiola incana* L.) is a Mediterranean plant (winter annual) that has been cultivated since the 16th century. Due to its stem length and quality raceme, it is used in landscape and as a cut flower. Most of the cultivars either have single or double florets (El-Ghait et al., 2022). Biostimulants are naturally organic metabolic inducers which stimulates plant developmental stages, induce stress resistance and enhance nutrient use efficiency. Cuticle absorption of nutrients in the plant vascular system and ion uptake are both influenced by application mode (Fernandez et al., 2021). Foliar application of biostimulants is more beneficial to boost up growth, quality, mineral use efficiency and change the soil texture (Niu et al., 2021).

Indiscriminate use of chemical fertilizers has led to negative impacts on the environment. To mitigate this, biostimulants have emerged as a supplement to mineral fertilizers and hold a promise to improve the yield as well as quality of the crop under protected conditions. Biostimulants are a rich source of vitamins, poly-oligosaccharides, plant growth regulators (PGRs), and humic acid. These diverse biostimulants work to mitigate the impact of biotic and abiotic stresses, ultimately enhancing the

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physiological processes in plants. Biostimulants are used in horticultural crops like flowers, fruits and vegetables. Plant extracts, humic substances, hydrolyzed proteins, microorganisms and some inorganic substances also perform biostimulant action (Roupheal and Colla, 2020; Franzoni et al., 2022). Seaweed extracts, fulvic and humic acids, protein hydrolysates, and chitosan are instances of non-microbial biostimulants. (Eiansary et al., 2020). In order to boost plant growth and development in a synergistic way, formulated biostimulant products frequently incorporate both microbial and non-microbial components collectively with multiple active chemical substances (Lin et al., 2022). Therefore, it is need of the time to use alternative of chemical fertilizers that are not as harmful as chemical fertilizers to the soil and environment and have better effect on crop production. However, there is quite limited research on the impact of biostimulants on the growth and yield of flower crops particularly, lisianthus and stock to produce high quality stems and to maintain their quality after harvest. This research aimed to examine the effectiveness of different biostimulants in improving the growth, yield, and quality of *Eustoma grandiflorum* L. and *Matthiola incana* L. It was hypothesized that the application of different biostimulants would improve the growth, yield and quality of lisianthus and stock by improving nutrient uptake, increasing photosynthesis, enhancing tolerance to biotic and abiotic factors and improving plant health to produce best quality cut flowers and also would lower the use of traditional chemical fertilizers to lower cost of production and enhance profit margins.

Materials and Methods

The present study was conducted at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, during 2022-23, to evaluate the efficacy of biostimulants on the quality production of lisianthus (*Eustoma grandiflorum* L.) and stock (*Matthiola incana* L.). Different biostimulants, viz., Corteva XYZ, humic acid and Isabion (an amino acid based biostimulant product marketed by Syngenta), were compared to improve the growth, yield, nutrient uptake and quality attributes of lisianthus and stock. Seeds were imported from Pan American Seeds (USA), and transplants were raised in 128 cell plastic plug trays containing peat moss, UAF Gro, and coco coir (1:1:1; v/v/v) as growing substrate. Seedlings were kept in a greenhouse under polythene tunnels at 25 ± 3 °C temperature with 60% - 70% relative humidity. Soil was thoroughly pulverized, leveled and laid out. A basal dose of granular fertilizer Nitrophos NP (23:23), and K (50) kg ha^{-1} was applied during soil preparation. Before transplanting a pre-emergence herbicide, viz. Dual Gold® 5 mL L^{-1} was applied to control weeds. At 2 - 4 true leaf stage, healthy seedlings were transplanted into thoroughly prepared soil on raised beds at a spacing of 15 × 15 cm for both species, lisianthus and stock. Experiments were laid out in a randomized complete block design (RCBD) individually for each species with three replications of 20 plants each. All other cultural practices, like fertilization, irrigation, weeding, integrated pest management (IPM), etc. were similar for all treatments during the entire period of study. Biostimulants, viz., Corteva XYZ (3 mL L^{-1}), humic acid (0.4%) and Isabion (3 mL L^{-1}), were applied in the form of foliar sprays individually, three times each, at 15-day intervals after transplanting these seedlings.

Data collection

Data were recorded at harvest on production time (days) by counting the days from the transplanting date to the first flower/floret opening, which was the time to harvest the first marketable stem. Ten plants from each replication were randomly selected and their average was computed. Lisianthus stems were harvested when 2 - 3 flowers were open, while stock stems were harvested when lower 5 - 6 florets open stage. Plant height (cm) was measured using a meter rod, which was positioned from the base of the plant to the top of the plant/raceme. The resulting measurements were recorded in centimeters. The number of leaves per stem (n°) was counted at the time of harvest, while stem diameter (mm) and flower/floret diameter (cm) of both species were measured from 15 cm above the ground level and fully opened flower/florets using digital caliper (Electronic digital vernier caliper 150 mm 6"). Fresh weight and dry weight (g) of a stem was recorded using electric weighing balance (Model; HK-DC-320 AS). Leaf area (cm^2) of fully matured young leaves of *Eustoma grandiflorum* and *Matthiola incana* was measured by recording length and maximum breadth from center of leaf with meter rod and leaf area (cm^2) was calculated using following formula (Carleton and Foote, 1965):

Leaf area (cm^2) = Length x Maximum breadth x 0.68 (Constant factor)

Data for leaf total chlorophyll contents (SPAD) were collected using chlorophyll meter (at leaf FT+ GREEN LLC USA). A mature leaf from the middle of the stem (30 cm from ground level) was selected and inserted between the pit knob of chlorophyll meter, three readings were taken from the single leaf and their average was computed. Flower quality of *Eustoma grandilflorum* and *Matthiola incana* was assessed on a rating scale of 1 to 9, where 9 = best quality, 5 = medium quality and 1= poor quality stems (Cooper and Spokas, 1991) by three independent judges and their averages were calculated. The length of the raceme was measured when stems were ready for harvest, using a meter rod exclusively during the harvest period for the stock. The number of marketable stems were counted on the basis of flower quality and stem length of lisianthus. Ten stems were recut from both species at uniform stem length, placed in Commercial Floriculture Laboratory at 22 ± 2°C temperature in distilled water until termination and vase life (days) was recorded. Stems were considered ready to terminate when they exhibited >50% of any termination symptoms such as stem necrosis, leaf wilting, stem bending and petal wilting.

Statistical analysis

The data were analyzed statistically using the analysis of variance (ANOVA) technique, following Fisher's analysis of variance (Statistix 8.1). Treatment means were compared using the Least Significant Difference (LSD) test at $p \leq 0.05$ (Steel et al., 1997).

Results and Discussion

Growth Indices

Plants supplied with 3 mL L^{-1} Isabion produced stems with the tallest plant height (60.3 cm) for lisianthus and with Corteva XYZ, for stock (88.7 cm). The least production time was recorded for lisianthus (59 days) when sprayed with 3 mL L^{-1} Corteva XYZ and for stock (75.6 days) with 3 mL L^{-1} Isabion. These findings are in line with the impact of *Ascophylum nodosum* biostimulant at 5 mL L^{-1} of water, sprayed on (*Dendranthema grandiflora* Tzvelev.), resulted in tallest plants (72.1 cm), greatest flower diameter (7.48 cm) and overall plant development (Suchitha, et al., 2023). Better plant height might be due to increased nutrients and water uptake under the influence of biostimulants and in turn, improving nutrient metabolism of the plant system. Polyamines, applied exogenously or synthesized endogenously, can have beneficial impact on plant growth, productivity and stress resilience (Tyagi et al., 2023). Furthermore, as the concentration of Isabion increased, there was a corresponding increase in height of the 'Cheerful White' stock plants (Ali et al., 2022). The active components present in bio-fertilizers are microorganisms, which play a crucial role in facilitating the synthesis of organic compounds and other biotic substances essential for plant growth, development and quality (Ali et al., 2022). Highest stem fresh weight (77.6 g) was recorded in lisianthus when sprayed with 3 mL L^{-1} Isabion while, (86.6 g) was recorded in stock when plants were supplied with 3 mL L^{-1} Corteva XYZ. Statice (*Limonium sinuatum* L.) plants when supplied with biostimulants application enhanced plant fresh and dry weights along with the highest root weight. A study on combined effect of vermicompost and bioslurry resulted in the tallest quality stems compared to control (Nyakeyo et al., 2023).

Yield Indices

While longest production time (79.4 and 90.1 days) was recorded where no biostimulant (water spray) only application was carried out (Fig. 1). These results are similar with findings that production time for *Salvia splendens* and *Matthiola incana* plants was reduced when plants were sprayed with biostimulants rather than the plants were where no additional nutrient were applied (Yildrim et al., 2021). Greatest flower diameter (39.8 and 4.2 mm) was recorded when plants were supplied with 3 mL L^{-1} Isabion for lisianthus and stock, respectively. These results are similar with studies on gerbera stems when sprayed with commercial biostimulant (seaweed), resulted in significant improvements in flower diameter, number of leaves, and stem size. Foliar application of humic acid at 350 ppm in gladiolus and 1% in China aster resulted

in bigger corm size, increased plant vegetative and reproductive growth in gladiolus and China aster, respectively (El-Kot et al., 2020; Vidya et al., 2022). Amino acids present in Isabion encourage, plant development and growth by improving assimilation of enzymes (Trejo et al., 2018). Total leaf chlorophyll contents (60.8 and 80.8 SPAD) and number of buds (20.1 and 12.0) in lisianthus and stock were highest when sprayed with 3 mL L^{-1}. Corteva XYZ and Isabion for lisianthus (Table 1) and for stock (Table 2), respectively. The effect of izomen and humus as biostimulant resulted in highest plant height, number of leaves and leaf chlorophyll contents in freesia flowers (Abdulla, 2019). Highest stem fresh weight (77.6 and 86.7 g) and stem dry weight (16.9 and 22.6 g) were recorded when plants were sprayed with 3 mL L^{-1} Isabion for lisianthus and Corteva XYZ, each for lisianthus and stock, respectively. Stem fresh weight and dry weight of scarlet sage significantly increased by the application of biostimulants (Zeljković et al., 2010). As biostimulants produced positive results by enhancing plant metabolic activities and nutrient uptake which in sequence increase stem dry weight (Zeljković et al., 2023). Data regarding number of flowers in lisianthus had no significant differences and averaged 1.9. For stock, greatest raceme length (17.9 cm) was recorded when 3 mL L^{-1} Corteva XYZ was sprayed followed by 0.4% humic acid (16.4 cm). Gladiolus plants when supplied with amino acids, produced greatest number of florets than the plants supplied with no additional nutrient. Marigold plants when applied with amino acid and farmyard manure produced flowers earlier compared to control. Algal extracts of *Ascophyllum nodosum* may be used as natural biostimulator on marigold for improving seed germination, root growth and seedling development, while fish amino acids improved flowering sand yield of marigold (Tavares et al., 2020; Sivasankar et al., 2021).

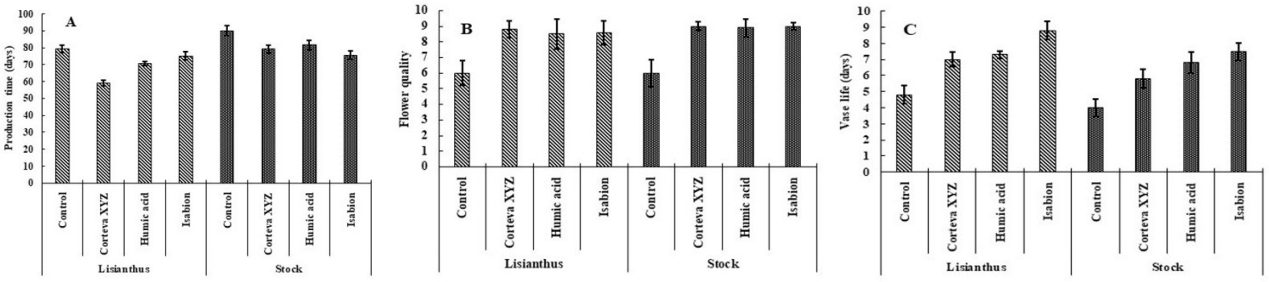

Fig. 1. Effect of biostimulants on production time (A), flower quality (B) and vase life (C) of lisianthus and stock. Bars represents means of 30 plants ± S.E.

Table 1. Effect of biostimulants on plant height (cm), floret diameter (mm), leaf area (cm^2), stem diameter (mm), leaf total chlorophyll contents (SPAD), buds (no.), stem fresh weight (g) and stem dry weight (g) of *Eustoma grandiflorum* L. Data represent means of 30 plants.

Treatments	Plant height (cm)	Flower diameter (mm)	Leaf area (cm^2)	Stem diameter (mm)	Leaf total chlorophyll contents (SPAD)	Buds (n^o)	Stem fresh weight (g)	Stem dry weight (g)
Control (water spray)	37.0 d[z]	26.1 d	11.2 c	3.8 bc	53.2 c	10.2 c	42.5 c	11.2 b
Corteva XYZ (3 mL L^{-1})	49.4 c	36.1 a	27.7 a	4.1 a	60.8 b	20.1 a	51.4 b	12.8 b
Humic acid (0.4%)	51.6 b	30.6 c	22.8 b	3.6 c	58.0 bc	13.6 b	52.5 b	12.9 b
Isabion (3 mL L^{-1})	60.3 a	39.8 a	26.2 ab	4.0 ab	75.3 a	19.9 a	77.6 a	16.9 a
Significance[y]	<0.0001	0.0003	<0.0001	0.0199	0.0008	0.0003	<0.0001	0.0003

[z]Means separation within columns by Fisher's LSD at $p < 0.05$.

[y]P values were obtained using general linear model (GLM) procedures of statistix (version 8.1) for significant effects of biostimulants on growth, yield and quality of lisianthus.

Table 2. Effect of biostimulants on plant height (cm), floret diameter (mm), leaf area (cm^2), stem diameter (mm), leaf total chlorophyll contents (SPAD), buds (no.), stem fresh weight (g) and stem dry weight (g) of *Matthiola incana* L. Data represent means of 30 plants.

Treatments	Plant height (cm)	Flower diameter (mm)	Leaf area (cm^2)	Stem diameter (mm)	Leaf total chlorophyll contents (SPAD)	Buds (n^o)	Stem fresh weight (g)	Stem dry weight (g)
Control (water spray)	38.5 c[z]	2.2 c	16.9 c	3.7 c	51.6 d	6.2 c	43.4 b	15.9 b
Corteva XYZ (3 mL L^{-1})	88.7 a	3.5 b	32.9 a	4.3 bc	73.8 b	8.3 b	86.7 a	22.6 a
Humic acid (0.4%)	77.7 b	4.3 a	28.2 a	4.9 b	64.8 c	12.0 a	81.6 a	20.4 a
Isabion (3 mL L^{-1})	79.6 b	4.2 a	32.2 b	6.1 a	80.8 a	12.0 a	86.1 a	15.1 b
Significance[y]	<0.0001	<0.0001	<0.0015	<0.0028	<0.0001	0.0002	<0.0001	0.0015

[z]Means separation within columns by Fisher's LSD at $p < 0.05$.

[y]P values were obtained using general linear model (GLM) procedures of statistix (version 8.1) for significant effect of biostimulants on growth, yield and quality of stock.

Application of biostimulants resulted in highest number of flowers and leaves in French marigold plants by improving the development and blooming characteristics of the aboveground mass of the plant (Zeljkovič et al., 2023). Best flower quality (8.8 and 9.0) was recorded when 3 mL L^{-1} Corteva XYZ or Isabion were applied for lisianthus and stock, respectively. Use of bio-fertilizers and algae extract resulted in tallest and best quality cut freesia stems (AL-Shareefi et al., 2019; Bhupenchandr et al, 2020). Moreover, longest vase life (8.8 and 7.5 days) was recorded for stems treated with 3 mL L^{-1} Isabion, for both lisianthus and stock, respectively. Plants treated with water spray (control) exhibited stunted growth with shorter poor-quality stems having shortest flower diameter (26.1 and 2.2 mm), leaf area (11.2 and 16.9 cm^2), lowest number of buds (10.2 and 6.2), least chlorophyll contents (53.2 and 51.6 SPAD) and shortest vase life (4.8 and 4.0 days), for lisianthus and stock, respectively.

Biostimulants mostly enhance seed and transplant vigor, stimulate vegetative growth, improve nutrient acquisition and antioxidative capacity of plant tissues, contributing to higher stress tolerance and improved plant yield and flower quality (Shahrajabian et al., 2021).

Quality Indices

Longest vase life (8.8 and 7.5 d) was recorded in plants sprayed with Isabion at 3 mL L^{-1} and humic acid 0.4% for lisianthus and stock, respectively. The results are in line with Yildrim et al. (2021) who reported that foliar application of 0.2% humic acid increased the postharvest quality characters, the longevity of cut spike in tap water and in 2% sucrose were greatly influenced by the same treatment. Combined effect of bioslurry and vermicompost resulted in longest vase life (22.9 days) compared to control (12.2 days) in Limonium flower (Nyakeyo et al., 2023). Growth and quality of geranium was improved by using moringa leaf extract (MLE) compared to control (Ali et al., 2018). Begonia seeds treated with Radifarm promoted plant growth and nutrient uptake in leaves. Combined impact of bio-fertilizer with spermine enhanced flower quality and neck girth in gerbera. PGRs alongwith amino acids like gibberellins enhanced flower stalk length and vase life of cut gerbera stems (Giri and Beura, 2021). In African marigold, vegetative parameters, chemical composition and floral traits were attained by applying humic and gibberellic acid (Alziyituni, 2023). Biostimulants when applied to plants assist in rapid seedling emergence in directly seeded crops by overcoming transplanting shocks (Prisa, 2024). Accumulation of phenolic compounds in lettuce leaves also increased by foliar application of pig blood derived protein hydrolates to plants at different growth stages (Wadas and Dziugiel, 2020; Zhou et al., 2022). Humic and fulvic acids also assist in promoting photosynthesis, amino acids, carbohydrates, protein, nucleic acid synthesis and enzyme activities and iron uptake and growth when applied on foliage cuticle by sprays but their overdose may cause root problems and stunted growth (Yucel et al., 2020; Kisvarga et al., 2022). Market for plant biostimulants is expanding rapidly. These compounds have a number of beneficial effects, such as boosting nutrient intake, accelerating photosynthesis, regulating the synthesis of plant hormones, improving plant tolerance to abiotic stress, and boosting crop quality and yield (Shahrajabian et al., 2023; Pathania et al., 2023).

Conclusions

Foliar application of 3 mL L^{-1} of Isabion performed best for enhancing growth and yield of both lisianthus and stock and may be used for ensuring high quality sturdy stem production of *Eustoma grandiflorum* L., and *Matthiola incana* L. commercially.

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Author Contribution

IA: Conceptualization, Methodology, Supervision, Writing – Review & Editing. **HA**: Data Curation, Investigation, Writing – Original Draft. **JMD**: Methodology, Project Administration, Writing – Review & Editing. **TH:** Formal Analysis, Software, Writing – Original Draft. **HS:** Validation, Writing – Review & Editing. **AJ:** Validation, Writing – Review & Editing.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

All the the research data is contained in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to edit the text of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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