

RESEARCH ARTICLE

HUMIC AND FULVIC ACID-BASED BIOSTIMULANT ENHANCES RESILIENCE OF BOK CHOY (*BRASSICA RAPA* SUBSP. *CHINENSIS* (L.) HANELT) UNDER SEVERE DROUGHT STRESS

Nik Nur Izzah Atirah Binti Nek Ramlan^a, Christina Seok Yien Yong^{a*}, Azrul Afiq Bin Azmi Murad^a, Chin Kam Ngui^b, Kee Wee Ng^b

^a Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

^b Advansia Seeds and Biotech Pte Ltd, Jalan Sungai Pinang 5/7, 42920 Port Klang, Selangor, Malaysia.

*Corresponding Author Email: chrisyong@upm.edu.my

This is an open access article distributed under the Creative Commons Attribution License CC BY 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ARTICLE DETAILS

Article History:

Received 26 January 2026
Revised 20 February 2026
Accepted 25 February 2026
Available online 06 March 2026

ABSTRACT

Drought is one of the major abiotic stresses impacting global agricultural productivity, and leafy vegetables are among the most vulnerable crops. In this study, we evaluated the effectiveness of a humic and fulvic acid-based biostimulant in enhancing the resilience of a widely consumed vegetable, bok choy (*Brassica rapa* subsp. *chinensis*) under drought condition. Humic and fulvic acid biostimulant-treated plants (AR10R1 group) and -untreated control plants (NT10R1 group) were subjected to a simulated prolonged severe drought condition in greenhouse setting. Growth metrics including shoot and root growth, number and size of leaves, and wet and dry biomass of above and below ground parts were quantified. The survival and recovery rates of the experimental plants were also assessed. Our results revealed significant differences ($p < 0.05$) in nearly all plant growth metrics, as well as the survival and recovery outcomes between the two groups. The AR10R1 group showed superior growth, greater biomass accumulation, and substantially improved survival rate compared to the NT10R1 group under drought condition. Remarkably, the AR10R1 plants exhibited a recovery rate ten-fold higher than that of the NT10R1 plants. The biostimulant was applied before commencement of the drought experiment. This suggests that the biostimulant has a long-lasting effect, as it was able to alleviate drought-induced damage in the AR10R1 plants, enhancing their survival throughout the prolonged severe drought condition (10% soil moisture level for 20 days), and subsequently their recovery during the rehydration stage. Our study provides useful insights into the efficacy of humic and fulvic acid biostimulants in mitigating drought-induced stress in bok choy. We believe the integration of humic and fulvic acid-based biostimulant in agriculture is a promising and sustainable strategy to mitigate drought-induced yield losses in bok choy production, and it can potentially be optimized to enhance resilience in other leafy crops in the face of climate change.

KEYWORDS

Climate change, pak choy, post-drought recovery, survival rate, sustainable agriculture

1. INTRODUCTION

Climate change intensifies the frequency and severity of drought events globally, particularly in tropical regions. The El Niño Southern Oscillation is one of the most significant drivers of annual global climate volatility. It causes dramatic precipitation shifts in many parts of the world leading to drought events in different regions, but most intensely in the tropics, especially South Asia and Southeast Asia, threatening food security and the livelihood of millions of people in the regions (World Health Organization, 2023). Approximately 15 to 25% of Southeast Asia's population resides in drought hotspots, including Malaysia (Economic and Social Commission for Asia and the Pacific, 2020; Luhaim et al., 2021).

Malaysia suffers a series of severe drought events recurrently associated with El Niño. Between 1986 to 2016, Malaysia has experienced 12 El Niño events of various gravities (Khor et al., 2021). In 1997/98, the El Niño event brought prolonged dry conditions and reduced rainfall across the country, which had led to significant deficits in rice production necessitating the importation of over a million tons of rice to mitigate food shortages at the time (Amin and Alam, 2016). The oil palm plantation suffered notable yield losses due to strong El Niño events that happened in 1997/98 and 2015/16 (Khor et al., 2023). In the 2023/2024 El Niño

incident, the dry weather caused critical water shortages and heatwave in Malaysia, adversely affecting the yields of rice. Consequently, paddy production in Malaysia fell by eight percent below the average (Food and Agriculture Organization of the United Nations, 2025). These events highlight the vulnerability of the agricultural sector to climate change, and the significant impact of drought on food security and economy.

Drought stress disrupts essential biological processes, and elicits negative morphological and physiological changes in plants (Seleiman et al., 2021). Agricultural drought is typically caused by meteorological drought, which results in inadequate precipitation. The lack of rainfall leads to dry soil, crop damage, and water shortages. Consequently, it reduces plant growth, crop productivity and agricultural yields (Adunya and Benti, 2020). Water constitutes 80-95% of plant biomass and plays vital roles in numerous physiological processes crucial for plant growth, development, and metabolism (Brodersen et al., 2019). Hence, water availability and plant's ability to uptake water are fundamentally important for its survival, particularly under drought conditions. Furthermore, the capacity of plants to mitigate drought-induced oxidative stress or damage is essential for their survival and subsequent recovery.

Many approaches have been used to reduce the effects of drought on agricultural crops, which include the adoption of water-saving irrigation

Quick Response Code



Access this article online

Website:
www.mjsa.com.my

DOI:
10.26480/mjsa.02.2026.54.61

and management such drip irrigation and water harvesting, implementation of soil conservation practice such as cover crops and minimum tillage, and the development of drought-tolerant varieties or planting of drought-tolerant species. In recent years, there has been a growing interest in improving plant growth and drought resilience sustainably through the application of biostimulants (Franzoni et al., 2022; Papa et al., 2022). According to the European Biostimulants Industry Council (2019), biostimulants are generally defined as substances and/or microorganisms that can enhance nutrient uptake efficiency, abiotic and biotic stress tolerances, and quality of crops, regardless of their nutrient content. They are typically classified into two types, microbial and non-microbial. A microbial plant biostimulant encompasses a single microorganism or a consortium of microorganisms (Castiglione et al., 2021); while non-microbial biostimulants include humic and fulvic acids, protein hydrolysate, seaweed extract, biopolymers, and inorganic compounds (Francesca et al., 2021; Jacomassi et al., 2022).

Humic and fulvic acids provide many agronomic benefits by improving soil health, stimulating root growth and enhancing nutrient uptake in plants. Studies have demonstrated positive correlations between plant growth and the application of humic and fulvic acids in crops such as barley, broccoli and yarrow (Alsudays et al., 2024; Ibrahim et al., 2024; Bayat et al., 2021). In addition, studies have also reported the potential of humic acid and fulvic acid in supporting plant growth under drought conditions, including in tomato, foxtail millet and tea tree (Aytac et al., 2024; Shen et al., 2020; Sun et al., 2020). These studies highlight the potential of humic acid and fulvic acids to enhance plant resilience. However, there is still a paucity of scientific evidence concerning its effects on leafy crops, which are highly vulnerable to drought conditions.

In this study, we investigated the effects of a humic and fulvic acid-based biostimulant in sustaining growth and enhancing resilience of bok choy (*Brassica rapa* subsp. *chinensis* L.) exposed to a simulated prolonged severe drought condition in a greenhouse setting. We report the effects of humic and fulvic acid-based biostimulant on the above- and below ground growth metrics of bok choy including shoot growth, root growth and plant biomass. We also evaluated the survival of the plants during and after drought. We further accessed the recovery rate of drought-stressed plants following a rehydration treatment.

2. MATERIAL AND METHODS

2.1 Experiment Location and Condition

The greenhouse pot experiment was conducted at the Department of Biology, Faculty of Science, Universiti Putra Malaysia (3°00'05.0N, 101°42'17.8E) from May 4, 2023 to July 29, 2023. The temperatures in the greenhouse were dependent on the ambient conditions, which fluctuated between 26.0 – 36.0 °C throughout the experiment period. Radiation was provided by sunlight with an average daylight of 12 hours. The experiment was conducted in a greenhouse setting to facilitate precise controlling of soil moisture levels to simulate prolonged and severe drought condition during study.

2.2 Materials, Seed Germination and Plant Cultivation

The *Brassica rapa* subsp. *chinensis* seeds (i.e. variety Alicious-211 F1A) and a commercial biostimulant (i.e. Axta Roots) used in this study were obtained from Advansia Seeds and Biotech Pte Ltd (Malaysia). The biostimulant contains high quality humic acids (13.20% w/v) and fulvic acids (3.30% w/v). The seeds were imbibed in water for two hours prior to germination in a medium containing mixture of compost soil and peat moss (1:3). The seedlings were grown in germination trays for seven days and watered daily before being transplanted into experimental planting pots containing 1.5 kg of compost soil and peat moss (3:1). The water-holding capacity of the planting medium was determined to be approximately 60.0 ± 1.0%. Watering was performed manually and all plants in the pots were well watered before the drought assay. The normal soil moisture level was set at 30.0 ± 2% and monitored daily using a YY-1000 soil moisture meter. A total of 60 plants were cultivated for 48 days under normal moisture level prior to the drought assay.

2.3 Grouping of Plants and Treatments

The plants were divided into two groups, a control group (i.e. NT10R1, n=20), which was not treated with Axta Roots Humic and Fulvic acid biostimulant (ARHFB), and a treatment group (i.e. AR10R1, n=20), which was treated with ARHFB. For the application of ARHFB, the Axta Roots stock solution was diluted with tap water to a concentration of 0.40% (v/v) following manufacturer's instruction, and 30ml was applied directly to the soil of each AR10R1 plant once on days 40, 42, 44, 46 and 48 prior to the drought experiment. To ensure the consistency of plant's responses to the treatment, ARHFB was applied consistently at 9.00 in the morning

across the five treatment days. The ARHFB treatment was halted once drought experiment started on day 49. The average cost of ARHFB application for one plant is approximately US\$0.004 (detailed cost calculation is provided in Appendix A). A separate set of 20 plants grown under normal watering and without Axta Roots application were included in this experiment to ensure the variations observed in the growth parameters and drought responses of the control and treatment groups were not caused by condition in the greenhouse. All the plants were arranged in randomized block design to minimize uncontrolled variability that could obscure treatment effects.

2.4 Drought Stress Experiment

To evaluate the growth, survival rate and the recovery rate of the plants under drought stress, the experiment was divided into two phases, (a) a drought phase and (b) a rehydration phase. During the drought phase, all 40 plants were subjected to a simulated drought condition by lowering and maintaining the soil moisture content at $10.0 \pm 2.0\%$ for a consecutive of 20 days (from day 49 to 68). A soil moisture content of 10% is categorized as severe drought (Aliarab et al., 2020), while a persistent and prolonged drought is generally defined by 20 consecutive days of inadequate rainfall and irregular precipitation (Liu et al., 2021). Watering was carried out manually, and the moisture level of the soil in all experimental pots was monitored daily using the YY-1000 soil moisture meter. The data of above-ground growth parameters, including plant height, leaf length, leaf width, number of green leaves and number of surviving plants were recorded daily throughout the drought phase. At the end of the drought experiment, three surviving plants from each group were selected randomly for the observation of below-ground morphology (i.e. root). The root wet weight was measured using a Mettler Toledo laboratory balance; while the dry weight was determined by oven-drying the sample at 70°C for approximately 34 hours or until a constant dry weight was reached (Coves et al., 2023).

2.5 Post-Drought Rehydration Experiment

The remaining surviving plants were subsequently subjected to a 15-day rehydration phase (days 69 - 83) by restoring the soil moisture to a normal level ($30 \pm 2\%$). The inclusion of a post-drought rehydration phase in the experimental design allowed the comparison of plant recovery rates between the treatment (AR10R1) and control (NT10R1) groups. All surviving plants were harvested after the rehydration phase was complete. The data of plant growth metrics including plant height, leaf length, leaf width, number of leaves, number of wilted leaves, number of surviving plants, plant weight, shoot weight, root weight, and longest root length were recorded. The dry weights of all harvested plants were determined by oven-drying method.

2.6 Statistical Analysis

An independent t-test ($P < 0.05$) was performed to compare the mean values of each growth parameter between the treatment group (AR10R1) and the control group (NT10R1), following confirmation of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). The non-parametric test (Mann-Whitney U test) was used to compare the distributions of the two groups when data deviated from a normal distribution (Perme et al., 2018). The plant survival and recovery rates of each group were calculated after the drought phase and rehydration phase, respectively (formulas are provided in Appendix B).

3. RESULT AND DISCUSSION

3.1 Comparison of Plant Growth Between the AR10R1 and NT10R1 Groups Under a Simulated Drought Condition

Our results demonstrate significant positive effects of ARHFB application on bok choy plants exposed to prolonged severe drought condition. The treatment group (AR10R1) outperformed the control group (NT10R1) in all aerial plant growth metrics including the height, leaf width and length, and number of green leaves (Figure 1, Supplementary Table 1). The NT10R1 group suffered more substantial reduction in plant growth. For instance, between Day-1 and Day-20 of drought phase, the mean heights of NT10R1 and AR10R1 plants decreased by 55.3% and 44.2%, respectively, indicating greater loss of turgidity and more severe retardation in the NT10R1 plants. Statistical analysis showed significant differences ($p < 0.05$) in growth reduction between the two groups (Supplementary Table 2), confirming the positive effect of ARHFB application in sustaining the above ground growth of AR10R1 plants during drought. The complete datasets of plant height, leaf length and width, and number of leaves recorded from each individual plant throughout the drought phase are provided in Supplementary Table 3.

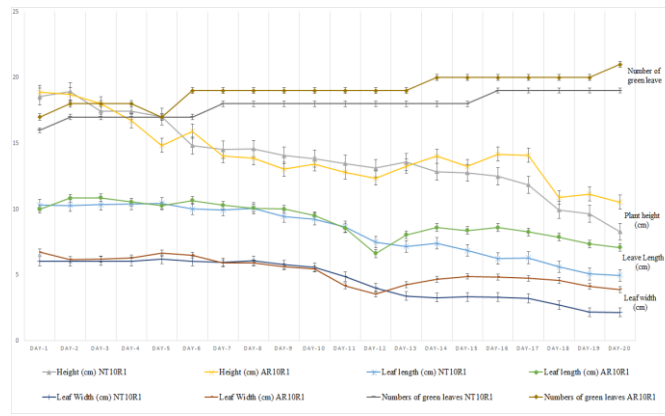


Figure 1: Comparison of aerial plant growth performances between the AR10R1 and NT10R1 groups throughout the 20-day drought phase.

At the end of the 20 days drought experiment, both groups had the same number of surviving plants (n=19) remained. Nonetheless, substantial differences in morphology and overall health conditions were evident between the two groups. Despite having the same number of surviving plants, the NT10R1 group showed more noticeable drought-induced injuries, such as browning of leaves and stems, more severe wilting and smaller leaves, and shorter stature (Figure 2(A) and Supplementary Table

1). The NT10R1 group also displayed a significantly higher (p-value = 0.018) average count of wilted leaves (n=14), in contrast to AR10R1 group (n=11) on the final day of the drought experiment. The AR10R1 plants were affected by the drought-induced stress to a lesser extent and exhibited better overall health, less browning, having more green leaves and taller stature relatively (Figure 2(B) and Supplementary Table 1).

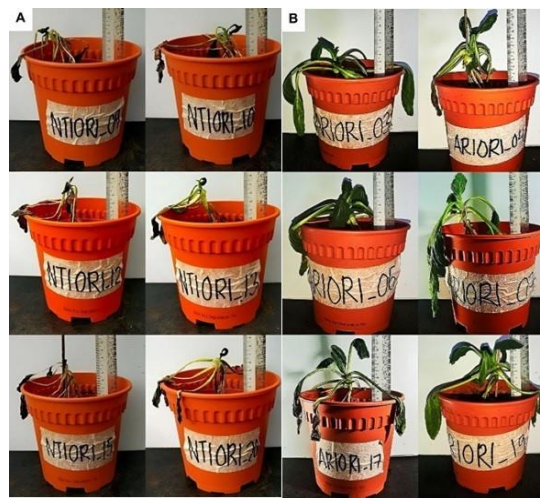


Figure 2: Morphological conditions of NT10R1 (control) and AR10R1 (treatment) plants on the final day of the 20-day drought experiment. (A) NT10R1 plants (left panel) and (B) AR10R1 plants (right panel), showed pronounced differences in appearance and overall health status.

Out of the 19 surviving plants in each group, three plants were randomly selected and deracinated at the end of the drought experiment to compare the root growth. Morphological observations on the root organs of the two groups revealed a denser network of lateral roots in the AR10R1 group (Figure 3(A)) compared to the NT10R1 group (Figure 3(B)). The AR10R1 group exhibited a more developed lateral root system, with the heaviest

root wet weight weighing 5.6 g compared to 2.8 g in the NT10R1 group. The AR10R1 plants also displayed a thicker primary root than the NT10R1 group (based on crude observation). These results suggest that the application of ARHFB prior to drought assay might have stimulated root development in AR10R1 group. However, there were no significant statistical differences in the root dry weights.

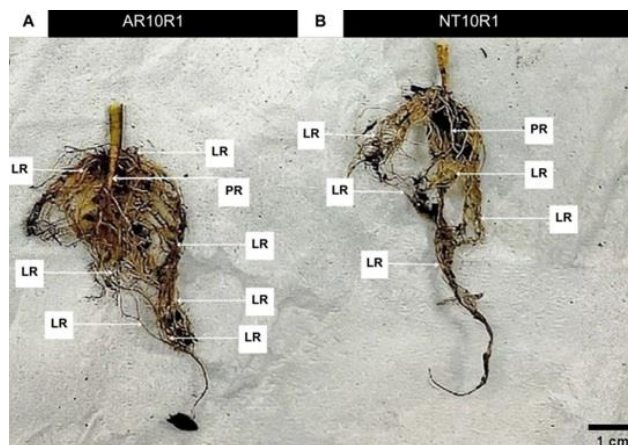


Figure 3: Root organs of AR10R1 (A) and NT10R1(B) plants after the 20-day drought experiment, showing the root network, primary root (PR) and the lateral root (LR).

Humic and fulvic acids are the key components of soils and organic materials that can promote plant growth by improving cell membrane permeability, photosynthesis, and nutrient uptake (Shen et al., 2020; Mindari et al., 2024). Acting as exogenous growth hormones, they mimic

the action of auxins to regulate plant growth by stimulating plasma membrane (PM) H⁺-ATPase and nitric oxide (NO) production, both of which are essential for promoting growth (Nardi, 2021; Moura et al., 2023). As evidenced in our study, the AR10R1 plants were able to maintain

better growth than the NT10R1 plants throughout the 20-day drought phase. Drought stress, often manifests as browning and severe wilting of leaves due to chlorophyll degradation, can significantly disrupt photosynthesis, a vital process for plant survival. Chlorophyll degradation reduces photosynthetic efficiency and compromises chloroplast function (Anjum et al., 2011; Shen et al., 2020). In addition, drought can alter the chlorophyll a/b ratio and deplete carotenoids, further hindering photosynthesis and transpiration due to limited carbon dioxide uptake and stomatal closure (Yang et al., 2021). Previous studies have revealed the potential roles of humic and fulvic acids in preserving chlorophyll content in some crops under drought such as millet (Shen et al., 2020). Humic acids was also demonstrated to be effective in mitigating the adverse effects of drought on chlorophyll content, as shown by its ability to increase the chlorophyll content in both maize and sorghum plants under drought (Ria et al., 2024). On the other hand, fulvic acids can increase chlorophyll concentration and enhance drought resilience by promoting photosynthesis and boosting antioxidant defences, as shown in crops such as in tea plants and maize (Qiu et al., 2021; Anjum et al., 2011). These mechanisms likely contributed to the healthier status and more number of green leaves in the AR10R1 plants than the NT10R1 plants during the drought phase.

Drought-induced oxidative stress caused by the accumulation of reactive oxygen species (ROS) can damage cellular components such as the nucleus, proteins, and cell membranes (Rehman et al., 2022). Humic acids help to mitigate this stress by activating antioxidants that neutralize ROS and reduce cellular damage. Humic acid can also improve soil nutrient availability, soil fertility and structure, and aids in water retention (Cordeiro et al., 2011; Campos, 2021). Similarly, fulvic acid alleviates drought symptoms by increasing leaf water content, enhancing the activity of antioxidant enzymes, reducing ROS accumulation, preserving chloroplast and mesophyll cell integrity, and increasing the expression of drought-tolerance genes,

thereby mitigating the adverse effects of drought on plants (Fang et al., 2020). These mechanisms might have diminished drought-induced damage and enhanced the survival of the AR10R1 plants throughout the drought phase in this study.

3.2 Comparison of Plant Growth Parameters Between the AR10R1 and NT10R1 Groups During and After a 15-Day Rehydration Phase

The AR10R1 plants and NT10R1 plants displayed remarkable differences in their recovery rates after a 15-day of rehydration treatment. At the end of the rehydration phase, only one plant (6.25%) out of 16 NT10R1 plants survived, while 10 plants (62.5%) in the AR10R1 group recovered successfully (Figure 4). This represents a ten-fold improvement in recovery for the treated group. Statistical analysis revealed a significantly higher recovery rate in the AR10R1 group ($p < 0.001$) compared to the NT10R1 group. Majority of the NT10R1 plants failed to recover even after the soil moisture was restored to normal level ($30.0 \pm 2\%$) for 15 days, suggesting that the drought-induced damage was irreparable in most NT10R1 plants without the ARHFB treatment.

The median survival values further exemplify the disparity between the two groups. The NT10R1 group had a median survival of two plants, while the AR10R1 group had a median survival of 13 plants. In terms of temporal patterns, it was observed during the early phase (Days 1-5), the NT10R1 group suffered a steep decline from 16 to 6 plants, whereas the AR10R1 group experienced minimal loss, retaining 14 plants. During the mid-phase (Days 6-10), the NT10R1 group continued to decline with only 2 plants surviving on Day-10; while the AR10R1 group exhibited a gradual reduction retaining 12 plants. By the late phase (Days 11-15), the NT10R1 group reduced to a single surviving plant on Day-11; whereas the AR10R1 group stabilized at 10 plants on Day-12 demonstrating enhanced recovery from drought-induced damage. These findings highlight a strong positive influence of the humic and fulvic acids biostimulant application on the recovery of bok choy following a prolonged severe drought condition.

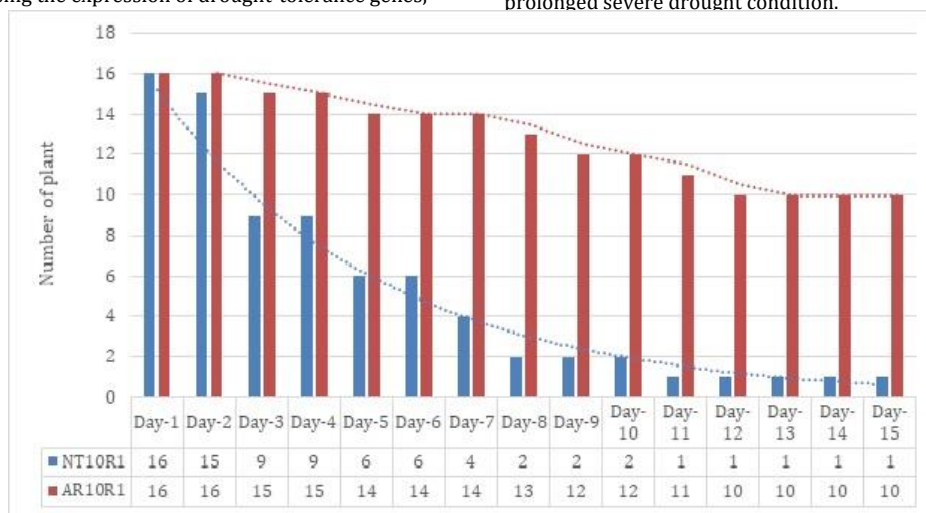


Figure 4: The number of surviving plants in the AR10R1 and NT10R1 groups throughout the 15-day rehydration phase. The NT10R1 plants show a more drastic decrease in the number of surviving plants compared to the AR10R1 plants.

Statistical analysis revealed significant differences ($p < 0.05$) in all above-ground growth parameters of the surviving plants (NT10R1, $n=1$; AR10R1, $n=10$) in the two groups after a 15-day of rehydration (Table 1). Notable differences in the plant heights, leaf sizes, wet and dry shoot weights between the two groups indicate greater turgidity and biomass recovery in the AR10R1 plants compared to the NT10R1 plant. For instance, the shoot mean wet and dry weights of the AR10R1 plants were 11x and three times heavier than that of the NT10R1 plant, respectively. The AR10R1 plants were also more than twice as tall as the NT10R1 plant. In addition, plants in the AR10R1 group exhibited better morphology condition compared to the NT10R1 plant (Figure 5). The NT10R1 plant showed poor recovery with nearly all wilted and brownish colour leaves (Figure 5(A)); while the AR10R1 plant showed

satisfactory recovery and appeared healthier with greener and larger leaves (Figure 5(B)). The recovery of crops after drought stress is an aspect that has been largely overlooked in previous studies, but our study has demonstrated a direct correlation between the ARHFB treatment and post-drought recovery in bok choy. Furthermore, since the ARHFB was only applied to the experimental plants prior to the drought assay, this demonstrated that the biostimulant has a long-lasting effect as it enhanced survival of the plants during the 20-day drought phase and their recovery during the 15-day rehydration phase. The complete set of plant height, leaf length and width, and number of leaves data collected from each individual plant throughout the rehydration phase is provided in Supplementary Table 3.

Table 1: The mean values of above-ground growth data for NT10R1 and AR10R1 plants after 15 days of rehydration phase, and the corresponding P -values.

Group	Plant Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Number of leaves	Shoot Wet Weight (g)	Shoot Dry Weight (g)
NT10R1 (n=1)	7.50	5.50	2.50	19	1.25	0.435

Table 1 (cont): The mean values of above-ground growth data for NT10R1 and AR10R1 plants after 15 days of rehydration phase, and the corresponding *P*-values.

AR10R1 (n=10)	16.13	9.69	6.06	24	14.00	1.506
<i>P</i>-value	<0.001	0.009	0.003	0.026	0.020	0.004

Note: n = The number of surviving plants in each group



Figure 5: Comparison of plant conditions between the NT10R1 and AR10R1 groups after 15 days of rehydration phase: (A) the sole surviving NT10R1 plant in pot and the whole plant after uprooting, and (B) one representative of surviving AR10R1 plants in pot and the whole plant after uprooting.

Minimal disparity was observed in the longest root length and root dry weight between the two groups (Table 2). Both parameters showed no significant differences statistically. Nonetheless, qualitative observation revealed that the AR10R1 plants (Figure 6B and 6D) displayed a noticeably denser root network compared to the NT10R1 plant (Figure 6A and 6C). This observation was supported by the root wet weight measurements,

where AR10R1 plants displayed a mean root wet weight approximately four times heavier than the NT10R1 plant though it was not significant statistically. This finding suggests that the application of ARHFB stimulated roots proliferation and increased the root biomass in the AR10R1 plants, which might have enhanced their recovery during the rehydration phase.

Table 2: The mean values of root length, root wet and dry weights of the NT10R1 and AR10R1 groups after 15 days of rehydration phase.

Group	Mean Longest Root Length (cm)	Mean Root Weight (g)	
		Dry	Wet
NT10R1 (n=1)	5.80	0.030	0.090
AR10R1 (n=10)	6.60	0.097	0.400
<i>P</i>-Value	0.712	0.067	0.023

Note: n = The number of surviving plants in each group

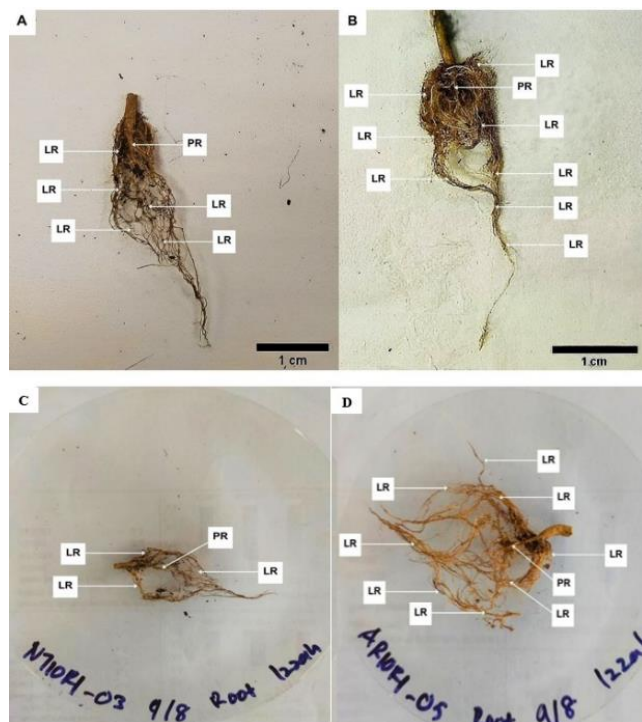


Figure 6: The wet and dry root biomass of NT10R1 and AR10R1 plants after 15 days of rehydration, showing the primary root (PR) and lateral root (LR). Root organs of NT10R1(A) and AR10R1(B), before drying; Dried root biomass of NT10R1(C) and AR10R1 (D), after drying.

Plant resilience to drought involves not only surviving during drought but also effective recovery after drought. Drought-induced oxidative stress is characterized by the accumulation of ROS such as O_2^- , H_2O_2 and $-OH$ radicals, which causes membrane damage, increased lipid peroxidation, enzyme deactivation and nucleic acid damage, leading to cell death (Anjum et al., 2011; Zykova et al., 2018). The AR10R1 plants displayed a significantly higher recovery rate than the NT10R1 group, accentuating the effectiveness of humic and fulvic acid-based application in enhancing the recovery of leafy vegetables after drought. In contrast, the NT10R1 group, which suffered greater damage during drought had a significantly lower recovery rate. We speculate that the application of ARHFB efficiently reduced the severity of drought-induced damages in the AR10R1 plants by improving their ROS scavenging capacity, and acting as antioxidants to neutralize excessive ROS, thus substantially increased their recovery. Humic acids act as electron donors or acceptors can increase peroxidase activity and cell proline levels, and decrease H_2O_2 levels, thereby maintaining redox homeostasis and preventing oxidative damage (Silva et al., 2023). While, fulvic acids improve the scavenging capacity of ROS in plants and serve as antioxidants to neutralize excessive ROS accumulation and/or act as signaling molecules to promote the production of endogenous antioxidants (Wang et al., 2019, Fang et al., 2020).

The health and survival of plants are closely linked to their root system, which plays a crucial role in nutrient and water uptake (Kalra, 2023). Roots are the most drought-sensitive plant organ that respond directly to changes in soil conditions. Drought impedes root growth, causing symptoms such as abnormal root elongation, thinning of lateral roots, shortening of root life span, and increased cell death, which directly impairing the plant's ability to uptake water (Xiao et al., 2020; Kang, 2022). This is evidenced in the thinner root mass observed in the NT10R1 plants in our study. Well-developed roots improve a plant's ability to absorb and utilize water from the soil, thus improving its resilience to drought stress (Gowda et al., 2011). Humic and fulvic acids can stimulate the development of roots indirectly by improving the mineral supply in soil (Shah et al., 2018). Humic acids were found to stimulate lateral root formation and overall root development by modulating auxin and cytokinin biosynthesis, resulting in an increase in root length, surface area and mass in wheat (Rathor et al., 2024). Humic acids can also improve nutrient transfer, increase antioxidant levels and promotes root growth under limited water conditions (Zhang et al., 2021). While Yu et al. (2023) revealed that fulvic acids stimulate root elongation and optimize root morphology, which improves nitrogen uptake in apple trees. Fulvic acids also promote root growth in germinated seeds and increase both the root length and number of lateral root tips in hydroponic tomato plants (Zhang et al., 2021). Consistent with previous findings, the increase in root biomass in our study is likely one of the key factors that enhanced the survival and recovery of the AR10R1 plants during drought and rehydration phases.

The ten-fold improvement of post-rehydration recovery rate observed in AR10R1 plants signifies successful mitigation of drought-induced yield losses in bok choy.

Leafy vegetables are highly susceptible to drought due to their large surface area, leading to high transpiration rate and water loss. They require significant amount of water intake to maintain large leaf structures for optimal photosynthesis activity. Consequently, drought stress can impact the development and function of leaves, reduced photosynthetic efficiency and yield in leafy vegetables (Abbas et al., 2023; Li et al., 2023). Hence, innovative solutions are necessary to enhance the resilience of leafy vegetables, particularly under prolonged and severe drought stress. However, not only the enhancement of drought resilience in leafy crops that requires immediate attention, the sustainability of the proposed solutions must also be emphasized to minimize the negative impact of agriculture on the environment facing climate change. In this regard, humic and fulvic acids-based plant biostimulant is a potential sustainable solution to enhance leafy crop resilience to abiotic stress.

4. CONCLUSIONS

The application of humic and fulvic acids-based biostimulants significantly enhanced the survival, recovery and resilience of bok choy under prolonged severe drought. The higher post-drought and post-rehydration overall above- and below-ground biomass observed in the AR10R1 plants compared to the NT10R1 plants supports benefits to the entire plant. Our findings support the adoption of humic and fulvic acid biostimulant is a useful strategy to tackle drought-related challenges in leafy crop cultivation. We believe the integration of humic and fulvic acids-based biostimulant in agriculture is a practical, affordable and sustainable adaptive agronomic strategy to enhance resilience and ensure long-term yield stability of leafy vegetables under drought stress. Nonetheless, field trial is necessary to validate their effectiveness, and studies to understand the molecular and physiological changes that drive these positive responses need to be conducted.

ACKNOWLEDGMENTS

We thank Miss Nurul Asnaashilla Binti Shaari and Mr. Muhammad Irfan Bin Mohamad Nadzim for their assistance in data collection and plant harvesting. We also thank Dr. Benjamin Lau for proofreading the manuscript.

FUNDING

This work was supported by Advansia Seeds and Biotech Pte Ltd.

REFERENCES

- Abbas, K., Li, J., Gong, B., Lu, Y., Wu, X., Lü, G., and Gao, H., 2023. Drought stress tolerance in vegetables: The functional role of structural features, key gene pathways, and exogenous hormones. *Int. J. Mol. Sci.* 24(18), 13876. <https://doi.org/10.3390/ijms241813876>.
- Adunya, T., and Benti, F., 2020. The impacts of climate-induced agricultural drought on four cereal crops: A case study in Bako Tibe District, Oromia National Regional State, Ethiopia. *Caraka Tani: Journal of Sustainable Agriculture.* 35(1), 135–146. <http://dx.doi.org/10.20961/carakatani.v35i1.35749>.
- Aliarab, A., Vazifekah, E.O., and Sadati, S.E., 2020. Effect of soil moisture content and nitrogen fertilizer on survival, growth and some physiological characteristics of *Platycladus orientalis* seedlings. *J. For. Sci.* 66(12), 511–523. <https://doi.org/10.17221/34/2020-JFS>.
- Alsudays, I.M., Alshamary, F.H., Alabdallah, N.M., Alatawi, A., Alotaibi, M.M., Alwutayd, K.M., Alharbi, M.M., Alghanem, S.M.S, Alzuibr, F.M., Gharib, H.S., and Allah, M.M.A.A., 2024. Applications of humic and fulvic acid under saline soil conditions to improve growth and yield in barley. *BMC Plant Biol.* 24(1), 191. <https://doi.org/10.1186/s12870-024-04863-6>.
- Amin, A.Q.A., and Alam, G.M., 2016. Impact of El-Niño on agro-economics in Malaysia and the surrounding regions: An analysis of the events from 1997–98. *Asian Journal of Earth Sciences.* 9(1), 1–8. <https://doi.org/10.3923/AJES.2016.1.8>.
- Anjum, S.A., Wang, L., Farooq, M., Xue, L., and Ali, S., 2011. Fulvic acid application improves the maize performance under well-watered and drought conditions. *Journal of Agronomy and Crop Science.* 197(6), 409–417. <https://doi.org/10.1111/j.1439-037X.2011.00483.x>.
- Aytaç, E., Ünlü, H.Ö., Erkan, İ.E., and Akçay, U.Ç., 2024. Humic acid mitigates drought stress in tomato. *Bilge International Journal of Science and Technology Research.* 8(1), 27–37. <https://doi.org/10.30516/bilgesci.1421304>.
- Bayat, H., Shafie, F., Aminifard, M.H., and Daghighi, S., 2021. Comparative effects of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (*Achillea millefolium* L.). *Scientia Horticulturae.* 279(1), 109912. <https://doi.org/10.1016/j.scienta.2021.109912>.
- Brodersen, C.R., Roddy, A.B., Wason, J.W., and McElrone, A.J., 2019. Functional status of xylem through time. *Annu Rev Plant Biol.* 70(1), 407–433. <https://doi.org/10.1146/annurev-arplant-050718-100455>.
- Campos, R.H., Robles, C., and García, A.C., 2021. Humic acids effects on plant growth and protection against water stress in selected native maize populations from Mexico. *Rev. Fitotec. Mex.* 44(4), 561–569.
- Castiglione, A.M., Mannino, G., Contartese, V., Bertera, C.M., and Ertani, A., 2021. Microbial biostimulants as response to modern agriculture needs: Composition, role and application of these innovative products. *Plants.* 10(8), 1533. <https://doi.org/10.3390/plants10081533>.
- Cordeiro, F.C., Catarina, C.S., Silveira, V., and Souza, S.R.D., 2011. Humic acid effect on catalase activity and the generation of reactive oxygen species in corn (*Zea mays*). *Biosci Biotechnol Biochem.* 75(1), 70–74. <https://doi.org/10.1271/bbb.100553>.
- Coves, S., Soengas, P., Velasco, P., Fernández, J.C., and Cartea, M.E., 2023. New vegetable varieties of *Brassica rapa* and *Brassica napus* with modified glucosinolate content obtained by mass selection approach. *Front. Nutr.* 10. <https://doi.org/10.3389/fnut.2023.1198121>.
- Economic and Social Commission for Asia and the Pacific, 2020. Ready for the dry years: Building resilience to drought in Southeast Asia (accessed 20 August 2024).
- European Biostimulants Industry Council (EBIC), 2019. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 (accessed 11 August 2024).
- Fang, Z., Wang, X., Zhang, X., Zhao, D., and Tao, J., 2020. Effects of fulvic acid on the photosynthetic and physiological characteristics of *Paeonia ostii* under drought stress. *Plant Signaling and Behavior.* 15(7), 1774714. <https://doi.org/10.1080/15592324.2020.1774714>.
- Food and Agriculture Organization of the United Nations (FAO), 2025. Global information and early warning system on food and agriculture (accessed 28 February 2025).
- Francesca, S., Cirillo, V., Raimondi, G., Maggio, A., Barone, A., and Rigano, M.M., 2021. A novel protein hydrolysate-based biostimulant improves tomato performances under drought stress. *Plants.* 10(4), 783. <https://doi.org/10.3390/plants10040783>.
- Franzoni, G., Cocetta, G., Prinsi, B., Ferrante, A., and Espen, L., 2022. Biostimulants on crops: Their impact under abiotic stress conditions. *Horticulturae.* 8(3), 189. <https://doi.org/10.3390/horticulturae8030189>.
- Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E., and Serraj, R., 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research.* 122(1), 1–13. <https://doi.org/10.1016/j.fcr.2011.03.001>.
- Ibrahim, E.A., Ebrahim, N.E.S., and Mohamed, G.Z., 2024. Mitigation of water stress in broccoli by soil application of humic acid. *Scientific Reports.* 14(1), 2765. <https://doi.org/10.1038/s41598-024-53012-4>.
- Jacomassi, L.M., Viveiros, J.D.O., Oliveira, M.P., Momesso, L., Siqueira, G.F.D., and Crusciol, C.A.C., 2022. A seaweed extract-based biostimulant mitigates drought stress in sugarcane. *Front. Plant Sci.* 13. <https://doi.org/10.3389/fpls.2022.865291>.
- Kalra, A., Goel, S., and Elias, A.A., 2023. Understanding role of roots in plant response to drought: Way forward to climate-resilient crops. *Plant Genome.* 17(1), e20395. <https://doi.org/10.1002/tpg2.20395>.
- Kang, J., Peng, Y., and Xu, W., 2022. Crop root responses to drought stress: Molecular mechanisms, nutrient regulations, and interactions with microorganisms in the rhizosphere. *Int. J. Mol. Sci.* 23(16), 9310. <https://doi.org/10.3390/ijms23169310>.
- Khor, J.F., Ling, L., Yusop, Z., Chin, R.J., Lai, S.H., Kwan, B.H., and Ng, D.W.K., 2023. Impact comparison of El Niño and ageing crops on Malaysian oil palm yield. *Plants.* 12(3), 424. <https://doi.org/10.3390/plants12030424>.
- Khor, J.F., Ling, L., Yusop, Z., Tan, W.L., Ling, J.L., and Soo, E.Z.X., 2021. Impact of El Niño on oil palm yield in Malaysia. *Agronomy.* 11(11), 2189. <https://doi.org/10.3390/agronomy11112189>.
- Li, J., Abbas, K., Wang, L., Gong, B., Hou, S., Wang, W., Dai, B., Xia, H., Wu, Z., Lu, G., and Gao, H., 2023. Drought resistance index screening and evaluation of lettuce under water deficit conditions on the basis of morphological and physiological differences. *Front. Plant Sci.* 14, 1228084. <https://doi.org/10.3389/fpls.2023.1228084>.
- Liu, B., Liu, Y., Wang, W., and Li, C., 2021. Meteorological drought events and their evolution from 1960 to 2015 using the daily SWAP index in Chongqing, China. *Water.* 13(14), 1887. <https://doi.org/10.3390/w13141887>.
- Luhaim, Z., Tan, M.L., Tanggang, F., Zulkafli, Z., Chun, K.P., Yusop, Z., and Yaseen, Z.M., 2021. Drought variability and characteristics in the Muda River Basin of Malaysia from 1985 to 2019. *Atmosphere.* 12(9), 1210. <https://doi.org/10.3390/atmos12091210>.
- Mindari, W., Chakim, M., Widjajani, B., Sasongko, P., Aditya, H., Pazi, A., and Gandaseca, S., 2024. The optimization of biosilica and humic acid to increase soil nutrient availability and nutrient uptake in rice plant in sandy soil. *Caraka Tani: Journal of Sustainable Agriculture.* 40(1), 18–33. <https://doi.org/10.20961/carakatani.v40i1.89018>.
- Moura, O.V.T.D., Berbara, R.L.L., Torchia, D.F.D.O., Silva, H.F.O.D., Castro, T.A.V.T.D., Tavares, O.C.H., Rodrigues, N.F., Zonta, E., Santos, L.A., and Garcia, A.C., 2023. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops: A review. *Journal of the Saudi Society of Agricultural Sciences.* 22(8), 493–513. <https://doi.org/10.1016/j.jssas.2023.05.001>.
- Nardi, S., Schiavon, M., and Francioso, O., 2021. Chemical structure and biological activity of humic substances define their role as plant growth promoters. *Molecules.* 26(8), 2256. <https://doi.org/10.3390/molecules26082256>.
- Papa, S., Fusco, G.M., Ciriello, M., Formisano, L., Woo, S.L., Pascale, S.D., Rouphael, Y., and Carillo, P., 2022. Microbial and non-microbial

- biostimulants as innovative tools to increase macro and trace element mineral composition of tomato and spinach. *Horticulturae*. 8(12), 1157. <https://doi.org/10.3390/horticulturae8121157>.
- Perme, M.P., and Manevski, D., 2018. Confidence intervals for the Mann-Whitney test. *Stat Methods Med Res*. 28(12), 3755-3768. <https://doi.org/10.1177/0962280218814556>.
- Qiu, C., Sun, J., Shen, J., Zhang, S., Ding, Y., Gai, Z., Fan, K., Song, L., Chen, B., Ding, Z., and Wang, Y., 2021. Fulvic acid enhances drought resistance in tea plants by regulating the starch and sucrose metabolism and certain secondary metabolism. *Journal of Proteomics*. 247, 104337. <https://doi.org/10.1016/j.jpro.2021.104337>.
- Rathor, P., Upadhyay, P., Ullah, A., Gorim, L.Y., and Thilakarathna, M.S., 2024. Humic acid improves wheat growth by modulating auxin and cytokinin biosynthesis pathways. *AoB Plants*. 16(2), plae018. <https://doi.org/10.1093/aobpla/plae018>.
- Rehman, T., Tabassum, B., Yousaf, S., Sarwar, G., and Qaisar, U., 2022. Consequences of drought stress encountered during seedling stage on physiology and yield of cultivated cotton. *Front. Plant Sci*. 13, 906444. <https://doi.org/10.3389/fpls.2022.906444>.
- Ria, M.E.A., Elghareeb, E.M., Shukry, W.M., Hamed, S.A.A., and Ibraheem, F., 2024. Mitigation of drought stress in maize and sorghum by humic acid: Differential growth and physiological responses. *BMC Plant Biol*. 24(1), 514. <https://doi.org/10.1186/s12870-024-05184-4>.
- Seleiman, M.F., Suhaibani, N.A., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Wajid, H.H.A., and Battaglia, M.L., 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*. 10(2), 259. <https://doi.org/10.3390/plants10020259>.
- Shah, Z.H., Rehman, H.M., Akhtar, T., Alsamadany, H., Hamooh, B.T., Mujtaba, T., Daur, I., Zahrani, Y.A., Alzahrani, H.A.S., Ali, S., Yang, S.H., and Chung, G., 2018. Humic substances: Determining potential molecular regulatory processes in plants. *Front. Plant Sci*. 9, 263. <https://doi.org/10.3389/fpls.2018.00263>.
- Shen, J., Guo, M.J., Wang, Y.G., Yuan, X.Y., Wen, Y.Y., Song, X.E., Dong, S.Q., and Guo, P.Y., 2020. Humic acid improves the physiological and photosynthetic characteristics of millet seedlings under drought stress. *Plant Signal Behav*. 15(8), 1774212. <https://doi.org/10.1080/15592324.2020.1774212>.
- Silva, R.M.D., Canellas, N.A., Olivares, F.L., Piccolo, A., and Canellas, L.P., 2023. Humic substances trigger plant immune responses. *Chem. Biol. Technol. Agric*. 10, 123. <https://doi.org/10.1186/s40538-023-00468-7>.
- Sun, J., Qiu, C., Ding, Y., Wang, Y., Sun, L., Fan, K., Gai, Z., Dong, G., Wang, J., Li, X., Song, L., and Ding, Z., 2020. Fulvic acid ameliorates drought stress-induced damage in tea plants by regulating the ascorbate metabolism and flavonoids biosynthesis. *BMC Genomics*. 21, 411. <https://doi.org/10.1186/s12864-020-06815-4>.
- Wang, Y., Yang, R., Zheng, J., Shen, Z., and Xu, X., 2019. Exogenous foliar application of fulvic acid alleviate cadmium toxicity in lettuce (*Lactuca sativa* L.). *Ecotoxicol Environ Saf*. 167, 10-19. <https://doi.org/10.1016/j.ecoenv.2018.08.064>.
- World Health Organization, 2023. El Niño Southern Oscillation (ENSO) (accessed 16 August 2024).
- Xiao, S., Liu, L., Zhang, Y., Sun, H., Zhang, K., Bai, Z., Dong, H., and Li, C., 2020. Fine root and root hair morphology of cotton under drought stress revealed with RhizoPot. *J. Agro. Crop. Sci*. 206(6), 679-693. <https://doi.org/10.1111/jac.12429>.
- Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., and Chen, S., 2021. Response mechanism of plants to drought stress. *Horticulturae*. 7(3), 50. <https://doi.org/10.3390/horticulturae7030050>.
- Yu, B., Wang, L., Cui, D., Gao, W., Xue, X., and Nie, P., 2023. Effects of fulvic acid on growth and nitrogen utilization efficiency in M9T337 seedlings. *Plants*. 12, 3937. <https://doi.org/10.3390/plants12233937>.
- Zhang, P., Zhang, H., Wu, G., Chen, X., Gruda, N., Li, X., Dong, J., and Duan, Z., 2021. Dose-dependent application of straw-derived fulvic acid on yield and quality of tomato plants grown in a greenhouse. *Front. Plant. Sci*. 12, 736613. <https://doi.org/10.3389/fpls.2021.736613>.
- Zykova, M.V., Schepetkin, I.A., Belousov, M.V., Krivoshchekov, S.V., Logvinova, L.A., Bratishko, K.A., Yusubov, M.S., Romanenko, S.V., and Quinn, M.T., 2018. Physicochemical characterization and antioxidant activity of humic acids isolated from peat of various origins. *Molecules*. 23(4), 753. <https://doi.org/10.3390/molecules23040753>.

